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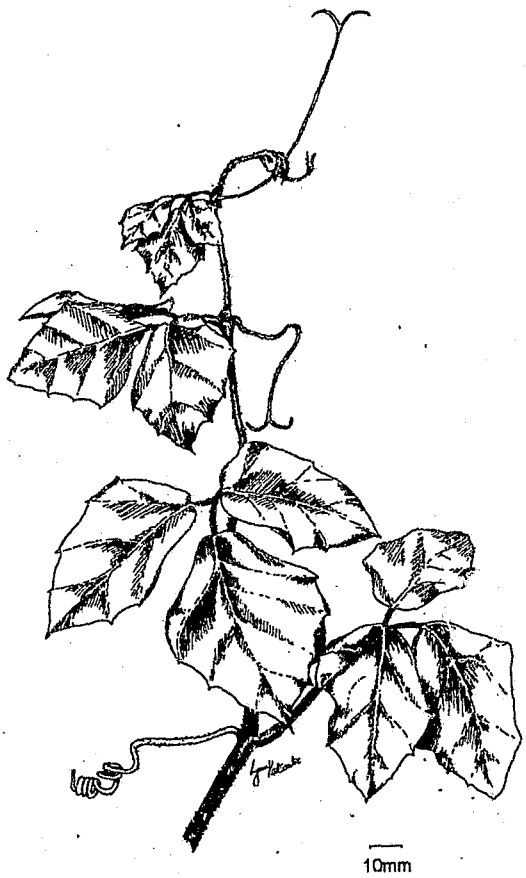
The pharmacological actitvity of Rhoicissus tridentata subsp cuneifolia in relation to parturition

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THE PHARMACOLOGICAL ACTIVITY OF *RHOICISSUS TRIDENTATA* SUBSP *CUNEIFOLIA* IN RELATION TO PARTURITION



Lynn Coleen Katsoulis

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree
of
Doctor of Philosophy

Johannesburg, 2000

DECLARATION

I, Lynn Coleen Katsoulis declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

.....

..... day of ....., 2000

This thesis is dedicated to my loving husband, Terry.

His unending encouragement and support, gave me the confidence to apply to university and also carried me through to completing this thesis.

May my precious children achieve far more.

PUBLICATIONS AND PRESENTATIONS

1.1. Publications

L.C. Katsoulis, D.J.H. Veale and I. Havlik (1999) The pharmacological action of *Rhoicissus tridentata* on isolated rat uterus and ileum. Phytotherapy Research (in press).

L.C. Katsoulis (1999) *Rhoicissus tridentata* subsp *cuneifolia*: the effect of geographical distribution and plant storage on rat uterine contractile activity. South African Journal of Botany **65**(4):

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K.B. Brookes, O.V. Doudoukina, L.C. Katsoulis & D.J.H. Veale (1999) Uteroactive traditional medicines: *Combretum kraussii*. South African Journal of Chemistry (In press)

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1.2. Oral presentations

L.C. Katsoulis, D.J.H. Veale and I. Havlik (1997) Seasonal and distributional variation in the contractile activity of *Rhoicissus tridentata* subsp *cuneifolia* on isolated rat uterus and ileum. South African Pharmacology Society Annual Conference. Drakensberg

B. Brookes, O. Doudoukina, D.J.H. Veale and L.C. Katsoulis (1997) Isolation of compounds from C. kraussi, uteroactive traditional medicine. Joint annual symposium of the South African Societies for Microbiology and Biochemistry.

L.C. Katsoulis, D.J.H. Veale and I. Havlik (1998) Variation in the contractile response to *Rhoicissus tridentata* subsp *cuneifolia* with different plant parts used from different seasons – ecological implications. South African Association of Botanists 24th Annual conference. Cape Town

L.C. Katsoulis, I. Havlik (1999) The Pharmacological Action of *Rhoicissus tridentata* on isolated rat uterus and ileum. University of the Witwatersrand Medical School Research Day. Johannesburg

B. Brookes, O. Doudoukina, L.C. Katsoulis and D.J.H. Veale (2000) Uteroactive components in traditional medicine: Isihlambezo. Natural Products Symposium.

L.C. Katsoulis (2000) Plants used in pregnancy-related remedies in the Gauteng. Indigenous Plant Use Forum. Nelspruit

1.3. Poster presentations

L.C. Katsoulis, D.J.H. Veale, N. Butkow and I. Havlik (1996) Preliminary studies on the effects of *Rhoicissus tridentata* subsp *cuneifolia* on isolated rat uterus and ileum. The First International Conference on Pharmaceutical and Pharmacological Sciences (South Africa). Midrand

B. Brookes, O. Doudoukina, D.J.H. Veale and L.C. Katsoulis (1996) Biologically active components from plants used in traditional pregnancy related herbal medicine or "isihlambezo". 33rd convention of the South African Chemistry institute.

L.C. Katsoulis (1999) The necessity of standardising herbal remedies: An example using *Rhoicissus tridentata*. Herbal Medicine into the Next Millennium, Conference Proceedings. Lismore, Australia

L.C. Katsoulis, D.J.H Veale, I. Havlik (1999) The Pharmacological Action of *Rhoicissus tridentata* on isolated rat uterus and ileum. The Indigenous Plant Use Forum, Richards Bay, South Africa.

Award

Outstanding Research from South African Pharmacology Society Annual 1997

ABSTRACT

Decoctions and infusions of *Rhoicissus tridentata* subsp. *cuneifolia* (Vitaceae) roots and lignotubers are widely used as traditional medicine by South African women during pregnancy and childbirth. Pharmacological studies using isolated rat uterus and ileum were done to determine whether there is any pharmacological grounding for the use of the remedies to induce labour. Initial studies showed a large variation in the contractile activity of the extracts, so investigations were done to determine whether the contractile activity varied according to the season in which plant material was harvested, the location in which the plant grew, or the length of storage of harvested plant material.

Plant material harvested from Umlazi (KwaZulu-Natal) and the Suikerbos Nature Reserve (Gauteng) was used for studies on the mechanism of contractile activity. Seasonal variation was investigated by harvested three plants from Suikerbosrand for two years at approximately three monthly intervals. The distributional effect was done using material harvested from around South Africa. Material from Suikerbosrand was stored for either three months or a year to determine whether storage altered the contractile activity. After each harvesting, the different parts of the plants were separated, dried, milled and boiled for approximately an hour. The solutions were allowed to settle overnight at 4°C, after which the supernatant was siphoned off, then frozen and lyophilised. All lyophilised end products were kept frozen until use.

Oestrogenized virgin Sprague-Dawley rats euthanazed with CO_2. Uterine and ileal tissue was dissected out and mounted in 50 ml organ baths containing Tyrode solution, aerated with 5% CO_2 in O_2. After a resting period the organs were challenged with cumulative doses of reference agonist or herbal extract, or pretreated with the herbal extract before adding the reference drugs.

The pharmacological action of an aqueous extract of *R. tridentata* subsp. *cuneifolia* was investigated using isolated rat uterus and ileum. The results showed that the extract directly stimulates concentration dependent contractions of uterus and ileum. Preincubation of the organs with the plant extract had no effect on the maximal uterine response to the cumulative addition of acetylcholine or oxytocin, and slightly depressed the response to serotonin and noradrenaline. The maximal ileal response to acetylcholine was depressed where the response to

maximal concentrations of serotonin was unchanged. Pretreatment with atropine and indomethacin both blocked the initial response to the *Rhoicissus* extract which indicates that the muscarinic receptors and prostaglandin synthesis could be involved in the contractile response to the extract. Methysergide and prazosin had no effect on the direct action of the extract which infers that serotonin receptors and α-adrenoceptors do not play a role in mediating the smooth muscle response to the plant extract.
Cellular toxicity of *R. tridentata* extracts from Umlazi and Suikerbosrand was determined using MTT assays, and enzyme immunoassays were used to determine the effect of the extracts on cellular prostaglandin E_2 production.

Results from the pharmacological studies indicate that the contractions of isolated rat uterus and ileum seem to be mediated by muscarinic receptors and the synthesis of cyclooxygenase products. The contractility of the plant extract appears to be mediated predominantly by muscarinic M_4 receptors. The other muscarinic receptor subtypes play less of a role. The contractions seem to be devoid of serotonergic, adrenergic, histaminergic or nicotinic activity.

Studies on the variation in contractile activity suggest that the activity of the extract does vary according to the season or location of material harvesting. The extracts form material harvested during summer or autumn was more active than material harvested during winter or spring. The lignotubers yielded the most active extracts. Extracts of plants from most geographic areas stimulated contractions although the response varied in magnitude, however, the extract of a plant from Mondeor, acted in an opposite manner, inhibiting acetylcholine induced contractions. Storing dried plant material did not alter the activity of the extracts.

The plant extracts, up to a concentration of 1mg/ml, appear not to be toxic to human kidney epithelial cells, human hepatoma cells, human histiocytoma cells or mouse leydig cells. The production of PGE_2 by human histiocytoma cells was stimulated by 1mg/ml *R. tridentata* extract confirming that contractions are possible mediated by the synthesis of cyclooxygenase products.

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NOMENCLATURE

μM	- micromolar
5-HT	- serotonin
AC	- adenylyl cyclase
ACh	- acetylcholine
ATP	- adenosine triphosphate
Ca^{2+}	- calcium ions
cAMP	- cyclic adenosine monophosphate
carbogen	- 5% carbon dioxide, 95% oxygen
CNS	- central nervous system
COX	- cyclooxygenase
COX-1	- cyclooxygenase isoform 1
COX-2	- cyclooxygenase isoform 2
DAG	- diacylglycerol
DMSO	- dimethylsulphoxide
EDRF	- endothelium-derived relaxing factor
IL-1alpha	- interleukin-1alpha
IP_3	- inositol-1,4,5-*tris*phosphate
K^+	- potassium ions
M_1	- muscarinic subtype 1 acetylcholine receptors
M_2	- muscarinic subtype 2 acetylcholine receptors
M_3	- muscarinic subtype 3 acetylcholine receptors
M_4	- muscarinic subtype 4 acetylcholine receptors
mM	- millimolar
mRNA	- messenger ribonucleic acid
MTT	- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NA	- noradrenaline
Na^+	- sodium ions
nM	- nanomolar
NO	- nitric oxide
PBS	- Dulbecco phosphate buffer
PG	- prostaglandin
PGE	- prostaglandin E

$PGF_{2\alpha}$	- prostaglandin $F_{2\alpha}$
PGI_2	- prostacyclin
PKC	- protein kinase C
PLA_2	- phospholipase A_2
PLC-β	- phospholipase C-β
STD	- sexually transmitted diseases
TBA	- traditional birth attendant
tbsp	- tablespoon
tsp	- teaspoon
TXA_2	- thromboxane A

CHAPTER 1: INTRODUCTION

1.1. Traditional and Herbal Medicine

The medical use of herbs is deeply rooted in the history and folklore of virtually all cultures (Garty 1993; Dubick 1986). Even though modern medicine has dominated the medical field for most of this century, recently there has been an exponential increase in the sale of herbs around the world (Rosti *et al.* 1994). Herbal medicine is the most common form of all alternative treatments in western countries (Angell & Kassirer 1998), which are used predominantly by the more affluent and more highly educated portion of the population, between the ages of 25-49 (Eisenberg *et al.* 1993; Blackmore 1999). This return to herbal remedies is probably due to the misconception that because herbs are 'natural' they are safe as well as the efficacy of herbal remedies in the treatment of simple and uncomplicated illnesses (Dubick M.A. 1986; Pillans 1994). This misconception is coupled with dissatisfaction with modern health care, rising costs (Taylor 1996) and the often hurried and impersonal care delivered by conventional physicians (Angell & Kassirer 1998). The situation in third world countries is very different to that of developing countries. Many communities still do not have the privilege of access to evidence based medicine and therefore have no choice but to rely on traditional medicines. Traditional medicine is the principle and often the only form of health care for many Africans (Patel 1993), especially in the rural areas.

1.1.1. Traditional medicine in South Africa

Traditional practitioners are widely spread throughout South Africa. It is estimated that approximately 200 000 traditional healers practice in South Africa compared with 25 000 doctors of modern medicine (Abdool Karim *et al.* 1992). In sub-Saharan Africa the ratio of traditional healers to people is estimated at 1:100-1000, whereas the ratio of medically qualified doctors to people is estimated at 1:10 000-100 000 (Unterhalter 1982). In South Africa, 66% of the population lives in a rural setting yet only 20% of doctors are located in rural areas (Unterhalter 1982). This low doctor-patient ratio in rural black communities means that the traditional healer is often the first, nearest

or even only contact for the rural black African to medical care (Buchmann *et al.* 1989). As many as 80% of black South Africans visit a traditional healer before visiting an allopathic doctor (Gumede 1990). Traditional healers are also very popular because they are culturally familiar, speak the same language and provide culturally familiar ways of explaining the cause of ill-health and its relationship to the people's social and supernatural worlds (Chipfakacha 1994).

The theory underlying traditional medicine in the many black ethnic groups in South Africa is essentially similar (Kale 1995). Most blacks are superstitious, with deep-rooted traditions which make no provision for modern medicine and its derivatives. Scientific medicine is and has always been a foreign cultural idea. For example, the "germ" theory is not understood or appreciated. To the African diseases fall into three major categories: (i) those due to magic or evil spirits; (ii) those due to conditions for which causes have been empirically determined; and (ii) those due to psychological phenomena (Chipfakacha 1994).

1.1.2. Types of healers

Inyangas are herbalists who possess extensive knowledge about curative herbs and medicines of animal origin. Ninety percent of inyangas are male.

Isangomas are diviners who determine the cause of illness by using ancestral spirits, and 90% are female.

Umthandazi are faith healers who are professed Christians belonging to one of the many independent African churches. They heal by prayer, using holy water or ash, or by touching the patient (Conco 1972; Kale 1995).

Traditional birth attendants (TBA) are not healers, but deliver babies born in their villiage. They are usually elderly woman who are respected in society for their skills. If a complication occurs, the birth attendant seeks the advice of an inyanga (Kale 1995).

1.1.3. Registration of traditional healers in South Africa

In South Africa there are no recognised institutes for the training and registering of traditional healers, thus no standardisation between traditional

healers exists. There have been attempts by national traditional healer associations to set examinations testing the competence of healers with the aim of setting up a registry for traditional healers. This would help regulate the standards of healers, as minimum qualifications and levels of training could be stipulated (Schuster Campbell 1998; Hutchings 1999). However, this process is still in its initial stages. Currently there is no way of measuring the competence of a traditional healer. A registry for traditional healers in Zimbabwe was set up in 1980, yet a survey conducted in 1995 showed that nearly half of traditional healers in the suburbs of Harare were not registered with any formal organisation (Winston *et al.* 1995). It appears that incentives would have to be offered, or the registration and training would have to benefit the healer before the general level of competence of healers could be raised.

At the moment, the traditional healer in most parts of central, southern and eastern Africa practices his art in an empirical manner, relying on personal insight and experience for success (Harries & Cullinan 1994). The thought of testing the efficacy of a certain remedy in sufficient numbers of patients and controls is a completely foreign concept to most traditional healers (Healers. Pers. comm.) Since there is no reference against which to compare a healer, the opportunities for irresponsible quackery and reckless profiteering are completely open (Bodenstein 1977). Even if traditional healers have scientific training, or have the will to learn more, very little work has been done on the toxicity and pharmacological activity of the South African flora (Huxtable 1990). More importantly, there is no institute in this country providing sound training in herbalism as is the case in the United States, Europe, China and India.

Traditional healers have become more acceptable since the change of government in 1994. This is manifest in the agreements by Medical Aid Societies such as the NBC Medical Aid Society which has agreed with the African National Healer's Association to honour claims for its members who have consulted sangomas (Kanhema 1993).

1.1.4. Reports of adverse effects of African traditional medicines

Many adverse effects of African traditional remedies have been reported in the literature. A few of these reports are as follows. Traditional medicines caused 51.7% (31/60) of fatal poisonings admitted to Ga-Rankuwa Hospital during the period from 1981 to 1985 (Joubert 1990). Acute dichromate caused 7 cases of poisoning after the use of traditional purgatives obtained from local inyanga. Symptoms of poisoning included renal failure and abnormal liver function tests and fatalities (Wood *et al.* 1990; Michie *et al.* 1991). Potassium dichromate used as enemas caused patients to suffer from severe colonic complications (Dunn *et al.* 1991). Inappropriate use of traditional medicines resulted in numerous fatalities, invariably in children (Bye & Dutton 1991). Cardiac glycosides were present in 44% of autopsies where deaths were presumed to have been caused by traditional remedies, where the most common clinical presentation was gastrointestinal irritation which occurred in 54% of the patients (McVann *et al.* 1992). Traditional eye medicines are used frequently in African traditional medicine, which have been found to be used by 33% of the patients presenting with corneal ulcers (Courtright *et al.* 1994; Lewallen & Courtright 1995). The risk of oesophageal cancer associated with regular consumption of *Solanum nigrum* as a food is higher than the risk associated with smoking (relative-risks 3.6 and 2.6 respectively). Even though the above severe and fatal complications due to traditional medicines have been described in the literature, the overall incidence of herbal poisonings in South Africa is not known (Kale 1995).

An indirect, and presumably more common problem caused by traditional medicine is the delayed presentation of patients to clinics or hospitals. The reason black patients present to western doctors late is thought to be due to initial recourse to traditional healers coupled with inadequate or expensive orthodox medical care (Onuba 1992). Unfortunately in many cases, valuable time is lost while a certain disease is in its initial stages and still curable. A patient waits until the disease is severe, often until irreversible damage has been caused before turning to orthodox medicine. Often no help can be given at this stage. This in turn decreases the faith black patients have in orthodox medicine, making it appear as if orthodox medicine is as effective as

traditional medicines. Therefore it is a vicious circle. Two examples of this scenario are given below.

Two children were taken to traditional healers for apparently minor ailments, one with a lesion that had developed around the point of entry of a thorn in the hand and the other with a lesion in the knee. It took close on a year for each of the children to be presented to hospitals by which time secondary conditions had developed and both lost the use of a limb. Over the year, the child with a thorn in the hand developed chronic osteomyelitis which then progressed to a point of irreversible damage, requiring removal of the radius resulting in the loss of use of his right hand. The other child presented with a malignant lesion requiring an above-knee amputation of the left leg (Chipfakacha 1994). In both cases the limbs could have been saved if the children had been referred to orthodox medicine early in the progression of their diseases.

Closer collaboration between traditional healers and western doctors together with basic medical training of traditional healers enabling them to recognise when their medical system will fail, could avoid the perpetual repetition of cases such as described above.

1.1.5. Advantages of traditional health care systems

Despite many shortcomings of traditional and herbal medicines, African traditional medicine offers several advantages compared to conventional orthodox medicine. These include: *(i)* the whole family is involved in the treatment. The focus of attention is not only the patient but also the reaction of the family and close relatives; *(ii)* traditional healing has a closeness, a shared view, informality, and the use of everyday language in the consultations; and *(iii)* traditional healing both reinforces and articulates the values of the community to which the healer belongs (Chipfakacha 1994). Modern doctors in contrast, are separated from their patients by social class, language, economic position, specialised education and cultural background. Most physicians usually interrupt their patients only 18 seconds after they begin

describing their problem, while traditional healers depend on good communication (McKenzie 1994).

Many traditional healers only charge a patient once the patient has been healed (McKenzie 1994), even if the treatment involved weeks to months of daily consultations (Healers, Pers. comm.). However, the fees may sometimes be exorbitant, for instance a cow (Kale 1995). This system seems to be changing as many traditional healers adopt the western practitioners' custom of charging per consultation (Healers, Pers. comm.).

1.1.6. Integration of traditional healers into primary health care

Since the Alma Ata conference held by the WHO/UNICEF there has been a growing impetus towards either integrated systems or the beneficial co-existence of traditional and modern medical systems (Pretorius 1991). Closer collaboration between the two medical systems has been proposed in many countries such as Zimbabwe, Malawi, Swaziland, Tanzania and Mali (Freeman & Motsei 1992; Chana *et al.* 1994; Berger *et al.* 1994; Mbura *et al.* 1985). It is believed that all interested groups stand to gain from such a liaison: the authorities responsible for health care delivery, the western-trained health care workers, the traditional healers and the users of these services (Pretorius 1991).

Providing traditional healers are educated to recognize illnesses which they cannot and should not treat (e.g. tuberculosis and eye disease) traditional healers fulfil three of Morrell's (1986) four primary health care objectives, being that primary health care: *(i)* should be acceptable to the people; *(ii)* should be accessible to the people; and *(iii)* should identify those medical needs of the population which can be prevented, modified or treated. Incorporating traditional healers into modern health services would fulfil Morrell's fourth objective of primary health care, that primary health care should make maximum use of the available manpower and resources to meet the medical needs of the people (Chipfakacha 1994).

With regard to the developing world, doctors should reconsider the flaw in the attitudes that systems developed in the West are inherently preferable to the local alternatives (Patel 1993). Western medicine should not ignore, devalue or ban traditional practice. To do so would jeopardise black South Africans suffering from depression, mental handicaps or AIDS as well as any other poor person for whom the traditional healer is often the only and best relief. Traditional remedies are also believed to be effective in diarrhoea, headaches, other pains, and for sedating a patient. It is thought that traditional health care is more cost effective than official health care for some health problems such as psychiatric disorders (Patel 1993), and the success in treating psychological disorders is well known and often recognised (Kale 1995).

Edwards (1986) investigated the diagnosis and treatment of a group of psychiatric patients by Zulu healers and modern clinical psychologists. The results indicated that while traditional and modern healers worked from different theoretical orientations, there was significant agreement in the diagnosis and treatment by the healers and psychologists. The patients perceived both traditional and modern practitioners as being more or less equally helpful.

Western medicine should embrace, train and collaborate with traditional healers, while encouraging traditional healers to administer safe, hygienic treatments for conditions that are more amenable to the healers' type of practice. Dialogue should be conducted on an equal footing as the sooner some mutual understanding is achieved the better (Harries & Culling 1994). Incorporation of traditional healers into the modern health care system would help identify diseases before they reach an advanced stage. Before the patient becomes disabled or dies because of the loss of valuable time. Traditional healers should be encouraged to refer patients they are incapable of curing or treating (Chipfakacha 1994). However, Chief Zungu, leader of the Traditional Healers Association of South Africa, argues that traditional healers have been referring patients to mainstream doctors and hospitals for years, yet physicians will never refer a patient to a traditional healer. He states that this is a very unfair relationship (McKenzie 1994).

Skepticism about the possibility of western medicine being able to collaborate with any type of traditional healer has been expressed by Kiernan (1978). In his opinion, medical doctors could collaborate with sangomas as they are competent in the physical and social realms and therefore their practices are complementary to modern medicine. The same does not apply to inyangas as he feels that inyangas practice in opposition to modern medicine and therefore collaboration with inyangas would not be possible or desirable.

1.2. Researching South African Traditional Medicinal Plants

Traditional healers claim that when they undergo training, ancestral spirits are their principal teachers (Gumede 1990; Schuster Campbell 1998). Thus there are no standard treatment regimens for specific indications. A wide variety of plants are prescribed for the same condition by different practitioners making research on the impact of traditional medicine an almost impossible task as there are insufficient resources and manpower to determine the efficacy and safety of every plant used by traditional healers. Over a thousand species are used by the Zulu people alone (Hutchings *et al.* 1996). Part of the solution to this problem lies in collaboration between traditional healers and researchers. This process would be more likely to succeed if traditional healers could be persuaded to cooperate with orthodox physicians. Traditional healers need to identify the plants most commonly used and those that pose the greatest risk to the patients. Then scientific researchers need to focus their research on this selected group of plants. Results should then be simplified and made accessible to traditional healers.

Previous work has shown that traditional healers are not merely custodians of tradition, but are innovative and willing to learn from their Western counterparts (Simon 1991; Green *et al.* 1995). Traditional healers are therefore likely to heed advice given to them. However, healers seem more likely to accept positive findings than negative ones, as was apparent when results on the toxicity of impila (*Callilepis laureola*) were presented to some traditional healers (Hutchings 1999).

1.3. Pregnancy Related Traditional Remedies

Pregnancy-related traditional and herbal remedies are used in many countries throughout Africa (Mbura *et al.* 1985), as well as other countries such as China, Japan, India, Melanesia, Hawaii, North and South America (Varga & Veale 1997). In rural South Africa, up to 75% of pregnant Zulu women visit traditional healers to request remedies specifically for pregnancy (Morris & Mdlalose 1991). There is also a renewed interest in these remedies in the United States and Europe (*Bunce 1987; Ehudin-Pagano et al. 1987*).

1.3.1. South African pregnancy related traditional remedies

Fertility is a dominant theme in the culture of black South Africans as it ensures the preservation and propagation of the tribe. A large family is regarded as insurance against hunger and want in old age, with childbearing and infant care being two sacred duties of women in African society (Gumede 1978). Barrenness is regarded as a disgrace, as procreation is expected to automatically follow marriage. Barrenness has been identified as a cause of emotional stress in black patients living according to black customs and may lead to typical clinical presentation of stress (Ramasuvha 1982). Girls as young as 16 years visit doctors complaining of sterility, as the boyfriend wants proof of womanhood (namely a baby) before marriage (Gumede 1978). Pregnancy is therefore an event of great importance, which is manifested in the number of traditional remedies used for pregnancy related indications, and the number of customs and taboos that have been developed around pregnancy.

African folklore teaches that many of the complications during pregnancy are caused by spells cast by the "abathakathi" (sorcerers). The role of the African traditional healer is to diagnose the source of the problem and to prescribe a remedy to ensure the well-being of the foetus (Harries & Cullinan 1994; Conco 1972).

1.3.2. Classification of pregnancy related traditional remedies

Four groups of pregnancy related remedies are prescribed. The first group is *isihlambezo* which comprises the most common antenatal traditional remedies.

These remedies are prescribed to enhance foetal growth, strengthen the mother and the foetus, aid the normal physiological functioning of the mother, ensure an easy and complication free delivery, speed up delivery, produce a clean baby with no vernix, and remove excess fluid from the abdomen (Gerstner 1941; Gumede 1978; Gumede 1990; Morris & Mdlalose 1991; Varga & Veale 1997). The routine use of these remedies usually commences from the 7th lunar month and continues through to delivery (Morris & Mdlalose 1991; Veale *et al.* 1992). *Imbiza* is another group of remedies which have cleansing properties (Varga & Veale 1997). These are not specific to pregnancy, but are commonly prescribed to pregnant women at any stage of pregnancy. The third group of remedies is known as *umsekelo* (Bryant 1966; Veale *et al.* 1992). These remedies are used specifically to prevent miscarriages and premature deliveries. The last group, known as either *imbelekisane* or *inembe* is used specifically to induce or augment labour. Components of these remedies are thought to have strong uterotonic properties (Conco 1972; Gumede 1990; Varga & Veale 1997; Gumede 1978). Some healers use an additional type of remedy. After the birth, the mother is given an infusion (e.g. ugobho - *Gunnera perpensa)* to aid the involution and healing of the uterus (Larsen *et al.* 1983).

1.3.3. Composition of pregnancy related traditional remedies

A literature review compiled by Veale *et al.* (1992) revealed that 57 plant species were reported to be used in herbal remedies during pregnancy and childbirth, of which *Rhoicissus tridentata* is one of the plants prescribed. Of the 57 plant species, 16 (28%) are known to be potentially toxic, and four (7%) are known to have caused fatal human poisonings.

Most traditional healers mix two or three components in one decoction (Varga & Veale 1997). The mixing of 57 different ingredients in a variety of permutations yields a tremendous diversity between different kinds of isihlambezo, inembe, imbelekisane or imbiza, yet most research done on these remedies treats pregnancy-related remedies as an homogenous group (Bullough 1981, Larsen *et al.* 1983, Mbura *et al.* 1985, Mitri *et al.* 1987; Morris & Mdlalose 1991). This is no different to treating all scientific medicines as homogenous. If specific components of remedies are not identified the results have very little

applicability, as the different components of the remedies are chemically diverse and will therefore have diverse pharmacological effects. The pharmacology and chemistry of each plant needs to be investigated individually before the effect of these remedies on pregnancy can be elucidated enabling predictions to be made.

1.3.4. Transplacental transfer of drugs

The placenta is an organ with many physiological functions, several involving exchange of various endogenous and exogenous substances between the maternal and foetal circulation (Bourget *et al.* 1995). Most drugs taken by pregnant woman can cross the placenta and expose the developing embryo and foetus to their pharmacological and teratogenic effects (Koren 1998). The potential toxicity to the foetus is greater than to the mother due to the increased blood-brain-barrier permeability and the poorer liver enzyme conjugating function in the foetus (Norman 1992). Critical factors affecting the intensity of placental drug transfer and drug effects on the foetus (Seller 1965; Norman 1992) include the following: *(i)* the physicochemical properties of the drug; *(ii)* the duration of exposure to the drug; *(iii)* distribution characteristics in different foetal tissues; *(iv)* the effects of the drugs used in combination; *(v)* the surface area of the exchange membrane; *(vi)* the thickness of the endothelio-syncytial membrane; *(vii)* the maternal blood flow and hydrostatic pressure in the intravillous chamber; *(viii)* the blood pressure in foetal capillaries; and *(ix)* the difference between maternal and foetal osmotic pressures. As term approaches, foetal-maternal exchanges intensify, in part because of thinning of the membranes.

Passive transfer is the predominant form of exchange, however, active transport, facilitated diffussion, transfer by molecular binding, endocytosis and, passage by effraction also occur. The placenta contains microsomal enzymes which metabolise many exogenous substances. The result is to produce more polar metabolites that are more readily excreted. This process usually results in detoxification activity, however, theoretically the possibility remains that metabolism could produce a substance that is more foetotoxic than the parent compound (Bourget *et al.* 1995).

A pH gradient could affect the transplacental passive transport of drugs since only non-ionised or slightly ionised compounds cross membranes in this manner (Reynolds & Knott 1990). It has been established that if the pH of foetal plasma falls, the foeto-maternal ratio of basic compounds increases (Biehl *et al.* 1978; Kennedy *et al.* 1979). This so-called ion-trapping effect is believed to be more pronounced in the case of strong rather than weak bases (Reynolds & Knott 1990).

1.3.5. Altered maternal pharmacokinetics during pregnancy

Plasma protein binding of drugs may fall during pregnancy because of a oestrogen induced fall in plasma albumin concentrations by up to 20%. Therefore, there may be a fall in the bound fraction of drugs normally extensively bound to plasma albumin. This will normally lead to increased clearance of the drug, even though the concentration of the free drug does not fall to the same extent (Grahame-Smith & Aronson 1992; Norman 1992).

Metabolism of drugs is also altered during pregnancy. The functions of different enzymes are affected differently during pregnancy. During late stages of pregnancy, the conjugation of drugs with glucuronic acid is reduced to about half the normal level. This is probably due to the high levels of progesterone and pregnanediol, which are known to inhibit glucuronyl transferases *in vitro* (Robson & Stacey 1968). Certain oxidative metabolic transformations of drugs are also inhibited, where the activity of mono-oxygenase is increased (Norman 1992). That is, compounds that undergo conjugation with glucorinic acid or oxidation will be metabolised at a reduced rate leading to increased plasma concentrations of these compounds, in turn causing more of the compound to pass into the foetal circulation.

Excretion. Renal blood flow doubles by the third trimester, and the glomerular filtration rate increases by about 70%. Thus drugs which are mainly eliminated by renal excretion will be cleared more rapidly. In contrast the clearance of drugs with a high hepatic extraction ratio show no change in the clearance rate.

This is because there is no change in the blood flow to the liver during pregnancy (Norman 1992).

Nutrition is also an important factor in drug metabolism. Dingell *et al.* (1966) showed that rats maintained on a diet deficient in protein and calcium have lowered capacities to metabolise drugs, both by oxidative and reductive pathways, due to decreased activity of the microsomal enzymes of the liver. Animals on diets deficient in protein and magnesium have been shown to be more prone to acetylsalicylic acid toxicity (West 1964).

The increased plasma concentrations of effected compounds during pregnancy could be further accentuated if the mother has a nutrient deficient diet. The diet of the majority of subeconomic and/or rural black South Africans has been shown to consist of mainly carbohydrates and is deficient in protein, as well as certain vitamins and minerals (Coetzee 1974; Clarke & Ngobese 1975; Bembridge 1987). This is especially apparent in communities with insufficient land to cultivate enough food and who rely on bought refined meals rather than eating whole grains grown themselves. There is also a custom of discouraging pregnant women from consuming milk and meat from the family herd and from eating eggs and fish during pregnancy (Larsen *et al.* 1983; Sindiga 1995). Therefore, herbal remedies metabolised by microsomal enzymes have the potential of being more toxic during pregnancy.

The complex interactions influencing the pharmacokinetics of drugs during pregnancy, with either possible increases or decreases in plasma levels of drugs, have led to the general rule in modern medicine to avoid drugs as far as possible during pregnancy. If drugs cannot be avoided the drug concentration should be kept in the lower third of the therapeutic range. This rule should be extended to the use of herbal remedies during pregnancy, however, the use of herbs during pregnancy is so deeply entrenched in black African culture, that it is unlikely that caution will be exercised for many years to come.

1.3.6. Altered pharmacokinetics in the foetus and neonate

In general, foetal and newborn animals have a limited capacity to metabolise drugs. The microsomal enzymes of the liver which oxidatively metabolise drugs, including cytochrome P-450, are more or less absent in the foetus, but increase rapidly after birth, reaching adult levels at about 30 days in rats and 8 weeks in humans.

Reactions which are diminished or impaired in foetuses and new-born infants are: the reduction of nitro and azo compounds; the conjugation of drugs and their metabolites; conjugation with glucuronic acid, glycine and glutathione (mercapturic acids). Acetylation and sulphate reactions however occur at normal adult levels. This lowered rate of drug metabolism usually leads to a decreased ability to deactivate drugs, and consequently to prolonged pharmacological activity and increased toxicity. On the other hand, when metabolism is responsible for converting an inactive prodrug into an active drug, the effects are reversed - diminished pharmacological activity and lowered toxicity would be expected (Robson and Stacey 1968).

Evaluation of foetal effects and efficacy of modern drugs during pregnancy is a field that has been minimally investigated (Bourget *et al.* 1995). This is even more apparent with investigations on the safety and efficacy of herbal remedies during pregnancy. No clinical studies on the safety of herbal remedies during pregnancy could be located. The only data on the use of herbs during pregnancy are in the form of case reports or animal studies (Keeler & Crowe 1984; Panter *et al.* 1990, Lemonica & Alvarenga 1994).

1.3.7. Teratogenicity

The thalidomide disaster in the early 1960's where there was a dramatic increase in the incidence of a rare birth defect including phocomelia, a condition involving the shortening or complete absence of limbs, horrified the western world, highlighting the dangers of ingesting medicines during pregnancy. It is estimated that 10 000 children were born with birth defects because of maternal exposure to this one agent (Berkowitz & Katzung 1998). Other allopathic drugs have been identified to possess teratogenic properties which include cytotoxic

agents, retinoic acid, phenytoin, carbamazepine, sodium valproate, warfarin, gentamicin and lithium (Norman 1992). Million of dollars are spent by pharmaceutical companies to determine the safety of drugs during pregnancy. It is unknown whether spending the same amount researching the safety of herbal remedies during pregnancy would also yield results showing that certain herbs are teratogenic. Interviews with traditional birth attendants showed that they are aware that alcohol and cigarettes should be avoided during pregnancy (Sindiga 1995), but no mention is made of possible dangers of traditional herbal remedies.

Organogenesis of the foetus occurs within the first trimester of pregnancy, therefore the greatest risk of causing major congenital malformation is during the first trimester. However, malformations in sexual differentiation, and neurological development may occur if a harmful compound is ingested during the second or third trimester.

Undoubtedly teratogenic compounds do occur in higher plants, but no plant species has been shown as having been responsible for congenital malformations in humans. However, experiments on laboratory animals, where large doses of plants not usually experienced by humans were incorporated into the food source, have demonstrated that the potential for certain plants to be teratogenic exists (Evans 1996). Some plant species or genera used in South African traditional medicines that have been shown to have caused malformations in laboratory animals include: *Senecio* (Asteraceae), *Indigofera spicata* (Solanaceae), *Lobelia* (Campanulaceae), *Mimosa* (Mimosaceae), *Lupinus sericeus* (Fabaceae), *Conium maculatum* (Umbelliferae), *Veratrum californicum* (Liliaceae) (Evans 1996). *Cinnamonium zeylanicum* has been shown to be embryofetotoxic (Lemonica & Macedo 1994).

Despite all the potential dangers listed above associated with drug ingestion during pregnancy, less than one percent of congenital malformations are thought to be caused by the use of modern drugs during pregnancy (Norman 1992).

1.3.8. Lactation

Most drugs pass into breastmilk. As mentioned above the hepatic metabolism of many drugs is reduced in neonates making neonates particularly prone to toxic effects of herbs ingested by lactating mothers.

Two breastfed neonates were admitted to hospital in Italy after their mothers drank copious amounts (more than 2*l* per day) of a herbal mixture to stimulate lactation. The remedies contained liquorice, fennel, anise and *Galega officinalis*. The neonates showed neurological symptoms of hypotonia, lethargy, emesis, weak cry, poor sucking and torpid reactions to painful stimuli. Once the teas were discontinued the condition of the neonates improved within 24-36 hrs. Essential oils (anetholes and related compounds) within the anise and fennel were suspected to have been the causative agents in the poisonings (Rosti *et al.* 1994).

Two species of *Senecio* containing pyrrolizidine alkaloids were linked to fatal hepatic veno-occlusive disease in a newborn infant of a woman, who again was drinking copious amounts of a herbal tea as a lactagogue (Roulet 1988). The symptoms of pyrrolizidine poisoning mimic Reye's syndrome (Fox *et al.* 1978).

1.3.9. Pregnancy-related remedy usage

South African traditional healers generally do not administer isihlambezo during the first trimester. The use of isihlambezo normally commences from the beginning of the third month, or whenever the patient consults the healer. Treatment continues on a daily basis, or whenever needed, until the end of the eighth month or until birth (Varga & Veale 1997). Morris & Mdlalose (1991) found that up to 75% of rural Zulu women use traditional remedies specifically for pregnancy. In Tanzania, where approximately 50% of the women use traditional remedies during pregnancy, remedies are used throughout pregnancy, with the highest usage rate being during the first trimester, followed by usage during labour. The usage rate increases with age, indicating that young people are being swayed towards accepting western culture and abandoning traditional practices as "uncivilized". It is the older women who tend to keep cultural habits including traditional medicines (Mbura *et al.* 1985).

1.3.10. Traditional Birth Attendants (TBA)

Traditional birth attendants are characteristic of most developing countries. They are usually older women who are respected in their communities for their skills for assisting during confinement. The conditions for becoming a traditional birth attendant usually include having at least two children and an apprenticeship lasting for anytime up to 15-20 years. Birth attendants do not charge for their services but may accept gifts (Kale 1995).

60-80% of all babies in the developing world are delivered at home by either trained or untrained birth attendants (Islam *et al.* 1982b; Cosminsky 1983; Bullough 1981; Fajemilehin 1995; Wollast *et al.* 1993). Home delivery is especially common in rural areas where long-held superstitions are observed. Tetanus is the most common danger associated with home deliveries, which accounts for 20-40% of neonatal deaths in developing countries. Neonatal tetanus is mainly associated with the use of unsterile instruments for cutting the umbilical cord, unsanitary surroundings and the prevalent practice of dressing the umbilical stump with substances such as cow-dung, herbs, clay or ash (Islam *et al.* 1982a; Buchmann *et al.* 1989; Fajemilehin 1995).

South Africa has a lower rate of home deliveries than that reported from other developing countries. Buchmann *et al.* (1989) reported that 46% of woman in the Mosveld health ward (rural northern Kwazulu-Natal) delivered at home, of which only 47% were attended by traditional birth attendants. These lower proportions of home-births are despite this region having a particularly high density of traditional healers (Buchman, Pers. comm.). The reasons given for home deliveries were predominantly shortage of transport and sudden or unexpected labour. Of the women who intentionally gave birth at home 94% were multiparas (Buchman *et al.* 1989).

Larsen *et al.* (1983) reported the following practices of TBAs after close interactions with 5 Zulu traditional birth attendants.

Antenatal care: No physical examinations are done on pregnant women. The TBA serves more as an educator. Pregnant women are advised to avoid eating eggs and meat or milk from the family herd. The TBA may recommend

or prescribe isihlambezo. Pregnant woman are advised to be as active as possible, with no tradition that heavy manual labour may possibly be harmful. TBA were shown to have little knowledge about complications such as eclampsia or antepartum haemorrhage.

Labour: TBAs are usually only called late in the first stage of labour. If labour is prolonged (primigravida >24hrs and multigravida >12 hrs), the woman in labour is given imbelekisane. TBAs were shown to be unable to detect foetal distress, meconium staining or postpartum haemorrhage.

Care of the neonate: Care is taken to keep the neonate warm. Once the cord is cut with a razor blade the cord stump is treated with various traditional remedies to help it dry out quickly. Colostrum is regarded with the same revulsion as pus. Therefore, babies are only put to the breast after 12-24 hours while the colostrum is expressed and thrown away. During this time babies are given sugar water. Enemas are also very commonly used in the first week if the baby does not pass a stool or cries persistently or is restless with colic. Tachypnoea is treated with traditional medicines. If there is no improvement the baby is referred to the nearest clinic. Jaundiced and vomiting neonatal infants are usually referred immediately.

The TBAs were found to be eager to learn and to assist in the better care of their people. This is similar to what was found with traditional healers as mentioned earlier.

As with other traditional health workers there have been suggestions and attempts to incorporate TBAs into the primary health care system. TBAs would have to be trained to identify high-risk mothers and to refer these mothers to adequately staffed and equipped health facilities. This would reduce birth-related injuries or illnesses and improve these mothers' chances of survival. By TBAs encouraging birth spacing, breast feeding, sanitary practices and appropriate weaning and feeding practices, infant deaths can be reduced (Sindiga 1995).

Wollast *et al.* (1993) reported the results of giving 280 TBAs such training. The TBAs were given a one month training course, during which they

attended about 20 deliveries in prenatal clinics, followed by a two week refresher course in the second year. The training focused on sanitary practices, simple obstetrical manipulations, and recognizing the criteria for referral and evacuation in the course of the pregnancy and delivery. The results showed that the training was successful, and the traditional birth attendants were able to recognise high-risk patients who they did refer to clinics. Evacuation criteria were also heeded. However, the training focused on antenatal care neglecting postpartum care. This led to a high maternal mortality (27/6129) which occurred postpartum, and was thought to have been caused mainly by delayed evacuation and/or the referral centers being insufficiently equipped to receive these cases. Even though the program was a success, they concluded that the training courses needed to be carefully constructed.

1.3.11. Adverse effects of pregnancy-related traditional remedies

Anecdotal observations on the effects of traditional remedies used to induce or augment labour (Baragwanath Nursing Sisters, Pers. Comm.) indicate that the risks are similar to the risks of labour induction using oxytocin. These risks include foetal distress, cephalopelvic disproportion, uterine hyperstimulation and low Apgar scores (Liston & Campbell 1974; Knutzen *et al.* 1977; Davies *et al.* 1973). Frequent contractions of the uterus in response to oxytocin stimulation can interfere with the uterine blood flow and so produce distress in the foetus (Liston & Campbell 1974). Labour induction using oxytocin has been linked to increased risk of neonatal jaundice (Davies *et al.* 1973), which has also been shown to occur with the use of Chinese herbal remedies during pregnancy. Two commonly used Chinese herbal medicines, *Coptis chinensus* ("chuen-lin") and *Artemisia scoparia* ("yin-chen") have been shown to displace bilirubin from serum proteins and this increases the risk of hyperbilirubinaemia (Chan 1994).

Oxytocin has little to no effect on the nondilated uneffaced cervix and does not alter cervical ripening. Stimulation of myometrial activity while the cervix is undilated causes increased intrauterine pressure increasing stress on the foetus, while simultaneously decreasing cervical compliance (Olah *et al.* 1993). It is assumed that certain herbal oxytocics may have the potential to cause

similar effects. In extreme cases the increased uterine pressure may cause rupturing of the uterus. A study on maternal deaths occurring in all ethnic groups throughout South Africa between 1980 and 1982 revealed that 2.3% were caused by uterine rupture. Of the overall maternal deaths, 96% of these foetuses died (Boes 1987a & b). In the central region of Malawi in 1975, 105 cases of uterus ruptures were reported of which 20 were reported fatal. It is not known whether herbal remedies contributed towards these figures or not. The use of herbal remedies was implicated in the rupturing of some uteri in Cameroon (Nasah & Drouin 1978).

Deaths from acute renal failure associated with the use of herbal medicine have been reported in Zambian pregnant women (Loventhal *et al.* 1974). Accidental poisonings with herbal remedies caused 15/109 maternal deaths in Malawi (Bullough 1981). Fatal herbal poisonings are commonly caused by hepatorenal failure (Huxtable 1990; Pillans 1994; Bodenstein 1977; Wainwright *et al.* 1977). Fatal oesophageal strictures in Nigeria have also been associated with the use of herbal remedies (Mbura *et al.* 1985). Certain childhood disorders such as congenital malformations, malnutrition and tumours were also thought to be caused by toxic or carcinogenic constituents of herbal remedies taken during pregnancy (Shoental 1972).

The use of *isihlambezo* during pregnancy has been associated with premature delivery and growth retarded babies. However, a small sample size was used in this study which prevented any conclusions being made (Morris & Mdlalose, 1991). The ingestion of large quantities of imbelekisane was implicated in several cases of unexplained foetal death resulting from premature labour (Broster 1981). Mitri *et al.* (1987) found that women who had recently taken isihlambezo had an increased incidence of foetal meconium passage, which in turn was correlated to a significant increase in the incidence of caesarean section and decreased Apgar scores. An increased incidence of meconium passage in foetuses of drug-dependent mothers has been ascribed to the intestinal hyperperistalsis effect of drug withdrawal (Ostrea *et al.* 1982). The pathophysiology of meconium passage may reflect: i) a response to foetal hypoxemia, which could cause contractions in foetal gut, peristalsis and anal

sphincter relaxation; ii) an increased vagal activity from *in utero* stresses (eg. cord compression) without concomitant hypoxemia; iii) a normal physiological activity related to foetal gut maturity; or iv) a combination of the above (Houlihan & Knuppel 1994). Both i) and ii) are caused by uterine hypertonia, which has been shown to be induced *in vitro* by the ingestion of imbelekisane infusions (Larsen *et al.* 1983) and the components of pregnancy-related remedies *Triumfetta rhomboidea, Gunnera perpensa, Clivia miniata, Pentanisia prunelloides and Agapanthus africanus* (*Larsen et al.* 1983; Veale *et al.* 1989; Osore 1982, Kaido *et al.* 1997). The latter three plant infusions also stimulate ileal contractions (Veale *et al.* 1989; Kaido *et al.* 1997). Provided that the active constituent(s) of these plant extracts pass into foetal circulation, the possibility exists that these plants may increase the incidence of meconium staining through increased intestinal peristalsis caused by direct stimulation of intestinal muscle.

Despite the potential hazardous effects of isihlambezo and imbelekisane, together with their wide usage, there is considerable lack of research in this area. Note that no plant species were identified in any of the clinical studies represented above. Even though some pharmacological studies have been done, very few active ingredients of the plants utilised have been identified, and the pharmacological actions of most remedies are generally unknown. Since the molecular size and lypophilicity of the components are unknown, it is impossible to postulate which of the compounds are absorbed, what their volumes of distribution would be, or which are likely to cross the placenta and enter foetal circulation.

Even though there is this risk of teratogenicity, the effects of herbal remedies on the uterus and labour dominate the literature, and will be the focus of this thesis.

1.3.12. Westerners' approach to pregnancy

In western civilization, despite the advancement of medical science, pregnancy is shrouded by "wives' tales" and traditions passed down from previous generations. From personal experience, the community treats a pregnant woman with far more concern and interest than they treat a non-

pregnant woman. Throughout her pregnancy she is told about traditional practices on how to determine whether she is carrying a boy or a girl, how to overcome nausea or discomfort, how to ensure the well-being of the baby, or is told about a remedy or practice which will ease labour. Once the child is born, more emphasis is placed on orthodox medical treatment of the baby.

Medical training does not immunise one against irrationalism. The magical and religious handling of sickness for centuries has not vanished in the west. In the presence of sickness, suffering, crippling and death, religion must speak, often in the form of alternative or complementary medicine (Levin 1996). With the renewed interest in traditional or alternative health care in the West has come an increase in the formalised use of herbal remedies during pregnancy. The Journal of Nurse-Midwifery provides recipes for the preparation of herbal remedies for use during pregnancy, giving indications very similar to those of isihlambezo. They are intended to enhance maternal well-being and to ease labour (Bunce 1987; Ehudin-Pagano *et al.* 1987). Books such as Roberts (1986) are also written for the general public specifically on the use of herbs during pregnancy.

Herbs used in western herbalism considered to be unsafe in the first two trimesters because of abortifacient action, but are used during the third trimester, especially from week 36 onwards to increase uterine tone and to regulate contractions at birth include (Grieve 1971; Mills 1988; McIntyre 1994):

Acorus calamus (Calamus)
Alchemilla vulgaris (Lady's mantle)
Artemisia spp. (Wormwood)
Capsicum frutescens (Cayenne)
Cassia acutifolia (Senna)
Caulophyllum thalictroides (Blue Cohosh)
Chrysanthemum parthenium (Feverfew)
Cimicifuga racemosa (Black Cohosh)
Dryopteris filix-mas (Male fern)
Foeniculum vulgare (Fennel)
Glycyrrhiza glabra (Licorice)
Hydrastis canadensis (Goldenseal)
Linum usitatissimum (Flax seed)
Mentha pulegium (Pennyroyal)
Mitchella repens (Partridge Berry)
Passiflora incarnata (Passion Flower)
Phytolacca americana (Poke root)
Podophyllum peltatum (American Mandrake, Mayapple)
Prunus serotina (Wild Cherry)

Rheum spp. (Rhubarb)
Rubus idaeus (Red Raspberry leaf)
Salvia officinalis (Sage)
Sanguinaria canadensis (Bloodroot)
Tanacetum vulgare (Tansy)
Thuja occidentalis (Thuja)
Thymus vulgaris (Thyme)
Vinca rosea (Periwinkle)
Viscum album (Mistletoe)

No clinical trials on these plants in relation to labour have been reported in the literature. A case report by Jones & Lawson (1998) showed that maternal consumption of *Caulophyllum thalictroides* was implicated as the cause of acute nonfatal neonatal myocardial infarction associated with profound congestive heart failure and shock. The authors confirmed that *C. thalictroides* was the causative agent using laboratory assays of the vasoactive glycoside extracted from the plant, which was shown to have toxic effects on the myocardium in the laboratory animals.

1.4. Herbal Medicine

1.4.1. Secondary plant metabolites

Secondary metabolites are thought to be the compounds possessing pharmacological activity within the plant. Even though they have been the subject of investigation for most of the past century, there is no clear definition of the term secondary metabolite or for their role within plants. It is still difficult to separate primary metabolites from secondary metabolites especially when considering several plant hormones and storage compounds. (Mothes 1980). The middle of this century saw a tremendous increase in the interest in secondary metabolites as the pharmaceutical industry became interested in secondary metabolites as a source of compounds in their search for antibiotics. This was initiated by the discovery of penicillin and by the investigation of cancer-inhibiting substances. The search led to the discovery and structure elucidation of nearly 800 alkaloids of the tryptamine-monoterpene type from the *Gentianales* alone (Mothes 1980). However, these

screening programs led to disappointment, despite impressive pharmacological histories of certain groups of compounds (Gordon 1994). Today the focus of drug discovery is on chemically synthesising compounds and on the genetic basis of many diseases.

Plants synthesise a greater array of secondary compounds than animals. Insects and molluscs produce more secondary metabolites than higher animals but this is still much lower than that found in plants. The reason for the greater array of secondary compounds in plants (as opposed to animals) is thought to be the inability of plants to move and their lack of excretory organs. The first theory leads to the supposition that secondary metabolites confer an ecological advantage to the plant, such as defence from attack from micro-organisms and/or animals. This is related to the irritant, toxic or unpalatable characteristics of several secondary metabolites (Bell 1980). For example, physiologically active compounds such as histamine, acetylcholine and 5-hydroxytryptamine are present in the stinging cells of the European stinging nettle (*Urtica dioica*). Another possible role of secondary metabolites is that they may increase the ability of one plant species to compete with another species in a given environment (Bell 1980). For these reasons, there has been speculation that plants under stress, both from unfavourable environmental conditions and herbivores, will produce more secondary metabolites than unstressed plants.

Apart from a source of pharmaceutical compounds, secondary metabolites are also useful as phylogenetic tools. The discipline of chemotaxonomy was developed in the 1960's and is still being used to clarify the taxonomy of plants. Since chemical compounds are genetically coded only certain species or even races of a plant species synthesise specific secondary metabolites. Secondary metabolites tend to vary qualitatively between taxa whereas primary metabolites vary quantitatively and are subject to both environmental and genetic control (Bell 1980).

1.5. Shortcomings of Traditional Herbal Medicine

1.5.1. Lack of evidence

Alternative medicine as a whole has received much criticism from the western medical fraternity for their lack of hard evidence on the efficacy of their remedies and medical practices. (Pantowitz 1996, Levin 1996). It is this lack of evidence which drives the wedge between orthodox medical practitioners and alternative or traditional medicines (Richards 1996). Alternative medicine relies on anecdotes and theories. On the whole, claims and anecdotes about alternative therapies are published in books and magazines for the general public rather than peer-reviewed scientific journals (Angell & Kassirer 1998).

Modern practitioners are not always justified in their accusations against alternative therapies, especially in the case of herbal medicines, as many clinical trials have proven the efficacy of herbal remedies. Orthodox physicians however are unaware of these studies (Blumenthal 1998).

Until the 20th century, most remedies worldwide were botanicals. A few were found through trial and error to be helpful. Therapeutic successes with botanicals came at great human cost. The indications for using given botanicals were ill defined, dosage was arbitrary because the concentrations of the active ingredients were unknown, and all manner of contaminants were often present. Many of the remedies simply did not work, and some were harmful or even deadly (Angell & Kassirer 1998). This is no different to the stage at which traditional herbal medicine in South Africa is today. Unfortunately, no clinical studies have been done on South African herbals to determine which species are toxic, or which are effective. We only have case reports on the adverse effects of certain species.

1.5.2. Availability and trade of medicinal plant material

In urban areas such as Johannesburg, there are very few areas where medicinal plants can be harvested. Even though considerable work is being done to encourage traditional healers to grow their own medicinal plants

(Mander *et al.* 1998), it is not yet the custom of traditional healers to grow their own plants. There are myths such as the planting of a plant which has been collected from the wild next to one's house attracts lightning to strike the house.

Traditional healers within and around Johannesburg rely on periodic visits to rural areas to harvest plant material, or plant collectors harvest traditionally used plants from all around South Africa and its neighbouring countries and transport them to the cities. The collectors then sell the plant material to herb vendors in urban Herbal Markets who in turn sell the plants to traditional healers or the end user (Cunningham 1988). It is very seldom that the end user of the traditional remedy knows where the plant grew or how long the plant has been stored (Healers, Pers. comm).

1.5.3. Standardisation of herbal products

A problem facing the herbal industry world-wide is that the potency of different plants of the same species may be highly variable, as the concentration of the pharmacologically active principles in plants varies according to the growth environment, the part used, time of harvesting, conditions and length of storage (Huxtable 1990). In the United States, analyses of ginseng products showed that the concentration of the active ingredient in each pill varied by as much as a factor of 10 among brands that were labelled as containing the same amount. Some brands contained none at all (Angell & Kassirer 1998). Germany, Europe's leading importer of herbal medicinal products has prepared monographs defining quality standards and potency tests for over 350 single plant drugs to monitor these herbal products. Known as the Commission E monographs, they include descriptions of uses, contraindications, side effects and dosages (Taylor 1996). Herbal products produced by reputable herbal companies undergo rigorous processes of standardisation (Mills 1999), based on criteria stipulated in the Commission E.

However, in Africa and other developing countries herbal products are seldom standardised, since herb vendors in urban areas sell plants from a wide variety of localities. This causes a problem as the plants will vary genetically and phenotypically and traditional healers have no way of determining the concentration of the active ingredients with the plants they dispense. This poses a risk of accidentally overdosing patients. In rural areas, traditional healers usually harvest their plants from the same location. Therefore, they are not exposed to as high a risk of variation in the potency and efficacy of the plants as there will be an inherent form of standardisation in the growth environment or race of the plant. However, the plant constituents may vary qualitatively and/or quantitatively over time as the seasons change.

1.5.4. Identification and contamination of plant material

Another problem with herbal medicine is the misidentification of plant material. The possibility exists of a pharmacologically inert plant being substituted by a highly toxic plant (Huxtable 1990). For example, the confusion between comfrey and digitalis has been documented (Roulet *et al.* 1988). In South African urban areas this is highly possible as collectors sell plant material to traditional healers without diagnostic leaves or flowers, or the material is chopped or ground before being sold. This makes it difficult for healers to verify the identity of plant material, especially to a species level.

There have been warnings that herbal products sold in western countries containing Plantain have been contaminated with *Digitalis lanata* cardiac glycosides (Slifman *et al.* 1998). Lead poisoning has also been reported after ingestion of Indian diabetic herbal remedy (Beigel *et al.* 1998). Similar contaminations are highly possible in the unregulated and largely primitive plant markets throughout South Africa.

1.5.5. Temporal variation in constituents of medicinal plants

Seasonal variation in chemical constituents and biological activity of plants is a well documented phenomenon (Bos *et al.* 1998; Dolling *et al.* 1994; Brackenbury & Appleton 1997). The levels of solanesol and castaprenols in horsechestnut leaves (*Aesculus hippocastrum*) are 5 and 80 times greater in

October than they are in April (Threlfall 1980). The expression of genes coding for chemical constituents of plants are under the control of various factors, such as the developmental stage of the organism, duration and intensity of light, nutrient supply, triggering of internal signals and sequential expression of genes coding for secondary metabolites (Luckner 1980). All the above factors vary temporally which in turn cause seasonal fluctuations in the levels of both primary and secondary metabolites within plant tissues.

1.5.6. Spatial variation

As with the temporal variation in levels of chemical constituents within plants, factors affecting the nature and quality of secondary metabolites can be affected by temperature, rainfall, aspect, length of day, light quality and altitude (Evans 1996). These factors all vary spatially, whether it be on a micro or macro level.

Soil types also vary considerably from one location to another. The different particle sizes of the soils impact on the water content of the soils (Raven *et al.* 1986). The neighbouring plant species composition also vary which impact on the competitive impact on the plant and on the amount and composition of the micro- and macronutrients recycled to the soil. The pH of the soils also differ which impacts on the availability of the various nutrient elements within the soil (Taiz & Zeiger 1991). The microorganisms, (bacteria, fungi and protozoa) also differ both in species composition and number. All these factors alter the water and nutrient availability to the plant in a certain locality and therefore alter the chemical composition of the individual plant. This in turn alters the concentrations of primary and secondary metabolites within the plants to an extent which is dependant on the homeostatic mechanisms of the specific synthetic pathway of the relevant chemical compound within the plant.

Different populations of a particular plant species may differ genetically. This has been shown in the Tea Tree oil plants. The plant populations in and around Ballina (New South Wales, Australia) were shown to produce tea tree oil of a higher quality and quantity than anywhere else in Australia. These

differences are caused by genetic differences in the different chemical races of the plant (Leach and Bell 1999).

1.5.7. Stability of plant constituents

The marketing system of African medicinal plants in urban areas requires the healers to store plant material for considerable lengths of time under uncontrolled conditions. Once harvested, the plant material is subject to bacterial and fungal degradation. For practical reasons linked to the expense of packaging, plant material sold in South African herbal markets is normally sold as whole plant parts, and is not ground before selling. Therefore, the water content of the material is relatively high increasing the possibility that the plant material is colonised by microorganisms. The plant material is not only prone to being degraded by the microbe, but the possibility also exists of the microbe excreting pharmacologically active exudates into the plant material. The conditions in which the plant material is stored is also not conducive to protecting the stability of the chemical constituents of the plant. Plant material was often seen stored in direct sunlight, or close to roadsides. Not only do the warm conditions, particularly in the humid environment of Kwazulu-Natal, favour the growth of bacteria and fungi, but the storage of plants on roadsides, increases the possibility that the plant material is contaminated with heavy metals, such as lead, or other pollutants from the road.

1.6. *Rhoicissus tridentata*

1.6.1. Classification

Rhoicissus tridentata is a polymorphic species that has undergone numerous nomenclature revisions over the last two centuries. Originally the species was divided and classified as a number of species in the genus *Rhus*. The species were later reclassified in the genus *Cissus*. *Rhoicissus* was classified as a separate genus by Planchon (1887), but again there was confusion on the delineation between species. Later Wild and Drummond (1963) combined three different species, *R. cuneifolia, R. erythrodes* and *R. cirrhiflora* and classified them as *R. tridentata*. Their work only dealt with species collected north of the Zambezi river and thus did not consider species such as

R. pauciflora and *R. dimidiata*. Urton *et al.* (1986) classified all the above mentioned *Rhoicissus* species as *Rhoicissus tridentata*, but divided the species into two subspecies, being subspecies *tridentata* and subspecies *cuneifolia*. The subspecies are divided according to their leaflet margins. If the leaflets have no dentations or crenations on the leaflet margins, or if the number of dentations or crenations is four or less they are classified as subspecies *tridentata*. If the leaflets have more than four dentations or crenations they are classified as subspecies *cuneifolia* (Figure 1.1 a & b). The subspecies cuneifolia is more prevalent having a wider distribution, extending from the eastern Cape in the south to the Northern limits of South Africa, where subspecies *tridentata* is limited from Riversdale in the west to Port St Johns in the east and extending inland into the Karoo (Figure 1.2 a & b).

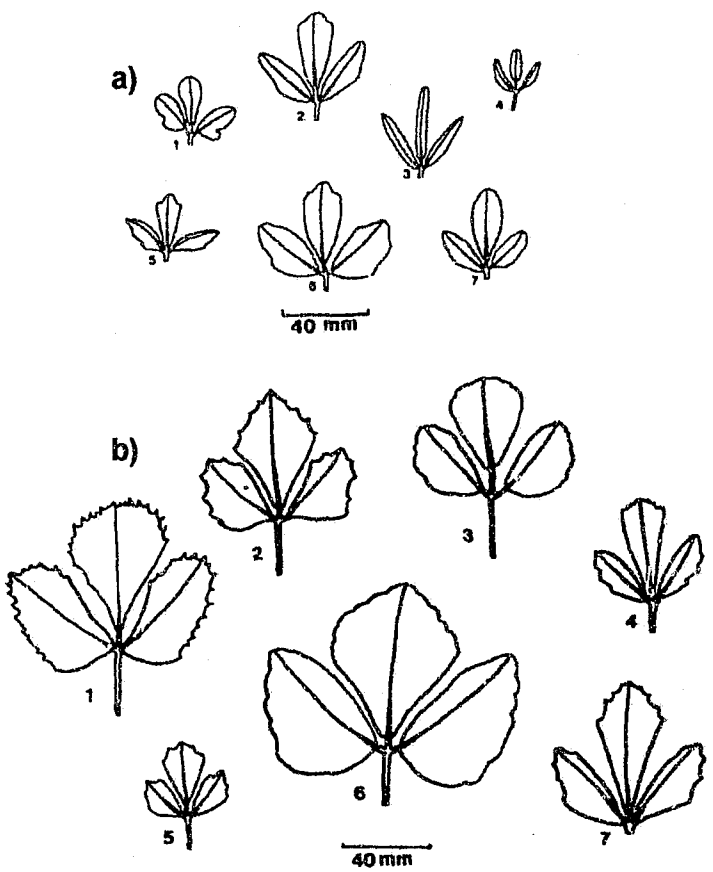


Figure 1.1. Comparison of leaf shape between *R. tridentata* subspecies a) *tridentata* and b) *cuneifolia* (Urton *et al.* 1986).

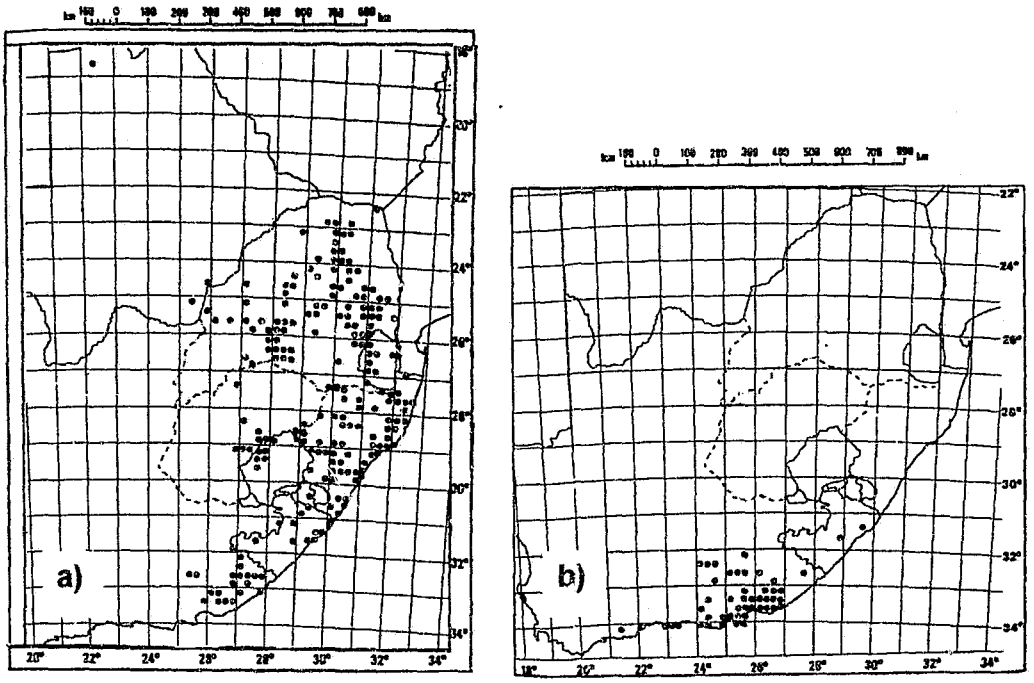


Figure 1.2. Distribution of a) *Rhoicissus tridentata* subsp. *cuneifolia* and b) *R. tridentata* subsp. *tridentata* (Urton *et al.*1986).

1.6.2. Characteristics of *Rhoicissus tridentata* subsp *cuneifolia*

Rhoicissus tridentata (L.f.) Wild & Drum. subsp *cuneifolia* (Eckl & Zeyh). N.R. Urton, is a deciduous shrubby creeper in the family Vitaceae, commonly known as wild grape (Smith 1966) or isiNwazi (Zulu) (Gerstner 1941). It has branches spreading outwards from a thick woody base. The leaves are trifoliate with wedge-shaped leaflets, each having a serrated margin. The plant bears small inconspicuous green flowers followed by small grape-like bunches of small brownish red berries. Lignotubers ranging in size from 5 to 30 cm in diameter are attached to the roots. If damaged the tubers reveal a blood red colour beneath the brown epidermis (Urton *et al.* 1986; van Wyk 1997).

The overall form of the plant varies according to its habitat. If growing in forests, it forms a woody climber of up to 10m or more. In high bush it scrambles freely

over other plants. In open grassveld it occurs as an erect shrub of up to 2m or more (Urton *et al.* 1986).

1.6.3. Zulu usage and preparation of remedies containing *Rhoicissus*

Decoctions and infusions of *Rhoicissus tridentata* roots or lignotubers are taken orally or as enemas in many South African traditional herbal remedies. Varga & Veale (1997) found that *Rhoicissus tridentata* is commonly used by Zulu women in pregnancy related remedies in the Durban region (Kwazulu-Natal). Decoctions are also used as enemas to relieve painful menstruation or taken orally to treat impotence and infertility (Watt & Breyer-Brandwijk 1962). The roots or tubers are used for stomach ailments, kidney and bladder complaints (Bryant 1966; Pujol 1990; Hutchings 1996). The tubers are also used for cattle diseases (Pujol 1990). The usage of *Rhoicissus* spp. by other African tribal groups or countries is given in Table 1.1.

The focus of this thesis is on the use of *R. tridentata* subsp. *cuneifolia* during pregnancy

1.6.4. Scientific findings on *R. revoilii* and *R. tridentata*

R. revoilii has been found to display antifungal activity inhibiting trichophyton, mentagrophytes and *Candida albicans* (Sawhney 1978b), but was found to be inactive against the bacteria *Neisseria gonorrhoea* (Sawhney 1978a). *R. tridentata* harvested in Rwanda was found to lack *in vitro* acaricidal activity against the insects *Appendiculatus* and *Rhipicephalus* (Van Puyvelde 1985).

1.6.5. Toxicity of *R. tridentata*

R. tridentata has been reported to be toxic, and has been implicated in a number of fatalities caused by CNS depression followed by respiratory failure (Watt & Breyer-Brandwijk 1962; Brandt & Muller 1995). Deaths of swine from ingestion of tubers have been reported in Tanzania and the toxicity of the tubers has been confirmed in rabbits (Hutchings 1996). No differentiation between the two subspecies were made in these reports.

Table 1.1. Medicinal usage of the *Rhoicissus* genus by other African tribal groups. Indications related to reproduction are coloured blue.

Species	Ethnic group	Plant part used	Route of admin.	Preparation	Indication	Reference
R. tridentata	Zulu	roots / lignotubers	oral	decoction	impotence infertility stomach ailments kidney and bladder complaints	Watt & B-Brandwijk 1962 Bryant 1966 Pujol 1990 Hutchings *et al.* 1996
			enema	decoction	dysmenorrhoea	
	Sotho	tubers			colds infertility	Guillarmod 1971
	Venda	roots	oral	decoctions	stomach ailments causing blood in the faeces infertility stomach pain in children	Arnold & Gulimian 1984
				cold water extract	pulsating anterior fontanel in babies	Arnold & Gulimian 1984
		leaves	oral	cold water extract	antiemetic for children	Arnold & Gulimian 1984
	Botswana	tuber			abdominal distension in pregnant woman, and in those who recently gave birth	Hutchings *et al.* 1996
	Lobedu				epilepsy	Watt & B-Brandwijk 1962
	East Africa	root	oral	decoction	painful menstruation	Kokwaro 1976
	Masai				nerve stimulant	Watt & B-Brandwijk 1962

Species	Ethnic group	Plant part used	Route of admin.	Preparation	Indication	Reference
	Tanzania	leaves	oral	infusion	menorrhagia	Chhabra *et al.* 1993
		dried leaves	oral	decoction	excessive menstruation painful menstruation	Chhabra *et al.* 1984
		root	oral		indigestion, abdominal pain	Chhabra *et al.* 1984
	Kenya	tuber	oral	infusion	fever malaria	Gakunju *et al.* 1995
R. revoilii	Tanzania	root	oral	decoction	hernia indigestion, abdominal pains and swelling	Chhabra *et al.* 1993
				juice	prevent abortions	Sawhney *et al.* 1978b
		root bark	oral	decoction	menstrual disorders	Chhabra *et al.* 1984
		root / sap / stems	external	decoctions / fresh	wounds, cuts, sores and burns Fresh sap has anaesthetic properties	Sawhney *et al.* 1978b Chhabra *et al.* 1984 Chhabra *et al.* 1993 Hedberg *et al.* 1983
	East Africa	roots	oral	decoctions	avoid abortion wound healing	Hedberg *et al.* 1983
				raw as food	feed calves of cows who produce insufficient milk	Hedberg *et al.* 1983
	Kenya	tuber	oral	decoction	infertility venereal disease cathartic -used for stomach problems	Johns *et al.* 1990
R. erythrodes	Malawi	tuber	oral		barrenness, both sexes	Msonthi & Magombo 1983

The above table shows that tribal groups throughout Africa use *Rhoicissus* for indications relating to either the reproductive tract or the hypothallamic axis.

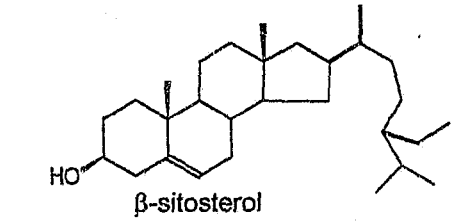
1.6.6. Chemistry of *R. tridentata* and *R. revoilii*

Chhabra *et al.* (1984) found *R. tridentata* leaves to contain anthocyanins, coumarins, essential oils, flavanoids, saponins, sterols and triterpenes. Whereas *R. revoilii* root bark was found to contain anthocyanins, coumarins, flavanoids, quinones, sterols and tannins.

1.6.7. Identification of pharmacologically active components

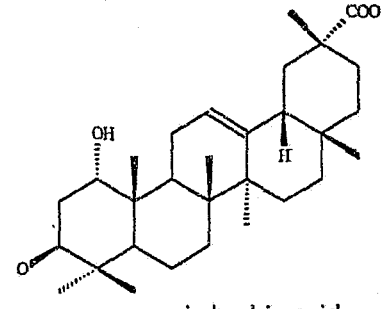
Isolation of the uterotonic components of *R. tridentata* subsp. *cuneifolia* has been done by Bridget Brookes (Chemistry Department, Mangosuthu Technikon, Durban) and myself. B. Brookes fractionated extracts from the plant, and the pharmacological activity of the fractions were tested by myself. Currently, the active fractions are being characterised and the results will form part of her PhD thesis.

Preliminary findings suggest that β-sitosterol (24β-ethylcholest-5-en-3β–ol), β-sitosterol-3-O-β-D-gycoside, imberbic acid (1-α,3-β-dihydroxyolean-12-en-29-oic acid) as well as an unidentified sugar appear to account for most of the contractile activity. The structures are given below (Figure 1.3).



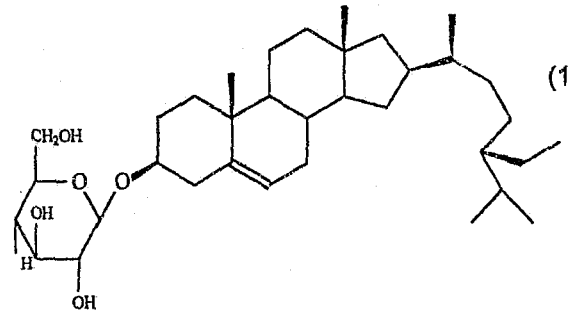
β-sitosterol

(24β-ethylcholest-5-en-3β–ol)



imberbic acid

(1-α,3-β-dihydroxyolean-12-en-29-oic acid)

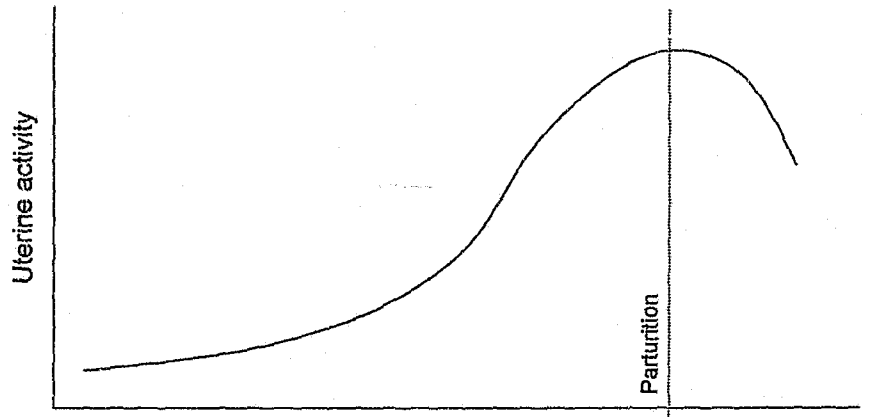


β-sitosterol-3-O-β-D-glycoside

Figure 1.3. Compounds isolated from *Rhoicissus tridentata* subsp. *cuneifolia*.

1.7. Physiology of Labour

Labour is the physiological process by which a foetus is expelled from the uterus to the outside world. Labour is divided into three phases according to uterine activity. Phase 0 is the quiescent stage during which there is minimal uterine activity, mainly mild uncoordinated Braxten-Hicks contractions. Activation of uterine activity occurs during Phase 1 causing a linear increase in uterine activity, followed by an exponential increase in uterine activity during phase 2. The third phase is characterised by a decrease in uterine activity, during which time the placenta is discharged and involution of the uterus takes place. Each stage of labour is subject to different set of chemical controls (Figure 1.4).



Phase 0	Phase 1	Phase 2	Phase 3
Quiescence	*Activation*	*Stimulation*	*Involution*
Progesterone	Oestrogen	Prostaglandins	Oxytocin
Prostacyclin	Prostaglandins	Oxytocin	Thrombin ?
Relaxin	CRH		
Nitric oxide	Progesterone ?		
PHRH			
CRH ?; HPL ?			

Figure 1.4. Regulation of uterine activity during pregnancy and labour. Question marks indicate a possible influence. PHRH = Parathyroid hormone-related hormone; CRH = Corticotropin releasing hormone; HPL = human placental lactogen (Norwitz *et al.* 1999).

1.7.1. Myometrial contraction

Uterine smooth muscle shows a high degree of spontaneous contractility which increases greatly at puberty and varies thereafter with the ovulatory cycle, stage of gestation, degree of stretch and region of the uterus. Waves of depolarization of the myometrial cell membrane, with superimposed spike activity are associated with contraction. Cell-to-cell spread of excitation occurs, but electrical conduction is slow and decremental in nature. Low-resistant contacts (gap junctions) greatly facilitate the spread of excitation (Graves 1995).

The uterine contraction is initiated by either neurotransmitter or hormonal stimulation resulting in the influx of Na^+, depolarisation of the membrane and an action potential. The action potential leads to a rise in intracellular calcium which in turn causes the actin and myosin filaments to interact by means of an ATP-dependent mechanism resulting in myometrial contraction (Steer & Johnson 1998). In smooth muscle, contraction results from the relatively slow process of phosphorylation of light chains of myosin, a reaction that is catalysed by myosin light-chain kinase, a calcium and calmodulin-dependant enzyme (Graves 1995).

Oxytocin and prostaglandins E_2 and $F_{2\alpha}$ are the most important compounds controlling contractions during labour. Both prostaglandins and oxytocin stimulate contractions by activating excitatory membrane bound receptors. The two compounds interact in a complementary and sometimes synergistic manner (Graves 1995).

The uterus is also innervated with both parasympathetic and sympathetic neurones. The former by way of the pelvic nerve and the latter by way of the postganglionic fibres from the inferior mesenteric ganglia. Both can elicit increased activity in the mature human uterus, but denervation causes little change in uterine motor activity. Adrenergic innervation includes both excitatory α-receptors and inhibitory β_2-receptors (Bulbring & Tomita 1987). The relative dominance of these receptors is altered by hormonal status (Steer & Johnson 1998). Muscarinic receptors are also expressed on the surface of myometrial cells which when stimulated cause contraction of myometrial cells (Ehlert *et al.*

1997). Other receptors mediating myometrial or other smooth muscle contractions include oxytocic, prostanoid, serotonergic and histaminergic receptors (Blattner *et al.* 1978; Graves 1995; Steer & Johnson 1998) (Figure 1.5).

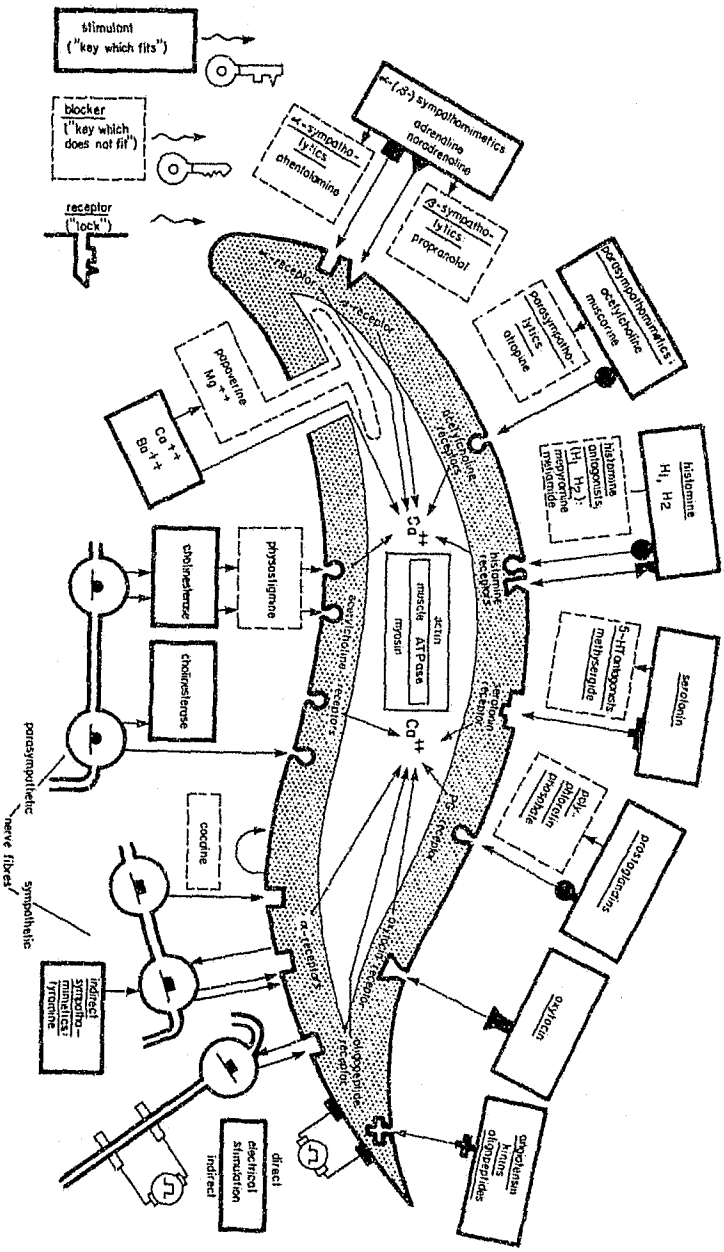


Figure 1.5. Pharmacology of the smooth muscle cell.

1.7.2. Oxytocin

Oxytocin is a neurohypophyseal hormone. Oxytocin is synthesised in the supraoptic and paraventricular nuclei of the hypothalamus, and is stored in nerve endings in the neurohypophysis (Graves 1995). Oxytocin is released from the posterior pituitary in a pulsatile manner. The pulse frequency increases as labour advances (Fuchs *et al.* 1991). Oxytocin release is stimulated by reflex sensory stimuli from the cervix, vagina and breasts, and is inhibited by ovarian relaxin. The secretion of oxytocin is also suppressed by ethanol which is the basis for the use of ethanol as a tocolytic agent in the past (Graves 1995). Other possible sources of oxytocin are the foetus and the placenta itself. However, whether or not oxytocin is released from these sites in amounts sufficient to yield a contractile response is still uncertain (Bossmar 1998).

The sensitivity of the uterus to oxytocin is dependent on both gestational age and the level of spontaneous uterine activity (Caldeyro-Barcia & Peiros 1959, Theobald *et al.* 1969). In early to mid-pregnancy the uterus is insensitive to oxytocin, but from week 30 of gestation onwards the sensitivity to oxytocin increases markedly until week 40 when the uterus displays the maximal levels of sensitivity to the hormone (Seitchik & Castillo 1983). The increased sensitivity of the myometrium and decidua to oxytocin is caused by an increase in the number of oxytocin receptors (Forsling 1979). Prolonged exposure to oxytocin causes a decrease in the myometrial content of oxytocin receptors, and a subsequent decrease in the uterine sensitivity to the peptide (Phaneuf *et al.* 1994). This correlates with the clinical observations that oxytocin is a less potent stimulant in later stages of delivery than at the beginning of labour (Bossmar *et al.* 1994). Oxytocin is present in both maternal and foetal circulation during late gestation (Forsling 1979). However, parturition may occur in the absence of oxytocin, although labour may be prolonged under these circumstances. In addition, mechanical stimulation of the foetal membranes or cervix can induce labour with little change in plasma levels of oxytocin. While maternal oxytocin may not be the sole trigger for the onset of labour, it can be considered to play a vital facilitatory role in parturition (Graves 1995).

1.7.3. Prostaglandins and myometrial contraction

Prostaglandins are autacoids derived from membrane phospholipids (Figure 1.5). They can be considered to be local hormones since, with a few exceptions, they exert their effects and are inactivated in the tissues or organs in which they are synthesised. Prostaglandins are extremely prevalent and have been detected in almost every tissue and body fluid. Their production increases in response to diverse stimuli and they produce a wide array of effects on the different biological systems. With regard to myometrial contractility and parturition, prostaglandins E_2 and $F_{2\alpha}$ are considered to be the important prostaglandins (Graves 1995).

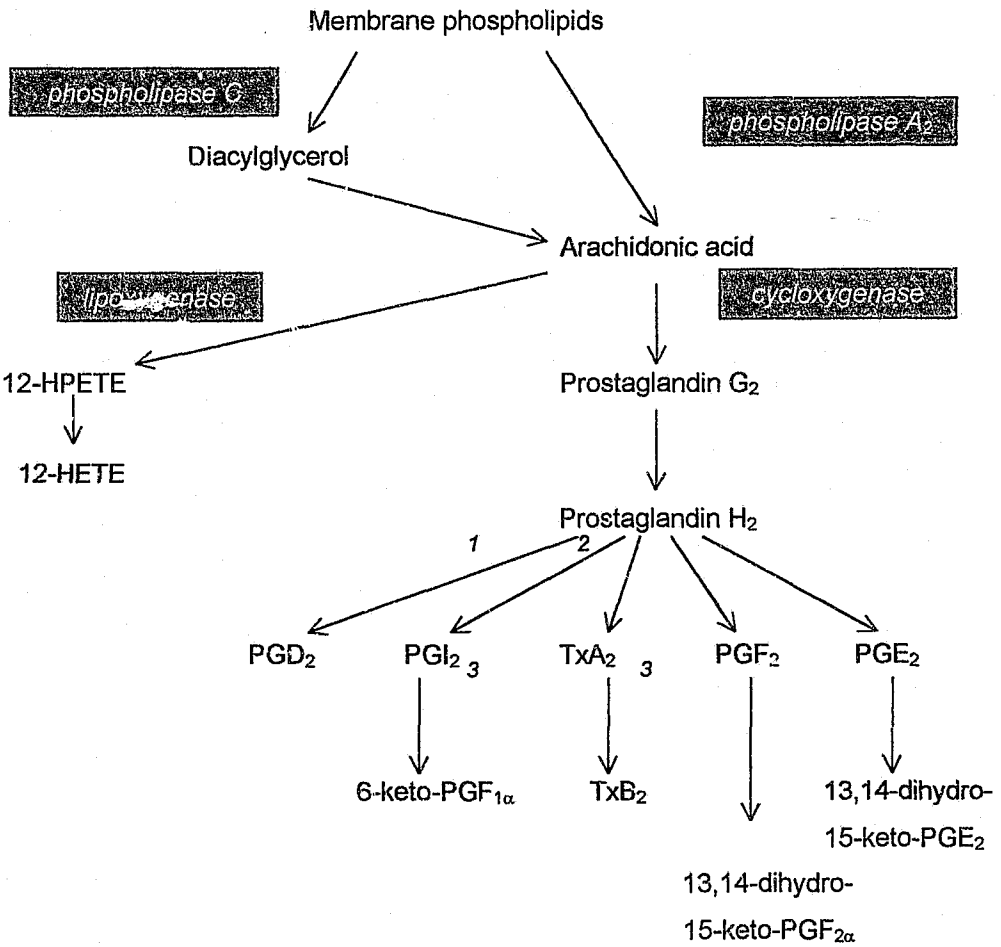


Figure 1.6. Metabolic pathway of phospholipids in the cascade of prostaglandin synthesis (*1* = reductase, *2* = isomerase, *3* = oxidation).

The specific activity of phospholipases that catalyse the rate limiting step in the formation of prostaglandins increases in human amnion late in gestation. The foetal membrane also possesses large amounts of cyclooxygenase and phospholipids that contain arachidonic acid (Figure 1.5). The level of PGE_2 and $PGF_{2\alpha}$ increases in the maternal uterine vein with the onset of labour which has lead to the hypothesis that prostaglandins are involved in the onset of labour (Davidson *et al.* 1987). It is not certain whether the increased levels of prostaglandins during labour is a major determinant in the onset of labour, or whether they only serve to sustain contractions that have been initiated by oxytocin (Cambell & Halushka 1995). The formation of prostaglandins by the amnion may increase progressively during the later stages of pregnancy as a result of the accumulation of substances derived from the foetus, especially platelet activating factor (PAF) (Graves 1995). These increased levels of prostaglandins may be sufficient to stimulate contractions during labour in the absence of any change in the number of prostaglandin receptors expressed (Carsten & Miller 1987). In addition to causing the elaboration of prostaglandins, PAF can initiate uterine contractility directly. Since PAF-hydrolase in the maternal plasma declines during the last trimester, it is possible that the amounts of PAF and prostaglandins that reach the myometrium may be sufficient to initiate labour (Angle & Johnston 1989).

The contractile effects of PGE_2 and $F_{2\alpha}$ are mediated through their ability to increase intracellular calcium. This intracellular increase in calcium is mediated by both cell surface and sarcoplasmic reticulum receptors (Kurachi *et al.* 1989). Table 1.2 shows the diversity of prostaglandin receptors to which the above two prostaglandins bind thus affecting smooth muscle tone. The affinity of the PGE receptor seems to be 10-20 times that of the $PGF_{2\alpha}$ receptor (Carsten & Miller 1987). Prostaglandins together with oestrogens enhance the formation of gap junctions between myometrial cells (Garfield & Hayashi 1981; Mackenzie & Garfield 1985) which appear around the time of onset of labour (Beyer *et al.* 1989). Gap junctions connect the interiors of neighbouring cells providing low resistance connections between cells allowing the rapid

propagation of electrical activity throughout the uterus. This enables the myometrium to contract synchronously (Bleasdale 1990).

Table 1.2 Diversity of prostaglandin (PG) receptors that affect smooth muscle tone (Campbell & Halushka 1995).

PROSTAGLANDIN	PG RECEPTOR SUBTYPE	SMOOTH MUSCLE TONE	SECOND MESSENGER
PGE, $PGF_{2\alpha}$	EP_1	+	$IP_3/DAG/Ca^{2+}$
PGE	EP_2	-	cAMP (↑)
PGE	EP_3	+	cAMP (↓ / ↑)
$PGF_{2\alpha}$	FP	+	$IP_3/DAG/Ca^{2+}$

The expression of the various prostaglandin receptors differs between pregnant on nonpregnant uteri. The expression of relaxant prostaglandin E receptor subtype two (EP(2)) and contractile prostaglandin F receptor (FP) mRNA in the rat uterus is changed during various states of pregnancy and is regulated by steroid hormones, thus, steroid hormones regulate uterine activity during pregnancy and labour. The expression of EP(2) receptors in the rat uterus is three times higher during pregnancy than in the nonpregnant state and decreases during labour at term where it is a third that of the nonpregnant state. The expression of FP receptors in rat uterus increases three fold during pregnancy and reaches maximal levels during labour when the expression is 5 times higher than in the nonpregnant uterus (Dong & Yallampalli 2000).

The local instillation of prostaglandins can induce cervical ripening at doses that do not affect uterine motility. They can also produce softening of the cervix late in the first trimester of pregnancy, by which time the major structural changes in the cervical collagen have occurred. This has been shown by administering intravaginal misoprostol (PGE_1 analogue) which causes ripening of the cervix and induces labour at term (Fletcher *et al.* 1993).

Other metabolites of arachidonic acid are prostacyclin (PGI_2) and thromboxane A_2 (TXA_2). In relation to parturition, PGI_2 is largely confined to

the uterine, umbilical and foetal vasculature, where it may serve to ensure an adequate flow of blood and a patent ductus arteriosus (Grave 1995). PGI_2 may have a direct effect on uterine smooth muscle whereas there is no evidence that TXA_2 is involved in myometrial activity. Thromboxane and prostacyclin have potent platelet aggregatory and antiaggregatory effects respectively. These two autocoids may be of more importance in the control of factors, such as haemostasis after delivery of the placenta, than in stimulating uterine contractility during labour (Vane 1978). Low dose aspirin (50 mg/day) strongly inhibits maternal TXA_2 but not PGI_2 production and thus shifts the balance between PGI_2/TXA_2 to the dominance of the vasodilatory, anti-aggregatory side. This is manifested by improved foetal haemodynamic performance and reduces the need of intensive neonatal care (Viinikka *et al.* 1993).

Women who ingest analgesic doses of aspirin (a cycloxygenase inhibitor) towards the end of pregnancy have pregnancies prolonged by about 7 days compared to controls (Lewis & Schulman 1973). This prolongation of pregnancy also occurs with other prostaglandin synthesis inhibitors, such as indomethacin. However, these drugs should not be used routinely because of the possible adverse effects, notably postpartum haemorrhage and intrauterine closure of the ductus arteriosus (Creasy 1987; Insel 1996).

Foetal membranes are involved in the synthesis and metabolism of prostaglandins in late pregnancy (Schwarz *et al.* 1975a; Schwarz *et al.* 1975b). The enzymes involved in degrading prostaglandins are more active in the chorion than in the amnion (Duchesne *et al.* 1978). The metabolic rates of prostaglandins are not different before or after the onset of labour (Keirse *et al.* 1976).

Foetal membranes are the source of the primary prostaglandins PGE_2 and $PGF_{2\alpha}$ (Keirse *et al.* 1977). The amnion has the greatest capacity to synthesize prostaglandins and it increases markedly towards term (Okazaki *et al.* 1981). The synthesis of cycloxygenase-2 (COX-2) increases markedly with

advancing gestation (Fuchs *et al.* 1999) and the onset of labour (Smieja *et al.* 1993; Slater *et al.* 1994; Dong *et al.* 1996), whereas only low concentrations of cycloxygenase-1 (COX-1) mRNA are present in myometrium at term (Fuchs *et al.* 1999). Endogenous oxytocin is a major factor in the induction of COX-2 expression and $PGF_{2\alpha}$ release at term and during parturition[1] (Fuchs *et al.* 1999). The availability of arachidonic acid in the foetal membranes does not seem to change with the onset of labour (Aitkin *et al.* 1990). It is unclear whether these prostaglandins have access to the myometrium, or whether they are metabolized as they cross the chorion and amnion (Nackla *et al.* 1986). It appears that some cells lack prostaglandin dehydrogenase, and thus allow the transfer of prostaglandins to the myometrium (Challis *et al.* 1991).

1.7.4. Prostaglandins as abortifacients

Therapeutic uses of prostaglandins in relation to gestation and parturition include the use of prostaglandins as abortifacients and cervical ripening agents. Abortions during the first trimester are usually accomplished by means of suction curettage or the use of the contraversial drug, RU 486 (mifepristone, a progesterone antagonist), together with a prostaglandin. The combination causes an abortion in 99% of the cases. Misoprostol (PGE_1 analogue) together with methotrexate (antifolate drug) given in early pregnancy induce abortions in 96% of cases (Hausknecht 1995).

Beyond the first few weeks of the second trimester vaginal suppositories of dinoprostone (PGE_2 analogue) are effective. In other circumstances, such as following membrane rupturing without the elimination of the uterine contents, carboprost (15-methyl $PGF_{2\alpha}$) has been effective (Graves 1995).

1.7.5. Control of the onset of labour

Labour is defined as a increase in myometrial activity, or more precisely, a switch in the pattern of myometrial contractility from irregular contractions (long-lasting, low frequency activity) to regular contractions (high-intensity,

[1] Experiments done on cows, PGE_2 concentrations were not measured.

high frequency activity), resulting in effacement and dilatation of the uterine cervix (Nathanielsz *et al.* 1997). In normal labour, there seems to be a time-dependent relation between the biochemical changes in the connective tissue in the cervix that usually occur before the spontaneous rupture of the foetal membranes (Duff *et al.* 1984). Considerable evidence suggests that the foetus controls the timing of labour and thus its birth, but exactly how is still unknown (Norwitz *et al.* 1999).

Throughout pregnancy contraction of the uterus needs to be inhibited to prevent the detachment of the placenta from the uterine wall. In contrast, at the onset of labour the uterus needs to become sensitive to contraction inducing stimuli, .i.e. the uterus needs to undergo numerous biochemical and physiological changes towards the end of pregnancy.

Labour at term is thought to result from a decreas. in factors which inhibit myometrial activity throughout pregnancy (López Bernal *et al.* 1995). This is demonstrated by removing quiescent myometrial tissue obtained from a uterus at term and placing it in an isotonic solution. The tissue will contract vigorously and spontaneously without added stimuli (López Bernal *et al.* 1995; Garrioch 1978). *In vivo,* however, it is likely that both the removal of inhibitory influences together with an increase in uterine stimulation are important (Norwitz *et al.* 1999).

In sheep, parturition is initiated by a surge of cortisol secretion from the foetal adrenal cortex, which acts on placental enzymes to activate the biosynthetic pathway causing progesterone to be converted to oestrogens. Thus, placental secretion of progesterone falls and oestrogen secretion rises as progesterone is increasingly metabolized to oestrogens. This rapid change in steroid hormone ratios stimulates the release of prostaglandins from both the placenta and the myometrium, and increases the sensitivity of the myometrium to oxytocin which initiates uterine contractions that become

powerful enough to expel the foetus, provided that softening and dilatation of the cervix have occurred (Steer & Johnson 1998).

The situation is much less clear in humans. We are still not certain whether the human foetal adrenal gland plays any role in the initiation of labour. Experiments suggest that increasing cortisol secretion plays a role in initiating labour. The exact mechanisms are unclear as the biosynthetic pathways for oestrogen synthesis in sheep and humans are very different. In sheep oestrogen synthesis is predominantly a placental event whereas in the human not only the placenta but also the foetal and maternal adrenal cortexes are involved (Steer & Johnson 1998).

Oestrogen concentrations in maternal plasma and amniotic fluid increase during the later stages of pregnancy, especially in the final two or three weeks. Progesterone concentrations may decrease simultaneously. A progesterone-binding protein accumulates in foetal membranes that may further decrease the effective concentrations of progesterone in these structures. The progressive dominance by oestrogen is thought to be responsible for the increases in myometrial excitability (owing to increases in slow Na^+ channels and gap junctions), the myometrial sensitivity to oxytocin, and the capacity to elaborate prostaglandins in the foetal membranes. The changing ratios of progesterone and oestrogen have also been thought to be responsible for the ripening of the uterine cervix, which is partly caused by a progressive decrease in the collagen content. These alterations are thought to be crucial in preparation for the softening, dilatation, and effacement that occur during normal labour and delivery (Graves 1995).

1.7.6. Gustavii's hypothesis on the onset of labour

Decidual cells which degenerate in late pregnancy contain lysosomes containing phospholipase A_2, and oestrogens can labilize the membranes of

these lysosomes (Gustavii 1975). It is thought that the rising oestrogen levels coupled with the falling progesterone concentrations at the end of pregnancy act as lysosomal labilizers (Gustavii 1975). The released phospholipase A_2 acts on the membrane phospholipids causing the release of arachidonic acid, which in turn gets converted to primary PGE_2 and $PGF_{2\alpha}$ (Figure 1.6).

This hypothesis also explains the mechanism of midtrimester abortion following intramniotic injection of hypertonic saline which stimulates the release of prostaglandins into the amniotic fluid. Missed abortions are also explained by Gustavii's hypothesis, as the maintained high levels of progesterone and falling oestrogen levels delays the degeneration of decidual cells, and thus suppresses prostaglandin synthesis (Gustavii 1975).

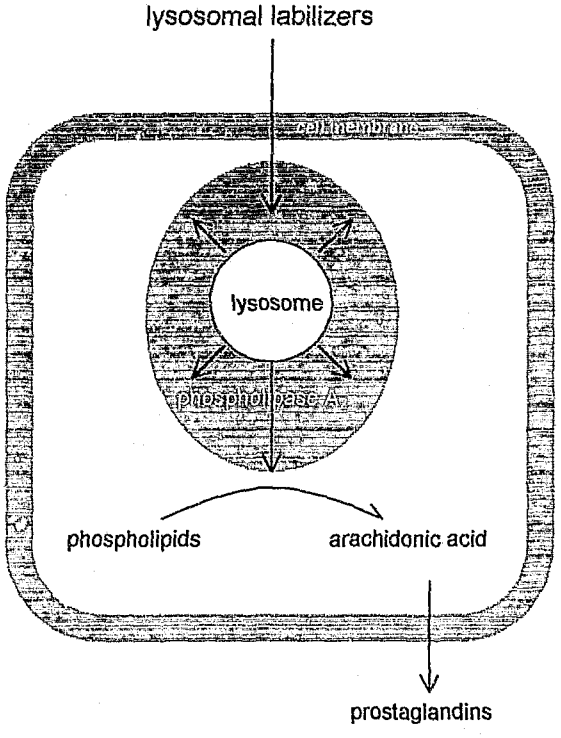


Figure 1.7. A scheme for the involvement of phospholipase containing lysosomes in the synthesis of prostaglandins.

1.7.7. Other physiological effects of prostaglandins

Cyclooxygenase (COX) exists in two isoforms: constitutive COX-1 and inducible COX-2. These enzymes catalyze the same reaction but differ in terms of regulation of expression. The constitutive isoform COX-1 is responsible for the production of PGs involved in prostanoid-mediated physiological functions such as gastric cytoprotection, maintenance of renal homeostasis, and maintenance of normal platelet functions. The second isoform, COX-2, has been demonstrated to be highly expressed in response to inflammatory or mitogenic stimuli. Thus, it is proposed that COX-2 is responsible for the production of PGs associated with inflammatory conditions (Chan *et al.* 1999). Both COX-1 and COX-2 have been found to be expressed in the following human tissues: lung, uterus, testis, brain, pancreas, kidney, liver, thymus, prostate, mammary gland, stomach and small intestine (O Neill & Hutchinson 1993).

a) *Reproduction.* Seminal fluid has very high concentrations of prostaglandins of which substantial amounts are absorbed by the vagina following coitus. It is thought that the prostaglandins may play a role in conception through actions on the cervix, uterine body, fallopian tubes and semen transport. However, their precise role still remains obscure (Campbell & Halushka 1995).
b) *Menstruation.* Menstruation is coupled with disruption of uterine membranes, the release of arachidonate and the stimulation of prostaglandin synthesis. Concentrations of prostaglandins in menstrual fluid are elevated, which are thought to contract uterine and gastrointestinal smooth muscle and sensitise afferent pain fibres and thereby contribute towards the symptoms of primary dysmenorrhoea. This is the reason why cyclooxygenase inhibitors are more effective relievers of these symptoms than narcotic analgesics (Ylikokala & Dawood 1978).
c) *Vascular and pulmonary smooth muscle.* Locally generated PGE_2 and PGI_2 modulate vascular tone. PGI_2 seems to counteract the effects of circulating vasoconstrictor autacoids, to maintain blood flow to vital organs. PGE_2 and PGI_2 play a role in the hypotension related to septic

shock. PGE_2 causes bronchodilation whereas $PGF_{2\alpha}$, TXA_2 and leukotriene C_4 cause bronchocontriction. All these autocoids are released in allergic constriction of the airways (Campbell & Halushka 1995).

d) *Kidney.* Prostaglandins modulate renal blood flow and may serve to regulate urine formation by both renovascular and tubular effects. The elaboration of PGE_2 and PGI_2 is increased by factors that reduce renal blood flow, such as stimulation of sympathetic nerves and angiotensin.

e) *Inflammatory and immune response.* Prostaglandins and leukotrienes are released by a host of mechanical, thermal, chemical, bacterial, and other insults. They contribute importantly to the genesis of the signs and symptoms of inflammation. They serve as potent chemoattractants for polymorphonuclear leukocytes and can promote exudation of plasma by mobilizing this source of additional inflammatory mediators. PGE_2 and PGI_2 do not appear to have a direct effect on vascular permeability, but they enhance oedema formation and leukocyte infiltration by promoting blood flow in the inflamed region. They also potentiate the pain producing activity of bradykinin and other autacoids (Campbell & Halushka 1995).

1.8. Muscarinic Receptors

1.8.1. The autonomic nervous system

The autonomic nervous system regulates the activities of structures that are not under voluntary control and that are functions below the level of consciousness. Thus, respiration, circulation, digestion, body temperature, metabolism, sweating and the secretions of certain endocrine glands are regulated in part or entirely by the autonomic nervous system. The sympathetic and parasympathetic nervous systems have contrasting functions in regulating the internal environment.

The sympathetic system, and its associated adrenal medulla are not essential to life in a controlled environment. It is continuously active but the degree of

activity varies from moment to moment and from organ to organ. The sympathetic system acts as a unit during rage and fright when the sympathetically innervated structures throughout the body are affected simultaneously. Heart rate is accelerated, blood pressure rises, red blood cells are poured into circulation from the spleen, blood flow is shifted from the skin and splanchnic region to the skeletal muscles, blood glucose rises, the bronchioles and pupils dilate, and the whole system is better prepared for "fight and flight". Adrenaline and noradrenaline function as the sympathetic hormone and neurotransmitter, respectively. The adrenergic receptors mediate the transmission of adrenergic sympathetic impulses.

The parasympathetic system is organised mainly for discrete and localised discharge and is concerned primarily with conservation of energy and maintenance of organ function during periods of minimal activity. It slows the heart rate, lowers the blood pressure, stimulates gastrointestinal movements and secretions, aids absorption of nutrients, protects the retina from excessive light, and empties the urinary bladder and rectum. All these effects are mediated by muscarinic receptors which are activated by the endogenous neurotransmitter acetylcholine.

Muscarinic receptors maintain a contractile state of smooth muscle of the ciliary muscle, iris sphincter, bronchi, bladder and gastrointestinal tract, and cause relaxation of the esophageal sphincter, penile blood vessels and other organs (Ehlert *et al.* 1997).

1.8.2. Muscarinic receptors and labour

Even though muscarinic receptors mediate contractions of equal magnitude to oxytocin (see Figure 4.1a), muscarinic receptors are not thought to play a role in the onset or progression of labour and are never included in any review of the physiology or management of labour. The only role they could play in parturition is the enhancement of Ca^{2+}-induced myometrial contraction, producing additional contractile force with the available amount of intracellular calcium (Izumi *et al.* 1996) which may enhance the expelling forces of oxytocin and prostaglandins.

Muscarinic receptors may play a role in mediating intrauterine emptying of the foetal rectum, if exogenous muscarinic agonists are administered to the mother and they pass into either foetal circulation or sufficiently high levels in the amniotic fluid to enable stimulation of intestinal muscarinic receptors. This would increase the possibility of intrauterine meconium staining of the liquor. No experimental or clinical evidence could be found in the literature proving this.

1.8.3. Subtypes of muscarinic receptors

Traditionally muscarinic receptors were classified primarily on the basis of the effect and relative potencies of selective agonists and antagonists. The effects of acetylcholine that are mimicked by the plant alkaloid muscarine, and selectively antagonised by atropine were termed muscarinic effects and were found to be mediated by muscarinic receptors. Other effects of acetylcholine are mimicked by nicotine, but are not antagonised by atropine but rather by tubocurarine. These were termed nicotinic effects and were found to be mediated by nicotinic receptors (Ross 1995).

As the diversity and selectivity of drugs increased, it became apparent that multiple subtypes of receptors exist within this system. Molecular cloning as a tool has revealed further differences between closely related subtypes of receptors. The use of pharmacological methods to distinguish one subtype of muscarinic receptor from another has identified four subtypes (M_1-M_4), whereas five subtypes can be differentiated using molecular cloning (m_1-m_5) (Ehlert *et al.* 1997).

More than one subtype of muscarinic receptor is expressed in the smooth muscle of various tissues. Typical locations of receptor subtypes and their responses are given in table 1.3. Competitive binding studies demonstrated the abundance of M_2 receptors in most smooth muscle. In most smooth muscle, including guinea pig uterus and longitudinal rat and guinea pig ileum, M_2 receptors account for 80% of the total receptor density (Ehlert *et al.* 1997). Hybridization techniques on whole guinea pig ileum detected M_2 mRNA, a

small amount of M_3 mRNA and a trace amount of M_1 mRNA (Maeda *et al.* 1988).

Despite M_2 receptors being the dominant population, contractions have been shown to be mediated predominantly by the M_3 receptor population. M_3 muscarinic receptors induce a contractile response through stimulation of phosphoinositide hydrolysis. In guinea pig ileum, muscarinic receptors inhibit voltage dependent calcium currents. Muscarinic m_2 and m_3 receptor subtypes were found to be expressed on intestinal smooth muscle. The m_3 receptors induce smooth muscle contraction and secretion from exocrine glands (Bernstein & Haga 1990). The antagonism of M_2 receptors causes an increase in heart rate whereas antagonising M_3 had no effect on heart rate (Lin *et al.* 1997).

Table 1.3 Typical locations of the various cholinergic receptor subtypes and the second messengers mediating the cellular response to each subtype.

Receptor name	Typical locations	Result of ligand binding
Muscarinic M_1	CNS neurons Sympathetic postganglionic neurons Some presynaptic sites	Formation of IP_3 and DAG Increased intracellular Ca^{2+}
Muscarinic M_2	Myocardium Smooth muscle Some presynaptic sites	Opening K^+ channels Inhibition of adenylyl cyclase
Muscarinic M_3	Exocrine glands Smooth muscle and endothelium	Formation of IP_3 and DAG Increased intracellular Ca^{2+}
Muscarinic M_4		Opening K^+ channels Inhibition of adenylyl cyclase
Muscarinic M_5		
Nicotinic N_N	Postganglionic neurones Some presynaptic cholinergic terminals	Opening of Na^+, K^+ channels, depolarization

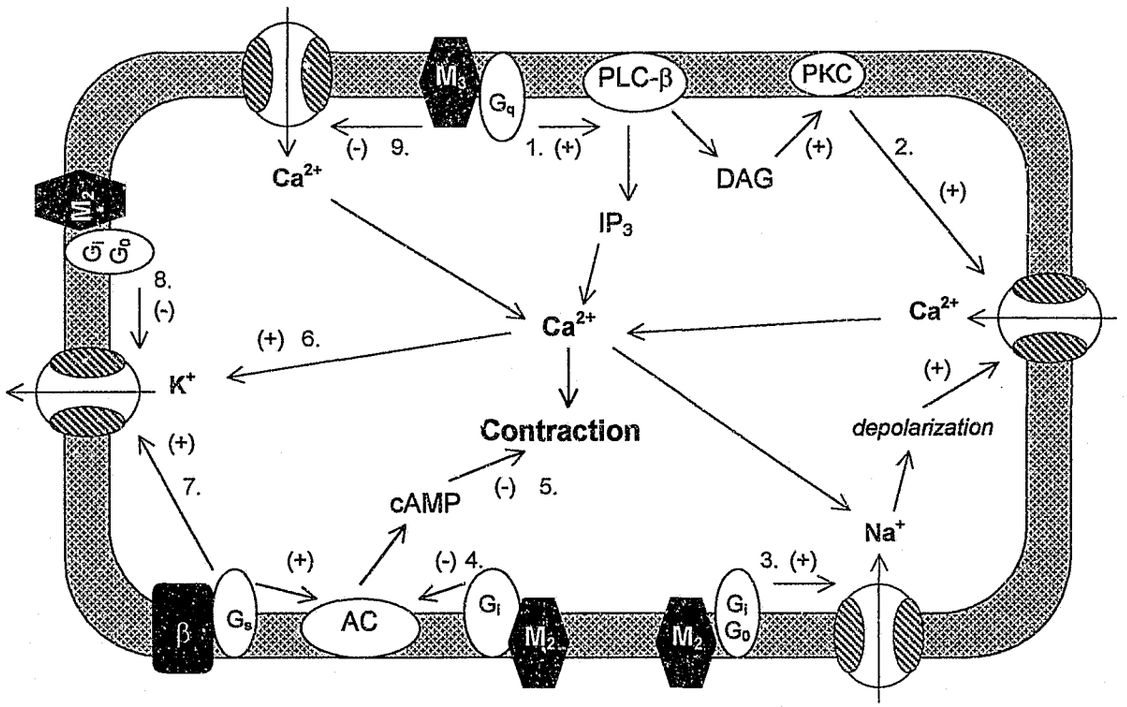


Figure 1.8 Contractile mechanisms for muscarinic receptor subtypes in smooth muscle. All the mechanisms may not exist simultaneously in the same smooth muscle cell, but rather to a varying extent in different cells depending upon the expression of the various signaling proteins (Ehlert *et al.* 1997). The second messengers for M_1 and M_4 subtypes are similar to those of M_3 and M_2 respectively.
Abbreviations: PLC-β = phospholipase C-β; IP_3 = inositol-1,4,5-*tris*phosphate; DAG = diacylglycerol; PKC = protein kinase C; AC = adenylyl cyclase; β = a subtype of the β-adrenergic receptor family; cAMP = cyclic AMP.

1. M_3 receptors stimulate phosphoinositide hydrolysis.
2. In the colon and stomach, muscarinic receptors potentiate the voltage dependent calcium current through activation of PKC.
3. Muscarinic receptors stimulate a non-selective cation conductance (mainly sodium) that triggers depolarization and an influx of calcium through voltage dependent calcium channels. This conductance is likely to be mediated by the M_2 receptor and is dependent on calcium to varying degrees in different types of smooth muscle.
4. M_2 muscarinic receptors inhibit adenylyl cyclase activity and inhibit the increase in cAMP elicited by forskolin, the β-adrenergic receptor and other receptors that signal through G_s.
5. β-adrenergic receptors, forskolin and other agents cause relaxation through cAMP, and the M_2 receptor can oppose this relaxation by inhibiting the increase in cAMP.
6. Many types of smooth muscle cells contain calcium-activated potassium channels that act to restore membrane potential following depolarization and an influx of calcium.
7. β-adrenergic receptors may have an alternatate, non-cAMP-dependent mechanism for causing relaxation in the trachea.
8. Muscarinic receptors suppress the calcium-activated potassium conductance. This conductance is pertussis toxin-sensitive; consequently, we suggest that it is mediated through the M_2 receptor by signaling through a G protein of the G_i and G_o family.
9. In the guinea pig ileum, muscarinic receptors inhibit the voltage dependent calcium current.

1.9. Aims

1. To document the ingredients and the various methods of preparations of pregnancy-related remedies in Gauteng.

South African traditional healers prepare almost all their remedies using tap water. Pregnancy-related remedies containing Rhoicissus are usually boiled in water. The preparation of extracts used in this study will all be prepared mimicking the preparation techniques of the traditional healers as the aim of the study is to determine the pharmacological activity of the extracts used by local pregnant women.

2. Pharmacology of crude aqueous decoctions of Rhoicissus tridentata subsp. cuneifolia.

   a. To determine the mechanism of contractile activity of R. tridentata subsp. cuneifolia decoctions on isolated rat uterus and ileum.

   b. To determine whether the plant extracts are cytotoxic on mammary cells, hepatocytes and fibroblasts.

   c. To investigate the effect of the R. tridentata subsp. cuneifolia decoctions on prostaglandin synthesis in cultured cells.

   d. To determine whether the contractile response to R. tridentata subsp. cuneifolia decoctions exhibits seasonal variation.

   e. To determine whether the contractile response to R. tridentata subsp. cuneifolia decoctions exhibits distributional variation.

   f. To determine whether the uterotonic activity of decoctions of different plant parts of R. tridentata subsp. cuneifolia varies.

   g. To determine whether the activity of the extracts is altered by storing dried R. tridentata subsp. cuneifolia material for a year as is common practice amongst traditional healers.

Rationale for this research

- The pharmacological assays of *R. tridentata* subsp. *cuneifolia* will provide scientific evidence to determine whether the use of *R. tridentata* subsp. *cuneifolia* has the potential to induce or augment labour, and whether the plant has the potential to cause birth complications caused by uterine hypertonia.

- Once the receptor system involved in mediating the contractile response is identified potential adverse effects of *R. tridentata* subsp. *cuneifolia* remedies may be postulated.

- The results on possible sources of variation in the effect of the *R. tridentata* subsp. *cuneifolia* extracts will indicate whether the plant remedies need to be standardised and will give an indication of when the plant is the most potent.

Information gathered will be published in refereed scientific journals and simplified versions will be published in the "Indigenous Plant Use Forum newsletter" which is circulated to traditional healers and other interested parties throughout the country.

CHAPTER 2: METHODS

2.1. Interviews

Three traditional healer associations in Johannesburg were consulted. After much persistence and patience, names of individual healers were obtained from the presidents of the associations. Healers were interviewed either at their own homes or consulting rooms, or they were brought to the Medical School premises where they were interviewed.

Traditional healers were interviewed using open ended questions to determine where they were trained, how long they were practising, and what plants they prescribe for what indications during pregnancy. In order to make our conversations a two-way flow of information, any questions the healers had about basic biology were answered and time was taken to inform them about findings from this study.

Traditional healers used vernacular names (usually Zulu) of the plants. The corresponding botanical names were determined from the following texts: Gerstner (1941), Watt & Breyer-Brandwyk (1962), Hutchings (1996), Van Wyk (1997).

2.2. Harvesting regimens for *Rhoicissus tridentata* used for all studies

2.2.1. *Material for investigations into the role of different receptor systems in mediating the contractile response to R. tridentata decoctions.*

R. tridentata subsp. *cuneifolia* roots were collected from Treasure Beach Grasslands, Umlazi, Kwazulu-Natal in July and October 1995. The roots were harvested by Bridget Brookes (a colleague from Mangosuthu Technikon who worked on the chemistry of *Rhoicissus*) and identified by Geoff Nichols (Horticulturist from Durban Municipality). (Voucher specimen number - 086931, C.E. Moss Herbarium). Details on the location are given in Table 2.1.

2.2.2. *Material for receptor subtype investigations.*

Lignotubers harvested in Autumn (April 1997) from Suikerbosrand Nature Reserve, 60 km south of Johannesburg, were used for the investigation into the involvement of the various receptor subtypes in the mediation of the contractile response to aqueous extracts of *R. tridentata* subsp. *cuneifolia.*

2.2.3. *Material to investigate seasonal variation in contractile activity.*

R. tridentata subsp. *cuneifolia* plant material was collected from Suikerbos Nature Reserve. The plants were collected within a thicket on a North East facing slope of the grassland. Three plants approximately 10m apart were harvested every three months from May 1996 to April 1998. Plants which were sufficiently far apart to be different individuals were chosen to allow a replicate of plants. The identity of the plants was validated by L. Katsoulis and voucher specimens were lodged in the C.E. Moss Herbarium. Voucher specimen numbers 086928, 082929 and 082930.

2.2.4. *Material for investigations into the contractile activity of extracts from different plant parts.*

The same plants harvested for the study of seasonal variation, from Suikerbos Nature Reserve, were used to determine whether there was any variation in response to different parts of the plant.

2.2.5. *Material to investigate the distributional variation in contractile activity.*

Lignotubers are interspersed along the roots which usually grow within crevices between rocks making them very difficult to locate. Stems, as opposed to roots or leaves, were shown to have the uterotonic activity most similar to that of the lignotubers. Therefore, stems were chosen as the plant part to be investigated for distributional variation in uterotonic activity.

Plant material was always collected during summer. The plant material was harvested from around the south-eastern part of South Africa, from a variety of different habitats (Table 2.1).

Table 2.1 Collection details of plants harvested to determine the effect of distribution on the contractile activity of aqueous extracts.

LOCATION	PROVINCE	VEGETATION TYPE *	SEASON	PART	Altitude (m)+	MAR# (mm)+
Suikerbosrand Nature Reserve	Gauteng	Mixed grassveld	Autumn	stems	1541	698
Mondeor	Gauteng	Sour grassveld	Autumn	stems	1541	698
Magaliesburg Mountains	Gauteng	Bushveld gorge	Summer	stems	1157	659
Umlazi	Kwazulu-Natal	Coast-belt scrub forest	Summer	roots	8	1022
Durban	Kwazulu-Natal	Coast-belt scrub forest	Summer	roots	8	1022
Morgenzon Bos	Mpumalanga	Climax forest in sourveld gorge	Autumn	stems	1525	905.8

* Vegetation classification according to Acocks 1988

+ Supplied by the South African Weather Bureau

MAR = mean annual rainfall

2.2.6. *Harvesting of material to investigate the effect of storage on the contractile activity of the decoctions.*

Stems and lignotubers of *R. tridentata* subsp. *cuneifolia* collected from Suikerbos Nature Reserve were used to test whether storing the plant material altered the response to aqueous extracts of the plant.

2.3. *R. tridentata* subsp. *cuneifolia* decoction preparation

All plant material harvested was prepared in the same manner. Different plant parts were separated, washed and dried overnight at approximately 60°C. Woody material (roots, branches and lignotubers) was cut into small pieces approximately 1cm^3 using an electrical band saw. The material was milled in a rotomill and sieved through a 1mm mesh. The milled sample was then boiled in round bottom flasks fitted with a condenser. The sample was boiled at a

ratio of about 100g plant material per litre distilled water for an hour mimicking the traditional healer's preparation methods. The solids were allowed to settle in measuring flasks overnight at 4°C. The supernatant was siphoned off, frozen and lyophilised. Filters were not used as they blocked within a couple of minutes, whereas the particulate matter precipitated out sufficiently if left to stand overnight. All lyophilised products were stored at -20°C until use. The mean percentage yield of lyophilate as a percentage of dry weight was 13.3% ± 7.5%.

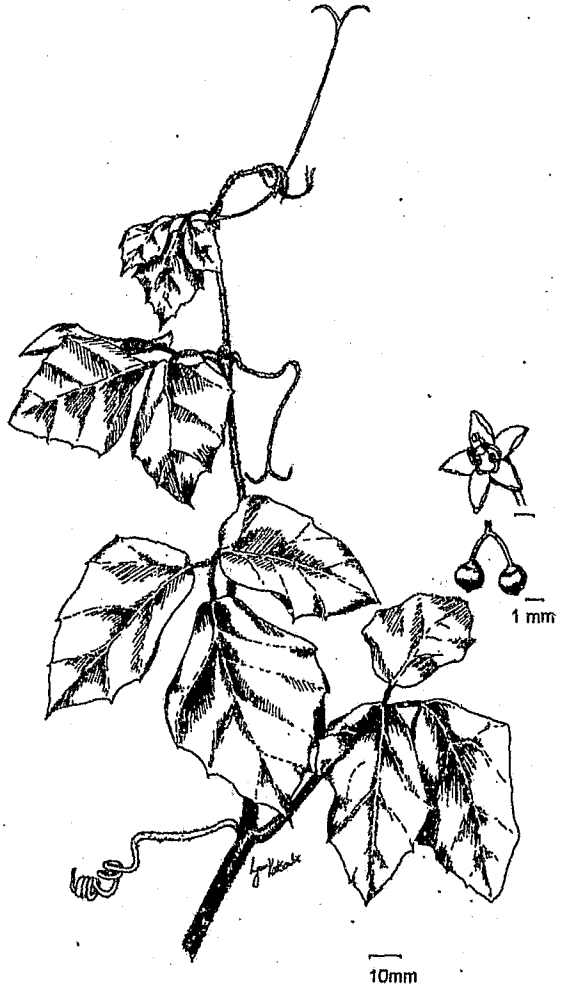


Figure 2.1. Aerial parts of *Rhoicissus tridentata* subsp. *cuneifolia* harvested from Suikerbosrand.



Figure 2.2 *Rhoicissus tridentata* subsp *cuneifolia* growing at Suikerbosrand Nature Reserve. a) overall growth habit b) leaf stucture c) & d) underground parts showing a small lignotuber.

2.4. Animals and isolated organ preparations

Virgin Sprague-Dawley rats weighing about 250g were oestrogenized by injection with stilboestrol (10µg/100g) (Maybaker (SA)) i.p. 24 hours before being euthanased with CO_2. Portions of uterus, approximately 1.5cm long, were dissected from the central portion of each uterine horn. The uterine portions were trimmed of fat and connective tissue, opened longitudinally then mounted in 50 ml organ baths containing Tyrode's solution (136 mM NaCl, 2.68 mM KCl, 1.80 mM $CaCl_2$, 1.05mM $MgCl_2$, 11.9mM $NaHCO_3$, 0.42 mM NaH_2PO_4, 5.55mM glucose). Tyrode's buffers the pH at approximately pH 7. The baths were aerated with 5% CO_2 in O_2 and the temperature maintained at 26°C to decrease spontaneous contractility (Kumagai *et al.* 1952).

Two 1.5cm sections of ileum, approximately 4cm from the ileocaecal junction, were dissected out of the same rat the uterus was obtained from. The tissues were trimmed of connective tissue, then mounted in the organ baths as for the uterus. The bath temperature was maintained at 37°C.

Both the organs were allowed to equilibrated suspended in the organ baths filled with Tyrode solution for at least 30 min, during which time the baths were rinsed frequently. Isotonic contractions of the organs were measured against 1g resistance and recorded electronically using potentiometer recorders. The organs were challenged with cumulative doses of reference agonists or herbal extract, or pretreated 5 min before adding the reference drug with either an antagonist, the herbal extract, or both, according to Van Rossum (1963). Standard curves using the relevant reference agonist alone were run at the start of each experiment and between each test challenge. Repeat experiments were carried out using tissues from different rats.

The response to each concentration of drug plotted by the potentiometer recorder was measured using a ruler, then expressed as a percentage of the maximal response to the reference drug control curve run prior to the test curve. The graphs were drawn using GraphPad Prism®, and the curves converged using the variable slope sigmoidal dose-response or Boltzmann

sigmoidal equations. Student-t tests were used to analyse all data for significant differences using Systat®.

2.5. Agonists and antagonists

Table 2.2 Reference drugs used to elucidate the pharmacological action of the *R. tridentata* extract on isolated rat uterus and ileum.

Chemical used	Pharmacological action	Source
acetylcholine chloride	muscarinic receptor agonist	Sigma
atropine sulphate	muscarinic receptor antagonist	BDH
pirenzepine 2HCl	M_1-receptor antagonist	RBI
gallamine triethiodide	M_2-receptor antagonist	RBI
4-DAMP mustard HCl	M_3-receptor antagonist	RBI
tropicamide	M_4-receptor antagonist	RBI
oxytocin (Syntocinon)	oxytocin receptor agonist	Sandoz
noradrenaline bitartrate	adrenoceptor agonist	Sigma
propanolol	β_2-adrenoceptor antagonist	Sigma
prazosin	α_1-adrenoceptor antagonist	RBI
yohimbine	α_2-adrenoceptor antagonist	RBI
serotonin creatinine sulphate	5-HT receptor agonist	Sigma
methysergide maleate	5-HT receptor antagonist	RBI
ketanserin	5-HT_1 receptor antagonist	RBI
tropisetron	5-HT_2 receptor antagonist	RBI
indomethacin	cyclooxygenase inhibitor	Sigma
NS-398	COX-2 inhibitor	RBI
pyrilamine maleate	H_1 histamine receptor antagonist	RBI
hexamethonium dichloride	ganglion blocker	RBI
mecamylamine HCl	nicotinic antagonist	RBI
cholera toxin	G_α-protein inhibitor	RBI
pertussis toxin	islet-activating protein	RBI

2.6. Analysis of Inorganic Ion Content

The concentration of potassium, sodium and calcium ions of the plant extracts was determined using ion selective electrodes in a Beckman CX3 automatic analyzer.

2.7. Prostaglandin Synthesis in Cultured Cells

Choice of cell type: The results from the organ bath studies indicated that products of cyclooxygenase, such as prostaglandins, are involved in the mediation of the smooth muscle contractile response to the *R. tridentata* extracts. Cellular studies were done to confirm that the plant extract stimulates the synthesis of prostaglandin E_2 which is one of the two prostaglandins that cause myometrial contractions.

This investigation could have been done by either exposing the plant extract to cyclooxygenase itself, to test whether the plant has any direct action on the enzyme, or by using cells that produce prostaglandins. Cellular studies were chosen to determine whether the plant extract was able to stimulate cellular production of prostaglandins, either by penetrating the cells or activating an extracellular receptor.

Ideally uterine cells should have been used for these studies. Considering this study of cellular production of prostaglandin E_2 was a small adjunct to the project, together with the high cost of quantifying prostaglandin levels, a human lung histiocytoma cell line previously shown to produce prostaglandins was purchased, rather than using a uterine cell line that at that stage had not been shown to produce prostaglandins.

Molecular studies have shown that COX-2 mRNA expression is higher than COX-1 mRNA expression during parturition (Dong *et al.* 1996; Fuchs *et al.* 1999). Similarly COX-2 mRNA has been found to be overexpressed in human non-small cell lung cancers (Ochiai *et al.* 1999). Therefore, human lung histiocytoma cells (giant cell tumour cells), shown to produce prostaglandin E_2 (ATCC catalogue number TIB-223), were used to determine whether the

Rhoicissus extract increases the production of PGE_2. As only radioimmunoassays were available to detect $PGF_{2\alpha}$ levels, and as I was pregnant at the time, no $PGF_{2\alpha}$ levels were measured.

Plant extracts from material harvested from both Suikerbosrand and Durban displaying low, medium or high levels of contractile activity were used in both experiments outlined below.

Cells were plated out into 96 well microtitre plates at a concentration of 13 500 cells per well, where they were exposed to the plant extract. The plates were incubated at 36°C and aerated with carbogen. The concentrations of prostaglandins in each well were determined by transferring 50µl cell media to plates coated with goat anti-mouse IgG against PGE_2 provided in the "Biotrak prostaglandin E_2 enzymeimmunoassay system" (Code: RPN 222). Instructions provided were followed to determine the levels of PGE_2 in each well (Figure 2.3). Standard curves were used to extrapolate PGE_2 levels. The layouts of the microtitre plates are given in appendix A4.

The first experiment done was to determine the time frame in which prostaglandins are detectable. Cells were exposed to 1mg/ml plant extract for 5, 10, 15, 30, 60, 120 and 180 minutes.

Once the optimal time span for the experiments was determined dose response curves were constructed after cells in 96-well microtitre plates were exposed to 6 concentrations of *Rhoicissus* extract (0.1µg/ml - 1mg/ml).

An outline of the method used to detect PGE_2 levels: 100µL assay buffer was pipetted into the non-specific binding (NSB) wells, and 50µL assay buffer was pipetted into the zero standard wells. 50µL of each standard (2.5, 5, 10, 20, 40, 80, 160 and 320pg/50µl) and unknown sample was added to the relevant wells. Diluted antibodies (50µl) were added to all wells except the blank (B) and NSB wells. Diluted conjugate (50µl) was added to all wells except the blank. The plate was then covered and incubated at room temperature for 1

hour on a microtitre plate shaker. All the wells were then washed four times with 300µL washing buffer. Residual wash buffer was removed by blotting the plate on tissue paper. Enzyme substrate (150µL) was added to all wells. The plate was covered and shaken on a microtitre plate shaker for 30 minutes at room temperature. The absorbance of the blue colour that developed was read at 620nm. A confirmation reading was taken after the reaction was halted by adding 100µL of 1M sulphuric acid to each well, which converts the colour to yellow with proportionally similar optical densities. The plates were shaken for 30 seconds and the absorbance was reread at 450nm within 10 minutes of adding the sulphuric acid.

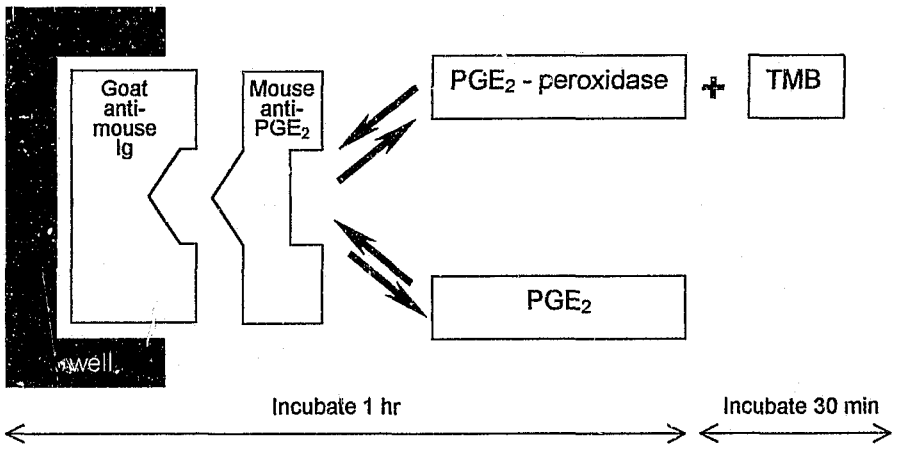


Figure 2.3. The principle of the prostaglandin E_2 enzyme immunoassay. The wells are precoated with goat anti-mouse immunoglobulin. The wells are then treated with PGE_2 antibodies which bind to the immunoglobulins. Once the unknown samples are added to the wells either PGE_2 or PGE_2-peroxidase binds to the PGE_2-antibodies. The wells are then rinsed thoroughly and the 3,3',5,5'tetramethylbenzidine (TMB) is added which is converted into a blue product by the peroxidase, and can be read on the multiwell scanning spectrophotometer. That is, the intensity of the colour is inversely proportional to the amount of PGE_2 present in the well.

2.8. Cytotoxicity

The toxicity of the *Rhoicissus* extracts should have been tested doing LD50 tests using either rats or mice. Unfortunately the application to do this was turned down by the Animal Ethics Screening Committee of the University. A second resort was to test the effect of the plant extracts on either mammalian cells or on crustaceans, such as brine shrimps. These latter methods could not be used to show the respiratory depressing effect the *Rhoicissus* has been reported to have (Brandt & Muller 1995).

2.8.1. Cytotoxicity using the MTT assay method.

The effect of the plant extracts on the viability of four mammalian cell lines was determined using MTT (tetrazolium) assays according to Mosmann (1983). The assay is based on the principle that colourless tetrazolium rings are cleaved by various dehydrogenase enzymes in active mitochondria yeilding coloured lipophilic formazan that can be measured using a scanning multiwell spectrophotometer. Since only active mitochondria are able to cleave the tetrazolium salt the reaction only occurs in living, metabolically active cells and the amount of formazan generated is directly proportional to the active cell number (Mosmann 1983). Mosmann developed the technique to avoid any washing steps to cut down on running times. He added isopropanol to the culture media in the final step of the experiment to dissolve the formazan crystals. In preliminary assays this was problematic as rigorous mixing was required and the results were highly variable. Therefore, the crystals were dissolved with DMSO according to López de Cerrain *et al* (1996), where all the media was removed before adding the organic solvent (DMSO) to dissolve the crystals. This was more time consuming but the formazan crystals dissolved more consistently. Accordingly, the absorbance was read at 540nm only. The absorbance at 620nm was not subtracted from the 540nm reading as unlike Mosmann's method, the growth media had been removed from the wells, and did not have to be accounted for.

The cytotoxicity of *Rhoicissus* extracts was determined using four cell lines: a) Graham's cells (human kidney epithelial cells), b) human hepatocytoma cells,

c) human histiocytoma cells and d) mouse leydig cells. Growth media used for the different cell lines are given in Appendix A.2. Single cell suspensions were obtained by trypsinization of the confluent cells. The trypsin was inactivated by adding growth media containing 5% Fetal Calf Serum. The viable cells were quantified using a haemocytometer after staining a sample of the cell suspension with trypan blue in saline. Viable cells exclude trypan blue by means of an energy dependent mechanism, so unstained cells were presumed to be viable. Cells were only used if the percentage viability was above 90%. The cell concentration was corrected to 0.15 million cells/ml, before plating 90µL into each well of 96-well microtitre plates yielding 13 500 cells per well. The cells were then incubated at 37°C and aerated with carbogen for 24 hours, allowing the cells to stabilize before adding the plant extracts.

Ultracentrifuged plant extract (10µL) which had been filtered through a 2µm microfilter was added to six test wells for each concentration of plant extract. Cells were exposed to extract concentrations ranging from 0.1µg/ml to 10mg/ml. Equal volumes of the relevant culture media were added to six untreated wells. The cells were then incubated for a further 24 hours. 10µL 5mg/ml MTT (3-(4,5,-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma) in PBS (Dulbecco phosphate buffer) was added to each well and incubated as above, for a further 4 hours. The growth media was then removed using a multichannel pippette and replaced with 100 µL pure DMSO to solubilize the MTT-formazan product (López de Cerain *et al.* 1996). The plates were left for an hour to allow the MTT to solubilize. The plates were then shaken by the microplate reader (Labsystems, EMS Reader MF) and the absorbance was read at 540nm. Each experiment was done duplicate.

The viability of the cells exposed to each dilution of plant extract was calculated relative to the survival of untreated cells. The absorbance of treated wells was divided by the absorbance of the untreated well in the same column and expressed as a percentage viability.

CHAPTER 3: Results Section 1

3. Results from Open Ended Interviews

No firm conclusions can be made from results presented in this section as only six traditional healers were extensively interviewed, and a further eight healers were casually questioned about certain aspects relating to plant remedies.

Making contact with traditional healers was extremely difficult and time consuming. Only a small percentage of arrangements were honoured by the traditional healers. Appointments were usually made at venues deep in crime ridden areas. On numerous occasions the journey to the particular venue was made only to find that the traditional healer was not there. The frustration in trying to meet with traditional healers and the risk on my life caused me to stop trying to increase the sample size.

Traditional healers were contacted through the presidents of three traditional healer associations in Johannesburg or Soweto (Traditional Healers' Association; African Skilled Herbalists Association; Traditional Healers Organisation). Using this method of contacting healers allows one to speak to a healer having the confidence of the healer from the beginning of an interview. Healers contacted in this manner were willing to discuss any information relevant to the study. None of the healers appeared to be protecting age-old secrets as is the common perception people have of healers. Each interviewing session was treated as a two way learning process. More than half our time together was always spent teaching them about basic medical principles. All healers spoken to were very aware of their inadequate training and lack of knowledge, and each healer had questions about some medical issue. Indications from all the healers were that they were willing to learn and wanted more rigorous methods of training.

3.1. Training of traditional healers

While interviewing traditional healers, ten were asked about their levels of education and about the training procedure they went through to become

traditional healers. None of the healers went through years of rigorous training and apprenticeship as is the general perception of how traditional healers are trained. Out of ten traditional healers, 5 had not completed primary education (grade 7), three had completed matric (grade 12) of which two had worked as clerks for a number of years, and one worked as a journalist for over 15 years. The remaining two traditional healers had tertiary education qualifying as midwives who had worked in hospitals for a number of years. The two healers who previously worked as clerks in banks left their professions to become traditional healers after the change of government. Both said that perceptions on traditional medicines had changed since the change of government in 1994, making traditional healers far more acceptable. This change caused them to follow previous callings to become traditional healers.

The duration of training varied from 6 months to 3 years. Each traditional healer spoke about ancestral spirits as being their primary teachers on the medical use of plants. Their living tutors" served more as mentors and taught them how to divine the spirits and how to interpret dreams. Three of the healers were trained in Soweto, whereas the remaining healers went to a rural setting to undergo initiation and to receive part of their training.

3.2. Preparation of remedies containing *Rhoicissus tridentata*

Fourteen traditional healers were asked how they prepare *Rhoicissus* containing remedies for any indication (not specific to pregnancy). All ground dried tubers or roots of *Rhoicissus* by hand and then prepared the decoction by boiling the sample in water for between thirty minutes and an hour. There was a difference between the manner in which they dispensed the remedies. Four of the traditional healers prepared the decoctions mixing three to four different ingredients chosen according to the specific complaints of the patient. These remedies were sold as bottled decoctions (Figure 3.3). The rest of the healers sold mixtures of dried ingredients (usually wrapped in newspaper) and gave instructions on how the remedy should be prepared. The dosages they prescribed differed. Most commonly measurements were in handfuls. None of the healers used standard measuring items such as cups, bottle tops, or scales. When the suggestion was made that they should use a scale, most healers were accepting of the concept, but none of them started

Some healers instructed patients to drink a set dose per day, where others told the patients to drink the decoction as required. No concerns were voiced about possible contamination of the decoctions or about fermentation or degradation of the "muties" over time.

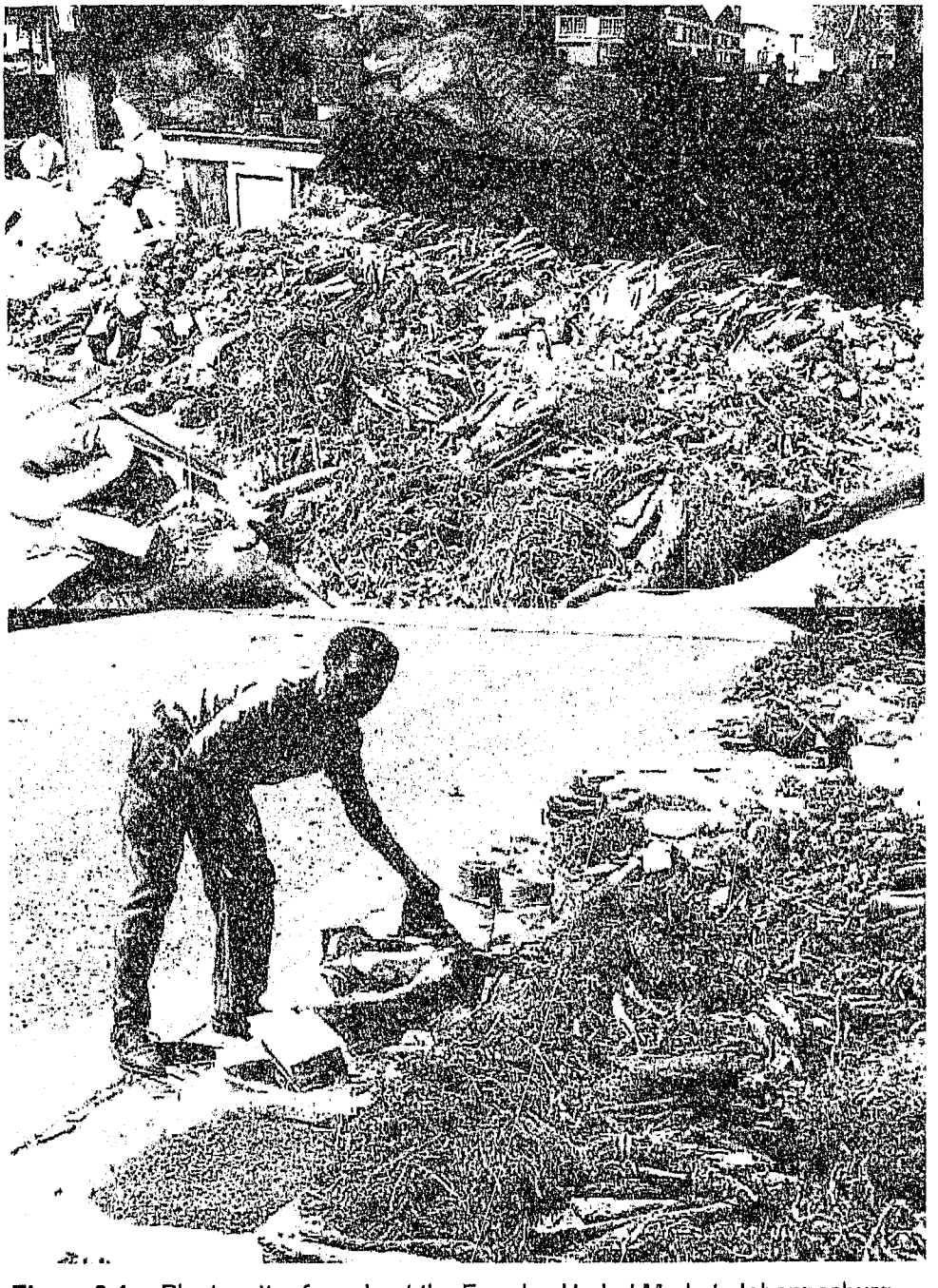


Figure 3.1 Plant matter for sale at the Faraday Herbal Market, Johannesburg.

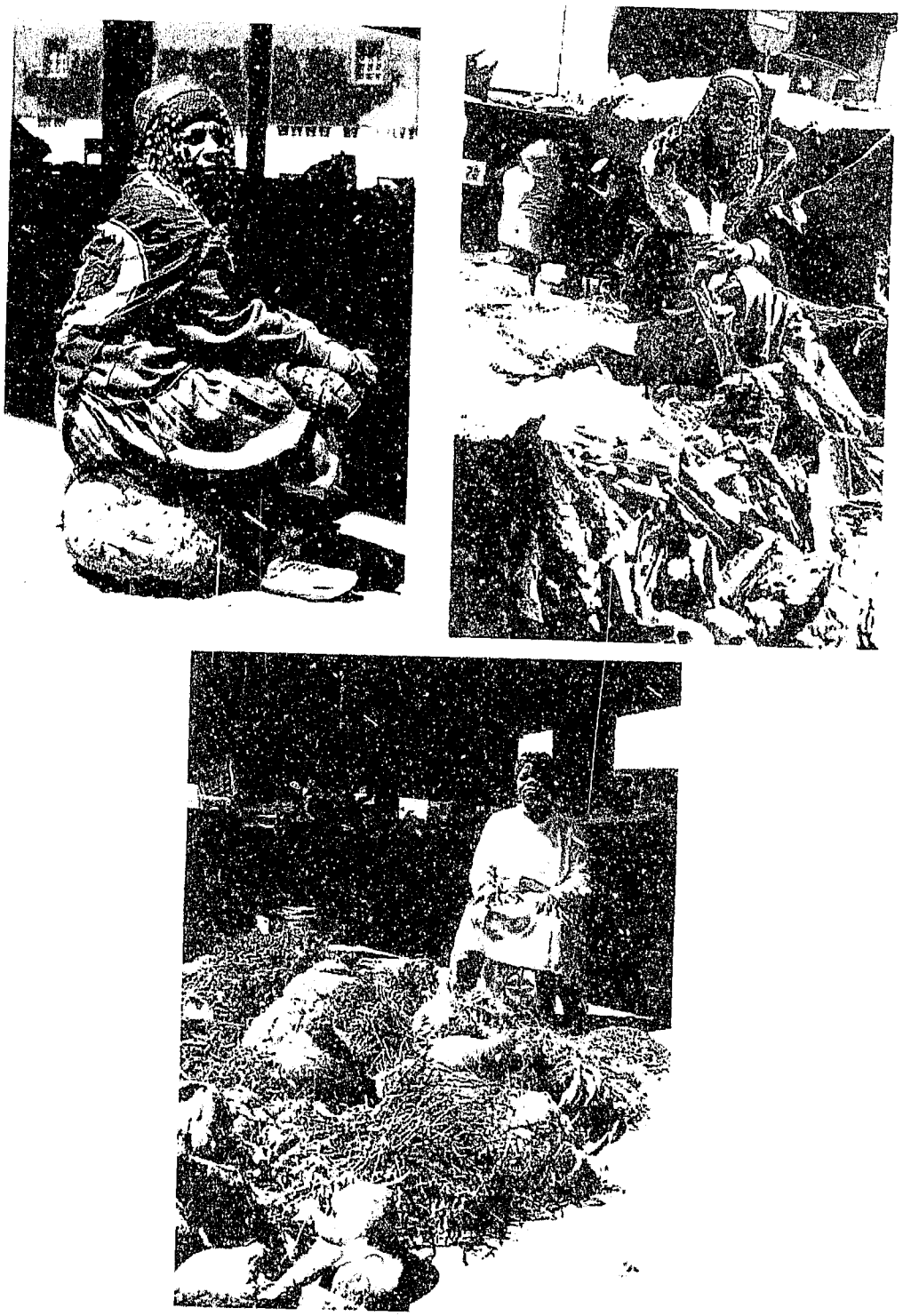


Figure 3.2 Plant vendors selling their merchandise at Faraday Herbal Market.



Figure 3.3 A plant vendor and traditional healer. The mixture (front left) is his personal "imbiza" remedy which he sells either as a dry mixture wrapped in newspaper or as a bottled decoction (behind the dried mix).



Figure 3.4 *Rhoicissus* lignotubers for sale.

3.3. Dangers of herbal remedies during pregnancy

None of the healers questioned with respect to the possible dangers of prescribing herbal remedies during pregnancy were aware of possibly teratogenic effects of the remedies. Healers were aware that the imbelekisane remedies were potentially dangerous and should be used with care.

3.4. Duration of therapy

Remedies were usually prescribed for varying durations starting from the first through to the third trimesters. The remedies were usually prescribed for a certain complaint of the patient, such as backache, fatigue or fear of a recurrent miscarriage. Thus the healers would prescribe a relevant isihlambezo for the particular indication. The duration of therapy usually lasted until the particular complaint dissipated. When the remedy was being taken to ensure an easy delivery, the remedy was taken from the beginning of the third trimester until delivery. Some traditional healers differentiate between imbelekisane and isihlambezo, while others do not.

3.5. Plants prescribed by the traditional healers in pregnancy related remedies.

The six traditional healers that were extensively interviewed prescribed between 1 and 16 plants in their pregnancy related remedies. A total of 48 species were prescribed (Table 3.1, Page 75), of which 14 (29%) were listed in the literature review on pregnancy related remedies compiled by Veale *et al.* (1992). A further 11 species (total of 52%) were documented in other literature sources (Table 3.3, Page 84). Of the plants prescribed by each healer, 50 to 100% have previously been documented to be used during pregnancy (Figure 3.5, Page 80). Of the 48 plant species prescribed, 18 species (37.5%) have been reported to be potentially poisonous, and 10 (20.8%) have been reported to have caused fatal poisonings in either livestock or humans (Table 3.4, Page 87).

3.6. Toxicity of *Rhoicissus tridentata*

Despite reports in the literature implicating *R. tridentata* in fatal poisonings (Watt & Breyer-Brandwijk, 1962; Brandt & Muller, 1995), none of 14 traditional healers questioned about the safety of 'Isinwasi" were aware of any toxic effects of the plant. None of the traditional healers were aware of seasonal or distributional differences in the effects of the plant. All material obtained by the healers from plant collectors were treated as being the same, and were dispensed in equal quantities.

Table 3.1 Ingredients prescribed by the traditional healers interviewed for use during pregnancy, or pregnancy related indications. Superscripts after the botanical names refer to whether the plants are reported to be toxic (T) or to be used in pregnancy related remedies (P) in the scientific literature.

PP=plant part used; TH = name of traditional healer.

Vernacular	Botanical	Use	PP*	TH*
ALLIACEAE				
ubani	*Agapanthus africanus* (L.)Hoffmg.[T, P]	isihlambezo	bb	2
ishaqa	*Tulbaghia violacea* Harv.[T]	imbiza	rt	6
AMARYLLIDACEAE				
umayime	*Clivia miniata* Regel[T, P]	isihlambezo - reduces pain - eases delivery - revives nerves and veins of reproductive system umsekelo	rt bb	2;4
impompo	*Scadoxus puniceus* (L.) Friis & Nordal[T, P]	imbiza - cleansing labour induction if overdue	bb	4;6
ANACARDIACEAE				
marula	*Sclerocarya birrea* (A. Rich.) Hochst. subsp *caffra* (Sond.) Kokwaro[P]	isihlambezo	bk	5
ASPARAGACEAE				
isigobo	*Protasparagus africanus* (Lam.) Oberm.	isihlambezo - menstrual pains - stomach pains - skin rash - infertility - mental retardation - swollen nerves in lung - cleanses blood	bb	2
ASTERACEAE				
iphungula	*Berkeya rhapontica* (DC.) Hutch. & Burtt Davy subsp. *aristosa* R ösl. var. *aristosa*	isihlambezo - cleansing agent - eases backache - easy delivery	rt	2
uqadolo amalenjane	*Bidens pilosa* L.	isihlambezo - stops bladder, kidney and back pains - limits amniotic fluid	rt	2

Vernacular	Botanical	Use	PP*	TH*
		- heals sores in bladder - gives blood more tonic - maternal strength		
impila	*Callilepis laureola* T, P	isihlambezo - easy delivery - maternal and foetal health - calm down restless child - bone fusion - wound healing - embalming - exorcism (steam) - hayfever (steam)	rt	2;4
impepo	*Helichrysum decorum* DC.	isihlambezo - headache	lt	4
uhlunguhlungu	*Vernonia hirsuta* (DC.)Sch. Bip. *V. neocorymbosa* Hilliard T, P *Brachylaena ellyptica* (Thunb.) DC. P	imbiza - cleansing fertility - opens tubes and cleanses womb	rt	4
CAESALPINACEAE				
mosethla	*Peltophorum africanum* Sond.		bk	4
CAMPANULACEAE				
amazombe	*Roella glomerata* A. DC.		lt	2
CELASTRACEAE				
ingwavuma	*Cassine transvaalensis* (Burtt Davy) Codd	infant - helps growth	bk	2
CONVOLVULACEAE				
uboqom	*Convulvulus* sp P	isihlambezo	rt	6
CUCURBITACEAE				
intshungu	*Momordica foetida* Schumach. P	isihlambezo - reduces amniotic fluid - revives reproductive system	rt	2
CYPERACEAE				
ibhuma	*Cyperus* sp	isihlambezo - foetal strength		2
DRACAENACEAE				
mogaga	*Sanseviera hyacynthoides* (L.) Druce P	infertility caused by STD	bb	5

Vernacular	Botanical	Use	PP*	TH*
EUPHORBIACEAE				
umahlabekufeni	*Croton gratisimus* Burch. var. *gratissimus*[T]	imbiza - cleansing	wd	4
HALORAGACEAE				
ugobo	*Gunnera perpensa* L. [P]	isihlambezo - general isihlambezo - backache or tiredness imbiza - cleansing fertility - if menstruate too often or if has STD	rt	1;2; 6
HYACINTHACEAE				
ugibisila	*Bowiea volubilis* Harv. ex Hook. f. [T, P]	imbiza - cleansing	bb	4
umathunga	*Eucomis autumnalis* (mill.) Chitt. subsp. *clavata* (Bak.) Reyneke[T, P] *E. autumnalis* (mill.) Chitt. subsp. *autumnalis* [T, P]	fertility - open tubes sores in uterus imbiza - cleansing	bb	3;4
inguduza	*Scilla natalensis* Planch.[T,P]	imbiza - cleansing - sores in uterus	bb	3;4
umahlokoloza	*Urginea physodes* (Jacq.) Bak.[T, P]	fertility	bb	3
HYPOXIDACEAE				
modi	*Hypoxis acuminata*	isihlambezo	bb	7
ilabatekha	*Hypoxis colchicifolia* Bak.[T] *H. hemerocallidea* Fisch. & C.A. Mey.	isihlambezo isihlambezo - for headache fertility - if menstruate too often, or has STD	bb	1;5
LORANTHACEAE				
iphakama	*Tieghemia quinquenervia* (Hochst.) Baile	isihlambezo		2
MIMOSACEAE				
intolwane umdabu	*Elephantorrhiza elephantina* (Burch.) Skeels [T, P] *E.burkei* [T, P]	fertility - opens tubes imbiza - cleansing isihlambezo - loose muscles - easy delivery - maternal strength - urinate (keeps system	bk rt rt	3;6 5

Vernacular	Botanical	Use	PP*	TH*
		going) 6 months after birth - takes away clots	wd	3
MYRSINACEAE				
umaguqu	*Maesa alnifolia* Harv. *M. lanceolata* Forssk.[T]	isihlambezo - to settle foetus if too high or breech		4;3
ORCHIDACEAE				
imfe-yenkawu	*Ansiella africana* Lindl.	isihlambezo		2
amabelejonosi	*Eulophia angolensis* (Reichb. f.) Summerh. *E. cucullata* (Afzel. ex Swartz) Steud. [P] *E.speciosa* (R. Br. ex Lindl.) H.Bol. *E. streptopetala* Lindl.	isihlambezo - reduces pain		2
PAPILIONACEAE				
umsintsi	*Erythrina caffra* Thunb.	imbiza - cleansing	wd	4
RUBIACEAE				
morokolopuli	*Kohautia amatymbica* Eckl. & Zeyh. [P]	isihlambezo umsekelo menstrual pains	rt	5
icimamlilo	*Pentanisia prunelloides* (Eckl. & Zeyh.) Walp.[P]	isihlambezo - kills pain	bb	1
STERCULIACEAE				
patengaka	*Hermannia depressa* N.E. Br.	isihlambezo - backache		5
TILIACEAE				
ihlalanyathi	*Grewia caffra* Meisn. *G. occidentalis* L.[T, P]	isihlambezo - makes baby move strengthens nerves of reproductive system	bk	2
VITACEAE				
isinwazi	*Rhoicissus digitata* (L.f) Gilg & Brandt [P] *R. tomentosa* (Lam.) Wild & Drum.[T, P]	isihlambezo - strengthens reproductive system -protects mother and foetus from illness	rt bb	2;4

Vernacular	Botanical	Use	PP*	TH*
	R. tridentata (L.f.) Wild & Drum. subsp. *cuneifolia* (Eckl. & Zeyh.) N.R. Urton[T, P]	umsekelo		
mbola	*Parinari curatellifolia*	fertility - open tubes	bb	3
sehlulamanye	*Schrebera saundersiae*			4
sekaname	*Homeria pallida*	fertility	bb	3;5
NON-BOTANICAL INGREDIENTS				
iqaku leufene nempila	Baboon urine	isihlambezo - helps uterus - cystitis		1;2
skhupha sequanda	chicken egg yolk	isihlambezo - maternal and foetal strength		1
qanda lentshe	ground ostrich eggshell	isihlambezo - maternal and foetal strength		1;3
mtsansi we hashi	horse placenta	isihlambezo - easy delivery contractions, ease pain		1
	liquid soap & water (sunlight soap)	imbiza		1
	liquid mercury[T]	imbelekisane		1
	LP records	imbelekisane		4,7, 8
UNIDENTIFIED INGREDIENTS				
dumalala	Not identified	infant - makes baby sleep	lt	2
isobo	Not identified	imbiza - if not menstruating	rt	6
mokgalakane	Not identified	fertility		3
ndodaemadevu	Not identified	imbiza - if not menstruating	rt	6
udabulibayi	Not identified	isihlambezo		3
udonuzibovu	Not identified	imbiza - sores in uterus	bb	3
ukunyakaza	Not identified	isihlambezo - strengthens nerves		2
umeluze	Not identified	imbiza - cleansing	bb	4
umsongelo	Not identified	imbiza	rt	6

Key: bb = bulb; bk=bark; rt=root; wd=branch; lt=leaves and twigs
*TH = Traditional healers or midwives: 1 - Nomsa; 2 - Bouylix; 3 - Martha; 4 - Fiki; 5 - Moseki; 6 - Laymond; 7 - Baragwanath Nursing Sisters, 8 - Johannesburg General Hospital Nursing Sisters

3.7. Previous documentation of use of prescribed plants during pregnancy

The number of plants prescribed by each healer ranged between one species through to sixteen (Figure 3.2). The proportion of plants prescribed by the various healers which are documented to be used during pregnancy varies from 50-100%.

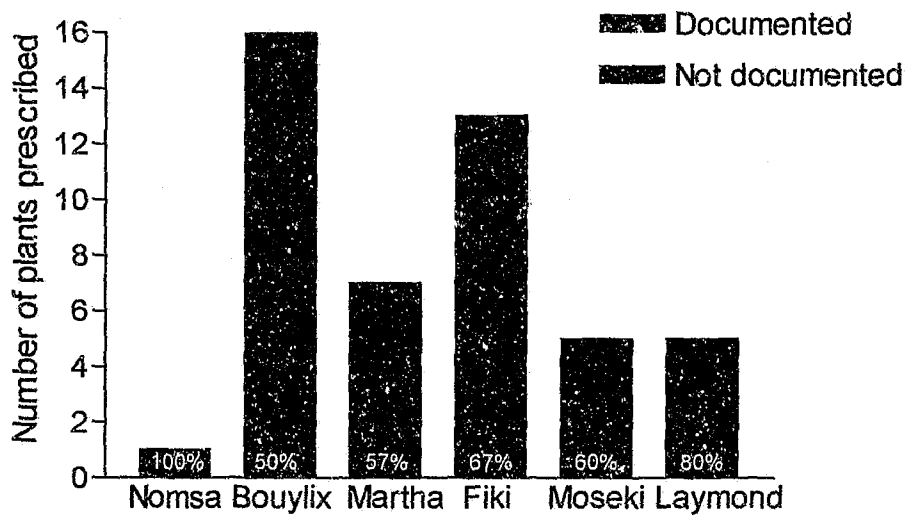


Figure 3.5. The number of plants prescribed by different traditional healers in pregnancy related remedies. The colours indicate the proportion of plants either documented or not documented in the scientific literature for use during pregnancy.

Table 3.2 Plants prescribed by traditional healers in pregnancy related remedies tabulated according to indications, and the number of healers using the plant for the specific indication.

Indication	Plant species	No.
Imbiza		
cleansing	*Protasparagus africanus*	1
	Berkeya rhapontica	1
	Bowiea volubilis	1
	Croton grattisimus	1
	Elephantorrhiza elephantina	1
	Erythrina caffra	1
	Gunnera perpensa	3
	Scadoxus puniceus	3
	Scilla natalensis	2
	Tulbaghia violacea	1
	Vernonia spp. / *Brachylaena ellyptica*	1
Isihlambezo		
menstrual pains	*Protasparagus africanus*	1
	Kohautia amatymbica	1
stomach pains	*Protasparagus africanus*	1
skin rash	*Protasparagus africanus*	1
cystitis	baboon urine	2
maternal/foetal	chicken egg yolk	1
strength	ground ostrich eggshell	2
	Bidens pilosa	1
	Callilepis laureola	2
	Cyperus sp.	1
	Elephantorrhiza elephantina / E. burkei	1
	Gunnera perpensa	3
to ease labour	Horse placenta	1
	Berkeya rhapontica	1
	Callilepis laureola	2
	Clivia miniata	2
	Elephantorrhiza elephantina / E. burkei	1
	Eulophia cucullata	1
	Pentanisia prunelloides	1

Indication	Plant species	No.
avoid repeated miscarriage	*Clivia miniata*	2
	Kohautia amatymbica	1
	Rhoicissus digitata / R. tomentosa / R. tridentata	2
to ease back pain	*Berkeya rhapontica*	1
	Bidens pilosa	1
	Gunnera perpensa	3
	Hermannia depressa	1
headache	*Hypoxis colchicifolia / H. hemerocallidea*	2
diuresis	*Elephantorrhiza elephantina / E. burkei*	1
to strengthen nerves of reproductive system	*Clivia miniata*	2
	Grewia occidentalis	2
	Momordica foetida	1
to make baby move	*Grewia occidentalis*	1
to make baby drop or turn if breech	*Maesa alnifolia / M.lanceolata*	2
to reduce amniotic fluid	*Momordica foetida*	1
general / unspecified	*Agapanthus africanus*	1
	Convulvulus sp.	1
	Gunnera perpensa	3
	Hypoxis colchicifolia / H. hemerocallidea	2
	Roella glomerata	1
	Sclerocarya birrea subsp *caffra*	1
	Schrebera saudersiae	1
	Tieghemia quinquenervia	1
Imbelikesane		
(labour induction)	baboon urine	2
	Horse placenta	1
	Scadoxus puniceus	2
Fertility		
unspecified	*Protasparagus africanus*	1
	Homeria pallida	2
	Urginea physodes	1
to open tubes	*Elephantorrhiza elephantina*	1
	Eucomis autumnalis	2
	Parinari curatellifolia	1
	Vernonia neocorymbosa	1
STD related	*Gunnera perpensa*	3
	Sanseviera hyacinthoides	1
	Hypoxis colchicifolia / H. hemerocallidea	2

Indication	Plant species	No.
frequent menstruation	*Gunnera perpensa*	3
	Hypoxis colchicifolia / H. hemerocallidea	2
Paediatrics		
calm restless child	*Callilepis laureola*	1
to make baby sleep	not identified	1
to help infant growth	*Maytenus heterophylla / Cassine transvaalensis*	1

3.8. Literature citings of plants used in pregnancy related remedies

The use of pregnancy related remedies is extensively reported in the literature. Table 3.3 gives references for citings of the various ingredients prescribed by the interviewed traditional healers and Table 3.4 gives the documented toxic effects of the plants.

Table 3.3 Documented pregnancy related uses of plants prescribed by the traditional healers interviewed.

Ingredient	Use / Effect	Reference*
Agapanthus africanus	isihlambezo; inembe	11
	expulsion of placenta	6
	stimulate contractions of isolated uterus and ileum	
	agonise muscarinic receptors and promote prostaglandin synthesis	12
Bowiea volubilis	bulbs - facilitate delivery	3
	procure abortions	13
	isihlambezo, inembe	11
Brachylaena ellyptica	procure abortions	2
	inembe	3; 11
Callilepis laureola	isihlambezo, inembe	3; 4
	infertility	5; 11
Clivia miniata	induce / facilitate birth	3; 11
	inembe, isihlambezo	5
	uterotonic on isolated rat uterus	10
Convulvulus sp *(C. sagittatus)*	unspecified medicine during pregnancy in Lesotho.	13
Cyperus sp	inembe	11
Elephantorrhiza elephantina *E.burkei*	womb cleansers soon after birth	9

Ingredient	Use / Effect	Reference *
Eucomis autumnalis	prevent premature birth (Lesotho)	13
	isihlambezo, inembe	11
Eulophia cucullata	impotence	1
	barrenness	5
Grewia occidentalis	facilitate or procure delivery	1
	spasmolytic on *in vitro* guinea-pig ileum and spasmogenic on rat and rabbit duodenum.	5
	isihlambezo, inembe	11
Gunnera perpensa	isihlambezo, inembe	1
	cystitis	11
	facilitate expulsion of the placenta in women and animals	2
	uterotonic on isolated rat uterus	11
		6
Hypoxis colchicifolia	impotency and barrenness	1
Kohautia amatymbica	sterility (Lesotho)	5
Momordica foetida	abortifacient and ecbolic (Uganda)	13
	antinicotinic and antimuscarinic action *in vitro*	5
Pentanisia prunelloides	facilitate placental expulsion	13
	isihlambezo, inembe	3
	stimulate contractions of isolated rat uterus and ileum	6
		11
Rhoicissus digitata	isihlambezo, inembe	5
R. tomentosa	dysmenorrhoea	11
R. tridentata		
Sanseviera hyacynthoides	prevent miscarriage (Western Cape)	13
	ease birth pains (Tswana)	5
	inhibits uterine contractions stimulated by oxytocin and serotonin *in vitro*	8
	no effect on isolated ileum	

Ingredient	Use / Effect	Reference *
Scadoxus puniceus	ensure safe delivery	3
	isihlambezo, inembe	11
Scilla natalensis	isihlambezo, inembe	3; 11
Sclerocarya birrea subsp *caffra*	isihlambezo, inembe	5
	menorrhagia (Zimbabwe)	11
	infertility (Vhavenda)	
Urginea physodes	isihlambezo, inembe	11; 2
Vernonia neocorymbosa	isihlambezo, inembe	3
	menstrual irregularities	11
	abortifacient	13
Baboon urine	isihlambezo	7
	oxytocic *in vitro*	
horse placenta	oxytocic *in vitro*	14
liquid mercury		

*** References:**

1. Bryant 1966
2. Gerstner 1939
3. Gerstner 1941
4. Gumede 1990
5. Hutchings *et al.* 1996
6. Kaido *et al.* 1997
7. Morris & Mdlalose 1991
8. Neuwinger 1996
9. Pujol 1990
10. Veale *et al.* 1989
11. Veale *et al.* 1992
12. Veale *et al.* 1999
13. Watt & Breyer-Brandwijk 1962
14. Onaguluchi & Ghasi 1996

Table 3.4 Potential toxic effects of plants prescribed by the traditional healers interviewed in pregnancy related traditional remedies [a].

Ingredient	Symptoms of poisoning	Toxic principle	Ref[b]
Agapanthus africanus	Haemolytic anaemia Sap causes severe mouth ulceration		5; 6
Bowiea volubilis	Haemolysis, severe vomiting and diarrhoea, salivation, cardiac failure, death.	bufadienolides, cardiac glycosides	2; 4; 6; 7
Callilepis laureola	Confusion, vomiting,, diarrhoea, convulsions, hypoglycaemia, hepatic centrilobar necrosis, and renal tubular necrosis, death.	atractyloside	3; 5; 9
Clivia miniata	Salivation, vomiting, diarrhoea, CNS depression	lycorine	4; 5
Croton grattisimus	Bark reputed to be highly toxic; Skin and mucosal irritation.	diterpenoids	4; 6
Elephantorrhiza elephantina *E.burkei*	Oral administration: constipation Parental administration: pulmonary oedema, loss of appetite, diarrhoea, exhaustion, liver degeneration, death.		3; 6
Erythrina caffra	Highly toxic	erysovine, erythraline (*Erythrina* alkaloids)	4
Eucomis autumnalis	Death in sheep Abdominal pain, diarrhoea, renal failure	haemolytic saponins	6, 7
Hypoxis colchicifolia	Corms reputed to be highly toxic		3
Maesa lanceolata	Reported to be toxic		6
Momordica sp.	Fruit caused death of dogs and sheep. Violent diarrhoea and vomiting		8
Protasparagus africanus	No reports		
P. exuvialis	High fever, confusion		8

Rhoicissus tomentosa	Severe colic and childhood diarrhoea.		3; 6
R. tridentata	CNS depression leading to respiratory arrest.		
Sanseviera sp.	Leaves act as neuromuscular blockers		8
Scadoxus puniceus	Dizziness, visual disturbances, CNS excitation or depression, death.	lycorine, haemanthidine	3; 5; 7; 8
Scilla natalensis	Death of experimental sheep with dyspnoea, weak quickened pulse.		5; 6; 7
Tulbaghia violacea	Abdominal pain, gastro-enteritis, acute inflammation, intestinal mucosa sloughing, inhibition of intestinal peristalsis, miosis, death.		1; 3; 6
Urginea physodes	3g/kg bulbs fed to sheep caused death within 22 hours.	cardiac glycosides eg. scillaren A	3; 4
Vernonia neocorymbosa	Fatal if used incorrectly (according to the Vhavenda)		3
Vernonia sp.	Used as arrow poison by Ndorba Decreases heart rate and causes CNS paralysis		8

[a] **Based on:**

1- Burton 1990
2- Gerstner 1941
3- Hutchings *et al.* 1996
4- Van Wyk *et al.* 1997
5- Veale *et al.* 1992
6- Watt and Breyer-Brandwijk 1962
7- Hutchings & Terblanche 1989
8- Neuwinger 1996
9- Watson *et al.* 1979

CHAPTER 4: Results section 2

4. PHARMACOLOGICAL ACTION OF *RHOICISSUS TRIDENTATA* ON ISOLATED RAT UTERUS AND ILEUM

4.1. Inorganic Ion Concentrations.

The concentrations of K^+, Ca^+ and Na^+ in the maximal doses of the *R. tridentata* extract (from roots harvested in Umlazi) added to the organ baths were 0.96 mmol/L, 0.15 mmol/L and 0.70 mmol/L respectively. That is a collective osmolarity of 1.81mmol/L. The maximal volume of plant extract added was 1.3ml in 50ml Tyrode. Therefore, the plant extract would not have affected the tonicity of the Tyrode solution surrounding the organs.

4.2. Comparison Between the Efficacy and Potency of the Different Reference Drugs.

The contractions caused by the various reference drugs were expressed relative to the contractions stimulated by acetylcholine on the same piece of tissue. All concentrations stated refer to final concentrations in the organ baths in all the *in vitro* experiments.

In the uterus (Figure 4.1a), acetylcholine and oxytocin exhibited similar efficacies, followed by serotonin then noradrenaline. The maximal contractions induced by noradrenaline were only about 28% of those caused by acetylcholine. This is the reason why (later in this chapter) the plant extract appears to be more effective when compared to contractions stimulated by noradrenaline. In the ileum, the maximal contractions stimulated by serotonin were half the size of those stimulated by acetylcholine (Figure 4.1b).

Oxytocin was the most potent agonist in the oestrogenised uterus, followed by serotonin, acetylcholine and then noradrenaline (Figure 4.1a), whereas in the ileum acetylcholine was more potent than serotonin (Figure 4.1b).

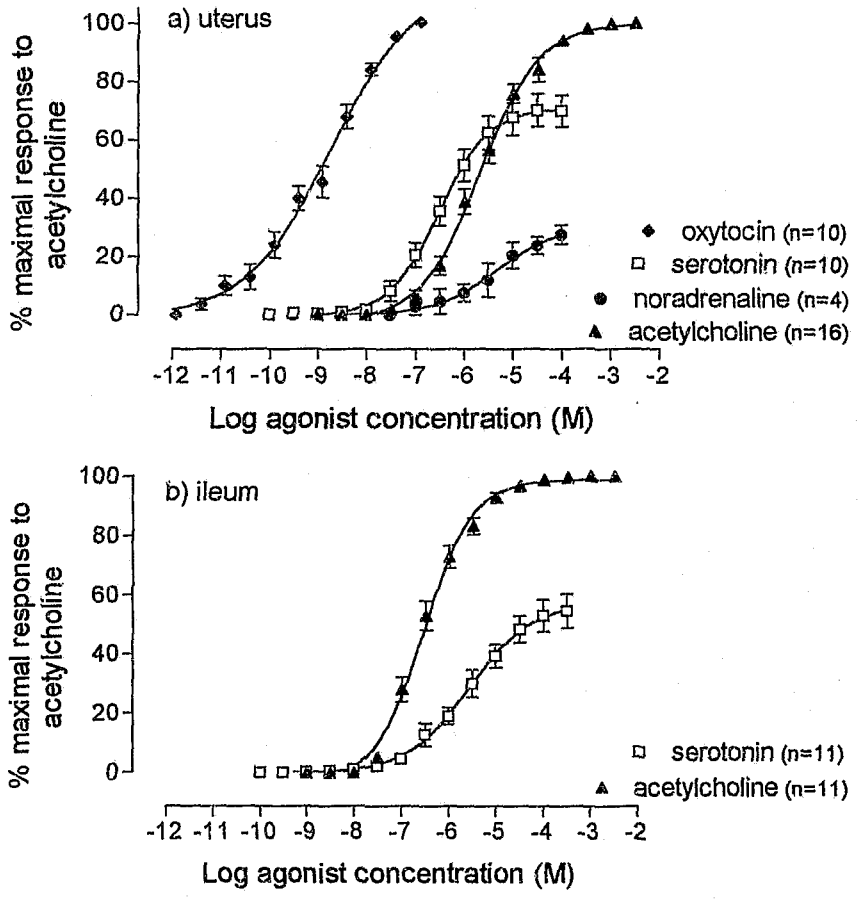


Figure 4.1 Comparison of the isolated rat, a) uterus and b) ileum responses to the cumulative addition of the different reference agonists used in this study. All values were calculated relative to the organ's maximal response to acetylcholine. Each point represents the mean with the error bars representing the standard error of the mean.

4.3. THE PHARMACOLOGY OF *RHOICISSUS TRIDENTATA* SUBSP. *CUNEIFOLIA* ON ISOLATED RAT UTERINE AND ILEAL SMOOOTH MUSCLE

4.3.1. Extracts used for the investigations of pharmacological action

Crude aqueous decoctions of *R. tridentata* subsp. *cuneifolia* were used for this study to determine the pharmacological action of the extracts ingested by women treated by local traditional healers. Organic solvents and various fractionation methods were avoided as these are not used by local healers.

Aqueous extracts of *R. tridentata* subsp. *cuneifolia* roots harvested in either Durban or Umlazi were used for the following investigations into the pharmacological action of the plant extracts presented in this chapter:

Cumulative additions of *R. tridentata*	(Figure 4.2)
Oxytocin	(Figure 4.5)
Noradrenaline and prazosin	(Figure 4.7)
Acetylcholine and atropine	(Figure 4.9)

Once the stock of the above extracts was depleted, all the other studies were done using aqueous extracts from tubers harvested during summer and autumn from Suikerbosrand. These extracts displayed the same pharmacological action as the roots harvested in KwaZulu-Natal. The following studies were done using extracts from tubers harvested from Suikerbosrand:

Indomethacin	(Figure 4.6)
Noradrenaline and yohimbine	(Figure 4.8)
Muscarinic subtype selective antagonists	(Figure 4.10)
Serotonin and methysergide	(Figure 4.11)
Serotonin and ketanserin	(Figure 4.12)
Serotonin and tropisetron	(Figure 4.13)
Hexamethonium, mecamylaine	(Figure 4.14)
Cholera toxin, pertussis toxin	(Figure 4.14)
pyrilamine	(Figure 4.15)
NS-398	(Figure 4.17)

4.3.2. The contractile action of *R. tridentata* subsp *cuneifolia*

The *Rhoicissus* extract stimulated concentration dependent contractions in both isolated rat uterus and ileum (Figure 4.2). In the doses given, the ileal dose response curve was sigmoidal, whereas the uterine curve was not. Higher doses could not have been given as explained later.

The crude aqueous extracts of *R. tridentata* at a bath concentration of 1.3mg/ml exhibited a wide range in the size of contractions in the different uteri and the ileui used (Figure 4.3). The range in the uterine and ileal responses, relative to acetylcholine, varied from 0-85% and 0-95% respectively.

It is not possible to determine the IC_{50} of the extract in the uterus since a maximal response was not obtained. The IC_{50} for the ileum was 0.8µg/ml.

Figure 4.4 shows the resulting contractions following the addition of accumulating doses of acetylcholine to the baths containing the isolated uterus (a) and ileum (b). After washing out the baths, plant extract was added to the baths, exposing the organs to 1.3 mg/ml plant extract. Both the uterine and ileal muscle contracted to about 50% of the maximum effect of acetylcholine. Without washing, accumulating doses of acetylcholine were added to the baths. In the case of the uterus, there was a slight increase in the contraction, but the increase in contraction was more marked in the case of the ileum. On washing out the baths, it was found that in the case of the ileum, the organ returned to its original length, however, the uterus did not relax fully irrespective of repeated washouts.

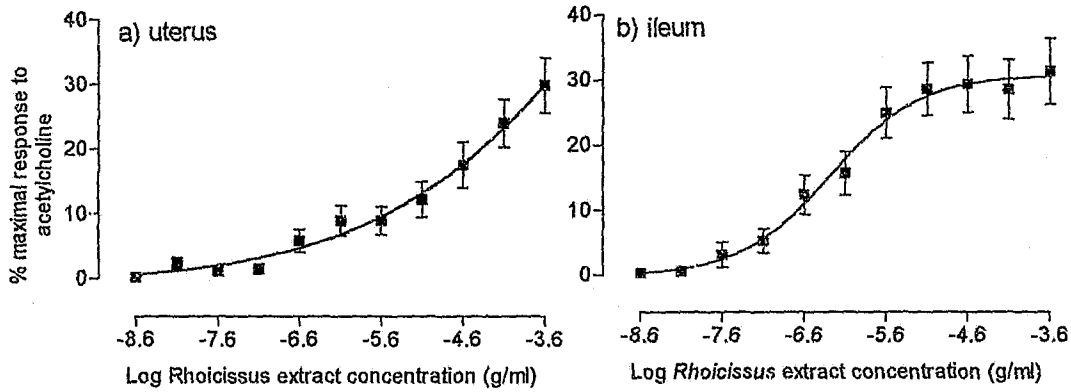


Figure 4.2 Direct action of *R.tridentata* subsp. *cuneifolia* aqueous root extracts on isolated rat a) uterus and b) ileum. All values were calculated relative to the maximal response obtained from a prior cumulative dose response curve to acetylcholine alone. Each point represents the mean response from seven rats with the SEM.

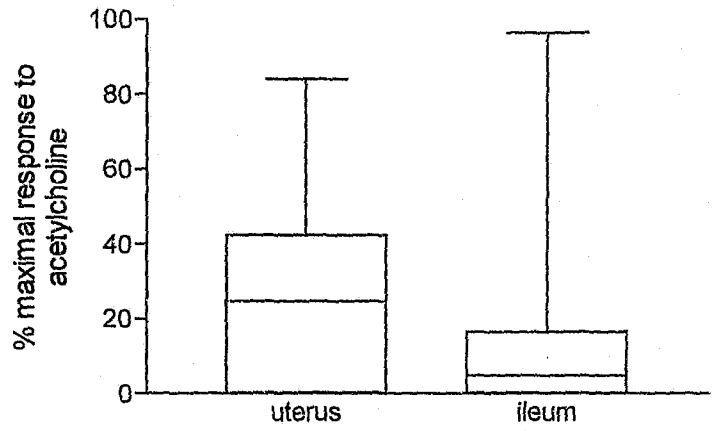


Figure 4.3 Box and whisker plot showing the range of maximal contractions stimulated by 1.3 mg/ml *R. tridentata* roots. The box extends from the 25th to the 75th percentile, with the horizontal line at the median. The whiskers show the range of the data.

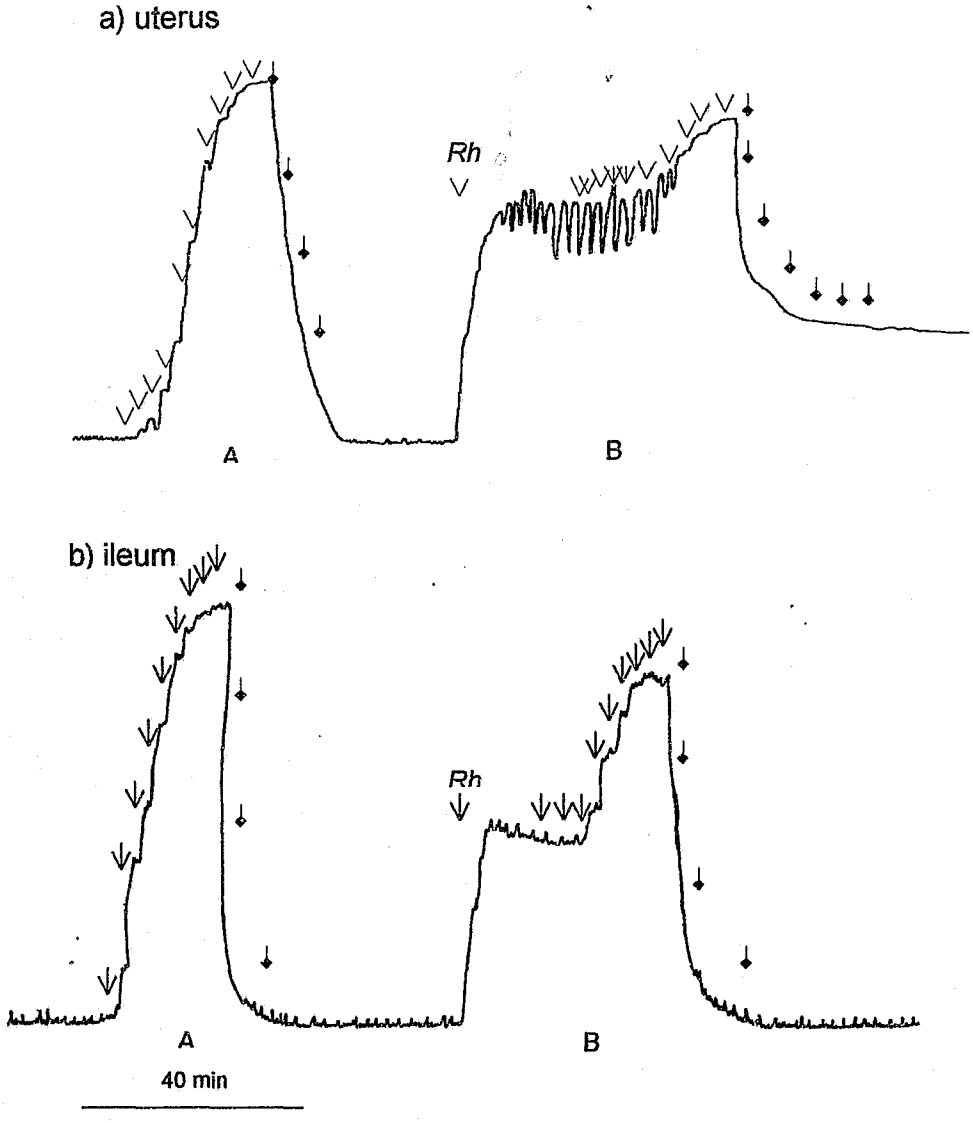


Figure 4.4 Tracing of the a) uterine and b) ileal contractions caused by (A) cumulative additions of acetylcholine, (B) 1.3 mg/ml *R. tridentata* Umlazi root extract (*Rh)* followed by cumulative additions of acetylcholine. The arrows (↓) indicate when doses of exponentially increasing concentrations of acetylcholine were added to the organ bath (0.32ng - 3mg/ml). The baths were rinsed between tests to allow the organs to relax (↓).

4.4. INVOLVEMENT OF DIFFERENT RECEPTOR SYSTEMS IN THE CONTRACTILE RESPONSE TO THE *RHOICISSUS* EXTRACT

All the dose response curves in the following section are presented in the same format. Each point represents the mean with the error bars representing the standard errors of the mean. The black curves represent the contractile response to the agonist alone and serve as references. The green curves represent the contractile response to *R. tridentata* root aqueous extracts (with no washing out of the organ baths) followed by the cumulative addition of the reference agonist. The red curves represent the response to the antagonist followed by the reference agonist that indicates to what extent the receptors are blocked at the concentration of antagonist used. The blue curves represent responses to the plant extract and reference agonist after the relevant receptors have been blocked by an antagonist. These curves indicate whether the contractile activity of the plant extract is altered if the relevant receptors are blocked. The sample size is given in the legends on the graphs for the different curves.

4.4.1. OXYTOCIC RECEPTOR SYSTEM

The effect of the *Rhoicissus* extract on the uterine response to oxytocin.

The direct contractile action of a final concentration of 1.3 mg/ml crude extract was 28.5% ± 14 (95% CI) of the maximal response to oxytocin (Figure 4.5). The extract had no effect on the maximal response to oxytocin. No oxytocic antagonists were available.

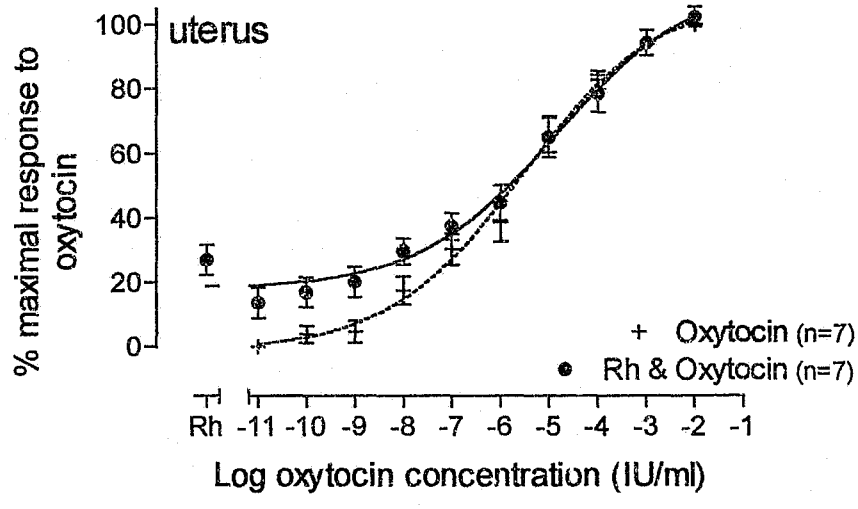


Figure 4.5 The isolated uterine response to oxytocin alone (+) compared to when the organs were pretreated with a final concentration of 1.3 mg/ml *Rhoicissus* extract for 5 minutes before the cumulative addition of oxytocin.

4.4.2. Prostaglandin synthesis

The effect of indomethacin on the response to the cumulative addition of acetylcholine was negligible. Preincubation with 5 μM indomethacin for 15 min significantly reduced the initial activity of 1.3 mg/ml plant extract in both the uterus and ileum ($p<0.001$), without affecting the organs' response to the acetylcholine following the plant extract (Figure 4.6).

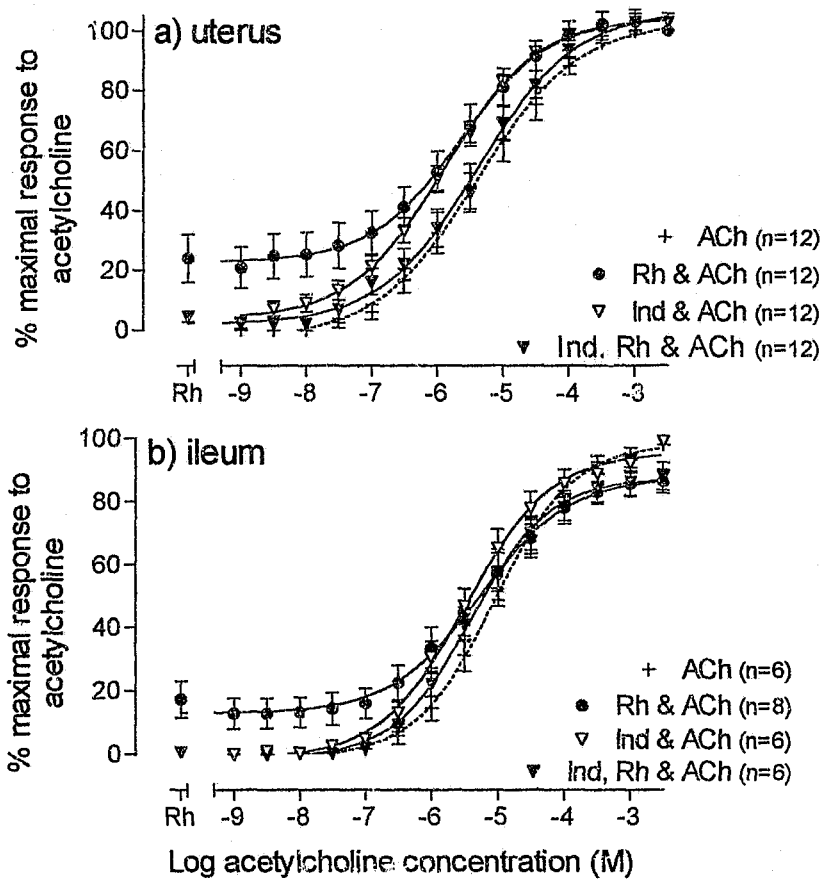


Figure 4.6 The contractile response of isolated rat a) uterus and b) ileum to acetylcholine (+) when pretreated with 5 μM indomethacin for 15 minutes (▽), 1.3 mg/ml *R. tridentata* extract for 5 minutes (●), and indomethacin followed by the plant extract before the cumulative additions of acetylcholine (▼).

4.4.3. Adrenergic Receptor System

The effect of the plant extract on the α-receptor response to noradrenaline.

The direct action of the plant extract increased the initial response to noradrenaline in the uterus once the β-adrenergic receptors had been blocked by 2.7μM propanolol. The direct action of the plant extract was 54.9% ± 5.5 (95% CI) of the maximal response to noradrenaline. The extract did not alter the response to maximal concentrations of noradrenaline (Figure 4.7). Prazosin, an α_1-adrenoceptor antagonist, did not alter the uterine response to the *Rhoicissus* extract, even though the same dose of prazosin shifted the dose response curve to noradrenaline to the right. Yohimbine, an α_2-adrenoceptor antagonist, at a concentration which shifted the dose response curve to noradrenaline to the right, also did not alter the contractile activity of the plant extract (Figure 4.8).

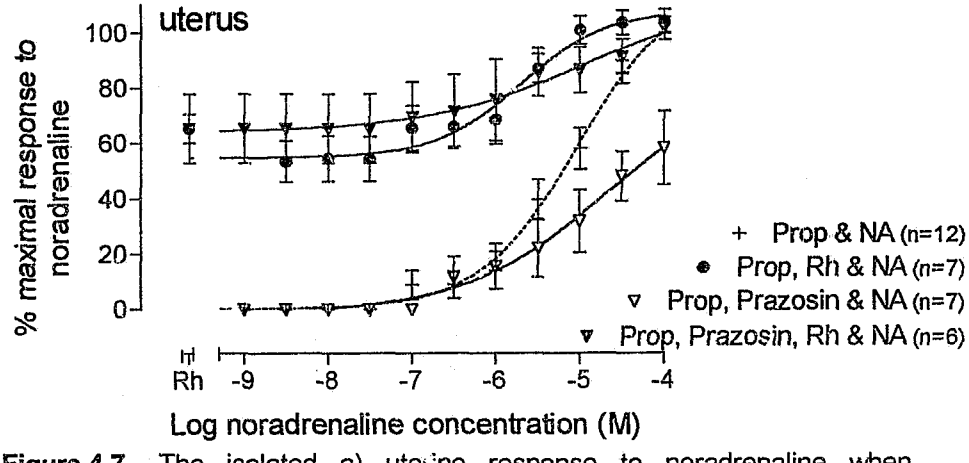


Figure 4.7 The isolated a) uterine response to noradrenaline when pretreated with 2.7μM propanolol for 5 minutes then 1.3 mg/ml *Rhoicissus* extract for 5 minutes (●), compared to the response to noradrenaline when pretreated with propanolol only (+), propanolol and 2μM prazosin (∇) or prazosin followed by the *Rhoicissus* extract (▼).

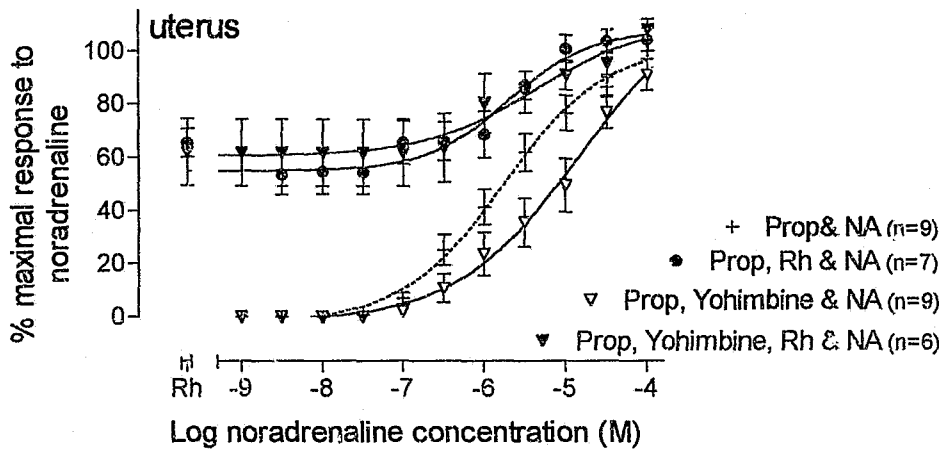


Figure 4.8 The isolated rat uterine response to noradrenaline when pretreated with 2.7 μM propanolol for 5 minutes followed by 1.3 mg/ml *Rhoicissus* aqueous extract for 5 minutes (●), compared to the response to noradrenaline when pretreated with propanolol only (+), propanolol and 2μM yohimbine (∇) or yohimbine followed by the *Rhoicissus* extract (▼).

4.4.4. Muscarinic receptor system

The effect of the *Rhoicissus* extract on the isolated uterus and ileum response to acetylcholine.

Pretreatment of the organs with 1.3 mg/ml plant extract directly stimulated contractions 18.3% ± 4.6 (95% CI) of the maximal contractions stimulated by acetylcholine in the uterus and 14.3 ± 4.3 (95% CI) in the ileum (Figure 4.9). The extract did not affect the uterine response to cumulative addition of acetylcholine, whereas the maximal response was inhibited in the ileum. Preincubation for 5 minutes with 0.4nM atropine, a non-specific muscarinic antagonist, shifted the acetylcholine curve to the right without blocking the response to maximal concentrations of acetylcholine, and completely blocked the direct action of the *Rhoicissus* in both the uterus and the ileum.

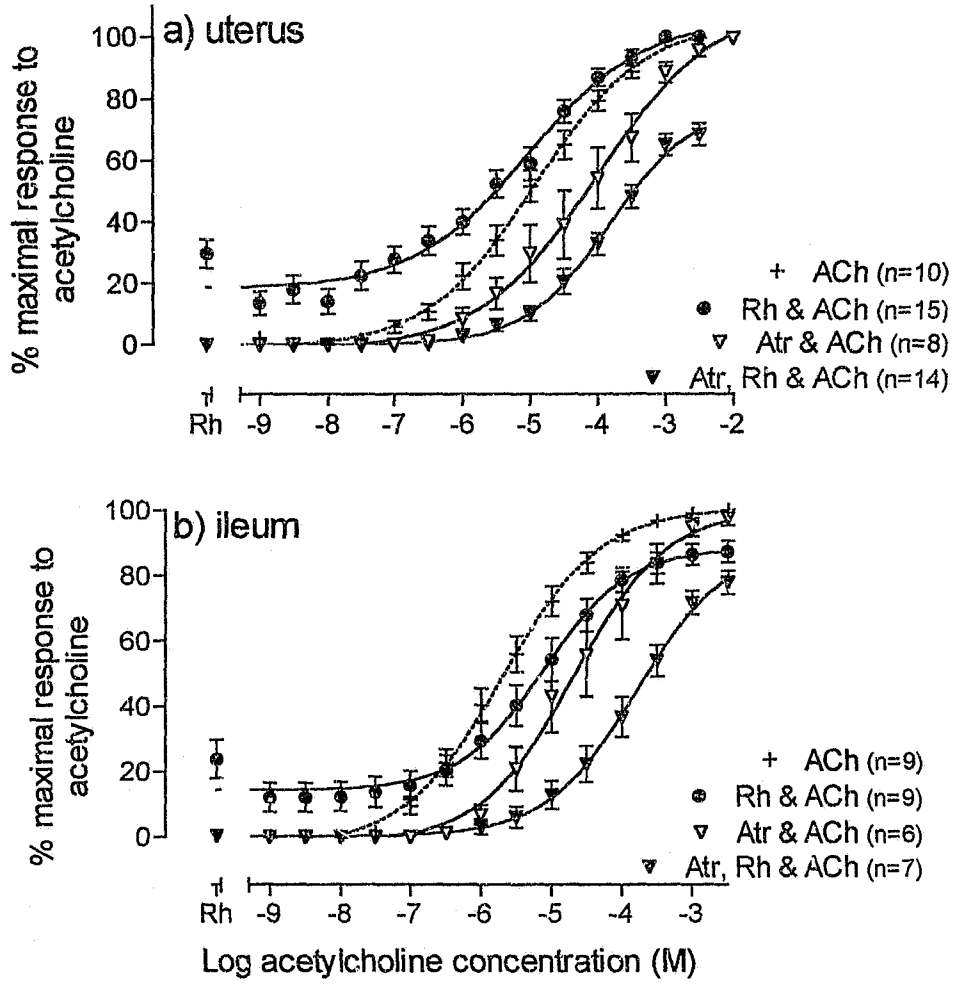


Figure 4.9 The a) uterine and b) ileal response to acetylcholine (+) compared to when the organs were pretreated with 1.3 mg/ml *R. tridentata* root extract (●) for 5 minutes before the addition of acetylcholine. The response of isolated rat uterus and ileum to acetylcholine when pretreated with $4x10^{-8}$M atropine for 5 minutes (▽) or atropine for 5 minutes followed by the plant extract for another 5 minutes before the cumulative addition of acetylcholine (▼).

The effect of muscarinic subtype specific antagonists on the uterine response to the extract and acetylcholine.

Pretreating the isolated rat uterus with the muscarinic antagonists pirenzepine (1.5μM), tropicamide (4.5μM) and 4-DAMP (2nM) which have affinities selective for muscarinic receptor subtypes M_1, M_3 and M_4 respectively, all shifted the dose response curve to acetylcholine to the right (Figure 4.10 a, b & d). Gallamine (2μM), a M_2 muscarinic receptor antagonist did not alter the uterine response to acetylcholine (Figure 4.10c). Pirenzepine and Gallamine did not alter the contractile activity of the plant extract, whereas tropicamide completely blocked the uterine response to *Rhoicissus*. 4-DAMP inhibited the uterine response to the *Rhoicissus* extract, and suppressed the uterine response to maximal concentrations of acetylcholine in the presence of the plant extract.

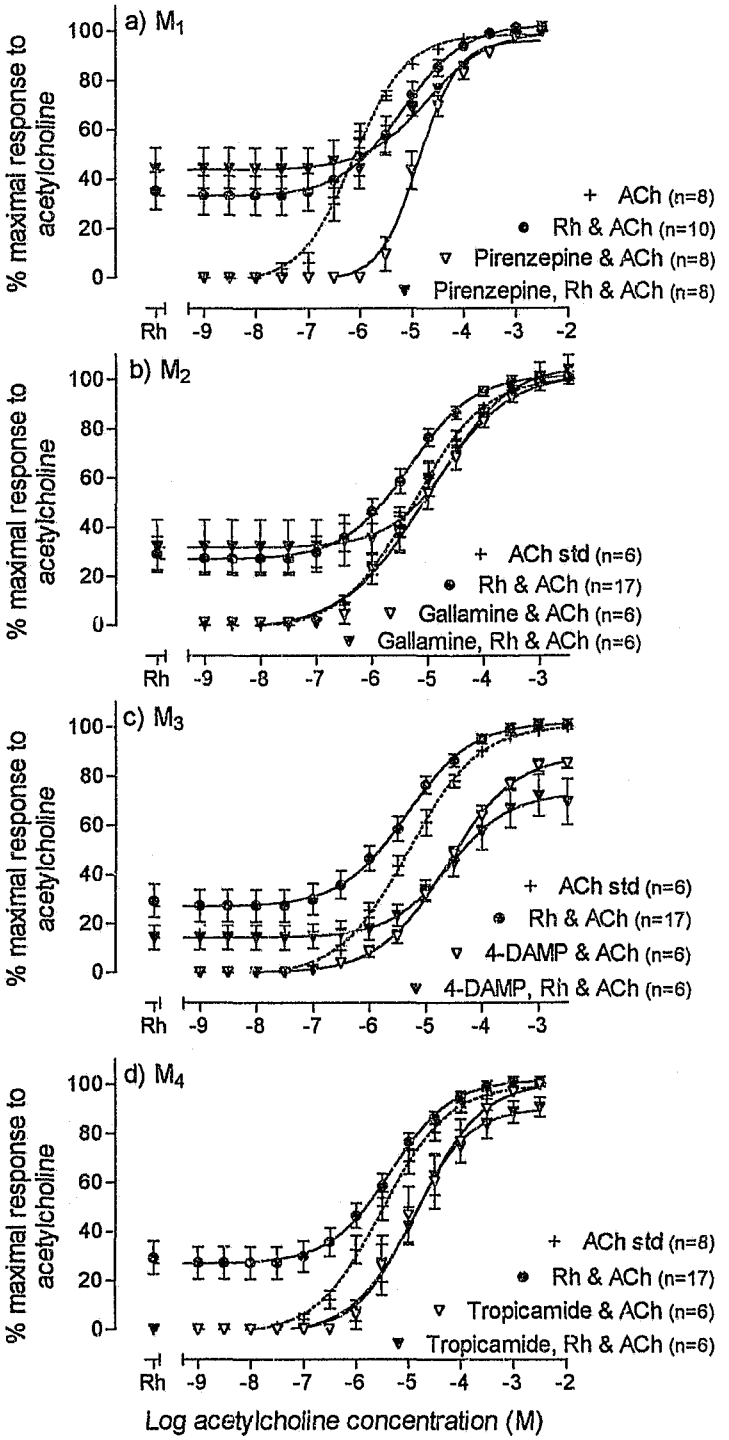


Figure 4.10 The effect of antagonists on muscarinic subtypes on the isolated rat uterine response to acetylcholine alone or after pretreatment with the *R. tridentata* extract.

4.4.5. Serotonergic receptor system

The effect of the plant extract on the response of the isolated organs to serotonin

The direct activity of the plant extract increased the initial response to serotonin in the uterus and ileum (Figure 4.11). The mean direct contractile activity of the *Rhoicissus* extract was 22% in the uterus and 18% in the ileum. The maximal response to serotonin was unchanged by the extract in the uterus but depressed in the ileum. Methysergide, a non-specific serotonin receptor antagonist, had no effect on the uterine response to the *Rhoicissus* extract, but increased the ileal response.

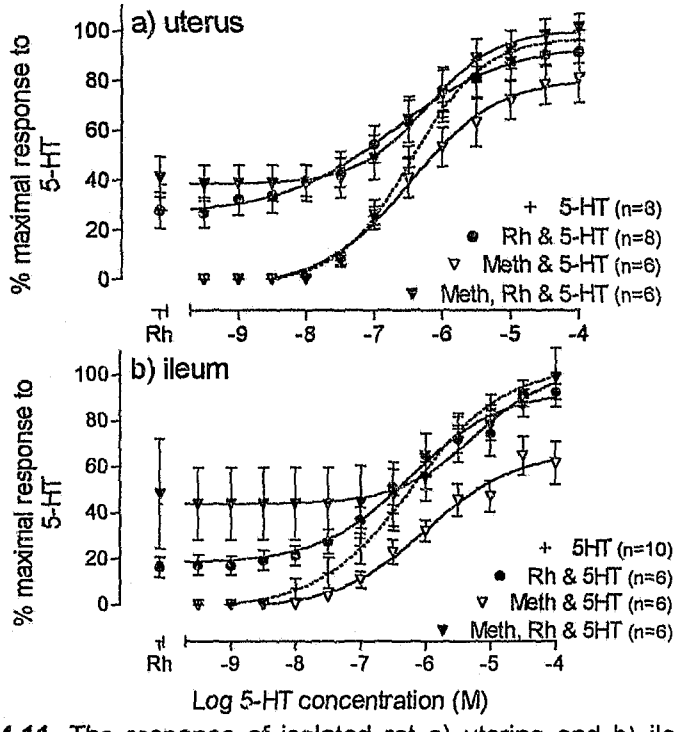


Figure 4.11 The response of isolated rat a) uterine and b) ileal muscle to serotonin (+) when pretreated with 1.3 mg/ml *R. tridentata* extract (●). The contractile response of the isolated rat uterus and ileum to serotonin when pretreated with 1μM methysergide for 5 minutes (▽), or methysergide followed by the plant extract and then cumulative additions of serotonin (▼).

The effect of serotonin receptor subtype antagonists on the uterine and ileal response to the plant extract and serotonin.

As with the methysergide response, ketanserin and tropisetron, antagonists specific for serotonin receptor subtypes 5-HT_1 and 5-HT_2 respectively, had no effect on the uterine response to the *R. tridentata* extract at concentrations which shifted the dose response curves to serotonin to the right (Figure 4.12a & 4.13a). However, both antagonists increased the direct ileal response to the extract (Figures 4.12b & 4.13b).

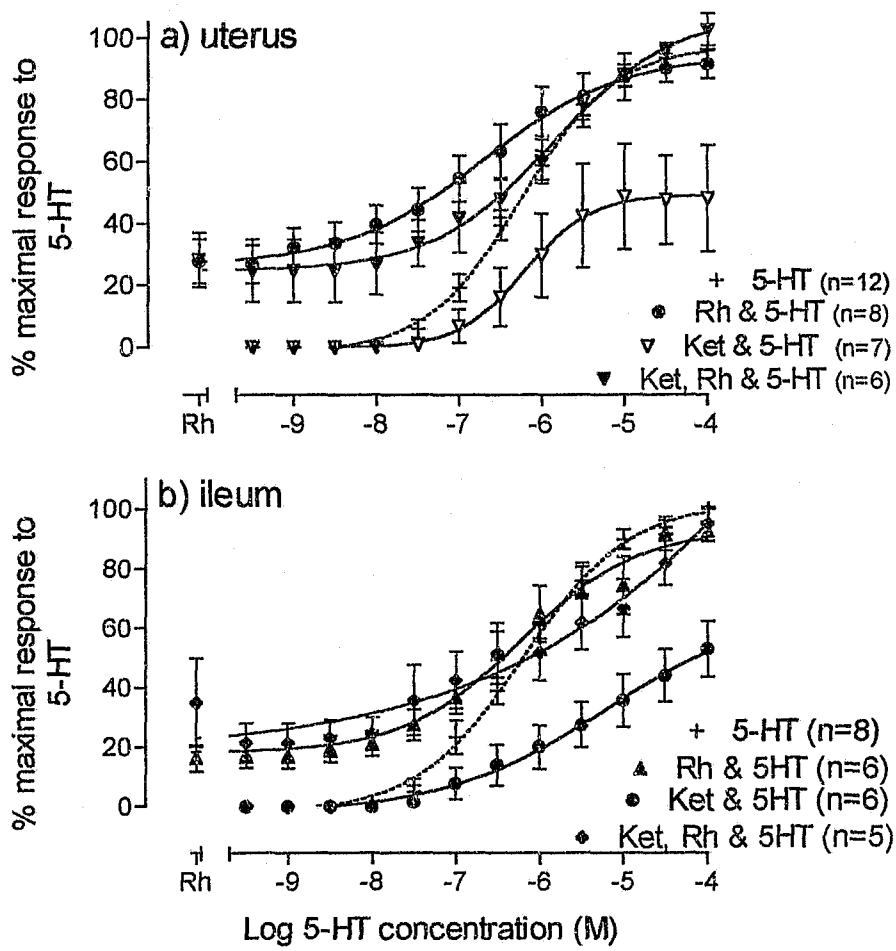


Figure 4.12 The effect of ketanserin, a 5-HT_1 receptor subtype antagonist, on the isolated rat a) uterine and b) ileal response to serotonin with or without the pretreatment of the *R. tridentata* extract.

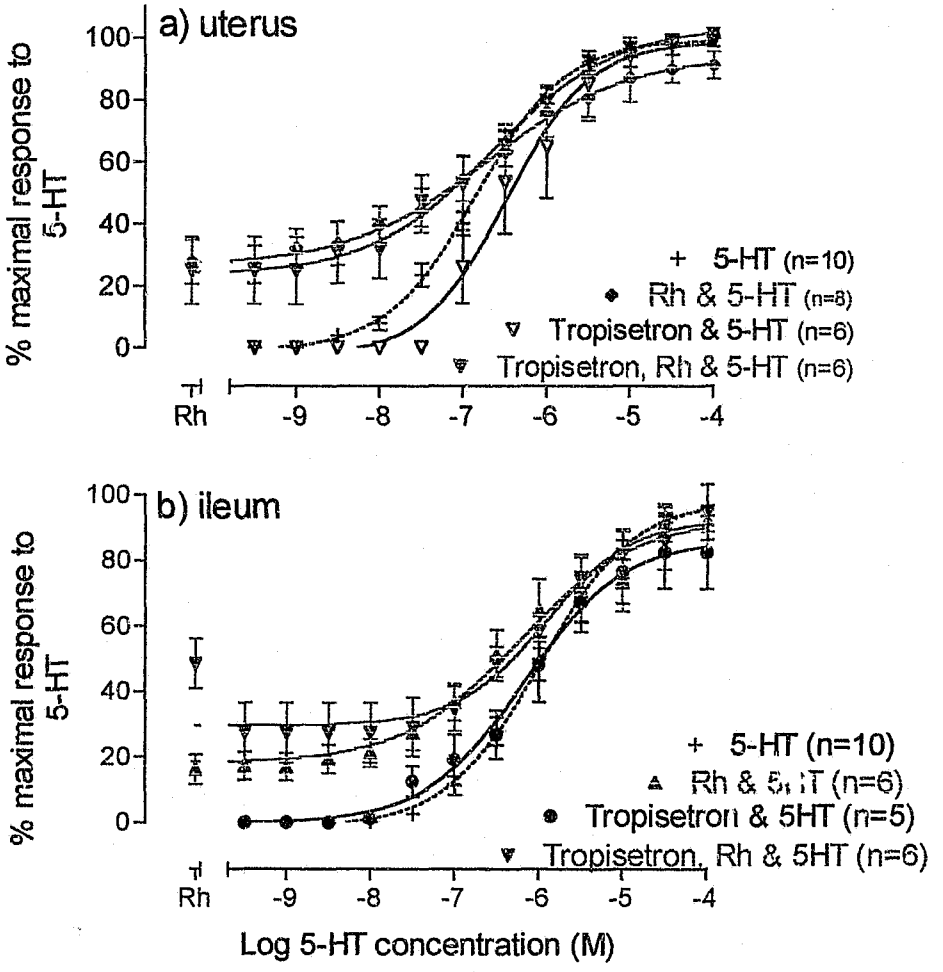


Figure 4.13 The effect of tropisetron, a 5-HT_2 receptor subtype antagonist, on the isolated rat a) uterine and b) ileal response to serotonin with or without the pretreatment of the *R. tridentata* extract.

Bar Graphs

The following bar graphs represent the direct response of the isolated organs to the plant extract alone, or to the plant extract after the organ had been exposed to an antagonist, i.e. the bars represent the first point on the dose response curves above. The error bars in all the bar charts represent the standard deviation from the mean, unlike earlier graphs which gave the standard error of the mean.

4.4.6. Ganglion and second messenger blockers

Hexamethonium (1.4μM) a ganglionic blocker, the nicotinic antagonist mecamylamine (2μM) and cholera toxin (1μM) a G_{α}-protein inhibitor all did not alter the response to the extract. Pertussis toxin (50μM) slightly inhibited the response to the extract but not significantly (Figure 4.14). Micromolar concentrations of these antagonists have been shown to block contractile responses mediated by stimulation of nicotinic receptors in many studies using isolated organs (Barthó *et al.* 1987; Okamura *et al.* 1995; Hopkins *et al.* 1996).

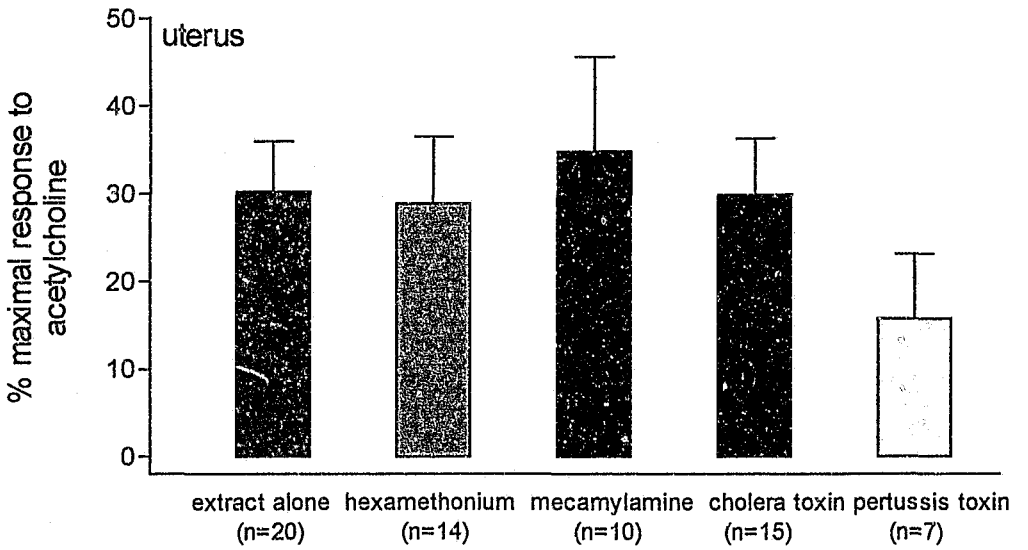


Figure 4.14 Bar graphs showing the maximal uterine responses to 1.3mg/ml *R. tridentata* extract after the organs had been pretreated with the antagonists below the x-axis.

4.4.7. Summary of results of non-specific antagonists

The direct contractile response of the isolated rat uterus to the *R. tridentata* extract was blocked by both the muscarinic blocker atropine (0.4nM) and the cyclooxygenase inhibitor indomethacin (5μM). The 5-HT antagonist methysergide (1μM), the α_1-adrenoceptor blocker prazosin (2μM), and the histamine antagonist pyrilamine (8μM) all had no effect on the direct activity of the extract (Figure 4.15). These antagonists have been shown to block the reference agonists in figures 4.6 to 4.9.

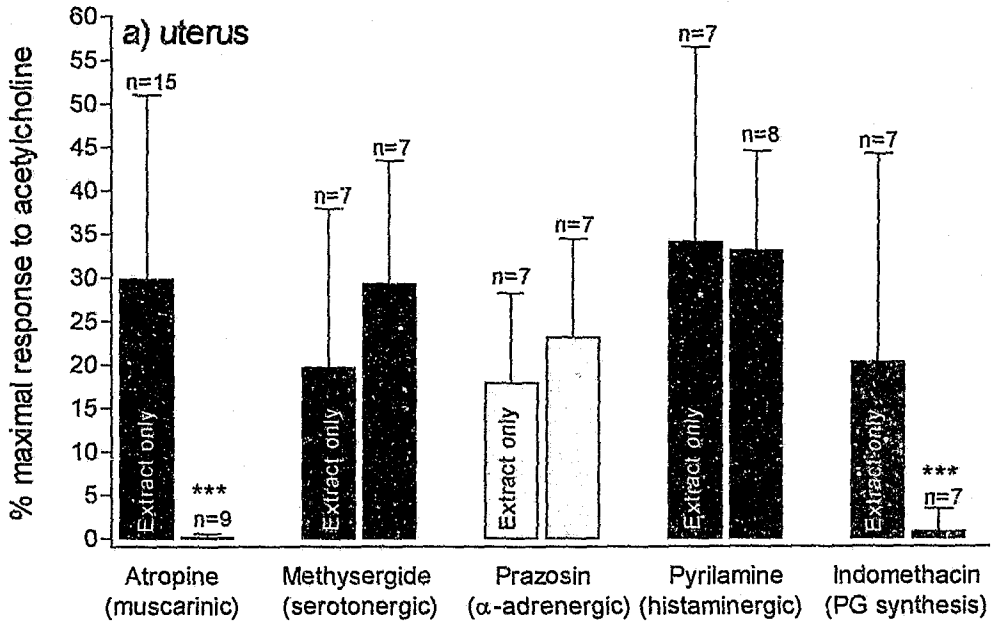


Figure 4.15 The bar graphs showing the mean maximal uterine responses to 1.3mg/ml *R. tridentata* extract after the organs had been pretreated with the antagonists given below the x-axis. The response to the extract alone is charted next to the response to the extract after pretreatment with the relevant antagonist. The stars represent significant differences from the contractile response to *R. tridentata* extract alone (*** = $p < 0.001$).

As with the uterus, the direct activity of the *R. tridentata* extract on the isolated rat ileum was blocked by both the muscarinic blocker atropine (0.4nM) and the cyclooxygenase inhibitor indomethacin (5μM) but not the 5-HT antagonist methysergide (1μM). (Figure 4.16).

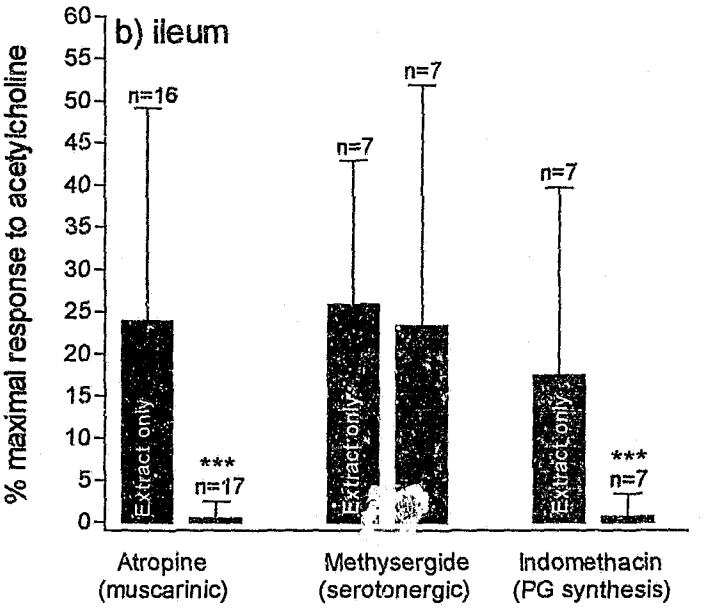


Figure 4.16 Bar graphs showing the mean maximal ileal responses to 1.3mg/ml *R. tridentata* extract after the organs had been pretreated with the antagonists below the x-axis. The stars represent significant differences from the contractile response to *R. tridentata* extract alone (*** = $p < 0.001$).

4.4.8. Prostaglandin synthesis inhibitors

Indomethacin, which inhibits both COX-1 and COX-2, almost completely blocked the contractile activity of the *R. tridentata* extract, whereas 1μM NS-398, a COX-2 selective inhibitor, reduced the contractile activity of the extract ($p<0.01$).

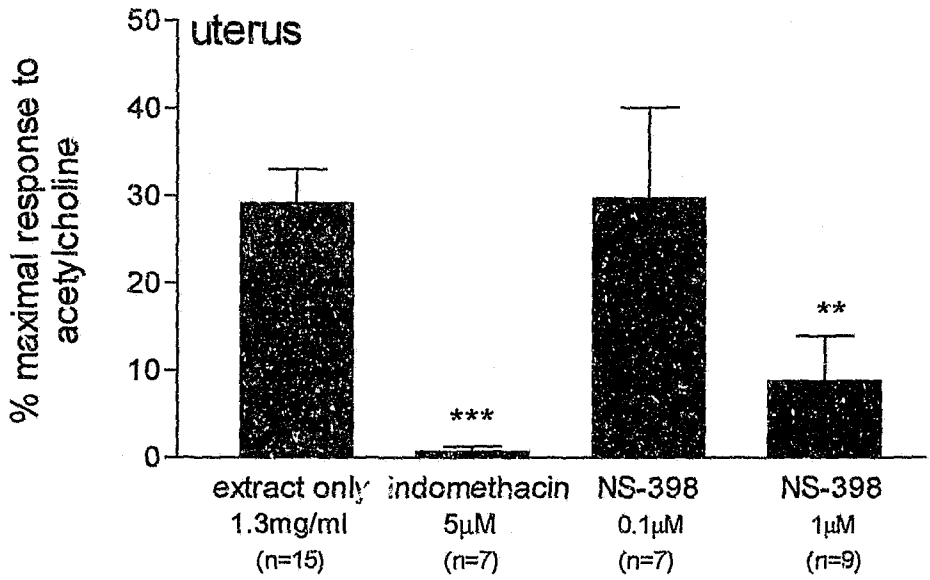


Figure 4.17 Bar graphs showing the mean maximal uterine responses to 1.3mg/ml *R. tridentata* extract after the organs had been pretreated by various cyclooxygenase inhibitors. The error bars represent the standard deviation from the mean. The stars represent significant differences from the contractile response to *R. tridentata* extract alone (*** = $p< 0.001$, **= $p<0.01$).

4.4.9. α-adrenoceptor antagonists

Pretreatment of uterine tissue with the α-adrenoceptor antagonists, prazosin and yohimbine did not alter the response to the *Rhoicissus* extract.

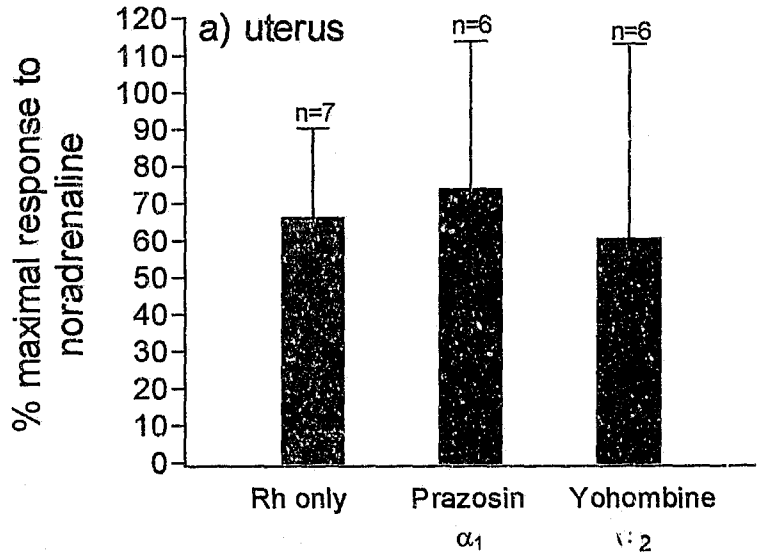


Figure 4.18 The bar graphs show the mean maximal uterine responses to 1.3mg/ml *R. tridentata* extract after the organs had been pretreated by prazosin and yohimbine. The error bars represent the standard deviation from the mean. The stars represent significant differences from the contractile response to *R. tridentata* extract alone (*** = p< 0.001, **= p<0.01).

4.4.10. Muscarinic receptor antagonists

Pretreatment of uterine tissue with the non-specific muscarinic antagonist, atropine, blocked the uterine response to the *R. tridentata* extract, whereas the antagonists on muscarinic subtypes M_1 and M_2, pirenzepine and gallamine respectively, did not inhibit the uterine response to the extract. 4-DAMP, an irreversible muscarinic antagonist specific for M_3 receptors, reduced the contractile response to the *Rhoicissus* extract. Tropicamide, a M_4 receptor antagonist blocked the contractile action of the plant extract.

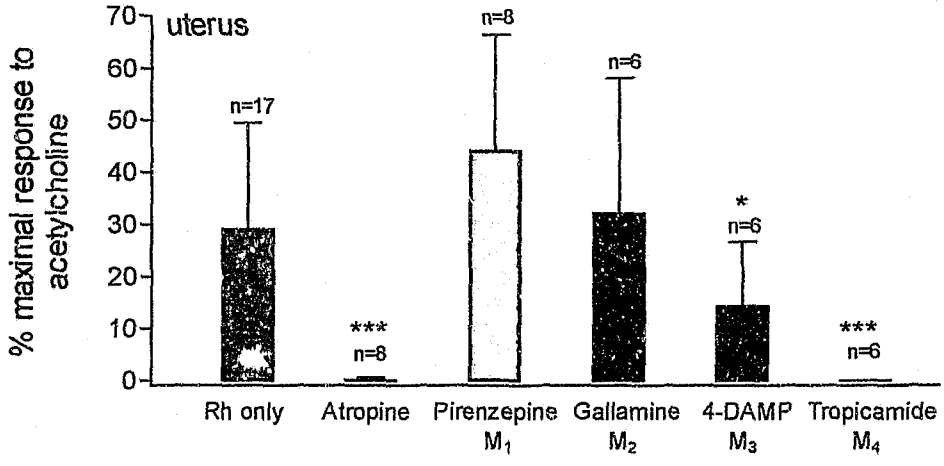


Figure 4.19 The bar graphs show the mean maximal uterine responses to 1.3mg/ml *R. tridentata* extract after the organs had been pretreated by various muscarinic antagonists. The stars represent significant differences from the contractile response to *R. tridentata* extract alone (*** = $p < 0.001$, * = $p < 0.05$).

4.4.11. Serotonin receptor antagonists

Pretreatment of both uterine and ileal tissue with the serotonergic receptor antagonists, methysergide, ketanserin and tropisetron did not alter the response to the *Rhoicissus* extract.

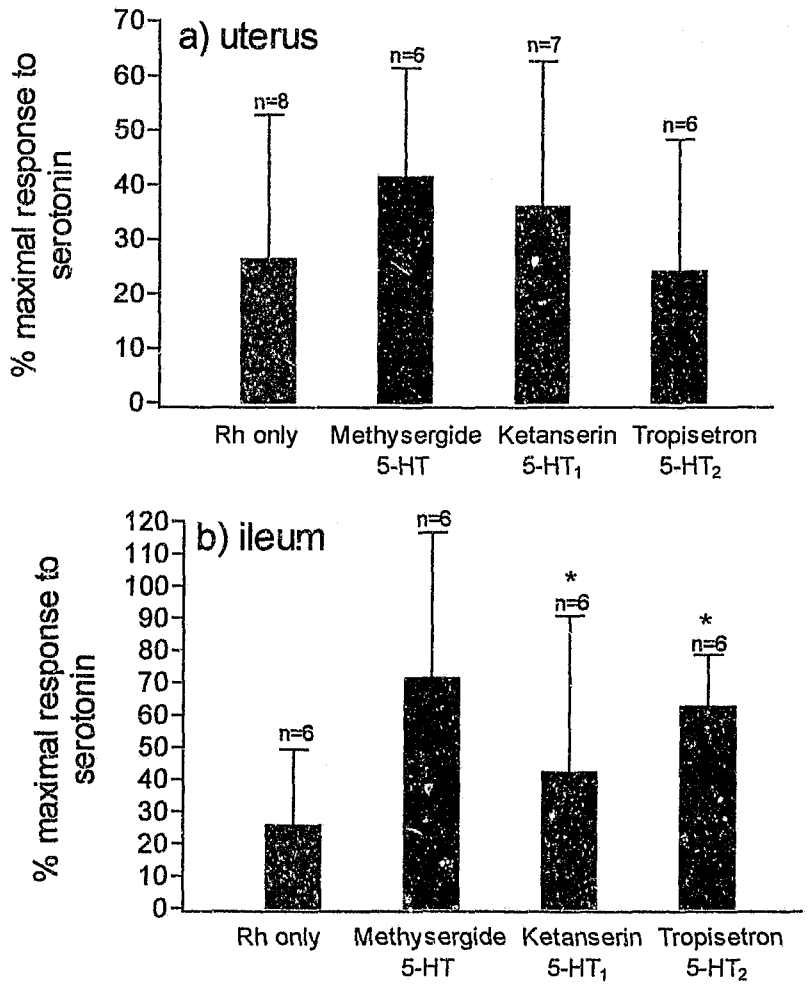


Figure 4.20 Bar graphs showing the mean maximal responses to 1.3mg/ml *R. tridentata* extract after the organs had been pretreated with various serotonin antagonists. The stars represent significant differences from the contractile response to *R. tridentata* extract alone (* = $p < 0.05$).

4.4.12. The effect of *R. tridentata* on the basal tone of the uterus

As mentioned at the start of this chapter, *Rhoicissus* extracts increase the basal tone of the uterus. This effect was apparent after the organs had been rinsed at the end of a test challenge that included incubating the uterus with the plant extract. The plant did not alter the basal tone of the ileum. The extent to which the basal tone is increased was not correlated to the extent to which the extract stimulated contractions, as shown in figure 4.21.

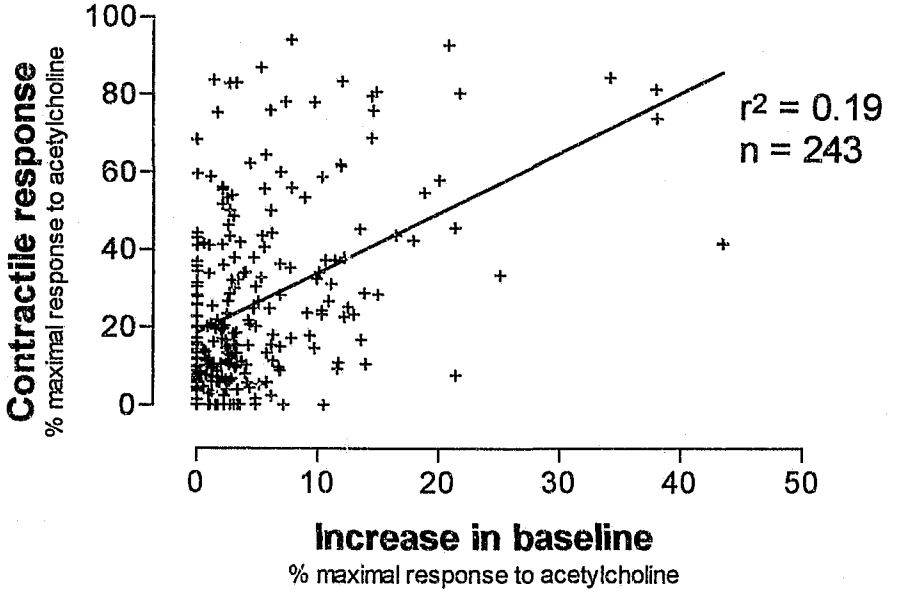


Figure 4.21 The correlation between the contractions of the isolated rat uterus stimulated by 1.3 mg/ml *R. tridentata* extract and the extent to which the baseline was raised after the organ had been rinsed and allowed to return to its resting state.

CHAPTER 5: Results Section 3

5. TEMPORAL AND SPATIAL VARIATION IN THE EXTENT OF CONTRACTILE RESPONSE INDUCED BY *R. TRIDENTATA*

Investigations on the factors influencing the contractile activity were done *using uterine* tissue. The same maximum dose of *R. tridentata* extract (*1.3mg/ml*) was given in all cases. Results obtained from extracts made from *Rhoicissus* plant material harvested during different seasons indicated that the contractile activity of the plant extracts is affected by the timing of harvesting. The results are arranged on the axes from two different perspectives. The first set of graphs (Figure 5.1) illustrate the extreme reactions from different parts of the plant showing the effect of seasonal differences in the timing of harvesting. The second set of graphs (Figure 5.2) is drawn from the same data, but the data are arranged according to seasons to show the differences resulting from the use of different plant parts.

5.1. Seasonal variation in the contractile activity of extracts from different plant parts of *Rhoicissus tridentata.*

Tuber extracts: The extracts responsible for stimulating the largest contractions were obtained from ne tubers harvested in summer and autumn whereas the extracts from tubers harvested during winter or spring displayed the lowest direct activity (Figures 5.1). At a 95% confidence level, the summer tubers were more active than the spring and winter tubers, and the autumn tubers were more active than the winter tubers. The uterine response to maximal concentrations of acetylcholine was not significantly increased by the summer, autumn and winter tuber extracts. The spring tuber extracts, however, increased the maximal responses significantly (13% increase, $P<0.05$).

Root extracts: The extracts of roots harvested in summer stimulated the largest contractions (27.2% relative to acetylcholine), whereas the extracts from roots harvested in autumn and spring stimulated the smallest contractions (6.6% and 5.5% relative to acetylcholine) (Figures 5.1). This is different from the pattern seen in the tuber extracts, where the autumn tubers yielded the most active extracts. The winter extracts gave a response between that of the summer and spring extracts. At a 95% confidence level, the contractions stimulated by the summer root extracts were significantly higher than the those stimulated by the spring and autumn root extracts, and the results from the winter extracts were higher than those from the autumn extracts. The maximal uterine response to acetylcholine was not increased by any roots extract, but the autumn root extract depressed the maximal response by 15% ($P < 0.01$).

Stem extracts: Summer stem extracts elicited the greatest contractile response (36.5%) whereas the smallest response was to the spring extract (8.7%) (Figure 5.1). The autumn and winter extracts gave similar initial responses which were midway between the responses to the summer and spring extracts. The only significant differences were between the summer and spring extracts ($P < 0.001$). The summer stem extract was the only stem extract to increase the maximal response to acetylcholine (112% relative to acetylcholine) ($P< 0.05$).

Leaf extracts: Since the plant is deciduous, leaves were only available in summer and autumn. Extracts from these two harvests directly stimulated contractions equal in size (summer: 18.1%; winter: 18.4%), but the effects on the maximal response to acetylcholine differed. The summer extract increased the maximal response to acetylcholine (106.3%) whereas the autumn extracts inhibited the maximal response (88.6%; $P<0.05$) (Figure 5.1).

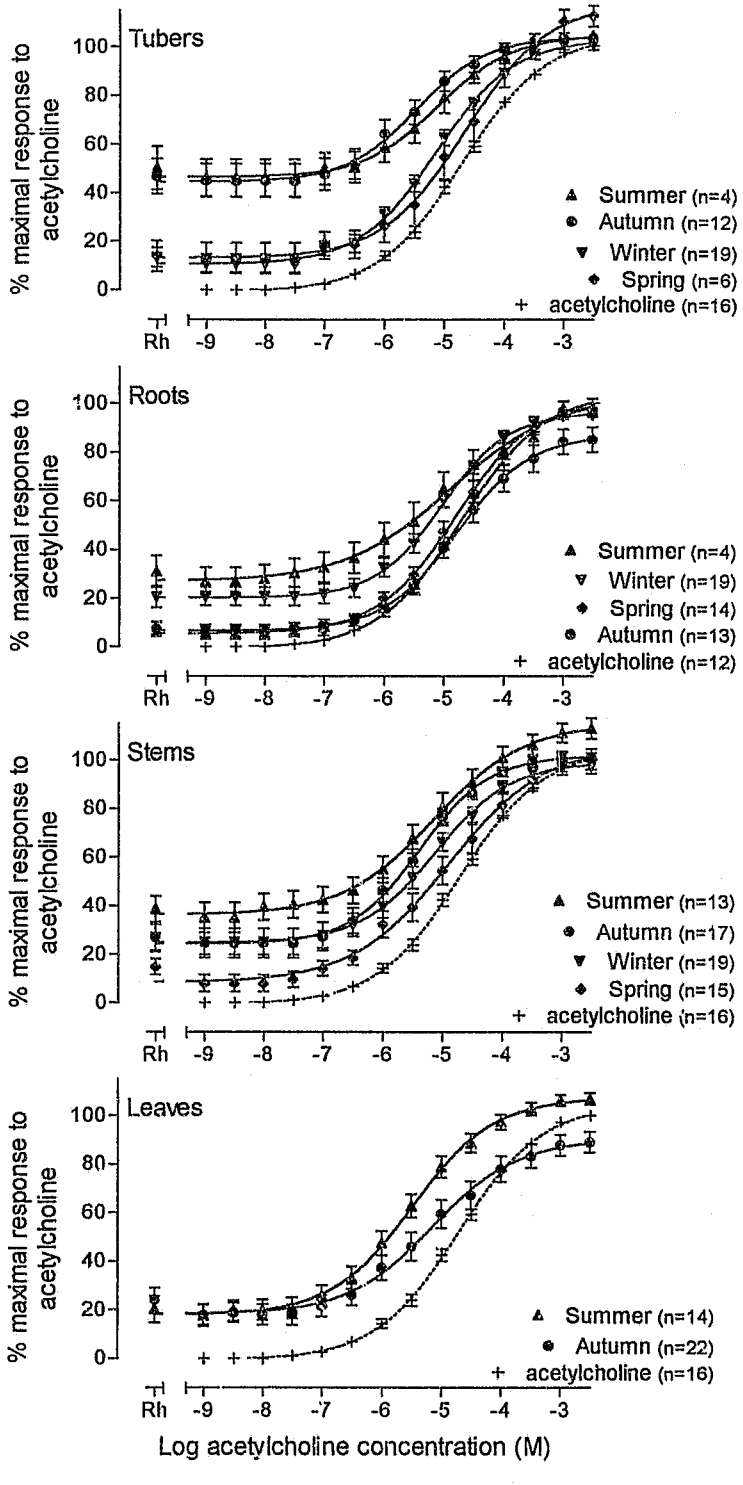


Figure 5.1. Dose response curves of isolated rat uterus when pre-treated with 1.3 mg/ml aqueous extracts of *Rhoicissus tridentata* showing the seasonal variation in the contractile response to different plant parts.

5.2. Variation in the contractile activity of extracts from different plant parts of *Rhoicissus tridentata.*

Spring extracts: Extracts from all parts of the plants harvested during spring displayed similar direct contractile activity (Figure 5.2). The stem and root extracts did not alter the maximal response to acetylcholine whereas the tuber extracts increased the maximal response (113.1%; $P<0.05$).

Summer extracts: The tuber extracts were the most potent, followed by the stems, and then the roots, with the leaf extracts being the least potent (Figure 5.2). The leaf extracts were significantly less active than the tuber and stem extracts ($P< 0.05$). Stem and leaf extracts increased the response to maximal concentrations of acetylcholine ($P<0.05$).

Autumn extracts: The most potent extract again was obtained from the tubers and the least potent extract was obtained from the roots (Figure 5.2). The direct contractile response to the stem, leaf and fruit extracts was similar, which was midway between the initial responses to the tuber and root extracts. The tuber extracts were significantly more active than the root (99.9% confidence level), stem and leaf extracts (95% confidence level), and the stems were more active than the roots (95% confidence level). The maximal response to acetylcholine was unchanged by the tuber and stem extracts but depressed by the root and leaf extracts (95% confidence level).

Winter extracts: As in the case of the response to the spring extracts, the initial responses to the stem, root and tuber extracts were similar. Root extracts depressed the maximal response to acetylcholine slightly ($P<0.01$).

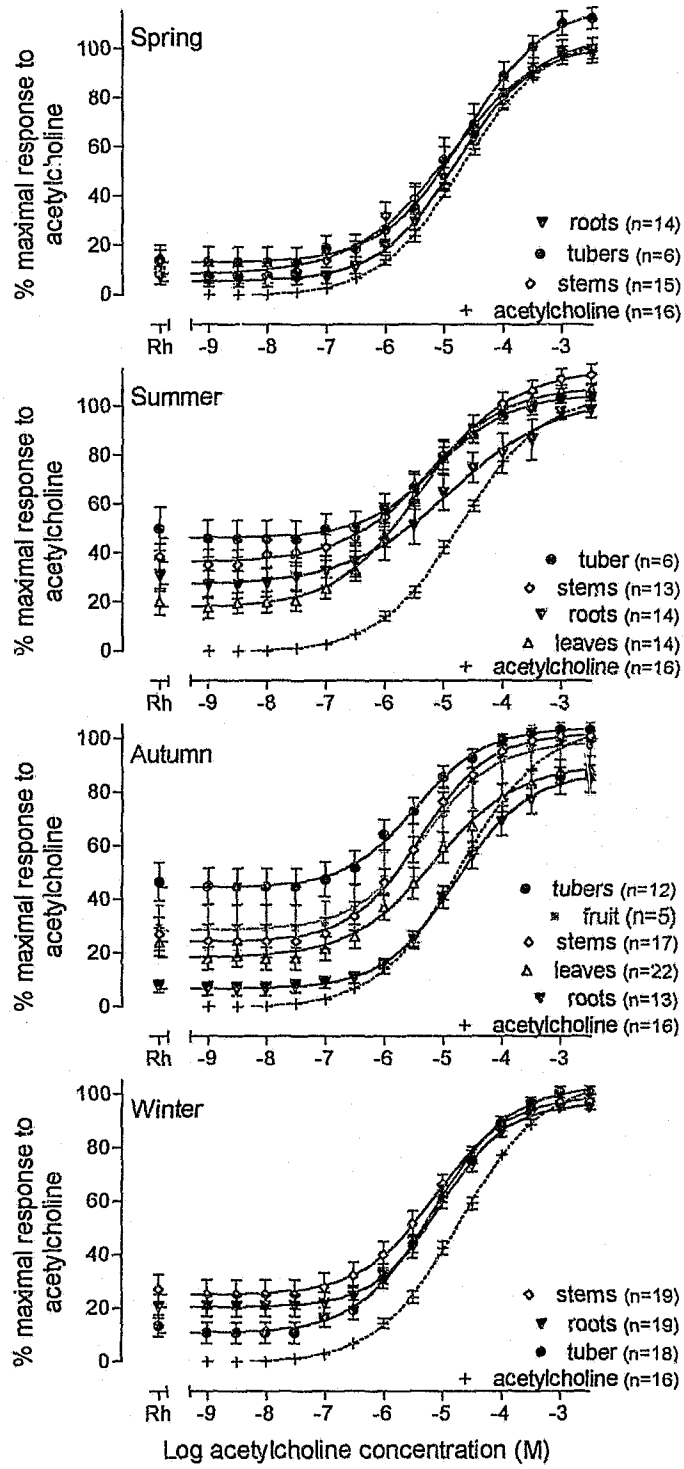


Figure 5.2. The effect of extracts of different parts of the plants harvested during the four seasons.

5.3. Box and whisker plots of range of seasonal data

The data range of the direct contractile activity stimulated by the tuber extracts is shown in figure 5.3. These graphs illustrate the wide range of responses to a single extract. The response to the crude autumn tuber extract ranged from 13.5% to 93.5% of the maximal response to pure acetylcholine.

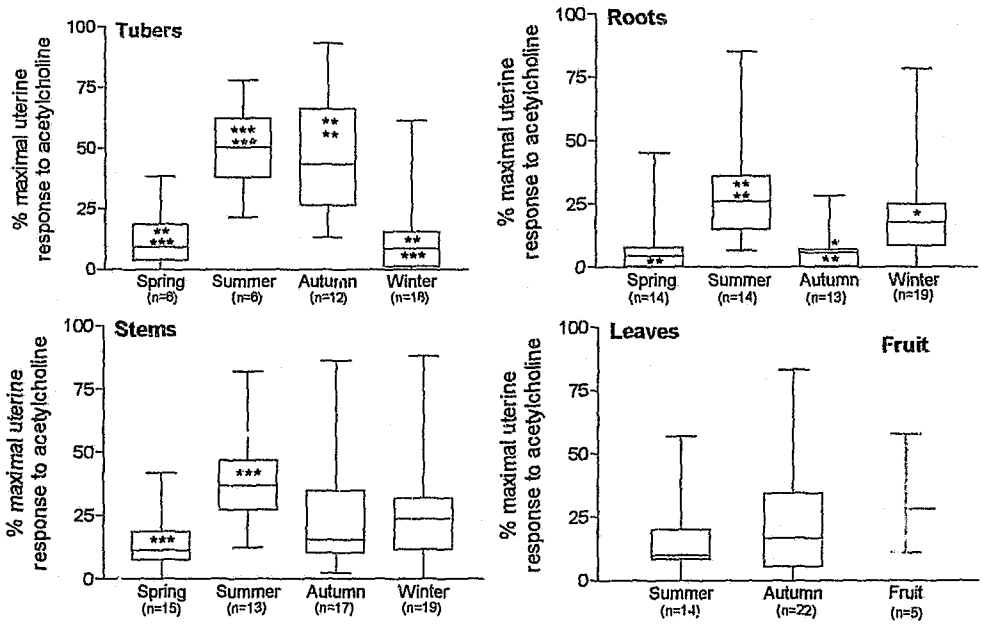


Figure 5.3. Box and whisker plots of the maximal direct contractile responses to 1.3 mg/ml extracts from *R. tridentata* harvested during different seasons. The box extends from the 25th to the 75th percentile, with the horizontal line at the median. The whiskers show the range of the data. The colour of the stars indicates the season significantly different from the data set.

Table 5.1 Statistical differences between the contractile activity of extracts from *Rhoicissus* from different plant parts harvested in different seasons.

Tubers		**Roots**	
spring and summer	**	spring and summer	**
spring and autumn	***	summer and autumn	**
summer and winter	***	autumn and winter	*
autumn and winter	**	**Stems**	
		spring and summer	***

Key: * = p<0.05, ** = p<0.01, *** = p<0.001

5.4. Seasonal variation in the increase in basal tone of isolated rat uterus.

It was shown in chapter 3 that there was no correlation between the contractile response to a *Rhoicissus* extract and the extent to which the basal tone of the uterus was increased. On the other hand Figure 5.4 shows that there are significant differences in the increase in basal tone between extracts from plant material harvested during different seasons. When all part plants are meaned the winter and summer extracts increase the basal tone to a higher extent than the spring extract do ($P<0.001$), and the winter extracts more than the autumn extracts ($P < 0.05$).

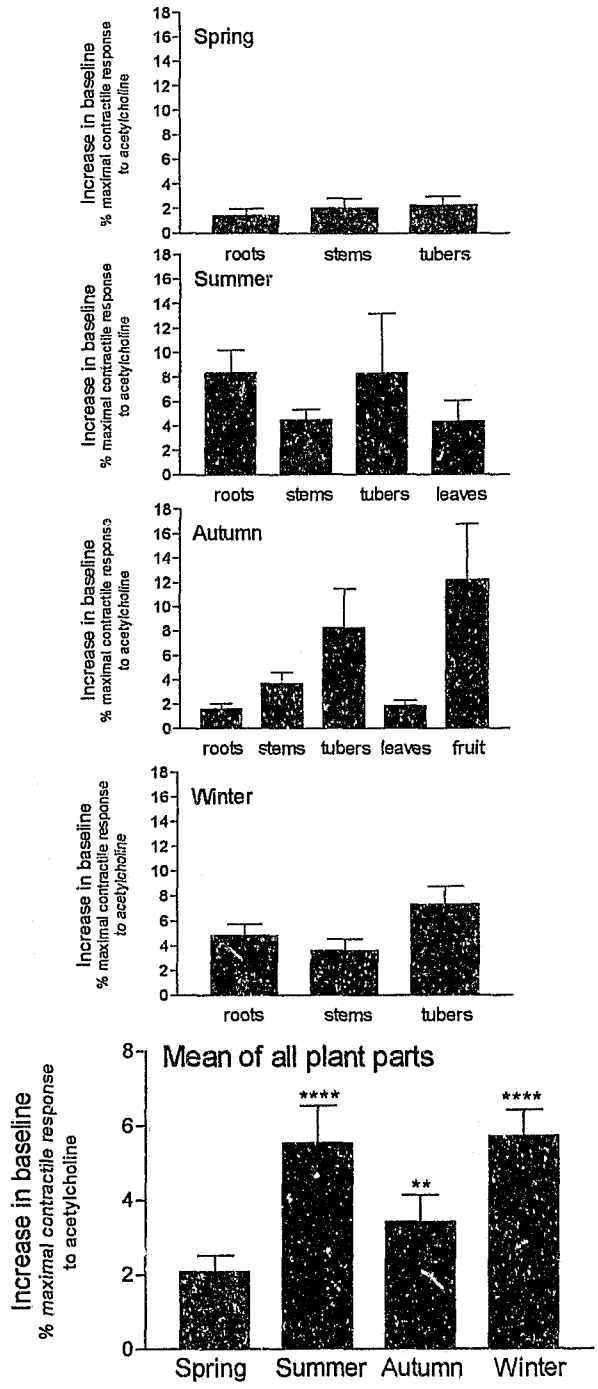


Figure 5.4. Variation in the increase in baseline (mean lowest point) once the organs had been rinsed at least five times after a test challenge which included 1.3 mg/ml *R. tridentata.* The stars represent significant differences where the colour of the stars indicates the data set from which that specific data set is different (e.g. the data from the summer and autumn extracts are significantly different from the data from spring extracts).

5.5. Distributional variation

The results show that there is a variation in the pharmacological response to aqueous extracts from *R.tridentata* stems and roots harvested from different localities (Figure 5.5). All the extracts stimulated contractions, but the magnitude of these contractions varied. The effect on the maximal response to acetylcholine is where the interesting differences occurred. The extract from the plant harvested in Mondeor was the only extract to shift the dose response curve to the right and inhibit the response to maximal concentrations of acetylcholine, i.e. most of the extracts from *R. tridentata* appeared to act as agonists whereas the extract from Mondeor had results similar to those expected from an antagonist. The Mondeor extract seemed to compete with acetylcholine in a non-competitive manner. This inhibition of acetylcholine was negated by rinsing the bath, suggesting that the inhibition is reversible.

The possibility of misidentification of the Mondeor sample was investigated by rechecking the herbarium specimens. The identification appeared to be correct. The only apparent morphological difference was that the stem lengths between the leaf axils were shorter than in other specimens, giving the specimen a much denser appearance.

Extracts from *R. tridentata* roots and stems harvested from Suikerbosrand during summer were previously shown to have similar contractile activity (Figure 5.2). The root extract from the plant harvested at Umlazi gave a response similar to that of extracts from stems harvested at Suikerbosrand. However, the roots harvested near Durban yielded an extract that stimulated contractions significantly higher than the contractions stimulated by the Suikerbosrand stem extracts.

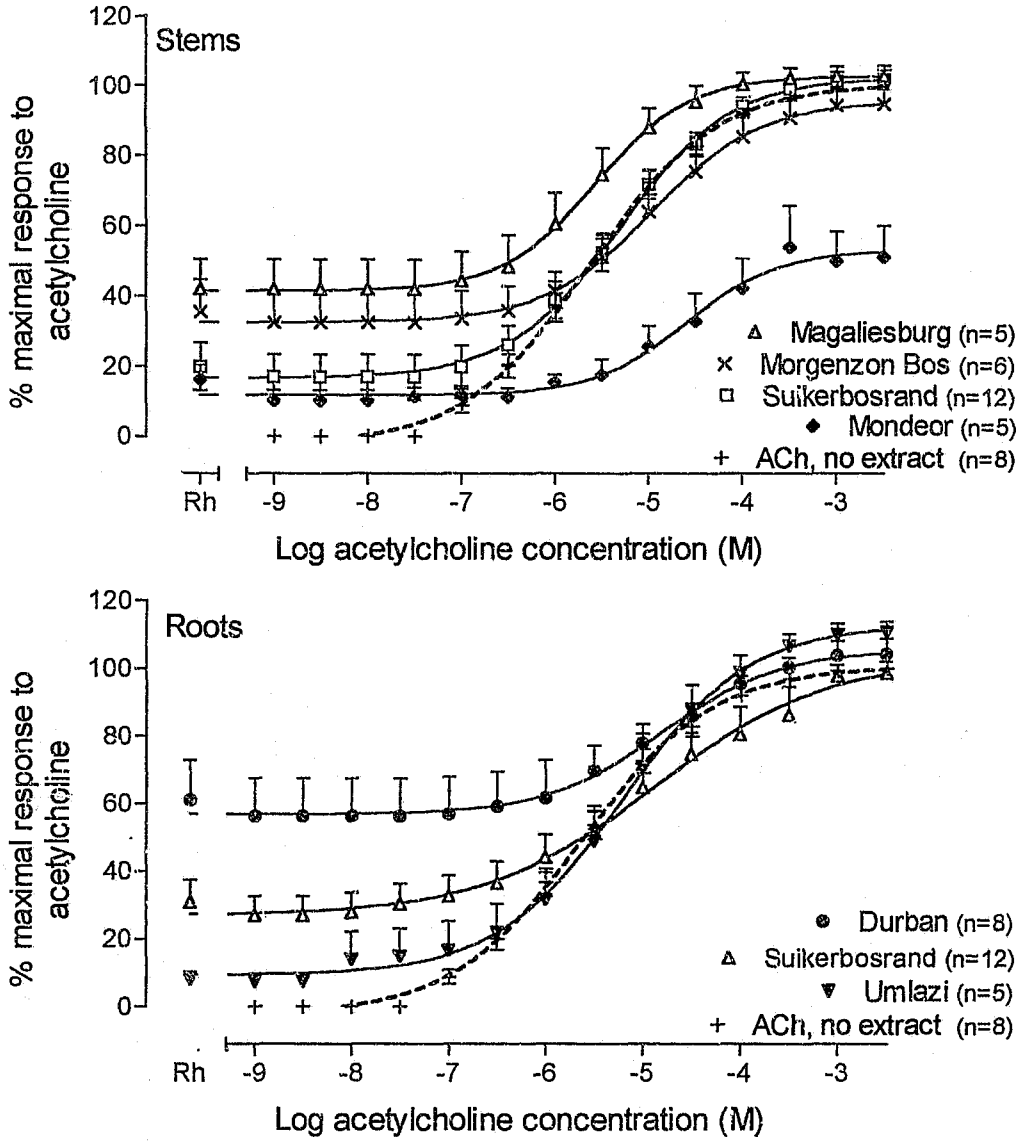


Figure 5.5. The contractile response of isolated uterine smooth muscle to aqueous extracts of *R. tridentata* stems and roots harvested from different localities around South Africa. The tissues were incubated with 1.3 mg/ml crude aqueous extracts for 5 minutes before the cumulative addition of acetylcholine.

5.6. The effect of storing dried plant material

The contractile responses o *Rhoicissus* stem and tuber extracts that had been stored for either one or twelve months yielded identical contractile responses from isolated rat uterus (Figure 5.6). The extracts stored for one month or twelve months directly stimulated uterine contractions 27% ± 4 and 26% ± 5 (95% CI) respectively, relative to the maximal contractions to acetylcholine. Neither of the extracts altered the response to maximal concentrations of acetylcholine.

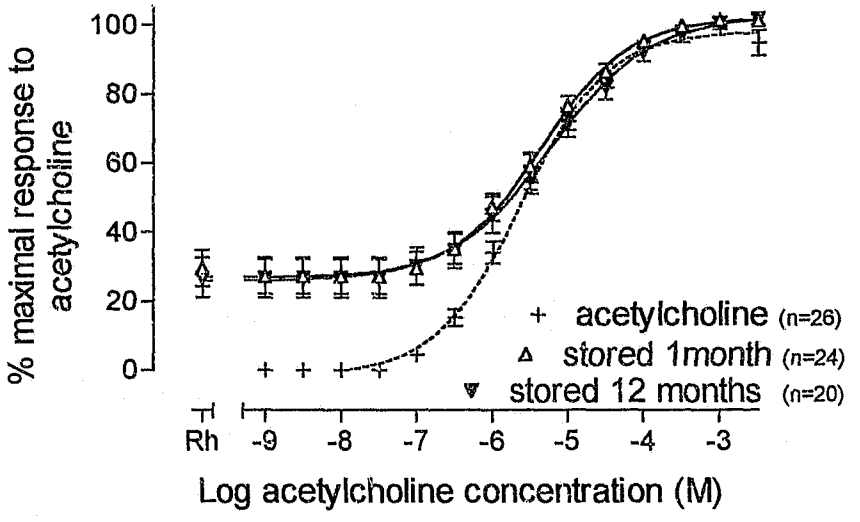


Figure 5.6. Uterine contractile response to *R. tridentata* extracts from plant material that had been stored for 1 month (Δ) compared to extracts from plant material that had been stored for 12 months (▼). The tissues were incubated with 1.3 mg/ml of plant extract before the cumulative addition of acetylcholine.

CHAPTER 6: Results section 4

RESULTS OBTAINED FROM CELL CULTURE EXPERIMENTS

6.1. Effect of *R. tridentata* Extracts on Prostaglandin Synthesis in Histiocytoma Cell

As mentioned earlier, it would have been preferable to use a uterine cell line for these following experiments, but no uterine cell line was available that had been shown to produce prostaglandins at that time.

Extensive literature searches and discussions with scientists from all over the world could not reveal in what time span prostaglandins were synthesized. Therefore, the first step was to determine what incubation time should be used to detect the maximum levels of prostaglandins. Cells were incubated with 1 mg/ml of plant extract, compared to 1.3 mg/ml used in the isolated organ experiments.

6.1.1. Prostaglandin synthesis over time

Once challenged with *R. tridentata* extract, the histiocytoma cells start excreting prostaglandins into the cell medium almost immediately. The levels of extracellular prostaglandin E_2 reach a maximum at 15 to 20 minutes, after which there is a rapid drop in the extracellular concentrations of the prostaglandins (Figure 6.1).

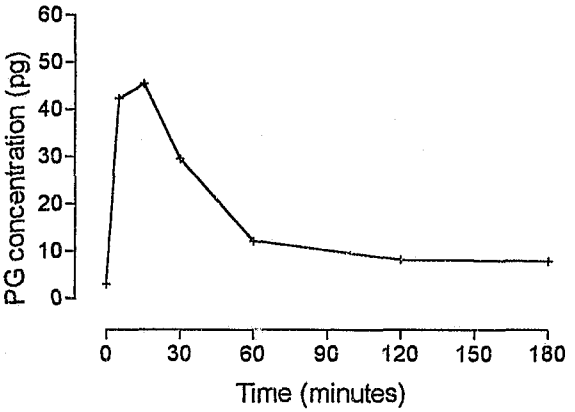


Figure 6.1. Prostaglandin E_2 synthesis over time by histiocytoma cells stimulated with 1 mg/ml *Rhoicissus* extract.

6.1.2. Dose-response curves of the effect of *R. tridentata* on prostaglandin synthesis by histiocytoma cells

R. tridentata extracts only stimulated minimal amounts of prostaglandin E_2 synthesis at plant extract concentrations of 0.1 µg/ml to 0.1mg/ml, whereas 1mg/ml caused a 7.8 fold increase in the amount of extracellular prostaglandins compared to untreated cells (Figure 6.2). This significant increase in prostaglandin synthesis was not evident in intracellular prostaglandin E_2.

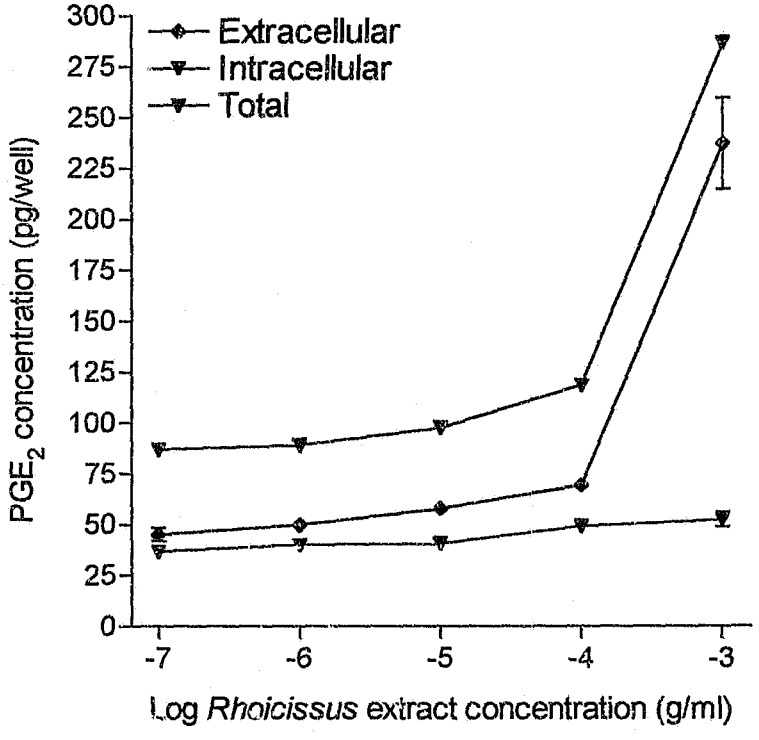


Figure 6.2. Dose response curve of intracellular, extracellular and total prostaglandin concentrations after histiocytoma cells were incubated with varying concentrations of four different *Rhoicissus* extracts for 20 minutes.

6.2. Toxicity assays

6.2.1. Parameters for the MTT Assays

Different numbers of human histiocytoma cells were incubated with MTT for four hours, without the addition of any plant extract to determine what would be the optimal number of cells to have per well. Figure 6.3 shows that the absorbance at three wavelengths, 405nm, 540nm and 620nm, all increased exponentially between 690 and 5 500 cells per well. The absorbance tapers off, increasing linearly at a cell concentration above 5 500. The vertical dotted line marks 13 500 cells per well which is the concentration of cells used for the MTT assays for all cell lines.

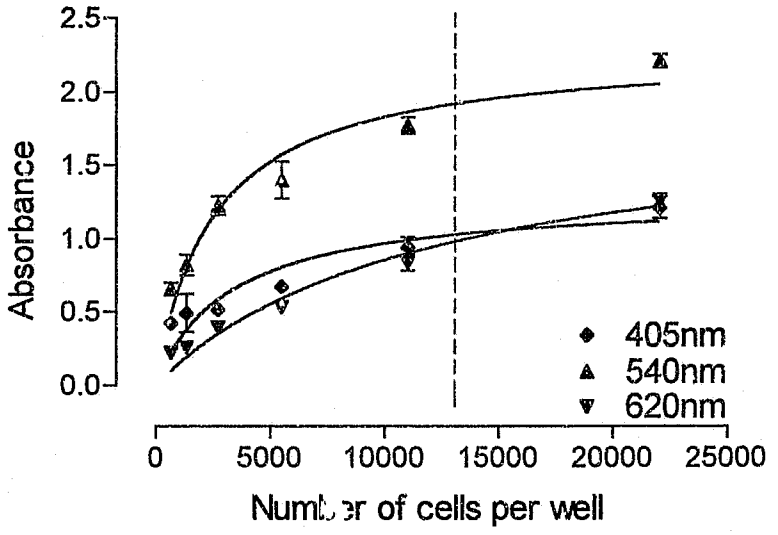


Figure 6.3. The optical density at different wavelengths corresponding to different concentrations of histiocytoma cells per well.

The absorbance spectrum from 340nm to 690nm for the different concentrations of histiocytoma cells per well, which have been incubated with MTT for four hours, is shown in Figure 6.4. The absorbance either peaks or is close to the peak at 540nm for all cell concentrations. Similarly, when 13 500 cells per well are treated

with varying concentrations of *R. tridentata* extract for 24 hours, followed by treatment with MTT for 4 hours, the absorbance also peaks at around 540nm, as indicated by the vertical dotted line (Figure 6.5). Based on the above results, the absorbance was read at 540 nm for all the following MTT assays.

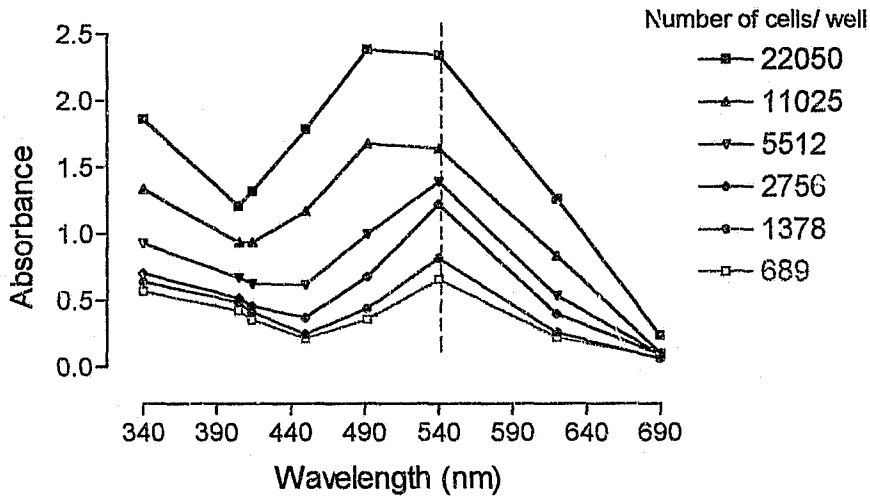


Figure 6.4. The absorbance spectra from wells with different numbers of histiocytoma cells.

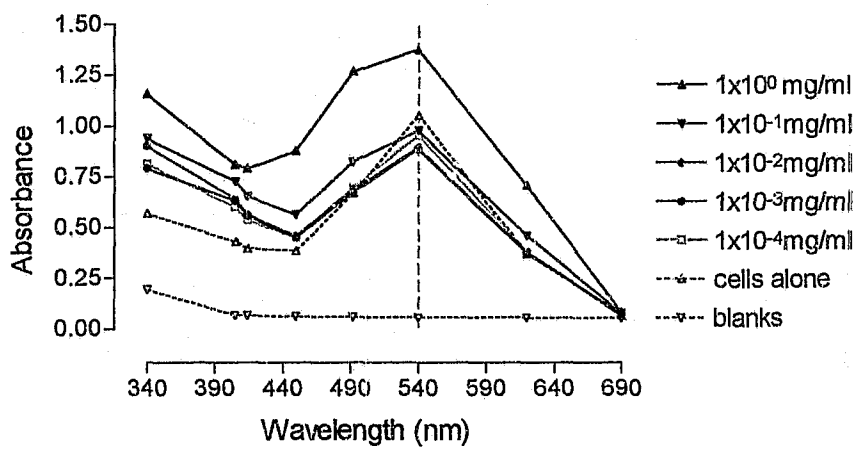


Figure 6.5. The absorbance spectra from wells containing 13 500 histiocytoma cells and treated with different concentrations of *R. tridentata* extract.

6.2.2. Reason for method modifications

Mossman *et al.* (1983) developed the MTT assay for the rapid screening of large numbers of samples. They analyzed their results by subtracting the absorbance at 620nm from the absorbance read at 570nm. The absorbance at 620nm represents the absorbance of the culture media and the sample being tested. The assay was designed to save time by avoiding the removal of media, and therefore they had to account for the background absorbance. In preliminary assays for this study, it was found that removing the culture medium and dissolving the formazan crystals with DMSO yielded far more consistent results, and avoided the step of having to mix the solutions within the wells using a multichannel pipette. Since, in this modified method, all the culture media and plant extract are being removed it was no longer necessary to account for the absorbance caused by these two components. It was therefore decided to represent cellular viability as the absorbance obtained from the test wells in relation to the absorbance obtained from the control wells. The results using the different calculations are given in Figure 6.6. The results from the highest concentrations of plant extract were discarded, as a large proportion of the plant extract lysed the cells as the osmolarity was too high, which accounts for the discrepancies in the results using the different calculations at a plant concentration of Log -2 g/ml (0.01g/ml).

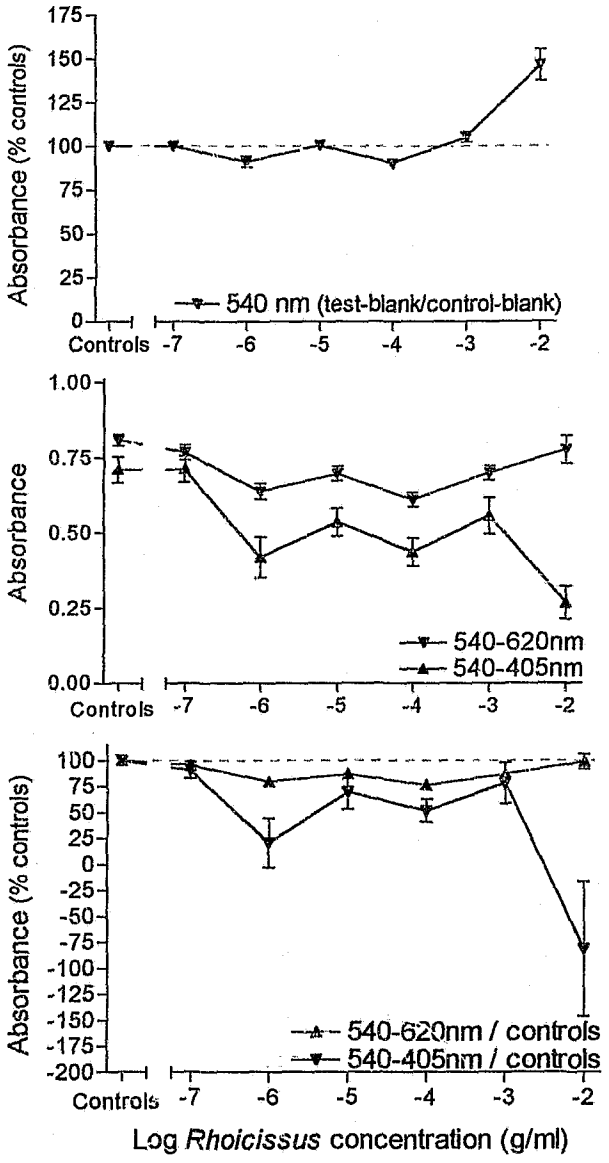


Figure 6.6. Dose response curves of the results from the MTT assays using different calculations to analyze the results. The top graph represents results calculated from the absorbance at 540 nm, where the absorbance from the test wells was calculated as a percentage of the control. The middle graph represents the absorbance of the results, where either the absorbance at 620nm or 405nm is subtracted from the absorbance at 540nm. The bottom graph displays the results in the middle graph as a percentage of the controls.

The curves in the following section represent the results from MTT assays used to measure the cellular viability after the four different cell types were exposed to a range of concentrations of aqueous extracts of *R. tridentata* harvested at Suikerbosrand and Durban. The samples were chosen to represent a wide range of contractile activities. The points on the graphs represent the mean and the error bars represent the standard errors of the mean. Stars on the graphs indicate significant differences between that value and the control (* = $P < 0.05$; ** = $P<0.01$; *** = $P<0.001$).

6.2.3. MTT assay results from different cell lines

The results from the cytotoxicity assays using *R. tridentata* extracts from all plant parts and different seasons, on the four cell lines show that the extracts do not reduce the cellular viability to below 90% relative to the controls in any of the cell lines. On the contrary, the absorbance at 540nm for most of the assays on human kidney, human hepatoma and mouse leydig cells, was above that of the controls for all concentrations of the plant extracts (Figure 6.7). Significant differences between the absorbance of the test wells and that of the controls wells were only evident at the higher concentrations of plant extract in the hepatoma, leydig and histiocytoma cells, but not in the kidney epithelial cells. In the histiocytoma cells, the absorbance of the wells with lower concentrations of plant extract was below that of the control wells. However, the decreases were minimal, the mean absorbance was always above 90% of that of the control wells. The results from student-t tests are given in the appendices (Table A.4).

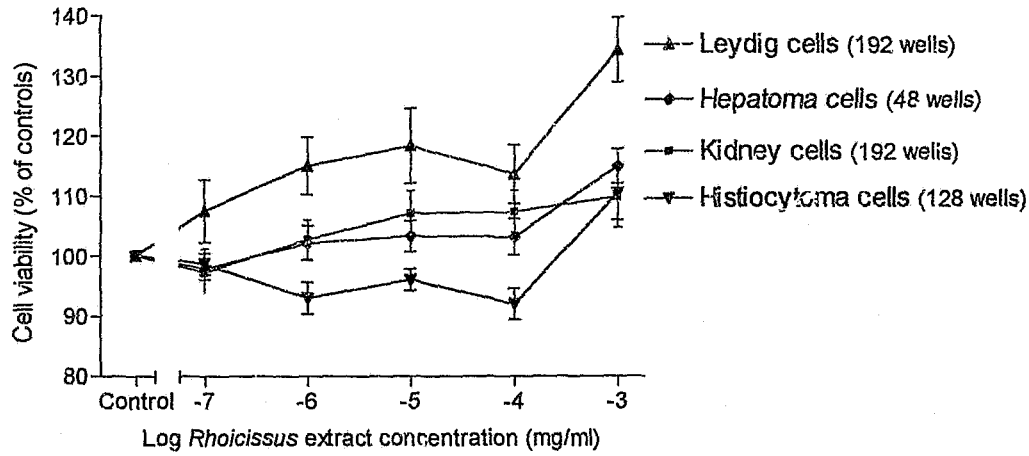


Figure 6.7. Dose response curves showing the cellular viability after the cells were incubated with varying concentrations of *Rhoicissus* extracts for 24 hours. The graph represents the mean results from extracts tested in the number of wells given in the legends. Significant differences between the absorbance of test wells and control wells are shown in later graphs.

6.2.4. *Kidney epithelial cells*

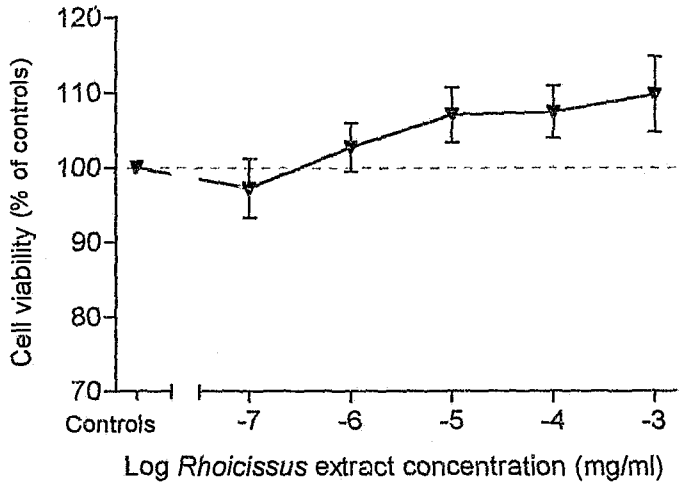


Figure 6.8. Dose response curve of cell viability after being incubated with varying concentrations of *Rhoicissus* extracts for 24 hours. The graph represents the mean results from extracts of 16 different plant samples tested in 12 wells each. No significant differences between the absorbance of test wells and control wells occurred.

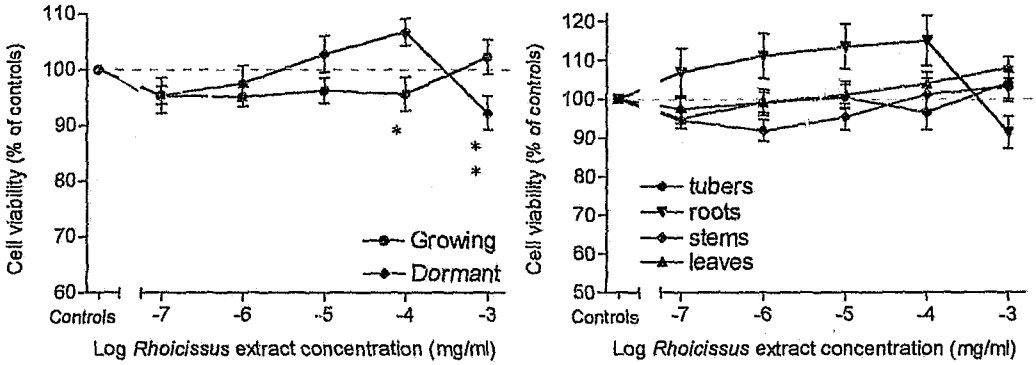


Figure 6.9. The effect of *Rhoicissus* extracts from a) different seasons and b) different plant parts on the viability of human kidney epithelial cells. Stars indicate significant differences between the extracts.

6.2.5. Hepatoma cells

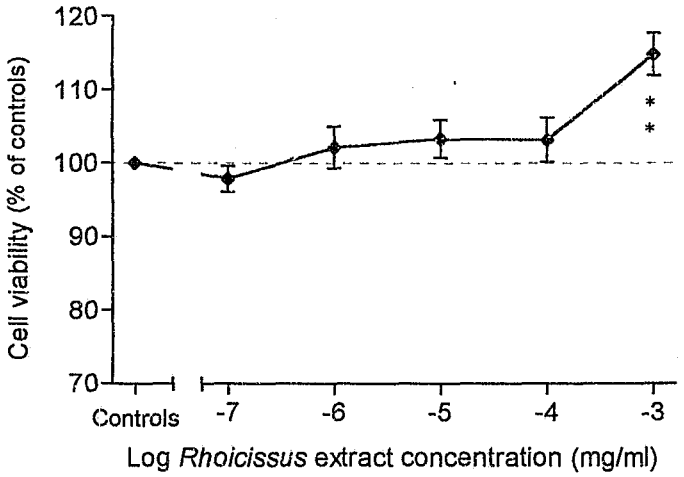


Figure 6.10. The cellular viability of human hepatoma cells after being incubated with aqueous extracts of *Rhoicissus* for 24 hours. The curves represent results from 8 plant extracts tested in 6 wells each. Stars represent significant differences between the absorbance of test and control wells.

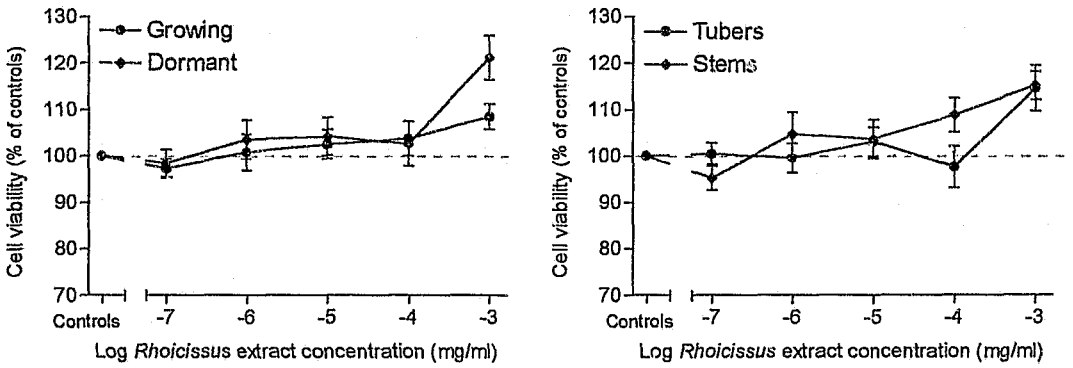


Figure 6.11. Comparisons between the effect of *Rhoicissus* extracts from different a) seasons and different b) plant parts on the viability of human hepatoma cells after being exposed to the extracts for 24 hours. There were no significant differences.

6.2.6. Mouse leydig cells

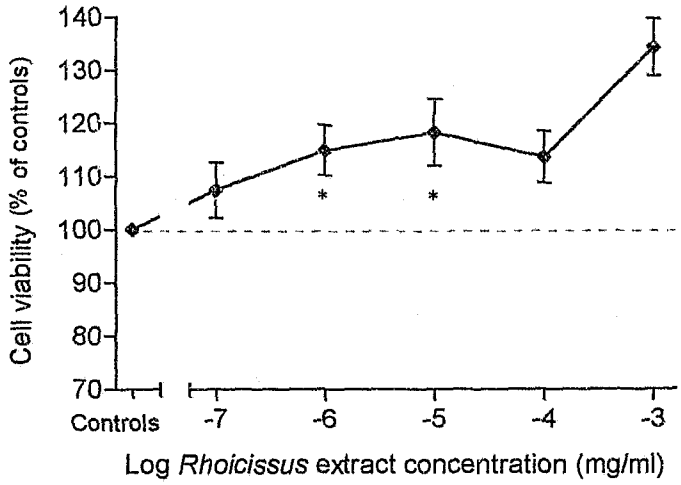


Figure 6.12. Results from the MTT assay testing the effect of *Rhoicissus* aqueous extracts on the viability of mouse leydig cells after being exposed to the extracts for 24 hours. Each point represents the mean of extracts from 16 plant samples each tested in 12 wells.

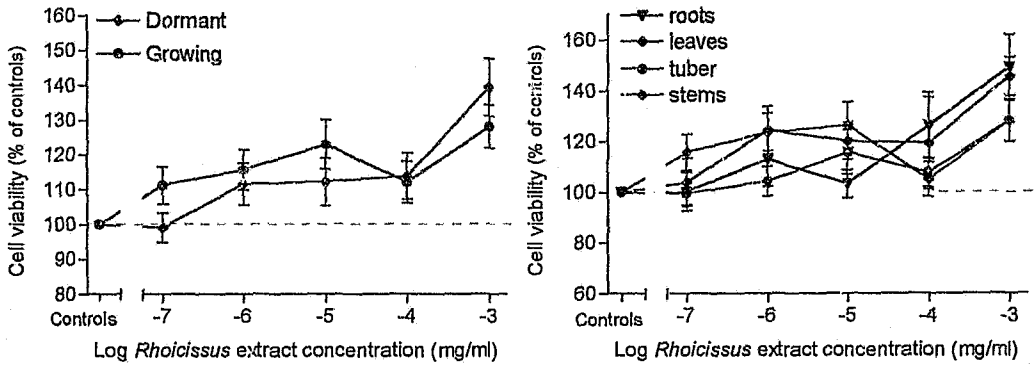


Figure 6.13. Comparisons between the effect of *Rhoicissus* extracts from different a) seasons and different b) plant parts on the viability of mouse leydig cells after being exposed to the plant extracts for 24 hours.

6.2.7. Human histiocytoma cells

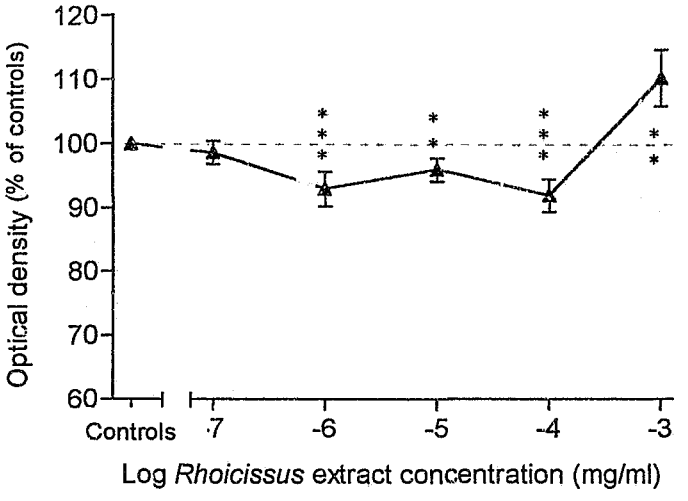


Figure 6.14. Results from the MTT assay testing the effect of *Rhoicissus* aqueous extracts on the viability of human histioctytoma cells after being exposed to the extracts for 24 hours. Each point represents the mean of extracts from 16 plant samples each tested in 8 wells.

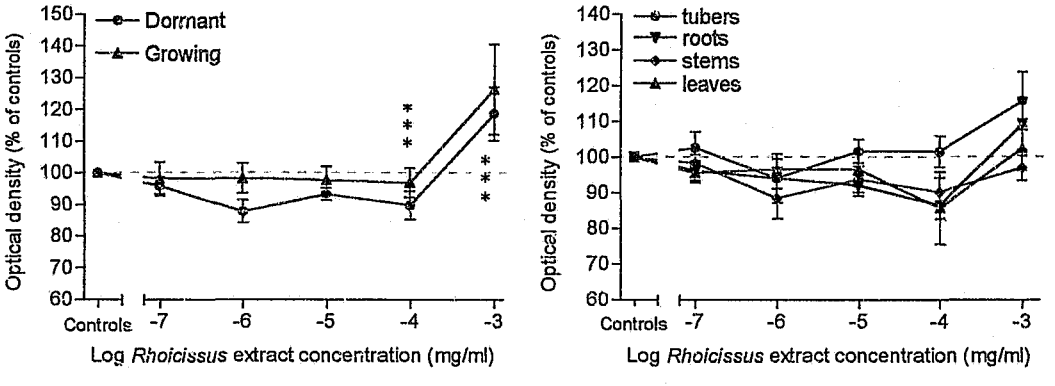


Figure 6.15. Comparisons between the effect of *Rhoicissus* extracts from different a) seasons and different b) plant parts on the viability of human histiocytoma cells after being exposed to the plant extracts for 24 hours.

6.2.8. Seasonal differences

The cellular viability of the hepatoma and leydig cell lines after being exposed to *R. tridentata* extracts obtained from material harvested during either the dormant or growing seasons yielded similar results (Figures 6.11a & 6.13a). The only differences in the kidney epithelial and histiocytoma cells occurred at the higher concentrations of plant extract (Figures 6.9a & 6.15a). At the highest concentrations of plant extract, extracts obtained from dormant plant material yield a lower absorbance than extracts obtained from growing plant material. However, the differences are negligible. Results from the statistical analyses are given in the appendices (Table A.5).

6.2.9. Plant parts used

There were no significant differences between the results from tuber extracts and those from stem extracts in the hepatoma cell line (Figure 6.11b; Table A6). At a confidence level of 99%, there were no differences between the cellular viability after leydig cells had been exposed to a range of extract concentrations from different parts of *R. tridentata* (Figure 6.13b; Table A.8). Minor differences in the viability of kidney epithelial and histiocytoma cells occurred after exposure to exposed to extracts from different parts of *R. tridentata*. The viability of kidney epithelial cells after being exposed to root extracts was about 10% lower than the viability after being exposed to either tuber or stem extracts (1 mg/ml; $P< 0.001$; Figure 6.6b; Table A.7). The viability of histiocytoma cells was slightly lower after exposure to stem extracts than was the case after exposure to either tuber or root extracts (1 mg/ml; $P<0.001$; Figure 6.15b; Table A.9).

6.3. Relationship Between Contractile Activity and Effect of Cellular Viability

The following graph shows the relationship between the contractile activity and the toxicity of *R. tridentata* extracts. The data from the previous experiments were analysed to determine whether the contractile activity was correlated to the cellular toxicity of the extracts.

There is a very poor correlation between the direct contractile activity of *R. tridentata* extracts on isolated rat uterine tissue and the effect of the same extract on cellular viability in all cell types tested (Figure 6.16).

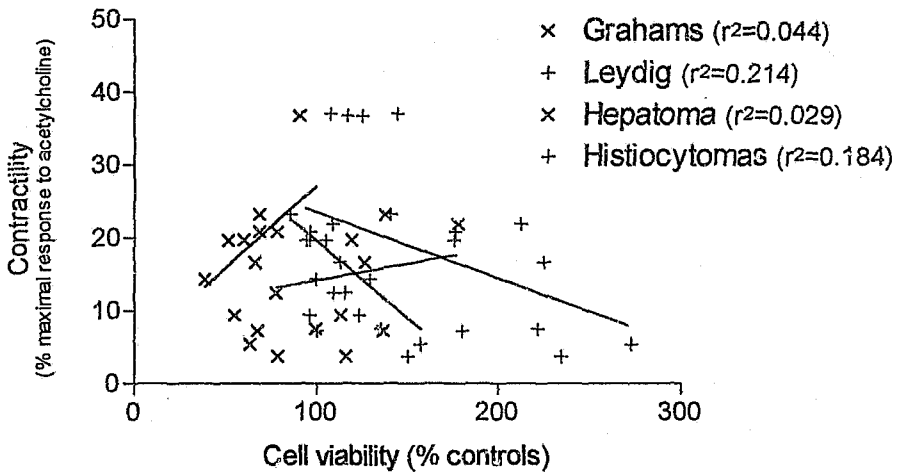


Figure 6.16. Correlation between the direct contractile activity of 1.3 mg/ml *R. tridentata* extract on isolated rat uterine tissue and the viability of all cell types tested after exposure to 1mg/ml *R. tridentata* extract for 24 hours.

CHAPTER 7: DISCUSSION AND CONCLUSIONS

7.1. Interviews with traditional healers

7.1.1. Training of traditional healers

With Gauteng being a center of immigration from rural areas all over Southern Africa, one would expect the traditional medicines used to represent a mixture of plants used by the different cultures. However, most of the healers spoken to were of Zulu origin and cited Zulu names for the plants. This could be a feature of the Faraday market (Central Johannesburg) and could indicate that the traditional healer associations contacted were biased to Zulu origins. This does not necessarily indicate that there are more Zulu remedies than remedies from other cultures. Anecdotally, the general perception in Gauteng is that most traditional remedies are of Zulu origin.

When describing the methods of training to become an inyanga, all traditional healers described how the mentor would teach the pupil how to interpret dreams. None of the healers were taught about what plant to use for specific indications. The trainee would then report back to the mentor, telling the mentor details of dreams which included what plants should be used for a particular ailing patient. Some mentors would write down the plant names together with the relevant indication without ever teaching the trainee healer about the medicinal properties of the plants. The transfer of knowledge appeared not to follow a logical direction. Two of the healers spoke about their training with deep dissatisfaction and discontent. They would have preferred more rigorous, thorough and perhaps more academic training. None of the healers underwent long periods of apprenticeship, contrary to the common perception of how traditional healers are trained. The minimum training period was three months and the maximum was three years. These results do not mean that no traditional healers ever undergo several years of training. Reports in the literature about traditional healers undergoing years of thorough training are numerous (Schuster Campbell 1998), but these healers are usually located in rural areas (Gericke 1999). However, these results do

show that there are many inadequately trained traditional healers within urban areas who are prescribing plants containing pharmacological compounds that are potentially very toxic.

Most healers and plant vendors spoken to were very keen for their plants to be tested in the laboratory to determine whether their plants are effective for a specific indication. On the whole, they were pleased to hear that "isinwazi" (*Rhoicissus* spp.) does cause contractions of isolated uterine tissue. This willingness of traditional healers to accept positive results is consistent with previous reports (Simon 1991; Green *et al.* 1995). However, when told that the plant had on occasions caused fatal poisonings, or that the contractile activity of the species was highly variable, they either paid little attention or openly showed their disapproval of what they were being told. This opposition to negative implications of traditional medicines is consistent with the approach of traditional healers when told of the number of childhood deaths caused by "impila" (*Callilepis laureola)* (Hutchings 1999).

The traditional healers' ignorance of the toxic potential of *R. tridentata* despite reports implicating the plant in fatal poisonings (Watt & Breyer-Brandwijk 1962; Brandt & Muller 1995) suggests that perhaps only certain plant samples are toxic. The possibility exists that the depressing effects of the plant extracts on the CNS fluctuate seasonally, similar to the fluctuations seen in the contractile action. This would explain why the traditional healers are generally unaware of the toxic potential of *Rhoicissus tridentata.* Another possibility is that the plant only contains sufficiently high concentrations of the toxic compound(s) when harvested from a specific location. Further investigation should be done to determine whether the poisonings that have occurred are clustered around the harvesting of plant material during a certain season or from a specific location. (Unfortunately clinical records of the poisonings reported by Brandt & Muller 1995 could not be located by the staff at Garankua hospital.)

The toxicity of *R. tridentata* may be disguised by the healers using the plant in mixtures of two or three ingredients. The other remedy ingredients may act as antidotes to the toxic effects of *Rhoicissus*, or *Rhoicissus* may be diluted, thus

avoiding toxic effects. The combination of factors may explain why the healers don't recognise *Rhoicissus* as the poisonous ingredient.

7.1.2. Plants used during pregnancy

Insufficient traditional healers were interviewed to determine conclusively how many plants are being used during pregnancy by black women in Gauteng. However, the results do give an indication of which plants are being used.

The traditional healers interviewed mentioned 46 ingredients which could be identified using published lists of plant names (Gerstner 1941; Watt & Breyer Brandwijk 1962; Hutchings *et al.* 1996). A further 9 ingredients were not included in any of the lists. 40 ingredients were plants, 4 were animal products and 2 were non-organic (Table 3.1; Page 75). Of the identified plant species, 23 were documented in the literature as being used in pregnancy-related remedies (Table 3.3; Page 84), 19 were documented to be toxic, of which 12 have been reported to have caused fatal poisonings to either humans, dogs or sheep (Table 3.4; Page 87).

Eleven plants were being used in general "cleansing" remedies which is consistent with the perception that it is the African traditional healers' role to diagnose the source of a problem occurring during pregnancy and that the foetus needs to be protected from harm while in the womb. These remedies are used for both spiritual and physical catharsis. There were many specific indications mentioned by the traditional healers (Table 3.2; Page 81). The highest number of plants were used for aiding maternal and foetal strength throughout pregnancy and for easing labour.

Rhoicissus tridentata and *Clivia miniata* were both mentioned as being used to prevent miscarriages, yet both have been shown to cause uterine contractions *in vitro*. Therefore, these two plants are more likely to increase the chances of a spontaneous abortion than to prevent it.

Most citings in South African literature on the use of plants during pregnancy do not stipulate specific indications for using herbal remedies during

pregnancy. However, during interviews traditional healers seldom used the terms isihlambezo or imbelekisane. They spoke of specific pregnancy-related indications for each plant. This is consistent with Mbura *et al.* (1985) who interviewed Tanzanian women in antenatal clinics on the reasons why they used traditional remedies during pregnancy. They also listed the use of plants according to specific pregnancy-related complaints.

Callilepis laureola was reported to be used during pregnancy to enhance foetal and maternal health as well as to ease labour i.e. the plant may be prescribed at any stage of gestation. The plant is also used specifically to calm a restless child, yet *C. laureola* has been implicated in many fatal childhood poisonings in South Africa (Stewart *et al.* 1998). The toxic principles are atractylosides which often kill the patient within 24 hours, often before they are able to reach a hospital. Atractylosides cause hypoglycaemia which in turn inhibits oxidative phosphorylation. The symptoms of poisoning usually include centrilobar hepatic necrosis, acute renal failure, disturbed consciousness and convulsions (Steenkamp *et al.* 1999).

The prevalence of remedies used specifically for children could reflect the morbidity and mortality patterns typical of a poverty-stricken population where the main burden of disease is on children within the first 5 years of life (Unterhalter 1982).

7.2. Pharmacological action of *R. tridentata* on isolated organs

7.2.1. Inorganic ion concentrations

The ion content of the plant extract once diluted in the Tyrode solution in the organ baths was too low to have any direct effect on the isolated smooth muscle preparation. This indicates that the contractions were stimulated by pharmacologically active ingredient(s) within the plant extract, and not the action of ions on the cell membranes.

7.2.2. Reference drugs

Uterus: Oxytocin was the most potent and efficacious agonist on the oestrogenised non-pregnant isolated uterus. The efficacy of acetylcholine was equal to that of oxytocin, but the potency was approximately three orders of magnitude lower. Adrenaline had only half the efficacy of acetylcholine and an even lower potency (Figure 4.1a; Page 90).

Ileum: The efficacy of acetylcholine was double that of serotonin while the potency on a molecular concentration was a hundred times that of serotonin (Figure 4.1b; Page 90).

7.2.3. Direct activity

The direct activity of the *Rhoicissus* extract (Figures 4.2a & b; Page 93) shows that the plant is able to stimulate concentration dependent contractions of both isolated uterus and ileum *in vitro*. The ileal response was sigmoidal and reached a maximal effect plateau at 7.9 μg/ml, whereas the uterine response was hyperbolic, i.e. the uterine response to the doses given was increasing exponentially at a plant concentration three orders of magnitude higher than that yielding the maximum ileal response. The Tyrode solution was virtually opaque when the highest concentrations of the plant extract were used. To add even higher concentrations of plant extract would not only have depleted the stocks of plant material but would have also changed the ionic concentrations of the Tyrode solution.

The crude extract stimulated contractions of approximately 30% of the maximal contractions stimulated by acetylcholine in both the uterus and ileum. Figure 4.3 (Page 93) shows that there was a wide range in the contractile response to the plant extract. The wide range in responses seems to be particular to *R. tridentata,* as this was also evident while doing antimalarial tests on the plant extracts (data not included). Simultaneous dosing of two isolated uterus or ileum preparations, made from the same animal, in different organ baths alongside one another with the same extract could yield significantly different responses. One preparation could not respond at all

whereas the other could contract 40% or more relative to the maximal response to acetylcholine. The maximum responses to the *Rhoicissus* extract in the isolated rat uterus and ileum were 81.7% and 98.1% of the maximal response to acetylcholine, respectively. These are exceptionally high responses considering that the plant extracts were crude aqueous extracts.

The crude aqueous extract of *R. tridentata* directly stimulates contractions of both isolated uterus and ileum, yet a permanent increase in the baseline is only seen in the uterus (Figures 4.4a & b; Page 94). The mechanism by which this occurs is not known.

The results obtained from these *in vitro* experiments cannot be directly applied to the clinical setting as it is not known whether the active component(s) in the plant extract is(are) absorbed from the gastrointestinal lumen and whether or not the compound(s) are resistant to first pass metabolism. However, these data provide pharmacological evidence that should the active ingredients in the plant extracts be absorbed, then they will be able to stimulate contractions of ileal and uterine smooth muscle, and therefore there is the potential that they are able to stimulate contractions *in vivo*.

Assuming that the active component(s) of the plant is(are) able to stimulate contractions *in vivo*, the plant extract would increase uterine contractions, and thus augment labour and increase the risk of birth complications associated with uterine hypertonia, such as foetal distress. The extract can also stimulates ileal contractions and therefore has the potential of stimulating the foetal intestinal wall, directly increasing the incidence of meconium staining.

7.2.4. Oxytocic receptor system

The plant extract did not alter the response of isolated uterus to oxytocin, suggesting that the plant extract does not possess antagonistic or non-competitive agonist activity on oxytocin receptors (Figure 4.5; Page 96). No deductions can be made on whether the plant extract is able to competitively agonise the oxytocin receptors or not, as no oxytocin antagonists were available. Atosiban is an oxytocin antagonist that could be used in the

laboratory (Bossmar 1998). A source for this compound, however, could not be located.

7.2.5. Prostaglandin synthesis

The direct action of the plant extract was significantly inhibited by pretreatment of the organs with the cyclooxygenase inhibitor indomethacin in both the uterus and the ileum (Figure 4.6a & b, Figure 4.17; Pages 97 & 110). This suggests that products of cyclooxygenase, such as prostaglandins or thromboxane A_2, may be involved in the contractile response to the plant extract.

As mentioned in the introduction, prostaglandins are thought to play a vital role in the onset and progress of labour. Prostaglandins increase the formation of gap junctions facilitating rapid transfer of action potentials between cells, and directly stimulate uterine contractions. If the *Rhoicissus* extract is able to induce prostaglandin synthesis *in vivo,* the plant extract has the potential of contributing towards the onset of labour and of augmenting contractions during labour.

COX-1 and COX-2 proteins are both expressed in rat uteri. They are primarily localised to epithelial cells of the endometrium and smooth muscle cells in the circular layers of the myometrium (Dong *et al.* 1996). NS-398 has been shown to prevent the augmentation of prostaglandins produced by IL-1alpha in oestrogenised rat uterus (Franchi *et al.* 1998). Indomethacin inhibits COX-1 and COX-2 activity to the same degree (Futaki *et al.* 1994), whereas NS-398 is a COX-2 specific inhibitor (Futaki *et al.* 1994). At a concentration of 0.1μM, NS-398 did not alter the activity of the crude *Rhoicissus* extract whereas 1μM inhibited the contractile response (Figure 4.17; Page 110). These data suggest that *R. tridentata* promotes the synthesis of prostaglandins through COX-1 and COX-2.

An increase in plasma levels of prostaglandin synthesis has been found to occur when other labour inducing agents have been used. Orally administered castor oil (60ml) which was found to induce labour in 75% of patients with

prematurely ruptured membranes, as opposed to 58% of controls. After administration of the castor oil in these patients, it was found that there were increased levels of circulating prostaglandin E (Davis 1984). *Agapanthus africanus* leaves also stimulate uterine contractions by promoting prostaglandin synthesis (Veale *et al.* 1999).

7.2.6. Adrenergic receptor system

The adrenergic system is not thought to play a crucial role in the progression of labour, but rather in the inhibition of labour. β-receptors mediate myometrial relaxation. This aspect of β-receptor activity is utilised therapeutically in the treatment of premature labour.

Noradrenaline had less than half the efficacy of acetylcholine causing the *R. tridentata* extract to appear more active than when compared to acetylcholine or oxytocin (Figure 4.1a; Page 90 & Figure 4.7; Page 98). The contractile response of the uterus to the plant extract when compared to acetylcholine is 18% ± 4 (SEM) whereas when it is compared to noradrenaline the response is 65% ± 8 (SEM). If the response to the extract relative to noradrenaline is corrected according to the response of noradrenaline relative to acetylcholine, the response to the plant extract works out to be 17 ± 2 (SEM) which is the same as the response when compared to acetylcholine.

The response to the plant extract after blocking the β-adrenoceptors with propanolol is the same as without propanolol (graph not given). This suggests that the plant extract does not stimulate the β-adrenoceptors. The plant extract did not alter the isolated uterus response to noradrenaline once the β-adrenoceptors were inactivated by propanolol, leading to the inference that the plant extract has neither agonistic nor antagonistic activity on β-adrenergic receptors.

Pretreating the uterine tissue with the α_1-adrenoceptor antagonist prazosin (2μM) or the α_2-adrenoceptor antagonist yohimbine (2μM) both successfully antagonised the α-adrenoceptors as is evident in the shift of the uterine dose

response curves of noradrenaline to the right, and the suppression of the maximal response to noradrenaline (Figure 4.7 & 4.8; Pages 98 & 99). These doses of prazosin and yohimbine did not alter the organs' response to the plant extract alone or the dose response curve to noradrenaline once the tissue had been pretreated with the plant extract. These data suggest that the plant extract does not have any agonistic activity on either of the α-adrenoceptor subtypes.

7.2.7. Muscarinic receptor system

Uterus and ileum: The contractile response to the plant extract of both uterus and ileum was completely blocked by pretreatment of the organs with the muscarinic antagonist atropine (40nM) (Figure 4.9a & b; Page 101). The same concentration of atropine shifted the dose-response curve to acetylcholine to the right, without significantly reducing the maximal response to acetylcholine. These results suggesting that muscarinic receptors are involved in mediating the contractile response to the plant extract.

Preincubation of the organs with both atropine and the plant extract caused further inhibition of the response to acetylcholine, shifting the dose response curve further to the right. In both organs the response to maximal doses of acetylcholine was suppressed by pretreatment with atropine (40nM) and the plant extract (1.3 mg/ml).

Muscarinic innervation is of greater clinical significance in the intestinal wall than in the uterus. Figures 4.2b & 4.4b (Pages 93 & 94) show that the aqueous extracts of *R. tridentata* stimulate contractions of the isolated rat ileum. The contractions appear to be mediated by muscarinic receptors (Figure 4.9b; Page 101). Provided that the active component of the plant extract which stimulates muscle contraction is absorbed into systemic circulation in a pharmacologically active form, and is able to pass into foetal circulation, *Rhoicissus* extracts have the potential of directly stimulating muscarinic receptors in the foetus, which in turn may increase the incidence of foetal meconium staining. This provides possible pharmacological evidence

for the correlation drawn by Mitri *et al.* (1987) that ingestion of herbal remedies towards the end of gestation increases the incidence of foetal meconium staining.

Muscarinic receptors are present in many different smooth muscle tissues. Again, assuming that the active component(s) of the plant is (are) absorbed into systemic circulation and are able to withstand first-pass metabolism, the plant extract may stimulate these muscarinic receptors, resulting in many effects not related to parturition, such as, decreased heart rate, increased salivation, abdominal cramps, diarrhoea, urinary urgency and miosis.

7.2.8. Muscarinic receptor subtypes and the uterine response

The M_1, M_3 and M_4 muscarinic receptor antagonists, pirenzepine (1.5μM), 4-DAMP (2nM) and tropicamide (4.5μM), respectively, all shifted the uterine dose-response curves to acetylcholine to the right, whereas the M_2 receptor antagonist gallamine (1.5μM) had no effect on the uterine response to acetylcholine (Figures 4.10 a-d; Page 103). These results suggest that contractions in rat uterus are mediated by M_1, M_3 and M_4 receptors.

Muscarinic receptor populations in different animals

A first glance at the above results could lead to the conclusion that they are incorrect, as since M_2 receptors account for 80% of the total receptor density in most smooth muscle (Ehlert *et al.* 1997), one would expect the M_2 receptors to mediate uterine contractions. However, the prevalence and efficacy of different muscarinic receptors differ in different animal species as well as in the different smooth muscles within the species.

No reports on the muscarinic receptor subtype populations of rat uterus could be found, but in rat and pig ileum, these three receptor subtypes were found to be present as minor receptor populations (Ehlert *et al.* 1997). In guinea-pig ileum, M_3 receptors were found to be the principle receptors mediating

contractions (Boxall *et al.* 1998). M_1 receptors were detectable, but only in trace amounts and M_4 were not detected at all (Maeda *et al.* 1988).

In many smooth muscle tissues a minor M_3 muscarinic receptor population mediates contraction, despite M_2 outnumbering M_3 receptors by a factor of about four (Boxall *et al.* 1998; Ehlert *et al.* 1999). Pretreating the organs with gallamine only, blocks M_2 receptors but leaves M_3 receptors unopposed. In order to test the role of M_2 receptors, the M_3 receptors have to be inactivated with a M_3 antagonist such as 4-DAMP together with histamine and a relaxing agent such as isoproterenol or forskolin (Ehlert *et al.* 1999).

Interpretation of results

Pretreatment with pirenzepine and gallamine (M_1 and M_2 antagonists respectively) did not alter the uterine response to the *R. tridentata* extract, even though pirenzepine antagonised acetylcholine. These results suggest that in the case of pirenzepine, the active component(s) of the extract has (have) a very low affinity for the M_1 receptors, or that the intrinsic activity is low.

The uterine response to the extract in the presence of gallamine is unaltered. Despite the M_2 receptor population being the most dense, M_2 receptors appear not play a prominent role in mediating the contractile response to the extract.

Pretreatment with 4-DAMP (2nM; M_3 antagonist) partially blocked the contractile activity of the extract whereas tropicamide (4.5μM; M_4 antagonist) completely blocked the contractile activity of the *R. tridentata* extract. Both antagonists caused a suppression of the maximal response of the uterus to acetylcholine in the presence of the extract. These results suggest that the contractile response to the extract is mediated by both M_3 and M_4 receptors.

No investigations on the involvement of receptor subtypes in the ileum were done.

7.2.9. Serotonergic receptors

Serotonin receptors are also not important in labour, but are important in the contraction of the intestinal wall. Pretreating the organs with 1.3 mg/ml plant extract does not alter the uterine or ileal response to serotonin (Figure 4.11; Page 104). This suggests that the plant extract does not have an affinity for serotonin receptors.

The serotonin antagonist, methysergide, at a concentration that shifts the serotonin dose-response curve to the right, does not alter the uterine or ileal response to the plant extract alone, or to serotonin after pretreatment with the plant extract. These results suggest that the contractile response of the organs to the plant extract does not involve serotonergic receptors.

This is confirmed by data represented in figures 4.12 and 4.13 (Pages 105 & 106) which show that ketanserin and tropisetron, which are antagonists on 5-HT_1 and 5-HT_2 receptors respectively, do not alter the response of the uterus or ileum to the plant extract.

7.2.10. Histamine receptors

Pyrilamine (8μM) did not alter the direct contractile response of the isolated rat uterus to the aqueous *R. tridentata* extract (Figure 4.15; Page 108). This suggests that histamine receptors are not involved in the contractile response to the extract. No experiments on ileum were done.

7.2.11. Ganglionic receptors

Hexamethonium and mecamylamine (in micromolar concentrations) have been shown to block contractile responses mediated by stimulation of nicotinic receptors in many studies using isolated organs (Barthó *et al.* 1987; Okamura *et al.* 1995; Hopkins *et al.* 1996). In this study the ganglionic blocker,

hexamethonium (1.4μM) and the non-competitive nicotinic antagonist, mecamylamine (2μM) both did not alter the contractile activity of the plant extract on the uterine smooth muscle (Figure 4.14; Page 107). These results indicate that the uterine contractile activity of the plant extract does not involve nicotinic receptors.

7.2.12. Second messengers

The islet-activating protein, pertussis toxin (50μM) inhibited the direct uterine contractile activity of the plant extract from 30.3% to 18.7% of the maximal response to acetylcholine (Figure 4.14; Page 107). That is a 38% reduction in the contractile activity. However, this reduction in activity was not significant. These results are surprising as tropicamide completely blocked the contractile response suggesting that M_4 receptors are involved in the mediation of the contractile response, yet M_4 receptors mediate pertussis toxin sensitive inhibition of adenylate cyclase and subsequent accumulation of cAMP (Ehlert *et al.* 1997). However, the contractile activity of the plant extract also seems to be mediated by M_3 receptors which stimulate contractions by potently activating phosphatidyl inositol hydrolysis and stimulating large, rapid and transient chloride currents by a pertussis toxin insensitive G protein pathway (Lechleiter *et al.* 1991; Ehlert *et al.* 1997).

PGE_2 and $PGF_{2\alpha}$ have also been shown to inhibit adenylate cyclase through a pertussis sensitive mechanism in the pregnant rat myometrium (Goureau *et al.* 1990).

These data suggest that adenylate cyclase is not a major mediator of the contractile action of the *R. tridentata* extract suggesting that the M_3 receptors mediate a large portion of the response to the plant extract. The pertussis toxin induced reduction in the contractile activity of the extract may indicate the proportion of the response which is mediated through either M_2 or M_4 receptors or through the activation of prostanoid receptors.

Pertussis toxin acts by inhibiting the inhibitory nucleotide regulatory protein mediating the signal transmission to adenylate cyclase, whereas cholera toxin inhibits the stimulatory nucleotide regulatory protein on the same pathway (Lux & Schultz 1986), known as the Gα protein. Cholera toxin (1μM) also did not alter the uterine response to the plant extract. These results suggest that the G_α protein is not involved in the mediation of the contractile response to the extract.

7.2.13. Blocking of the action of the plant extract by atropine and indomethacin

The results from the isolated organ studies indicate that the plant extract stimulates smooth muscle contractions through two mechanisms: the activation of muscarinic receptors and through promoting the synthesis of cyclooxygenase products. The involvement of these seemingly independent mechanisms of contraction has also been found to mediate smooth muscle contractility in response to *Agapanthus africanus* aqueous extracts (Veale *et al.* 1999) as well as to venom from the soldierfish (Gymnapistes marmoratus) (Hopkins *et al.* 1997).

Tobacco smoking by pregnant women increases the frequency of spontaneous abortions and preterm births. The mechanism by which this is thought to occur is also similar to the pharmacological activity of *R. tridentata* extracts. Nicotine and its major metabolite cotinine both activate PLA_2, thereby increasing the production of PGE_2. Nicotine also activates nicotinic receptors in the placenta causing the release of acetylcholine which in turn activates muscarinic receptors (Sastry *et al.* 1999).

Myometrium: $PGF_{2\alpha}$, PGE_2, and muscarinic agonists all enhance Ca^{2+}-induced contraction of myometrium in late gestation (Izumi *et al.* 1996). These contractile elements produce additional contractile force with the same amount of intracellular calcium, thus providing additional expelling forces for delivery of a foetus (Izumi *et al.* 1996). The activation of M_2 receptors also

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inhibits the relaxant effects of β-adrenoceptor activation (Ehlert *et al.* 1999) which would otherwise impair labour.

Endothelial cells of blood vessels: In addition to increasing uterine contractility, activation of muscarinic receptors of endothelial cells results in the release of endothelium-derived relaxing factor (EDRF), and possibly the smooth muscle relaxing factor, nitric oxide (NO) (Sastry *et al.* 1999). Therefore, activation of muscarinic receptors counteracts the vasoconstrictive effects of PGE_2 through the release of EDRF / NO and therefore counteracts the effects of PGE_2 on the placental vascular resistance which would usually decrease foetal blood flow through the placenta, i.e. *R. tridentata* would produce opposing effects on the placental vasculature. The potentiation of prostaglandin synthesis would increase vascular resistance, whereas activation of the muscarinic receptors would decrease vascular resistance.

Gastrointestinal tract: As mentioned earlier, muscarinic agonists have the potential of directly stimulating intrauterine meconium passage through stimulating foetal intestinal motility. Prostanoids have also been found to be diarrhoeagenic (Weiler *et al.* 1990) by increasing the fluid content of the intestines (Keith *et al.* 1992) and by a receptor mediated increase in smooth muscle tone (Campbell & Halushka 1995). That is, *R. tridentata* theoretically has the potential of increasing meconium staining of the liquor by two independent mechanisms.

Further links between muscarinic receptors and prostaglandins have been identified. PGI_2 (prostacyclin) facilitates acetylcholine release from intramural nerves in the guinea-pig ileum, possibly by increasing the excitability of cholinergic cell bodies (Gaion & Trento 1983). However, this is a pertussis toxin sensitive mechanism. Contractile responses to M_1 and M_4 receptors in the iris sphincter have been found to involve the formation of prostanoids, that partially inhibit the contractile response. It is thought that this is a possible mechanism in other types of smooth muscle (Ehlert 1997).

One possible mechanism by which the plant extract is acting is by stimulating the production of cyclooxygenase products, which in turn stimulate the release of acetylcholine from the nerve endings which then stimulates the postsynaptic muscarinic receptors. The reverse interaction is also possible. This would explain why indomethacin and atropine both completely block the action of the plant extract, and why either doesn't only partially block the contractile activity.

A method to test this hypothesis is to block the release of acetylcholine from nerve terminals using tetrodotoxin. Owing to local legislation, tetradotoxin was unavailable and this experiment could not be conducted.

Figure 7.1 (Page 156) illustrates the proposed mechanisms by which the *Rhoicissus* extract is stimulating contraction of the isolated rat uterus and ileum.

7.2.14. Increase in the baseline of uterus following the initial dose of *R. tridentata*

Figure 4.21 (Page 114) shows the relationship between the contractile response of 243 test challenges on the uterus with *Rhoicissus* aqueous extracts, plotted against the extent to which the same challenge caused an increase in the basal length of the uterine tissue, following repeated washing out of the Tyrode solution. The graph shows that there is no correlation between the contractile response to the extract and the extent to which the baseline is raised. The r^2 is 0.19 indicating that there is no direct relationship between the two responses of the uterus to the plant extract.

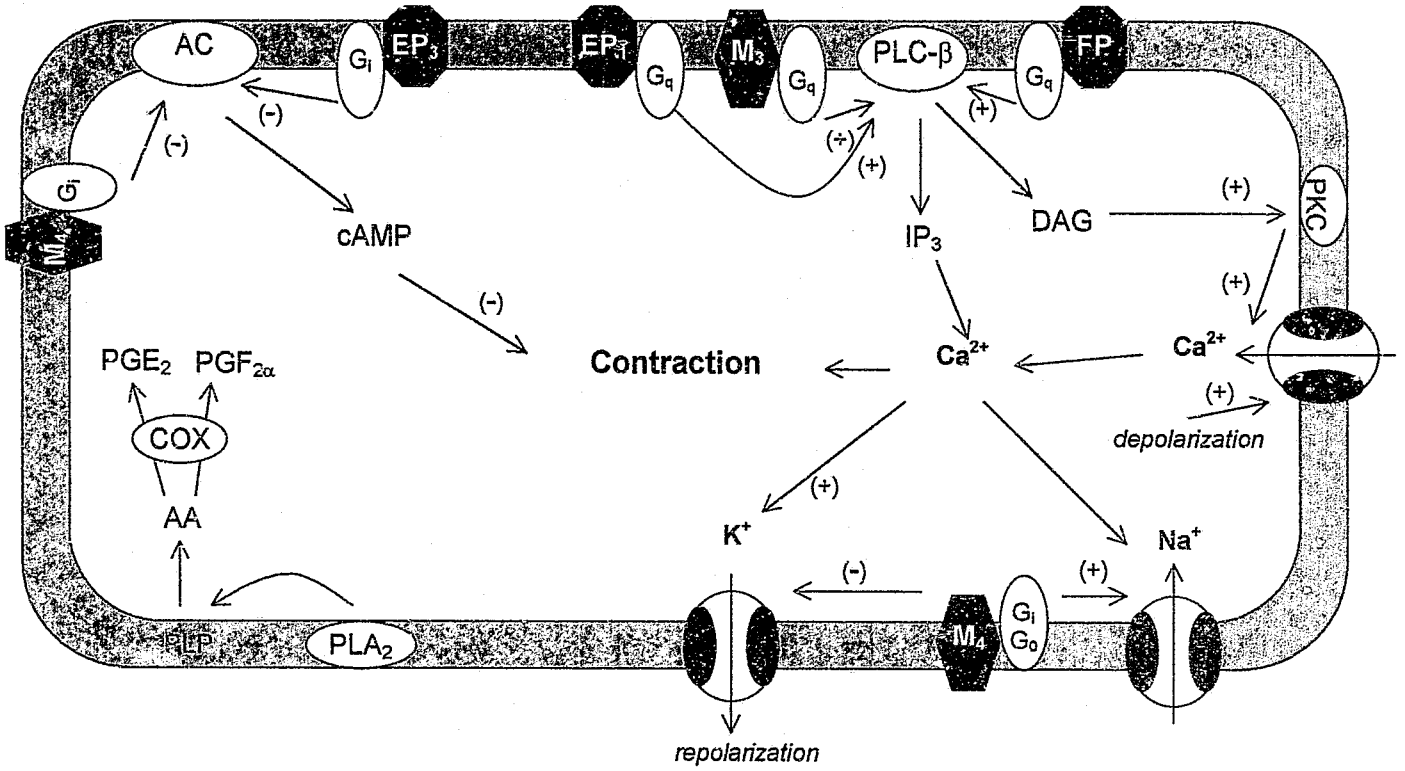


Figure 7.1 Summary of the contractile mechanism of *R. tridentata* aqueous extracts in isolated rat uterus and ileum. Muscarinic receptors are represented by blue pentagons, where prostanoid receptors are represented by violet octagons.

Abbreviations: PLC-β = phospholipase C-β; IP_3 = inositol-1,4,5-*tris*phosphate; DAG = diacylglycerol; PKC = protein kinase C; AC = adenylyl cyclase; cAMP = cyclic AMP, PLP = phospholipids; PLA_2 = phospholipase A_2; COX = cyclooxygenase; AA = arachidonic acid.

7.3. Variation in contractile activity

7.3.1. Seasonal Variation

Figure 5.1 (Page 117) illustrates how the various parts of the plants harvested in winter and spring all yield aqueous extracts with low levels of contractile activity. On the contrary, there is considerable variation between the extracts from the various plant parts harvested during summer and autumn. During summer the tubers yield the most active aqueous extracts, followed by the stems then roots and leaves. The autumn plants exhibit a similar pattern of variation except that the roots are the least active. The autumn and summer tubers are 4 and 5 times more active than the winter and spring extracts. The stems display similar seasonal variability.

The root and stem extracts show a similar pattern of seasonal variation in uterotonic activity to that of the tuber extracts. That is, the activity of the extracts increases when the plants are harvested after the first rains. The activity increases throughout the growing (or rainy) season reaching a peak in autumn. The activity of the extracts then falls as the plants lose their leaves and become dormant during winter. The rise in activity as the rains start follows the statement of Birch (1960) that an improved uptake of minerals from the soil follows the mineralization flush after the first rains. This usually leads to a sharp rise in the nitrogen content within the plant.

The variability in the contractile activity of the extracts could be caused by *i)* altered concentrations of the active principle(s), *ii)* a change in the quality or nature of the active principle(s), or *iii)* changes in the production of compounds such as saponins which may alter the extraction of the pharmacologically active component(s).

The extent to which the extracts increased the basal tone of the uterus did not change significantly between extracts from different plant parts (Figure 5.4; Page 122). After pooling all the results according to season, extracts from plant material harvested during summer and winter increased the basal length more than extracts from plant material harvested during spring ($p<0.001$).

7.3.2. Plant part used

All parts of the plant harvested in summer and autumn are able to directly stimulate contractions of isolated uterus. The traditional healers usually use the lignotubers, which yield the most uterotonically active extracts. The activity of the tuber extracts is the highest during the growing season and decreases during the seasons when the plant is dormant. The stems are the next most active parts of the plant, followed by either the roots or leaves, depending on the season.

7.3.3. Relevance to conservation

The stem extracts yield a similar response to the most active part, the lignotubers. This is very significant from an environmental perspective, as harvesting stems for the trade in traditional remedies would have a much lower impact on the plant populations of *Rhoicissus tridentata* than harvesting the lignotubers and roots. With the current use of the lignotubers, the plant has to be uprooted for lignotubers to be harvested resulting in a decrease in the population size. Unfortunately, lignotubers are far easier to transport and to market compared to the stems and branches which have to be packaged. Secondly, the stems and branches and are not as easily identified. Therefore, it is unlikely that herb vendors would revert to selling stems rather than tubers. This could only be implemented if the herbal market could advance to a stage of selling remedies packaged in boxes, or as bottled liquid remedies.

7.4. Distributional variation in contractile activity

The results show that there is variation in the pharmacological response to aqueous extracts from *R.tridentata* plants harvested from different localities. All the extracts stimulated uterine contractions and either slightly increased or inhibited the maximal response to acetylcholine (Figure 5.5; Page 124). The extract from the plant harvested in Mondeor was the exception, in that it stimulated minimal uterine contractions but significantly inhibited the maximal response. That is, this is the only extract that competed with acetylcholine in a non-competitive manner.

Turning to the activity of plant parts from Suikerbosrand, the root extracts were less active than the extracts from lignotubers or stems (Figure 5.2; Page 119). In the investigation of distributional variation, the extract from the roots harvested from Umlazi gave a response similar to that of the extracts from roots harvested from Suikerbosrand, whereas the roots harvested from Durban yielded an extract with an unexpectedly high level of activity.

Even though these results have illustrated the variation with distribution, no conclusions can be made about what factors may be responsible for this variation. Soil samples, the mean rainfall, temperature and the rate of photosynthesis prior to harvesting were not obtained. Further studies using a controlled environment, such as in a phytotron, would be necessary to ascertain which factors influence the composition of compounds within the plant that are extracted when making the decoctions.

Further taxonomic studies should be done to validate the deliniation of the subspecies proposed by Urton et al (1986). The occurrence of certain secondary metabolites is sometimes restricted to a few species or even to a single chemical race, as they are sometimes formed at certain stages of the individual's development (so-called idiophase) (Mothes 1980).

Chemotaxonomic studies could test whether there are chemical components which could be used to characterise further deliniations at the species or subspecies level within the *Rhoicissus* genus. Perhaps the difference in contractile response is a manifestation of taxonomic differences.

There is also the possibility that the contractile component of the plant extracts are only produced when *R. tridentata* is colonised by a fungus which is restricted to certain regions. This has been shown to occur with compounds such as phytoalexins, which are only produced under a stimulus such as the invasion of the plant tissue by a fungus (Bell 1980).

7.4.1. Clinical relevance of variation in contractile activity

The data presented have highlighted two independent variations in the contractile activity of the plant extracts, viz. temporal and distributional variation. These variations highlight a serious flaw in the use of traditional herbal remedies. Collectors and vendors have no way of determining the chemical composition of the plant material harvested. Nine traditional healers in and around Johannesburg were questioned on whether they were aware of any seasonal variation in the efficacy of *R. tridentata*, and none of them seemed aware of the concept.

The distributional variation in contractile activity appears to be more significant than seasonal variation as it was only the extent of the response that varied seasonally, whereas the type of response varied distributionally, i.e. the plant extracts acted either agonistically or antagonistically. The traditional healers have no way of determining what the effect of a specific *R. tridentata* sample will be. This is extremely worrying, especially considering that the crude extracts yielded contractile responses of up to 98.1% of the maximal response to acetylcholine. The variability in pharmacological activity is more than likely the reason why traditional healers have not identified *R. tridentata* as a potentially toxic plant. One can assume that the plant is more often not toxic.

The variability in the pharmacological activity of *Rhoicissus* also reiterates the urgent need to establish standardisation criteria for herbal remedies, particularly southern African traditional remedies. The results also support the suggestion made by Tobler (1994) that there should be a holistic approach to standardising the production of medicinal plants. That is, the entire production of medicinal plants must be controlled, starting from the cultivation, harvesting and plant part selection, and continuing through the entire process of remedy formulation.

7.5. The effect of storage on contractile activity

The results of investigations into the effect of storing dried plant material indicate that the active components of the plant that are extracted by water are stable at room temperature and away from sunlight for at least a year (Figure 5.6; Page 125). This is relevant to the traditional healer, as dried plant material is stored by medicinal plant vendors and by the traditional healers themselves.

7.6. The effect of *R. tridentata* extracts on prostaglandin synthesis in human histiocytoma cells

The extracellular concentrations of PGE_2 in the histiocytoma cell cultures increased almost immediately once the cells were exposed to the plant extracts, reaching a peak at approximately 15 to 20 minutes (Figure 6.1; Page 126). This is similar to what is seen in the isolated organs, where the initial responses to the plant occur almost immediately after dosing and the response increases continuously, reaching a maximum response in 15 to 20 minutes. The sudden drop in the amounts of extracellular PGE_2 suggests that the cells produce enzymes to metabolise prostaglandins that rapidly degrade extracellular prostaglandins.

The sudden increase in the amounts of PGE_2 once the plant extract concentration is increased above 0.1mg/ml (Figure 6.2; Page 127) is also consistent with results obtained from the contractile response of the isolated rat ileum where the response increases exponentially (Figure 4.2b; Page 93). However, the isolated uterus responded to lower concentrations of the *R. tridentata* extract. The ileum and cells responded to 2.5µg/ml and 1mg/ml respectively.

These results confirm the results obtained from the isolated organs, indicating that aqueous *R. tridentata* extracts stimulate the production of cyclooxygenase products, in particular prostaglandin E_2. There still is the possibility that other cyclooxygenase products are also increased by the plant extracts.

7.7. Effect of *R. tridentata* on cell culture survival

7.7.1. Toxicity on different cell lines

To test the toxicity of the plant extracts LD50 tests should ideally have been done. However, since the Animal Ethics Committee of the University of Witwatersrand does not allow LD50 tests to be done, MTT assays on a variety of cell lines were done to test whether the plant extracts affect the viability of cells. Results from these studies give no indication on whether the plant extracts are able to cause respiratory depression or cardiac function for example.

The plant extracts did not significantly reduce the absorbance of all the cell lines consistently, but rather there was a consistent increase in the absorbance of test wells relative to the control wells. These results suggest that the plant extracts, in the concentrations tested, are not toxic on any of the cell lines used, being human kidney epithelial cells, mouse leydig cells, human hepatoma cells and human histiocytoma cells. The first two are non-cancerous lines whereas the latter two are cancerous lines.

The increased reduction of the tetrazolium salt (MTT) by the cells to form the formazan product does not necessarily indicate that there is an increase in the number of viable cells in the test wells relative to the control wells. The increase in the formation of the formazan product could indicate that the plant extracts activate the mitochondria, or the mitochondrial enzymes directly. Mosmann *et al.* (1983) found that metabolically active cells produce more formazan than resting cells. There is also the possibility of a direct interaction between the plant extract and the MTT. For future experiments it is suggested that the cell media is removed and the cells washed with PBS before the addition of MTT, to cancel out any possibility of this interaction.

7.7.2. Seasonal differences in the effect of the plant extracts on cellular viability

The effect of the plant extract from plant material harvested in either dormant or growing seasons did not show a great degree of variation in the effect on cellular viability. The only differences that were found were in the assays on the kidney epithelial cells and the histiocytoma cells (Figure 6.9 & 6.15; Pages 134 & 137). In the kidney cells, 10mg/ml plant extracts from dormant plant material reduced the cellular viability of the treated cells by approximately 10% relative to the controls, whereas the same extract concentration from plant material harvested during growing seasons did not alter the cellular viability. In the histiocytoma cells, concentrations of up to 0.1µg/ml of extracts from dormant plant material reduced the cellular viability to approximately 90% relative to the controls, whereas extracts from plant material harvested during the growing seasons did not alter the cellular viability.

These results suggest that there are only minor differences in the cytotoxic effects of extracts from different seasons, unlike the contractile activity of the plant extracts which appear to vary seasonally. Extracts from plant material harvested from dormant plants are slightly more cytotoxic to kidney epithelial and histiocytoma cells than extracts from plant material harvested while growing.

7.7.3. The effect of extracts from different plant parts on cellular viability

There were only minor differences in the effect of extracts from different plant parts on the cellular viability of the four cell lines on which the plant extracts were tested, suggesting that there are no consistent differences in the cytotoxic effects of extracts from different plant parts (Figure 6.9, 6.11, 6.13 & 6.15; Pages 134 - 137).

These results are not consistent with results obtained by Martina Geheeb-Keller (Pers. comm.; unpublished results) who found that aqueous root extracts from *R. tridentata* reduced the cellular viability of a HepG2 cell line (human hepatoma cells), whereas extracts from any other plant part did not alter cellular viability.

7.7.4. Correlation between contractile activity and cytotoxicity

When the contractile activity on isolated rat uterus of each extract was plotted against the effect of the extract on cellular viability, there was a very poor correlation between the two variables (Figure 6.16; Page 139).

7.8. CONCLUSIONS

7.8.1. Ingredients used in pregnancy-related remedies

The traditional healers interviewed used a total of 46 ingredients in their pregnancy-related remedies. 40 of these were plant material, 4 were animal products and 2 were non-organic. 19 of these plant species have been reported to be toxic, of which 12 have caused fatal poisonings. The traditional healers interviewed all had very limited training as herbalists, but were all willing and open for further training or opportunities to increase their knowledge.

7.8.2. Contractile activity of *R. tridentata*

Rhoicissus tridentata was found to directly stimulate concentration dependant contractions of both isolated rat uterus and ileum. The baseline length of the uterus was increased to varying degrees after exposure to the plant extract, whereas the ileal baseline length remained unchanged. The extent to which the uterine basal contraction was increased was not a function of the original contractile response.

7.8.3. Pharmacological activity of *R. tridentata*

The contractile response in the isolated rat uterus and ileum appeared to be mediated through muscarinic receptors and through the stimulation of prostaglandin synthesis, and did not involve serotonergic receptor activity. The role of oxytocin receptors in the contractile response to the aqueous extract of *R. tridentata* was inconclusive. The uterine response also did not involve either adrenergic or histamine receptors. Furthermore, the contractile response appeared to be pertussis toxin insensitive, i.e. it did not involve adenylate cyclase, or nicotinic receptors.

7.8.4. Variation in the contractile activity of *R. tridentata*

The contractile activity of *R. tridentata* varies seasonally. Extracts of the plant are most active when prepared from plant material harvested during summer and autumn, and least active during winter and spring. The activity of extracts

from all plant parts varies in a similar pattern. Generally, the lignotubers yield the most active extracts followed by the stems, roots and leaves. The contractile response is influenced by the locality where the plant was grown. All but one extract tested stimulated uterine contractions to varying degrees whereas one plant yielded extracts that antagonised the activity of acetylcholine. Storing dry plant material for a year does not effect the contractile activity of decoctions made from the plant material.

7.8.5. Cytotoxicity of aqueous extracts of *R. tridentata*

Aqueous extracts of *R. tridentata* appeared not to reduce cellular viability of human kidney epithelial cells, human hepatoma cells, human histiocytoma cells and mouse leydig cells. The effect of the extracts on the cells did not appear to display the seasonal variation evident in the contractile activity, neither were there marked differences in the cellular responses to different plant parts.

7.8.6. The effect of aqueous extracts of *R. tridentata* on prostaglandin synthesis by human histiocytoma cells

Aqueous extracts from *R. tridentata* increased extracellular levels of prostaglandin E_2 in cultures of human histiocytoma cells. The PGE_2 levels peaked at approximately 20 minutes following exposure of the cells to *R. tridentata* extract.

7.9. Recommendations for further work

7.9.1. Herbal remedies used during pregnancy

Further interviews on both pregnant women and traditional healers in Gauteng need to be done before we shall have a complete list of plants being used during pregnancy by local women. An accurate reflection on what plants are being used would be very useful if clinical studies on the impact of different herbal remedies were ever to be done.

Interviews of traditional healers should be more structured, asking what they would consider to be isihlambezo, inembe, imbiza, imbelikesane or umsekelo. Traditional healers should be asked to fill in questionaires on what plants they would prescribe for the common complaints during pregnancy.

In certain areas, especially the more affluent areas, alternative medicines have become popular and acceptable to both patients and midwives. This means that patients would admit to using herbal remedies. Case control studies could easily be done on women who are using popular herbal products (as listed on page 22) to determine whether there are any differences in the birth outcomes or labour durations of these births.

7.9.2. Absorption of active components from gastrointestinal tract

Animal experiments should be done, where plasma concentrations of the pharmacologically active ingredients of *R. tridentata* are measured following the administration of the active components. These experiments could identify which components are absorbed and whether the active components are able to withstand first pass metabolism.

7.9.3. Pharmacological action of *Rhoicissus tridentata*

In vivo pharmacological investigations on the plant in relation to pregnancy are not justified until there is conclusive proof that this plant is causing an impact in the clinical setting.

Further *in vitro* tests should be done to determine whether an oxytocin antagonist such as atosiban or tetrodotoxin alters the uterine response to the plant extracts, as well as tests on possible mechanisms of respiratory depression. Opioid receptors have been shown to be present on ileal tissue. Therefore isolated ileum preparations can be used to determine whether the plant is able to agonise opioid receptors. Isolated ileal muscle needs to be electrically stimulated, and then the effects of the plants need to be tested, using opioid receptor agonists as references and testing whether opioid antagonists such as naloxone alter the dose response curves to the plant extract.

Further work on the relationship between prostaglandin synthesis and muscarinic receptor activation will clarify the mechanism of the contractile activity of *Rhoicissus*. Either, these are two independent systems mediating contractions or possibly the one process stimulates the other.

Results from investigations done by Ribeiro *et al.* (1999) suggest that there is an interaction among epidermal growth factor, nitric oxide (NO) and PGs and that in this interrelationship are involved COX-II and iNOS. This mechanism might be important during implantation and labor. Chaud *et al.* (1998) also found NO to be involved in the synthesis of PGs in the uterus. They suggest that NO is an important intermediate in the stimulation of PG synthesis caused by platelet activating factor.

Clearly, PG synthesis in the uterus is a complex process. At this stage it is unclear how the *Rhoicissus* extracts interact in this process.

7.9.4. Screening of plants used during pregnancy

A large scale screening project on the plants listed in the review of plants documented to be used in pregnancy-related remedies, and the plants listed in table 3.1, should be done to determine which of the plants are uterotonic

and which are not. The plants which are uterotonic should be tested for muscarinic activity and the effect of cyclooxygenase on the direct activity of the plant. These effects may possibly be a common mechanism of action among many of the uterotonic plants.

7.9.5. Variation in the contractile activity of *Rhoicissus tridentata*

Inconclusive findings from work done for this thesis indicate that the contractile activity of *R. tridentata* extracts varies depending on the plant size or age. Investigations could be done to determine whether this is possibly a further factor affecting the variation in contractile activity.

The possibilities of *Rhoicissus tridentata* ever being marketed in a controlled market are very small. Not only is the possibility of controlling the herbal market small, but *Rhoicissus tridentata* has been reported to be a toxic plant and if the market is controlled, this species should not be included as a permissible plant. Therefore, further studies on which environmental factors affect the composition of active components of the plant would not be applied, and would therefore be of academic value only. The results from this study simply highlight that the contractile activity of the species is highly variable, and measures should be taken to minimise the factors which affect the variablity as far as possible.

7.9.6. Long term studies on the clinical impact of traditional remedies used during pregnancy

The question of the impact of traditional medicine on the outcome of pregnancy can be investigated in two ways, by analysing the pharmacological action of individual plant species, or by doing clinical investigations. However, *in vitro* pharmacological studies on the extracts only provide indirect evidence and always raise the question of whether the active components are absorbed or not. Clinical evidence gives direct evidence negating questions of whether compounds are absorbed and whether they can withstand first pass metabolism. Since many patients are taking these plants, clinical studies on the effects of the plants would be possible.

The use of traditional remedies is regarded by black South Africans as very private. Medical professionals also often frown on the use of traditional remedies. Therefore, it is ineffective to question women in a clinical setting on whether they use traditional remedies or not. If they are willing to divulge their use of traditional remedies it is very seldom that they are aware of what plant species they used, or which plant species were used to mix up a concoction of a number of plant species.

Therefore, it would be necessary to devise another mechanism to determine what plant species was ingested. This could be done with chemical analyses of urine. Foukaridis *et al.* (1994) demonstrated that this is possible using HPLC diode array. With the advancement of chemical analytical equipment, equipment such as HPLC-MS would give a fingerprint for each plant species overcoming the obstacle of conditions within the column affecting the elution time of different substances.

Clinical investigations using the urine of children suspected of ingesting impila (*Callilepis laureola*) have shown that this method is possible (Steenkamp *et al.* 1999). HPLC and GC-MS were used for these studies.

Clinical studies using urine analytical techniques would identify which plants increase the incidence of adverse birth outcomes, and which possibly improve birth outcome, and would determine whether pregnancy-related remedies do impact on the clinical outcome of birth.

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A. APPENDICES

A.1. Tyrode solution formula

Stock Solution 1	
NaCl	1000g
KCl	25g
$CaCl_2.2H_2O$	33g
$MgCl_2.6H_2O$	27g

Stock Solution 2	
$NaHCO_3$	252g
NaH_2PO_4	12.5g

Add 800 ml of solution 1 and 400 ml of solution 2 to approximately 10 L of distilled water. Add 30 g glucose and make up to 20 L.

Stilboestrol used to oestrogenise rats:
Stilboestrol in arachis oil (0.2mg/ml)
dose 0.15ml/300g animal weight

A.2. Details of cell cultures used in project

a) Graham cells

ATCC number:	CRL-1573.1
Organism:	human
Designation:	293 / SF
Tissue:	kidney; transformed with adenovirus 5 DNA
Age / Stage:	embryo
Morphology:	epithelial
Gender:	male
Tumorigenic:	yes, in nude mice
Growth properties:	monolayer
Receptors expressed:	vitronectin

Culture medium	
HAM F10	90%
Foetal bovine serum	10%

b) Human fibrous histiocytoma cells

ATCC number:	TIB-223
Designation:	GCT (giant cell tumor)
Tissue:	histiocytoma, fibrous, from metastatic site, lung
Age:	29 years
Gender:	male

Tumorigenic:	yes
Growth properties:	adherent
Products:	prostaglandin E_2
	plasminogen activator
	colony stimulating activity
	erythroid enhancing activity
Receptors expressed:	luteinizing hormone
	epidermal growth factor
	androgen
	oestrogen
	progesterone

Culture medium

McCoy's 5a medium with 1.5 mM L-glutamine 90%
Foetal bovine serum 10%

c) Mouse Testis (leydig) cells

ATCC number:	CRL-1714
Designation:	TM3
Strain:	BALB/c nu/+
Tissue:	normal; testis; leydig cells
Age:	11-13 days
Gender:	male
Morphology:	epithelial
Tumorigenic:	no
Growth properties:	adherent
Products:	prostaglandin $F_{2\alpha}$
Receptors expressed:	luteinizing hormone
	epidermal growth factor
	androgen
	oestrogen
	progesterone

Cell Culture Medium (Modified Dulbecco's medium)

DMEM w/HEPES (10mM)	100 ml
L-Glutamine (100x)	1 ml
Solution 1	1 ml
Non-essential amino acids (100x)	1 ml
NCTC 135	10 ml
Foetal bovine Serum	12 ml
Gentamicin	1.4 ml

Solution 1 (100x)
1320 mg oxalacetic acid (100 mM, MW 132)
80 mg crystalline bovine insulin (20 units/ml, 25 units/mg)
Add Na pyruvate 550 mg (50 mM, FW 110)

Mix the oxalacetic acid and bovine insulin together, stir at 37°C. Add the Na pyruvate. Bring up to 100 ml with distilled water. Stir at 37°C until solution dissolves. Filter, aliquot and store frozen.

A.3. Results of statistical analyses of all data

A.3.1 Effect of the antagonists on the direct activity of the *Rhoicissus* extracts

Table A.1 P-values from student-t tests done comparing the contractile response of isolated rat uterus to the *Rhoicissus* extract alone as compared to the response to the extract after the organs had been treated with an antagonist.

			uterus	ileum
Rh extract	vs	Indomethacin	0.000	0.000
		NS-398	0.687	
		NS-398 2	0.003	
	vs	Prazosin	0.343	
		Yohomibine	0.882	
	vs	Atropine	0.000	0.000
		Pirenzepine	0.054	
		Gallamine	0.939	
		4-DAMP	0.016	
		Tropicamide	0.000	
	vs	Methysergide	0.920	0.066
		Tropisetron	0.145	0.022
		Ketanserin	0.690	0.022
		Hexamethonium	0.440	
		Mecamylamine	0.928	
		Cholera toxin	0.767	
		Pertussis toxin	0.094	
		Pyrilamine	0.794	

A.3.2 Seasonal differences in contractile activity

Table A.2 P-values from student-t tests comparing the seasonal differences in the direct contractile activity of *Rhoicissus* extracts on isolated rat uterus.

			tubers	roots	stems	leaves
spring	vs	summer	0.000	0.006	0.000	
spring	vs	autumn	0.007	0.767	0.266	
summer	vs	autumn	0.704	0.004	0.060	0.591
spring	vs	winter	0.765	0.077	0.058	
summer	vs	winter	0.000	0.190	0.138	
autumn	vs	winter	0.002	0.041	0.554	

Table A.3 P-values from student-t tests comparing the isolated rat uterine maximal contractile response to acetylcholine after the organs had been pretreated with 1.3mg/ml of various *Rhoicissus* extracts for 5 minutes.

			tubers		roots		stems		leaves	
			DF	P value	DF	P value	DF	P value	DF	P value
summer	vs	autumn	5	0.478	12	0.061	11	0.082	13	0.003
summer	vs	winter	5	0.382	13	0.302	11	0.021		
autumn	vs	winter	11	0.495	12	0.102	12	0.615		
summer	vs	spring	5	0.132	13	0.903	11	0.042		
autumn	vs	spring	5	0.076	12	0.104	12	0.533		
winter	vs	spring	5	0.134	13	0.464	14	0.912		
summer	vs	control	5	0.054	13	0.709	11	0.012	13	0.012
autumn	vs	control	11	0.039	12	0.013	12	0.405	21	0.021
winter	vs	control	17	0.715	18	0.008	18	0.411		
spring	vs	control	5	0.033	13	0.617	14	0.933		

A.3.3 Contractile activity of extracts from different plant parts

Table A.4 P-values from student-t tests comparing the direct contractile activity of *Rhoicissus* extracts from different plant parts harvested in different seasons on isolated rat uterus.

Seasons			spring	summer	autumn	winter
tubers	vs	stems	0.680	0.407	0.050	0.044
tubers	vs	roots	0.287	0.187	0.000	0.213
stems	vs	roots	0.023	0.309	0.019	0.371
tubers	vs	leaves		0.007	0.017	
stems	vs	leaves		0.021	0.665	
roots	vs	leaves		0.090	0.093	

A.3.4 Cytotoxicity of *R. tridentata* on different cell lines

Table A.5 P-values from student-t tests comparing the absorbance at 540nm from the MTT assays performed using different concentrations of *Rhoicissus* extract on the four cell lines.

		Hepatoma	Leydig	Histiocytoma (DF=127)	Graham (DF=144)
0.01g/ml	vs controls	0.259	0.001	0.000	0.030
1mg/ml	vs controls	0.001	0.083	0.001	0.134
0.1mg/ml	vs controls	0.645	0.095	0.000	0.081
0.01mg/ml	vs controls	0.306	0.031	0.002	0.317
1ug/ml	vs controls	0.872	0.756	0.000	0.687
0.1ug/ml	vs controls	0.775	0.042	0.053	0.562

A.3.5 Seasonal differences in cytotoxicity

Table A.6 P-values from student-t tests comparing the absorbance at 540nm from the MTT assays performed using *Rhoicissus* extracts from dormant (D) and growing (G) seasons on the four cell lines. Highlighted blocks show P-values less than 0.05.

		Hepatoma	Leydig	Histiocytoma (DF=71)	Graham (DF=77)
D controls	vs G controls	0.948	0.092	0.959	0.299
D 0.01g/ml	vs G 0.01g/ml	0.135	0.522	0.465	0.001
D 1mg/ml	vs G 1mg/ml	0.396	0.619	0.694	0.011
D 0.1mg/ml	vs G 0.1mg/ml	0.943	0.350	0.616	0.618
D 0.01mg/ml	vs G 0.01mg/ml	0.597	0.066	0.969	0.109
D 1µg/ml	vs G 1µg/ml	0.596	0.945	0.000	0.566
D 0.1µg/ml	vs G 0.1µg/ml	0.318	0.475	0.000	0.836

A.3.6 Cytotoxicity of extracts from different plant parts

Table A.7 P-values from student-t tests comparing the absorbance at 540nm from the MTT assays performed using *Rhoicissus* extracts from tubers (T) and stems (S) on the hepatoma cells.

		p-value
T controls	vs S controls	0.274
T 0.01g/ml	vs S 0.01g/ml	0.678
T 1mg/ml	vs S 1mg/ml	0.302
T 0.1mg/ml	vs S 0.1mg/ml	0.579
T 0.01mg/ml	vs S 0.01mg/ml	0.184
T 1µg/ml	vs S 1µg/ml	0.623
T 0.1µg/ml	vs S 0.1µg/ml	0.080

Table A.8 P-values from student-t tests comparing the absorbance at 540nm from the MTT assays performed using *Rhoicissus* extracts from different plant parts on the Graham cell line. Highlighted blocks show P-values less than 0.05.

Tuber controls	vs	Root controls	0.217
Tuber 0.01g/ml	vs	Root 0.01g/ml	0.429
Tuber 1mg/ml	vs	Root 1mg/ml	0.001
Tuber 0.1mg/ml	vs	Root 0.1mg/ml	0.040
Tuber 0.01mg/ml	vs	Root 0.01mg/ml	0.678
Tuber 1ug/ml	vs	Root 1ug/ml	0.685
Tuber 0.1ug/ml	vs	Root 0.1ug/ml	0.792
Tuber controls	vs	Stem controls	0.830
Tuber 0.01g/ml	vs	Stem 0.01g/ml	0.453
Tuber 1mg/ml	vs	Stem 1mg/ml	0.701
Tuber 0.1mg/ml	vs	Stem 0.1mg/ml	0.099
Tuber 0.01mg/ml	vs	Stem 0.01mg/ml	0.161
Tuber 1ug/ml	vs	Stem 1ug/ml	0.313
Tuber 0.1ug/ml	vs	Stem 0.1ug/ml	0.877
Tuber controls	vs	Leaves controls	0.802
Tuber 0.01g/ml	vs	Leaves 0.01g/ml	0.000
Tuber 1mg/ml	vs	Leaves 1mg/ml	0.458
Tuber 0.1mg/ml	vs	Leaves 0.1mg/ml	0.074
Tuber 0.01mg/ml	vs	Leaves 0.01mg/ml	0.206
Tuber 1ug/ml	vs	Leaves 1ug/ml	0.255
Tuber 0.1ug/ml	vs	Leaves 0.1ug/ml	0.174
Root controls	vs	Stem controls	0.131
Root 0.01g/ml	vs	Stem 0.01g/ml	0.014
Root 1mg/ml	vs	Stem 1mg/ml	0.000
Root 0.1mg/ml	vs	Stem 0.1mg/ml	0.774
Root 0.01mg/ml	vs	Stem 0.01mg/ml	0.074
Root 1ug/ml	vs	Stem 1ug/ml	0.092
Root 0.1ug/ml	vs	Stem 0.1ug/ml	0.606
Root controls	vs	Leaves controls	0.263
Root 0.01g/ml	vs	Leaves 0.01g/ml	0.001
Root 1mg/ml	vs	Leaves 1mg/ml	0.081
Root 0.1mg/ml	vs	Leaves 0.1mg/ml	0.374
Root 0.01mg/ml	vs	Leaves 0.01mg/ml	0.670
Root 1ug/ml	vs	Leaves 1ug/ml	0.378
Root 0.1ug/ml	vs	Leaves 0.1ug/ml	0.423
Stem controls	vs	Leaves controls	0.445
Stem 0.01g/ml	vs	Leaves 0.01g/ml	0.671
Stem 1mg/ml	vs	Leaves 1mg/ml	0.535
Stem 0.1mg/ml	vs	Leaves 0.1mg/ml	0.932
Stem 0.01mg/ml	vs	Leaves 0.01mg/ml	0.882
Stem 1ug/ml	vs	Leaves 1ug/ml	0.467
Stem 0.1ug/ml	vs	Leaves 0.1ug/ml	0.664

Table A.9 P-values from student-t tests comparing the absorbance at 540nm from the MTT assays performed using *Rhoicissus* extracts from different plant parts on leydig cells. Highlighted blocks show P-values less than 0.05.

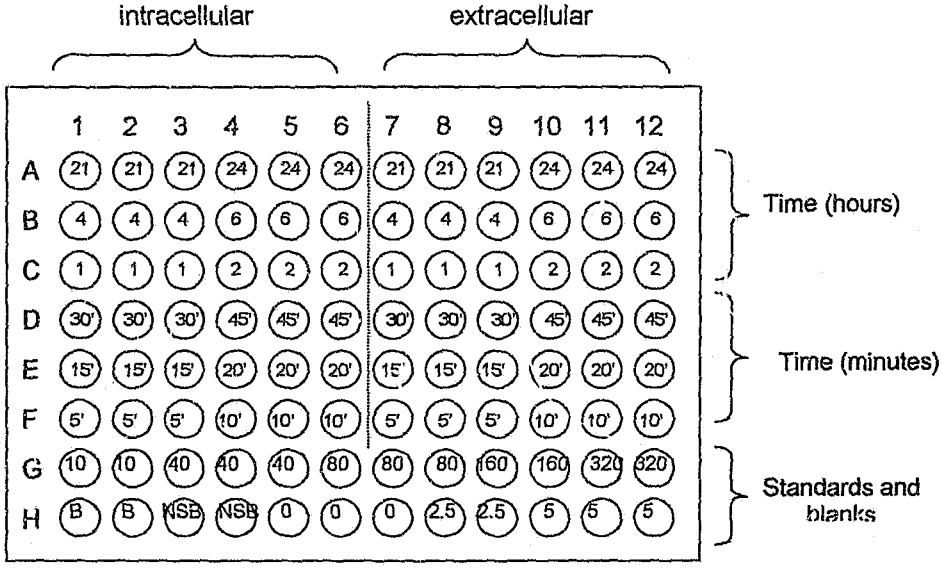
Tuber controls	vs	Root controls	0.235
Tuber 0.01g/ml	vs	Root 0.01g/ml	0.814
Tuber 1mg/ml	vs	Root 1mg/ml	0.222
Tuber 0.1mg/ml	vs	Root 0.1mg/ml	0.113
Tuber 0.01mg/ml	vs	Root 0.01mg/ml	0.004
Tuber 1ug/ml	vs	Root 1ug/ml	0.026
Tuber 0.1ug/ml	vs	Root 0.1ug/ml	0.315
Tuber controls	vs	Stem controls	0.942
Tuber 0.01g/ml	vs	Stem 0.01g/ml	0.208
Tuber 1mg/ml	vs	Stem 1mg/ml	0.666
Tuber 0.1mg/ml	vs	Stem 0.1mg/ml	0.239
Tuber 0.01mg/ml	vs	Stem 0.01mg/ml	0.207
Tuber 1ug/ml	vs	Stem 1ug/ml	0.006
Tuber 0.1ug/ml	vs	Stem 0.1ug/ml	0.147
Tuber controls	vs	Leaves controls	0.815
Tuber 0.01g/ml	vs	Leaves 0.01g/ml	0.057
Tuber 1mg/ml	vs	Leaves 1mg/ml	0.265
Tuber 0.1mg/ml	vs	Leaves 0.1mg/ml	0.143
Tuber 0.01mg/ml	vs	Leaves 0.01mg/ml	0.628
Tuber 1ug/ml	vs	Leaves 1ug/ml	0.003
Tuber 0.1ug/ml	vs	Leaves 0.1ug/ml	0.696
Root controls	vs	Stem controls	0.461
Root 0.01g/ml	vs	Stem 0.01g/ml	0.740
Root 1mg/ml	vs	Stem 1mg/ml	0.892
Root 0.1mg/ml	vs	Stem 0.1mg/ml	0.068
Root 0.01mg/ml	vs	Stem 0.01mg/ml	0.006
Root 1ug/ml	vs	Stem 1ug/ml	0.204
Root 0.1ug/ml	vs	Stem 0.1ug/ml	0.016
Root controls	vs	Leaves controls	0.216
Root 0.01g/ml	vs	Leaves 0.01g/ml	0.371
Root 1mg/ml	vs	Leaves 1mg/ml	0.038
Root 0.1mg/ml	vs	Leaves 0.1mg/ml	0.137
Root 0.01mg/ml	vs	Leaves 0.01mg/ml	0.506
Root 1ug/ml	vs	Leaves 1ug/ml	0.975
Root 0.1ug/ml	vs	Leaves 0.1ug/ml	0.675
Stem controls	vs	Leaves controls	0.285
Stem 0.01g/ml	vs	Leaves 0.01g/ml	0.335
Stem 1mg/ml	vs	Leaves 1mg/ml	0.030
Stem 0.1mg/ml	vs	Leaves 0.1mg/ml	0.027
Stem 0.01mg/ml	vs	Leaves 0.01mg/ml	0.151
Stem 1ug/ml	vs	Leaves 1ug/ml	0.707
Stem 0.1ug/ml	vs	Leaves 0.1ug/ml	0.312

Table A.10 P-values from student-t tests comparing the absorbance at 540nm from the MTT assays performed using *Rhoicissus* extracts from different plant parts on histiocytoma cells. Highlighted blocks show P-values less than 0.05.

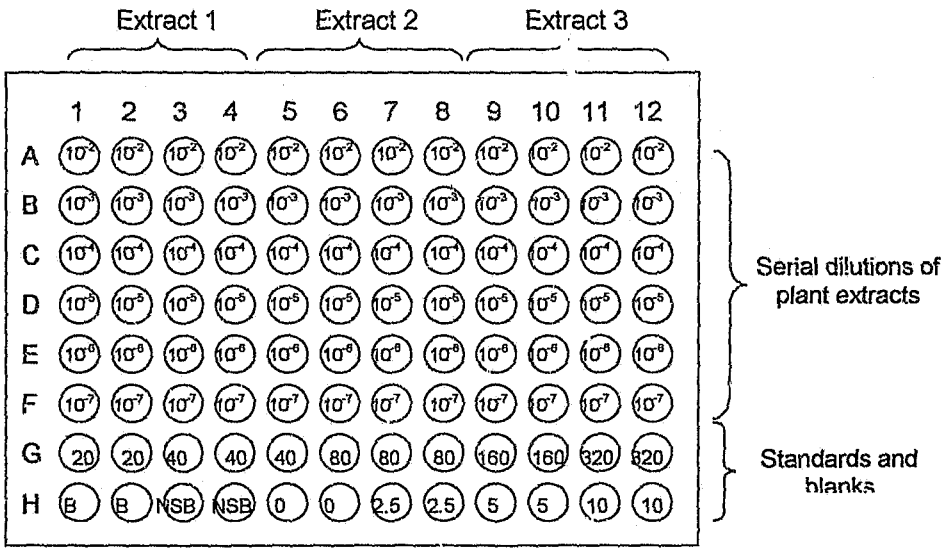
Tuber controls	vs	Root controls	0.142
Tuber 0.01g/ml	vs	Root 0.01g/ml	0.134
Tuber 1mg/ml	vs	Root 1mg/ml	0.048
Tuber 0.1mg/ml	vs	Root 0.1mg/ml	0.050
Tuber 0.01mg/ml	vs	Root 0.01mg/ml	0.387
Tuber 1ug/ml	vs	Root 1ug/ml	0.064
Tuber 0.1ug/ml	vs	Root 0.1ug/ml	0.678
Tuber controls	vs	Stem controls	0.674
Tuber 0.01g/ml	vs	Stem 0.01g/ml	0.000
Tuber 1mg/ml	vs	Stem 1mg/ml	0.000
Tuber 0.1mg/ml	vs	Stem 0.1mg/ml	0.001
Tuber 0.01mg/ml	vs	Stem 0.01mg/ml	0.084
Tuber 1ug/ml	vs	Stem 1ug/ml	0.581
Tuber 0.1ug/ml	vs	Stem 0.1ug/ml	0.697
Tuber controls	vs	Leaves controls	0.044
Tuber 0.01g/ml	vs	Leaves 0.01g/ml	0.094
Tuber 1mg/ml	vs	Leaves 1mg/ml	0.135
Tuber 0.1mg/ml	vs	Leaves 0.1mg/ml	0.514
Tuber 0.01mg/ml	vs	Leaves 0.01mg/ml	0.256
Tuber 1ug/ml	vs	Leaves 1ug/ml	0.189
Tuber 0.1ug/ml	vs	Leaves 0.1ug/ml	0.258
Root controls	vs	Stem controls	0.336
Root 0.01g/ml	vs	Stem 0.01g/ml	0.000
Root 1mg/ml	vs	Stem 1mg/ml	0.000
Root 0.1mg/ml	vs	Stem 0.1mg/ml	0.812
Root 0.01mg/ml	vs	Stem 0.01mg/ml	0.364
Root 1ug/ml	vs	Stem 1ug/ml	0.048
Root 0.1ug/ml	vs	Stem 0.1ug/ml	0.501
Root controls	vs	Leaves controls	0.650
Root 0.01g/ml	vs	Leaves 0.01g/ml	0.502
Root 1mg/ml	vs	Leaves 1mg/ml	0.475
Root 0.1mg/ml	vs	Leaves 0.1mg/ml	0.008
Root 0.01mg/ml	vs	Leaves 0.01mg/ml	0.170
Root 1ug/ml	vs	Leaves 1ug/ml	0.398
Root 0.1ug/ml	vs	Leaves 0.1ug/ml	0.672
Stem controls	vs	Leaves controls	0.584
Stem 0.01g/ml	vs	Leaves 0.01g/ml	0.397
Stem 1mg/ml	vs	Leaves 1mg/ml	0.037
Stem 0.1mg/ml	vs	Leaves 0.1mg/ml	0.090
Stem 0.01mg/ml	vs	Leaves 0.01mg/ml	0.816
Stem 1ug/ml	vs	Leaves 1ug/ml	0.301
Stem 0.1ug/ml	vs	Leaves 0.1ug/ml	0.672

A.4. Layout of microtitre plates for prostaglandin assays

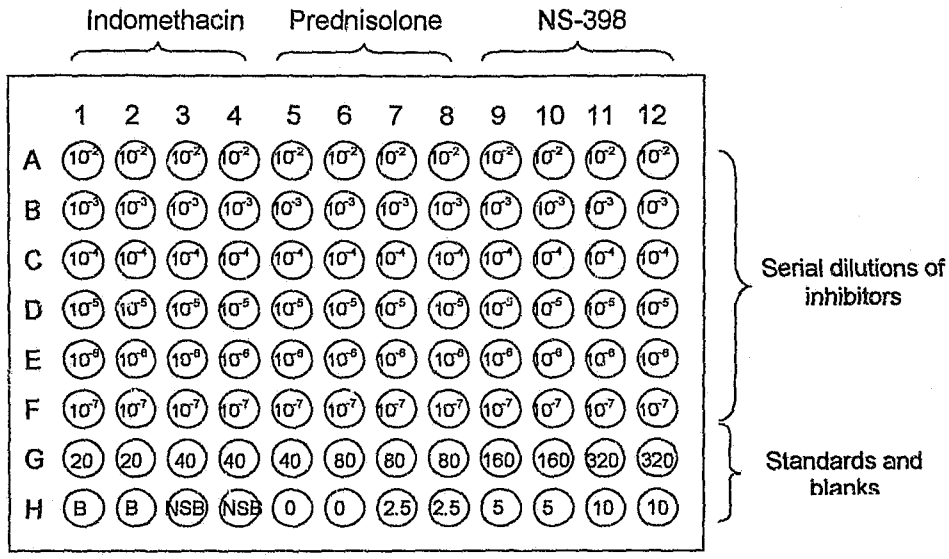
A.4.1 Prostaglandin synthesis over time



A.4.2 Dose-response experiment



A.4.3 Effect of indomethacin and hydrocortisone



A.5. Raw data collected from open-ended interviews with traditional healers

Notes taken from interviews with six of the traditional healers are given below. In most cases the terms used by the traditional healers are given.

NOMSA

Sangoma
Ex nursing sister, midwife
Trained under Mr Nkosi, Deepdale, Swaziland who now has a shop in Mayi Mayi Bazaar, Jeppe Street, Johannesburg

Pregnancy

- 6-7 months
  Labatega (1/2 bulb) - make way for baby
  Gobho (1/2 bulb)
  Baboon urine (5th fingernail size) - good for uterus and cystitis
  Boil mixture for ~ 15 minutes, and give the patient the prepared extract.
  Drink ½ glass/day

- Last two weeks of 8TH month
  Qarda lentshe (ground ostrich shell) ~2cm^2 -strengthens mother and foetus
  Skhupha seguarda (chicken egg yolk)
  Mercury (small droplet) - cause contractions
  1 tsp of remedy once a week

- 9TH month
  Sunlight soap and water enema once a week

Labour (once bleeding has started)

As for 8th month plus
Mtsansi we hashi (horse placenta; paperlike; 1cm^2) – easy delivery, helps contractions & baby, eases pain
Mix all together, 1 tbsp.

Postnatal

Labatega (1/2 bulb) - clears womb
Cimamlilo (1/2 bulb) - kills pain

Usually sends patients to hospital to deliver, but delivered three babies at home as there was not enough time to get to a hospital.

BOUYLIX

Trained in Soweto
Makes all medicines in the same way as described below.
Normally single plants used.
Dosage: normally 1 handful of plant material, powdered.
Brought to the boil in water
Patients given liquid extract and takes (1/2 glass/day (after straining); babies 1 tsp/day).
No set isihlambezo regime – plants prescribed according to patient's needs.

Pregnancy

- 2-3 months after menstruation stops
  Ubani (*Agapanthus africanus*) 1 handful; enema - strengthens foetus
  Iqaku lenifene nempila (baboon urine)
  Impila *(Callilepis laureola)*

- 3-7 months after menstruation stops
  Amabelejongosi (*Lissochilus arenarius*) - reduces pain
  Ugadolo/Amaleyane (*Bidens pilosa*) roots - stops pain from bladder, kidneys
  - helps in limiting amniotic fluid
  - kills scres in bladder
  - gives blood more tonic
  - strengthens body
  Ibhuma (*Cyperus* sp / *Typha* spp) - strengthens foetus

- 6 months to delivery
  Iphengulula (Iphingula – *Berkheya rhapontica*) roots – cleanses bladder
  - cleanses kidneys
  - helps ease backache
  - helps baby's growth
  - easy delivery

  Mix with ostrich shell; nutrients for baby growth; 3 x/day

Medicines for baby
Impila
Ingwavuma (bark) - helps baby's growth
Dumalala – pinch of ground twigs and leaves placed in babies bath water
- makes baby sleep

Other plants used

- If no movement of baby:
  Hlanyathi ihlalanyathi (*Grewia occidentalis*) bark

- helps baby move
- strengthens nerves and veins of reproduction system

Intshingu (*Momordica foetida*) roots
- takes out unneeded amniotic fluid
- revives female reproduction system

Amazombe (*Gynandropsis gynandra*) leaves
(*G. pentaphylla*)

Ukunyakaza
- gives strength to all the nerves and veins of the body

Isinwasi (*Rhoicissus cuneifolia* or *Cissus quandrangularis*) roots
- strengthens female reproductive system

Umayime (*Clivia miniata*) roots
- reduces pain allowing easy delivery (use from 3–9 month)
- revives nerves and veins of reproductive system
- snake antivenom

Iblucu (roots)
Ipahatana
Ghobo (isighobo - *Asparagus* sp)
- menstrual pains
- stomach pains
- skin rash
- infertility
- mental retardation
- swollen nerves in lungs
- cleanses blood

1 pinch ashed roots; lick off palm of hand

Infe-yentawu (*Ansiella humilis*)

Causes of birth complications according to Bouylix
- early induction of labour using isihlambezo (this may cause extreme pains and rupturing of the womb)
- protein in urine causes high blood pressure
- traces of blood in urine, caused by bad insertion of catheter
- umbilical cord may wrap around babies neck, and if it tightens it may suffocate or strangle the baby.

MARTHA

Zulu Chemist along Louis Botha; grandparents (deceased) taught her.

Isihlambezo

- 1-7 months
  powered roots; ½ handful in 1 litre water
  Boil 30 minutes, sieve, give extract; ¼ cup, 2 x/day
  Strengthens baby, makes baby move, takes away pains

- 8-9 months
  ground bulb; 1 litre water; 2 flat tbsp.
  Boil 30 minutes; sieve; give extract; ½ cup 3 x1 day
  - easy delivery, opens cervix
  - makes breech baby turn

Labour induction
  Ostrich egg – ½ tsp powdered
  Burnt record – 1 tsp powdered ash
  Mix in ½ cup water and drink (warm)
  Baby will be born within about 3 hours

Fertility
- For woman who has periods but cannot fall pregnant
  Mahlokoloze (½ bulb)
  Sekaname (½ bulb)
  Mokgalakane (1 cm^3)
  Mix in 2 litres water, simmer for 3 hours, sieve, ½ cup 2 x/day
  Drink a total of 5 litres

- Blocked tubes
  Mathunga (½ bulb)
  Mbola (½ bulb)
  Itolwane (~ 15 x 3cm bark)
  Mix in 2 litres water, simmer 1 hour; make up to ~ 1 litre; ½ cup 1 x /day

- Sores in uterus (drop; VD)
  Inguduza (5cm^3 bulb)
  Udonguzibovu (1/2 bulb)
  Umathunga (1/2 bulb)
  2 litres water; simmer 1 hour; enema; 1 cup once a week

Remedy to take away clots six months after birth
  Umdabu (1 handful, branch)
  Itolwane (1 handful, branch)
  Udabulibayi – chemist, 1 pinch
  1 ½ litres; boil 30 minutes; ½ cup 2 x/day – only 1 litre
  Clean womb-bleeding

FIKI

Trained in 1980 in Meadowlands ~ 1 year.
Worked as nurse assistant in Johannesburg General, then assistant for a gynaecologist in Hillbrow. Currently in "River" training.
Now waiting for spirits to call. They are asked for their cow which means she has probably almost finished.

Infertility

isinwazi (tuber) - cleansing
Boil 30cm, in 1 litre ¼ cup/day (1st day full cup)
Husband and wife drink 1 litre in total. "like DNC, settling womb"

Mpopo (1 bulb) – cleansing
Boil 30cm in 1 litre. Both drink 1 cup 1st day, then ¼ cup/day, till litre finished. Take off tops, leave bottoms on while they drink, helps conception.
If don't conceive, throw bones to find out reason.
Reasons eg. – use 'prevent' earlier (contraception not favoured)
Spirits may want something e.g. want the new bride presented to the ancestor's grave.

Remedies used during pregnancy

- 3 – 8 months
  isinwazi – as before; no danger
  Lady takes as she feels, max ½ cup/ day
  She may miss a day
  Protects baby and mother from other illnesses (makes them healthy)

- 8 – 9 months
  Nothing

- Once deep into labour, just before going to hospital
  Sehlula ananye ~ level tsp
  isinwazi pinch
  Put powder above lighted charcoal
  Kneel with it between legs

Won't give any other medicines during pregnancy, even if ill.

The only one is:
Impepe (grass)
Light it and breath fumes (same as if not pregnant) for headache
Bile – common cause of headaches and stomach aches.

Remedies for baby

Impila – use if child is restless – or add to imbiza
1 tsp boiled in cup of water – calms the child.

If woman previously miscarried

Umayime, isinwazi and unagugu are mixed together

Umaguqu – to settle child if too high or breech.

Womb cleansing remedy

Hlunguhlung umathung

- burn to reduce potency
- roll ash in vaseline – small amounts
- place in vagina – opens tubes and clears womb, then give imbiza

MOSEKI

Traditional healer for ± 30 years
Trained in Bekkersdal.

Infertility

- Infertility caused by VD
  Ghobo (1 flat tsp)
  Sekaname (¼ bulb)
  Magaga (¼ bulb)
  Labeteka (¼ bulb)
  Boil 1 hour in 2 litres of water, make up to 2 litres.
  ½ cup after breakfast, lunch and supper. First day 1 cup.
  When pain gone ½ cup 2 x /day.

- Menstruate too often
  Labetega
  Gobo
  ½ cup 3 x / day
  Also massage as womb may not be in good condition

Pregnancy

Don't give medicines routinely. Usually only massage.

Isihlambezo:

Intolani (1 dsp roots)
Marula (1 dsp bark)
Boil 1 hour in 2 litres water, ½ cup 3 x / day

Backache:

patheyengaka

Menstrual pains & during pregnancy if miscarried before

Morokalapudi (1 tsp roots)
Boil 1 hour in 2 litres; ½ cup 3 x / day.

Ntolwani – makes woman urinate, keeps system going; loosens muscles allowing an easy delivery;maternal strength

Morning sickness
1 green apple in morning
Mageuo (soft porridge)
2 cups water.

Overdue pregnancy - massage to induce labour.

LAYMOND

Zulu
Has shop in Soweto
Inyanga and sangoma but practices as an inyanga.
Previously journalist for World News
Used to dig out plants for his grandfather
Trained under 2 people – 1 inyanga and 1 sangoma.

Imbiza
Umdabou (1 dsp root) boil in ½ litre and cool
Use as an enema or drink ¼ cup 3 x day – no side effects
Ishaqa (1 dsp root in ½ litre)
Umsongelo (1 dsp root in ½ litre)

If not menstruating – imbiza
Gobo (1 dsp root)
Ndoda emadevu (1 dsp root)
Boil, or just pour on hot water

Pregnancy - isihlambezo
First finds out how mother-to-be feels and diagnoses according to symptoms
Isihlambezo - *used to make new baby grow perfectly*
Start giving from 1st month

- *If woman feeling well*
  Ubogo 2 dsp / l (root)
  Boil/pour on hot water, ½ cup 3 x /day.
  Drinks it when babies not moving or feels she needs it.
  Eaten as porridge.

- Backache or tired
  Ugobho (root) grows in wet area 2 dsp/litre

- Headache
  Labatega - 1 dsp/litre ¼ cup 3 / day.

If labour overdue
Impompo 1 tsp ½ litre
Induces vomiting. Drinks 2 sp / hr x Spyt. Gives birth same day without fail.

A.6. Harvesting details

Rh A roots
Harvesting: Umlazi grasslands, 5/2/96 (from Bridget)

Sample prep: Came as a shredded sample which I milled and boiled in distilled water for approximately an hour, filtered through itself and then lyophilized.

Organ Response: variable. Four responses
initial augmentation, no suppression of the maximal response
initial augmentation, suppression of the max
no initial augmentation, no supression of the max
no initial suppression, suppression of the max

Did many runs as results were variable. The high n value elicited the different responses to the same samples run on different organs simultaneously. That is with all variables held constant four distinct responses were evident.
This could be explained by there being different compounds in the extract which all have similar intrinsic rates of activity and dissociation constants. The response of a particular dosing would be dependant on the order in which the different compounds bound to the receptors or which compound dominated the dose. The second option is unlikely as the lyophilized matter was mixed in relatively large quantities and then decanted into eppindorff tubes to ensure all constituents of the extract were randomly distributed through different dosing fractions.

Rh B: Autumn, 2/5/96, Suikerbosrand, in a thicket. (1st patch)
No tuber could be found while digging.

Sample prep: Dried sample away from direct light for two weeks. Cut the large portions into pieces about $1cm^3$ then milled the sample through rotomill in the pharmacy department.

Rh C: spring, bone dry. 2/9/96, Suikerbosrand, in a thicket. (1st patch)
There were no leaves on the plant yet, just a few emerging leaves.

Rh D: spring, bone dry. 2/9/96, Suikerbosrand, in a thicket. (2nd patch)
Plant was approximately 10m away from Rh B and C.
No tuber was found. There were no leaves on the plant yet, just a few emerging leaves.

Rh E: early summer, before first rains. 3/11/96, Suikerbosrand, in a thicket (1st patch).
Rh F: mid summer, after a very wet period. 3/12/96, Suikerbosrand, in a thicket (1st patch).

Rh G: mid summer, after a very wet period. 3/12/96, Suikerbosrand in a thicket (2nd patch).

Rh H: Tonquani Gorge, Magaliesburg Mountains, Halfway up side of gorge in dry, humus rich soil below a dense canopy.

Rh I: Umlazi, Durban, 10/96 (collected by Bridget). May be the same as Rh A

Rh J: Garden in Mondeor, 3/97 (Leonie's Garden).

Rh K: Suikerbosrand, late summer - autumn, after a very wet period, 1/4/97 (1st patch).

Rh L: Suikerbosrand, late summer - autumn, after a very wet period, 1/4/97 (2nd patch).

Rh M: Suikerbosrand, late summer - autumn, after a very wet period, 1/4/97 (3rd patch).

Rh N: Mt Sheba, 12/6/97 - winter, but plant still growing. The plant was growing in a fairly sunny part of the gorge, in the crevices of a large rock close to the stream at the bottom of the gorge.

Rh O: Suikerbosrand, mid-winter, 1st patch (1st patch)

Rh P: Suikerbosrand, mid-winter, 1st patch (2nd patch)

Rh Q: Suikerbosrand, mid-winter, 1st patch (3rd patch)

A.7. Raw data from all experiments

Raw data are presented in the following order:
(Excel was used to tabulate the data, which does not have the facility to number pages)

Isolated organ experiments with the following reference drugs:
(Data from uterine tissue is always given before data from ileal tissue)

Experiments testing the mechanism of action:

- All Reference drugs
- Oxytocin
- Indomethacin
- Atropine
- Pirenzepine
- Gallamine
- 4-DAMP
- Tropicamide
- Prazosin
- Yohimbine
- Methysergide
- Ketanserin
- Tropisetron
- All bar graphs
- Correlation between baseline and contraction

Experiments testing the variation in contractile response:

- ACh standards
- Different plant parts and seasons
- Plant extract alone - box & whisker plots
- Stability of harvested plant material

MTT Assays:

- Graham's cells (Kidney epithelial cells)
- Leydig cells
- Hepatocyoma cells
- Histiocytoma cells

Uterine contractile response to reference drugs relative to acetylcholine

Acetylcholine

ACh conc	33.1.b	33.1.f	33.2.a	34.1.a	34.1.e	36.1.a	36.2.a	39.1.b	39.3.a	39.3.b	39.4.a	39.4.b	105.2.a	106.1.a	106.2.a	107.1.a	107.2.a	108.1.a	108.2.a
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.3	0.0	18.3	0.0	0.0	0.0
-7	0.0	0.0	1.4	0.0	14.6	0.0	0.0	28.4	0.0	0.0	0.0	0.0	0.0	22.9	2.3	25.0	13.1	8.5	2.4
-6.5	4.1	12.1	1.8	17.9	22.9	25.3	0.0	34.7	5.1	3.2	3.6	1.0	9.8	38.1	16.1	33.8	26.4	25.5	41.4
-6	30.2	32.2	8.7	48.0	57.6	60.8	11.2	31.6	33.0	28.9	16.2	14.9	47.4	61.0	33.3	53.6	64.5	33.0	71.0
-5.5	42.9	47.0	19.6	68.3	75.7	81.2	37.1	38.6	50.6	48.1	33.5	35.9	73.7	79.7	54.0	75.2	80.2	50.0	81.7
-5	65.7	66.4	43.5	85.4	88.9	94.9	73.5	59.8	73.9	70.3	60.9	63.4	91.0	86.4	75.9	88.5	90.9	75.5	89.3
-4.5	77.6	82.6	13.8	91.1	94.4	98.0	88.2	74.9	85.2	83.4	81.2	80.0	96.2	94.1	86.2	93.8	94.2	88.3	92.3
-4	91.4	91.6	82.6	95.1	98.3	100.0	94.7	86.0	96.6	92.0	90.4	92.1	97.0	94.9	95.4	99.1	96.7	97.9	97.3
-3.5	97.1	97.3	91.3	98.0	100.0	100.0	100.0	96.7	98.9	97.9	94.9	99.0	99.6	97.5	99.4	100.0	98.3	100.0	98.8
-3	99.6	100.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.5	100.0	100.0	99.2	100.0	100.0	100.0	100.0	100.0
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Oxytocin

	103.1.d	103.2.d	104.1.d	104.2.d	28.1	29.1	29.2	29.3	29.4	31.1	31.2	31.3	31.4
-13.4	0.0	0.0	0.0	0.0									
-12.9	0.0	0.0	0.0	0.0									
-12.4	0.0	0.0	0.0	0.0									
-11.9	0.0	0.0	0.0	0.0									
-11.4	20.3	11.0	14.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-10.9	24.6	27.0	21.4	24.7	0.0	0.0	0.0	0.0	2.7	0.0	0.0	8.4	23.5
-10.4	28.8	24.6	33.1	38.3	0.0	0.0	0.0	0.0	4.6	4.6	0.0	2.3	32.2
-9.9	39.0	27.8	44.4	48.7	11.5	26.5	0.0	20.8	11.0	24.0	0.0	20.2	39.1
-9.4	44.1	38.1	69.4	60.4	23.0	44.7	22.5	34.7	26.6	32.4		34.8	49.7
-8.9	49.2	43.7	77.4	70.8	32.7	58.0	24.4	56.2	48.4	19.7	12.0	42.2	58.7
-8.4	62.7	64.3	83.1	77.9	64.1	82.7	46.9	82.6	73.1	70.7	32.5	69.3	75.0
-7.9	78.8	81.7	97.6	87.0	81.0	93.6	71.9	87.6	89.0	87.8	73.0	79.1	85.8
-7.4	92.4	93.7	104.8	98.1	92.3	99.5	90.6	96.1	92.5	101.6	95.0	90.4	95.9
-6.9	96.6	95.2	111.3	102.6	95.5	102.0	103.1	100.4	98.9	107.0	107.0	96.5	90.0

Noradrenaline

NA conc	36.1.c	36.2.c	39.1.c	39.3.c	39.4.c
-9.5	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0
-8	24.6	0.0	0.0	0.0	0.0
-7.5	32.1	0.0	0.0	0.0	0.0
-7	31.4	11.2	0.0	0.0	0.0
-6.5	34.8	17.6	0.9	0.0	0.0
-6	32.8	12.9	12.6	1.1	3.8
-5.5	32.1	19.1	24.2	4.4	0.0
-5	26.6	28.8	27.0	16.9	9.5
-4.5	38.2	24.7	28.4	26.8	15.0
-4	34.8	21.8	36.5	27.9	24.0

Serotonin

5-HT conc	33.1.c	33.1.e	33.2.c	34.1.b	34.1.d	104.1.e	104.2.e	105.2.c	106.1.c	106.2.c	107.1.c	107.2.c	108.1.c	108.2.c
-10	0.0	0.0	0.0	0.0	0.0			0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9.5	0.0	6.0	0.0	0.0	0.0	35.5	44.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	2.4	0.0	0.0	0.0	0.0	50.0	48.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	10.6	0.0	0.0	0.0	0.0	51.6	5[illegible].2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8		0.0	5.8	0.0	0.0	54.8	54.5	12.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	20.8	6.7	3.3	0.0	0.0	49.2	56.5	21.1	37.3	9.2	0.0	0.0	0.0	0.0
-7	19.6	11.4	10.1	35.8	0.0	66.1	66.2	41.4	42.4	25.3	1.8	26.4	10.6	21.9
-6.5	41.2	8.7	17.0	50.4	22.2	71.8	70.1	65.4	44.9	43.7	13.3	46.3	29.8	44.4
-6	55.5	27.4	25.4	69.5	47.6	83.1	76.0	88.0	46.6	48.3	26.5	66.9	51.1	63.9
-5.5	70.6	33.4	32.6	78.9	63.9	87.9	79.9	93.6	51.7	52.9	39.8	78.5	74.5	78.1
-5	73.1	35.5	41.3	88.6	71.5	90.3	80.5	94.0	52.5	57.5	41.6	83.5	88.3	85.8
-4.5	73.9	40.8	44.2	87.8	73.3	90.3	80.5	94.0	57.6	62.1	45.1	83.5	91.5	86.4
-4	70.6	44.1	40.6	82.9	71.5	90.3	81.8	94.0	61.0	62.1	48.4	83.5	93.6	87.0

Ileal contractile response to reference drugs relative to acetylcholine

Acetylcholine

ACh conc	33.4.b	33.4.h	34.3.a	34.4.a	34.4.f	35.3.e	35.4.e	105.3.a	105.4.a	106.3.a	106.4.a	107.3.a	107.4.a	108.3.a	108.4.a
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	3.2	10.3	0.0	0.0	3.7	5.0	18.9	8.7	14.7	2.9	6.7	0.8	0.0	1.1	1.8
-7	8.1	31.2	6.1	4.9	37.8	21.8	53.2	34.8	60.3	30.9	38.3	11.4	30.4	25.1	26.4
-6.5	18.6	55.3	22.0	31.1	62.2	48.1	79.3	41.8	79.4	60.3	67.5	39.6	71.4	62.3	57.3
-6	43.2	77.5	37.7	64.0	75.6	69.0	88.3	76.6	86.8	79.4	85.0	68.3	83.9	80.9	77.3
-5.5	67.6	86.5	55.4	73.2	86.6	83.7	94.6	83.6	91.2	88.2	91.7	79.2	90.2	87.4	86.4
-5	86.5	96.0	73.2	90.2	96.3	100.0	99.1	92.3	95.6	94.1	96.7	90.1	94.6	92.9	92.7
-4.5	94.6	98.8	84.9	96.3	99.4	100.0	100.0	97.5	100.0	97.1	100.0	94.6	97.3	95.1	95.5
-4	98.9	100.0	94.6	98.8	100.0	100.0	100.0	99.2	100.0	97.1	100.0	98.0	99.1	98.4	98.2
-3.5	100.0	100.0	98.5	98.8	100.0	100.0	100.0	100.0	100.0	98.5	100.0	100.0	99.6	99.5	99.1
-3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Serotonin

5-HT conc	33.4.c	33.4.e	33.4.g	34.3.b	34.4.b	34.4.e	35.3.d	35.4.d	105.3.c	105.4.c	106.3.f	106.4.e	107.3.e	107.4.c	108.3.c	108.4.c
-10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	1.0	0.0	0.0	1.3	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	1.1	0.0	2.0	0.0	2.4	1.3	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8		1.1	0.8	2.9	4.9	3.7	1.3		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	1.6	0.8	5.9	11.0	8.5	1.3	4.5	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	2.2	0.8	6.8	15.9	12.2	1.7	11.7	0.0	4.4	0.0	1.7	0.0	0.9	8.7	1.4
-6.5	0.5	3.2	1.6	11.7	26.8	15.9	3.8	22.5	1.7	57.4	2.9	18.3	7.9	2.2	13.1	3.6
-6	2.2	7.6	5.1	20.5	34.1	20.7	7.9	30.6	23.1	30.9	7.4	18.3	31.7	5.4	32.8	9.1
-5.5	4.9	12.4	8.6	59.5	52.4	25.0	14.6	45.0	39.7	42.6	10.3	30.0	46.5	7.1	42.6	13.6
-5	7.0	18.9	49.0	49.8	56.1	32.9	22.0	55.0	47.9	42.6	33.8	40.0	58.4	14.3	51.4	18.2
-4.5	10.3	22.2	49.0	49.0	56.0	32.0	35.6	65.3	54.5	47.1	64.7	65.0	81.2	26.8	57.4	20.9
-4	11.4	22.0	49.0	49.0	56.0	32.0	49.8	81.5	57.9	47.1	80.9	76.7	81.2	27.0	61.2	23.6
-3.5	13.0	22.0	49.0	49.0	56.0	32.0	61.9	94.6	57.9	47.1	80.9	76.7	81.2	27.0	61.2	23.6

The effect of the Rhoicissus extract on the organ responses to Oxytocin

Oxytocin std

	28.1	29.1	29.2	29.3	29.4	31.1	31.2	31.3	31.4
-11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-10	0.0	0.0	0.0	0.0	2.7	0.0	0.0	8.5	23.5
-9	0.0	0.0	0.0	0.0	4.6	2.4	0.0	2.5	32.2
-8	12.1	25.8	0.0	20.7	11.1	27.2	0.0	20.9	39.1
-7	24.1	43.8	21.8	34.6	26.9	35.7	0.0	35.7	49.7
-6	34.4	56.9	23.6	56.0	48.9	14.7	11.2	43.7	58.7
-5	67.1	80.9	45.5	82.3	73.9	66.6	30.4	71.8	75.0
-4	84.9	91.6	69.7	87.2	89.9	84.5	68.2	81.9	85.8
-3	96.6	97.5	87.9	95.7	93.5	95.7	88.8	93.6	95.9
-2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Rhoicissus **extract and oxytocin**

	28.1.d	29.1.c	29.1.f	29.2.c	29.2.f	29.3.c	29.3.f	29.4.c	29.4.f	28.1.b	31.1.c	31.2.c	31.3.c	31.4.c
-11.5	17.1	23.3	28.4	17.6	11.6	29.9	25.7	9.1	0.0	32.0	69.0	24.0	40.0	49.0
-11	0.0	23.3	0.0	0.0	0.0	24.3	24.3	4.8	0.0	0.0		12.0	40.0	49.0
-10	22.0	23.3	0.0	0.0	0.0	20.9	24.3	4.8	0.0	0.0	39.8	12.0	41.0	49.0
-9	22.0	23.3	0.0	18.3	0.0	19.4	24.6	13.4	5.2	0.0	56.5	13.0	37.0	49.0
-8	20.7	54.3	33.0	18.3	15.4	47.4	30.9	28.2	12.4	12.0	57.5	15.0	37.0	33.0
-7	24.4	57.4	48.9	23.2	22.1	63.1	34.6	45.2	27.1	19.0	52.0	14.0	47.0	45.5
-6	29.3	64.3	53.2	28.9	1.7	69.8	59.6	59.4	44.0	33.0	61.0	14.0	52.0	56.0
-5	42.7	86.0	82.6	47.2	36.0	87.3	86.0	72.0	74.1	38.0	91.5	23.0	75.0	69.0
-4	59.1	93.8	92.9	59.9	60.5	93.3	90.4	86.0	77.7	65.0	118.0	31.5	89.0	83.0
-3	89.0	98.4	96.8	85.6	84.3	97.8	95.6	94.1	93.4	79.0	134.5	70.0	107.0	94.0
-2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	87.0	139.0	96.0	113.0	99.0

Effect of indomethacin on the uterine response to the *Rhoicissus* extract

Acetylcholine std

Ach std	97.1.g	98.4.g	99.3.d	99.3.g	101.4.g	102.4.g
-9	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	3.3	0.0	0.0	0.0
-6.5	8.8	2.8	4.9	1.6	6.6	0.0
-6	13.2	9.7	8.2	4.9	10.5	9.7
-5.5	22.0	30.6	21.3	16.4	21.1	19.4
-5	39.6	41.7	41.0	31.1	42.1	30.6
-4.5	50.5	62.5	54.1	47.5	61.8	58.3
-4	76.9	83.3	73.8	70.5	85.5	79.2
-3.5	92.3	97.2	89.3	86.9	96.1	90.3
-3	98.9	100.0	100.0	98.4	100.0	100.0
-2.5	100.0	100.0	100.0	100.0	100.0	100.0

Indomethacin and acetylcholine

Ach conc	46.1	46.2	47.1	47.2	48.1	48.2	49.1	49.2	50.1	50.2	51.1	51.2
-9.5	40.2	21.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	2.6	3.3
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	21.4	0.0	0.0	0.0	64.6	0.0	8.0	0.0	0.0	0.5	0.0	0.5
-8	0.0	0.0	0.0	0.0	26.6	0.0	9.6	0.0	0.0	0.0	0.0	0.0
-7.5	22.6	9.6	9.0	0.0	39.6	0.0	9.9	0.0	0.0	3.9	0.0	3.6
-7	43.6	17.1	0.0	0.0	37.5	2.1	10.4	0.6	0.0	8.7	3.0	0.0
-6.5	53.6	21.1	12.7	0.0	42.7	7.6	10.7	4.3	6.5	8.2	4.2	3.0
-6	63.7	30.0	22.3	4.0	54.5	17.0	19.7	7.2	19.8	12.5	12.6	8.7
-5.5	78.0	41.8	48.8	15.6	68.3	30.3	26.4	13.5	33.9	20.5	27.8	11.9
-5	90.6	63.8	57.2	31.1	85.5	54.8	51.3	26.3	58.0	39.7	47.1	21.4
-4.5	96.2	80.5	72.3	50.7	92.0	62.6	70.4	51.6	74.6	57.9	66.1	32.4
-4	97.4	89.9	85.5	75.8	97.6	86.9	85.1	75.2	86.4	80.0	85.9	58.6
-3.5	99.1	96.3	93.4	89.0	98.4	95.8	94.1	85.9	96.2	92.5	94.8	81.7
-3	100.0	99.3	100.0	98.6	100.0	99.0	97.7	96.1	98.5	99.6	99.5	97.2
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Rhoicissus extract and acetylcholine

Ach conc	78.2.b	78.4.b	79.1.l	79.4.l	80.2.l	80.4.l
-9.5	85.0	25.9	30.6	6.6	75.8	15.6
-9	66.0	25.9	26.9	0.0	71.2	15.6
-8.5	66.0	25.9	26.9	0.0	71.2	15.6
-8	66.0	33.3	26.9	0.0	71.2	15.6
-7.5	66.0	35.2	26.9	0.0	71.2	15.6
-7	66.0	53.7	26.9	0.0	71.2	21.9
-6.5	66.0	59.3	26.9	7.2	71.2	28.1
-6	66.0	75.9	26.9	14.5	71.2	48.4
-5.5	66.0	87.0	26.9	19.1	72.7	68.8
-5	68.0	98.1	26.9	43.4	77.3	82.8
-4.5	73.0	101.9	37.3	59.2	87.9	90.6
-4	87.0	104.6	49.3	77.6	93.9	95.3
-3.5	99.0	108.3	53.0	85.5	97.0	97.7
-3	104.0	108.3	65.7	89.5	100.0	99.2
-2.5	106.0	108.3	64.2	92.1	100.0	100.0

Indomethacin, *Rhoicissus* extract and acetylcholine

Ach conc	97.1.h	98.4.h	99.3.e	101.4.h	102.4.h	50.1.g	51.1.d	51.2.d
-9.5	8.5	6.7	0.0	0.0	0.0	16.9	0.0	10.1
-9	8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	8.5	0.0	0.0	0.0	0.0	19.7	0.0	0.0
-7	8.5	0.0	3.3	0.0	0.0		.0	10.6
-6.5	8.5	3.3	3.3	7.9	0.0	28.7	.0	0.0
-6	12.8	10.8	6.6	11.8	17.1	25.8	11.9	12.1
-5.5	13.8	26.7	18.0	17.1	20.0	41.0	49.7	26.6
-5	29.8	59.2	39.3	39.5	45.7	58.1	83.4	57.8
-4.5	44.7	84.2	62.3	52.6	62.9	68.0	97.4	83.4
-4	68.1	108.3	91.8	68.4	88.6	87.6	100.0	88.4
-3.5	87.2	127.5	109.0	81.6	94.3	89.9	100.7	89.4
-3	91.5	133.3	116.4	82.9	94.3	100.0	100.0	99.5
-2.5	91.5	133.3	116.4	92.9	94.3	100.0	100.0	100.0

Effect of indomethacin on the ileal response to the *Rhoicissus* extract

Acetylcholine std

Ach conc	105.3.a	105.4.a	103.3.a	106.4.a	107.3.a	107.4.a	108.3.a	108.4.a	Rh G	46.3	46.4	47.3	48.3	49.3	49.4	50.3	50.4	51.3	51.4
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	1.8	2.2	0.0	0.0	0.0	0.0	0.0
-8	8.7	14.7	2.9	6.7	0.8	0.0	1.1	1.8		0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0
-7.5	34.8	60.3	30.9	38.3	11.4	30.4	25.1	26.4		1.1	0.0	0.0	10.3	4.3	0.0	0.9	0.0	0.0	0.0
-7	41.8	79.4	60.3	67.5	39.6	71.4	62.3	57.3		7.9	1.3	2.4	27.2	12.7	2.0	2.3	0.0	0.0	2.2
-6.5	76.6	86.8	79.4	85.0	68.3	83.9	80.9	77.3		22.5	9.2	9.7	39.0	27.9	5.0	12.5	4.9	0.0	14.6
-6	83.6	91.2	88.2	91.7	79.2	90.2	87.4	86.4		50.6	25.0	21.4	51.1	43.3	17.6	38.6	14.6	8.5	37.7
-5.5	92.3	95.6	94.1	96.7	90.1	94.6	92.9	92.7		75.3	48.7	53.6	66.9	64.7	34.3	60.0	25.8	26.1	69.9
-5	97.5	100.0	97.1	100.0	94.6	97.3	95.1	95.5		88.8	71.1	71.8	82.1	78.0	36.9	80.9	48.8	47.1	86.9
-4.5	99.2	100.0	97.1	100.0	98.0	99.1	98.4	98.2		95.5	89.5	79.7	89.4	84.3	72.7	89.9	69.5	60.4	93.7
-4	100.0	100.0	98.5	100.0	100.0	99.6	99.5	99.1		100.0	97.4	87.9	97.1	93.2	85.9	97.4	86.6	70.4	100.0
-3.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0		100.0	100.7	93.7	97.8	95.5	93.0	97.6	92.7	80.1	100.0
-3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0		100.0	100.0	98.2	97.7	98.7	96.5	99.6	95.1	94.3	100.0
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Indomethacin and acetylcholine

Ach conc	46.3	46.4	47.3	48.3	49.3	49.4	50.3	51.3	51.4	50.3	50.4	51.3	51.4	50.4
-9.5	0.0	0.0	0.0	0.0	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.3
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	2.1	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.0
-8	0.0	0.0	0.0	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	8.1	12.9	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	14.0
-7	8.1	1.9	0.0	12.2	19.4	0.0	6.9	0.0	1.2	0.0	0.0	0.0	0.0	18.6
-6.5	35.0	8.3	3.9	22.7	41.9	2.0	18.5	0.0	11.6	3.7	8.6	0.0	5.1	22.1
-6	64.1	26.5	20.4	42.5	64.5	10.0	46.8	9.9	44.2	25.9	15.5	4.5	29.1	25.6
-5.5	82.2	51.9	39.5	61.5	73.4	26.0	67.1	22.9	64.0	50.0	22.4	7.5	51.9	34.9
-5	91.9	77.2	72.4	80.8	82.3	52.0	82.1	44.3	83.7	69.4	36.2	10.5	77.2	53.5
-4.5	96.8	91.7	93.4	89.3	88.7	68.0	89.0	65.6	94.2	77.8	48.3	34.6	91.1	62.8
-4	98.8	98.8	97.4	94.5	91.9	80.0	93.6	79.4	98.8	87.0	63.8	45.1	97.5	74.4
-3.5	99.8	100.0	99.0	96.4	90.3	88.0	97.1	84.0	100.0	89.8	69.0	57.1	100.0	74.4
-3	100.0	100.0	100.0	97.0	88.7	92.0	99.4	90.1	100.0	90.7	79.3	75.2	100.0	76.7
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	87.2	100.0	100.0

***Rhoicissus* extract and acetylcholine**

Ach std	22.3.c	22.4.e	23.3.c	23.4.c	25.4.h	30.3.e	30.4.c	30.4.e	33.3.h	33.4.l	33.4.j	34.3.h	34.4.g	36.3.e	36.4.e
-9.5	53.0	82.4	19.7	16.8	10.7	0.0	1.4	16.1	0.0	5.5	5.9	11.4	20.7	4.3	12.5
-9	22.0	73.6	19.7	0.0	10.7	0.0	0.0	9.1	0.0	3.6	4.7	11.4	20.7	4.3	12.5
-8.5	22.0	73.6	19.7	0.0	10.7	0.0	0.0	10.9	0.0	3.6	4.7	11.4	20.7	4.3	12.5
-8	22.0	73.6	19.7	0.0	10.7	0.0	0.0	15.0	0.0	4.0	4.7	11.4	20.7	4.3	12.5
-7.5	22.0	79.3	19.7	0.0	10.7	0.0	3.4	20.2	0.0	5.9	5.1	11.4	20.7	4.3	12.5
-7	22.0	73.6	19.7	0.0	10.7	1.2	13.4	32.6	0.0	8.3	5.1	11.4	24.4	5.7	12.5
-6.5	22.0	76.2	19.7	0.0	10.7	10.5	38.4	53.4	0.0	18.2	5.1	21.9	36.6	12.9	14.4
-6	22.0	80.3	19.7	0.0	10.7	34.9	61.0	67.4	0.0	38.3	6.7	40.0	59.8	32.9	32.6
-5.5	34.5	84.5	19.7	3.2	13.7	59.3	82.2	87.6	11.3	56.1	4.3	50.5	69.5	45.0	52.3
-5	53.0	90.2	19.7	8.9	28.0	80.2	100.0	93.3	24.5	72.7	11.5	61.9	81.7	60.0	69.7
-4.5	68.5	93.8	38.9	33.7	56.3	90.7	98.6	90.2	45.3	80.2	28.9	64.6	84.1	70.0	82.6
-4	81.3	95.3	73.2	66.3	66.2	100.0	98.6	90.2	58.5	84.6	48.2	61.9	84.1	72.9	89.4
-3.5	85.7	99.0	88.5	87.4	69.2	100.0	98.6	90.2	58.5	83.8	69.2	60.0	81.7	77.1	95.5
-3	88.7	100.0	99.4	96.8	74.7	100.0	98.6	90.2	58.5	81.0	76.3	60.0	81.0	80.0	98.5
-2.5	89.0	100.0	100.0	100.0	78.0	100.0	98.6	90.2	58.5	84.6	76.7	60.0	81.0	80.0	100.0

Indomethacin, *Rhaicissus* extract and acetylcholine

Ach conc	105.3.b	105.4.b	106.3.b	106.4.b	107.3.b	107.4.b	108.3.b	108.4.b
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	2.7	0.0	0.0
-7	1.7	4.4	0.0	0.0	0.0	8.0	0.0	0.0
-6.5	26.4	22.1	0.0	0.0	14.9	24.1	5.5	11.8
-6	49.6	54.4	2.2	8.3	39.6	50.0	29.5	39.1
-5.5	80.2	83.8	38.2	33.3	60.4	73.2	60.1	67.3
-5	100.8	97.1	55.9	51.7	75.2	81.3	76.5	82.7
-4.5	107.4	108.8	75.0	69.2	88.1	88.4	88.5	94.5
-4	110.7	117.6	86.8	80.0	95.0	91.1	95.1	100.0
-3.5	114.0	120.6	92.6	87.5	102.0	94.6	100.5	106.4
-3	112.4	124.3	94.1	90.8	105.0	96.4	102.7	107.3
-2.5	112.4	126.5	95.6	93.3	107.9	96.4	103.8	108.2
	112.4	126.5	95.6	93.3	107.9	97.3	109.3	110.0

46.3	46.4	47.3	48.3	49.3	49.4	49.3	49.4	50.3	50.4	50.3	50.4
0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	12.1
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.9	0.5	0.9	1.3	0.0	0.0	0.0	0.0	5.2
0.0	0.0	0.0	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	0.0	0.0	7.2	2.0	1.8	5.1	0.0	0.0	0.0	1.5	10.3
9.9	1.2	1.3	11.9	5.0	1.8	17.9	1.1	1.3	0.0	13.2	13.8
31.7	6.2	5.3	20.8	26.0	5.3	30.8	2.3	8.8	2.7	17.0	16.4
59.4	21.0	18.4	34.0	42.5	31.6	48.7	6.8	21.3	5.4	18.9	19.0
78.2	42.0	34.2	49.9	54.0	40.4	57.7	13.6	93.8	10.8	34.0	25.9
91.1	69.1	65.8	68.5	63.0	57.0	62.8	23.9	71.3	21.6	49.1	39.7
96.0	77.2	81.6	82.2	69.0	68.4	67.9	36.4	80.0	28.4	54.7	46.6
99.0	79.0	86.8	88.5	73.0	73.7	74.4	59.1	87.5	37.8	58.5	55.2
100.0	77.8	88.2	90.7	76.0	78.9	70.5	61.4	88.8	45.9	58.5	55.2
100.0	76.5	88.2	91.8	79.0	78.9	76.9	63.6	85.0	66.2	54.7	56.9
100.0	72.8	84.2	90.0	81.0	78.9	80.8	72.7	85.0	73.0	69.8	74.1

The effect of atropine on the uterine response to the *Rhoicissus* extract

Acetylcholine standard

Exp No.	22.1	22.2	23.1	23.2	24.1	24.2	25.1	25.2	26.1	26.2	27.1	27.2	33.1	34.1	34.2	103	104
	0	0	0	0	17.4	16.8	3.3	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	4.7	0	0	0	0	0	0
	0	0	2.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-7.5	4.5	0	0	4.4	0	0	1	1.1	4.7	0	8.3	0	0	0	3	1.304348	0
-7	0	3	0	0	20.7	25.3	2.5	2.3	0	0	7.3	0	0	14.6	4.9	18.43296	2.05617
-6.5	7.1	5.6	0	0	22.6	23.7	10.3	1.8	19.5	3.2	8.3	0	12	22.9	0	33.55112	13.33091
-6	14.3	19.3	1	6.3	26.2	32.5	14.1	5.8	28.6	8	13	6.3	32.1	57.6	16.2	60.85588	43.21947
-5.5	28	32.6	8.1	15	37.7	46.9	22.1	10.8	36.3	14.6	20.5	26.4	46.8	75.7	32.9	77.78094	47.17566
-5	51.4	59.1	26	33.8	53.4	62.3	38.9	23	57	27.4	34.7	30.2	66.2	88.9	54.9	88.37169	83.79379
-4.5	70.2	76.1	42.9	51.9	70.8	73	53.9	35.2	69.6	44.8	49.8	38.1	82.3	94.4	72.6	93.96899	91.0519
-4	83.6	90.3	62.9	71.3	80.9	86.7	70.5	59.4	81.2	68.2	66.2	57.3	91.3	98.3	89	98.88132	98.5738
-3.5	90.7	92.7	78.6	86.3	91	92.8	83	80.6	90.2	86.3	82	68.8	97	100	94.5	99.17944	99.67532
-3	99.1	99.6	93	98	97.7	98.5	98.8	98.3	98	108.6	95.6	100	100	100	98.5	100	100
-2.5	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Atropine and Acetylcholine

Exp No.	58	59	62	63	33.1	34.1	34.2	103.1	103.2	104.1	104.2
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.8	0.0	2.4	1.4
-6	0.0	0.0	12.8	0.0	0.0	0.0	0.0	23.0	30.6	8.1	11.6
-5.5	11.6	0.0	18.4	0.6	0.0	0.0	0.0	33.6	41.1	30.9	32.0
-5	4.5	3.8	23.1	13.2	0.0	0.0	0.0	62.8	71.0	57.7	61.9
-4.5	18.7	8.5	29.9	21.2	0.0	0.0	0.0	80.5	84.7	75.6	72.8
-4	38.2	30.1	38.0	32.2	36.0	23.7	10.0	88.5	91.9	90.2	89.1
-3.5	54.0	44.1	53.0	50.5	58.7	64.1	36.0	96.5	96.0	95.9	93.2
-3	83.0	81.9	81.1	69.3	87.0	82.4	90.0	99.1	100.0	100.0	97.3
-2.5	92.4	92.8	92.1	82.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0
-2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

***Rholdsaus* extract then acetylcholine**

Exp No.	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	22	23	25	29	33	34
-9.5	21.5	0.1	57.9	0.7	26.6	76.5	38.1	9.9	31.4	58.5	46.2	21.1	25.7	57.6	50.7	18.5	26.8	18.1	4.8	29.1	7.7
-9	25.4	0.0	11.0	0.0	0.0	0.0	34.1	0.0	33.6	56.5	0.0	0.0	0.0	46.3	33.8	17.0	10.2	12.3	4.8	0.0	0.0
-8.5	25.4	0.0	82.6	0.0	38.0	0.0	34.1	0.0	33.6	56.5	0.0	0.0	0.0	46.3	33.8	17.0	11.8	12.3	4.8	3.9	0.0
-8	27.1	0.0	11.0	0.0	0.0	0.0	34.1	0.0	33.6	56.5	0.0	0.0	0.0	55.7	33.8	17.0	10.8	12.3	4.8	0.0	0.0
-7.5	26.5	7.8	61.5	0.0	48.4	0.0	41.6	12.0	34.5	56.5	0.0	0.0	0.0	56.9	45.1	17.0	10.8	12.3	4.8	30.7	8.8
-7	31.2	0.7	17.6	0.0	58.7	52.0	48.9	15.5	37.8	56.5	42.3	33.4	20.2	52.9	43.7	24.4	10.8	12.3	24.9	0.0	1.1
-6.5	34.3	1.2	56.0	26.7	54.3	74.5	63.1	35.6	44.8	59.8	42.3	28.8	28.5	50.9	40.8	25.9	10.8	13.3	4.8	0.0	20.0
-6	35.9	14.5	13.2	28.0	59.8	78.4	73.3	53.1	49.5	62.2	47.4	48.3	41.4	51.5	40.8	30.1	10.8	20.5	32.0	24.4	27.8
-5.5	55.2	17.0	83.7	40.1	74.5	89.2	82.7	69.9	63.2	70.7	59.0	55.5	56.1	57.8	54.9	36.2	11.2	25.6	42.8	36.2	42.0
-5	70.7	40.2	24.2	55.0	81.5	93.6	92.1	0.0	74.0	76.8	76.3	67.9	78.5	64.2	81.7	48.4	21.4	34.9	60.9	45.7	57.5
-4.5	89.5	66.8	62.6	59.0	88.0	94.1	96.3	95.0	82.3	85.0	87.2	80.2	89.6	76.4	94.4	67.9	37.8	45.1	75.5	59.1	68.2
-4	100.6	82.0	65.9	75.0	91.3	99.0	99.0	100.0	92.2	91.2	94.9	88.4	97.0	88.7	104.2	84.6	65.6	61.2	95.5	72.4	83.9
-3.5	101.1	93.5	71.4	80.1	95.7	101.5	98.8	100.2	96.9	96.8	97.4	94.6	99.6	95.4	111.3	98.5	83.8	79.6	102.1	76.0	100.0
-3	103.9	99.5	92.3	90.7	99.5	101.5	99.8	101.5	100.3	99.2	99.0	99.5	100.7	100.6	115.5	101.8	97.0	100.5	108.9	96.1	100.7
-2.5	103.9	99.5	92.3	90.7	99.5	101.5	99.8	101.5	100.3	99.2	99.0	99.5	100.7	100.6	115.5	101.8	97.0	100.5	108.9	96.1	100.7

Atropine; extract then acetylcholine

	Br (extract code)		Cr				Ct				Dr				Gr						
Exp No.	58.3	60.1	60.3	61.4	62.2	62.4	60.1	60.3	61.1	61.3	77.2	77.4	61.1	61.3	77.2	77.4	58.4	60.2	60.4	78.2	75.4	79.2	79.4
Rh	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0
Ach conc																							
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0
-7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2	0.0	2.2	0.0	0.0	0.0	0.0
-6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	2.2	0.0	0.0	0.0	0.0
-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.5	0.0	2.2	0.0	0.0	0.0	0.0
-5.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	28.1	0.0	0.0	2.8	26.1	0.0	0.0	1.3	0.0	3.3	0.0	0.0	0.0	0.0
-5	0.0	0.0	0.0	5.0	24.4	5.0	1.0	5.5	5.7	28.7	2.2	0.0	5.7	28.7	2.2	0.0		4.9	0.9	4.3	18.5	0.0	0.0
-4.5	9.8	15.2	0.0	7.5	24.4	5.7	3.9	5.5	8.5	27.0	5.4	4.7	8.5	27.0	5.4	4.7	6.7	5.6	3.6	9.8	49.1	0.0	0.0
-4	22.0	28.1	6.0	11.3	26.2	6.3	17.6	15.4	8.5	29.8	10.2	29.1	8.5	29.6	10.2	29.1	12.1	87.0	9.4	21.7	59.3	0.0	0.0
-3.5	24.4	44.4	14.3	17.5	34.1	16.4	31.4	29.7	14.5	43.5	45.2	55.8	14.5	43.5	45.2	55.8	23.5	40.3	25.9	41.3	75.9	6.7	15.2
-3	35.4	64.3	32.1	23.8	44.5	28.4	49.0	51.6	24.8	55.7	55.9	79.1	24.8	55.7	55.9	79.1	34.9	59.7	43.8	63.0	85.2	29.8	40.0
-2.5	53.7	78.4	46.4	41.3	60.4	42.8	58.8	71.4	40.4	72.2	75.3	93.0	40.4	72.2	75.3	93.0	52.3	79.9	73.2	79.3	90.7	52.9	60.7
-2	62.8	84.2	53.0	43.8	70.1	55.3	66.7	73.6	61.7	82.6	35.5	93.0	61.7	82.6	35.5	93.0	61.7	87.7	76.3	85.9	92.6	55.8	63.4

Effect of Atropine on the ileal contractile response to the extract

Acetylcholine standard

Ach conc	22.3	22.4	23.3	23.4	24.3	24.4	25.4	26.3	26.4	27.3	27.4	33.4	34.3	34.4	36.3	36.4
-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-8.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-8	0	0	0	0	0.9	0	0	0	0	0	0	0	0	0	0	0
-7.5	0	1.7	0	0	1.5	0	2	0.8	2	0	4.4	10.3	0	3.7	0	0
-7	3	8.2	1.3	3.1	9.1	4	8	4.2	8.8	0	14.5	31.2	11.4	37.8	2.9	4.4
-6.5	2	27.1	5.2	7	30.7	15.3	17.5	11.5	18	8.3	22.9	55.3	29.7	62.2	25.7	23.9
-6	23.8	52.5	14.8	12.8	53.7	25.1	42.9	30.9	37.3	13.9	28.6	77.5	57.1	75.6	54.3	47.8
-5.5	51.2	72.2	27.9	21.8	68.5	35.8	61.5	47.8	54.2	22.2	42.6	88.5	75.4	86.6	71.4	68.6
-5	75	83.5	47.2	38.9	81	61.5	78.9	71.2	72.4	38.9	62.8	96	91.4	96.3	80	80.5
-4.5	85.1	90.5	64.7	55.3	88.5	77.9	86.2	83.9	84.3	63.9	83.8	98.8	100	99.4	92.9	87.4
-4	94	95	82.6	75.8	94	90.7	93.1	93.3	92.4	81.9	95.5	100	100	100	95.7	93.7
-3.5	97.9	97.5	93.2	88.6	96.5	94.9	96.3	98.4	96.4	94.4	100	100	100	100	98.6	96.5
-3	99.4	99.6	98.9	97.1	99.3	98.8	97.4	98.7	97.6	100	100	100	100	100	100	96.9
-2.5	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Atropine and acetylcholine

Ach conc	34.3.j	34.4.i	36.4.g	36.3.g	103.3.b	103.4.b	104.3.b	104.4.b
-9.5								
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-6.5	0.0	3.7	0.0	0.0	2.5	0.0	4.1	0.0
-6	4.0	7.3	0.0	0.0	22.2	2.0	18.4	0.0
-5.5	20.0	11.0	0.0	0.0	51.2	12.2	44.9	27.6
-5	24.0	49.4	1.5	0.0	77.8	49.0	78.6	65.8
-4.5	40.0	61.0	8.2	0.0	86.4	81.6	87.8	81.6
-4	64.0	73.2	31.3	23.9	91.4	98.0	92.9	92.1
-3.5	80.0	79.3	65.7	53.7	97.5	100.0	96.9	96.1
-3	100.0	81.7	92.5	85.1	100.0	100.0	100.0	100.0
-2.5	100.0	81.7	100.0	100.0	100.0	100.0	100.0	100.0

Extract and acetylcholine - Ileum

Ach conc	20.3	20.4	21.3	21.4	22.3	22.4	23.3	23.4	25.4	30.3	30.4	33.3	33.4	33.4	34.3	34.4	36.3
-9.5	44.4	71.3	51.9	11.5	53.0	82.4	19.7	16.8	10.7	0.0	8.7	0.0	5.7	5.9	11.4	20.7	4.3
-9	18.7	31.1	36.2	5.1	22.0	73.6	5.1	0.0	1.1	0.0	4.5	0.0	4.2	4.7	3.8	6.1	0.0
-8.5	18.7	31.1	36.2	5.1	22.0	73.6	5.1	0.0	1.1	0.0	5.4	0.0	4.2	4.7	3.8	6.1	0.0
-8	18.7	31.1	36.2	5.1	22.0	73.6	4.5	0.0	1.1	0.0	7.5	0.0	4.3	4.7	3.8	6.1	0.0
-7.5	23.5	31.8	36.2	6.4	22.0	79.3	3.8	0.0	1.1	0.0	11.8	0.0	5.5	5.1	3.8	13.4	0.7
-7	23.5	34.2	36.2	6.4	22.0	73.6	3.8	0.0	1.1	1.2	23.0	0.0	6.7	5.1	10.5	24.4	5.7
-6.5	24.3	34.2	40.0	7.7	22.0	76.2	1.9	0.0	2.7	10.5	45.9	0.0	11.7	5.1	21.9	36.6	12.9
-6	26.5	34.8	50.9	14.1	22.0	80.3	1.9	0.0	8.2	34.9	64.2	0.0	22.5	6.7	40.0	59.8	32.9
-5.5	36.7	44.8	66.9	31.4	34.5	84.5	3.8	3.2	13.7	59.3	84.9	11.3	30.2	4.3	50.5	69.5	45.0
-5	60.8	64.0	81.8	51.3	53.0	90.2	13.4	8.9	28.0	80.2	96.6	24.5	42.1	11.5	61.9	81.7	60.0
-4.5	82.2	80.8	90.8	64.1	68.5	93.8	38.9	33.7	56.3	90.7	94.4	45.3	54.5	28.9	64.8	84.1	70.0
-4	93.7	96.8	97.6	70.5	81.3	95.3	73.2	66.3	66.2	100.0	94.4	58.5	66.4	48.2	61.9	84.1	72.9
-3.5	100.0	95.8	100.0	75.6	85.7	99.0	88.5	87.4	69.2	97.7	94.4	58.5	76.5	69.2	60.0	81.7	77.1
-3	100.0	96.3	100.0	75.6	88.7	100.0	99.4	96.8	74.7	97.7	94.4	58.5	78.7	76.3	60.0	81.0	80.0
-2.5	100.0	100.0	100.0	76.3	89.3	100.0	100.0	100.0	78.0	97.7	94.4	58.5	80.6	76.7	60.0	81.0	80.0

Atropine, extract then acetylcholine

Ach conc	34.3	36.4	23.3	23.4	24.4	23.3	23.4	24.3	26.3	24.3	27.4	34.4	36.3	24.4	25.4	26.4	27.3
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4	0.0	12.2	0.0	0.0	0.0	4.1	0.0
-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	41.5	0.0	0.0	0.9	4.7	0.0
-5.5	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.8	8.7	3.5	57.9	0.0	0.0	2.3	8.1	8.6
-5	3.8	0.0	0.0	0.0	0.0	0.0	0.0	1.2	21.8	28.9	19.3	67.1	0.0	2.5	7.8	20.3	28.6
-4.5	9.7	1.3	4.2	2.0	0.0	0.0	3.1	3.0	36.2	47.0	39.0	75.6	4.3	9.3	26.0	37.8	50.0
-4	19.0	3.1	23.2	8.0	10.6	12.9	16.7	18.7	59.6	64.5	47.8	82.9	21.4	27.8	55.3	54.7	57.1
-3.5	25.7	16.4	48.6	34.0	31.7	43.6	38.0	45.2	82.1	71.7	57.9	82.9	47.1	54.9	76.3	64.9	68.6
-3	31.4	44.0	75.2	68.0	74.2	77.6	65.6	82.5	98.1	75.0	60.5	85.4	72.9	67.9	83.6	70.3	74.3
-2.5	32.4	61.6	79.7	86.0	91.9	76.7	76.6	94.0	100.0	78.3	60.0	85.0	84.3	75.3	84.2	71.6	77.1

Effect of pirenzepine on the uterine response

Acetylcholine std

Ach conc	130.1.a	130.2.a	130.3.a	130.4.a	130.1.c	130.2.c	130.3.c	130.4.c	131.1.a	131.2.a	131.3.a	131.4.a	131.1.c	131.2.c	131.3.c	131.4.c
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.4	12.8	5.0	0.0	0.0	0.0
-7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.4	13.9	0.0	0.0	0.0	0.0
-6.5	74.5	72.4	69.2	33.0	0.0	16.7	64.6	52.5	50.0	3.6	5.6	23.4	38.1	0.0	0.0	16.7
-6	75.8	71.4	71.7	56.4	74.2	68.0	75.2	66.2	70.6	51.4	45.6	52.0	60.5	40.0	38.2	49.6
-5.5	80.9	82.1	78.5	66.3	75.5	77.7	79.2	68.3	81.9	76.2	62.0	72.7	83.3	70.8	58.6	65.0
-5	90.9	90.2	88.3	77.6	81.7	87.7	87.7	84.1	92.1	81.6	93.8	82.4	93.5	87.0	81.3	84.4
-4.5	96.7	96.7	95.5	95.0	86.4	92.5	95.1	89.6	95.2	94.5	86.2	91.2	95.2	95.5	89.0	89.9
-4	99.9	100.0	95.5	99.3	92.0	97.3	98.1	95.8	98.4	97.7	93.6	95.4	98.8	98.0	93.8	96.0
-3.5	99.8	100.0	100.0	100.0	94.6	99.9	100.0	100.0	99.9	100.0	97.1	98.1	100.0	100.0	96.4	97.4
-3	100.0	100.0	100.0	100.0	98.1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.6	99.5
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Pirenzepine and acetylcholine

Ach conc	130.1.b	130.2.b	130.3.b	130.4.b	131.1.b	131.2.b	131.3.b	131.4.b
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-5.5	0.0	53.0	22.5	0.0	0.0	0.0	0.0	0.0
-5	56.4	72.0	56.2	38.5	55.4	0.0	33.8	35.6
-4.5	82.7	78.8	80.9	76.9	64.5	65.9	51.2	57.4
-4	89.5	86.4	87.6	90.4	83.5	80.3	69.5	77.2
-3.5	94.0	90.9	89.9	94.2	95.0	93.2	83.3	89.1
-3	100.0	98.5	94.4	98.1	100.0	96.2	92.9	97.4
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

***Rhoicissus* extract and Acetylcholine**

Ach conc	81.2.b	81.4.b	82.2.h	82.4.h	83.1.e	83.3.e –K	81.1.e - L	81.3.e	82.1.b	82.3.b	83.1.h	83.3.h - L	81.1.b–M	81.3.b	82.1.h	82.3.h	83.2.e	83.4.e
-9.5	82.9	44.0	62.6	26.0	11.9	21.8	10.1	9.9	17.6	13.1	7.9	7.2	22.2	2.3	11.7	5.9	86.3	43.5
-9	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-8.5	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-8	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-7.5	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-7	77.8	47.3	67.6	26.7	11.9	24.3	13.2	9.9	21.2	13.1	13.3	11.8	15.0	3.8	0.0	14.6	79.7	40.2
-6.5	77.8	56.0	68.2	33.3	23.9	36.0	15.3	17.9	27.1	24.6	15.2	16.0	19.8	13.1	17.6	18.5	79.7	47.3
-6	77.8	76.7	69.3	65.3	26.7	52.3	25.4	40.3	40.0	46.3	18.2	24.1	19.8	34.6	30.2	44.9	79.7	64.1
-5.5	88.0	88.0	72.6	78.7	28.4	69.5	41.3	57.8	55.3	59.0	34.5	34.6	24.0	52.3	43.9	62.9	83.0	79.3
-5	96.8	96.7	86.0	89.3	60.8	84.5	63.0	75.3	84.7	75.8	60.6	50.6	50.3	76.2	68.3	78.0	91.5	87.5
-4.5	100.0	101.3	88.3	94.7	72.7	93.7	77.2	88.2	103.5	84.4	70.3	68.4	71.3	87.7	79.0	88.3	94.1	92.4
-4	102.5	103.3	94.4	98.7	83.0	100.4	88.4	93.9	117.6	91.0	87.9	81.9	92.8	96.2	90.2	97.6	96.7	95.7
-3.5	104.4	104.0	96.1	100.0	90.3	103.8	93.7	96.6	125.9	95.1	89.7	88.6	106.0	100.0	96.6	101.5	98.7	97.3
-3	104.4	102.7	98.3	100.0	92.6	104.2	97.9	97.3	127.1	95.9	94.5	92.8	112.0	100.0	100.0	103.4	99.3	97.3
-2.5	104.4	101.3	92.7	100.0	93.2	103.8	97.8	97.3	127.1	96.7	94.5	97.0	113.2	100.0	100.0	103.4	99.4	97.3
	100.0	100.0	100.0	100.0	100.0	100.0												

Pirenzepine, *Rhoicissus* extract and acetylcholine

Ach conc	130.1.d	130.2.d	130.3.d	130.4.d	131.1.d	131.2.d	131.3.d	131.4.d
-9.5	71.6	48.2	69.2	36.6	57.6	25.1	1.5	46.0
-9	71.6	48.2	69.2	36.6	57.6	25.1	1.5	46.0
-8.5	71.6	48.2	69.2	36.6	57.6	25.1	1.5	46.0
-8	71.6	48.2	69.2	36.6	57.6	25.1	1.5	46.0
-7.5	71.6	48.2	69.2	36.6	57.6	25.1	1.5	46.0
-7	71.6	48.2	69.2	36.6	57.6	25.1	1.5	46.0
-6.5	71.6	48.2	69.2	60.3	57.6	25.1	1.5	46.0
-6	71.6	48.2	69.2	36.6	57.6	25.1	1.5	46.0
-5.5	71.6	63.3	70.2	63.1	57.6	25.1	47.0	52.0
-5	71.6	73.6	76.8	68.7	70.8	60.7	53.1	73.3
-4.5	81.4	75.8	81.8	72.5	81.5	76.2	67.5	77.5
-4	84.9	82.8	85.7	80.6	92.4	87.0	85.2	87.5
-3.5	89.5	89.9	91.1	88.7	96.6	93.3	92.1	92.3
-3	98.9	96.8	98.8	95.6	98.5	96.8	96.7	96.6
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
		100.0	100.0	100.0				

82.4.h	83.1.e	83.3.e –K	81.1.e - L	81.1.b–M	81.1.e - L	81.1.b–M
26.0	11.9	21.8	10.1	22.2	10.1	22.2
20.0	11.9	21.8	10.1	15.0	10.1	15.0
20.0	11.9	21.8	10.1	15.0	10.1	15.0
20.0	11.9	21.8	10.1	15.0	10.1	15.0
20.0	11.9	21.8	10.1	15.0	10.1	15.0
26.7	11.9	24.3	13.2	15.0	13.2	15.0
33.3	23.9	36.0	15.3	19.8	15.3	19.8
65.3	26.7	52.3	25.4	19.8	25.4	19.8
78.7	28.4	69.5	41.3	24.0	41.3	24.0
89.3	60.8	84.5	63.0	50.3	63.0	50.3
94.7	72.7	93.7	77.2	71.3	77.2	71.3
98.7	83.0	100.4	88.4	92.8	88.4	92.8
100.0	90.3	103.8	93.7	106.0	93.7	106.0
100.0	92.6	104.2	97.9	112.0	97.9	112.0
100.0	93.2	103.8	97.8	113.2	97.8	113.2
100.0	100.0	100.0			100.0	

Effect of gallamine on the uterine response

Acetylcholine std

Ach conc	116.2.c	116.3.c	116.4.a	117.1.c	117.3.c	117.4.a	118.1.c	118.1.e	118.3.c	118.3.e	11_.1.c	119.3.c
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	0.0	4.8	0.0	0.0	6.8	2.7	0.0	0.0	0.0	14.7
-6.5	3.2	6.2	0.0	23.4	0.0	0.0	18.2	5.3	26.1	5.6	1.6	20.6
-6	13.5	23.5	6.8	36.3	2.4	7.3	37.2	40.0	43.2	28.7	30.3	26.5
-5.5	38.7	49.4	10.9	54.0	14.3	23.8	64.2	54.0	62.2	41.7	41.0	35.3
-5	65.8	72.8	28.7	70.2	34.9	46.7	79.1	74.7	80.2	70.4	56.4	54.4
-4.5	78.7	82.1	54.2	82.3	57.1	66.2	89.9	84.7	87.4	85.2	67.6	72.1
-4	92.3	91.4	80.3	91.1	80.2	79.5	95.3	93.3	95.5	93.5	82.4	85.3
-3.5	97.4	96.9	93.7	97.6	90.5	92.1	97.3	98.7	99.1	96.3	93.1	92.6
-3	100.0	98.8	99.3	99.2	97.6	98.0	98.6	100.0	100.0	99.1	97.3	95.6
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Gallamine and acetylcholine

Ach conc	116.2.d	117.4.b	118.1.d	118.3.d	119.3.d	127.2.d
-9	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0
-7	3.2	0.0	0.0	0.0	5.0	0.0
-6.5	12.1	0.9	5.0	15.3	20.5	0.0
-6	19.7	6.9	29.6	42.3	34.8	4.6
-5.5	36.3	17.4	48.7	56.8	48.5	17.1
-5	57.3	33.0	61.2	75.7	53.0	41.4
-4.5	73.9	50.2	74.3	85.6	62.1	63.2
-4	85.4	74.2	85.5	90.1	81.8	78.9
-3.5	94.3	88.8	94.1	96.4	89.4	91.4
-3	98.1	97.4	98.0	100.0	97.0	97.4
-2.5	100.0	100.0	100.0	100.0	100.0	100.0

Rhoicissus extract and Acetylcholine

Ach conc	81.2.b	81.4.b	82.2.h	82.4.h	83.1.e	83.3.e –K	81.1.e - L	81.3.e	82.1.b	82.3.b	83.1.h	83.3.h - L	81.1.b–M	81.3.b	82.1.h	82.3.h	83.2.e	83.4.e
-9.5	82.9	44.0	62.6	26.0	11.9	21.8	10.1	9.9	17.6	13.1	7.9	7.2	22.2	2.3	11.7	5.9	86.3	43.5
-9	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-8.5	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-8	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-7.5	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-7	77.8	47.3	67.6	26.7	11.9	24.3	13.2	9.9	21.2	13.1	13.3	11.8	15.0	3.8	0.0	14.6	79.7	40.2
-6.5	77.8	56.0	68.2	33.3	23.9	36.0	15.3	17.9	27.1	24.6	15.2	16.0	19.8	13.1	17.6	18.5	79.7	47.3
-6	77.8	76.7	69.3	65.3	26.7	52.3	25.4	40.3	40.0	46.3	18.2	24.1	19.8	34.6	30.2	44.9	79.7	64.1
-5.5	88.0	88.0	72.6	78.7	28.4	69.5	41.3	57.8	55.3	59.0	34.5	34.6	24.0	52.3	43.9	62.9	83.0	79.3
-5	96.8	96.7	86.0	89.3	60.8	84.5	63.0	75.3	84.7	75.8	60.6	50.6	50.3	76.2	68.3	78.0	91.5	87.5
-4.5	100.0	101.3	88.3	94.7	72.7	93.7	77.2	88.2	103.5	84.4	70.3	68.4	71.3	87.7	79.0	88.3	94.1	92.4
-4	102.5	103.3	94.4	98.7	83.0	100.4	88.4	93.9	117.6	91.0	87.9	81.9	92.8	96.2	90.2	97.6	96.7	95.7
-3.5	104.4	104.0	96.1	100.0	90.3	103.8	93.7	96.6	125.9	95.1	89.7	88.6	106.0	100.0	96.6	101.5	98.7	97.3
-3	104.4	102.7	98.3	100.0	92.6	104.2	97.9	97.3	127.1	95.9	94.5	92.8	112.0	100.0	100.0	103.4	99.3	97.3
-2.5	104.4	101.3	92.7	100.0	93.2	103.8	97.8	97.3	127.1	96.7	94.5	97.0	113.2	100.0	100.0	103.4	99.4	97.3
	100.0	100.0	100.0	100.0	100.0	100.0												

Gallamine, *Rhoicissus* extract and acetylcholine

Ach conc	117.1.d	117.3.d	118.1.f	118.3.f	119.1.d	119.4.d
-10	2.4	0.0	19.4	27.2	0.0	0.0
-9.5	5.6	7.9	48.4	15.5	68.4	47.2
-9	5.6	7.9	48.4	15.5	68.4	47.2
-8.5	5.6	7.9	48.4	15.5	68.4	47.2
-8	5.6	7.9	48.4	15.5	68.4	47.2
-7.5	5.6	7.9	48.4	15.5	68.4	47.2
-7	5.6	9.5	48.4	15.5	68.4	47.2
-6.5	12.1	9.5	48.4	15.5	68.4	52.8
-6	13.7	14.3	48.4	15.5	68.4	52.8
-5.5	17.7	24.6	48.4	15.5	68.4	58.3
-5	31.5	46.0	69.0	70.9	74.7	66.7
-4.5	41.1	69.8	74.2	78.6	75.8	75.0
-4	64.5	96.8	82.6	87.4	85.8	91.7
-3.5	82.3	114.3	92.9	93.2	92.1	94.4
-3	87.9	128.6	95.8	98.1	97.9	97.2
-2.5	91.9	132.5	100.0	100.0	100.0	100.0
	100.0	100.0	100.0	100.0	100.0	100.0

Effect of 4-DAMP on the uterine response

Acetylcholine std

Ach conc	109.2.a	109.4.a	110.1.c	110.3.a	110.4.a	112.1.a	112.2.a	113.3.a	113.4.a	114.3.a	114.4.a	116.1.c	116.4.c	117.2.d	117.4.c
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	3.3	0.0	0.0	0.0	0.3	0.0	0.4	0.0	6.0	9.9	2.6	4.3	7.5	0.0	0.0
-6.5	10.6	3.1	1.6	5.0	6.2	0.0	8.2	6.8	8.0	24.6	10.3	18.3	11.6	18.4	4.1
-6	24.4	18.5	4.9	30.0	26.2	0.0	28.1	27.1	26.0	39.4	36.1	35.7	22.5	40.8	19.4
-5.5	44.7	42.5	18.7	50.0	51.5	0.0	50.9	44.1	46.0	56.6	53.5	54.8	36.4	61.2	39.6
-5	69.1	64.6	34.1	70.0	75.4	0.0	73.0	54.4	66.0	66.3	72.9	72.2	60.7	81.0	56.7
-4.5	82.9	80.6	51.2	74.0	88.5	74.7	83.9	64.7	80.0	78.3	84.7	83.5	75.7	91.2	73.7
-4	91.9	92.9	74.8	90.0	95.4	90.1	96.4	75.0	92.0	93.7	93.1	92.2	89.0	97.3	88.0
-3.5	97.6	96.0	94.3	98.0	99.2	98.9	100.0	85.3	96.0	96.0	95.8	97.4	96.0	100.0	94.5
-3	100.0	97.3	100.0	100.0	100.0	99.5	100.0	91.2	96.0	96.0	97.2	99.1	100.0	100.0	98.6
-2.5	100.0	100.0	100.0	100.0	100.0	99.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

4-DAMP and acetylcholine

Ach conc	110.1.d	112.1.b	113.3.b	114.4.b	116.4.d	117.2.e
-9	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	0.0	0.0	6.4	0.0
-6.5	4.1	0.0	3.6	0.0	10.4	5.4
-6	8.1	2.2	14.3	4.2	15.0	6.8
-5.5	12.2	6.6	23.2	5.0	22.0	14.3
-5	34.1	29.7	32.1	20.8	41.6	32.7
-4.5	40.7	51.6	48.2	39.6	56.1	54.4
-4	50.4	70.3	66.1	52.8	74.0	70.1
-3.5	73.2	81.3	78.6	65.3	82.1	79.6
-3	88.6	89.0	83.9	73.6	87.9	83.0
-2.5	89.0	90.1	84.0	77.1	88.0	83.0
	100.0	100.0	100.0	100.0	100.0	100.0

Rhoicissus extract and Acetylcholine

Ach conc	81.2.b	81.4.b	82.2.h	82.4.h	83.1.e	83.3.e --K	81.1.e - L	81.3.e	82.1.b	82.3.b	83.1.h	83.3.h - L	81.1.b--M	81.3.b	82.1.h	82.3.h	83.2.e	83.4.e
-9.5	82.9	44.0	62.6	26.0	11.9	21.8	10.1	9.9	17.6	13.1	7.9	7.2	22.2	2.3	11.7	5.9	86.3	43.5
-9	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-8.5	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-8	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-7.5	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-7	77.8	47.3	67.6	26.7	11.9	24.3	13.2	9.9	21.2	13.1	13.3	11.8	15.0	3.8	0.0	14.6	79.7	40.2
-6.5	77.8	56.0	68.2	33.3	23.9	36.0	15.3	17.9	27.1	24.6	15.2	16.0	19.8	13.1	17.6	18.5	79.7	47.3
-6	77.8	76.7	69.3	65.3	26.7	52.3	25.4	40.3	40.0	46.3	18.2	24.1	19.8	34.6	30.2	44.9	79.7	64.1
-5.5	88.0	88.0	72.6	78.7	28.4	69.5	41.3	57.8	55.3	59.0	34.5	34.6	24.0	52.3	43.9	62.9	83.0	79.3
-5	96.8	96.7	86.0	89.3	60.8	84.5	63.0	75.3	84.7	75.8	60.6	50.6	50.3	76.2	68.3	78.0	91.5	87.5
-4.5	100.0	101.3	88.3	94.7	72.7	93.7	77.2	88.2	103.5	84.4	70.3	68.4	71.3	87.7	79.0	88.3	94.1	92.4
-4	102.5	103.3	94.4	98.7	83.0	100.4	88.4	93.9	117.6	91.0	87.9	81.9	92.8	96.2	90.2	97.6	96.7	95.7
-3.5	104.4	104.0	96.1	100.0	90.3	103.8	93.7	96.6	125.9	95.1	89.7	88.6	106.0	100.0	96.6	101.5	98.7	97.3
-3	104.4	102.7	98.3	100.0	92.6	104.2	97.9	97.3	127.1	95.9	94.5	92.8	112.0	100.0	100.0	103.4	99.3	97.3
-2.5	104.4	101.3	92.7	100.0	93.2	103.8	97.8	97.3	127.1	96.7	94.5	97.0	113.2	100.0	100.0	103.4	99.4	97.3
	100.0	100.0	100.0	100.0	100.0	100.0												

4-DAMP, *Rhoicissus* extract and acetylcholine

Ach conc	110.2.d	112.2.b	113.4.b	114.3.b	116.1.d	117.4.d
-9.5	8.3	0.0	17.2	26.3	30.4	3.7
-9	8.3	0.0	17.2	26.3	30.4	3.7
-8.5	8.3	0.0	17.2	26.3	30.4	3.7
-8	8.3	0.0	17.2	26.3	30.4	3.7
-7.5	8.3	0.0	17.2	26.3	30.4	3.7
-7	11.1	0.0	17.2	26.3	30.4	4.1
-6.5	11.8	1.5	17.2	26.3	33.9	5.8
-6	12.5	6.0	17.2	26.9	34.8	9.2
-5.5	15.3	20.9	19.7	27.5	42.6	13.8
-5	24.3	40.3	23.8	38.1	47.8	28.6
-4.5	31.3	57.5	28.7	45.6	60.9	43.1
-4	34.7	77.6	36.9	60.0	73.9	62.2
-3.5	45.1	82.1	39.3	73.8	82.6	78.8
-3	55.6	88.1	38.5	78.1	88.7	84.8
-2.5	47.2	86.6	34.4	78.1	88.7	82.0

Effect of tropicamide on the uterine response

Acetylcholine std

Ach conc	127.1.a	127.1.c	127.2.a	127.3.a	127.3.c	127.4.a	128.1.a	128.1.c	128.2.a	128.2.c	128.1.a	128.1.c	129.2.a	129.1.a	129.1.c	129.1.e	129.2.a	129.2.c
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	17.5	0.0	3.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	3.5	0.0	0.0	0.0	35.1	0.0	9.9	0.0	13.4	0.0	14.0	0.0	0.0	0.0	7.8	0.0
-6.5	1.2	3.7	7.0	0.0	11.4	0.0	61.9	20	33.0	4.5	28.6	0.0	27.1	4.1	0.0	0.8	29.3	2.3
-6	6.0	6.3	25.9	60.7	45.5	0.0	84.5	58.2	60.7	29.5	54.5	19.5	58.1	13.5	0.8	3.4	52.1	25.7
-5.5	10.6	17.4	46.2	73.8	71.6	46.0	93.8	76.9	71.7	52.5	76.8	42.5	77.0	27.9	8.5	13.4	70.7	40.9
-5	27.1	36.9	66.4	85.9	87.5	64.6	96.9	91.8	86.4	72.0	90.2	74.3	91.6	65.8	26.2	29.4	74.3	62.0
-4.5	49.4	65.3	79.0	91.7	94.3	77.0	98.5	96.3	92.1	83.0	93.8	84.1	96.3	81.1	49.2	58.0	82.6	77.2
-4	77.1	91.7	92.3	96.4	98.9	86.7	99.0	99.3	96.3	94.0	99.1	92.0	99.5	91.9	73.8	69.7	85.0	90.1
-3.5	91.6	95.8	97.2	100.0	100.0	95.6	100.0	99.3	99.0	96.5	100.0	96.5	100.0	94.6	87.7	80.7	88.6	94.2
-3	98.8	100.0	100.0	100.0	100.0	99.1	100.0	99.6	100.0	99.0	100.0	99.1	100.0	99.1	96.9	95.0	98.2	98.8
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Tropicamide and acetylcholine

Ach conc	127.3.b	128.1.b	128.2.b	128.1.b	128.2.b	129.1.b
-9	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	0.0	0.0	0.0	0.0
-6.5	0.0	0.0	0.0	0.0	0.0	0.0
-6	9.4	15.3	11.4	0.0	0.0	0.0
-5.5	63.2	56.9	34.8	1.8	0.0	0.0
-5	72.5	78.1	57.2	34.2	32.8	4.8
-4.5	84.2	88.3	66.7	57.0	53.6	12.9
-4	93.6	98.4	79.1	75.4	80.7	35.3
-3.5	99.4	99.3	87.6	87.7	93.8	72.6
-3	100.0	100.0	96.5	95.6	97.9	91.9
-2.5	100.0	100.0	100.0	100.0	100.0	100.0
	100.0	100.0	100.0	100.0	100.0	100.0

Rhoicissus extract and Acetylcholine

Ach conc	81.2.b	81.4.b	82.2.h	82.4.h	83.1.e	83.3.e –K	81.1.e - L	81.3.e	82.1.b	82.3.b	83.1.h	83.3.h - L	81.1.b–M	81.3.b	82.1.h	82.3.h	83.2.e	83.4.e
-9.5	82.9	44.0	62.6	26.0	11.9	21.8	10.1	9.9	17.6	13.1	7.9	7.2	22.2	2.3	11.7	5.9	86.3	43.5
-9	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-8.5	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-8	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-7.5	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0	5.9	79.7	40.2
-7	77.8	47.3	67.6	26.7	11.9	24.3	13.2	9.9	21.2	13.1	13.3	11.8	15.0	3.8	0.0	14.6	79.7	40.2
-6.5	77.8	56.0	68.2	33.3	23.9	36.0	15.3	17.9	27.1	24.6	15.2	16.0	19.8	13.1	17.6	18.5	79.7	47.3
-6	77.8	76.7	69.3	65.3	26.7	52.3	25.4	40.3	40.0	46.3	18.2	24.1	19.8	34.6	30.2	44.9	79.7	64.1
-5.5	88.0	88.9	72.6	78.7	28.4	69.5	41.3	57.8	55.3	59.0	34.5	34.6	24.0	52.3	43.9	62.9	83.0	79.3
-5	96.8	95.7	86.0	89.3	60.8	84.5	63.0	75.3	84.7	75.8	60.6	50.6	50.3	76.2	68.3	78.0	91.5	87.5
-4.5	100.0	101.3	88.3	94.7	72.7	93.7	77.2	88.2	103.5	84.4	70.3	68.4	71.3	87.7	79.0	88.3	94.1	92.4
-4	102.5	103.3	94.4	98.7	83.0	100.4	88.4	93.9	117.6	91.0	87.9	81.9	92.8	96.2	90.2	97.6	96.7	95.7
-3.5	104.4	104.0	96.1	100.0	90.3	103.8	93.7	96.6	125.9	95.1	89.7	88.6	106.0	100.0	96.6	101.5	98.7	97.3
-3	104.4	102.7	98.3	100.0	92.6	104.2	97.9	97.3	127.1	95.9	94.5	92.8	112.0	100.0	100.0	103.4	99.3	97.3
-2.5	104.4	101.3	92.7	100.0	93.2	103.8	97.8	97.3	127.1	96.7	94.5	97.0	113.2	100.0	100.0	103.4	99.4	97.3
	100.0	100.0	100.0	100.0	100.0	100.0												

Tropicamide, *Rhoicissus* extract and acetylcholine

Ach conc	127.3.d	128.1.d	128.2.d	129.1.d	129.2.b	129.2.d
	0.0	0.0	0.0	0.0	0.0	0.0
-9.5	0.0	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	0.0	0.0	0.0	0.0
-6.5	0.0	0.0	0.0	0.0	0.0	0.0
-6	0.0	0.0	0.0	0.0	35.6	0.0
-5.5	13.6	16.5	38.8	1.6	42.4	49.1
-5	37.5	38.5	58.7	8.8	54.8	54.5
-4.5	62.5	66.1	79.1	25.6	78.5	65.5
-4	71.6	78.9	88.8	44.0	89.3	75.8
-3.5	77.3	87.2	93.9	58.4	97.2	88.5
-3	78.4	91.7	96.9	72.0	100.0	93.3
-2.5	78.4	96.3	98.5	78.4	100.0	93.3

Effect of Tropicamide on the ileal response to the extract

Ach std (Percentage of the maximum)

	127.3.a	127.3.c	127.4.a	127.4.c	128.3.c	128.4.a	128.4.c	129.3.a	129.3.c	129.4.a	129.4.c
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	6.7	10.8	7.7	16.1	3.1	0.0	0.0	11.5	1.3	3.8	0.9
-7	16.7	19.4	18.0	29.5	11.6	3.8	1.2	37.2	12.5	21.0	3.4
-6.5	35.0	24.7	36.1	38.3	34.1	11.5	5.6	69.2	38.8	46.6	14.5
-6	63.3	35.5	67.0	49.0	66.7	43.6	13.7	85.9	65.0	82.9	45.3
-5.5	80.0	49.5	79.4	61.1	[illegible]	73.1	46.6	94.9	78.8	89.5	71.8
-5	86.7	65.5	94.8	73.2	8[illegible]	89.7	65.8	100.0	87.5	98.1	87.2
-4.5	91.7	74.2	100.0	81.2	86.[illegible]	92.3	73.9	100.0	91.3	99.0	91.5
-4	96.7	82.8	100.0	87.9	93.8	93.6	78.3	100.0	93.8	99.0	97.4
-3.5	99.2	90.3	100.0	90.6	97.7	100.0	83.9	100.0	95.0	100.0	96.6
-3	100.0	96.8	100.0	91.9	100.0	100.0	96.3	100.0	98.8	100.0	94.0
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Tropicamide and acetylcholine

	127.3.b	127.4.b	128.3.b	128.4.b	129.3.b	129.4.b
-9	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	0.0	0.0	0.0	0.0
-6.5	0.0	0.0	0.0	0.0	0.0	0.0
-6	2.2	11.4	6.0	1.7	7.6	3.4
-5.5	15.1	27.1	20.0	5.0	34.2	17.8
-5	28.0	45.7	55.0	21.8	70.9	60.2
-4.5	52.7	62.9	71.0	50.4	83.5	83.1
-4	73.1	82.1	87.0	73.1	93.7	96.6
-3.5	84.9	90.7	92.0	84.0	97.5	100.0
-3	95.7	96.4	98.0	91.6	97.5	101.7
-2.5	102.2	100.0	100.0	91.0	100.0	107.6
-2	105.4	100.0	100.0	91.6	10.0	107.0

Extract and acetylcholine - ileum

Ach conc	20.3	20.4	21.3	21.4	22.3	22.4	23.3	23.4	25.4	30.3	30.4	33.3	33.4	33.4	34.3	34.4	36.3	36.4
-9.5	44.4	71.3	51.9	11.5	53.0	82.4	19.7	16.8	10.7	0.0	8.7	0.0	5.7	5.9	11.4	20.7	4.3	12.1
-9	18.7	31.1	36.2	5.1	22.0	73.6	5.1	0.0	1.1	0.0	4.5	0.0	4.2	4.7	3.8	6.1	0.0	2.3
-8.5	18.7	31.1	36.2	5.1	22.0	73.6	5.1	0.0	1.1	0.0	5.4	0.0	4.2	4.7	3.8	6.1	0.0	2.3
-8	18.7	31.1	36.2	5.1	22.0	73.6	4.5	0.0	1.1	0.0	7.5	0.0	4.3	4.7	3.8	6.1	0.0	3.8
-7.5	23.5	31.8	36.2	6.4	22.0	79.3	3.8	0.0	1.1	0.0	11.8	0.0	5.5	5.1	3.8	13.4	0.7	4.5
-7	23.5	34.2	36.2	6.4	22.0	73.6	3.8	0.0	1.1	1.2	23.0	0.0	6.1	5.1	10.5	24.4	5.7	7.6
-6.5	24.3	34.2	40.0	7.7	22.0	76.2	1.9	0.0	2.7	10.5	45.9	0.0	11.7	5.1	21.9	36.6	12.9	14.4
-6	26.5	34.8	50.9	14.1	22.0	80.3	1.9	0.0	8.2	34.9	64.2	0.0	22.5	8.7	40.0	59.8	32.9	32.6
-5.5	36.7	44.8	66.9	31.4	34.5	84.5	3.8	3.2	13.7	59.3	84.9	11.3	30.2	4.3	50.5	69.5	45.0	52.3
-5	60.8	64.0	81.8	51.3	53.0	90.2	13.4	8.9	28.0	80.2	96.6	24.5	42.1	11.5	61.9	81.7	60.0	69.7
-4.5	82.2	80.8	90.8	64.1	68.5	93.8	38.9	33.7	56.3	90.7	94.4	45.3	54.5	28.9	64.8	84.1	70.0	82.6
-4	93.7	96.8	97.6	70.5	81.3	95.3	73.2	66.3	66.2	100.0	94.4	58.5	66.4	48.2	61.9	84.1	72.9	89.4
-3.5	100.0	95.6	100.0	75.6	85.7	99.0	88.5	87.4	69.2	97.7	94.4	58.5	76.5	69.2	60.0	81.7	77.1	95.5
-3	100.0	96.3	100.0	75.6	88.7	100.0	99.4	96.8	74.7	97.7	94.4	58.5	78.7	76.3	60.0	81.0	80.0	98.5
-2.5	100.0	100.0	100.0	76.3	89.3	100.0	100.0	100.0	78.0	97.7	94.4	58.5	80.6	76.7	60.0	81.0	80.0	100.0

Tropicamide, extract then acetylcholine

Ach conc	127.3.d	127.4.d	128.3.d	128.4.d	129.3.d	129.4.d
-9.5	4.3		3.1	0.0	0.0	0.8
-9	4.3		3.1	0.0	0.0	0.8
-8.5	4.3		3.1	0.0	0.0	0.8
-8	4.3		3.1	0.0	0.0	0.8
-7.5	4.3		3.1	0.0	0.0	0.8
-7	4.3		3.1	0.0	0.0	0.8
-6.5	5.4		3.1	0.0	0.0	0.8
-6	11.8		3.1	0.6	12.3	6.8
-5.5	23.7	34.9	4.7	2.5	37.0	22.0
-5	39.8	40.3	14.7	11.8	70.4	55.1
-4.5	50.5	50.3	26.4	32.3	84.0	79.7
-4	58.1	67.8	36.4	48.4	95.1	98.3
-3.5	69.9	77.2	40.3	55.3	97.5	102.5
-3	80.6	81.2	43.4	64.6	100.0	105.9
-2.5	88.2	88.6	50.4	80.7	100.0	117.8
-2	93.5	94.0	58.1	106.2		

The effect of Prazosin on the uterine response to the *Rhoicissus* extract

Propanolol and noradrenaline std

	36.A	39.A	39B	40.A	40.B	42.A	42.B
-10	6.5	0.0	0.0	0.0	0.0	0.0	0.0
-9.5	0.5	0.0	0.0	0.0	0.0	16.0	1.0
-9	1.5	0.0	0.0	0.0	0.0	10.6	32.4
-8.5	17.6	0.0	0.0	0.0	0.0	11.1	34.4
-8	22.5	0.0	0.0	0.0	0.0	33.8	51.2
-7.5	32.4	0.0	9.2	0.0	8.9	42.3	39.4
-7	43.9	37.6	11.7	0.0	8.9	54.2	37.8
-6.5	72.0	59.3	3.6	0.0	15.9	65.1	37.4
-6	87.6	74.3	22.3	84.8	49.3	80.9	81.3
-5.5	92.4	78.7	41.7	85.0	84.8	90.4	89.0
-5	100.0	86.5	61.6	94.9	100.0	96.1	100.0
-4.5	100.0	97.0	90.7	100.0	100.0	98.5	100.0
-4	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Propanolol, Prazosin and acetylcholine

NA conc	110.2.e	110.3.e	114.1.d	114.2.c	119.3.e	132.1.e	132.1.h	132.2.e	133.1.g	133.3.g	134.3.g	134.4.g
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	4.9	8.3	3.9	30.0	60.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-6.5	14.6	44.4	3.9	43.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-6	29.3	63.9	23.4	53.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-5.5	53.7	88.9	34.4	73.3	46.7	48.6	4.3	28.8	40.0	0.0	22.1	34.9
-5	73.2	80.6	87.2	100.0	73.3	0.0	51.1	48.6	56.7	46.4	28.3	72.1
-4.5	100.0	83.3	9.0	100.0	96.7	97.3	76.6	96.0	73.3	61.9	60.0	89.8
-4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Propanolol, *Rhoicissus* extract and noradrenaline

	36.1.d	36.2.d	39.1.d	39.4.d	39.4.f	40.1.b	40.3.b	40.4.b	42.3.e	42.4.e	42.1.f	42.3.f
-10.5	52.4	28.6	76.3	41.2		82.9	73.5	72.2	65.2	80.6	35.5	74.7
-10	60.2	28.6	93.6	20.6		63.8	50.0	55.6	47.8	80.6	31.8	18.2
-9.5	40.8	28.6	87.0	20.6		63.8	50.0	55.6	47.8	80.6	31.8	18.2
-9	73.8	28.6	87.0	12.4		63.8	50.0	44.4	47.8	80.6	31.8	22.2
-8.5	69.9	28.6	87.0	12.4		63.8	50.0	44.4	47.8	80.6	57.9	22.2
-8	89.3	28.6	87.0	12.4		63.8	41.2	44.4	47.8	80.6	57.9	22.2
-7.5	83.5	28.6	87.0	12.4		67.6	41.2	44.4	47.8	80.6	57.9	22.2
-7	95.1	47.6	95.0	24.7		63.8	41.2	44.4	53.6	86.1	57.9	94.9
-6.5	95.1	45.2	95.0	24.7		63.8	41.2	58.3	53.6	69.4	78.5	80.8
-6	95.0	45.2	100.0	24.7		79.0	17.6	58.3	68.1	77.8	86.0	80.8
-5.5	100.0	33.3	117.0	72.2	18.8	94.3	64.7	69.4	81.2	100.0	77.6	94.9
-5	110.0	14.3	117.0	72.2	45.8	118.1	97.1	100.0	81.2	120.0	100.0	93.9
-4.5	110.0	16.7	117.0	72.2	60.4	118.0	94.1	100.0	100.0	120.0	100.0	105.0
-4	110.0		117.0	72.2	60.0	118.0	100.0	100.0	100.0	120.0	100.0	105.0

Propanolol, prazosin, *Rhoicissus* extract and noradrenaline

NA conc	112.4.c	114.1.e	132.1.l	132.2.l	133.1.h	134.3.h	
-10.5	75.9	59.4	127.7	7.0	83.3	90.7	76.0
-9	75.9	59.4	127.7	7.0	83.3	90.7	76.0
-8.5	75.9	59.4	127.7	7.0	83.3	90.7	76.0
-8	75.9	59.4	127.7	7.0	83.3	90.7	76.0
-7.5	75.9	59.4	127.7	7.0	83.3	90.7	76.0
-7	81.5	78.9	127.7	7.0	83.3	90.7	76.0
-6.5	94.4	78.1	127.7	7.0	83.3	90.7	76.0
-6	113.0	78.1	127.7	7.0	90.0	90.7	76.0
-5.5	107.4	76.6	148.9	48.0	100.0	93.6	88.8
-5	100.0	79.7	148.9	50.0	106.7	94.3	88.8
-4.5	100.0	82.0	148.9	70.0	113.3	95.0	88.8
-4	100.0	87.5	176.6	105.0	116.7	95.7	105.0

The effect of yohimbine on the uterine response to the *Rhoicissus* extract

Propanolol and noradrenaline

NA conc	109.2.d	109.2.f	109.4.d	109.4.f	110.3.e	110.4.c	110.4.e	111.1.b	111.2.b	113.3.d	113.4.d	114.3.c	114.3.e	114.4.c	133.2.g	134.2.g
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	12.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	14.6	17.6	10.5	0.0	8.3	0.0	24.6	0.0	0.0	0.0	0.0	14.3	21.1	0.0	0.0	0.0
-6.5	48.8	32.4	34.2	84.1	44.4	17.5	45.9	31.3	13.5	0.0	10.3	21.4	21.1	0.0	0.0	0.0
-6	80.5	50.0	53.9	87.8	63.9	42.1	75.4	47.9	30.4	14.0	33.3	25.0	31.6	25.6	0.0	0.0
-5.5	126.8	61.8	68.4	93.9	88.9	68.4	82.0	70.8	60.8	60.5	51.3	28.6	47.4	42.7	20.7	18.7
-5	119.5	70.6	81.6	9[illegible]	80.6	100.0	91.8	87.5	82.4	100.0	100.0	42.9	52.6	51.3	51.8	20.6
-4.5	85.4	85.3	92.1	100.0	83.3	100.0	98.4	93.8	85.1	100.0	100.0	50.0	63.2	76.9	68.9	100.0
-4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Propanolol, yohimbine and noradrenaline

NA conc	109.2.d	109.4.e	110.3.d	111.1.c	111.2.c	113.3.e	114.3.d	133.2.h	134.2.h
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	17.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-6.5	28.6	44.1	17.1	0.0	0.0	0.0	7.5	0.0	0.0
-6	54.3	55.9	43.8	34.1	1.7	5.9	12.5	0.0	0.0
-5.5	68.6	70.6	61.0	47.7	3.4	29.4	15.0	24.0	0.0
-5	88.6	85.3	65.9	53.6	22.0	58.8	30.0	32.1	0.0
-4.5	94.3	100.0	78.0	88.6	71.2	64.7	45.0	59.2	91.6
-4	94.3	100.0	100.0	100.0	100.0	100.0	50.0	100.0	75.7

Propanolol, *Rhoicissus* extract and noradrenaline

	36.1.d	36.2.d	39.1.d	39.4.d	39.4.f	40.1.b	40.3.b	40.4.b	42.3.e	42.4.e	42.1.f	42.3.f
-10.5	52.4	28.6	76.3	41.2		82.9	73.5	72.2	65.2	80.6	35.5	74.7
-10	60.2	28.6	93.6	20.6		63.8	50.0	55.6	47.8	80.6	31.8	18.2
-9.5	40.8	28.6	87.0	20.6		63.8	50.0	55.6	47.8	80.6	31.8	18.2
-9	73.8	28.6	87.0	12.4		63.8	50.0	44.4	47.8	80.6	31.8	22.2
-8.5	69.9	28.6	87.0	12.4		63.8	50.0	44.4	47.8	80.6	57.9	22.2
-8	89.3	28.6	87.0	12.4		63.8	41.2	44.4	47.8	80.6	57.9	22.2
-7.5	83.5	28.6	87.0	12.4		67.6	41.2	44.4	47.8	80.6	57.9	22.2
-7	95.1	47.6	95.0	24.7		63.8	41.2	44.4	53.6	86.1	57.9	94.9
-6.5	95.1	45.2	95.0	24.7		63.8	41.2	58.3	53.6	69.4	78.5	80.6
-6	95.0	45.2	100.0	24.7		79.0	17.6	58.3	68.1	77.8	86.0	80.8
-5.5	100.0	33.3	117.0	72.2	18.8	94.3	64.7	69.4	81.2	100.0	77.6	94.9
-5	110.0	14.3	117.0	72.2	45.8	118.1	97.1	100.0	81.2	120.0	100.0	93.9
-4.5	110.0	16.7	117.0	72.2	60.4	118.0	94.1	100.0	100.0	120.0	100.0	105.0
-4	110.0		117.0	72.2	60.0	118.0	100.0	100.0	100.0	120.0	100.0	105.0
-3.5												
-10.5	80.0	40.0	3.2	46.4	123.8	109.6	80.0	40.0	3.2	46.4	123.8	109.6
-9	78.9	40.0	3.2	46.4	123.8	109.6	78.9	40.0	3.2	46.4	123.8	109.6
-8.5	78.9	40.0	3.2	46.4	123.8	109.6	78.9	40.0	3.2	46.4	123.8	109.6
-8	78.9	40.0	3.2	46.4	123.8	109.6	78.9	40.0	3.2	46.4	123.8	109.6
-7.5	78.9	40.0	3.2	46.4	123.8	109.6	78.9	40.0	3.2	46.4	123.8	109.6
-7	78.9	40.0	3.2	46.4	123.8	109.6	78.9	40.0	3.2	46.4	123.8	109.6
-6.5	87.7	41.5	3.2	46.4	123.8	109.6	87.7	41.5	3.2	46.4	123.8	109.6
-6	89.5	51.2	27.0	67.9	138.1	109.6	89.5	51.2	27.0	67.9	138.1	109.6
-5.5	89.5	65.9	42.9	82.1	121.4	105.8	89.5	65.9	42.9	82.1	121.4	105.8
-5	89.5	78.0	63.5	92.9	121.4	101.9	89.5	78.0	63.5	92.9	121.4	101.9
-4.5	93.0	87.8	76.2	92.9	121.4	101.9	93.0	87.8	76.2	92.9	121.4	101.9
-4	93.0	100.0	100.0	117.9	133.3	103.8	93.0	100.0	100.0	117.9	133.3	103.8

Propanolol, yohimbine, *Rhoicissus* extract and noradrenaline

NA conc	110.4.d	111.1.d	111.2.d	112.2.c	113.4.e	114.4.d	110.4.d	111.1.d	111.2.d	112.2.c	113.4.e	114.4.d
-10.5	80.0	40.0	3.2	46.4	123.8	109.6	80.0	40.0	3.2	46.4	123.8	109.6
-9	78.9	40.0	3.2	46.4	123.8	109.6	78.9	40.0	3.2	46.4	123.8	109.6
-8.5	78.9	40.0	3.2	46.4	123.8	109.6	78.9	40.0	3.2	46.4	123.8	109.6
-8	78.9	40.0	3.2	46.4	123.8	109.6	78.9	40.0	3.2	46.4	123.8	109.6
-7.5	78.9	40.0	3.2	46.4	123.8	109.6	78.9	40.0	3.2	46.4	123.8	109.6
-7	78.9	40.0	3.2	46.4	123.8	109.6	78.9	40.0	3.2	46.4	123.8	109.6
-6.5	87.7	41.5	3.2	46.4	123.8	109.6	87.7	41.5	3.2	46.4	123.8	109.6
-6	89.5	51.2	27.0	67.9	138.1	109.6	89.5	51.2	27.0	67.9	138.1	109.6
-5.5	89.5	65.9	42.9	82.1	121.4	105.8	89.5	65.9	42.9	82.1	121.4	105.8
-5	89.5	78.0	63.5	92.9	121.4	101.9	89.5	78.0	63.5	92.9	121.4	101.9
-4.5	93.0	87.8	76.2	92.9	121.4	101.9	93.0	87.8	76.2	92.9	121.4	101.9
-4	93.0	100.0	100.0	117.9	133.3	103.8	93.0	100.0	100.0	117.9	133.3	103.8

The effect of methysergide on the uterine response to the *Rhoicissus* extract

5-HT std

5HT conc	120.1.a	120.2.a	121.1.a	121.1.e	121.1.g	121.2.a	121.2.e	121.2.g	126.2.c	126.2.e	130.1.a	130.1.c	130.1.e	130.2.a	130.2.c	131.1.a	131.1.c	131.2.a	131.2.c
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	2.2	0.9	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	3.6	1.8	0.0	0.0	0.0	2.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	5.6	0.0	5.6	0.0	1.9	0.0
-7.5	20.1	14.8	4.1	0.0	27.9	15.2	2.5	0.0	7.8	2.8	8.9	0.0	0.0	19.2	0.0	10.1	0.0	14.7	2.6
-7	41.0	37.5	15.8	9.2	35.2	31.8	41.8	7.1	56.5	40.2	31.4	2.6	2.9	35.4	5.1	20.2	13.2	29.5	30.5
-6.5	66.9	61.1	34.9	19.5	55.8	54.3	67.2	40.0	68.7	69.2	59.1	13.2	5.9	64.5	56.4	31.5	24.2	60.9	53.0
-6	85.6	69.0	43.2	74.3	78.8	73.5	88.5	56.5	81.7	86.0	70.9	42.1	41.2	79.6	70.5	50.6	30.8	78.8	72.8
-5.5	100.0	88.4	67.1	88.1	87.3	87.4	91.8	70.6	90.4	95.3	86.6	57.9	70.6	89.5	92.3	64.0	57.1	89.7	94.0
-5	100.0	100.0	75.3	98.2	92.7	92.7	97.5	83.5	100.0	97.2	87.4	76.3	73.5	92.1	92.3	69.7	71.4	93.6	98.7
-4.5	100.0	100.0	100.0	100.0	96.4	97.4	99.2	94.1		100.0	94.5	86.8	82.4	98.7	98.7	86.5	79.1	94.9	99.3
-4					100.0	100.0	100.0	100.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Methysergide and 5-HT

	121.1.d	121.2.d	126.2.d	130.1.b	131.1.b	131.2.a	121.2.f	126.2.f	130.2.b
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.9	0.0
-7.5	14.5	21.9	13.9	0.0	15.2	3.2	0.0	3.6	0.0
-7	25.7	41.1	60.9	11.0	19.2	31.8	0.0	22.6	17.1
-6.5	40.2	67.5	73.0	23.6	23.2	50.3	1.6	58.9	28.3
-6	52.0	81.5	87.0	33.1	31.3	68.2	22.1	67.0	36.2
-5.5	60.9	88.7	91.3	41.7	47.5	89.2	9.0	89.3	50.0
-5	73.2	92.1	97.4	52.8	60.6	94.3	30.3	92.9	58.6
-4.5	94.4	94.0	98.3	59.8	76.8	98.7	30.0	93.0	59.2
-4		96.7		85.0	100.0	100.0	30.0	93.0	61.2

Rhoicissus extract and 5-HT

5HT	30.1.b	32.1.b	33.1.d	34.1.c	34.2.c	35.1.b	43.1.b	43.2.b	44.1.b	45.1.d	45.3.d	44.1.e	45.1.b
-10	52.7	30.6	43.8	21.6	0.0	77.7	46.9	0.0	0.0	0.0	15.8	56.1	15.3
-11	25.8	35.0	24.7	0.0	0.0	63.8	46.9	5.0	0.0	0.0	5.0	42.0	8.6
-10.5	25.8	38.2	24.7	0.0	0.0	63.8	46.9	0.0	0.0	0.0	5.0	42.0	8.6
-10	25.8	38.2	37.1	0.0	0.0	64.6	46.9	0.0	0.0	26.5	11.9	42.0	8.6
-9.5	41.3	39.2	42.1	0.0	0.0	64.6	46.9	0.0	0.0	25.2	14.9	44.6	30.1
-9	53.2	40.1	44.9	39.6	0.0	72.3	48.4	0.0	0.0	30.3	14.9	42.0	33.7
-8.5	62.0	42.0	50.6		4.5	71.2	37.5	0.0	0.5	33.9	15.8	42.7	42.9
-8	62.0	42.7	57.3	58.6		71.2	18.8	0.0	17.6	40.0	20.8	40.8	46.0
-7.5	66.1	48.4	75.3	66.7	6.0	75.8	35.9	0.0	24.8	44.2	22.8	43.9	67.5
-7	79.6	58.0	75.3	81.1	8.2	77.7	60.9	0.0	49.6	59.4	31.7	50.3	76.7
-6.5	90.4	65.6	84.3	93.7	20.9		71.9	0.0	56.5	75.2	43.6	61.1	96.9
-6	98.1	87.3	91.0	100.0	58.2		90.6	0.0	67.2	85.5	68.3	68.5	100.0
-5.5	95.6	92.4	91.0	100.0	67.2	100.0	89.8	0.0	83.5	88.5	72.3	69.7	100.0
-5	96.6	100.0	100.0	100.0	100.0	100.0	90.6	0.0	90.4	89.7	95.0	71.3	100.0
-4.5	96.6	100.0	100.0	100.0	100.0	100.0	93.8	43.8	93.6	89.7	83.2	71.0	100.0
-4	96.6	100.0	100.0	100.0	100.0	100.0	93.8	43.8	93.6	89.7	100.0	71.0	100.0

Methysergide, *Rhoicissus* extract and serotonin

5HT	121.1.h	121.2.h	130.1.d	130.2.d	131.1.d	131.2.d
-10	52.1	57.5	37.7	27.8	9.9	62.4
-9.5	52.1	49.0	37.7	27.8	9.9	56.1
-9	52.1	49.0	37.7	27.8	9.9	56.1
-8.5	52.1	49.0	37.7	27.8	9.9	56.1
-8	53.3	49.0	37.7	27.8	9.9	56.1
-7.5	59.4	55.0	37.7	27.8	9.9	56.1
-7	61.2	66.0	37.7	63.9	9.9	56.1
-6.5	66.1	71.5	71.0	84.3	19.8	73.2
-6	79.4	80.0	85.5	97.2	26.4	81.5
-5.5	89.7	83.5	100.0	108.3	54.9	96.8
-5	92.1	82.0	100.0	113.9	61.5	104.5
-4.5	93.9	98.0	100.0	119.4	71.4	106.4
-4	95.2	100.0	105.8	120.4	80.2	106.4

The effect of methysergide on the ileal response to the *Rhoicissus* extract

Serotonin std

	127.3.e	127.4.e	128.3.f	128.4.h	130.3.a	130.3.c	130.4.a	130.4.c	131.3.a	131.3.c	131.4.a	131.4.c	132.3.g	132.4.g
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	1.7	0.0	8.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	5.6	0.0	0.0	3.4	7.0	57.6	25.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	8.3	0.0	8.6	10.2	17.4	75.8	67.6	1.4	0.0	1.1	1.0	0.0	0.0	0.0
-7	11.1	13.3	17.3	27.1	29.6	90.9	87.3	37.8	2.1	22.8	4.2	9.4	0.0	4.2
-6.5	22.2	53.3	34.6	49.2	51.3	92.4	91.5	59.5	4.2	41.3	6.3	31.3	13.5	8.3
-6	38.9	66.7	43.2	74.6	62.6	89.4	94.4	70.3	44.2	59.8	13.5	53.1	40.5	33.3
-5.5	58.3	83.3	60.5	83.1	88.7	95.5	100.0	71.6	77.9	94.6	26.0	68.8	97.3	83.3
-5	88.9	100.0	69.1	93.2	100.0	97.0	100.0	71.6	91.6	97.8	37.5	75.0	100.0	91.7
-4.5	94.4	100.0	86.4	93.2		100.0		100.0	100.0	103.3	58.3	93.8		100.0
-4	100.0		100.0	100.0					100.0	100.0	100.0	100.0		

Methysergide and serotonin

	130.3.b	130.4.b	131.3.b	131.4.b	36809.0	120.4	127.3	127.4	128.3	128.4
-9.5	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0
-7.5	4.2	0.0	0.0	0.0		6.4	0.0	0.0	9.9	11.9
-7	13.3	0.0	5.7	1.0		11.5	2.8	12.5	34.6	15.3
-6.5	20.8	2.9	21.4	20.8		16.7	11.1	33.3	59.3	20.3
-6	31.7	16.2	44.3	29.2		25.6	13.9	41.7	56.8	30.5
-5.5	36.7	17.6	77.1	41.7		69.2	22.2	66.7	44.4	33.9
-5	39.2	19.1	85.7	42.7		69.0	30.6	58.3	42.0	37.3
-4.5	90.8	100.0	90.0	61.5		69.0	33.3	58.0	42.0	37.3
-4		100.0		85.0		69.0	38.9	58.3	42.0	37.3

***Rhoicissus* and serotonin**

5HT	32.3.c	32.4.c	33.4.d	33.4.f	34.3.c	34.3.c	35.4.c	44.3.b	45.2.b	45.4.d	44.3.e	45.2.d	45.4.f
-12	15.5	12.9	25.6	17.7	7.7	30.0	65.2	21.6	8.9	22.1	54.3	19.1	35.4
-11	4.2	3.5	10.3	5.2	7.7	22.5	26.1	9.7	3.8	4.4	54.3	7.2	16.7
-10.5	5.6	5.9	10.3	5.2	7.7	23.8	26.1	9.7	3.8	4.4	54.3	7.2	16.7
-10	5.6	8.2	10.3	5.2	11.5	25.0	27.2	8.3	3.8	4.4	54.3	7.2	16.7
-9.5	9.2	9.4	16.7	5.2	11.5	25.0	27.2	8.3	3.8	4.4	54.3	7.2	16.7
-9	9.2	8.2	15.4	5.2	11.5	27.5	27.2	8.3	3.8	4.4	51.9	7.2	16.7
-8.5	12.0	8.2	15.4	5.2	30.8	30.0	28.3	8.3	3.8	4.4	51.9	7.2	16.7
-8	21.1	9.4	15.4	5.2	30.8	30.0	30.4	16.5	3.8	4.4	51.9	7.2	16.7
-7.5	33.8	14.1	23.1	7.3	34.6	31.3	32.6	34.0	4.5	4.4	67.9	8.6	16.7
-7	52.1	28.2	25.6	13.5	61.5	47.5	40.8	50.5	8.3	9.6	58.0	11.8	18.8
-6.5	80.3	47.1	38.5	19.8	76.9	62.5	48.9	86.9	8.3	16.2	66.7	15.8	22.9
-6	100.0	80.0	70.5	100.0	92.3	92.5	63.0	100.0	14.6	27.9	76.5	20.4	26.0
-5.5	100.0	88.2	100.0	100.0	100.0	100.0	66.8	100.0	16.6	33.8	76.5	24.3	28.1
-5	100.0	100.0	100.0	100.0	100.0	100.0	66.8	100.0	21.0	39.7	77.8	29.6	29.2
-4.5	100.0	100.0	100.0	100.0	100.0	100.0	71.7	100.0	41.4	52.9	100.0	53.3	38.5
-4	100.0	100.0	100.0	100.0	100.0	100.0	83.7	100.0	78.3	82.4	100.0	79.6	70.8

Methysergide, *Rhoicissus* and serotonin

	Ktuber	Ktuber	Groots	Froots	Froots	Froots
	130.3	130.4	131.3	131.4	132.3	132.4
-10	0.0	80.0	68.3	140.6	324.3	173.5
-9.5	0.0	80.0	68.3	78.1	81.1	100.0
-9	0.0	80.0	68.3	78.1	81.1	100.0
-8.5	0.0	80.0	68.3	78.1	81.1	100.0
-8	0.0	80.0	68.3	78.1	81.1	100.0
-7.5	0.0	80.0	68.3	78.1	81.1	100.0
-7	0.0	82.0	71.4	78.1	81.1	100.0
-6.5	0.0	84.0	63.5	78.1	81.1	100.0
-6	20.0	88.0	63.5	78.1	81.1	100.0
-5.5	67.5	94.0	63.5	78.1	81.1	100.0
-5	85.0	96.0	63.5	78.1	105.4	100.0
-4.5	86.3	100.0	63.5	78.1	105.4	100.0
-4	95.0	100.0	63.5	78.1	183.8	100.0

The effect of ketanserin on the uterine response to the *Rhoicissus* extract

Serotonin standard

5HT	105.1.c	105.2.c	106.1.c	106.1.e	106.2.c	106.2.e	107.1.c	107.1.e	107.2.c	107.2.e	108.1.c	108.1.e	108.2.c	108.2.e	122.2.a	123.2.e	126.1.c	126.2.c	126.1.c	130.1.f	130.2.f	132.1.a	132.2.a
-10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	12.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.1	3.3	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	22.4	61.1	0.0	14.8	0.0	0.0	0.0	0.0	4.2	0.0	0.0	0.0	0.0	26.0	0.0	5.1	3.3	0.0	0.0	0.0	0.0	0.0
-7	17.9	44.0	69.4	0.0	40.7	0.0	3.7	20.0	31.7	40.0	11.4	0.0	25.3	0.0	49.5	3.7	31.8	45.5	0.0	0.0	2.7	0.0	4.4
-6.5	45.3	69.6	73.6	71.7	70.4	53.7	27.4	38.9	55.4	47.4	31.8	20.4	51.4	0.0	59.9	9.8	54.1	58.1	24.1	14.8	6.3	5.4	22.1
-6	79.2	93.6	78.4	65.0	77.8	70.7	54.8	40.0	80.2	73.7	54.5	44.4	74.0	9.2	78.6	61.0	69.4	75.6	35.4	24.1	46.4	21.6	43.4
-5.5	84.9	99.6	84.7	65.0	85.2	85.4	82.3	52.3	94.1	93.7	79.5	57.4	90.4	52.3	86.5	76.2	93.9	84.6	49.4	46.3	58.9	40.5	70.8
-5	100.0	100.0	88.1	78.7	92.6	85.4	85.9	60.0	100.0	100.0	94.3		99.3		94.3	91.5	98.0	95.1	75.9	68.5	77.7	60.8	86.7
-4.5	100.0	100.0	94.4	90.0	100.0	87.6	93.2	100.0	100.0		97.7		100.0		96.4	97.0	100.0	98.7	82.3	81.5	80.4	78.4	93.6
-4		100.0	100.0	100.0	100.0	100.0	100.0	100.0			100.0	87.0		78.0	99.0	99.4	100.0	100.0	91.1	90.7	84.6	90.5	95.6
												100.0		100.0	100.0	100.0		100.0	100.0	100.0	100.0	100.0	100.0

Ketanserin and serotonin

	107.2.d	108.1.d	108.2.d	130.1.g	130.2.g	132.1.b	132.2.b
-11	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-10	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	4.8	0.0	0.7	0.0	0.0	0.0	0.0
-7	33.3	6.3	1.7	0.0	0.0	0.0	0.0
-6.5	55.2	32.8	9.6	0.0	0.0	0.0	0.0
-6	73.3	60.9	41.1	0.0	0.0	0.0	2.7
-5.5	88.6	85.9	61.6	12.0	2.7	0.0	3.5
-5	100.0	87.5	68.5	22.2	6.3	0.0	7.1
-4.5		86.1	73.3	24.1	35.7	0.0	15.9
-4		100.0	77.4	23.1	11.6	4.1	28.3

***Rhoicissus* extract and 5-HT**

5HT	30.1.b	32.1.b	33.1.d	34.1.c	34.2.c	35.1.b	43.1.b	43.2.b	44.1.b	45.1.d	45.3.d	44.1.e	45.1.b
-10	52.7	30.6	43.8	21.6	0.0	77.7	46.9	0.0	0.0	0.0	15.8	56.1	15.3
-11	25.8	35.0	24.7	0.0	0.0	63.8	46.9	5.0	0.0	0.0	5.0	42.0	8.6
-10.5	25.8	38.2	24.7	0.0	0.0	63.8	46.9	0.0	0.0	0.0	5.0	42.0	8.6
-10	25.8	38.2	37.1	0.0	0.0	64.6	46.9	0.0	0.0	28.5	11.9	42.0	8.6
-9.5	41.3	39.2	42.1	0.0	0.0	64.6	46.9	0.0	0.0	25.2	14.9	44.8	30.1
-9	53.2	40.1	44.9	39.6	0.0	72.3	46.4	0.0	0.0	30.3	14.9	42.0	33.7
-8.5	62.0	42.0	50.6		4.5	71.2	37.5	0.0	0.6	33.9	15.8	42.7	42.9
-8	62.0	42.7	57.3	58.6		71.2	18.6	0.0	17.8	40.0	20.8	40.8	46.0
-7.5	66.1	48.4	75.3	66.7	6.0	75.8	35.6	0.0	24.8	44.2	22.8	43.9	67.5
-7	79.8	58.0	75.3	81.1	8.2	77.7	60.9	0.0	49.6	59.4	31.7	50.3	76.7
-6.5	90.4	65.6	84.3	93.7	20.9		71.9	0.0	58.5	75.2	43.6	61.1	96.9
-6	96.1	87.3	91.0	100.0	58.2		90.6	0.0	67.2	85.5	66.3	68.5	100.0
-5.5	95.6	92.4	91.0	100.0	67.2	100.0	89.8	0.0	83.5	88.5	72.3	69.7	100.0
-5	96.6	100.0	100.0	100.0	100.0	100.0	90.6	0.0	90.4	89.7	95.0	71.3	100.0
-4.5	96.6	100.0	100.0	100.0	100.0	100.0	93.6	43.6	93.6	89.7	83.2	71.0	100.0
-4	96.6	100.0	100.0	100.0	100.0	100.0	93.8	43.6	93.6	89.7	100.0	71.0	100.0

Ketanserin, *Rhoicissus* and serotonin

5HT	106.1.f	107.1.f	107.2.f	108.1.f	108.2.f	122.2.b
-10	58.3	14.5	1.1	11.1	37.6	19.6
-9.5	58.3	0.0	0.0	11.1	34.9	19.6
-9	58.3	0.0	0.0	11.1	34.9	19.6
-8.5	58.3	0.0	0.0	11.1	34.9	19.6

-6	58.3	0.0	0.0	11.1	34.9	30.7
-7.5	58.3	30.7	0.0	11.1	34.9	33.5
-7	80.0	20.5	0.0	18.5	38.5	51.4
-8.5	61.7	45.8	5.3	25.9	53.2	53.6
-8	70.0	53.6	22.1	35.2	71.6	69.8
-5.5	75.0	79.5	40.0	75.9	82.6	81.8
-5	76.7	84.3	45.3	90.7	98.2	88.3
-4.5	90.0	97.6	49.5	100.0	100.9	89.9
-4	91.7	100.0	70.5	120.4	107.3	93.3

The effect of ketanserin on the ileal response to the *Rhoicissus* extract

Serotonin std

5HT	105.3.c	105.4.c	106.3.f	106.4.e	107.3.e	107.4.c	107.4.e	108.3.c	108.3.e	108.3.g	108.4.c	108.4.g	122.1.a	122.1.c	124.3.d	124.3.f	124.4.c	124.4.e	125.3.c	125.4.c	126.4.e	132.3.a	132.4.a
-10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	21.6	8.5	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0	0.0
-7.0	0.0	9.4	0.0	1.9	0.0	3.3	0.0	14.3	0.0	0.0	5.8	0.0	32.2	39.4	7.2	4.0	13.3	9.1	4.2	0.0	14.1	4.6	0.0
-6.5	2.9	121.9	3.6	21.2	9.8	8.3	1.1	21.4	10.6	2.4	14.8	25.7	43.6	54.9	55.1	5.3	23.3	47.5	8.3	12.1	23.9	9.9	8.1
-6.0	40.0	65.8	9.0	21.2	39.0	20.0	4.4	53.6	25.9	5.9	37.0	28.6	67.9	63.4	59.9	12.0	56.7	54.5	22.9	78.6	50.7	43.5	34.7
-5.5	68.6	90.6	12.6	34.6	57.3	26.7	8.8	69.6	32.9	11.6	55.6	31.4	81.5	67.6	78.0	45.3	93.3	75.8	56.3	84.6	77.5	75.8	62.2
-5.0	82.9	90.6	41.4	46.2	72.0	53.3	17.6	83.9		64.7	74.1	40.0	91.3	87.3	93.4	66.7	100.0	100.0	79.2	97.0	94.4	99.2	91.8
-4.5	94.3	100.0	78.3	75.0	100.0	100.0	47.3	93.8		85.9	85.2	48.6	96.7	91.5	93.4	100.0	100.0	100.0	89.6	100.0	94.4	100.0	100.0
-4.0	100.0	100.0	99.1	88.5	100.0	100.0	63.7	100.0	60.0	88.2	96.3	74.3	100.0	97.2	97.2	100.0	100.0	100.0	95.8	100.0	97.9	100.0	100.0
	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Ketanserin and serotonin

	108.3	107.3	108.4	107.4	106.4
-11.0	0.0	0.0	0.0	0.0	0.0
-10.5	0.0	0.0	0.0	0.0	0.0
-10.0	0.0	0.0	0.0	0.0	0.0
-9.5	0.0	0.0	0.0	0.0	0.0
-9.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	2.1
-8.0	0.0	11.8	0.0	0.0	5.3
-7.5	16.7	38.2	0.0	2.3	17.0
-7.0	21.7	55.3	0.0	2.3	17.0
-6.5	23.3	48.7	0.0	5.8	18.1
-6.0	25.0	48.7	0.0	15.1	24.5
-5.5	28.3	53.9	0.0	46.5	54.3
-5.0	48.3	76.3	4.5	62.8	81.7
-4.5	91.7		10.4	81.4	89.4

***Rhoicissus* extract and serotonin**

5HT	32.3.c	32.4.c	33.4.d	33.4.f	34.3.c	34.3.e	35.4.c	44.3.b	45.2.b	45.4.d	44.3.e	45.2.d	45.4.f
-12.0	15.5	12.9	25.6	17.7	7.7	30.0	65.2	21.6	8.9	22.1	54.3	19.1	35.4
-11.0	4.2	3.5	10.3	5.2	7.7	22.5	26.1	9.7	3.8	4.4	54.3	7.2	16.7
-10.5	5.6	5.9	10.3	5.2	7.7	23.8	26.1	9.7	3.8	4.4	54.3	7.2	16.7
-10.0	5.6	8.2	10.3	5.2	11.5	25.0	27.2	8.3	3.8	4.4	54.3	7.2	16.7
-9.5	9.2	9.4	18.7	5.2	11.5	25.0	27.2	8.3	3.8	4.4	54.3	7.2	16.7
-9.0	9.2	8.2	15.4	5.2	11.5	27.5	27.2	8.3	3.8	4.4	51.9	7.2	16.7
-8.5	12.0	8.2	15.4	5.2	30.8	30.0	28.3	8.3	3.8	4.4	51.9	7.2	16.7
-8.0	21.1	9.4	15.4	5.2	30.8	30.0	30.4	16.5	3.8	4.4	51.9	7.2	16.7
-7.5	33.8	14.1	23.1	7.3	34.6	31.3	32.6	34.0	4.5	4.4	67.9	6.6	16.7
-7.0	52.1	28.2	25.6	13.5	61.5	47.5	40.8	50.5	8.3	9.6	58.0	11.8	18.6
-6.5	80.3	47.1	38.5	19.8	76.9	62.5	48.9	66.9	8.3	16.2	66.7	15.5	22.9
-6.0	100.0	80.0	70.5	100.0	92.3	92.5	63.0	100.0	14.6	27.9	76.5	20.4	26.0
-5.5	100.0	88.2	100.0	100.0	100.0	100.0	66.8	100.0	16.6	33.6	76.5	24.3	28.1
-5.0	100.0	100.0	100.0	100.0	100.0	100.0	66.8	100.0	21.0	39.7	77.8	29.6	29.2
-4.5	100.0	100.0	100.0	100.0	100.0	100.0	71.7	100.0	41.4	52.9	100.0	53.3	38.5
-4.0	100.0	100.0	100.0	100.0	100.0	100.0	83.7	100.0	78.3	82.4	100.0	79.6	70.8
	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Ketanserin, *Rhoidssus* and serotonin

	107.3	108.3	108.4	106.3	107.4
-11.5	3.3	17.6	13.8	12.0	4.4
-11.0	3.3	17.6	13.8	12.0	4.4
-10.5	3.3	17.6	13.8	12.0	4.4
-10.0	3.3	17.8	13.8	12.0	4.4
-9.5	3.3	17.6	13.8	12.0	4.4
-9.0	4.2	21.2	17.2	12.0	6.6
-8.5	6.3	21.2	20.7	12.0	11.0
-8.0	10.0	34.1	22.4	16.8	11.0
-7.5	32.5	40.0	27.6	47.0	13.2
-7.0	71.7	42.4	31.0	49.5	14.3
-6.5	66.7	44.7	32.8	51.0	17.6
-6.0	85.8	52.9	36.2	54.0	31.3
-5.5	95.8	55.3	39.7	59.0	58.2
-5.0	108.3	78.8	62.1	79.0	71.4
-4.5	111.7	98.8	100.0	113.0	86.8

The effect of tropisteron on the uterine response to the *Rhoicissus* extract

Serotonin std

5HT conc	122.3.a	123.1.a	123.1.c	123.1.e	123.2.a	123.2.c	123.2.e	124.1.a	124.1.c	124.2.a	124.2.c	125.1.a	125.1.c	125.2.a	125.2.c	126.1.a	126.2.a	131.1.e	131.2.e
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	13.1	0.0	0.0	0.0	0.0	0.0	0.0	10.8	0.0	12.3	24.2	4.1	7.0	3.3	1.5	0.0	0.0	0.0
-8.0	0.0	23.5	0.0	0.0	0.6	0.6	0.0	16.3	20.6	2.0	18.5	30.3	5.1	8.3	3.3	11.9	7.5	0.0	0.0
-7.5	0.0	31.4	10.0	6.1	19.8	1.9	3.7	25.5	43.1	32.4	27.7	40.9	31.6	47.8	45.5	27.8	41.0	6.3	5.4
-7.0	22.0	45.1	17.7	7.8	46.3	39.2	9.8	42.9	48.0	52.7	45.4	53.8	54.1	61.8	56.1	46.6	60.4	21.9	32.6
-6.5	41.5	69.3	55.4	53.9	74.0	70.3	61.0	69.4	65.7	76.4	91.5	72.0	69.4	80.3	75.6	75.8	79.9	32.8	54.3
-6.0	59.8	83.0	75.4	63.5	87.8	88.6	76.2	84.7	77.5	87.8	93.8	87.1	93.9	87.9	84.6	87.2	91.0	57.8	78.3
-5.5	81.7	92.8	87.7	81.7	96.0	95.6	91.5	91.8	86.3	93.2	97.7	97.7	98.0	91.7	95.1	99.5	97.0	85.9	89.1
-5.0	90.2	99.3	92.3	93.9	98.3	98.1	97.0	92.9	90.2	93.2	100.0	97.7	100.0	93.0	96.7	100.0	99.3	100.0	98.9
-4.5	96.3	100.0	94.6	96.5	99.4	98.7	99.4	94.9	97.1	93.9		98.5	100.0	93.6	100.0	100.0	99.6	100.0	99.5
-4.0	100.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0		100.0		100.0	100.0		100.0	100.0	100.0

Tropisetron and serotonin

5HT conc	123.2.b	124.1.d	124.2.f	125.1.b	126.2.b	131.1.f	131.2.f
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.0	0.0	0.0	0.0	26.3	0.0	0.0	0.0
-7.5	0.3	0.0	0.0	0.0	0.8	0.0	0.0
-7.0	2.9	24.7	31.3	39.5	34.8	16.4	33.6
-6.5	66.5	37.1	69.5	56.1	75.0	27.4	44.0
-6.0	78.0	57.3	85.9	63.2	79.5	32.9	58.4
-5.5	91.9	73.0	100.0	86.0	92.4	67.1	86.4
-5.0	96.5	93.3	100.0	91.2	97.7	87.7	96.8
-4.5	97.7	91.0	100.0	96.5	98.5	100.0	102.4
-4.0	100.0	94.4	100.0	100.0	100.0	100.0	100.8

Rhoicissus **extract and serotonin**

5HT	30.1.b	32.1.b	33.1.d	34.1.c	34.2.c	35.1.b	43.1.b	43.2.b	44.1.b	45.1.d	45.3.d	44.1.e	45.1.b
-10.0	52.7	30.6	43.8	21.6	0.0	77.7	46.9	0.0	0.0	0.0	15.8	56.1	15.3
-11.0	25.8	35.0	24.7	0.0	0.0	63.8	46.9	5.0	0.0	0.0	5.0	42.0	8.6
-10.5	25.8	38.2	24.7	0.0	0.0	63.8	46.9	0.0	0.0	0.0	5.0	42.0	8.6
-10.0	25.8	38.2	37.1	0.0	0.0	64.6	46.9	0.0	0.0	28.5	11.9	42.0	8.6
-9.5	41.3	39.2	42.1	0.0	0.0	64.6	46.9	0.0	0.0	25.2	14.9	44.6	30.1
-9.0	53.2	40.1	44.9	39.3	0.0	72.3	48.4	0.0	0.0	30.3	14.9	42.0	33.7
-8.5	62.0	42.0	50.6		4.5	71.2	37.5	0.0	0.6	33.9	15.8	42.7	42.9
-8.0	62.0	42.7	57.3	58.6		71.2	18.8	0.0	17.6	40.0	20.8	40.8	46.0
-7.5	66.1	48.4	75.3	66.7	6.0	75.8	35.9	0.0	24.8	44.2	22.8	43.9	67.5
-7.0	79.6	58.0	75.3	81.1	8.2	77.7	60.9	0.0	49.6	59.4	31.7	50.3	76.7
-6.5	90.4	65.6	84.3	93.7	20.9		71.9	0.0	56.5	75.2	43.6	61.1	96.9
-6.0	96.1	87.3	91.0	100.0	58.2		90.6	0.0	67.2	85.5	68.3	68.5	100.0
-5.5	95.6	92.4	91.0	100.0	87.2	100.0	89.8	0.0	83.5	88.5	72.3	69.7	100.0
-5.0	96.8	100.0	100.0	100.0	100.0	100.0	90.6	0.0	90.4	89.7	95.0	71.3	100.0
-4.5	96.6	100.0	100.0	100.0	100.0	100.0	93.8	43.8	93.6	89.7	83.2	71.0	100.0
-4.0	96.6	100.0	100.0	100.0	100.0	100.0	93.8	43.8	93.6	89.7	100.0	71.0	100.0

Tropisetron, *Rhoicissus* extract and serotonin

5HT conc	123.1.d	123.2.d	124.1.b	124.2.b	125.2.b	126.1.b
-10.0	6.2	41.1	17.6	14.7	70.5	0.0
-9.5	6.2	41.1	17.6	14.7	70.5	0.0
-9.0	6.2	41.1	17.6	14.7	70.5	0.0
-8.5	36.2	41.1	23.1	14.7	70.5	0.0
-8.0	29.2	41.1	31.5	20.0	70.5	0.0
-7.5	63.1	41.1	43.5	54.7	70.5	12.7
-7.0	70.8	41.1	43.5	70.0	73.1	19.0
-6.5	74.6	70.8	43.5	79.3	80.1	45.2
-6.0	91.5	85.7	64.8	86.7	85.9	65.2
-5.5	104.6	97.6	82.4	92.7	92.3	86.9
-5.0	103.9	99.4	84.3	100.0	95.5	95.9
-4.5	105.4	100.0	94.4	100.0	95.5	96.8
-4.0	106.2	100.0	100.0		100.0	100.0

The effect of tropisteron on the ileal response to the *Rhoicissus* extract

Serotonin std

5HT conc	124.3.a	124.4.a	125.3.a	125.4.a	126.3.c	126.4.a	130.3.f	130.4.f	131.3.e	131.4.e	132.3.d	132.4.d
-9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.0	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	2.4	8.9	2.5	5.0	5.0	0.0	5.6	0.0	0.0	2.0	0.0	0.0
-7.0	9.4	13.3	5.0	32.5	12.5	25.6	11.1	0.0	11.5	13.3	0.0	0.0
-6.5	47.2	42.2	7.5	35.0	35.0	51.2	25.9	2.8	19.7	19.4	25.0	23.1
-6.0	69.3	77.8	11.3	62.5	65.0	69.8	55.6	5.5	33.1	49.0	55.0	46.2
-5.5	98.4	88.9	27.5	82.5	80.0	90.7	85.2	9.2	58.6	74.5	70.0	61.5
-5.0	100.0	100.0	31.3	100.0	92.5	100.0	95.4	12.8	63.7	98.0	100.0	69.2
-4.5	98.4	84.4		92.5	100.0	100.0	100.0	100.0	88.5	100.0	95.0	76.9
-4.0			100.0	90.0	97.5	97.7	92.6	100.0	100.0	100.0	90.0	100.0

Tropisetron and serotonin

	124.4.b	125.4.b	126.3.b	130.3.g	130.4.g	131.3.f
-9.5	0.0	0.0	0.0	0.0	0.0	0.0
-9.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0			0.0	0.0
-8.0	0.0	0.0	2.0		4.6	0.0
-7.5	51.4	8.5	13.3	30.0	5.5	5.5
-7.0	60.0	11.9	53.3	41.7	7.3	15.9
-6.5	67.1	25.4	73.3	45.8	10.1	25.9
-6.0	67.1	40.7	84.0	59.2	12.8	45.3
-5.5	70.0	72.9	89.3	86.7	28.4	57.2
-5.0	77.1	89.8	94.7	92.5	31.2	74.1
-4.5	80.0	96.6	97.3	92.5	29.4	100.0
-4.0	91.4	100.0	97.3	90.0	27.5	89.6

Rhoicissus extract and serotonin

5HT	32.3.c	32.4.c	33.4.d	33.4.f	34.3.c	34.3.c	35.4.c	44.3.b	45.2.b	45.4.d	44.3.e	45.2.d	45.4.f
-12.0	15.5	12.9	25.6	17.7	7.7	30.0	65.2	21.6	8.9	22.1	54.3	19.1	35.4
-11.0	4.2	3.5	10.3	5.2	7.7	22.5	26.1	9.7	3.8	4.4	54.3	7.2	16.7
-10.5	5.6	5.9	10.3	5.2	7.7	23.8	26.1	9.7	3.8	4.4	54.3	7.2	16.7
-10.0	5.6	8.2	10.3	5.2	11.5	25.0	27.2	8.3	3.8	4.4	54.3	7.2	16.7
-9.5	9.2	9.4	16.7	5.2	11.5	25.0	27.2	8.3	3.8	4.4	54.3	7.2	16.7
-9.0	9.2	8.2	15.4	5.2	11.5	27.5	27.2	8.3	3.8	4.4	51.9	7.2	16.7
-8.5	12.0	8.2	15.4	5.2	30.8	30.0	28.3	8.3	3.8	4.4	51.9	7.2	16.7
-8.0	21.1	9.4	15.4	5.2	30.8	30.0	30.4	16.5	3.8	4.4	51.9	7.2	16.7
-7.5	33.8	14.1	23.1	7.3	34.6	31.3	32.6	34.0	4.5	4.4	67.9	8.6	16.7
-7.0	52.1	28.2	25.6	13.5	61.5	47.5	40.8	50.5	8.3	9.6	58.0	11.8	18.8
-6.5	80.3	47.1	38.5	19.8	76.9	62.5	48.9	86.9	8.3	16.2	66.7	15.8	22.9
-6.0	100.0	80.0	70.5	100.0	92.3	92.5	63.0	100.0	14.6	27.9	76.5	20.4	26.0
-5.5	100.0	88.2	100.0	100.0	100.0	100.0	66.8	100.0	16.6	33.8	76.5	24.3	28.1
-5.0	100.0	100.0	100.0	100.0	100.0	100.0	66.8	100.0	21.0	39.7	77.8	29.6	29.2
-4.5	100.0	100.0	100.0	100.0	100.0	100.0	71.7	100.0	41.4	52.9	100.0	53.3	38.5
-4.0	100.0	100.0	100.0	100.0	100.0	100.0	83.7	100.0	78.3	82.4	100.0	79.6	70.8

Tropisetron, *Rhoicissus* extract and serotonin

	124.3.b	125.5.b	126.4.b	131.4.f	132.3.e	132.4.e
-10.0	89.8	30.0	53.3	66.3	45.0	53.3
-9.5	47.8	20.0	0.0	38.8	5.0	53.3
-9.0	47.8	20.0	0.0	38.8	5.0	53.3
-8.5	47.8	20.0	0.0	38.8	5.0	53.3
-8.0	47.8	20.0	0.0	38.8	5.0	53.3
-7.5	47.8	30.0	0.0	38.8	5.0	53.3
-7.0	47.8	30.0	33.3	38.8	5.0	53.3
-6.5	47.8	70.0	40.0	38.8	45.0	53.3
-6.0	75.8	71.7	43.3	46.3	55.0	60.0
-5.5	83.4	98.3	56.7	52.5	80.0	73.3
-5.0	91.1	100.0	63.3	58.8	80.0	100.0
-4.5	110.0	100.0	60.0	61.3	85.0	100.0
-4.0	120.0	105.0	60.0	85.0	100.0	100.0

Data for all bar graphs showing the pharmacological activity of *Rhoicissus*

Uterus

Prostaglandin synthesis

Rhoic only	21.5	0.1	57.9	0.7	26.6	76.5	36.1	9.9	31.4	58.5	46.2	21.1	25.7	57.6	50.7	18.5	26.8	18.1	4.9	29.1	7.7	37.4	21.7	31.1
Indomethacin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	12.1			
NS-398	53.8	7.3	63.2	0.0	35.9	42.3	0.0																	
NS-398 dose 2	0.0	0.0	0.0	35.6	0.0	7.3	0.0	0.0	35.9															

Muscarinic receptors

Rhoic only	28.3	50.5	58.0	0.0	56.7	66.2	0.0	1.7	42.3	0.0	15.6	0.0	52.9	55.6	48.4	5.5	51.7	59.1	6.9	5.8				
Atropine	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	
Pirenzepine	73.0	55.3	76.6	30.8	72.6	38.0	10.3	57.9																
Gallamine	5.8	7.9	48.4	15.5	68.4	47.2																		
4-DAMP	8.3	0.0	17.2	28.3	30.4	3.7																		
Tropicamide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0																

Adrenergic receptors

Rhoic only	60.2	28.6	93.6	41.2	70.2	73.5	72.2	57.8	35.5	65.2	74.7	116.7	60.2	28.6	93.6	41.2	82.9	73.5	72.2	57.8	65.2	116.7	35.5	74.7
Prazosin	75.9	59.4	127.7	7.0	83.3	90.7																		
Yohimbine	78.9	0.0	3.2	46.4	123.8	109.6																		

Serotonergic receptors

Rhoic only	52.7	30.6	43.8	21.6	0.0	77.7	46.0	0.0	0.0	0.0	15.8													
Methysergide	52.1	57.5	37.7	27.8	9.9	62.4																		
Ketanserin	58.3	14.5	1.1	11.1	37.6	19.6	81.6	43.9	55.8															
Tropisetron	6.2	41.1	17.6	14.7	70.5	0.0	17.6																	

Histaminergic receptors

Rhoic only	28.3	50.5	58.0	0.0	56.7	66.2	0.0	1.7	42.3	0.0	15.6	0.0	52.9	55.6	48.4	5.5	51.7	59.1	6.9	5.8				
Pyrilamine		30.5	30.0	30.0	20.0	27.0	35.4	32.5	59.1															

Ganglion and second messenger blockers

Hexamethonium	93.9	0.0	0.0	0.0	47.8	18.5	18.3	23.6	64.2	35.3	0.9	7.0	38.8	56.1										
Mecamylamine	31.5	0.0	57.1	0.0	2.3	38.5	95.6	57.1	66.7	0.0														
Cholera toxin	38.8	13.3	72.7	42.0	37.9	21.1	19.1	2.0	50.0	1.3	8.4	64.0	48.2	0.0										
Pertussis toxin	20.0	23.7	0.0	3.1	46.2	1.4	58.1																	

Ileum

Proetaglandin synthesis - ileum

Rhoic only	53.0	82.4	19.7	16.8	10.7	0.0	1.4	16.1	0.0	5.5	5.9	11.4	20.7	4.3	12.5									
Indomethacin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	12.1			

Muscarinic receptors - ileum

Rhoic all	44.4	71.3	51.9	11.5	53.0	82.4	19.7	16.8	10.7	0.0	8.7	0.0	5.7	5.9	11.4	20.7	4.3	12.1						
Atropine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.6						

Serotonergic receptors - ileum

Rhoic only	15.5	12.9	25.6	17.7	7.7	30.0	65.2	21.6	8.8	22.1	0.0	80.0												
Methysergide	0.0	80.0	68.3	140.8	80.0	60.0																		
Ketanserin	3.3	17.8	13.8	80.0	133.3	43.2	4.4																	
Tropisetron	59.8	68.3	53.3	66.3	45.0	53.3																		

Correlation between the increase in baseline and the magnitude of the contractile response

Baseline	3.4	0	0	4.9	10.5	2.8	10.9	14.6	37.9	6.9	2.3	1.7	2.5	3.1	13.9	1.3	11.4	38	2.6	0.6	0	0	0	4.2
Contraction	0	0	8.5	0	0	0	26.6	75.7	81.1	59.9	0	0	15.2	37.8	28.7	25.3	37.1	73.6	46.2	41.4	59.4	40.9	4.4	5.6
Baseline	0	0	0	10.6	0	0	2.7	2.5	4.3	4	0.8	0	5.6	0	1.3	1.3	0	0	2.9	6.3	3.6	3.1	1.7	0.3
Contraction	10.1	101.6	5.7	37.2	9.8	0	6	11.3	21.6	34.1	11.3	0	40.6	6.9	7	6.1	13.9	0	6.6	11.3	41.8	15.4	6.9	8.4
Baseline	0.9	21.7	10.3	1	2.1	6.1	8.2	1.2	0	0	2.1	3	0	5.6	21.4	3.2	1.4	7.8	6.9	0	0	14.5	1	0.9
Contraction	10	80.2	24.1	41	6.9	75.8	15.4	20.7	44.2	25.7	51.6	18.3	36.8	55.6	7.7	9.6	10	55.8	28.2	19.8	42.9	68.7	33.7	20.1
Baseline	4.4	3.3	2.7	0	6.9	5.8	0	1.7	2.2	0	3.1	2.9	0	1.3	2.1	3	34.1	21.3	9.7	11.1	1.6	2.3	6.9	12
Contraction	20.6	13.2	10.3	10	14.9	13.3	7.9	7.2	36	15.3	30	53.9	22.7	3.3	41.1	48.5	84.1	45.6	77.9	31.1	19.6	2.3	36.1	83.3
Baseline	7.3	6	12.5	0	0	4.4	11.9	6.2	4.7	3.1	15	4.9	2.2	2.1	0	3.4	0	4.9	0	0	7.2	14	0	25
Contraction	78	24.7	25	0	13.9	4.4	61.4	2.3	24.7	0	28.3	1.8	19.6	19.7	8.7	32.1	20	30.3	6.3	30.3	0	10.5	28.2	33.3
Baseline	0	0	0	2.3	1.6	0	2.4	3.7	3.3	2.3	0.6	4.1	0	1.7	2.5	3.3	0	0	5.8	1.7	0	2.4	1.1	20.8
Contraction	7.8	27.8	35.7	0	0	4	6.5	11.1	15.2	0	4.7	10.2	8.5	75.2	3.8	18.5	15.3	9.7	5.8	6.1	14.2	14.6	0	92.5
Baseline	2.4	4	9.9	0	11.9	1.4	0	2.6	0	16.5	0	13	6.9	43.4	6.2	7.7	1.9	1	14.9	10.1	1.7	0.8	0.6	1.4
Contraction	16.5	8	32.4	31.3	61.9	16.2	11.7	26.5	13.6	43.7	8.3	23.2	8.8	41.5	44.2	35.2	19.4	11.8	80.5	34.1	8.5	7.6	14.1	83.6
Baseline	18.8	5.4	3.3	10.3	8.9	0	0	0	0	2.7	0	3.9	6.8	14.5	17.9	4.4	0	0	1.7	3.3	0	2.7	3.4	7.8
Contraction	54.5	43.5	10	58.6	53.3	1.5	5.8	13.5	8.6	28.4	13.3	33.8	9.7	79.4	42.3	62.2	26	11.9	21.7	82.8	34.3	43.3	3.9	93.9
Baseline	1.9	5.3	2.1	0	4.7	0	1.3	2.1	2.5	6.1	4.9	5.7	0.7	1.1	2.1	10.3	9.1	1	5.3	12.2	2.7	9.3	2.9	0
Contraction	5.8	86.8	55.9	8.3	37.8	3.6	9.4	55.3	13.3	50	20.1	64.3	13.6	58.7	17	23.1	23.6	2.9	32.7	37.8	12.9	17.8	11.8	68.3
Baseline	13.6	1	2.5	0	3.6	12.2	7.8	5.2	2.7	0	4.3	0	2.5	2.9	13.5	11.6	11.7	0	2.9	2.3	2.1	2	5.1	2.7
Contraction	16.7	0	23.3	0	0	22.6	17.1	5.2	50	15.9	15.2	17.1	53.2	32	45.2	9.3	10.9	0	12.5	20.2	20.2	10.5	26.5	82.7
Baseline	20	9.7	6.3																					
Contraction	57.8	14.5	17.9																					

Ach std - seasonal and spatial variation

Ach std	56.1	56.2	56.3	56.4	57.1	57.2	57.3	57.4	58.1	58.2	58.3	58.4	60.1	60.2
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	3.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	2.5	0.0	3.3	5.2	2.4	0.0	2.3	2.0	0.0	0.0	3.0	3.4
-6.5	3.5	1.4	6.4	4.3	4.5	7.7	7.5	6.2	11.7	10.4	2.7	1.3	8.5	8.4
-6	11.9	12.0	16.4	6.9	9.4	13.7	11.9	13.0	17.7	20.5	13.8	7.0	22.0	21.9
-5.5	27.7	24.2	28.0	17.5	15.0	23.4	18.1	19.2	23.7	28.0	22.5	16.6	38.8	39.0
-5	49.0	39.0	43.0	33.1	26.4	44.4	27.5	34.2	34.5	41.8	40.9	31.3	63.7	59.1
-4.5	66.8	61.4	60.2	51.2	45.2	64.5	52.7	50.7	49.0	55.9	56.4	46.7	76.0	74.1
-4	83.3	78.5	77.1	75.9	71.4	81.0	72.8	69.9	66.3	73.1	76.0	66.1	87.0	85.5
-3.5	93.2	91.0	90.7	86.6	86.2	91.9	88.5	82.9	81.4	85.5	83.9	82.9	95.4	93.8
-3	98.5	97.0	96.6	97.6	99.5	97.6	97.2	94.5	94.1	96.7	96.3	93.7	98.8	98.1
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Ach std	60.3	60.4	61.1	61.2	61.3	61.4	62.1	62.3	62.4	77.2	77.4	80.1.k	80.3.h	80.3.k
-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-7.5	0.0	0.0	0.0	0.0	0.0	0.0	22.2	7.2	0.0	0.0	0.0	0.0	0.0	0.0
-7	0.0	0.0	4.3	5.9	7.2	2.2	26.1	5.1	0.0	0.0	0.0	0.0	0.0	0.0
-6.5	0.0	0.0	11.4	12.1	19.5	6.8	30.0	19.9	7.0	0.0	0.0	0.9	0.0	0.0
-6	0.0	0.0	19.1	21.4	35.7	14.8	39.6	28.9	17.4	0.7	11.2	4.7	0.0	8.8
-5.5	11.9	0.0	30.3	33.0	53.9	28.9	45.4	16.3	29.5	3.9	38.5	7.5	10.0	19.6
-5	38.1	50.1	37.0	55.8	68.2	51.3	52.2	61.7	46.0	11.6	63.2	27.1	27.8	33.0
-4.5	59.5	67.3	49.9	71.1	77.5	62.3	67.6	77.4	58.4	31.9	76.6	38.3	44.4	63.7
-4	78.6	81.2	73.7	84.5	87.6	80.3	83.1	86.6	75.3	54.7	90.6	72.0	66.7	82.4
-3.5	91.1	93.1	85.1	91.8	93.8	92.5	85.5	93.3	84.4	80.6	96.7	83.2	81.1	92.3
-3	96.4	99.6	95.4	98.2	99.3	100.0	98.1	97.1	93.3	99.5	100.0	100.0	94.4	100.0
-2.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Contractile response to extracts from different plant parts

Spring roots

Ach conc	56.1.b	56.3.b	57.1.l	57.3.l	61.4.b	62.2.c	62.4.b	80.3.l–C	56.2.h - - E	56.4.h	58.2.b	58.4.b	60.2.f	60.4.f - - D
-9.5	9.7	0.0	4.3	14.2	0.0	0.0	4.0	4.4	0.0	0.0	45.3	8.4	7.0	6.5
-9	9.7	0.0	4.1	0.0	0.0	0.0	4.0	4.4	0.0	0.0	36.7	6.5	7.0	6.5
-8.5	9.7	0.0	4.1	0.0	0.0	0.0	4.0	4.4	0.0	0.0	36.7	6.5	7.0	6.5
-8	9.7	0.0	4.1	0.0	0.0	0.0	4.0	4.4	0.0	0.0	36.7	6.5	7.0	6.5
-7.5	9.7	0.0	4.1	0.0	0.0	19.2	4.0	4.4	0.0	0.0	36.7	6.5	7.0	6.5
-7	9.7	10.6	4.1	6.0	0.0	0.0	4.0	4.4	0.0	0.0	36.7	6.5	9.5	8.4
-6.5	18.1	15.4	4.1	0.0	0.0	17.4	5.0	6.7	14.8	7.4	36.7	7.8	13.3	8.4
-6	29.2	26.4	9.0	13.4	24.7	17.4	12.0	15.6	23.4	16.7	45.3	16.9	19.6	8.8
-5.5	42.4	38.5	16.4	23.9	38.7	25.6	19.0	22.2	33.6	25.0	58.6	23.4	31.6	15.3
-5	63.9	54.8	20.1	37.3	61.3	36.6	31.0	44.4	50.8	41.2	81.3	55.8	51.9	34.4
-4.5	77.8	73.1	32.0	55.2	74.2	44.2	44.0	61.1	70.3	62.0	92.2	79.2	68.4	57.7
-4	87.5	82.7	41.0	87.3	86.0	65.7	59.0	77.8	82.0	82.9	101.6	98.7	80.4	77.2
-3.5	95.6	92.3	68.9	95.5	91.4	76.7	69.0	87.8	93.8	96.8	107.0	111.7	90.5	91.6
-3	100.0	98.1	71.3	100.0	94.1	83.1	73.5	96.7	101.6	107.9	111.3	120.8	98.1	104.2
-2.5	100.0	100.0	71.3	100.0	94.0	83.0	75.0	97.8	103.9	107.8	111.7	122.1	100.0	107.0

Summer roots

Ach conc	74.1.g	74.3.g	75.2.b	75.4.b	76.2.b	76.4.b	77.2.b	77.4.b - -F	74.2.g - G	74.4.f	75.2.g	75.2.j	80.2.l	80.4.l
-9.5	27.5	20.4	14.1	46.4	7.6	26.2	8.3	41.5	85.0	25.9	30.6	6.6	75.8	15.6
-9	18.7	20.4	14.1	38.1	7.6	23.0	8.3	41.5	66.0	25.9	26.9	0.0	71.2	15.6
-8.5	18.7	20.4	14.1	38.1	7.6	23.0	8.3	41.5	66.0	25.9	26.9	0.0	71.2	15.6
-8	18.7	20.4	14.1	38.1	7.6	23.0	8.3	47.2	66.0	33.3	26.9	0.0	71.2	15.6
-7.5	18.7	26.5	14.1	38.1	7.6	45.9	8.3	50.9	66.0	35.2	26.9	0.0	71.2	15.6
-7	18.7	26.5	14.1	38.1	7.6	47.5	8.3	56.6	66.0	53.7	26.9	0.0	71.2	21.9
-6.5	20.3	34.7	14.1	38.1	9.0	63.9	8.3	64.2	66.0	59.3	26.9	7.2	71.2	28.1
-6	20.3	34.7	18.7	46.4	15.2	84.4	20.4	73.6	66.0	75.9	26.9	14.5	71.2	48.4
-5.5	24.2		18.7	57.7	24.1	95.1	26.9	81.1	66.0	87.0	26.9	19.1	72.7	68.8
-5	44.5		32.8	79.4	44.8	104.9	46.1	89.6	68.0	98.1	26.9	43.4	77.3	82.8
-4.5	57.7		48.0	90.7	60.0	108.2	63.0	94.3	73.0	101.9	37.3	59.2	87.9	90.6
-4	0.0		68.7	99.0	77.9	111.5	83.3	98.1	87.0	104.6	49.3	77.6	93.9	95.3
-3.5	0.0		85.9	104.1	86.2	113.1	92.6	98.1	99.0	108.3	53.0	85.5	97.0	97.7
-3	101.1		100.0	105.7	91.0	113.1	93.5	100.0	104.0	108.3	65.7	89.5	100.0	99.2
-2.5	103.8		104.0	106.2	91.0	113.1	94.4	100.0	106.0	108.3	64.2	92.1	100.0	100.0

Autumn roots

Ach conc	52.1.e	53.2.l	53.3.l	54.2.b	55.2.f	56.1.h	56.3.h	57.2.c	57.4.d	58.1.b	58.3.b	60.1.f	60.3.f
-9.5	3.7	0.0	15.9	28.1	0.0	0.0	0.0	6.5	11.1	6.9	15.2	5.7	4.7
-9	2.4	0.0	15.9	28.1	0.0	0.0	0.0	6.5	11.1	0.0	15.2	5.7	0.0
-8.5	2.4	0.0	15.9	28.1	0.0	0.0	0.0	6.5	11.1	0.0	15.2	5.7	0.0
-8	2.4	0.0	15.9	28.1	0.0	0.0	0.0	6.5	11.1	0.0	15.2	5.7	0.0
-7.5	9.1	0.0	15.9	28.1	0.0	0.0	0.0	6.5	11.1	6.9	15.2	5.7	0.0
-7	10.7	0.0	15.9	28.1	0.0	4.8	0.0	6.5	11.1	8.0	15.2	11.5	4.7
-6.5	12.2	0.0	15.9	28.1	5.7	4.8	6.0	6.5	11.1	9.7	19.6	14.9	4.7
-6	12.8	10.9	24.6	28.1	9.1	14.3	19.9	8.1	11.1	18.3	22.8	20.7	7.0
-5.5	13.7	20.0	38.9	32.3	17.6	30.2	27.9	12.1	22.2	27.4	45.7	25.3	14.0
-5	16.2	29.1	50.8	54.2	36.4	55.6	49.8	18.5	29.6	40.0	64.1	52.9	32.6
-4.5	21.3	51.8	69.0	71.9	50.0	73.0	68.7	25.8	45.7	49.1	79.3	69.0	57.0
-4	33.8	68.2	84.9	85.9	58.0	85.7	87.6	36.3	53.1	56.0	91.3	82.2	76.2
-3.5	45.1	80.0	92.1	95.8	64.2	92.1	93.5	43.5	61.7	60.6	94.6	92.0	89.5
-3	61.0	89.1	98.4	100.0	70.5	98.4	100.0	50.0	70.4	64.0	98.9	95.4	98.3
-2.5	65.9	93.6	100.0	100.0	61.4	100.0	100.0	51.6	74.1	64.6	100.0	96.6	98.3

Winter roots

Ach conc	97.4.b	93.3.h	99.1.h	100.4.h	101.2.b	102.4.e - -	96.1.b - P	97.4.g	98.3.b	99.3.h	100.2.h	101.2.h - P	97.2.e - -Q	97.4.h	
-9.5	37.8	5.2	78.4	12.9	17.8	11.8	0.0	25.0	25.0	1.6	19.6	0.0	29.2	18.8	
-9	37.8	5.2	75.7	12.9	17.8	11.8	0.0	25.0	25.0	0.0	19.6	19.7	29.2	18.8	
-8.5	37.8	5.2	75.7	12.9	17.8	11.8	0.0	25.0	25.0	0.0	19.6	19.7	29.2	18.8	
-8	37.8	5.2	75.7	12.9	17.8	11.8	0.0	25.0	25.0	0.0	19.6	19.7	29.2	18.8	
-7.5	37.8	5.2	75.7	12.9	17.8	11.8	0.0	25.0	25.0	0.0	19.6	19.7	29.2	18.8	
-7	40.8	5.2	75.7	13.4	19.5	11.8	0.0	26.2	25.0	1.6	25.2	19.7	29.2	19.5	
-6.5	45.9	6.5	75.7	17.2	21.2	13.7	1.0	32.1	25.0	1.6	28.1	19.7	29.2	22.1	
-6	61.2	14.4	75.7	33.3	37.3	20.6	17.7	45.2	27.9	3.3	37.0	23.4	37.5	27.3	
-5.5	75.5	22.2	75.7	43.5	54.7	29.4	21.9	57.1	33.8	13.1	57.0	36.2	48.2	36.4	
-5	85.7	40.5	77.0	67.7	72.9	52.0	50.0	75.0	48.5	31.1	77.8	54.8	64.0	59.1	
-4.5	90.8	56.2	78.4	79.6	83.1	69.6	71.9	83.3	60.3	48.4	88.1	71.3	76.7	74.0	
-4	95.9	75.8	89.2	89.2	91.5	77.5	88.5	90.5	77.9	70.5	94.1	79.8	90.1	83.1	
-3.5	98.0	87.6	95.9	93.0	100.0	83.3	94.8	94.0	86.8	82.0	96.3	85.1	94.9	88.3	
-3	100.0	90.2	100.0	94.6	100.8	84.3	99.0	96.4	91.2	95.1	96.3	87.2	96.4	90.9	
-2.5	100.0	94.1	100.0	94.6	100.9	84.3	100.0	96.4	91.2	95.1	96.3	87.3	96.4	90.9	

Winter roots - continued

Ach conc	98.1.b	99.4.b	100.4.b	101.2.e	102.2.h
-9.5	20.2	17.1	5.2	50.0	15.9
-9.0	20.2	17.1	5.2	38.2	15.9
-8.5	20.2	17.1	5.2	38.2	15.9
-8.0	20.2	17.1	5.2	38.2	15.9
-7.5	20.2	17.1	5.2	38.2	15.9
-7.0	23.3	17.4	7.2	38.2	16.7
-6.5	24.8	21.3	20.6	38.2	16.7
-6.0	31.8	34.1	33.5	38.2	21.2
-5.5	40.3	41.1	53.6	43.6	24.2
-5.0	58.9	68.6	71.1	55.5	40.9
-4.5	74.4	88.4	83.5	66.8	56.1
-4.0	88.4	106.2	93.3	79.1	72.7
-3.5	96.1	111.6	97.4	86.4	80.3
-3.0	100.0	111.7	100.0	87.7	80.3
-2.5	100.0	111.7	100.0	87.7	80.3

Spring tubers

Ach conc	56.2.e	56.4.e	57.1.g	57.3.f	61.1.b	61.3.b
-9.5	0.0	0.0	7.3	11.3	26.1	38.4
-9.0	0.0	0.0	6.5	11.3	25.2	36.6
-8.5	0.0	0.0	6.5	11.3	25.2	36.6
-8.0	0.0	0.0	6.5	11.3	25.2	36.6
-7.5	0.0	0.0	6.5	11.3	25.2	36.6
-7.0	15.1	17.1	6.5	11.3	25.2	39.7
-6.5	15.1	6.7	9.8	12.5	25.2	43.8
-6.0	21.9	23.8	14.6	15.0	25.2	58.9
-5.5	30.9	27.6	17.1	20.6	39.1	75.9
-5.0	50.6	47.6	38.2	31.3	69.6	92.0
-4.5	64.9	64.8	55.3	45.0	87.8	98.2
-4.0	80.8	91.4	84.6	68.8	101.7	105.4
-3.5	92.5	105.7	101.6	83.8	111.3	109.8
-3.0	98.9	119.0	118.7	92.5	120.0	112.5
-2.5	100.0	120.0	122.0	99.4	121.3	112.5

Summer tubers

Ach conc	78.2.h	78.4.h	79.2.b	79.4.b	83.2.h	83.4.h
-9.5	21.5	44.4	68.7	56.0	77.9	30.8
-9	21.5	38.9	59.0	56.0	69.0	29.7
-8.5	21.5	38.9	59.0	56.0	69.0	29.7
-8	21.5	38.9	59.0	56.0	69.0	29.7
-7.5	21.5	38.9	59.0	56.0	69.0	29.7
-7	25.3	40.7	60.2	57.8	69.0	45.1
-6.5	26.6	44.4	60.2	60.6	69.0	41.8
-6	32.9	51.9	60.2	66.1	71.0	68.1
-5.5	40.5	63.0	62.7	76.1	80.0	74.7
-5	49.4	81.5	75.9	89.9	89.0	88.5
-4.5	72.2	92.6	85.5	94.5	92.4	93.4
-4	82.3	100.0	97.6	98.2	98.6	96.7
-3.5	87.3	103.7	104.8	100.1	100.0	97.8
-3	103.8	104.6	108.4	102.8	102.1	98.9
-2.5	108.9	104.6	109.6	101.8	102.1	98.9

Autumn tubers

Ach conc	81.2.h	81.4.h	82.1.e	82.3.e	83.1.b	83.3.b - K	81.2.e – L	81.4.e	82.2.b	82.4.b	83.2.h	83.4.h – L
-9.5	71.0	61.3	33.7	21.4	20.7	13.2	41.1	48.5	93.3	45.6	77.9	30.8
-9	63.8	59.7	31.7	21.4	20.7	13.2	41.1	48.5	93.3	45.6	69.0	29.7
-8.5	63.8	59.7	31.7	21.4	20.7	13.2	41.1	48.5	93.3	45.6	69.0	29.7
-8	63.8	59.7	31.7	21.4	20.7	13.2	41.1	48.5	93.3	45.6	69.0	29.7
-7.5	63.8	59.7	31.7	21.4	20.7	13.2	41.1	48.5	93.3	45.6	69.0	29.7
-7	63.8	59.7	31.7	23.8	20.7	15.7	44.3	54.5	93.3	48.1	69.0	45.1
-6.5	63.8	62.1	31.7	29.5	27.9	20.7	60.7	60.6	93.3	60.0	69.0	41.8
-6	67.4	63.7	35.6	53.3	33.5	42.6	75.0	77.3	94.1	87.5	71.0	68.1
-5.5	74.6	79.0	46.2	62.9	41.3	59.5	85.0	86.4	96.6	88.8	80.0	74.7
-5	81.9	91.9	50.5	81.0	76.0	78.5	90.7	96.2	99.2	105.0	89.0	88.5
-4.5	89.9	96.8	61.5	91.4	86.0	90.9	95.7	99.2	103.4	110.0	92.4	93.4
-4	94.2	100.8	90.4	100.0	93.9	96.7	97.9	101.5	109.2	113.1	98.6	96.7
-3.5	96.4	101.6	97.1	105.2	100.6	100.8	99.3	101.5	110.9	114.4	100.0	97.8
-3	97.8	101.6	100.0	107.1	103.4	102.5	100.0	101.5	111.3	114.4	102.1	98.9
-2.5	97.8	101.6	100.0	107.1	103.9	102.5	100.0	101.5	111.4	114.4	102.1	98.9

Winter tubers

Ach conc	97.3.b	98.1.h	99.2.h	100.3.h	101.1.b	102.3.e – I	96.4.b – P	97.1.b	98.4.b	99.4.h	100.1.h	101.3.h	102.1.h – I	98.3.e - Q
-9.5	25.0	0.0	0.0	4.5	61.4	2.3	0.0	10.4	15.8	0.0	24.0	0.0	8.0	15.4
-9	25.0	0.0	0.0	4.5	61.4	0.0	0.0	10.4	11.5	0.0	0.0	0.0	0.0	12.8
-8.5	25.0	0.0	0.0	4.5	61.4	0.0	0.0	10.4	11.5	0.0	0.0	0.0	0.0	12.8
-8	25.0	0.0	0.0	4.5	61.4	0.0	0.0	10.4	11.5	0.0	0.0	0.0	0.0	12.8
-7.5	25.0	0.0	0.0	4.5	61.4	0.0	0.0	10.4	11.5	0.0	0.0	0.0	0.0	12.8
-7	25.0	0.0	8.9	4.5	61.4	9.1	0.0	13.9	16.5	18.6	32.6	7.5	4.0	12.8
-6.5	28.6	7.6	13.4	5.6	61.4	15.2	4.3	17.4	17.3	15.5	38.6	8.8	10.0	12.8
-6	37.5	12.1	23.2	14.5	61.4	27.3	46.4	27.8	28.8	21.6	45.5	18.1	15.0	16.7
-5.5	49.1	25.8	44.6	27.9	61.4	47.7	63.8	40.0	40.3	28.9	56.7	37.5	25.0	25.6
-5	67.9	52.3	66.1	49.2	71.3	64.4	81.2	57.4	63.3	45.4	75.5	58.8	46.0	35.9
-4.5	82.1	69.7	76.8	67.0	76.2	77.3	89.9	73.0	75.5	58.8	85.8	77.5	66.0	56.4
-4	96.4	87.9	88.4	81.0	83.2	87.1	95.7	85.2	85.6	79.4	94.4	96.3	82.0	79.5
-3.5	105.4	106.1	92.9	91.6	86.1	94.7	98.6	93.9	90.6	85.6	97.0	111.3	83.0	91.0
-3	108.9	114.4	99.1	96.6	86.2	97.0	100.0	99.1	94.2	92.8	101.3	116.9	89.0	92.3
-2.5	110.7	114.4	100.0	96.6	86.2	97.0	100.0	100.0	94.3	92.7	101.3	116.9	89.0	92.3

Winter tubers - continued

Ach conc	99.2.b	100.2.b	101.3.b	102.3.b
-9.5	45.5	9.3	7.3	10.9
-9.0	39.5	9.3	7.3	10.9
-8.5	39.5	9.3	7.3	10.9
-8.0	39.5	9.3	7.3	10.9
-7.5	39.5	9.3	7.3	10.9
-7.0	39.5	15.0	11.5	17.2
-6.5	39.5	0.0	18.8	31.3
-6.0	45.8	37.9	24.0	50.8
-5.5	49.0	60.0	38.5	69.5
-5.0	73.1	80.0	57.3	84.4
-4.5	83.0	89.3	81.3	93.8
-4.0	90.9	95.0	96.9	100.8
-3.5	98.0	97.1	112.5	107.0
-3.0	100.0	97.9	116.7	108.6
-2.5	100.0	97.8	116.7	108.6

Spring stems

Ach conc	56.1.e	56.3.e	57.2.f	57.4.g	62.1.b	62.3.b – C	56.2.b - D	56.4.b	57.1.d	57.3.c	58.1.e	58.3.e	60.2.b	60.3.b	80.1.l
-9.5	9.9	8.1	10.2	5.7	41.5	20.7	13.8	0.0	6.6	11.3	41.8	15.4	17.6	20.0	0.0
-9	9.9	0.0	0.0	0.0	33.8	0.0	0.0	0.0	4.4	0.0	41.8	13.6	17.6	0.0	0.0
-8.5	9.9	0.0	0.0	0.0	33.8	0.0	0.0	0.0	4.4	0.0	41.8	13.6	17.6	0.0	0.0
-8	9.9	0.0	0.0	0.0	33.8	0.0	0.0	0.0	4.4	0.0	41.8	13.6	17.6	0.0	0.0
-7.5	9.9	0.0	0.0	3.8	33.8	7.6	0.0	0.0	4.4	3.8	41.8	13.6	17.6	9.4	0.0
-7	9.9	6.1	4.7	13.2	33.8	10.9	17.1	5.2	7.3	8.2	43.6	16.0	21.4	16.5	0.0
-6.5	12.7	10.1	7.8	15.1	33.8	23.9	20.7	11.5	7.3	13.6	45.5	19.8	34.0	22.9	0.0
-6	19.7	32.3	15.6	20.8	33.8	37.0	31.6	25.0	10.9	23.9	49.1	28.4	60.4	90.6	6.4
-5.5	32.4	35.4	15.6	24.5	35.7	44.6	40.7	36.5	19.7	35.2	54.5	33.3	75.5	97.6	9.6
-5	47.9	42.4	25.0	39.6	47.8	59.8	61.8	65.6	36.5	55.3	65.5	66.7	85.5	103.5	14.4
-4.5	66.9	62.6	37.5	54.7	51.2	69.6	76.0	88.0	55.5	74.2	75.5	72.2	95.0	114.1	20.8
-4	81.7	81.8	51.6	67.9	68.6	78.3	86.5	107.3	71.5	85.5	84.5	86.4	102.5	125.9	41.6
-3.5	90.1	94.9	68.0	86.8	81.2	84.2	98.2	122.9	86.1	89.3	93.6	92.6	105.7	127.1	57.6
-3	94.4	100.0	78.1	98.1	90.8	87.0	98.9	134.4	98.5	98.1	99.1	100.0	106.3	130.0	77.6
-2.5	100.0	99.0	76.6	100.0	88.9	88.0	100.0	138.5	100.0	100.0	100.0	100.0	106.9	130.0	77.6

Summer stems

Ach conc	78.1.h	78.3.h	79.1.b	79.3.b	80.2.b	80.4.b –F	78.4.g - G	79.2.a	79.2.d	79.2.g	79.2.k	79.4.a
-9.5	46.7	39.8	19.8	12.2	81.9	34.1	28.1	44.2	29.9	53.3	25.9	46.7
-9	46.7	36.0	19.8	12.2	79.5	34.1	18.0	44.2	29.9	53.3	0.0	46.7
-8.5	46.7	36.0	19.8	12.2	79.5	34.1	18.0	44.2	29.9	53.3	0.0	46.7
-8	46.7	36.0	19.8	12.2	79.5	34.1	18.0	44.2	29.9	53.3	53.8	46.7
-7.5	46.7	36.0	19.8	13.3	79.5	34.1	18.0	44.2	33.3	53.3	60.8	46.7
-7	46.7	36.0	20.8	18.4	79.5	43.5	18.0	51.3	34.5	53.3	60.8	46.7
-6.5	47.5	41.0	23.8	23.5	79.5	55.1	18.0	53.1	34.5	55.4	66.4	57.6
-6	48.4	53.4	39.1	38.8	79.5	73.9	21.3	61.9	34.5	57.6	76.2	73.9
-5.5	60.7	72.0	63.4	54.1	85.4	87.0	34.3	72.6	34.5	60.9	89.2	95.7
-5	76.2	89.4	79.2	78.6	91.2	97.1	39.9	83.2	42.5	73.9	104.2	107.6
-4.5	86.1	101.2	89.6	88.8	95.9	100.0	59.0	92.0	52.9	84.8	117.5	119.6
-4	94.3	109.3	97.5	95.4	100.0	101.4		95.6	71.3	92.4	127.6	123.9
-3.5	100.0	111.8	101.0	101.0	101.8	102.2		97.3	90.8	98.9	137.1	127.2
-3	104.1	113.0	102.0	103.1	101.8	102.2	126.1	99.1	109.2	102.2	141.3	127.2
-2.5	105.7	113.7	102.0	104.1	101.8	102.2	136.0	100.0	116.1	103.3	143.4	127.2

Autumn stems

Ach conc	81.2.b	81.4.b	82.2.h	82.4.h	83.1.e	83.3.e –K	81.1.e - L	81.3.e	82.1.b	82.3.b	83.1.h	83.3.h - L	81.1.b–M	81.3.b	82.1.h
-9.5	82.9	44.0	62.6	26.0	11.9	21.8	10.1	9.9	17.6	13.1	7.9	7.2	22.2	2.3	11.7
-9	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0
-8.5	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0
-8	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0
-7.5	77.8	44.0	62.6	20.0	11.9	21.8	10.1	6.1	17.6	13.1	7.9	7.2	15.0	0.0	0.0
-7	77.8	47.3	67.6	26.7	11.9	24.3	13.2	9.9	21.2	13.1	13.3	11.8	15.0	3.8	0.0
-6.5	77.8	56.0	68.2	33.3	23.9	36.0	15.3	17.9	27.1	24.6	15.2	16.0	19.8	13.1	17.6
-6	77.8	76.7	69.3	65.3	26.7	52.3	25.4	40.3	40.0	46.3	18.2	24.1	19.8	34.6	30.2
-5.5	88.0	88.0	72.6	78.7	28.4	69.5	41.3	57.8	55.3	59.0	34.5	34.6	24.0	52.3	43.9
-5	96.8	96.7	86.0	89.3	60.8	84.5	63.0	75.3	84.7	75.8	60.6	50.6	50.3	76.2	68.3
-4.5	100.0	101.3	88.3	94.7	72.7	93.7	77.2	88.2	103.5	84.4	70.3	68.4	71.3	87.7	79.0
-4	102.5	103.3	94.4	98.7	83.0	100.4	88.4	93.9	117.6	91.0	87.9	81.9	92.8	96.2	90.2
-3.5	104.4	104.0	96.1	100.0	90.3	103.8	93.7	96.6	125.9	95.1	89.7	88.6	106.0	100.0	96.6
-3	104.4	102.7	98.3	100.0	92.6	104.2	97.9	97.3	127.1	95.9	94.5	92.8	112.0	100.0	100.0
-2.5	104.4	101.3	92.7	100.0	93.2	103.8	97.8	97.3	127.1	96.7	94.5	97.0	113.2	100.0	100.0

Autumn stems - continued

Ach conc	82.3.h	83.2.e	83.4.e
-9.5	5.9	86.3	43.5
-9.0	5.9	79.7	40.2
-8.5	5.9	79.7	40.2
-8.0	5.9	79.7	40.2
-7.5	5.9	79.7	40.2
-7.0	14.6	79.7	40.2
-6.5	18.5	79.7	47.3
-6.0	44.9	79.7	64.1
-5.5	62.9	83.0	79.3
-5.0	78.0	91.5	87.5
-4.5	88.3	94.1	92.4
-4.0	97.6	96.7	95.7
-3.5	101.5	98.7	97.3
-3.0	103.4	99.3	97.3
-2.5	103.4	99.4	97.3

Winter stems

Ach conc	96.4.e	97.2.b	98.2.h	99.1.e	100.1.e	102.1.b - C	97.3.e - P	98.2.b	99.2.e	100.2.e	101.3.e	102.1.e - P	97.1.e - Q	98.4.e	99.3.b
-9.5	23.8	35.4	12.4	88.2	78.0	27.5	8.6	32.1	20.0	30.3	7.2	24.7	15.2	14.6	10.5
-9	9.5	35.4	12.4	88.2	78.0	27.5	8.6	32.1	20.0	30.3	3.3	24.7	15.2	14.6	10.5
-8.5	9.5	35.4	12.4	88.2	78.0	27.5	8.6	32.1	20.0	30.3	3.3	24.7	15.2	14.6	10.5
-8	9.5	35.4	12.4	88.2	78.0	27.5	8.6	32.1	20.0	30.3	3.3	24.7	15.2	14.6	10.5
-7.5	9.5	35.4	12.4	88.2	78.0	27.5	8.6	32.1	20.0	30.3	3.3	24.7	15.2	14.6	10.5
-7	9.5	35.4	20.2	88.2	78.0	32.7	8.6	34.8	27.8	30.3	11.1	32.7	23.9	14.6	14.6
-6.5	30.2	39.6	23.3	88.2	78.9	35.7	16.1	37.8	29.6	34.8	17.8	32.7	30.4	14.6	17.5
-6	38.1	52.1	41.9	88.2	79.7	57.2	17.2	60.8	40.9	58.6	23.3	34.0	14.1	18.3	29.8
-5.5	57.1	63.9	55.0	89.2	85.4	72.1	24.7	69.6	55.7	77.9	38.9	44.0	41.3	22.0	33.9
-5	69.8	77.1	73.6	91.2	81.3	81.0	49.5	79.7	68.7	95.1	58.9	66.0	65.2	40.2	45.6
-4.5	81.0	85.4	83.7	92.2	87.0	92.9	66.7	89.2	78.3	100.4	80.0	74.0	73.9	56.1	64.9
-4	86.5	90.3	90.7	102.0	92.7	101.9	83.9	95.9	88.7	108.2	95.6	80.7	93.5	75.6	93.0
-3.5	90.5	93.1	94.2	104.9	96.3	107.1	93.5	98.6	92.6	110.7	107.8	85.3	104.3	80.5	109.9
-3	90.5	93.1	95.0	105.9	96.3	110.0	98.9	100.0	95.7	110.7	114.4	82.0	109.8	80.5	110.5
-2.5	90.5	93.1	95.0	105.9	96.3	110.0	100.0	100.0	95.7	110.7	116.1	82.0	109.7	80.5	110.5

Winter stems - continued

Ach conc	100.3.b	100.3.e	101.1.e	102.2.e
-9.5	0.0	0.0	53.2	31.9
-9.0	0.0	0.0	40.5	27.1
-8.5	0.0	0.0	40.5	27.1
-8.0	0.0	0.0	40.5	27.1
-7.5	0.0	0.0	40.5	27.1
-7.0	0.0	0.0	40.5	27.1
-6.5	3.8	11.5	40.5	28.0
-6.0	12.7	20.2	40.5	31.9
-5.5	33.8	38.9	43.0	33.8
-5.0	50.8	69.2	43.0	53.6
-4.5	73.1	77.8	48.1	63.8
-4.0	93.1	84.6	57.0	73.4
-3.5	97.7	95.1	65.8	78.3
-3.0	106.9	100.0	65.8	78.3
-2.5	110.8	100.0	65.8	78.3

Summer leaves

Ach conc	71.1.b	71.3.b	74.1.b	74.3.b	74.4.b	75.3.b - F	84.2.b - G	84.4.b	85.1.b	85.3.h	87.1.b	87.3.b	88.2.b	88.4.b
-9.5	8.5	7.8	0.0	8.2	10.2	12.2	55.6	7.7	56.8	11.6	9.4	10.0	55.8	28.2
-9	8.5	7.8	0.0	8.2	10.2	12.2	26.7	7.7	50.0	11.6	9.4	10.0	55.8	28.2
-8.5	8.5	7.8	14.1	22.4	10.2	12.2	26.7	7.7	50.0	11.6	9.4	10.0	55.8	28.2
-8	8.5	7.8	14.1	26.5	10.2	12.2	26.7	7.7	50.0	11.6	9.4	10.0	55.8	28.2
-7.5	9.4	8.5	19.0	30.6	10.2	13.3	26.7	7.7	50.0	11.6	9.4	10.0	55.8	28.2
-7	12.8	15.5	19.6	38.8	14.8	13.3	38.9	13.1	50.0	12.8	18.0	13.6	58.4	39.6
-6.5	21.4	35.7	24.5	44.9	15.6	14.4	64.4	24.4	50.0	16.3	29.3	20.0	63.0	41.1
-6	29.1	58.9	27.6	65.3	26.6	24.4	75.6	48.8	50.0	29.7	49.2	40.7	77.9	59.4
-5.5	42.7	77.5	39.9	87.8	42.2	43.3	85.6	74.4	50.0	50.0	64.1	64.3	85.7	69.3
-5	58.1	91.5	49.7	102.0	69.5	71.1	94.4	94.0	56.1	69.8	77.3	85.7	96.1	87.1
-4.5	68.4	98.4	63.8	110.2	86.7	87.8	97.8	103.6	65.2	83.7	86.7	97.1	98.7	92.1
-4	85.5	102.3	77.9	116.3	103.1	95.6	102.2	108.9	80.3	93.6	92.2	105.0	101.9	97.0
-3.5	93.2	102.3	90.8	118.4	113.3	111.1	102.8	111.9	84.8	101.2	96.1	112.1	101.9	98.0
-3	99.1	102.3	100.0	118.4	118.8	120.0	102.8	112.5	95.5	103.5	97.7	114.3	102.6	100.0
-2.5	100.0	102.3	100.0	118.4	121.1	122.2	102.8	112.5	100.0	105.8	97.7	114.3	100.0	100.0

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