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IN VITRO PHARMACOLOGICAL PROPERTIES AND COMPOSITION OF LEAF ESSENTIAL OILS AND EXTRACTS OF SELECTED INDIGENOUS *PELARGONIUM* (GERANIACEAE) SPECIES

Jacqueline Yolande Yvette Lalli

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Pharmacy.

Johannesburg, 2005.

DECLARATION

I, Jacqueline Yolande Yvette Lalli declare that this dissertation is my own work. It is being submitted for the degree of Master of Pharmacy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature: .....

..... day of ....., 2006.

DEDICATION

To my parents

Pier-Antonio and Patrizia Lalli.

For all the sacrifices you have made in life,

I hope this work can be a token of my deep appreciation.

‘*You must understand the whole of life, not just one little part of it.*

That is why you must read, that is why you must look at the skies,

that is why you must sing and dance, and write poems, and suffer,

and understand, for all that is life.’

J. Krishnamurti

PRESENTATION

Jacqueline Y. Lalli, Alvaro M. Viljoen, Sandy F. van Vuuren, Hüsnü C. Başer. 2004. Aromatic Pelargoniums – their Essential Oils and Pharmacological Properties. Podium presentation at the Botany Symposium, University of Johannesburg, South Africa (Abstract, see Appendix B).

ABSTRACT

Despite commercial interest and ethnobotanical data, the chemical composition and pharmacological activities of a number of indigenous *Pelargonium* species remain unexplored. Twenty-one *Pelargonium* species, from the section *Pelargonium*, were included in this study.

The volatile compounds of 13 species were extracted by hydrodistillation and their chemical compositions determined by gas chromatography coupled to mass spectroscopy (GC-MS). The essential oil data was chemotaxonomically informative confirming taxonomic relationships between *P. graveolens* and *P. radens; P. papilionaceum* and *P. vitifolium* and between *P. panduriforme* and *P. quercifolium.* New chemical affinities were established among *P. betulinum, P. hispidum* and *P. scabrum*; *P. capitatum* (provenance WSBG), *P. glutinosum* and *P. quercifolium* (provenance SBG) and among *P. graveolens*, *P. radens* and *P. tomentosum*. The non-volatile compounds were extracted with acetone and the extracts were analysed using high performance liquid chromatography (HPLC). The representative flavonoid patterns of the *Pelargonium* species indicated that *P. betulinum*, *P. capitatum*, *P. graveolens*, *P. hispidum, P. panduriforme* and *P. vitifolium* have numerous similarities in their chemical profiles. *Pelargonium scabrum* and *P. sublignosum* share definite chemical patterns. The HPLC fingerprints of *P. papilionaceum* and *P. vitifolium* were chemically diverse.

A microdilution bioassay was performed on the acetone extracts and the essential oils to assess their antimicrobial (both bacterial and fungal) potential. The essential oils and extracts were more selective for the Gram-positive test pathogens than for the Gram-negative bacterium. The crude extracts of *P. glutinosum* (provenance SBG), *P. pseudoglutinosum*, *P. scabrum* and *P. sublignosum* exhibited considerable antimicrobial activity against the Gram-positive bacteria (*B. cereus* and *S. aureus*) with *P. pseudoglutinosum* exerting the highest activity (MIC = 0.039 mg/ml). The essential oils showed reduced antimicrobial activity compared to the plant extracts. Using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, exceptional anti-oxidant activity was observed for the crude extracts of *P. betulinum* and *P. crispum* (IC_{50} values of 4.13 µg/ml and 4.49 µg/ml, respectively, compared to ascorbic acid, IC_{50} = 4.72 µg/ml). The essential oils of *P. quercifolium* showed the greatest inhibition of 5-lipoxygenase activity (IC_{50} = 33.24 µg/ml

- 38.67 µg/ml). The antimalarial activity of the non-volatile extracts was evaluated against the choloroquine-resistant Gambian FCR-3 strain of *Plasmodium falciparum* using the hypoxanthine incorporation assay. *Pelargonium panduriforme* (provenance SBG) exerted the greatest activity (IC_{50} = 1.34 ± 0.29 µg/ml). Other species possessing similarly potent antimalarial activity included *P. citronellum* (provenance NBG), *P. citronellum* (provenance SBG), *P. quercifolium* (provenance SBG) and *P. radens.*

A microculture tetrazolium salt reduction (MTT) assay was used to determine the cellular toxicity of the acetone extracts and essential oils against transformed human kidney epithelium (Graham) cells. The acetone extracts of *P. sublignosum* and *P. citronellum* (provenance NBG) displayed the highest toxicities (IC_{50} = 11.89 ± 1.54 µg/ml and 19.14 ± 0.98 µg/ml, respectively). *Pelargonium vitifolium* (IC_{50} = 178.48 ± 5.44 µg/ml) and *P. tomentosum* (provenance SBG) (IC_{50} = 195.13 ± 7.90 µg/ml) appeared to be non-toxic. The *Pelargonium* essential oils proved to be considerably toxic (IC_{50} ≤0.10 µg/ml - 30.30 ± 1.81 µg/ml).

The flavonoid derivatives detected in the *Pelargonium* acetone extracts may have contributed to their positive biological activities. The results from the MTT assay suggested that the antimicrobial and antimalarial activity of the extracts may be ascribed to general cytotoxic effects. The pharmacological properties manifested by the extracts and essential oils of certain *Pelargonium* species substantiates their use in traditional medicines and validates their commercial exploitation in the perfumery, cosmetic, food and pharmaceutical industries; however, their toxicity profiles must be considered.

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

AIDS: Acquired Immune Deficiency Syndrome

ATCC: American Type Culture Collection

ca.: circa, approximately

CFU: colony forming unit

COX: cyclo-oxygenase

˚C: degrees Celsius

DMSO: dimethyl sulfoxide

DNA: deoxyribonucleic acid

DPPH: 2,2-diphenyl-1-picrylhydrazyl

EDTA: ethylenediaminetetraacetic acid

FCS: foetal calf serum

GC: gas chromatography

GC-MS: gas chromatography-mass spectroscopy

h: hour

HEPES: N-2-hydroxyethyl-piperazine-N′-2-ethane-sulfonic acid

HETE: hydroxyeicosatetraenoic acid

HPETE: hydroperoxyeicosatetraenoic acid

HPLC: high performance liquid chromatography

HPLC-UV: high performance liquid chromatography-ultraviolet

IC_{50}: inhibitory concentration 50%

INT: p-iodo-nitrotetrazolium violet

ISO: International Standards Organisation

KCl: potassium chloride

KH_2PO_4: potassium dihydrogenphosphate

λ: lambda, wavelength

LOX: lipoxygenase

max: maximum

mg: milligram

MIC: minimum inhibitory concentration

min: minutes

ml: millilitre

MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide

m/z: mass-to-charge ratio

µCi: microcurie

µg: microgram

µl: microlitre

µM: micromole

n: number of experimental runs

NaCl: sodium chloride

NADH: nicotinamide adenine dinucleotide

NADPH: nicotinamide adenine dinucleotide phosphate

$NaHCO_3$: sodium hydrogencarbonate/sodium bicarbonate

$Na_2HPO_4.2H_2O$: di-sodium hydrogenphospahte dihydrate

NBG: National Botanical Garden (Kirstenbosch)

NCTC: National Collection of Type Cultures

NHLS: National Health Laboratory Services

nm: nanometre

NSAID: non-steroidal anti-inflammatory drugs

OTU: operational taxonomic unit

PBS: phosphate buffer solution

pH: potential hydrogen

RBC: red blood cell

R_f : retention factor

RNA: ribonucleic acid

rpm: revolutions per minute

RPMI: Roswell Park Memorial Institute

RRI: relative retention indices

SBG: Stellenbosch Botanical Garden

SDH: succinate-dehydrogenase

sp.: species

spp.: species

subsp.: subspecies

suppl.: supplement

syn.: synonym

TLC: thin layer chromatography

UV-VIS: ultraviolet-visible

var.: variant

v/v: volume per volume

WHO: World Health Organization

WSBG: Walter Sisulu Botanical Garden (Johannesburg)

w/v: weight per volume

CHAPTER 1: GENERAL INTRODUCTION

1.1 Plants provide therapeutic benefits

The medical systems of the world have always valued the use of medicinal plants (Heinrich *et al.*, 2004). The plant kingdom has provided therapeutic benefits to man dating back as far as the earliest recorded knowledge obtained from countless prehistoric references from ancient Egypt, Assyria, China and India. Early explorers of the New World documented the first findings of medicinal plants for many parts of the world (Heinrich *et al.*, 2004).

Throughout human evolution, religious practices and rituals have influenced the treatment of disease and the use of plants (Lawless, 1995). The unwritten purposes for which medicinal plant substances were used by the ancients are analogous to the purposes for which they are utilized nowadays; we have managed to decipher the secret *modus operandi* of Nature. Today, knowledge regarding the biosynthesis and biological effects of secondary plant metabolites has broadened extensively (Raskin *et al*., 2002).

Bioscientific studies of the virtues and chemical properties of indigenous remedies used by native people throughout the world have resulted in the production of many therapeutic agents used in modern, conventional medicine (Heinrich *et al.*, 2004). Today, opium from *Papaver somniferum* is used as an analgesic, aspirin compounds from *Salix nigra* are used for their analgesic, antipyretic and anti-inflammatory properties, digoxin from *Digitalis purpurea* is used for heart failure treatment, vincristine and vinblastine from Madagascan Periwinkle (*Catharanthus roseus*) are used as antineoplastic agents, taxol from Yew (*Taxus baccata*) is used for the treatment of ovarian and breast cancers, quinine from *Cinchona* species is used in the treatment of malaria, and caffeine from *Coffea arabica* is used as a stimulant (Dweck, 2001; Raskin *et al*., 2002).

1.2 Drug development over the years

1.2.1 From 'Natural' to synthetic

Plant extracts were the first drugs, followed by natural compounds of which the chemical structure was known, followed by inorganic compounds (Chadwick and Marsh, 1990). The focus of the 17th and 18th centuries was to account for the clinical usefulness of medicinal plants. The introduction of synthetic acetyl salicylic acid to the medicinal world in 1897, unveiled the remarkable connection between plants and human wellbeing (Raskin *et al.*, 2002). The transformation of plant-derived chemical compounds to chemically defined drugs began in the early 19th century. Drug discovery and manufacturing advanced with the advent of synthetic chemistry in the 1940's and 1950's. Vinblastine and vincristine, marketed in 1961 and 1963, respectively, were the last useful plant-derived drugs; plant extracts used to be a source of discovery for bioactive entities (Raskin *et al.*, 2002). The latter is the result of pharmaceutical companies setting aside their research and development budgets towards the synthesis of compounds.

The 20th century marked the change over from extraction processing to manufacturing of synthetic analogues (Raskin *et al.*, 2002). Synthetic compounds, identical in chemical structure and biological properties have partially substituted naturally-derived drug constituents. Due to cost and inefficiency as well as to fully achieve the therapeutic claims, natural extracts are eventually being replaced by synthetic molecules which have no association with the naturally-derived entity. Furthermore, combinational chemistry and computational drug design has decreased the discovery of natural drug products (Raskin *et al.*, 2002).

1.2.2 Back to 'Natural'

There has been an increased movement towards a more "green" ideology. In recent years, due to renewed interest in herbal and homeopathic medicines, plant-derived pharmaceuticals have become prominent in the market place, making it a favoured healthcare choice. Great interest has been focused on natural foods, cosmetics and drugs due to concerns of the toxicity and safety of modern compounds as well as concerns regarding aspects of ecology. The increased popularity of plant-based medicines can also be attributed to the general perception that products from Nature are inherently 'safer' due to the history of human use and long association of plants and humans. Religious and

philosophical views may also play a role. Today, there is a wealth of products on the market which contain active plant materials (Raskin *et al*., 2002). Market research data reveal high levels of expenditure on phytomedicines. Phytopharmaceutical products, including prescription and over-the-counter products, account for 50% of the medicine market (Raskin *et al.*, 2002).

1.3 The rationale for the use of botanically-derived compounds

A plant may be considered as a complex biosynthetic laboratory, synthesizing many diverse bioactive chemical compounds exerting curative effects. The plant's defence system against predation, herbivores, microbial and viral infection is provided by such bioactive compounds. Only one third of all diseases can be treated efficiently and so after more than 100 years of pharmaceutical research, innovative and effective lead compounds are still needed (Mulzer and Bohlmann, 2000). The idea that complex biologically active compounds in plants have been selected and perfected by evolution for longer than drug companies must be realized (Raskin *et al.*, 2002).

Complex diseases are often treated with a single drug. The problem of resistance to anticancer, antimalarial and antimicrobial drugs coupled with the multifaceted nature of complex diseases brings to light the consideration that a combination of therapeutic agents should be used for the treatment of certain diseases. The traditional medicinal system uses the approach of combining botanical and non-botanical remedies for the treatment of complex diseases and in this way provides a more holistic approach to disease treatment and prevention. Plants produce an array of compounds to prevent the development of resistance and thus to protect themselves against pathogens. This suggests that the treatment of certain diseases with botanically-derived agents are less likely to lead to resistance problems as the biological effects exerted by plant products maybe the result of the multiple and synergistic effects caused by the interactions between many different biochemical compounds.

The increasing cost of energy and chemical raw materials, pharmaceutically related environmental concerns as well as the fact that plants are the most abundant and renewable resource makes them more compatible as a source of drug discovery for the future. Furthermore, advances in metabolic engineering, biochemical genomics, chemical

separation, molecular characterization and pharmaceutical engineering, have better equipped us to exploit the therapeutics of plants. Numerous bioactive compounds derived from plants are used as prototypes, where their structures are elucidated and can be used as templates for structural modification for the development of better and newer drugs (structure optimization programs) (Raskin *et al.*, 2002; Heinrich *et al.*, 2004).

1.4 Plant species contain active constituents of medicinal value yet to be discovered

It is mostly the developed countries which derive much benefit from the modern pharmaceutical products. About 65-80% of the world's population (developing countries) uses traditional plant-based medicines for their primary health care due to the poverty and lack of access to modern medicines (Calixto, 2005). Governments of Third World countries advocate the movement towards traditional medical practices where plants are the main source of drug therapy (Evans, 1989). About 60% of South Africans seek advice from traditional healers in addition to using modern medical services (van Wyk *et al.*, 2002b). However, within the domain of the traditional drug discovery process, plants have received dwindled importance (Raskin *et al.*, 2002). Very few plants can be bioscientifically and clinically recommended due to lack of extensive scientific research (Heinrich *et al.*, 2004). Of the quarter million plant species in the world, only a small percentage has been chemically or physiologically studied. About 119 plant-derived compounds of known structure are used as commercial drugs. These 119 drugs are obtained from only 90 plant species out of the conservative estimates of about 250 000 plants species on earth (Chadwick and Marsh, 1990).

A good place to commence a systematic investigation of a plant is to first consider plants that have been claimed to be useful as folk medicines for particular pathological ailments i.e. the plant has been subjected to some human screening (Evans, 1989). In this way, credibility in terms of its effectiveness can be provided as a result of human experience in the past and present. One of the indigenous plant groups which have been used traditionally are members of the Geraniaceae. Root extracts of plants of the Geraniaceae family have been used for many years as native herbal medicines in South Africa (Seidel and Taylor, 2004). In the late 1800's and early 1900's there were several reports

concerning the curative properties of Geraniaceae species (Maberley, 1897; Laidler, 1928; Watt and Warmelo, 1930; Watt and Breyer-Brandwijk, 1962).

1.5 The systematic classification of the genus *Pelargonium*

As a means of taxonomic classification, all plants are divided into 'families'. The plant species used in this study belong to the Geraniaceae family which is subdivided into five genera, namely *Erodium* (heron's bill), *Geranium* (crane's bill), *Monsonia*, *Pelargonium* (stork's bill) and *Sarcocaulon* (Figure 1.1). The genus *Pelargonium* contains about 280 species and contains an array of variation in floral morphology and life forms. This genus was classed as the third largest angiosperm genus in the Cape Floristic Region (CFR) (Goldblatt and Manning, 2000). *Pelargonium* is a good example of the impressive evolutionary angiosperm radiations typical for the CFR (Bakker *et al*., 2004). The *Pelargonium* species are subgrouped into 16 recognized sections (Bakker *et al*., 2004) which are phylogenetically arranged. van der Walt (1985) taxonomically revised the section *Pelargonium*, which contains 24 species (Figure 1.1).

1.5.1 The phylogenetic relationships in the genus *Pelargonium*

Bakker *et al*. (2004) used DNA sequences from nuclear, chloroplast and mitochondrial encoded regions as sources of phylogenetic markers in order to reconstruct phylogenetic relationships among representatives of all currently recognized sections of *Pelargonium.* The use of DNA sequence data from the three different genomic regions provides the advantage of three separately evolving sets of characters in phylogenetic reconstruction and is useful for reconstructing deep-level phylogenies. The analysis included 153 taxa, four of which were not *Pelargonium* species. The datasets contained cpDNA *trnl-F* sequences for 152 taxa, nrDNA ITS sequences for 55 taxa and mtDNA *nadl* b/c exons for 51 taxa. The phylogenetic hypotheses obtained from the three different genomic regions were generally similar with all incongruencies occurring only within some of the main clades and not between them. An overall phylogenetic hypothesis integrating the information in the three datasets was obtained for the genus *Pelargonium* L'Hérit. (Bakker *et al*., 2004). It was found that the genus consists of five main clades (A1, A2, B, C1 and C2) (Figure 1.1) some with different evolutionary patterns regarding biogeographic distributions, dispersal capacity, pollination biology and karyological diversification (Bakker *et al*., 2004).

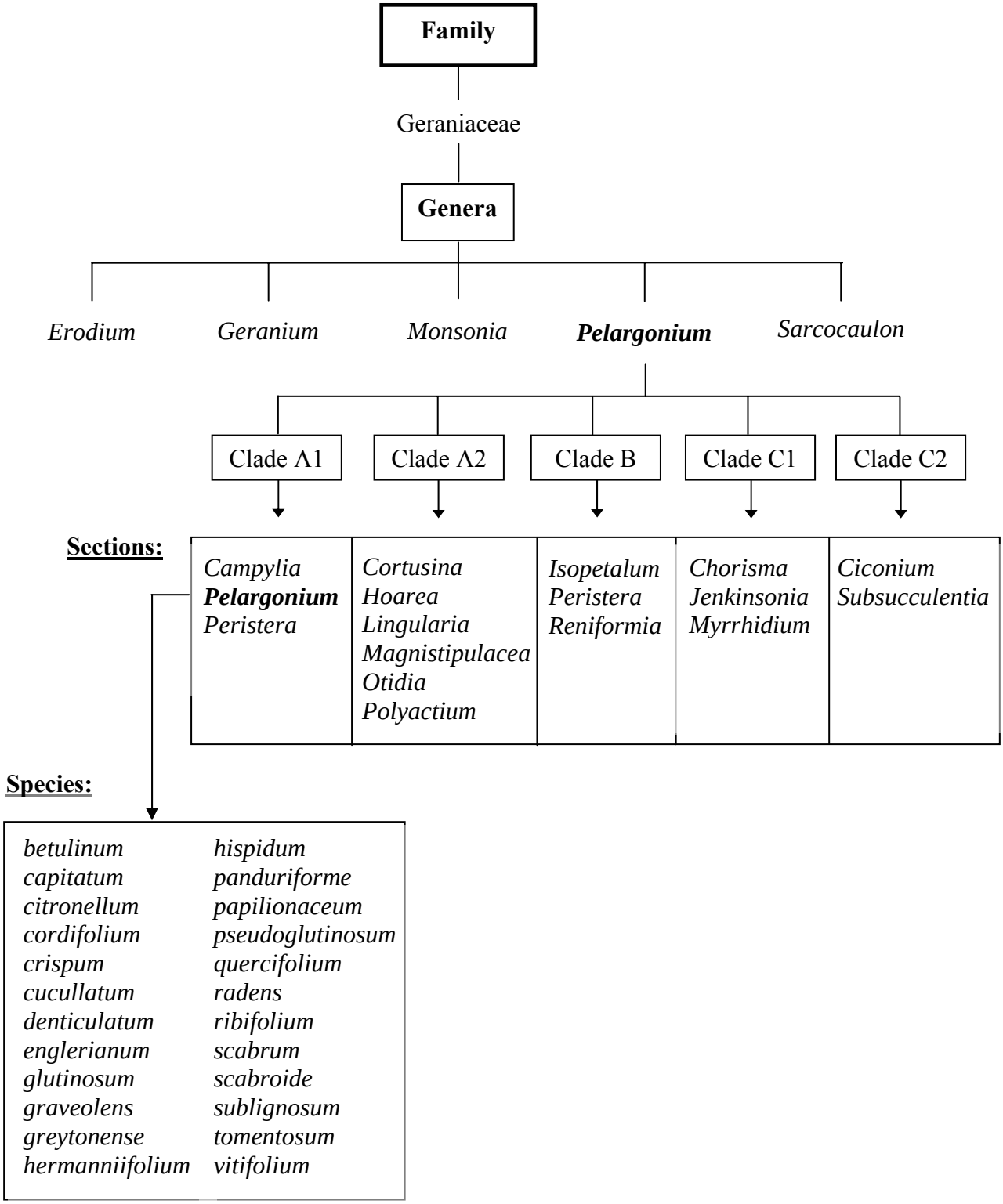

Figure 1.1: Outline of the taxonomic classification of the family Geraniaceae (van der Walt, 1985; Bakker *et al*., 2004). This study is restricted to species contained in section *Pelargonium*.

The five main clades are largely in agreement with the current sectional classification (Bakker *et al*., 2005) and are grouped into two subgeneric clades correlating with chromosome size, and further supported by evidence from hybrid formation (Gibby *et al*., 1996), but not by any known morphological characters. In brief, clade A and B reside in the "small chromosome clade" which comprises about 80% of the currently described 280 species, mostly with chromosomes of <1.5 µm in length (Albers and van der Walt, 1984; Gibby and Westfold, 1986; Gibby, 1990). The remainder of the genus contains about 55 species with chromosomes of 1.5-3.0 µm long (Albers and van der Walt, 1984; Gibby and Westfold, 1986; Gibby, 1990).

Clade A (the "winter rainfall clade") contains over 70% of all currently described *Pelargonium* species and is largely confined to the South African Cape winter rainfall region (Bakker *et al*., 1999). It includes woody shrubs, shrubs (*Ligularia* and *Pelargonium* clades), stem succulents (*Cortusina* grade, *Otidia* clade), geophytes (*Hoarea*, *Polyactium* clades) and herbaceous annuals (*Peristera* grade). Clade A contains two main subclades, clade A1 and clade A2.

Clade A1 comprises mainly woody evergreen diploid shrubs of the type section *Pelargonium*, confined mainly to the winter rainfall regions. Only *P. radens* and *P. graveolens* (both with *2n* = 88), are found in the summer rainfall region. Species of the section *Pelargonium* clade are commonly infertile and natural hybrids are known (Albers and van der Walt, 1984), as also shown by the three independent incongruencies between the cpDNA and rDNA topologies. However, morphological and phytochemical characters also strongly support monophyly of the section *Pelargonium* clade. Clade A1 also contains the small herbaceous rosette subshrubs of section *Campylia*, mainly distributed in the Western Cape winter rainfall region, and *P. nanum* (section *Peristera*).

Clade A2 (the "xerophytic" clade) comprises in total almost half of all *Pelargonium* species and six of its sections, namely *Cortusina, Hoarea, Ligularia*, *Magnistipulacea, Otidia* and *Polyactium*. The *Ligularia*/*Hoarea* clade consists of woody shrubs and geophytes. The species currently assigned to section *Ligularia* (Albers *et al*., 2000) form a paraphyletic group with regard to the *Hoarea* clade. However, only 12 of the 78 species representing the section *Hoarea* were included in this study. It will become evident whether this relationship holds once complete sampling of the *Hoarea* clade has been

carried out since taxonomic sampling is significant in determining relationships. The *Polyactium*/*Cortusina/Otidia* clade includes stem succulents and geophytes and is morphologically well-characterized. The species currently assigned to section *Cortusina* form a paraphyletic group with regard to the *Otidia* and *Polyactium* clades. The *Otidia* clade consists of a small radiation of stem and leaf succulents, mainly occurring in the dry area of the Western Cape winter rainfall region and the Northern Cape coastal region. Nested within clade A2 is a radiation consisting of about 80 species from the section *Hoarea*, all of which are characterized by a series of tunicate tubers. Within clade A, the karyological diversification has been limited, with two independent reductions from $x = 11$ to 10 in clade A1 and from $x = 11$ to 10 and 9 in the *Hoarea* clade of clade A2 (Gibby *et al*., 1996). In clade A, polyploidy has occurred independently several times. It has been suggested that clade A2 represents an adaptive radiation, probably in response to late-Miocene and Pliocene aridifications.

Clade B consists mostly of "weedy", dwarf, herbaceous annual species, previously assigned to section *Peristera*. It also comprises section *Reniformia*, seven species from Australia and New Zealand, and *P. cotyledonis* (section *Isopetalum*) from St. Helena Island. The abovementioned large distributions indicate high dispersal capacity of species of the *Peristera* clade (Bakker *et al*., 1998). Within clade B, the karyological diversification has been more profound, with one reduction from $x = 11$ to 8 in the *Reniformia* clade, and five different chromosome numbers in the *Peristera* clade including polyploid series (Hellbrügge, 1997).

Clade C comprises the *Pelargonium* species with large-sized chromosomes presently assigned to five different sections, distributed in Western and Eastern Cape, tropical eastern Africa, Madagascar and Asia Minor. Clade C1 includes the sections *Chorisma, Jenkinsonia* and *Myrrhidium*; the species are (sub)shrubs typically with wooden stems. Only *P. redactum* and *P. senecioides* (section *Jenkinsonia*) and *P. myrrhifolium* (section *Chorisma*) are annuals or short-lived perennials (Albers *et al*., 1995; van der Walt *et al*., 1997). Clade C1 consists of two basic chromosome numbers ($x = 11$ in the *Myrrhidium*/*Chorisma* clade and $x = 9$ in the *Jenkinsonia* clade) among which two independent transitions were reconstructed. Clade C2 consists of species presently assigned to sections *Subsucculentia* (van der Walt *et al*., 1995) and *Ciconium*, as well as a clade consisting of the presumed allopolyploid species from Asia Minor, *P. endlicherianum* and

P. quercetorum, the tetraploid *P. caylae* from Madagascar and *P. karooicum* (diploid and polyploid) from the Cape winter rainfall area. Clade C2 comprises three different basic chromosome numbers ($x = 9$, $x = 10$ and $x = 17$).

1.5.2 Confusion over the nomenclature of *Pelargonium*

Confusion has resulted from the vernacular use of the name geranium for a plant which should be correctly referred to as a pelargonium (van der Walt, 1977). The confusion between the genera *Geranium* and *Pelargonium* has existed before Linnaeus (1753) and his binomial system of classification where both genera were placed under the genus *Geranium* (Lis-Balchin, 2002d). In the seventeenth century when first introduced into Europe, all pelargoniums were called geraniums, most probably because of the similar fruit structure. About 200 years ago, botanists noted distinct differences and the two genera were separated (Miller, 2002). Although Sweet (1820) and other botanists reclassified them under two genera, acceptance by the general public and nurserymen is still low (Lis-Balchin, 2002d). During the twentieth century, the name 'geranium' has become re-established as a vernacular name for pelargoniums especially for the zonal cultivars (Miller, 2002). Our modern cultivated varieties are derived from *Pelargonium* species and not *Geranium* species. From the systematic classification shown in Figure 1.1, it is clear that *Geranium* exists as one genus and *Pelargonium* as another, both genera of which differ markedly from one another (van der Walt, 1977).

1.6 Distribution of *Pelargonium* species

The first *Pelargonium* species, *Pelargonium triste*, was collected in 1632 by John Tradescant at the Cape (Vorster, 1990). In the early 1700's plants were brought to Europe and England to be planted in estate gardens. One of the first *Pelargonium* species to be introduced to England was *P. triste* (van der Walt, 1977). *Pelargonium cucullatum* was collected by Paul Hermann in 1672 from the slopes of Table Mountain (Vorster, 1990). This species was established in the Royal Botanical Gardens at Kew, England by 1690 and the gardens of Leyden had 10 species of *Pelargonium* by 1687 (van der Walt, 1977).

Most Pelargoniums are found in the Southern Hemisphere, where almost the entire genus (about 200 species) is found in South Africa (Vorster, 1990). Most species occur in the wide coastal region stretching from the Namib Desert (North) to the Cape (South) and

along the coast towards the eastern seashore (Figure 1.3). Most species occur as irregular, isolated groups distributed within the western coastal belt (van der Walt and Vorster, 1983). The genus is a fundamental part of the Cape flora (van der Walt, 1977), where some species grow on the edge of scrubland, stony and sandy areas and others scramble through shrubs. The various microhabitats of the south-western Cape, which favour speciation, is the reason for the rich diversity of species in this region.

The other 50 *Pelargonium* species occur throughout eastern tropical Africa, Arabia, Syria, western India, Australia and New Zealand and on the islands of Madagascar, St. Helena and Tristan da Cunha (Vorster, 1990) (Figure 1.3). *Pelargonium* species are also found growing naturally in Spain, California and Italy. It is speculated that the wide distribution of the genus is a result of continental drift (van der Walt and Vorster, 1988).

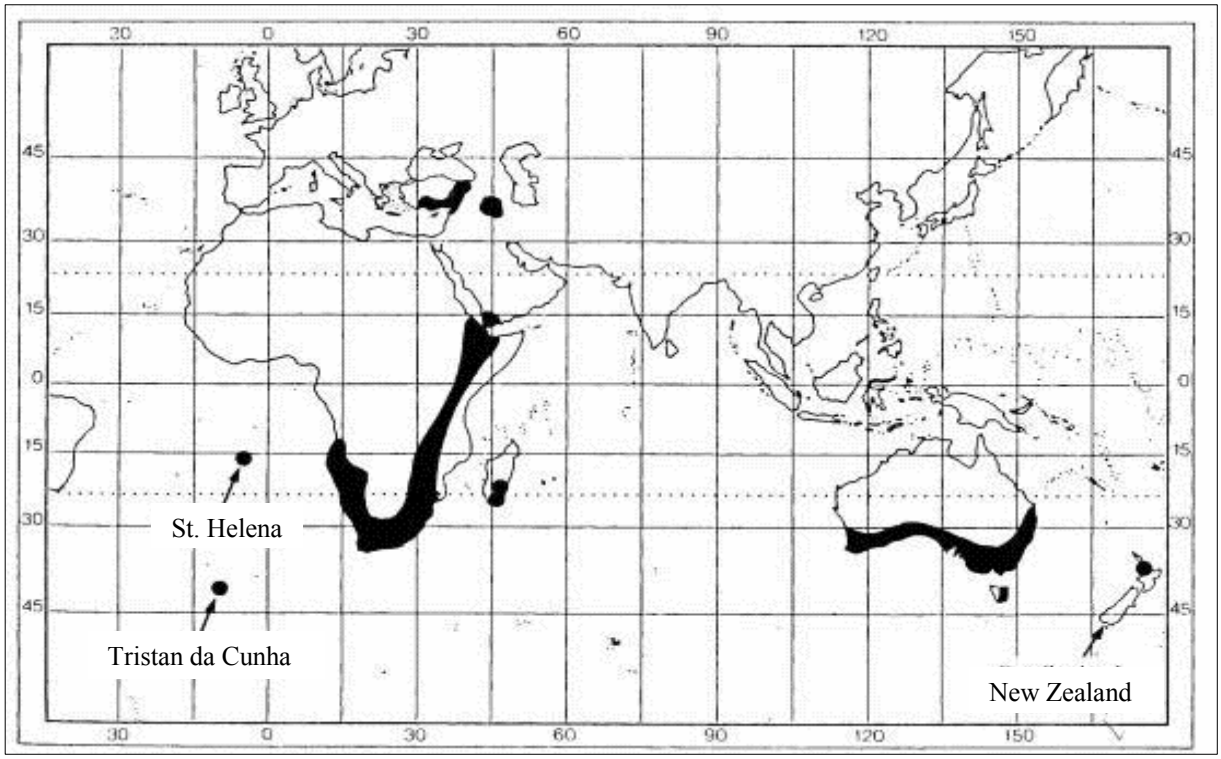

Figure 1.2: The global distribution of the genus *Pelargonium* (van der Walt and Vorster, 1988).

1.7 General characteristics of the genus *Pelargonium*

The genus *Pelargonium* is a group of annual or perennial herbs, shrublets or shrubs which are characteristically erect to decumbent, branched to many-branched. The stems are often woody at the base, soft-wooded or subsucculent, frequently viscid and aromatic, usually glandular and variously hairy (van der Walt, 1977). The stems bear irregularly marked, non-aromatic to aromatic leaves (Webb, 1984; van der Walt, 1985). All the species have petiolate leaves with stipules (Miller, 2002) and almost every lobed shape can be found; a few species have entire leaves (Webb, 1984). The obtuse to acute leaf margins are finely to coarsely dentate or serrate. Some leaves are soft and flannelly due to small, silky hairs and occasionally the leaves are sticky. Fine hairs give the green leaf a grey appearance (Webb, 1984).

The flowers have five petals and are zygomorphic. The two posterior spathulate or obovate petals are larger and broader than the three anterior ones. Flower colour ranges from white, pink, pinkish-purple to purple. Dark purple, wine-red or dark red markings occur on the posterior petals (van der Walt, 1985). Not more than seven stamens out of the ten bear fertile pollen (Miller, 2002). The adnate nectar tube is the most characteristic feature of the flower (Webb, 1984). A valuable tool for identification is the presence of aromatic oils in many species. A few species emit a scent from their flowers, but only at night (Miller, 2002). A common feature of all five genera is the shape of the schizocarp (elongated fruit) which resembles a stork's head or bill just before it is ripe and ready to disperse (van der Walt, 1977).

Figure 1.3: The stork-shaped fruit and zygomorphic flowers of *Pelargonium capitatum.*

1.7.1 The general morphology of the section *Pelargonium*

This is a large section characterized by shrubs and subshrubs with woody, branched or very branched stems, variegated leaves (rarely entire and often lobed or palmately partite) and five-petalled flowers (van der Walt, 1977). The foliage is frequently aromatic and sometimes viscid (Miller, 2002). The posterior two petals, marked with darker spots or lines, are larger and broader than the anterior three (Webb, 1984; Miller, 2002). The flowers are white, pink or purple and have seven fertile stamens. The section has a basic chromosome number of $x = 11$ and the chromosomes, in comparison with those of the other sections of the genus, are relatively small (van der Walt, 1985).

1.8 The essential oil of *Pelargonium* ('geranium oil')

Many of the natural *Pelargonium* species and the hybrids and cultivars derived from them have scented leaves producing essential oil (Williams and Harborne, 2002). The species particularly rich in essential oil belong to the sections *Pelargonium*, *Polyactium* and *Cortusina* (Webb, 1984). High quality oil is contained in the leaves, stems and stalks of scented pelargoniums. Just before the plants bloom, they are harvested and processed to obtain the essential oil. *Pelargonium* essential oil is incorrectly declared as geranium oil; the species involved in the production of the oil are all *Pelargonium* and not *Geranium*. The geranium oil obtained from *Geranium macrorrhizum* in Bulgaria is both chemically and medicinally different to the commercial 'geranium oil' from *Pelargonium* species (Lis-Balchin, 2002b). 'Geranium oil', as described by the International Standards Organisation (ISO 4731), is 'the essential oil obtained by steam distillation from the herbaceous parts of *Pelargonium graveolens* L'Héritier ex Aiton, *P. roseum* Willdenow and their cultivars and hybrids' (Lis-Balchin, 1990).

Since the early 1800's, the rose-scented 'geraniums' belonging to the genus *Pelargonium* have been cultivated commercially for their essential oil (Widmer and Collins, 1991). Commercial 'geranium oil' is derived from various *Pelargonium* cultivars growing mainly in Réunion, China, Egypt and Morocco (Hart and Lis-Balchin, 2002). *Pelargonium capitatum*, *P. graveolens* and *P. radens* are considered to have been used in cultivation programs to create and ennoble the rose 'geranium' cultivars. A very important commercial essential oil producer is the cultivar known as cv. Rosé, often referred to as *P. graveolens*, obtained from the hybridization of *P. capitatum* x *P. radens* (Demarne and van

der Walt, 1989). Cultivars of *P. capitatum* x *P. graveolens* are less important commercial essential oil sources. *Pelargonium odoratissimum*, with a definite apple-like scent, is often incorrectly quoted as a source of rose-scented 'geranium oil' (Lis-Balchin and Roth, 2000).

1.9 The traditional uses of *Pelargonium* species

Pelargoniums are mentioned in old apothecary manuals. In South Africa, both the scented and unscented *Pelargonium* species were used as traditional remedies by the Sotho, Xhosa, Khoi-San and Zulus to treat various ailments (Watt and Breyer-Brandwijk, 1962). Some of the folk medicines were also used by the Dutch settlers who exported them back to Holland. The locals and Dutch commonly used *P. grossularioides* (Lis-Balchin, 1996). The fleshy tubers of Pelargoniums are commonly used to make infusions and decoctions. However, a traditional method of using *Pelargonium* roots is to boil the tuber in milk. The roots may also be chewed or powdered and mixed with food (Latté and Kolodziej, 2004).

The ability of Pelargoniums to provide relief in cases of diarrhoea and dysentery was known to the earliest South African tribes (van der Walt, 1977); this was the main use of Pelargoniums in the past (Lis-Balchin, 1996). The large tubers of *P. antidysentericum* were used by the Khoi-San as a remedy for dysentery and anaemia (Miller, 2002). Infusions of *Pelargonium luridum* tubers are used to treat diarrhoea and dysentery. Numerous other species with similar fleshy underground parts are also antidiarrhoetic (van Wyk *et al*., 2002b). The presence of highly astringent tannins may be responsible for the antidiarrhoeal effects (van Wyk *et al*., 2002b). The more tannin-containing roots of numerous *Pelargonium* species were used for syphilis (Lis-Balchin, 2002c).

In South Africa, various *Pelargonium* species were employed as remedies for wounds, abscesses, fever reduction, colic, nephritis and suppression of urine, colds and sore throats, haemorrhoids, gonorrhoea, stimulating milk-production, anthelmintic infections and were also used as an insecticide (Lis-Balchin, 1996). Decoctions of the roots of *Knowltonia vesicatoria* (Ranunculaceae) were mixed with *Pelargonium* roots to treat colds and influenza (van Wyk *et al*., 2002b). The powdered leaves of *Pelargonium inquinans* were used to relieve headaches and common cold symptoms and were used as a deodorant by native tribesman (van der Walt, 1977). In traditional South African medicine, a variety of ailments (gastrointestinal disorders, hepatic disorders and respiratory tract infections) are

treated with "Umckaloabo" (Seidel and Taylor, 2004). This traditional drug originates from the root material of *P. sidoides* and *P. reniforme* (Kolodziej, 2002). "Umckaloabo" is also claimed to cure menstrual complaints such as dysmenorrhoea. The aerial parts of *P. sidoides* and *P. reniforme* are used in wound healing (Kolodziej, 2002). In folk medicine, the fresh flowers of *P. endlicherianum* have been used as an anthelmintic (Bozan *et al*., 1999). The essential oils of some *Pelargonium* species are employed by the native population in southern Africa due to their effective insect repellent properties (Kolodziej, 2002). *Pelargonium radens* leaves have been used as a folk remedy for splanchnic neoplasm (Hirose and Shibata, 1978). Ethnobotanical data, presented in Table 1.1, concerning *P. betulinum, P. cucullatum, and P. papilionaceum* (included in this study), provides information as to the type of formulations that were used, the methods by which they were administrated and the conditions treated.

Table 1.1: The traditional medicinal uses of three species belonging to the section *Pelargonium* (Watt and Breyer-Brandwijk, 1962; van der Walt, 1977; May, 2000; Lawrence and Notten, 2001).

Botanical name	**Common name**	**Traditional use**	**Distribution**
P. betulinum	Birch-leaf pelargonium, Camphor-scented pelargonium, 'Kanferblaar', 'Maagpynbossie', 'Suurbos'.	Fresh leaves were placed in boiling water and the camphoraceous vapour was inhaled to relieve coughs and other chest problems (van der Walt, 1977). The leaves were used in ointments as a wound-healing emollient. The plant was also used for the relief of flatulence and stomach pain (Lawrence and Notten, 2001).	West coast to the east coast of the Western Cape. It is mostly confined to coastal areas.

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Botanical name	Common name	Traditional use	Distribution
P. cucullatum	Hooded-leaf pelargonium, Tree pelargonium, 'Wilde malva'.	Traditionally, the roots and leaves were used as an astringent for colic and diarrhoea, as a wound-healing emollient and as an antispasmodic (van der Walt, 1977). The leaves were used as a poultice for bruises, stings and abscesses. This plant was also employed to cure kidney ailments, fevers, coughs and chest problems (May, 2000).	South-western Cape, well represented in the Cape Peninsula.
P. papilionaceum	Butterfly pelargonium, 'Rambossie'.	The leaves were used as an alternative to tobacco in South Africa and smoked possibly for its medicinal effects (Watt and Breyer-Brandwijk, 1962).	South-western, southern and eastern Cape.

1.10 Anecdotal information

'Geranium oil' is one of the most commonly used oils in aromatherapy (Lis-Balchin *et al*., 1996a). Many properties of 'geranium oil' have been quoted in aromatherapy books and are summarized in Table 1.2. Many of these properties are directly transcribed from Culpepper's Herbal account (Culpepper, 1985) of real geranium for example *Geranium robertianum*, which has a completely different chemical composition (Lis-Balchin *et al.*, 1996a). Not all the claims made in aromatherapy of the supposed uses or properties of 'geranium oil' are supported by scientific evidence (Lis-Balchin *et al.*, 1996a). The scientific proof that inhalation of a pleasant odour and its action via the limbic system produces a relaxing effect should be accepted, as has massage (Lis-Balchin, 2002c). Theoretically, when the two are used together, several stress-related conditions such as dermatitis, asthma and intestinal problems could be relieved (Lis-Balchin, 2002c). The essential oil is used as a nervinum and expectorant (Steinegger and Hänsel, 1992).

Table 1.2: The reported therapeutic uses of 'geranium oil' obtained from *Pelargonium graveolens* (Jackson, 1993; Lawless, 1995; Curtis, 1996).

Characteristics	The greenish-olive Bourbon oil has a rosy-sweet, minty scent. 'Geranium oil' has an all-over balancing effect which extends to the skin, emotions and the hormone system.
Principle components	Citronellol, geraniol, linalool, isomenthone, menthone, phellandrene, sabinene, limonene.
Skin care	'Geranium oil' is a popular ingredient in skin care preparations. Due to its antiseptic and anti-inflammatory properties, it is useful in treating acne and other skin infections. It is known to relieve shingles (neuralgia), herpes blisters, bruises, burns, broken capillaries, cuts, dermatitis, eczema, ringworm, ulcers and infected wounds. It is appropriate for all skin types and has been reported to delay the process of skin aging. It is used as a gentle cleansing tonic and has a regulating action on sebum secretion and thus suitable for dry or oily (congested) skin. Dry, inflamed skin is relieved by its soothing, moistening and cooling or refreshing properties. It helps to prevent the formation of scar tissue and stretch marks as it has cicatrizing (healing) properties.
Circulation, muscles and joints	'Geranium oil' can be used as a circulatory tonic to increase poor circulation. As a lymphatic stimulant, it is detoxifying. Through the stimulatory action on various elimination processes, toxins and residues from metabolic processes which can cause diseases are removed from the system. This effect plays an integral role in many chronic diseases such as arthritis and helps to reduce cellulite and clear skin conditions. Through its detoxifying effects, it exerts uplifting and strengthening properties. 'Geranium oil' is also used for breast engorgement and oedema as it has diuretic properties.
Respiratory system	Relieves colds, tonsillitis, laryngitis and bronchitis.

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Gastrointestinal system	The cooling properties of the oil assist in the relief of symptoms occurring from heat in the abdomen. Such symptoms include constipation with dryness or burning and yellow diarrhoea. Gastritis and peptic ulcers are relieved by its anti-inflammatory properties. 'Geranium oil' is known to have an antispasmodic action indicating its usefulness in relieving flatulence and diarrhoea. It is also used as a vermifuge (expels worms from the intestine).
Genito-urinary and endocrine systems	'Geranium oil' influences the hormone secretion of the adrenocortical glands and so is used to treat hormonally-related symptoms of menopause such as hot flushes and vaginal dryness. It also relieves period pains. Cystitis with burning urination is relieved by its anti-inflammatory properties.
Nervous system	Pain associated with neuralgia and shingles is relieved by its analgesic properties. In aromatherapy, it is used to treat palpitations, panic attacks and anxiety as the scent has physical and emotional "balancing" properties. It has antidepressant and revitalizing properties. 'Geranium oil' is either sedative or stimulating depending on the individual.
Other use	The strong smell of the oil acts as a repellent to mosquitoes and head lice.

1.11 *In vitro* pharmacological studies involving *Pelargonium* essential oils and extracts

1.11.1 Experiments involving guinea-pig ileum preparations

The traditional use of Pelargoniums in diarrhoea and dysentery signifies a potential antispasmodic action on smooth muscle. This possibility has been researched using isolated ileum and other muscle preparations and many species, scented and unscented, have been studied. The results suggest a definite antispasmodic action in some fractions (Lis-Balchin, 1996).

The majority of *Pelargonium* oils and commercial 'geranium oils' have a spasmolytic action on intestinal smooth muscle and decrease the response to electrical stimulation (Hart and Lis-Balchin, 2002). It was found that the essential oils of *P. odoratissimum*, *P. exstipulatum* and *P.* x *fragrans* have a postsynaptic spasmolytic effect on guinea-pig smooth muscle which was mediated via cyclic adenosine monophosphate (cAMP) in *P. odoratissimum* and *P.* x *fragrans* (Lis-Balchin and Roth, 2000). The involvement of cAMP in the mode of action of the latter two essential oils shows a similarity with commercial 'geranium oil' and with other floral *Pelargonium* species (Lis-Balchin and Roth, 2000). Due to the relaxant effect of the oils of *P. odoratissimum*, *P. exstipulatum* and *P.* x *fragrans*, they have potential for use in aromatherapy products (Lis-Balchin and Roth, 2000). Many essential oil fractions from various species have been found to be initially spasmogenic (contractile effect) followed by an antispasmodic activity (Lis-Balchin *et al*., 1996c). In a study carried out by Lis-Balchin *et al*. (1997) all the essential oils tested, which were obtained by steam distillation from scented-leaf *Pelargonium* species, hybrids and cultivars, were spasmolytic. The results from this investigation support the exploitation of different *Pelargonium* oils for their spasmolytic actions and possibly other functions (Lis-Balchin *et al*., 1997).

Citronellol, citronellal and geraniol (components of *Pelargonium* oil) have a spasmolytic action on guinea-pig ileum (Reiter and Brandt, 1985; Lis-Balchin and Hart, 1997). Geranyl acetate has been reported to have spasmolytic activity (Sticher, 1977). However, *P. grossularioides* oil contracts the tissue but upon washout (when tone returns to normal) it inhibits momentarily the contractions induced electrically (Lis-Balchin and Hart, 1994). Geranyl formate contracts guinea-pig ileum (Lis-Balchin *et al*., 1996b), as does citronellyl formate (Hart and Lis-Balchin, 2002). Alpha- and β-pinene cause an initial spasmogenic effect on intestinal smooth muscle (guinea-pig ileum) and then produce a spasmolytic effect (Lis-Balchin *et al*., 1999). According to temporary findings, α-pinene does not act via muscarinic cholinceptors or histamine receptors and the two enantiomers of α-pinene have different pharmacological activities (Lis-Balchin *et al*., 1999).

The non-scented pelargoniums studied included several zonals, regals and ivy-leaved pelargoniums. The methanolic and water-soluble extracts of the leaves and roots were tested and all were spasmolytic on guinea-pig ileum. However, the *P. grossularioides* water-soluble and ethanolic extracts did not produce a spasmolytic action (Hart and Lis-

Balchin, 2002). The alkaloid extracts of the zonals were all spasmolytic (Lis-Balchin, 1997). The methanolic and water-soluble extracts and alkaloid fractions of *P. luridum* (root) and the leaves of *P. inquinans* and *P. cucullatum* were also spasmolytic. Studies conducted on freshly-prepared hydrophilic and methanolic extracts have indicated that numerous scented *Pelargonium* species and cultivars produce an initial spasmogenic effect followed by a relaxation action. This suggests that the spasmogenic effect may be caused by phenolic compounds (Hart and Lis-Balchin, 2002).

In conclusion, experiments conducted on isolated guinea-pig ileum revealed that the majority of *Pelargonium* oils and their components cause smooth muscle relaxation. This action is mediated through a mechanism that involves the enzyme adenylate cyclase and an increase in the concentration of the second messenger, cAMP. There is some verification of calcium channel blockade but only at levels higher than those necessary to produce significant spasmolytic effect. No conclusive results have been obtained with regard to the pharmacology of the central actions of *Pelargonium* as few studies have been carried out. Some evidence indicates that a few methanolic extracts use calcium channels at normal concentrations. However, numerous extracts have shown no such activity; this is in accordance with the mode action of their corresponding essential oils (Hart and Lis-Balchin, 2002).

1.11.2 Experiments involving uterine preparations

Pelargonium grossularioides may be an abortifacient as its essential oil and water soluble extracts caused an isolated rat uterus to contract (Lis-Balchin and Hart, 1994). Uterine activity is reduced by both 'geranium oil' and geraniol at concentrations which are spasmolytic on intestinal muscle (Lis-Balchin and Hart, 1997); however, the mechanism of action has not been investigated.

1.11.3 Experiments involving bronchial preparations

Guinea-pig tracheal muscle is generally used to study the activity on bronchial muscle. Reiter and Brandt (1985) used an alternative preparation where less cartilage was involved (Hart and Lis-Balchin, 2002). Reiter and Brandt (1985) reported that the components of *Pelargonium* oil, namely citronellal, citronellol, geraniol and linalool, are all spasmolytic, with intestinal smooth muscle being more responsive than tracheal smooth muscle. Using

guinea-pig ileum and trachea, linalool was found to be spasmolytic (Brandt, 1988). Lis-Balchin and Hart (2000) found that α- and β-pinene also relax guinea-pig tracheal muscle.

1.11.4 Experiments involving cardiac preparations

Lis-Balchin and Hart (1994) reported that *P. grossularioides* essential oil reduces the force of contraction of a spontaneously beating perfused isolated heart.

1.11.5 Experiments involving skeletal muscle preparations

Two successful preparations, for the study of the activity on skeletal muscle, are the chick biventer cervices and the rat phrenic nerve hemi-diaphragm. It was found that *P. grossularioides* essential oil increases the rat diaphragm tone and reduces the size of the contraction when the phrenic nerve is stimulated and when the muscle is directly stimulated (Lis-Balchin and Hart, 1994). Hence, the oil has a myogenic action; however, the mechanism has not been investigated (Hart and Lis-Balchin, 2002). Using the chick biventer muscle preparation, commercial 'geranium oil' decreased the tone and did not change the size of contraction. Geraniol showed a spasmolytic action on the skeletal muscle.

1.11.6 Receptor binding studies

The binding of glutamate, an excitatory neurotransmitter in the brain, to its receptors on membranes prepared from the cerebral cortex of the rat is dose-dependently inhibited by linalool (Elisabetsky *et al*., 1995). The latter authors suggested that this action is in accordance with their findings that linalool produces sedative effects in animals.

1.11.7 Antimicrobial activity

Positive bactericidal and fungicidal activities were shown from the screening of crude extracts of certain species and cultivars belonging to Geraniaceae (Lis-Balchin, 1990). The traditional use of Pelargoniums in wound healing may be attributable to their antimicrobial action (Lis-Balchin, 1996) which has been well documented. Commercial 'geranium oil', numerous *Pelargonium* species and cultivars have proven to be strong antimicrobial agents (Lis-Balchin and Roth, 2000).

Wollmann *et al*. (1973) reported that the essential oil of some *Pelargonium* species have an antimicrobial effect. Fifty commercial essential oils were studied at four different concentrations against a variety of 25 bacterial genera (Deans and Ritchie, 1987). 'Geranium oil' was most effective against the dairy product organism *Brevibacterium linens* and the toxin-producing *Yersinia enterocolitica*. 'Geranium oil' was tested by Pattnaik *et al*. (1996) for antimicrobial activity by disc diffusion; only 12 bacterial strains out of a total of 22 (Gram-positive cocci and rods, Gram-negative rods) were inhibited. However, all 12 of the fungi tested (3 yeast-like, 9 filamentous) were inhibited. The leaves of *Pelargonium* x *hortorum* were found to be most active against *Candida albicans*, *Trichophyton rubrum* and *Streptococcus mutans*. These latter organisms cause common dermal, mucosal or oral infections in humans (Heisey and Gorham, 1992). Chaumont and Leger (1992) investigated the antifungal effects of vapours of essential oils and some volatile compounds. It was found that the vapours of 'geranium' Bourbon, citral, geraniol and citronellol inhibited the four different strains of fungi tested. It was reported by Chandravadana and Nidry (1994) that the antifungal activity of *P. graveolens* oil against *Colletotrichum gloesporoides* (a post-harvest fruit anthracnose fungus) was greater than that of citronellol or geraniol alone.

An investigation of the bioactivity of 16 commercial 'geranium oil' samples from Réunion, China, Egypt and Morocco, showed variable antimicrobial activity between samples of oil (Lis-Balchin *et al.,* 1996a). It was found that 8 to 19 out of 25 different bacterial species were inhibited. *Clostridium sporogenes*, *Bacillus subtilis* and *Brevibacterium linens* were inhibited by all the samples. *Acinetobacter calcoacetica* and *S. aureus* were inhibited by all but one sample. For all the other organisms tested, there were differences in inhibition. This study was in agreement with Deans and Ritchie (1987) where a 'geranium oil' sample inhibited 19 out of 25 bacteria. The anti-*Listeria* action of the 'geranium oil' samples was also variable; 3 to 16 out of the possible 20 *Listeria monocytogenes* strains were affected (Lis-Balchin *et al.,* 1996a). The same study showed variable antifungal activity against three filamentous fungi. The samples were generally more effective against the two *Aspergillus* species (*A. niger* and *A. ochraceus*) than against *Fusarium culmorum*. The results indicated that the wide variability in bioactivity between commercial samples could not be directly correlated with the country of origin or the chemical composition.

Lis-Balchin *et al*. (1996b) found that 16 'geranium oil' samples were active against 8 to 18 of the bacteria tested and 3 to 16 samples were active against 20 *Listeria monocytogenes* varieties. The same study involving three filamentous fungi indicated a wide range of activities within the group of samples when considering that the samples should be very similar. In the presence of 1 µl of 'geranium oil' per 1 ml of YES broth (yeast extract, glucose and amino acid supplement), the spoilage organism *Aspergillus niger* was inhibited by 0-94%, the mycotoxigenic *A. ochraceus* by 12-95% and the plant pathogenic *Fusarium culmorum* by 40-86%. *Fusarium culmorum* was less affected, with two exceptions, than the two *Aspergilli.* The growth of *A. niger* was more inhibited, with two exceptions, than *A. ochraceus*, whilst antifungal activity was poor (<40% inhibition) (Deans, 2002).

The results of an earlier study (Lis-Balchin *et al*., 1996c), where the antimicrobial activity of 24 cultivars were tested against 25 bacteria and *Aspergillus niger*, were in line with the findings of the aforementioned study (Lis-Balchin *et al*., 1996b). The most sensitive bacteria were a group of mainly Gram-positive micro-organisms, while the least sensitive bacteria were a group of mostly Gram-negative organisms. Inhibition against *A. niger* ranged from 3-98%; growth enhancement was caused by two extracts. The activity in some cultivars was due to the level of monoterpenes present.

Dorman and Deans (2000) studied several individual oil compounds for antibacterial activity. The ranking order of activity of 'geranium oil' components was linalool (mean inhibition zone diameter of 12.5 mm) > geranyl acetate (9.4 mm) > nerol (8 mm) > geraniol (7 mm) > menthone (6.8 mm) > β-pinene (6.3 mm) > limonene (6.1 mm) > α-pinene (5.8 mm). These activities are somewhat modest in comparison with more phenolic compounds. Bacteria exhibiting the greatest level of inhibition included *Clostridium sporogenes* (11.5 mm) > *Lactobacillus plantarum* (10.5 mm) > *Citrobacter freundii* (9.9 mm) > *Escherichia coli* (9.6 mm) > *Flavobacterium suaveolens* (9 mm). The mean inhibition zone diameters of the other bacteria tested were <9 mm (Deans, 2002).

Pelargonium graveolens oil produced by hydrodistillation of dried 'geranium' leaves was evaluated against 25 different genera of bacteria selected for their agricultural, economic and health significance (Dorman and Deans, 2004). The oil displayed inhibitory activity against most of the micro-organisms tested. However, the oil did not inhibit the growth of

A. hydrophila, *A. faecalis*, *E. carotovora*, *E. coli*, *L. plantarum*, *Moraxell*a sp., *P. vulgaris* and *Y. enterocolitica*.

Pelargonium essential oils added to quiche filling as a model food system showed effective antimicrobial activity comparable with that of commercial thyme, clove, 'geranium' and coriander oils (Lis-Balchin *et al*., 1998a). In a study conducted to assess the antimicrobial activity of *Pelargonium* essential oils and their corresponding hydrosols in a model food system, the results suggested the potential application of certain *Pelargonium* essential oils as food antimicrobial agents (Lis-Balchin *et al*., 2003).

Several lipophilic *Pelargonium* extracts displayed pronounced antimicrobial effects (Lis-Balchin *et al*., 1996c). The methanolic extracts (hydrophilic fraction) of certain *Pelargonium* species and cultivars were assessed for bioactivity against 25 test bacteria. All the extracts produced considerable antibacterial activity (Lis-Balchin and Deans, 1996). Bacteria with the greatest level of growth inhibition included *Clostridium sporogenes*, *Yersinia enterocolitica*, *Serratia marcescens*, *Micrococcus luteus*, *Flavobacterium suaveolens*, *Bacillus subtilis*, *Alcaligenes faecalis*, *Enterococcus faecalis*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The individual extracts were ranked by activity as follows: *P. zonale* (15.5 mm) > *P. acraem* (15.2 mm) > *P. inquinans* (13.7 mm) > *P. scandens* (12.9 mm) > 'White Boar' (12.6 mm) > *P. hybridum* (10.5 mm) > *P. cucullatum* (8.2 mm) > *P. capitatum* (5.6 mm). However, the antifungal activity of the extracts was poor (Deans, 2002).

Lis-Balchin *et al*. (1998b) assessed the *in vitro* antibacterial activity of the volatile oils, petroleum spirit and methanolic extracts from a number of *Pelargonium* species and cultivars. Their activity was evaluated against *Bacillus cereus*, *Proteus vulgaris*, *Staphylococcus aureus* and *Staphylococcus epidermidis.* Considerable variation in activity occurred both between the cultivars and the different extraction techniques. The substantial antibacterial activity recorded indicated that *Pelargonium* essential oils and solvent extracts could be used as effective novel food or cosmetic antimicrobial agents (Lis-Balchin *et al*., 1998b). In a study carried out by Lis-Balchin and Roth (2000), the antimicrobial activity of the essential oils of *P. odoratissimum* and *P.* x *fragrans* against *B. cereus*, *P. vulgaris*, *S. aureus* and *S. epidermidis* was similar. Furthermore, their microbiological activity was comparable to that of commercial 'geranium oil'. The results indicated that *P.*

odoratissimum and *P.* x *fragrans* essential oils could be employed in food preservation or household products (Lis-Balchin and Roth, 2000).

1.11.8 Anti-oxidant activity

The traditional uses for which *Pelargonium* species were employed may be related to their anti-oxidant action. It has been reported that *Pelargonium* species and the commercial 'geranium oil' have anti-oxidant properties (Fukaya *et al*., 1988; Youdim *et al*., 1999; Dorman *et al*., 2000).

The anti-oxidant values for 16 different commercial samples of 'geranium oil' showed variation (Lis-Balchin *et al*., 1996a), suggesting that anti-oxidants may have been added to some of the oils. 'Geranium oil' was evaluated for its anti-oxidant capacity using a procedure known as the thiobarbituric acid reactive substances determination, where egg yolk, one-day-old chicken livers or muscle from mature chickens were used as the medium. 'Geranium oil' demonstrated anti-oxidant activity; however, such activity was weak in comparison with that of oregano, thyme and nutmeg oils (Dorman *et al*., 1995). The *in vitro* anti-oxidant property of *Monarda citriodora* var. *citriodora*, *Myristica fragrans*, *Origanum vulgare* ssp. *hirtum*, *Pelargonium* sp. and *Thymus zygis* oils was evaluated by Dorman and Deans (2004). The least active oils were those of *Pelargonium* sp. and *O. vulgare* at the top concentration levels.

Lis-Balchin *et al.* (1996a) found no direct correlation between the source of 'geranium oils', their main chemical constituents and any of the bioactivities studied. Thus, the beneficial effects accredited to 'geranium oil' may be a result of the action of its odour via the limbic system.

The methanolic extracts of representative species and cultivars of *Pelargonium* (Geraniaceae) were found to exert pronounced anti-oxidant actions; this together with the antibacterial activity suggests that *Pelargonium* extracts could be used in the food, cosmetic or other industries (Lis-Balchin and Deans, 1996). Tepe *et al*. (2005) carried out a study in which the methanolic extracts of *P. endlicherianum*, *Verbascum wiedemannianum*, *Sideritis libanotica* subspecies *linearis*, *Centaurea mucronifera* and *Hieracium cappadocicum* from Turkish flora were screened for *in vitro* anti-oxidant

activity by two test systems, namely the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and the β-carotene/linoleic acid assay. In both these aforementioned assays, the extract of *P. endlicherianum* demonstrated greater anti-oxidant activity than the other plant extracts tested. The results from this study support the use of *P. endlicherianum* in food as an additive and in anti-aging remedies (Tepe *et al*., 2005).

1.11.9 Antitumour activity

Pharmacological studies performed on three *P. graveolens* oil samples revealed that some of the major chemical compounds, namely citronellol, citronellyl formate, geraniol and citronellyl acetate, possess marginal antitumour activities (Fang *et al*., 1989).

1.11.10 Anthelmintic activity

The fresh and dried flowers of *P. endlicherianum* have been investigated for anthelmintic activity. The water distillate of the fresh flowers exhibited such activity (Baytop and Tarcan, 1962).

1.11.11 Insecticidal activity

It has been reported for many years that 'geranium oils' possess bactericidal, fungicidal as well as insecticidal properties (Saksena, 1984; Deans and Ritchie, 1987). The insecticidal activity of 'geranium oil' and some of its compounds (citronellol and geraniol) has been well researched (Wirtz and Turrentine, 1980; Dale and Saradamma, 1982). It was found that many *Pelargonium* species have an antifeedant action on insects (Lis-Balchin, 1996); insect larvae stopped eating vegetation and died when extracts were sprayed onto the vegetation.

Methanolic leaf extracts of *P. citrosa* were tested by Jeyabalan *et al*. (2003) for their biological, larvicidal, pupicidal, adulticidal, antiovipositional activity, repellency and biting deterrency against *Anopheles stephensi*, a malaria vector. *Pelargonium citrosa* leaf extracts displayed substantial toxicity to the malarial vector. Larval mortality was dose dependent; the highest dose (4%) caused 98% mortality. The larval, pupal and adult developments were completely inhibited by the treatment. Oviposition and egg hatchability were also affected and the extracts inhibited the growth of the larvae. All the concentrations of the extract used in the study showed good repellent action against the adult mosquito. The leaf

extracts exerted a detrimental effect on *A. stephensi* at 4%; this suggests that repellents can act as insecticides when concentrations exceed a certain threshold. The leaf extracts were also proven to have significant biting deterrency. The highly bioactive compounds of *P. citrosa* leaf extracts could be used to develop naturally occurring insecticides; an alternative to the expensive and environmentally harmful organic insecticides (Jeyabalan *et al*., 2003).

1.12 *In vivo* pharmacological studies involving *Pelargonium* essential oils

Tisserand and Balacs (1995) reported that 'geranium oil' and the components geraniol, linalool and citronellol cause a decrease in blood pressure in experimental animals. However, reliable results from human experiments are not available (Hart and Lis-Balchin, 2002).

The overall activity of mice is affected by the inhalation of vapour from essential oils and the 'geranium oil' components geraniol, linalyl acetate and citronellal are sedative (Buchbauer *et al*., 1993).

In humans, certain brain waves, contingent negative variation (CNV), are affected by exposure to essential oil vapours. Oil vapours which depress CNV are considered to be sedative and those that increase CNV are stimulant (Lis-Balchin, 2002c); however, with regards to *Pelargonium* oil, both depression and stimulation have been reported from CNV studies in man (Torii *et al*., 1988; Manley, 1993).

1.13 Clinical studies

Pelargonium oil has not been the focus of well controlled clinical trials for either the inhalation of the vapour or application through massage (Hart and Lis-Balchin, 2002). Practically no trials have been carried out using 'geranium oil', since the choice essential oil has almost always been lavender oil (Lis-Balchin, 2002c).

At an Aromatherapy conference, a study carried out at the London clinic concerning the use of essential oils for the treatment of chemotherapy-induced side effects of patients undergoing high dose chemotherapy was reported (Gravett *et al*., 1995); however, no scientific publications followed. Groups of patients were not randomly allocated and

double-blinding was not attempted (Lis-Balchin, 2002c). The treatment of mucositis (damage to the mouth lining due to chemotherapy) with mouth washes often produces a burning sensation. The latter treatment was changed to one drop tea tree, one drop bergamot and one drop 'geranium' in half a glass of boiled warm water five times daily. Gargling with swallowing was allowed. The findings from this study did not indicate a statistically significant difference (Lis-Balchin, 2002c). Another group of patients suffering from chemotherapy-induced diarrhoea were treated with Buscopan® and 'aromatherapy' which consisted of 15 drops 'geranium', 10 drops German chamomile, one drop patchouli and 10 drops turmeric phytol mixed in sweet almond oil (50 ml). This was initially applied twice daily by abdominal massage but due to nausea and diarrhoea this was substituted by oral administration of small doses in an alcoholic vehicle. The findings did not indicate statistically significant differences between groups (Lis-Balchin, 2002c).

A number of clinical studies have been performed (Heil and Reitermann, 1994; Dome and Schuster, 1996; Haidvogl *et al*., 1996) to provide credibility to the claimed efficacy and safety of the herbal product "Umckaloabo." Clinical studies have shown that this herbal medicine has efficacy in ear, nose and throat (ENT) infections and respiratory tract infections. An extract of *P. reniforme* and *P. sidoides* may be a safe herbal product considering the mild to moderate side effects in patients treated with "Umckaloabo" proven by clinical studies (Kolodziej, 2002). Matthys *et al*. (2003) and Chuchalin *et al*. (2005) conducted a randomised, double-blind, placebo-controlled trial to evaluate the efficacy and safety of a *Pelargonium sidoides* preparation (EPs® 7630) in the treatment of acute bronchitis in adults. The results from both studies showed that EPs® 7630 was superior in efficacy compared to the placebo in the treatment of acute bronchitis in adults. The severity of symptoms was reduced and the duration of working inability was shortened for almost two days (Matthys *et al*., 2003). In addition, all adverse events were assessed as non-serious. Therefore treatment with EPs® 7630 may be an effective alternative to antibiotics for the treatment of acute bronchitis.

1.14 Toxicity of 'geranium oil'

From the toxicological aspect, 'geranium oil' has been implicated rarely and all references are attributable to contact dermatitis and sensitization. Most references are due to geraniol, a main component of 'geranium oil' (Lovell, 1993). Patch tests to geraniol were negative;

however, a few studies have indicated dermatitis to perfumes containing 'geranium oil' (Klarmann, 1958). Recent reports from Japanese studies (Nakayama, 1998) provided a list of Class A fragrances classified as common cosmetic sensitizers and primary sensitizers; 'geranium oil' was included in this class.

1.15 The commercial value of *Pelargonium* essential oils and extracts

There is an increasing growth of the commercial use of natural products in all industrial sectors. Export earnings for producers come from the industrial utilization of medicinal plants. 'Geranium oil' is among the top twenty of essential oils and the annual world production is worth almost seven million pounds (Williams and Harborne, 2002).

Due to its pleasant scent, 'geranium oil' is used in various cosmetic preparations such as soaps, creams and perfumes. 'Geranium oil' is employed in toiletries for its herbal character to reinforce the 'Natural' concept (Wells and Lis-Balchin, 2002). 'Geranium oil' and mixtures containing its constituents have been used for many years to make artificial rose oil (Wells and Lis-Balchin, 2002). *Pelargonium graveolens* is used as a substitute for the expensive attar of roses (van der Walt and Vorster, 1988). *Pelargonium* oils contain various monoterpenoids such as (+)-isomenthone and are extensively used in the production of perfumes (van Wyk *et al.,* 2002b). 'Geranium oil' has green herbal, fresh and earthy characteristics and is often used in masculine fragrances. It is also used in women's fragrances: Ivoire, Balmain (1980) contained 'geranium' as a heart note; Giorgio, Armani (1981) is a mixture of mandarin and 'geranium' and Paris, Yves St. Laurent (1983) contained 'geranium' as a top note (Wells and Lis-Balchin, 2002), just to name a few. Many scented-leaf pelargoniums have potential for use in the flavour and fragrance market (Lis-Balchin, 1990). The leaves of a few scented species are used as infusions to condition the hair and to freshen the skin. The leaves are also used as a basis for pot-pourri.

Pelargoniums have found a culinary function, due to the growing popularity of the use of herbs. Several species with scented foliage are possible sources of aromatic oils which may be used for culinary flavouring (Miller, 2002). The food industry employs small amounts of 'geranium oil' in products such as non-alcoholic drinks, ice-creams, candy, baked goods, gelatines and puddings (Lis-Balchin, 1990). It is also used in most major food categories and in alcoholic and soft drinks as a flavouring agent. The various aromas of the scented-leaf pelargoniums are potentially useful in the food industry (Lis-Balchin, 1990).

The aromas of some Pelargoniums are pleasantly floral and fresh, reminiscent of rosaceous fragrances and others are tropical fruit-like e.g. *Pelargonium grossularioides* and *P. mollicomum* and certain *P. odoratissimum* and *P.* x *fragrans* cultivars. Others have a rather balsamic smell e.g. *P. exstipulatum* and *P.* x *fragrans* (Lis-Balchin and Roth, 2000). Among the raw aromatic material employed in the food sector, aromatic substances containing a mint scent occupy a favoured position (Greenhalgh, 1979). *Pelargonium tomentosum* produces a strong and pleasant peppermint-scented essential oil (Demarne and van der Walt, 1990). Numerous *Pelargonium* essential oils and solvent extracts are strongly antimicrobial and could be used commercially in food processing (Deans, 2002).

Pelargonium reniforme and the closely related *P. sidoides* are used as ingredients in a German remedy called "Umckaloabo", which is used to treat bronchitis in children. The presence of umckalin and structurally related coumarins may be partly responsible for the activity of the medicine prepared from *P. reniforme* (van Wyk *et al*., 2002b).

Various *Pelargonium* species have been proclaimed to be used as antispasmodics, antidysenterics and astringents, yet these species have not been commercially exploited (Lis-Balchin, 1990).

The pharmacological properties of the extracts and oils of certain *Pelargonium* species validate their commercial exploitation in the cosmetic, food/beverage and pharmaceutical sectors.

1.16 Rationale

Studies involving Pelargoniums have intensively focused on the chemical composition of their essential oils (Williams and Harborne, 2002). Much research has revolved around *P. capitatum*, *P. graveolens* and *P. radens* oils and their hybrids, while the other indigenous oil-bearing *Pelargonium* species, of the section *Pelargonium*, have received little attention. There is still a great deal to be learnt about the non-volatile compounds present in the *Pelargonium* species. Furthermore, published investigations have revealed that various *Pelargonium* species possess positive pharmacological activities and since species within a family are chemotaxonomically related, there is the possibility that some of the species

belonging to the section *Pelargonium* which hitherto remain unexplored may contain bioactive constituents.

In addition, there is the increasing utilization of natural products in all industrial sectors and studies revealing potential bio-therapeutic properties of several *Pelargonium* oils and extracts have supported their potential niche within the realms of the food, cosmetic and pharmaceutical industries. The other unexplored species of the section *Pelargonium* are deserving of thorough investigation by conducting bioassays and chemical analyses. In this way, we are better equipped to further develop the therapeutic application and commercialization of *Pelargonium* species.

1.16.1 Study objectives

The aims of the study were:

- To determine the chemical composition of selected *Pelargonium* essential oils using GC and GC-MS analysis and to assess if the compositions are chemotaxonomically informative. Phylogenetic relationships of certain *Pelargonium* species within section *Pelargonium* were evaluated by correlation with the chemotaxonomical appraisal of their essential oils.
- To analyse the chemical composition of selected *Pelargonium* solvent extracts (non-volatiles) using HPLC.
- To investigate the *in vitro* bioactivities of the essential oils and solvent extracts. The activities investigated included antimicrobial, anti-oxidant, anti-inflammatory and antimalarial activity. Through the evaluation of these properties, the use of Pelargoniums in traditional medicines can be verified.
- To determine which phytoconstituents are possibly responsible for the investigated activities displayed by the oils and solvent extracts.
- To conduct *in vitro* toxicity studies to assess the feasibility of using the active oils and solvent extracts in commercial preparations.

CHAPTER 2: PLANT MATERIAL COLLECTION AND SAMPLE PREPARATION

2.1 Plant material collection

The *Pelargonium* species (section *Pelargonium*) used in this study were chosen according to the availability of fresh plant material from the botanical gardens (SBG, NBG and WSBG) and according to the authenticity of taxonomic identification. Twenty-one species out of a total of 24 currently recognized species were included in the study. The studied species, their source and voucher details are given in Table 2.1. Preference was given to the collection from the Stellenbosch Botanical Garden of Stellenbosch University. The Department of Botany and Zoology at the University of Stellenbosch has been researching the taxonomy of the genus *Pelargonium* for several years. Plant material in their collection has been authenticated and the taxonomy confirmed.

Table 2.1: The specific localities and voucher numbers of the *Pelargonium* species selected for the study.

Species	Source	Voucher no.
P. betulinum	NBG	A Viljoen 941 (WITS)
P. capitatum	WSBG	A Viljoen 918 (WITS)
P. capitatum	Strand	A Viljoen 951 (WITS)
P. citronellum	NBG	A Viljoen 943 (WITS)
P. citronellum	SBG	A Viljoen 1172 (WITS)
P. cordifolium	NBG	A Viljoen 1173 (WITS)
P. cordifolium	WSBG	A Viljoen 1174 (WITS)
P. crispum	NBG	A Viljoen 1175 (WITS)
P. cucullatum	SBG	A Viljoen 1176 (WITS)
P. cucullatum	WSBG	A Viljoen 1177 (WITS)
P. glutinosum	SBG	A Viljoen 939 (WITS)
P. glutinosum	WSBG	A Viljoen 1178 (WITS)
P. graveolens	SBG	A Viljoen 947 (WITS)
P. graveolens	WSBG	A Viljoen 920 (WITS)

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Species	Source	Voucher no.
P. graveolens	WSBG	A Viljoen 920 (WITS)
P. greytonense	SBG	A Viljoen 1179 (WITS)
P. hermanniifolium	NBG	A Viljoen 1180 (WITS)
P. hermanniifolium	SBG	A Viljoen 1181 (WITS)
P. hispidum	SBG	A Viljoen 946 (WITS)
P. panduriforme	SBG	A Viljoen 949 (WITS)
P. panduriforme	WSBG	A Viljoen 926 (WITS)
P. papilionaceum	NBG	A Viljoen 944 (WITS)
P. pseudoglutinosum	NBG	A Viljoen 1182 (WITS)
P. quercifolium	SBG	A Viljoen 940 (WITS)
P. quercifolium	WSBG	A Viljoen 921 (WITS)
P. radens	NBG	A Viljoen 942 (WITS)
P. scabroide	SBG	A Viljoen 1183 (WITS)
P. scabrum	SBG	A Viljoen 948 (WITS)
P. sublignosum	SBG	A Viljoen 1184 (WITS)
P. tomentosum	SBG	A Viljoen 950 (WITS)
P. tomentosum	WSBG	A Viljoen 919 (WITS)
P. vitifolium	SBG	A Viljoen 945 (WITS)

Where: - NBG = National Botanical Garden (Kirstenbosch), SBG = Stellenbosch Botanical Garden, WSBG = Walter Sisulu Botanical Garden (Johannesburg). Voucher specimens of all the material collected are housed in the herbarium of the Department of Pharmacy and Pharmacology, University of Witwatersrand (WITS), Johannesburg.

Table 2.2: The total taxa in section *Pelargonium* and an indication of the sampling carried out in this study.

Species	Species sampled for essential oil testing				Species sampled for extract testing				
	GC-MS analysis	**MIC assay**	**5-LOX assay**	**MTT assay**	**HPLC analysis**	**MIC assay**	**DPPH assay**	**Antimalarial assay**	**MTT assay**
P. betulinum	✓	-	-	-	✓	✓	✓	✓	✓
P. capitatum	✓	-	-	-	✓	✓	-	✓	✓
P. citronellum	✓	✓	✓	✓	✓	✓	✓	✓	✓
P. cordifolium	-	-	-	-	✓	✓	✓	✓	✓
P. crispum	-	-	-	-	✓	✓	✓	✓	✓
P. cucullatum	-	-	-	-	✓	✓	✓	✓	✓
P. denticulatum	-	-	-	-	-	-	-	-	-
P. englerianum	-	-	-	-	-	-	-	-	-
P. glutinosum	✓	-	-	-	✓	✓	✓	✓	✓
P. graveolens	✓	✓	✓	✓	✓	✓	✓	✓	✓
P. greytonense	-	-	-	-	✓	✓	-	✓	✓
P. hermanniifolium	-	-	-	-	✓	✓	✓	✓	✓
P. hispidum	✓	-	-	-	✓	✓	✓	✓	✓
P. panduriforme	✓	✓	✓	✓	✓	✓	✓	✓	✓
P. papilionaceum	✓	-	-	-	✓	✓	✓	✓	✓
P. pseudoglutinosum	-	-	-	-	✓	✓	✓	✓	✓
P. quercifolium	✓	-	✓	✓	✓	✓	✓	✓	✓
P. radens	✓	✓	✓	✓	✓	✓	-	✓	✓
P. ribifolium	-	-	-	-	-	-	-	-	-
P. scabroide	-	-	-	-	✓	✓	-	✓	✓
P. scabrum	✓	-	✓	✓	✓	✓	✓	✓	✓
P. sublignosum	-	-	-	-	✓	✓	✓	✓	✓
P. tomentosum	✓	✓	✓	✓	✓	✓	✓	✓	✓
P. vitifolium	✓	-	-	-	✓	✓	-	✓	✓

Where: - GC-MS = gas chromatography coupled to mass spectroscopy; MIC = minimum inhibitory concentration; 5-LOX = 5-lipoxygenase; MTT = 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; HPLC = high performance liquid chromatography; DPPH = 2,2-diphenyl-1-picrylhydrazyl.

2.2 Sample preparation

2.2.1 Essential oils

Distillation was performed either on the same day of harvesting or the day after harvesting. The aerial parts (i.e. leaves, stalks and stems) of the fresh plant material were cut into small pieces and the essential oil was extracted by hydrodistillation using a modified Clevenger's glass apparatus. This extraction process was carried out at room temperature for 3 hours, after which the essential oils extracted were collected in amber vials and refrigerated prior to analysis.

2.2.2 Non-volatile extracts

Fresh plant material (stems and leaves) of each species was dried in the oven set at 30°C. The yield of extractives is increased by the pre-drying of the plant material as this leads to rupture of the cell structure and subsequently improved solvent access (Harborne *et al*., 1975). Once dried, the plant material was pulverized using a pestle and mortar. This powdered material was subjected to solvent extraction with acetone for 3 hours in a water bath set at 40°C. The extractant material and solvent were filtered (Whatman® filter paper no 41, pore size 20-25 μm) to remove any plant material debris. Following this, the plant material was rinsed twice with acetone and filtered after each rinse. The extracts were reduced under vacuum using a rotary evaporator (Büchi rotavapor R-114, waterbath B-480) at 35°C. The resultant acetone extracts were further reduced in the fumehood and once concentrated to dryness kept at 4°C prior to analysis.

CHAPTER 3: ESSENTIAL OIL COMPOSITION AND CHEMOTAXONOMY

3.1 Introduction

Since the early 1800's, the rose-scented 'geraniums' belonging to the genus *Pelargonium* have been cultivated commercially for their essential oil. Much research has been done to clarify the chemical composition of these cultivars (Widmer and Collins, 1991). 'Geranium oil' is a complex mixture of more than 120 monoterpenes and sesquiterpenes and other low molecular weight aromatic compounds (Lis-Balchin, 2002a). These are commercially important plant products employed extensively in the perfume, pharmaceutical and flavour sectors (Brown and Charlwood, 1986). Due to the difference in cultivars used, the climate, the country of origin, the time of the harvest, fertilizers used, etc., the chemical composition of the oil is variable (Lis-Balchin, 2002a). However, citronellol, geraniol and linalool, either free or in ester combination, are the major compounds making up about 60-70% of the oil. The following terpenoids occur in oils from all localities: isomenthone, menthone, nerol, *cis*-rose oxides and *trans*-rose oxides, α-terpineol, α-pinene, myrcene and β-phellandrene (Williams and Harborne, 2002). Only a few investigations have been carried out to determine the volatiles present in other scented *Pelargonium* species, which are not used commercially (Widmer and Collins, 1991).

In addition to correlating the data obtained in this study with previously reported analyses concerning the well-known Pelargoniums, the chemical compositions of those *Pelargonium* essential oils which have received little attention were investigated. Thirteen *Pelargonium* species (18 samples) out of a total of 24 (section *Pelargonium*) were selected for investigation of their essential oil composition. This study aims to broaden the existing knowledge concerning the volatile *Pelargonium* constituents and explores the possible chemotaxonomic application of the essential oil data of selected species belonging to the section *Pelargonium*.

3.1.1 The current hypothesis of relationships in section *Pelargonium*

Bakker *et al.* (2004) derived phylogenetic hypotheses for the genus *Pelargonium* L'Hérit. based on DNA sequence data from nuclear, chloroplast and mitochondrial encoded regions. The datasets contained cpDNA *trnl-F* sequences for 152 taxa, nrDNA ITS sequences for 55 taxa and mtDNA *nadl* b/c exons for 51 taxa. Following total evidence analysis of the three genomic DNA regions, a single hypothesis was constructed which revealed that the genus consists of five main clades (A1, A2, B, C1 and C2). Of particular interest, A1 is divided into two clusters, the lower one of which contains mostly the analysed species of section *Pelargonium*. This lower branch is further divided into two groups; the upper group contains, in addition to the analysed species of *Campylia*, *P. denticulatum* and *P. quercifolium* which are closely associated with one another. The lower group contains four subclusters, encompassing the other analysed *Pelargonium* species of section *Pelargonium*. The first subcluster contains only *P. hispidum*; in the second subcluster, *Pelargonium tomentosum* and *P. papilionaceum* are closely related; in the third, *P. graveolens*, *P. radens* and *P. vitifolium* are also closely associated. The karyological similarity ($2n$ = 88) between *P. graveolens* and *P. radens* was reported by Albers and van der Walt (1984). The fourth subcluster comprises the remainder of the analysed *Pelargonium* species. Within this subcluster, *P. alpinum*, *P. grandiflorum* and *P. sublignosum* were grouped together and further, *P. scabrum* was clustered with two species from section *Campylia*, namely *P. incarnatum* and *P. setulosum*.

Furthermore, the constructed hypothesis following optimization of the cpDNA *trnl-F*/nrDNA ITS/mtDNA datasets indicated that *P. hispidum* is more closely associated to *P. papilionaceum* and *P. tomentosum* than to the other analysed species. Within the clustering of *P. graveolens*, *P. radens* and *P. vitifolium*, the latter two species are more closely related. With respect to the fourth subcluster and taking into account only those species which were included in our essential oil analysis, *P. betulinum*, *P. citronellum* and *P. scabrum* reside in this subcluster. Bakker *et al*. (2004) indicated that all the analysed species of section *Pelargonium* have a chromosome number of x = 11.

An evaluation was carried out to assess if the essential oil compositions were chemotaxonomically informative by comparison to the latest species-level phylogeny for the section *Pelargonium*. The current phylogenetic relationships that were tested in this study included the occurrence of *P. quercifolium* as an outlying species, the association of

P. hispidum with respect to the other analysed species, the relationship between *P. tomentosum* and *P. papilionaceum*, the close grouping of *P. graveolens*, *P. radens* and *P. vitifolium* and the clustering of *P. betulinum*, *P. citronellum* and *P. scabrum*.

3.2 Materials and methods

3.2.1 Thin layer chromatography

Thin layer chromatography (TLC) was used for the qualitative analysis of the selected *Pelargonium* essential oils. It was performed as a preliminary study to provide information as to whether a chemical pattern of variation or uniformity exists between and among the selected species.

3.2.1.1 Principle of the method

TLC is a chromatographic technique of fractionating crude mixtures into their components by the movement of a solvent on a thin layer of suitable adsorbent (Smith and Feinberg, 1972). It is viewed as a crude method for the identification of the separated chemical substances. It has the advantages of speed, low cost and simplicity (Jork *et al.*, 1990).

3.2.1.2 Protocol

The essential oils were diluted with hexane in the ratio 1: 7. For each test sample, using a micro-pipette, spots of 2 µl each were applied 2 cm from the bottom of the silica gel plates (Alugram Sil G/UV_{254}). When each spot was applied, the solvent was allowed to evaporate before another spot was applied. The mobile phase used was toluene: ethyl acetate (9.3: 0.7). The atmosphere of the TLC tank was given time to become saturated with the vapour of the developing solvent. The plate was placed vertically in this solvent, without immersing the spots themselves. Once the solvent reached the solvent front (1 cm from the top edge of the TLC plate), the plate was removed from the tank and dried. Vanillin-sulphuric acid spray reagent was prepared in the following way: 1 g vanillin was dissolved in 100 ml ethanol to make solution I and 10 ml sulphuric acid was added to 90 ml ethanol to make solution II. The plate was sprayed with 10 ml of solution I followed immediately by 10 ml of solution II. The plate was placed in the oven at 110°C for 5-10 minutes under observation and the separated compounds were evaluated visually (Wagner and Bladt, 1996).

3.3 Results and discussion

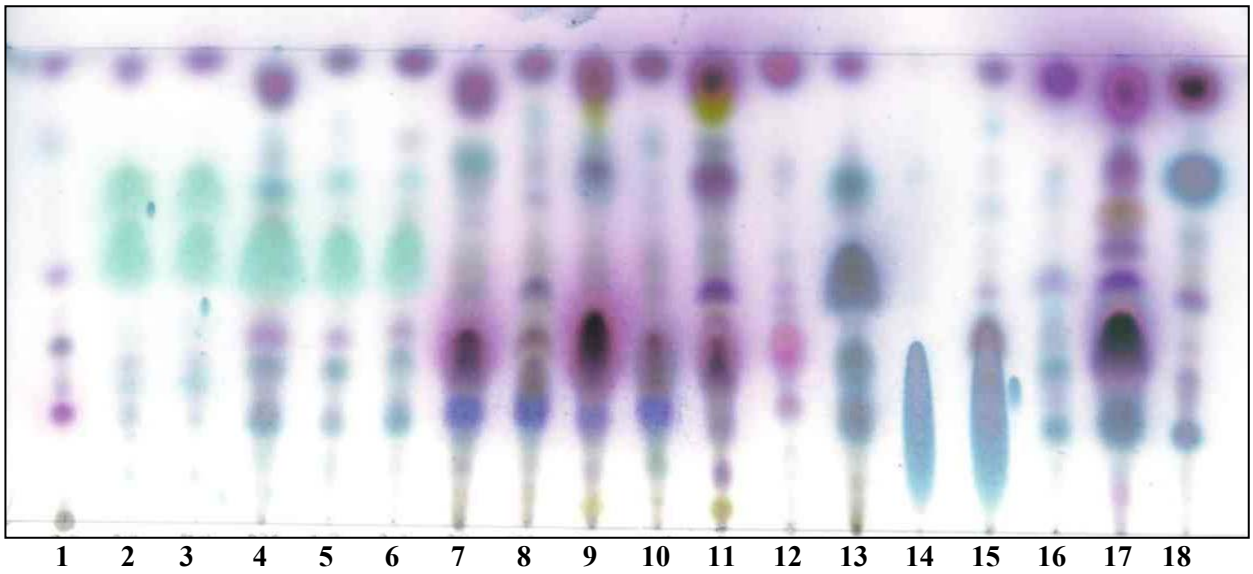


Figure 3.1: Vanillin sprayed-TLC plate of the essential oils of selected *Pelargonium* species. The track numbers of the species are given in Table 3.1.

Table 3.1: TLC plate track number and corresponding species name and locality.

TLC plate track number	Species	Locality
1	*P. capitatum*	WSBG
2	*P. tomentosum*	WSBG
3	*P. tomentosum*	SBG
4	*P. graveolens*	WSBG
5	*P. radens*	NBG
6	*P. graveolens*	SBG
7	*P. quercifolium*	WSBG
8	*P. panduriforme*	WSBG
9	*P. quercifolium*	SBG
10	*P. panduriforme*	SBG
11	*P. glutinosum*	SBG
12	*P. betulinum*	NBG
13	*P. citronellum*	NBG
14	*P. papilionaceum*	NBG
15	*P. vitifolium*	SBG
16	*P. hispidum*	SBG
17	*P. scabrum*	SBG
18	*P. capitatum*	Strand

NBG = National Botanical Garden (Kirstenbosch), SBG = Stellenbosch Botanical Garden, WSBG = Walter Sisulu Botanical Garden (Johannesburg).

TLC demonstrated the most characteristic constituents present in the selected *Pelargonium* essential oils. Chemical variation appears to occur between the oil samples of *P. capitatum* (track 1 and 18); this may be due to the separation of different quantities. The profiles produced by the two samples of *P. quercifolium* (track 7 and 9) are similar. *Pelargonium glutinosum* (track 11) appears to be most comparable to *P. quercifolium* (track 9). Some degree of compositional variation exists between the *P. panduriforme* samples (track 8 and 10). *Pelargonium quercifolium* (track 7) and *P. panduriforme* (track 10) produce analogous profiles. The two samples of *P. graveolens* (track 4 and 6) produce identical chemical compositions as do the two samples of *P. tomentosum* (track 2 and 3). The TLC profile of *P. radens* (track 5) is similar to those of the *P. graveolens* oils (track 4 and 6). Furthermore, the *P. tomentosum* oils (track 2 and 3) show similarities with *P. radens* (track 5) and *P. graveolens* (track 4 and 6). *Pelargonium betulinum* (track 12) displays a chromatographic profile different from those of the other species as does *P. citronellum* (track 13). *Pelargonium scabrum* (track 17) appears to have some compounds in common with *P. quercifolium* (track 7 and 9) and *P. glutinosum* (track 11). *Pelargonium hispidum* (track 16) shares some similarities with *P. capitatum* (track 18).

TLC indicated an unusual compound in the essential oils of *P. papilionaceum* (track 14) and *P. vitifolium* (track 15). Lis-Balchin and Roth (1999) found citronellic acid in only two *Pelargonium* species, *P. papilionaceum* and *P. vitifolium* and one cultivar, 'Sweet Rosina'. This characteristic compound was verified as being citronellic acid by running TLC with a standard of citronellic acid and obtaining a retention factor (R_f) value. The TLC profiles of *P. papilionaceum* (track 14) and *P. vitifolium* (track 15) are evidently similar to one another and are anomalous compared to those of the other species.

Since essential oils are complex mixtures of many different constituents, other techniques of analysis were used to provide a more detailed profile of their composition.

3.4 Materials and methods

3.4.1 Gas chromatography (GC) and gas chromatography coupled to mass spectroscopy (GC-MS)

In order to characterize the patterns of chemical diversity and similarity displayed in the preliminary TLC study, GC analysis was performed on all essential oil samples. GC-MS analysis was carried out for further identification of the individual components in the oil mixtures. The method of choice for the identification of terpenoids, fatty acids and various other volatile compounds in complex plant extracts is GC coupled with mass spectroscopy (Spring, 2000).

3.4.1.1 Principle of the method

Gas chromatography is a separating device. Retention times of the chromatogram peaks are compared with those of reference compounds run under identical conditions to tentatively identify the peaks. Gas chromatography is also appropriate for quantitative analysis. Mass spectroscopy is a method of analysis which determines the molar mass of a substance and provides information about the molecular structures. The mass spectrum of a substance is unique to that substance. In this way one can compare the mass spectrum of a substance to that of previously recorded spectra resulting in the identification of that substance. Mass spectroscopy is used for positive peak identification (Gillespie *et al*., 1994).

3.4.1.2 Protocol

Analytical gas chromatography was performed using a Shimadzu GC-17A system fitted with J & W-DB1 column (30 m x 0.25 mm with 0.25 µm film thickness). Helium was used as the carrier gas (1 ml/min). The column temperature was programmed at 60°C for 1 min and then raised to 180°C for the total analysis time of 80 min. The injector port and flame ionization detector (FID) were set at 250°C.

Identifications of the compounds in the oils were carried out by GC-MS analysis using a Hewlett-Packard 1800A GCD system. The system was equipped with an Innowax FSC column (60 m x 0.25 mm with 0.25 µm film thickness) and helium was used as the carrier gas at a flow rate of 1 ml/min. The following parameters were used: the GC oven temperature was kept at 60°C for 10 min and programmed to increase to 220°C at a rate of

4°C/min, then kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Alkanes were used as reference points in the calculation of relative retention indices (RRI). Split flow was adjusted at 50 ml/minute. The injector and detector temperatures were set at 250°C. MS were taken at 70 eV. Mass range was from 35 to 425 *m/z*. Component identifications were done by comparing their mass spectra and retention indices using Wiley GC-MS Library and BAŞER Library of Essential Oil Constituents. The latter is an in-house database generated with authentic standards. The percentage amounts were obtained from electronic integration measurements using flame ionization detection (FID).

3.4.2 Cluster analysis

Essential oil components can be useful taxonomic markers and cluster analysis, a multivariate statistical procedure, based on chemical composition facilitates this establishment of chemotaxonomical relationships between or among selected species. Cluster analysis was performed on the GC-MS data of 18 essential oil samples (13 species) to ascertain potential patterns of shared and diverse chemistry between and among certain *Pelargonium* species.

3.4.2.1 Principle of the method

Cluster analysis is performed on the combined matrix of qualitative and quantitative phytochemical data. Through this method of analysis a branching diagram referred to as a dendrogram is generated, which represents the degree of phenetic similarity among the species analysed by GC-MS. In this way relationships can be observed between and among species. An operational taxonomic unit (OTU) is the lowest taxonomic unit that can be classified in a study. The tips of the dendrogram are represented by the tips of the OTUs. The OTUs are clustered into groups according to similarity. The greater the coefficient value (r), the higher the level of similarity.

3.4.2.2 Protocol

Using quantitative and qualitative data from GC-MS analysis, a combined data matrix was created. The data for each species was tabulated into a matrix with the individual species as operational taxonomic units, where n = 18 were assigned as columns of individual species and n = 315 were assigned as rows of compounds. The data matrix was analysed by a phenetic program, NTSYS-pc software package, version 2.0 (Rohlf, 1998). Correlation was selected as a measure of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition.

3.5 Results and discussion

The percentage amounts of the compounds identified in the selected *Pelargonium* essential oils are presented in Table 3.2. In addition to the summary given in Table 3.2, the essential oil composition of each species is included in the monograph section which has been added as an appendix to this dissertation. Figure 3.2 shows the dendrogram generated from the statistical analysis performed on a total of 315 different chemical compounds. Quantitative and qualitative chemical variability and similarity occurs between and among the analysed species.

Table 3.2: The chemical composition of the hydrodistilled essential oils of selected *Pelargonium* species.

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
1014	tricyclene						0.1	tr			0.6	tr			0.2	0.1	0.4	0.3	
1032	α-pinene	0.1	0.2				0.2	0.1	tr	0.6	0.3	tr		0.1	0.3	0.1	0.5	0.3	
1035	α-thujene									1.0	0.1			0.2		tr			
1048	2-methyl-3-buten-2-ol				0.1														
1076	camphene								tr	tr	0.1				tr	tr	0.1	tr	
1100	undecane					tr			tr			tr							
1112	anhydrolinalool oxide				tr				tr										
1118	β-pinene						0.1	tr	tr	0.1	tr			tr	0.1	0.3	tr	tr	
1132	sabinene									0.5	0.2			0.1	tr	tr	tr	tr	
1136	thuja-2,4(10)-diene									0.1									
1146	δ-2-carene																	tr	
1159	δ-3-carene									0.3				tr					
1174	myrcene				tr		0.4	0.3				tr			0.9	1.4	0.8	0.4	
1176	α-phellandrene								0.1	4.3	8.8		0.8	1.1					
1188	α-terpinene								tr	0.2	0.1								
1195	dehydro-1,8-cineole				0.2														
1202	3-hexanol		0.1																
1203	limonene				0.1	tr	0.6	0.6	tr	2.2	3.0	tr	0.3	0.2	0.8	0.2	1.5	1.3	
1213	1,8-cineole			tr		0.1			0.2			tr				1.3			0.1

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
1216	3-methylcyclopentanone						0.1	tr							tr		tr	tr	
1218	β-phellandrene						tr	tr	tr	1.4	4.0		0.3	0.2	0.1		0.6	0.1	
1225	(Z)-3-hexenal								tr			tr							
1230	butyl butyrate												0.1						
1246	(Z)-β-ocimene								tr	tr	2.4	0.3		tr			tr		
1255	γ-terpinene								tr	1.0	0.3	tr	0.3	tr					
1266	(E)-β-ocimene								tr	tr	0.2	0.1		tr			tr		
1280	p-cymene	0.2	0.1		0.1	0.5	0.9	0.9	0.1	45.4	37.6	tr	6.2	54.9	1.1	1.0	1.1	0.8	0.2
1290	terpinolene								tr	0.1		tr		tr					
1327	3-methylcyclohexanone						0.1	tr							0.1				
1337	geijerene								0.1			tr							tr
1348	6-methyl-5-hepten-2-one				0.4							tr							tr
1353	*cis*-rose oxide		0.6				tr	tr	tr			0.1				0.1			tr
1365	*trans*-rose oxide		0.3					tr				tr				tr			
1384	α-pinene oxide							tr							tr		tr	tr	
1384	heptyl acetate	0.1																	
1385	allo-ocimene*										0.1	tr							
1391	(Z)-3-hexenol				0.1		tr		0.1			tr				tr			
1398	2-nonanone						tr		2.5							0.1			
1400	nonanal								tr			tr							
1400	(Z)-2-hexenol								tr			tr				0.1			

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
1402	heptyl isobutyrate			0.5															
1406	fenchone																tr	tr	
1413	rosefuran										0.1								
1419	photocitral B				0.2														
1429	perillene				0.1		tr	0.1		tr	0.1	tr		0.2	0.1	tr	tr	tr	
1429	(E)-2-octenal								tr										
1434	hexyl butyrate					3.2							8.2						
1450	*trans*-linalool oxide (furanoid)				0.1		0.1	tr	0.1			tr			0.1	0.1			tr
1451	β-thujone									tr				0.1					
1452	1-octen-3-ol				tr				tr										tr
1452	α,p-dimethylstyrene									1.4	0.2		0.2	0.5			tr		
1458	*cis*-1,2-limonene epoxide						tr	tr							tr			tr	
1459	acetic acid			0.3	tr	0.3						tr							tr
1464	(E)-2-hexenyl butyrate			0.5	tr	2.7							6.3						
1466	α-cubebene		0.5			0.2					0.1	tr				tr			tr
1473	(Z)-3-hexenyl 2-methylbutyrate				tr				0.1							tr			
1475	menthone		0.9			0.7	5.1	1.0	0.1						1.9		41.1	36.1	
1478	*cis*-linalool oxide (furanoid)				0.1					0.5						0.1			
1481	(Z)-3-hexenyl isovalerate							tr	0.2							tr			
1482	*cis*/*cis*-photocitral				0.3														

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
1487	citronellal																		tr
1491	octyl acetate	5.7																	tr
1492	cyclosativene												0.2						
1493	α-ylangene					0.1								0.1					tr
1497	α-copaene	1.4	0.7		0.1				0.1	0.1	0.2		0.1			0.2			0.4
1503	isomenthone		1.9		0.1	1.4	65.8	83.3	0.3		0.3	tr			84.5	tr	49.3	56.6	
1503	hexyl valerate					0.1							0.1						
1506	*trans*/*trans*-photocitral				0.6														
1508	heptyl butyrate					0.4							0.2						
1509	2-nonanol								0.2										
1528	α-bourbonene	0.2	0.1																tr
1532	camphor			0.1		tr			tr							tr			
1535	β-bourbonene	2.8	1.5		tr	0.3	tr	tr									tr	tr	0.1
1541	benzaldehyde				0.1					0.1		tr				0.1	tr	tr	
1545	*cis*-α-bergamotene															tr			
1549	β-cubebene	0.2				0.1					0.3								tr
1553	linalool		0.3	0.1	1.8	0.1	0.3	0.3	0.7	4.4		tr			0.6	0.7	tr	0.1	0.2
1553	octanol	0.1	0.2	0.1				tr	tr			tr							tr
1556	8,9-limonene epoxide*																	tr	
1560	*trans*-dihydro-α-terpineol									0.2									
1562	isopinocamphone			0.3															

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
1568	*trans*-α-bergamotene															0.3			
1569	neoisopulegol											tr							0.3
1570	methyl citronellate											tr							0.1
1571	*trans*-p-menth-2-en-1-ol							tr			0.6	tr		0.2	tr		tr	0.1	
1572	β-ylangene	0.1													0.2				
1574	hexadecane		0.2																
1576	pentyl hexanoate					0.3													
1577	α-cedrene	0.6			0.5											0.3			
1580	*cis*-isopulegone							0.1							tr				
1583	isopulegol											tr					tr		0.3
1584	*trans*-isopulegone																		tr
1586	pinocarvone							tr											
1586	cyperene																	tr	
1587	methyl decanoate						0.1		0.1							4.2			
1588	β-copaene		tr																tr
1589	*cis*-dihydro-α-terpineol									tr									
1589	isocaryophyllene		1.0						0.1										
1592	neomenthol																0.3	0.1	
1596	α-guaiene																tr		
1597	bornyl acetate		0.1	0.4						0.1									
1600	β-elemene							0.1					0.1				tr	0.1	

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
1602	6-methyl-3,5-heptadien-2-one				tr							tr							
1604	2-undecanone								tr										
1604	thymol methyl ether									tr									
1606	p-menth-3-en-8-ol						tr	tr											
1608	octyl butyrate			2.1									0.4						
1611	terpinen-4-ol			tr						0.6				0.2					
1612	β-caryophyllene	5.3		0.2	0.6	2.5			8.6		0.2	0.1	0.1			3.9	0.4		0.2
1612	citronellyl formate		31.1				tr	tr											0.1
1613	β-cedrene	tr			tr														
1614	hexyl tiglate					0.8							0.2						
1615	p-menth-1-en-9-al								tr										
1616	hotrienol									0.2									
1617	guaia-6,9-diene		0.5			0.3				0.1	0.1		0.1	tr	tr				
1618	cadina-3,5-diene					0.3			0.1										
1620	β-cyclocitral											tr							
1620	ethyl decanoate								tr										
1624	*cis*-β-terpineol															tr			
1626	2-methyl-6-methylen-3,7-octadien-2-ol															0.2			
1628	aromadendrene				tr	0.7				0.3	0.2		0.1	0.1					0.1
1632	neoisomenthol						0.4								0.2		tr	tr	
1637	menthol					0.1	0.1	0.2	0.1						0.1		0.6	0.2	

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
1638	*cis*-p-menth-2-en-1-ol							0.3								tr	0.1	0.1	
1639	*trans*-p-mentha-2,8-dien-1-ol				0.1														
1647	(E)-2-decenal					0.1						tr							
1651	sabina ketone									0.1									
1661	allo-aromadendrene	0.6	0.6			0.7				0.3	0.1		0.8	0.1	tr	tr			0.1
1662	isomenthol						0.1								tr				
1664	1-nonanol	0.1																	
1664	*trans*-pinocarveol									tr									
1668	citronellyl acetate		0.9						tr			tr				0.1			
1668	(Z)-β-farnesene	0.2					tr	0.1							tr	0.2			tr
1674	γ-gurjunene		0.3												tr				
1677	epi-zonarene										0.1								
1678	*cis*-p-mentha-2,8-dien-1-ol																	tr	
1679	heptadecane		0.1						tr										
1681	(Z)-3-hexenyl tiglate								0.1										
1682	δ-terpineol															tr			
1683	carvotanacetone									0.1	0.1			tr					
1685	isovaleric acid																		tr
1687	α-humulene	1.5				0.2			0.4			tr			tr	0.3	0.1	tr	
1688	selina-4,11-diene										tr								
1689	*trans*-piperitol				0.1														

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
1690	cryptone		0.2				0.3	0.3		0.7	2.9		tr	1.4	0.3		0.2	0.3	
1693	β-acoradiene	0.5														0.1			
1694	neral				17.4														
1696	β-alaskene															0.1			
1698	geranyl formate		0.5																
1704	γ-muurolene	1.9	0.4				0.1	tr	0.1							0.2			0.2
1706	α-terpineol		0.3	0.3	0.2	0.2	0.1	0.2	0.5	0.5		0.3			0.1	2.8	0.1	0.1	0.2
1707	ledene					0.9			tr	0.7			1.8	0.4			0.1	tr	tr
1714	(Z,E)-α-farnesene								tr										
1719	borneol			1.0				tr							0.1		tr	tr	
1725	verbenone			0.7											tr				
1726	germacrene D	0.2									tr		0.1				0.1		
1730	δ-guaiene												tr	0.3					
1740	valencene												0.1		tr				0.1
1740	α-muurolene	2.4	1.4	0.6		tr		0.3	0.1		0.3		0.5	0.2		tr			0.4
1740	geranial				27.2														
1741	β-bisabolene						0.5										0.1		
1743	α-cadinene	0.2														tr			
1744	α-selinene		0.3																
1748	piperitone						0.4	0.3	0.1			tr		tr	0.6		0.5	0.4	0.2
1750	β-dihydroagarofuran			0.4		0.4			tr										0.1

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
1751	phellandral										0.7			0.3					
1751	carvone						0.1	0.1			0.4			0.4	0.1		tr	tr	
1755	*cis*-dihydroagarofuran			0.2															
1755	bicyclogermacrene												0.1						
1758	*cis*-piperitol									tr	0.1			tr			tr	tr	
1758	(E,E)-α-farnesene								0.2										
1763	naphthalene					0.5													
1765	geranyl acetate		0.2									tr							
1766	1-decanol	0.5		0.2					tr										
1772	citronellol		9.9				0.4	0.4	0.3			0.1			0.2				2.2
1773	δ-cadinene	2.6			0.1		tr	tr	0.3	0.1	0.7		1.2	0.5		0.7			
1776	γ-cadinene	tr			tr	tr			tr	tr	0.1		0.1	tr		0.6			
1783	drimenene					0.1													
1783	p-methylacetophenone				0.2		0.1	0.1		0.8	0.7			0.7	0.1		tr	tr	
1786	ar-curcumene	0.1			0.2											0.7			
1798	methyl salicylate				tr		0.1								0.1	tr			
1799	cadina-1,4-diene								tr										
1800	octadecane		0.1																
1802	cuminaldehyde						tr	0.1		0.7	1.2		0.1	0.8	tr		tr	0.1	
1805	tridecanal			0.3															
1808	nerol				0.6				tr										

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
1809	citronellyl butyrate		0.2	0.2				0.1	0.1	0.1		0.3			tr				
1811	p-mentha-1,3-dien-7-al																	tr	
1815	valeric acid																		0.1
1823	p-mentha-1(7),5-dien-2-ol*									0.8	1.1		0.6	1.0			tr		
1825	geranyl propionate											tr							
1827	(E,E)-2,4-decadienal										0.1								
1829	octyl hexanoate												0.1						
1834	citronellyl isovalerate															0.1			
1837	myrtanol*			0.1															
1845	*trans*-carveol		tr				0.1		0.1	tr	0.3				0.1		tr	tr	
1853	*cis*-calamenene	1.0	1.6	0.5		1.3	tr	tr	tr		0.4		0.1	0.2		0.2			0.1
1857	geraniol				1.0				0.1	tr		tr				0.1			tr
1864	p-cymen-8-ol		0.4		0.7		0.1	0.1	tr	4.0	2.9	tr	1.0	2.8	0.1	0.1	tr	tr	tr
1865	isopiperitenone										0.1			0.1					
1868	(E)-geranyl acetone		0.4			0.2	tr		tr			tr							0.1
1871	*trans*-calamenene	0.1																	
1882	*cis*-carveol								tr										
1885	2-phenylethyl propionate							0.2							0.1		tr		
1886	geranyl butyrate				0.1	0.3				0.1		0.1		0.2					
1889	ascaridole						tr												
1896	2-phenylethyl isobutyrate							0.1							0.1			tr	

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
1898	1,11-oxidocalamenene	0.1																	tr
1900	epi-cubebol										0.2								
1916	α-agarofuran			2.8		0.1					1.1								0.5
1918	β-calacorene	0.1																	
1937	2-phenylethyl alcohol				0.1			0.1											
1941	α-calacorene	0.4			0.1	0.2	0.1		0.1	tr	0.1		0.4	0.1	tr	0.3			tr
1941	citronellyl hexanoate							0.1		0.1				0.1					
1949	(E)-jasmone								0.1										
1949	piperitenone						tr										0.1		
1953	palustrol					0.2				tr									
1965	citronellyl propionate														0.1				
1965	3-phenylpropyl acetate	3.0																	
1966	(E)-12-norcaryophyll-5-en-2-one								tr										
1969	(Z)-jasmone															0.1			
1980	2-phenylethyl butyrate											tr			tr				
1981	heptanoic acid															0.1			
1984	γ-calacorene	0.2					tr				0.1		0.1			0.1			tr
1988	2-phenylethyl 2-methylbutyrate						tr								tr				
1992	2-phenylethyl isovalerate														tr				
1998	furopelargone A		0.1																
2001	isocaryophyllene oxide	0.7		0.3	0.2	0.8										0.4	tr	0.1	tr

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
2008	caryophyllene oxide	2.8	2.8	10.0	0.9	6.3	0.1	0.1	0.5	0.1	0.4	tr			tr	4.2	0.2	0.2	0.6
2012	maaliol					0.2													
2020	caryophylla-8(14)-en-5-one															0.3			
2030	norbourbonone		0.5				tr	tr							tr				
2030	methyl eugenol				0.1	0.3					0.1			tr		0.3			
2033	epi-globulol			0.6															
2040	octyl octanoate												0.2						
2045	humulene epoxide I	tr		0.1									tr						
2050	(E)-nerolidol	0.1			tr	0.2	tr	0.5	0.2				2.8		0.4	0.9			0.1
2057	ledol	0.1	0.2			0.4				0.3	0.1			0.2				tr	
2071	humulene epoxide II	0.8	0.5	0.1	tr	0.6					0.2				tr	0.1	tr	tr	0.1
2080	cubenol	2.5		0.6		1.0	tr	0.1		0.1	0.4			tr	0.1	0.3			
2084	octanoic acid								0.1			tr							
2088	1-epi-cubenol	0.6		0.5		0.2	0.1	0.1			0.3					0.1			
2096	neryl hexanoate													tr					
2096	elemol									0.6									
2098	globulol			0.1	0.2	0.9												tr	
2104	viridiflorol	0.3		24.0	tr	8.9				7.9			36.6	13.0			tr		
2105	geranyl hexanoate													tr					
2110	furopelargone B		6.1						tr		0.1				tr				
2113	cumin alcohol							tr		0.1	tr			tr			tr	tr	

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
2127	10-epi-γ-eudesmol			6.9		0.3			0.1	0.1	6.3			tr					7.1
2131	hexahydrofarnesylacetone	tr	0.5				tr					tr				tr	tr		0.1
2140	2-phenylethyl valerate							0.2							0.1				
2143	cedrol	5.4		0.3				tr								0.7			
2144	spathulenol		0.4		0.6	6.9				3.8	1.9		6.2	5.9	tr		tr		
2170	β-bisabolol															tr			
2179	1-tetradecanol				0.1							tr	0.3						
2184	nonanoic acid								0.4			tr					tr		0.1
2184	*cis*-p-menth-3-ene-1,2-diol													tr					
2185	γ-eudesmol			0.3						2.0			0.3						
2187	T-cadinol	18.9	0.5					tr	tr					0.9		6.1			0.9
2193	β-betulenal												0.2			5.6			
2196	ambrox					2.3							0.4						
2198	thymol						tr							0.3			tr		
2204	clovenol															0.4			
2205	eremoligenol			0.5															
2209	T-muurolol	1.7	0.3	0.1			tr	0.1			tr			tr	tr	0.2		tr	
2210	agarospirol			0.4							0.5								0.4
2211	2-phenylethyl tiglate						1.3	1.9							0.8		tr	tr	0.3
2219	α-muurolol	0.8	0.4	0.3							tr								tr
2228	valerianol			0.9							0.8								tr

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
2239	carvacrol				0.2		0.1			0.9	1.3		0.9	1.5	tr		tr		
2247	*trans*-α-bergamotol	tr			0.1	1.2					0.2		0.6	tr					
2249	cadalene		1.1				0.1	0.3					0.3						
2250	α-eudesmol									0.9									
2253	4α-hydroxy-dihydroagarofuran			2.2							3.8								
2254	citronellic acid		tr		0.1		0.1	tr	0.8			96.2					tr	tr	74.7
2255	α-cadinol	4.5			0.1								0.3	0.1	0.1		tr	tr	
2257	decyl decanoate								tr										
2257	β-eudesmol			2.9					tr	0.9	tr								
2272	14-acetoxy-β-caryophyllene															0.1			
2273	1-pentadecanol				0.1									tr					
2273	selin-11-en-4α-ol			0.2															
2279	sandaracopimaradiene					1.0											tr		
2281	decanoic acid		0.3	0.1			12.9	0.4	47.0						0.1	6.4			
2287	8,13-epoxy-15,16-dinor-labdane					0.4													
2287	8,13-epoxy-15,16-dinor-labd-12-ene					2.3							1.4						
2300	tricosane			0.1															
2308	nerolic acid				0.3														
2316	caryophylladienol I		0.2													0.2			
2324	caryophylladienol II		0.3		tr	0.1										2.3			

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
2340	10-hydroxy-calamenene	0.5		tr			tr												0.2
2349	geranic acid				36.0							0.2	0.1						
2357	14-hydroxy-β-caryophyllene		0.3										0.2			27.9			
2376	manoyl oxide			0.6		1.8							0.5						
2384	1-hexadecanol									tr			0.2						
2384	farnesyl acetone		0.4																
2392	caryophyllenol II				0.1				0.1							0.9			0.1
2396	8α-13-oxo-14-en-epi-labdane					1.8							0.5						
2407	hydroxy-α-calacorene*	tr					0.1	0.1											
2411	2-decenoic acid*						3.9	0.1	31.3						0.1				
2415	14-hydroxy-4,5-dihydro-β-caryophyllene															1.2			
2438	kaur-16-ene					0.7													
2478	14-hydroxy-α-humulene															0.7			
2500	pentacosane		0.2	0.2				tr				tr							
2509	dodecanoic acid		0.5	0.1			0.1	tr	0.1				0.2			0.9			
2538	2-undecenoic acid*								0.1										
2609	14-hydroxy-4,5-epoxy-β-caryophyllene (β,β form)															0.3			
2617	tridecanoic acid			0.2				tr					0.2						
2622	phytol											tr		tr					
2663	14-hydroxy-4,5-epoxy-β-															1.1			

RRI	Compound	% Amounts of identified compounds																	
		bet	**cap**	**cap**	**cit**	**glu**	**gra**	**gra**	**his**	**pan**	**pan**	**pap**	**que**	**que**	**rad**	**sca**	**tom**	**tom**	**vit**
		N	**St**	**W**	**N**	**S**	**S**	**W**	**S**	**S**	**W**	**N**	**S**	**W**	**N**	**S**	**S**	**W**	**S**
	Oil yield (%)	**0.04**	**0.02**	**0.02**	**0.36**	**0.02**	**0.22**	**0.06**	**0.02**	**0.48**	**0.35**	**0.17**	**0.07**	**0.26**	**0.69**	**0.12**	**0.27**	**0.21**	**0.05**
	caryophyllene (β,α form)																		
2670	tetradecanoic acid		0.9					0.1					0.4	tr		tr			
2700	heptacosane		0.1	tr				tr				0.2						tr	
2822	pentadecanoic acid												tr						
2900	nonacosane	0.1						0.1	tr			0.1					tr	tr	
2931	hexadecanoic acid	0.7	0.6	1.1				0.1	0.1			tr	tr					0.1	0.2
	Total	**73.0**	**75.3**	**65.6**	**92.8**	**59.2**	**95.7**	**94.1**	**97.3**	**92.0**	**90.5**	**98.1**	**84.2**	**89.7**	**95.1**	**83.3**	**98.3**	**97.9**	**91.3**

Where: - RRI = relative retention indices; tr = less than 0.05%; *correct isomer not identified; N = National Botanical Garden (Kirstenbosch), S = Stellenbosch Botanical Garden, St = Strand region, W = Walter Sisulu Botanical Garden (Johannesburg); bet = *Pelargonium betulinum* (L.) L'Hérit., cap = *Pelargonium capitatum* (L.) L'Hérit., cit = *Pelargonium citronellum* J.J.A. van der Walt., glu = *Pelargonium glutinosum* (Jacq.) L'Hérit., gra = *Pelargonium graveolens* L'Hérit., his = *Pelargonium hispidum* (L.f.) Willd., pan = *Pelargonium panduriforme* Eckl. & Zeyh., pap = *Pelargonium papilionaceum* (L.) L'Hérit., que = *Pelargonium quercifolium* (L.f.) L'Hérit., rad = *Pelargonium radens* H.E. Moore, sca = *Pelargonium scabrum* (Burm. f.) L'Hérit., tom = *Pelargonium tomentosum* Jacq., vit = *Pelargonium vitifolium* (L.) L'Hérit.

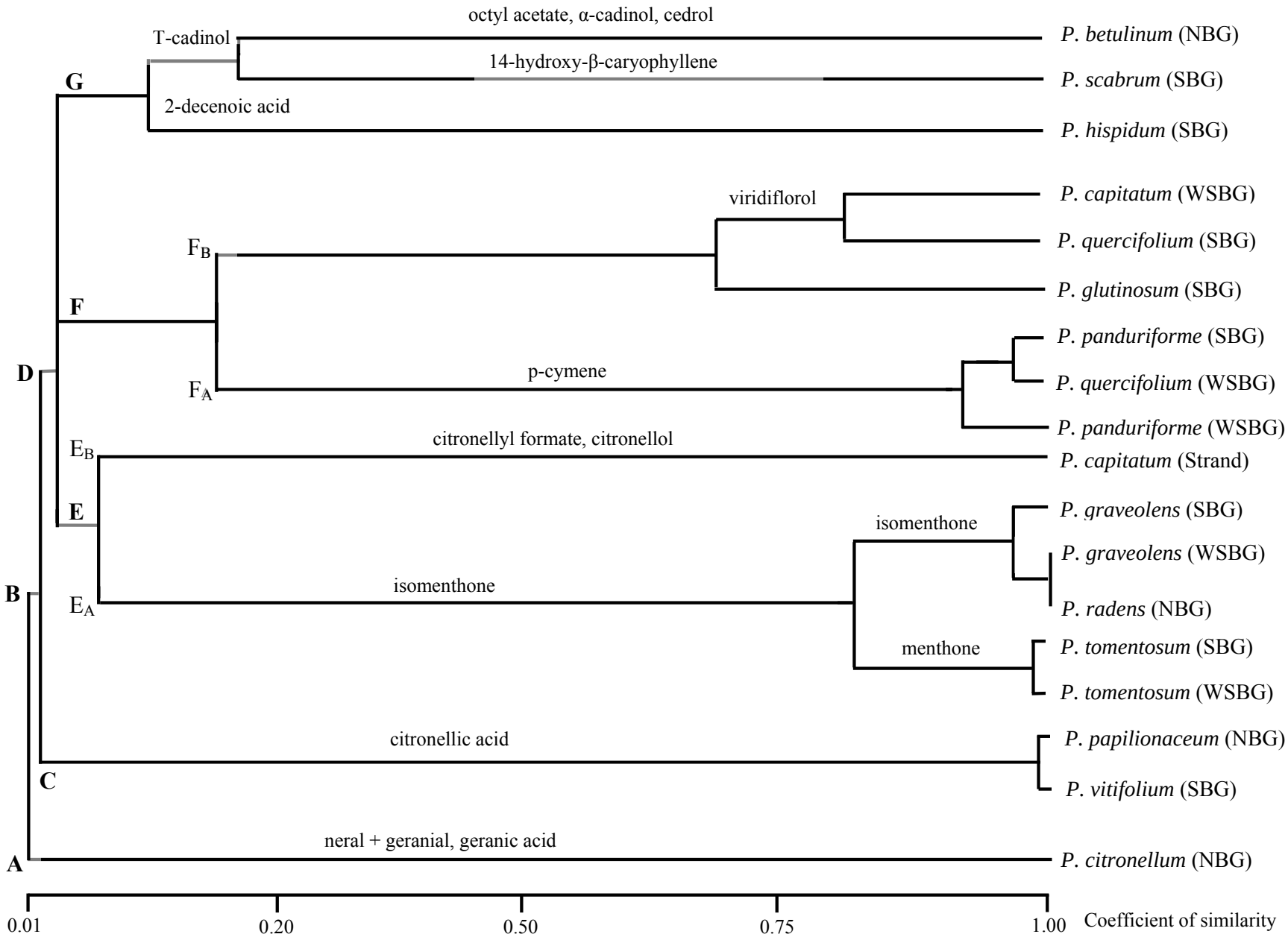


Figure 3.2: A dendrogram constructed from the essential oil composition of 18 *Pelargonium* samples.

Pelargonium citronellum (NBG) exists as an outlier from the other clustered species, resulting in the first division where cluster (B) comprises 17 OTUs. A previous study, involving the analysis of the essential oil of four natural populations of *P. citronellum* from different localities in South Africa, revealed that the oil mainly contains a mixture of aldehydes with a lemon scent (Demarne and van der Walt, 1993). The aldehydes detected in the latter study include (E)-2-hexenal, photocitral B, epi-photocitral A, photocitral A, neral, geranial and other unidentified aldehyde compounds. The mixture of aldehydes detected in *P. citronellum* (NBG) oil includes photocitral B (0.2%), *cis*/*cis*-photocitral (0.3%), *trans*/*trans*-photocitral (0.6%) and benzaldehyde (0.1%). Furthermore, the aldehydes, neral (17.4%) and geranial (27.2%) occur as major constituents. These citral isomers were only detected in this species and are present in very high concentrations. However, geranic acid is the most abundant (36.0%) essential oil constituent in *P. citronellum* (NBG). Geranic acid occurs at greatly reduced levels in *P. papilionaceum* (0.2%) and *P. quercifolium* (SBG) (0.1%). The monoterpenes geraniol (1.0%) and linalool (1.8%) are present in lower concentrations in *P. citronellum* (NBG). The oxygenated sesquiterpene caryophyllene oxide occurs at a level of 0.9%.

In the study carried out by Demarne and van der Walt (1993), small quantities of geranic acid (<0.5-4.7%) and large quantities of neral (26.9-37.4%) and geranial (35.8-47.9%) were found in *P. citronellum*. In the latter study, geranial always occurred at a higher concentration than neral, this was also noted in the analysis of *P. citronellum* (NBG). Demarne and van der Walt (1993) further documented that the monoterpenoids geraniol (<0.5-1.7%) and linalool (0.5-1.3%) were present at low levels. These identified amounts of geraniol and linalool are in agreement with this study. The eight main compounds encountered in the *P. citronellum* essential oil of three of the clones obtained from different origins in South Africa included linalool, photocitral A, "A" of lemongrass, neral, geraniol, geranial, geranic acid and germacrene D. The aforementioned compounds collectively represented 80% of the composition of these oils (Vorster, 1990). The sum of these compounds in the *P. citronellum* (NBG) sample, excluding photocitral A, "A" of lemongrass and germacrene D (since they are absent), is 83.4%. It was concluded by Demarne and van der Walt (1993) that only neral, geranial and sometimes geranic acid are present at more than 5%. However, in the *P. citronellum* oil sample analysed by Lis-Balchin (2002a), citronellol (30.5%) and geranial (42.6%) were identified as the two major compounds. Neral occurred at lower levels of 2.6%. Linalool and geraniol contributed

3.7% and 0.1%, respectively. Citronellol was not detected in the *P. citronellum* essential oils analysed by Demarne and van der Walt (1993) nor was it present in the *P. citronellum* (NBG) sample.

Pelargonium citronellum appears to be a uniform species with respect to its essential oil composition. Within the genus *Pelargonium*, the only species which produce such large amounts of neral and geranial are *P. citronellum* and *P. crispum* (Demarne and van der Walt, 1993). Lis-Balchin (1991) recorded high levels of citral (neral and geranial) in the "citrus group" of scented 'geraniums' ('Mabel Grey', 'Frensham' and 'Citronella').

Cluster (B) is subdivided into cluster (C) and (D). *Pelargonium papilionaceum* (NBG) and *P. vitifolium* (SBG) are chemically anomalous from the species grouped in cluster (D), since cluster (C) exists as an outlier. This second division is attributed to the predominance of citronellic acid in *P. papilionaceum* (NBG) and *P. vitifolium* (SBG). Other compounds common to both *P. papilionaceum* and *P. vitifolium* occur at much lower levels. *Pelargonium vitifolium* is closely related to *P. capitatum* and *P. papilionaceum* (van der Walt, 1977). The GC-MS results provide chemical evidence to support the taxonomic relationship established between *P. papilionaceum* and *P. vitifolium.* The degree of similarity between *P. papilionaceum* (NBG) and *P. vitifolium* (SBG) is very high (i.e. coefficient of similarity is almost 1). The GC profiles of *P. papilionaceum* and *P. vitifolium* are shown in Figure 3.3. The GC-MS results do not confirm a close association between *P. vitifolium* and *P. capitatum*.

Figure 3.3: Comparison of the GC profiles of the hydrodistilled essential oils of (a) *P. papilionaceum* (NBG) and (b) *P. vitifolium* (SBG).

It was reported by Demarne and van der Walt (1992) that among the Pelargoniums only *P. vitifolium* synthesizes large amounts of citronellic acid. In this analysis, citronellic acid is the principal compound encountered in the oil profiles of *P. papilionaceum* (NBG) and *P. vitifolium* (SBG) (Figure 3.3) and contributes 96.2% and 74.7% to their total oil compositions, respectively. Of all the *Pelargonium* species analysed in this study, only *P. papilionaceum* (NBG) has such a high concentration of a single component. Citronellic acid occurs at 0.8% in *P. hispidum* (SBG) and at 0.1% in *P. citronellum* (NBG) and *P. graveolens* (SBG). Trace amounts were detected in *P. capitatum* (Strand), *P. graveolens* (WSBG) and both samples of *P. tomentosum*. About 85.9-89.3% citronellic acid was found in *P. papilionaceum* essential oil (Lis-Balchin and Roth, 1999) and 76.9-83.9% occurred in *P. vitifolium* essential oil (Demarne, 1989). These values correlate with those obtained in this study. The vernacular name 'Rambossie' given to *P. papilionaceum* is derived from the distinctive he-goat-like odour (fruity lemon scent) of the leaves. *Pelargonium vitifolium* and the cultivar 'Sweet Rosina' also emit this acrid, persistent odour (Houghton and Lis-Balchin, 2002). The citronellic acid produced by *P. papilionaceum* and *P. vitifolium* species and cultivars could possibly be used as a strong fixative in perfumes (Houghton and Lis-Balchin, 2002). The majority of the other compounds detected in *P. papilionaceum* (NBG) occur in trace amounts.

Citronellic acid is derived from the oxidation of citronellal which is a reduction product of geraniol (Wuryatmo *et al*., 2003). Trace amounts of the monoterpene alcohol geraniol were detected in both *P. vitifolium* and *P. papilionaceum.* Trace amounts of the aldehyde citronellal was found in *P. vitifolium* and was absent in *P. papilionaceum*. These amounts may be an indication that almost complete reduction of geraniol and almost complete oxidation of citronellal took place to yield large amounts of the end-product citronellic acid.

Pelargonium vitifolium contains an essential oil profile which typifies this species (Demarne and van der Walt, 1992). Citronellic acid, citronellol and 10-epi-γ-eudesmol were the only three compounds occurring in amounts greater than 1% in the oil samples obtained from *P. vitifolium* plant material collected from five different localities in South Africa (Demarne and van der Walt, 1992). Furthermore, citronellic acid (77-85%) was consistently found to be the principal component. These findings correlate with the analysis of *P. vitifolium* (SBG); citronellol occurs at 2.2%, 10-epi-γ-eudesmol at 7.1% and

citronellic acid at 74.7%. Very low levels of citronellol (0.1%) are present in *P. papilionaceum* (NBG), whereas 10-epi-γ-eudesmol is absent. Citronellol occurred at 2.6-6.3% in *P. vitifolium* (Demarne, 1989) and at 0.2-1.6% in *P. papilionaceum* (Lis-Balchin and Roth, 1999). *Pelargonium capitatum* (Strand) contains 9.9% of citronellol. *Pelargonium capitatum* (WSBG) and *P. panduriforme* (WSBG) contain similar levels of 10-epi-γ-eudesmol, 6.9% and 6.3%, respectively. Lis-Balchin and Roth (1999) identified 0.1-5.0% geranic acid in *P. papilionaceum,* but it was absent in *P. vitifolium*. The latter is in agreement with the results obtained in this analysis. *Pelargonium vitifolium* is not considered for commercial essential oil production as its oil composition is not appealing and its oil yield is low (Demarne and van der Walt, 1992).

Fifteen OTUs associate to form cluster (D), which contains three major clusters, (E), (F) and (G) (Figure 3.2). These three distinct groups diverge at a low degree of similarity in the dendrogram. The delimitation of these three separate clusters implies that the species grouped within each cluster have somewhat different chemical profiles to those of the species grouped in the other clusters, although some similarities may also occur.

Cluster (E) contains two branches, (E_A) and (E_B), due to the quantitative differences of isomenthone and citronellyl formate and citronellol. These two branches separate at a low similarity coefficient. The subgroup (E_A) is defined by the accumulation of high levels of isomenthone (>49.0%). The similarity in the compositions of the *P. graveolens* samples is represented by their close association within the cluster dendrogram (Figure 3.2). This is also true for the *P. tomentosum* samples. The dendrogram reflects a close relationship among *P. graveolens, P. radens* and *P. tomentosum*; quantitative and qualitative similarities occur in the chemical profiles of the aforementioned species. Common compounds of the latter species include α-pinene, p-cymene, menthone, isomenthone, linalool, cryptone, α-terpineol, p-cymen-8-ol and caryophyllene.

Pelargonium tomentosum (WSBG) has an essential oil dominated by menthone (36.1%) and isomenthone (56.6%). The monoterpenic compounds limonene and p-cymene are present at 1.3% and 0.8%, respectively. Similarly, the majority of *P. tomentosum* (SBG) oil is accounted for by menthone (41.1%) and isomenthone (49.3%). Limonene (1.5%) and p-cymene (1.1%) occur at levels similar to those in *P. tomentosum* (WSBG). There are also quantitative and qualitative resemblances in the minor compounds of the two *P.*

tomentosum samples. The occurrence of menthone and isomenthone as the main compounds in *P. tomentosum* oil has been previously reported (Demarne *et al*., 1986; Lis-Balchin, 2002a). *Pelargonium tomentosum* oil consists almost entirely of menthone and isomenthone (Williams and Harborne, 2002). *Pelargonium tomentosum* accumulated equal amounts of menthone and isomenthone (Lis-Balchin, 2002a). Similar levels of these two compounds occur in *P. tomentosum* (SBG). However, isomenthone occurs at a somewhat higher level than menthone in *P. tomentosum* (WSBG) as was found by Demarne *et al*. (1986). The strong peppermint scent is due to the presence of both these ketones in large quantities (Vorster, 1990). *Pelargonium tomentosum* has a culinary use due to its peppermint flavour (Williams and Harborne, 2002).

In the *P. tomentosum* oil sample analysed by Lis-Balchin (2002a), limonene and p-cymene were present in similar relative amounts to those found in the *P. tomentosum* samples of this study (1.3-1.5% and 0.8-1.1%, respectively). Demarne *et al*. (1986) found limonene at 3.9%, and p-cymene at lower levels of 0.2%. α-Phellandrene (2.8%), tricyclene (1.0%), α-pinene (1.0%) and myrcene (1.4%) were detected above 0.9% (Demarne *et al*., 1986). α-Phellandrene is absent, but tricyclene (0.3-0.4%), α-pinene (0.3-0.5%) and myrcene (0.4-0.8%) occur at lower levels in both the oil samples in this analysis. The composition of *P. tomentosum* oil is very uniform, irrespective of the growing conditions (Vorster, 1990).

It has been previously reported that not only is *P. radens* and *P. graveolens* morphologically and karyologically similar but they are also biochemically analogous (van der Walt and Demarne, 1988). Hence it was not surprising that the dendrogram (Figure 3.2) revealed a very close affinity between these two species. *Pelargonium graveolens* (SBG) has a close chemical association with *P. radens* but not to the same extent as *P. graveolens* (WSBG) demonstrated (Figure 3.2). The coefficient of similarity for *P. graveolens* (WSBG) and *P. radens* is 1.00, this value being indicative of virtually identical chemical characteristics. This degree of similarity is demonstrated in their GC profiles (Figure 3.4), which are almost mirror images of one another.

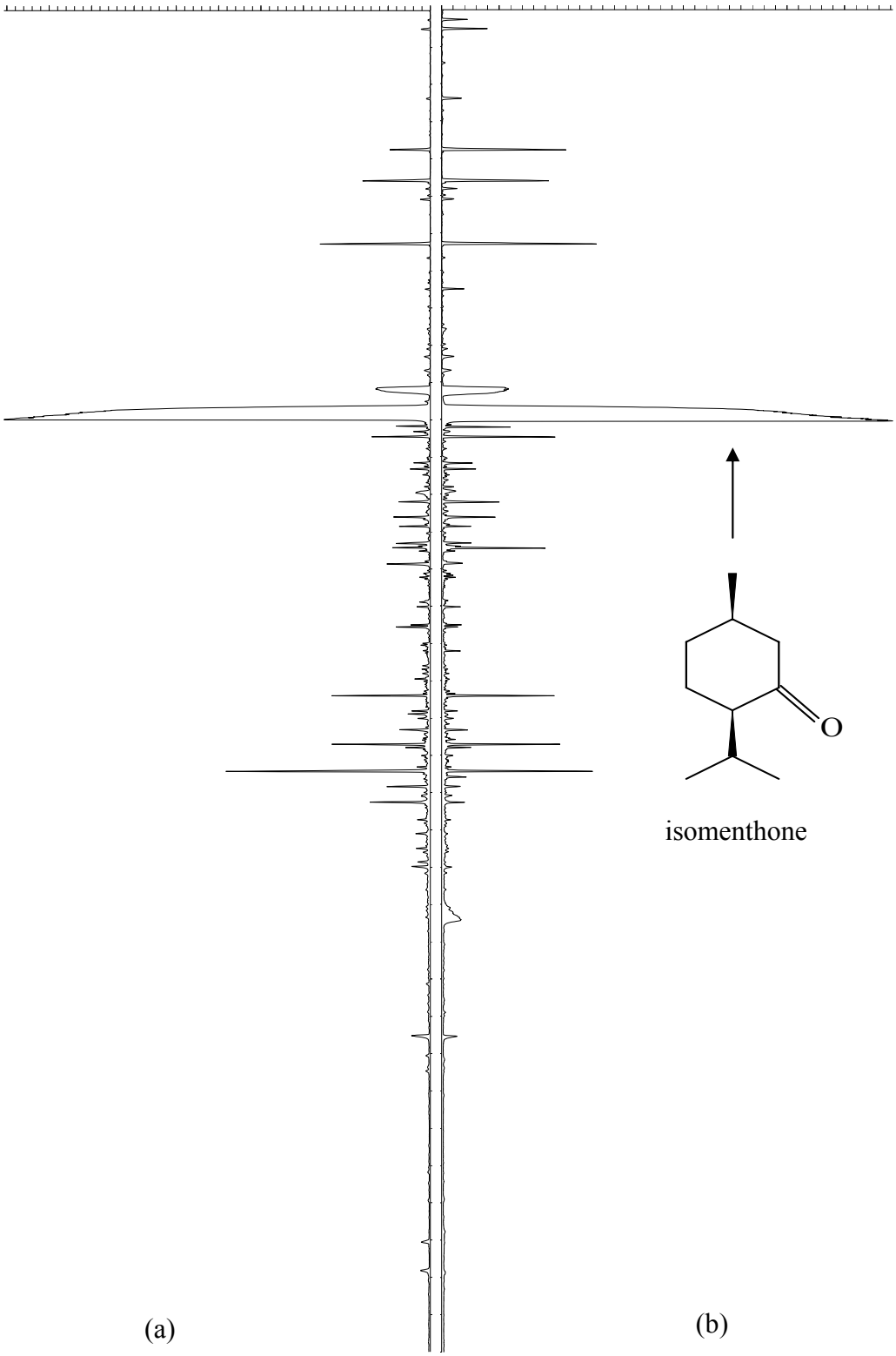


Figure 3.4: Comparison of the GC profiles of the hydrodistilled essential oils of (a) *P. graveolens* (WSBG) and (b) *P. radens* (NBG).

The major monoterpene identified in *P. radens* (NBG) is isomenthone (84.5%). Menthone (1.9%) and p-cymene (1.1%) are the only other two compounds present above 1%. With regards to the two samples of *P. graveolens*, the monoterpenic ketone isomenthone (65.8-83.3%) also occurs as the principal component, menthone (1.0-5.1%) and p-cymene (0.9%) are also present. The relative concentration of isomenthone in *P. radens* oil is slightly higher than that in *P. graveolens* oil (Vorster, 1990).

Other compounds common to the *P. graveolens* oil samples and *P. radens* (NBG), above 0.5%, include limonene (0.6-0.8%) and 2-phenylethyl tiglate (0.8-1.9%). *Pelargonium graveolens* (SBG) contains 3.9% of 2-decenoic acid and substantial amounts of decanoic acid (12.9%); these compounds occur in low amounts (0.1-0.4%) in *P. graveolens* (WSBG) and *P. radens*. The compounds common to *P. graveolens* (WSBG) and *P.* radens (NBG) and which are not present in *P. graveolens* (SBG) exist in minor or trace amounts.

Isomenthone represents more than 80% of the total oil yield for *P. graveolens* (WSBG) and *P. radens* (NBG). This was also found to be true for the essential oil of *P. graveolens* and *P. radens* analysed by van der Walt and Demarne (1988). Isomenthone occurred at 32.8-81.8% and at 7.0-81.5% in the *P. radens* and *P. graveolens* oils, respectively, analysed by Lis-Balchin (2002a). Large quantities of isomenthone (± 80%) in the oils of *P. graveolens* and *P. radens* are responsible for their mint-scent (van der Walt and Demarne, 1988). The latter authors detected other major compounds in these oils, namely menthone (1.6-2.3%), myrcene (3.0-5.0%), (Z)-β-ocimene (1.4-6.0%), limonene (0.9-1.8%) and α-phellandrene (0.9-1.3%). (Z)-β-Ocimene and α-phellandrene are absent in the *P. graveolens* and *P. radens* oil samples of this study, menthone (1.0-5.1%), myrcene (0.3-0.9%) and limonene (0.6-0.8%) are present. The same authors reported that the isomeric terpenes citronellol, geraniol, linalool and nerol are either absent or present in very low amounts (always <0.5% of the total yield) in the oils of *P. graveolens* and *P. radens*. This is true for the analyses of these oils in this study; citronellol (0.2-0.4%) is present in the oil samples in very small quantities, geraniol and nerol are absent. Linalool occurs in small amounts (0.3%) in the *P. graveolens* samples, but occurs in slightly higher amounts of 0.6% in *P. radens* (NBG). However, citronellol has been found as a major compound (53.7%) in *P. radens* and at substantial levels (17.6%) in *P. graveolens* (Lis-Balchin, 2002a). Linalool (1.2-12.8%) and geraniol (19.1-41.9%) were also found in high amounts in *P. graveolens* (Lis-Balchin, 2002a). Geraniol is always partially transformed into its isomer linalool during steam

distillation. The total amount of geraniol and linalool remains constant in the essential oil (Demarne and van der Walt, 1989).

Citronellyl esters and geranyl esters are completely lacking in *P. graveolens* and *P. radens* (Demarne and van der Walt, 1989). Citronellyl formate (11.2%) and geranyl formate (0.7-4.2%) were detected in *P. graveolens* (Lis-Balchin, 2002a). Geranyl esters are absent in the oil samples of *P. graveolens* and *P. radens* (NBG) analysed in this study. Citronellyl formate occurs in trace amounts in both the *P. graveolens* samples and is absent in *P. radens*. Other citronellyl esters either occur in small quantities or are absent in the *P. graveolens* and *P. radens* (NBG) oils.

Although *P. radens* and *P. graveolens* are chemically similar there are some differences which characterize these oils. The compounds which occur in the profiles of the *P. graveolens* oil samples but not in *P. radens* (NBG) and visa versa are present at very low levels. The presence of 2-phenylethyl tiglate in *P. graveolens* (0.6-0.8%) but not in *P. radens* was noted by van der Walt and Demarne (1988). 2-Phenylethyl tiglate is present at 0.8% in the oil of *P. radens* (NBG) and occurs in an amount of 1.3% and 1.9% in *P. graveolens* (SBG) and *P. graveolens* (WSBG), respectively. van der Walt and Demarne (1988) reported that β-bourbonene (0.1-0.2%) and guaia-6,9-diene (0.1-0.2%) seemed to occur in *P. radens* and not in *P. graveolens*. However, guaia-6,9-diene (6.0%) has been detected in *P. graveolens* (Lis-Balchin, 2002a). Guaia-6,9-diene is present in trace amounts in *P. radens* (NBG) and is absent in the *P. graveolens* samples. However, β-bourbonene is absent in *P. radens* (NBG) but present in trace amounts in the *P. graveolens* oils.

When comparing the GC-MS results of the *P. graveolens* samples in this study with the work by Lis-Balchin (1991), distinct differences are noted with regard to the major constituents. The major compounds in a *P. graveolens* cultivar were citronellol and geraniol (Lis-Balchin, 1991). β-Caryophyllene, geranyl formate and geranyl butyrate occurred at substantial levels in the latter *P. graveolens* cultivar, but are absent in the *P. graveolens* oil samples analysed in this study. Compounds common to the *P. graveolens* cultivar and the *P. graveolens* samples differ considerably in concentration, with higher levels for all the common compounds except isomenthone occurring in the *P. graveolens* cultivar. Hybrids or cultivars are developed to express certain desirable traits and some caution needs to be taken when extrapolating results obtained in this study which is based

on authentic species to data previously reported for plants produced in cultivation programs. Different cultivars have been analysed by different authors (Lis-Balchin, 1991). There is often great variation in essential oil data between authors due to the confusion with regards to the authentication of the scented pelargoniums (Lis-Balchin, 1991). It is obvious that authentic species will have differences in their chemical compositions in comparison to hybrids or cultivars. Many *Pelargonium* hybrids bear no similarity to either parent (Houghton and Lis-Balchin, 2002).

Rana *et al*. (2002) investigated the *P. graveolens* oil obtained from plants growing in the Himalayan region of Uttaranchal and found citronellol and geraniol to be the principal components. Other major compounds included linalool, p-menthone, citronellyl formate, α-humulene and α-selinene. In this study, the *P. graveolens* oil samples (SBG) and (WSBG) have very low levels of citronellol (0.4%), citronellyl formate (<0.05%) and linalool (0.3%). Furthermore, geraniol, α-humulene and α-selinene were not present. Isomenthone, the principle compound in *P. graveolens* oil samples (SBG) and (WSBG) was not detected in the *P. graveolens* oil analysed by Rana *et al*. (2002). These findings indicate that oils of the same authentic species growing in different areas can differ in their chemical composition. These differences may be attributed to the environment, which is made-up of climatic and soil factors, in which the plant grows and develops (Richardson *et al*., 1954). Growth regulators, shading, distillation, storage, weeds, leaf ontogeny, drying and seasons all influence the chemical composition of *Pelargonium* species oil (Rana *et al*., 2002). Hence, such appreciable differences in chemical characters noted between the present data and published data may be attributed to the different environmental parameters associated with the different localities from where the plants were collected. It is important to note that the chemical differences could be accounted for by the different genetic make-up of the plants analysed; the genetic component being far more pronounced than the environmental effects.

Putievsky *et al*. (1990) carried out a study to determine the influence of different intervals between the last irrigation and harvest on the essential oil content and composition. It was found that a short interval between irrigation and summer harvest leads to a higher percent of geraniol than of citronellol in *P. graveolens*. The levels of citronellol become higher than that of geraniol at a longer interval between the last irrigation and harvest. A longer interval creates a situation of water stress for the plant. In this study, the samples of *P.*

graveolens have a high percentage of citronellol and are devoid of geraniol. It is thought that in 'geranium', citronellol and related dihydro derivatives of geraniol are formed by enzymatic reduction of geraniol (Putievsky *et al*., 1990). The activity of this enzyme is affected by water stress and other environmental factors and responds by increasing the content of citronellol in the essential oil (Putievsky *et al*., 1990).

A Réunion-type and an African-type 'geranium oil' are known in commerce. The African-type oil has a higher concentration of 10-epi-γ-eudesmol than of guaia-6,9-diene (Bhattacharya *et al*., 1993). The latter authors revealed that an essential oil of rose-scented 'geranium' (*Pelargonium* sp.) cultivated under the semiarid tropical climate of Andhra Pradesh, South India possessed a composition similar to the African-type 'geranium'. In this study, the oils from the species which are considered to have been used in cultivation programs to create and ennoble the rose 'geranium' cultivars, namely *P. capitatum*, *P. graveolens* and *P. radens,* were evaluated to determine which type of oil they could be classified as. The two samples of *P. graveolens* are devoid of both 10-epi-γ-eudesmol and of guaia-6,9-diene. *Pelargonium capitatum* (WSBG) has a substantial quantity of 10-epi-γ-eudesmol (6.9%) and is devoid of guaia-6,9-diene. The latter compound is present in low amounts (0.5%) in *P. capitatum* (Strand) and in trace amounts in *P. radens* (NBG). 10-Epi-γ-eudesmol is absent in both *P. capitatum* (Strand) and *P. radens* (NBG). Since *P. capitatum* (WSBG) oil has a high content of 10-epi-γ-eudesmol, this oil may be classified as an African-type 'geranium oil'. It cannot be assumed that *P. capitatum* (Strand) and *P. radens* (NBG) are similar in composition to the Réunion 'geranium oil' as this oil is characterized by large quantities of guaia-6,9-diene (Bhattacharya *et al*., 1993).

Demarne (1989) carried out a comprehensive study of the essential of *P. capitatum.* It was found that stable differences exist in the composition of several *P. capitatum* oils from five different origins in South Africa. Further, the work by Viljoen *et al*. (1995) established a high degree of variability in the composition of the analysed *P. capitatum* oils. The GC-MS results of the two *P. capitatum* oil samples analysed in this study also displayed variation; their OTUs are located in separate cluster groups, namely (E) and (F) which diverge at a low degree of similarity. The major essential oil constituents detected in *P. capitatum* (WSBG) include caryophyllene oxide (10%), viridiflorol (24%) and 10-epi-γ-eudesmol (6.9%). Compounds present in amounts of 1.0-2.9% include octyl butyrate, borneol, α-agarofuran, β-eudesmol, 4α-hydroxy-dihydroagarofuran and hexadecanoic acid.

Pelargonium capitatum (Strand) is represented by the upper branch (E_B) of cluster (E). Citronellyl formate (31.1%) and citronellol (9.9%) are most abundant in this latter oil. Citronellol is employed by the food industry in citrus beverages (Lis-Balchin, 1990). Furopelargone B (6.1%) also occurs as a major constituent. This oil is also characterized by isomenthone (1.9%), β-bourbonene (1.5%), isocaryophyllene (1.0%), α-muurolene (1.4%), *cis*-calamenene (1.6%), caryophyllene oxide (2.8%) and cadalene (1.1%). Geranyl formate (0.5%) was only detected in *P. capitatum* (Strand) essential oil. The oxygenated sesquiterpene caryophyllene oxide is the only common compound in *P. capitatum* (Strand) and *P. capitatum* (WSBG) which occurs above 1.6%.

The principal compounds detected in *P. capitatum* (Strand) correlate with previous published work. Citronellol and citronellyl formate were identified as the main compounds in three of the four main chemotypes of *P. capitatum* recognized (Demarne and van der Walt, 1989). α-Pinene was the main component in the other chemotype. α-Pinene is present at 0.2% in *P. capitatum* (Strand), but is absent in *P. capitatum* (WSBG). The latter oil was devoid of citronellol and citronellyl formate. Substantial amounts of geraniol, geranyl formate, guaia-6,9-diene, β-caryophyllene and germacrene D occurred in the chemotypes (Demarne and van der Walt, 1989). The aforementioned compounds are either absent or exist at lower levels in the two samples analysed in this study. Different chemotypes cause patterns of variation to occur in oil composition. Viljoen *et al*. (1995) identified eight chemotypes in a study performed on forty natural populations of *P. capitatum* selected along the coast of South Africa. It was concluded that several biochemical pathways exist in *P. capitatum* (Viljoen *et al*., 1995). The analysed oil samples in this study may represent different chemotypes of *P. capitatum*.

Cluster (F) is divided into two subgroups (F_A) and (F_B). *Pelargonium panduriforme* (SBG) and *P. quercifolium* (WSBG) are grouped closely with *P. panduriforme* (WSBG) to form cluster (F_A). Among all the oils analysed, the two samples of *P. panduriforme* and the sample of *P. quercifolium* (WSBG) contain the highest levels of p-cymene (37.6-54.9%). *Pelargonium panduriforme* (SBG) and *P. quercifolium* (WSBG) contain substantial amounts (7.9-13.0%) of viridiflorol. The above partly explains the particular assemblage of these samples within subcluster (F_A) (Figure 3.2). Furthermore, the following compounds: α-phellandrene (1.1-8.8%), p-cymen-8-ol (2.8-4.0%) and spathulenol (1.9-5.9%) are present in these three samples, above 1%.

p-Cymene (37.6-45.4%) is the main constituent in both samples of *P. panduriforme.* In addition to the compounds common to the three samples mentioned above, limonene (2.2-3.0%) and β-phellandrene (1.4-4.0%) are other major compounds common to both the *P. panduriforme* samples. Another major component present in *P. panduriforme* (WSBG) includes 10-epi-γ-eudesmol (6.3%) followed by lesser amounts of (Z)-β-ocimene (2.4%), cryptone (2.9%), cuminaldehyde (1.2%), p-mentha-1(7),5-dien-2-ol (1.1%), p-cymen-8-ol (2.9%), α-agarofuran (1.1%), carvacrol (1.3%) and 4α-hydroxy-dihydroagarofuran (3.8%). The other major compound detected in *P. panduriforme* (SBG) is the oxygenated sesquiterpene viridiflorol (7.9%). Compounds occurring in quantities of 1.0% and more in this oil sample include α-thujene (1.0%), γ-terpinene (1.0%), linalool (4.4%), α,p-dimethylstyrene (1.4%) and γ-eudesmoḷ (2.0%). Linalool is employed by the food industry in citrus beverages (Lis-Balchin, 1990).

The *P. quercifolium* oils have several common compounds present at high levels. The major component in *P. quercifolium* (WSBG) is the monoterpene p-cymene (54.9%). Other major compounds include α-phellandrene (1.1%), cryptone (1.4%), p-mentha-1(7),5-dien-2-ol (1.0%), p-cymen-8-ol (2.8%), viridiflorol (13.0%), spathulenol (5.9%) and carvacrol (1.5%). *Pelargonium quercifolium* (SBG) oil is characterized by a high concentration of the sesquiterpene viridiflorol (36.6%). The following compounds collectively contribute 35.1% to the total oil composition of *P. quercifolium* (SBG): p-cymene (6.2%), the esters hexyl butyrate (8.2%) and (E)-2-hexenyl butyrate (6.3%), ledene (1.8%), δ-cadinene (1.2%), p-cymen-8-ol (1.0%), (E)-nerolidol (2.8%), 8,13-epoxy-15,16-dinor-labd-12-ene (1.4%) and spathulenol (6.2%).

The findings obtained in this study for the two *P. quercifolium* oil samples are in accordance with that of Widmer (1988) where p-cymene was present in high concentrations and good quantities of hexyl butyrate and viridiflorol occurred in *P. quercifolium.* Furthermore, *P. quercifolium* was characterized by large amounts of α-phellandrene and good amounts of β-phellandrene, γ-terpinene and *cis*-3-hexenyl butyrate (Widmer, 1988). However, very low levels of β-phellandrene (0.2-0.3%) and γ-terpinene (<0.05-0.3%) were present in *P. quercifolium* (SBG) and *P. quercifolium* (WSBG) and *cis*-3-hexenyl butyrate was absent in both samples. The main compounds reported in *P. quercifolium* oil by Lis-Balchin (2002a) were α-terpineol and β-pinene. p-Cymene occurred in substantial amounts (Lis-Balchin, 2002a). β-Pinene is absent in *P. quercifolium*

(SBG) but present in trace amounts in *P. quercifolium* (WSBG), α-terpineol is absent in both the *P. quercifolium* oil samples. Furthermore, *trans*-ocimene, nerol and neral occurred above 1.0% (Lis-Balchin, 2002a); however, these compounds were not detected in the *P. quercifolium* oil samples analysed in this study.

The two hydrodistilled essential oil samples of *P. quercifolium* produced moderately incongruent chemical patterns in this study. *Pelargonium quercifolium* (SBG) has a chemical profile more similar to that of *P. capitatum* (WSBG) and *P. glutinosum* (SBG) than to that of *P. quercifolium* (WSBG). *Pelargonium quercifolium* (WSBG) is grouped in (F_A) and *P. quercifolium* (SBG) is situated in (F_B). These two subgroups diverge at a low similarity coefficient value (<0.20). However, the OTUs belonging to the two samples of *P. quercifolium* still reside within the same cluster group (F). The chemical compositions of the oil samples may differ with the location where the plant is grown. Different local growth conditions such as soil, temperature, sunlight can influence the oil composition producing variations (Lis-Balchin, 1991). Moreover, genetic differences produce chemotypes depending on the condition of its growth and dominant constituents. Further investigations should be carried out to determine whether the chemical variation observed between the two samples is due to genetic or environmental factors. Plant composition is dependent on a complex interrelationship between many chemical, physical and biological factors associated with the environment (Richardson *et al*., 1954).

With reference to cluster (F_B), *P. capitatum* (WSBG) is more closely associated with *P. quercifolium* (SBG) than with *P. glutinosum* (SBG). This is possibly due to the quantitative difference of viridiflorol; *P. capitatum* (WSBG) and *P. quercifolium* (SBG) are characterized by high viridiflorol content (24.0-36.6%). A lower amount of 8.9% is present in *P. glutinosum.* Other compounds common to both *P. capitatum* (WSBG) and *P. quercifolium* (SBG) include octyl butyrate (0.4-2.1%), β-caryophyllene (0.1-0.2%), α-muurolene (0.5-0.6%), *cis*-calamenene (0.1-0.5%), humulene epoxide I (<0.05-0.1%), γ-eudesmol (0.3%), manoyl oxide (0.5-0.6%), dodecanoic acid (0.1-0.2%), tridecanoic acid (0.2%) and hexadecanoic acid (<0.05-1.1%). Generally, these common compounds occur in similar relative quantities in the two oil samples. The grouping of *P. capitatum* (WSBG) and *P. quercifolium* (SBG) has the same coefficient of similarity as the cluster (E_A).

The oil of *P. glutinosum* (SBG) is characterized by its richness in oxygenated sesquiterpenes, namely caryophyllene oxide (6.3%), spathulenol (6.9%) and viridiflorol (8.9%). The sum of the following compounds explains 22.5% of the essential oil composition: hexyl butyrate (3.2%), (E)-2-hexenyl butyrate (2.7%), isomenthone (1.4%), β-caryophyllene (2.5%), *cis*-calamenene (1.3%), cubenol (1.0%), ambrox (2.3%), *trans*-α-bergamotol (1.2%), 8,13-epoxy-15,16-dinor-labd-12-ene (2.3%), manoyl oxide (1.8%), 8α-13-oxo-14-en-epi-labdane (1.8%) and sandaracopimaradiene (1.0%). Lis-Balchin (2002a) identified two sesquiterpenic compounds in large amounts in *P. glutinosum* essential oil, the one occurred at 23.5-25.0% and the other at 20.7-26.4%. A previous analysis of *P. glutinosum* indicated large quantities of hexyl butyrate and (E)-2-hexenyl butyrate and substantial amounts of α-phellandrene, p-cymene, β-phellandrene and *cis*-ocimene (Vorster, 1990). Substantial amounts of α-phellandrene, p-cymene, hexyl butyrate and hexenyl butyrate were detected by Lis-Balchin (2002a). In *P. glutinosum* (SBG), α-phellandrene, β-phellandrene and *cis*-ocimene are absent and p-cymene occurs at a level of 0.5%. Myrcene, limonene and citronellol were detected in *P. glutinosum* in substantial amounts (Lis-Balchin, 2002a), only limonene was present in trace amounts in the *P. glutinosum* (SBG) oil sample.

The following findings are responsible for the observed correlation of *P. glutinosum* (SBG) with *P. capitatum* (WSBG) and *P. quercifolium* (SBG). Caryophyllene oxide exists in its highest amounts (6.3-10.0%) in *P. capitatum* (WSBG) and *P. glutinosum* (SBG). Hexyl butyrate (3.2-8.2%) and 8,13-epoxy-15,16-dinor-labd-12-ene (1.4-2.3%) were only detected in *P. quercifolium* (SBG) and *P. glutinosum* (SBG) essential oils. Substantial levels (2.7-6.3%) of (E)-2-hexenyl butyrate occur in *P. quercifolium* (SBG) and *P. glutinosum* (SBG). The largest quantities (6.2-6.9%) of spathulenol were detected in the latter species. Compounds common to *P. capitatum* (WSBG), *P. quercifolium* (SBG) and *P. glutinosum* include β-caryophyllene, α-muurolene, *cis*-calamenene and manoyl oxide.

Interesting to note is the presence of ambrox (2.3%) in the oil of *P. glutinosum* (SBG). This compound occurs at lower levels (0.4%) in *P. quercifolium* (SBG). Ambergris is a pathological metabolite of the spermwhale (*Physeter macrocephalus L.*) which accumulates as concretions in the intestinal tract. Since antiquity, ambergris has been one of the most highly valued materials used in perfumery (Bolster *et al*., 2001). It has a subtle odour reminiscent of seaweed, wood and moss but with a peculiar sweet, yet dry undertone

of persistence. Ambergris is also valued for its supposed restorative and aphrodisiac properties (Sell, 1990). The commercially most important constituent of the scarce natural ambergris is (−)-Ambrox® (Figure 3.5), which is considered as the prototype of what perfumers understand as an ambery (ambergris) note and the release of ambergris scent is strongly related to structural features of the tetra-*nor*-labdane skeleton (Bolster *et al*., 2001). (−)-Ambrox® has the character of the no longer employed natural ambergris and is fundamental in creating fragrances (Castro *et al*., 2002). An example of a perfume using (−)-Ambrox® is Extravagance D'Amarige Perfume by Givenchy. Today, (−)-Ambrox® is synthesized from the diterpene (−)-sclareol, the only practical starting material. Due to the high cost of (−)-Ambrox®, there is the ongoing search for new syntheses of (−)-Ambrox® from inexpensive, abundantly available labdanes (Bolster *et al*., 2001).

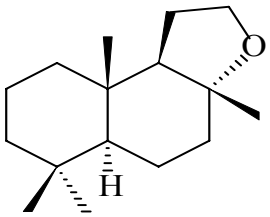


Figure 3.5: The chemical structure of ambrox.

A vast proportion of *P. hispidum* (SBG) oil consists of the fatty acids decanoic acid (47.0%) and 2-decenoic acid (31.3%). The sesquiterpene β-caryophyllene (8.6%) together with 2-nonanone (2.5%) represent a substantial proportion (11.1%) of the total oil composition. A previous study which involved three clones of *P. hispidum* from South Africa, concluded that their oil compositions were vastly different (Vorster, 1990). This previous analysis indicated that α-pinene was a dominant compound in the one clone, isomenthone in another and α-phellandrene, p-cymene, β-phellandrene and the alcohols *cis*-p-menthene-2 ol-1 and *trans*-p-menthene-2 ol-1 were major compounds in the third clone. These aforementioned compounds are either absent or occur at very low levels in the *P. hispidum* (SBG) oil sample of this study. It has been previously suggested that several chemotypes exist in *P. hispidum* (Vorster, 1990).

The oil hydrodistilled from *P. betulinum* (NBG) is sesquiterpenoid-rich in nature. It is characterized by the oxygenated sesquiterpene T-cadinol occurring as the main compound (18.9%). Other major sesquiterpenes identified in *P. betulinum* (NBG) oil include β-bourbonene (2.8%), β-caryophyllene (5.3%), α-humulene (1.5%), γ-muurolene (1.9%), δ-cadinene (2.6%), caryophyllene oxide (2.8%), α-cadinol (4.5%), α-copaene (1.4%), cubenol (2.5%), α-muurolene (2.4%), *cis*-calamenene (1%), cedrol (5.4%) and T-muurolol (1.7%). Other main components identified include the esters, octyl acetate (5.7%) and 3-phenylpropyl acetate (3.0%). Most of the compounds mentioned above occur in their highest amounts in *P. betulinum* (NBG). *trans*-Calamenene and 3-phenylpropyl acetate were only found in this oil sample. Interesting to note is the substantial amount (5.4%) of cedrol in *P. betulinum* (NBG). Several odourants with ambery odour characteristics have been developed from cedrene/cedrol such as Cedramber®
(http://www.bojensen.net/EssentialOilsEng/EssentialOils01/EssentiaOils01.htm).

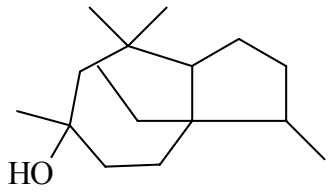


Figure 3.6: The chemical structure of cedrol.

The main sesquiterpenic compounds detected in *P. scabrum* (SBG) include β-caryophyllene (3.9%), caryophyllene oxide (4.2%), T-cadinol (6.1%) and 14-hydroxy-β-caryophyllene (27.9%). Approximately 11% of the total oil composition is collectively made-up by p-cymene (1.0%), 1,8-cineole (1.3%), myrcene (1.4%), α-terpineol (2.8%), 14-hydroxy-4,5-epoxy-β-caryophyllene (β,α form) (1.1%), 14-hydroxy-4,5-dihydro-β-caryophyllene (1.2%) and caryophylladienol II (2.3%). Decanoic acid (6.4%) and its acetate, methyl decanoate (4.2%) are present at substantial levels. Interesting to note is that 14-hydroxy-β-caryophyllene was the major component in the essential oils from buds of five *Betula* species growing in Turkey (Demirci *et al*., 2000). Furthermore, β-betulenal was isolated from *Betula* essential oils (Demirci *et al*., 2000). The aldehyde β-betulenal is present at 5.6% in this oil sample. β-Betulenal also occurs at a low level (0.2%) in *P. quercifolium* (SBG). The main compounds previously reported in *P. scabrum* were

geranial and citronellol which contributed 32.5-44.6% and 5.7-18.1%, respectively to the total oil composition (Lis-Balchin, 2002a). Other compounds detected include terpinen-4-ol (0-13.9%), neral (1.4-5.0%) and geraniol (0.1-3.9%). In the *P. scabrum* (SBG) sample of this study, geraniol is present in very low amounts (0.1%) and all of the other aforementioned compounds are absent. Myrcene, p-cymene and linalool occur in the same relative amounts in *P. scabrum* (SBG) as was detected in the sample analysed by Lis-Balchin (2002a), but isomenthone occurs in trace amounts and γ-terpinene is absent in the *P. scabrum* (SBG) sample. There appears to be some variation with regard to the composition of *P. scabrum* essential oil.

Cluster (G) represents a diverse entity. *Pelargonium hispidum* (SBG) branches off from the grouping of *P. betulinum* (NBG) and *P. scabrum* (SBG) at a low degree of similarity. This separation is due to the abundance of 2-decenoic acid (31.3%) in *P. hispidum* and the absence of this compound in *P. betulinum* and *P. scabrum.* A substantial amount of 2-decenoic acid (3.9%) occurs in *P. graveolens* (SBG). The highest concentrations of T-cadinol (6.1-18.9%) were detected in *P. betulinum* and *P. scabrum*, whereas trace amounts occur in *P. hispidum*; this also contributes to the partitioning of cluster (G). Furthermore, caryophyllene oxide occurs in *P. betulinum* and *P. scabrum* in good quantities (2.8% and 4.2%, respectively). *Pelargonium hispidum* shares some chemical similarities with *P. betulinum* and *P. scabrum.* β-Caryophyllene, present in substantial quantities (3.9-8.6%), is common to *P. betulinum*, *P. hispidum* and *P. scabrum*. There are numerous other compounds common to all three of these oil samples, but which occur at lower levels.

The dendrogram indicates that discrepancies exist in the coupled chemical profiles of *P. betulinum* (NBG) and *P. scabrum* (SBG). *Pelargonium hispidum* and *P. scabrum* both contain decanoic acid in high amounts (6.4-47.0%), but is absent in *P. betulinum.* 2-Nonanone (2.5%) is found in *P. hispidum* and is present in *P. scabrum* at low amounts (0.1%), but is absent in *P. betulinum*. The high levels of octyl acetate (5.7%), α-cadinol (4.5%) and cedrol (5.4%) characterize *P. betulinum* oil, these compounds are either absent or present at very low levels (<0.05-0.7%) in the other oils analysed. The large content of 14-hydroxy-β-caryophyllene (27.9%) typifies *P. scabrum.* This compound is detected in *P. capitatum* (Strand) and *P. quercifolium* (SBG) at very low levels (0.2-0.3%). Methyl decanoate (4.2%) and β-betulenal (5.6%) further distinguish *P. scabrum* oil as these are not

widespread compounds and further do not exist in such high levels in those oils in which they were detected.

The latest phylogenetic relationships pertaining to the section *Pelargonium* (Bakker *et al*., 2004) were evaluated with respect to the chemotaxonomic assessment of the essential oil compositions. This chemical evaluation of the essential oils did not indicate *P. quercifolium* as an outlying species, but was associated with other species. In this study, *P. hispidum* was grouped with *P. betulinum* and *P. scabrum* and was not closely associated with *P. tomentosum* or *P. papilionaceum*. The dendrogram did not indicate a close relationship between *P. tomentosum* and *P. papilionaceum*. The chemical analysis indicated a close relationship between *P. graveolens* and *P. radens*; this is in agreement with the findings of Bakker *et al*. (2004). However, *P. vitifolium* was not closely grouped with these two aforementioned species. With regards to the clustering of *P. betulinum*, *P. citronellum* and *P. scabrum*, *P. betulinum* and *P. scabrum* were grouped together; however, *P. citronellum* occurred as an outlier. Overall, incongruencies occurred between the chemotaxonomic appraisal and the current phylogenetic hypotheses of relationships.

GC analysis is an indispensable tool for chemotaxonomic studies of essential oils (Harborne, 1998). GC-MS data obtained in this study was used to generate a dendrogram which inturn established clusters of those entities exhibiting chemical affinities. GC-MS analysis confirmed many of the chemical similarities between and among certain species indicated by TLC analysis.

The taxonomic affinity of *P. graveolens* and *P. radens* as suggested by karyological and morphological similarities (e.g. floral structure) was further supported by their correlating essential oil chemistry (van der Walt and Demarne, 1988). This study confirmed that *P. graveolens* and *P. radens* are closely related species. The close relationship reported between *P. panduriforme* and *P. quercifolium* (van der Walt and Vorster, 1988) was confirmed in this study. *Pelargonium papilionaceum* and *P. vitifolium* have many features in common (van der Walt, 1977). The chemical composition of their essential oils was found to be similar in this study. Furthermore, new affinities were revealed between certain species based on the preponderance of several common compounds. Chemical similarities were established among *P. betulinum, P. hispidum* and *P. scabrum*; *P. capitatum*, *P. glutinosum* and *P. quercifolium* and among *P. graveolens*, *P. radens* and *P. tomentosum*.

A growing demand for natural raw materials has been created by the perfumery, cosmetic and flavour industries. Many natural fragrance plant materials for perfumery products have healing properties and thus are used in aromatherapy and in cosmetic products (De Silva, 1995). The constituents detected in the *Pelargonium* essential oils may be of interest to the flavour, fragrance and cosmetic industries.

CHAPTER 4: CHEMICAL COMPOSITION OF THE NON-VOLATILE EXTRACTS

4.1 Introduction

4.1.1 The non-volatile chemical constituents previously found in *Pelargonium* species (section *Pelargonium*)

The flavonoids myricetin and quercetin predominate in the species from the sections *Hoarea* and *Pelargonium* (Bate-Smith, 1973). Williams *et al*. (2000) established, from a survey of 58 *Pelargonium* species from 19 sections, that flavonols are the major leaf vacuolar flavonoid constituents in the genus *Pelargonium*. This study included *P. crispum* 'Whiteknights', *P. cucullatum, P. graveolens*, *P. quercifolium* and *P. tomentosum* which were found to contain the flavonols myricetin, quercetin and kaempferol. In addition, isorhamnetin was found in *P. crispum* 'Whiteknights' and *P. tomentosum*, quercetin 3-methyl ether occurred in *P. quercifolium* and *P. tomentosum* and the flavone luteolin was detected in *P. graveolens*, *P. quercifolium* and *P. tomentosum.*

Glandular hairs on the leaf surface of many *Pelargonium* species not only produce terpenoid constituents but also flavonoid aglycones (Williams and Harborne, 2002). The major leaf exudate constituent in *P. crispum* is the flavone chrysin (5,7-dihydroxyflavone), accompanied by a *C*-methyl derivative of the corresponding 5,7-dihydroxyflavanone. Several flavonol (quercetin and kaempferol) methyl ethers were detected in *P. quercifolium* and *P. tomentosum* (Williams *et al.*, 1997). Exudate flavonoids are usually present in plants which grow in alpine, arid or semi-arid conditions and it is generally assumed that they serve as a UV-screen to help in the adaptation of plants to such conditions (Williams *et al*., 1997).

Both hydrolysable (ellagitannins) and non-hydrolysable (proanthocyanidins) tannins are produced in large quantities in many species of the genus *Pelargonium* (Williams and Harborne, 2002). *Pelargonium cucullatum, P. graveolens*, *P. quercifolium* and *P. tomentosum* contain proanthocyanidins and *P. crispum* contains proanthocyanidins, ellagitannins and free ellagic acid (Williams *et al.*, 2000). Okuda *et al*. (1980) found that geraniin, a characteristic ellagitannin of *Geranium* species, does not occur in the several

Pelargonium taxa surveyed which included *P. graveolens* and *P. tomentosum*. Thorough chemical investigations of the *Pelargonium* tannins need to be carried out (Williams and Harborne, 2002). Another characteristic constituent of the genus *Pelargonium* is tartaric acid (Williams and Harborne, 2002).

The Geraniaceae is not known as an alkaloid-producing family (Houghton and Lis-Balchin, 2002) although alkaloids have been detected in *Erodium* (Medina *et al*., 1977; Mossa *et al*., 1983; Lis-Balchin and Guittonneau, 1995). A main chemotaxonomic division in the genus *Pelargonium*, based on the presence or absence of alkaloids, was indicated by the study of representative species of all sections of the genus (Lis-Balchin, 1997). The species of the section *Pelargonium* included in the aforementioned study and which did not contain alkaloids were *P. betulinum*, *P. capitatum*, *P. citronellum*, *P. cucullatum* subspecies *tabulare*, *P. denticulatum*, *P. graveolens*, *P. hispidum*, *P. tomentosum, P. vitifolium*, *P.* '*viscossimum*' and *P.* '*filicifolium*.'

This study aims to broaden the knowledge regarding the non-volatile chemical constituents of *Pelargonium* species and also attempts to indicate possible relationships between and among species.

4.1.2 Flavonoids

Flavonoids are a ubiquitous group of polyphenolic substances which occur in most plants and make-up a significant percentage of the chemical constituents of many species (Miller, 1996). Flavonoids occur as normal metabolites in plant tissues, concentrating in seeds, fruit skin or peel, bark and flowers. The most apparent function of flavonoids in the plant kingdom is to act as pigments to attract birds and insect pollinators. These compounds are also involved in the control of plant growth and development and defence against infectious plant diseases (Harborne *et al*., 1975). Flavonoids have been reported to exert multiple biological activities including antibacterial, anti-inflammatory, anti-allergic, antimutagenic, antiviral, antineoplastic, antithrombotic and vasodilatory activities (Di Carlo *et al*., 1999). The anti-oxidant function of flavonoids underlies many of the abovementioned actions in the body (Di Carlo *et al*., 1999). Two benzene rings on either side of a three-carbon ring are common to flavonoid molecules. Various classes of flavonoids are created by the many combinations of hydroxyl groups, sugars, oxygens and

methyl groups attached to the ring systems (Miller, 1996). Compared to most other plant compounds, flavonoids are more readily separated and detected on two-dimensional chromatograms and can be identified by simple methods i.e. by a spectrophotometer. Of all the plant constituents, flavonoids are favoured as taxonomic markers; there is the possible utility of flavonoids for chemical pattern comparison between species. Flavonoids are preferred to the terpenoids and alkaloids in systematic studies since they are universally distributed in vascular plants, they exhibit significant structural diversity, their chemical stability enables their detection in herbarium tissue and they are easily identified (Harborne *et al*., 1975).

4.2 Materials and methods

4.2.1 High performance liquid chromatography (HPLC) analysis

HPLC analysis with ultraviolet detection (HPLC-UV) was carried out on the acetone extracts of 21 *Pelargonium* species. HPLC is used for the analysis of compounds not adequately volatile for analysis by gas chromatography (Hamilton and Sewell, 1977). High reproducibility, ease of automation and the ability to analyse the numerous constituents in botanicals, makes liquid chromatography advantageous (Ong, 2004). HPLC is well established for the analysis of plant extracts; it is ideally suited to both the qualitative and quantitative chromatographic analysis of non-volatile compounds (Harborne *et al*., 1975). HPLC can be a useful tool in chemosystematics where it assists in the characterization of species on the basis of their secondary metabolite contents (de Oliveira *et al*., 2001).

4.2.1.1 Principle of the method

A chromatograph uses a non-polar solid phase and a polar liquid phase. HPLC separates compounds on the basis of their interaction with the solid particles of a tightly packed column and the solvent of the mobile phase (Lindsay, 1987). High pressures are required for the elution of the analytes through the column before they pass through a diode-array detector (DAD) which measures the absorption spectra of the analytes for purposes of identification. HPLC-DAD screening, based on the ultraviolet-visible (UV-VIS) monitoring, is well suited to screening for metabolites which contain significant chromophores (Mulzer and Bohlmann, 2000).

Photodiode-array (PDA) based instruments are the most powerful UV-VIS absorbance detectors in use today; very rapid collection of data over a selected spectral range is afforded (http//www.laballiance.com/la_info/support/hplc4.htm). Many compounds absorb UV radiation including those having π-bonding electrons and those with unshared electrons, e.g. olefins, aromatics and compounds having >C=O, >C=S, –N=O and –N=N– (Hamilton and Sewell, 1977). HPLC analysis provides information concerning the quantity of each chemical compound present and the UV spectrum further enables one to tentatively distinguish the class of compound under investigation (qualitative information).

4.2.1.2 Protocol

The samples were analysed using a Walters 2690 HPLC system (Phenomenex Aqua C18 column, 250 mm x 2.1 mm at 80°C) equipped with a 996 photodiode array (PDA) detector. The samples were dissolved in methanol to obtain a concentration of 50 mg/ml and 10 µl of this solution was injected under the following conditions: mobile phase flow rate: 0.2 ml/min, gas flow through the nebulizer: 30 L/h, nebulizer temperature: 80°C, expansion region temperature: 90°C and source temperature: 225°C. The initial mobile phase was 10% acetonitrile in 10 mM aqueous formic acid and the solvent ratio was changed through a linear gradient to 90% acetonitrile, 10% 10 mM aqueous formic acid at 40 minutes. This ratio was maintained for 10 minutes where after the solvent ratio was changed back to the initial starting conditions. Final analysis involving integration and identification was performed using the Empower® software program. The chromatographic profiles produced for each species were evaluated spectrophotometrically for the presence of flavonoids. The different classes of flavonoids were tentatively identified by a comparison of their UV spectra against characteristic absorbance spectra of previously identified flavonoids (Markham, 1982). Most flavonoids have UV spectra with two major absorption maxima, one occurs in the range of 240-285 nm (band II) and the other in the range of 300-400 nm (band I). The band II absorption may have originated from the A-ring benzoyl system and the band I from the B-ring cinnamoyl system (Harborne, 1967).

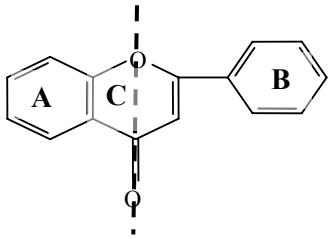
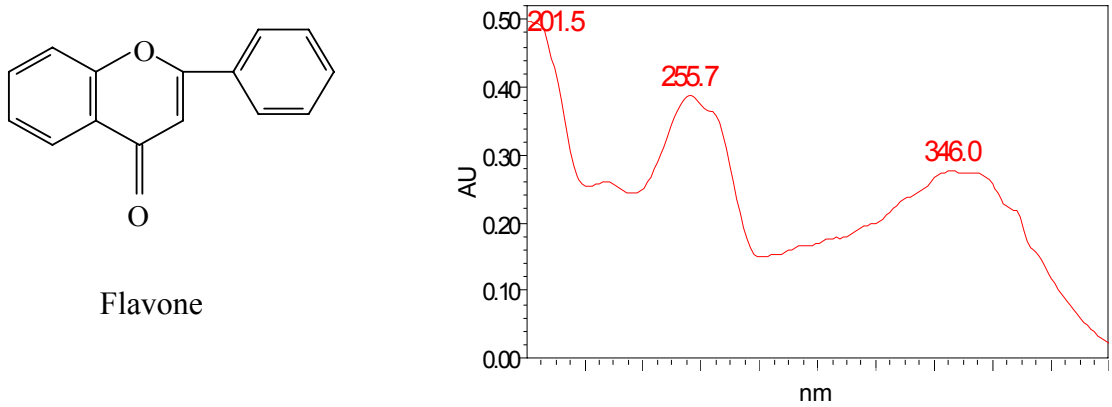


Figure 4.1: The structure of a flavonoid molecule showing the ring systems.

Flavones and flavonols have very similar spectra. They are identified by a characteristic absorbance maximum in the region 240-270 nm (band II). The position of the band I absorption indicates the type of flavonoid under investigation. The flavones absorb between 304-350 nm (Figure 4.2, (a)) while flavonols absorb between 352-385 nm. The UV spectra of isoflavones, flavanones and dihydroflavonols have a low intensity band I absorption which usually occurs as a shoulder to the band II absorption. Isoflavones have a band II between 245-270 nm and the band I shoulder occurs in the 300-340 nm region. Flavanones exhibit high absorbances at 270-295 nm and a broad shoulder at about 330 nm (Harborne *et al*., 1975) (Figure 4.2 (b)).

(a)



(b)

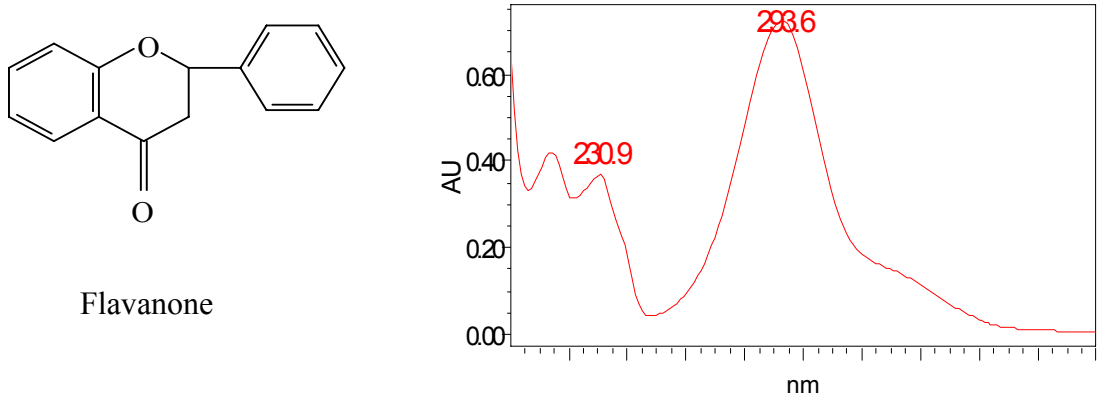
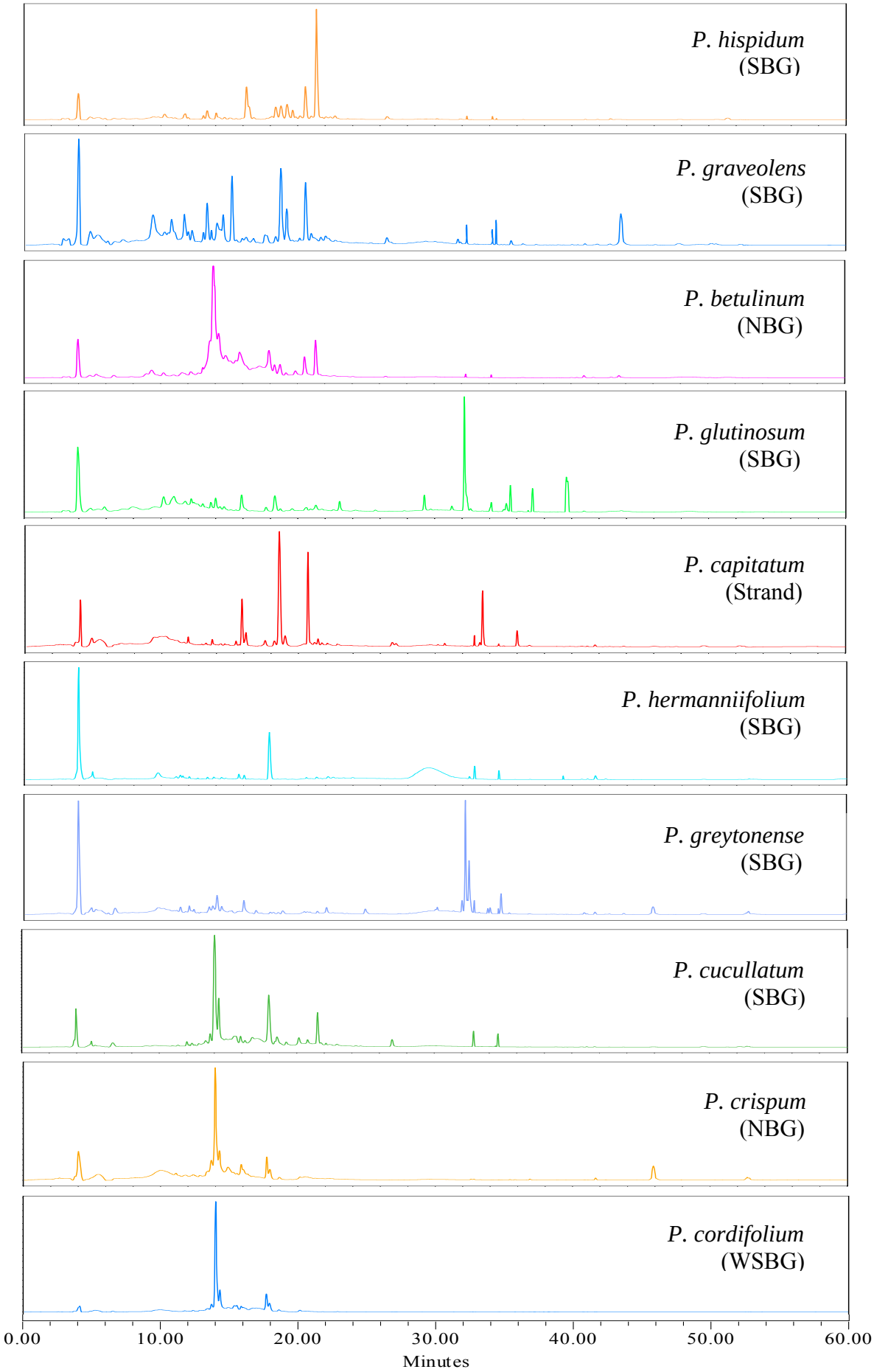


Figure 4.2: The chemical structure and corresponding UV spectrum of (a) flavone and (b) flavanone.

4.3 Results and discussion

The HPLC chromatograms of the *Pelargonium* acetone extracts and their respective tables with retention times, UV absorbance maxima, tentative identifications and percentage integrations of the major peaks detected for each species are shown in the monographs (Appendix A). Figure 4.3 shows the HPLC profiles of the analysed species in a stacked format for easy comparison.

Comparison of the HPLC profiles revealed chemical diversity and similarity among several species. UV spectra resembling the flavonoid class of compounds were identified. It is assumed that such spectra are indicative of derivatives of compounds from this class. Flavonoid derivatives were detected in all the extracts analysed. Due to their easily identifiable UV spectra, flavonoids were used as a means to compare the species. Table 4.1 highlights the numerous flavonoid derivatives detected in the *Pelargonium* extracts. The representative flavonoid patterns identified in the *Pelargonium* species provide valuable data for taxonomic purposes as such patterns can be used for exploring relationships between species and can be used to identify significant correlations between chemistry and taxonomy. The data suggests that the following taxa: *P. betulinum* (NBG), *P. capitatum* (Strand), *P. graveolens* (SBG), *P. hispidum* (SBG), *P. panduriforme* (SBG) and *P. vitifolium* (SBG) share rather characteristic chemical profiles. The similar flavonoid patterns of *P. capitatum* (Strand) and *P. vitifolium* (SBG) provide chemical evidence that these two species are related (van der Walt, 1977). *Pelargonium sublignosum* is distantly related to *P. scabrum* (van der Walt and Vorster, 1988). Their flavonoid patterns suggest that these two species are chemically related.



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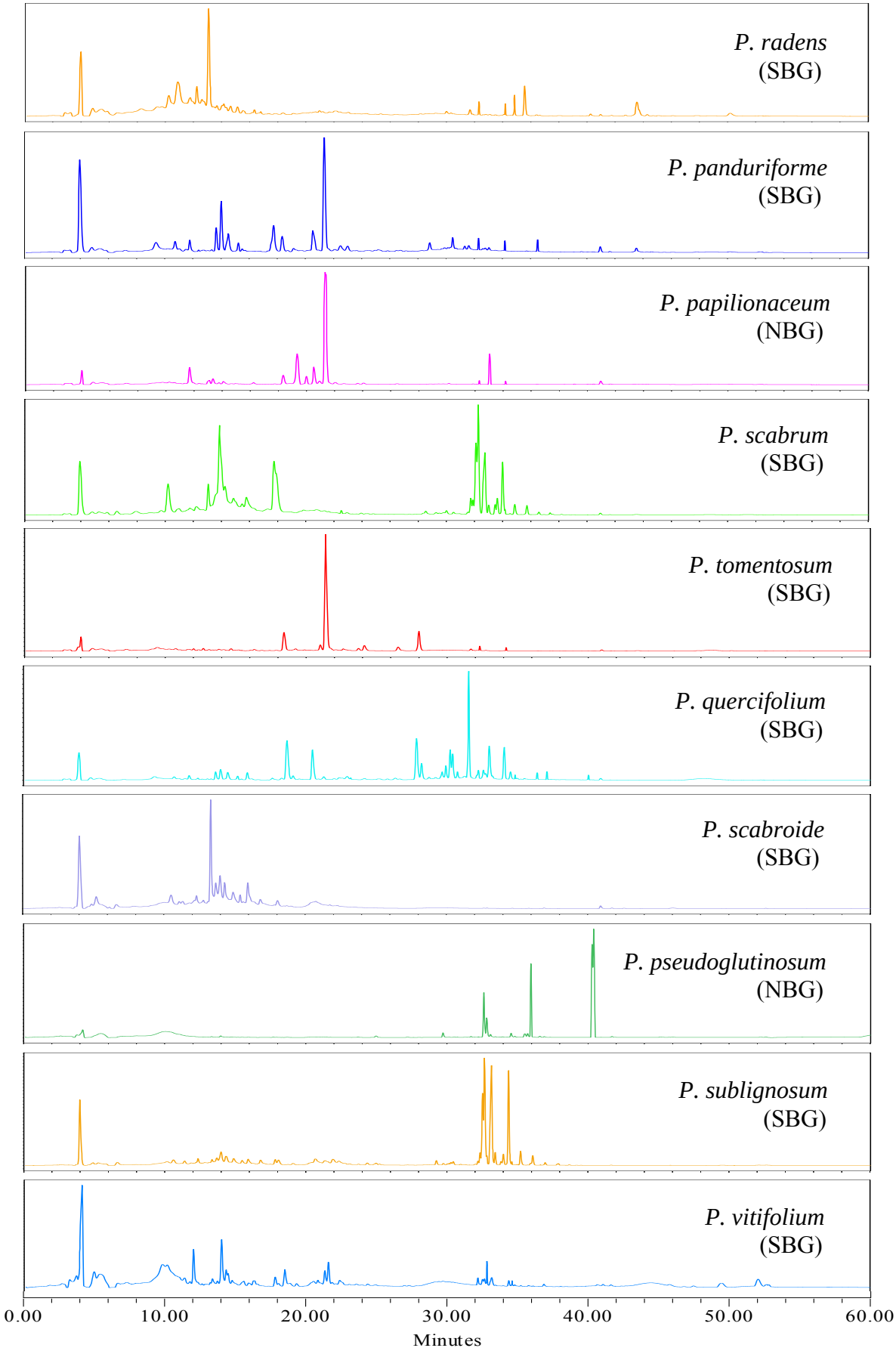


Figure 4.3: HPLC profiles of the acetone extracts of selected *Pelargonium* species of the section *Pelargonium*.

Table 4.1: HPLC-UV results of the tentatively identified flavonoid derivatives present in the acetone *Pelargonium* extracts.

Rt (min)	UV λ_{max} (nm)	Ft	bet	cap	cit	cor	cri	cuc	glu	gra	gre	her	his	pan	pap	pse	quer	rad	scab	sca	sub	tom	vit
			N	St	N	W	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S
11.07	260.4	D			+																		
12.38	278.0	C						+															
13.12	279.3	C			+																		
13.71	283.9	C		+														+					
14.31	278.0	C																	++				+
15.19	255.7, 353.2	A								++													
15.44	265.0, 351.6	A		+																			
15.46	272.1	C				+																	
15.87	260.3, 357.3	A		++					+			+					+						
15.93	255.7, 343.6	B								+					+								
16.25	255.7, 355.6	A		+						+			+++										+
16.46	255.7, 353.2	A											+										
17.74	255.6, 354.0	A		+	+	++	++		+	+		+++		++						+++	+		+
18.25	261.5, 348.0	B		+																			
18.40	262.7, 348.4	B	+						+	+			+	+	+							++	++
18.57	253.2	D						+															
18.61	255.6, 354.0	A	+	+++						++			++				+++						++
19.22	255.7, 354.4	A		+						+			++										
19.48	287.6	C			+																		
19.63	255.7, 350.8	B											+										
20.56	255.7, 354.4	A	+	+++						++			+++	++	++		++						
20.97	271.0	C			+																		
21.35	255.7, 346.0	B	+	+				++	+				+++	+++	+++							+++	+
21.82	272.2	C			+																		
22.07	243.8, 346.0	B													+								

Rt (min)	UV λ_{max} (nm)	Ft	bet	cap	cit	cor	cri	cuc	glu	gra	gre	her	his	pan	pap	pse	quer	rad	scab	sca	sub	tom	vit
			N	St	N	W	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S
22.09	269.7	**D**									+												
22.98	280.5	**C**			+									+									
23.06	262.7, 350.8	**B**							+													+	
24.14	254.5, 348.4	**B**																				+	
26.93	255.6, 366.8	**A**						+					+									+	
27.52	284.1	**C**			+																		
27.87	255.7, 359.0	**A**															+++					++	
28.23	260.4, 337.7	**B**															+						
29.24	267.5, 336.5	**B**			+				+							+							
29.26	291.0	**C**																			+		
29.68	249.7, 347.2	**B**															+						
30.16	268.7, 346.0	**B**			+												+	+					
30.26	254.5, 355.6	**A**															+						
30.77	262.7, 363.9	**A**															+						
31.56	260.4, 338.9	**B**															+++						
31.73	288.8	**C**																		+			
31.89	275.8	**C**																		+			
32.17	268.7, 335.3	**B**							+++								+						
32.23	288.6	**C**			+						+++									++	+		
32.49	267.4, 312.3	**D**									+++	+											
32.53	272.1	**C**																			++		
32.62	268.7, 331.7	**B**														++							
32.83	267.4, 336.1	**B**												+		+							
33.01	292.4	**C**																		+	+		
33.02	254.5, 354.4	**A**		++										+			++						
33.16	266.2, 338.5	**D**																		++	+++		
33.62	288.8	**C**																		+	+		

Rt (min)	UV λ_{max} (nm)	Ft	bet	cap	cit	cor	cri	cuc	glu	gra	gre	her	his	pan	pap	pse	quer	rad	scab	sca	sub	tom	vit
			N	St	N	W	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S
34.38	292.2	**C**									+									++	+++		
34.53	253.3, 352.0	**A**															+						
34.56	268.5, 343.2	**B**														+							
34.86	292.4	**C**																		+			
35.28	290.0	**C**			+															+	+		
35.56	267.5, 346.0	**B**																+					
36.00	267.4, 345.6	**B**		+																			
36.09	292.2	**C**																			+		
37.03	290.0	**C**			+																		

Where: - Rt = retention time (minutes); Ft = Flavonoid type: **A** = flavonol, **B** = flavone, **C** = flavanone, **D** = isoflavone; bet = *P. betulinum,* cap = *P. capitatum*, cor = *P. cordifolium*, cri = *P. crispum*, cuc = *P. cucullatum*, glu = *P. glutinosum*, gra = *P. graveolens*, gre = *P. greytonense*, her = *P. hermanniifolium*, his = *P. hispidum*, pan = *P. panduriforme*, pap = *P. papilionaceum*, pse = *P. pseudoglutinosum*, que = *P. quercifolium*, rad = *P. radens*, scab = *P. scabroide*, sca = *P. scabrum*, sub = *P. sublignosum*, tom = *P. tomentosum*, vit = *P. vitifolium*; N = National Botanical Garden (Kirstenbosch), S = Stellenbosch Botanical Garden, St = Strand region, W = Walter Sisulu Botanical Garden (Johannesburg).

+ = low concentration (≥0.2-5.0%), ++ = medium concentration (>5.0-10.0%), +++ = high concentration (>10.0-52.3%).

A particular compound with a UV absorbance maximum in the 206.2-218.0 nm region occurs, in varying concentrations, in all the HPLC chromatograms at a retention time of about 3.98 min (Figure 4.4). This compound appears to be characteristic of the *Pelargonium* species. It is the major compound present in *P. hermanniifolium*, *P. graveolens*, *P. greytonense* and *P. vitifolium*.

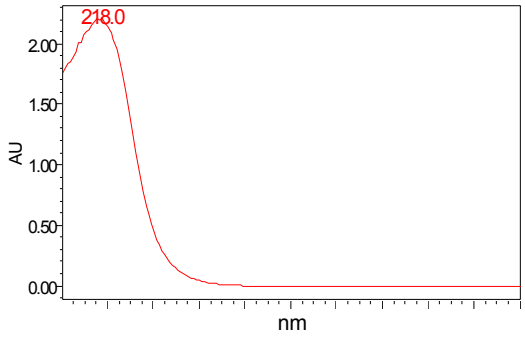


Figure 4.4: UV absorbance spectrum of the major compound (Rt = 3.98 min) identified in *P. hermanniifolium*, *P. graveolens*, *P. greytonense* and *P. vitifolium*.

λ_{max} values observed for the major peak of *P. scabroide* at Rt = 13.32 min and of *P. radens* at Rt = 13.09 min are similar.

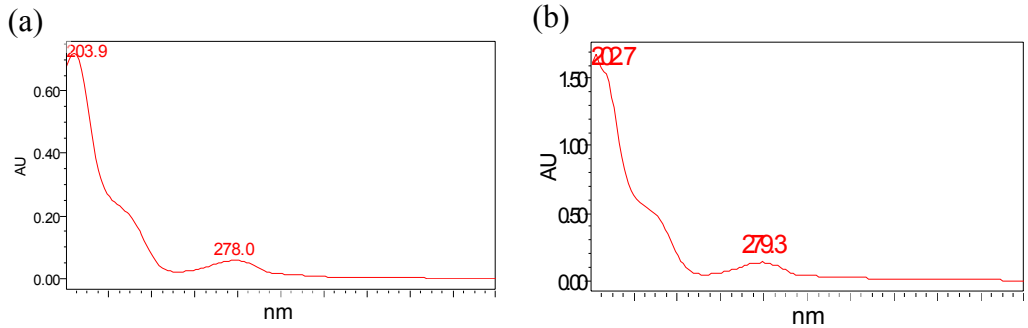


Figure 4.5: The UV spectrum of the major compound in (a) *P. scabroide* and (b) *P. radens*.

The main compound in *P. scabrum* (Rt = 32.38 min; 266.3 nm and 359.0 nm) appears to be similar to the main compound present in *P. sublignosum* (Rt = 32.67 min; 267.4 nm and 359.4 nm). *Pelargonium pseudoglutinosum* has a major compound at Rt = 40.41 min. A similar compound occurs in *P. glutinosum* at Rt = 39.62 min.

Pelargonium betulinum (NBG), *P. cordifolium* (WSBG), *P. crispum* (NBG) and *P. cucullatum* (SBG) and *P. scabrum* (SBG) share some similarities in their chemical fingerprints. The major peak (2) (Figure 4.6) for each of the aforementioned species occurs near a Rt of 13.95 min and the λ_{max} values observed for these major peaks are similar (220.3 nm and 278.0 nm). The compound occurring at Rt = 13.98 min in *P. sublignosum* appears to be similar to the compounds above. A number of other similar compounds occurred in all five abovementioned species (Figure 4.6 and Table 4.2).

Table 4.2: Retention times and UV data of the similar major compounds of *P. betulinum* (NBG), *P. cordifolium* (WSBG), *P. crispum* (NBG), *P. cucullatum* (SBG) and *P. scabrum* (SBG).

Peak number	**Retention time (min)**	**UV λ_{max} (nm)**
1	13.61	221.5, 281.5
2	13.95	220.3, 278.0
3	14.24	221.5, 278.0
4	15.78	221.5, 278.0
5	17.92	220.3, 278.1

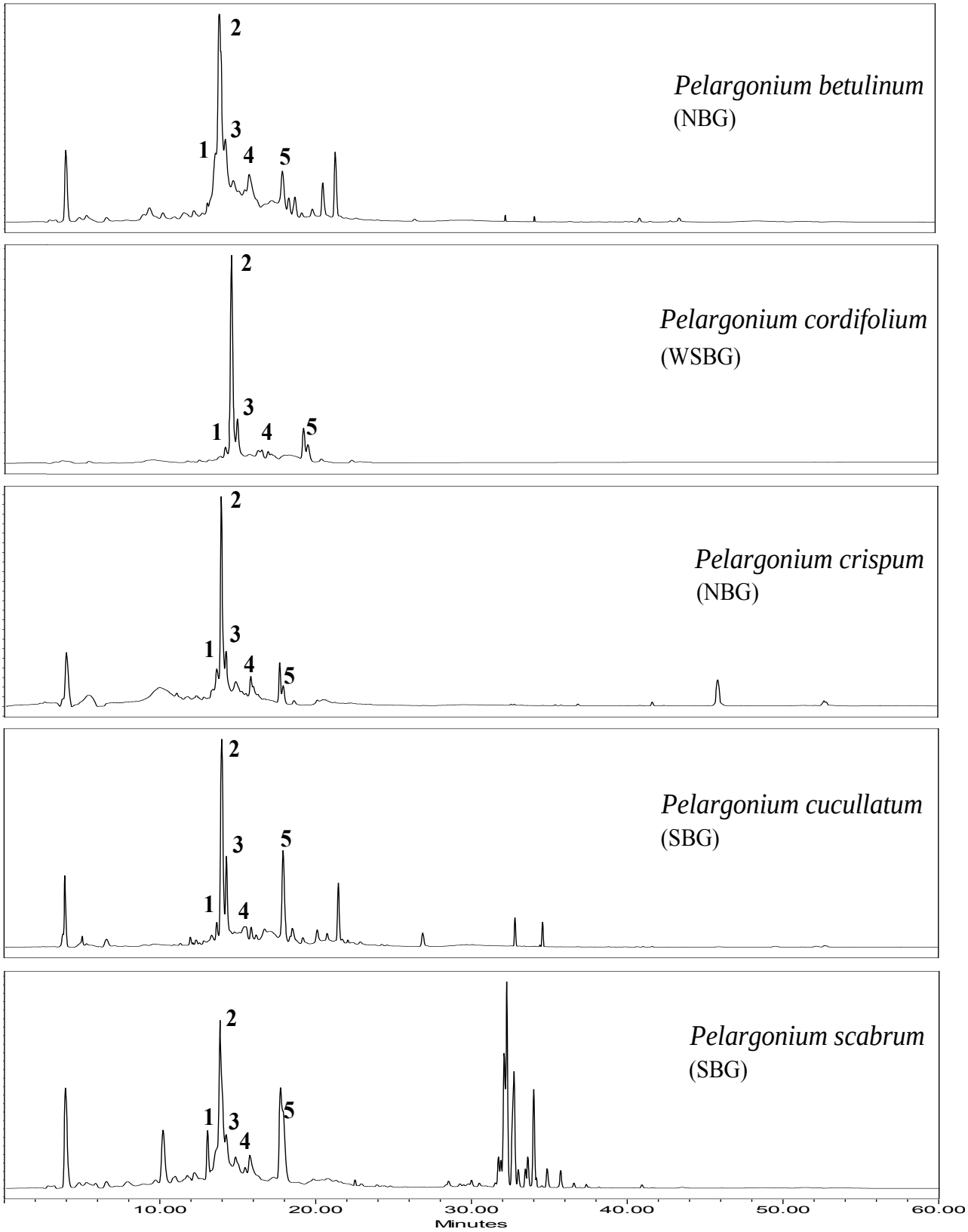


Figure 4.6: HPLC profiles of *P. betulinum* (NBG), *P. cordifolium* (WSBG), *P. crispum* (NBG), *P. cucullatum* (SBG) and *P. scabrum* (SBG).

P. vitifolium and P. panduriforme also accumulate high concentrations of a compound at about Rt = 14.01 min. It was confirmed through UV spectral analysis, that the compound occurring at this retention time in *P. vitifolium* is similar to the major compound present in the abovementioned species (Rt = 13.95 min). However, this is not true for *P. panduriforme* as the compound at Rt = 14.01 min produced a different absorbance spectrum; an absorbance maximum was observed at 209.7 nm. Figure 4.7 represents this comparison spectrophotometrically.

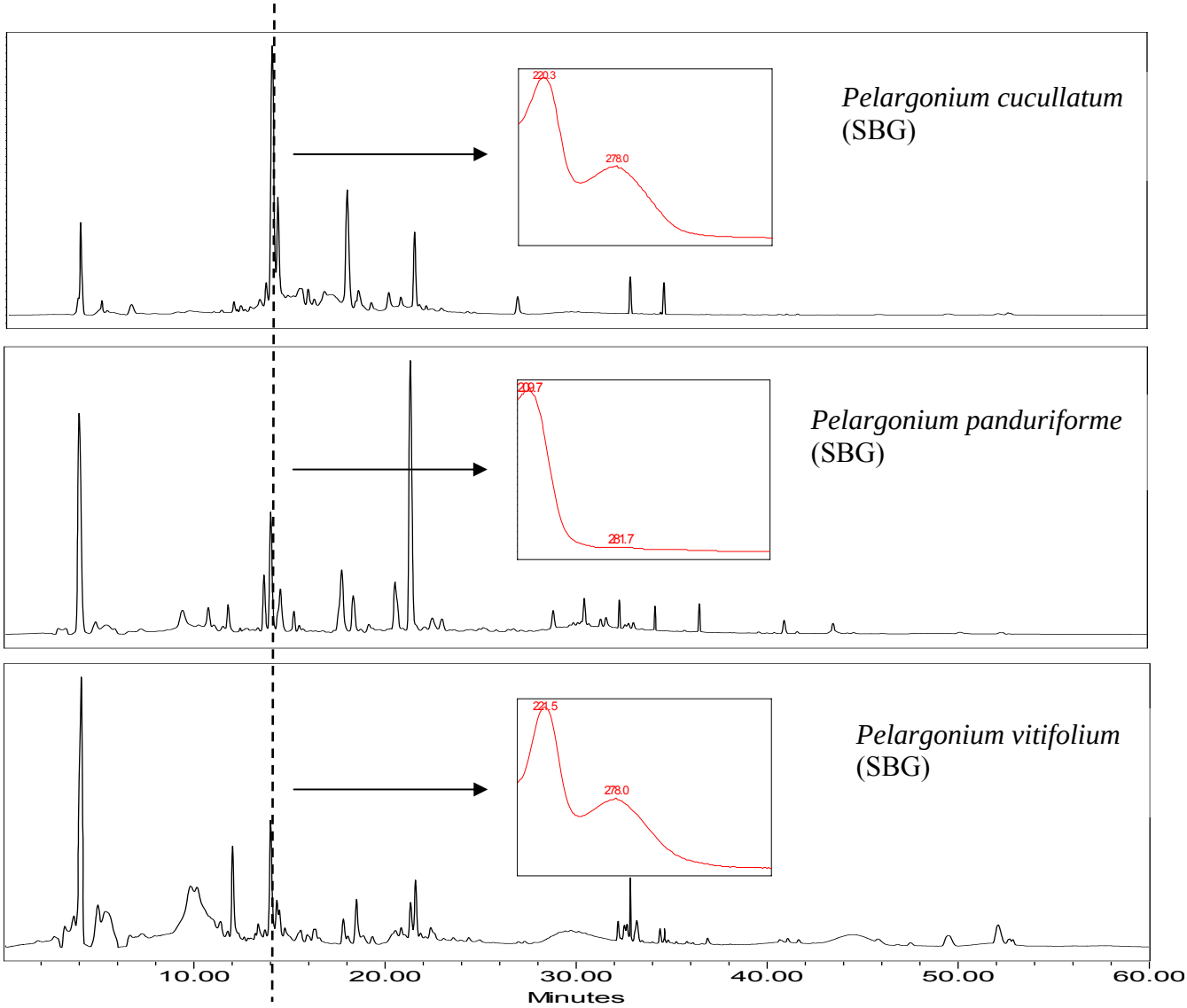


Figure 4.7: HPLC chromatograms of *P. cucullatum* (SBG), *P. panduriforme* (SBG) and *P. vitifolium* (SBG) with their respective UV absorbance spectra corresponding to the Rt = 13.95 min. Where: - SBG = Stellenbosch Botanical Garden.

The flavonoid derivatives at Rt = 17.74 min in *P. crispum*, at Rt = 17.60 min in *P. cordifolium* and at Rt = 17.75 min in *P. scabrum* appear to be similar to one another. Inspection of the UV data of the major compound of *P. hermanniifolium* at Rt = 17.89 min (maxima occurring at 255.6 nm and 354.0 nm) revealed this compound to be similar to the abovementioned flavonol compounds. In addition, *P. panduriforme* also contains a substantial amount of a similar flavonol compound at Rt = 17.74 min.

Pelargonium hispidum (SBG), *P. panduriforme* (SBG), *P. papilionaceum* (NBG) and *P. tomentosum* (SBG) have a similar main compound at Rt = 21.35 min (peak 2 in Figure 4.8) with UV absorbance maxima at 255.7 nm and 346.0 nm. This compound could possibly be a flavone as typical flavone UV spectra exhibit two major absorption peaks in the region 240-400 nm (Mabry *et al*., 1970). A substantial amount of a similar compound was noted in *P. betulinum* (Rt = 21.33 min), *P. cucullatum* (SBG) (Rt = 21.51 min) and *P. vitifolium* (Rt = 21.59 min). Another two compounds, one at Rt = 18.40 min (1) and the other at Rt = 34.22 min (3), were found to be common among *P. hispidum, P. panduriforme* (SBG)*, P. papilionaceum* and *P. tomentosum*. The compound at peak (1) was identified as a flavone. The flavone derivative which occurs at Rt = 18.40 min appears to also occur in *P. betulinum*, *P. glutinosum, P. graveolens* and *P. vitifolium.*

The HPLC fingerprints of *P. papilionaceum* and *P. vitifolium* (Figure 4.9) were assessed in tandem to determine if there was chemical evidence to confirm the taxonomic relationship established between *P. papilionaceum* and *P. vitifolium* by van der Walt (1977). The retention times and corresponding absorbance spectra of the major peaks of *P. papilionaceum* were obtained. The HPLC profile of *P. vitifolium* was assessed to ascertain whether or not peaks occurred at the same retention times as the major peaks found in *P. papilionaceum.* It was found that the absorbance data of these mutual peaks were different, except at Rt = 11.68 min the compound in *P. papilionaceum* is similar to that found in *P. vitifolium* (Rt = 12.01 min) as is demonstrated in Figure 4.9, peak (1). As mentioned before, the principal compound of *P. papilionaceum* occurs at Rt = 21.33 min and was identified as a flavone with UV absorbance maxima at 255.7 nm and 346.0 nm. The main compound of *P. vitifolium* occurs at Rt = 4.10 min with UV absorbance maximum at 208.6 nm. Such findings noted from HPLC analysis are in disparity with the results obtained from the essential oil chemistry analysis which provided evidence of a chemotaxonomic relationship between the two species.

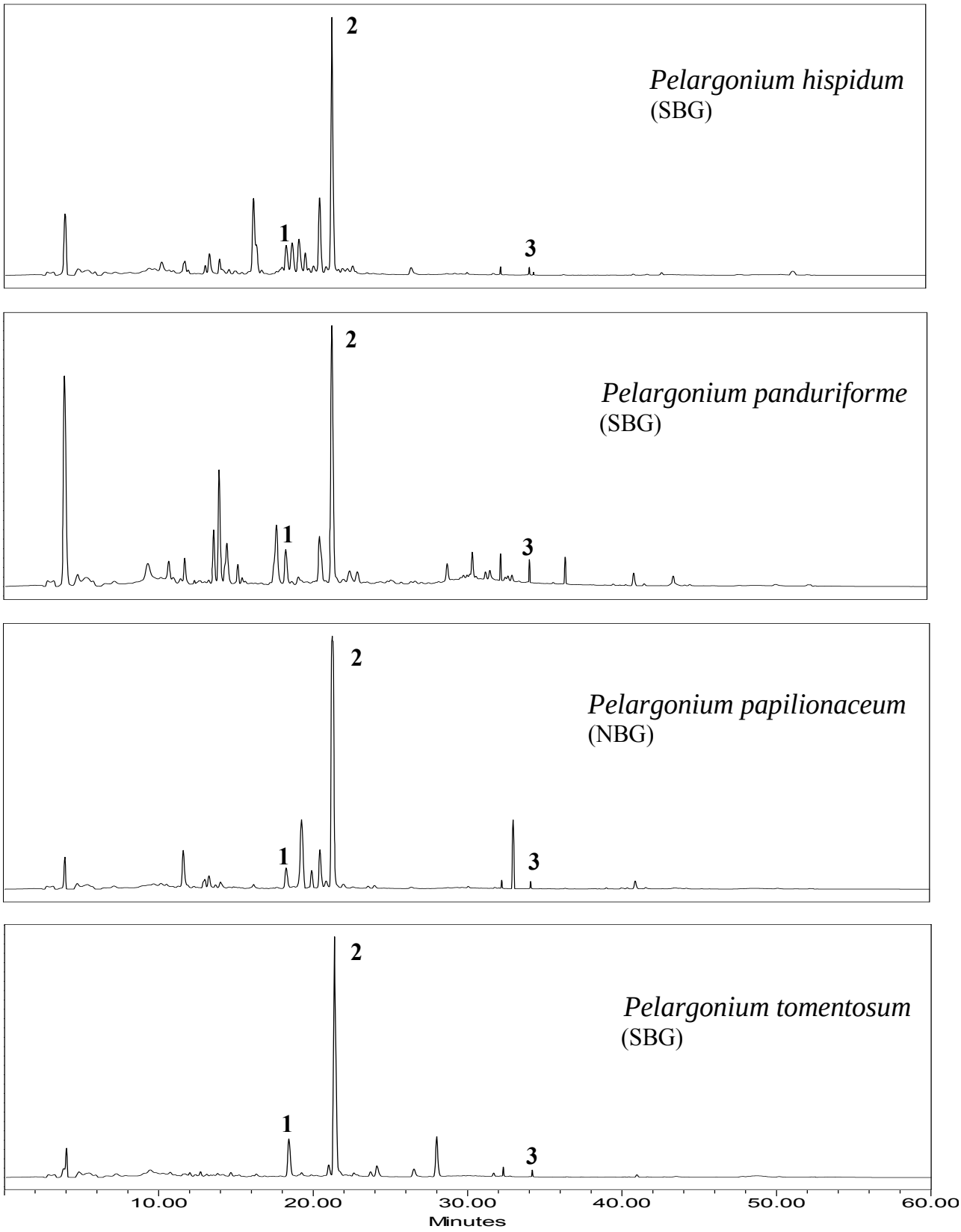


Figure 4.8: HPLC profiles of *P. hispidum* (SBG), *P. panduriforme* (SBG), *P. papilionaceum* (NBG) and *P. tomentosum* (SBG).

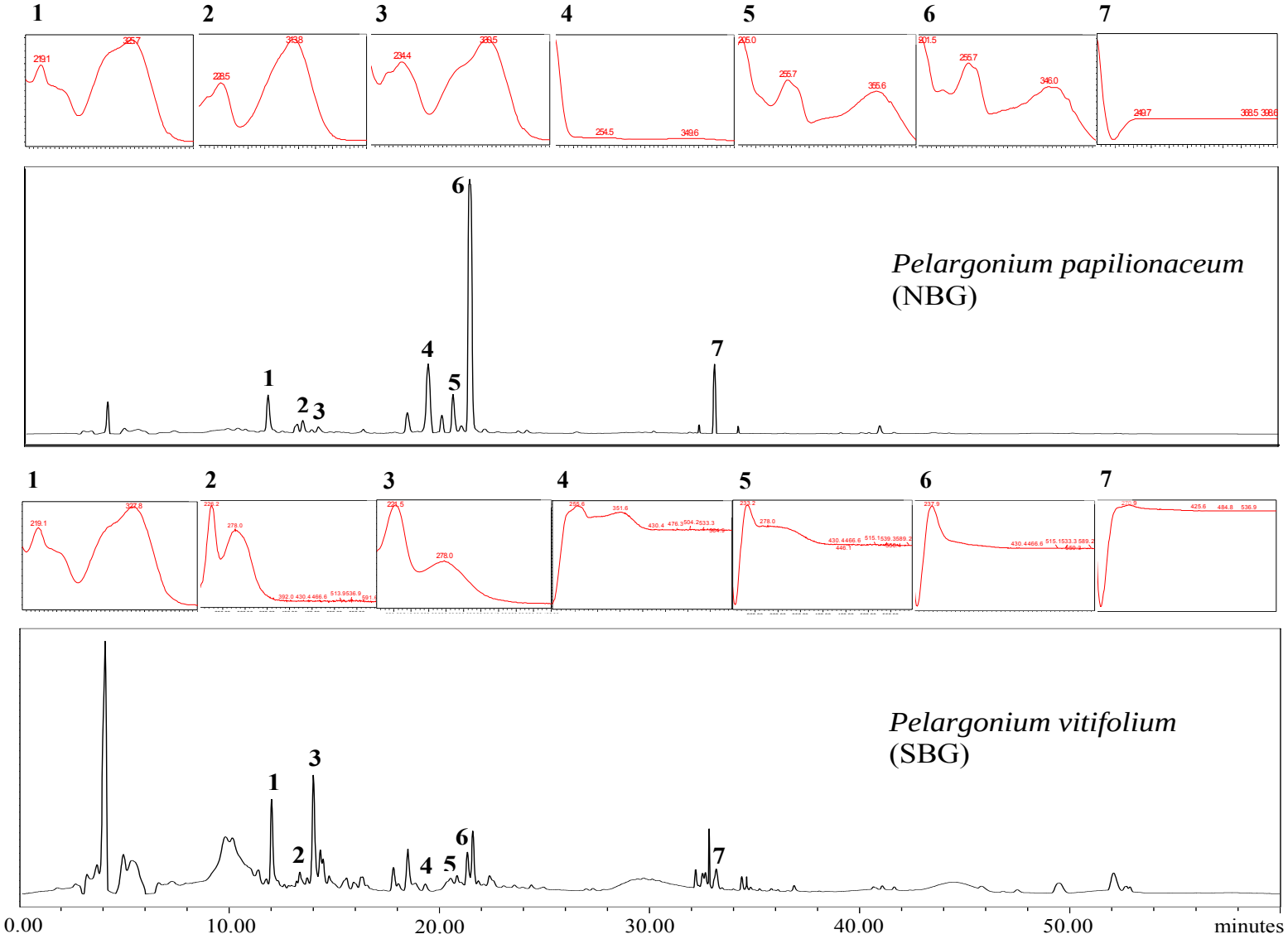


Figure 4.9: Comparison of the HPLC-UV data of *P. papilionaceum* and *P. vitifolium*. (1) Rt = 11.68 min, (2) Rt = 13.35 min, (3) Rt = 14.10 min, (4) Rt = 19.35 min, (5) Rt = 20.54 min, (6) Rt =21.33 min and (7) Rt = 33.05 min.

HPLC is gaining increasing importance for the analysis of plant extracts (de Oliveira *et al*., 2001). It is apparent from this study that HPLC can be of valuable use in plant chemosystematics assisting to characterize species on the basis of their secondary metabolite contents (de Oliveira *et al*., 2001).

The HPLC assignments mentioned in the monograph section are tentative only. If these tentative identifications are confirmed then certain *Pelargonium* species can be utilized as a source for the isolation of natural flavonoids. The HPLC analysis results could form the basis of a more detailed study of leaf phenolics in *Pelargonium*. Flavonoids exert multiple biological activities (Di Carlo *et al*., 1999); a mixture of the aforesaid metabolites may afford the *Pelargonium* acetone extracts with positive *in vitro* biological effects which will be tested in the succeeding chapters.

CHAPTER 5: ANTIMICROBIAL ACTIVITY

5.1 Introduction

Plants have been used for centuries to treat infections. The antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900's (Martindale, 1910; Hoffman and Evans, 1911). In recent years, a large number of plant extracts and in some cases their constituents have been investigated for their antimicrobial properties against various bacteria and fungi (Moleyar and Narasimham, 1992; Urzua *et al*., 1998; Hammer *et al*., 1999; Inouye *et al*., 2001; Kalemba and Kunicka, 2003; Duarte *et al*., 2005).

5.1.1 Resistance to antibiotics

Microbial resistance to antibiotics (products of micro-organisms or their synthesized derivatives) has increased even though a number of antibiotics have been introduced by pharmaceutical industries in the past thirty years (Nascimento *et al*., 2000). It was found that two or three antibiotics of micro-organism origin were introduced each year (Clark, 1996). Following a decrease in this development rate, the pace again quickened when scientists realized that the life span of any antibiotic is limited and so it is understood that conventional antibiotics have become less effective and new ailments are becoming unmanageable with these conventional medicines. The fact that bacteria have the genetic ability to transmit and acquire resistance to therapeutic agents (Cohen, 1992) is a concern considering the number of immunosuppressed hospitalized patients, many of which are multi-resistant. Higher mortalities are thus a result of new infections occurring in hospitals (Nascimento *et al*., 2000).

In order to abate the problem of drug resistance the following needs to be considered: the uncontrolled usage of antimicrobials and a greater insight into mechanisms of resistance. Furthermore, the continuation for the search of natural or synthetic compounds, which could potentially be sources for the development of novel and efficient antimicrobials, is integral to curtail the problem of drug resistance.

However, since the discovery of penicillins, not many lead compounds have had the capacity for the development of drugs to be employed for treating human infections (Pauli, 2003). In addition, the use of botanical extracts as antimicrobial therapeutics dwindled almost completely due to the introduction of antibiotics in the 1950's (Cowan, 1999). Due to the reliance on bacterial and fungal sources for compounds with antimicrobial properties, very few dispensed pharmaceuticals having higher-plant origins are actually intended for use as antimicrobial agents.

5.1.2 Plants as therapeutic antimicrobials

In the late 1990's, plant-derived therapeutics regained popularity. There are a number of reasons for the renewed interest in plant antimicrobials. In recent times, the public has become aware of the problems related to overprescribing as well as to the incorrect usage of conventional antibiotic medicines. Moreover, many people are practicing autonomy in terms of their medical care and with many botanical therapeutics (plant extracts, mixtures and single plant compounds) being easily attainable over-the-counter from commercial outlets such as herbal or health food stores, widespread self-medication with these products is carried out by the lay community (Cowan, 1999).

The rapid pace of extinction of plant species is also driving this trend towards plant-derived therapeutics (Lewis and Elvin-Lewis, 1995). Botanical extracts possessing known antimicrobial activities can afford great benefits to treatments. Thus, before the potentially bioactive phytochemicals which could be synthesized chemically, is lost, the knowledge concerning the traditional medicinal usage of plants gained by the indigenous people must be acquired (Borris, 1996). This would have particular benefits for developing countries with limited access to more expensive conventional antimicrobials.

5.1.3 Secondary plant metabolites

Plants synthesize aromatic compounds, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). Many of these compounds protect the plant against micro-organisms, insects and herbivores; the antimicrobial compounds are the plant's defence system against predation. The numerous glandular hair components may play some role in protecting *Pelargonium* plants from herbivore or microbial attack in the natural environment (Williams and Harborne, 2002). Plants may prove to be valuable

sources of novel antimicrobials in the treatment of resistant microbial strains due to the antimicrobial plant compounds which could possibly inhibit bacteria by a different mechanism to that of the currently used antibiotics.

5.1.4 The use of botanical antimicrobials as preservatives in the food, cosmetic and pharmaceutical industries

The presence of micro-organisms in consumer products constitutes a potential hazard. Firstly, deterioration of the product may occur. Micro-organisms possess a metabolic versatility whereby almost any formulation ingredient may undergo degradation in the presence of a suitable micro-organism. Secondly, the presence of micro-organisms may constitute an infection hazard to the consumer or patient (Bloomfield *et al*., 1988). Essential oils are often incorporated into food, pharmaceutical, cosmetic and household products, thus it is important to investigate whether natural substances possess inhibitory effects against micro-organisms (Pauli, 2001) as these substances could provide the dual function of flavouring/perfuming and antimicrobial activity.

5.1.5 Reported antimicrobial properties of Pelargoniums

Acute and chronic bronchitis, asthma and acute sinusitis have been treated by the inhalation of essential oils (Inouye *et al.*, 2001). Ethnobotanical data indicates that the vapours from the boiled leaves of *P. betulinum* were inhaled to relieve coughs and other chest problems (van der Walt, 1977). *Pelargonium cucullatum* was also traditionally used for this purpose (May, 2000). It has been found that through the inhalation of certain essential oils the respiratory tract fluid output increases and an anti-inflammatory effect is exerted on the trachea (Frohlich, 1968; Boyd and Sheppard, 1970; Burrow *et al.,* 1983; Shubina *et al.*, 1990). However, the use of the aforementioned *Pelargonium* species for respiratory conditions may have also been due to their antimicrobial properties. Many plant-derived essential oils are known to exhibit antimicrobial activity against a wide range of bacteria and fungi (Cox *et al*., 2001).

Pelargonium species were used as traditional remedies to treat various ailments (Watt and Breyer-Brandwijk, 1962), many of which involve bacteria or fungi (Lis-Balchin and Deans, 1996). Numerous studies have concluded that 'geranium oil' possesses antimicrobial activity (Lis-Balchin *et al*., 1996a; 1996b; 1996c; 1998a; 1998b). Most of the

Pelargonium oils studied by Lis-Balchin *et al.* (2003) showed potential against several micro-organisms in some food systems. Pattnaik *et al*. (1996) tested the antifungal effect of 'geranium oil'; all twelve of the fungi strains tested were inhibited by the oil. Four different strains of fungi were inhibited by the vapours of 'geranium' Bourbon (Chaumont and Leger, 1992). Results from an investigation carried out on a number of *Pelargonium* species and cultivars suggested that *Pelargonium* essential oils and solvent extracts could be used as antibacterial agents (Lis-Balchin *et al.*, 1998b). Lis-Balchin *et al*. (1996c) established that the antimicrobial property of several lipophilic *Pelargonium* extracts was very pronounced. The hydrophilic extracts of representative species and cultivars of *Pelargonium* displayed considerable antibacterial activity (Lis-Balchin and Deans, 1996).

5.2 Materials and methods

The potential antimicrobial activity of the non-volatile extracts (26 samples) and essential oils (7 samples) was evaluated against four standard micro-organism strains which included two Gram-positive bacteria, *Staphylococcus aureus* (ATCC 12600) and *Bacillus cereus* (ATCC 11778), one Gram-negative bacterium, *Klebsiella pneumoniae* (NCTC 1633) and one fungal strain, *Candida albicans* (ATCC 10231). The reference stock cultures were obtained from the National Health Laboratory Services (NHLS), Johannesburg and were maintained viable in the Department of Pharmacy and Pharmacology, University of the Witwatersrand.

5.2.1 Minimum inhibitory concentration assay

After the preliminary screening of the plant samples using the disc diffusion assay (Janssen *et al*., 1987), a more comprehensive assessment of their antimicrobial effects was carried out using the micro-well serial dilution assay (Eloff, 1998a). Such an assay was performed in order to quantify the antimicrobial activity for the test pathogens.

5.2.1.1 Principle of the method

Microbial growth is indicated by the presence of a reddish colour which is produced when p-iodo-nitrotetrazolium violet (INT), a dehydrogenase activity detecting reagent, is reduced by metabolically active micro-organisms to the corresponding intensely coloured formazan (Eloff, 1998a). The minimum inhibitory concentration (MIC) is defined as the lowest concentration that produces an almost complete inhibition of visible micro-organism growth in liquid medium. Thus, the MIC value is the concentration in the first well in which no colour change was observed (Baker and Tenover, 1996).

5.2.1.2 Protocol

The essential oils and plant extracts were dissolved in acetone to obtain stock solution concentrations of 128 mg/ml and 64 mg/ml, respectively. Sterile water (100 µl) was added to all the 96 wells of the microtitre plate. Aliquots of 100 µl from the stock solutions of the essential oils or plant extracts were added to the wells of row A. Serial dilutions (1: 1) of the initial concentrations were prepared vertically in the plate. The excess volume of 100 µl was discarded from the wells in row H. Culture (100 µl) containing a final density of 1 x 10^6 CFU/ml was added to each well, thereby achieving final well concentrations ranging from 32 mg/ml to 0.250 mg/ml for the essential oils and 16 mg/ml to 0.125 mg/ml for the crude extracts.

For each micro-organism, columns were reserved for the positive control and the negative control. Culture supplemented with the antibiotics ciprofloxacin (Oxoid) or amphotericin B (Oxoid) was used as the positive control for bacteria and fungi, respectively. Ciprofloxacin (Oxoid) and amphotericin B (Oxoid) were prepared in sterile water and DMSO, respectively, to produce a stock concentration of 1 mg/ml. The antibiotics were then diluted with sterile water to obtain a starting concentration of 0.01 mg/ml. For the observation of normal bacterial and fungal growth, control wells containing acetone (100 µl) and no plant extract (negative control) were prepared.

Sterile plate sealers were used to cover the microtitre plates to reduce evaporation of the well contents. The plates were incubated under normal atmospheric conditions, at 37°C for 24 hours for bacteria inoculum and for 48 hours for yeast inoculum. After the incubation period, 40 µl of 0.4 mg/ml INT (SIGMA) was added to all the wells and left to stand for 6

hours for the plates containing bacteria and 24 hours for the plates with yeast. The MIC was then visually assessed.

The crude extracts producing an MIC ≤0.125 mg/ml were retested, the stock solution was diluted with acetone to obtain a lower starting concentration. Acetone did not inhibit the bacterial or fungal growth at the tested concentrations.

5.3 Results and discussion

Essential oils contain mixtures of terpenoids, particularly monoterpenes and sesquiterpenes, and various aliphatic hydrocarbons (Dorman and Deans, 2000). Terpenoids are active against bacteria and fungi (Cowan, 1999). The antibacterial properties of essential oils are mainly attributable to the phenolic components (Cosentino *et al*., 1999). Essential oils and perfumes have been widely investigated as antimicrobial agents (Bloomfield *et al*., 1988). Maruzzella and Bramnick (1961) found that 20% of a variety of perfumery chemicals at a dilution of 1: 500 inhibited at least one of the four test bacteria. The main groups of chemicals were ranked in order of decreasing activity as follows: aldehydes, alcohols, eugenol, acids, lactones, ethers, ketones, esters and acetals. Alcohols possess bactericidal activity by acting as protein denaturing agents, solvents or dehydrating agents (Dorman and Deans, 2000) and further, have antifungal activity (Pauli, 2001). The *Pelargonium* essential oils tested in this assay contain a mixture of the aforementioned compounds so one would expect the essential oils to exert good antimicrobial activity. However, the *Pelargonium* essential oils tested displayed generally low antimicrobial activity (Table 5.1).

The non-volatile extracts showed promising antimicrobial activity (Table 5.2). Low antimicrobial activity (MIC = 8 mg/ml) was only observed for the extract of *P. papilionaceum* against *B. cereus* and for that of *P. quercifolium* (SBG) against *K. pneumoniae.* A similar trend was found by Lis-Balchin *et al*. (1998b) where methanolic and petroleum spirit extracts of *Pelargonium* species were more potent antibacterial agents than the steam-distilled volatile samples. A possible explanation for the reduced activity of the essential oils in comparison to the activity of crude extracts is that the essential oil constituents are volatile and so there is a resulting loss of active constituents which may be considerable due to long incubation periods (Pauli, 2001).

Table 5.1: *In vitro* antimicrobial activity of selected *Pelargonium* essential oils.

Plant species	**Locality**	**MIC (mg/ml)**			
		Antibacterial activity			**Antifungal activity**
		***Klebsiella pneumoniae* NCTC 1633**	***Bacillus cereus* ATCC 11778**	***Staphylococcus aureus* ATCC 12600**	***Candida albicans* ATCC 10231**
P. citronellum	NBG	16	4	4	4
P. graveolens	SBG	r	8	8	2
P. panduriforme	SBG	r	8	8	2
P. panduriforme	WSBG	r	8	4	4
P. radens	NBG	r	16	8	8
P. tomentosum	SBG	r	8	8	8
P. tomentosum	WSBG	r	16	8	8
Control	-	2.5×10^{-3} (a)	3.13×10^{-4} (a)	2.5×10^{-3} (a)	1.25×10^{-3} (b)

Where: - MIC = minimum inhibitory concentration (mg/ml); Microbial Strains: NCTC = National Collection of Type Cultures, ATCC = American Type Culture Collection; NBG = National Botanical Garden (Kirstenbosch), SBG = Stellenbosch Botanical Garden, WSBG = Walter Sisulu Botanical Garden (Johannesburg); r = resistant, MIC value >32 mg/ml; (a): ciprofloxacin; (b): amphotericin B; n = 3: triplicate measurements were performed for all samples.

Table 5.2: *In vitro* antimicrobial activity of *Pelargonium* acetone extracts.

Plant species	**Locality**	**MIC (mg/ml)**			
		Antibacterial activity			**Antifungal activity**
		***Klebsiella pneumoniae* NCTC 1633**	***Bacillus cereus* ATCC 11778**	***Staphylococcus aureus* ATCC 12600**	***Candida albicans* ATCC 10231**
P. betulinum	NBG	1	0.33	1.13	2
P. capitatum	Strand	1	1	2	1.5
P. citronellum	NBG	3	0.25	0.16	1
P. citronellum	SBG	3	0.41	0.16	0.5
P. cordifolium	NBG	1.5	0.25	0.75	0.75
P. cordifolium	WSBG	nd	nd	nd	nd
P. crispum	NBG	2	0.38	0.56	1.33
P. cucullatum	SBG	1.5	0.25	1	1
P. cucullatum	WSBG	3.33	0.5	2	2
P. glutinosum	SBG	2	0.078	0.078	1
P. glutinosum	WSBG	2	0.25	0.5	2
P. graveolens	SBG	2	2	4	3.33
P. graveolens	WSBG	nd	nd	nd	nd
P. greytonense	SBG	3.2	0.25	0.5	1.5
P. hermanniifolium	SBG	1.5	3	1.5	1.5
P. hispidum	SBG	2	4	4	3
P. panduriforme	SBG	2	2	2	2
P. panduriforme	WSBG	2	0.5	0.5	1
P. papilionaceum	NBG	4	8	2	1.19
P. pseudoglutinosum	NBG	2	0.039	0.039	0.54
P. quercifolium	SBG	8	0.33	0.16	2
P. quercifolium	WSBG	2	1	1	1
P. radens	SBG	2.5	0.5	2	2
P. scabroide	SBG	2	0.25	1	1
P. scabrum	SBG	2	0.059	0.078	0.38
P. sublignosum	SBG	2	0.13	0.078	0.5
P. tomentosum	SBG	2	2	2	2.33
P. tomentosum	WSBG	nd	nd	nd	nd
P. vitifolium	SBG	4	1	2	2
Control	-	2.5×10^{-3} (a)	3.13×10^{-4} (a)	2.5×10^{-3} (a)	1.25×10^{-3} (b)

Where: - MIC = minimum inhibitory concentration (mg/ml); Microbial strains: NCTC = National Collection of Type Cultures, ATCC = American Type Culture Collection, NBG = National Botanical Garden (Kirstenbosch), SBG = Stellenbosch Botanical Garden, WSBG = Walter Sisulu Botanical Garden (Johannesburg); r = resistant, MIC value >32 mg/ml; nd = not determined due to insufficient extract for testing; (a): ciprofloxacin, (b): amphotericin B; n = 3: triplicate measurements were performed for all samples.

The results further indicate that both the essential oils and extracts have a more selective antibacterial action against the Gram-positive bacteria. This is in accordance with other studies which reported Gram-positive bacteria to be more sensitive to both the essential oils and extracts (Cosentino *et al*., 1999; Karaman *et al*., 2003). Gram-negative bacteria possess an outer membrane, containing hydrophilic polysaccharide chains (Figure 5.1), which acts as a barrier to hydrophobic compounds (Tassou and Nychas, 1995; Mann *et al.,* 2000), hence the microbial resistance of *K. pneumoniae* to the tested *Pelargonium* essential oils. *Klebsiella pneumoniae* was not resistant to *P. citronellum* (NBG) essential oil; however, this latter oil displayed very weak antimicrobial activity (MIC = 16 mg/ml) against this Gram-negative bacterium.

Not all studies on essential oils have concluded that Gram-positives are more susceptible (Wilkinson *et al*., 2003). Zaika (1988) proposed that Gram-positive bacteria are less sensitive to the volatile oil antibacterial properties as compared to the Gram-negative bacteria.

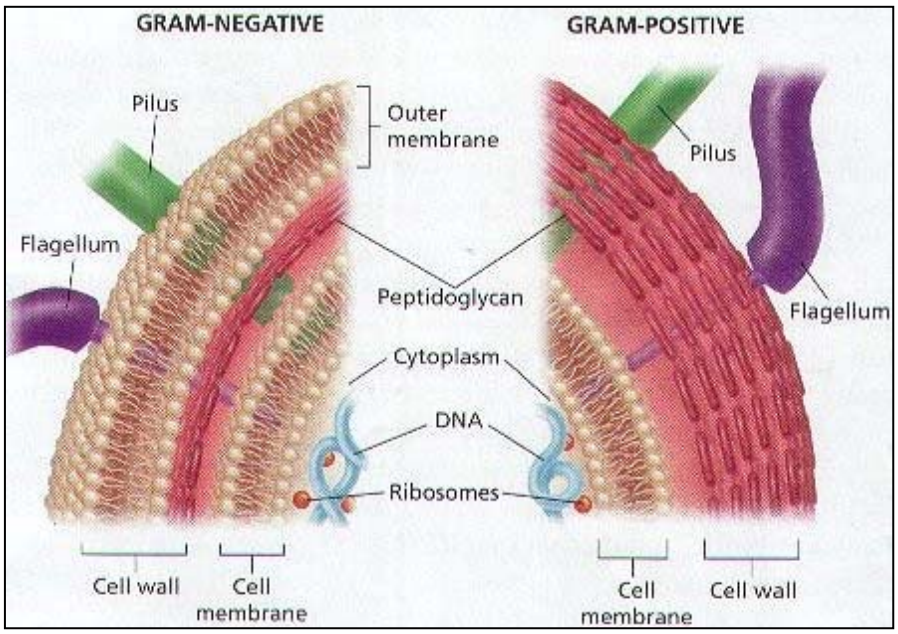
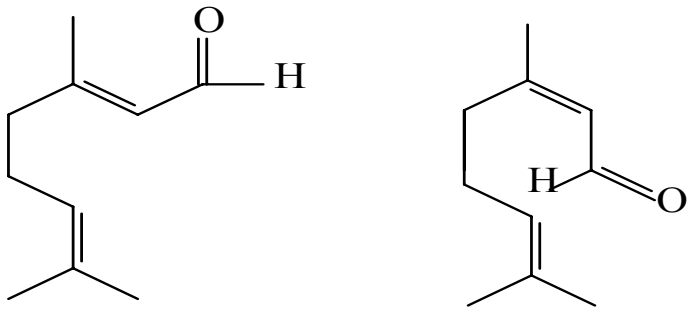


Figure 5.1: A comparison of the cell walls of the Gram-negative and Gram-positive bacteria. The Gram-negative bacterium's outer membrane with polysaccharide chains is shown (Johnson, 2005).

5.3.1 Essential oils

The bioactivity of the essential oils would be assumed to correlate to the respective composition of the plant essential oils (Dorman and Deans, 2000). Isomenthone, a ketone, occupies a large portion (49.3-84.5%) of the oil composition of *P. graveolens* (SBG), *P. radens* (NBG) and the *P. tomentosum* samples. Substantial amounts (36.1-41.1%) of the ketone menthone also occur in the *P. tomentosum* oils. Ketone ranked low among other compounds in terms of antimicrobial activity (Maruzzella and Bramnick, 1961). This may explain the low antimicrobial activity of the abovementioned oils.

Amongst the essential oils tested, *P. citronellum* (NBG) exerted the greatest activity against the bacteria, with *P. panduriforme* (WSBG) having the same MIC value of 4 mg/ml against *S. aureus*. The antimicrobial activity of *P. citronellum* (NBG) may be attributed to the two major compounds, namely neral (17.4%) and geranial (27.2%) (Figure 5.2), which were only detected in *P. citronellum* (NBG) oil. In a study conducted by Dorman and Deans (2000), the aldehydes *cis* and *trans* citral (geranial and neral) displayed moderate activity against the test micro-organisms (which included *K. pneumoniae* and *S. aureus*). It has been proposed that the highly electronegative arrangement of an aldehyde group conjugated to a carbon to carbon double bond (Moleyar and Narasimham, 1986) may inhibit the growth of micro-organisms by disturbing biological processes involving electron transfer and by reacting with protein and nucleic acid components. Geranic acid (36.0%) is the principle compound in *P. citronellum* (NBG) oil. As mentioned previously, acids among other perfumery chemicals have antibacterial activity (Maruzzella and Bramnick, 1961).



geranial neral

Figure 5.2: The chemical structures of the two major aldehydes, geranial and neral, present in *Pelargonium citronellum* (NBG) essential oil.

In a study involving *Cymbopogon citratus*, with major components being geranial and neral, noteworthy antibacterial and antifungal activities were recorded (Chalchat *et al*., 1997). In addition, Araujo *et al*. (2003) found that the main component of *M. officinalis* oil, namely citral (geranial and neral: 58.3%), showed a marked fungitoxic effect. However, in a study by Ngassapa *et al*. (2003), geranial, neral, limonene, germacrene D, camphor, linalool, β-caryophyllene and myrcene were identified as the main components of the two samples of *Lippia javanica* (Burm. f.), which were found to be inactive against the fungi tested. The implication is that the compounds present in high concentrations in *Lippia javanica* (Burm. f.) oil may be interacting with one another in a way which antagonizes the antifungal activity of neral and geranial. This assumption is further supported by the fact that the major compounds in *Lippia javanica* (Burm. f.) oil (except for geranial, neral and linalool) are either present in small amounts or are absent in *P. citronellum* (NBG) oil which has antifungal activity (MIC = 4 mg/ml for *C. albicans*). *Pelargonium citronellum* (NBG) also contains the oxygenated monoterpene linalool (1.8%) known to possess good antimicrobial activity (Carson and Riley, 1995).

Lis-Balchin *et al*. (1998b) found that *Pelargonium* essential oils showed substantial *in vitro* activity against *S. aureus*, *Proteus vulgaris*, *B. cereus* and *S. epidermidis* and concluded that *Pelargonium* essential oils could be used as novel food or cosmetic antimicrobial agents. The antibacterial activity of *P. citronellum* (NBG) against *B. cereus* and *S. aureus* suggest that *P. citronellum* (NBG) oil may delay microbial contamination. It is interesting to note that *Pelargonium citronellum* is a strongly aromatic shrub, with lemon scented leaves and is grown as a culinary herb (van der Walt and Vorster, 1988). *Pelargonium citronellum* (NBG) with its additional aromatic and flavouring properties could be employed as a natural food preservative. It also has the potential as a cosmetic or pharmaceutical preservative. However, in studies by Münzing and Schels (1972) and Blakeway (1986) the major restriction for the use of essential oils as preservatives was the high concentration of oil required to afford effective preservative action. At the required concentrations, cosmetic and toiletry preparations would have a 'medicinal' smell and so these required concentrations gave an olfactive limitation on the application of the oils. Moreover, oil toxicity and cost are the main limitations for the use of essential oils in pharmaceutical products. The toxicity of *P. citronellum* oil was evaluated (Chapter 10) to validate its potential use as an antimicrobial agent.

The essential oil of *P. graveolens* was more active against the Gram-positive bacteria than the Gram-negative bacterium, this is in agreement with a previous report by Dorman and Deans (2000). In addition, *P. graveolens* inhibited *K. pneumoniae* and *S. aureus* to the same extent in a disc diffusion assay study carried out by Dorman and Deans (2000). The quantitatively determined results from the MIC assay in this study show that *P. graveolens* (SBG) inhibits *S. aureus* whereas it is resistant to *K. pneumoniae.* The different culture strains used in the two studies could be causing this difference in activity towards *K. pneumoniae*. Hammer *et al*. (1999) tested various essential oils and plant extracts for their antimicrobial effects. A variety of test micro-organisms were used including *K. pneumoniae*, *S. aureus* and *C. albicans*. The essential oil of *P. graveolens* showed greater activity against *C. albicans* than against *S. aureus* and failed to inhibit *K. pneumoniae* at the highest concentration. These findings correlate with the MIC results obtained for the sample of *P. graveolens* (SBG) essential oil.

The antibacterial activity of essential oils is most probably attributable to several targets and mechanisms due to the many different chemical compounds present in essential oils. Figure 5.3 represents the locations and mechanisms in the bacterial cell as possible sites of action for essential oil compounds. The fact that essential oils are lipophilic enables them to partition in the lipids of the bacterial cell membrane and mitochondria, causing disruption of the structures rendering them more permeable. Following this, leakage of ions and other cell contents can occur. Ultimately death of the bacterial cell results from the extensive loss of cell content or the leakage of significant molecules and ions (Burt, 2004). The precise mode of action and antibacterial activity of individual essential oil compounds is influenced by their chemical structure (Dorman and Deans, 2000).

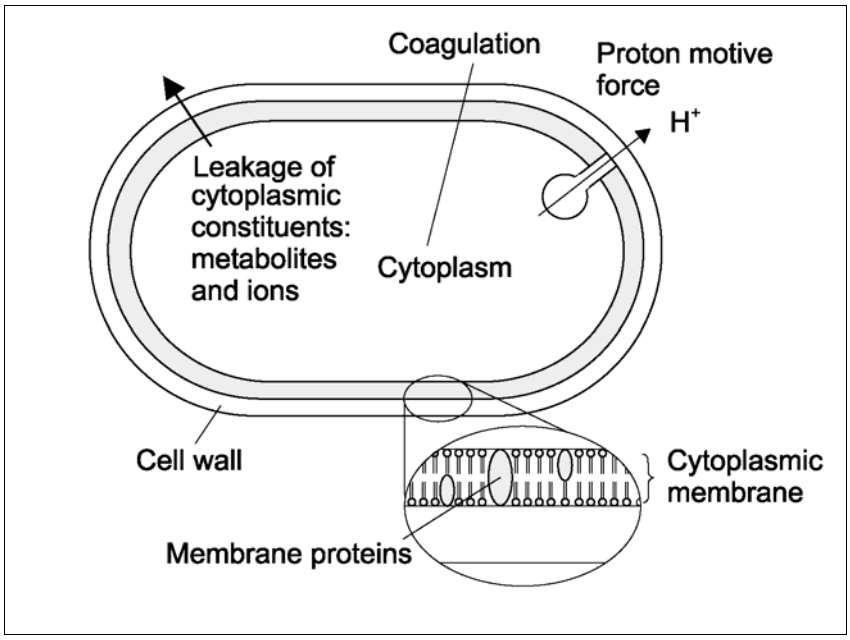


Figure 5.3: Locations and mechanisms in the cell of the bacterium which may act as sites of action for essential oil compounds (Burt, 2004).

The yeast *C. albicans* was most sensitive to the oils of *P. graveolens* (SBG) and *P. panduriforme* (SBG), both oils producing an MIC value of 2 mg/ml. The essential oil of *P. graveolens* and its main components, geraniol and citronellol, exhibited strong activity against six fungal species of *Trichophyton* (Shin and Lim, 2004). Minor compounds play an important role in antimicrobial activity, probably by producing a synergistic or potentiating effect between other compounds (Burt, 2004). Citronellol (0.4%) in *P. graveolens* (SBG) may contribute to its observed fungal activity. Limonene, linalool and menthol, which have been reported to possess antimicrobial properties (Duarte *et al.*, 2005), are also present in this sample. Terpinen-4-ol has been shown to possess good antimicrobial activity against *C. albicans* (Williams, 1996). The monoterpenes linalool and limonene possess antimicrobial activity (Carson and Riley, 1995; Dorman and Deans, 2000). *Pelargonium panduriforme* (SBG) contains 0.6% terpinen-4-ol, this compound does not exist in the other oils tested. *Pelargonium panduriforme* (SBG) essential oil also contains the highest amount of linalool (4.42%) in comparison to the other oil samples. The combined effects of terpinen-4-ol, linalool, limonene (2.2%) and other compounds in minor amounts may contribute towards the good antifungal activity (MIC = 2 mg/ml)

displayed by this oil. *Pelargonium graveolens* (SBG) and *P. panduriforme* (SBG) samples may be sources of effective anti-*Candida* agents.

With reference to the dendrogram (Chapter 3, Figure 3.2), *P. citronellum* (NBG), generally the most active oil, is clustered separately from the other essential oils. *Pelargonium graveolens* (SBG) and *P. panduriforme* (SBG) produced identical MIC values for all the test micro-organisms. However, these two oils have markedly different chemical compositions as is reflected in the dendrogram (Chapter 3, Figure 3.2). *Pelargonium graveolens* (SBG) and *P. radens* (NBG) produced similar MIC values for *K. pneumoniae* and *S. aureus*. These oils have a high degree of similarity. *Pelargonium radens* (NBG) and *P. tomentosum* (WSBG) are closely associated in the cluster dendrogram, this similarity in chemical composition is portrayed in their antimicrobial activities. The *P. tomentosum* duplicate samples are more similar in their chemistry than the *P. panduriforme* duplicate samples. This degree of similarity extends to their antimicrobial activity, where the *P. tomentosum* samples produced different activities against one micro-organism while the *P. panduriforme* samples differed in their activities against two micro-organisms.

The MIC technique evaluated the essential oils in liquid medium and does not allow the effect of the vapour phase to be tested. One must consider that essential oils are highly volatile at room temperature and so the evaluation of the vapour activity would then provide a better indication of whether these oils are effective inhalation therapeutics. Investigation of the antimicrobial capacity of the vapours of the essential oils would provide an indication of their possible usage as disinfectants in hospital rooms and work environments. The vapours of essential oils can exert their antimicrobial activity by gaseous contact. Inouye *et al.* (2001) introduced a new parameter, minimal inhibitory dose (MID), as a measure of the vapour activity on exposure. Moleyar and Narasimham (1986) showed that the antimicrobial activity of volatile compounds results from the combined effect of direct vapour absorption on micro-organisms and indirect effect through the medium that absorbed the vapour (Inouye *et al*., 2001).

5.3.2 Non-volatile extracts

Several species showed substantial activity against the two Gram-positive bacteria. Inhibition was observed to be greater against *B. cereus* than against *S. aureus* i.e. *B. cereus* appears to be the more sensitive bacterium. Of particular importance are the acetone extracts of *P. glutinosum* (SBG), *P. pseudoglutinosum*, *P. scabrum* and *P. sublignosum* (Table 5.2). The acetone extract of *P. pseudoglutinosum* exerted the highest antimicrobial activity against *B. cereus* and *S. aureus* (MIC = 0.039 mg/ml). These findings highlight the potential use of the latter species as antimicrobial agents. In general, the crude extracts exhibited reduced activity towards the Gram-negative bacterium *K. pneumoniae* and to the yeast *C. albicans* (Table 5.2). The extracts of *P. betulinum* and *P. capitatum* were most active against *K. pneumoniae. Candida albicans* was found to be least resistant to *P. scabrum* which was the second most active test substance against *B. cereus* (MIC = 0.059 mg/ml). Other extracts with substantial activity against *C. albicans* include *P. citronellum* (SBG), *P. pseudoglutinosum* and *P. sublignosum*.

Equal activities against *K. pneumoniae* were noted for the duplicate samples of *P. citronellum, P. glutinosum* and *P. panduriforme*. *Pelargonium citronellum* samples also produced equal activities against *S. aureus*. In general, the duplicate samples showed varying antimicrobial activities. This suggests that the composition of extracts from different collections of the same species can differ. Plant composition is dependent on a complex interrelationship between many chemical, physical and biological factors associated with the environment (Richardson *et al*., 1954).

It was found that *P. betulinum* (NBG), *P. capitatum* (Strand), *P. graveolens* (SBG), *P. hispidum* (SBG), *P. panduriforme* (SBG) and *P. vitifolium* (SBG) extracts share very characteristic chemical profiles (Chapter 4). Of these species, *P. graveolens* (SBG) and *P. hispidum* (SBG) produced the most similar MIC values. The two related species, *P. capitatum* and *P. vitifolium* (van der Walt, 1977), produced the same MIC values against the Gram-positive micro-organisms. Similar promising antimicrobial activity was displayed by *P. scabrum* and *P. sublignosum*, which have very similar flavonoid patterns (Chapter 4). *Pelargonium vitifolium* and *P. papilionaceum* produced similar MIC values except with respect to *B. cereus*. However, their HPLC profiles proved to be different (Chapter 4).

The *Pelargonium* species tested by Lis-Balchin and Deans (1996) showed pronounced antibacterial activity, but poor antifungal activity. However, *P. capitatum* and *P. cucullatum* in particular showed no activity against *K. pneumoniae* and *S. aureus,* respectively. In this study, *P. capitatum* (Strand) and the *P. cucullatum* samples showed promising activity against the bacteria as well as *C. albicans.* In the study performed by Lis-Balchin and Deans (1996) the hydrophilic (methanolic) fractions were tested, whereas in this study the acetone extracts were tested. In the study performed by Lis-Balchin and Deans (1996) the lipophilic fractions were removed from the test extracts so that the potency of the hydrophilic fractions could be assessed. Acetone is a very useful extractant, it is able to extract both hydrophilic and lipophilic compounds (Eloff, 1998b). The presence of the lipophilic compounds in the acetone extracts may be responsible for the greater antimicrobial activity observed. Lis-Balchin *et al*. (1996c) established that the antimicrobial property of several lipophilic *Pelargonium* extracts was very pronounced. A further possible reason for the discrepancy between the two studies is the testing method used. The agar-well method used in the study by Lis-Balchin and Deans (1996) is useful as a preliminary check for antimicrobial activity prior to more detailed studies such as the MIC assay which provides a quantitative analysis of the antimicrobial activity.

Phenolic compounds have been reported to exert antimicrobial properties. Flavonoids are abundant in all Pelargoniums (Bate-Smith, 1973). *Pelargonium* plant material was extracted with acetone which isolates flavonoid glycosides and the more polar aglycones such as hydroxylated flavones, flavonols, biflavonyls, aurones, chalcones and anthocyanidin (Harborne *et al*., 1975). HPLC-UV analysis (Chapter 4) confirmed the presence of flavonoid derivatives in the crude extracts of the studied *Pelargonium* species. Plants synthesize flavonoids in response to microbial infection (Dixon *et al*., 1983). Thus it is not surprising that they have been found to exert *in vitro* antimicrobial activity against many micro-organisms (Cowan, 1999). Green tea (*Camellia sinensis*) has been found to be active against multiple types of micro-organisms and contains the flavonoid catechin. The flavones and other phenolics in Thyme (*Thymus vulgaris*) have activity against bacteria and fungi. Tree bard (*Podocarpus nagi*) contains the flavonol totarol active against Gram-positive bacteria (Cowan, 1999). Powers (1964) investigated the effects of more than 20 flavonoids on bacteria. No compound was devoid of inhibitory activity toward one or more of the ten bacteria studied. The presence of flavonoids in the non-volatile extracts may account for their observed antimicrobial activity.

Flavonoids are hydroxylated phenolic compounds occurring as a C_6–C_3 unit attached to an aromatic ring. It has been reported that the more hydroxylation, the greater the antimicrobial activity (Sato *et al.,* 1996). However, the finding that flavonoids with no hydroxyl groups on their β-rings are more active against micro-organisms than those with hydroxyl groups (Chabot *et al.*, 1992) provides an indication that the membrane of the microbe is the microbial target of flavonoids. Flavonoids with more lipophilic properties may also disrupt membranes of the micro-organisms (Tsuchiya *et al.,* 1996). Flavonoids have the ability to complex with bacterial cell walls, with possible targets in the microbial cell being surface-exposed adhesions, cell wall polypeptides and membrane-bound enzymes (Cowan, 1999). A possible mechanism of action of the flavonoids is irreversible complexation with nucleophilic amino acids in extracellular and soluble proteins. This leads to inactivation and loss of function of the proteins. Such a mechanism of action provides considerable antimicrobial effects (Cowan, 1999).

Furthermore, even though it has been reported that the solvents water, ethanol or methanol are used for the extraction of tannins (Cowan, 1999), it must be realized that tannins are present in Pelargoniums (Bate-Smith, 1973) and that this class of compound possesses antimicrobial effects (Smith *et al*., 2003), which have been widely recognized. They are toxic to filamentous fungi, yeasts and bacteria (Cowan, 1999). The physical and chemical properties of the polyphenolic skeleton of tannins are responsible for the anticipated interaction with biological systems (De Bruyne *et al*., 1999). Interesting to note is that acetone received the highest overall rating, among a variety of other extractants examined, for their ability to solubilize antimicrobials from plants (Eloff, 1998b).

Staphylococcus aureus is commonly found on human skin and mucosa and can infect wounds. It is one of the most persistent infectious micro-organisms and is commonly found in nosocomial infections (Steenkamp *et al*., 2004). Even though the process of healing occurs by itself, an infection can hamper this process (Priya *et al*., 2002). Topical antimicrobial therapy is the most important part of wound management to prevent infection. The profound antimicrobial effects of Pelargoniums may be responsible for their ability to assist in wound-healing (Lis-Balchin, 1996). The extracts of *P. glutinosum* (SBG), *P. pseudoglutinosum*, *P. scabrum* and *P. sublignosum*, which displayed considerable activity against *S. aureus* (Table 5.2), may be potential sources of topical

antimicrobial agents for the treatment of wound infections and may be sources of effective agents for nosocomial infections.

The traditional therapeutic uses of *P. betulinum* (van der Walt, 1977; Lawrence and Notten, 2001), *P. cucullatum* (van der Walt, 1977; May, 2000) and *P. papilionaceum* (Watt and Breyer-Brandwijk, 1962) may be validated by their antimicrobial effects as demonstrated by the acetone extracts. The antimicrobial properties of the *Pelargonium* species recorded in this study need to be assessed as being a result of selective or non-selective triggering of cell death. The cytotoxic effects of the extracts towards human kidney epithelial cells are presented in Chapter 10. The *Pelargonium* extracts with promising antimicrobial activity should be investigated further for the isolation of the antimicrobial compounds. A potentially effective formulation may be developed by combining the identified active components in an appropriate manner. Through the use of bioactive plant entities, novel ethno-therapeutic treatments of infections could be launched.

CHAPTER 6: THE ROLE OF ESSENTIAL OILS IN THE ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS

6.1 Introduction

The aminoglycoside antibiotics are protein synthesis inhibitors. An aminoglycoside passively diffuses across the outer membrane via bacterial porin channels and then is actively transported across the bacterial cell membrane into the cytoplasm where it binds irreversibly to 30S-subunit ribosomal proteins (Katzung, 2001). Aminoglycoside transport is enhanced by cell wall synthesis inhibitors such as the penicillins. The concept of improving a drug's antimicrobial action by enhancing its transport into the bacterium by concomitant administration of another drug was used as the premise to formulate a hypothesis: "essential oils enhance the antimicrobial efficacy of non-volatile compounds."

The results from Chapter 5 showed that the non-volatile extracts have greater antimicrobial activity than the essential oils. These extracts were obtained by acetone solvent extraction and were not completely devoid of essential oil constituents. This study was performed to determine if a pharmacological interaction (e.g. synergism or antagonism) exists between volatile and non-volatile components.

6.1.1 Principle

Essential oils are non-polar and thus are able to partition in the lipids of the micro-organism cell wall and cause disruption of the structures rendering the cell wall more permeable (Burt, 2004) to the more polar non-volatile compounds (e.g. flavonoid glycosides). These compounds are able to move through the cell wall and into the micro-organism where they can exert their activity. In this way the activity of the non-volatile compounds is enhanced. To test this hypothesis, the following was determined:

1) The antimicrobial activity of the essential oil.
2) The antimicrobial activity of the non-volatile extract (devoid of most essential oil constituents).
3) The antimicrobial activity of the plant extract containing both essential oil compounds and non-volatile compounds.

6.2 Materials and methods

6.2.1 Protocol

Fresh plant material of *P. graveolens*, *P. quercifolium* and *P. tomentosum* was collected from Walter Sisulu Botanical Garden (Johannesburg). For each species four samples were prepared:

(1) Essential oil (EO) by hydrodistillation,

(2) solvent extract of non-volatile compounds (NV) from the material remaining in the Clevenger apparatus after obtaining the essential oil in (1) above,

(3) soxhlet extract of fresh plant material (FMS) and

(4) soxhlet extract of air-dried plant material (ADS).

For each species the samples were prepared in the following way:

(1) Fresh plant material was hydrodistilled to obtain the essential oil. Hydrodistillation was carried out as described in Chapter 2, Section 2.2.1.

(2) The biomass (plant material and liquid) remaining after hydrodistillation was dried in the oven at 40°C. After drying, approximately 15 g of this material was subjected to soxhlet extraction with equal volumes of methanol and chloroform for 3-4 hours. A total solvent volume of 250 ml was used. This was performed to obtain a plant extract (NV) containing mostly non-volatile compounds (polar and non-polar) and devoid of the essential oil compounds liberated during hydrodistillation.

(3) The same method as described in (2) above was carried out on approximately 25 g of fresh plant material to obtain a plant extract (FMS) containing the non-volatile compounds and to a lesser extent volatile compounds.

(4) The same method as described in (2) above was carried out on approximately 15 g of air-dried plant material to obtain a plant extract (ADS) containing the non-volatile compounds and to a lesser extent volatile compounds.

The soxhlet method was used to extract the fresh plant material and the air-dried plant material as opposed to the usual cold extraction method. The rationale for this is that the soxhlet method is a heat extraction method like hydrodistillation, thus making extracts obtained through (2), (3) and (4) more comparable.

6.2.1.1 Soxhlet extraction method

The plant material was pulverized, pressed into an extraction thimble and weighed, compensating for the weight of the thimble. In a round bottom flask, the solvent mixture (250 ml) of 1: 1 methanol and chloroform was heated to a temperature higher than the boiling points of each of the two solvents (Figure 6.1). The vapours moved through the outer chamber of the soxhlet apparatus and into the condenser. Following condensation, the liquid flowed back into the bottom of the soxhlet chamber. As this distilled solvent rose in the soxhlet chamber, it permeated through the cellulose extraction thimble, holding the *Pelargonium* plant material. This extraction was evident from making the observation that the solvent in the soxhlet chamber had a different colour than it had in its pure form in the round bottom flask. The solution was forced through the small inner tube as the solvent level rose. Due to a siphoning-effect the chamber was flushed (Figure 6.1). The flushed solvent, containing the extracted compounds, returned to the round bottom flask. Extraction with fresh solvent was repeated, the solvent was redistilled from the solution in the round bottom flask, condensing in the chamber. The solution in the round bottom flask became more concentrated each time the process was repeated since more was being extracted from the plant material. The extraction process was complete when the solution in the soxhlet chamber was the same colour as the pure solvent. The extraction process was carried out for about 3 to 4 hours. Once allowed to cool down, the extract in the round bottom flask was poured into petri-dishes to allow for the evaporation of the solvent mixture.

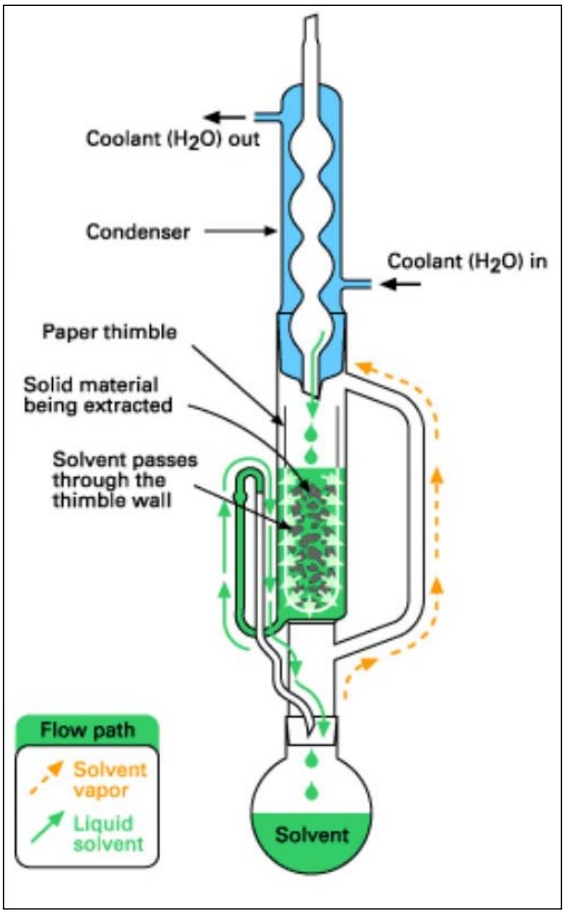


Figure 6.1: The process of soxhlet extraction (http://www.aquaculture.ugent.be//ATA/analysis/crudprot.htm).

6.2.2 Phytochemical verification

6.2.2.1 Thin layer chromatography

TLC was carried out on all the crude extracts for each species to confirm if essential oil compounds were absent in the NV extracts and to verify their presence in the FMS and ADS extracts. The essential oil samples, obtained through (1), were used as references and were diluted with hexane in the ratio 1: 7. The crude extract samples were prepared using methanol. The solvent system toluene: ethyl acetate (9.3: 0.7) was used to develop the TLC plates. The TLC plates were sprayed with anisaldehyde-sulphuric acid reagent which was prepared in the following way: 0.5 ml anisaldehyde was mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid, in that order (Wagner and Bladt, 1996). The anisaldehyde-sulphuric acid spray reagent detects

terpenoids amongst several other compounds and thus was used to verify the presence of essential oil compounds in the extracts. After spraying, the TLC plates were heated at 100°C for 5-10 min and were evaluated visually and under UV-365 nm.

TLC analysis was again carried out on all the crude extracts for each species so that a comparison of the chemical patterns of the non-volatile compounds of the three types of crude extracts could be made. This was performed to determine whether the rigorous extraction process of hydrodistillation caused decomposition of the non-volatile compounds present in the biomass from which the NV extract was obtained. The ADS extracts were used in the study to verify the results produced by the FMS extracts.

The NV, FMS and ADS extracts were dissolved in methanol to obtain a concentration of 50 mg/ml and 5 µg of this solution was applied to silica gel plates (Alugram Sil G/UV_{254}). TLC plates were developed in two different solvent systems to determine which system would produce the best separation of the compounds. The two solvent systems used were methanol: water: acetone: ethyl acetate: chloroform (1: 0.8: 3: 4: 1.2) and toluene: dioxane: acetic acid (9: 2.5: 1). The TLC plates were sprayed with two different reagents to determine which one would produce better visualization of the compounds. The first spray, anisaldehyde-sulphuric acid reagent was prepared as described above. After spraying, the plates were heated at 100°C for 5-10 minutes and viewed visually and under UV-365 nm. This reagent detects terpenoids, propylpropanoids, pungent and bitter principles and saponins. The second spray used was natural products-polyethylene glycol reagent (NP/PEG), which is used for the detection of flavonoids. The plates were sprayed first with 10 ml of solvent A (diphenylboric acid-β-ethylamino ester in 1% methanol) and then with 8 ml of solvent B (polyethylene glycol-4000 (PEG) in 5% ethanol) for colour development (Wagner and Bladt, 1996). The plates were evaluated under UV-365 nm.

6.2.2.2 HPLC analysis

The chromatographic profiles of the three types of crude extracts for each species were also obtained using HPLC analysis. This method is more sensitive than TLC and so provides more accurate data from which better chemical comparisons among the crude extracts can be made. The protocol as described in Chapter 4, Section 4.2.1.2 was used to analyse the samples.

6.2.3 Antimicrobial activity

The MIC assay as described in Chapter 5, Section 5.2.1.2 was carried out on all four samples of each species to assess the antimicrobial activity. The microbial test organisms used included *K. pneumoniae* (NCTC 1633), *B. cereus* (ATCC 11778), *S. aureus* (ATCC 12600) and *C. albicans* (ATCC 10231).

6.3 Results and discussion

6.3.1 Phytochemical verification

6.3.1.1 Thin layer chromatography

The presence or absence of essential oil compounds in the crude extract samples could not be verified as it appears that TLC is not a sensitive enough method for the detection of such compounds in these extracts. GC analysis should therefore be performed.

Hydrodistillation selectively removes the essential oil from the plant material resulting in the latter material being devoid of most essential oil constituents. Thus it is assumed that the NV extract, obtained after soxhlet extraction of the remaining biomass, did not contain many essential oil compounds. The ADS and the FMS extracts can be expected to contain essential oil compounds as the plant material used to obtain these extracts were not subjected to the process of hydrodistillation prior to soxhlet extraction. The ADS material may contain less essential oil constituents than the FMS extract as the plant material was air-dried prior to subjecting it to soxhlet extraction.

With regards to the analysis of the non-volatile compounds of the crude extracts, good separation was achieved using the solvent system toluene: dioxane: acetic acid (9: 2.5: 1) and the spots were more easily visualized using the NP/PEG reagent. Fluorescent spots were observed under UV-365 nm indicating the presence of flavonoids in the extracts (Figure 6.2).

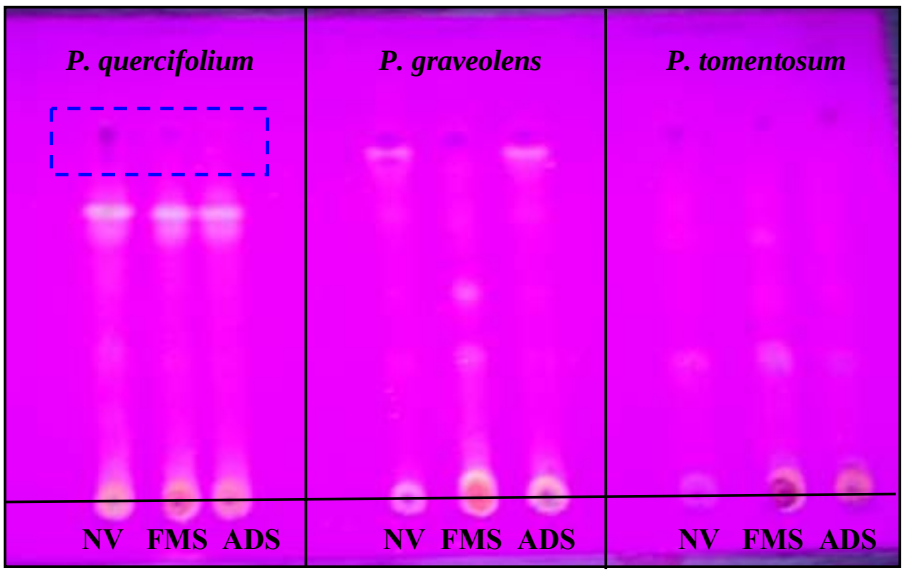


Figure 6.2: The natural products-polyethylene glycol sprayed-TLC plate viewed at UV-365 nm shows the presence of flavonoids in the crude extract samples. Where: - NV: solvent extract of non-volatile compounds; FMS: soxhlet extract of fresh plant material; ADS: soxhlet extract of air-dried plant material. All species were obtained from Walter Sisulu Botanical Garden.

The chemical pattern of the ADS and FMS extracts were compared to that of the NV extract for each species. Intense fluorescent spots (UV-365 nm) were observed for *P. quercifolium* in all the extracts. In *P. graveolens*, the NV and ADS extracts both contain a flavonoid at the same R_f value. Two flavonoids are observed at lower R_f values in the FMS extract. In *P. tomentosum*, a flavonoid at the same retention factor (R_f) value occurs in all three extracts. It was noted that the compound/s occurring at the same R_f value in the NV and FMS extracts of *P. quercifolium* did not occur in the ADS extract (highlighted by the dashed box in Figure 6.2). Consistency was generally observed in the chemical patterns of the three types of crude extracts.

6.3.1.2 HPLC analysis

The HPLC profiles of the three types of crude extracts for each species are shown in Figure 6.3, 6.4 and 6.5. The retention times, UV absorbance maxima, tentative identifications and percentage areas of the major peaks detected in each extract are summarized in Tables 6.1-6.3.

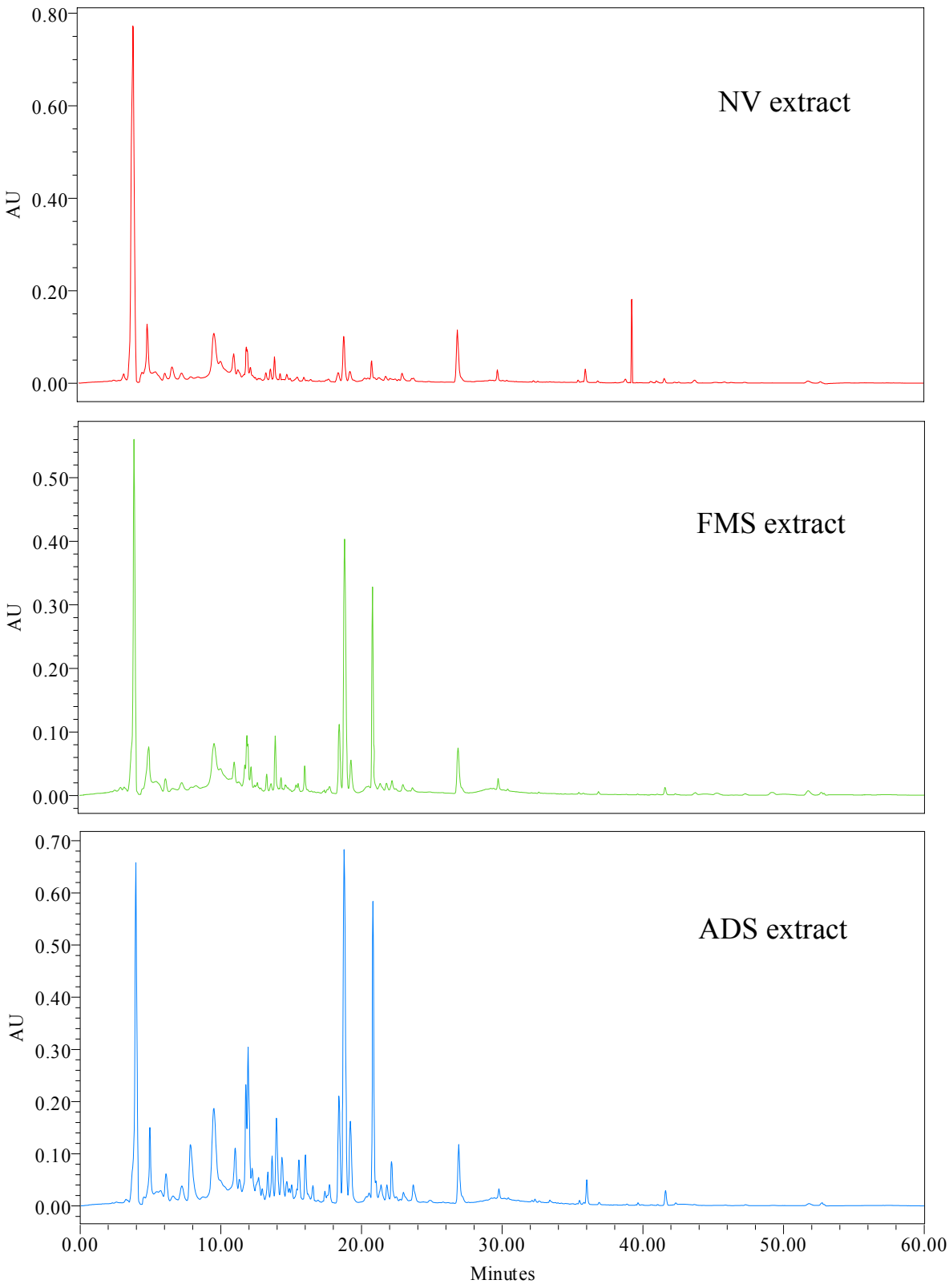


Figure 6.3: HPLC chromatograms of the three crude extracts of *P. graveolens* (WSBG). Where: - NV: solvent extract of non-volatile compounds; FMS: soxhlet extract of fresh plant material; ADS: soxhlet extract of air-dried plant material.

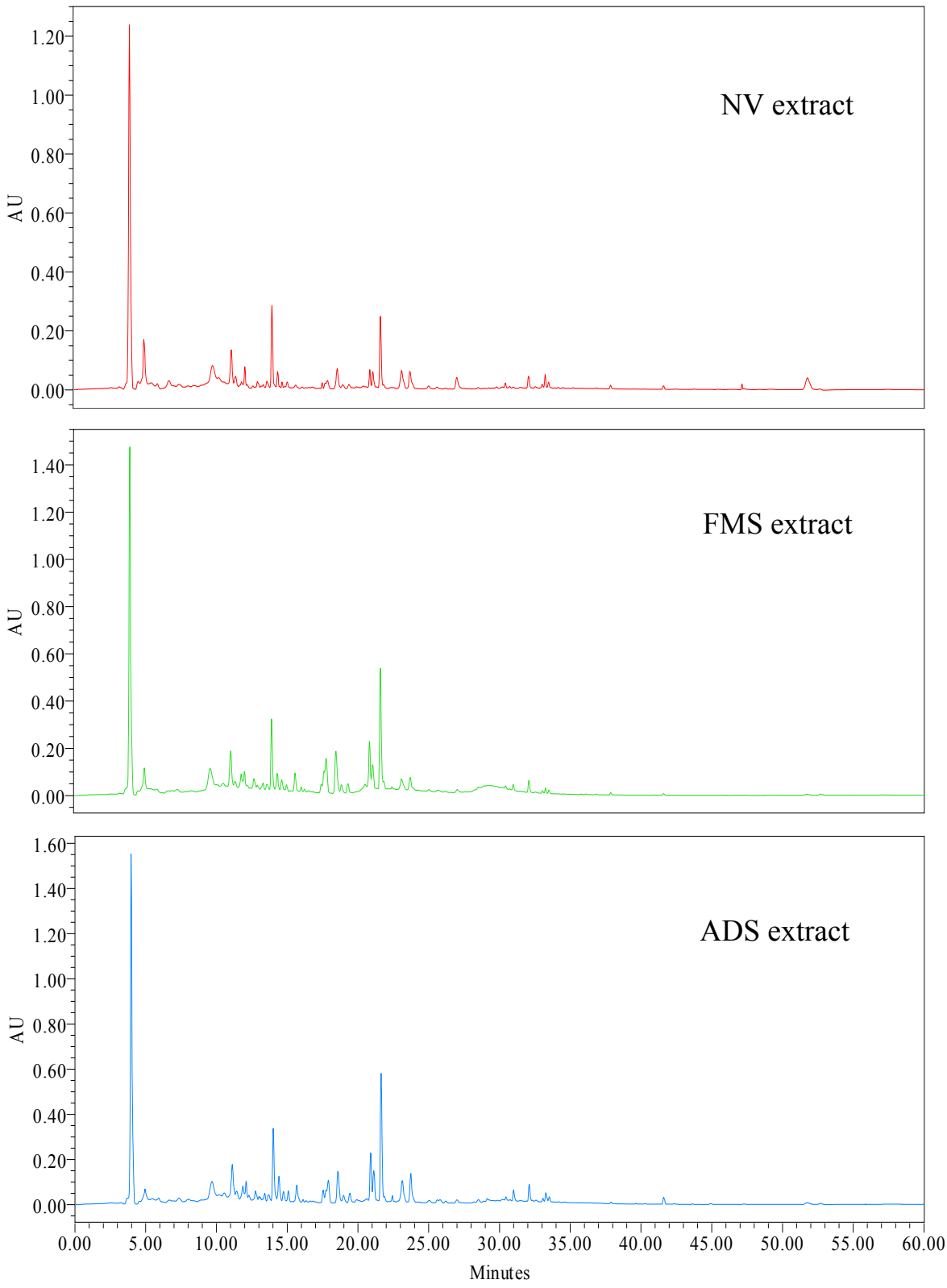


Figure 6.4: HPLC chromatograms of the three crude extracts of *P. quercifolium* (WSBG). Where: - NV: solvent extract of non-volatile compounds; FMS: soxhlet extract of fresh plant material; ADS: soxhlet extract of air-dried plant material.

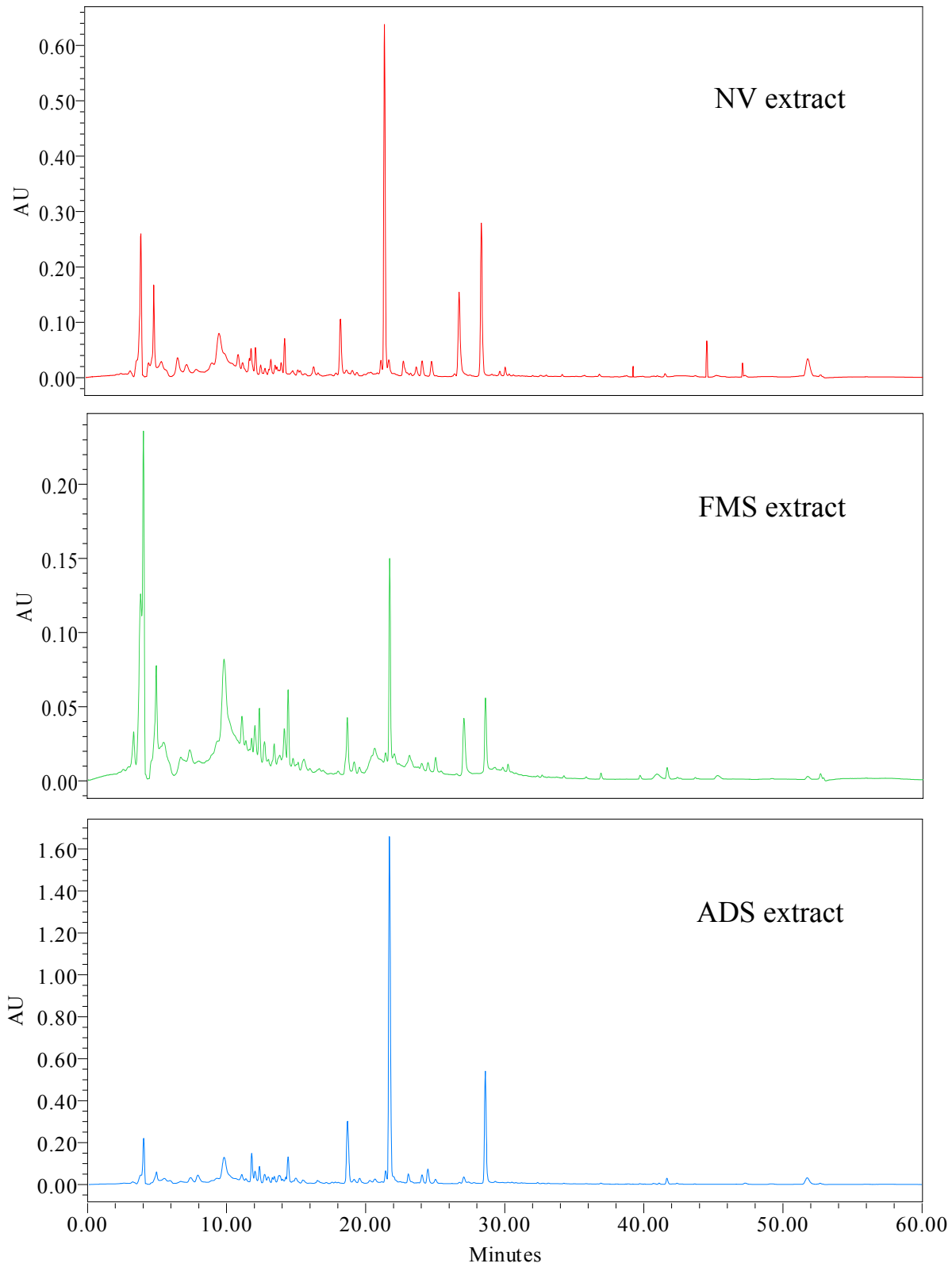


Figure 6.5: HPLC chromatograms of the three crude extracts of *P. tomentosum* (WSBG). Where: - NV: solvent extract of non-volatile compounds; FMS: soxhlet extract of fresh plant material; ADS: soxhlet extract of air-dried plant material.

Table 6.1: HPLC-UV data of the major peaks detected in the HPLC chromatograms of the NV, FMS and ADS extracts of *P. graveolens* (WSBG).

Retention time (min)	**UV absorbance maxima (nm)**	**Tentative identification**	**% Integration**		
			NV extract	**FMS extract**	**ADS extract**
3.97	212.1		55.84	28.15	15.59
4.59	260.3		0.96	-	2.69
5.02	274.4		5.85	4.43	-
6.21	283.9		0.86	1.07	1.13
6.73	214.5, 273.3		2.05	-	-
7.36	201.6, 247.3		0.92	0.65	1.04
7.85	256.7		-	-	4.14
9.64	207.4, 317.1		6.66	4.86	6.40
11.07	203.9, 278.0		2.04	1.40	1.90
11.82	218.0, 278.0		-	1.23	3.47
11.97	219.1, 319.4		2.89	4.23	5.77
12.27	209.8, 320.6		-	1.14	1.15
12.68	294.5		-	-	1.36
13.39	221.5, 281.5		-	0.86	0.93
13.71	227.4, 313.5		0.86	-	1.57
13.99	268.5		1.68	3.11	3.40
14.41	278.0, 307.6		-	0.92	1.96
14.70	233.2, 308.8		-	-	0.78
15.55	301.6, 337.3		-	-	1.94
16.07	256.7, 357.5	flavonol	-	1.25	1.87
17.73	2555.6, 354.0	flavonol	-	-	0.82
18.52	262.6, 348.0	flavone	1.13	5.15	4.60
18.91	255.6, 354.0	flavonol	4.77	22.08	17.24
19.36	255.6, 352.8	flavonol	1.01	2.67	3.84
20.89	257.9, 352.8	flavonol	1.22	11.17	9.95
21.40	265.0, 346.8	flavone	-	-	0.82
22.27	266.2, 345.6	flavone	-	0.63	1.31
23.04	250.9, 372.8	flavonol	-	0.71	-
26.94	255.6, 369.2	flavonol	5.62	3.71	2.33
29.79	265.0, 363.4	flavonol	0.75	0.59	-
36.03	267.4, 344.4	flavone	1.05	-	0.69
39.33	307.6		3.84	-	-

Where: - WSBG = Walter Sisulu Botanical Garden (Johannesburg).

Table 6.2: HPLC-UV data of the major peaks detected in the HPLC chromatograms of the NV, FMS and ADS extracts of *P. quercifolium* (WSBG).

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration		
			NV extract	FMS extract	ADS extract
3.97	213.3		42.76	34.81	31.89
4.99	274.4		6.64	3.09	1.88
6.75	214.5, 272.1		1.18	-	-
9.64	207.4, 317.1		3.67	4.27	3.41
11.09	201.6, 278.0		4.32	4.31	4.16
11.45	206.3, 260.3, 293.4		1.24	-	0.85
11.82	216.8, 287.4		-	1.19	1.59
12.06	219.1, 282.7, 317.1		1.51	1.37	1.43
12.72	201.6, 283.9		-	1.13	1.06
13.97	268.5		7.76	6.28	6.51
14.37	206.3, 282.7		1.35	1.82	2.53
14.69	278.0		-	1.25	1.01
15.09	220.3, 270.9	flavanone	-	-	0.98
15.64	207.4, 352.8	flavonol	-	1.82	2.02
17.54	207.4, 234.4		-	-	1.21
17.67	255.6, 354.0	flavonol	-	1.96	-
17.82	255.6, 354.0	flavonol	1.73	4.33	3.69
18.52	254.4, 348.0	flavone	2.90	5.59	3.95
18.91	255.6, 348.0	flavone	-	1.02	0.88
19.36	255.6, 352.8	flavonol	-	0.97	1.09
20.89	255.6, 354.0	flavonol	1.60	4.72	4.31
21.11	201.6, 230.9		1.89	2.91	3.53
21.66	255.6, 348.0	flavone	7.34	12.59	12.10
23.16	234.4, 363.4		3.21	1.74	2.93
23.77	233.2, 278.0	flavanone	2.70	1.74	3.32
27.05	255.6, 372.8	flavonol	1.77	-	-
31.00	256.7, 351.6	flavonol	-	-	0.90
32.12	243.8, 336.1	flavone	1.20	1.08	1.39
33.29	267.4, 348.0	flavone	1.12	-	0.80
41.60	408.8		-	-	0.60
51.79	261.5		3.08	-	-

Where: - WSBG = Walter Sisulu Botanical Garden (Johannesburg).

Table 6.3: HPLC-UV data of the major peaks detected in the HPLC chromatograms of the NV, FMS and ADS extracts of *P. tomentosum* (WSBG).

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration		
			NV extract	FMS extract	ADS extract
3.34	268.5		-	2.56	-
3.84	263.8		1.47	15.68	-
4.04	206.3		8.43	20.67	6.28
4.60	257.9		-	0.78	-
4.95	261.5		6.39	6.70	2.09
5.53	278.0		3.69	-	0.91
6.74	214.5, 272.1		3.10	-	-
7.37	247.3		2.07	1.21	0.93
7.94	257.9		1.60	-	1.33
9.36	209.8, 255.6, 293.4		3.69	-	-
9.82	206.3, 317.1		14.69	9.12	6.27
11.12	205.1, 278.0		2.24	1.71	-
11.41	209.8, 261.5, 293.4		1.49	-	-
11.82	214.5, 278.0		1.08	0.53	2.88
12.05	208.6, 308.8		1.82	1.84	1.36
12.37	208.6, 308.8		1.54	2.65	1.65
12.74	308.8		-	1.24	1.16
13.00	255.0, 281.5		-	-	0.82
13.44	221.5, 285.1		1.35	0.97	-
13.76	205.1, 312.3		-	-	1.32
14.15	213.3, 319.4		-	2.10	-
14.44	208.6, 283.9		1.68	3.74	2.73
15.57	229.7, 289.8		-	1.18	-
18.69	262.6, 349.2	flavone	3.76	4.15	8.78
19.17	233.2, 314.7		-	0.92	-
21.44	236.7		-	0.46	1.18
21.72	255.6, 348.0	flavone	17.61	9.77	40.84
22.01	256.7, 308.8	flavone	1.38	-	-
23.03	285.1	flavanone	1.41	-	0.98
23.15	237.9, 368.0	flavonol	-	1.43	-
24.05	263.8, 342.0	flavone	-	-	1.00
24.43	254.4, 348.0	flavone	1.10	-	1.76
25.02	246.1, 289.8		1.09	0.91	-
27.05	254.4, 368.0	flavonol	5.79	4.30	0.81
28.60	255.6, 357.5	flavonol	9.01	4.44	12.71
36.90	275.6		-	0.30	-
41.65	278.0, 411.2		-	0.62	0.63
51.78	261.5		2.55	-	1.57

Where: - WSBG = Walter Sisulu Botanical Garden (Johannesburg).

Quantitative and qualitative similarities were found among the three extracts of each species. For each species, many of the flavonoid derivatives and other non-volatile compounds present in the FMS and ADS extracts were present in the NV extract. Some compounds present in the FMS extracts were not detected in either the NV or the ADS extracts. Similarly, some compounds present in the ADS extracts were not detected in either the NV or the FMS extracts. This implies that the absence of these compounds in the NV extracts was not due to the influence of the process of hydrodistillation. In this way, the ADS extracts were used to show that when the plant material is processed by soxhlet extraction only, to obtain the FMS or ADS extract, some quantitative and qualitative chemical variation in the non-volatile component of the extract can still occur.

The TLC and HPLC results therefore indicate that hydrodistillation did not cause decomposition of the non-volatile compounds of the biomass used to obtain the NV extract. Due to the consistency established in the chemical patterns of the NV, FMS and ADS extracts, it can be assumed that the NV extract is representative of the non-volatile component of the FMS and ADS extracts. The antimicrobial activity produced by the NV extract gives some indication of the antimicrobial activity expected to be produced by the non-volatile component of the FMS and ADS extracts. The latter extracts contain the volatile component in addition to the non-volatile component and having some idea of the expected antimicrobial activity of the non-volatile component of the FMS and ADS extracts assists in the evaluation of the effect of the volatile component on the non-volatile component of these extracts.

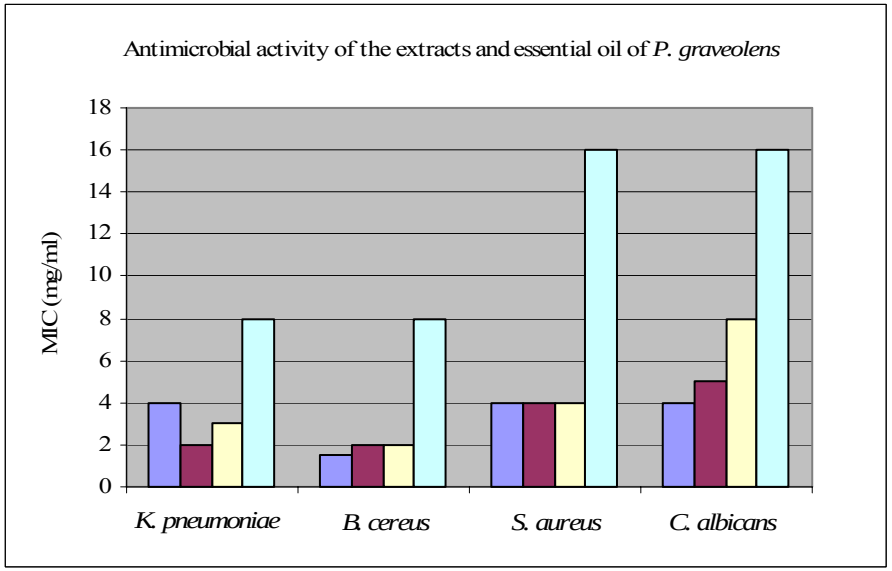
6.3.2 Antimicrobial activity

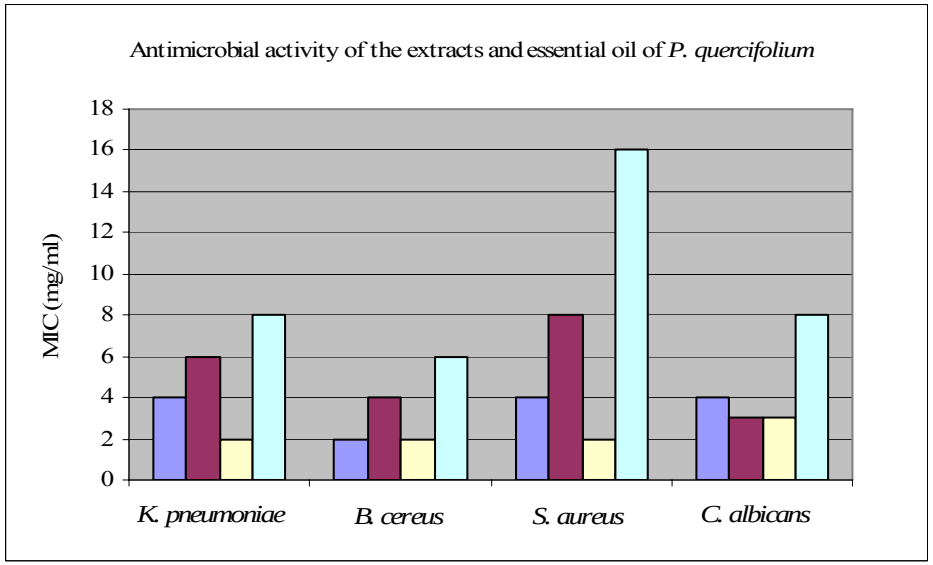
The MIC values obtained are summarized in Table 6.4 and graphically presented in Figure 6.6. All the extracts of each species exerted antimicrobial activity against all the test pathogens. It is evident from the results that the extracts containing predominately non-volatile compounds i.e. the NV extracts do not require the presence of essential oil compounds to exert antimicrobial effects.

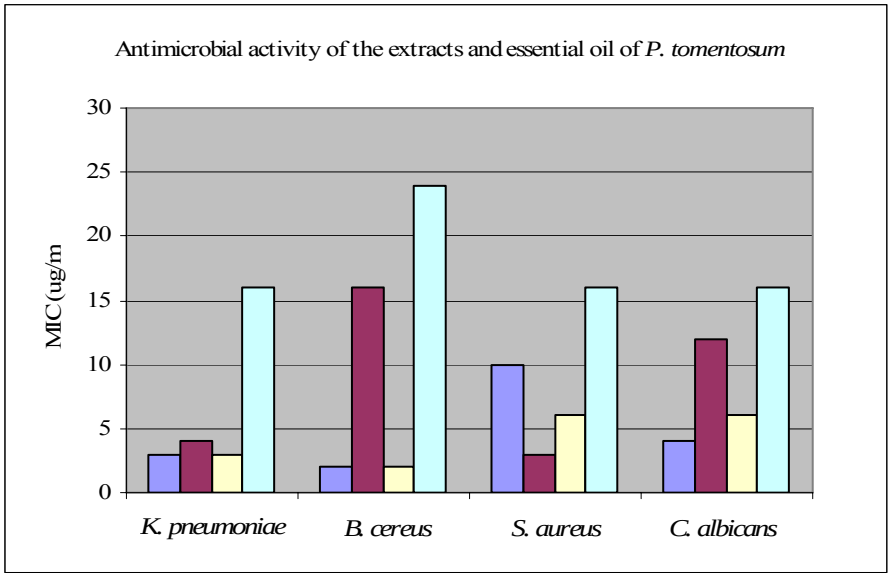
Table 6.4: The MIC values (mg/ml) recorded for the extracts and essential oil of *P. graveolens*, *P. quercifolium* and *P. tomentosum*.

Species	***Klebsiella pneumoniae* NCTC 1633**				***Bacillus cereus* ATCC 11778**				***Staphylococcus aureus* ATCC 12600**				***Candida albicans* ATCC 10231**			
	NV	**FMS**	**ADS**	**EO**	**NV**	**FMS**	**ADS**	**EO**	**NV**	**FMS**	**ADS**	**EO**	**NV**	**FMS**	**ADS**	**EO**
P. graveolens (WSBG)	4	2	3	8	1.5	2	2	8	4	4	4	16	4	5	8	16
P. quercifolium (WSBG)	4	6	2	8	2	4	2	6	4	8	2	16	4	3	3	8
P. tomentosum (WSBG)	3	4	3	16	2	16	2	24	10	3	6	16	4	12	6	16
Controls	2.5×10^{-3} (a)				3.13×10^{-4} (a)				2.5×10^{-3} (a)				1.25×10^{-3} (b)			

Where: - MIC = minimum inhibitory concentration (mg/ml); n = 3, values were conducted in triplicate; Microbial strains: NCTC = National Collection of Type Cultures, ATCC = American Type Culture Collection; NV: solvent extract of non-volatile compounds, FMS: soxhlet extract of fresh plant material, ADS: soxhlet extract of air-dried plant material, EO: essential oil; WSBG = Walter Sisulu Botanical Garden (Johannesburg); (a): ciprofloxacin, (b): amphotericin B.







Key to the graphs:

- NV: solvent extract of non-volatile compounds
- FMS: soxhlet extract of fresh plant material
- ADS: soxhlet extract of air-dried plant material
- EO: essential oil

Figure 6.6: The MIC values (mg/ml) of the extracts and essential oil of three *Pelargonium* species.

The NV, FMS and ADS extracts of *P. graveolens* were equally active against *S. aureus*. Identical MIC results were produced by the NV and ADS extracts of *P. tomentosum* for *K. pneumoniae* and *B. cereus*. The MIC values obtained for the NV and ADS extracts of *P. quercifolium* for *B. cereus* were the same. These findings suggest that the essential oils did not have an influence on the antimicrobial activity of the non-volatile compounds or extract.

The FMS and ADS extracts were expected to consistently produce the same results as they both contain some essential oil compounds in addition to the non-volatile compounds. The antimicrobial activity of both the FMS and ADS extracts of *P. graveolens* against *K. pneumoniae*, of *P. quercifolium* against *C. albicans* and of *P. tomentosum* against *S. aureus* were greater than the activity of their respective NV extracts. The presence of the essential oil compounds in the abovementioned FMS and ADS extracts could be responsible for their improved antimicrobial activity. However, there is little evidence from the results to support this. The FMS extracts generally exerted lower antimicrobial activity than the NV extracts.

All the FMS and ADS extracts produced greater antimicrobial activity than the corresponding essential oils obtained by distillation. This suggests that the non-volatile compounds improve the antimicrobial property of volatile compounds. Essential oils extracted by hexane exhibit greater antimicrobial activity than the corresponding steam distilled essential oils. The difference in antimicrobial properties indicates a difference in the composition of oils obtained by solvent extraction as opposed to distillation (Burt, 2004). It can be concluded that in the case of *Pelargonium*, essential oils do not enhance the antimicrobial activity of non-volatile extracts, since the ADS and FMS extracts did not consistently produce greater antimicrobial properties than the NV extracts. In most cases, comparison of the MIC values of the NV and FMS extracts suggest that the essential oils are decreasing the antimicrobial activity of the non-volatile extracts. However, the ADS extract produced lower antimicrobial activity compared to the NV extract in only three of the tested samples. One must consider that not all the essential oil components present in the EO sample are present in the FMS extract and ADS extract. In addition, the essential oil constituents present in the latter extracts may not occur in sufficient concentrations to produce improved activity via disruption of the lipophilic structures of the micro-organism cell wall.

CHAPTER 7: ANTI-OXIDANT ACTIVITY

7.1 Introduction

7.1.1 Definition of a free radical

A free radical is any chemical species which contains one or more unpaired valence electrons (Halliwell and Gutteridge, 1989).

7.1.2 Free radicals in the body

During normal aerobic metabolism free radicals are produced endogenously, and can react with almost all of the cell constituents. Superoxide radicals ($O_2•^-$) and other active oxygen species are products of several cell redox systems such as xanthine oxidase and membrane-related nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The oxidases are produced by various cells in order to accomplish useful bodily functions. The superoxide anion (low reactivity) can diffuse across large distances in the cell and in the presence of iron or copper a metal-catalyzed reaction, known as the Haber-Weiss reaction, can occur. The hydroxyl radical (•OH) is produced from this reaction (Halliwell and Gutteridge, 1989).

Through the formation of hydroxyl radicals a process known as lipid peroxidation occurs. In the outer atomic orbital of normal oxygen species, two electrons are present and this confers stability to the molecule. However, the presence of only one of these electrons in the outer orbital results in a highly reactive and unstable molecule. In order to complete their orbital pair, free oxygen radicals attract electrons from other molecules. The highly reactive hydroxyl radical (•OH) reacts with nearby biological molecules which are fundamental cellular components (Willson, 1978). The hydroxyl radicals interact with the polyunsaturated fatty acid side chain of membrane lipids which results in the production of lipid-derived free radicals, namely conjugated dienes and lipid hydroperoxides. These radicals cause oxidative damage or breakdown of membranes. The hydroxyl radicals also react with the amino acyl chains and thiol groups of proteins or the bases and sugar residues within nucleotides and nucleic acids resulting in DNA chain breakage (Repetto and Llesuy, 2002) and in this way molecular and cellular damage occurs.

7.1.3 The body's protective mechanisms against free radicals

A certain physiological level of reactive oxygen species (ROS) is fundamental for regulation of cell function. An elaborate endogenous anti-oxidant system compensates for the production of ROS and other free radicals during metabolism.

7.1.3.1 Intrinsic enzymatic anti-oxidant detoxifying mechanisms

Several intracellular enzymes protect the cell against damaging oxygen-radical reactions by scavenging reactive oxygen intermediates (ROI):

1. Superoxide dismutases (SOD) prevent the accumulation of the superoxide anion by catalyzing the dismutation of $O_2\bullet^-$ to hydrogen peroxide.
2. Glutathione peroxidase and catalase prevent the hydrogen peroxide from forming the hydroxyl radical (catalase catalyzes the reduction of H_2O_2 to water, glutathione peroxidase catalyzes the removal of H_2O_2 at the expense of glutathione oxidation).
3. Glutathione lipoperoxidase prevents the accumulation of cytosolic lipid peroxides (Halliwell and Gutteridge, 1989; Winyard *et al*., 1994).

7.1.3.2 Intrinsic non-enzymatic anti-oxidant detoxifying mechanisms

1. A non-catalytic mechanism which protects the cells from the damaging effects of oxygen radicals involves the chelation of iron by transferrin. Due to this mechanism, the concentration of low molecular mass chelates of iron in normal human serum, capable of catalyzing the Haber-Weiss reaction, is effectively zero. Thus the formation of the hydroxyl radical is inhibited (Winyard *et al*., 1994).
2. The breakdown of polyunsaturated fatty acids mediated by radicals is inhibited by vitamin E (α-tocopherol) and β-carotene, both of which are lipid-soluble anti-oxidants. Water-soluble molecules such as thiols, vitamin C (ascorbic acid) and uric acid scavenge oxygen radicals ($O_2\bullet^-$ and •OH). In addition, reduced vitamin C regenerates oxidized vitamin E, and prevents oxidation of thiols in the cytosol.

7.1.4 Imbalance between pro-oxidants and anti-oxidants in the body

Free radicals are involved in several biochemical mechanisms *in vivo*, but when free radicals are produced in excess of local anti-oxidant defence mechanisms, they become highly reactive causing oxidative damage to the cell membrane and ultimately cell death if

the damage continues. Many environmental, lifestyle and pathological factors cause a disturbance in the pro-oxidant/anti-oxidant balance with subsequent accumulation of excess free radicals resulting in oxidative stress (Rice-Evans and Burdon, 1994; Alfonso *et al*., 2003). These factors may increase the process of lipid peroxidation through the formation of excess hydroxyl radicals. Phagocytic cells upon stimulation have increased oxygen uptake and utilize the NADPH oxidase to release superoxide anions into extracellular fluid (Kleblanoff, 1982). Xanthine oxidase containing tissues, during ischaemia and reperfusion, appear to produce superoxide anions (McCord, 1985).

7.1.5 Free radical-mediated pathology

The free radical involvement in cell damage forms part of a more multifaceted deleterious process (Rice-Evans and Burdon, 1994). The aetiology and pathogenesis of various degenerative diseases such as neurodegenerative disorders, cardiovascular disease, inflammation and other chronic diseases have been linked to oxidative stress and hence associated with the oxidation of polyunsaturated fatty acids, lipoproteins and membrane lipids (Repetto and Llesuy, 2002; Willcox *et al*., 2004). The key oxygen-radical species mediating biochemical tissue damage in various pathological conditions is thought to be the hydroxyl free radical (Halliwell and Gutteridge, 1989).

Human inflammatory fluids have been found to contain oxidized molecules which are products of free radical reactions (Winyard *et al*., 1994). In acute or chronic inflammation, the biological metabolic balance of the production and removal of oxygen radicals may be disturbed by a pathogenetic factor ultimately leading to cellular damage. The inflammatory process is another potential source of free radicals (peroxy and alkoxy radicals) (Jackson *et al*., 1983; Duncan, 1991). Due to the high reactivity of peroxy and alkoxy radicals with other polyunsaturated fatty acids, they can initiate the non-enzymatic lipid peroxidation process, whereby membrane fatty acids are degraded. Damaged cell membranes lead to further inflammation. Hence free radicals and inflammation are intimately linked.

7.1.6 Anti-oxidants

An anti-oxidant is any substance which can delay or prevent the oxidation of a substrate when present in small amounts relative to that oxidizable substrate (Rice-Evans and Burdon, 1994). Several different stages occur in the process of lipid peroxidation. Thus anti-oxidants can act in the five following ways in the oxidative sequence (Halliwell and Gutteridge, 1989).

Anti-oxidants:

1) Decrease localized oxygen concentrations;
2) Prevent chain initiation by scavenging initiating radicals;
3) Bind catalysts such as metal ions to prevent initiating radical generation;
4) Decompose peroxides so that they cannot be reconverted to initiating radicals;
5) Break chains to prevent continued hydrogen abstraction by active radicals.

The activity of the endogenous anti-oxidant systems may be reinforced by anti-oxidants and in this way provides additional protection against oxidative stress. Lipid peroxidation and other free radical-mediated processes are inhibited by anti-oxidants and thus protect the body from several free radical-related diseases (Repetto and Llesuy, 2002). Anti-oxidants of synthetic or natural origin may have a significant role to play in the maintenance of the pro-oxidant/anti-oxidant balance. Of current interest is the potential anti-oxidant protective effect of natural compounds on affected tissues. Research indicates that through sufficient anti-oxidant defence, protection can be afforded against free radical damage associated with specific diseases and lifestyle patterns and processes such as cancer, aging, circulatory conditions, arthritis, pollution and strenuous exercise (Packer, 1991).

7.2 Materials and methods

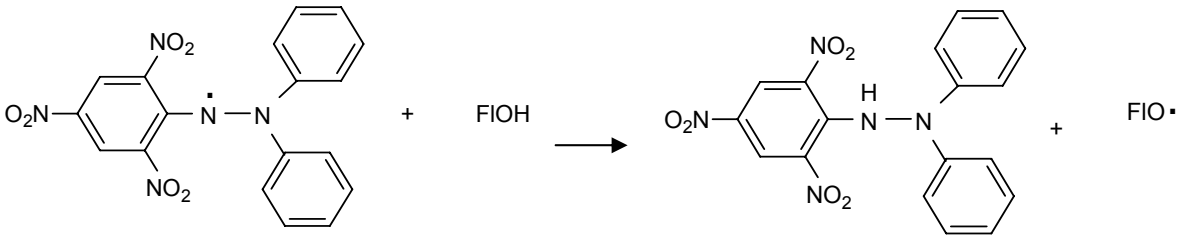
7.2.1 The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay

HPLC analysis provides standardized information with regards to the concentration of individual anti-oxidants in plant extracts. The prediction of a mixture's anti-oxidant potency based on compositional data is difficult since the anti-oxidant pattern is rather complex. Thus the use of specific assays to assess the anti-oxidant activity of plant extracts is necessary (Schwarz *et al*., 2001). Test systems that evaluate the radical scavenging ability of anti-oxidants aim to simulate basic mechanisms involved in lipid oxidation by

measuring either the reduction of stable radicals or radicals generated by radiolysis, photolysis, or the Fenton reaction (Schwarz *et al*., 2001). The anti-oxidant activity of compounds can be evaluated accurately, conveniently and rapidly using the DPPH method (Prakash, 2001). The quantitative measurement of the *in vitro* hydrogen donating capacity of 23 *Pelargonium* acetone extracts was carried out using the DPPH assay adopted from the colourimetric method described by Shimada *et al*. (1992).

7.2.2 Principle of the method

The DPPH molecule is a stable free radical with a low deterioration rate and low reactivity towards most compounds. As a result, only good hydrogen atom donors are likely to react with the stable radical (Schwarz *et al*., 2001). The DPPH radical has a dark violet colour due to the delocalization of an unpaired electron. Briefly, the stable DPPH radical has a strong absorption band at 550 nm, but upon reduction by an anti-oxidant or radical species its absorption decreases (Pinelo *et al.,* 2004) and so bleaching of the purple coloured DPPH occurs at 550 nm (Figure 7.1).



DPPH radical (purple) **Reduced DPPH (yellow)**

Figure 7.1: The reduction of the DPPH radical by a hydrogen-donating compound.

7.2.3 Protocol

DPPH (Fluka) was dissolved in analytical grade methanol (Ultrafine Ltd.) to obtain a 96 µM solution which was stored in a volumetric flask wrapped in foil and kept in the fridge at +4ºC. An initial stock solution of 10 000 µg/ml was prepared by dissolving 10 mg of plant extract in 1 ml of dimethyl sulfoxide (DMSO) (Saarchem). A 500 µg/ml solution was prepared, whereby 50 µl of the 10 000 µg/ml stock solution was diluted with 950 µl DMSO.

For the primary screening of the extracts, each sample was tested at an initial concentration of 100 µg/ml. Fifty microlitres of the 500 µg/ml solution were added to wells 1B, 1C, 1D, 2B, 2C, 2D. Eleven other test samples were plated out in the same way. For the control readings, 50 µl of DMSO was added to each well of rows A and H. DPPH solution (200 µl) was added to the wells in column 1, 3, 5, 7, 9, 11. Analytical grade methanol (200 µl) was added to the wells in columns 2, 4, 6, 8, 10, 12; the reaction mixtures that contained extract and methanol were used to compensate for coloured extracts. The plates were shaken for two minutes using an automated microtitre plate reader (Labysystems Multiskan RC). After allowing the plates to stand for a period of 30 minutes at room temperature in the dark, the decrease in absorbance at 550 nm was measured using an UV-VIS spectrophotometer linked to a computer equipped with GENESIS® software. The percentage decolourisation as a measure of the free radical-scavenging activity of the extracts was calculated according to the following equation:

$$\% \text{ Decolourisation} = \frac{[\text{Av controls} - (\text{Av sample}_{DPPH} - \text{Av sample}_{methanol})] \times 100}{\text{Av controls}}$$

Where: - Av controls = average absorbance of all DPPH control wells – average absorbance of all methanol control wells; Av sample_{DPPH} = average absorbance of sample wells with DPPH and Av $\text{sample}_{methanol}$ = average absorbance of sample wells with methanol.

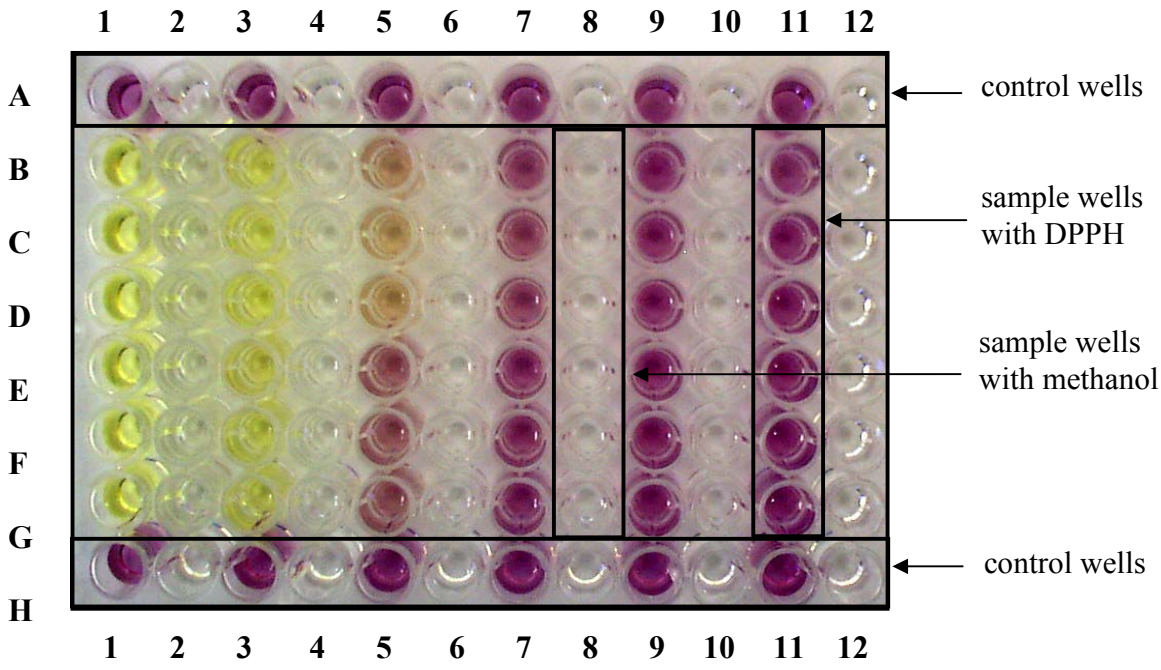


Figure 7.2: A 96-well microtitre plate prepared for use in the DPPH assay. Purple wells indicate the absence of anti-oxidant substances and yellow wells signify anti-oxidant activity.

For those extracts with ≥50% decolourisation at the starting concentration of 100 µg/ml, retesting of the samples was carried out at lower concentrations using serial dilutions. Five stepwise 1: 1 serial dilutions were prepared from each 500 µg/ml solution of plant extract using DMSO. Percentage decolourisation was plotted against the concentration of the sample and the IC_{50} values were determined using Enzfitter® version 1.05 software. IC_{50} values indicate the concentration of sample required to scavenge 50% of the DPPH radical. Each experiment was done in triplicate. Vitamin C (L-ascorbic acid) was used as a positive control.

7.3 Results and discussion

The anti-oxidant activity of the *Pelargonium* acetone extracts are summarized in Table 7.1 and presented in Figure 7.3. Lis-Balchin and Deans (1996) concluded that the anti-oxidant activity of methanolic extracts of representative species and cultivars of *Pelargonium* were very pronounced. This was found to be true for the acetone extracts of numerous species in this study. *Pelargonium betulinum* (IC_{50} = 4.13 ± 0.14 µg/ml) and *P. crispum* (IC_{50} = 4.49 ± 0.18 µg/ml) displayed greater anti-oxidant activity than the positive control, vitamin C (IC_{50} = 4.72 ± 0.14 µg/ml). Other species showing remarkable anti-oxidant ability include *P. cordifolium* (NBG and WSBG), *P. cucullatum* (WSBG) and *P. scabrum* (Table 7.1). In the study conducted by Lis-Balchin and Deans (1996), the methanolic extracts of *P. cucullatum* showed pronounced anti-oxidant activity, this is in agreement with the result obtained in this study for the acetone extract of *P. cucullatum* (WSBG) (IC_{50} = 10.91 ± 0.54 µg/ml). *Pelargonium glutinosum* (SBG), *P. graveolens* (SBG), *P. hermanniifolium*, *P. hispidum*, *P. quercifolium* (SBG) and *P. sublignosum* produced substantial activity. *Pelargonium citronellum* (SBG), *P. panduriforme* (WSBG), *P. papilionaceum* (NBG) showed limited activity (Figure 7.3.).

Table 7.1: *In vitro* anti-oxidant activity (µg/ml) of *Pelargonium* acetone extracts.

Species	**Locality**	**DPPH IC_{50} (µg/ml)**	**s.d.**
P. betulinum	NBG	4.13	0.14
P. capitatum	Strand	nd	nd
P. citronellum	NBG	23.70	3.68
P. citronellum	SBG	84.01	16.08
P. cordifolium	NBG	5.01	0.55
P. cordifolium	WSBG	5.31	0.24
P. crispum	NBG	4.49	0.18
P. cucullatum	SBG	40.18	5.65
P. cucullatum	WSBG	10.91	0.54
P. glutinosum	SBG	16.41	0.33
P. glutinosum	WSBG	29.17	0.78
P. graveolens	SBG	14.49	0.46
P. graveolens	WSBG	26.81	1.80
P. greytonense	SBG	nd	nd
P. hermanniifolium	SBG	13.50	0.73
P. hispidum	SBG	12.78	0.45
P. panduriforme	SBG	nd	nd
P. panduriforme	WSBG	91.58	4.45
P. papilionaceum	NBG	81.24	13.44
P. pseudoglutinosum	NBG	52.38	0.67
P. quercifolium	SBG	17.15	0.58
P. quercifolium	WSBG	61.87	3.19
P. radens	SBG	nd	nd
P. scabroide	SBG	nd	nd
P. scabrum	SBG	7.15	0.11
P. sublignosum	SBG	17.61	3.18
P. tomentosum	SBG	28.16	2.65
P. tomentosum	WSBG	21.73	0.99
P. vitifolium	SBG	nd	nd
Vitamin C	-	4.72	0.14

Where: - NBG = National Botanical Garden (Kirstenbosch), SBG = Stellenbosch Botanical Garden, WSBG = Walter Sisulu Botanical Garden (Johannesburg); nd = not determined due to insufficient extract for testing; s.d. = standard deviation; n = 3: triplicate measurements were performed for all samples.

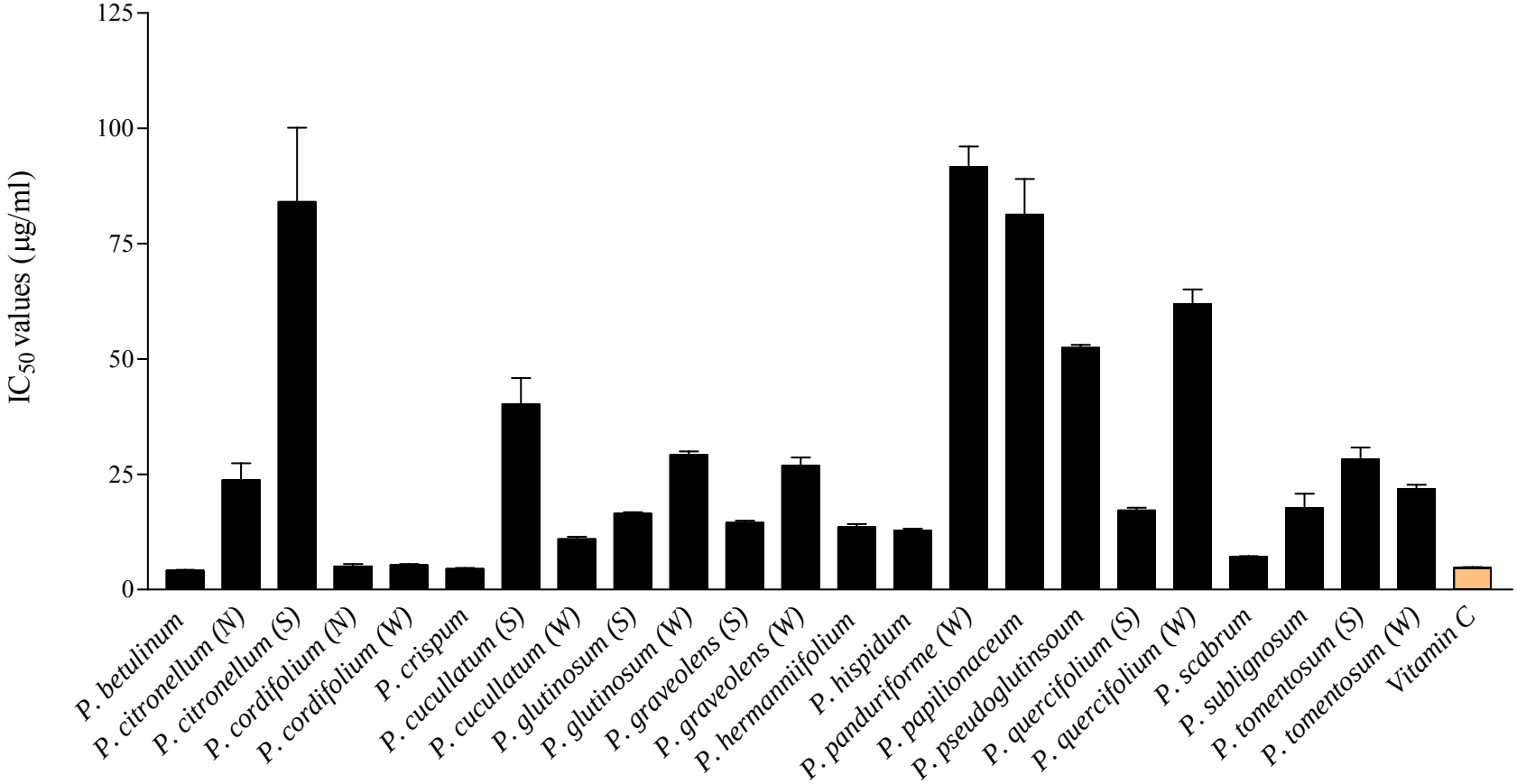


Figure 7.3: The comparative DPPH radical-scavenging abilities of *Pelargonium* acetone extracts in terms of IC_{50} values. Where: - (S) = Stellenbosch Botanical Garden, (N) = National Botanical Garden (Kirstenbosch), (W) = Walter Sisulu Botanical Garden (Johannesburg).

An attempt was made to relate those species with similar anti-oxidant activity on a chemical level. *Pelargonium betulinum, P. cordifolium* (WSBG), *P. crispum* and *P. cucullatum* (SBG) and *P. scabrum* extracts share some similarities in their chemical fingerprints (Chapter 4). *Pelargonium betulinum, P. cordifolium* (WSBG), *P. crispum* and *P. scabrum* demonstrated exceptional anti-oxidant activity (IC_{50} = 4.13 ± 0.14 µg/ml – 7.15 ± 0.11 µg/ml). However, *P. cucullatum* (SBG) displayed moderate anti-oxidant activity (IC_{50} = 40.18 ± 5.65 µg/ml). The representative flavonoid patterns identified in the *Pelargonium* species suggested that *P. betulinum* (NBG), *P. capitatum* (Strand), *P. graveolens* (SBG), *P. hispidum* (SBG), *P. panduriforme* (SBG) and *P. vitifolium* (SBG) share very characteristic chemical profiles (Chapter 4). Due to insufficient extract, *P. capitatum* (Strand), *P. panduriforme* (SBG) and *P. vitifolium* (SBG) were not tested in the DPPH assay. It was found that *P. graveolens* (SBG) and *P. hispidum* (SBG) produced almost similar IC_{50} values. *Pelargonium scabrum* and produced greater anti-oxidant activity than *P. sublignosum*; these species had similar chemical profiles.

The two samples of *P. cordifolium* displayed similarly high anti-oxidant activities (Figure 7.3). *Pelargonium tomentosum* (SBG) and *P. tomentosum* (WSBG) displayed analogous IC_{50} values of 28.16 ± 2.65 µg/ml and 21.73 ± 0.99 µg/ml, respectively. The similar activity of the duplicate samples of the same species may be due to their similar chemical compositions. Notable differences were observed in the activities of the duplicate samples of *P. citronellum*, *P. cucullatum* and *P. quercifolium*. Such diverse radical-scavenging abilities may be attributable to the different chemistry of the duplicate samples of the same species. Further investigations involving HPLC analysis of the duplicate samples would allow for a comparison of their chemical compositions in order to gain a better understanding of the similarities and differences observed in activity.

Flavonoids have been shown to be effective scavengers of ROS (Rusak *et al.,* 2005). The superoxide anion ($O_2^{\bullet -}$), hydroxyl (•OH), peroxyl (ROO•) and alcohoxyl (RO•) radicals are trapped via the anti-oxidant activity of flavonoids (Repetto and Llesuy, 2002). Flavonoids act as anti-oxidants *in vivo*; they stabilize the membranes by decreasing their permeability and also bind to free fatty acids (Svoboda and Hampson, 1999). Flavonoids are potential therapeutics for free radical mediated pathologies (Repetto and Llesuy, 2002).

The health benefits related to flavonoids have increased the significance of natural anti-oxidants (Schwarz *et al*., 2001).

The composition of the extracts and thus their anti-oxidant activity is greatly influenced by the extraction methods (Schwarz *et al*., 2001). *Pelargonium* plant material was extracted with acetone which isolates flavonoid glycosides and the more polar aglycones such as hydroxylated flavones, flavonols, biflavonyls, aurones, chalcones and anthocyanidin (Harborne *et al*., 1975). HPLC-UV analysis confirmed the presence of flavonoid derivatives in the *Pelargonium* acetone extracts (Chapter 4). These compounds may contribute to the anti-oxidant activity of the *Pelargonium* extracts. The presence of flavonoids is mainly responsible for the medicinal properties of folk plants. However, other organic and inorganic compounds such as coumarins, phenolic acids and anti-oxidant micronutrients e.g. Cu, Mn, Zn may also contribute to the medicinal properties of plants (Repetto and Llesuy, 2002).

The *Pelargonium* species, in particular *P. betulinum, P. crispum P. cordifolium* and *P. scabrum*, are possible sources of anti-oxidant compounds. It is projected that these compounds may have potential use in the development of therapeutics for free radical-mediated pathologies. Sources of natural anti-oxidants are significant in the chemoprevention of diseases resulting from lipid peroxidation, since such anti-oxidant compounds protect against oxidative stress (Nakatami, 2000).

Vitamins, minerals and botanical extracts are amongst some of the most popular substances incorporated into topical preparations used to defy skin aging. Natural products are used in cosmetic preparations due to their low mammalian toxicity (Aburjai and Natsheh, 2003). The daily application of skincare products containing anti-oxidant agents can mitigate and partially reverse the skin aging effects of free radicals. The appearance of fine lines and wrinkles can be improved by using vitamin C and E containing creams. Vitamin C restores the skin's elasticity and resiliency by stimulating the production of collagen (Rabat, 2003). *Pelargonium betulinum, P. crispum P. cordifolium* and *P. scabrum* in this study showed similar anti-oxidant activity to that of vitamin C. Isolation of the active anti-oxidant compounds from these *Pelargonium* species may be potential ingredients for use in cosmetic preparations where they may play a role in the preservation and rejuvenation of the skin through their anti-oxidant action.

The major bestower to skin aging is ultraviolet radiation; this is known as photo-aging. Flavonoids have considerable absorption between 200-380 nm and so function to screen the plants from UV radiation (Harborne *et al*., 1975). This suggests the possible utilization of flavonoids as UV-screen compounds to protect the human skin. In addition, the flavonoids may protect against UV radiation-induced peroxidation, probably by scavenging oxygen free radicals generated by UV irradiations (Bonina *et al*., 1996). Furthermore, phenolic substances enhance the solubility characteristics of compounds and the phenols are easily moved across biological membranes (Parke, 1968). This further advocating the potential use of *Pelargonium* extracts, containing many flavonoids, as ingredients in skin care preparations where they may have the dual action of anti-aging and sun protection.

Oxygen may be highly damaging to food if not properly controlled. ROS initiates oxidative rancidity (lipid oxidation) of foods (Alfonso *et al*., 2003). Synthetic anti-oxidants as food additives are consumed in large amounts. There is increasing evidence that these agents have been associated with toxicity problems. Interest in the development of natural anti-oxidants as food preservatives is escalating due to consumer preference for natural ingredients and concerns regarding toxic effects of synthetic anti-oxidants.

The food industry requires the anti-oxidative properties of botanical extracts. Food with added natural anti-oxidants can be referred to as functional foods since the consumer is afforded greater health benefits by these products (Alfonso *et al*., 2003). Tocopherols, ascorbic acid, rosemary extracts, lycopene and some flavonoids are natural anti-oxidants which are available to be added to food in place of synthetic agents (Alfonso *et al*., 2003). Scavenging by stable radicals is a suitable method for predicting the inhibition, by botanical extracts, of primary oxidation product formation in food systems (Schwarz *et al*., 2001). Such a prediction needs to be verified by assessing the anti-oxidant activity in a model food system. This verification would possibly highlight certain *Pelargonium* species as having a definite place in the food industry.

CHAPTER 8: ANTI-INFLAMMATORY ACTIVITY

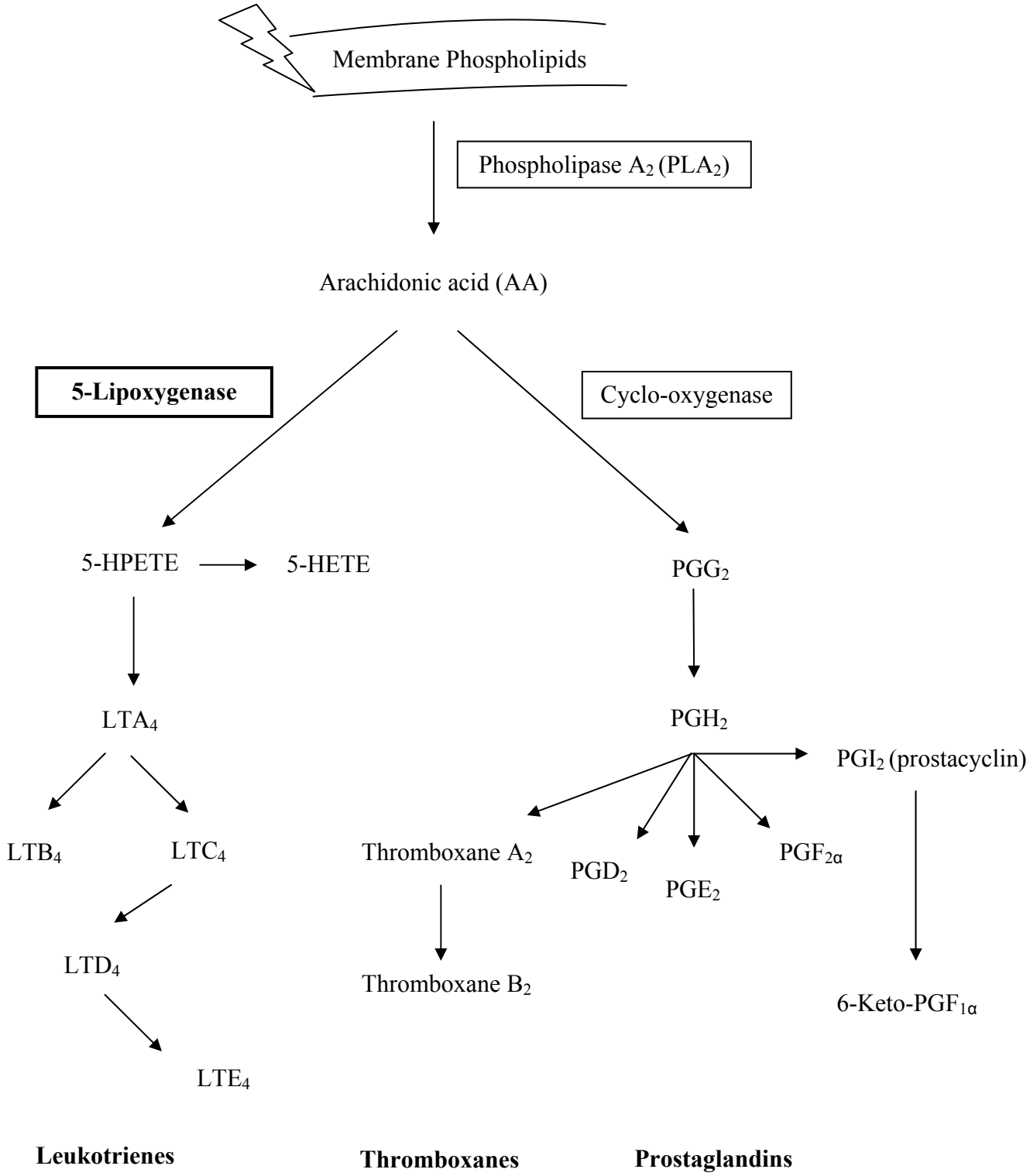
8.1 Introduction

Inflammation comprises a complex cascade of adaptive responses to tissue injury which are both local and systemic. The local responses involve recruitment of phagocytic cells and removal of foreign or endogenous material. These processes occur more efficiently through the changes brought on by the systemic responses. There is immense replication of functions amongst the biochemical mediators and regulators of inflammation due to the importance of this response to the survival of the organism (Whicher and Evans, 1992). When this response is exaggerated it can be the initiator of various ailments (Baylac and Racine, 2003).

8.1.1 The biosynthesis of eicosanoids

The hormone-like compounds produced by tissue cells and tumour-infiltrating leukocytes are referred to as eicosanoids, which are biosynthesized from the fatty acid components of the phospholipid structure of cell membranes, mainly from arachidonic acid (AA). Eicosanoids are classified into three classes, namely prostaglandins (PGs), leukotrienes (LTs) and thromboxanes, and have powerful activities in cell proliferation and tissue repair, blood clotting, blood vessel permeability, inflammation and immune cell behaviour (Fischer and Slaga, 1985).

In the event of trauma, phospholipase A_2 (PLA_2) generates AA near the cell wall surface which is acted upon by enzymes (Figure 8.1). The synthesis of eicosanoids occurs via three enzymatic pathways. The first pathway is catalyzed by cyclo-oxygenase (COX) which produces endoperoxides that are instantly converted into series-2-prostaglandins and thromboxanes (Figure 8.1). The lipoxygenase (LOX) group of enzymes is involved in the second pathway, these enzymes create hydroperoxyeicosatetraenoic acids (HPETEs). HPETEs are converted into series-4 LTS and different hydroxyeicosatetraenoic acids (HETEs) such as 5-HETE, 12-HETE and 15-HETE. The third pathway involves cytochrome P450 which catalyzes the formation of 12-HETE and 16-HETE (Wallace, 2002).



5-HPETE = 5-hydroperoxyeicosatetraenoic acid, 5-HETE = 5-hydroxyeicosatetraenoic acid; LTA_4 = leukotriene A_4, LTB_4 = leukotriene B_4, LTC_4 = leukotriene C_4, LTD_4 = leukotriene D_4, LTE_4 = leukotriene E_4; PGG_2 = prostaglandin G_2, PGH_2 = prostaglandin H_2, PGD_2 = prostaglandin D_2, PGE_2 = prostaglandin E_2, $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$, 6-Keto-$PGF_{1\alpha}$ = 6-Keto-prostaglandin $F_{1\alpha}$.

Figure 8.1: Metabolism of arachidonic acid (modified from Celotti and Durand, 2003).

8.1.2 The role of 5-lipoxygenase in inflammation

The 5-lipoxygenase (5-LOX) enzyme is present in macrophages and mast cells, but is expressed mainly in leukocytes. It is a soluble monomeric enzyme containing a non-heme iron atom which is in the ferrous form in the resting state. During catalysis the iron cycles between ferrous and ferric states and so the enzyme is converted from an inactive reduced Fe (II) state to the active oxidized Fe (III) state.

5-LOX is the initial key enzyme in the second enzymatic cascade; once stimulated by the 5-lipoxygenase activating protein (FLAP), it catalyzes the first two steps in LT biosynthesis from AA. Arachidonic acid is first converted into 5-HPETE (Figure 8.1) by the insertion of oxygen into the C-5 of AA (dioxygenase activity) and then through a dehydration step to the short lived epoxide LTA_4 (epoxygenase activity). LTA_4 is a common unstable intermediate converted by LTA_4 hydrolase into the dihydroxy leukotriene B_4 or converted via leukotriene C_4 synthase, a specific glutathione-transferase enzyme, into LTC_4, LTD_4 and LTE_4 which make-up the mixture previously known as the slow-reacting substance of anaphylaxis (SRSA) (Wallace, 2002).

8.1.2.1 The pathophysiological role of leukotrienes

Borgeat and Samuelsson (1979) discovered LKs more than twenty years ago, but only in recent years has the inflammatory role of LKs been fully identified (Celotti and Durand, 2003).

LKs are powerful mediators of inflammatory and allergic responses through the modulation of the smooth muscle tone and vascular permeability. Activation of neutrophil migration and chemotaxis and stimulation of superoxide production and lysosomal enzyme secretion is brought on by LTB_4. Rheumatoid arthritis, psoriasis and inflammatory bowel disease have been associated with increased levels of this 5-LOX by-product (Tsuji *et al*., 1998). 5-LOX compounds induce the activation of leukocytes resulting in tissue damage and hyperplasia. The pathophysiological role of leukotriene molecules has been well defined in various inflammatory and allergic conditions including asthma and allergic rhinitis (Rioja *et al*., 2002). Knock-out animal studies revealed a definite contribution of 5-LOX and its products to the inflammatory response; mice deficient in 5-LOX exhibited a significant decrease in several inflammatory parameters (Celotti and Durand, 2003).

In the early 1980's, several global pharmaceutical companies realized that novel anti-inflammatory agents could be developed from the discovery of 5-LOX inhibitors or from particular leukotriene receptor antagonists (Young, 1999).

8.1.3 The role of cyclo-oxygenase enzyme

There are two isoforms of cyclo-oxygenase. Cyclo-oxygenase-1 (COX-1) is constitutively expressed in most mammalian tissues and produces prostanoids important in homeostatic functions such as gastric cytoprotection, maintenance of renal function, and platelet stabilization and activity. Cyclo-oxygenase-2 (COX-2) is an immediate early response gene product in inflammatory and immune cells and synthesizes high levels of prostaglandins (Rioja *et al*., 2002). COX-2 synthesizes series-2-prostaglandins (e.g. PGE_2, $PGF_{2\alpha}$) that contribute to pain, inflammation and swelling (Wallace, 2002). Previous research indicates important roles of COX-2 in several physiological processes such as wounds and ulcer healing, cardiovascular protection, regulation of bone resorption, renal function and female reproductive physiology (Celotti and Durand, 2003).

8.1.4 Current anti-inflammatory agents

The effectiveness of conventional non-steroidal anti-inflammatory drugs (NSAIDs) for the chronic treatment of inflammatory diseases is curtailed by the high occurrence of the serious side-effects of gastric ulceration, perforation or obstruction (Fries, 1996). The selective COX-2 inhibitors (e.g. Celebrex®, Vioxx®) were developed as anti-inflammatory agents in the hope of producing a new class of compounds with a reduced side effect profile. However, it has been found that cardiovascular concerns can emerge particularly if selective COX-2 inhibitors are used in patients with underlying heart diseases. Furthermore, there are potential kidney, liver or gastro-intestinal related complications due to selective COX-2 inhibition (Celotti and Durand, 2003). The above highlights the need for the development of safe anti-inflammatory agents.

8.1.5 Botanical anti-inflammatory agents

It has been reported that several botanical agents have the ability to modulate the inflammatory process. Plants with traditional use as anti-inflammatory agents include feverfew, *Tanacetum parthenium*; devil's claw, *Harpagophytum procumbens*; hops, *Humulus lupulus*; Asian ginseng, *Panax ginseng* and milk thistle, *Silybum marianum,* just

to name a few (Wallace, 2002). Pharmacological and pharmacognostic investigations have resulted in the development of many conventional anti-inflammatory agents such as salicylic acid which is a derivative of salicin obtained from the *Salix* and *Populus* subspecies (Trease and Evans, 1978).

The application of multiple natural agents offers considerable synergistic effects due to their different compounds providing multiple effects at varying points in the inflammatory cascade. In addition, several botanical agents can be selected and combined to provide both COX and LOX inhibition (Wallace, 2002).

Numerous essential oils with reported anti-inflammatory properties are used for the treatment of various disease states. Essential oils contain lipid soluble compounds which are able to permeate through the skin barrier and interfere with enzymatic pathways occurring in the epidermis and beyond (Baylac and Racine, 2003).

8.1.6 Anti-inflammatory agents within the realm of cancer therapy

Anti-inflammatory agents may have a potential therapeutic application as adjuncts to conventional cancer treatments such as chemotherapy, radiation and surgery. Emerging research is relating cancer initiation, promotion, progression, angiogenesis and metastasis to inflammatory effects. LOX by-products, the LTs and HETEs (LTB_4, 5-HETE and 12-HETE), have been implicated in tumour growth and advancement (McCarty, 2001). It has been proposed by limited research that LTs may exert a greater role than PGs in stimulating tumour growth, depending on the type of cancer (Wallace, 2002).

Previously presented evidence suggests that the inhibition of lipoxygenase and its by-products is a fundamental component in integrative cancer approaches (Wallace, 2002). Thus, the goal of integrative cancer therapies is not only to destroy the affected cells, but is also to incorporate molecular management of cancer physiopathology by employing a key strategy which appears to be inflammatory deregulation particularly modulated by dietary, nutritional and botanical agents.

Numerous phytomedicines are COX as well as LOX inhibitors and so decrease the generation of PGE_2, LTB_4, 5-HETE and 12-HETE and other inflammatory compounds,

which have demonstrated definite roles in cancer. The following botanical agents have demonstrated *in vitro* and/or *in vivo* anti-inflammatory actions and anticancer properties: boswellic acids (*Boswellia serrata*), bromelain (*Ananas comosus*), curcumin (*Curcuma longa*) and quercetin (Wallace, 2002).

8.2 Materials and methods

8.2.1 5-lipoxygenase (soybean) assay

The *in vitro* anti-inflammatory activity of the essential oils of seven *Pelargonium* species was assessed using the method of Sircar *et al*. (1983) as modified by Evans (1987).

8.2.1.1 Principle of the method

5-lipoxygenase catalyses the oxidation of unsaturated fatty acids containing 1-4 pentadiene structures. Within the body, arachidonic acid is the biological substrate of 5-LOX, but the enzyme also accepts linoleic acid, which was used in this assay. *In vitro*, 5-lipoxygenase oxidizes linoleic acid into a conjugated diene which absorbs at 234 nm. 5-LOX activity is evaluated by the spectrophotometric measurement of this by-product at 234 nm. The initial reaction rate was measured spectrophotometrically and the decrease of this initial reaction rate is a measure of the samples inhibitory activity (Baylac and Racine, 2003).

8.2.1.2 Protocol

The assay was carried out in phosphate buffer which is an aqueous medium and so a non-ionic surfactant Tween® 20 was employed to disperse the essential oil in the buffer. Linoleic acid, rather than arachidonic acid, was used due to its ease of handling and stronger affinity for the 5-lipoxygenase enzyme resulting in greater UV absorbance readings (Baylac and Racine, 2003).

Samples were dissolved in DMSO (Saarchem) and Tween® 20 (Merck) to produce a starting concentration of 100 µg/ml. The standard assay mixture was prepared in a 3 ml cuvette maintained at 25°C in a thermostated waterbath. The standard assay mixture contained 10 µl of plant extract solution, 2.95 ml of prewarmed (25°C) 0.1 M potassium phosphate buffer (pH 6.3) prepared with analytical grade reagents and 45 µl of 100 µM

linoleic acid (≥99%) from Fluka. For the negative control the same mixture was prepared as above but a blank consisting of DMSO and Tween® only (no plant extract) was used.

The enzymatic reaction was initiated by the addition of 100 units isolated 5-lipoxygenase (Cayman), diluted with equal volume 0.1 M potassium phosphate buffer (pH 6.3) maintained at 4°C. A single beam UV-VIS spectrophotometer (Analytikjena Specord 40) connected to a computer equipped with Winaspect® software recorded the increase in absorbance at 234 nm for 10 minutes at 37°C and so the enzyme activity was followed spectrophotometrically. When the enzyme was added to linoleic acid, stabilized with the buffer, the rate of the enzyme-catalysed conversion of the substrate to 9-hydroperoxy-10,12,15-octa-decatrienoic was maximal. The transformation of the unsaturation site of linoleic acid (from diene 1-4 to diene 1-3) caused the increase in absorption at 234 nm (Baylac and Racine, 2003).

For those test samples which displayed activity at 100 µg/ml, different dilutions were prepared using DMSO and Tween® 20. The initial reaction rate was determined from the slope of the straight line portion of the curve. The percentage inhibition of enzyme activity attributable to the sample was calculated by comparison with the negative control. Percentage enzyme activity was plotted against concentration of plant extract. Thereafter, the IC_{50} values (the concentration of essential oil which produced 50% inhibition) were determined from the dose-response curves using Enzfitter® version 1.05 software.

Nordihydroguaiaretic acid (NDGA, Cayman), a synthetic 5-LOX inhibitor, was used as a positive control (reference compound) in this assay. NDGA has been reported to exert strong inhibitory effects on the 5-LOX enzyme (Abad *et al*., 1995).

8.3 Results and discussion

The 5-LOX assay revealed that 5-lipoxygenase, the key enzyme involved in the biosynthesis of LTs, is a potential site of action for certain *Pelargonium* essential oils (Table 8.1). The oils of *P. citronellum* (NBG), *P. panduriforme* (SBG and WSBG), *P. quercifolium* (SBG and WSBG) and *P. scabrum* dose-dependently inhibited the activity of 5-LOX. As the concentration of oil increases, there is concomitant decrease in the initial reaction rate determined from the slope of the straight line portion of the curve. The two samples of *P. quercifolium* showed the greatest inhibition of 5-LOX enzyme activity (IC_{50} = 33.24 µg/ml - 38.67 µg/ml). *Pelargonium citronellum* (NBG), *P. panduriforme* (SBG and WSBG) and *P. scabrum* also displayed promising inhibitory activity (Figure 8.2). *Pelargonium graveolens* (SBG), *P. radens* (NBG) and *P. tomentosum* (SBG and WSBG) did not exert 5-LOX inhibitory activity (IC_{50} >100 µg/ml).

Table 8.1: The *in vitro* 5-LOX inhibitory activity of the *Pelargonium* essential oils.

Species (essential oil)	**Locality**	**5-LOX inhibitory activity IC_{50} (µg/ml)**	**Standard error**
P. citronellum	NBG	50.04	7.06
P. graveolens	SBG	>100	-
P. panduriforme	SBG	45.58	1.13
P. panduriforme	WSBG	45.39	9.13
P. quercifolium	SBG	33.24	7.66
P. quercifolium	WSBG	38.67	4.31
P. radens	NBG	>100	-
P. scabrum	SBG	54.68	5.29
P. tomentosum	SBG	>100	-
P. tomentosum	WSBG	>100	-
Control	-	5	

Where: - NBG = National Botanical Garden (Kirstenbosch), SBG = Stellenbosch Botanical Garden, WSBG = Walter Sisulu Botanical Garden (Johannesburg).

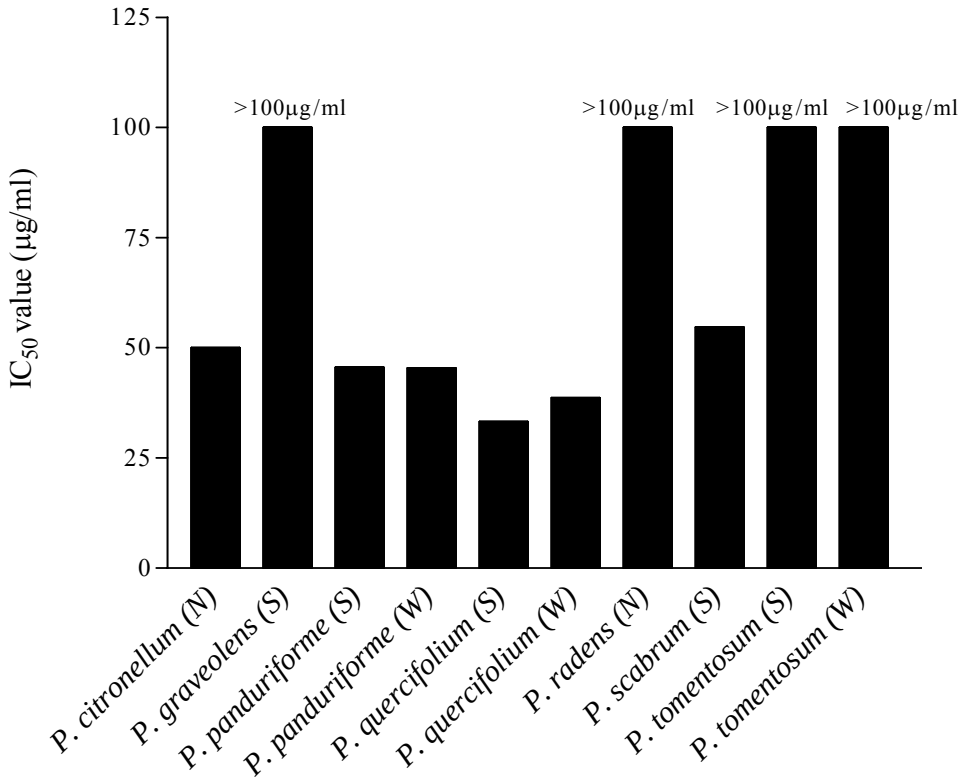


Figure 8.2: The IC_{50} values (µg/ml) in terms of 5-LOX inhibitory activity of the *Pelargonium* essential oils.

With reference to the dendrogram (Chapter 3, Figure 3.2), the essential oils exhibiting enzyme inhibition are found in different clusters, (A), (F) and (G). The two samples of *P. panduriforme* have the same anti-inflammatory potencies. This is expected since these oil samples are closely associated in the cluster dendrogram. The *P. quercifolium* samples exerted similar inhibitory effects; however, they have a moderately low degree of similarity in the dendrogram. The difference in the IC_{50} values of the duplicate samples of *P. panduriforme* and of that of *P. quercifolium* was assessed and evident from the dendrogram; the *P. quercifolium* samples were not as closely associated with one another as the *P. panduriforme* samples. *Pelargonium quercifolium* (WSBG) oil showed reasonably similar activity to that of the *P. panduriforme* samples and these species are grouped in the same cluster subgroup (F_A).

Pelargonium citronellum (NBG) and *P. scabrum* produced similar IC_{50} values; however, they are not chemically similar. *Pelargonium citronellum* (NBG) occurs in cluster (A) and *P. scabrum* is located in cluster (G). This indicates that oils with varying constituents can produce similar anti-inflammatory activities.

Pelargonium graveolens (SBG), *P. radens* (NBG), *P. tomentosum* (SBG) and *P. tomentosum* (WSBG) are all grouped into one cluster, namely subgroup (E_A) (Chapter 3, Figure 3.2). The chemical similarity shared by these oil samples was demonstrated by their results obtained in this assay. Isomenthone occurred in very high amounts (49.3-84.5%) in subgroup (E_A). This compound may exert antagonistic effects on any anti-inflammatory compounds which may be present in these oils, as in the other clusters containing the active species, *P. citronellum* (NBG), *P. panduriforme*, *P. quercifolium and P. scabrum*, isomenthone is either absent or present at very low levels.

One is inherently inclined to think that the major compounds are responsible for the biological activities displayed by a species. Several chemical compounds have been reported by Baylac and Racine (2003) to produce *in vitro* inhibition of the 5-LOX enzyme, some of these compounds are present at low levels in the active oils (Table 8.2). These compounds may act collectively in a synergistic manner and may contribute to the anti-inflammatory activity of *P. citronellum* (NBG), *P. panduriforme* (SBG and WSBG), *P. quercifolium* (SBG and WSBG) and *P. scabrum.*

Table 8.2: The percentage amounts of the compounds, with proven *in vitro* inhibitory action on the 5-LOX enzyme, present in the active oils.

Compound	% Amounts					
	cit NBG	**pan SBG**	**pan WSBG**	**que SBG**	**que WSBG**	**sca SBG**
Terpenic hydrocarbons						
γ-terpinene	-	0.1	0.3	0.3	tr	-
α-pinene	-	0.6	0.3	-	0.1	0.1
Sesquiterpenic hydrocarbons						
β-caryophyllene	0.6	-	0.2	0.1	-	3.9
germacrene D	-	-	tr	0.1	-	-
Sesquiterpenic alcohol						
(E)-nerolidol	tr	-	-	2.8	-	0.9
Esters						
citronellyl acetate	-	-	-	-	-	0.1
bornyl acetate	-	0.1	-	-	-	-

Where: - cit: *P. citronellum*; pan: *P panduriforme;* que: *P. quercifolium;* sca: *P. scabrum.* SBG = Stellenbosch Botanical Garden, NBG = National Botanical Garden (Kirstenbosch), WSBG = Walter Sisulu Botanical Garden; tr = trace: <0.05%.

In a study carried out by Baylac and Racine (2003) it was found that essential oils rich in citral could not be spectrophotometrically assessed due to the strong absorption of citral (neral and geranial) at 234 nm. This phenomenon was observed when the 5-LOX assay was conducted on *P. citronellum* (NBG) which contains 44.6% citral (17.4% neral and 27.2% geranial), the second most predominate compound present in the oil. *Pelargonium citronellum* (NBG) showed moderate inhibition of the enzyme (IC_{50} = 50.04 µg/ml) and as reported by Baylac and Racine (2003), essential oils rich in citral such as lemongrass and *Litsea cubeba* have acclaimed anti-inflammatory activity in aromatherapy.

‘Geranium oil’ (*P. graveolens*) is used in aromatherapy for its anti-inflammatory virtues (Lawless, 1995). In this assay, an IC_{50} value greater than 100 µg/ml was recorded for *P. graveolens* (SBG). This indicates that there is maybe another mode of action responsible for its reported anti-inflammatory activity. The numerous beneficial effects of ‘geranium oil’, which are accredited to ‘geranium oil’ regardless of its chemical composition, may be due to its action as an odour via the limbic system (Lis-Balchin *et al*., 1996a).

Ethnobotanical data reported that the vapours from boiled fresh leaves of *P. betulinum* were inhaled to treat coughs and other chest problems (van der Walt, 1977). *Pelargonium cucullatum* was also documented as a remedy for coughs and related-chest problems (May, 2000). The essential oils of *P. betulinum* and *P. cucullatum* may explain their traditional use. LTs play a key role in respiratory ailments such as asthma (Rioja *et al*., 2002). Unfortunately, insufficient essential oil was available for testing so as to determine whether their use in respiratory conditions is related to their anti-inflammatory effects mediated through the inhibition of the 5-LOX enzyme.

The results obtained from the *in vitro* 5-LOX assay suggest that certain *Pelargonium* essential oils may have a physiological role in modulating the 5-LOX pathway. The pathophysiological effects of LTs can be antagonized or avoided by targeting LT production through 5-LOX pathway inhibition (Prasad *et al*., 2004) and so *P. quercifolium*, *P. panduriforme*, *P. scabrum* and *P. citronellum* may have promising therapeutic applications in various inflammatory and allergic conditions. Further studies are required to elucidate the exact mechanism of inhibitory action of selected *Pelargonium* essential oils on the 5-LOX enzyme. The aforementioned *Pelargonium* essential oils may be useful ingredients in cosmetic products as the oils not only possess pleasant aromatic qualities but may offer skin soothing activity, through their inhibition of the 5-LOX enzyme (Baylac and Racine, 2003).

Investigations involving different combinations of selected *Pelargonium* essential oils should be carried out to assess whether synergistic or antagonistic effects are produced. The synergistic effects of the essential oils can be tested by combining the IC_{50} concentration of one essential oil with that of another essential oil. These mixtures of essential oils can be tested on 5-LOX as described above. The resultant effect can then be analysed by comparing the total percent 5-LOX inhibition, obtained from the sum of the

inhibition of the individual samples of essential oil, to the percent inhibition produced in the assay.

The benefit which certain *Pelargonium* essential oils can contribute to pharmaceutical anti-inflammatory agents should be assessed. Natural anti-inflammatory approaches offer a non-toxic and comprehensive strategy for controlling inflammatory eicosanoids. Development of these approaches, which control the responses that lead to tissue damage in the inflamed area, could provide an alternative to the conventional NSAID therapy for the modulation of various inflammatory diseases with reduced adverse gastric effects (Rioja *et al*., 2002).

Those *Pelargonium* oils with demonstrated inhibitory action on 5-LOX may have potential anticancer actions and so may have applications in integrative cancer therapies. Research directed in evaluating the effect that natural anti-inflammatory strategies exert in cancer patients is necessary (Wallace, 2002).

CHAPTER 9: ANTIMALARIAL ACTIVITY

9.1 Introduction

One of the greatest health burdens facing Africa is malaria. Malaria is one of the top three killers among communicable diseases as it has been estimated that at least 300 million people die from the disease each year (Clarkson *et al*., 2004).

9.1.1 The life cycle of the malaria parasite

Malaria is caused by parasitic protozoa of the genus *Plasmodium* and is transmitted from one human to another by the bite of infected female *Anopheles* mosquitoes (Figure 9.1). Following this, sporozoites are released into the bloodstream and move to the liver where they mature into schizonts. These rupture and release merozoites which enter the bloodstream and infect red blood cells (RBCs). The merozoites undergo asexual multiplication in the RBCs, which rupture within 48 to 72 hours infecting more RBCs. Some of these parasites may develop into gametocytes which can be ingested be a mosquito during a blood meal. In the mosquito's gut, the parasite undergoes another cycle resulting in sporozoites which migrate to the salivary glands, in preparation for the mosquito's next blood meal (William, 2004).

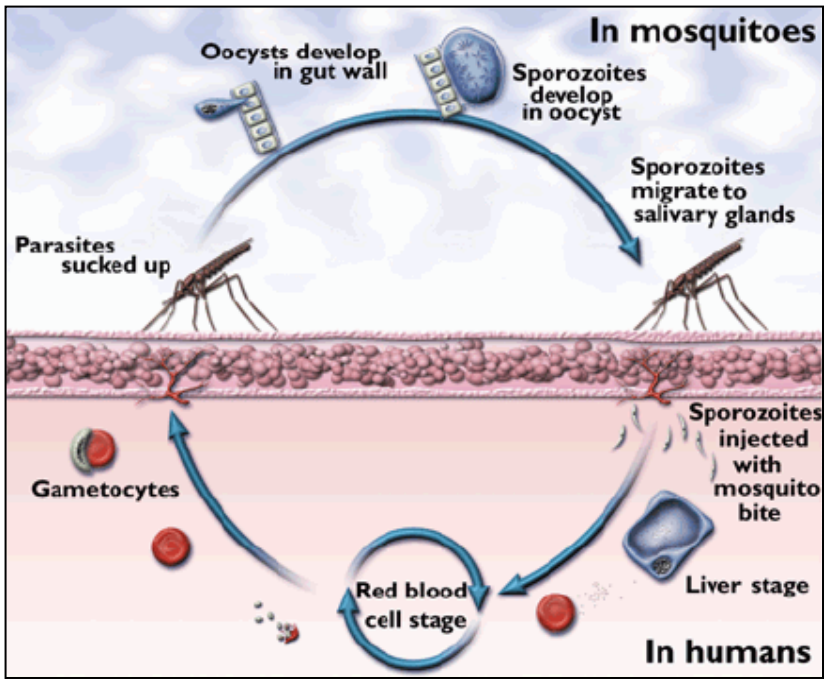


Figure 9.1: The life cycle of the malaria parasite (http://www.ncmls.kun.nl/molbio/mal.asp).

The first symptoms usually occur 10 days to 4 weeks after infection. The typical symptom of malaria is a violent fever lasting 6-8 hours, recurring every two or three days. As the disease progresses, anaemia and enlargement of the spleen develop (William, 2004).

9.1.2 Antimalarial chemotherapy

The first treatment used for malaria was found in South America. For centuries, the bark from the *Cinchona* tree has been used to treat fevers. It was found that the bark which contains the alkaloid quinine has antimalarial properties (William, 2004). *Cinchona pubescens* and several other species offered the only effective treatment for malaria, for more than 300 years (van Wyk *et al*., 2000b). Medicinal plants or structures modelled on plant lead molecules have been sources for the majority of antimalarial agents. Such agents include the quinoline-based antimalarial drugs, artemisinin and its derivatives (Clarkson *et al*., 2004).

In the last 30 years, few antimalarial drugs have been developed (Ridley, 2002). The increasing resistance of vectors to insecticides and the increasing prevalence of drug resistant strains of *Plasmodium falciparum* have caused an ongoing recrudescence of malaria. These abovementioned developments, the difficulty of developing efficient vaccines and the fact that the control and treatment of malaria depends on relatively few chemoprophylactic and chemotherapeutic drugs emphasize the necessity for novel, affordable antimalarial compounds (Clarkson *et al*., 2004; Zirihi *et al*., 2005). The search for new antimalarial drugs from traditional remedies has been prompted by the isolation of artemisinin, from Chinese traditional medicine, which has a different chemical structure and mechanism of action to that of quinine (Qinghaosu Antimalarial Coordinating Research Group, 1979). Research is now being focused on obtaining drugs with different structural features, since present antimalarial agents, due to parasites developing resistance to them, have become ineffective (Peters, 1998).

Within the South African traditional healthcare system, treatments of various illnesses including malaria and its associated symptoms are mainly based on the use of herbal plant remedies (Watt and Breyer-Brandwijk, 1962). It is essential to obtain more scientific information with regards to the efficacy and safety of the herbal medicines in use. The validation of traditional practices could lead to innovative strategies in malaria control. A

range of medicinal plants, from other parts of Africa, have been tested for antiplasmodial activity and have shown potential as effective Western medicines. Many plants related to these tested plants occur in South Africa and not much is known about their antiplasmodial activity (Prozesky *et al*., 2001).

9.1.3 Insect deterrent property of *Pelargonium* species

Insect repellents protect humans and animals from haematophagus insects e.g. mosquitoes, ticks, fleas, blackflies and cockroaches (Ahmad *et al*., 1995). By reducing the contact between man and insect vectors, repellents hinder the transmission of diseases. The avoidance movements of insects are caused by the action of repellents on the olfactory, chemoreceptor or gustatory senses of insects (Jeyabalan *et al*., 2003).

The essential oils of some *Pelargonium* species are employed by the native population in southern Africa due to their effective insect repellent properties (Kolodziej, 2002). In particular, the essential oils of *P. graveolens*, *P. capitatum*, *P. odoratissimum* and *P. radula* (Cav.) L'Hérit. (syn. *P. radens* H.E. Moore) used in perfumes can be used to deter insects (Simmonds, 2002). The repellent properties of certain essential oil compounds such as α-pinene, limonene, carvone and β-myrcene explain the reported insect deterrent properties of certain *Pelargonium* species (Kolodziej, 2002). The strong smell of 'geranium oil' acts as a repellent to mosquitoes (Lawless, 1995). The insecticidal activity of 'geranium oil' and some of its compounds (citronellol and geraniol) has been well researched (Wirtz and Turrentine, 1980; Dale and Saradamma, 1982). Due to insufficient quantities of the *Pelargonium* oil samples in this study, testing could not be carried out to assess if the oils have, in particular, an inhibitory effect on the malaria protozoa.

Very little information exists with regard to the effect of 'geranium'-derived compounds on non-'geranium' feeding insects. However, existing information indicates that active molecules are present in Pelargoniums. The herbal medicine of "Umckaloabo" originating from *P. sidoides* and *P. reniforme* has shown activity against the intracellular protozoan *Leishmania donovani* (Kolodziej, 2002). Jeyabalan *et al*. (2003) studied the effects of *P. citrosa* leaf extracts on *Anopheles stephensi*. The results of this study showed that *P. citrosa* leaf extract has potent mosquitocidal, repellent and growth regulatory and ovicidal activity (Jeyabalan *et al*., 2003). *Pelargonium citrosa* leaf extracts may provide a natural

alternative for malaria vector control (Jeyabalan *et al*., 2003). In order to minimize human contact with vector and nuisance mosquitoes, the use of protection measures i.e. the application of repellents to exposed skin have long been supported (Gupta and Rutledge, 1994). *Pelargonium citrosa* leaf extract could be employed in a similar capacity.

The pressing need for new antimalarial compounds substantiates the investigation of *Pelargonium* species for possible antimalarial activity.

9.2 *In vitro* antiplasmodial activity

The *in vitro* semi-automated microdilution assay technique which measures the ability of the extracts to inhibit the incorporation of [^{3}H]-hypoxanthine into the malaria parasite was used (Desjardins *et al*., 1972; van Zyl and Viljoen, 2002). Acetone extracts were tested against the choloroquine-resistant Gambian FCR-3 strain of *Plasmodium falciparum.*

9.3 Materials and methods

9.3.1 Principle of the method

Nucleotides are activated precursors of the nucleic acids DNA and RNA and are intermediates in biosynthetic pathways. Nucleotides also play a role in many biochemical processes occurring in the mammalian host and in the parasite, particularly those pathways involving energy transfer and biosynthesis. Intra-erythrocytic-stage plasmodia are unable to synthesize purines *de novo* (Gero and O'Sullivan, 1990). The parasite relies on host-derived hypoxanthine as a source of purine precursors for nucleic acid synthesis and energy metabolism (Olliaro and Yuthavong, 1999). Hence, parasitic growth was estimated by the *in vitro* parasitic uptake of radioactive [^{3}H]-hypoxanthine.

9.3.2 Protocol

9.3.2.1 Cell culture

The parasite culture was maintained in 25 ml of culture medium consisting of 10.4 g/L RPMI-1640 with L-glutamine, 5.9 g/L HEPES buffer (N-2-hydroxyethyl-piperazine-N′-2-ethane-sulfonic acid), 4.0 g/L D-glucose, 44 mg/L hypoxanthine and 50 mg/ml gentamicin sulphate dissolved in 1 L autoclaved milliQ water (Millipore®) and filtered through a

Sterivex-GS 0.22 µm filter unit. The culture medium was further supplemented with 10 ml 10% (v/v) human plasma and 4.2 ml 5% (w/v) $NaHCO_3$. Every alternate day, human erythrocytes were added to the culture. The human plasma (South African Blood Transfusion Services) used in the assay was heat inactivated at 56°C in the water bath for 2 hours, centrifuged at 3000 rpm for 20 minutes and aliquoted into sterile 50 ml tubes and stored at 20°C until required. The donated blood was collected in citrate phosphate dextrose adenosine-1-containing tubes to prevent coagulation and stored at 4°C for a maximum of two weeks. Before use, the whole blood was washed three times in PBS (phosphate buffer solution, pH 7.4) by centrifuging it at 2000 rpm for 5 minutes to remove the plasma and other blood cells. The PBS solution contained 8.0 g NaCl, 0.3 g KCl, 0.73 g $Na_2HPO_4.2H_2O$ and 0.2 g KH_2PO_4 dissolved in 1 L sterile water and the solution was autoclaved at 120°C for 20 minutes and stored at 4°C. Following the washing step, the erythrocytes were resuspended in experimental medium to avoid dehydration and stored at 4°C. The experimental medium was prepared in the same way as the culture medium; however, it did not contain gentamicin sulphate and hypoxanthine. Cultures were gassed with 3% O_2, 5% CO_2 and 92% N_2 for approximately 30 seconds before being incubated at 37°C.

9.3.2.2 Assessment of parasite growth

The parasitaemia and morphology of the parasites were assessed daily to evaluate parasitic growth. A thin smear was prepared in the following way: a small amount of blood, from the bottom of the culture flask, was placed on and smeared across a clean slide. Methanol was used to fix the smear after which it was dried and placed in Giemsa stain diluted with Giemsa buffer (1: 10) for 20 minutes. The Giemsa buffer solution contained 3.5g KH_2PO_4 and 14.5 g $Na_2HPO_4.12H_2O$ dissolved in 1 L of distilled water and autoclaved at 120ºC for 20 minutes. The slide was washed with water and once dry, the parasitic morphology was examined microscopically using the oil immersion objective (1000x) and the percentage parasitaemia was calculated using the following equation:

$$\% \text{ Parasitaemia} = \frac{\text{Number of infected cells x 100}}{\text{Total cells (infected + uninfected cells)}}$$

5% (w/v) D-sorbitol was used to synchronize the cultures every second day when the parasites were mostly in the ring stage (Lambros and Vanderberg, 1979). The assay was conducted when the parasitaemia was between 2 to 3%.

9.3.2.3 Hypoxanthine incorporation assay

Each extract was prepared in DMSO to obtain a stock concentration of 10 mg/ml which was serially diluted with experimental medium. The following dilutions were made: 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.1 mg/ml, 0.01 mg/ml, 0.001 mg/ml and 0.0001 mg/ml. For the drug controls, chloroquine and quinine, various concentrations (10 µM to 0.00001 µM) were prepared using experimental medium. The various concentrations of plant extracts (25 µl) were plated out in triplicate in a 96-well plate. The synchronous parasite suspension (200 µl), predominately in the ring stage, was adjusted to a 0.5% parasitaemia and 1% final haematocrit and added to the wells containing the various concentrations of plant extract. Eight wells of 200 µl untreated parasite and four wells of uninfected red blood cells were used to obtain control values. Plates were maintained for 24 h in a candle jar under micro-aerobic (3% O_2, 5% CO_2, 92% N_2) and humidified conditions at 37°C.

Labelled [^{3}H]-hypoxanthine (5 mCi, Amersham Pharmacia Biotech, UK) stabilized in ethanol: water (1: 1 v/v) was adjusted to 18 µCi with experimental medium and 25 µl of this was then added to each well and parasites were maintained for a further 24 hours under humidified conditions at 37°C. The labelled parasitic DNA was harvested onto a glass-fibre GFB-filtermat by a Titertrek® cell harvester. Once the filtermats were dry, liquid beta scintillation fluid was added and the incorporated [^{3}H]-hypoxanthine radio-active counts determined. The radioactivity incorporated into the treated parasites was compared with that in the control parasites maintained on the same plate in order to determine the growth inhibition for each extract concentration. The percentages of parasite growth, calculated using equation (1) below, were plotted against their respective concentrations. The concentrations causing 50% inhibition of parasite growth (IC_{50}) were calculated from the log sigmoid dose-response curves generated by Enzfitter® software. Chloroquine and quinine, antimalarial agents, were used as positive controls.

$$\%\ \text{Parasite growth} = \frac{(\text{Test sample})\,\text{cpm} - (\text{Mean RBC control})\,\text{cpm}}{(\text{Mean parasite control})\,\text{cpm} - (\text{Mean RBC control})\,\text{cpm}} \times 100 \qquad (1)$$

Where: - cpm = counts per minute; RBC = red blood cells.

9.4 Results and discussion

All the acetone extracts displayed considerable antimalarial activity, with IC_{50} values ranging from 1.34 ± 0.29 µg/ml to 22.46 ± 3.21 µg/ml (Table 9.1). Most of the IC_{50} values obtained fall below 15.00 µg/ml (Figure 9.2). *Pelargonium panduriforme* (SBG) showed the greatest activity with an IC_{50} value of 1.34 ± 0.29 µg/ml which is 22 and 45 times greater than the IC_{50} values of chloroquine and quinine, respectively (Figure 9.3). Other species possessing similarly potent antimalarial activity include *P. citronellum* (NBG), *P. citronellum* (SBG), *P. quercifolium* (SBG) and *P. radens. Pelargonium cucullatum* (SBG) and *P. pseudoglutinosum* produced very similar activities (Figure 9.2). *Pelargonium crispum* and *P. graveolens* (SBG) extracts were found to exert the lowest activity (IC_{50} = 21.00 ± 4.33 µg/ml and 22.46 ± 3.21 µg/ml, respectively). *Pelargonium crispum* and *P. graveolens* (SBG) are 350 and 374 fold less active than the chloroquine control, respectively and are 700 and 749 fold less active than the quinine control, respectively.

Table 9.1: The *in vitro* antimalarial activity of selected *Pelargonium* acetone extracts against the *P. falciparum* chloroquine-resistant Gambian FCR-3 strain.

Species	**Locality**	**Antimalarial activity IC_{50} (µg/ml)**	**s.d.**
P. betulinum	NBG	16.23	2.75
P. capitatum	Strand	9.81	0.34
P. citronellum	NBG	1.58	0.65
P. citronellum	SBG	1.74	0.79
P. cordifolium	NBG	10.84	0.75
P. cordifolium	WSBG	10.17	2.43
P. crispum	NBG	21.00	4.33
P. cucullatum	SBG	7.42	0.35
P. cucullatum	WSBG	nd	-
P. glutinosum	SBG	11.07	1.15
P. glutinosum	WSBG	nd	-
P. graveolens	SBG	22.46	3.21
P. graveolens	WSBG	9.48	1.17
P. greytonense	SBG	13.21	1.89
P. hermanniifolium	SBG	13.54	3.95
P. hispidum	SBG	12.23	1.93
P. panduriforme	SBG	1.34	0.29
P. panduriforme	WSBG	nd	-
P. papilionaceum	NBG	5.15	0.36
P. pseudoglutinosum	NBG	7.93	0.75
P. quercifolium	SBG	2.66	0.36
P. quercifolium	WSBG	nd	-
P. radens	SBG	1.90	0.39
P. scabroide	SBG	15.94	0.16
P. scabrum	SBG	16.10	0.02
P. sublignosum	SBG	9.01	1.22
P. tomentosum	SBG	17.62	4.84
P. tomentosum	WSBG	13.83	0.61
P. vitifolium	SBG	9.18	1.61
Chloroquine	-	0.06	0.003
Quinine	-	0.03	0.002

Where: - NBG = National Botanical Garden (Kirstenbosch), S = Stellenbosch Botanical Garden, Strand = Strand region, WSBG = Walter Sisulu Botanical Garden (Johannesburg); s.d. = standard deviation; nd = not determined due to insufficient extract for testing; n = 3: triplicate measurements were performed for all samples.

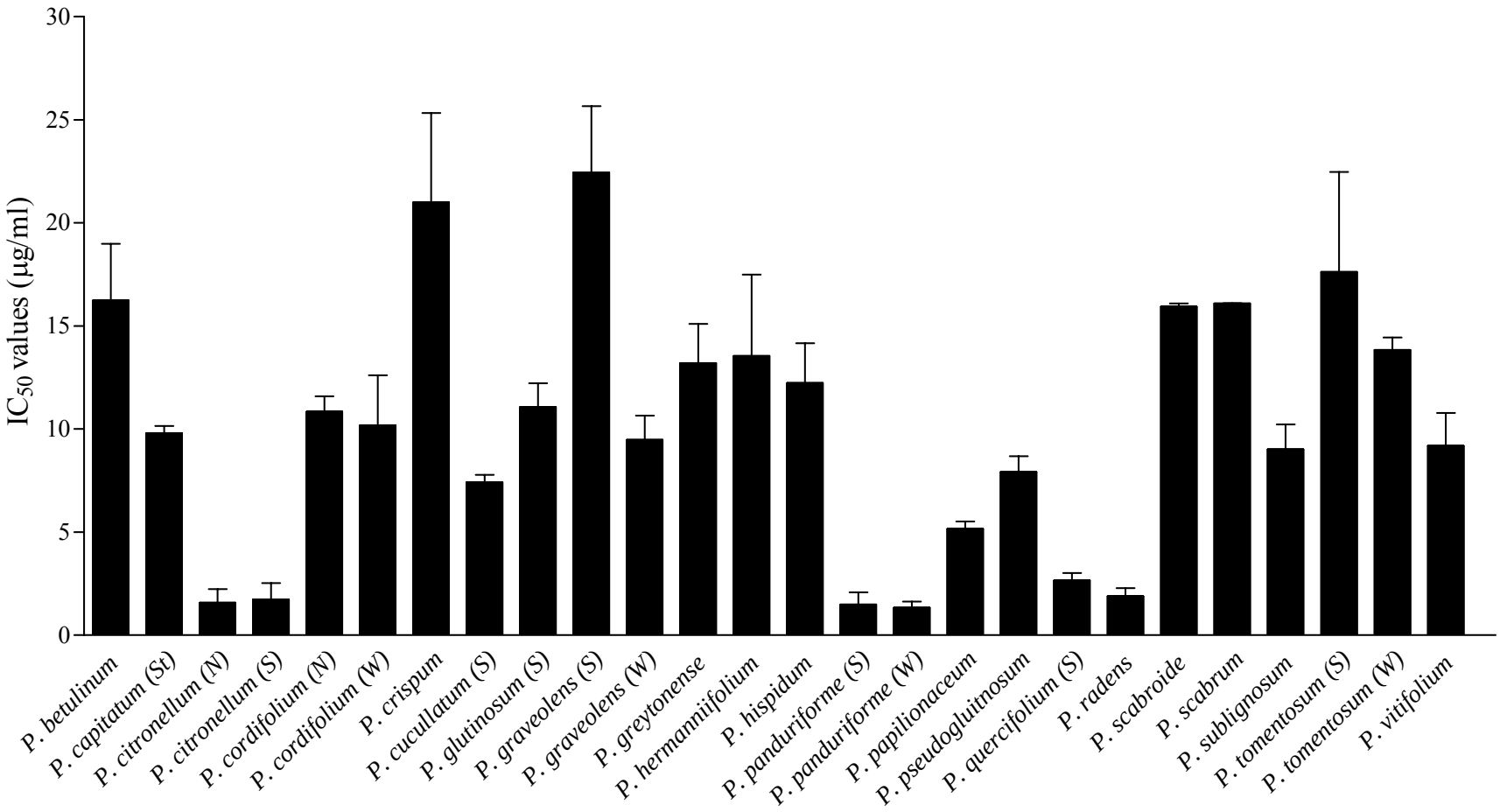


Figure 9.2: The *in vitro* antimalarial activity of selected *Pelargonium* acetone extracts. Where: - (N) = National Botanical Garden (Kirstenbosch), (S) = Stellenbosch Botanical Garden, (St) = Strand region, (W) = Walter Sisulu Botanical Garden (Johannesburg).

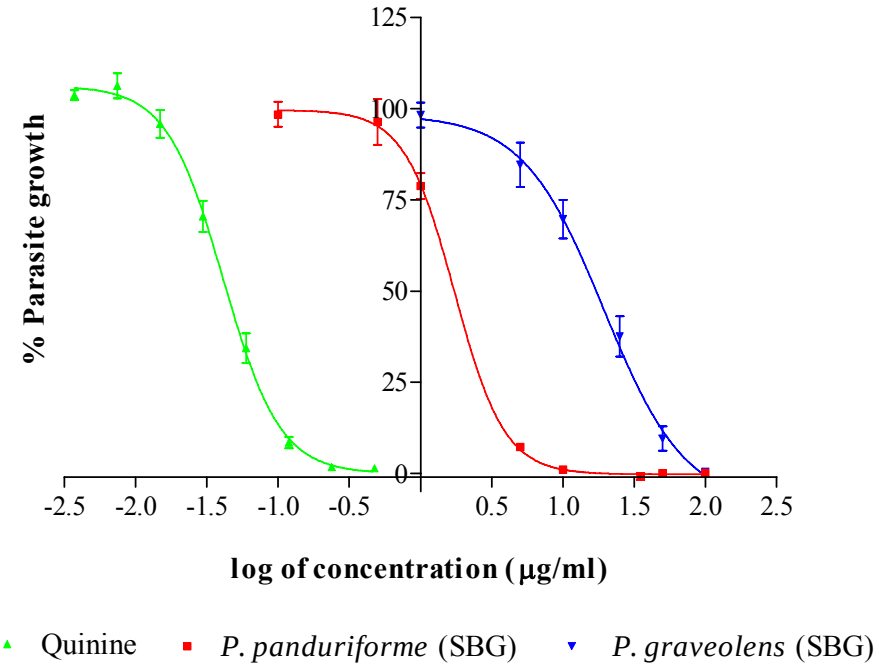


Figure 9.3: Dose-response curves for *P. graveolens* (SBG), *P. panduriforme* (SBG) and quinine.

Substantial evidence supports the fact that hypoxanthine, formed by the host during ATP catabolism, is the main source of purines for *P. falciparum*. The antimalarial activity of the extracts could be due to their inhibitory effects on the parasite-specific enzymes utilized in purine salvage. Hypoxanthine salvage is a potential target for new drug discovery (Olliaro and Yuthavong, 1999). *Pelargonium* species may exert their antimalarial activities through other mechanisms which may involve the principle antimalarial chemotherapeutic targets (Figure 9.4) which have been classified into three categories (Olliaro and Yuthavong, 1999):

1) Targets responsible for processes occurring in the *Plasmodium* digestive vacuole; these mainly include haemoglobin digestion and haem detoxification. The membrane of the digestive vacuole appears to be involved in drug accumulation and extrusion.

2) Enzymes involved in macromolecular and metabolite synthesis. This includes nucleic acid metabolism, phospholipids metabolism, glycolysis and tubulin assembly.

3) Targets responsible for membrane processes (including trafficking and drug transport) and signalling.

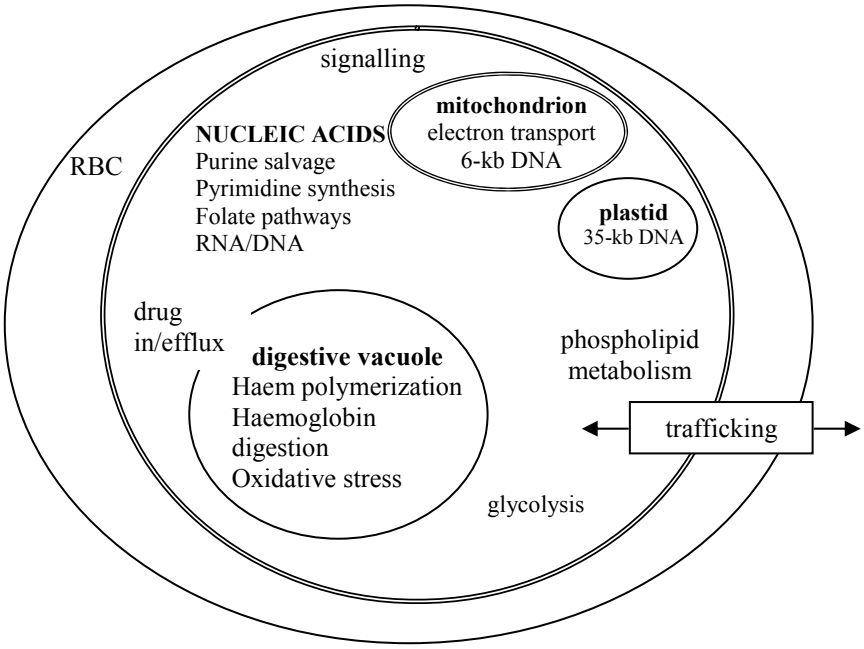


Figure 9.4: The principal chemotherapeutic targets in *Plasmodium protozoa* (Olliaro and Yuthavong, 1999).

Bidens pilosa L. (Asteraceae) is among the numerous plants used in Brazil to treat malaria. An investigation showed that the *in vivo* activity of the ethanol roots extract against *P. falciparum* depends on the presence of polyacetylene and flavonoids (Oliveira *et al*., 2004). The antimalarial properties of the *Pelargonium* extracts may be attributable to the various flavonoid derivatives detected in the extracts (Chapter 4). The major flavonoids identified in each species should be isolated and tested for antimalarial activity.

The samples of the same species collected from different localities produced very similar antimalarial activities. However, the *P. graveolens* samples produced different IC_{50} values; *P. graveolens* (SBG) is about two fold less active than *P. graveolens* (WSBG).

It was concluded from Chapter 4 that *P. betulinum* (NBG), *P. cordifolium* (WSBG), *P. crispum* (NBG) and *P. cucullatum* (SBG) and *P. scabrum* (SBG) acetone extracts exhibit several similarities in their HPLC profiles (Chapter 4, Figure 4.6). The aforementioned species each contain a dominant compound (Chapter 4, peak 2 in Figure 4.6) at retention time of approximately 13.95 minutes with similar λ_{max} values (220.3 nm and 278.0 nm).

Pelargonium betulinum (16.23 ± 2.75 µg/ml) and *P. scabrum* (16.10 ± 0.02 µg/ml) exert almost identical antimalarial activities. *Pelargonium cucullatum* (SBG) and *P. cordifolium* (WSBG) share similar IC_{50} values of 7.42 ± 0.35 µg/ml and 10.17 ± 2.43 µg/ml, respectively.

A flavone occurs as the main compound in *P. hispidum* (SBG), *P. panduriforme* (SBG), *P. papilionaceum* (NBG) and *P. tomentosum* (SBG) at a retention time of about 21.35 minutes with UV absorbance maxima occurring at approximately 255.7 nm and 346.0 nm (Chapter 4, peak 2 in Figure 4.8). *Pelargonium hispidum* (SBG) and *P. tomentosum* (SBG) exhibited similar activities (IC_{50} = 12.23 ± 1.93 µg/ml and 17.62 ± 4.84 µg/ml, respectively). However, of these species, the IC_{50} values of *P. panduriforme* (SBG) and *P. papilionaceum* were found to be most similar (IC_{50} = 1.34 ± 0.29 µg/ml and 5.15 ± 0.36 µg/ml, respectively).

The similar flavonoid patterns of *P. capitatum* (Strand) and *P. vitifolium* (SBG) (Chapter 4) provide chemical evidence that these two species are related (van der Walt, 1977). It was found that *P. capitatum* (Strand) and *P. vitifolium* (SBG) have very similar antimalarial activities. *Pelargonium sublignosum*, with a similar flavonoid pattern to *P. scabrum*, produced an IC_{50} value almost 2-fold greater than that of the latter species. *Pelargonium glutinosum* (SBG) has a substantial amount of a compound similar to the major compound present in *P. pseudoglutinosum* (Chapter 4). These species produced similar IC_{50} values of 11.07 ± 1.15 µg/ml and 7.93 ± 0.75 µg/ml, respectively. *Pelargonium papilionaceum* and *P. vitifolium,* with different chemical compositions (Chapter 4), exerted similar antimalarial activities (Table 9.1).

Pelargonium citronellum (NBG), *P. citronellum* (SBG), *P. panduriforme* (SBG), *P. quercifolium* (SBG) and *P. radens* (SBG) extracts have the potential to be employed as effective plant-based medicines for the treatment of malaria. The antimalarial activity of the crude extracts may be due to their general cytotoxicity effects. For the therapeutic potential of these extracts to be considered, they must then have greater selectivity for the *Plasmodium* parasite than for human cells. In order to obtain a better insight into the possible mode of antimalarial action of the *Pelargonium* extracts, the MTT assay was carried out on the crude extracts. The toxicity data obtained is presented in Chapter 10.

CHAPTER 10: TOXICITY

10.1 Introduction

From the earliest of times, plants have been used to cure sickness and alleviate suffering. However, our ancestors discovered that some plants have the ability to produce various ailments such as skin irritation and that some can even result in death.

10.1.1 History of toxicology

Toxicology is derived from "tox" meaning 'bow' or 'arrow'. Poisoned weapons have been and are still being used for the procurement of food, for the protection against wild animals and in tribal warfare. Through mans search for remedies he discovered toxic substances which when applied to the point of a weapon were able to elicit desirable effects e.g. curare. As man attempted to make his weapons more lethal to his enemies and prey, he experimented with plant juices and in so doing, discovered that the names given to plants and herbs correspond to the toxic effects they exert on animal life. For example, cowbane (water hemlock) has a lethal effect on cattle. Highly toxic plant principles were combined to produce an effective hunting poison. Plants are the main providers of active ingredients for African poisons (Neuwinger, 1996).

All herbalists, during the medieval and Renaissance European era wrote about toxic plants. Centuries ago, poisoning was considered a fine art and to be poisoned was as ordinary as becoming ill with a disease. The members of primitive tribes, in order to exert power over their enemies, kept to themselves their acquired knowledge of plants, in particular of those plants which caused stupor, delirium and death (Thompson, 1931). We owe much of our attained knowledge of the properties and uses of many vegetable poisons by primitive races to archaeological research. However, little or no information regarding other botanical substances has been obtained. Tribes guard their knowledge of poisons which is only known by certain chiefs or by the tribal medicine men that pass on the knowledge to their successors. Poisonous plants form an integral part of South Africa's flora (van Wyk *et al*., 2002a). Our knowledge of the active principles of plants indigenous to South Africa is limited. A great deal of knowledge of the plants employed by man for their toxic effects on animals and humans remains to be discovered.

10.1.2 Plant toxins

Plants are factories and store-houses of a variety of chemicals with more than 200 known plant-derived amino acids compared to only 30 in mammals. There are probably thousands of sensitizing chemicals in plants (Mitchell and Rook, 1979). Toxic substances present in a large number of plants worldwide can either cause skin irritation or when ingested in sufficient quantities over a short or a prolonged period of time can exert detrimental effects on the systems of animals or man and can even result in death.

Plant toxins have no role in the growing or fruiting process. Three main hypotheses have been put forward to explain the physiological significance of the toxic principles present in plants. One or more of the following may be accountable for the production of plant toxins. Toxic substances are (a) products of catabolism (b) a protection to deter people and animals from eating the plants (c) intermediates in the anabolic processes in plants (Steyn, 1934). One should consider why some plants and not others protect themselves from being eaten. If this were the case then those plants most eaten by stock would most likely produce poison. In addition, plants would have concentrated poisons in the parts growing above ground in order to protect themselves. However, many poisonous plants, concentrate a large portion in their roots or bulbs. The hypothesis that these substances are excretory products is supported by the fact that they are concentrated in the bark. The hypothesis that these substances are normal products of the metabolic process is supported by the fact that poisons disappear from certain plants when they are grown in darkness (Steyn, 1934).

10.1.3 Toxic components as therapeutics

Most poisonous plants have found a function within traditional and modern medicine. The dangerous effects that certain plants exert on bodily functions are what make those plants valuable therapeutics. Arrow poisons have provided a tool in research for the unearthing of effective therapeutics. For example, ouabain and *k*-strophanthin are used for acute cardiac insufficiency, physostigmine is used for the treatment of glaucoma and myasthenia gravis, *d*-tubocurarine is used in anaesthesia for its muscle-relaxant properties, reserpine is used as an antihypertensive drug and ajmaline for disturbances of cardiac rhythm (Neuwinger, 1996).

Ergot is an unusual form of the fungus *Claviceps purpurea* which grows in the ovaries of certain varieties of grasses, but is today mainly found in rye. The consumption of rye contaminated with ergot was recognized at the end of the 18th century to be the cause of a dreadful disease which is known today as ergotism. The symptoms of this disease include dementia, prolonged vasospasm which may result in gangrene and stimulation of the uterine smooth muscle which in pregnancy may result in abortion. Today, ergot is utilized in medicines for migraines (dihydroergotamine), hyperprolactinaemia (bromocriptine) and postpartum haemorrhage (ergonovine maleate) (Katzung, 2001). Through scientific research, what once caused terrible agony and death through its toxic properties has now been employed into a medicinal agent of great worth.

The tobacco plant (*Nicotiana tabacum*) yields nicotine, one of the most poisonous substances, yet it is used daily by millions of people worldwide for its pleasurable stimulant effects.

10.1.4 Traditional medicines

The most fundamental aspect of health care is to do no harm (Oath of Maimonides); to assure that what is taken by humans for medicinal purposes is safe (Cordell and Colvard, 2005). It is considered by many users of traditional medicines that such medicines are safe for human consumption. This assumption is partly based on widespread prior field experience (Cordell and Colvard, 2005). Due to the amazing floral and cultural diversity in South Africa, a large number of plant species are used for their medicinal properties (Light *et al*., 2005). However, few plants have been scientifically studied for their efficacy and safety (Calixto, 2005). The number of phytotherapeutic products from various traditional medicine systems has considerably increased and these products are entering the commercial market in developed and developing countries. Due to the lack of clear and agreeable quality control and marketing regulations, the issues of safety and efficacy are being both understated and ignored (Cordell and Colvard, 2005).

When biological activity is identified, through the screening of a plant, such activity is classified according to potency; the 'most active' is usually selected for isolation. However, often the therapeutic window between effectiveness and toxicity (Figure 10.1) is too narrow and so the compound falls short of expected therapeutic values at the stage of

toxicity trials. In animal studies, the therapeutic index of a drug is used to estimate the potential benefit of the drug in humans (Katzung, 2001).

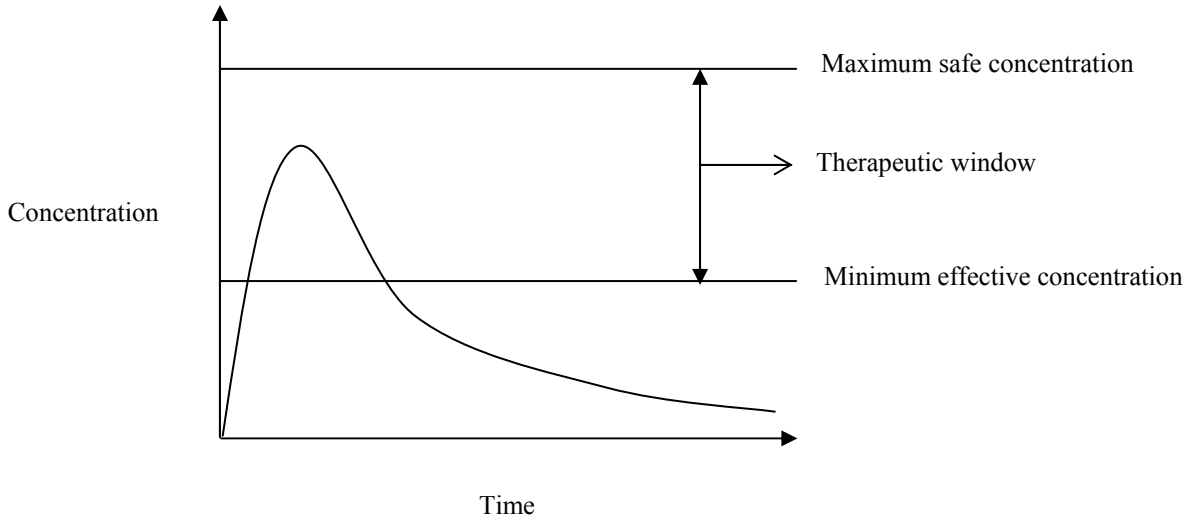


Figure 10.1: The therapeutic window of a plasma concentration-time curve following a single extravascular dose of a drug (modified from Aulton, 1999).

Medicinal plants, like orthodox medicines, have to be biologically active to be efficacious, it follows then that overdosing leads to the possibility of poisoning (van Wyk *et al*., 2002a). "Only the dose determines that a thing is not a poison", states the Paracelsian dictum (Neuwinger, 1996). Steenkamp *et al*. (2000) showed that traditional medicines containing pyrrolizidine alkaloids can cause poisoning and fatality. Several of the deaths occurred in children who were given the same dosage as adults. This information is vital and needs to be channelled to traditional healers. Researchers need to sideline the pursuit of new drug leads and focus on comprehensive toxicity studies of traditional medicinal plants (Jäger, 2005).

10.1.5 Toxicity of *Pelargonium* species

There is no published literature regarding the toxicity of *Pelargonium* extracts (Lis-Balchin and Deans, 1996). However, there have been several reports of dermatitis due to *Pelargonium*-derived 'geranium oil'.

Keil (1947) reported that geraniol, a compound found in 'geranium oil', causes sensitization. 'Oil of geranium' has been reported to cause contact dermatitis and cheilitis

(Klarmann, 1958). Cosmetic preparations containing 'geranium oil' may cause dermatitis in hypersensitive individuals (Mitchell and Rook, 1979). The species of this genus have been reported to cause dermatitis; one case of dermatitis from picking scented pelargonium leaves has been reported (Lis-Balchin and Deans, 1996). According to Agrup (1969) patch testing with the leaf of *Pelargonium* species can cause irritant reactions and reactions registered as allergic should be accepted with caution. In a study conducted by Hjorth (1968), four out of 52 patients tested showed a positive reaction. However, in another study using 400 people, none of the persons tested, showed a positive reaction (Fregert and Hjorth, 1968). The reports of dermatitis from species of this genus are lacking in adequate detail to affirm an allergenic effect (Mitchell and Rook, 1979).

Ethnobotanical information indicates that *P. betulinum* and *P. cucullatum* were used to relieve coughs and other chest problems, in preparations for wound-healing and for the relief of gastro-intestinal related problems (van der Walt, 1977; May, 2000; Lawrence and Notten, 2001). The leaves of *P. papilionaceum* were smoked for their medicinal properties (Watt and Breyer-Brandwijk, 1962). The implied methods of administration include inhalation, local superficial application to the skin and ingestion of the preparations. A plant maybe harmless when absorbed through the skin and lungs but harmful when ingested (van Wyk *et al*., 2002a). Most traditional remedies are used as teas or as alcoholic essences (Lis-Balchin and Deans, 1996). Thus it is important to perform toxicity studies on the volatile and non-volatile *Pelargonium* extracts as such studies will provide an indication of the possible toxic effects these extracts will exert if used internally.

The results obtained from preceding Chapters revealed that *Pelargonium* crude extracts have potentially important biological properties related to antimicrobial (Chapter 5), free radical scavenging (Chapter 7) and antimalarial (Chapter 9) effects and that certain *Pelargonium* essential oils have promising anti-inflammatory activity (Chapter 8). Hence, Pelargoniums are potentially valuable therapeutic agents. The toxicity profiles of selected *Pelargonium* species were determined in order to appraise their potential for medicinal use. Furthermore, an attempt has been made to relate the antimicrobial (Chapter 5) and antiplasmodial (Chapter 9) activities of the *Pelargonium* acetone extracts to their cytotoxicity.

10.2 Materials and methods

10.2.1 Microculture tetrazolium assay

Pelargonium acetone extracts and essential oils were assessed for *in vitro* cytotoxicity using microculture tetrazolium (MTT) colourimetry as described by Mosmann (1983). Transformed human kidney epithelium (Graham) cells were used in the assay.

10.2.2 Principle of the method

A mitochondrial enzyme, succinate-dehydrogenase (SDH), present in living cells cleaves the tetrazolium ring of the yellow tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (MTT) and reduces it to an insoluble purple formazan reaction product (Figure 10.2) (Mosmann, 1983). When solubilized, the formazan product can be measured spectrophotometrically. The absorbance recorded is directly proportional to the viable cell number per well which inturn is directly proportional to the amount of formazan produced (Alley *et al*., 1988).

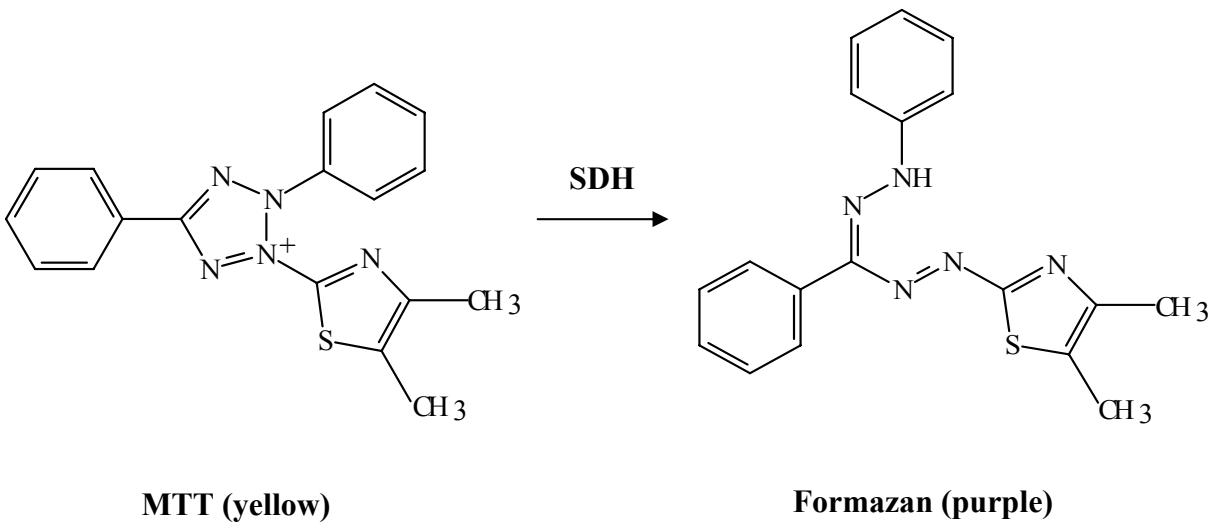


Figure 10.2: The structure of MTT and the formazan reaction product.

10.2.3 Protocol

Culture medium consisting of HAM F10 solution, 5% (v/v) foetal calf serum (FCS) and 0.5 mg/ml gentamicin sulphate was used to maintain transformed human kidney epithelium (Graham) cells. The HAM F10 solution consisted of 9.38 g of HAM F10 medium (Highveld Biological, South Africa) and 1.18 g of $NaHCO_3$ in a total volume of 1 L of sterile water. The FCS was inactivated at 56ºC for 2 hours, before use. The culture medium was replaced every second day and once the cells reached confluency, they were trypsinised with 4 ml of 0.25% Trypsin / 0.1% Versene EDTA (Highveld Biological, South Africa) at ambient temperature to obtain single cell suspensions of the Graham cells. The cells were centrifuged at 1500 rpm for 5 minutes to remove the trypsin. Once the cells were resuspended in experimental medium, 1 ml of the resuspended cells was used to seed a new culture and the remaining cell suspension was used in the MTT assay. The experimental medium was prepared in the same way as the culture medium except no gentamicin sulphate was added. Following this, 50 µl of the trypsinised cell suspension was stained with 0.2% (w/v) Trypan blue in a 1: 1 ratio and the number of cells per ml of cell suspension was determined using a haemocytometer. Before using the cells, ≥95% cell viability was confirmed.

The cell suspension was then adjusted with experimental medium to 0.25 million cells/ml and 180 µl of the adjusted cell suspension was dispensed in 96-well microtitre plates and incubated under humidified conditions at 37ºC in 5% CO_2 for six hours to allow the cells to adhere to the bottom of the wells. Thereafter, the various concentrations of plant samples (a total volume of 2 µl of essential oils and 20 µl of acetone extracts) were plated out in triplicate in 96-well microtitre plates. The essential oil samples were prepared as follows: a stock solution of 10% was prepared by dissolving 10 µl of the test essential oil in 90 µl of DMSO and a 1: 10 serial dilution was performed to produce concentrations ranging from 1% to 1 x 10^{-11} %. Experimental medium was used to prepare concentrations of the acetone extracts and control drugs ranging from 10 µM to 1.0 x 10^{-5} µM.

HAM solution (18 µl) was added to each well containing essential oil sample to ensure a total volume of 200 µl per well. Experiments for each test compound were carried out in triplicate. For each plate, 11 peripheral wells were utilized for the untreated cell controls and one peripheral well for the blank cell-free control (Figure 10.3). Chloroquine and quinine were used as positive controls. The plates were incubated under humidified

conditions at 37ºC in 5% CO_2. MTT (USB™) solution (12 mM) was prepared using phosphate buffered saline (pH 7.4) and then sterile filtered and stored at 4ºC. At 44 hours of incubation, 40 µl of MTT was added to each well and the plates were incubated for a further four hours. Experimental media supernatant (180 µl) was removed from each well by slow aspiration and 150 µl DMSO was added to each well to stop the reaction and to solubilize the formazan crystals. After allowing the plates to stand for 3-4 minutes, they were shaken at 1020 rpm for four minutes using a plate shaker to obtain thorough formazan solubilization. The absorbance was then read at a test wavelength of 540 nm and a reference wavelength of 690 nm using a microtitre plate reader (Labsystems iEMS Reader MF) connected to a computer equipped with Ascent® software.

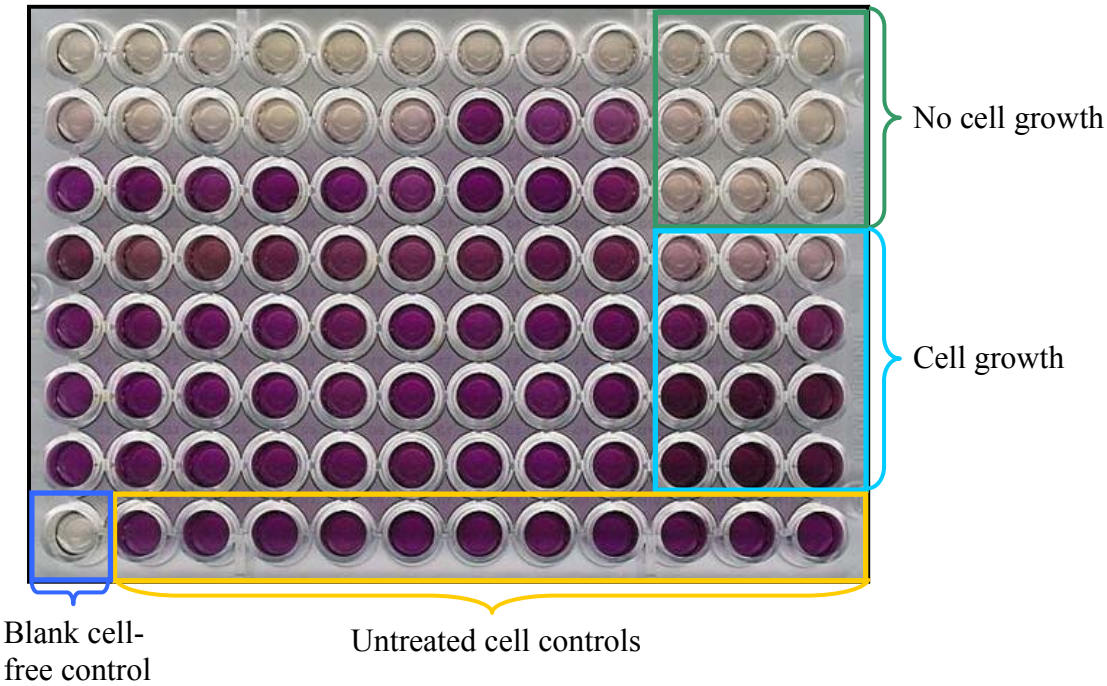


Figure 10.3: A 96-well microtitre plate prepared for use in the MTT assay. The clear wells indicate inhibition of cell growth whereas the purple wells indicate cell growth.

The results were expressed as percentage cellular viability of the drug and cell-free controls, using equation (1). The percentage cellular viability data was plotted against their respective concentrations and after logarithmic transformation of the concentration, sigmoidal dose-response curves were generated by the Enzfitter® software. The concentration which inhibits 50% of cellular growth (IC_{50} value) was determined from the log sigmoid dose-response profile.

$$\% \text{ Cellular viability} = \frac{\text{Drug treated abs}_{(540\text{-}690)} - \text{Mean abs of cell - free control}_{(540\text{-}690)}}{\text{Mean abs of untreated cell control}_{(540\text{-}690)} - \text{Mean abs of cell - free control}_{(540\text{-}690)}} \times 100 \quad (1)$$

Where: - abs = absorbance

The safety index relates the dose of a drug required to produce a desired effect to that which causes an undesired effect. The toxicity of the acetone extracts was compared to their antimalarial activity, using the safety index equation (2) below, to determine the degree of selectivity of the extracts for the *Plasmodium* parasite.

$$\text{Safety Index} = \frac{\text{Toxicity}}{\text{Activity}} \quad (2)$$

10.3 Results and discussion

10.3.1 Acetone extracts

Pelargonium acetone extracts showed a wide spectrum of toxicity, IC_{50} values varied from 11.89 µg/ml to 195.13 µg/ml (Table 10.1). Chloroquine inhibits 50% cell growth at a concentration of 125.56 ± 5.04 µg/ml and quinine at 136.06 ± 4.06 µg/ml. The acetone extracts of *P. sublignosum* and *P. citronellum* (NBG) displayed the highest toxicities (IC_{50} values of 11.89 ± 1.54 µg/ml and 19.14 ± 0.98 µg/ml, respectively). *Pelargonium sublignosum* is about 11-fold more toxic than the controls. Species exhibiting toxicity values close to that of the controls include *P. capitatum* (Strand), *P. cordifolium* (WSBG) and *P. cucullatum* (WSBG) (Figure 10.4). The IC_{50} values of *P. vitifolium* (178.48 ± 5.44 µg/ml) and *P. tomentosum* (SBG) (195.13 ± 7.90 µg/ml) are higher than those reported for the controls (Figure 10.5).

Table 10.1: *In vitro* toxicity results, antimalarial activity and safety index values for *Pelargonium* acetone extracts.

Species (Extracts)	Locality	Toxicity (µg/ml)		Antimalarial activity (µg/ml)		Safety Index
		IC_{50}	s. d.	IC_{50}	s. d.	
P. betulinum	NBG	88.55	1.51	16.23	2.75	5.46
P. capitatum	Strand	101.59	1.75	9.81	0.34	10.36
P. citronellum	NBG	19.14	0.98	1.58	0.65	12.11
P. citronellum	SBG	59.94	2.33	1.74	0.79	34.45
P. cordifolium	NBG	74.70	2.17	10.84	0.75	6.89
P. cordifolium	WSBG	116.88	8.01	10.17	2.43	11.49
P. crispum	NBG	74.02	2.30	21.00	4.33	3.52
P. cucullatum	SBG	73.81	2.62	7.42	0.35	9.95
P. cucullatum	WSBG	118.89	5.32	nd	nd	-
P. glutinosum	SBG	31.44	1.27	11.07	1.15	2.84
P. glutinosum	WSBG	46.29	0.81	nd	nd	-
P. graveolens	SBG	83.31	2.56	22.46	3.21	3.71
P. graveolens	WSBG	80.48	0.94	9.48	1.17	8.49
P. greytonense	SBG	39.65	1.18	13.21	1.89	3.00
P. hermanniifolium	SBG	46.47	1.38	13.54	3.95	3.43
P. hispidum	SBG	84.30	1.16	12.23	1.93	6.89
P. panduriforme	SBG	32.93	3.75	1.34	0.29	31.83
P. panduriforme	WSBG	42.65	0.90	nd	nd	-
P. papilionaceum	NBG	53.76	1.60	5.15	0.36	10.44
P. pseudoglutinosum	NBG	30.54	5.46	7.93	0.75	3.85
P. quercifolium	SBG	85.61	1.93	2.66	0.36	32.18
P. quercifolium	WSBG	48.69	1.04	nd	nd	-
P. radens	SBG	30.81	1.23	1.90	0.39	16.22
P. scabroide	SBG	53.76	1.57	15.94	0.16	3.37
P. scabrum	SBG	37.78	1.72	16.10	0.02	2.35
P. sublignosum	SBG	11.89	1.54	9.01	1.22	1.32
P. tomentosum	SBG	195.13	7.90	17.62	4.84	11.07
P. tomentosum	WSBG	50.71	1.17	13.83	0.61	3.67
P. vitifolium	SBG	178.48	5.44	9.18	1.61	19.44
Chloroquine	-	125.56	5.04	0.06	0.003	2092.67
Quinine	-	136.06	4.06	0.03	0.002	4535.33

Where: - NBG = National Botanical Garden (Kirstenbosch), SBG = Stellenbosch Botanical Garden, Strand = Strand region, WSBG = Walter Sisulu Botanical Garden (Johannesburg); s.d. = standard deviation; nd = not determined due to insufficient plant extract for testing; n = 3: triplicate measurements were performed for all samples.

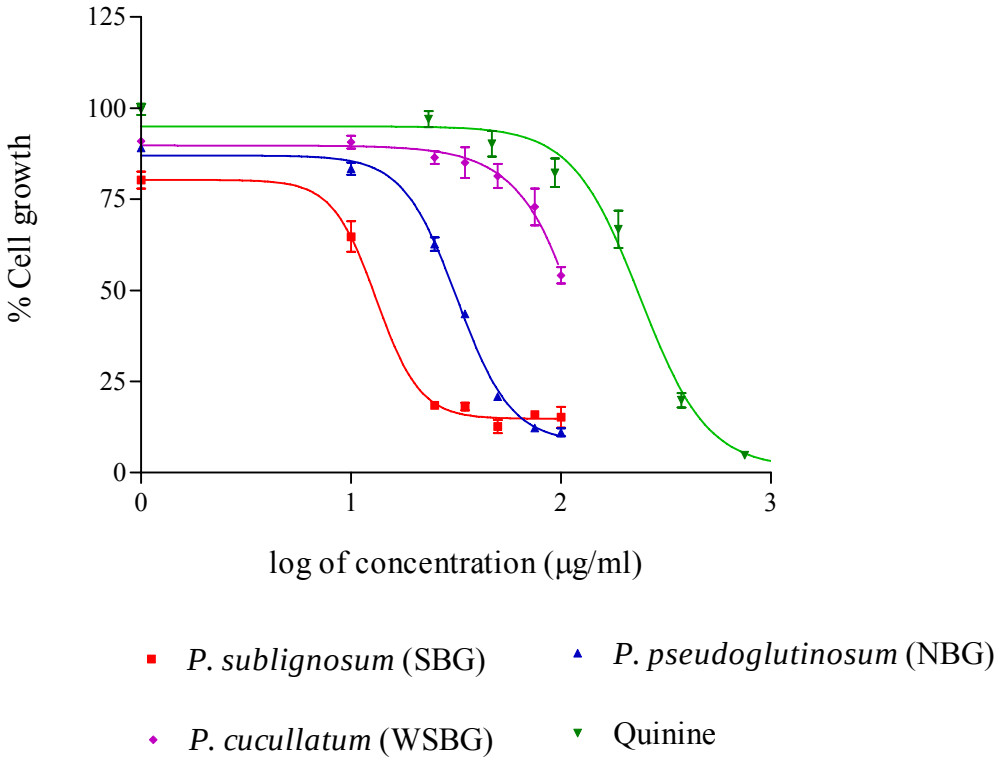


Figure 10.4: Sigmoid dose-response curves for *P. sublignosum* (SBG), *P. pseudoglutinosum* (NBG), *P. cucullatum* (WSBG) and quinine. Where: - (NBG) = National Botanical Garden (Kirstenbosch), (SBG) = Stellenbosch Botanical Garden, (WSBG) = Walter Sisulu Botanical Garden (Johannesburg).

Extracts from plant material of the same species collected from different localities (duplicate samples) which showed reasonably similar toxicity profiles include the *P. glutinosum* and *P. panduriforme* samples. In particular, the *P. graveolens* samples produced almost identical toxicity results (Figure 10.5).

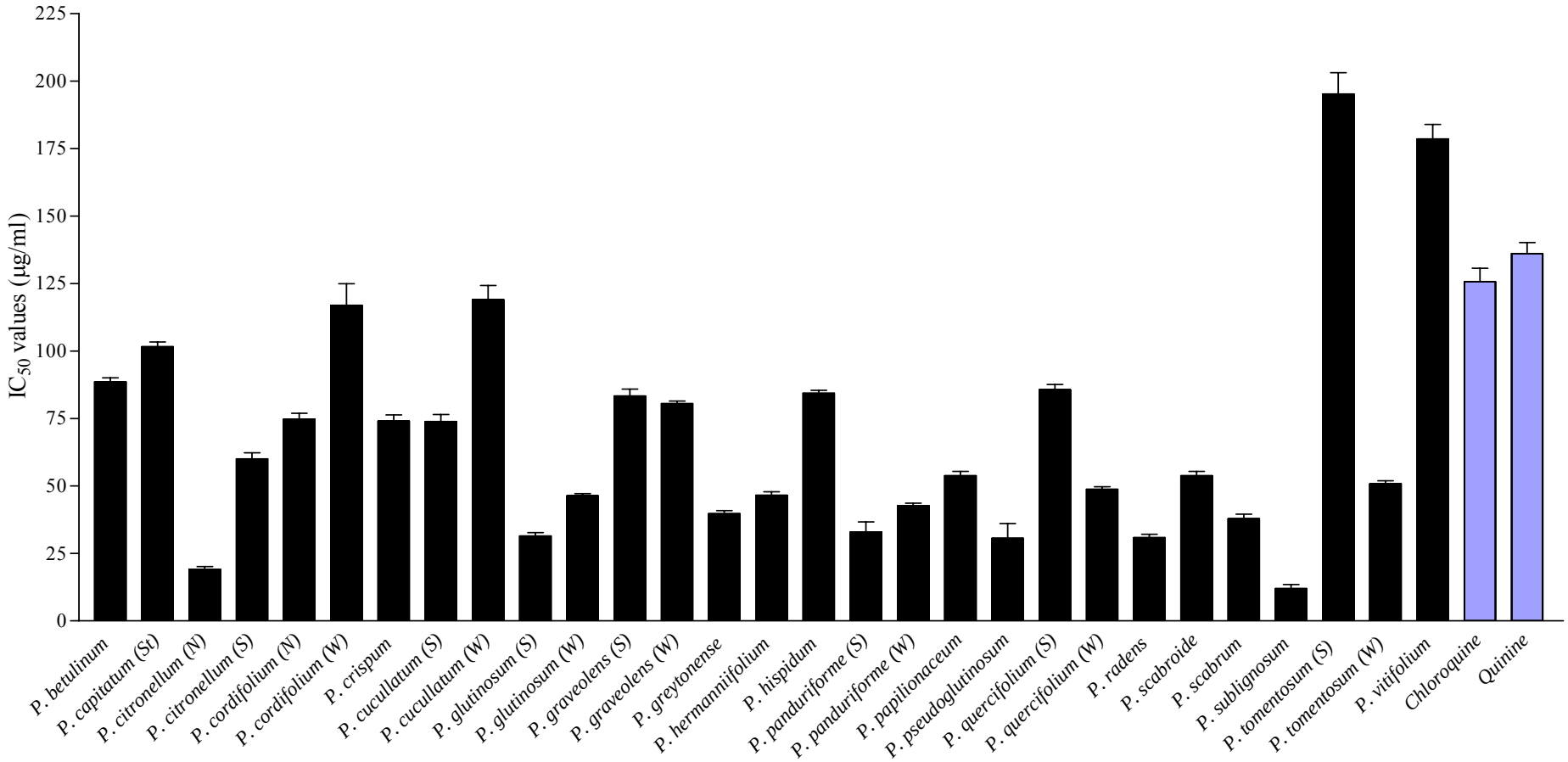


Figure 10.5: The toxicity profile of the *Pelargonium* acetone extracts. Where: - (N) = National Botanical Garden (Kirstenbosch), (S) = Stellenbosch Botanical Garden, (St) = Strand region, (W) = Walter Sisulu Botanical Garden (Johannesburg).

It is well-known that the toxicity can vary considerably for members of the same species of a plant growing in different areas; the geographical variation influences the composition and ratio of constituents. Moreover, members of the same species of a plant growing near each other can also vary considerably in toxicity (Steyn, 1934). In particular, *P. tomentosum* (SBG) and *P. tomentosum* (WSBG) produced greatly different IC_{50} values of 195.13 ± 7.90 µg/ml and 50.71 ± 1.17 µg/ml, respectively; *P. tomentosum* (WSBG) is almost four-fold more toxic than *P. tomentosum* (SBG). *Pelargonium citronellum* (NBG) is about three times more toxic than *P. citronellum* (SBG) and the samples of *P. cordifolium* (NBG) and *P. cucullatum* (SBG) produced similar toxicity profiles and are about 1.6 times more toxic than their respective duplicate samples from Walter Sisulu Botanical Garden. *Pelargonium quercifolium* (WSBG) is almost twice as toxic as *P. quercifolium* (SBG) (Figure 10.5). The chemical constitution of the duplicate samples should be compared as this is fundamental in understanding the observed discrepancy in their toxicities. Variations in growing conditions such as solar radiation and water content can contribute to the synthesis of different related compounds in aromatic plants (Nuńez and De Castro, 1992).

It was concluded from Chapter 4 that *P. betulinum* (NBG), *P. cordifolium* (WSBG), *P. crispum* (NBG) and *P. cucullatum* (SBG) and *P. scabrum* (SBG) acetone extracts have several similarities in their HPLC profiles. These species produced low toxic effects except for *P. scabrum* (SBG) (IC_{50} = 37.78 ± 1.72 µg/ml). *Pelargonium crispum* (NBG) and *P. cucullatum* (SBG) produced almost identical toxicity profiles (IC_{50} = 74.02 ± 2.30 µg/ml and 73.81 ± 2.62 µg/ml, respectively). HPLC analysis established that the chemical compositions of the *P. papilionaceum* and *P. vitifolium* acetone extracts are different (Chapter 4), this is further confirmed by their toxicity values. *Pelargonium papilionaceum* is about three times more toxic than *P. vitifolium*. *Pelargonium glutinosum* (SBG) has a similar compound, in substantial amounts, to the major compound present in *P. pseudoglutinosum* (Chapter 4). These species produced similar IC_{50} values of 31.44 ± 1.27 µg/ml and 30.54 ± 5.46 µg/ml, respectively.

Pelargonium cucullatum was employed to cure kidney ailments (May, 2000). This traditional use is considered safe since low cytotoxic effects (IC_{50} = 73.81 ± 2.62 µg/ml – 118.89 ± 5.32 µg/ml) were produced by the two samples tested in this assay which used the human kidney epithelial (Graham) cell line. In addition, the toxicity profiles of *P.*

betulinum and *P. cucullatum* (SBG and WSBG) indicate that they have the potential to be used for their reported benefits in wound-healing and gastro-intestinal related problems. *Pelargonium papilionaceum* appears to exert greater toxic effects (IC_{50} = 53.76 ± 1.60 µg/ml) (Figure 10.5).

Botanically-derived medicines are based upon the idea that they contain compounds which possess potent pharmacological activities (Kaur *et al*., 2005). However, the very compounds which promote health and relieve illness could be toxic to the human body. It was concluded from HPLC analysis (Chapter 4) that the *Pelargonium* acetone extracts contain numerous flavonoids. The antitumour effect of flavonoids on various tumour cell lines has been well documented. Flavonoids have been shown to arrest cell growth at more than one stage of the cell cycle (Pedro *et al*., 2005). Induction of apoptosis is one of a combination of biologic effects which could contribute to the antiproliferative activity of flavonoids (Rusak *et al*., 2005). Flavonoid derivatives may have contributed to the cytotoxicity recorded for the crude extracts. Furthermore, sesquiterpene lactones, iridoids and tannins produce long-term toxic effects (Neuwinger, 1996). The toxicity of plants may be ascribed to the presence of substances such as glucosides, resins, bitters, picrotoxins, toxalbumins, alcohols, lactones and organic acids (Steyn, 1934). Possibly, these aforementioned compounds impart toxic attributes to the *Pelargonium* extracts. Although, it must be noted that the *Pelargonium* plant material was extracted with acetone which mainly extracts flavonols (Cowan, 1999).

It was concluded on the basis of the flavonoid patterns of *P. betulinum* (NBG), *P. capitatum* (Strand), *P. graveolens* (SBG), *P. hispidum* (SBG), *P. panduriforme* (SBG) and *P. vitifolium* (SBG) that these species share very characteristic flavonoid patterns (Chapter 4). *Pelargonium betulinum* (NBG), *P. graveolens* (SBG) and *P. hispidum* (SBG) produced similarly low toxic values (IC_{50} = 83.31 ± 2.56 µg/ml – 88.55 ± 1.51 µg/ml). *Pelargonium capitatum* (Strand) also displayed low toxicity (IC_{50} = 101.59 ± 1.75 µg/ml) and *P. vitifolium* was non-toxic (Figure 10.5). It could be argued, from the assumption mentioned previously, that since numerous flavonoids were identified in these latter species, they should produce low IC_{50} values as did *P. panduriforme* (SBG) (32.93 ± 3.75 µg/ml). However, flavonoids are not a primary source of toxicity in mammals (Neuwinger, 1996).

Pelargonium scabrum and *P. sublignosum*, with similar characteristic flavonoid patterns, both produced low toxicity profiles.

Pelargonium betulinum, P. crispum, P. cordifolium (NBG and WSBG) and *P. scabrum* displayed remarkable anti-oxidant activities (Chapter 7). These extracts did not produce considerably toxic profiles except for the *P. scabrum* extract (Figure 10.5). Theoretically, the implication of this is that *P. betulinum, P. crispum, P. cordifolium* (NBG and WSBG) may produce anti-oxidant effects without producing considerable toxic effects. Plant remedies should offer a margin of efficacy over toxicity. This finding prompts further studies of these anti-oxidant active species, with regards to the isolation and elucidation of their anti-oxidant compound/s, followed by toxicity testing of these isolates with the potential of being utilized as therapeutic agents.

The toxicity results were evaluated together with the MIC results (Chapter 5). *Pelargonium citronellum* (NBG) *P. glutinosum* (SBG), *P. pseudoglutinosum* (NBG), *P. quercifolium* (SBG), *P. scabrum* (SBG) and *P. sublignosum* (SBG) exerted considerable antimicrobial effects against the Gram-positive bacteria *B. cereus* and *S. aureus*. The latter species, except for *P. quercifolium* (SBG), produced considerable toxic effects, which were from about 3.5 to 11 times greater than the toxic effects produced by the controls. The results from the MTT assay suggest that the antimicrobial action of the extracts may be ascribed to general cytotoxic effects. For all the species tested, the toxicity IC_{50} values are lower than the concentrations required to produce an antimicrobial effect. This may be indicating that the mammalian cell membrane is more permeable to the effects of the extracts compared to Gram-positive and Gram-negative bacterial cell walls and the fungal cell wall. The greater selectivity of *Pelargonium* extracts for human kidney epithelial cells indicates that the toxic effects of botanical extracts need to be considered if such substances have the potential to be used as therapeutic antimicrobials in humans. However, *P. cordifolium* (NBG), *P. crispum* (NBG), *P. quercifolium* (SBG) have a niche in therapeutics due to their promising activity against both Gram-positive micro-organisms and their reasonably low toxicity profiles. However, one must consider that the results from *in vivo* toxicity studies may be very different.

The antimalarial activity of the extracts may be due to their general toxic effects towards living cells. When comparing the toxicity and antimalarial data of the crude extracts (Table 10.1) it can be seen that they are more selective for the *Plasmodium* parasite. The safety indices of the crude extracts and controls are presented in Table 10.1. The safety index of chloroquine and quinine is 2092.67 and 4535.33, respectively. The safety indices of the controls are appreciably higher than those of the tested extracts which range from 1.32 to 34.45. The safety indices indicate that the extracts have a low degree of selectivity for the *Plasmodium* parasite. *Pelargonium citronellum* (SBG), *P. panduriforme* (SBG) and *P. quercifolium* (SBG) exhibited considerable antimalarial activity and appear to have the greatest selectivity for the *Plasmodium* parasite since these extracts demonstrated the highest safety indices (34.45, 31.83 and 32.18, respectively) among all the extracts tested (Table 10.1). Further investigations performed on *P. citronellum* (SBG), *P. panduriforme* (SBG) and *P. quercifolium* (SBG) should involve the isolating and testing of the active antimalarial compound/s. If several different compounds are responsible for each of their antimalarial activities recorded, then a drug containing a combination of such compounds could be developed to afford potentially potent antimalarial activity.

10.3.2 Essential oils

The MTT assay revealed that various *Pelargonium* essential oils are considerably more toxic to the human kidney epithelium cell line than the crude extracts (Table 10.2). *Pelargonium graveolens* (SBG) and the two *P. panduriforme* samples are extremely toxic (IC_{50} ≤0.10 µg/ml) in the test system used. These species are about 1256 and 1361 times more toxic than chloroquine and quinine, respectively. The two *P. quercifolium* samples and *P. tomentosum* (SBG) also produced substantial toxicity (IC_{50} ≤1.00 µg/ml – 8.81 ± 1.46 µg/ml). *Pelargonium tomentosum* (WSBG) oil is the least toxic (IC_{50} = 30.20 ± 1.81 µg/ml) of all the oils tested. This species is about four-fold more toxic than chloroquine and quinine. The order of decreasing toxicity of the essential oils is as follows: *P. graveolens* (SBG), *P. panduriforme* (SBG), *P. panduriforme* (WSBG) > *P. tomentosum* (SBG) > *P. quercifolium* (SBG) > *P. quercifolium* (WSBG) > *P. scabrum* > *P. citronellum* (NBG) > *P. radens* > *P. tomentosum* (WSBG) (Figure 10.6).

Table 10.2: *In vitro* cytotoxicity (µg/ml) of various *Pelargonium* essential oils.

Species (Essential oils)	**Locality**	**Toxicity (µg/ml)**		
		IC_{50}	**s.d.**	**n**
P. citronellum	NBG	22.40	4.77	3
P. graveolens	SBG	≤0.10	-	3
P. panduriforme	WSBG	≤0.10	-	3
P. panduriforme	SBG	≤0.10	-	3
P. quercifolium	SBG	3.34	2.79	3
P. quercifolium	WSBG	8.81	1.46	3
P. radens	NBG	27.20	4.76	3
P. scabrum	SBG	17.80	3.55	3
P. tomentosum	SBG	≤1.00	-	3
P. tomentosum	WSBG	30.20	1.81	3
Chloroquine	-	125.56	5.04	3
Quinine	-	136.06	4.06	3

Where: - s.d. = standard deviation; n = 3: triplicate measurements were performed for all samples; NBG = National Botanical Garden, SBG = Stellenbosch Botanical Garden, WSBG = Walter Sisulu Botanical Garden.

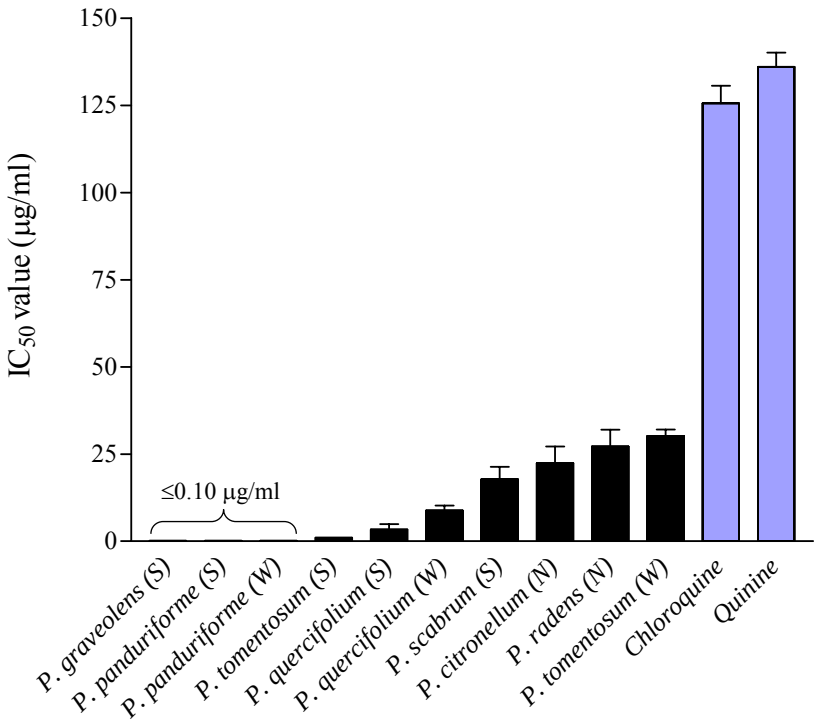


Figure 10.6: The decreasing order of toxicity of the various tested *Pelargonium* essential oils and the IC_{50} values of the controls.

The *P. quercifolium* essential oil samples produced promising anti-inflammatory activity (IC_{50} = 33.24 – 38.67 µg/ml) in the 5-LOX assay (Chapter 8). *Pelargonium graveolens* (SBG) and *P. panduriforme* (SBG) oils produced promising anti-fungal activity (MIC = 2 mg/ml) (Chapter 5). Due to the aromatic, flavouring and antimicrobial properties of *P. citronellum* (NBG) it was anticipated that this oil may have application in the food, cosmetic and pharmaceutical industry (Chapter 5). The cytotoxicity IC_{50} values of these oils are lower than the concentrations required to produce pharmacological effects. This indicates that caution is required when using these oils for therapeutic benefits.

The GC-MS data of the essential oils (Chapter 3, Table 3.2) were evaluated to gain insight as to the compounds possibly contributing to the toxic effects observed. *Pelargonium citronellum* (NBG) produced a similar toxicity profile to *P. scabrum* and to *P. radens* yet the cluster dendrogram (Chapter 3, Figure 3.2) did not show a close association of *P. citronellum* (NBG) with either of these two species. The *P. panduriforme* samples produced the same toxicity values (IC_{50} ≤0.10 µg/ml), these samples were clustered in the same cluster subgroup (F_A). *Pelargonium graveolens* (SBG) is equally toxic; however, it is grouped in cluster (E) which diverges from cluster (F) at a low similarity coefficient. The two *P. quercifolium* samples, with similar IC_{50} values of 3.34 ± 2.79 µg/ml and 8.81 ± 1.46 µg/ml, are grouped within the same cluster i.e. (F) but within different subgroups. *Pelargonium quercifolium* (WSBG) is found in the subgroup that contains the *P. panduriforme* samples (F_A), but it is *P. quercifolium* (SBG) which produces an IC_{50} value more similar to that of the *P. panduriforme* samples, yet occurs in subgroup (F_B). The essential oils of *P. radens and P. tomentosum* (WSBG) produced similar toxicity profiles (Figure 10.6). This similarity is not surprising as these species are closely related chemically. Interesting to note is that the two *P. tomentosum* samples produced different toxicity profiles (IC_{50} ≤1.00 µg/ml and IC_{50} = 30.20 ± 1.81 µg/ml), yet the dendrogram demonstrates that they are chemically similar, their OTUs diverge at a high degree of similarity. *Pelargonium graveolens* (SBG) is chemically similar to *P. tomentosum* (SBG), both these oils were extremely toxic (IC_{50} ≤0.10 µg/ml and IC_{50} ≤1.00 µg/ml, respectively).

The "plant laboratory" generates secondary metabolites and often occurring in high concentrations are those constituents with remarkable properties (Neuwinger, 1996). p-Cymene, isomenthone, menthone, viridiflorol, 14-hydroxy-β-caryophyllene, geranic acid, geranial and neral occur as main compounds in the various essential oils tested. Some of

these compounds mentioned also occur at lower concentrations. In particular, p-cymen-8-ol was present as a common compound at low levels. A single active constituent or several structurally related compounds could collectively be causing the toxicity of the plants (van Wyk *et al*., 2002a).

Seatlholo *et al*. (2006, *In press*) conducted a study on the biological activities of twenty essential oil constituents. The toxicity study revealed that nerolidol was the most toxic compound with an IC_{50} value of 1.00 ± 0.30 µg/ml. (E)-Nerolidol occurs at 2.8% in *P. quercifolium* (SBG). Linalool which occurs in *P. citronellum* (NBG) (1.8%) and in *P. panduriforme* (SBG) (4.4%) was also found to be toxic (IC_{50} = 20.00 ± 10.00 µg/ml) (Seatlholo *et al*., 2006, *In press*). However, menthone a major compound in the *P. tomentosum* samples (36.1-41.1%) showed low toxicity (IC_{50} = 120.00 ± 3.00 µg/ml) as did p-cymene (IC_{50} = 110.00 ± 10.00 µg/ml) which was the major compound in *P. panduriforme* (SBG) (45.4%), *P. panduriforme* (WSBG) (37.6%) and *P. quercifolium* (WSBG) (54.9%). This implies that various arrangements of compounds at low levels and not the major compounds identified maybe contributing to the toxic effects of the tested *Pelargonium* oils.

'Geranium oil' has been reported to cause contact dermatitis (Klarmann, 1958; Mitchell and Rook, 1979). Geraniol, a compound found in 'geranium oil', causes sensitization (Keil, 1947). Seatlholo *et al*. (2006, *In press*) showed that geraniol is toxic to human kidney epithelial cells (IC_{50} = 20.00 ± 1.00 µg/ml). The sensitizing or toxic compounds producing negative effects on the skin could be partly responsible for the toxic effects demonstrated by the oils towards human kidney epithelial cells. Geraniol occurs in low amounts in the oil samples of *P. citronellum* (NBG), *P. panduriforme* (SBG and WSBG) and *P. scabrum.*

Three volatile oils from *P. graveolens* were assessed for anti-tumour action (Fang *et al*., 1989). The compounds citronellol, citronellyl formate, geraniol and citronellyl acetate exhibited marginal anti-tumour activity. Such activity may be due to the general cytotoxic effects of the compounds. The sample of *P. graveolens* (SBG), proven to be toxic in the MTT assay (IC_{50} ≤0.10 µg/ml), contains 0.4% citronellol and trace amounts of citronellyl formate.

The toxicity of a plant in humans cannot be accurately predicted by a specific test (van Wyk *et al*., 2002a). Additional human cell lines should be used to assess whether the toxicity values of the *Pelargonium* extracts and essential oils obtained in this study are consistent with those produced when testing with other cell lines and whether greater selectivity for certain human cell types exists. The human kidney epithelial cell line used in the MTT assay serves as a prototype for the other organ-cell types of the human body, thus the toxic effects produced by the acetone extracts and essential oils in this study provides some idea of the results one can anticipate when using other cell lines.

The next step would be to investigate the toxic effects of the individual major compounds common to the oils, using standards of these compounds. Once this has been performed, the identified toxic compound/s can be isolated from the plant and chemical and biological investigations can be performed on the toxin/s. In this way, knowledge of those active principles possessing toxic properties can be broadened. Toxicity studies involving skin preparations would provide an indication of the toxicity profile of the extracts and oils if they were to be employed in topical dermatological preparations for cosmetic use. *In vivo* studies should be conducted on both the extracts and essential oils as although *in vitro* cellular toxicity was indicated by the MTT assay, this may not be relevant *in vivo*.

CHAPTER 11: GENERAL CONCLUSION

ESSENTIAL OIL COMPOSITION AND CHEMOTAXONOMY

- Cluster analysis defined patterns of similarity between and among certain *Pelargonium* samples based on their essential oil data. Definite chemotaxonomic relationships were apparent between *P. papilionaceum* and *P. vitifolium*; *P. graveolens* and *P. radens* and between *P. panduriforme* and *P. quercifolium.* In particular, the chemical composition of *P. graveolens* (WSBG) and *P. radens* was almost identical, this is in line with the work of van der Walt and Demarne (1988). New chemical affinities were established among *P. betulinum, P. hispidum* and *P. scabrum*; *P. capitatum* (WSBG), *P. glutinosum* and *P. quercifolium* (SBG) and among *P. graveolens*, *P. radens* and *P. tomentosum*. Overall, incongruencies occurred between the chemotaxonomic appraisal and the current phylogenetic hypotheses of relationships. Only the close relationship between *P. graveolens* and *P. radens* as indicated by Bakker *et al*. (2004) was found to occur in the chemical analysis.

- The two oil samples of *P. panduriforme*, collected from different localities, were chemically similar as were the duplicate samples of *P. graveolens* and *P. tomentosum*. The GC-MS results of the *P. tomentosum* oil samples were in general agreement with published data; this indicated that the composition of the oil is relatively stable. GC-MS analysis of the *P. capitatum* oil samples confirmed the findings of previous studies which indicated variability in the oil composition. Viljoen *et al*. (1995) concluded that several biochemical pathways exist in *P. capitatum*. Comparison of the present data of *P. graveolens* with a previous study (Rana *et al*., 2002), indicated that chemical variation pertaining to its oil can occur. The two hydrodistilled essential oil samples of *P. quercifolium* produced moderately incongruent chemical patterns in this study. Different local conditions can influence the oil composition (Lis-Balchin, 1991) producing variations between the same species growing in different areas. The genetic component may account for chemical variations noted; this component being far more pronounced than the environmental effects. Growth regulators, shading, distillation, storage, weeds, leaf ontogeny, drying and seasons all influence the chemical composition of

Pelargonium species oil (Rana *et al*., 2002). The essential oil compositions of *P. papilionaceum, P. vitifolium* and *P. citronellum* were different from those of the other analysed species.

- Ambrox (2.3%) was detected in the oil of *P. glutinosum.* The commercially most important constituent of the scarce natural ambergris is (−)-Ambrox® (Bolster *et al*., 2001), it has the nature of the no longer employed natural ambergris and is fundamental in creating fragrances (Castro *et al*., 2002). An amount of 5.4% of cedrol was detected in *P. betulinum.* Several odourants with ambery odour characteristics have been developed from cedrol such as Cedramber®. A mixture of aldehyde compounds were detected in *P. citronellum*. The latter species is known for its strong lemon scent. Very high quantities (36.1-41.1%) of menthone were detected in the *P. tomentosum* samples. Large quantities (49.3-84.5%) of isomenthone were detected in the oils of *P. graveolens*, *P. radens* and *P. tomentosum*. These ketones have a mint-scent. Good quantities of citronellol (9.9%) and linalool (4.4%) were detected in *P. capitatum* (Strand) and *P. panduriforme* (SBG), respectively. Both citronellol and linalool are employed by the food industry in citrus beverages (Lis-Balchin, 1990). The *Pelargonium* essential oils may be a source of several other constituents of interest to the flavour and fragrance industries.

CHEMICAL COMPOSITION OF THE NON-VOLATILE EXTRACTS

- A particular compound (UV absorbance maximum: 206.2-218.0 nm, retention time: ± 3.98 minutes) appeared to be characteristic of the *Pelargonium* species. It was the major compound present in *P. hermanniifolium*, *P. graveolens*, *P. greytonense* and *P. vitifolium*. HPLC analysis confirmed the presence of flavonoid derivatives in the acetone extracts. The tentative flavonoid patterns of the *Pelargonium* species were used as a means to compare the species chemically. The data suggested that the following taxa: *P. betulinum* (NBG), *P. capitatum* (Strand), *P. graveolens* (SBG), *P. hispidum* (SBG), *P. panduriforme* (SBG) and *P. vitifolium* (SBG) have numerous similarities in their chemical profiles. *Pelargonium capitatum* and *P. vitifolium* are related (van der Walt, 1977) and so are *P. sublignosum* and *P. scabrum* (van der Walt and Vorster, 1988); their similar flavonoid patterns confirmed this. The HPLC fingerprints of *P. papilionaceum* and *P. vitifolium* were

chemically diverse. Such findings noted from HPLC analysis were in disparity with the results obtained from the essential oil chemistry analysis which provided evidence of a chemotaxonomic relationship between these two species.

ANTIMICROBIAL ACTIVITY

- The *Pelargonium* essential oils and acetone extracts showed a more selective antibacterial action against the test Gram-positive micro-organisms *Bacillus cereus* and *Staphylococcus aureus*. The essential oils displayed reduced antimicrobial activity compared to the acetone extracts. The antibacterial activity of *P. citronellum* (NBG) against *B. cereus* and *S. aureus* suggested that *P. citronellum* (NBG) oil may delay microbial contamination. *Pelargonium citronellum* (NBG) oil with its additional aromatic and flavouring properties could be employed as a natural food preservative. It also has the potential as a cosmetic or pharmaceutical preservative. The Gram-negative bacterium, *Klebsiella pneumoniae*, showed microbial resistance to all the tested essential oils except to *P. citronellum* (NBG); however, this latter oil displayed very weak anti-Gram-negative bacterial activity. The yeast *Candida albicans* was most susceptible to the oils of *P. graveolens* (SBG) and *P. panduriforme* (SBG). These latter oils may be potential sources of effective anti-*Candida* agents.

- The *P. pseudoglutinosum* acetone extract displayed the greatest antimicrobial activity against the Gram-positive bacteria. The acetone extracts of *P. glutinosum* (SBG), *P. scabrum* and *P. sublignosum* also produced remarkable anti-Gram-positive activity. *Pelargonium betulinum* and *P. capitatum* (Strand) extracts were most active against *K. pneumoniae*. *Candida albicans* was found to be least resistant to *P. scabrum* which was the second most active test substance against *B. cereus*. The presence of flavonoids in the non-volatile extracts may account for their observed antimicrobial activity.

- The non-volatile *Pelargonium* extracts are potentially good sources of antimicrobial compounds which may have the potential to be utilized in the development of novel, efficient antimicrobial drugs. Furthermore, they may provide additional benefits to conventional antimicrobial treatments. In particular, extracts of *P. glutinosum*, *P. pseudoglutinosum, P. scabrum* and *P. sublignosum* may

contain active compounds which offer some preservative benefit and thus may have applications in the food, cosmetic or pharmaceutical industries. Furthermore, the abovementioned species, may be potential sources of topical antimicrobial agents for the treatment of wound infections involving *S. aureus* and may be sources of effective agents for nosocomial infections. However, one must not consider that natural products are the answer to the problem of microbial resistance. Klimek *et al.* (1948) found that *S. aureus* was able to develop resistance to allicin, a natural compound from garlic (*Allium sativum*).

- The antimicrobial results obtained for the acetone extracts support the traditional use of various *Pelargonium* species for the treatment of various ailments such as wounds, abscesses, coughs, colds, sore throats and gonorrhoea. However, the antimicrobial data obtained for the essential oils in this study, did not correlate with previous reports documenting effective antimicrobial activity of 'geranium oils'. Evaluation of the vapour of the essential oils may provide a better indication of their antimicrobial activity.

- It was concluded that in the case of *Pelargonium*, essential oils do not enhance the antimicrobial activity of non-volatile extracts. However, the results suggested that the non-volatile compounds may improve the antimicrobial property of volatile compounds.

- The results from the MTT assay suggested that the antimicrobial action of the extracts may be ascribed to general cytotoxic effects. The greater selectivity of the *Pelargonium* extracts for human kidney epithelial cells indicates that the toxic effects of those extracts with considerable antimicrobial activity need to be considered if such substances have the potential to be used as *in vivo* therapeutic antimicrobials. However, *P. cordifolium* (NBG), *P. crispum* (NBG), *P. quercifolium* (SBG) have a niche in therapeutics due to their promising activity against both Gram-positive micro-organisms and their reasonably low toxicity profiles.

ANTI-OXIDANT ACTIVITY

- *Pelargonium betulinum, P. crispum, P. cordifolium* (NBG and WSBG) and *P. scabrum* displayed exceptional DPPH-scavenging activity. The flavonoid derivatives present in the *Pelargonium* species may contribute to their *in vitro* anti-oxidant activities. Extracts of numerous species may have a potential role in reducing or preventing oxidative stress which has been associated with several degenerative diseases. These extracts may thus be potential sources of natural anti-oxidant supplements. There is the possibility for extracts of specific *Pelargonium* species to be employed in the skin care regimen to slow down skin ageing via its anti-oxidant properties. However, such statements require further evaluation in that the anti-oxidant function of *Pelargonium* extracts should be assessed in biological systems (*in vivo*). In addition, the food industry requires the anti-oxidative properties of botanical extracts; certain *Pelargonium* species may be of importance in this industry.

- The abovementioned species did not produce considerable toxic profiles except for the *P. scabrum* extract. This prompts further studies of these anti-oxidant active species, with regards to the isolation and structure elucidation of their anti-oxidant compound/s, followed by toxicity testing of these isolates with the potential of being utilized as therapeutic agents.

ANTI-INFLAMMATORY ACTIVITY

- *Pelargonium quercifolium* essential oil was the most active oil in the *in vitro* 5-lipoxygenase assay. The bioactivity of the essential oils would be assumed to correlate to the respective composition of the plant essential oils, the structural configuration and the functional groups of the chemical constituents and interactions between components (Dorman and Deans, 2000). Several chemical compounds, previously reported to produce *in vitro* inhibition of the 5-lipoxygenase enzyme (Baylac and Racine, 2003), were present at low levels in the active oils. These compounds may act collectively in a synergistic manner and in this way may contribute to their anti-inflammatory activity. Numerous terpenic and sesquiterpenic hydrocarbons may contribute to the activity of *P. quercifolium*. *Pelargonium quercifolium*, *P. panduriforme*, *P. scabrum* and *P. citronellum* may have promising therapeutic applications in various inflammatory and allergic

conditions. The aforementioned *Pelargonium* essential oils may be useful ingredients in cosmetic products as the oils not only possess pleasant aromatic qualities but may offer skin soothing activity, through their inhibition of the 5-lipoxygenase enzyme.

ANTIMALARIAL ACTIVITY

- General cytotoxicity may explain the antimalarial activity of the extracts. *Pelargonium citronellum* (SBG), *P. panduriforme* (SBG) and *P. quercifolium* (SBG) exhibited considerable antimalarial activity and these extracts produced the highest safety indices. This indicated that the latter extracts, compared to the other extracts, had greater selectivity for the *Plasmodium* parasite than for the human kidney epithelium (Graham) cells. The latter species deserve further investigations in terms of isolating and testing of their active antimalarial compound/s. If several different compounds are responsible for each of their antimalarial activities recorded, then a drug containing a combination of such compounds could be developed to afford potentially potent antimalarial activity.

- *Pelargonium pseudoglutinosum* extract produced considerable antimalarial activity and exerted the greatest activity against the Gram-positive bacteria. However, it produced a low safety index value. This species requires further investigations with respect to the isolation of the active compounds. Such compounds in isolated form where other compounds do not exert an influence could be non-toxic. If this is the case then the active compounds could serve as templates for the development of therapeutic agents with novel modes of antimalarial and antimicrobial actions.

TOXICITY

- The acetone extracts of *P. sublignosum* and *P. citronellum* (NBG) displayed the highest toxicities. Species exhibiting very low toxic profiles included *P. capitatum* (Strand), *P. cordifolium* (WSBG) and *P. cucullatum* (WSBG). *Pelargonium tomentosum* (SBG) and *P. vitifolium* were non-toxic. Flavonoid derivatives may have contributed to the cytotoxicity recorded for the acetone extracts.

- The essential oils showed high cytotoxicity towards the human cell line used in the MTT assay. *Pelargonium graveolens* (SBG) and the two *P. panduriforme* samples proved to be the most toxic. *Pelargonium tomentosum* (WSBG) oil was the least toxic. Various compounds (geraniol, nerolidol and linalool) previously reported to be toxic were present at low levels in the tested *Pelargonium* oils. Such compounds maybe contributing to their cytotoxicity. The cytotoxicity of the essential oils appears to be selective as the essential oils exerted poor antimicrobial activity. The greater selectivity of the oils shown for the human cells in this study needs to be further substantiated by using other human cell lines. Furthermore, other methods of testing the antimicrobial activity of the essential oils may provide a better indication of this selectivity.

- The *P. quercifolium* essential oil samples produced promising anti-inflammatory activity in the 5-LOX assay. *Pelargonium graveolens* (SBG) and *P. panduriforme* (SBG) oils produced good anti-fungal activity. Due to the aromatic, flavouring and antimicrobial properties of *P. citronellum* (NBG) it was anticipated that this oil may have application in the food, cosmetic and pharmaceutical industry. The toxicity IC_{50} values of these oils are lower than the concentrations required to produce pharmacological effects. This indicates that the toxicity of these oils must be considered in accordance with their potential applications.

This study intends to make a contribution towards the knowledge base of plant species with therapeutic potential. This study confirmed the presence of bioactive compounds in *Pelargonium* species and showed that the properties of *Pelargonium* species correspond to the traditionally held views. The food, cosmetic and pharmaceutical industries have recognized the value of natural ingredient utilization and have shifted their focus towards investigating and manufacturing alternative, more natural antimicrobials, antibiotics and anti-oxidants. The antimicrobial results together with the concomitant anti-oxidant activity make a compelling case for the potential use of certain non-volatile *Pelargonium* extracts in the aforesaid industries. However, studies involving well-designed human trials need to be conducted for further affirmation. A great deal still needs to be learned with regard to the precise mode of action of the compounds of those *Pelargonium* species demonstrating considerable activities.

A quote by Juritz in 1915 remains relevant today: "South Africa still remains as indifferent as in Pappe's day to the pharmacological possibilities of its almost inexhaustible flora" (Vorster, 1990). Many plant-derived substances have been fundamental in relieving human suffering which justifies the continued search for new and improved plant-derived drugs. Many more beneficial drug entities from the plant kingdom are yet to be discovered; much wealth and wonder is still held by Nature's secrets.

RECOMMENDATIONS FOR FURTHER RESEARCH

- **Phytochemical investigations**

It should be noted that for taxonomic reasons and due to time constraints plant material was obtained from botanical gardens. It is recommended that a thorough geographical variation study is performed on each species to explain the diversity within and between natural populations.

Research on aromatic Pelargoniums has been restricted to the volatile compounds. The pharmacological results clearly indicate that the non-volatile compounds warrant further study as the HPLC assignments mentioned in the monograph section are tentative only. If these tentative identifications are confirmed then certain *Pelargonium* species can be utilized as a source for the isolation of natural flavonoids. A compound with a typical flavone UV spectrum occurred in high amounts in *P. hispidum* (SBG), *P. panduriforme* (SBG), *P. papilionaceum* (NBG) and *P. tomentosum* (SBG). Isolation, subsequent structural elucidation and biological testing of this compound should be carried out.

In order to make a comprehensive validation of the differences in biological activities produced by some duplicate samples, their HPLC profiles need to be obtained and evaluated.

- **Antimicrobial activity**

Investigations to determine the antibacterial and antifungal effects of the vapours of the essential oils of Pelargoniums should be carried out. If such findings prove to be positive then there is the potential use of *Pelargonium* oils in the disinfection of hospital rooms and work environments. Before the clinical practice of vapour therapy of *Pelargonium* essential oils can be applied in whole-organism systems, their effectiveness in terms of parameters such as minimum exposure time for the therapeutic effect to occur and bactericidal vapour concentration should be subjected to animal and human studies (Inouye *et al*., 2001).

Further antimicrobial investigations involving different micro-organisms should be performed on the non-volatile *Pelargonium* extracts.

The non-volatile extract of *P. pseudoglutinosum* showed considerable activity against the Gram-positive bacteria. Further investigations of its antimicrobial activity should be performed. The rapidity of a bactericidal effect or the duration of a bacteriostatic effect can be determined by time-kill analysis (survival curve plot). In this analysis, the number of viable cells remaining in broth after the addition of the extract is plotted against time. The compound/s responsible for the antibacterial activity of *P. pseudoglutinosum* should be isolated. Once isolated other biological activities can be evaluated.

Historically, tuberculosis is the largest single cause of death of the human species. Following a long period of declining incidence, there has been a reappearance of the disease over the last two decades. The uncontrolled usage of the currently available antimycobacterial drugs has resulted in the emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* (Seidel and Taylor, 2004). "Umckaloabo" is highly valued by the southern African native population for its curative and palliative effects (Kolodziej, 2002). This traditional drug originates from the root material of *P. sidoides* and *P. reniforme* and decoctions are used for the treatment of tuberculosis (Miller, 2002). Seidel and Taylor (2004) reasoned that the supposed *in vivo* efficacy of "Umckaloabo" may be related to both direct antibacterial effects on pathogenic mycobacteria and to indirect immunomodulating properties of components of the extracts. Other *Pelargonium* species may prove to be valuable sources of new antimycobacterial drugs.

- **Anti-oxidant activity**

The exceptional anti-oxidant activities of *P. betulinum*, *P. cordifolium*, *P. crispum* and *P. scabrum* indicate that they possess bioactive compounds. Pursuing the isolation and biological studies of these active compounds in the future is justified.

- **Anti-inflammatory activity**

Pelargonium essential oils should be assessed for inhibitory activity towards the two isoforms of cyclo-oxygenase (COX-1 and COX-2). In this way, we will be able to screen for potential balanced inhibitors of 5-LOX and COX pathways. Such substances are important for the advancement of effective and safe anti-inflammatory therapy.

The major compounds detected in the essential oils of *P. citronellum* (NBG), *P. panduriforme* (SBG and WSBG), *P. quercifolium* (SBG and WSBG) and *P. scabrum*

(SBG), should be assessed individually to determine their contribution to the anti-inflammatory activity displayed by the whole oil.

- **Antimalarial activity**

The antimalarial findings raise some interesting expectations about these plant extracts, indicating that further studies on the chemical and biological properties of their active components should be carried out.

- **Anticancer activity**

The anticancer potential of *Pelargonium* species should be investigated. Those *Pelargonium* species demonstrating both anti-inflammatory and anticancer properties, with a low toxicity profile, deserve consideration as adjuncts in cancer treatment or chemoprevention.

- **Antiviral activity**

The ever increasing number of people infected with the human immunodeficiency virus (HIV) has highlighted the importance of the development of novel antiretroviral agents. Investigations have focused on plant sources for possible novel therapeutics. The herpes simplex virus (HSV) is responsible for various life threatening diseases. There is the need to search for new and more effective antiviral agents for HSV infections since the chemotherapeutic agents available are either low in quality or have limited efficiency (Vijayan *et al*., 2004). The treatment of viral infections with current antiviral drugs often leads to the problem of viral resistance. Investigating *Pelargonium* species for potential antiviral activity is validated by the increasing need for substances with antiviral activity.

- **Other activities requiring investigation**

There are various other properties of *Pelargonium* species requiring further research (van Wyk *et al*., 2002b). These include wound-healing, antispasmodic and analgesic effects (Watt and Breyer-Brandwijk, 1962). Research into these other properties of Pelargoniums is well-founded as their importance within the realms of the cosmetic and pharmaceutical industries may be further developed.

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<http://www.ibiblio.org/pfaf/cgi-bin/arr_html?Pelargonium+graveolens>
<http://www.ibiblio.org/pfaf/cgi-bin/arr_html?Pelargonium+quercifolium>
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APPENDIX A: MONOGRAPHS OF SELECTED *PELARGONIUM* SPECIES

Appendix A contains the monographs of the 21 *Pelargonium* species (section *Pelargonium*) included in this study. The species were selected according to the availability of fresh plant material from the botanical gardens (SBG, NBG and WSBG) and according to authenticity of taxonomic identification.

1. *Pelargonium betulinum* (L.) L'Hérit.

Synonym:

P. georgense Knuth (van der Walt, 1985).

Common names:

Birch-leaf pelargonium, Camphor-scented pelargonium, 'Kanferblaar', 'Maagpynbossie', 'Suurbos' (van der Walt, 1977; Lawrence and Notten, 2001).

Botanical description:

Pelargonium betulinum is a small upright or sprawling, bushy shrublet or shrub measuring 0.3-1.3 m in height. The branches are woody and the leaves are small, asymmetrically oval or ovate, hard and leathery. The leaves resemble those of the European Birch trees (*Betula* species); this explains the derivation of the species name '*betulinum*'. The leaf blades are either almost hairless or covered with fine hairs. The leaf border is dentated with red-tipped teeth. The leaf margin of the plants from the Mossel Bay - Knysna regions is coarser than that of plants from the south-western Cape. The umbel-like inflorescence generally contains three to four flowers and sometimes up to six flowers. The plant is noticeable when in full bloom as the flowers are large and attractive. The flowers are most commonly pink and purple in colour, a white form is also found. Dark purplish streaks occur on the petals, particularly on the posterior two petals which are broader and darker than the three anterior ones. The seven-fertile stamens contain orange anthers (van der Walt, 1977; Lawrence and Notten, 2001).

Distribution and habitat:

Pelargonium betulinum is mostly located in the coastal areas of the winter rainfall region of South Africa, from Yzerfontein to Knysna (west coast to the east coast of the Western Cape). *Pelargonium betulinum* grows on sandy dunes and on flat landscapes. *Pelargonium betulinum* is frequently found growing with *P. cucullatum*. Hybrids of these two species are therefore common (van der Walt, 1977).

Figure 1.1: *Pelargonium betulinum.*

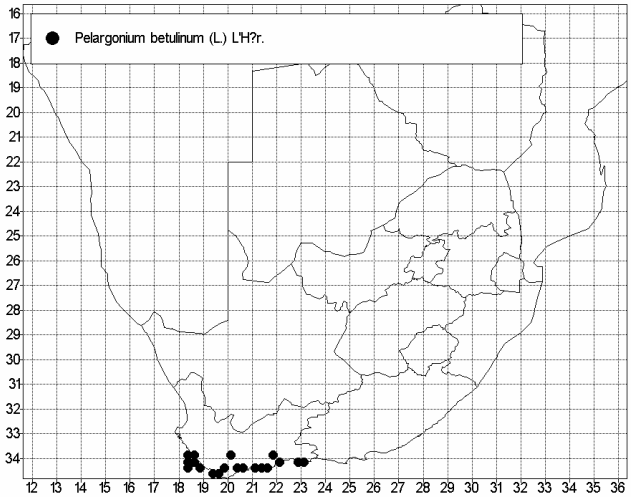


Figure 1.2: The geographical distribution of *Pelargonium betulinum* (All maps purchased and included with permission from SANBI).

Traditional uses:

The vapours (essential oils) obtained from the steamed leaves were inhaled to treat coughs and other chest-related problems (van der Walt, 1977). The leaves were used in wound healing ointments. The common name 'Maagpynbossie' (stomach-pain bush) was derived from the fact that this plant was used for the relief of stomach pain and flatulence (Lawrence and Notten, 2001).

Remarks:

The vernacular name 'Kanferblaar' (camphor leaf) or camphor-scented pelargonium is derived from the camphor-like odour produced when the leaves are crushed. The name 'Suurbos' (sour bush) refers to the taste of the leaves (Lawrence and Notten, 2001).

Chemical composition of the essential oil:

Fresh plant material was collected from the National Botanical Garden (Kirstenbosch) and subjected to hydrodistillation for 3 hours. The oil yield was 0.04%.

GC-MS profile and analysis

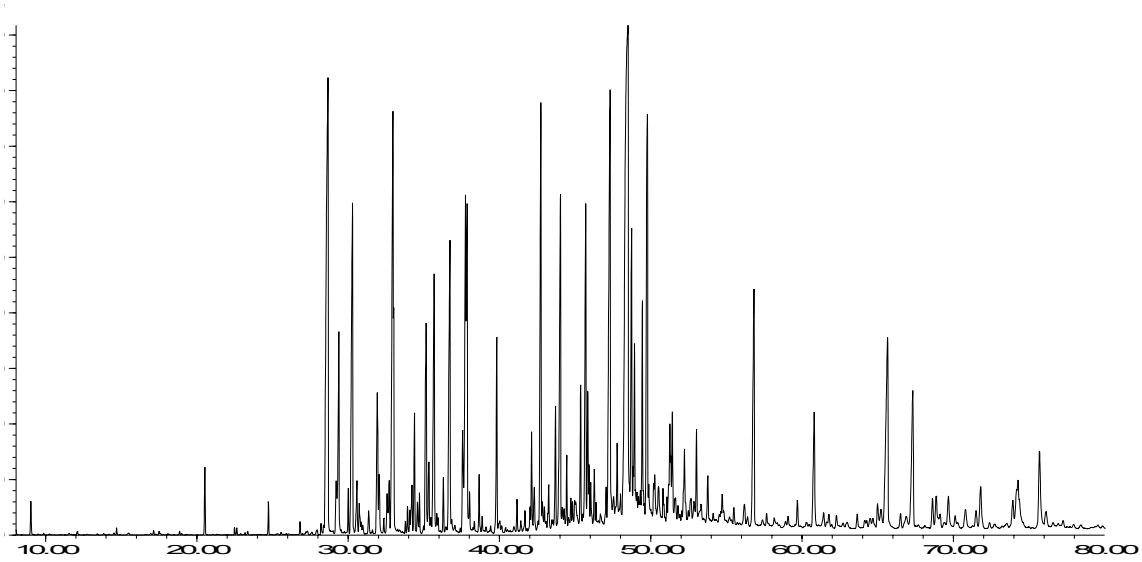


Figure 1.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium betulinum* (NBG).

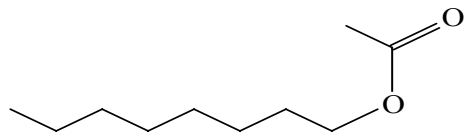
Table 1.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium betulinum* (NBG).

RRI	Compound	Area %
1032	α-pinene	0.13
1280	p-cymene	0.24
1384	heptyl acetate	0.09
1491	**octyl acetate**	**5.74**
1497	α-copaene	1.41
1528	α-bourbonene	0.15
1535	β-bourbonene	2.76
1549	β-cubebene	0.20
1553	octanol	0.12
1572	β-ylangene	0.05
1577	α-cedrene	0.60
1612	**β-caryophyllene**	**5.28**
1613	β-cedrene	trace
1661	allo-aromadendrene	0.64
1664	1-nonanol	0.14
1668	(Z)-β-farnesene	0.16
1687	α-humulene	1.45
1693	β-acoradiene	0.45
1704	γ-muurolene	1.89
1726	germacrene D	0.23

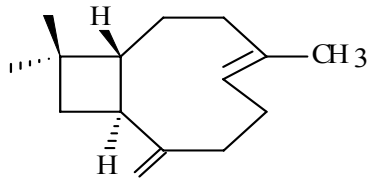
RRI	Compound	Area %
1740	α-muurolene	2.44
1743	α-cadinene	0.24
1766	1-decanol	0.51
1773	δ-cadinene	2.59
1776	γ-cadinene	trace
1786	ar-curcumene	0.14
1853	*cis*-calamenene	0.99
1871	*trans*-calamenene	0.05
1898	1,11-oxidocalamenene	0.07
1918	β-calacorene	0.09
1941	α-calacorene	0.40
1965	3-phenylpropyl acetate	2.99
1984	γ-calacorene	0.18
2001	isocaryophyllene oxide	0.65
2008	caryophyllene oxide	2.82
2045	humulene epoxide I	trace
2050	(E)-nerolidol	0.09
2057	ledol	0.10
2071	humulene epoxide II	0.76
2080	cubenol	2.51
2088	1-epi-cubenol	0.58
2104	viridiflorol	0.25
2131	hexahydrofarnesylacetone	trace
2143	**cedrol**	**5.40**
2187	**T-cadinol**	**18.94**
2209	T-muurolol	1.72
2219	α-muurolol	0.78
2247	*trans*-α-bergamotol	trace
2255	**α-cadinol**	**4.45**
2340	10-hydroxy-calamenene	0.53
2407	hydroxy-α-calacorene*	trace
2900	nonacosane	0.10
2931	hexadecanoic acid	0.69
	Total	**72.79%**

*correct isomer not identified; trace: <0.05%; (NBG) = National Botanical Garden (Kirstenbosch).

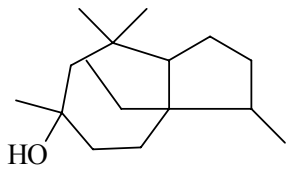
T-cadinol, a sesquiterpene, is the principle compound (18.94%) identified in the essential oil. Octyl acetate, β-caryophyllene, cedrol and α-cadinol are other major compounds, collectively making-up 20.87% of the total oil composition.



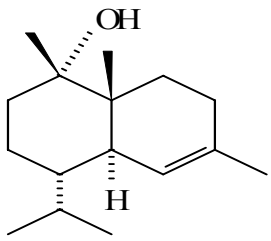
octyl acetate



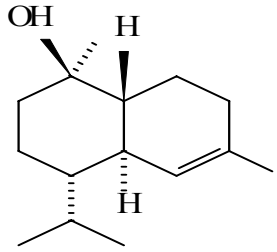
β-caryophyllene



cedrol



T-cadinol



α-cadinol

Figure 1.4: Chemical structures of the major compounds identified in the essential oil of *Pelargonium betulinum* (NBG).

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium betulinum* collected from the National Botanical Garden (Kirstenbosch).

HPLC profile and analysis

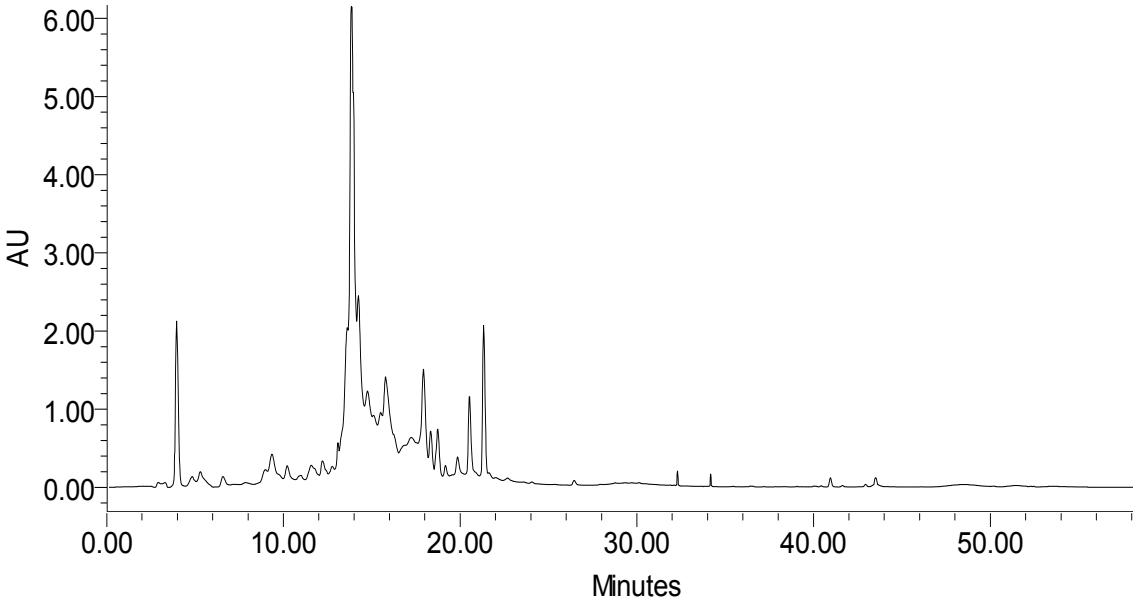


Figure 1.5: HPLC profile of the acetone extract of *Pelargonium betulinum* (NBG).

Table 1.2: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium betulinum* (NBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
4.01	203.8		5.72
4.85	207.3, 280.5		0.65
5.27	214.4, 279.3		1.19
6.56	215.6, 271.0		0.56
9.36	207.3, 315		2.79
10.22	206.2, 271.0		1.40
11.61	219.1, 284.1		2.02
12.25	219.1, 280.5		1.53
13.70	222.6, 281.7		26.92
14.30	222.6, 281.7		10.22
14.75	222.6, 280.5		6.37
15.78	222.6, 278.1		10.85
17.24	220.3, 280.5		7.90
17.93	220.3, 278.1		6.70
18.34	263.9, 350.8	flavone	2.04
18.74	255.7, 355.6	flavonol	2.37
19.84	222.6, 280.5		1.93
20.52	255.7, 354.4	flavonol	3.67

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
21.39	255.7, 350.8	flavone	4.89
32.30	261.6		0.15
34.18	265.1		0.11

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium betulinum* in this study include the following:

- The acetone extract produced promising antimicrobial activity. The extract was most active against the Gram-positive micro-organism *B. cereus* (MIC = 0.33 mg/ml).

- The *Pelargonium betulinum* extract displayed exceptional anti-oxidant activity (IC_{50} = 4.13 µg/ml) in the DPPH assay. Of all the extracts tested, this species displayed the highest anti-oxidant activity.

- *Pelargonium betulinum* extract displayed antimalarial activity; an IC_{50} value of 16.23 ± 2.75 µg/ml was obtained from the hypoxanthine incorporation assay.

- The acetone extract exhibited low toxicity in the MTT assay (IC_{50} = 88.55 ± 1.51 µg/ml).

2. *Pelargonium capitatum* (L.) L'Hérit.

Synonym:

Pelargonium drummondii (van der Walt, 1985).

Common name:

Rose-scented pelargonium (van der Walt, 1977).

Botanical description:

Pelargonium capitatum is a shrubby or bushy, low growing (0.25-1 m tall) plant with side-branches which can reach a length of 60 cm. The sprawling or upright stems are soft wooded. Long soft hairs cover the stems and leaves, which when crushed release a rose-like aroma. The creased, velvety leaves have a cordate base and are deeply 3-6-lobed. The segments may also be lobed with a toothed leaf margin. The flower heads seem to be quite condensed; this is due to the short flower pedicles and the large number of flowers. Eight to twenty flowers are borne on each capitate (head-like) inflorescence. Generally, the petals are cyclamen-purple coloured with beetroot-purple stripes on the two posterior and larger petals. Pale pink and dark pink-purple coloured flowers can also be found. Each flower consists of seven fertile stamens (van der Walt, 1977).

Distribution and habitat:

The distribution of *P. capitatum* stretches from Lambert's Bay (Western Cape) on the Atlantic coast southwards and then eastwards to Richards Bay (KwaZulu-Natal) on the Indian Ocean coast. *Pelargonium capitatum* is found growing on dunes or low sandy hill-sides near the coast. Like other Pelargoniums, it usually grows in disturbed areas (van der Walt, 1977).

Figure 2.1: *Pelargonium capitatum* in flower.

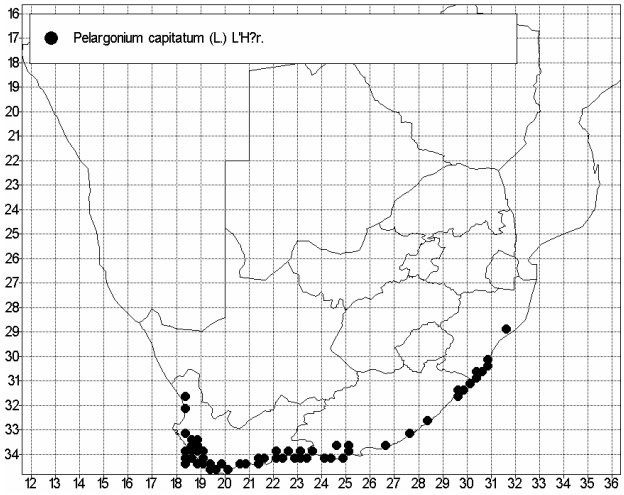


Figure 2.2: The geographical distribution of *Pelargonium capitatum.*

Medicinal uses:

The whole plant is a soothing, emollient herb (Bown, 1995). All plant parts are astringent (Grieve, 1984). It is taken internally to treat minor digestive ailments as well as kidney and bladder disorders. It is used for the relief of rashes and cracked skin (Bown, 1995).

Other uses:

Pelargonium capitatum is one of the Pelargoniums cultivated for its essential oil (van der Walt, 1977) which has the scent of roses. The essential oil is used in aromatherapy, skin care and is employed in perfumes. The leaves are used in pot-pourri (Bown, 1995).

Remark:

Pelargonium capitatum is closely related to *P. vitifolium* (van der Walt, 1977).

Chemical composition of the essential oil:

Hydrodistillation was carried out on fresh plant material for 3 hours.

1. *Pelargonium capitatum* from Strand region yielded 0.02% oil,

2. *Pelargonium capitatum* from Walter Sisulu Botanical Garden yielded 0.02% oil.

GC-MS profiles and analyses

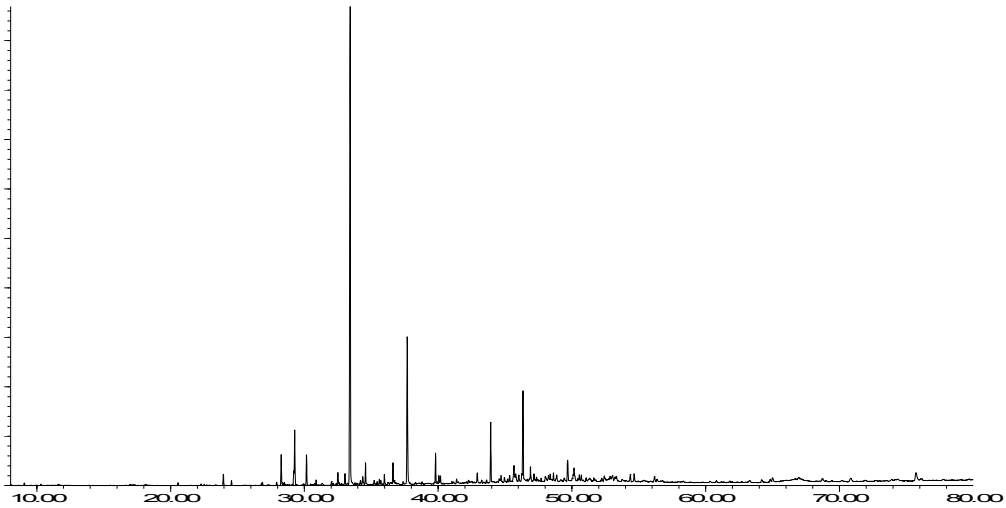


Figure 2.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium capitatum* (Strand).

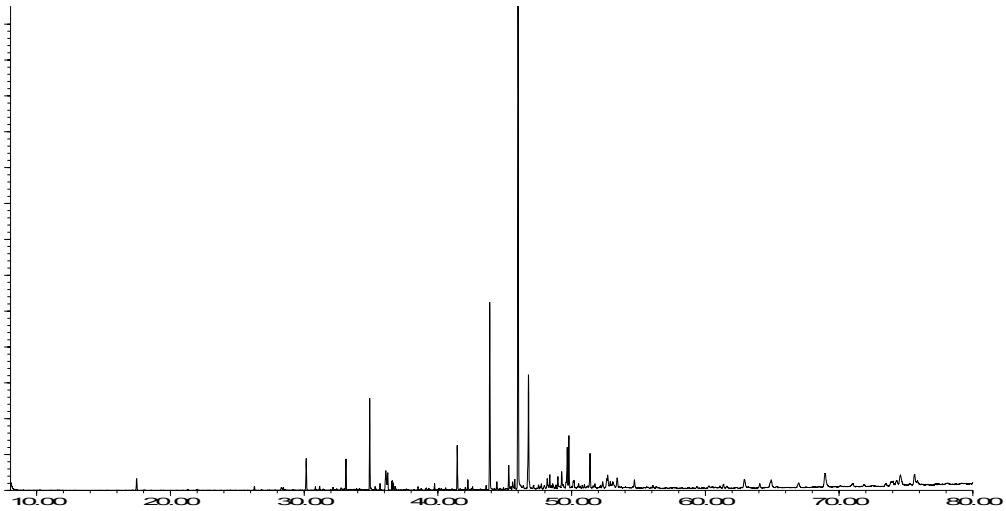


Figure 2.4: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium capitatum* (WSBG).

Table 2.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium capitatum.*

RRI	Compound	Area %	
		Strand	**WSBG**
1032	α-pinene	0.19	-
1202	3-hexanol	0.07	-
1213	1,8-cineole	-	trace
1280	p-cymene	0.07	-
1353	*cis*-rose oxide	0.60	-
1365	*trans*-rose oxide	0.27	-
1402	heptyl isobutyrate	-	0.48
1459	acetic acid	-	0.26
1464	(E)-2-hexenyl butyrate	-	0.47
1466	α-cubebene	0.51	-
1475	menthone	0.89	-
1497	α-copaene	0.71	-
1503	isomenthone	1.93	-
1528	α-bourbonene	0.13	-
1532	camphor	-	0.07
1535	β-bourbonene	1.52	-
1553	linalool	0.29	0.12
1553	octanol	0.24	0.09
1562	isopinocamphone	-	0.28
1574	hexadecane	0.15	-
1588	β-copaene	trace	-
1589	isocaryophyllene	1.01	-
1597	bornyl acetate	0.13	0.39
1608	**octyl butyrate**	-	**2.08**
1611	terpinen-4-ol	-	trace
1612	**citronellyl formate**	**31.14**	-
1612	β-caryophyllene	-	0.19
1617	guaia-6,9-diene	0.53	-
1661	allo-aromadendrene	0.58	-
1668	citronellyl acetate	0.92	-
1674	γ-gurjunene	0.32	-
1679	heptadecane	0.09	-
1690	cryptone	0.19	-
1698	geranyl formate	0.51	-
1704	γ-muurolene	0.44	-
1706	α-terpineol	0.25	0.28
1719	borneol	-	0.97
1725	verbenone	-	0.73
1740	α-muurolene	1.35	0.58
1744	α-selinene	0.26	-
1750	β-dihydroagarofuran	-	0.37
1755	*cis*-dihydroagarofuran	-	0.16
1765	geranyl acetate	0.15	-

RRI	Compound	Area %	
		Strand	WSBG
1766	1-decanol	-	0.15
1772	**citronellol**	**9.85**	-
1800	octadecane	0.13	-
1805	tridecanal	-	0.29
1809	citronellyl butyrate	0.23	0.24
1837	myrtanol*	-	0.09
1845	*trans*-carveol	trace	-
1853	*cis*-calamenene	1.59	0.52
1864	p-cymen-8-ol	0.44	-
1868	(E)-geranyl acetone	0.39	-
1916	**α-agarofuran**	-	**2.78**
1998	furopelargone A	0.08	-
2001	isocaryophyllene oxide	-	0.28
2008	**caryophyllene oxide**	**2.77**	**9.97**
2030	norbourbonone	0.52	-
2033	epi-globulol	-	0.57
2045	humulene epoxide I	-	0.14
2057	ledol	0.24	-
2071	humulene epoxide II	0.47	0.13
2080	cubenol	-	0.56
2088	1-epi-cubenol	-	0.47
2098	globulol	-	0.12
2104	**viridiflorol**	-	**24.00**
2110	**furopelargone B**	**6.13**	-
2127	**10-epi-γ-eudesmol**	-	**6.88**
2131	hexahydrofarnesylacetone	0.54	-
2143	cedrol	-	0.29
2144	spathulenol	0.39	-
2185	γ-eudesmol	-	0.28
2187	T-cadinol	0.46	-
2205	eremoligenol	-	0.48
2209	T-muurolol	0.31	0.08
2210	agarospirol	-	0.35
2219	α-muurolol	0.38	0.26
2228	valerianol	-	0.87
2249	cadalene	1.14	-
2253	**4α-hydroxy-dihydroagarofuran**	-	**2.23**
2254	citronellic acid	trace	-
2257	**β-eudesmol**	-	**2.93**
2273	selin-11-en-4α-ol	-	0.19
2281	decanoic acid	0.25	0.09
2300	tricosane	-	0.08
2316	caryophylladienol I	0.16	-
2324	caryophylladienol II	0.27	-
2340	10-hydroxy-calamenene	-	trace

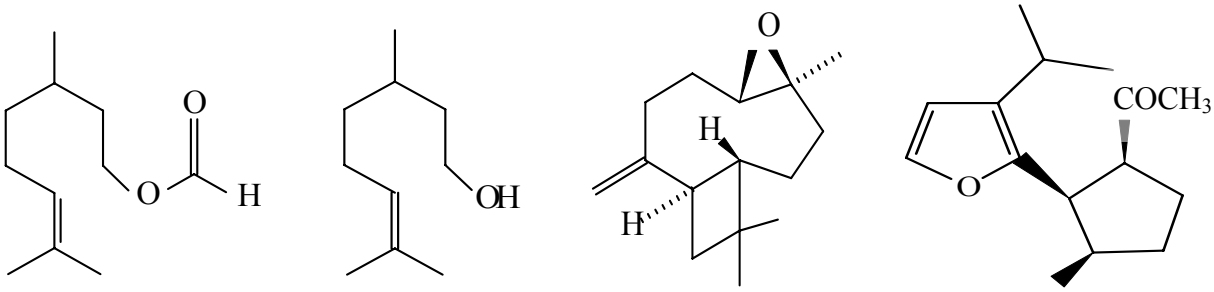
RRI	Compound	Area %	
		Strand	WSBG
2357	14-hydroxy-β-caryophyllene	0.29	-
2376	manoyl oxide	-	0.56
2384	farnesyl acetone	0.35	-
2500	pentacosane	0.22	0.17
2509	dodecanoic acid	0.46	0.14
2617	tridecanoic acid	-	0.20
2670	tetradecanoic acid	0.94	-
2700	heptacosane	0.08	trace
2931	hexadecanoic acid	0.60	1.05
	Total	**75.12%**	**64.96%**

*correct isomer not identified; trace: <0.05%; WSBG = Walter Sisulu Botanical Garden.

Citronellyl formate, the major compound identified in *P. capitatum* (Strand), contributes 31.14% to the total oil composition. Citronellol, a monoterpene, contributes 9.85% and the sesquiterpenes caryophyllene oxide and furopelargone B represent 2.77% and 6.13%, respectively.

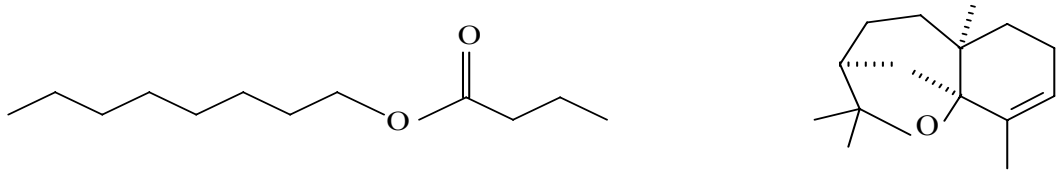
The principle compound in *P. capitatum* (WSBG) is the sesquiterpene viridiflorol which occupies 24.00% of the total oil composition. Other major compounds include caryophyllene oxide (9.97%) and 10-epi-γ-eudesmol (6.88%). Collectively, octyl butyrate, α-agarofuran, β-eudesmol and 4α-hydroxy-dihydroagarofuran make-up 10.02% of the total oil composition.

There is considerable chemical variation between the two oil samples of *P. capitatum*. Caryophyllene oxide is the only major compound common to both the oil samples with a higher percentage occurring in *P. capitatum* (WSBG).

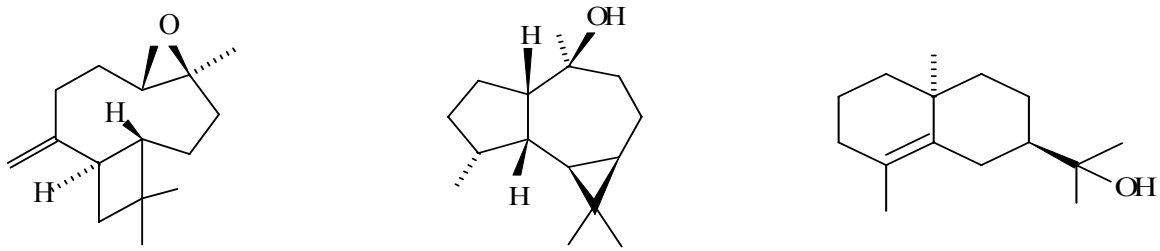


citronellyl formate citronellol caryophyllene oxide furopelargone B

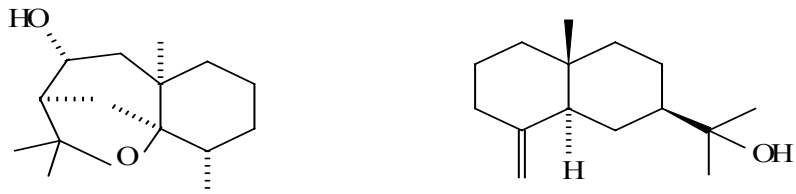
Figure 2.5: Chemical structures of the major compounds identified in the essential oil of *Pelargonium capitatum* (Strand).



octyl butyrate α-agarofuran



caryophyllene oxide viridiflorol 10-epi-γ-eudesmol



4α-hydroxy-dihydroagarofuran β-eudesmol

Figure 2.6: Chemical structures of the major compounds identified in the essential oil of *Pelargonium capitatum* (WSBG).

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium capitatum* collected from the Strand region.

HPLC profile and analysis

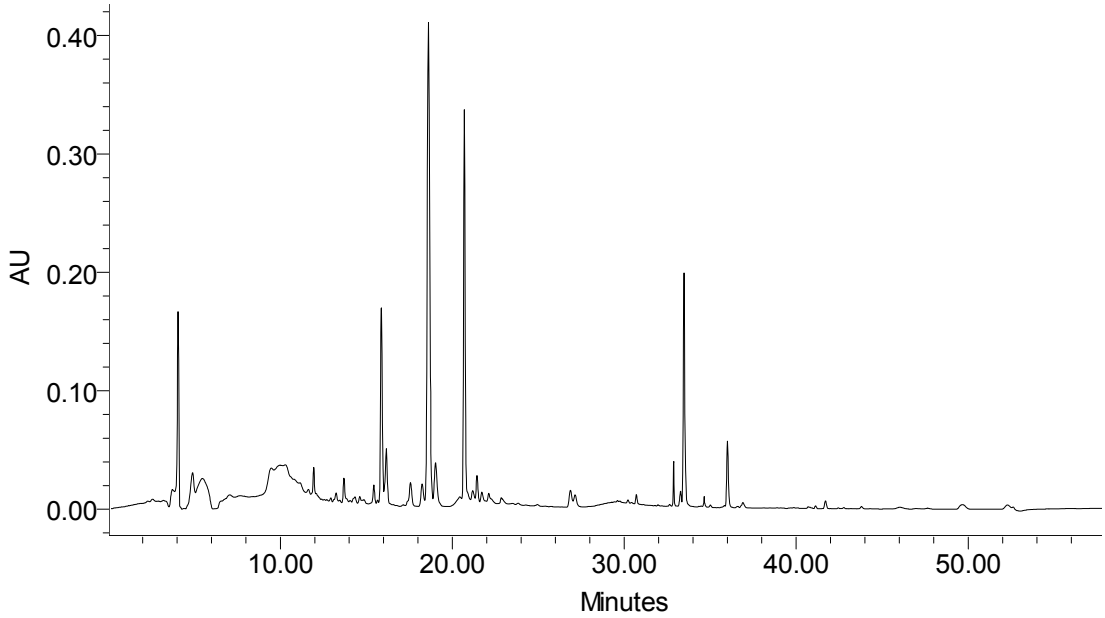


Figure 2.7: HPLC profile of the acetone extract of *Pelargonium capitatum* (Strand).

Table 2.2: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium capitatum* (Strand) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
4.05	207.4		8.21
4.92	202.8, 261.5		3.54
5.51	205.1		7.59
10.22	207.4		1.41
11.94	220.3, 278.0, 314.7		1.03
13.71	283.9	flavanone	1.23
15.44	265.0, 351.6	flavonol	0.36
15.88	260.3, 357.5	flavonol	8.51
16.17	255.6, 351.6	flavonol	2.89
17.57	255.6, 357.5	flavonol	1.55
18.25	261.5, 348.0	flavone	1.37
18.61	255.6, 354.0	flavonol	30.29
19.03	255.6, 351.6	flavonol	2.93

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
20.70	255.6, 354.0	flavonol	15.60
21.43	254.4, 348.0	flavone	1.10
32.88	261.5		0.87
33.48	254.4, 354.0	flavonol	8.86
36.00	267.4, 345.6	flavone	2.65

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium capitatum* in this study include the following:

- *Pelargonium capitatum* (Strand) acetone extract produced MIC values ranging from 1 - 2 mg/ml.

- The *P. capitatum* (Strand) extract displayed substantial antimalarial activity (IC_{50} = 9.81 ± 0.34 µg/ml).

- The acetone extract (Strand) produced a low toxicity profile (IC_{50} = 101.59 ± 1.75 µg/ml).

3. *Pelargonium citronellum* J. J. A. van der Walt

Common name:

Lemon-scented pelargonium (van der Walt and Vorster, 1988).

Botanical description:

Pelargonium citronellum is a densely branched shrub and can reach a height of 2 m. The stems are herbaceous when young and hirsute with many glandular hairs. The lower surfaces of the leaves are conspicuously veined. The leaves are sparsely covered with rough, stiff hairs with glandular hairs interspersed. The margins of the leaves are irregularly serrate-dentate. Leaf characteristics are intermediary to those of *P. scabrum* and *P. hispidum*. *Pelargonium citronellum* leaves compared to those of *P. scabrum* are less scabrous, but more strongly aromatic. The lemon scent of the leaves and stems is attributed to the chemical substance, citronella, hence the epithet '*citronellum*'. Five to six and sometimes up to eight flowers are borne on the pseudo-umbel inflorescence. The flowers are pinkish to purple. The posterior two petals have characteristic dark purple markings and are spathulate to obovate and the anterior three petals are spathulate. Seven fertile stamens are present (van der Walt and Vorster, 1988).

It is likely that *P. citronellum* evolved from the southern Cape form of *P. scabrum*, since the latter form resembles *P. citronellum* to a greater extent than the Western Cape form. *Pelargonium scabrum* can hybridize easily with other species and so it is also highly likely that *P. citronellum* originated from the hybridization of *P. scabrum* and *P. hispidum* (van der Walt and Vorster, 1988).

Distribution and habitat:

Pelargonium citronellum is restricted to the one degree square which includes the town of Ladismith in the Western Cape. It is commonly found growing on the northern foothills of the Langeberg range between Muiskraal and Herbertsdale. This distribution area receives most of its rainfall during winter months. *Pelargonium citronellum* is found near streams or rivulets (van der Walt and Vorster, 1988).

Figure 3.1: *Pelargonium citronellum.*

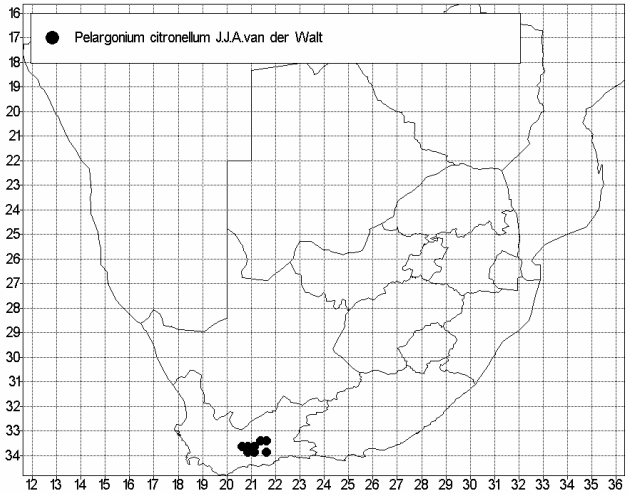


Figure 3.2: The geographical distribution of *Pelargonium citronellum.*

Use:

Pelargonium citronellum is grown as a culinary herb (van der Walt and Vorster, 1988).

Chemical composition of the essential oil:

Fresh plant material collected from the National Botanical Garden (Kirstenbosch) was hydrodistilled for 3 hours and yielded 0.36% of an orange-yellow essential oil.

GC-MS profile and analysis

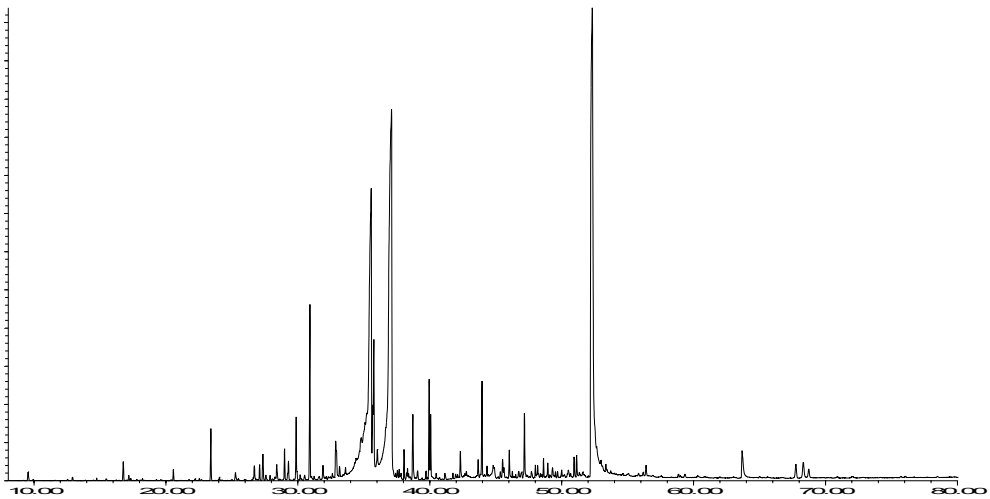


Figure 3.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium citronellum* (NBG).

Table 3.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium citronellum* (NBG).

RRI	Compound	Area %
1048	2-methyl-3-buten-2-ol	0.12
1112	anhydrolinalool oxide	trace
1174	myrcene	trace
1195	dehydro-1,8-cineole	0.20
1203	limonene	0.05
1280	p-cymene	0.11
1348	6-methyl-5-hepten-2-one	0.43
1391	(Z)-3-hexenol	0.12
1419	photocitral B	0.17
1429	perillene	0.05
1450	*trans*-linalool oxide (furanoid)	0.14
1452	1-octen-3-ol	trace
1459	acetic acid	trace
1464	(E)-2-hexenyl butyrate	trace
1473	(Z)-3-hexenyl 2-methylbutyrate	trace
1478	*cis*-linalool oxide (furanoid)	0.14
1482	*cis/cis*-photocitral	0.28
1497	α-copaene	0.05
1503	isomenthone	0.06
1506	*trans/trans*-photocitral	0.56
1535	β-bourbonene	trace
1541	benzaldehyde	0.06
1553	**linalool**	**1.78**
1577	α-cedrene	0.54
1602	6-methyl-3,5-heptadien-2-one	trace
1612	β-caryophyllene	0.59
1613	β-cedrene	trace
1628	aromadendrene	trace
1639	*trans*-p-mentha-2,8-dien-1-ol	0.10
1689	*trans*-piperitol	0.07
1694	**neral**	**17.42**
1706	α-terpineol	0.19
1740	**geranial**	**27.21**
1773	δ-cadinene	0.09
1776	γ-cadinene	trace
1783	p-methylacetophenone	0.15
1786	ar-curcumene	0.22
1798	methyl salicylate	trace
1808	nerol	0.63
1857	geraniol	0.98
1864	p-cymen-8-ol	0.65
1886	geranyl butyrate	0.07
1937	2-phenylethyl alcohol	0.06

RRI	Compound	Area %
1941	α-calacorene	0.06
2001	isocaryophyllene oxide	0.16
2008	caryophyllene oxide	0.89
2030	methyl eugenol	0.11
2050	(E)-nerolidol	trace
2071	humulene epoxide II	trace
2098	globulol	0.23
2104	viridiflorol	trace
2144	spathulenol	0.61
2180	1-tetradecanol	0.08
2239	carvacrol	0.24
2247	*trans*-α-bergamotol	0.08
2254	citronellic acid	0.05
2255	α-cadinol	0.09
2273	1-pentadecanol	0.06
2308	nerolic acid	0.26
2324	caryophylladienol II	trace
2349	**geranic acid**	**35.95**
2392	caryophyllenol II	0.08
	Total	**92.24 %**

trace: <0.05%; NBG = National Botanical Garden (Kirstenbosch).

Geranic acid was identified as the main compound (35.95%) in the essential oil. The aldehydes, neral and geranial, collectively make-up 44.63% of the total oil composition. Linalool, a monoterpene, contributes 1.78% to the total oil composition.

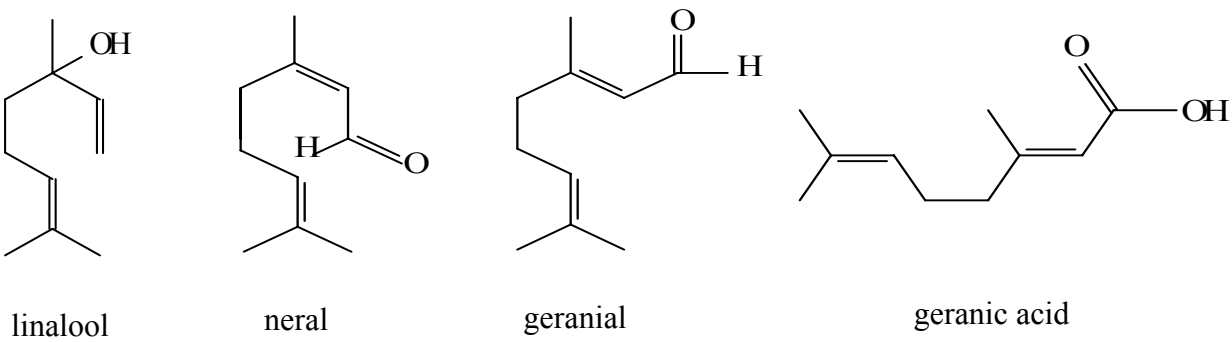


Figure 3.4: Chemical structures of the major compounds identified in the essential oil of *Pelargonium citronellum* (NBG).

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium citronellum* collected from the National Botanical Garden (Kirstenbosch).

HPLC profile and analysis

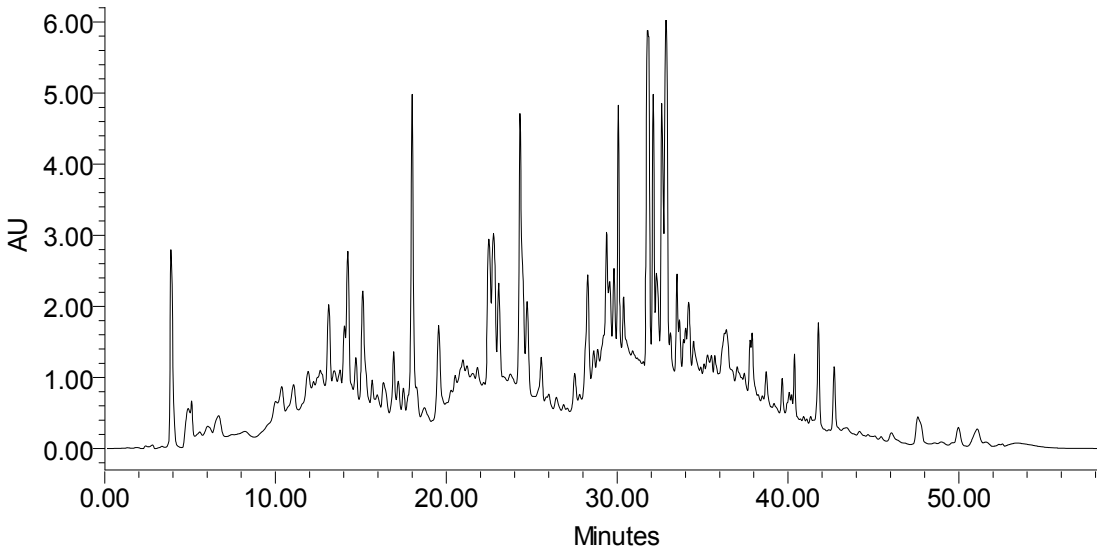


Figure 3.5: HPLC profile of the acetone extract of *Pelargonium citronellum* (NBG).

Table 3.2: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium citronellum* (NBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
4.02	209.7		1.83
10.02	206.2, 273.4		1.61
11.07	206.2, 260.4	isoflavone	1.79
11.92	206.2, 268.7		2.05
12.62	206.2, 280.5		1.42
13.12	206.2, 279.3	flavanone	1.98
14.32	220.3		2.42
14.97	207.3, 277.0		2.65
16.32	284.1		1.28
17.88	206.2, 266.3, 353.2	flavonol	3.67
19.48	209.7, 287.6	flavanone	2.33
20.97	209.7, 271.0	flavanone	1.80
21.82	209.7, 272.2	flavanone	1.36
22.37	220.3		2.68

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
22.90	220.3, 279.3		2.72
22.98	280.5	flavanone	1.86
23.75	209.7, 274.6		1.72
24.49	220.3, 277.0		4.45
24.73	220.3, 272.2		2.04
25.57	220.3, 277.0		1.64
27.52	284.1	flavanone	1.24
28.22	222.6, 2829		2.58
28.63	287.6		1.25
29.38	267.5, 349.6	flavone	3.38
29.67	209.7, 282.9		1.57
29.72	206.2, 274.6, 363.9		1.75
30.16	268.7, 346.0	flavone	2.78
30.46	209.7, 272.2, 349.6		3.02
30.90	279.3		1.47
31.65	287.6		5.11
32.02	272.2, 397.4	flavanone	2.50
32.30	29.7, 268.7		1.97
32.52	275.8		2.98
33.01	268.7, 290.0, 343.6		4.72
33.12	236.8		1.40
33.50	343.6		1.53
34.18	201.5, 273.4		1.69
34.47	222.6, 272.2		2.08
35.28	232.0, 290.0	flavanone	1.08
35.72	235.6, 290.0, 342.4		1.22
36.38	230.9, 273.4, 344.8		4.38
37.03	234.4, 290.0	flavanone	1.70
37.90	234.4, 300.7, 349.6		1.41
38.73	239.1, 269.9, 338.9		1.31
41.78	236.8, 301.9, 349.6		1.51
42.70	237.9, 277.0		1.05

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium citronellum* in this study include the following:

- Both *P. citronellum* acetone extracts showed substantial antibacterial activity against the Gram-positive bacteria. In particular, an MIC value of 0.16 mg/ml was produced by both the extract samples against *S. aureus. Pelargonium citronellum* (NBG) essential oil displayed moderate antimicrobial activity (MIC = 4 mg/ml) against the Gram-positive bacteria and the yeast strain.

- The *P. citronellum* (SBG) extract produced low anti-oxidant activity (IC_{50} = 84.01 ± 16.08 µg/ml) whereas the *P. citronellum* (NBG) extract demonstrated promising anti-oxidant activity (IC_{50} = 23.70 ± 3.68 µg/ml).

- *Pelargonium citronellum* essential oil (NBG) displayed moderate anti-inflammatory activity (IC_{50} = 50.04 ± 7.06 µg/ml).

- Both *P. citronellum* extract samples (NBG and SBG) showed pronounced antimalarial activity (IC_{50} = 1.58 ± 0.65 µg/ml and 1.74 ± 0.79 µg/ml, respectively).

- The *P. citronellum* (NBG) extract produced substantial cytotoxicity (IC_{50} = 19.14 ± 0.98 µg/ml), as did the essential oil (NBG) which produced an IC_{50} value of 22.40 ± 4.77 µg/ml in the MTT assay. The *P. citronellum* extract (SBG) produced a lower toxicity profile (IC_{50} = 59.94 ± 2.33 µg/ml).

4. *Pelargonium cordifolium* (Cav.) Curtis

Synonym:

P. cordatum L'Hérit. (van der Walt, 1977).

Common name:

Heart-leaved pelargonium (van der Walt, 1977).

Botanical description:

Pelargonium cordifolium is a branching shrub reaching a height of 1-1.75 m. The main stem is woody at the base and has herbaceous side-branches covered with hairs. The leaves are cordate and flat or curled. The margin of the leaf is finely to coarsely toothed and unlobed or shallowly lobed. The lower surface of the leaf is lighter in colour and has a velvety texture due to the soft, wool-like hairiness. The extent of hairiness varies greatly. Plants found in the region of Humansdorp have leaves bearing many hairs, whereas some plants growing in the George area have leaves almost devoid of hairs. Three varieties have been documented on the basis of leaf characteristics. The branched inflorescence terminates in several 4-8-flowered umbel-like groups. The flowers resemble those of *P. hispidum* and *P. papilionaceum*, the posterior two petals being larger than the three anterior ones. The posterior pink or purple petals have dark purple veins. The anterior petals are lightly coloured. Seven fertile stamens are present (van der Walt, 1977; 1985).

Distribution and habitat:

Pelargonium cordifolium grows mainly near the coast in southern and eastern Cape. It grows in damp places in Fynbos or at forest margins or in forests (van der Walt, 1977).

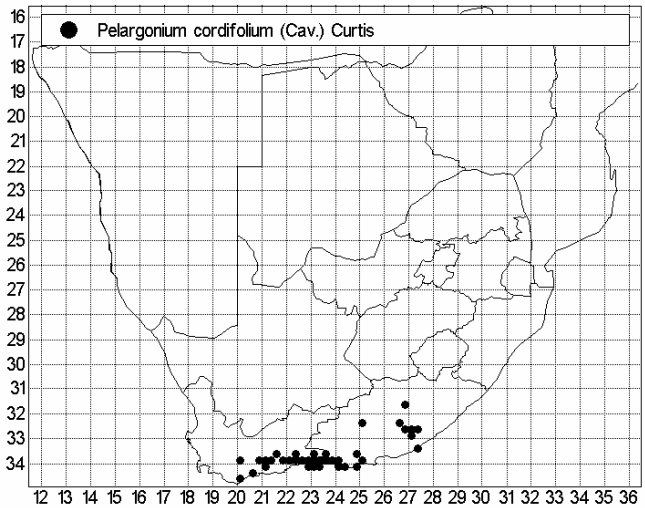


Figure 4.1: *Pelargonium cordifolium.*

Figure 4.2: The geographical distribution of *Pelargonium cordifolium.*

The essential oil of *P. cordifolium* was not obtained due to insufficient plant material.

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium cordifolium* collected from the Walter Sisulu Botanical Garden.

HPLC profile and analysis

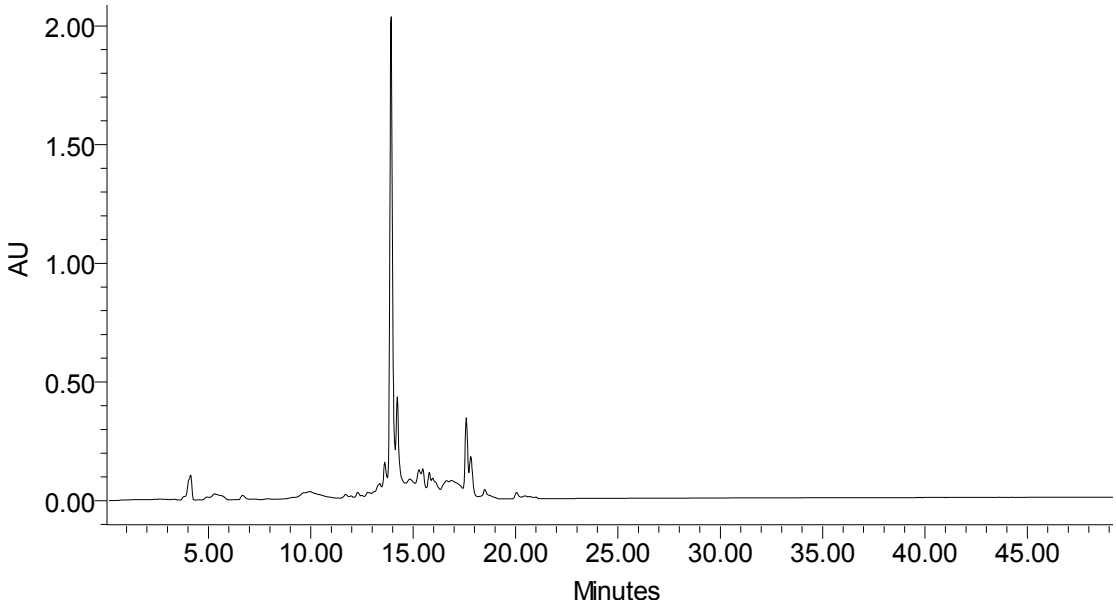


Figure 4.3: HPLC profile of the acetone extract of *Pelargonium cordifolium* (WSBG).

Table 4.1: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of *Pelargonium cordifolium* (WSBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
4.13	209.8		4.25
13.62	221.5, 281.5		4.45
13.92	221.5, 278.0		52.24
14.23	221.5, 278.0		14.41
15.29	222.7, 279.2		4.41
15.46	272.1, 351.6	flavanone	3.50
15.79	223.8, 275.6		3.10
17.59	255.6, 354.0	flavonol	8.69
17.82	221.5, 279.2		4.95

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium cordifolium* in this study include the following:

- The acetone extract (NBG) produced promising antimicrobial activity. The extract was most active against *B. cereus* (MIC = 0.25 mg/ml).

- Both extract samples (NBG and WSBG) exerted pronounced anti-oxidant activity in the DPPH assay with IC_{50} values of 5.01 ± 0.55 µg/ml and 5.31 ± 0.24 µg/ml, respectively.

- Substantial antimalarial activity was displayed by both acetone extracts (IC_{50} = 10.17 ± 2.43 µg/ml – 10.84 ± 0.75 µg/ml).

- *Pelargonium cordifolium* extract (NBG) produced an IC_{50} = 74.70 ± 2.17 µg/ml in the MTT assay. *Pelargonium cordifolium* (WSBG) extract produced a lower toxicity profile (IC_{50} value of 116.88 ± 8.01 µg/ml).

5. *Pelargonium crispum* (P. J. Bergius) L'Hérit.

Common names:

Crisped-leaf pelargonium (van der Walt and Vorster, 1988), Lemon geranium (http://www.ibiblio.org/pfaf/cgi-bin/arr_html?Pelargonium+crispum).

Botanical description:

Pelargonium crispum is an erect to decumbent shrubby species and has a height ranging from 0.2-0.75 m. The densely pubescent to strigose slender, branching stems become woody and brownish with age. The stems are interspersed with glandular hairs. The leaves are arranged alternatively along the stem. The slightly glabrous or pubescent leaf blades are somewhat trilobed and curled, dentate and cuneate at their bases. Leaf margins are coarsely dentate-serrate. The species name '*crispum*' was derived from the evidently crisped (curly) leaves. The crushed leaves release a strong lemon scent much like lemon verbena (Genders, 1994). The inflorescence consists of 1-2(-3)-flowered pseudo-umbels. Petal colour ranges from white to dark pink-purple. The posterior two broadly spathulate, obovate petals have a carmine-rose colouring with dark red to dark purple markings veining towards the clawed petal base. The anterior rose or pale rose petals are spathulate with narrow claws. Each flower bears seven fertile stamens (Webb, 1984; van der Walt and Vorster, 1988).

Distribution and habitat:

Pelargonium crispum is found in the south-western winter rainfall region of the Cape. It grows on the lower slopes of mountains or on hills where the rainfall is low compared to the areas higher up on the mountains. It is found growing in sandy soil in the shelter of sandstone boulders (van der Walt, 1985; van der Walt and Vorster, 1988).

Figure 5.1: *Pelargonium crispum* in flower.

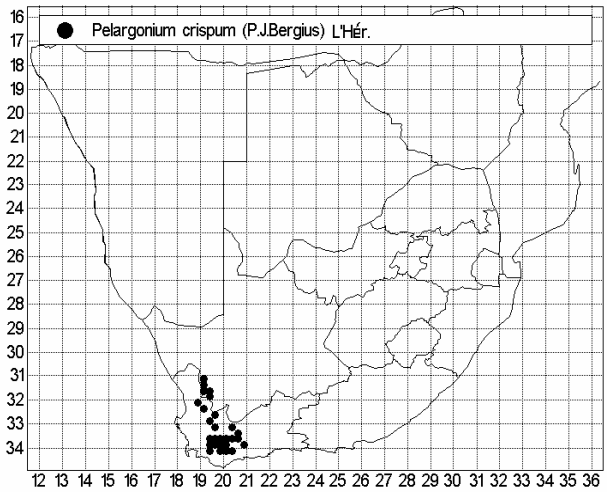


Figure 5.2: The geographical distribution of *Pelargonium crispum.*

Medicinal uses:

All parts of the plant are astringent (Grieve, 1984). When required it can be harvested and used fresh (Bown, 1995).

Edible uses:

Due to the lemon aroma of the leaves, they are crushed and added as a flavourant to soups, fruit dishes, jellies, sorbets, ice cream and cakes (Facciola, 1990; Bown, 1995). The leaves can be used to line cake pans and in this way the essence infuses into the pastry (Facciola, 1990). A tea can be made from the infusions of the leaves (Bown, 1995).

Other uses:

The strawberry and citral-scented essential oil extracted from the leaves and young shoots is used in the perfumery and soap-making industry (Usher, 1974). Dried leaves are used for pot-pourri and for making herb pillows (Bown, 1995).

Remark:

Pelargonium crispum is closely related to *P. hermanniifolium* (Berg.) Jacq. (Webb, 1984).

The essential oil of *P. crispum* was not obtained due to insufficient plant material.

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium crispum* collected from the National Botanical Garden (Kirstenbosch).

HPLC profile and analysis

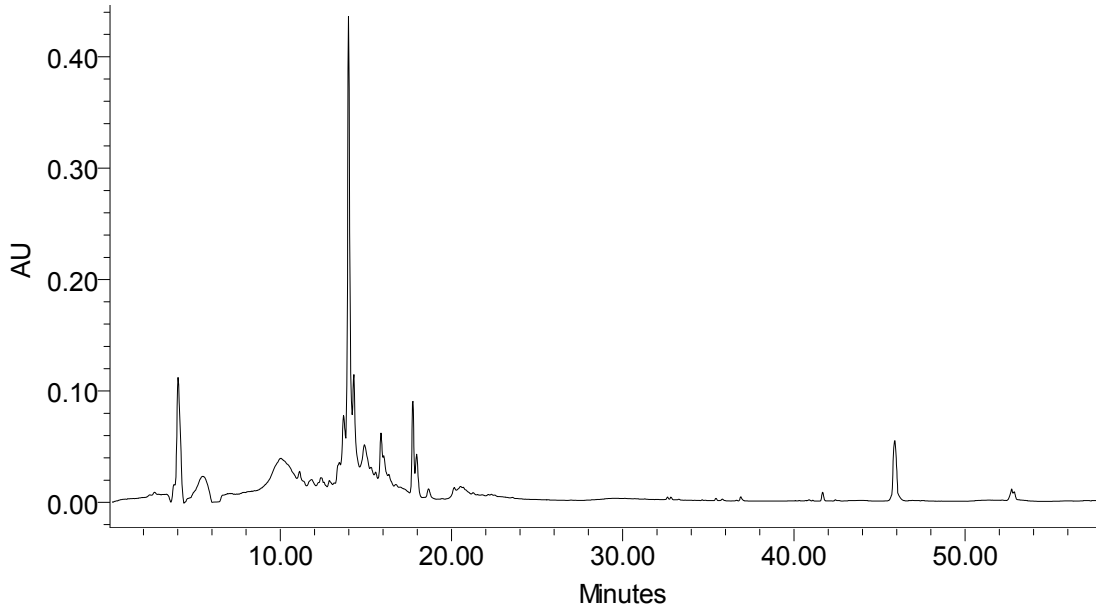


Figure 5.3: HPLC profile of the acetone extract of *Pelargonium crispum* (NBG).

Table 5.1: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium crispum* (NBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
4.03	212.1		15.31
5.52	203.9		7.58
10.03	205.1, 278.0		6.23
13.69	221.5, 278.0		6.39
13.98	220.3, 278.0		34.87
14.30	221.5, 278.0		8.92
14.91	222.7,278.0		2.78
15.88	225.0, 278.0		4.43
17.74	205.1, 255.6, 354.0	flavonol	5.82
17.96	223.8, 278.0		1.49
45.88	293.4		6.19

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium crispum* in this study include the following:

- The *P. crispum* (NBG) extract showed promising antimicrobial activity. MIC values of 0.38 mg/ml and 0.56 mg/ml were produced for the Gram-positive micro-organisms, *B. cereus* and *S. aureus*, respectively.

- *Pelargonium crispum* (NBG) was the second most active species in the DPPH assay, the acetone extract produced an $IC_{50} = 4.49 \pm 0.18$ µg/ml.

- The acetone extract (NBG) produced promising antimalarial activity ($IC_{50} = 21.00 \pm 4.33$ µg/ml).

- The *P. crispum* (NBG) acetone extract produced low cytotoxicity ($IC_{50} = 74.02 \pm 2.30$ µg/ml) in the MTT assay.

6. *Pelargonium cucullatum* (L.) L'Hérit.

Common names:

Hooded-leaf pelargonium, Tree pelargonium, 'Wilde malva' (van der Walt, 1977).

Botanical description:

This species can grow to a height of more than 2 m making it one of the tallest *Pelargonium* shrubs. It is a widespread, sprawling, branched shrub with the base of the main stem half-woody. The younger parts and side branches are succulent. The species name comes from the Latin word '*cucullatus*' meaning "hood" and refers to the characteristic shape of the cupped leaves. The leaves of the variety from the Table Mountain region are round or kidney-shaped and covered with long soft hairs. The margins of the leaves are slightly incised, irregularly toothed and have reddish tips. The leaves of plants from other areas along the coast are less cupped, have angularly incised margins, fewer hairs and characterize a transitional form to the leaves of the closely related, inland growing *P. angulosum*. The leaves of some forms release a strong billy goat-like scent when bruised. Four to ten flowers are borne on umbel-like inflorescences. The flowers are large and slightly sweet scented. There is a resemblance between the flowers of *P. cucullatum* and *P. betulinum.* Flower colour ranges from dark purple to light mauve. White-petalled forms also occur, but are rarely found. The posterior two petals are larger than the anterior three. Purple or red streaked veins occur on all five petals, but are more prominent on the posterior two petals. Seven fertile stamens are present (van der Walt, 1977; van der Walt and Vorster, 1981; Webb, 1984).

Distribution and habitat:

This species is the most conspicuous of the Pelargoniums growing in the south-western Cape (van der Walt, 1977). It occurs near the coast between Saldanha and Elim (west of Bredasdorp) (van der Walt, 1977). It has a continuous distribution from Gordon's Bay in the west to the area of Gansbaai in the east. A few isolated populations in the Cape Peninsula are separated from the rest by False Bay. The plants grow from near the high water mark on the narrow coastal flats to the lower foothills of the mountains (van der Walt, 1985). This species grows on sandy and granite slopes (May, 2000).

Figure 6.1: *Pelargonium cucullatum* in flower.

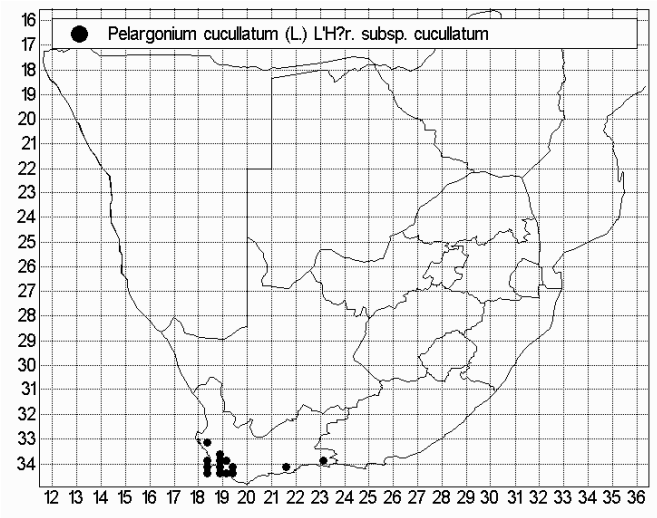


Figure 6.2: The geographical distribution of *Pelargonium cucullatum.*

Traditional uses:

Pelargonium cucullatum was traditionally used to treat kidney ailments, colic, diarrhoea, coughs and fevers (van der Walt, 1977; May, 2000). For the treatment of bruises, stings and abscesses, the leaves were used as a poultice (May, 2000). It was also used as an emollient and as an antispasmodic (van der Walt, 1977).

Remarks:

Pelargonium cucullatum is one of the principal ancestors of regal pelargoniums (Webb, 1984). Natural hybrids occur between *P. cucullatum* and *P. betulinum* (van der Walt, 1977).

The essential oil of *P. cucullatum* was not obtained due to insufficient plant material.

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium cucullatum* collected from the Stellenbosch Botanical Garden.

HPLC profile and analysis

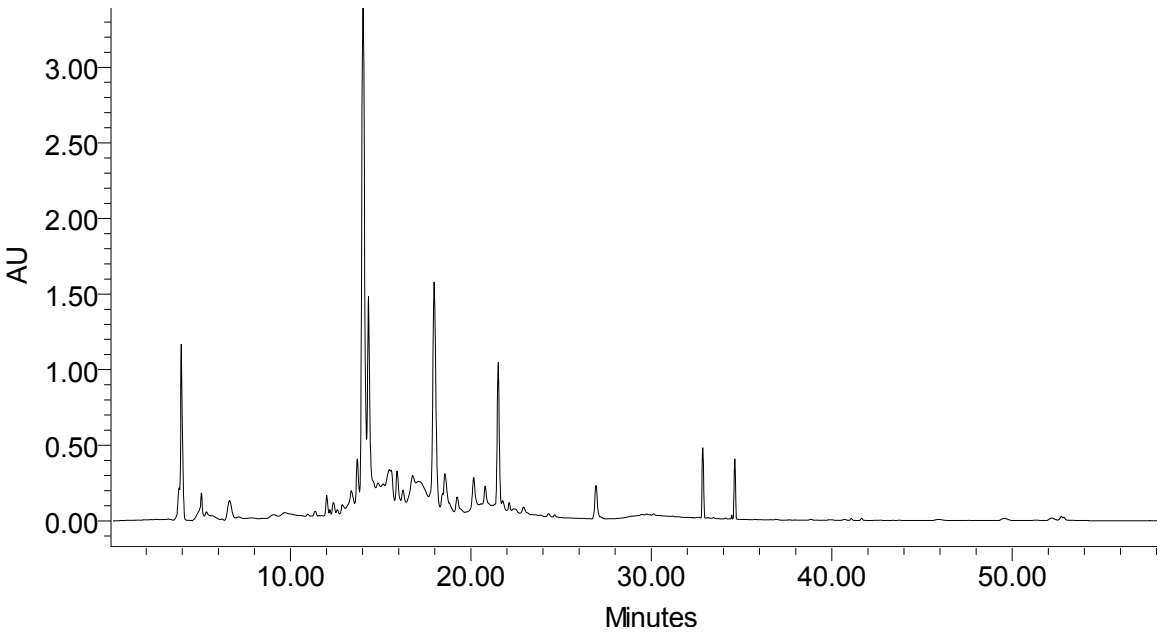


Figure 6.3: HPLC profile of the acetone extract of *Pelargonium cucullatum* (SBG).

Table 6.1: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium cucullatum* (SBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
3.95	213.3		5.14
5.07	278.0		1.11
6.61	215.6, 270.9		1.28
12.02	218.0, 327.8		0.67
12.38	278.0	flavanone	0.54
12.87	221.5, 278.0		0.63
13.36	220.3, 278.0		2.02
13.71	220.3, 281.5		2.27
14.10	221.5, 279.2		24.39
14.33	221.5, 278.0		10.52
14.83	220.3, 278.0		2.05
15.52	221.5, 280.3		4.07
15.92	221.5, 278.0		2.24
16.23	221.5, 268.5, 349.2		1.43
16.76	220.3, 278.0		3.73
17.20	221.5, 280.3		5.11
17.97	220.3, 278.0		12.80

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
18.57	253.2, 363.4	isoflavone	3.44
19.25	207.4, 255.6, 351.6		1.20
20.16	221.5, 278.0		2.57
20.79	207.4, 256.7, 351.6		2.39
21.52	255.6, 348.0	flavone	5.81
26.93	255.6, 366.8	flavonol	1.46
32.85	261.5		1.72
34.63	265.0		1.41

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium cucullatum* in this study include the following:

- Both *P. cucullatum* (SBG) and *P. cucullatum* (WSBG) extracts showed greater selectivity for the Gram-positive micro-organism *B. cereus* (MIC = 0.25 mg/ml and 0.5 mg/ml, respectively).

- The *P. cucullatum* (WSBG) extract produced considerable anti-oxidant activity (IC_{50} = 10.91 ± 0.54 µg/ml), whereas the *P. cucullatum* (SBG) extract produced moderate anti-oxidant activity (IC_{50} = 40.18 ± 5.65 µg/ml).

- The *P. cucullatum* (SBG) extract displayed substantial antimalarial activity (IC_{50} = 7.42 ± 0.35 µg/ml).

- The acetone extracts (SBG and WSBG) produced low toxicity profiles (IC_{50} = 73.81 ± 2.62 µg/ml and 118.89 ± 5.32 µg/ml, respectively) in the MTT assay.

7. *Pelargonium glutinosum* (Jacq.) L'Hérit.

Synonyms:

Geranium viscosum, *Geranium crataegifolium*, *Pelargonium erectum* (van der Walt and Vorster, 1988).

Common name:

Pheasant's foot geranium
(http://www.ibiblio.org/pfaf/cgi-bin/arr_html?Pelargonium+glutinosum).

Botanical description:

Pelargonium glutinosum is an erect, branching shrub growing up to 1.8 m tall. It is noted for the glaucous appearance of its foliage. The shape of the hairs which cover the plant cause it to be sticky (the species name '*glutinosum*' means very sticky). This species emits a pungent balm scent. The young stems are herbaceous and green becoming woody and brownish with age. The leaves are glabrous and palmately 5-lobed. The lobes have large, pinnately incised points. The lamina is cordiform in outline. Short, sharp-pointed hairs occur on the finely to coarsely dentate-serrate margins. One to eight flowers occur on each pseudo-umbel. Flower colour ranges from pale pink to dark pink. Dark pink-purplish markings and a large carmine spot occur on the posterior spathulate petals. The anterior three petals are spathulate with long narrow claws. The flowers have seven fertile stamens (Webb, 1984; van der Walt and Vorster, 1988).

Distribution and habitat:

It occurs from Piquetberg in the south-western Cape to the Kei River in the Eastern Cape. Its distribution pattern seems to be mainly associated with mountain ranges. In the Karoo regions where the annual rainfall is low, it is restricted to a mountainous habitat with rainfall very much higher compared to the nearby lower-lying regions. It grows in dry rocky places and in moist areas usually near running water such as streams (van der Walt and Vorster, 1988; Phillips and Rix, 1998).

Figure 7.1: *Pelargonium glutinosum*.

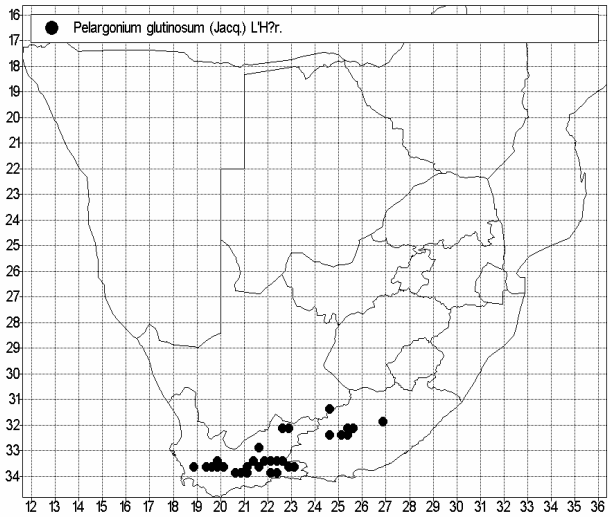


Figure 7.2: The geographical distribution of *Pelargonium glutinosum.*

Medicinal use:

All parts of the plant are astringent (Grieve, 1984).

Other use:

The essential oil has a labdanum fragrance (Uphof, 1959).

Remark:

The leaves of *P. panduriforme* Eckl. & Zeyh. and *P. quercifolium* (L.f.) L'Hérit. are also sticky and release the same balm scent as *P. glutinosum* (van der Walt and Vorster, 1988).

Chemical composition of the essential oil:

Fresh plant material collected from Stellenbosch Botanical Garden was subjected to hydrodistillation for 3 hours. The essential oil yield was 0.02%.

GC-MS profile and analysis

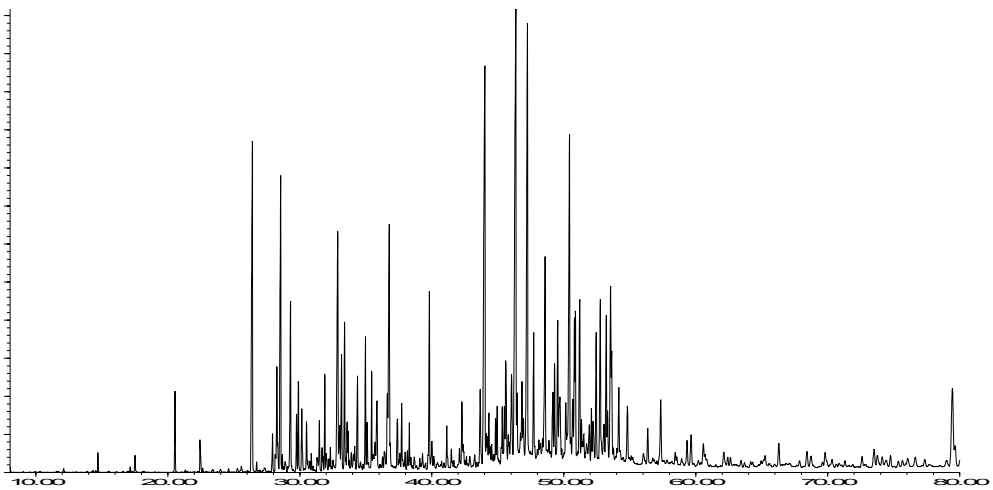


Figure 7.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium glutinosum* (SBG).

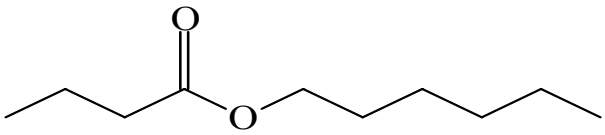
Table 7.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium glutinosum* (SBG).

RRI	Compound	Area %
1100	undecane	trace
1203	limonene	trace
1213	1,8-cineole	0.12
1280	p-cymene	0.46
1434	**hexyl butyrate**	**3.22**
1459	acetic acid	0.27
1464	(E)-2-hexenyl butyrate	2.72
1466	α-cubebene	0.24
1475	menthone	0.67
1493	α-ylangene	0.13
1503	isomenthone	1.42
1503	hexyl valerate	0.10
1508	heptyl butyrate	0.41
1532	camphor	trace
1535	β-bourbonene	0.33
1549	β-cubebene	0.08
1553	linalool	0.14
1576	pentyl hexanoate	0.28
1612	β-caryophyllene	2.51
1614	hexyl tiglate	0.83
1617	guaia-6,9-diene	0.32

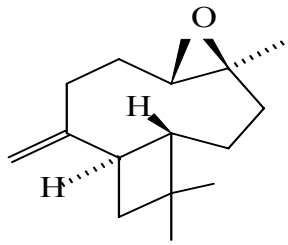
RRI	Compound	Area %
1618	cadina-3,5-diene	0.32
1628	aromadendrene	0.70
1637	menthol	0.13
1647	(E)-2-decenal	0.14
1661	allo-aromadendrene	0.71
1687	α-humulene	0.19
1706	α-terpineol	0.22
1707	ledene	0.87
1740	α-muurolene	trace
1750	β-dihydroagarofuran	0.43
1763	naphthalene	0.51
1776	γ-cadinene	trace
1783	drimenene	0.14
1853	*cis*-calamenene	1.30
1868	(E)-geranyl acetone	0.18
1886	geranyl butyrate	0.31
1916	α-agarofuran	0.14
1941	α-calacorene	0.18
1953	palustrol	0.17
2001	isocaryophyllene oxide	0.77
2008	**caryophyllene oxide**	**6.32**
2012	maaliol	0.18
2030	methyl eugenol	0.27
2050	(E)-nerolidol	0.23
2057	ledol	0.44
2071	humulene epoxide II	0.60
2080	cubenol	1.00
2088	1-epi-cubenol	0.21
2098	globulol	0.86
2104	**viridiflorol**	**8.88**
2127	10-epi-γ-eudesmol	0.34
2144	**spathulenol**	**6.92**
2196	ambrox	2.32
2247	trans-α-bergamotol	1.18
2249	8,13-epoxy-15,16-dinor-labdane	0.39
2279	sandaracopimaradiene	1.02
2305	8,13-epoxy-15,16-dinor-labd-12-ene	2.32
2324	caryophylladienol II	0.08
2376	manoyl oxide	1.83
2396	8α-13-oxo-14-en-epi-labdane	1.81
2438	kaur-16-ene	0.73
	Total	**59.59%**

trace: <0.05%; (SBG) = Stellenbosch Botanical Garden.

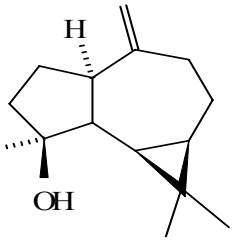
Viridiflorol makes-up 8.88% of the total oil composition. Caryophyllene oxide and spathulenol contribute 6.32% and 6.92%, respectively. Ambrox, an important constituent of the scarce natural ambergris, represents 2.32%.



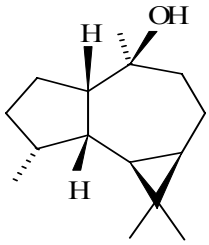
hexyl butyrate



caryophyllene oxide



viridiflorol



spathulenol

Figure 7.4: Chemical structures of the major compounds identified in the essential oil of *Pelargonium glutinosum* (SBG).

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium glutinosum* collected from Stellenbosch Botanical Garden.

HPLC profile and analysis

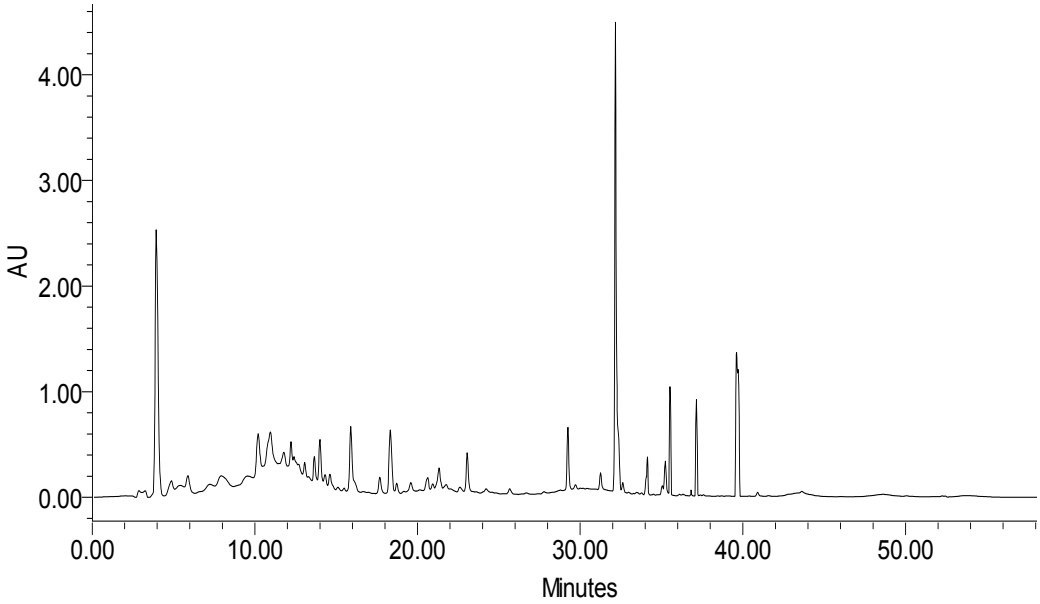


Figure 7.5: HPLC profile of the acetone extract of *Pelargonium glutinosum* (SBG).

Table 7.2: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium glutinosum* (SBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
4.07	212.0		13.38
4.87	205.0		1.33
5.43	203.8		1.41
5.88	203.8, 269.9		1.63
7.24	205.0		1.78
7.94	207.3		4.04
9.55	207.3		4.16
10.21	205.0, 271.0		5.03
10.97	207.3		9.58
11.82	207.3, 271.0, 307.8		4.66
12.24	207.3, 272.2		3.01
12.41	202.7, 281.7, 313.8		3.11
12.73	200.5, 328.1		1.11
13.07	205.0, 280.5		1.71
13.65	272.2		1.81
14.01	210.9, 281.7		2.47
14.62	200.5, 268.7		1.18
15.90	255.7, 355.6	flavonol	3.35
18.34	262.7, 349.6	flavone	3.05

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
21.32	255.7, 349.6	flavone	1.14
23.06	262.7, 350.8	flavone	1.44
29.25	267.5, 336.5	flavone	2.08
32.26	268.7, 329.3	flavone	13.64
34.15	250.9, 268.7, 343.6		1.10
35.23	241.5		1.14
35.53	261.6		2.58
37.14	261.6		2.46
39.62	268.7		6.63

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium glutinosum* in this study include the following:

- The *P. glutinosum* (SBG) extract displayed pronounced antibacterial activity (MIC = 0.078 mg/ml) against *B. cereus* and *S. aureus*. The MIC values produced by the *P. glutinosum* acetone extract (WSBG) against *B. cereus* and *S. aureus* were 0.25 mg/ml and 0.5 mg/ml, respectively.

- *Pelargonium glutinosum* (SBG) produced promising anti-oxidant activity (IC_{50} = 16.41 ± 0.33 µg/ml) in the DPPH assay. The *P. glutinosum* (WSBG) extract displayed lower activity (IC_{50} = 29.17 ± 0.78 µg/ml).

- Substantial antimalarial activity was produced by the acetone extract (SBG) (IC_{50} = 11.07 ± 1.15 µg/ml) in the hypoxanthine incorporation assay.

- Both extracts (SBG and WSBG) proved to be cytotoxic (IC_{50} = 31.44 ± 1.27 µg/ml – 46.29 ± 0.81 µg/ml) in the MTT assay.

8. *Pelargonium graveolens* L'Hérit.

Synonyms:

Geranium radula, Pelargonium terebinthinaceum, Pelargonium asperum, Pelargonium intermedium (van der Walt and Vorster, 1988).

Common names:

Pelargonium roseum (O'Brien, 1983), Rose-scented pelargonium (van der Walt and Vorster, 1988), Rose geranium, Old fashion rose geranium, Sweet-scented geranium (http://www.ibiblio.org/pfaf/cgi-bin/arr_html?Pelargonium+graveolens).

Botanical description:

Pelargonium graveolens is a much-branched shrub growing up to 1.3 m high. It is strongly aromatic having a fine, true-rose scent ('graveolens' is the Latin word meaning heavily scented). The stems are herbaceous and green when young, becoming woody and brownish with age. The stems are densely interspersed with glandular hairs. The leaves are grey-green and slightly hairy. The leaf blades are ovate to depressed ovate, pinnatipartite or even almost palmati-partite-cordate at the bases. The two large basal segments are usually further subdivided into five lobes. The peduncle has an umbel which bears 2-7 flowers. The petals have narrowly obovate bases, rounded tops and are entire in shape. The petals are white to pinkish-purple (rose-coloured). The two posterior petals are spathulate and each have a large carmine spot in the centre. The anterior petals are spathulate to oblanceolate with narrow claws. The flowers have seven fertile stamens (Webb, 1984; van der Walt and Vorster, 1988).

Distribution and habitat:

Pelargonium graveolens has two areas of distribution in southern Africa. One area of distribution is in Limpopo and the other in the south-eastern region of the Cape. In Limpopo, it occurs from Blouberg in the west to Wolkberg near Pilgrim's Rest in the east. In the Cape, its distribution occurs from near George in the west to Grahamstown in the east. It is found near the Hex River in the south-western Cape (Webb, 1984). It is speculated that in earlier times, *P. graveolens* had a distribution which was continuous along mountain ranges from Zimbabwe to the Cape. It is found growing in moist places which are semi-shaded (van der Walt and Vorster, 1988).

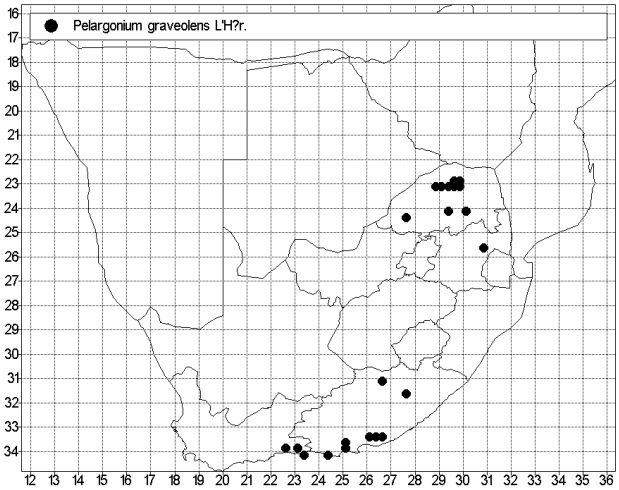


Figure 8.1: *Pelargonium graveolens.*

Figure 8.2: The geographical distribution of *Pelargonium graveolens.*

Culinary uses:

The raw flowers are added to salads (Facciola, 1990). The rose-scented leaves are added to desserts, jellies and vinegars. The fresh leaves are brewed to make a tea (Facciola, 1990; Bown, 1995).

Medicinal uses:

This plant has relaxant, anti-depressant and antiseptic effects. It reduces inflammation and controls bleeding (Bown, 1995). All parts of the plant are astringent (Grieve, 1984). Internally, it is used to treat pre-menstrual and menopausal problems, nausea, tonsillitis and poor circulation. It is used externally for the treatment of acne, haemorrhoids, eczema, bruises, ringworm and lice (Bown, 1995). The essential oil is applied locally in cervical cancer (Duke and Ayensu, 1985).

Other uses:

The 'geranium oil' obtained from *P. graveolens* is used in aromatherapy and in skin care. It is also used as a flavouring agent. The leaves are used in pot-pourri (Bown, 1995).

Remark:

The similar floral features and scent indicate a close relationship between *P. graveolens* and *P. radens.* Leaf characteristics distinguish these two species from one another (van der Walt and Vorster, 1988).

Chemical composition of the essential oil:

Hydrodistillation was carried out on fresh plant material for 3 hours.

1. *Pelargonium graveolens* from Stellenbosch Botanical Garden yielded 0.22% oil,
2. *Pelargonium graveolens* from Walter Sisulu Botanical Garden yielded 0.06% oil.

GC-MS profiles and analyses

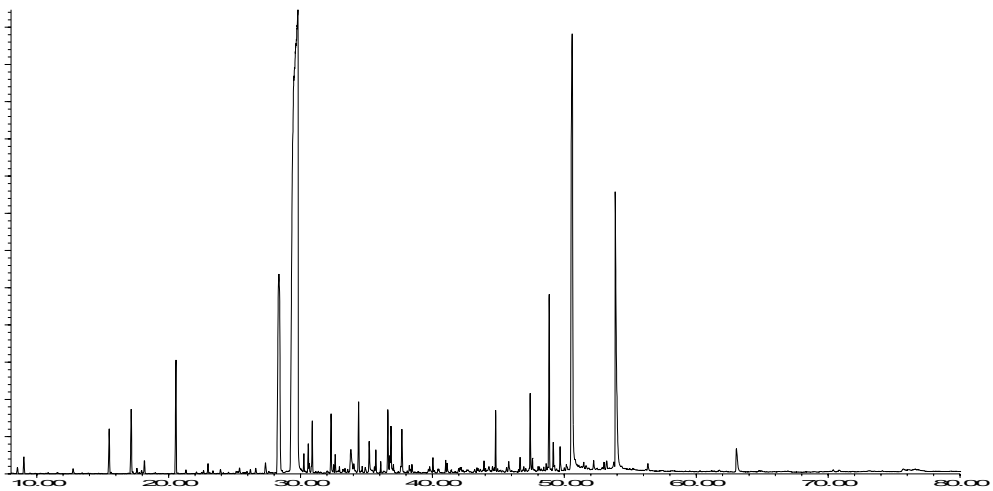


Figure 8.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium graveolens* (SBG).

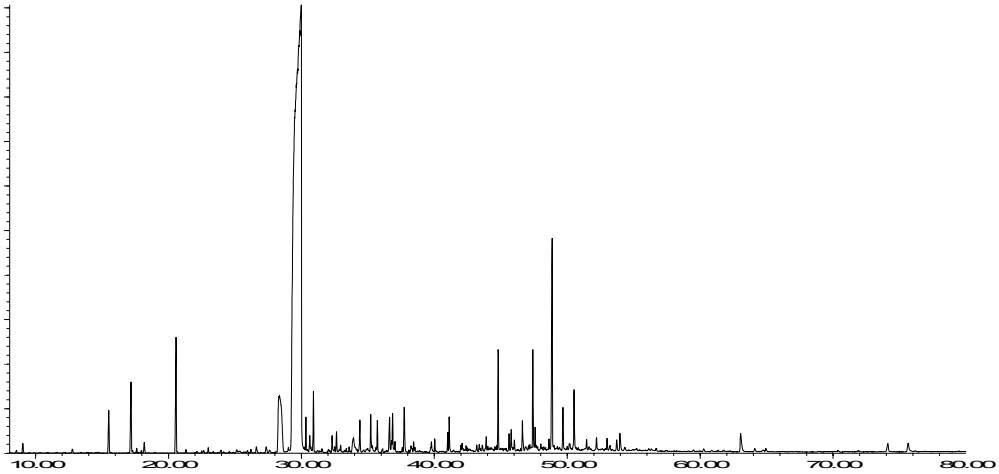


Figure 8.4: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium graveolens* (WSBG).

Table 8.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium graveolens*.

RRI	**Compound**	**Area %**	
		SBG	**WSBG**
1014	tricyclene	0.07	trace
1032	α-pinene	0.16	0.09
1118	β-pinene	0.05	trace
1174	myrcene	0.38	0.34
1203	limonene	0.56	0.57
1216	3-methylcyclopentanone	0.12	trace
1218	β-phellandrene	trace	trace
1280	p-cymene	0.87	0.85
1327	3-methylcyclohexanone	0.08	trace
1353	*cis*-rose oxide	trace	trace
1365	*trans*-rose oxide	-	trace
1384	α-pinene oxide	-	trace
1391	(Z)-3-hexenol	trace	-
1398	2-nonanone	trace	-
1429	perillene	trace	0.05
1450	*trans*-linalool oxide (furanoid)	0.06	trace
1458	*cis*-1,2-limonene epoxide	trace	trace
1475	**menthone**	**5.12**	**1.03**
1481	(Z)-3-hexenyl isovalerate	-	trace
1503	**isomenthone**	**65.78**	**83.30**
1535	β-bourbonene	trace	trace
1553	linalool	0.34	0.34
1553	octanol	-	trace

RRI	Compound	Area %	
		SBG	WSBG
1571	*trans*-p-menth-2-en-1-ol	-	trace
1580	*cis*-isopulegone	-	0.11
1586	pinocarvone	-	trace
1587	methyl decanoate	0.10	-
1600	β-elemene	-	0.14
1606	p-menth-3-en-8-ol	trace	trace
1612	citronellyl formate	trace	trace
1632	neoisomenthol	0.38	-
1637	menthol	0.12	0.24
1638	*cis*-p-menth-2-en-1-ol	-	0.26
1662	isomenthol	0.07	-
1668	(Z)-β-farnesene	trace	0.06
1690	cryptone	0.27	0.28
1704	γ-muurolene	0.05	trace
1706	α-terpineol	0.14	0.16
1719	borneol	-	trace
1740	α-muurolene	-	0.25
1741	β-bisabolene	0.46	-
1748	piperitone	0.35	0.27
1751	carvone	0.08	0.08
1772	citronellol	0.35	0.38
1773	δ-cadinene	trace	trace
1783	p-methylacetophenone	0.07	0.06
1798	methyl salicylate	0.06	-
1802	cuminaldehyde	trace	0.06
1809	citronellyl butyrate	-	0.05
1845	*trans*-carveol	0.07	-
1853	*cis*-calamenene	trace	trace
1864	p-cymen-8-ol	0.11	0.10
1868	(E)-geranyl acetone	trace	-
1885	2-phenylethyl propionate	-	0.20
1889	ascaridole	trace	-
1896	2-phenylethyl isobutyrate	-	0.09
1937	2-phenylethyl alcohol	-	0.08
1941	citronellyl hexanoate	-	0.05
1941	α-calacorene	0.06	-
1949	piperitenone	trace	-
1984	γ-calacorene	trace	-
1988	2-phenylethyl 2-methylbutyrate	trace	-
2008	caryophyllene oxide	0.05	0.12
2030	norbourbonone	trace	trace
2050	(E)-nerolidol	trace	0.51
2080	cubenol	trace	0.09
2088	1-epi-cubenol	0.08	0.13
2113	cumin alcohol	-	trace
2131	hexahydrofarnesylacetone	trace	-

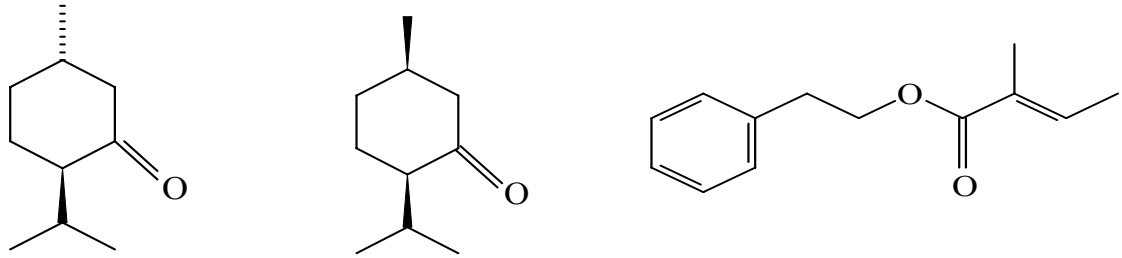
RRI	Compound	Area %	
		SBG	WSBG
2140	2-phenylethyl valerate	-	0.23
2143	cedrol	-	trace
2187	T-cadinol	-	trace
2198	thymol	trace	-
2209	T-muurolol	trace	0.07
2211	**2-phenylethyl tiglate**	**1.32**	**1.90**
2239	carvacrol	0.06	-
2249	cadalene	0.12	0.30
2254	citronellic acid	0.09	trace
2281	**decanoic acid**	**12.93**	0.42
2340	10-hydroxy-calamenene	trace	-
2407	hydroxy-α-calacorene*	0.07	0.11
2411	**2-decenoic acid***	**3.93**	0.12
2500	pentacosane	-	trace
2509	dodecanoic acid	0.06	trace
2617	tridecanoic acid	-	trace
2670	tetradecanoic acid	-	0.06
2700	heptacosane	-	trace
2900	nonacosane	-	0.14
2931	hexadecanoic acid	-	0.11
	Total	**95.04%**	**93.80%**

*correct isomer not identified; trace: <0.05%.

Isomenthone is the major essential oil compound in *P. graveolens* (SBG) contributing 65.78%. Decanoic acid made-up 12.93% of the total oil composition. Collectively, menthone, 2-phenylethyl tiglate and 2-decenoic acid contributed 10.37% to the total oil composition.

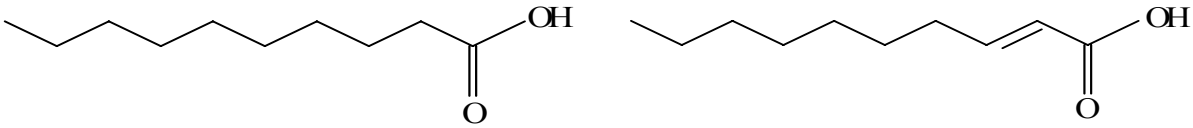
Isomenthone is the major compound (83.30%) in *P. graveolens* (WSBG) oil. Menthone and 2-phenylethyl tiglate occur at 1.03% and 1.90%, respectively.

The two oil samples display quantitative and qualitative similarities in their essential oil compositions. The major compound isomenthone is present in both the oil samples. Furthermore, menthone and 2-phenylethyl tiglate occur in substantial amounts in both the oil samples.



menthone isomenthone 2-phenylethyl tiglate

Figure 8.5: Chemical structures of the major compounds common to both essential oil samples of *Pelargonium graveolens.*



decanoic acid 2-decenoic acid

Figure 8.6: Chemical structures of the other major compounds identified in the essential oil of *Pelargonium graveolens* (SBG).

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium graveolens* collected from the Stellenbosch Botanical Garden.

HPLC profile and analysis

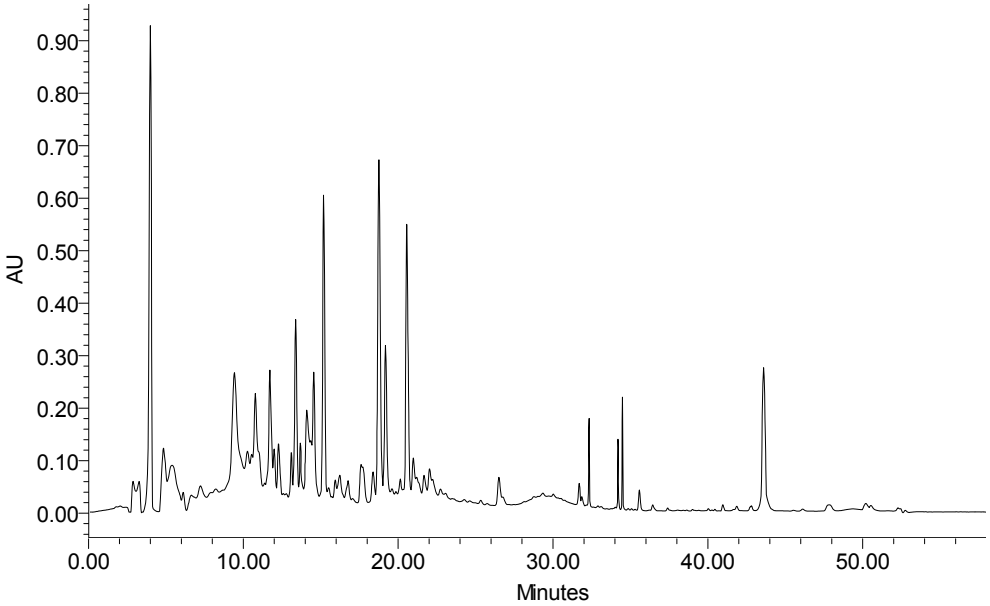


Figure 8.7: HPLC profile of the acetone extract of *Pelargonium graveolens* (SBG).

Table 8.2: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium graveolens* (SBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
2.88	206.2		1.12
3.29	206.2		1.11
4.01	210.9		12.88
4.85	206.2		2.91
5.42	206.2		4.39
7.23	248.6		0.96
9.41	206.2, 316.2		8.26
10.28	206.2, 269.9		1.82
10.78	205.0, 286.5		4.54
11.70	217.9, 326.9		3.81
12.27	206.2, 279.3		1.38
13.12	284.1		0.54
13.38	228.5, 312.6		4.42
13.69	227.3, 277.0		1.18
14.10	282.9,326.9		4.22
14.54	221.5, 272.2		3.38
15.19	255.7, 353.2	flavonol	7.30
15.93	229.1, 343.6	flavone	0.21
16.25	255.7, 352.0	flavonol	1.07
17.61	255.7, 354.4	flavonol	1.74

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
18.76	255.7, 354.4	flavonol	9.77
19.19	255.7, 354.4	flavonol	4.29
20.55	255.7, 354.4	flavonol	7.14
20.98	240.3		1.15
21.68	239.1, 268.7, 336.5		0.33
22.04	240.3, 316.2		1.04
26.51	254.5, 367.3		1.05
31.69	242.7		0.31
32.34	261.6		0.78
34.20	265.1		0.50
34.48	248.6		0.97
35.59	267.5		0.40
43.60	293.6		5.05

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium graveolens* in this study include the following:

- The *P. graveolens* (SBG) extract displayed moderate antimicrobial activity (MIC = 2 mg/ml – 4 mg/ml). *Pelargonium graveolens* (SBG) essential oil displayed poor antibacterial properties, but showed good activity against *C. albicans* (MIC = 2 mg/ml).

- The *P. graveolens* (SBG) extract showed promising anti-oxidant activity (IC_{50} = 14.49 ± 0.46 µg/ml). The *P. graveolens* (WSBG) extract produced a lower IC_{50} value of 26.81 ± 1.80 µg/ml.

- The essential oil (SBG) did not produce anti-inflammatory activity (IC_{50} >100 µg/ml) in the 5-lipoxygenase assay.

- *Pelargonium graveolens* (SBG) extract produced the lowest antimalarial activity (IC_{50} = 22.46 ± 3.21 µg/ml) of all the species tested. *Pelargonium graveolens* (WSBG) extract displayed substantial antimalarial activity (IC_{50} = 9.48 ± 1.17 µg/ml).

- The acetone extracts produced low toxicity profiles (IC_{50} = 80.48 ± 0.94 µg/ml - 83.31 ± 2.56 µg/ml) in the MTT assay. The essential oil (SBG) was extremely toxic (IC_{50} ≤0.10 µg/ml).

9. *Pelargonium greytonense* J. J. A. van der Walt

Botanical description:

Pelargonium greytonense is an erect, much-branched shrub growing up to 1 m high. This species is non-aromatic to aromatic. The young, herbaceous stems become woody, hirtellous with long soft hairs and glandular hairs interspersed. The leaves are shallowly 3–(5-8)-palmatilobate to palmatipartite, the base is cordate and the apices of the lobes are obtuse. The lamina is cordiform in outline. The leaf margin is coarsely dentate. The evidently veined leaves have long soft hairs and numerous glandular hairs interspersed. Two to nine flowers occur on each pseudo-umbel. The flowers are white to pale pink. Dark red markings occur on the posterior two spathulate to obovate petals, the apices of which are obtuse. The anterior three petals are much narrower than the posterior two (van der Walt, 1985; van der Walt and Vorster, 1988).

Pelargonium greytonense gives the impression of a rather young species due to the considerable morphological variation (particularly of leaf characteristics) that it exhibits. Many features of *P. greytonense* are intermediary between *P. hermanniifolium* (Berg.) Jacq. and *P. papilionaceum* (L.) L'Hérit. *Pelargonium greytonense* is probably a hybrid of these two species (van der Walt and Vorster, 1988).

Distribution and habitat:

Its distribution is confined to a small area in the south-western Cape. It is usually found on the southern slopes of the Riviersonderend Mountains. *Pelargonium greytonense* is plentiful in the village of Greyton, hence the epithet '*greytonense*' (van der Walt and Vorster, 1988). The habitat requirements of *P. greytonense* are between those of the two reputed parent species. It is often found in ravines (van der Walt and Vorster, 1988).

Figure 9.1: *Pelargonium greytonense* (van der Walt and Vorster, 1988).

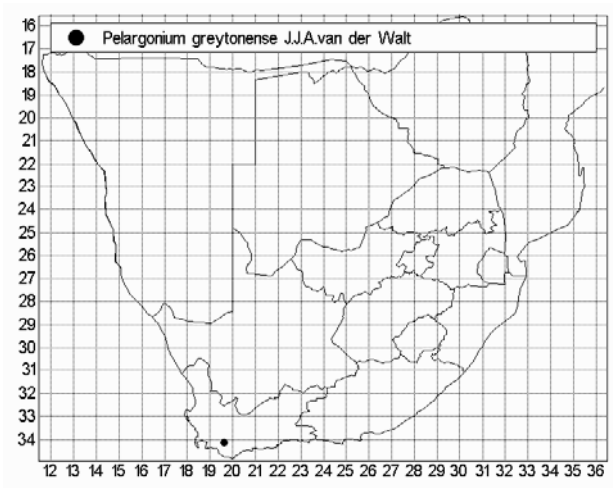


Figure 9.2: Geographical distribution of *Pelargonium greytonense.*

The essential oil of *P. greytonense* was not obtained due to insufficient plant material.

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium greytonense* collected from Stellenbosch Botanical Garden.

HPLC profile and analysis

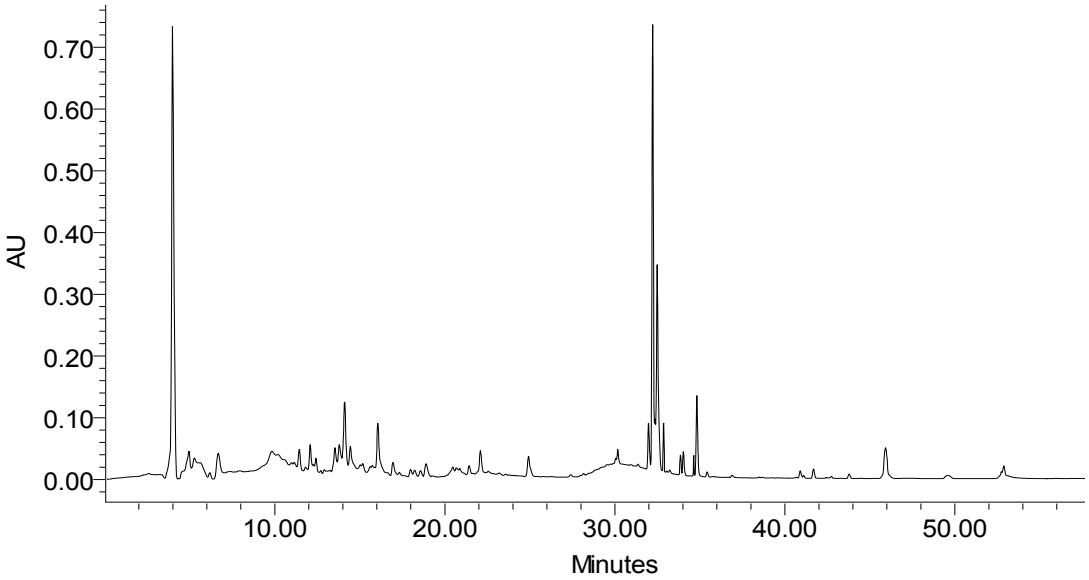


Figure 9.3: HPLC profile of the acetone extract of *Pelargonium greytonense* (SBG).

Table 9.1: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium greytonense* (SBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
3.98	213.3		31.44
4.94	208.6, 278.0		1.76
6.68	214.5, 269.7		2.32
11.43	206.3, 260.3, 293.4		1.16
12.09	219.1, 327.8		1.91
13.54	223.8, 278.0		1.93
13.79	222.7, 278.0		2.42
14.10	221.5, 278.0		5.40
14.44	222.7, 255.6, 278.0		2.78
16.06	225.0, 278.0		3.73
22.09	269.7	isoflavone	1.37
24.92	270.9, 313.5		1.57
31.98	240.3, 278.0		2.10
32.23	288.6	flavanone	20.21
32.49	267.4, 313.5	isoflavone	10.94
32.86	243.8, 268.5		1.45
34.03	288.6		1.04
34.82	287.4	flavanone	3.65
45.91	292.2		2.83

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium greytonense* in this study include the following:

- The acetone extract (SBG) showed promising activity against *B. cereus* (MIC = 0.25 mg/ml) and *S. aureus* (MIC = 0.5 mg/ml).

- Substantial antimalarial activity (IC_{50} = 13.21 ± 1.89 µg/ml) was produced by the acetone extract (SBG).

- The *P. greytonense* (SBG) extract is cytotoxic (IC_{50} = 39.65 ± 1.18 µg/ml).

10. *Pelargonium hermanniifolium* (Berg.) Jacq.

Botanical description:

Pelargonium hermanniifolium is an erect, often many-stemmed shrub and reaches a height of up to 1 m. It is usually non-aromatic. The stems are herbaceous when young but soon become woody, densely hirsute with glandular hairs interspersed, green but become brownish with age. The leaves resemble those of *Hermannia*, a genus of the family Sterculiaceae. The distichous leaves are sparsely strigose to strigose with glandular hairs interspersed. The lamina is narrowly to broadly obovate, 3-palmatilobate to 3-palmatisect with variably incised segments, often crisped. The base of the lamina is cuneate and the apex obtuse to acute. The leaf margin is crenate-serrate. The pseudo-umbels have one to two, sometimes up to three flowers each. The flowers are relatively large and are white to pink. Dark red markings occur on the posterior two, broadly spathulate petals, the apices of which are sometimes emarginate to cleft. The anterior three, spathulate petals have narrow claws (van der Walt and Vorster, 1981; van der Walt, 1985).

Distribution and habitat:

Pelargonium hermanniifolium grows in the Cape, from Worcester southwards to Caledon and eastwards to Swellendam. *Pelargonium hermanniifolium* is associated with a mountainous habitat, commonly found on the Riviersonderend Mountains. It is found on the higher slopes of mountains where precipitation is higher (van der Walt and Vorster, 1981; van der Walt, 1985).

Remark:

Pelargonium hermanniifolium species is closely related to *P. crispum* (Berg.) L'Hérit. (van der Walt and Vorster, 1981).

Figure 10.1: *Pelargonium hermanniifolium* in flower.

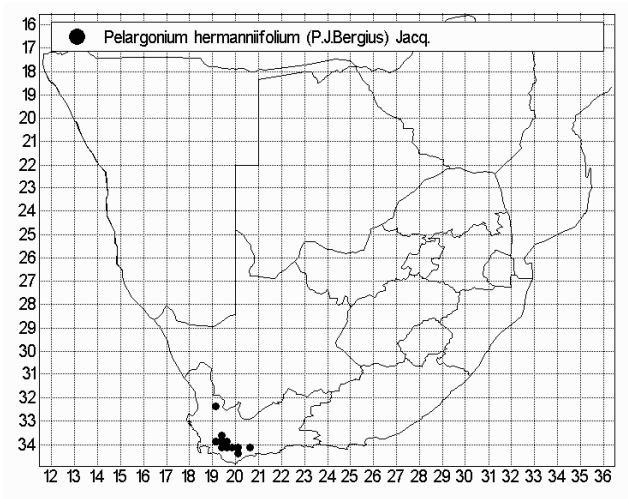


Figure 10.2: Geographical distribution of *Pelargonium hermanniifolium.*

Essential oil was not obtained for *P. hermanniifolium*.

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium hermanniifolium* collected from Stellenbosch Botanical Garden.

HPLC profile and analysis

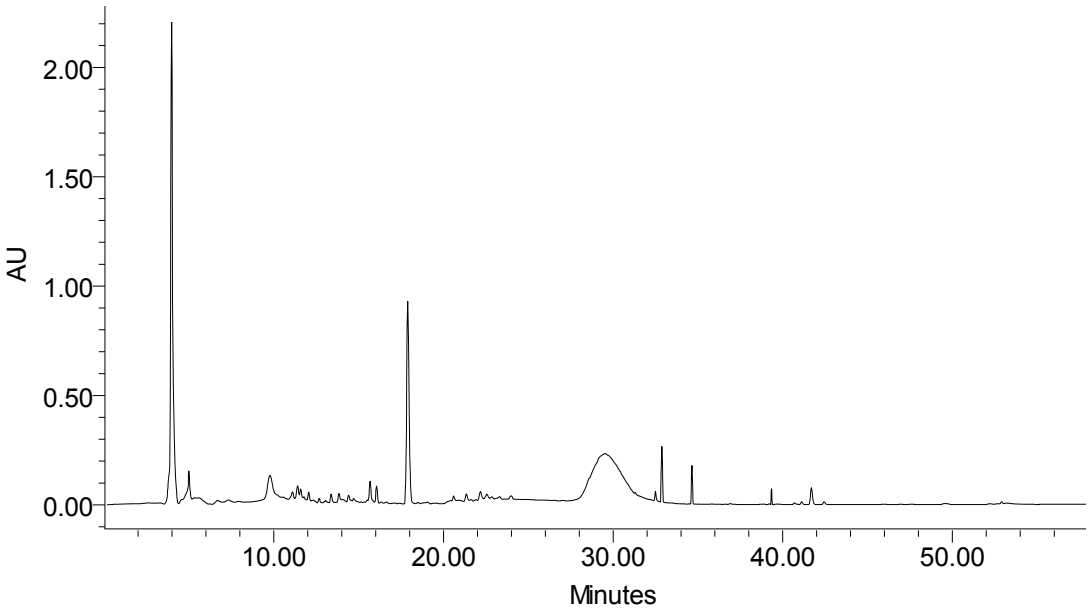


Figure 10.3: HPLC profile of the acetone extract of *Pelargonium hermanniifolium* (SBG).

Table 10.1: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium hermanniifolium* (SBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
3.97	218.0		30.36
4.99	210.9		3.26
9.77	207.4, 315.9		3.77
11.40	206.3, 259.1, 293.4		0.96
11.59	207.4, 313.5		0.64
15.67	261.5, 354.0	flavonol	1.25
16.05	222.7, 278.0		0.83
17.90	255.6, 354.0	flavonol	14.41
29.62	278.0		36.98
32.50	267.4, 308.8	isoflavone	0.22
32.87	243.8		2.11
34.64	265.0		1.30
39.33	278.0		0.37
41.69	278.0, 325.4		1.00

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium hermanniifolium* in this study include the following:

- The acetone extract (SBG) produced moderate antimicrobial activity (MIC = 1.5 mg/ml – 3 mg/ml).

- Substantial anti-oxidant activity (IC_{50} = 13.50 ± 0.73 µg/ml) was displayed by the *P. hermanniifolium* (SBG) extract.

- An IC_{50} value of 13.54 ± 3.95 µg/ml was produced by the acetone extract (SBG) in the antimalarial activity.

- The acetone extract (SBG) proved to be moderately toxic (IC_{50} = 46.47 ± 1.38 µg/ml) in the MTT assay.

11. *Pelargonium hispidum* (L. f.) Willd.

Common names:

Hispid pelargonium, 'Grofharige pelargonium' (van der Walt and Vorster, 1981).

Botanical description:

Pelargonium hispidum is an erect, branched, slightly lemon-scented shrub, growing up to 2.5 m high. The stems are woody at their bases, but above, they are herbaceous. The epithet '*hispidum*' (which is the Latin word meaning covered with coarse, rigid, erect hairs which are harsh to the touch) refers to the indumentum of the leaves and stems. The numerous pubescent to hirsute leaves, occurring alternatively on the stems, have long petioles. The leaf blades are palmately 5-7-lobed and the lobes are tri-angled or ovate, sharp and denticulate. The flowering branches are profusely branched. Six to twelve flowers occur on each pseudo-umbel. The bases of the posterior two petals are narrow cuneate, obovate round or entire and wine-red feather-like markings run from the base to the central area of the petal. The three anterior petals are spathulate with long, narrow claws and are of a pale carmine colour, with darker pigmentation towards the bases. Petal colour ranges from deep carmine to pale carmine. The flowers have seven fertile stamens (Webb, 1984; van der Walt, 1985). Inflorescence and floral features indicate a close association among *P. hispidum*, *P. cordifolium*, *P. papilionaceum* and *P. tomentosum* (van der Walt, 1985).

Distribution and habitat:

Pelargonium hispidum commonly grows in the south-western Cape mountains, as well as in the Swartberg Range in the southern Cape from Piketberg eastwards to Meiringspoort (Oudtshoorn district). It is commonly found growing on the lower slopes of mountains, where it occurs in shady ravines, often near streams between boulders or on scree (van der Walt, 1985).

Figure 11.1: The flowers of *Pelargonium hispidum.*

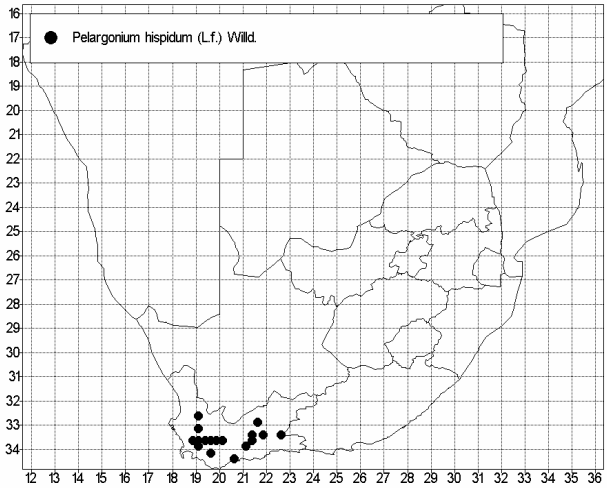


Figure 11.2: The geographical distribution of *Pelargonium hispidum.*

Chemical composition of the essential oil:

The fresh plant material collected from Stellenbosch Botanical Garden was subjected to hydrodistillation for 3 hours. An oil yield of 0.02% was obtained.

GC-MS profile and analysis

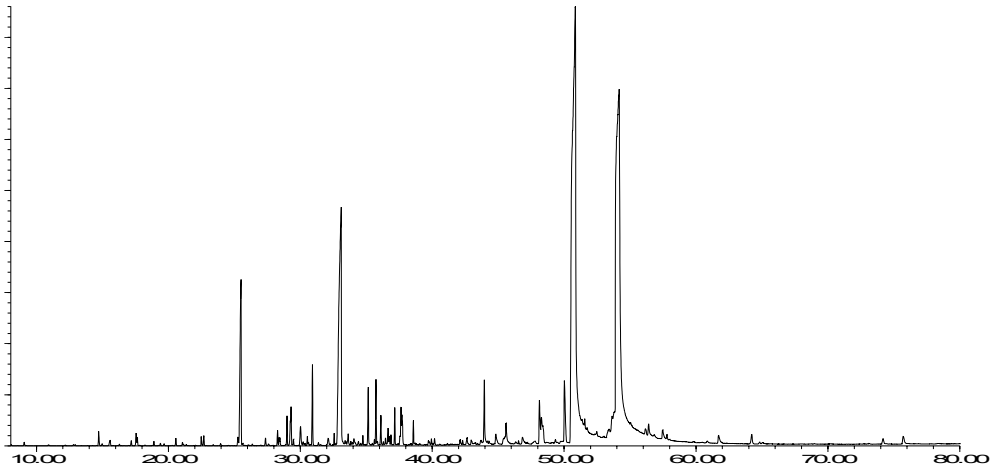


Figure 11.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium hispidum* (SBG).

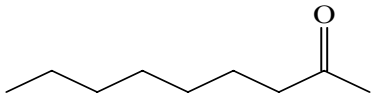
Table 11.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium hispidum* (SBG).

RRI	Compound	Area %
1032	α-pinene	trace
1076	camphene	trace
1100	undecane	trace
1112	anhydrolinalool oxide	trace
1118	β-pinene	trace
1176	α-phellandrene	0.07
1188	α-terpinene	trace
1203	limonene	trace
1213	1,8-cineole	0.17
1218	β-phellandrene	trace
1225	(Z)-3-hexenal	trace
1246	(Z)-β-ocimene	trace
1255	γ-terpinene	trace
1266	(E)-β-ocimene	trace
1280	p-cymene	0.06
1290	terpinolene	trace
1337	geijerene	0.08
1353	*cis*-rose oxide	trace
1391	(Z)-3-hexenol	0.11
1398	**2-nonanone**	**2.53**
1400	nonanal	trace
1400	(Z)-2-hexenol	trace
1429	(E)-2-octenal	trace
1450	*trans*-linalool oxide (furanoid)	0.05
1452	1-octen-3-ol	trace
1473	(Z)-3-hexenyl 2-methylbutyrate	0.06
1475	menthone	0.12
1481	(Z)-3-hexenyl isovalerate	0.15
1497	α-copaene	0.14
1503	isomenthone	0.28
1509	2-nonanol	0.22
1532	camphor	trace
1553	linalool	0.65
1553	octanol	trace
1587	methyl decanoate	0.05
1589	isocaryophyllene	0.08
1604	2-undecanone	trace
1612	**β-caryophyllene**	**8.55**
1615	p-menth-1-en-9-al	trace
1618	cadina-3,5-diene	0.06
1620	ethyl decanoate	trace
1637	menthol	0.08
1668	citronellyl acetate	trace
1679	heptadecane	trace

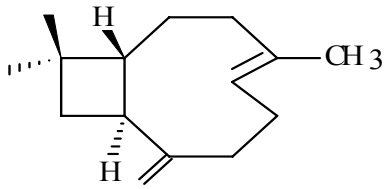
RRI	Compound	Area %
1681	(Z)-3-hexenyl tiglate	0.05
1687	α-humulene	0.39
1704	γ-muurolene	0.05
1706	α-terpineol	0.47
1707	ledene	trace
1714	(Z,E)-α-farnesene	trace
1740	α-muurolene	0.11
1748	piperitone	0.06
1750	β-dihydroagarofuran	trace
1758	(E,E)-α-farnesene	0.19
1766	1-decanol	trace
1772	citronellol	0.28
1773	δ-cadinene	0.28
1776	γ-cadinene	trace
1799	cadina-1,4-diene	trace
1808	nerol	trace
1809	citronellyl butyrate	0.10
1845	*trans*-carveol	0.06
1853	*cis*-calamenene	trace
1857	geraniol	0.05
1864	p-cymen-8-ol	trace
1868	(E)-geranyl acetone	trace
1882	*cis*-carveol	trace
1941	α-calacorene	0.08
1949	(E)-jasmone	0.05
1966	(E)-12-norcaryophyll-5-en-2-one	trace
2008	caryophyllene oxide	0.48
2050	(E)-nerolidol	0.16
2084	octanoic acid	0.11
2110	furopelargone B	trace
2127	10-epi-γ-eudesmol	0.10
2184	nonanoic acid	0.42
2187	T-cadinol	trace
2254	citronellic acid	0.81
2257	decyl decanoate	trace
2257	β-eudesmol	trace
2281	**decanoic acid**	**47.01**
2392	caryophyllenol II	0.08
2411	**2-decenoic acid***	**31.26**
2509	dodecanoic acid	0.10
2538	2-undecenoic acid*	0.11
2900	nonacosane	trace
2931	hexadecanoic acid	0.11
	Total	**96.48%**

*correct isomer not identified; trace: <0.05%; (SBG) = Stellenbosch Botanical Garden.

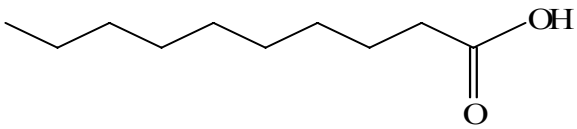
Decanoic acid occupies 47.01% of the total oil composition. 2-Decenoic acid also occupies a large portion (31.26%) of the oil. 2-Nonanone and β-caryophyllene contribute 2.53% and 8.55%, respectively.



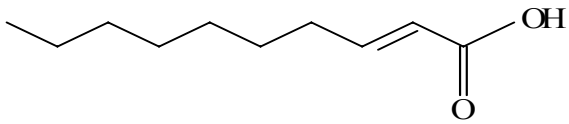
2-nonanone



β-caryophyllene



decanoic acid



2-decenoic acid

Figure 11.4: Chemical structures of the major compounds identified in the essential oil of *Pelargonium hispidum* (SBG).

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium hispidum* collected from Stellenbosch Botanical Garden.

HPLC profile and analysis

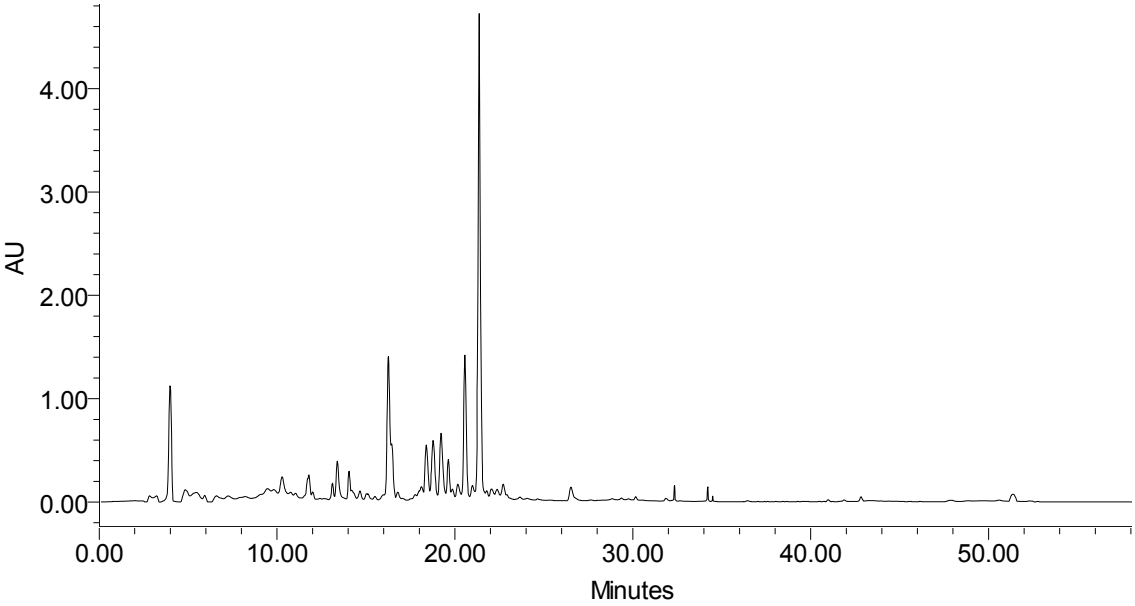


Figure 11.5: HPLC profile of the acetone extract of *Pelargonium hispidum* (SBG).

Table 11.2: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium hispidum* (SBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
3.98	212.0		8.83
10.28	206.2, 269.		3.54
11.79	220.3, 328.1		2.80
13.12	286.5		1.14
13.40	228.5, 313.8		3.55
14.05	212.0, 297.1, 325.7		2.12
16.26	255.7, 355.6	flavonol	13.88
16.46	255.7, 353.2	flavonol	1.27
18.40	262.7, 348.4	flavone	4.76
18.78	255.7, 354.4	flavonol	5.61
19.24	255.7, 354.4	flavonol	6.17
19.63	255.7, 350.8	flavone	2.83
20.56	255.7, 355.6	flavonol	10.57
21.24	256.8, 349.6	flavone	31.52
26.55	255.7, 360.5	flavonol	1.40

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium hispidum* in this study include the following:

- The *P. hispidum* (SBG) extract showed promising activity (MIC = 2 mg/ml) against *K. pneumoniae*.

- The acetone extract (SBG) displayed considerable anti-oxidant activity in the DPPH assay. An IC_{50} value of 12.78 ± 0.45 µg/ml was obtained.

- Substantial antimalarial activity (IC_{50} = 12.23 ± 1.93 µg/ml) was produced by the acetone extract (SBG) in the hypoxanthine incorporation assay.

- *Pelargonium hispidum* (SBG) extract displayed low toxicity in the MTT assay (IC_{50} = 84.30 ± 1.16 µg/ml).

12. *Pelargonium panduriforme* Eckl. & Zeyh.

Synonym:

Pelargonium panduraeforme (Webb, 1984).

Botanical description:

Pelargonium panduriforme is a strongly balm-scented, erect, branched, glaucous and occasionally viscid shrub reaching a height of up to 1.75 m. The stems are fleshy and woody with long spreading short, dense hairs. Later, the stems become almost smooth, terete, erect and leafless below with branches similar to the stems. The leaves are usually soft to the touch and closely packed. The lamina is panduriform (fiddle-shaped) to cordiform in outline, pinnatilobate to pinnatipartite with two broad obtuse lobes at the base, and the middle lobe very large, usually coarsely incised. When dry, the finely toothed lobes curl at the margins. Two to twenty large pale pink to pink flowers are borne in umbel-like inflorescences. The two posterior pink petals are narrowly spathulate, obovate and entire. These petals are striped and spotted with deep crimson along the veins. The three anterior petals with long narrow claws are smaller, narrower and paler. Seven fertile stamens are present (Webb, 1984; van der Walt and Vorster, 1988). *Pelargonium panduriforme* is closely related to *P. quercifolium* (van der Walt and Vorster, 1988).

Distribution and habitat:

Pelargonium panduriforme is found growing in the Eastern Cape, where it occurs from Antoniesberg in the area of Willowmore eastwards close to Riebeeck East. It has also been collected near Engcobo. It grows abundantly on the Baviaanskloof and Kouga Mountains. *Pelargonium panduriforme* grows in areas of bushveld and on the lower foothills or ravines, close to streams (van der Walt and Vorster, 1988).

Figure 12.1: *Pelargonium panduriforme* in flower.

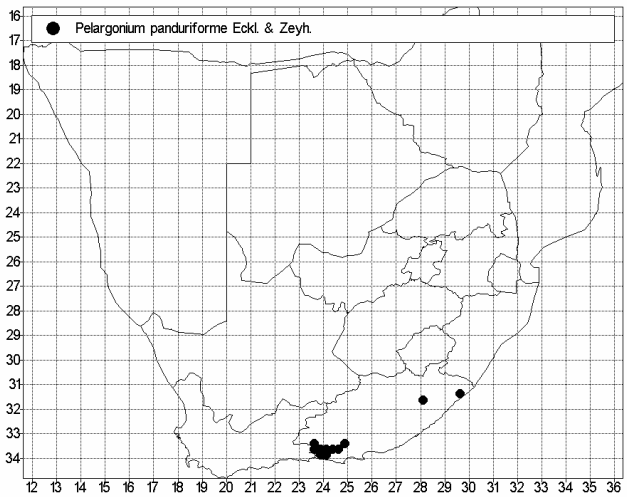


Figure 12.2: The geographical distribution of *Pelargonium panduriforme.*

Chemical composition of the essential oil:

Hydrodistillation was carried out on fresh plant material for 3 hours.

1. *Pelargonium panduriforme* from Stellenbosch Botanical Garden yielded 0.48% oil,
2. *Pelargonium panduriforme* from Walter Sisulu Botanical Garden yielded 0.35% oil.

GC-MS profiles and analyses

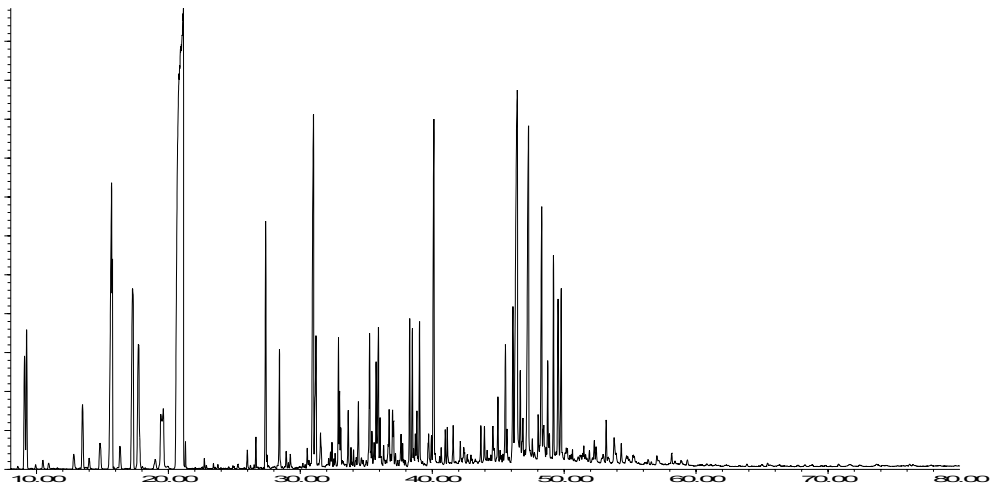


Figure 12.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium panduriforme* (SBG).

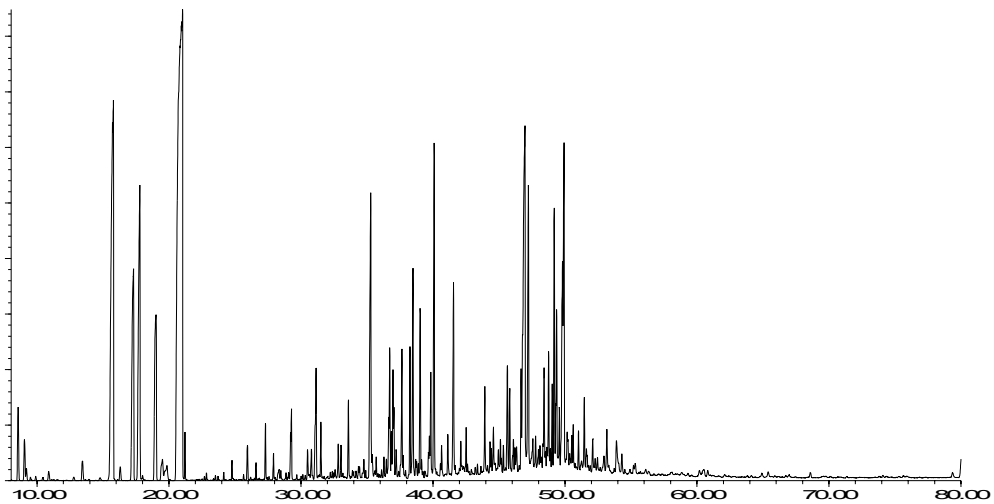


Figure 12.4: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium panduriforme* (WSBG).

Table 12.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium panduriforme*.

RRI	Compound	Area %	
		SBG	WSBG
1014	tricyclene	-	0.62
1032	α-pinene	0.60	0.28
1035	α-thujene	1.00	0.08
1076	camphene	trace	0.08
1118	β-pinene	0.13	trace
1132	sabinene	0.51	0.17
1136	thuja-2,4(10)-diene	0.10	-
1159	δ-3-carene	0.25	-
1176	**α-phellandrene**	**4.31**	**8.81**
1188	α-terpinene	0.19	0.10
1203	limonene	2.15	2.99
1218	**β-phellandrene**	1.38	**3.95**
1246	(Z)-β-ocimene	trace	2.38
1255	γ-terpinene	1.01	0.31
1266	(E)-β-ocimene	trace	0.23
1280	**p-cymene**	**45.38**	**37.59**
1290	terpinolene	0.08	-
1385	allo-ocimene*	-	0.09
1413	rosefuran	-	0.14
1429	perillene	trace	0.07
1451	β-thujone	trace	-
1452	α,p-dimethylstyrene	1.40	0.24

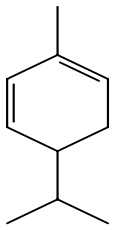
RRI	Compound	Area %	
		SBG	WSBG
1466	α-cubebene	-	0.11
1478	*cis*-linalool oxide (furanoid)	0.48	-
1497	α-copaene	0.07	0.15
1503	isomenthone	-	0.31
1541	benzaldehyde	0.13	-
1549	β-cubebene	-	0.25
1553	**linalool**	**4.42**	-
1560	*trans*-dihydro-α-terpineol	0.23	-
1571	*trans*-p-menth-2-en-1-ol	-	0.60
1589	*cis*-dihydro-α-terpineol	trace	-
1597	bornyl acetate	0.11	-
1604	thymol methyl ether	trace	-
1611	terpinen-4-ol	0.60	-
1612	β-caryophyllene	-	0.16
1616	hotrienol	0.20	-
1617	guaia-6,9-diene	0.10	0.14
1628	aromadendrene	0.26	0.17
1651	sabina ketone	0.06	-
1661	allo-aromadendrene	0.30	0.11
1664	*trans*-pinocarveol	trace	-
1677	epi-zonarene	-	0.06
1683	carvotanacetone	0.12	0.10
1688	selina-4,11-diene	-	trace
1690	cryptone	0.71	2.89
1706	α-terpineol	0.52	-
1707	ledene	0.73	-
1726	germacrene D	-	trace
1740	α-muurolene	-	0.25
1751	phellandral	-	0.72
1751	carvone	-	0.35
1758	*cis*-piperitol	trace	0.11
1773	δ-cadinene	0.08	0.69
1776	γ-cadinene	trace	0.07
1783	p-methylacetophenone	0.75	0.67
1802	cuminaldehyde	0.66	1.20
1809	citronellyl butyrate	0.11	-
1823	p-mentha-1(7),5-dien-2-ol*	0.76	1.13
1827	(E,E)-2,4-decadienal	-	0.09
1845	*trans*-carveol	trace	0.32
1853	*cis*-calamenene	-	0.40
1857	geraniol	trace	-
1864	**p-cymen-8-ol**	**3.97**	2.90
1865	isopiperitenone	-	0.11
1886	geranyl butyrate	0.11	-
1900	epi-cubebol	-	0.19

RRI	Compound	Area %	
		SBG	WSBG
1916	α-agarofuran	-	1.12
1941	α-calacorene	trace	0.11
1941	citronellyl hexanoate	0.08	-
1953	palustrol	trace	-
1984	γ-calacorene	-	0.06
2008	caryophyllene oxide	0.11	0.38
2030	methyl eugenol	-	0.10
2057	ledol	0.30	0.13
2071	humulene epoxide II	-	0.22
2080	cubenol	0.10	0.44
2088	1-epi-cubenol	-	0.30
2096	elemol	0.60	-
2104	**viridiflorol**	**7.89**	-
2110	furopelargone B	-	0.11
2113	cumin alcohol	0.10	trace
2127	**10-epi-γ-eudesmol**	0.10	**6.26**
2144	**spathulenol**	**3.82**	1.89
2185	γ-eudesmol	1.98	-
2209	T-muurolol	-	trace
2210	agarospirol	-	0.48
2219	α-muurolol	-	trace
2228	valerianol	-	0.78
2239	carvacrol	0.90	1.32
2247	*trans*-α-bergamotol	-	0.21
2250	α-eudesmol	0.91	-
2253	**4α-hydroxy-dihydroagarofuran**	-	**3.83**
2257	β-eudesmol	0.88	trace
2384	1-hexadecanol	trace	-
	Total	**91.74%**	**90.12%**

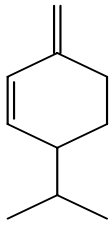
*correct isomer not identified; trace: <0.05%.

p-Cymene is the most abundant chemical constituent (45.38%) in the essential oil of *P. panduriforme* (SBG). Viridiflorol occurs at a substantial level of 7.89%. Other compounds contributing substantial amounts include α-phellandrene (4.31%), linalool (4.42%), p-cymen-8-ol (3.97%) and spathulenol (3.82%).

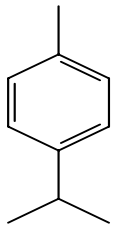
p-Cymene is the main compound (37.59%) in the hydrodistilled essential oil of *P. panduriforme* (WSBG). α-Phellandrene occupies 8.81% and 10-epi-γ-eudesmol represents 6.26%. β-Phellandrene and 4α-hydroxy-dihydroagarofuran contribute 3.95% and 3.83% to the total oil composition.



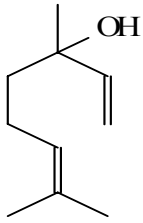
α-phellandrene



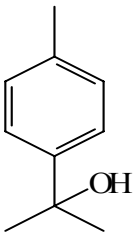
β-phellandrene



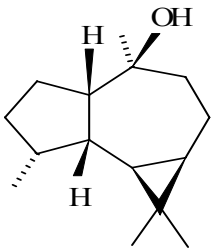
p-cymene



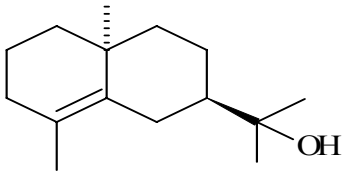
linalool



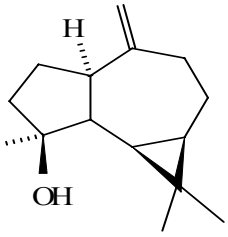
p-cymen-8-ol



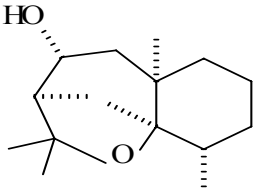
viridiflorol



10-epi-γ-eudesmol



spathulenol



4α-hydroxy-dihydroagarofuran

Figure 12.5: Chemical structures of the major compounds identified in the essential oil of *P. panduriforme* (SBG) and in the essential oil of *P. panduriforme* (WSBG).

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium panduriforme* collected from the Stellenbosch Botanical Garden.

HPLC profile and analysis

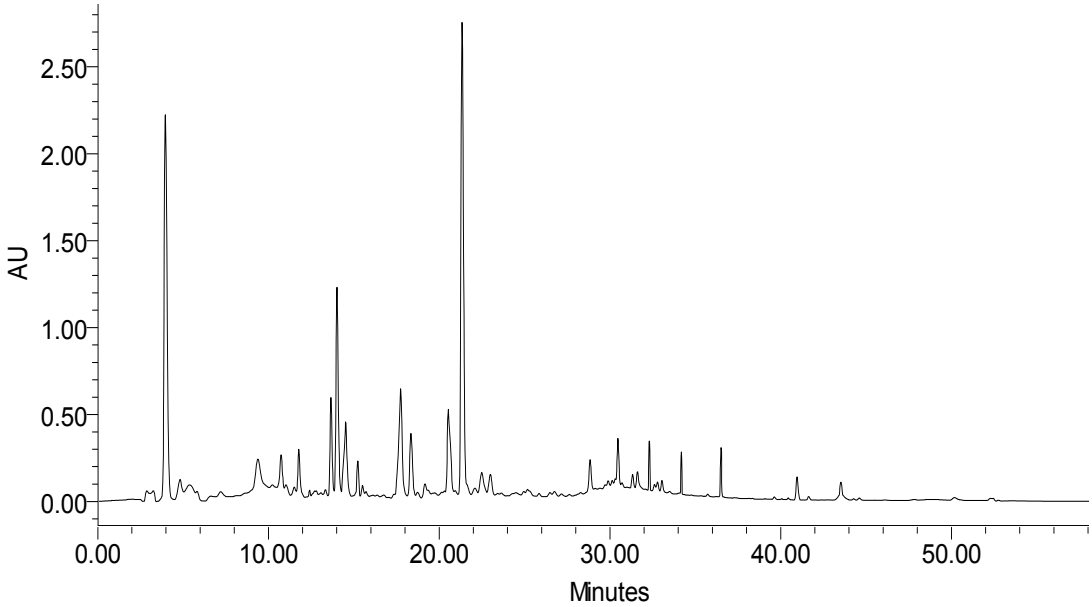


Figure 12.6: HPLC profile of the acetone extract of *Pelargonium panduriforme* (SBG).

Table 12.2: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium panduriforme* (SBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
3.81	210.9		20.42
9.38	207.3, 316.2		5.36
10.75	269.9		2.47
11.78	221.5, 275.8		1.96
13.67	269.9		3.77
14.02	209.7		8.61
14.51	221.5, 272.2		4.45
15.23	229.7, 277		1.39
17.74	255.7, 354.4	flavonol	6.97
18.34	262.7, 349.6	flavone	3.25
20.53	255.7, 354.4	flavonol	5.10
21.34	255.7, 349.6	flavone	22.83
22.50	241.5, 337.7		1.79
22.99	281.7	flavanone	1.31
28.84	254.5, 300.7, 347.2		1.69
30.47	256.8, 352.0		2.35
31.32	243.8		0.62
31.61	240.3, 330.5		0.85
32.31	247.4		1.02

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
34.18	265.1		0.76
36.51	273.4		1.12
40.96	273.4, 325.7		0.84
43.52	292.4		1.05

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium panduriforme* in this study include the following:

- The *P. panduriforme* (SBG) extract exhibited moderate antimicrobial activity (MIC values of 2 mg/ml were produced for all the test pathogens). The *P. panduriforme* (WSBG) extract showed improved activity against the Gram-positive micro-organisms (MIC = 0.5 mg/ml for both *B. cereus* and *S. aureus*). The essential oil samples showed reduced antimicrobial activity. *Pelargonium panduriforme* (SBG) essential oil produced an MIC value of 2 mg/ml with respect to *C. albicans*.

- Very low anti-oxidant activity was produced by the *P. panduriforme* (WSBG) extract in the DPPH assay (IC_{50} = 91.58 ± 4.45 µg/ml).

- Both acetone extracts (SBG and WSBG) showed promising anti-inflammatory activity in the 5-lipoxygenase assay. IC_{50} values of 45.39 µg/ml – 45.58 µg/ml were obtained.

- Pronounced antimalarial activity (IC_{50} = 1.34 ± 0.29 µg/ml) was produced by the acetone extract (SBG). The *P. panduriforme* (SBG) extract was the most active in the hypoxanthine assay.

- The acetone extracts (SBG and WSBG) displayed cytotoxicity in the MTT assay. *Pelargonium panduriforme* (SBG) and *P. panduriforme* (WSBG) produced IC_{50} values of 32.93 ± 3.75 µg/ml and 42.65 ± 0.90 µg/ml, respectively. The essential oil samples (SBG and WSBG) were extremely toxic; IC_{50} values of ≤0.10 µg/ml were produced by both samples.

13. *Pelargonium papilionaceum* (L.) L'Hérit.

Common names:

Butterfly pelargonium, 'Rambossie' (van der Walt, 1977).

Botanical description:

Pelargonium papilionaceum is an upright, many-branched shrub growing more than 2 m high. The main stem is woody at the base and contains herbaceous villous side-branches. The sparsely villous, glandular, large leaves are cordate or roundly cordate; the upper leaves are entire and the lower ones shallowly 3-5-lobed. The leaf border can be described as finely toothed, serrulated or almost entire. The leaves of *P. papilionaceum* somewhat resemble those of *P. vitifolium*. However, the leaves of *P. papilionaceum* are more aromatic and more evidently veined on the upper side than the leaves of *P. vitifolium.* The vernacular name 'Rambossie' is derived from the unpleasant he-goat-like odour (fruity lemon scent) of the leaves. The inflorescence is umbel-like and consists of five to twelve flowers. The pedicels are long and covered with long soft hairs. The flowers contain two large posterior petals, which are reflexed, and three narrow anterior ones. 'Papilionaceus' is the Latin word meaning butterfly-like and the species name '*papilionaceum*' refers to the two large posterior petals of the flower which resemble a butterfly. Flower colour is variable, from light pink to carmine. The posterior petals contain a distinguishing red-purplish spot contrasting with an adjacent white spot. There are seven fertile stamens (van der Walt, 1977; 1985). *Pelargonium papilionaceum* could be confused with *P. tomentosum* and *P. hispidum* (van der Walt, 1985).

Distribution and habitat:

Pelargonium papilionaceum is found growing along the coast from Somerset West in the Western Cape stretching eastwards to Humansdorp in the Eastern Cape. The distribution of *P. papilionaceum* is associated with mountains. It is found in half-shady areas at the borders of indigenous forests, near streams and grows in kloofs or ravines (van der Walt, 1977; 1985).

Figure 13.1: *Pelargonium papilionaceum* in flower.

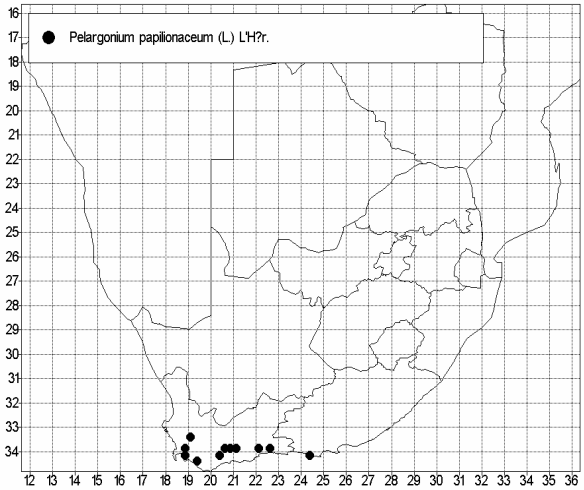


Figure 13.2: The geographical distribution of *Pelargonium papilionaceum.*

Traditional use:

The leaves were used as an alternative to tobacco and smoked for medicinal purposes (Watt and Breyer-Brandwijk, 1962).

Chemical composition of the essential oil:

Fresh plant material collected from the National Botanical Garden (Kirstenbosch) was subjected to hydrodistillation for 3 hours. The oil yield was 0.17%.

GC-MS profile and analysis

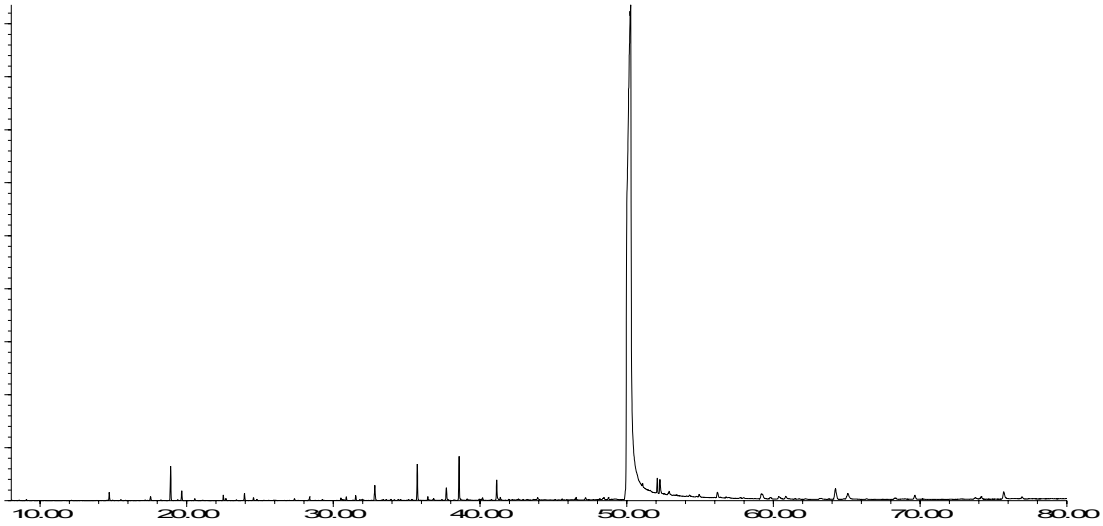


Figure 13.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium papilionaceum* (NBG).

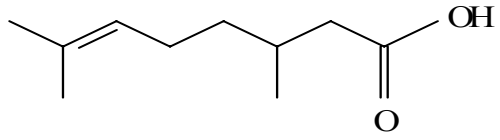
Table 13.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium papilionaceum* (NBG).

RRI	Compound	Area %
1014	tricyclene	trace
1032	α-pinene	trace
1100	undecane	trace
1174	myrcene	trace
1203	limonene	trace
1213	1,8-cineole	trace
1225	(Z)-3-hexenal	trace
1246	(Z)-β-ocimene	0.28
1255	γ-terpinene	trace
1266	(E)-β-ocimene	0.08
1280	p-cymene	trace
1290	terpinolene	trace
1337	geijerene	trace
1348	6-methyl-5-hepten-2-one	trace
1353	*cis*-rose oxide	0.05
1365	*trans*-rose oxide	trace
1385	allo-ocimene*	trace
1391	(Z)-3-hexenol	trace
1400	nonanal	trace
1400	(Z)-2-hexenol	trace
1429	perillene	trace
1450	*trans*-linalool oxide (furanoid)	trace

RRI	Compound	Area %
1459	acetic acid	trace
1466	α-cubebene	trace
1503	isomenthone	trace
1541	benzaldehyde	trace
1553	linalool	trace
1553	octanol	trace
1569	neoisopulegol	trace
1570	methyl citronellate	trace
1571	trans-p-menth-2-en-1-ol	trace
1583	isopulegol	trace
1602	6-methyl-3,5-heptadien-2-one	trace
1612	β-caryophyllene	0.12
1620	β-cyclocitral	trace
1647	(E)-2-decenal	trace
1668	citronellyl acetate	trace
1687	α-humulene	trace
1706	α-terpineol	0.26
1748	piperitone	trace
1765	geranyl acetate	trace
1772	citronellol	0.12
1809	citronellyl butyrate	0.29
1825	geranyl propionate	trace
1857	geraniol	trace
1864	p-cymen-8-ol	trace
1868	(E)-geranyl acetone	trace
1886	geranyl butyrate	0.14
1980	2-phenylethyl butyrate	trace
2008	caryophyllene oxide	trace
2084	octanoic acid	trace
2131	hexahydrofarnesylacetone	trace
2179	1-tetradecanol	trace
2184	nonanoic acid	trace
2254	**citronellic acid**	**96.16**
2349	geranic acid	0.18
2500	pentacosane	trace
2622	phytol	trace
2700	heptacosane	0.20
2900	nonacosane	0.05
2931	hexadecanoic acid	trace
	Total	**97.93%**

*correct isomer not identified; trace <0.05%; (NBG) = National Botanical Garden (Kirstenbosch).

Citronellic acid is the major compound (96.16%) in the essential oil.



citronellic acid

Figure 13.4: Chemical structure of the main compound, citronellic acid, identified in the hydrodistilled essential oil of *Pelargonium papilionaceum* (NBG).

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium papilionaceum* collected from the National Botanical Garden (Kirstenbosch).

HPLC profile and analysis

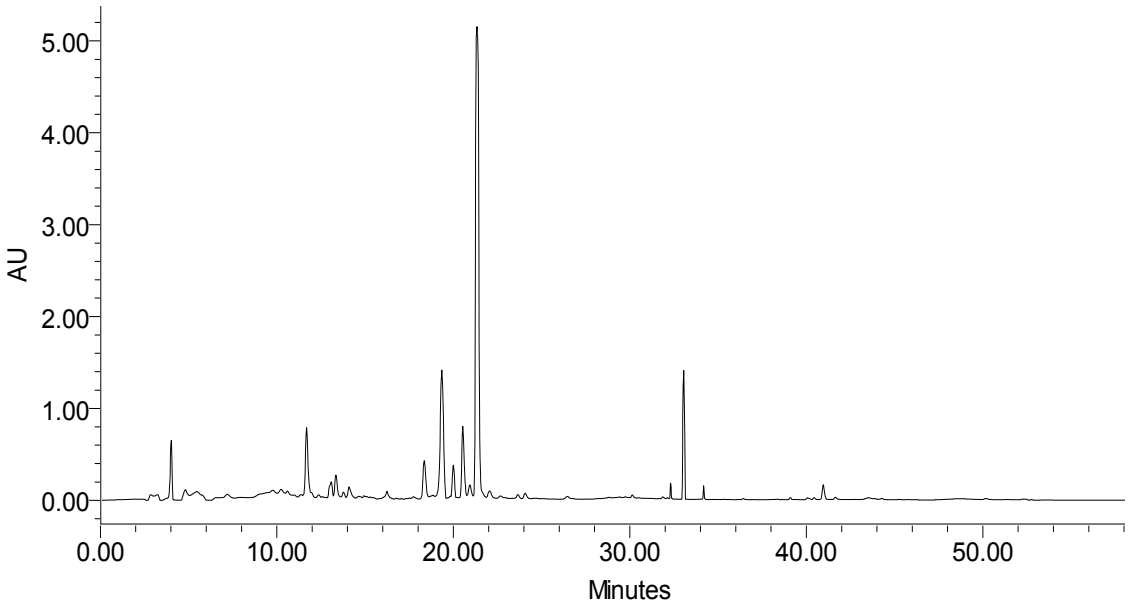


Figure 13.5: HPLC profile of the acetone extract of *Pelargonium papilionaceum* (NBG).

Table 13.2: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium papilionaceum* (NBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
4.02	206.2		3.50
4.82	206.2		1.38
11.68	219.1, 325.7		6.72
13.08	285.3		1.71
13.35	228.5, 313.8		2.10
13.79	229.7, 313.8		0.62
14.10	234.4, 330.5		1.42
16.24	254.5, 349.6	flavone	0.90
18.36	262.7, 346.0	flavone	3.39
19.35	254.5		13.16
20.01	235.6		2.55
20.53	255.7, 355.6	flavonol	5.42
20.94	237.9		1.29
21.22	254.5, 349.6	flavone	46.71
22.07	243.8, 346.0	flavone	0.78
32.31	261.6		0.38
33.05	249.7		6.71
34.19	265.1		0.24
40.97	273.4, 326.9		1.03

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium papilionaceum* in this study include the following:

- The acetone extract (NBG) displayed promising activity against *S. aureus* (MIC = 2 mg/ml) and *C. albicans* (MIC = 1.19 mg/ml).

- The *P. papilionaceum* (NBG) extract displayed low anti-oxidant activity (IC_{50} = 81.24 ± 13.44 µg/ml) in the DPPH assay.

- The acetone extract (NBG) produced considerable antimalarial activity (IC_{50} = 5.15 ± 0.36 µg/ml).

- The *P. papilionaceum* (NBG) extract displayed moderate toxicity (IC_{50} = 53.76 ± 1.60 µg/ml) in the MTT assay.

14. *Pelargonium pseudoglutinosum* R. Knuth

Synonym:

Pelargonium uniondalense (van der Walt and Vorster, 1988).

Botanical description:

Pelargonium pseudoglutinosum is an erect to decumbent, many-branched, non-aromatic, viscid shrub up to 1 m high. Stems are herbaceous when young but soon become woody and are green but with age change to wine-red and eventually brown. Glandular hairs are present on the glabrescent to sparsely strigose leaves. The upper surface of the leaves have larger, dark purple veins. The lamina is ovate in outline, pinnatipartite to pinnatisect with irregularly incised segments and is sticky. The base is cuneate to obtuse and the apices of the segments are acute. Short conical hairs occur on the dentate margins. The pseudo-umbels contain one to two pale pink to pink, relatively large flowers each. A dark pink blotch and purple markings occur on the posterior two, spathulate petals of which the apices are obtuse to emarginate. The anterior three, spathulate petals have long narrow claws (van der Walt, 1985; van der Walt and Vorster, 1988).

There is a resemblance between *P. pseudoglutinosum* and *P. glutinosum*, hence the epithet '*pseudoglutinosum*' ('pseudo' meaning false) (van der Walt and Vorster, 1988). The leaves of *P. pseudoglutinosum* could be confused with those of *P. glutinosum*; however, the latter species lacks the purple veins and has aromatic leaves. A reliable attribute to distinguish the two species from one another is the 1-2-flowered pseudo-umbels (*P. glutinosum* has more than two flowers per inflorescence) (van der Walt, 1985).

Distribution and habitat:

Pelargonium pseudoglutinosum has a restricted distribution in the Western Cape. It is known only from Mannetjiesberg, Keurboomsrivier, Uniondale and Prince Alfred's Pass. It grows in ravines near streams on well-drained soil (van der Walt, 1985; van der Walt and Vorster, 1988).

Figure 14.1: *Pelargonium pseudoglutinosum* in flower.

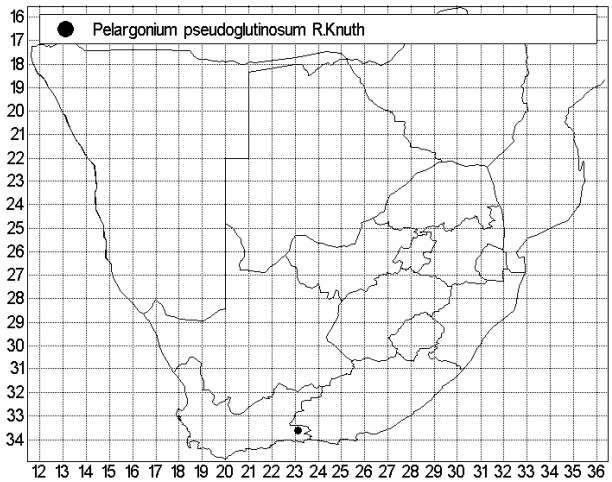


Figure 14.2: The geographical distribution of *Pelargonium pseudoglutinosum.*

The essential oil of *P. pseudoglutinosum* was not obtained.

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium pseudoglutinosum* collected from the National Botanical Garden (Kirstenbosch).

HPLC profile and analysis

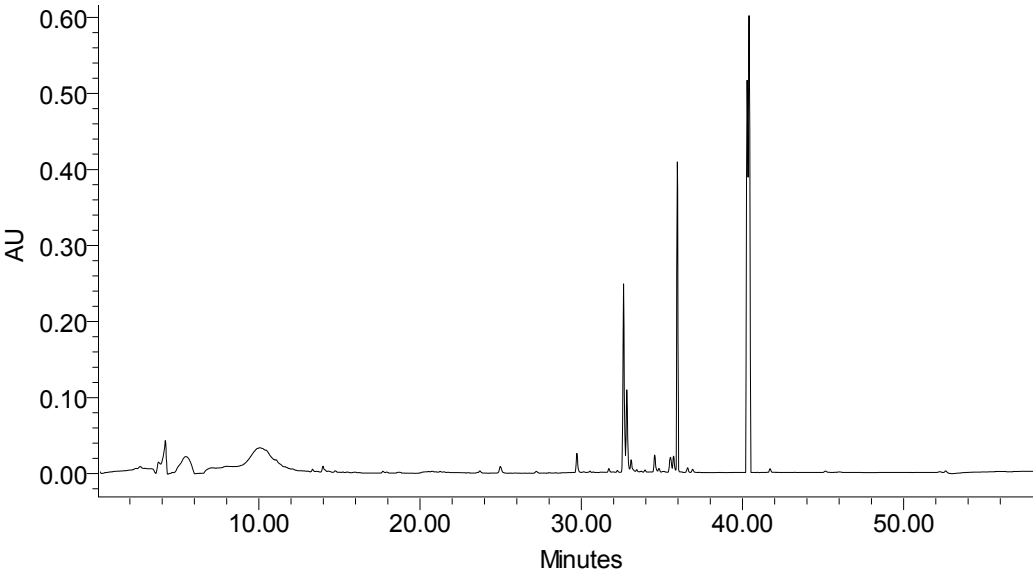


Figure 14.3: HPLC profile of the acetone extract of *Pelargonium pseudoglutinosum* (NBG).

Table 14.1: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium pseudoglutinosum* (NBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
3.77	206.3		7.39
4.19	207.4		3.84
5.48	207.4		5.25
10.08	207.4		6.01
29.73	267.4, 338.5	flavone	0.92
32.63	267.4, 329.0	flavone	9.41
32.82	267.4, 336.1	flavone	4.52
33.10	253.2, 343.2		0.39
34.56	268.5, 343.2	flavone	0.89
35.53	260.3, 329.0		0.77
35.73	245.0		0.61
35.96	261.5		13.56
40.23	278.0		19.22
40.51	278.0		27.22

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium pseudoglutinosum* in this study include the following:

- Of all the extracts tested, the *P. pseudoglutinosum* (NBG) extract displayed the greatest activity (MIC = 0.039 mg/ml) against *B. cereus* and *S. aureus*. Promising activity (MIC = 0.54 mg/ml) was displayed against *C. albicans*.

- The acetone extract (NBG) displayed moderate anti-oxidant activity (IC_{50} = 52.38 ± 0.67 µg/ml) in the DPPH assay.

- Considerably antimalarial activity was displayed by the acetone extract (NBG). An IC_{50} value of 7.93 ± 0.75 µg/ml was obtained in the hypoxanthine incorporation assay.

- Substantial cytotoxicity (IC_{50} = 30.54 ± 5.46 µg/ml) was recorded for the *P. pseudoglutinosum* (NBG) extract.

15. *Pelargonium quercifolium* (L. f.) L'Hérit.

Synonym:

Pelargonium karrooense (van der Walt and Vorster, 1988).

Common names:

Royal Oak (O'Brien, 1983), Oak-leaved geranium (http://www.ibiblio.org/pfaf/cgi-bin/arr_html?Pelargonium+quercifolium).

Botanical description:

Pelargonium quercifolium is a glaucous-green, erect, viscid shrub with stems which are herbaceous above, but woody at the base. It can grow up to 1.75 m high. This species has a somewhat unpleasant spicy, resinous scent (strong balm scent). The glossy, sticky leaves usually have a brown mark along the mid-rib and part of the veins. The leaves are almost scabrous, strigose with long soft hairs in between and densely interspersed with long glandular hairs. The lamina is 3-palmatisect to pinnatisect and the segments are irregularly pinnatifid to pinnatisect. The apices of the segments are acute to obtuse and the leaf margins are irregularly dentate-serrate. The epithet '*quercifolium*' refers to the resemblance of the leaves to those of some *Quercus* species (oaks). Two to six flowers occur on each pseudo-umbel. The petals are pale to dark rose in colour. Deep carmine veining occurs on the posterior two narrowly obovate or oblong obovate petals which have obtuse to emarginate apices. The anterior petals are smaller and paler with long narrow claws. There are seven fertile stamens (O'Brien, 1983; Webb, 1984; van der Walt, 1985; van der Walt and Vorster, 1988).

Distribution and habitat:

Pelargonium quercifolium grows in the Western and the Eastern Cape. It is commonly found growing along the road between Oudtshoorn and Willowmore (van der Walt and Vorster, 1988). *Pelargonium quercifolium* is usually associated with Fynbos vegetation and commonly grows on rocky hills or on mountain slopes in Rhenosterbosveld (van der Walt and Vorster, 1988) and along the sides of roads (Phillips and Rix, 1998).

Figure 15.1: *Pelargonium quercifolium* in flower.

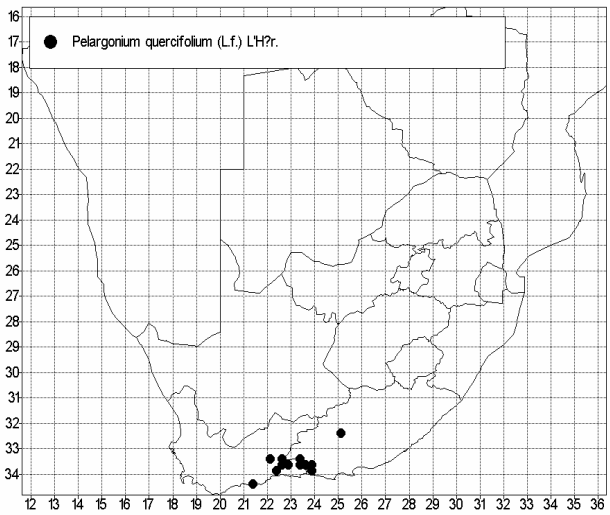


Figure 15.2: The geographical distribution of *Pelargonium quercifolium.*

Medicinal uses:

Pelargonium quercifolium is a stimulant herb with a resinous aroma (Bown, 1995). All parts of the plant are astringent (Grieve, 1984). The plant is used for the treatment of rheumatism, hypertension and heart disease. The plant is used fresh in infusions (Bown, 1995).

Other uses:

The essential oil has a labdanum fragrance (Uphof, 1959). The bruised leaves emit a warm strong aroma like incense (Genders, 1994). The dried leaves are used to make pot-pourri and are also added to insect-repellent sachets (Bown, 1995).

Remark:

Pelargonium quercifolium is a parent plant of many hybrids. *Pelargonium quercifolium* is closely related to *P. panduriforme* Eckl. & Zeyh. (van der Walt, 1985).

Chemical composition of the essential oil:

Hydrodistillation was carried out on fresh plant material for 3 hours. The oil yield was:

1. *Pelargonium quercifolium* from Stellenbosch Botanical Garden: 0.07%,
2. *Pelargonium quercifolium* from Walter Sisulu Botanical Garden: 0.26%.

GC-MS profiles and analyses

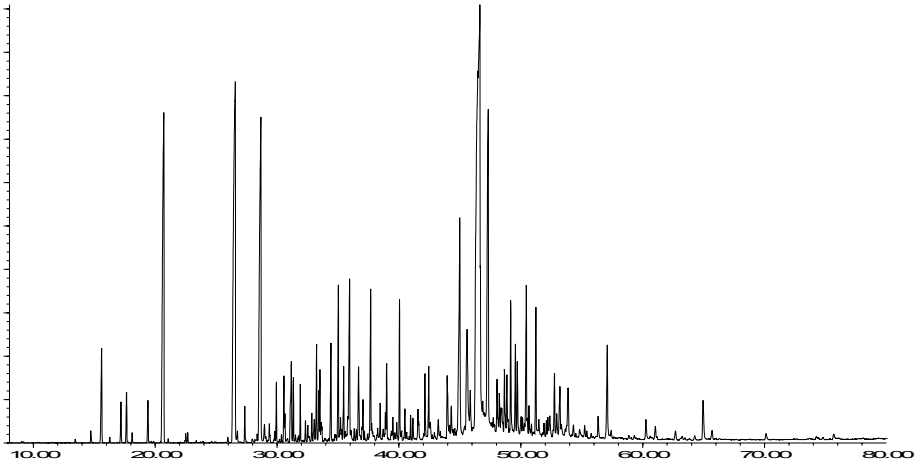


Figure 15.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium quercifolium* (SBG).

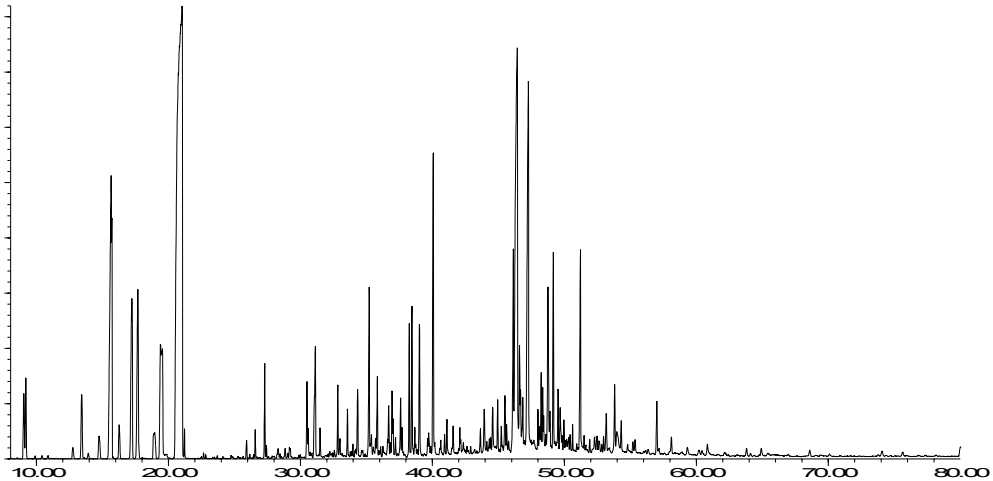


Figure 15.4: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium quercifolium* (WSBG).

Table 15.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium quercifolium.*

RRI	Compound	Area %	
		SBG	WSBG
1032	α-pinene	-	0.13
1035	α-thujene	-	0.19
1118	β-pinene	-	trace
1132	sabinene	-	0.10
1159	δ-3-carene	-	trace
1176	α-phellandrene	0.79	1.13
1203	limonene	0.27	0.22
1218	β-phellandrene	0.33	0.20
1230	butyl butyrate	0.05	-
1246	(Z)-β-ocimene	-	trace
1255	γ-terpinene	0.30	trace
1266	(E)-β-ocimene	-	trace
1280	**p-cymene**	**6.22**	**54.89**
1290	terpinolene	-	trace
1429	perillene	-	0.15
1434	**hexyl butyrate**	**8.16**	-
1451	β-thujone	-	0.09
1452	α,p-dimethylstyrene	0.18	0.49
1464	**(E)-2-hexenyl butyrate**	**6.34**	-
1492	cyclosativene	0.15	-
1493	α-ylangene	-	0.07
1497	α-copaene	0.10	-
1503	hexyl valerate	0.07	-
1508	heptyl butyrate	0.24	-
1571	*trans*-p-menth-2-en-1-ol	-	0.19
1600	β-elemene	0.09	-
1608	octyl butyrate	0.41	-
1611	terpinen-4-ol	-	0.23
1612	β-caryophyllene	0.10	-
1614	hexyl tiglate	0.22	-
1617	guaia-6,9-diene	0.12	trace
1628	aromadendrene	0.10	0.13
1661	allo-aromadendrene	0.77	0.05
1683	carvotanacetone	-	trace
1690	cryptone	trace	1.36
1707	ledene	1.77	0.42
1726	germacrene D	0.07	-
1730	δ-guaiene	trace	0.27
1740	valencene	0.07	-
1740	α-muurolene	0.45	0.18
1748	piperitone	-	trace
1751	phellandral	-	0.33
1751	carvone	-	0.41

RRI	Compound	Area %	
		SBG	WSBG
1755	bicyclogermacrene	0.08	-
1758	*cis*-piperitol	-	trace
1773	δ-cadinene	1.23	0.52
1776	γ-cadinene	0.06	trace
1783	p-methylacetophenone	-	0.68
1802	cuminaldehyde	0.08	0.84
1823	p-mentha-1(7),5-dien-2-ol*	0.56	1.04
1829	octyl hexanoate	0.08	-
1853	*cis*-calamenene	0.07	0.22
1864	p-cymen-8-ol	1.00	2.76
1865	isopiperitenone	-	0.05
1886	geranyl butyrate	-	0.23
1941	α-calacorene	0.36	0.13
1941	citronellyl hexanoate	-	0.09
1984	γ-calacorene	0.11	-
2040	octyl octanoate	0.15	-
2030	methyl eugenol	-	trace
2050	(E)-nerolidol	2.75	-
2057	ledol	-	0.19
2080	cubenol	-	trace
2096	neryl hexanoate	-	trace
2045	humulene epoxide I	trace	-
2104	**viridiflorol**	**36.55**	**13.01**
2105	geranyl hexanoate	-	trace
2113	cumin alcohol	-	trace
2127	10-epi-γ-eudesmol	-	trace
2144	**spathulenol**	**6.19**	**5.90**
2179	1-tetradecanol	0.33	-
2184	*cis*-p-menth-3-ene-1,2-diol	-	trace
2185	γ-eudesmol	0.27	-
2187	T-cadinol	-	0.85
2193	β-betulenal	0.22	-
2196	ambrox	0.43	-
2198	thymol	-	0.32
2209	T-muurolol	-	trace
2239	carvacrol	0.92	1.47
2247	trans-α-bergamotol	0.64	trace
2249	cadalene	0.55	-
2255	α-cadinol	-	0.05
2273	1-pentadecanol	-	trace
2305	8,13-epoxy-15,16-dinor-labd-12-ene	1.37	-
2349	geranic acid	0.07	-
2357	14-hydroxy-β-caryophyllene	0.24	-
2376	manoyl oxide	0.52	-
2384	1-hexadecanol	0.23	-

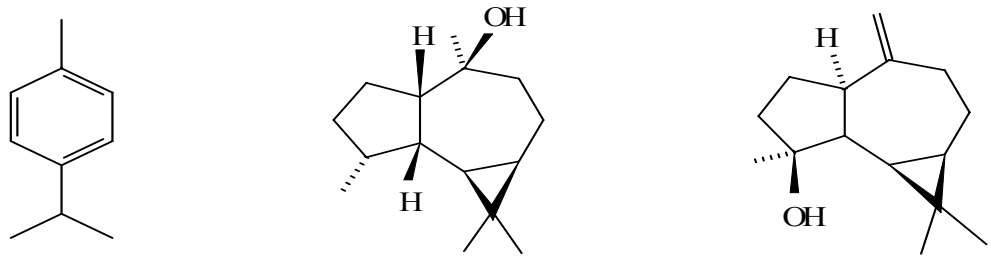
RRI	Compound	Area %	
		SBG	WSBG
2396	8α-13-oxo-14-en-epi-labdane	0.52	-
2509	dodecanoic acid	0.15	-
2617	tridecanoic acid	0.17	-
2622	phytol	-	trace
2670	tetradecanoic acid	0.35	trace
2822	pentadecanoic acid	trace	-
2931	hexadecanoic acid	trace	-
	Total	**83.62%**	**89.58%**

*correct isomer not identified; trace <0.05%.

Viridiflorol (36.55%) is the main compound in *P. quercifolium* (SBG). The following compounds also occur in high amounts in *P. quercifolium* (SBG) essential oil: p-cymene (6.22%), hexyl butyrate (8.16%), (E)-2-hexenyl butyrate (6.34%) and spathulenol (6.19%).

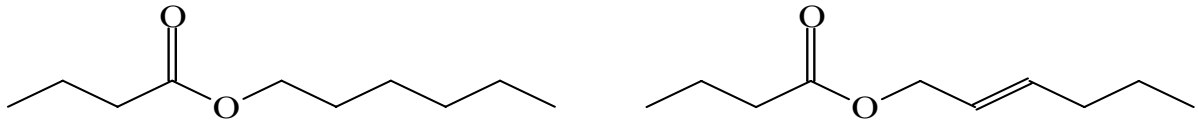
p-Cymene occupies 54.89% of the total oil composition of *P. quercifolium* (WSBG). Viridiflorol and spathulenol contribute 13.01% and 5.90%, respectively.

The major compounds common to both *P. quercifolium* (SBG) and *P. quercifolium* (WSBG) include p-cymene, viridiflorol and spathulenol. Hexyl butyrate and (E)-2-hexenyl butyrate were absent in *P. quercifolium* (WSBG).



p-cymene viridiflorol spathulenol

Figure 15.5: Chemical structures of the major compounds found in both *P. quercifolium* (SBG) and *P. quercifolium* (WSBG) essential oil samples.



hexyl butyrate (E)-2-hexenyl butyrate

Figure 15.6: Chemical structures of the other major compounds identified in *Pelargonium quercifolium* (SBG) essential oil.

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium quercifolium* collected from the Stellenbosch Botanical Garden.

HPLC profile and analysis

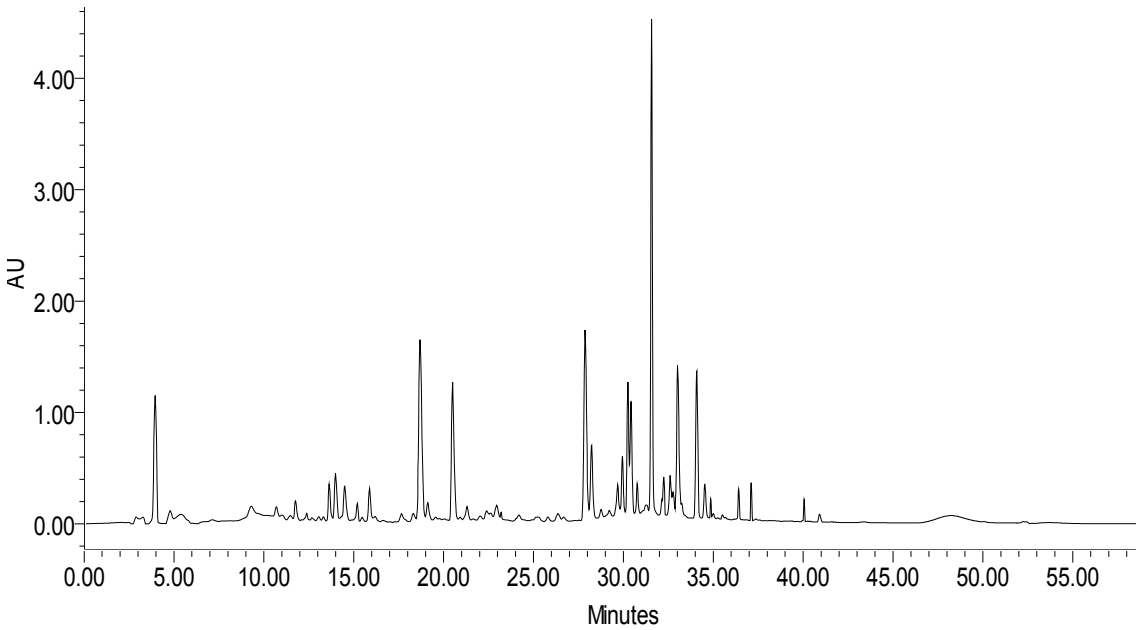


Figure 15.7: HPLC profile of the acetone extract of *Pelargonium quercifolium* (SBG).

Table 15.2: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium quercifolium* (SBG) acetone extract.

Retention time (min)	**UV absorbance maxima (nm)**	**Tentative identification**	**% Integration**
3.95	212.0		6.81
13.64	219.1, 271.0		1.20
13.98	210.9		2.33
14.50	221.5, 272.2		2.04
15.88	255.7, 355.6	flavonol	1.52
18.76	255.7, 355.6	flavonol	11.21
20.50	255.7, 355.6	flavonol	7.11
27.87	255.7, 359.0	flavonol	10.02
28.23	260.4, 337.7	flavone	3.65
29.68	249.7, 347.2	flavone	1.96
29.95	266.3, 350.8	flavone	2.60
30.26	254.5, 355.6	flavonol	4.98
30.43	258.0, 298.3, 352.0		4.94
30.77	262.7, 363.9	flavonol	1.62
31.63	260.4, 338.9	flavone	16.06
32.25	261.6		2.25
32.61	252.1, 350.8	flavone	2.00
33.02	254.5, 354.4	flavonol	7.62
34.08	249.7, 344.8		6.34
34.53	253.3, 352.0	flavonol	1.58
36.42	261.6		0.82
37.12	253.3		0.86
40.06	262.7		0.49

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium quercifolium* in this study include the following:

- The *P. quercifolium* (SBG) extract produced promising antibacterial activity against *B. cereus* (MIC = 0.33 mg/ml) and against *S. aureus* (MIC = 0.16 mg/ml). The *P. quercifolium* (WSBG) extract inhibited the latter micro-organisms to a lesser extent (MIC = 1.00 mg/ml) but showed improved activity against *K. pneumoniae* and *C.* albicans (MIC values of 2 mg/ml and 1 mg/ml were obtained, respectively).

- The *P. quercifolium* (SBG) extract produced substantial anti-oxidant activity (IC_{50} = 17.15 ± 0.58 µg/ml), whereas the *P. quercifolium* (WSBG) extract showed much lower anti-oxidant activity (IC_{50} = 61.87 ± 3.19 µg/ml).

- Of all the species tested, *P. quercifolium* (SBG and WSBG) produced the greatest inhibition of the 5-lipoxygenase enzyme (IC_{50} = 33.24 µg/ml – 38.67 µg/ml).

- *P. quercifolium* (SBG) extract displayed pronounced antimalarial activity (IC_{50} = 2.66 ± 0.36 µg/ml).

- The *P. quercifolium* (SBG) extract produced a lower toxicity profile (IC_{50} = 85.61 ± 1.93 µg/ml) than the *P. quercifolium* (WSBG) extract (IC_{50} = 48.69 ± 1.04 µg/ml). The essential oil samples proved to be very toxic. IC_{50} values of 3.34 ± 2.79 µg/ml and 8.81 ± 1.46 µg/ml were obtained for *P. quercifolium* (SBG) and *P. quercifolium* (WSBG), respectively.

16. *Pelargonium radens* H. E. Moore

Synonym:

Pelargonium radula (Cav.) L'Hérit. (van der Walt, 1977).

Common names:

Rasp-leaved pelargonium, Multifid-leaved pelargonium (van der Walt, 1977).

Botanical description:

Pelargonium radens is an upright, branching shrub reaching a height of 1 m. As the plant ages, the stem becomes woody toward the base. Rigid bristles are present on the herbaceous, slender side-branches. The leaves release a pungent rose and lemon scent when bruised. The leaves are triangular in outline, narrow and scabrid (deeply divided). Coarse, long, stiff, erect hairs are found on the upper surface making the leaves hard to the touch (almost scabrous). An important characteristic of the leaves are the margins of the narrow segments which are rolled under like a scraper. The species name '*radens*' (scraping) or '*radula*' (rasp or file) refers to the rasp-like leaves. Both the segments and apex are obtusely lobulate. At the base of the petioles are narrowly ovate or ovate stipules. The inflorescences are umbel-like and 2-6-flowered. The petals are pale purple or pink-purple in colour. The posterior petals have conspicuous beetroot purple veins and blotches and have an entire or slightly notched tip. The plant contains seven fertile stamens (van der Walt, 1977; Webb, 1984). *Pelargonium radens* is rather similar to *P. graveolens* (Huxley, 1992).

Distribution and habitat:

Pelargonium radens is confined to the eastern region of the Western Cape and the southern region of the Eastern Cape, where it occurs from near the vicinity of Barrydale eastwards along the coast to Engcobo (van der Walt, 1977). It grows on mountain sides and by rocky streams as well as in sheltered valleys (Phillips and Rix, 1998).

Figure 16.1: The flower of *Pelargonium radens.*

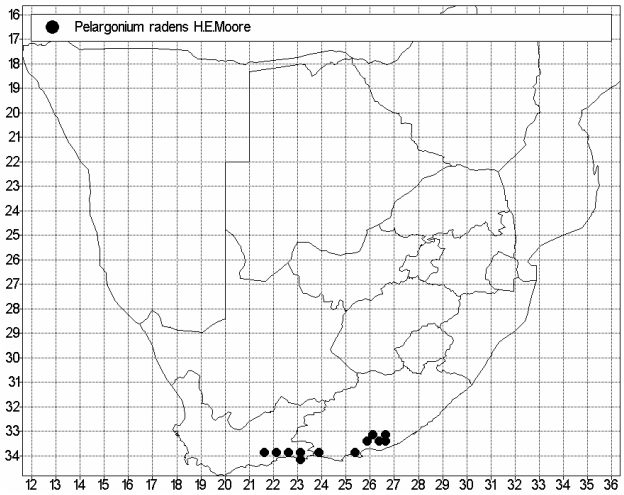


Figure 16.2: The geographical distribution of *Pelargonium radens.*

Medicinal uses:

All parts of the plant are astringent (Grieve, 1984). A rub made from the fresh leaves can be used externally to relieve aching feet or legs (Bown, 1995).

Other uses:

The lemony scented essential oil is used in perfumery (Uphof, 1959). The dried leaves are used in pot-pourri as a fixative for other perfumes. The dried leaves are also used in insect-repellent sachets (Bown, 1995).

Chemical composition of the essential oil:

The fresh plant material collected from the National Botanical Garden (Kirstenbosch) yielded 0.69% essential oil.

GC-MS profile and analysis

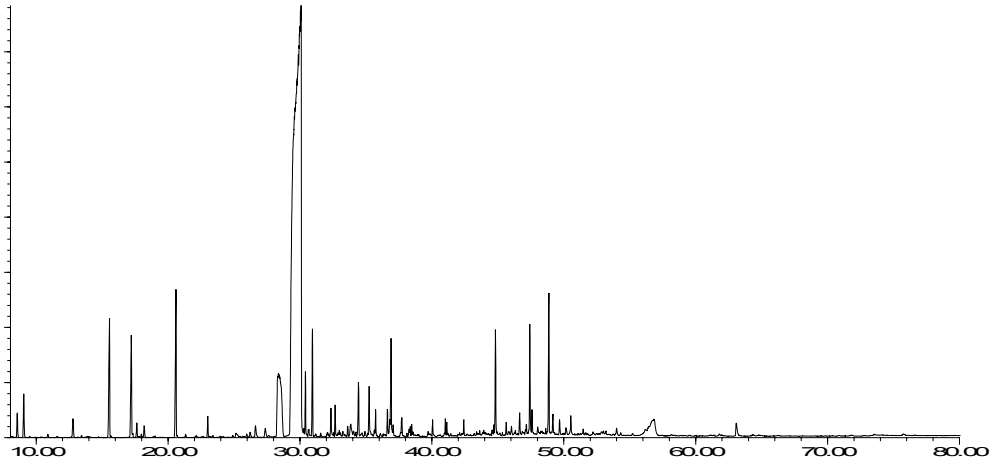


Figure 16.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium radens* (NBG).

Table 16.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium radens* (NBG).

RRI	Compound	Area %
1014	tricyclene	0.17
1032	α-pinene	0.28
1076	camphene	trace
1118	β-pinene	0.14
1132	sabinene	trace
1174	myrcene	0.90
1203	limonene	0.75
1216	3-methylcyclopentanone	trace
1218	β-phellandrene	0.10
1280	**p-cymene**	**1.06**
1327	3-methylcyclohexanone	0.13
1384	α-pinene oxide	trace
1429	perillene	0.10
1450	*trans*-linalool oxide (furanoid)	0.05
1458	*cis*-1,2-limonene epoxide	trace
1475	**menthone**	**1.88**
1503	**isomenthone**	**84.52**
1553	linalool	0.58
1571	*trans*-p-menth-2-en-1-ol	trace
1572	β-ylangene	0.15
1580	*cis*-isopulegone	trace
1617	guaia-6,9-diene	trace

RRI	Compound	Area %
1632	neoisomenthol	0.16
1637	menthol	0.05
1661	allo-aromadendrene	trace
1662	isomenthol	trace
1668	(Z)-β-farnesene	trace
1674	γ-gurjunene	trace
1687	α-humulene	trace
1690	cryptone	0.30
1706	α-terpineol	0.12
1719	borneol	0.05
1725	verbenone	trace
1740	valencene	trace
1748	piperitone	0.61
1751	carvone	0.07
1772	citronellol	0.16
1783	p-methylacetophenone	0.07
1798	methyl salicylate	0.06
1802	cuminaldehyde	trace
1809	citronellyl butyrate	trace
1845	*trans*-carveol	0.05
1864	p-cymen-8-ol	0.10
1885	2-phenylethyl propionate	0.07
1896	2-phenylethyl isobutyrate	0.08
1941	α-calacorene	trace
1965	citronellyl propionate	0.08
1980	2-phenylethyl butyrate	trace
1988	2-phenylethyl 2-methylbutyrate	trace
1992	2-phenylethyl isovalerate	trace
2008	caryophyllene oxide	trace
2030	norbourbonone	trace
2050	(E)-nerolidol	0.42
2071	humulene epoxide II	trace
2080	cubenol	0.05
2110	furopelargone B	trace
2140	2-phenylethyl valerate	0.11
2144	spathulenol	trace
2209	T-muurolol	trace
2211	2-phenylethyl tiglate	0.83
2239	carvacrol	trace
2255	α-cadinol	0.10
2281	decanoic acid	0.09
2411	2-decenoic acid*	0.05
	Total	**94.49%**

*correct isomer not identified; trace <0.05%; (NBG) = National Botanical Garden (Kirstenbosch).

Isomenthone makes-up 84.52% of the total oil composition. p-Cymene and menthone represent 1.06% and 1.88%, respectively.

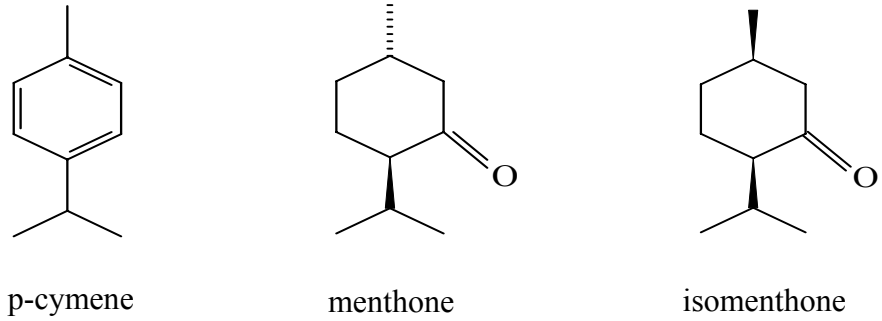


Figure 16.4: Chemical structures of the major compounds identified in the essential oil of *Pelargonium radens* (NBG).

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium radens* collected from the Stellenbosch Botanical Garden.

HPLC profile and analysis

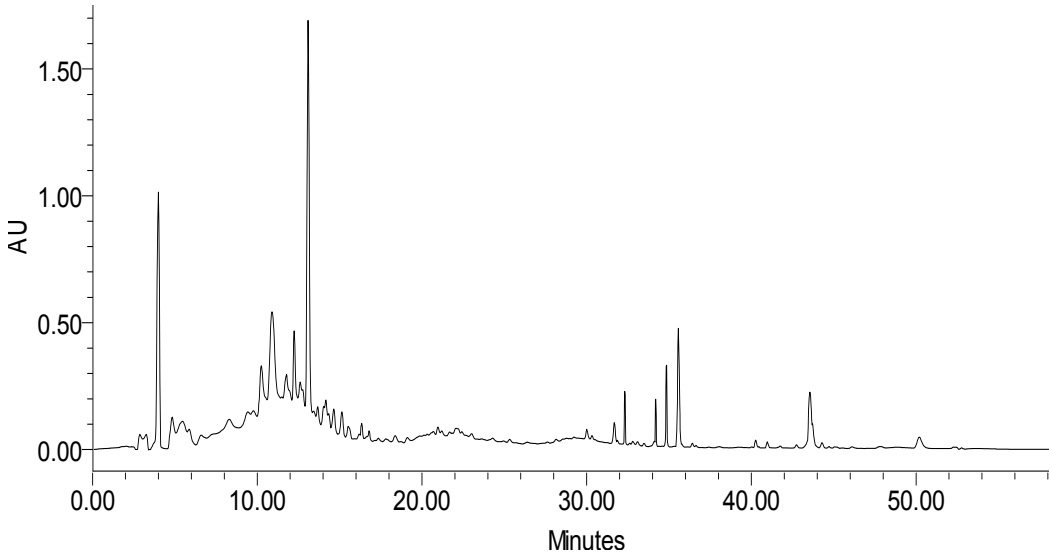


Figure 16.5: HPLC profile of the acetone extract of *Pelargonium radens* (SBG).

Table 16.2: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of *Pelargonium radens* (SBG) acetone extract.

Retention time (min)	**UV absorbance maxima (nm)**	**Tentative identification**	**% Integration**
3.99	210.9		11.04
4.85	205.0		2.29
8.29	205.0, 269.9		6.96
10.25	206.2, 269.9		7.93
10.92	207.3, 272.2		15.94
11.77	326.9		7.16
12.25	207.3, 272.2		6.09
12.61	206.2, 280.5, 325.7		4.32
12.77	207.3, 273.4		1.01
13.09	202.7, 279.3		18.01
13.67	278.1	flavanone	1.87
14.17	233.2, 328.1		1.54
14.65	219.1, 261.6		1.83
15.14	207.3, 287.6, 332.9		1.46
15.55	207.3, 267.5, 353.2		0.53
16.34	208.5, 233.2		0.37
30.01	267.5, 347.2	flavone	0.26
31.68	241.5		0.54
32.32	261.6		0.92
34.19	265.1		0.83
34.88	228.5		1.47
35.57	267.5, 346.0	flavone	3.91
43.55	293.6		3.71

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium radens* in this study include the following:

- The *P. radens* (SBG) extract was most active against *B. cereus* (MIC value of 0.5 mg/ml). Weak antimicrobial activity (MIC = 8 mg/ml to >32 mg/ml) was demonstrated by the *P. radens* (NBG) essential oil.

- The *P. radens* (NBG) essential oil did not exert anti-inflammatory activity in the 5-lipoxygenase assay (IC_{50} >100 µg/ml).

- Pronounced antimalarial activity (IC_{50} = 1.90 ± 0.39 µg/ml) was displayed by the *P. radens* (SBG) extract in the hypoxanthine incorporation assay.

- The *P. radens* (SBG) extract and essential oil (NBG) sample proved to be cytotoxic (IC_{50} = 30.81 ± 1.23 µg/ml and 27.20 ± 4.76 µg/ml, respectively).

17. *Pelargonium scabroide* R. Knuth

Botanical description:

This non-aromatic species is an erect to decumbent, branched subshrub reaching a height of up to 0.75 m. The young stems are herbaceous and wine-red becoming woody and greyish-brown with age. The stems are villous to hispid with small glandular hairs in between. The leaves are almost scabrous (hard to the touch), hence the epithet '*scabroide*'. The upper surface of the leaf is glabrescent whereas the lower surface is strigose to hispid with small glandular hairs. The leaves are 3-palmatisect to pinnatisect with irregularly incised segments. The lamina is cordiform in outline and the leaf margins are coarsely serrate-dentate and wine-red. The wine-red young stems, with long hairs and typical leaf structure, distinguish *P. scabroide* from other species of the section. The pseudo-umbels have three to five flowers each. Petals are white to pale pink-purple with darker markings; feather-like dark pink to wine-red markings occur on the posterior two, spathulate petals and a pink streak occurs on the anterior three, narrowly spathulate petals (van der Walt and Vorster, 1988).

Pelargonium scabroide and *P. sublignosum* Knuth have many features in common. Leaf characters are used to distinguish these two species from one another (van der Walt and Vorster, 1988).

Distribution and habitat:

Pelargonium scabroide has a small area of distribution. It grows on the mountains between Porterville and Touws River. It is also found in the south-eastern region of the Western Cape. It grows on sandy soil in the moist, shady habitats created by over-hanging sandstone boulders (van der Walt and Vorster, 1988).

Figure 17.1: *Pelargonium scabroide* (van der Walt and Vorster, 1988).

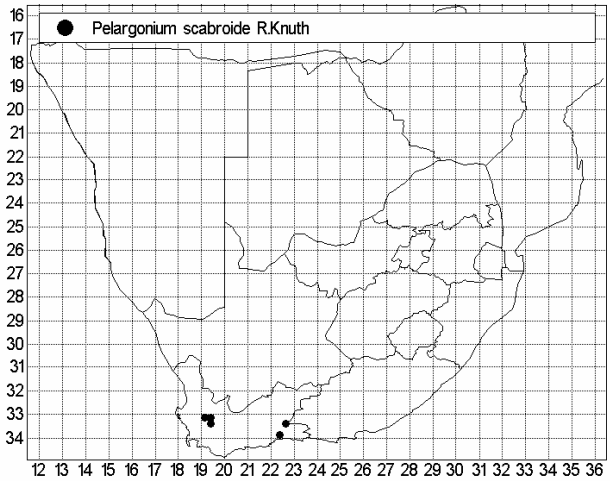


Figure 17.2: The geographical distribution of *Pelargonium scabroide.*

The essential oil of *P. scabroide* was not obtained.

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium scabroide* collected from the Stellenbosch Botanical Garden.

HPLC profile and analysis

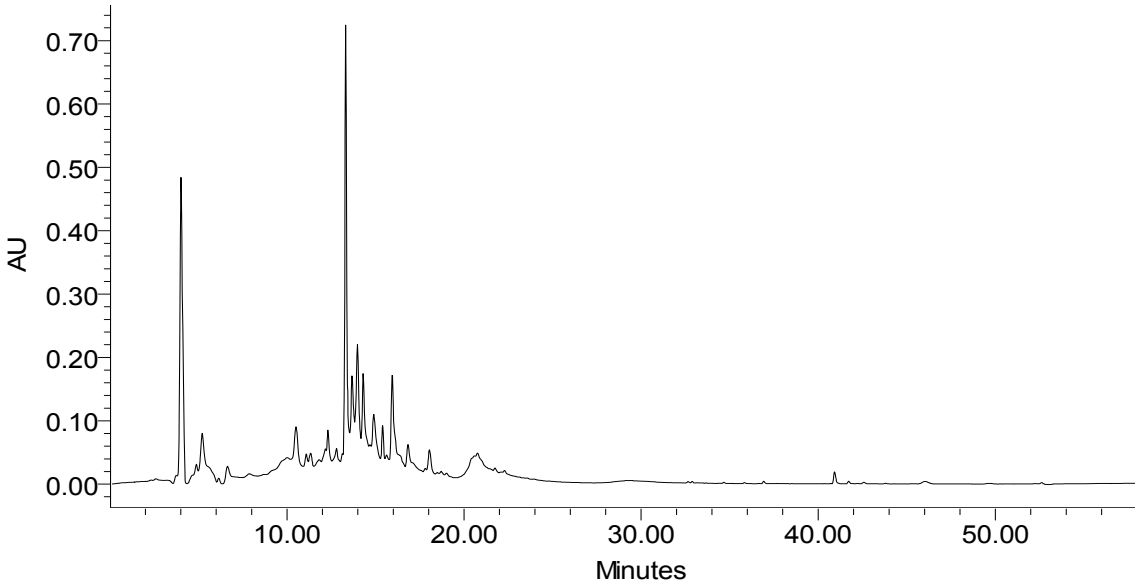


Figure 17.3: HPLC profile of the acetone extract of *Pelargonium scabroide* (SBG).

Table 17.1: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium scabroide* (SBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
4.02	213.3		21.16
5.22	215.6, 278.0		3.19
6.64	214.5, 273.3		0.95
10.51	286.3, 278.0		5.89
12.32	212.1, 278.0		0.90
13.32	203.9, 278.0		21.87
13.68	216.8, 278.0		6.83
13.98	219.1, 278.0		9.37
14.31	278.0	flavanone	7.47
14.88	209.8, 278.0		5.42
15.40	206.3, 278.0		1.25
15.94	222.7, 278.0		8.10
16.83	219.1, 278.0		1.08
18.05	221.5, 278.0		1.13
20.78	220.3, 278.0		5.40

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium scabroide* in this study include the following:

- The *P. scabroide* (SBG) extract showed substantial activity against *B. cereus* (MIC = 0.25 mg/ml). The extract produced an MIC value of 1 mg/ml with respect to *S. aureus* and *C. albicans* and an MIC value of 2 mg/ml with respect to *K. pneumoniae*.

- Promising antimalarial activity was displayed by the *P. scabroide* (SBG) extract (IC_{50} = 15.94 ± 0.16 µg/ml).

- The *P. scabroide* (SBG) extract produced moderate toxicity, an IC_{50} value of 53.76 ± 1.57 µg/ml was obtained in the MTT assay.

18. *Pelargonium scabrum* (Burm. f.) L'Hérit.

Common names:

Rough-leaved pelargonium, Three-pointed pelargonium (van der Walt, 1977).

Botanical description:

This woody, erect and much-branched shrub can grow as tall as 2 m. The presence of rigid hairs on the stems makes them scabrous (harsh and rough to the touch). When crushed the leaves emit a distinctive pleasant lemon scent; this varies according to the locality. The leaves are angularly tri-lobed, palmately veined and the leaf outline is rhomboid. The leaves of plants growing further inland in the Western Cape are more narrow-lobed. The leaves can vary from being almost glabrous to scabrous with glands always present. The margins of the leaves are finely to coarsely toothed. The umbel-like inflorescences bear two to six flowers each. The colour of the relatively small flowers can range from white to dark pink or mauve. Purple stripes are present on the two posterior petals which are twice as large as the anterior three. The flowers have seven fertile stamens (van der Walt, 1977; Webb, 1984). The leaves of *P. scabrum* are similar to those of *P. citronellum* J.J.A. v.d. Walt; however, the flowers of the latter species differ considerably (van der Walt, 1985).

Distribution and habitat:

Pelargonium scabrum has a large distribution area. It is found growing from Steinkopf (west of the Northern Cape) southwards along the western coastal area to the south-western Cape and eastwards to Willowmore in the Eastern Cape. Its distribution is associated with dry mountainous habitats and grows on well-drained, sandy soil (van der Walt, 1977; 1985).

Figure 18.1: The flower of *Pelargonium scabrum.*

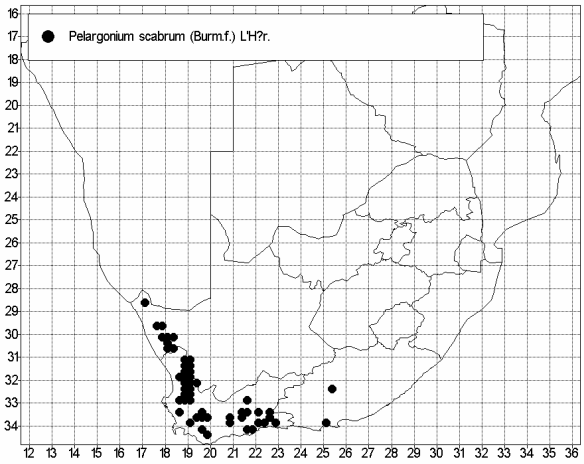


Figure 18.2: The geographical distribution of *Pelargonium scabrum.*

Chemical composition of the essential oil:

The fresh plant material collected from the Stellenbosch Botanical Garden was subjected to hydrodistillation for 3 hours. The oil yield was 0.12%.

GC-MS profile and analysis

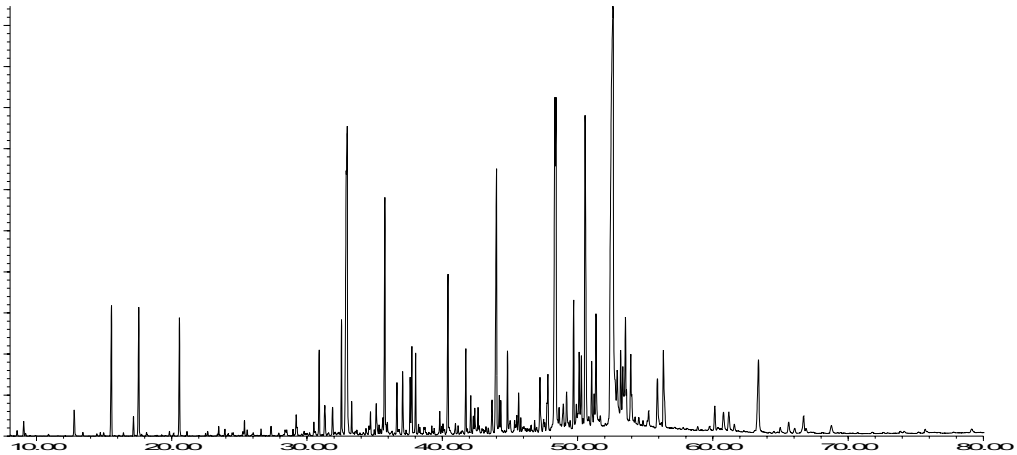


Figure 18.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium scabrum* (SBG).

Table 18.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium scabrum* (SBG).

RRI	Compound	Area %
1014	tricyclene	0.06
1032	α-pinene	0.13
1035	α-thujene	trace
1076	camphene	trace
1118	β-pinene	0.26
1132	sabinene	trace
1174	myrcene	1.37
1203	limonene	0.17
1213	1,8-cineole	1.26
1280	p-cymene	0.99
1353	*cis*-rose oxide	0.06
1365	*trans*-rose oxide	trace
1391	(Z)-3-hexenol	trace
1398	2-nonanone	0.08
1400	(Z)-2-hexenol	0.05
1429	perillene	trace
1450	*trans*-linalool oxide (furanoid)	0.05
1466	α-cubebene	trace
1473	(Z)-3-hexenyl 2-methylbutyrate	trace
1478	*cis*-linalool oxide (furanoid)	0.11
1481	(Z)-3-hexenyl isovalerate	trace
1497	α-copaene	0.22
1503	isomenthone	trace
1532	camphor	trace
1541	benzaldehyde	0.13
1545	*cis*-α-bergamotene	trace
1553	linalool	0.65
1568	*trans*-α-bergamotene	0.30
1577	α-cedrene	0.27
1587	**methyl decanoate**	**4.24**
1612	**β-caryophyllene**	**3.88**
1624	*cis*-β-terpineol	trace
1626	2-methyl-6-methylen-3,7-octadien-2-ol	0.24
1638	*cis*-p-menth-2-en-1-ol	trace
1661	allo-aromadendrene	trace
1668	citronellyl acetate	0.09
1668	(Z)-β-farnesene	0.17
1682	δ-terpineol	trace
1687	α-humulene	0.32
1693	β-acoradiene	0.10
1696	β-alaskene	0.07
1704	γ-muurolene	0.19
1706	α-terpineol	2.83

RRI	Compound	Area %
1740	α-muurolene	trace
1743	α-cadinene	trace
1773	δ-cadinene	0.69
1776	γ-cadinene	0.63
1786	ar-curcumene	0.65
1798	methyl salicylate	trace
1834	citronellyl isovalerate	0.08
1853	*cis*-calamenene	0.21
1857	geraniol	0.09
1864	p-cymen-8-ol	0.11
1941	α-calacorene	0.33
1969	(Z)-jasmone	0.05
1981	heptanoic acid	0.06
1984	γ-calacorene	0.12
2001	isocaryophyllene oxide	0.35
2008	**caryophyllene oxide**	**4.20**
2020	caryophylla-8(14)-en-5-one	0.33
2030	methyl eugenol	0.26
2050	(E)-nerolidol	0.90
2071	humulene epoxide II	0.07
2080	cubenol	0.30
2088	1-epi-cubenol	0.08
2131	hexahydrofarnesylacetone	trace
2143	cedrol	0.70
2170	β-bisabolol	trace
2187	**T-cadinol**	**6.08**
2193	**β-betulenal**	**5.64**
2204	clovenol	0.43
2209	T-muurolol	0.24
2272	14-acetoxy-β-caryophyllene	0.10
2281	**decanoic acid**	**6.36**
2316	caryophylladienol I	0.15
2324	caryophylladienol II	2.27
2357	**14-hydroxy-β-caryophyllene**	**27.93**
2392	caryophyllenol II	0.93
2415	14-hydroxy-4,5-dihydro-β-caryophyllene	1.23
2478	14-hydroxy-α-humulene	0.72
2509	dodecanoic acid	0.94
2609	14-hydroxy-4,5-epoxy-β-caryophyllene (β,β form)	0.34
2663	14-hydroxy-4,5-epoxy-β-caryophyllene (β,α form)	1.07
2670	tetradecanoic acid	trace
	Total	**82.93%**

trace <0.05%; (SBG) = Stellenbosch Botanical Garden.

14-Hydroxy-β-caryophyllene is the major compound (27.93%) in *P. scabrum* (SBG) essential oil. Other major compounds include methyl decanoate (4.24%), β-caryophyllene (3.88%), caryophyllene oxide (4.20%), T-cadinol (6.08%), β-betulenal (5.64%) and decanoic acid (6.36%).

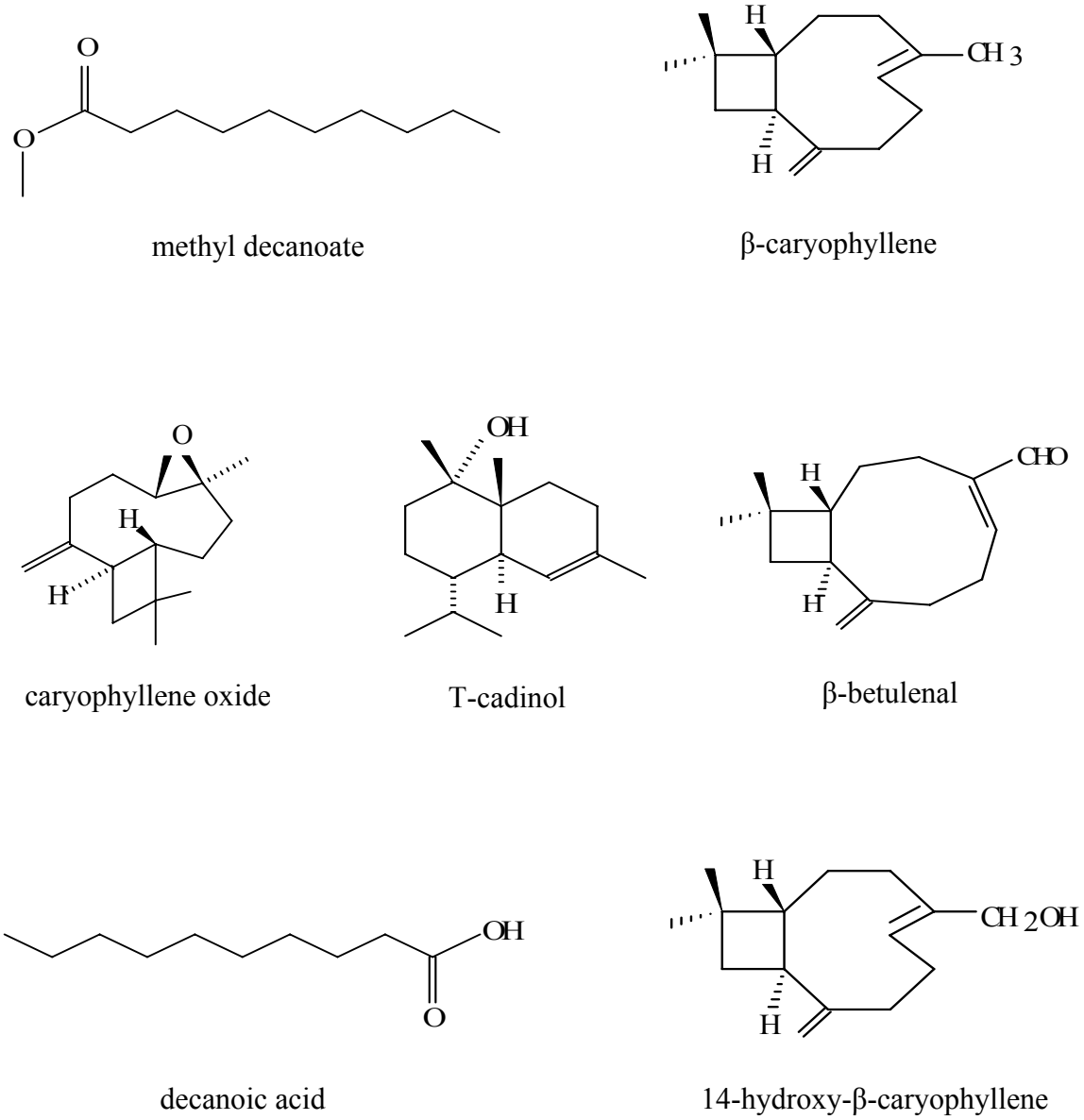


Figure 18.4: Chemical structures of the major compounds identified in the essential oil of *Pelargonium scabrum* (SBG).

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium scabrum* collected from the Stellenbosch Botanical Garden.

HPLC profile and analysis

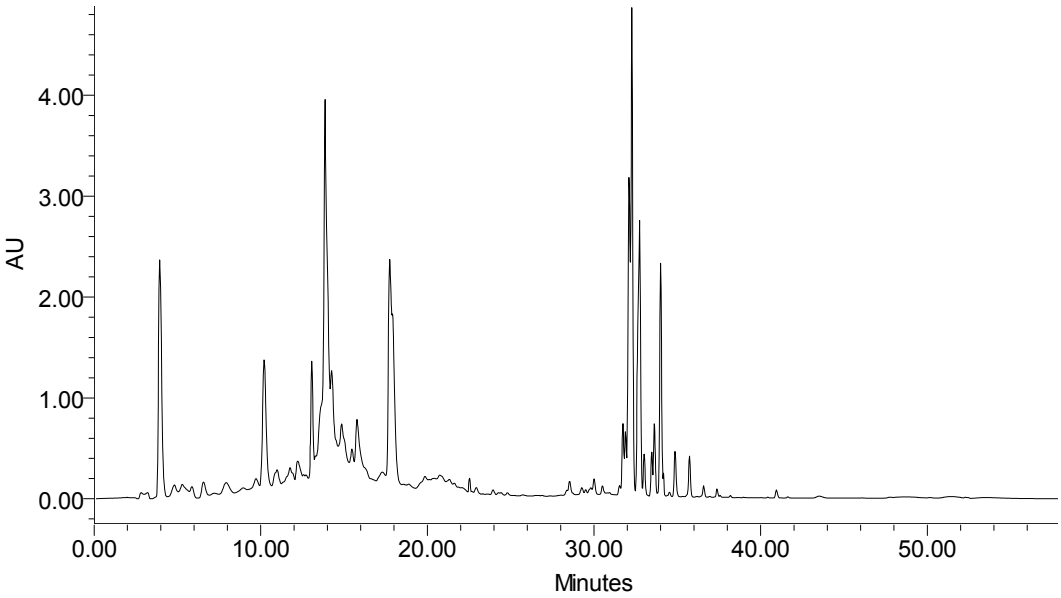


Figure 18.5: HPLC profile of the acetone extract of *Pelargonium scabrum* (SBG).

Table 18.2: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium scabrum* (SBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
4.07	212.0		7.81
10.21	205.0, 269.9		4.44
12.25	212.0, 279.3		0.49
13.06	205.0, 279.3		3.39
13.76	217.9, 278.1		18.85
14.26	219.1, 279.3		5.70
14.84	209.7, 279.3		4.75
15.78	222.6, 278.1		5.54
17.66	255.7, 353.2	flavonol	12.79
22.53	279.3		0.23
31.73	288.8	flavanone	1.34
31.89	275.8	flavanone	1.15
32.03	271.0	flavanone	6.70
32.36	239.1, 266.3, 359.0		9.37

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
32.80	266.3, 340.1	isoflavone	7.99
33.01	292.4	flavanone	0.81
33.47	236.8, 344.8		0.72
33.62	288.8	flavanone	1.41
34.06	293.6	flavanone	4.77
34.86	292.4	flavanone	0.92
35.73	292.4	flavanone	0.82

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium scabrum* in this study include the following:

- The *P. scabrum* (SBG) extract displayed considerable activity against *B. cereus* (MIC = 0.059 mg/ml) and *S. aureus* (MIC = 0.078 mg/ml). *Pelargonium scabrum* is the second most active species against *B. cereus*. Promising activity was produced with respect to *C. albicans* (MIC = 0.38 mg/ml).

- An IC_{50} value of 7.15 ± 0.11 µg/ml was obtained in the DPPH assay, representing substantial anti-oxidant activity.

- *Pelargonium scabrum* (SBG) essential oil displayed moderate anti-inflammatory activity (IC_{50} = 54.68 µg/ml) in the 5-lipoxygenase assay.

- Promising antimalarial activity (IC_{50} = 16.10 ± 0.02 µg/ml) was produced by the *P. scabrum* (SBG) extract.

- The *P. scabrum* (SBG) extract displayed toxic effects, an IC_{50} value of 37.78 ± 1.72 µg/ml was obtained in the MTT assay.

19. *Pelargonium sublignosum* R. Knuth

Botanical description:

Pelargonium sublignosum is an erect, branched shrub with an overall glaucous appearance. This species grows up to 1 m tall and is non-aromatic or strongly sweet-scented. The stem is woody, becoming completely wood-like with age (the epithet '*sublignosum*' refers to the woody stems). The stems are sparsely to densely villous interspersed with glandular hairs. The stems eventually become glabrous with a dark reddish-brown shining bark. The upturned branches are woody. The green to dull green leaves are sparsely hirtellous to villous, interspersed with glandular hairs. The lamina is cordiform in outline, and is deeply 3–(5)-lobed, the broad lobes being blunt or rounded with dentate to coarsely dentate and often reddish edges. Three to nine flowers occur on each pseudo-umbel. Flower colour is white, light pink or pink. The petals have a general linear-spathulate shape and are long-clawed at their bases. Feather-like pink-purplish markings occur on the posterior two petals (Webb, 1984; van der Walt 1985; van der Walt and Vorster, 1988).

The leaves of *P. sublignosum* are similar to those of *P. scabrum* (L.) L'Hérit., it is likely that *P. sublignosum* evolved from *P. scabrum*. The resemblance in the habit, leaves and indumentum of *P. sublignosum* and *P. scabroide* Knuth may imply a relationship between these two species. The latter species are more distantly related to *P. scabrum* (van der Walt and Vorster, 1988).

Distribution and habitat:

The distribution of *Pelargonium sublignosum* is confined to a small south-western region of the Western Cape. It is restricted to mountainous areas and has been obtained only on Piquetberg and on the mountain ranges between Porterville and Ceres. It grows in moist habitats such as in ravines, frequently between rocks (van der Walt and Vorster, 1988).

Figure 19.1: *Pelargonium sublignosum* in flower.

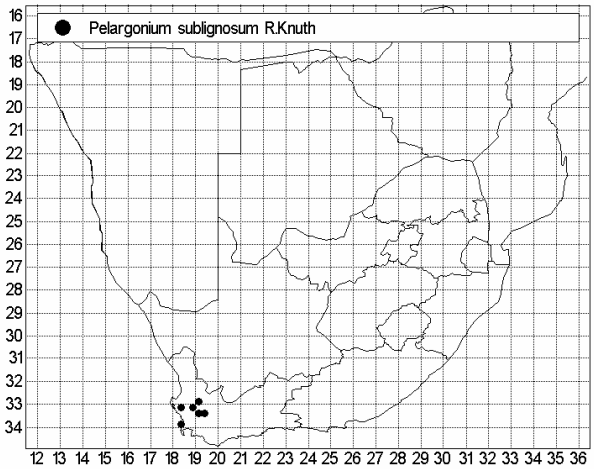


Figure 19.2: The geographical distribution of *Pelargonium sublignosum.*

The essential oil of *P. sublignosum* was not obtained.

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium sublignosum* collected from the Stellenbosch Botanical Garden.

HPLC profile and analysis

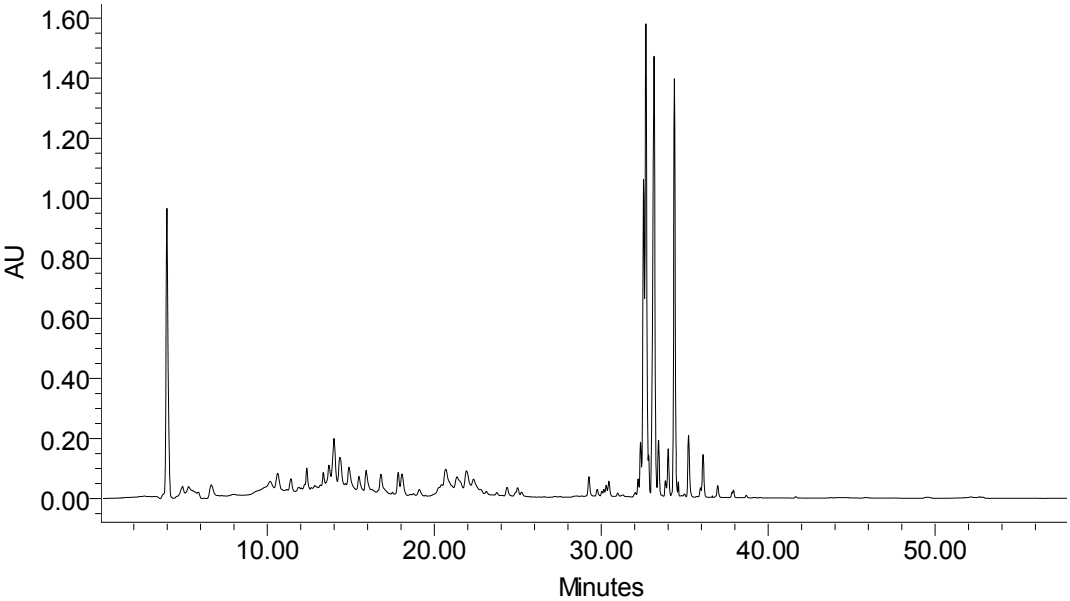


Figure 19.3: HPLC profile of the acetone extract of *Pelargonium sublignosum* (SBG).

Table 19.1: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium sublignosum* (SBG) acetone extract.

Retention time (min)	**UV absorbance maxima (nm)**	**Tentative identification**	**% Integration**
3.98	213.3		11.28
6.64	214.5, 272.1		0.74
10.18	210.9, 278.0		1.29
10.62	206.3, 272.1		1.54
11.41	208.6, 260.3		0.51
12.37	210.9, 275.6		1.20
13.35	207.4, 278.0		0.32
13.69	213.3, 278.0		1.47
13.99	219.1, 278.0		3.20
14.35	215.6, 278.0		2.17
14.89	209.8, 275.6		1.56
15.49	218.0, 275.6		0.51
15.91	222.7, 276.8		0.88
16.81	218.0, 278.0		1.14
17.83	255.6, 354.0	flavonol	0.54
18.06	221.5, 276.8		1.11
20.68	218.0, 278.0		2.36
21.36	220.3, 275.6		1.53
21.93	220.3, 275.6		1.54
29.26	291.0	flavanone	0.55
30.45	269.7, 351.6		0.31
32.21	288.6	flavanone	0.18
32.35	276.8	flavanone	1.51
32.53	272.1	flavanone	9.55
32.67	202.8, 267.4, 359.4		15.86
33.16	266.2, 338.5	isoflavone	18.02
33.44	292.2	flavanone	1.77
34.01	229.7, 288.6		1.53
34.38	292.2	flavanone	12.51
35.23	292.2	flavanone	1.98
36.09	292.2	flavanone	1.33

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium sublignosum* in this study include the following:

- The *P. sublignosum* (SBG) extract displayed considerable activity against *S. aureus* (MIC = 0.078 mg/ml). Substantial antimicrobial activity was also shown against *B. cereus* (MIC = 0.13 mg/ml). An MIC value of 0.5 mg/ml was obtained for *C. albicans*.

- Substantial anti-oxidant activity (IC_{50} = 17.61 ± 3.18 µg/ml) was displayed by the *P. sublignosum* (SBG) extract.

- Considerable antimalarial activity (IC_{50} = 9.01 ± 1.22 µg/ml) was produced by the *P. sublignosum* (SBG) extract.

- Of all the extracts tested in the MTT assay, the *P. sublignosum* (SBG) extract was the most toxic (IC_{50} = 11.89 ± 1.54 µg/ml).

20. *Pelargonium tomentosum* Jacq.

Synonym:

Pelargonium micranthum Eckl. & Zeyh. (van der Walt and Vorster, 1981).

Common name:

Pennyroya pelargonium, Peppermint-scented pelargonium, Piperitum (van der Walt and Vorster, 1981).

Botanical description:

Pelargonium tomentosum is a decumbent, bushy, strongly peppermint-scented plant with erect branches. It reaches a height of 0.5 m. Long, thick hairs cover the stem which is woody at the base, but fleshy above. The large grey-green soft leaves have a thick tomentose (felt-like) covering on all surfaces which give it that velvety touch. The leaf blades have a broadly cordate outline and are 3–(5)-palmatilobate to 3–(5)-palmatipartite, the base is cordately incised and the apices of the lobes are obtuse (the leaf is shaped much like a grape leaf). The leaf margins are irregularly crenate-serrate. Four to fifteen flowers occur on each pseudo-umbel. The five petals are white, entire in general shape and are very small. Prominent carmine-red marks occur on the petals. Posterior petals are elliptic to obovate with eared bases. The three anterior petals are longer and narrower than the two posterior ones. There are seven fertile stamens (van der Walt and Vorster, 1981; Webb, 1984; van der Walt, 1985). When not flowering, this species may be confused with *P. papilionaceum* (L.) L'Hérit. (van der Walt and Vorster, 1981).

Distribution and habitat:

Pelargonium tomentosum has been collected on the Hottentots Holland Mountains near Somerset West, on the Riviersonderend Mountains near Greyton and on the Langeberg range from Swellendam to Riversdale. It is rarely found on the Hottentots Holland Mountains but is abundant on the Langeberg range particularly near Swellendam. This species is restricted to mountains where it grows in semi-shaded, moist places (van der Walt, 1985). It is found on the margins of forests along streams (Phillips and Rix, 1998).

Figure 20.1: *Pelargonium tomentosum* in flower.

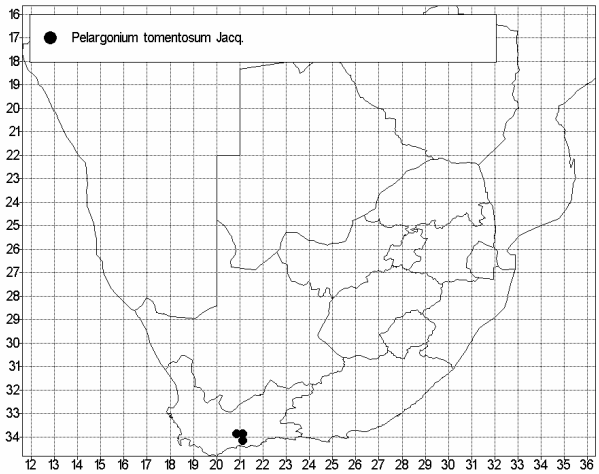


Figure 20.2: The geographical distribution of *Pelargonium tomentosum.*

Medicinal uses:

All parts of the plant are astringent (Grieve, 1984). The fresh leaves are used as a poultice for the relief of bruises and sprains (Bown, 1995).

Edible uses:

Often used as a culinary herb (van der Walt and Vorster, 1981). Due to their strong mint scent, the leaves and flowers are used to flavour cakes, puddings, pies, biscuits, etc. (Facciola, 1990; Bown, 1995). The fresh leaves are used to make a peppermint-flavoured tea (Bown, 1995).

Other uses:

A peppermint-scented essential oil is obtained from the plant (Chittendon, 1956). The dried leaves are used in pot-pourri (Bown, 1995).

Chemical composition of the essential oil:

Hydrodistillation was carried out on fresh plant material for 3 hours after which a pale yellowish-green oil was obtained. The oil yield was:

1. *Pelargonium tomentosum* from Stellenbosch Botanical Garden: 0.27%
2. *Pelargonium tomentosum* from Walter Sisulu Botanical Garden: 0.21%

GC-MS profile and analysis

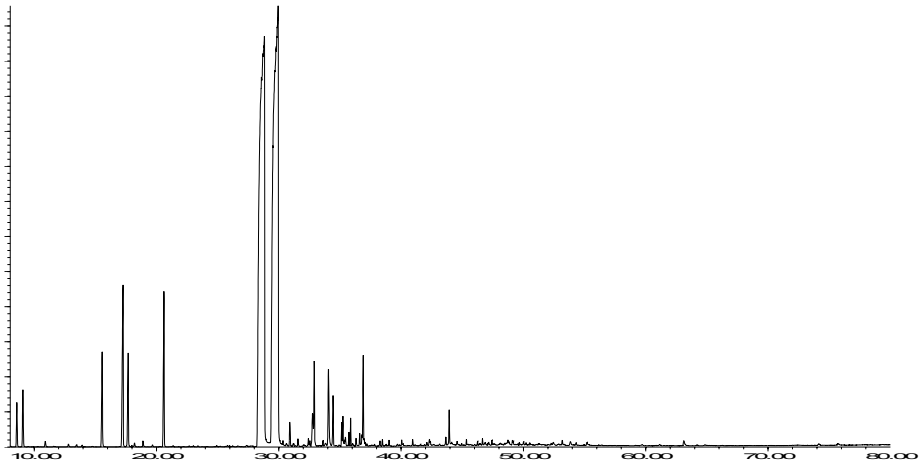


Figure 20.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium tomentosum* (SBG).

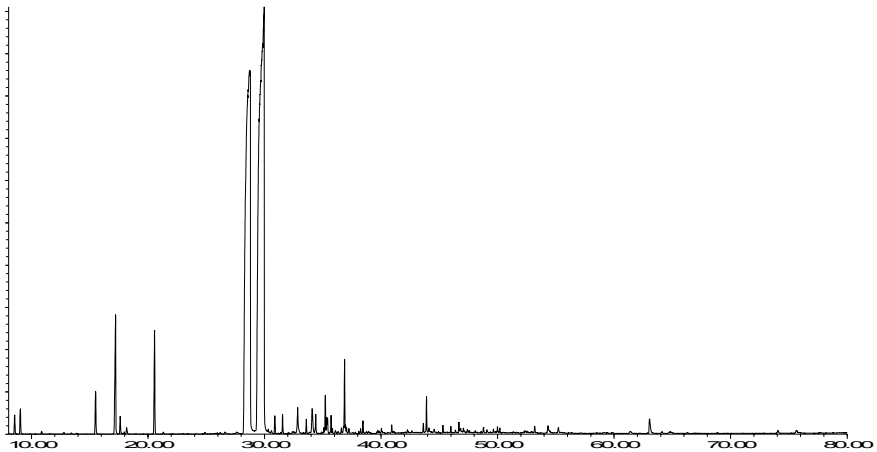


Figure 20.4: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium tomentosum* (WSBG).

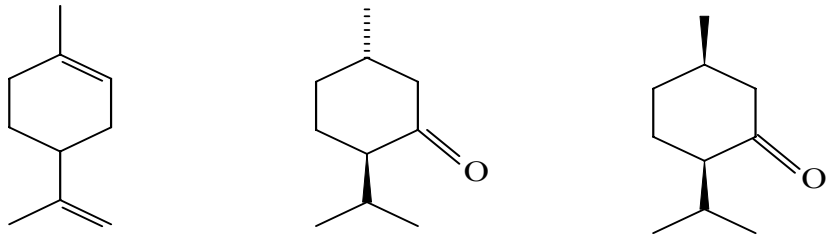
Table 20.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium tomentosum*.

RRI	Compound	Area %	
		SBG	WSBG
1014	tricyclene	0.44	0.25
1032	α-pinene	0.50	0.29
1076	camphene	0.05	trace
1118	β-pinene	trace	trace
1132	sabinene	trace	trace
1146	δ-2-carene	-	trace
1174	myrcene	0.76	0.41
1203	**limonene**	**1.48**	**1.25**
1216	3-methylcyclopentanone	trace	trace
1218	β-phellandrene	0.63	0.14
1246	(Z)-β-ocimene	trace	-
1266	(E)-β-ocimene	trace	-
1280	p-cymene	1.07	0.79
1384	α-pinene oxide	trace	trace
1406	fenchone	trace	trace
1429	perillene	trace	trace
1452	α,p-dimethylstyrene	trace	-
1458	*cis*-1,2-limonene epoxide	-	trace
1475	**menthone**	**41.14**	**36.06**
1503	**isomenthone**	**49.29**	**56.63**
1535	β-bourbonene	trace	trace
1541	benzaldehyde	trace	trace
1553	linalool	trace	0.12
1556	8,9-limonene epoxide*	-	trace
1571	*trans*-p-menth-2-en-1-ol	trace	0.10
1583	isopulegol	trace	-
1586	cyperene	-	trace
1592	neomenthol	0.29	0.10
1596	α-guaiene	trace	-
1600	β-elemene	trace	0.10
1612	β-caryophyllene	0.43	-
1632	neoisomenthol	trace	trace
1637	menthol	0.57	0.24
1638	*cis*-p-menth-2-en-1-ol	0.06	0.10
1678	*cis*-p-mentha-2,8-dien-1-ol	-	trace
1687	α-humulene	0.09	trace
1690	cryptone	0.16	0.27
1706	α-terpineol	0.06	0.11
1707	ledene	0.10	trace
1719	borneol	trace	trace
1726	germacrene D	0.06	-
1741	β-bisabolene	0.07	-
1748	piperitone	0.49	0.41

RRI	Compound	Area %	
		SBG	WSBG
1751	carvone	trace	trace
1758	*cis*-piperitol	trace	trace
1783	p-methylacetophenone	trace	trace
1802	cuminaldehyde	trace	0.06
1811	p-mentha-1,3-dien-7-al	-	trace
1823	p-mentha-1(7),5-dien-2-ol*	trace	-
1845	*trans*-carveol	trace	trace
1864	p-cymen-8-ol	trace	trace
1885	2-phenylethyl propionate	trace	-
1896	2-phenylethyl isobutyrate	-	trace
1949	piperitenone	0.05	-
2001	isocaryophyllene oxide	trace	0.05
2008	caryophyllene oxide	0.17	0.18
2057	ledol	-	trace
2071	humulene epoxide II	trace	trace
2098	globulol	-	trace
2104	viridiflorol	trace	-
2113	cumin alcohol	trace	trace
2131	hexahydrofarnesylacetone	trace	-
2144	spathulenol	trace	-
2184	nonanoic acid	trace	-
2198	thymol	trace	-
2209	T-muurolol	-	trace
2211	2-phenylethyl tiglate	trace	trace
2239	carvacrol	trace	-
2254	citronellic acid	trace	trace
2255	α-cadinol	trace	trace
2279	sandaracopimaradiene	trace	-
2700	heptacosane	-	trace
2900	nonacosane	trace	trace
2931	hexadecanoic acid	-	0.06
	Total	**97.96%**	**97.72%**

*correct isomer not identified; trace <0.05%.

Isomenthone and menthone are the major compounds present in both the *P. tomentosum* essential oil samples. Limonene contributes 1.48% to the *P. tomentosum* (SBG) oil sample and 1.25% to the *P. tomentosum* (WSBG) oil sample.



limonene menthone isomenthone

Figure 20.5: Chemical structures of the major compounds identified in the essential oil of *Pelargonium tomentosum* (SBG and WSBG).

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium tomentosum* collected from the Stellenbosch Botanical Garden.

HPLC profile and analysis

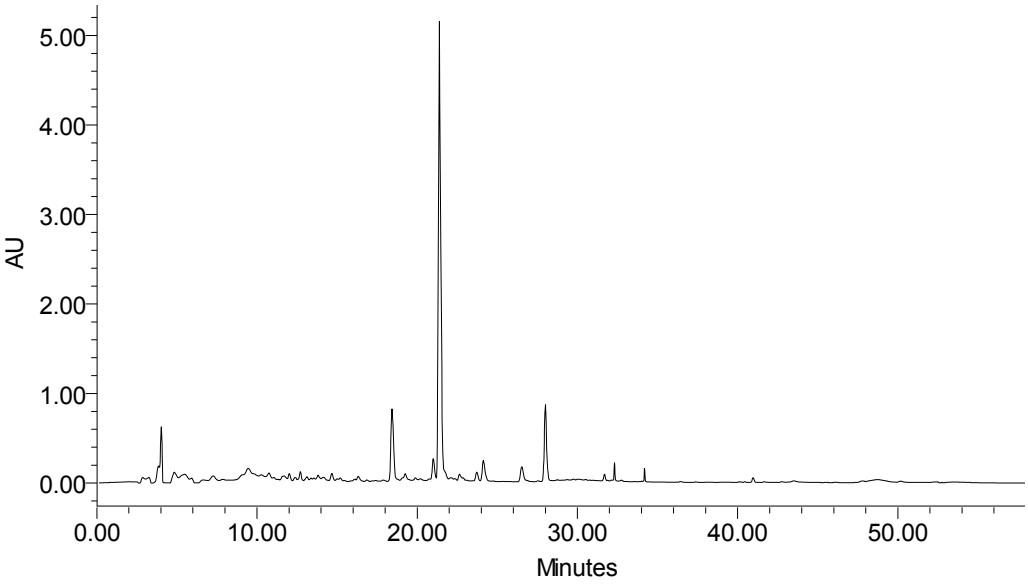


Figure 20.6: HPLC profile of the acetone extract of *Pelargonium tomentosum* (SBG).

Table 20.2: The retention times, UV absorbances maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium tomentosum* (SBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
4.03	207.3		4.49
4.84	205.0		2.22
5.50	280.5		2.97
7.23	248.6		1.26
9.48	206.2, 317.4		5.37
10.75	206.2		1.33
12.01	208.5, 317.4		0.33
12.70	227.3, 284.1		0.90
14.66	235.6		0.93
18.43	262.7, 348.4	flavone	9.50
21.01	241.5		2.59
21.54	256.8, 344.8	flavone	52.27
23.72	260.4, 337.7	flavone	0.98
24.14	254.5, 348.4	flavone	2.72
26.53	255.7, 373.3	flavonol	2.21
28.00	255.7, 359.0	flavonol	8.27
31.69	241.5		0.45
32.31	261.6		0.84
34.19	265.1		0.38

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium tomentosum* in this study include the following:

- *Pelargonium tomentosum* (SBG) extract produced moderate antimicrobial activity (MIC = 2 mg/ml - 2.33 mg/ml). The essential oil samples demonstrated poor antimicrobial activity (MIC = 8 mg/ml to >32 mg/ml).

- Promising anti-oxidant activity (IC_{50} = 28.16 ± 2.65 µg/ml - 21.73 ± 0.99 µg/ml) was displayed by the acetone extracts (SBG and WSBG).

- The *P. tomentosum* (SBG and WSBG) oil samples were inactive in the 5-lipoxygenase assay (IC_{50} >100 µg/ml).

- The acetone extracts (SBG and WSBG) produced promising antimalarial activities (IC_{50} = 13.83 ± 0.61 µg/ml - 17.62 ± 4.84 µg/ml).

- The *P. tomentosum* (SBG) extract proved to be non-toxic (IC_{50} = 195.13 ± 7.90 µg/ml). The *P. tomentosum* (WSBG) extract was moderately toxic (IC_{50} = 50.71 ± 1.17 µg/ml). *Pelargonium tomentosum* (SBG) essential oil was extremely toxic (IC_{50} ≤1.00 µg/ml). *Pelargonium tomentosum* (WSBG) essential oil was the least toxic of all the oil samples tested, but still produced substantial cytotoxicity (IC_{50} = 30.20 ± 1.81 µg/ml).

21. *Pelargonium vitifolium* (L.) L'Hérit.

Common names:

Vine-leaved pelargonium, Balm-scented pelargonium (van der Walt, 1977).

Botanical description:

An erect and strongly balm-scented shrub growing 0.5-1 m tall. At the base, the main stem is woody while the side branches are herbaceous and villous. The rough leaves are cordate in shape and are 5-lobed, but mostly 3-lobed. Stiff hairs cover the leaf blades making them rough to the touch. The leaf margins are irregularly toothed and coarse. The leaves of *P. vitifolium* resemble those of the vine, hence the epithet '*vitifolium*' (where 'viti-' is the Latin word meaning vine). Ovate stipules are present at the base of the petioles. The inflorescences resemble those of *P. capitatum* since they are capitate-like; however, they are less hairy and contain no more than twelve flowers. The colour of the petals range from light pink to carmine. Violet-purple flecks occur on the posterior two petals which are narrow at their base, obovate with rounded tops and entire. Seven fertile stamens are present (van der Walt, 1977; Webb, 1984).

Pelargonium vitifolium is closely related to *P. capitatum* and also has features in common with *P. papilionaceum*. Compared to *P. capitatum*, the leaves of *P. vitifolium* are more glandular and strongly aromatic, but not crinkled (van der Walt, 1977).

Distribution and habitat:

Pelargonium vitifolium is confined to the region of the south-western and southern Cape, occurring from the Cape Peninsula and eastwards along the coast to Knysna. Its distribution is associated with mountains, where it is specifically confined to the lower slopes, growing in half-shaded areas by streams between boulders or on scree (van der Walt, 1977; 1985).

Figure 21.1: *Pelargonium vitifolium* in flower.

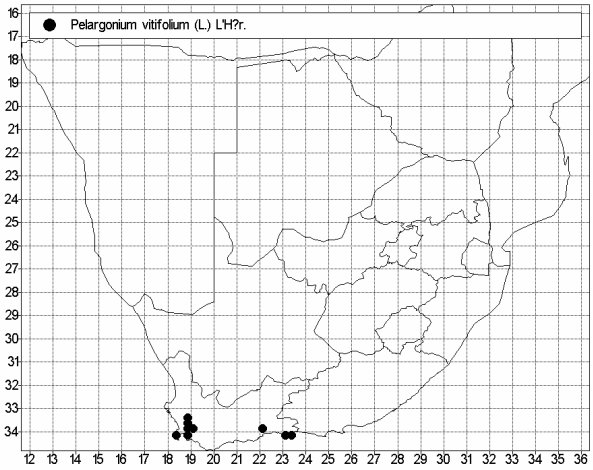


Figure 21.2: The geographical distribution of *Pelargonium vitifolium.*

Medicinal uses:

All parts of the plant can be used as an astringent (Grieve, 1994). A citronella-scented essential oil is obtained from the plant (Uphof, 1959).

Chemical composition of the essential oil:

Hydrodistillation (3 hours) of the fresh plant material collected from the Stellenbosch Botanical Garden yielded 0.05% oil.

GC-MS profile and analysis

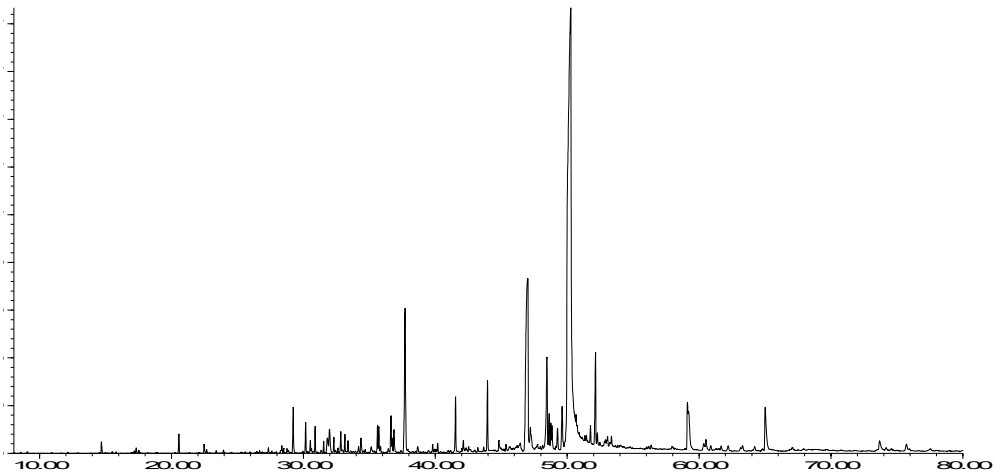


Figure 21.3: GC-MS profile (TIC) of the hydrodistilled essential oil of *Pelargonium vitifolium* (SBG).

Table 21.1: Compounds identified in the hydrodistilled essential oil of *Pelargonium vitifolium* (SBG).

RRI	Compound	% Area
1213	1,8-cineole	0.05
1280	p-cymene	0.16
1337	geijerene	trace
1348	6-methyl-5-hepten-2-one	trace
1353	*cis*-rose oxide	trace
1450	*trans*-linalool oxide (furanoid)	trace
1452	1-octen-3-ol	trace
1459	acetic acid	trace
1466	α-cubebene	trace
1487	citronellal	trace
1491	octyl acetate	trace
1493	α-ylangene	trace
1497	α-copaene	0.37
1528	α-bourbonene	trace
1535	β-bourbonene	0.07
1549	β-cubebene	trace
1553	linalool	0.20
1553	octanol	trace
1569	neoisopulegol	0.26
1570	methyl citronellate	0.07
1583	isopulegol	0.33
1584	*trans*-isopulegone	trace
1588	β-copaene	trace
1612	β-caryophyllene	0.18
1612	citronellyl formate	0.07
1628	aromadendrene	0.13
1661	allo-aromadendrene	0.13
1668	(Z)-β-farnesene	trace
1685	isovaleric acid	trace
1704	γ-muurolene	0.22
1706	α-terpineol	0.17
1707	ledene	trace
1740	valencene	0.05
1740	α-muurolene	0.35
1748	piperitone	0.15
1750	β-dihydroagarofuran	0.10
1772	**citronellol**	**2.24**
1815	valeric acid	0.05
1853	*cis*-calamenene	0.08
1857	geraniol	trace
1864	p-cymen-8-ol	trace
1868	(E)-geranyl acetone	0.06
1898	1,11-oxidocalamenene	trace
1916	α-agarofuran	0.54

RRI	Compound	% Area
1941	α-calacorene	trace
1984	γ-calacorene	trace
2001	isocaryophyllene oxide	trace
2008	caryophyllene oxide	0.57
2050	(E)-nerolidol	0.06
2071	humulene epoxide II	0.07
2127	**10-epi-γ-eudesmol**	**7.10**
2131	hexahydrofarnesylacetone	0.06
2184	nonanoic acid	0.05
2187	T-cadinol	0.92
2210	agarospirol	0.36
2211	2-phenylethyl tiglate	0.26
2219	α-muurolol	trace
2228	valerianol	trace
2254	**citronellic acid**	**74.65**
2340	10-hydroxy-calamenene	0.16
2392	caryophyllenol II	0.13
2931	hexadecanoic acid	0.16
	Total	**90.58%**

trace <0.05%; (SBG) = Stellenbosch Botanical Garden.

Citronellic acid makes-up a large portion (74.65%) of the total oil composition. Citronellol and 10-epi-γ-eudesmol represent 2.24% and 7.10%, respectively.

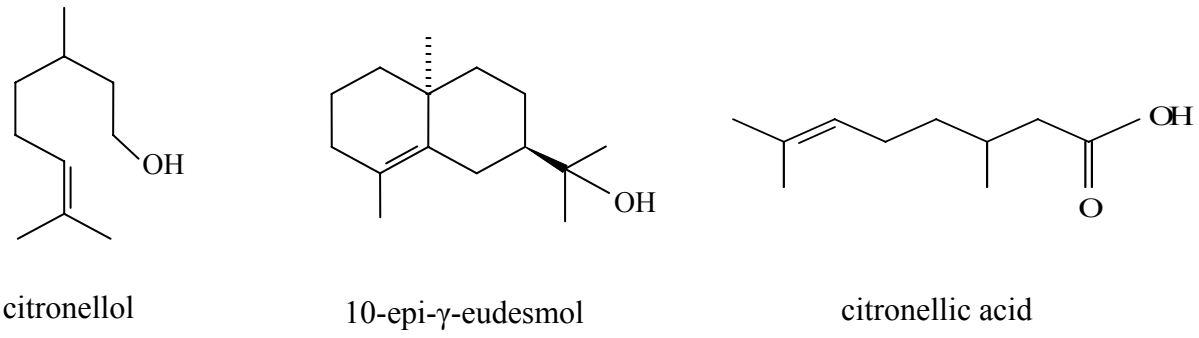


Figure 21.4: Chemical structures of the major compounds identified in the essential oil of *Pelargonium vitifolium*

Chemical composition of the acetone extract:

HPLC analysis was performed on the acetone extract of *Pelargonium vitifolium* collected from the Stellenbosch Botanical Garden.

HPLC profile and analysis

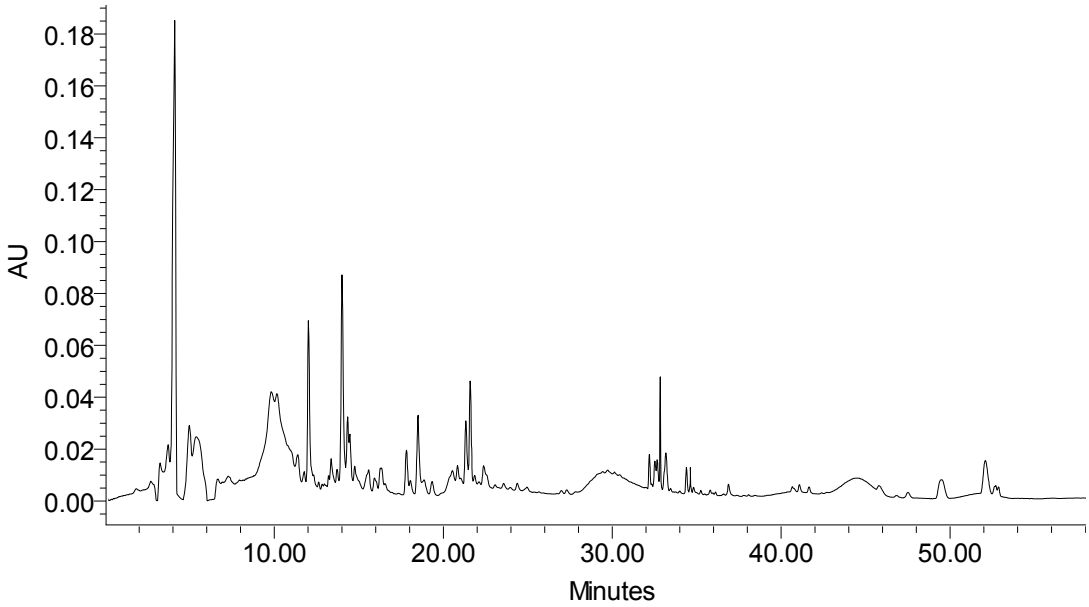


Figure 21.5: HPLC profile of the acetone extract of *Pelargonium vitifolium* (SBG).

Table 21.2: The retention times, UV absorbance maxima and percentage integrations of the major peaks identified in the HPLC profile of the *Pelargonium vitifolium* (SBG) acetone extract.

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
3.24	203.9		2.23
3.72	206.3		3.32
4.11	208.6		21.20
4.98	206.3, 263.8		4.91
5.40	206.3, 278.0		8.91
9.82	206.3, 320.6		1.78
11.39	216.8, 260.3, 293.4		0.77
12.02	219.1, 327.8		4.71
13.37	226.2, 278.0		1.12
13.72	227.4, 287.4		0.59
14.00	221.5, 278.0		7.54
14.34	285.1	flavanone	2.28
14.76	263.8, 349.2		1.37
15.59	228.5, 269.7, 332.5		1.21

Retention time (min)	UV absorbance maxima (nm)	Tentative identification	% Integration
15.92	233.2, 272.1		0.50
16.35	255.6, 351.6	flavonol	0.88
17.82	255.6, 354.0	flavonol	1.63
18.51	261.5, 349.2	flavone	2.51
21.34	257.9		2.01
21.59	255.6, 348.0	flavone	3.11
22.40	241.4		0.81
29.74	242.6		8.13
32.20	200.6		0.86
32.52	272.1		0.72
32.67	267.4, 356.3		0.83
32.84	261.5		1.36
33.18	266.2, 338.5		1.47
34.39	292.2		0.64
34.62	265.0		0.15
36.88	275.6		0.56
44.56	272.1		7.53
49.50	255.6		1.76
52.09	269.7		2.61

Summary of bioactivity results:

In vitro pharmacological activities recorded for *Pelargonium vitifolium* in this study include the following:

- The *P. vitifolium* (SBG) extract produced substantial antibacterial activity against *B. cereus* (MIC = 1 mg/ml).

- Considerable antimalarial activity (IC_{50} = 9.18 ± 1.61 µg/ml) was produced by the *P. vitifolium* (SBG) extract.

- The acetone extract (SBG) produced low cytotoxicity in the MTT assay, an IC_{50} value of 178.48 ± 5.44 µg/ml was obtained.

Glossary of monograph terms

Acute: leaves having a sharp but not extended point.

Carmine: a deep red with a tinge of purple; light crimson.

Cleft: divided into lobes by notches extending halfway, or further, from the margin to the midrib or to the base.

Cordate: heart-shaped.

Cordiform: heart-shaped.

Crenate: a leaf margin with rounded projections.

Cuneate: wedge shaped leaves with the point of the wedge forming the base of the lamina.

Cyclamen: any one of a group of plants with heart-shaped leaves and showy white, purple, pink, or crimson flowers, whose five petals bend backward.

Decumbent: describing a stem that lies along the ground.

Dentate: referring to a leaf margin that is toothed, having outward-pointing indents. Leaf margins finely toothed in this way are termed denticulate.

Distichous: leaves arranged alternately in two vertical rows on opposite sides of a stem.

Elliptic: oval in outline.

Emarginate: a leaf, petal or sepal that is indented at its tip.

Glabrous: a surface devoid of hairs or other projections.

Glaucous: surfaces having a waxy greyish-blue bloom.

Herbaceous: having stems that are usually soft and not woody.

Hirsute: covered with rather coarse hairs.

Hirtellous: minutely hirsute.

Hispid: covered with rough or stiff hairs.

Indumentum: a dense or sparse covering, usually of hairs.

Lamina: the flattened blade-like portion of the leaf.

Lobate: reference to a leaf that is divided into curved or rounded sections attached to each other by an entire central area.

Lobulate: having small lobes.

Oblanceolate: shaped like a lance head but with the tapering end at the base.

Obovate: inversely ovate (egg-shaped).

Obtuse: describing a leaf apex which is blunt or rounded.

Ovate: shaped like an egg, with the broadest part nearest the point of attachment.

Palmate: a leaf having four or more leaflets arising from a single point.

Palmatilobate: leaf margin palmately cut ⅛ to ¼ to the base of the leaf blade.

Palmatipartite: leaf margin deeply palmately cut but not to the base of the blade.

Palmatisect: leaf margin deeply palmately cut to the base of the blade.

Pandurate/ Panduriform: fiddle-shaped.

Partite: divided into parts.

Pinnate: having a series of leaflets, of a compound leaf, arranged on each side of a common stalk.

Pinnatifid: leaf margin pinnately cut to the midrib.

Pinnatilobate: leaf margin pinnately cut ⅛ to ¼ the way to the midrib.

Pinnatipartite: leaf margin deeply pinnately cut, but not to the midrib.

Pinnatisect: leaf margin deeply pinnately cut to the midrib.

Pubescent: general term indicating hairiness; with densely spaced, short, weak hairs.

Reflexed: describing a structure which is sharply bent backwards.

Reniform: kidney-shaped.

Scabrous: rough to the touch with small points of projections.

Serrate: referring to a leaf margin that is toothed, having forward-pointing indents.

Spatulate (spathulate): describes structures with a broad apex and a long narrow base.

Strigose: covered with stiff and straight bristles or hairs; hispid.

Terete: smooth, cylindrical and tapering.

Tomentose: a surface densely covered in short hairs.

Trichomes: any outgrowth from an epidermal cell.

Villous: describing something as having a hairy appearance due to a covering of long soft curly trichomes.

Viscid: covered with a sticky secretion.

(van der Walt, 1977; Tootill 1984; van der Walt and Vorster, 1988).

APPENDIX B

AROMATIC PELARGONIUMS – THEIR ESSENTIAL OILS AND PHARMACOLOGICAL PROPERTIES

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Introduction:

Pelargonium species belong to the Geraniaceae family which encompasses 5 genera. Of the 270 *Pelargonium* species, inhabiting the subtropical and temperate zones of the world, about 219 species occur in southern Africa. Many unscented and scented *Pelargonium* species have been used in folk medicines by the Sotho, Xhosa and Zulus to treat various ailments, many of which are caused by bacteria and fungi. A plenitude of research has been focused on Pelargoniums, namely *P. graveolens*, *P. capitatum* and *P. radens*, which are the main fragrant species used as the source of 'geranium oil'.

Research objectives:

To investigate the essential oil chemistry and to analyse the volatile and non-volatile plant extracts for positive *in vitro* biological activities.

Methodology:

The volatile compounds found within the leaves and stalks of selected *Pelargonium* species were extracted by hydrodistillation. The analytical assessment of gas chromatography coupled to mass spectroscopy (GC-MS) was used to investigate the composition of the *Pelargonium* essential oils. The non-volatile compounds were extracted by using acetone as the solvent. The hydrodistilled oils were assessed for anti-inflammatory activity by carrying out the *in vitro* 5-lipoxygenase assay. The acetone extracts were analysed for free radical-scavenging capacity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

Antimicrobial assays (disc diffusion and the microplate dilution method) were performed on the plant extracts and the essential oils.

Results:

Analogous chemical patterns were identified for some of the oils, confirming the taxonomic relationship between certain plant species; this being demonstrated for *P. papilionaceum* and *P. vitifolium.* The essential oils of *P. quercifolium* and *P. panduriforme* showed good inhibitory activity against the 5-lipoxygenase enzyme system with IC_{50} values of 33.24 – 38.67 µg/ml and 45.39 – 45.58 µg/ml, respectively. Exceptional anti-oxidant activity was displayed by the crude extracts of *P. betulinum* and *P. crispum* having IC_{50} values of 4.13 ± 0.14 µg/ml and 4.49 ± 0.18 µg/ml, respectively. The crude extracts of *P. glutinosum*, *P. scabrum* and *P. sublignosum* exhibited good antimicrobial activity against the test organisms, *B. cereus* and *S. aureus.* The essential oils showed reduced antimicrobial activity compared to the plant extracts.

Conclusion:

The positive biological activities manifested by the essential oils and crude extracts render concrete substantiation for the use of such species for traditional medicinal purposes. An inquest into these biological activities is well-grounded by the fact that pharmaceutical and cosmetic industries are investigating alternative, more natural antimicrobials and anti-oxidants.