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A PHYTOCHEMICAL AND PHARMACOLOGICAL STUDY OF TEN *COMMIPHORA* SPECIES INDIGENOUS TO SOUTH AFRICA

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A DISSERTATION SUBMITTED TO THE FACULTY OF HEALTH SCIENCES, UNIVERSITY OF THE WIWATERSRAND, JOHANNESBURG IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHARMACY

JANUARY, 2007



DECLARATION

I, Maria Penelope Paraskeva, declare that this dissertation is my own, unaided work except where acknowledged. It is being submitted in fulfilment 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 of Candidate)

…… day of ……, 2007

ABSTRACT

Commiphora species (from which myrrh is obtained) has been a source of several novel and bio-active natural compounds. Traditionally, *Commiphora* (Burseraceae) is used in southern Africa for the treatment of ulcers, fevers, and as a remedy for snake and scorpion bites. In western Africa, the macerated stem is used in the treatment of rheumatic conditions. The resin of some *Commiphora* species is applied topically to aid in wound healing. Documented uses include antibacterial and antifungal properties, as well as cytotoxic, cytostatic and anti-oxidant activity. The botanical diversity of this genus in South Africa warrants a study of this plant group, to provide scientific evidence for the traditional use of *Commiphora* species in African healing rites.

Ten *Commiphora* species were investigated. Fresh plant material of the selected species were identified and collected from natural populations in the Limpopo Province. Active compounds, viz. kaempferol and dihydrokaempferol, in *C. glandulosa* (stem) were isolated using bioassay-guided fractionation and identified using nuclear magnetic resonance spectroscopy. The stem and leaf extracts of each species were analysed for *in vitro* anti-oxidant, antimicrobial, anti-inflammatory, anticancer activity, as well as cytotoxicity. The anti-oxidant activity of the extracts was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the 2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) assays. Extracts generally exhibited poor anti-oxidant activity in the DPPH assay, with the exception of *C. schimperi* (stem), *C. neglecta* (stem), *C. tenuipetiolata* (stem and leaf), and *C. edulis* (stem), which possessed IC_{50} values ranging between 7.31 μg/ml and 10.81 μg/ml. Isolated compounds were subjected to the DPPH assay to determine the anti-oxidant potential of each compound, separately and in combination to establish possible synergistic, antagonistic or additive effects. The flavonol, kaempferol (IC_{50} = 3.32 μg/ml) showed exceptional radical scavenging activity, in contrast to the low activity displayed by dihydrokaempferol (IC_{50} = 301.57 μg/ml), their combination being antagonistic. Greater anti-oxidant activity was observed for most species in the ABTS assay when compared to the results obtained in the DPPH assay. The best activity was observed for the stem extracts of *C. neglecta* (IC_{50} = 7.28 μg/ml) and *C. mollis* (IC_{50} = 8.82 μg/ml).

In vitro antimicrobial efficacy was determined against Gram-positive and Gram-negative bacteria as well as yeasts using the MIC microtiter plate assay. A greater selectivity was exhibited by the extracts against the Gram-positive bacteria and yeast than against the Gram-negative bacteria. Using death kinetics studies (time-kill studies), the rate at which the antimicrobial agent kills pathogens over a 24-hour period was determined. The antibacterial activity of *Commiphora marlothii* (stem) was observed to begin at ca. 30 min of the exposure of *S. aureus* to the different concentrations of plant extract. All concentrations exhibited antibacterial activity, with a complete bactericidal effect achieved by all test concentrations by the 24th hour. *Commiphora pyracanthoides* (stem) displayed anti-inflammatory activity through good inhibition of the 5-LOX enzyme (IC_{50} = 27.86 μg/ml).

The ability of extracts and kaempferol to inhibit the *in vitro* growth of three human cancer cell lines, namely the colon adenocarcinoma (HT-29), breast adenocarcinoma (MCF-7), and the neuronal glioblastoma (SF-268), was evaluated using the sulforhodamine (SRB) antiproliferative assay. The most active *Commiphora* species against the HT-29 cells were *C. glandulosa* (leaf and stem) and *C. marlothii* (leaf). The MCF-7 cell line was the most sensitive to indigenous *Commiphora* species, with *C. edulis* (leaf and stem), *C. glandulosa* (leaf and stem), *C. marlothii* (leaf), *C. pyracanthoides* (leaf and stem), *C. schimperi* (stem), and *C. viminea* (stem) all possessing an inhibition greater than 80% at 100 μg/ml. *Commiphora glandulosa* (leaf and stem) and *C. pyracanthoides* (leaf and stem) were the two most active species against the SF-268 cells, with IC_{50} values ranging between 68.50 μg/ml and 71.45 μg/ml. The inhibition of the cancer cell proliferation by kaempferol in all three-cancer cell lines was determined, with IC_{50} values of 9.78 μg/ml in HT-29 cells, 20.21 μg/ml in MCF-7 cells and 43.83 μg/ml in SF-268 cells. The microculture tetrazolium cellular viability (MTT) assay was used to determine the cellular toxicity of the extracts against transformed human kidney epithelium (Graham) cells. *Commiphora glandulosa* (stem) proved to be most toxic (IC_{50} = 30.5 μg/ml). The IC_{50} values for all other extracts were in excess of 95 μg/ml suggesting low *in vitro* toxicity for the majority of the species.

A phytochemical investigation of the non-volatile constituents of the leaf and stems was conducted using high performance liquid chromatography (HPLC). The HPLC profiles and UV spectra of the stem extracts, and the representative flavonoid patterns in the leaf extracts of the species indicate that a similarity exists in their chemical fingerprint.

PRESENTATIONS

M. Paraskeva, A.M. Viljoen, S.F. van Vuuren, H. Davids and R.L. van Zyl, 2005. The pharmacological activity of 10 species of *Commiphora* indigenous to South Africa. Podium presentation, 5th Annual Meeting of the Indigenous Plant Use Forum (IPUF). Grahamstown, 27-30 June 2005. (Abstract in Appendix C)

M. Paraskeva, A.M. Viljoen, S.F. van Vuuren, H. Davids and R.L. van Zyl, 2005. The biological activity of 10 species of *Commiphora* indigenous to South Africa. Podium presentation at the University of Johannesburg Post Graduate Symposium. Johannesburg, 2 November 2005 (Abstract in Appendix C)

M. Paraskeva, S.F. van Vuuren, S. Drewes and A.M. Viljoen, 2006. The antibacterial and anti-oxidant activity of South African indigenous *Commiphora* species and the isolated compounds from *C. glandulosa.* Podium presentation at the University of the Witwatersrand, International Conference on Pharmaceutical and Pharmacological Sciences (ICPPS). Vereeniging, Johannesburg, 20-23 September 2006 (Abstract in Appendix C)

DEDICATION

I dedicate this dissertation to my parents,
Panagiotis and Irene Paraskeva

Thank you for your valuable guidance, patience and understanding.

"*I have few illusions, but I feel a responsibility to work towards the things I consider good and right. I don't know whether I will be able to change certain things for the better, or not at all. Both outcomes are possible. There is only one thing I will not concede and that is that it might be meaningless to strive in a good cause."*

President Vaclev Have of the Czech Republic

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This work was carried out at the University of the Witwatersrand, Johannesburg, Faculty of Health Sciences, Department of Pharmacy and Pharmacology, during the years 2005 and 2006.

Although I am the sole author of this dissertation, I am by no means the sole contributor. I would like to take this opportunity and use this as a platform to express my sincere gratitude to those who have contributed to my dissertation, to my education and to my life.

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To God, for always giving His fullest blessing.

The prayer of Jabez

'Oh, that you would bless me indeed,
and enlarge my territory,
that Your hand would be with me,
and that You would keep me from evil'

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

ABTS:	2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid)
AKT:	Activated protein kinase
AP-1:	Activator protein 1
ATCC:	American Type Culture Collection (Manassas, VA, USA)
ATP:	Adenosine Triphosphate
AR:	Androgen Receptor
AV:	Voucher specimen numbers (Alvaro Viljoen)
Av:	Average
Bax:	Bcl2-associated X protein
Bcl2:	B-cell lymphoma-2
C_2:	Carbon 2
C_3:	Carbon 3
C_6:	Carbon 6
ca.:	Circa (around; about)
CAL:	*Commiphora africana* leaves
CAS:	*Commiphora africana* stem
Cdk:	Cyclin-dependent kinase
CEL:	*Commiphora edulis* leaves
CES:	*Commiphora edulis* stem
cFLIP:	Cellular FADD-like interleukin-1-converting enzyme (FLICE) inhibitory protein
CFU:	Colony forming units
CGL:	*Commiphora glandulosa* leaves
CGS:	*Commiphora glandulosa* stem
clAP:	Cellular inhibitor of apoptosis protein
CM_AL:	*Commiphora marlothii* leaves
CM_AS:	*Commiphora marlothii* stem
CML:	*Commiphora mollis* leaves
CMS:	*Commiphora mollis* stem
CNL:	*Commiphora neglecta* leaves
CNS:	*Commiphora neglecta* stem

CO_2:	Carbon dioxide
CPL:	*Commiphora pyracanthoides* leaves
CPS:	*Commiphora pyracanthoides* stem
CSF:	Colony stimulating factors
CSL:	*Commiphora schimperi* leaves
CSS:	*Commiphora schimperi* stem
CTL:	*Commiphora tenuipetiolata* leaves
CTS:	*Commiphora tenuipetiolata* stem
CVL:	*Commiphora viminea* leaves
CVS:	*Commiphora viminea* stem
COX:	Cyclo-oxygenase
ºC:	Degrees Celsius
DMEM:	Dulbecco's Modified Eagle's Medium
DMSO:	Dimethyl sulfoxide
DNA:	Deoxyribonucleic acid
DPPH:	2,2-diphenyl-1-picrylhydrazyl
DSM:	Deutsche Sammlung von Mikroorganismen (culture collection; Braunschweig, Germany)
EDTA:	Ethylene diamine tetraacetic acid
EGF:	Epidermal growth factor
EGFR:	Epidermal growth factor receptor
Egr-1:	Early growth response 1
ELAM:	Endothelial leucocyte adhesion molecule
EpRE:	Electrophile responsive element
ER-α:	Estrogen receptor alpha
ER-β:	Estrogen receptor beta
FCS:	Foetal calf serum
FGF:	Fibroblast growth factor
FLAP:	5-Lipoxygenase activating protein
FTPase:	Farnesyl-protein transferase
FXR:	Farnesoid X receptor
g:	Gram

GC:	Gas chromatography
GPS:	Global positioning system
GST:	Glutathione S-transferase
GST-px:	Glutathione peroxidase
HER2:	Human epidermal growth factor receptor 2
HETE:	Hydroxyeicosatetraenoic acid
HL-60:	Leukemic cancer cell line
5-HPETE:	5-hydroxyperoxyeicosatetraenoic acid
HPLC:	High performance liquid chromatography
HPLC-UV:	High performance liquid chromatography-ultraviolet
HPRT:	Hypoxanthine guanine phosphoribosyl transferase
HT-29:	Colon adenocarcinoma cell line
H_2O_2:	Di-hydrogen Dioxide (Hydrogen peroxide)
OH:	Hydroxide
ICAM-1:	Intercellular adhesion molecule-1
IC_{50}:	Inhibitory concentration
IFN-γ:	Interferon gamma
IGF:	Insulin-like growth factor
IKK:	IkappaBalpha kinase
IL-1:	Interleukin
iNOS:	Inducible nitric oxide synthase
INT:	*p*-iodonitrotetrazolium
JAK2:	Janus kinase 2 protein kinase
JNK:	c Jun N-terminal kinase
KH_2PO_4:	Potassium di-hydrogenphosphate
$K_2S_2O_8$:	Potassium persulfate
λ:	Lambda (wavelength)
LOX:	Lipoxygenase
LPS:	Lipopolysaccharide
m:	Meters
Ma:	Million years ago

MAPK:	Mitogen-activated protein kinases
MCF-7:	Breast adenocarcinoma cell line
MDR:	Multiple drug resistance
mg:	Milligram
MIC:	Minimum inhibitory concentration
min:	Minutes
ml:	Milliliter
mM:	Millimolar
MMP:	Matrix metalloproteinase
MTT:	3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
µg:	Microgram
µl:	Microlitre
n:	Number of experimental runs
NaCl:	Sodium chloride
NADH:	Nicotinamide adenine dinucleotide
NADPH:	Nicotinamide adenine dinucleotide phosphate
$NaHCO_3$:	Sodium hydrogen carbonate
$Na_2HPO_4.2\ H_2O$:	Di-sodium hydrogenphosphate dehydrate
NCI:	National cancer institute
NCTC:	National collection of type cultures (Central Public Laboratory Service, London, UK)
NDGA:	Nordihydroguaiaretic acid
NF-κB:	Nuclear factor-kappa B
nm:	Nanometer
NMR:	Nuclear magnetic resonance
NR:	Nuclear receptors
Nrf2:	NF-E2-related Factor 2
NSAID:	Non-steroidal anti-inflammatory drugs
NW:	New world
OW:	Old world
O_2 :	Oxygen

PARP:	Polyadenosine-5′-diphosphate-ribose polymerase
PBS:	Phosphate buffer saline
PCA:	Principle component analysis
PDGF:	Platelet-derived growth factor
Pgp:	P-glycoprotein
pH:	Potential hydrogen
PKA:	Protein kinase A
PKC:	Protein kinase C
PLA_2:	Phospholipase A_2
PPARγ:	Peroxisome proliferator-activated receptor gamma
ppm:	Parts per million
p21/WAF:	Cyclin dependent kinase inhibitor complex
p27Kip/Cip:	Cyclin dependent kinase inhibitor complex
p53:	Tumour suppressor gene
R_f:	Retention factor
RNA:	Ribonucleic Acid
ROS:	Reactive oxygen species
rpm:	Revolutions per minute
RPMI 1640:	Roswell Park Memorial Institute Media 1640
s:	Seconds
SABS:	South African Bureau for Standards
SAR:	Structure-activity relationship
s.d.:	Standard deviation
SDG:	Succinate-dehydrogenase
SF-268:	Neuronal glioblastoma cancer cell line
spp.:	Species
SRB:	Sulphorhodamine
Src:	protein kinase
STAT:	Signal transducer and activator of transcription
subsp.:	Subspecies
syn.:	Synonym

TEAC:	Trolox equivalent anti-oxidant capacity
TGFα/β:	Transforming growth factor alpha/beta
TLC:	Thin layer chromatography
TNF:	Tumour necrosis factor
TRAF1:	Tumour necrosis factor receptor-associated factor
TSA:	Tryptone soya agar
TSB:	Tryptone soya broth
TYK2:	Tyrosine kinase 2
uPA:	Urokinase-type plasminogen activator
UV:	Ultra violet
UV-VIS:	Ultraviolet-visible
var:	Variant
VCAM:	Vascular cell adhesion molecule
VEGF:	Vascular endothelial growth factor
vs:	Versus
WHO:	World Health Organization
w/v:	Weight per volume
xlAP:	Inhibitor of apoptosis protein
↓:	Decrease
↑:	Increase