

Phytochemical study of Rhoicissus tomentosa

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**Thesis submitted in fulfillment of the requirements for the degree of
Masters of Science in the Department of Chemistry,
University of Western Cape**

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Abstract

For centuries, indigenous people in South Africa have used a variety of medicinal plants to treat chronic infections. This investigation focused on *Rhoicissus tomentosa*, belonging to the family, Vitaceae in an attempt to assess the phytochemistry of this plant which is widely used by traditional healers in South Africa to ensure the safe delivery during pregnancy and childbirth (Hutchings et al., 1996).

Phytochemical screening was undertaken to test for the presence of flavonoids, coumarins, phytosterols, aromatic acids, essential oils, terpenoides, saponins and resveratrol which was used as reference standard. Positive results were found for coumarins, flavonoids, phytosterols, essential oils, saponins, terpenoids, resveratrol and negative results were found for hydrolyzed tannins, condensed tannins, aromatic acids and alkaloids.

In addition, compounds identified from *R.tomentosa* by GC-MS analyses showed at least 30 active compounds tabulated in Tables 3 and 4. These compounds belonged to the chemical classes viz., coumarins, flavonoids, phytosterols, essential oils, saponins, and terpenoids including Resveratrol. Scientific information now documented can be used to enhance the overall knowledge of the biological activity and health benefits of *Rhoicissus tomentosa*.

Declaration

I declare that Phytochemical studies of *Rhoicissus tomentosa* is my own work and it has not been published for any degree or examination at any other university.

All the publications I have used have been recorded and acknowledged by complete referencing.

Full name: Nandipha Lucia Nqolo

Date: May 2008

Signed by:



Acknowledgements

I would like to thank Prof I Green for his mentorship, guidance, encouragement and effort he provided throughout this project.

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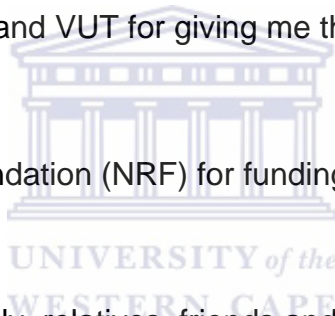
Huge level of gratitude to the following companies:

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Many thanks also to my family, relatives, friends and colleagues for their support and prayers

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Keywords

Anti-bacterial

Anti-fungal

Anti-inflammatory

Anti-microbial

Anti-oxidants

Medicinal plants

Phytoalexin

Pregnancy

Resveratrol

Rhoicissus

Rhoicissus tomentosa

South Africa

Traditional healers

Traditional medicine

Treatment



List of Abbreviations

Abs- absorbance

CC- Column Chromatography

DMF- dimethylformamide

DMSO- dimethylsulfoxide

EO -essential oils

EtOH- ethanol

EtOAc -ethyl acetate

Et₃N- triethylamine

GOK - Government of Kenya

GC-MS- Gas Chromatography Mass Spectroscopy

HepG₂- human hepatocellular liver carcinoma cell line

HIV- human immunodeficiency virus

H₂SO₄ - sulphuric acid

MAO- monoamine oxidase

NaH- sodium hydride

NAPPH- sodium triphenylphosphine

NMR- Nuclear Magnetic Resonance

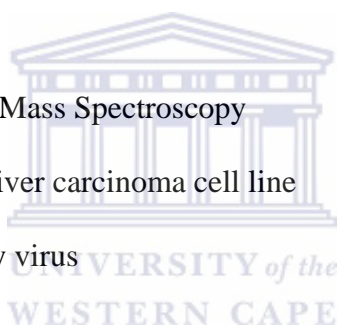
PLC- Preparative Layer Chromatography

PPh₃- Triphenyl phosphine

Pd(OAc)₂- palladium acetate

Rf- relative fractions

RSV- Resveratrol



SS- agars- *Salmonella Shigella* agars

TLC-Thin Layer Chromatography

UV- Ultra violet

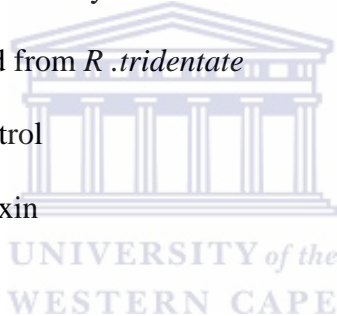
Vit E- Vitamin E

WHO- World Health Organization



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1. Introduction

Traditional medicinal plants have played an important role in South Africa, as they are used in the traditional treatment of various diseases on an empirical basis. These plants have been used by home users and traditional healers who are commonly known among Zulu people as izangoma, inyangas as well as herbalists and amaXhwele or amaGqirha by Xhosa communities.

The use of herbal remedies is becoming increasingly popular all over the world. It is estimated that approximately 80% of the South African population use a traditional remedy at some stage in their lives (Hutchings, 1989, Brand and Muller, 1995). Traditional plants and traditional remedies are part of the cultural and religious life of the African people. This broad use of traditional medicinal plants is attributable to its accessibility and affordability.

Rhoicissus tomentosa, isinwasi in Zulu or idiliya in Xhosa, wild grape in English from the family name Vitaceae is widely used by traditional healers in South Africa.

Rhoicissus tomentosa is a handsome, vigorous, evergreen tendril climber with ornamental, vine-like leaves and bunches of purple grape-like fruits (Fig 1).



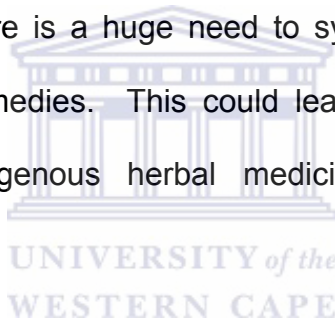
Fig.1 *Rhoicissus tomentosa*

The grape name Vitaceae is large with 1000 species spread throughout the world and is famous for its celebrated member, the grapevine called *Vitis vinifera*. The genus *Rhoicissus* is represented by 10 species *R.tomentosa*, *R.laetans*, *R.kougabergensis*, *R.microphylla*, *R.sessilifolia*, *R.digitata*, *R. Rhombedia*, *R. tridentate*, *R.revoilli* and *R.sekhukhuniensis* that occur in all the provinces of South Africa except the Northern Cape.

These species are widely used by traditional healers in South Africa to ensure safe delivery during pregnancy. (Hutchings et al., 1996; Table 1)

Their choice in herbal remedies is based on knowledge passed down from generation to generation. Although these plants can be used in different preparations, they are commonly prepared as a liquid solution or the leaves are decocted in water. South Africa, especially KwaZulu- Natal has a rich heritage of indigenous knowledge on the use of traditional medicinal plants (Hutchings et al., 1996; van Wyk et al., 1997). Zulu traditional plants medicine is widely used and

practised which is why Zulu medicinal plants are traditionally traded and used all over Southern Africa as well. Some further scientific investigation to validate traditional claims is needed to facilitate the acceptance of traditional healing as a valued resource in the South African Primary Health care system. Some scientific support for traditional treatment or potential usage of traditional plants has been shown (Hutchings et al., 1996; van Wyk et al., 1997). However there are still many plants that have not yet been screened. Scientific investigation and information of the therapeutic potential of the plant material is limited. With the possibility of traditional medicinal practitioners being integrated into the state health delivery system, there is a huge need to systematically evaluate plants used in Zulu traditional remedies. This could lead to new drug discovery or advance the use of indigenous herbal medicine for orthodox treatment (Hutchings et al., 1996).



Since the situation is now changing and the traditional healers are being recognized in South Africa, there is a great or active movement towards the integration of traditional healing into the official health care system again exemplifying the importance to evaluate the traditional methods of treatment. People like to recommend the medicine he /she used to his/her relatives and friends without any safety considerations. The general public and many of the traditional medicine practitioners also believe that the herbs are non-toxic and can be used in managing many health problems. Apparently, this cultural style or concept needs more attention in terms of drug safety education.

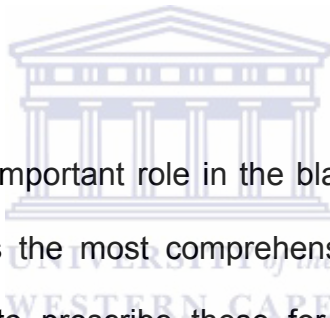
There is a an urgent need to comprehensively gather scientific information about the traditional plants, since the knowledge in using and preparing the remedies which have been passed from generation to generation has to be rigorously documented since the older generation will pass way. Consequently the planned remedial strategic plan must include the following:

- Detailed procedures for the preparation and listing of their ingredients, and for prescription of the traditional medicinal plants must be drawn up and documented.
- Proper training on these procedures should be available.
- Validation and verification of these procedures.
- Therapeutic uses and biological activities need to be described
- Poisonous, potential toxicity and side effects when overdose or over usage of these plants is applied must be documented with full details.
- Poisonous activities, toxicity and side effects must be fully documented.
- Pharmacology of the plant used in these traditional herbal remedies must be investigated.
- Traditional healers must work hand in hand with the investigators.

In an attempt to conserve traditional medicine knowledge, it is necessary that inventories of plants with therapeutic value are established and the knowledge related to their use is fully documented in systematic studies. These studies can

have other values too for society besides conserving traditional knowledge, for they can help to identify plants with market potential that can help generate incomes for local communities.

Many black South African women use traditional herbal remedies as antenatal medications to induce labour. This applies particularly to the rural areas where modern health care facilities are often lacking. The rural blacks are in many cases able to identify and harvest their own herbal remedies from the veld with the Xhosa, Zulu and Sotho tribes appearing to use similar remedies. In urban areas, black people are able to purchase traditional medicines from herbalist or 'Muti' shops.



Traditional healers play an important role in the black South African community or society and they possess the most comprehensive knowledge of medicinal plants which allows them to prescribe these for their patients. Fertility is a dominant theme in the culture of black South Africans as it ensures the preservation and propagation of the tribe. Children are regarded as an insurance against loneliness and poverty in old age and as a result a sterile woman is treated with contempt and pity (Krige, 1957; Gumedi, 1978; Brindley, 1985). Pregnancy is an event of great importance and many traditions and cultures have to be upheld in order to ensure successful confinement and the safe delivery of a healthy child. A literature review compiled by Veale et al. (1992) revealed that 57 plant species are used during pregnancy and child birth. It is estimated that the rate of infertility in Africa lies between 30 and 50% in some areas (Besley, 1976).

and it is estimated that 80% of the people in Zululand are dependent on traditional Zulu medicinal plants for health care (Gumede, 1989). In an effort to improve the efficiency of the practice and possibly to incorporate it into modern medicinal practices, researchers are increasingly turning their attention to evaluating the therapeutic potential of Zulu plant materials (Katsoulis, 1999; Ducan et.al., 1999; McGaw et al., 1997; Jager et al.,1996).

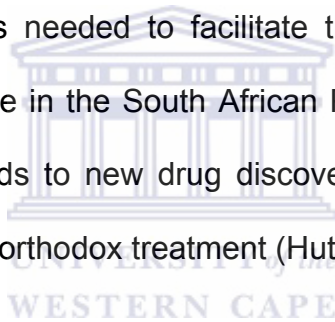
1.1 Literature review of health care practices in South Africa

Traditional healing is widely practiced in South Africa and it has been estimated that up to 80% of Zulu patients seen by medical practitioners also consult traditional healers (Gumede, 1989). Fortunately, traditional healers have officially been recognized by the authorities in South Africa. The situation is now changing and there has for some time been an active movement towards the integration of traditional healing into the official health care system (Holdstock, 1979; De Vos, 1988; Gumede, 1989; Pick, 1992)

In South Africa, most of the black population use traditional herbal medicine for their physical and psychological health needs. Medicinal plants have become the focus of an intense study recently in terms of conservation and as to whether their traditional uses are supported by actual pharmacological effects or merely based on folklore (Cunningham, 1988, Locher et al., 1995). It is estimated that 64% of the total world population depends on traditional medicine for their primary health care (Cotton, 1996). In Africa, where there is a severe shortage of qualified personnel in modern medicine (Mammem and Cloete, 1996) and

imported pharmaceuticals are expensive (Scott, 1993), this figure is even higher with 75% of the people relying on plant remedies prescribed by traditional healers (Hamilton, 1993).

In South African almost 75% of the population regularly consult traditional medical practitioners (Streak, 1995). The Zulus believe that the plant based medicines have power (amandla) and so traditional medical practices must also be seen in an historical and cultural perspective. With the increasing acceptance of traditional medicine as an alternative form of health care, the screening of medicinal plants for active compounds is very important. Further research to validate traditional claims is needed to facilitate the acceptance of traditional healing as a valued resource in the South African Primary Health Care system. This could also provide leads to new drug discovery or the use of indigenous herbal medicine to enhance orthodox treatment (Hutchings et al., 1996).

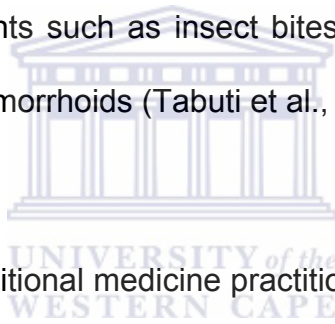


1.2 Literature review of health care practises

Internationally

Traditional medicine occupies a central place among rural communities of developing countries for the provision of health care in the absence of an efficient primary health care system (World Health Organization, 1995; Sheldon et al., 1997; The, 1998; Shrestha and Dhillon, 2003; Tabuti et al., 2003). Generation of incomes for local communities is seen as an important motivation for the conservation of local species (Shackleton, 2001). Studies related to herbal medicines can help to stimulate confidence in traditional medicine and enhance

appreciation of herbal medicine in local communities. Local communities will then have a higher appreciation of the value of their plant resources and make efforts to conserve them (Sheldon et al., 1997; Shackleton, 2001). According to Mr. M Wambuzi, the Medicinal Assistant in charge of Namwiwa sub-country health centre, the most common ailments of Bulamogi include malaria, respiratory tract infections, intestinal worms, diarrhea, diseases of the eye, anemia, measles, itchy skin rashes, fungal infections, jaundices, tonsillitis, hernias, gastroenteritis, pyomyositis, bubo, salpingitis, syphilis, ulcers, and false teeth in babies. Health care is provided by both orthodox and traditional medicine systems in which they use Vitaceae to treat ailments such as insect bites, bone settings, amoebiasis, spirits, ritual of twins and hemorrhoids (Tabuti et al., 2003).



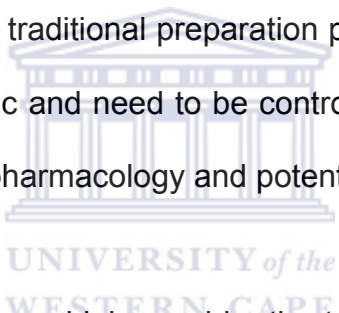
People routinely consult traditional medicine practitioners for chronic and psycho-spiritual illnesses (Tabuti et al., 2003a). Traditional medicine practitioners are usually elderly men, older than 30 years and are commonly registered with traditional healer associations. They have extensive experience of traditional healing, and learn the craft of healing by apprenticing under senior traditional medicine practitioners. Traditional medicine practitioners usually possess a modest education comprising of a primary level (Tabuti et al., 2003). In Kenya the literature survey reported that the health conditions are poor even by Kenian standards (GOK, 1992; Friis et al., 1998, 2002). Public health facilities are often short of staff, equipment and medicines, and few people can afford private practitioners. Much health care is relegated to the family, and home treatment is

common. Pharmaceuticals can be bought from any local shop and injections are often given by family fathers or village relatives rather than by professionals (Geissler et al 2000). The most prevalent illnesses, according to the government health statistics, are malaria and respiratory tract and intestinal infections (GOK, 1992), although these statistics are based upon outpatient data in government health facilities. Stomach problems might be also among the more prominent illnesses experienced by people. The studies show a high prevalence of malnutrition, anemia and infectious diseases among school-children (Friis et al 1998; Geissler et al., 1998a, b). A survey in Kenya found that 13 year old children already know most commonly used herbs for the treatment of common illnesses (Prince et al., 2001) and often use herbal medicines without adult consultation (Geissler et al., 2002). Children illnesses are taken care of within the family and treatment often employs self prepared herbal remedies, particularly the case for infants and young children, since the illness that concerns mothers the most at this age are not biomedically recognized and require long term herbal treatment in the home and all too often they cannot, or must not, be treated with pharmaceuticals (Geissler et al., 2000).

2. Traditional pharmacology and medicine

Herbal medicines are prepared in a variety of ways. Concoctions normally consist of mixtures of more than one species of plant, and are prepared in the form of decoctions and infusions whilst some are ground to powders, bathed and applied directly to skin or as ash. The validation should be ideally carried

out in two phases. Firstly, an evaluation of the claimed cures may be carried out by monitoring patients under the care of traditional medicine practitioners, as it was done in Mali (Diallo and Palsen, 2000). In Mali the Department of Traditional Medicine has been able to develop what they call improved drugs, some of which have been patented (Diallo and Paulsen, 2000). The Malian experience could be replicated in Uganda with obvious benefits. The next phase would be to subject promising herbal treatments to rigorous research and development encompassing laboratory analysis and clinical trials to determine their efficacy, safety and to determine dosages (World Health Organization, 2000). The traditional preparation practices of herbal medicines are sometimes unhygienic and need to be controlled for product safety. Very little is known about the pharmacology and potential toxicity of the plants used in these herbal remedies.




Species from the Vitaceae are widely used by the traditional healers in Africa to ensure the safe delivery of babies at the end of pregnancy and their therapeutic uses are listed in Table 1 as follows (Hutchings et al., 1996):

2.1 Therapeutic Uses (Table 1) of some *Rhoicissus* species

| Botanical name | Zulu name | Medicinal usage |
|------------------------------|-----------|--|
| <i>Rhoicissus - digitata</i> | Isinwasi | Roots are used as traditional medicine. They are probably used during pregnancy to facilitate delivery and for cattle disease in the same way as <i>R.tomentosa</i> (Hutchings et al., |

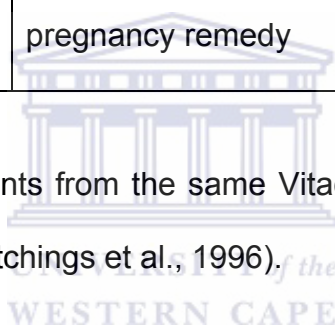
| | | | |
|-----------------------------------|---|----------|---|
| | | | 1996) |
| <i>Rhoicissus kougarbegensis</i> | - | Isinwasi | During pregnancy to ensure a safe delivery (Hutchings et al., 1996) |
| <i>Rhoicissus -laetans</i> | | sinwasi | During pregnancy to ensure a safe delivery (Hutchings et al., 1996) |
| <i>Rhoicissus microphylla</i> | - | Isinwasi | During pregnancy to ensure a safe delivery (Hutchings et al., 1996) |
| <i>Rhoicissus rhomboidea</i> | - | Isinwasi | Roots are used as traditional medicine. They are probably used during pregnancy to facilitate delivery and for cattle disease in the same way as <i>R.tomentosa</i> (Hutchings et al., 1996). |
| <i>Rhoicissus -revoilli</i> | | Isinwasi | During pregnancy to ensure a safe delivery (Hutchings et al., 1996) |
| <i>Rhoicissus sekhukhuniensis</i> | - | Isinwasi | During pregnancy to ensure a safe delivery (Hutchings et al., 1996) |
| <i>Rhoicissus sessilifolia</i> | - | Isinwasi | During pregnancy to ensure a safe delivery (Hutchings et al., 1996) |

| | | |
|-----------------------------|----------|--|
| <i>Rhoicissus-tomentosa</i> | Isinwasi | <p>The roots boiled in milk are given to calves to expel intestinal worms. They may also used during pregnancy to ensure safe delivery. Abdominal pains and swellings, anti-emetics in children, broken bones, cuts, epilepsy, convulsions, infertility and administered as an enema for delayed menstruation, dysmennorrhoea, menorrhagia, renal complaints, bladder and kidney complaints, sprained ankles, stomach ailments and sores.(Hutchings et al., 1996; Van Wyk et al., 1997;Veale et al. 1992)</p>  |
|-----------------------------|----------|--|

| | | |
|------------------------------|------------|---|
| <i>Rhoicissus tridentata</i> | - Isinwasi | <p>Chopped roots are administered as anthelmintics to calves, during pregnancy to ensure safe delivery (Hutchings et al., 1996). Used to feed children when mothers are not home (Tyiso and Bhat, 1998). Used to treat erectile dysfunction by the Vhavenda to care for many ailments including the treatment. Root bark powder and portions are mixed with Mabhundu, a traditional drink from water and different grains and the mixture is consumed immediately or within</p> |
|------------------------------|------------|---|

| | | |
|--|--|---|
| | | <p>two days. (Rakuambo et al., 2006).Used as ingredient in <i>inembe a</i>- a potent labour-inducing herbal mixture often used as an abortifacient and also in <i>Isihlambeso</i>, a decoction used by many Zulu women in South Africa as a preventative health tonic during pregnancy (Varga et al., 1997; Veale et al., 1997). Rootstock of <i>R- tridentata</i> can be toxic or potentially fatal if taken in large quantities and cause CNS paralysis and respiratory arrest. Toxic compound is Mezeleine Most commonly used for pregnancy remedy</p> |
|--|--|---|

The above medicinal plants from the same Vitaceae family are also used to treat cancer patients (Hutchings et al., 1996).



Anti- inflammatory, anti-microbial and antioxidant activities of

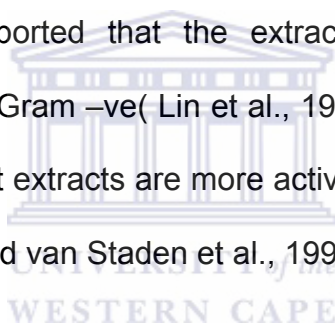
***Rhoicissus* species**

Results obtained from the scientific literature survey demonstrate that many of the *Rhoicissus* species from the family Vitaceae screened in the study possess potential anti-inflammatory and anti-microbial activities (Lin et al., 1999). The isolation of new and effective anti-inflammatory and anti-microbial compounds is important both for potential drug development and for the validation of the use of Zulu medicinal plants by traditional healers. Medicinal herbs are recognized as sources of natural antioxidants that can protect against oxidative stress and thus

play an important role in the chemoprevention of diseases that has their etiology and pathophysiology in reactive oxygen species. The medicinal properties of folk plants are mainly attributed to the presence of flavanoids, but may also be influenced by other organic and inorganic compounds such as coumarins, phenolic acids and antioxidant micronutrients e.g., Cu, Mn, Zn (Repetto and Llesuy, 2002).

Research reported on crude extracts of *Rhoicissus* from Vitaceae assayed for prostaglandin synthesis inhibition from the methanolic extracts of *R. rhomboidea*-root, *R. tomentosa*-leaf/stem, *R. digitata*-leaf, *R. tridentata*-root showed inhibitory activity against COX-1 compared to inhibition by the indomethacin standard. Extracts of *R. digitata* leaf and of *R. rhomboidea* roots exhibited the highest inhibition of prostaglandin synthesis with values of 53 and 56%, respectively. It was reported that none of the aqueous extracts showed any significant anti-inflammatory activity (Lin et al., 1999). Among these extracts that possessed higher anti-inflammatory activities, only *R. tridentata* has been used to treat pains, swellings, cuts and wounds by traditional practitioners. The results reported in the literature suggest that *Rhoicissus digitata* and *Rhoicissus rhomboidea* might have the potential to be used as anti-inflammatory agents. From the growth media which is an important pointer to the anti-microbial activity, none of the crude plant extract showed significant anti-microbial activity against growth of *S. aureus* (penicillin and erythromycin resistant strain) on blood agar plates (Lin et al., 1999). Muller-Hilton agar literature showed a significant capability to inhibit

the growth of *S.aureus*. Similar results were obtained when potato dextrose and SS agars were used to assay the inhibitory activity against *Candida albicans* and SS. Muller –Hilton agar was found to be the best medium to explicate the anti-microbial activity. It was reported that the methanolic extracts of *R. rhomboidea*-root and *R.tomentosa*-leaf/stem showed different levels of anti-microbial activities against almost all microorganisms tested (Lin et al., 1999). The methanolic extract of *R.digitata*-root mainly inhibited Gram +ve micro-organisms and Gram –ve micro-organisms such as *Pseudomonas* and *A.faecalis*. Most of the extracts showed poor inhibitory activity towards *Salmonella* and *Shigella* and none inhibited *E.coli*. It was reported that the extracts inhibited the Gram +ve microorganisms better than Gram –ve(Lin et al., 1999). From the previous study it was reported that the plant extracts are more active against the Gram +ve than Gram –ve bacteria (Rabe and van Staden et al., 1997; Vlietinck et al., 1995).



The anti-fungal activity was tested using *Candida albicans* and *S.cerevisiae*. The MeOH extract of *R.digitata*-leaf and *R.rhomboidea* exhibited the highest activity against *C.albicans*. It was reported that no significant inhibitory activity of the same extracts were observed against *S.cerevisiae*. However, all the plant extracts that demonstrated good anti-inflammatory activities were reported in the literature to also show better inhibitory activity against *Candida albicans*. The factors that seemed to affect the anti-microbial activity of the plant extracts were found to be the location and or the season in which the plant was collected and this may be significant and show a dramatic difference in anti -microbial activities

if it's collected in different locations and within a 3-month- interval (Lin et al., 1999). *R-tomentosa*, *R-digitata*, *R- rhomboidea*, *R- tridentate* and *R- tridentate* subsp. *cuneifolia* were evaluated to determine their therapeutic potential as antineoplastic agents. The antiproliferative activity *in vitro* against HepG2 cells was determined. The % inhibition of proliferation was calculated from the mean absorbencies of each dilution as follows:

$$\% I = \frac{\text{mean abs. of the control} - \text{mean abs. of the sample}}{\text{Abs. of control cells}}$$

Crude plant extracts showed activities ranging from 25% to 97% inhibition of proliferation when compared with the control which showed no inhibitory activity. Higher degrees of growth inhibition were found in aqueous root extracts in comparison with the methanol extracts of the same plant parts. The results found in the literature show potential antineoplastic activity, indicating some scientific validation for traditional usage (Opuku et al., 2000).

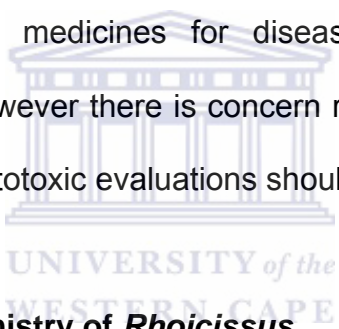
The aqueous root extract of *R. tridentate* subsp. *Cuneifolia* showed the highest antiproliferative with 96.27% inhibition of proliferation, whereas the methanol extract showed a lower antiproliferative activity of 87.01 % (Opuku et al., 2000). The crude extract of *R.tomentosa* exhibited 80.35% and 70.40% inhibition of proliferation respectively. The root extracts showed stronger inhibitory activities compared with the leaf and stem extracts with the exception of the stem extract of *R. rhomboidea*. The results from the literature suggest that *R.tridentate* subsp. *Cuneifolia* has a higher antineoplastic activity against the HepG2 cell line than the other crude plant extracts. *R digitata*, *R.rhomboidea*, *R.tridentate* and

R.tridentate subsp. *Cuneifolia*, showed potential antineoplastic activities. Since traditional healers mainly use aqueous decoctions it is possible that their preparations have been effective in some forms of cancer treatment. Resveratrol possesses antibacterial and antifungal activities and induces platelet hypo aggregation in rats; it protects liver from the lipid peroxidation and inhibits the oxidation of low density lipoproteins. And more recently its cancer preventive activity has been proven (Palomino et al., 2000).

As a natural polyphenolic substance, **resveratrol** shows a whole range of biological activities, viz., anti-oxidizing, anti-microbial features, anti-fungal activities and the ability to absorb free radicals.

Recently, **trans-resveratrol** has also been attributed anti-mutagen and chemo-protective features against cancer proliferation. It has been said to occur in red wine it decreases the risk of cardiovascular diseases when consumed in reasonable quantities (D. Buckaiova; V. Filip; I. Hanzilikova; K. Melzoch and J. Smidrkal, 2001). Grapes of *Vitis vinifera* and especially red wine represent its main source in human diet. Grape peels contain about 0.5-2.0 mg of **resveratrol/g** of dry weight and the average concentration in red wines of the world fluctuate between 1.0 and 3.0mg/l. Resveratrol determined in different parts of grapevine leaves varied from 6 to 490mg/kg of the dry material. The cluster stems have found to be the richest source of **resveratrol** (Opuku et al., 2000). The biochemical mechanism by which **resveratrol** inhibits cell proliferation was provided by studies in numerous human cell lines including

heptoblastoma HepG2 and colorectal tumor SW480 cells. The results show that **resveratrol** strongly inhibits cell proliferation at the micro molar range in a time- and dose-dependant manner. Therefore there is a need to assess how South Africa fares in the utilization of its enormous biodiversity resources, with particular reference to the antioxidant components of medicinal plants found on the continent. Hence the review aims to stimulate interest in all important research areas that will be of immense benefit to our people, who are plagued with several ailments and lack the technology found in economically advanced countries like the USA. About 40% of the SA population use alternative remedies, including herbal medicines for disease prevention and therapy (Eisenberg et al., 1998). However there is concern regarding the safety of these extracts, therefore further cytotoxic evaluations should be conducted.



3. An overview of the chemistry of *Rhoicissus*

The antineoplastic activities of some Zulu medicinal plants all belonging to the Vitaceae family have been investigated and reported to have as high as 97% inhibition of proliferation (Opuku et al., 2000). Recent reviews group cancer chemopreventive agents into blocking agents, antiproliferatives and antioxidants. The scientific literature reported that the methanolic extracts prepared separately from the roots, stems and leaves of four traditional Zulu Medicinal plants *R. tomentosa*, *R. digitata*, *R. rhomboidea* and *R. tridentata* were tested for their antioxidant activity. The extracts of *R. rhomboidea* and *R. tridentata* showed more than 50% antioxidant activity and inhibited the activities of the **1,1' diphenyl-2-**

picrylhydrazyl –NAPPH free radical, xanthine oxidase, and also prevented production of **thiobarbituric acid** reactive substances and free radical mediated DNA - deoxyribose sugar damage (Opuku et al., 2002). The extracts of *R. rhomboidea* and *R. tridentata* were reported to have a stronger chelating effect on Fe^{2+} ions than the antioxidant activity (Opuku et al., 2002). Fe^{2+} ions are known to stimulate the free radical reactions that are responsible for the decomposition of lipid peroxides into chain propagating alkoxy radicals and also reacting with H_2O_2 to produce hydroxyl radicals and other highly reactive oxygen species (Halliwell and Gutteridge, 1998).

R. tomentosa and *R. digitata* extracts possessed some prooxidative properties only at high concentrations. *R. rhomboidea* and *R. tridentata* were reported to be good free radical, especially of the peroxy type, inhibitors and have the ability to inhibit autoxidation of lipids, viz., in the treatment of liver diseases in which lipid peroxidation is an important component (Opuku et al., 2002). Plant constituents that have been reported to be free radical scavengers and antilipoperoxidants are mainly polyphenolic compounds (Constatino et al., 1992; Hamasaki et al., 1994). Polyphenolic compounds have the ability to chelate iron from ferritin (Boyer et al., 1988). However, it is known that the plant phenolics sometimes show pro-oxidative properties (Hodrick et al., 1986). Free radicals are known to cause various diseases in living tissue. The use of free radical scavengers for example Vit E in various treatment regimes is correlated with that organism having a longer life span (Opuku et al., 2002). Antioxidant activity of these Zulu

medicinal plants plays an important role in the healing of the various diseases and, given their ability to also inhibit cell proliferation, these plants emerge as the more promising species for research into substances that are effective against pathological conditions involving free radicals.(Opuku et al ., 2002).

Chhabra found *R. tridentate* leaves to contain anthocyanins, coumarins, essential oils, flavanoids, saponins, sterols and triterpenes; whereas *R. revouillii* root bark was found to contain anthocyanins, coumarins, flavanoids, quinines, sterols and tannins. (Katsoulis et al).

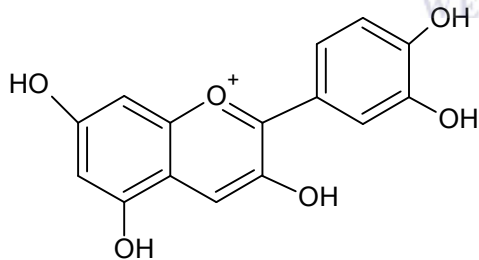
3.1 Tannins

Tannins are natural polyphenols or phenolic compound with molecular masses ranging from 500 to 3000-4000, usually classified into condensed tannins (proanthocyanidins) and hydrolysable tannins (gallo-and ellagitannins).Some tannin molecules(e.g., tea polyphenols) have anti-cancer or anti-carcinogenic or anti-mutagenic activity (Chung et al., 1998; Kaur et al., 1998). Chung et al. (1998) pointed out in their review that anti-carcinogenic or anti-mutagenic potential of tannins might be related to their anti-oxidative properties. Cactichin was described by Bruneton (1995) as a condensed form of tannin. It can be used to protect the skin layers against fluid losses and also has a vasoconstricting effect on small veins (Jansman, 1993). It kills bacteria by directly damaging the cell membrane, conforming that catechin is an antibiotic. Other studies have confirmed that catechin shows synergetic effects when combined with known antibiotics (Shimamura, 1999). Tannins have diverse effects on biological

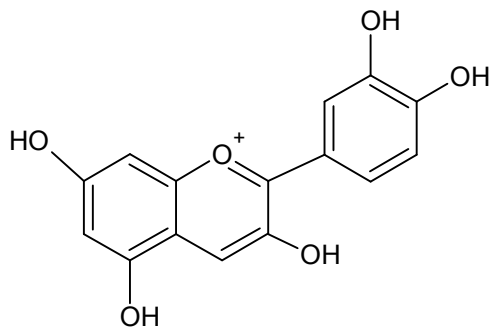
systems because they are potential metal ion chelators, protein precipitating agents and biological antioxidants (Freudenberg and Weinges, 1962; Hagerman et al., 1997). Moreover, tannins cause side effects in animals and man (Ihlenfeldt, 1994), and pharmaceutical companies do not place a high premium on tannins as potential primary health agents (Borris, 1996).

3.1.1 Anthocyanins

In other members of the family, particularly *Vitis* species (grapes), numerous phenolic compounds have been isolated and identified. Vine leaf is a traditional medicine in Europe, and it contains various polyphenols, anthocyanins and proanthocyanidins. Examples of two common anthocyanins- delphinidin and cyanidin are shown below (figure 2)



(a) Delphinidin



(b) Cyanidin

Fig 2

Anthocyanin containing drugs are traditionally used to treat the symptoms of capillary and venous fragility. Anthocyanins and polyphenols show numerous pharmacological activities and these compounds may be directly or indirectly responsible for the reported beneficial dietary value of wild grape (Hutchings et al., 1996). The traditional Zulu uses of the plant suggest analgesic effects, an aspect in need of further study (Hutchings et al., 1996).

3.2 Coumarins

Coumarins are lactones of *cis*-*o*-hydroxycinnamic acid derivatives, belonging to the phenolics with the basic skeleton of C₆ + C₃ (Xiao et al., 2000). Coumarins are present in plants in the free form and as glycosides (Y. Cai et al., 2004). Coumarins are reported in the folk medicine as traditional remedies for the treatment of respiratory tract disease (Rocha, 1945; Braga, 1976; Correa, 1984). Many pharmacological activities have been ascribed to coumarins such as anticlotting (Suttie, 1987), hypotensive (Huang et al., 1992), antimicrobial (Michaeli et al., 1970; Higgins et al., 1978) anti-inflammatory (Paya et al., 1992) and antitumor (Thornes and Sheehan, 1983; Gawron and Glowniak, 1987; Marshall et al., 1987). Research on the use of coumarin in the treatment of malignant melanoma (Gawron and Glowniak 1987) and of patients who have

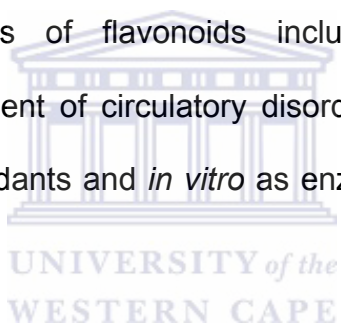
undergone mastectomy, and in the case of lymphoedema of the arms and legs has been published (Casley-Smith et al., 1993).

3.3 Flavonoids

Flavonoids are widely distributed in the plant kingdom, and have the basic skeleton of diphenylpropanes ($C_6 + C_3 + C_6$) with different oxidation levels of the central pyran ring. Flavonoids in plants are mainly present as glycosides with one or more sugar moieties linked through an O atom (O-glycosides) or through carbon-carbon bonds (C-glycosides) but some flavonoids are present as aglycones (Y. Cai et al., 2004). Flavonoids are naturally occurring substances in plants that are thought to have positive effects on human health. Studies on flavonoidic derivatives have shown a wide range of biological actions such as antibacterial, antiviral, anti-inflammatory, anticancer, and antiallergic activities (Di Carlo, Mascolo, Izzo, and Capasso, 1999; Havsteen, 1983). Flavonoids are natural products present in our diet and known to possess a number of biological activities including anti-cancer and anti-HIV infection (M. Dantuluri et al., 2005). Flavonoids were found to possess antitumor (Che et al., 1986; Patel et al., 1997) and antimicrobial properties (Ragasa et al., 2002). Flavonoids are also known to possess antibacterial properties (Hostettman et al., 1995).

Together with their biological activity, flavonoids are important components in the human diet, although they are generally considered as non-nutrients. Sources of flavonoids include foods, beverages, different herbal drugs and related phytomedicines (Aisling Aherne & O' Brien, 2002). The scientific evidence

reports that plant-based diets, in particular those rich in vegetable and fruits, protect against cancer and has been found to be strong and consistent by an expert panel (World Cancer Research Fund, 1997). A number of flavonoids have been shown to suppress carcinogenesis in various animal models (Yang, Landau, Huang, & Newmark, 2001). Flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals (Bravo, 1998) that are probably involved in several diseases. Flavonoids are common in leaves of plants and protect them against the damaging effects caused by UV-radiation and microbial infections. Pharmaceutical applications of flavonoids include the lowering of vein permeability and the treatment of circulatory disorders (Bruneton, 1995). They can also be used as antioxidants and *in vitro* as enzyme inhibitors (Smith et al., 1998).



Rutin (flavonoid) and its derivatives are combined with alkaloids for the treatment of senile cerebral defects (Vlahov, 1992). It also relieves micro trauma on tissue (Smith et al., 1998). Although rutin is relatively abundant in plants, only a small number of traditional medicines contain quantities sufficient for industrial extraction (Bruneton, 1995). Rutin can also help to improve the effectiveness of Vitamin C. This bioflavonoid can also help to strengthen veins, thus preventing bleeding (Kinghorn and Balandrin, 1993).

Neohesperidin (flavonoid) is less common than its unsaturated homologue, naringin, because most plant families accumulate their C-alkyl derivatives

(Markham, 1982) and nothing is known about its antibacterial properties. Hyperoside, quite common in a wide range of plants, contributes to the anti-inflammatory properties of these plants (Bruneton, 1995) and exhibits remarkable *in vitro* MAO inhibition. MAO is one of the two major enzymes involved in the catabolism of norepinephrine, epinephrine and dopamine within the brain. Ferulic acid is a natural protector against ultraviolet radiation known to cause skin disorders such as cancer and also to accelerate aging of the skin. The flavonoid is commonly used for the treatment of dyspepsia, as an herbal health supplement and as an herbal antioxidant (Bruneton, 1995).

3.4 Essential oils

Essential oils (EOs) used as ingredients in foods, perfumes, pharmaceuticals and natural medicine are susceptible to exert such activities. Some ingredients of essential oils have antimutagenic (anticarcinogenic) properties in bacterial and eukaryotic cells (F.Bakkali et al. 2006).

Several essential oils have been exported as fragrances, flavours and some plant extracts have potential to be developed as drugs and nutraceuticals (J. Manosroi et al., 2006). A mouth rinse prepared with the EO reduced plaque-bacteria growth in humans (Fernandes-Filho et al., 1998) and changed the clinical and histological response of the periodontium of dogs with marginal gingivitis (Girão et al., 2003).

3.5 Quinones

Quinones are also an important class of phenolics, occurring only in the medicinal herbs, but not in common vegetables and fruits. Natural quinones mainly have four classes, i.e. anthraquinones, phenanthraquinones, naphthoquinones and benzoquinones. Of these, anthraquinones are the most common in the medicinal herbs (Strack, 1997; Xiao et al., 2000).

3.6 Triterpenes and saponins

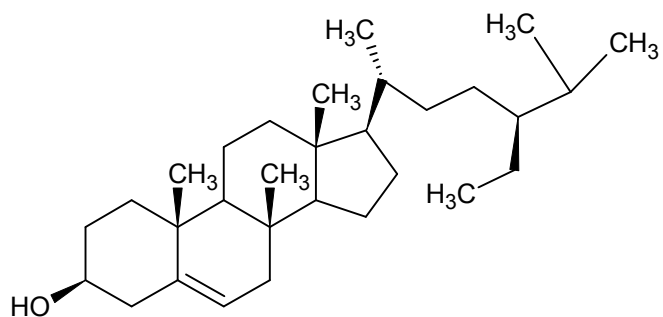
Many medicinal plants show in their chemical constitution triterpenoids which protect the stomach mucosa through the induction of gastroprotective mechanisms or acting as natural anti-oxidants (Gonzalez et al., 2001; Repetto and Llesuy, 2002; Kahraman et al., 2003). Saponins are an important class of natural products, which are structurally described as glycosylated triterpenoids or steroids. They are widely spread throughout the plant kingdom. Most saponins possess a variety of biological and pharmacological activities, such as cardiovascular, anti-fungal and hemolytic activities (M.Guo et al., 2006). Triterpenes and their glycoside saponins are large classes of secondary metabolites in plants. Triterpenes also exhibit a wide range of structural diversity and biological activity, and these glycoside saponins are of economical importance as natural medicines (Hostettmann and Marston 1995). In plants, physiological roles of triterpene saponins are the allelopathic (Waller et al., 1993), the antifungal (Papadopoulou et al., 1999) and anti-insect (Tava and Odoardi 1996) activities.

3.7 Phytosterols

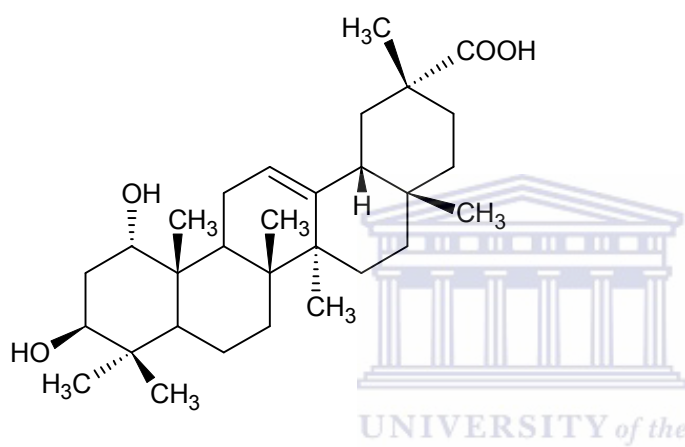
Phytosterols or plant sterols are compounds that occur naturally and bear close structural resemblance to cholesterol, but have different side chain configurations. More than 100 types of phytosterols have been reported in plant species, but the more abundant are sitosterols, stigmasterol and campesterol (Moreau et al., 2002; Berger et al., 2004; Kritchevsky and Chen, 2005). Other relevant phytosterols that can be found in plants in minor amounts are brassicasterol, Δ^5 -avenasterol, sitostanol and campestanol (Phillips et al., 2002). Sterols in plants exist in the form of free alcohols or fatty-acid esters, of steryl glycosides and of acylated steryl glycosides (Moreau et al., 2002; Phillips et al., 2002). Phytosterols play major roles in several areas, namely in pharmaceuticals (production of therapeutic steroids), nutrition (anti-cholesterol additives in functional foods, anti-cancer properties), and cosmetics, creams and lipstick (P. Fernandes, J.M.S, Cabral et al., 2007).

The preliminary findings of compounds isolated from *R. tridentate* suggest that β -Sitosterol, 24 β -ethylcholest-5-en-3 β -ol, β -sitosterol-3-O- β -D-glycoside, imberbic acid (1- α ,3- β -dihydroxyolean-12-en-29-oic acid) as well as unidentified sugars appear to account for most of the contractile activity. The structures are given below (Figure 3).

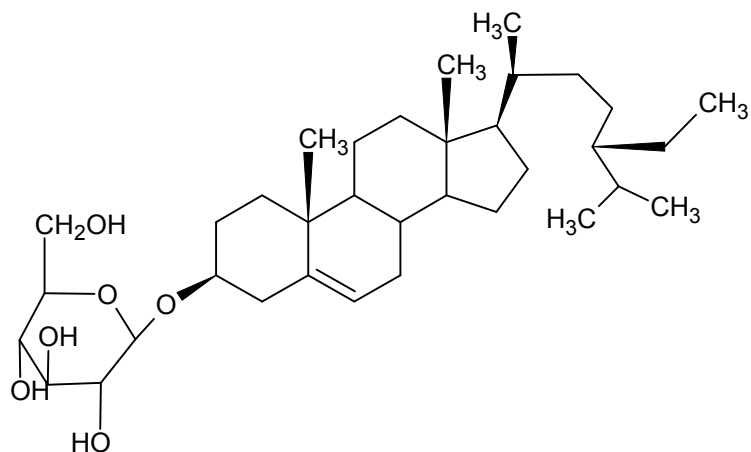
Fig. 3 Compounds isolated from *R. tridentate*



(a) 24 β -ethylcholest-5-en-3- β -ol (β -sitosterol)



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



(c) β -sitosterol-3-O- β -D-glycoside

3.8 Resveratrol

Resveratrol derived from *Vitis vinifera* which also belongs to the Vitaceae family possess anti-inflammatory and anti-mutagenous activities and is a potential cancer chemopreventive agent in humans (Jang et al., 1997). **Resveratrol** (3, 5, 4- trihydroxystilbene)

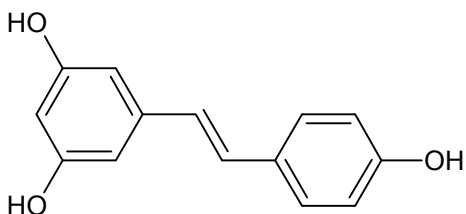


Fig. 4 *trans*- isomer **Resveratrol**

is present in only small amounts in *Vitis vinifera*, grapes and red wine and its quantity depends on the stress situation of the plant, as it occurs for all the other **phytoalexins**. For these reasons it cannot be obtained in large quantities by extractive procedures. In scientific literature **resveratrol** and **phytoalexin**

3.8.1 Structure of a phytoalexin

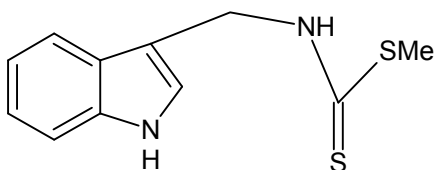
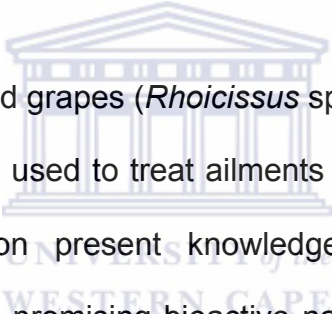


Fig. 5 Brassinin- a phytoalexin

Have been reported to have anti-cancer, anti-inflammation and anti-atherosclerotic (hardening of the arteries) antioxidant and anti-tumor activities. Many of these beneficial effects of **phytoalexin** require participation of the cells of the immune system. However, the effect of **resveratrol** on the development of immunological responses remains unknown. The natural product **resveratrol** is thought to have anti-HIV effects (A Heridia, Univ of Maryland, 2003). Unfortunately the exact dosage of **phytoalexins** in humans that achieves anti-HIV effects is unknown. **Phytoalexins** are antibiotic compounds produced by higher plants under the influence of chemical, physical or microbial stress factors.



Phytochemical studies on wild grapes (*Rhoicissus* species) of the Vitaceae family showed that they have been used to treat ailments in particular associated HIV-type 1 infection. Based on present knowledge in the literature survey, **resveratrol** appears to be a promising bioactive natural molecule with potential applications in phytotherapy, pharmacology or in nutriprotection (nutraceutical food) area. (Berlot et al., 2002; Delmas et al., 2002; Degrace et al., 2002; Jannin et al., 2002; Latruffe et al., 2002; Mali et al., 2002). **Phytoalexins** are benzo- γ -pyron derivatives which are ubiquitous in photosynthesizing cells. They have been used for centuries in folk medicine to treat human diseases such as inflammation, allergy, headache, parodontosis, viral and fungal infection, stomach or duodenal ulcers and even cancer (Adrian et al., 2000). **Resveratrol** has been proven to be remarkably efficient in preventing skin tumours forming

mice and in inhibiting the replication in vitro of human leukemia cells (Jang et al., 1997).

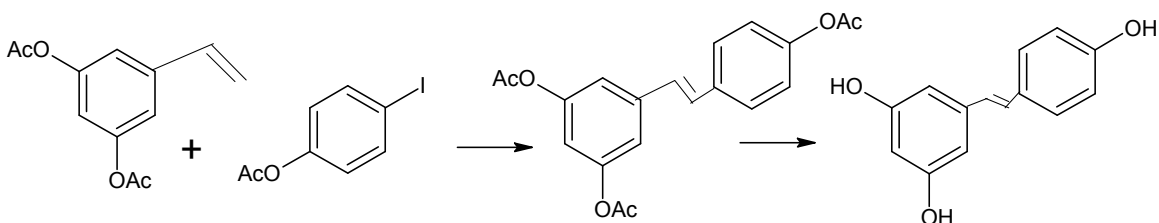
3. 8.2 Synthesis of Resveratrol

From the literature a great interest has arisen concerning **resveratrol** because of its biological properties such as heart protecting activity, platelet antiaggregation capability, and herpes simplex virus inhibition. Synthesis of resveratrol was carried out generally by using Wittig chemistry to form the ethylenic bridge. In the 1985 Moreno-Manas et al. achieved a synthesis of **resveratrol** by reacting *p*-hydroxy-benzaldehyde, previously protected as 4-trimethyl 1 silyloxy derivative, with the phosphonium ylid prepared from orcinol (Moreno-Manas et al., 1985)

The authors, by this way, obtained only the (*E*)-3, 4', 5-trihydroxy-stilbene, however there was a low yield of (10%). In 1997 Orsini et al. carried out a new synthesis by using the Wittig reaction between the 3, 5-bis-(tert-butyl)dimethylsilyloxy benzaldehyde and the phosphonium ylid obtained from (4-methoxybenzyl)-triphenyl-phosphonium chloride. The product of this reaction was a *Z/E* mixture, (ratio 2.3:1), of 3, 4', 5-trihydroxy-stilbenes (Orsini et al., 1997). In the same year Alonso et al. synthesized **resveratrol** by lithiation/condensation and silyl derivatives of 3, 5-dimethoxy-benzyl alcohol and the subsequent dehydration of the obtained product, gave only the *E* isomer. However the was low yield of 21 % (Alonso et al., 1997). In 1999 Wang et al. reported another synthesis in which 3, 5-dimethoxybenzaldehyde reacted with

the triethylphosphate, prepared from 4-methoxybenzyl bromide to give after deprotection and purification, a 45% yield of **resveratrol** (Wang et al., 1999).

From the literature a new synthetic strategy was reported which consists of coupling via the Heck reaction of a suitable styrene derivative with the *p*-iodo derivative shown in Scheme 1.



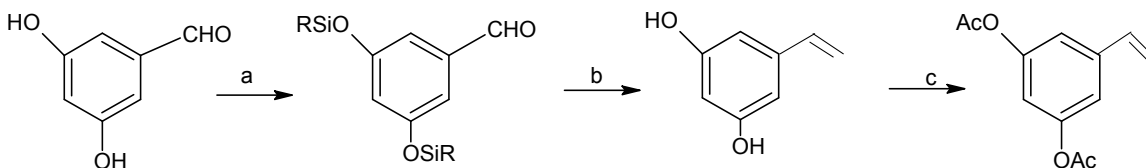
Scheme 1

The acetoxy derivative was utilized because it was well known that the Heck reaction gave better yields if phenolic functions are protected. Subsequently, compound was deacetylated to obtain **resveratrol** 1 as indicated in scheme 1 (M. Guiso et al. 2002).

The 3,5-diacetoxy-styrene (Scheme 2) was obtained by acetylation of the 3,5-dihydroxy-styrene which in turn was obtained via Wittig reaction between 3,5-dihydroxybenzaldehyde, previously protected as the di-tert-butyldimethylsilyl-ether and methylene triphenylphosphorane solution, prepared by suspending NaH in anhydrous DMSO and reacting this with the corresponding phosphonium salt as reported by Greenwald *et al* in Scheme 2 (Greenwald *et al.*, 1963).

The Heck reaction was carried out using 1 mol% of Pd (OAc)₂, based upon the aryl halide, as catalyst, PPh₃ as a ligand (P/Pd 3.5 mol), Et₃N as base and

acetonitrile as solvent. The reaction was carried out for about 17hrs and the fully acetylated **resveratrol** was obtained (70% yield) besides small amounts of partially deacetylated compounds (M. Guiso et al. 2002).



Scheme 2

(a) *N, N, N*-Diisopropyl-ethylamine, tert butyldimethylsilyl-chloride, anhydrous DMF; (b) $\text{Ph}_3\text{P}=\text{CH}_2$, anhydrous DMSO; (c) Ac_2O /Py.

4. Aims of thesis

The purpose of this study was to investigate the phytochemistry of the plant extract of *Rhoicissus tomentosa* by means of extraction, chromatographic separations and if possible, structural elucidation of isolated active compounds and, hopefully to and with the aid of GC-MS. It was further hoped to identify some of the components in the extracts with a reasonable degree of accuracy.

CHAPTER 2



Material and Methods

2.1 Plant material

Plant material was collected in April 2006, and verified as to its authenticity by Mr F Weitz at Botany Department at the University of Western Cape, South Africa. The collected plant material was cut into smaller pieces and leaves were separated from vine stems and dried at 40 °C in an oven for a week.

2.2 Preparation of extracts

The dried material was crushed into small pieces and loaded into a Soxhlet extraction apparatus. The dried leaves weighed 394g.



2.3 Solvent extraction

Solvents used were analytical grade and distilled prior to use.

Leaves were firstly extracted with hexane to remove the oily organic compounds followed by ethyl acetate extraction. Both extracts were evaporated to an oily residue in a Buchi Rotavapor (Labortechnik, Switzerland) at 40 °C. The crude mass of material obtained from the EtOAc extract was 88.62g and from hexane; 32.69g.

General experimental methods

The experimental methods used include: Hot extraction, TLC, CC, PLC, NMR Spectroscopy, HPLC and GC- MS.

2.4 Chromatography

Thin layer chromatography (TLC) was performed on aluminum plates coated with Merck Kieselgel 60 F₂₅₄ and fine silica gel 75-230 mesh was used for column chromatography. TLC and column chromatography employed EtOAc/Hexane (20% solution). Detection was carried out by spraying the plates with different sprays viz., vanillin (10% w/v in EtOH) from a 25:1 mixture v/v vanillin solution: concentrated H₂SO₄ which was prepared on the day of the experiment as it deteriorated relatively fast once acid was added and anisaldehyde/sulphuric acid: acetic acid (0.5:1:50). In both cases sulphuric acid was added slowly with stirring. The developed TLC plates had the components identified by UV light at 264 and 366nm wavelengths. TLC plates after spraying were then heated. Gravity column chromatography with the gradient elution was employed for the separation of various fractions. The column was run slowly overnight and the fractions collected where then identified by using TLC and those fractions that showed similar profiles were combined and rechromatographed. The eluents used were 10%, 20%, 30%, 40%, 50% and 60% EtOAc: hexane respectively, but 20% EtOAc/Hexane was found to be the preferred solvent system to effect separations.

2.6 Phytochemical analysis

Phytochemical analyses were carried out according to the methods of Latté (1999). The plant extracts were screened for the following compounds: coumarins, flavonoids, hydrolysable tannins, condensed tannins, alkaloids, terpenoides, phytosterols, essential oils, saponins and aromatic acids.



CHAPTER 3

Results and Discussion



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3. RESULTS AND DISCUSSION

Phytochemical analyses of *R- tomentosa* were performed according to the methods mentioned and results obtained are as follows:

Table 2. Phytochemical analysis of *R- tomentosa*

| Plant | Coum. | Flav. | H.Tan. | C.Tan. | Alk. | P.sterols | A.A. | EO. | Res. | Sap. | Terp. |
|---------------------|-------|-------|--------|--------|------|-----------|------|-----|------|------|-------|
| <i>R- tomentosa</i> | + | + | - | - | - | + | - | + | + | + | + |

Coum-coumarins; Flav- flavonoids; H.Tan- hydrolysable tannins; C.Tan- condensed tannins; Alk- alkaloids; P.sterols- phytosterols; A.A-aromatic acids; terp. Terpenoids; Res-Resveratrol (Reference material or Standard).

+ Positive

- Negative

Rhoicissus tomentosa tested positive for coumarins, flavonoids, phytosterols, essential oils, saponins, terpenoids and resveratrol and negative for hydrolysed tannins, condensed tannins, aromatic acids and alkaloids. One of the fractions in the test tubes appeared to crystallize as dark slender rods after chromatography. These fractions were collected and those with similar relative (R_f values) were combined. Further purification was done by PLC and the same solvent system viz., EtOAc:Hexane (1:4) was used for this purpose. Upon re-evaluation of these further purified fractions they tested positive for coumarins, flavonoids,

phytosterols, aromatic acids, essential oils, saponins, resveratrol and negative for hydrolysed tannins, condensed tannins and alkaloids.

The ^1H NMR spectrum demonstrated among other signals, three 1-proton signals at δ 9.51, 9.37 and 8.55 and aromatic doublets of doublets centered at δ 7.98 (J 8 Hz) and an interesting olefinic pattern between δ 6.15 and 6.32 in which a J of 17.5 was identified for a *trans* olefinic bond. A lone doublet (J = 5.2 Hz) at δ 5.32 is indicative of a deshielded set of protons viz., perhaps attached to a carbon with an oxygen atom but coupled to a similar doublet within the 3-proton signals at δ 6.2. A cluster of sharp signals at δ 3.88, 3.70, 3.42 and 3.26 allows for speculation that these protons are associated as being part of a saturated ring having some deshielding ability and then finally a complex signal system δ 2.20 to 0.69 which is due to saturated CH_2 and CH_3 groups in multi-ringed systems viz., steroids, terpenes. From this we could identify one component to be resveratrol.

Since the fractions were not absolutely pure, the integrations were not considered useable and thus a pure hexane and ethyl acetate:hexane (1:4) fraction of *R tomentosa* were submitted for a GC-MS analysis and to use the library comparative systems of the mass spectrometer in order to possibly identify the components in these samples.

Identification of the constituents of the hexane extract and 20% (ethyl acetate:hexane) eluted fraction of *R- tomentosa* was performed by comparison of their mass spectra, retention times and probabilities of compounds with those

reported in the literature of natural products. The GC-MS analysis of these fractions resulted in the detection of 30 possible components listed in Tables 3 and 4

Table 3

Compounds of *R-tomentosa* identified by GC-MS from the Hexane extract

| No. | Compounds* |
|-----|--|
| 1 | Carda-16, 20(22)-dienolide |
| 2 | 4, 4-dimethylcholestan-3-one |
| 3 | Heptadecane |
| 4 | 1-heptadecene |
| 5 | 9-hexylheptadecane |
| 6 | 11-hydroxypregn-4-ene-3, 20-dione |
| 7 | Lycoxanthin |
| 8 | Methyl tetraacetylmannopyranoside |
| 9 | 3, 12-oleandione |
| 10 | 2-octadecyl-1, 3, 5-trimethylcyclohexane |
| 11 | 6, 10, 14-trimethyl-2-pentadecanone |
| 12 | 7, 8, 12-tri-O-acetylingol |

Table 4

Compounds of *R-tomentosa* identified by GC-MS from ethyl acetate:hexane(1:4)

| eluted fraction | |
|-----------------|---|
| No. | Compounds* |
| 1 | 4-acetoxymethyl-1, 3, 3-trimethylcyclohexane |
| 2 | Canthaxanthin |
| 3 | Carotene |
| 4 | Cedran, 8S, 14-diol |
| 5 | Diepicedrene-i-oxide |
| 6 | 2, 3-dehydro-7, 7-dihydro-4-oxo-alpha-ionone |
| 7 | 4-ethyl-1-methylcyclohexenol |
| 8 | Eucalyptol |
| 9 | Globulol |
| 10 | Hedycaryol |
| 11 | 4-hydroxymethyl-1, 3, 3-trimethylcyclohexene |
| 12 | Isolongifolan-8-ol |
| 13 | Lycoxanthin |
| 14 | Resveratrol |
| 15 | Rhodopin |
| 16 | Terpineol |
| 17 | 6, 10, 14-trimethyl-2-pentadecanone |
| 18 | 1, 3, 3-trimethyl-2-oxabicyclo [2, 2, 2] octan-6-ol |

As it can be seen from the tables above, it may be concluded that compounds such as **heptadecane; 1-heptadecene; 9-hexylheptadecane; 6,10,14-trimethyl-2-pentadecanone;2-octadecyl-1,3,5-trimethylcyclohexane;4-ethyl-1-methylcyclohexenol;l;4-hydroxymethyl-1,3,3-trimethylcyclohexene; lycoxanthin, 4-acetoxymethyl-1,3,3-trimethylcyclohexane** are essential oils. Essential oils are any concentrated, hydrophobic liquid containing aromatic compounds from plants, which are called herbs or aromatic plants. They are used in perfumes, cosmetics and bath products, for flavoring food and drink, and for scenting incense and household cleaning products. Various essential oils have been used medicinally at different periods in history. Medical applications proposed by those who sell medicinal oils range from skin treatments or remedies for cancer, and are often based on historical use of these oils. Such claims are now subject to regulation in most countries, and have become correspondingly more vague, to stay within these regulation.

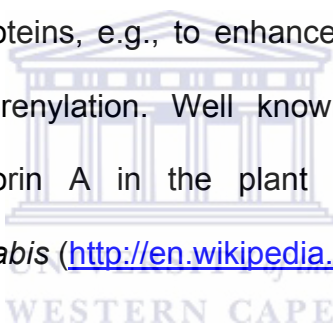
Interest in essential oils has been revived in recent years, with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by essential oils have curative effects. Oils are volatilized or diluted in carrier oil and used in massage, or burned as incense. EO's also used as aromatherapy which is a form of alternative medicine, in which healing effects are ascribed to the aromatic compounds in essential oils and other plant extracts. Many common essential oils have medicinal properties that have been applied in folk medicine since ancient times and are still widely used today.

For example, many essential oils have antiseptic properties

(http://en.wikipedia.org/wiki/Essential_oil).

Compounds such as **carda-16, 20(22)-dienolide; canthaxanthin; carotene; cedran- 8S-14-diol; diepicedrene-1oxide ,eucalyptol; globulol; hedycaryol; rhodopin and terpineol** are terpenoids.

Terpenoids are the largest group of natural products. Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Terpenoids contribute to the scent of eucalyptus, the flavors of cinnamon, cloves, ginger and the colour of yellow flowers. Sometimes terpenoids are added to proteins, e.g., to enhance their attachment to the cell membrane known as isoprenylation. Well known terpenoids include citral, menthol, camphor, Salvinorin A in the plant *Salvia divinorum*, and the cannabinoids found in *Cannabis* (<http://en.wikipedia.org/wiki/Terpenoid>).



Compounds such as **3,12-oleandione and isolongifolan-8-ol** are saponins. Saponins are used for hypercholestromia, hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory, weight loss and gentle blood cleanser. Soap nuts (*sapindus*), especially *Sapindus mukorossi*, are used medically as an expectorant, emetic, contraceptive, and for treatment of excessive salivation, epilepsy, chlorosis, and migraines. Soap nuts or *sapindus* are among the list of herbs and minerals in Ayurveda. They are popular ingredients in Ayurvedic shampoos and cleansers. They are used in Ayurvedic medicine as a treatment of eczema, psoriasis and for removing freckles. Saponins are believed to be useful

in the human diet for controlling cholesterol. The Maasai eat soup nuts laced with bitter bark and roots containing saponins. Heart disease is nearly nonexistent among the Maasai, and their cholesterol is one third lower than the average U.S citizen. It was reported that Urban Massai who do not eat the traditional soap nuts, do develop heart disease. Bile cholesterol is secreted into the intestine where much of it is later reabsorbed into the body. Saponins have the ability to bind to bile acids and cholesterol and thus prevent them from being reabsorbed. They are then removed from the heart muscle resulting in the heart pumping more efficiently. Saponins inhibit some kinds of cancer cell tumor growth in animals, particularly lung and blood cancers, without killing normal cells since cancer cells contain more cholesterol type compounds than normal cells, saponins bind to this type of cholesterol, interfering with cell growth and division. In the colon, bacteria metabolize primary bile acids, to make secondary bile acids, a highly carcinogen substance and a cause of colon cancer. Saponins bind to primary bile acids, preventing much of the secondary bile acids from forming (<http://en.wikipedia.org/wiki/Saponin>).

Compounds such as **11-hydroxypregn-4-ene-3, 20-dione** and **4,4-dimethylcholestan-3-one** were detected as phytosterol. Phytosterols are groups of steroid alcohols, phytochemicals naturally occurring in plants. They have many applications as food additives and in medicine and cosmetics. Plants contain a wide range of phytosterols. They act as a structural component in the cell membrane, a role which in mammalian cells is played by cholesterol. β -

Sitosterol can be used as a biomarker indicating the amount of terrestrially derived organic matter present in a sample. Phytosterols can be used to lower cholesterol levels, as a food ingredient or additive, and may act in cancer prevention. They occur naturally in small quantities in vegetable oils, especially sea buckthorn oil, corn oil and soybean oil. One such phytosterol complex isolated from vegetable oil is cholestatin, composed of campesterol, stigmasterol, and brassicasterol and is marketed as a dietary supplement. Sterols can reduce cholesterol in human subjects by up to 15 %

(<http://en.wikipedia.org/wiki/phytosterol>).

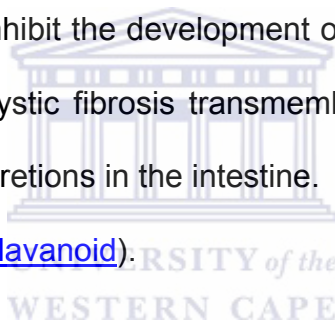
Compound **7, 8, 12-tri-O-acetylingol** was detected as a coumarin. Coumarins are toxins found in many plants notably in high concentration in tonka bean, woodruff, mullein, and bison grass. They have sweet scents, and readily recognized as the scent of newly mown hay, and have been used in perfumes since 1882. Coumarin has clinical medical value as the precursor for several anticoagulants, notably warfarin, and is used as a gain medium in some dye lasers (<http://en.wikipedia.org/wiki/coumarin>).

Compounds such as methyl **tetraacetylmannopyranoside**; **2, 3-dehydro-7, 7-dihydro-4-oxo-alpha-ionone** and **1, 3, 3-trimethyl-2-oxabicyclo [2,2,2] octan-6-ol** are flavonoids. Flavonoids are mostly commonly known for their antioxidant activity. However, it is now known that the health benefits they provide against cancer and heart disease are the results of other mechanisms. Flavonoids have been referred to as nature's biological response modifiers because of strong

experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. Flavonoids show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity.

Consumers and food manufactures have become interested in flavonoids for their medicinal properties, especially their potential role in the prevention of cancers and cardiovascular disease. The beneficial effects of fruit, vegetables and tea or even red wine have been attributed to flavanoid compounds rather than to known nutrients and vitamins. Flavonoids could also induce mechanisms that help kill cancer cells and inhibit tumor invasion. It can also help in the treatment of diarrhea; can inhibit the development of fluids that result in diarrhea by targeting the intestinal cystic fibrosis transmembrane conductance regulator which inhibits stimulated secretions in the intestine.

(<http://en.wikipedia.org/wiki/flavanoid>).



Resveratrol was also identified from the ethyl acetate: hexane fraction by GC-MS and it was used as a reference material. **Resveratrol** is a phytoalexin or polyphenolic produced naturally by several plants when under attack by bacteria or fungi, and is abundantly present in red wine.

Phytoalexins are antibacterial and antifungal chemicals produced by plants as a defense mechanism against infection by pathogens. Resveratrol has also been produced by chemical synthesis and is sold as a nutritional supplement derived primarily from Japanese knotweed. A number of beneficial health effects, such as anti-cancer, antiviral, neuroprotective, anti-aging, and anti-inflammatory effects

have been reported in-vitro(test tube) or in yeast, worms, fruit flies, fish, mice and rats. It was reported that Resveratrol found in the powdered root of *Polygonum cuspidatum*(*Polygonaceae*) is an active ingredient of Chinese and Japanese folk medicine and it has been used to cure diseases which contemporary medicine described as inflammation, allergy and hyperlipema (Baer-Dubowska and Ignatowicz, 2001).

It was reported that Resveratrol seems to increase the potency of some antiretroviral drugs against HIV *in vitro*. A cell culture study found that Resveratrol blocks the influenza virus from transporting viral proteins to the viral assembly site, hence restricting its ability to replicate. One study has theorized that it may stimulate the growth of human breast cancer cells, possibly because of its chemical structure, which is similar to a phytoestrogen. However other studies have found that it actually fights breast cancer. It was reported that Resveratrol is estrogenic and that some retailers advise that the compound may interfere the efficacy with oral contraceptives and that women who are pregnant or intending to become pregnant should not use the product, while others advise that it should not be taken by children or young adults under 18, as no studies have shown how it affects their natural development.

(<http://en.wikipedia.org/wiki/Resveratrol>).

CHAPTER 4



CONCLUSION AND RECOMMENDATION

In conclusion, identification of compounds contained in the hexane and ethyl acetate: hexane(1:4) fractions of *R-tomentosa* showed no fewer than 30 compounds documented in Tables 3 and 4. These compounds belong to the chemical classes' viz., coumarins, flavonoids, phytosterols, essential oils, saponins and terpenoids. Resveratrol which was used as reference standard was also present in the more polar fractions.

Phytochemical screening of *R-tomentosa* was positive for coumarins, flavonoids, phytosterols, essential oils, saponins, terpenoids and resveratrol and negative for hydrolyzed tannins, condensed tannins, aromatic acids and alkaloids which supported the findings of the GC-MS analysis.

Since there is a lack of knowledge about alternative therapies and herbal medications, it was important to assess the phytochemistry and possibly identify compounds in *Rhoicissus tomentosa*. There are no literature reports on the phytochemistry of *R-tomentosa* and thus this work is the first of its kind, which can be now be documented.

The results serve to meet the aims of this study and the scientific information that is now documented can be used to enhance the overall knowledge of *Rhoicissus tomentosa* and the role it plays when ingested by patients and to allow healthcare professionals to improve their impact on human health.

This preliminary work identified a large number of chemical compounds in *R-tomentosa* and further studies need to be done on structure elucidation of isolated active compounds for biological evaluations.



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