Validating the Use of *Solanum aculeastrum* For the Treatment of Cancer in the Eastern Cape, South Africa

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DEDICATION

My Guru "Raja Rao"
AND
Friends

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General Abstract

GENERAL ABSTRACT

Cancer was one of the most common and dreaded diseases of the 20th century. Today, the disease is still spreading with increasing incidence. Over 10 million new cases of cancer (excluding non-melanoma skin cancer) were reported in the year 2000 alone. A greater emphasis has recently been placed on research for complementary and alternative medicine that deals with cancer management. Several studies have been conducted on herbs under a multitude of ethnobotanical grounds. The use of medicinal plants for the treatment of cancer has become a common practice especially in the Eastern Cape Province of South Africa.

At the beginning of this programme, an ethnobotanical survey of plants used for the treatment of cancer was carried out in this Province and information on the names of plants, the parts used and the methods of preparation was collected. The study revealed that 17 plants species are commonly used for the treatment of this disease and *Solanum aculeastrum* was the most frequently used in the province.

The objective of this study was therefore, to investigate the anticancer properties of this plant. One of the studies included the investigation of its micro morphology and essential oils. The structure and distribution of foliar appendages on the leaves of *S. aculeastrum* were examined by scanning electron microscopy. Both glandular and non-glandular trichomes were observed. These differed from each other in morphology and location on the leaf. The GC-MS analyses of the volatile oils obtained by hydrodistillation of its

leaves yielded 31 compounds. The hexane fraction of the methanolic extract of the berries of the plant was also subjected to GC-MS analyses, yielding 16 compounds.

The crude extracts of the leaves and berries of the plant were investigated for antimicrobial activity against 10 bacterial and five fungal strains. The methanolic extracts of both the fruits and the leaves showed appreciable activity against Gram-positive and Gram-negative bacteria. The methanol extracts inhibited the growth of the fungi with percentage inhibition ranging from 60.26% to 100% and 56.0% to 100% on *Aspergillus flavus* and *Penicillium notatum* respectively. The acetone extracts were active against *A. flavus* and *P. notatum*. The antiproliferative activities were studied *in vitro* using three human tumour cell lines (HeLa, MCF7 and HT29). Methanolic extracts of the fruits had the highest activity with IC_{50} between 17.1 and 41.9 μg mL^{-1} while the activities of their aqueous extracts ranged between 27.9 and 48.5 μg mL^{-1}. The antioxidant activity of the crude methanol and water extracts had moderate antioxidant activity ranging from 53.1 to 65.5 μg mL^{-1}.

Tomatidine and solasodine are compounds, which were isolated from the berries of this plant. These compounds were tested for antitumour activities on HeLa, MCF7 and HT29 cancer cell lines. The IC_{50} values confirmed that tomatidine and solasodine had the greatest inhibitory effect on HeLa cells, the IC_{50} of the combined alkaloids (149.3 μM) was lower than the value for solasodine (252.5 μM) and unchanged from that of tomatidine (141.7 μM). The IC_{50} values of the two compounds combined was also lower in HT29 (169.0 μM) and MCF7 cells (126.9 μM) than for the individual compounds. The

alkaloids inhibited cell growth by blocking certain phase of the cell cycle after 24 h exposure.

S. aculeastrum exhibited low seed germination percentages under laboratory conditions, which might be due to innate dormancy. However, high temperatures of 100°C and 120°C applied to its green mature seeds for 45 to 60 min appears to break their innate dormancy, thus stimulating their subsequent germination to more than 85% while dry seeds showed very low germination and no germination was observed using smoke.

In general, the experiments and tests conducted in this study appear to have justified the use of *Solanum aculeastrum* for the treatment of cancer and make a substantial contribution to the knowledge base of the use of traditional medicine for the treatment of diseases such as cancer.

Keywords: Cancer, medicinal plants, ethnomedical, ethnobotanical, antimicrobial, anticancer, antioxidant, solasodine, tomatidine, *Solanum aculeastrum*, apoptosis.

Chapter 1

General Introduction

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GENERAL INTRODUCTION

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1.1 What is cancer?

Cancer is a class of diseases characterized by uncontrolled cell division and the ability of these cells to invade other tissues, either by direct growth into adjacent tissue (invasion) or by migration of cells to distant sites (metastasis). This unregulated growth is caused by a series of acquired or inherited mutations to DNA within cells, damaging genetic information that define the cell functions and removing normal control of cell division (Wu *et al.* 2002). These events, which may take years to materialize, cause the cell to become malignant. Once cancer cells have metastasized, they may continue growing and develop in different organs (Kenneth 2002). Cancers of the blood and blood-forming organs (bone marrow, lymphatic system, and spleen) usually do not form solid tumours, but instead circulate through other tissues where they can accumulate and form new cancers.

1.2 Cancer is a global problem

Cancer is one of the most common and dreaded diseases of the 20th century. The disease is spreading even with increasing incidence in the 21st century (Premalatha and Rajgopal 2005). Worldwide, over 10 million new cases of cancer (excluding non-melanoma skin cancer), resulting in more than six million deaths, were reported in the year 2000 (Parkin 2001; Parkin *et al.* 2001). Since 1990, there has been 22% increase in cancer incidence and mortality. While the four most common cancers are lung, breast, colorectal, and

stomach cancers, the four most deadly ones are lung, stomach, liver, and colorectal cancers (Parkin *et al.* 2001). In South Africa, incidences of cancer cases are increasing every year; breast cancer being the most common in women worldwide and the second most common cancer amongst South African women. The current statistics indicate that across all ethnic groups, one in every 31 women in this country is likely to get breast cancer (Human and Bajic 2002).

1.3 Western medicine for cancer treatment

Cancers originating in different tissues are genetically and biochemically distinct, therefore, different cancers require unique treatment patterns. Recent advances in radiation and computer technology have dramatically improved the ability to accurately detect many forms of cancers at their earliest stages of development. Early intervention is critical and may result in a cure. Once cancer has progressed, treatment approaches usually become more aggressive, combining surgical intervention with adjuvant chemotherapy, radiation therapy, and/or biologic therapy (Kenneth 2002; Kattlove and Winn 2003). Surgical resection is the most important aspect of treatment for patients with solid tumours. Surgery can also take on a preventive role for certain cancers (e.g., mastectomy for breast cancer, colectomy in colon polyp removal). Radiation therapy for cancer, often done on an outpatient basis, involves the delivery of an optimal dose of "ionizing" radiation (intense energy in the form of photons) to kill cancer cells and tumours, while trying to minimize damage to normal tissues (Kenneth 2002). Chemotherapy is the use of cytotoxic drugs for the treatment of cancer. The goal of chemotherapy for cancer treatment is to attack malignant cells while minimizing the damage to normal tissues that causes the side effects

often associated with chemotherapy (Tallberg 1998). Researchers have developed novel approaches to overcome immunosuppression and boost immunogenicity in some cases with biologic and immunologic therapy, the use of natural or synthetic biologic products based on molecules expressed by cancer cells or by living organisms in response to immunogenic challenges (Tallberg 1998). Biologic therapy employs proteins such as interleukin and interferon (also known as *cytokines*, or hormone-like molecules), tumour antigens (molecules present on the surface of tumour cells that can stimulate an immune response), and targeted monoclonal antibodies directed against tumour molecules (Tallberg 1998).

1.4 Phytomedicine for cancer treatment

Since the early 1950s, the identification and development of new lead compounds for anticancer chemotherapy has been partly driven by broad plant screening programs. In 1960, the National Cancer Institute of America initiated a plant collection program in collaboration with the United States Department of Agriculture (USDA) (Perdue 1976). To date, over 35,000 plant samples representing some 12,000 to 13,000 species have been collected by the USDA, mainly from temperate regions. Over 114,000 extracts have been tested for antitumour activity, primarily in the *in vivo* L1210 and P388 mouse leukaemia systems. While many active agents belonging to a wide variety of chemical classes were isolated and characterized (Cragg *et al.* 1993), few have satisfied the stringent requirements for preclinical and clinical development. These plant extracts are tested *in vitro* for selective cytotoxicity against panels of human cancer cell lines, such as leukaemia's, breast, central nervous system, colon, lung, ovarian, prostate and renal cancers (Boyd and Paull 1995). Plant extracts showing significant activity in screen are

subjected to bioassay-guided fractionation aimed at the isolation of the pure, active agents. Of the more than 44, 000 extracts tested so far in the *in vitro* human cancer cell line screen, less than 1% have shown some level of selective cytotoxicity (Cragg *et al.* 1996). In some instances, the patterns of differential cytotoxicity have been associated with known classes of compounds such as cardenolides, cucurbitacins, lignans, and quassinoids, but others appear to be new leads which are being investigated further (Cragg *et al.* 1996). Thus, despite over 30 years of extensive research into the synthesis and tissue culture production of the commercial anticancer drugs, vinblastine and vincristine, isolated from the source plant, *Catharanthus roseus*, grown in various regions of the world, remains the most economically viable method of large-scale production (Johnson *et al* 1963; Farnsworth 1982).

Several studies have been conducted on herbs under a multitude of ethno botanical grounds. For example, Hartwell (1969a; 1969b; 1969c; 1970a; 1970b; 1971a; 1971b; 1971c; 1971d) has collected data on about 3000 plants, some of which possess anticancer properties and have subsequently been used as potent anticancer drugs (Pandey 2002). Ayurveda, a traditional Indian medicine of plant drugs, has been successful from very early times in preventing or suppressing various tumours. (Premalatha and Rajgopal 2005). Drug discovery from medicinal plants has played an important role in the treatment of cancer. Indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last 50 years have been applied towards combating cancer (Newman *et al.* 2000, 2003; Butler 2004). Of all available anticancer drugs between 1940 and 2002, 40% were natural products or natural product-derived

with another 8% considered natural product mimics (Newman *et al.* 2003). In South Africa, many people still use phytomedicines as an alternative or to supplement modern western drugs (Van Wyk *et al.* 1997). This is not surprising considering South Africa's cultural diversity as well as its large floral biodiversity. The country is home to over 30,000 species of higher plants and 3000 of these species have been found to be used in traditional medicine across the country (Van Wyk *et al.* 1997). There are over 27 million users of indigenous medicine (Mander 1998) and an estimated 200,000 indigenous traditional healers (Van Wyk *et al.* 1997). However, little information is available on the treatment of cancer using plants in this country.

1.5 The choice of *Solanum aculeastrum* for this study

At the beginning of this study, an ethnobotanical survey of the plants used by herbalists, traditional healers and rural dwellers in the Eastern Cape for the treatment of various cancers was carried out. The results of the survey showed a total of 17 plants that are used in this area (Koduru et al., 2006c, 2006d). During the survey, *Solanum aculeastrum* Dunal was the most frequently mentioned plant by the members of the community, hence the species was chosen for further studies.

Solanum aculeastrum belongs to the family Solanaceae. It is commonly known as *mtuma* or goat bitter-apple. It is a multi-branched shrub or small tree which is 1-5 m high, usually heavily armed with large, sharp compressed prickles up to 15 mm long (Fig 1). In southern Africa, it is widely distributed and grows in areas with high rainfall of more than 700 mm per year and at altitudes from 275 to 1 780 m (Koduru *et al.* 2006a). It has been

recorded from gentle to steep slopes, on various soil types. Local healers use the extremely bitter berries and leaves for the treatment of various diseases in humans and domestic animals (Hutchings *et al.* 1996). The fresh and boiled ripe berries are used as a cure for jigger wounds and gonorrhoea respectively (Agnew and Agnew 1994). Prior to this study, Prof. A.J. Afolayan had interacted with a traditional healer in Umtata, (Eastern Cape) who claimed to have treated several cancer patients using the boiled and well macerated ripe berries of *S. aculeastrum*. Two of the patients actually confessed to have been cured using the herbal extract.

Figure A & B: *Solanum aculeastrum*

1.6 The aims and objectives of this study

1.6.1 Ethnobotanical information of plants used for the treatment of cancer

For many years traditional healers and herbalists in the Eastern Cape of South Africa have treated cancer patients using various medicinal plant species. Despite the long history of this practice in the province, the knowledge and experience of these herbalists have not been scientifically documented. According to Grierson and Afolayan (1999), information on traditional herbal practice in the province is passed from one generation to the other through oral tradition. Considering the rapid rate of deforestation and loss of biodiversity, there is a need for accurate scientific documentation of the knowledge and experience of these herbalists. One of the objectives of this study was to document information gathered from traditional healers and elder rural dwellers on the plants used in the province for the treatment of cancer.

1.6.2 Foliar micro-morphology

Trichomes are commonly found on the surfaces of leaves and some other plant organs. Scientific interest in plant trichomes is based on their functional importance and on the economic usefulness of some trichome-produced products (Valkama *et al.* 2003). Histochemical studies have indicated that the secretions from most trichomes contain terpenoids (essential oils) and flavonoid aglycones (Afolayan and Meyer 1995; Ascensao *et al.* 1999). Terpenes are reported to have anti-tumour activity (Aquino *et al.* 1990). The Solanaceae family includes a large number of species which are rich in alkaloids of medicinal value; some of these plants have great economic importance (Maiti *et al.* 2002). Trichome-produced compounds which showed anti-tumour activity have been

isolated from some members of Solanaceae (Guo and Wagner 1995). No information is available on the morphology and ultrastructure of the leaf appendages of *S. aculeastrum*. Therefore another objective of this study was to investigate the structure and distribution of different trichome types observed on the leaves of this plant, with a view to relating this to its anticancer property.

1.6.3 Isolation of volatile compounds

Plant species in the genus *Solanum* are known to be rich in steroidal glycoalkaloids and sesquiterpenoids (Cipollini and Levey 1997; Nagaoka *et al.* 2001; Shamim *et al.* 2004). Till now, there is no report on the type and structure of the volatile compounds from the leaves or berries of this plant. Yet, some of the pharmacological properties of *S. aculeastrum* may be due to its volatile components. One of the objectives of this study was to present the list of volatile compounds extracted and characterized from the leaves and berries of *S. aculeastrum*. Such information may be useful in the understanding of its anticancer property.

1.6.4 Antibacterial and antifungal activities of the crude extracts

Natural products from microorganisms have been the primary source of antibiotics, but with the increasing acceptance of herbal medicine as an alternative form of healthcare, the screening of medicinal plants for active compounds has become very important and may serve as promising sources of novel antibiotic prototypes (Meurer-Grimes *et al.* 1996; Rabe and Van Staden 1997). The antimycotic activity of *S. aculeastrum* has not been reported in the literature; yet, the members of the genus *Solanum* are known to be

rich in steroidal glycoalkaloids and sesquiterpenoids which have antibacterial, antimycotic and anticancer properties (Cipollini and Levey 1997; Nagaoka *et al.* 2001; Shamim *et al.* 2004). Some chronic inflammations such as gastritis and ulceration of the stomach or duodenum (peptic ulcer) which are caused by common microbes have been usually linked to a variety of cancer types in humans (Parsonnet *et al.* 1991, Conrad *et al.* 1995; American Cancer Society 2005). Another objective of this study was to investigate the antimicrobial activity of *S. aculeastrum* by preliminary bioassay screening of its extracts against 10 bacterial and five fungal strains. The selected bacterial strains consisted five Gram-positive; *Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus kristinae* and *Streptococcus pyogenes* and five Gram-negative; *Escherichia coli*, *Salmonella pooni*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* while the fungal species were *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum*, *Fusarium oxysporum* and *Candida albicans*.

1.6.5 Antitumour activity of crude extracts from S. aculeastrum

Over one million people are diagnosed annually with breast cancer which is one of the primary causes of deaths among women globally (Ferlay *et al.* 2001). The rates of increase of cancer incidence and lack of anticancer drugs have compelled scientists to embark on pharmacological and chemical investigations of medicinal plants for anticancer agents. Many results of the screening of plant extracts for antiproliferative activity have shown that higher plants are a potential source of antioncogenic agents which can compete favourably with chemotherapy and hormonal treatments (Pezzuto 1997; Wu *et al.* 2002). During the preliminary study of this plant, information collected

from the indigenous people of the Eastern Cape, revealed that the plant is used for the treatment of breast cancer (Koduru *et al.* 2006a, 2006b, 2006c, 2006d). However, there was no such report in the literature. Another important objective of this study was therefore, to investigate the *in vitro* cytotoxic properties of the crude extracts of the leaves and berries of this plant against three cancer cell lines viz. HeLa, HT29 and MCF7. This work was done in collaboration with the Department of Biochemistry and Microbiology of the Nelson Mandela Metropolitan University.

1.6.6 Antioxidant activity of crude extracts

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Free radical damage of cells or organs usually lead to cancer in some cases. Considerable laboratory evidence from chemical, cell culture, and animal studies indicates that antioxidants may slow or possibly prevent the development of cancer (Blot *et al.* 1993). Many plants in the Solanaceae family accumulate steroidal alkaloids (Tan *et al.* 2005). These compounds are nitrogen analogues of saponins. They are usually present as glycosides and are known to possess a variety of biological properties, including antifungal (Kusano *et al.* 1987), molluscicidal (Alzerreca and Hart 1982; Wanyonyi *et al.* 2003), teratogenic, embryotoxic (Friedman *et al.* 1992), and haemolytic (Dewick 1998) properties. They also exhibit strong antioxidant properties (Badami *et al.* 2005). Again, prior to this study, the antioxidant property of *S. aculeastrum* has not been reported in literature. In the present investigation, extracts of berries of this plant were screened for *in vitro* antioxidant activity. This work was done in

collaboration with the Programme for Phytomedicine, Department of Paraclinical Sciences, University of Pretoria.

1.6.7 Isolation, purification and identification of compounds and their anticancer activity

Considering the ethnomedical information on the anticancer property of *S. aculeastrum*, coupled with the antitumour activities of its crude extracts, it became essential to isolate and identify the active compound(s) in this plant and to examine the anticancer property of some of the pure compounds. Isolation of pure compounds from its extracts was achieved by precipitation with an alkali, and then subjected to hydrolysis. Two closely related bioactive compounds were identified using the NMR. Another objective of this study was to confirm and determine the IC_{50} of these compounds against the previously used three carcinoma cell lines. In addition, the compounds were tested for the induction of apoptosis in tumour cell lines. Again, this work was done in collaboration with the Department of Biochemistry and Microbiology of the Nelson Mandela Metropolitan University.

1.6.8 Improvement of seed germination

S. aculeastrum is increasingly being exploited in South Africa due to its widespread medicinal uses. There is, thus, an urgent need to conserve this species in the wild. Reinforcement of wild plant populations using individuals raised *ex-situ* is considered to be an important means of reducing the risk of extinction of overexploited plant populations (Bowes 1999). If the future demand for this plant is to be met, it is

imperative that this species be domesticated and commercially cultivated. Techniques for efficient low-cost cultivation practices are determined, to a large extent, by the germinability of the seeds. Requirements for seed germination in *S. aculeastrum* have not been investigated. This project was designed to evaluate the *ex-situ* requirements for optimal seed germination in *S. aculeastrum*. Specifically, the aim of this project was to investigate the effect of high temperature and smoke on the germination of its seeds under controlled environmental conditions.

1.7 The structure of the thesis

This thesis consists of chapters in the form of reprints of published articles and articles under review in various journals. The thesis is structured as follows: Ethnobotanical information of medicinal plants used for the treatment of cancer in the Eastern Cape is described in Chapter 2. Chapter 3 presents the foliar micro-morphology of *S. aculeastrum* and Chapter 4 reports on the isolation of volatile compounds from the leaves and berries. Crude extracts of the leaves and berries of this plant were tested for *in vitro* antibacterial and antifungal activity, with the results presented in Chapter 5. Chapter 6 describes the anticancer activity of the crude extracts of the leaves and berries on carcinoma cell lines, while the antioxidant activity of the berries is presented in Chapter 7. The isolation of bioactive compounds, their anticancer activities and induction of apoptosis on tumour cell lines is reported in Chapter 8. Chapter 9 describes the effects of temperature and smoke on the seed germination of *S. aculeastrum*. Finally, Chapter 10 deals with the general discussion and conclusions of the entire study, in an attempt to present a coherent picture of the results.

1.8 References

Afolayan, A.J & J.J.M. Meyer. 1995. Morphology and ultrastructure of secreting and non secreting foliar trichomes of *Helichrysum aureonitens* (Asteraceae). *Int J Plant Sci.* 156: 481-487.

Agnew, A.D.Q & S. Agnew. 1994. Upland Kenya Wild Flowers. A Flora of the Ferns and Herbaceous Flowering Plants of Upland Kenya. East Africa Natural History Society, Nairobi.

Alzerreca, A. & G. Hart. 1982. Molluscicidal steroid glycoalkaloids possessing stereoisomeric spirosolane structures. *Toxicol. Lett.* 12: 151-155.

American Cancer Society. 2005. Cancers linked to infectious disease. In: *Cancer Facts & Figures 2005.* Atlanta, Ga: American Cancer Society.

Aquino, R., De Simone, F., Vincieri, F., Pizza, C. & E. Gacs-Baltz. 1990. New polyhydroxylated triterpenes from *Uncaria tomentosa*. *J Nat Prod.* 53: 559-564.

Ascensao, L., Mota, L. & M.M. De Castro. 1999. Glandular trichomes on the leaves and flowers of *Plectranthus ornatus*: Morphology, Distribution and Histochemistry. *Annals of Botany* 84: 437-447.

Badami, S., Prakash Om, S.H. Dongre. & B. Suresh. 2005. In vitro antioxidant properties of Solanum pseudocapsicum leaf extracts. *Indian J Pharmacology* 37: 251-252.

Blot, W.J., J.Y. Li. & P.R. Taylor.1993. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst.* 85:1483-91.

Bowes, B.G. 1999. A Colour Atlas of Plant Propagation and Conservation.Manson Publishing Ltd, London.

Boyd, M.R. & K.D. Paull. 1995. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. *Drug Devel. Res.* 34: 91-109

Butler, M.S. 2004. The role of natural product chemistry in drug discovery. *J Nat Prod.* 67: 2141–2153.

Cipollini, M.L. & D.J. Levey. 1997. Antifungal activity of *Solanum* fruit glycoalkaloids: implications for frugivory and seed dispersal. *Ecology* 78: 799-809.

Conrad, M.K., J.T. Sinnott., Michael Albrink. & Pamela Sakalosky. 1995. Infections in Oncology: Gastric Cancer: An Infectious Disease? *Cancer Control Journal* 2(6).

Cragg, G.M., M.R. Boyd, J.H. Cardellina II, M.R. Grever, S.A. Schepartz, K.M. Snader. & M. Suffness. 1993. Role of plants in the National Cancer Institute Drug Discovery and Development Program. p. 80-95. In: D.A. Kinghorn and M.F. Balandrin (eds.), Human medicinal agents from plants. ACS Symposium Series 534. American Chemical Society, Washington DC.

Cragg, G.M., J.E. Simon, J.G. Jato. & K.M. Snader. 1996. Drug discovery and development at the National Cancer Institute: Potential for new pharmaceutical crops. p. 554-560. In: J. Janick (ed.), Progress in new crops. ASHS Press, Arlington, VA.

Dewick, P.M. 1998. *Medicinal Natural Products: A Biosynthetic Approach,* John Wiley & Sons Ltda: West Sussex, England.

Farnsworth, N.R. 1982. Rational approaches applicable to the search for and discovery of new drugs from plants. Pp. 27-59 in Memorias del 1er Symposium Latinoamericano y del Caribe de Farmacos Naturales, La Habana, Cuba, 21 al 28 de Junion, 1980.

Academia de Ciencias de Cuba y Comisin Nacional de Cuba ante la UNESCO, UNESCO Regional Office, Montevideo, Uruguay.

Ferlay, J., Bray, F., Pisani, P. & P.M. Parkin. 2001. *GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide. IARC Cancer Base No. 5. [1.0]* Lyon, France: IARC, 2001.

Friedman, M., J.R. Rayburn. & J.A. Bantle. 1992. Structural relationships and development toxicity of Solanum alkaloids in the frog embryo teratogenesis assay-Xenopus. *J. Agric. Food Chem.* 40: 1617-1624.

Grierson, D.S. & A.J. Afolayan.1999. An ethnobotanical study of plants used for the treatment of wounds in the Eastern Cape, South Africa. *J Ethnopharmacol.* 67: 327-32.

Guo, Z. & G.J. Wagner. 1995. Biosynthesis of cembratrienols in cell-free extracts from trichomes of *Nicotiana tabacum*. *Plant Science* 110: 1-10.

Hartwell, J.L. 1969a. Plants used against cancer. A survey. *Lloydia* 32: 247–296.

Hartwell, J.L. 1969b. Plants used against cancer. A survey. *Lloydia* 32: 153–205.

Hartwell, J.L. 1969c. Plants used against cancer. A survey. *Lloydia* 32: 78–107.

Hartwell, J.L. 1970a. Plants used against cancer. A survey. *Lloydia* 33: 288–392.

Hartwell, J.L. 1970b. Plants used against cancer. A survey. *Lloydia* 33: 97–194.

Hartwell, J.L. 1971a. Plants used against cancer. A survey. *Lloydia* 34: 386–425.

Hartwell, J.L. 1971b. Plants used against cancer. A survey. *Lloydia* 34: 204–255.

Hartwell, J.L. 1971c. Plants used against cancer. A survey. *Lloydia* 34: 310–361.

Hartwell, J.L. 1971d. Plants used against cancer. A survey. *Lloydia* 34: 103–150.

Human, S. & V.B. Bajic. 2002. Contribution to Skin Cancer Prevention in South Africa: Modelling the UV Index Utilizing Imprecise Data. *Austrian Journal of Statistics* 31(2,3):169-175.

Hutchings, A., Scott, A.H., Lewis, G. & A.B. Cunningham. 1996. *Zulu medicinal plants, an inventory.* University of Natal Press, Pietermaritzburg.

Johnson, I.S., Armstrong, J.G., Gorman, M. & J.P. Burnett Jr. 1963. The *Vinca* alkaloids: a new class of oncolytic agents. *Cancer Res.* 23:1390-1397.

Kattlove, H. & R.J. Winn. 2003. Ongoing care of patients after primary treatment for their cancer. *CA Cancer J Clin.* 53:172-196.

Kenneth A. C. 2002. Acupuncture and the cancer patient. *Medical Acupuncture Online Journal* 14(1):23-32.

Koduru Srinivas., D.S. Grierson. & A.J. Afolayan. 2006a. Effect of high temperatures on seed germination of *Solanum aculeastrum*. *Asian J Plant Sciences* 5(2): 353-356.

Koduru Srinivas., D.S. Grierson., M.A. Aderogba., J.N. Eloff. & A.J. Afolayan. 2006b. Antioxidant activity of *Solanum aculeastrum* (Solanaceae) berries. *International Journal of Pharmacology* 2(2): 262-264.

Koduru Srinivas., D.S. Grierson., M. van de Venter. & A.J. Afolayan. 2006c. *In vitro* antitumour activity of *Solanum aculeastrum* berries on three carcinoma cells. *International Journal of Cancer Research* 2(4): 397-402.

Koduru Srinivas., D.S. Grierson. & A.J. Afolayan. 2006d. Antimicrobial activity of *Solanum aculeastrum*. *Pharmaceutical Biology* 44(4):283-286.

Kusano, G., A. Takahashi., K. Sugiyama. & S. Nozol. 1987. Antifungal properties of solanum alkaloids. *Chem. Pharm. Bull.* 35: 4862-4867.

Maiti, R.K., L.R. Villarreal., A.V. Trevino. & M.C. Vallades-Cerda. 2002. Some aspects on pharmacognosy of ten species of the family solanaceae utilized in traditional medicine. *Caldasia* 24: 317-321.

Mander, M. 1998. Marketing of indigenous medicinal plants in South Africa, *A Case Study in Kwa-Zulu Natal*, Food and Agriculture Organization of the United Nations, Rome.

Meurer-Grimes, B., D.L. Mcbeth, B. Hallihan. & S. Delph. 1996. Antimicrobial activity in medicinal plants of the Scrophulariaceae and Acanthaceae. *Int J Pharma.* 34: 243-248.

Nagaoka, T., K. Goto., A. Watanabe., Y. Sakata. & T. Yoshihara. 2001. Sesquiterpenoids in Root Exudates of *Solanum Aethiopicum*. *Z Naturforsch* 56c: 707-713.

Newman, D.J., G.M. Cragg. & K.M. Snader. 2000. The influence of natural products upon drug discovery. *Natural Product Reports* 17(3): 215–234.

Newman, D.J., G.M. Cragg. & K.M. Snader. 2003. Natural products as sources of new drugs over the period 1981–2002. *Journal of Natural Products* 66(7): 1022–1037.

Pandey, G. 2002. Anticancer herbal drugs of India with special reference to Ayurveda. *New Delhi*: *Sri Satguru Publications*, 18–121.

Parkin D.M., F. Bray., J. Ferlay. & P. Pisani. 2001. Estimating the world cancer burden: Globocan 2000. *International Journal of Cancer* 94: 153–156.

Parkin, D.M. 2001. Global cancer statistics in the year 2000, *Lancet Oncology* 2: 533–543.

Parsonnet, J., Friedman, G.D. & D.P. Vandersteen. 1991. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med.* 325:1127-1131.

Perdue, Jr., R.E. 1976. Procurement of plant materials for antitumour screening. *Cancer Treat. Rpt*. 60:987-998

Pezzuto, J.M. 1997. Plant-derived anticancer agents. *Biochem Pharmacol*.53: 121-133.

Premalatha, B. & G. Rajgopal. 2005. Cancer-an ayurvedic perspective. *Pharmacological Research* 51:19-30.

Rabe, T. & J. Van Staden, 1997. Antibacterial activity of South African Plants used for medicinal purposes. *J Ethnopharmacol. 56*: 81-87.

Shamim, S., Ahmed, S.W. & I. Azhar. 2004. Antifungal activity of *Allium*, *Aloe*, and *Solanum* species. *Pharm Biol* 42(7): 491-498.

Tallberg, T. 1998. Biological Cancer Therapy, Its effect on inductional control, triggered by hormones and mediated by transformed mitochondria. *Journal of the Australasian College of Nutritional & Environmental Medicine* 17(1):17-24.

Tan, M.L., S.F. Suleiman, N. Najimuddin, M.R. Samian. & T.S. Tengku Muhammad. 2005. Methanolic extract of pereskia bleo (kunth) dc. (cactaceae) induces apoptosis in breast carcinoma, t47-d cell line. *J Ethnopharmacol*. 96(1-2): 287-294.

Valkama, E., Juha-Pekka, S., Julia, K. & P. Kalevi. 2003. Comparative analysis of leaf trichome structure and composition of epicuticular flavonoids in Finnish Birch Species. *Annals of Botany* 91: 643-655.

Van Wyk, B.E., B. Van Oudtshoorn. & N. Gericke. 1997. Medicinal Plants of South Africa, Briza Publications, Pretoria.

Wanyonyi, A.W., C.C. Sumesh, M. Gerald, M.N. Wilson. & K.T. Paul. 2003. Molluscicidal and antimicrobial activity of *Solanum aculeastrum*. *Fitoterapia* 74: 298-301.

Wu, J., Wu, Y. & B.B. Yang. 2002. Anticancer activity of *Hemsleya amabilis* extract. *Life Sciences* 71: 2161-70.

CHAPTER 2

ETHNOBOTANICAL INFORMATION OF PLANTS USED FOR THE TREATMENT OF CANCER IN THE EASTERN CAPE, SOUTH AFRICA

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Ethnobotanical information of medicinal plants used for the treatment of cancer by the people of the Eastern Cape Province, South Africa.

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Abstract

An ethnobotanical survey of plants used for the treatment of cancer was carried out in the Eastern Cape Province of South Africa. Information on the names of plants, the parts used and the methods of preparation was collected through questionnaire which was administered to the herbalists, traditional healers and rural dwellers. Information collected from these people has revealed 17 plant species that are used for the treatment of cancer in the province. These plants belong to13 families of which Hyacinthaceae and Hypoxidaceae are the most prominent. Roots, corms and bulbs are the commonest parts of plants used while decoctions and infusions are the main methods of preparation. *Solanum aculeastrum* was the most frequently and commonly used plant species for the treatment of cancer in the Province.

Keywords: Herbal medicine; Cancer; Medicinal plants; *Solanum aculeastrum*

Introduction

Over the past decade, herbal medicine has become a topic of global importance, making impact on both world health and international trade. Medicinal plants continue to play a central role in the healthcare systems of large proportions of the world's population[1]. This is particularly true in developing countries, where herbal medicine has a long and uninterrupted history of use. The recognition and development of the medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations[2]. The continuous usage of herbal medicine by a large proportion of the population of developing countries is largely due to the high cost of Western pharmaceuticals and health care. In addition, herbal medicines are more acceptable in these countries from their cultural and spiritual points of view[3]. The use of plants for medicinal remedies is an integral part of South African cultural life, a position that is unlikely to change to any significant degree in years to come[4]. It is estimated that 27 million South Africans use herbal medicines from more than 1020 plant and 150 animal species[5, 6, 7, 8].

Among the human diseases treated with medicinal plants is cancer which is probably the most important genetic disease. Every year, millions of people are diagnosed with cancer, majority of which has led to death. According to the American Cancer Society[9], deaths arising from cancer constitute 2-3% of the annual death record worldwide. In South Africa, cancer rates are increasing every year; breast cancer being the number one cancer in women worldwide and the second most common cancer amongst South African women. Current statistics indicate that across all ethnic groups, one in every 31 women in this country is likely to get breast cancer[10].

Many traditional healers and herbalists in the Eastern Cape of South Africa, for many years, have treated cancer patients using various medicinal plant species. Despite the long history of cancer treatment using herbal remedies in the province, the knowledge and experience of these herbalists have not been scientifically documented. According to Grierson and Afolayan[11], information on traditional herbal practice in the province is passed from one generation to the other through oral tradition. Considering the rapid rate of deforestation and loss of biodiversity, there is a need for accurate scientific documentation of the knowledge and experience of these herbalists. In this paper, we report the information gathered from traditional and elder rural dwellers on the plants used in the province for the treatment of cancer.

Methodology

The study area falls within latitudes 30°00'–34°15'S and longitudes 22°45'-30°15'E. It is bounded by the sea in the south and the drier Karoo (semi-desert vegetation) in the west. The altitude ranges from sea level in the south to approximately 2200 m in the north, and the vegetation type is thorn veld[12].

Information given in this paper was collected from herbalists, traditional healers and rural dwellers in the Province. Adopting the method of Jovel *et al.*[13], information was compiled through scientifically guided questionnaires, interviews and general conversations. Although informants were not scientifically literate but they were born in the region, where they have lived for most of their lives. The plants were initially identified by their vernacular names through consultations with the local people. Voucher specimens were prepared and deposited (Vedic Med 2005/1 –Vedic Med 2005/17) in the

Giffen Herbarium at the University of Fort Hare. Proper scientific identification of the plants and their uses in other communities were collected from the literature[14,15,16].

Results and discussion

The results of this study have revealed 17 plant species belonging to 13 families that are frequently used for the treatment of cancer by herbalist, traditional healers and people of the Eastern Cape Province of South Africa (Table 1). Out of these, members of Hypoxidaceae and Hyacinthaceae are the most commonly used plants. It was observed that some plants have more than one vernacular name. The reason for this is because the same plant is prepared in different ways in different communities to treat different ailments.

The methods of preparation vary. Decoctions and infusions are the most frequently used methods of preparation (Table 1). It was also observed that some plants were prepared using more than one method.

Roots, corms and bulbs were reported to be the most frequently used parts of the plants for the treatment of cancer, constituting about 54% of the preparations. This is followed by leaves and barks constituting 23% and 19% respectively, while the fruits, seeds and latex contribute about 4% of the herbal preparations.

This study has revealed that medicinal plants still play a very vital role in the primary health care of the people of this province. During the survey, it was observed that more than half of the total number of people questioned, regularly used medicinal plants to treat many ailments including cancer. Among the members of Solanaceae family, it was

observed that *Solanum aculeastrum* was the most commonly and frequently reported plant used for the treatment of cancer in the province. Based on this observation, work is in progress on the ethnopharmacological, phytochemical and pharmacological analysis of the plant. In conclusion, this study is important to preserve the knowledge of medicinal plants used by the people of the Eastern Cape Province of South Africa and also it is of important significance to exploit novel pharmacological agents in various treatments of diseases.

Medicinal plants used in local health traditions are gradually becoming extinct due to over utilization, population explosion and for other anthropogenic reasons. In order to reverse this trend, domestication of wild medicinal plants is of utmost importance. This would augment income of rural people and in turn help in the conservation of the species. Until the late 1980's little attention was paid in southern Africa to conservation issues relating to medicinal plant resources. Popular species which are slow-growing and slow-reproducing such as *Eucomis autumnalis* and *Scilla natalensis* (Hyacinthaceae), which are frequently used in traditional Zulu medicine are particularly threatened by over-exploitation and recognized by the healers as becoming scarce[17,18]. The main problem is the destructive harvesting of the underground parts of these plants or even the plant as a whole. The successful implementation of ways of alternative harvesting 'wild' plants sustainably is generally likely to involve agencies such as forest departments, usually working closely with local people. Encouragement of cultivation is likely to be useful, in order to take the pressure off wild stocks, thus helping to conserve genetic diversity. This could be through the development of small nurseries at each *in situ* site, so as to propagate the species and reintroduce them into nature where populations are low.

Reference

1. Akerele, O., Medicinal plants and primary health care: An agenda for action. *Fitoterapia*, 1988, 59, 355-63.
2. WHO., Regulatory Situation of Herbal Medicines. A Worldwide Review. Geneva, Switzerland, 1998. pp.1-5.
3. Cunningham, A. B., An Investigation of the Herbal Medicine Trade in Natal/KwaZulu. Investigational Report no. 29. Pietermaritzburg, Institute of Natural Resources, University of Natal, 1988.
4. Brandt, H. D., Osuch, E., Mathibe, L. and Tsipa, P., Plants associated with accidental poisoned patients presenting at Ga-Rankuwa Hospital, Pretoria. *Suid-Afrikaanse Tydskrif vir Wetenskap*, 1995, 91, 57-59.
5. Dauskardt, R. P. A., The changing geography of traditional medicine: Urban herbalism on the Witwatersrand. *Geojournal*, 1990, 22, 275-83.
6. Meyer, J. J. M., Afolayan, A. J., Taylor, M. B. and Engelbrecht, L., Inhibition of herpes simplex virus type 1 by aqueous extracts from shoots of *Helichrysum aureonitens*. *J Ethnopharmacol*, 1996, 52, 41-43.
7. Williams, V. L., The Witwatersrand Muti Trade, Veld and Flora, 1996. pp.12-14.
8. Mander, M., The Marketing of Indigenous Medicinal Plants in South Africa: A case study in Kwazulu-Natal, Institute of Natural Resources, Pietermaritzburg, 1997.
9. American Cancer Society. A biotechnology company dedicated to cancer treatment viewed on 25th January, 2006 <www.cancervax.com/info/index.htm>
10. Human, S. and V.B. Bajic, Contribution to Skin Cancer Prevention in South Africa: Modelling the UV Index Utilizing Imprecise Data. *Austrian J Statistics,* 2002, 31, 169-175.

11. Grierson, D. S. and Afolayan, A. J., An ethnobotanical study of plants used for the treatment of wounds in the Eastern Cape, South Africa. *J Ethnopharmacol*, 1999, 67, 327-32.

12. Acocks, J. P. H., Veld types of South Africa. *Memoirs of Botanical Survey of South Africa Botanical Research Institute.* Pretoria, Department of Agriculture and Water Supply, 1975. pp.57.

13. Jovel, E. M., Cabanillas, J. and Towers, G. H. N., An ethnobotanical study of the traditional medicine of the Mestize people of Suni Mirano, Loreto, Peru. *J Ethnopharmacol*, 1996, 53, 149-56.

14. Hedberg, I. and Stagard, F., Traditional Medical Plants. Ipeleng, Botswana, pp.324.

15. Roberts, M., Indigenous Healing Herbs. Southern Book Publishers, South Africa, 1990. pp.1-285.

16. Van Wyk, B. E., Van oudtshoorn, B. and Gercke, N., Medicinal plants of South Africa. Briza, Pretoria, 1997. pp.1-304.

17. Cunningham, A. B., Development of a conservation Policy on commercially exploited medicinal plants: a case study from Southern Africa. In: Akarele, O., Heywood, V., Synge, H. (Eds.), Conservation of Medicinal Plants. Cambridge University Press, 1991. pp. 337.

18. Zschocke, S., T. Rabe., J. L. S. Taylor., A. K. Jäger and J. van Staden., Plant part substitution-a way to conserve endangered medicinal plants? *J Ethnopharmacol*, 2000, 71, 281-292.

Table 1: Medicinal plants used for the treatment of cancer in the Eastern Cape, South Africa.

Family and botanical name	Local name	Part used	Preparation	Conservations status and endemicity
Hypoxidaceae				
Hypoxis hemerocallidea L.	inkomfe	Corms	Corms are pulverized, boiled in water and administered orally till the signs of relief are obvious.	nt/ne
Hypoxis argentea L.	Inongwe	Corms	Corms are stamped, boiled in water and administered orally till the patient is healed.	nt/ne
Hypoxis colchicifolia Bak	iLabatheka	Corms	Crushed corms are boiled in water and administered orally for several days or weeks.	nt/ne
Hyacinthaceae				
Merwilla plumbea (Lindl.) Speta	Inguduza	Bulbs	Decoctions are made from the bulbs, warmed gently and taken orally till the patient is cured.	nt/ne
Scilla natalensis Planch.	Blouslangkop	Bulbs	Decoctions are made from the gently warmed bulbs and taken orally till the signs of relief are obvious.	v/ne
Eucomis autumnalis (Mill.) Chitt	Umathunga	Bulbs	Decoctions of the warmed bulbs in water or milk are usually administered orally for several weeks.	v/ne
Solanaceae				
Solanum aculeastrum Dunal	Mtuma	Fruits, leaves, bark	This is the most commonly used plant in this area. The fruits are boiled until it burst into	nt/ne

		and roots	pieces. It is filtered, and the decoction administered once a day until the patient is cured.	
Alliaceae				
Tulbaghia violacea Harv.	Wild garlic, wildeknoflok	Bulbs	The fresh bulbs are boiled in water and the decoctions are taken orally for several weeks.	nt/ne
		Leaves	Crushed leaves are infused in water. It is then administered orally for several days.	
Gunneraceae				
Gunnera perpensa L.	River pumpkin, uGhobo	Rhizome	Aqueous infusions and decoctions of the gently warmed rhizomes are administered orally for three to four weeks.	nt/ne
Ranunculaceae				
Knowltonia capensis (L.) Huth	Blistering leaves, brandblare	Leaves	Crushed leaves are directly applied as poultices on the external tumours.	v/e
		Roots	Crushed leaves are boiled in water and taken orally.	
Cannabaceae				
Cannabis sativa L.	Nsangu, Umya	Leaves	Crushed leaves are administered orally every day until patient is cured.	nt/ne
Fabaceae				
Sutherlandia frutescens L. R.Br.	Umnwele	Branches, leaves,	Decoctions made from all parts of the plant are administered orally for internal cancer and	v/ne

		flowers and seeds	applied topically on external cancers.	
Pittosporaceae				
Pittosporum viridiflorum Sims	Umkhwenkwe, umVusamvu	Bark and roots	Decoctions or infusions are made with water from the stamped bark and roots and administered orally for several weeks.	nt/ne
		Bark and roots	Dried bark and roots are pulverized in to powder and taken orally with water.	
Cornaceae				
Curtisia dentata (Burm.f.) C.A.Sm.	umLahleni	Bark and leaves	Stamped and boiled in water to make a decoction. It is administered orally till signs of relief are obvious.	nt/ne
Euphorbiaceae				
Euphorbia ingens E.Mey. ex Boiss	Nkondze	Latex	Latex is applied topically on the external cancers every day until the tumour is healed.	nt/ne
Agapanthaceae				
Agapanthus africanus (L.) Hoffmanns	Mathunga, Agapanthus	Roots	Sun dried roots are powdered and infused in water and then taken orally until the patient is cured.	nt/e
Ulmaceae				
Celtis africana Burm.f.	umVumvu	Bark and roots	Sun dried bark and roots are powdered and infused in water or milk and taken orally every day till the signs of relief are obvious.	nt/ne

nt= neither rare nor threatened; v=vulnerable; e=endemic; ne=non-endemic.

Chapter 3

The foliar micro-morphology of *Solanum aculeastrum*, a medicinal plant of South Africa

CHAPTER 3

THE FOLIAR MICRO-MORPHOLOGY OF *SOLANUM ACULEASTRUM*, A MEDICINAL PLANT OF SOUTH AFRICA

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The Foliar Micro-morphology of *Solanum aculeastrum*, a Medicinal Plant of South Africa

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Abstract: *Solanum aculeastrum* is an important medicinal plant which is used for the treatment of several diseases including cancer in South Africa. The structure and distribution of foliar appendages on the leaves of this plant were examined by scanning electron microscope. Both glandular and non-glandular trichomes were observed, which differed from each other in morphology and location on the leaf. While short-stalked (SST) glandular trichomes were abundant on the adaxial leaf surface, single, multicellular and pointed stellate trichomes (ST) having 13-15 arms, were abundant on the abaxial surface of the leaf, together with long-stalked glandular trichomes (LST). We hypothesize that the bioactive therapeutic compounds secreted by *S. aculeastrum* are produced in these glandular trichomes.

Key words: *Solanum aculeastrum*, stellate trichomes, glandular hair

INTRODUCTION

Solanum aculeastrum (subsp. *aculeastrum*) Dunal, occurs from tropical Africa down to South Africa. It is a multi-branched shrub, 1-5 m high, heavily armed with large prickles and is wide-spread in South Africa. In southern Africa, it grows in areas with high rainfall of more than 700 mm per year and at altitudes from 275 to 1,780 m (Koduru *et al*., 2006). It has been recorded from gentle to steep slopes, on various soil types such as sandy soils, reddish brown clay-loam and brown sandy loam. *S. aculeastrum* has high medicinal value. Its berries and leaves are sometimes used as soap substitute; apparently because of its high saponin content. Local healers use the extremely bitter berries and leaves for the treatment of various diseases in humans and domestic animals. Both mature and immature berries contain the poisonous alkaloid, α-solanine (Hutchings *et al*., 1996). Other bioactive compounds that have been isolated from this plant include solaculine A (Wanyonyi *et al*., 2002) from the root bark and solamargine, beta-solamarine, solasonine and solasodine from the fruits (Watt and Breyer-Brandwijk, 1962; Drewes and Van Staden, 1995; Wanyonyi *et al*., 2002). The fresh and boiled ripe berries and leaves are used as a cure for jigger wounds and gonorrhoea, respectively (Agnew and Agnew, 1994). Moderate antioxidant activity of *Solanum aculeastrum* using crude extracts of berries have been previously reported (Koduru *et al*., 2006). Recent discussion with traditional healers of the Eastern Cape Province in South Africa revealed that the plant is used for the treatment of cancer, particularly breast cancer (Koduru *et al*., 2006).

Trichomes are commonly found on the surfaces of leaves and some other plant organs. Scientific interest in plant trichomes is based on their functional importance and on the economic usefulness of some trichome-produced products (Valkama *et al*., 2003). Histochemical studies indicated that the secretions from most trichomes contain terpernoids (essential oils) and flavonoid aglycones (Afolayan and Meyer, 1995; Ascensao *et al*., 1999). Terpenes are reported to have anti-tumour activity (Aquino *et al*., 1990; Atočka, 2003). The Solanaceae family includes a large number of species which are rich in alkaloids of medicinal value; some of these plants have great economic importance (Maiti *et al*., 2002). Trichome-produced compounds which showed anti-tumour activity have been isolated from some members of Solanaceae (Guo and Wagner, 1995).

No information is available on the morphology and ultrastructure of the leaf appendages of *S. aculeastrum*. We hypothesize that the bioactive therapeutic compounds of this plant are produced in the leaf trichomes. The objective of this study therefore was to investigate the structure and distribution of different trichome types observed on the leaves of *S. aculeastrum*, which could be the site of production of reported compounds that have been found to be biologically active.

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MATERIALS AND METHODS

Plant material: The leaves of *S. aculeastrum* were collected from plants naturally occurring in the wild at Kayalethu village in the Eastern Cape Province of South Africa (latitudes 30°00'- 34°15'S and longitudes 22°45'-30°15'E). The plant was identified at the Department of Botany, University of Fort Hare and a voucher specimen (Vedic Med 2005/16) was prepared and deposited in the Griffen Herbarium of the University.

Scanning electron microscopy: Fresh leaf pieces (10 × 10 mm^2) from *S. aculeastrum* were immersed in a fixative solution of 2.5% glutaraldehyde in 0.1 M phosphate buffer for 24 h. Samples were washed for 15-30 min with the buffer and dehydrated in graded ethanol series. Samples were then critical-point dried using CO_2, sputter coated with gold under vacuum and viewed with Hitachi (S-450) scanning electron microscope operating at 10 kV. Images were captured digitally with an Image Slave computer programme for Windows.

RESULTS AND DISCUSSION

The investigation of the adaxial and abaxial surfaces of the leaves of *S. aculeastrum* showed numerous glandular and non-glandular trichomes (Fig. 1-3). This is a natural phenomenon in most angiosperms (Fahn, 1967). Representative scanning electron micrographs of leaf sections are shown in Fig. 1A-3D. Two types of glandular trichomes, short-stalked (SST), long-stalked (LST) and one type of non-glandular, stellate trichomes were identified on the leaves. However, SST were more abundant on the adaxial leaf surface (Fig. 1A-C). They consist of a basal epidermal cell and a 3-tiered stalk with a large round head (Fig. 1B and C). The LST were present

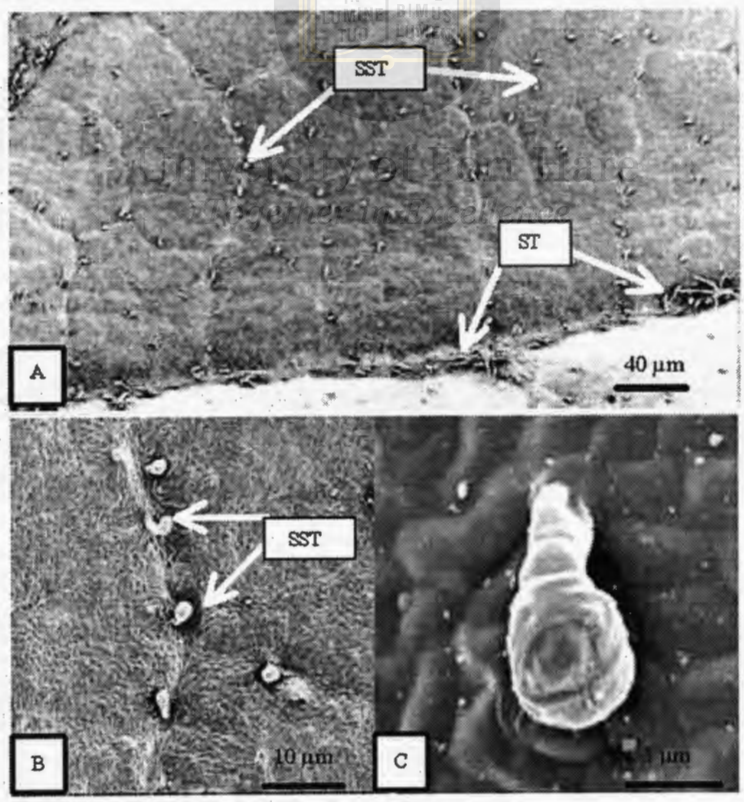

Fig. 1: SEM photographs. A: A portion of the adaxial surface of a leaflet of *S. aculeastrum* covered by glandular (SST) and non-glandular trichomes (ST). B: Glandular trichomes (SST) were distributed on the veins of the leaf. C: Glandular trichome consisting of stalk (St) bearing a secretory cell (Sc) at the tip. SST, Short-stalked trichome; SST, Non-glandular Trichome; ST, Stellate trichome; St, Stalk; Sc, Secretory cell. Scale bar in A = 40, in B = 10 and in C = 1 µm

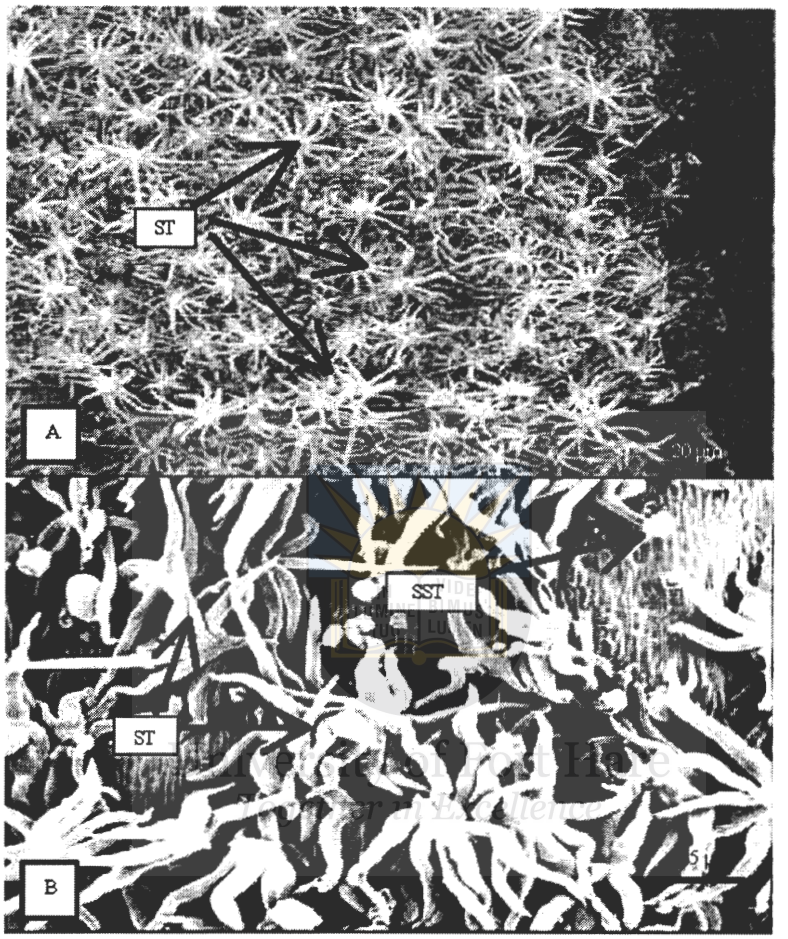

Fig. 2: SEM photographs of non-glandular trichomes on the abaxial surface of the leaf of *S. aculeastrum*. A. distribution of the Stellate Trichomes (ST). B. presence of Short-stalked Trichomes (SST) with ST. Bar in A = 20, in B = 5 μm

only on the abaxial surface of the leaf (Fig. 3A-D) along with the ST, the later were however, more abundant and densely distributed than on the upper surface of the leaves (Fig. 1A, 2A and B and 3A-D). Each ST appeared solitary but, multicellular and pointed with 13-15 arms (Fig. 2A-B).

Glandular trichomes are characterized by having 'heads' (glands) that release, on contact, sticky and/or toxic exudates that may entrap, irritate or potentially kill some pests (Simmons *et al*., 2003). These glands contain important secondary metabolites including terpenes, essential oils, flavonoids and lipophilic components (Levin, 1973; Dell and McComb, 1978; Wagner, 1991; Afolayan and Meyer, 1995; Ascensao *et al*., 1999). In most species, the source of these secondary metabolites has been attributed to the trichomes (Buta *et al*., 1993). The possession of glandular trichomes is characteristic of the genus *Solanum* and of many other members of Solanaceae, with the exception of *Nicotiana glauca* and *Solandra nitida* (Maiti *et al*., 2002). The two types of glandular trichomes identified on the leaves of *S. aculeastrum* might be responsible for the production, accumulation and release of volatile and secondary metabolites such as the saponins and steroid alkaloids reported by Drewes and Van Staden (1995). Although, micro-morphological studies alone do not provide the information required to establish sites of synthesis in cells (Afolayan and Meyer, 1995), it is plausible to assume that the therapeutic compounds in *S. aculeastrum* are produced by the glandular trichomes.

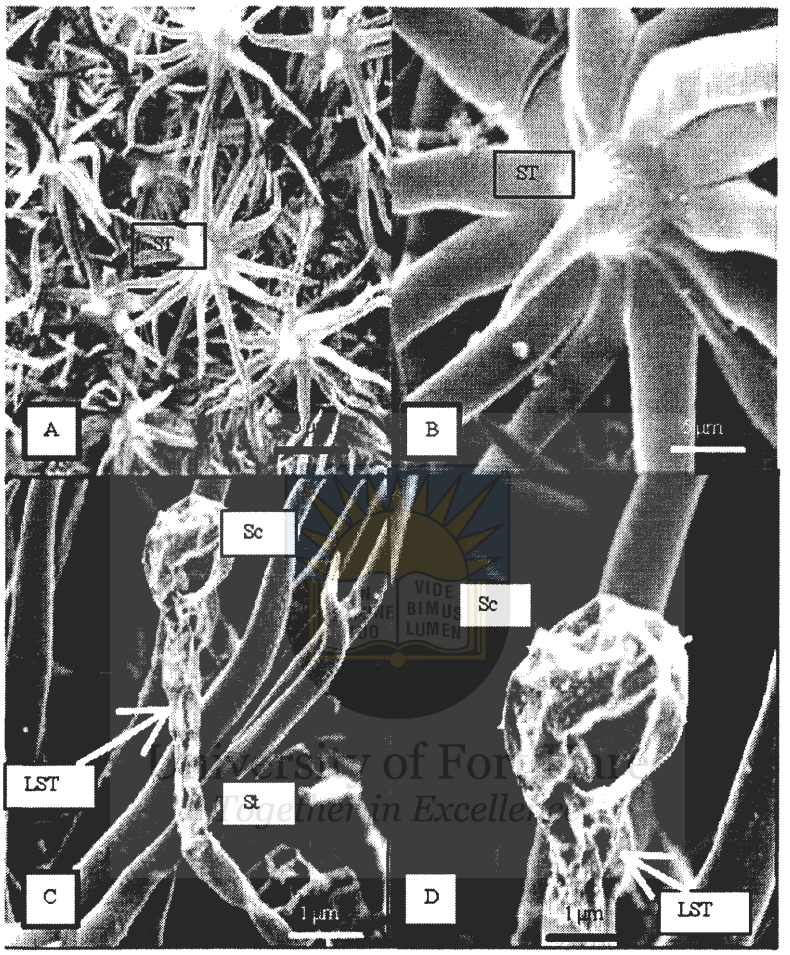

Fig. 3: SEM photographs of non-glandular trichomes on the abaxial surface of the leaf of *S. aculeastrum*. A and B. Stellate trichome distribution with arms (ST). C and D. Presence of Long-stalk Trichomes (LST) on the abaxial surface. Sc, Secretory cells. St, Stalk. Bar in A = 5, in B = 1, in C = 1 and in D = 1 μm

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REFERENCES

Afolayan, A.J. and J.J.M. Meyer, 1995. Morphology and ultrastructure of secreting and non secreting foliar trichomes of *Helichrysum aureonitens* (Asteraceae). Intl. J. Plant Sci., 156: 481-487.

Agnew, A.D.Q. and S. Agnew, 1994. Upland Kenya Wild Flowers. A Flora of the Ferns and Herbaceous Flowering Plants of Upland Kenya. East Africa Natural History Society, Nairobi.

Aquino, R., F. De Simone, F. Vincieri, C. Pizza and E. Gacs-Baltz, 1990. New polyhydrocylated triterpenes from *Uncaria tomentosa*. J. Nat. Prod, 53: 559-564.

Ascensao, L., L. Mota and M.M. De Castro, 1999. Glandular trichomes on the leaves and flowers of *Plectranthus ornatus*: Morphology, distribution and histochemistry. Ann. Bot., 84: 437-447.

Atočka, J., 2003. Biologically active pentacyclic triterpenes and their current medicine signification. J. Applied Biomed., 1: 7-12.

Buta, J.G., W.R. Lusby, J.W. Neal, R.M. Waters and G.W. Pittarelli, 1993. Sucrose esters from Nicotiana gossei active against the greenhouse whitefly Trialeuroides vaporariorum. Phytochemistry, 32: 859-864.

Dell, B. and J.A. McComb, 1978. Plant resins-their formation, secretion and possible functions. Adv. Bot. Res., 6: 276-316.

Drewes, F.E. and J. Van Staden, 1995. Aspects of the extraction and purification of solasodine from *Solanum aculeastrum* tissues. Phytochem. Anal., 6: 203-206.

Fahn, A., 1967. Plant Anatomy. Pergamon Press, Exeter, pp: 193-219.

Guo, Z. and G.J. Wagner, 1995. Biosynthesis of cembratrienols in cell-free extracts from trichomes of *Nicotiana tabacum*. Plant Sci., 110: 1-10.

Hutchings, A., A.H. Scott, G. Lewis and A.B. Cunningham, 1996. Zulu Medicinal Plants, An Inventory. University of Natal Press, Pietermaritzburg.

Koduru, S., D.S. Grierson, M.A. Aderogba, J.N. Eloff and A.J. Afolayan, 2006. Antioxidant activity of *Solanum aculeastrum* (Solanaceae) berries. Intl. J. Pharmacol., 2: 262-264.

Levin, D.A., 1973. The role of trichome in plant defense. Q. Rev. Biol., 48: 3-15.

Maiti, R.K., L.R. Villarreal, A.V. Trevino and M.C. Vallades-Cerda, 2002. Some aspects on pharmacognosy of ten species of the family solanaceae utilized in traditional medicine. Caldasia, 24: 317-321.

Simmons, A.T., G.M. Gurr, D. McGrath, H.I. Nicol and P.M. Martin, 2003. Trichomes of Lycopersicon spp. and their effect on Myzus persicae (Sulzer), Hemiptera: Aphidae. Austr. J. Entomol., 42: 373-378.

Valkama, E., S. Juha-Pekka, K. Julia and P. Kalevi, 2003. Comparative analysis of leaf trichome structure and composition of epicuticular flavonoids in Finnish Birch Species. Ann. Bot., 91: 643-655.

Wagner, G.J., 1991. Secreting Glandular Trichomes: More than Just Hairs. Plant Physiol., 96: 675-679.

Wanyonyi, A.W., C.C. Sumesh, M. Gerld, E. Udo and M.N. Wilson, 2002. Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum*. Phytochemistry, 59: 79-84.

Watt, J.M. and M.G. Breyer-Brandwijk, 1962. *Solanum nigrum* L. In: The Medicinal and Poisonous Plants of Southern and Eastern Africa. E. and S. Livingstone Ltd., Edinburgh, London, pp: 996-1000

CHAPTER 4

ISOLATION OF VOLATILE COMPOUNDS FROM *SOLANUM ACULEASTRUM* (SOLANACEAE)

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ISOLATION OF VOLATILE COMPOUNDS FROM *SOLANUM ACULEASTRUM* (SOLANACEAE)

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Isolation of Volatile Compounds from *Solanum aculeastrum* (Solanaceae)

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Abstract: The GC-MS analyses of the volatile oil obtained by hydrodistillation of the leaves of *Solanum aculeastrum* yielded 31 volatile compounds, representing 84.5% of the total oil composition. The oil consisted mainly of alkanes (17.5%), aldehydes (17%) and aromatic hydrocarbons (15.2%). The major compounds were n-nonane (12.4%) and o-phthalic acid (11.8%). The hexane fraction of the methanolic extract of the berries of the plant was also subjected to GC-MS analyses, yielding 16 compounds, which accounted for 87.1% of the total volatiles. The fraction was dominated by alkanes/alkenes with undecane (21.7%), tetradecane (10.8%) and tridecane (10.0%) being the most prominent.

Key Word Index: *Solanum aculeastrum*; hexane fraction; hydrodistillation; volatile compounds; undecane; *n*-nonane.

Introduction: Plant species in the genus *Solanum* are known to be rich in steroidal glycoalkaloids and sesquiterpenoids[1-3]. *Solanum aculeastrum* Dunal, known as goat bitter-apple, is a native of Africa and widely distributed in South Africa. It is a multi branched shrub, 1-5 m high, usually heavily armed with compressed prickles. The flowers are white, producing berries which are yellow-green in colour when ripe. It grows in the areas of high rainfall of more than 700 mm per year and at altitudes from 275 to 1780 m. The extremely bitter fruit of this species is used medicinally in various ways[4]. The fresh and boiled berries of the plant are used as a cure for jigger wounds, gonorrhea and for the treatment of acne[5,6]. Discussions with traditional healers of the Eastern Cape Province in South Africa revealed that the plant is used for the treatment of neoplasm particularly breast cancer. Phytochemical investigators of the plants have reported the presence of solaculine A, solamargine, β-solamarine, solasonine and solasodine, with molluscicidal activity[7-9].

Till now, there is no report on the type and structure of the volatile compounds from the leaves or berries of this plant. Yet, some of the pharmacological properties of *S. aculeastrum* may be due to its volatile components. This paper presents the list of volatiles extracted and characterized from the leaves and berries of *S. aculeastrum*.

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Experimental

Plant material: The berries and leaves of *S. aculeastrum* were collected from plants naturally occurring in the wild at Kayalethu village in the Eastern Cape Province of South Africa (latitudes 30°00'-34°15'S and longitudes 22°45'-30°15'E). The plant was identified at the Botany Department, University of Fort Hare and a voucher specimen (Vedic Med 2005/ 16) was prepared and deposited in the Griffen Herbarium of the University.

Preparation of extracts: A sample (300g) of fresh leaves was hydrodistilled for 3 hours using a Clevenger-type apparatus as reported earlier[10], and volatile compounds were collected in *n*-hexane. The dried, ground berries of *S. aculeastrum* were extracted by shaking in methanol at room temperature for 48 hrs. The extract was filtered and evaporated to a gummy mass in a rotary evaporator under reduced pressure at a maximum temperature of 40°C. The gummy mass was partitioned between water and *n*-hexane, and the *n*-hexane fraction subjected to column chromatography using gradient elution with solvents of increasing polarity to give several fractions. The hexane fraction eluted from 10-30% chloroform in *n*-hexane was subsequently subjected to GC-MS analyses[11].

GC-MS analysis: GC-MS analyses of the distilled compounds were performed with a Hewlett Packard 6890 Gas Chromatograph linked to Hewlett Packard 5973 mass spectrometer system equipped with a HP5-MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, Wilmington, DE, USA). The oven temperature was programmed from 70°C to 240°C at a rate of 5°C/min. The ion source was set at 240°C and ionization voltage 70 eV. Helium was used as a carrier gas. Spectra were analyzed using the Hewlett-Packard Enhanced Chem Station G1701 programme for Windows. The constituents were identified by matching their spectra with those recorded in the Wiley 275 (Wiley, New York) mass spectral library and literature[12] and their retention times and Kovats indexes.

Results and discussion: Extracts from the leaves and berries of *S. aculeastrum* contained mainly straight chain aliphatic hydrocarbons (C_{12}–C_{29}). Thirty one compounds were identified from the volatile oil obtained from the leaves of the plant (Table 1). This represented 84.5% of the total oil composition. The oil consisted of alkanes, aldehydes, aromatic hydrocarbons, terpenoids and some miscellaneous compounds, all representing 72% of the oil composition. The alkanes (17.5%) consisted predominantly of n-nonane (12.4%) and decane (3.5%). The aldehydes constituted about 17% of the total composition and consisted of nonanal (6.2%) and 9,17-Octadecadienal (4%). Aromatic hydrocarbons represented 15.2%. Terpenoids, notably δ-nerolidol and β-cyclocitral, represented 6.6%. Fatty acids, fatty acid esters, alcohols, diterpenes and ketones were also present (12.6%). O-phthalic acid (11.8%) was among the miscellaneous compounds present.

Sixteen compounds were identified in the hexane fraction of the methanolic extract of the berries of *S. aculeastrum* (Table 2). This represented 87.1% of the compounds. Undecane (21.7%), tetradecane (10.8%), tridecane (10%), and dodecane (7.9%) were the most predominant compounds. O-phthalic acid a miscellaneous compound was also present in signif-

cant quantity (14.9%). To the best of our knowledge the volatile compounds of the *S. aculeastrum* is being reported for the first time.

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References

1. **Cipollini, M.L. and Levey, D.J. (1997b).** Antifungal activity of *Solanum* fruit glycoalkaloids: implications for frugivory and seed dispersal. *Ecology,* 78: 799-809.
2. **Nagaoka, T., Got, K., Watanabe, A., Sakata, Y. and Yoshihara, T. (2001).** Sesquiterpenoids in Root Exudates of *Solanum Aethiopicum*. Z. Naturforsch. 56c: 707-713.
3. **Shamim S., Ahmed, S.W. and Azhar, I. (2004).** Antifungal activity of *Allium*, *Aloe*, and *Solanum* species, *Pharm. Biol.* 42: 491-498.
4. **Hutchings, A., Scott, A.H., Lewis, G. and Cunningham, A.B. (1996).** Zulu medicinal plants, an inventory. University of Natal Press, Pietermaritzburg.
5. **Kokwaro, J.O. (1993).** Medicinal plants of East Africa, East African Literature Bureau, Nairobi.
6. **Agnew, A.D.Q. and Agnew, S. (1994).** Upland Kenya wild flowers. A flora of the ferns and herbaceous flowering plants of upland Kenya, East Africa Natural History society, Nairobi.
7. **Drewes, F.E. and Van Staden, J. (1995).** Aspects of the extraction and purification of solasodine from *Solanum aculeastrum* tissues. *Phytochemical Analysis*, 6: 203-206.
8. **Wanyonyi, W.A., Sumesh, C.C., Gerld, M., Udo, E. and Wilson, M.N. (2002).** Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum*. Phytochemistry, 59: 79-84.
9. **Wanyonyi, W.A., Sumesh, C.C., Gerald, M., Wilson, N. and Paul, K.T. (2003).** Molluscicidal and antimicrobial activity of *Solanum aculeastrum*. Fitoterapia, 74: 298-301.
10. **Asekun, O. T., and O. Kunle. (2004).** The chemical constituents of the fruit essential oil of *Xylopia aethiopica* (Dunal) A. RICH from Nigeria. J. Essent. Oil Bearing Plants, 7: 186-188.
11. **Barbosa-Filho, J.M., Da-Cunha, E.V.L., Cornélio, M.L., Dias, C.S. and Gray, A.I. (1997).** Cissaglaberrimine, an aporphine alkaloid from Cissampelos glaberrima. *Phytochemistry,* 44: 959-961.
12. **Adams, R.P. (2001).** Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Corporation, Illinois, USA.

Table 1: Volatile compounds from the hydrodistillation of the leaves of *Solanum aculeastrum*

Compound	KI Value	% Composition
Terpenoids		
β-Cyclocitral	1219	1.2
Trans-β-Damascenone	1382	1.0
β-Ionone	1484	0.4
α-Farnesene	1503	1.0
δ-Nerolidol	1560	2.9
Alkanes		
n-Nonane	900	12.4
Decane	999	3.5
Undecane	1100	0.9
Eicosane	2000	0.7
Aldehydes		
2-Heptenal	945	0.8
Nonanal	1097	6.2
Decanal	1200	1.8
Decenal	1256	0.9
2, 4-Decadienal	1312	0.4
Undecenal	1359	0.4
Hexadecanal	1708	1.3
Pentadecanal	1912	1.2
9, 17-Octadecadienal	2116	4.0
Ketone		
Pentadecanone	1838	1.6
Alcohols		
N-Octanol	1060	1.8
2-Hexadecen-1-ol	1694	2.8
Fatty acids and esters		
Hexadecanoic acid	1954	1.6
9, 12-Octadecadienoic acid	2112	2.0
Octadecanoic acid	2135	0.6
Diterpenes		
Kaur-16-ene	2017	2.3
Aromatic hydrocarbons		
1, 2-Dimethyl-benzene	874	8.8
1, 4-Dimethyl-benzene	887	6.0
1,2-Dihydro-1,1,6-tri methyl-naphthalene	1192	0.4
Miscellaneous compounds		
2-Pentyl-Furan	983	1.5
Methyl salicylate	1192	2.3
O-Phthalic acid	2464	11.8

Table 2: Volatile compounds from the hexane fraction of the berries of *Solanum aculeastrum*

Compound	KI Value	% Composition
Alkanes/Alkenes		
Undecane	1100	21.7
Dodecane	1200	7.9
Tridecane	1300	10.0
2,7,10-Trimethyl dodecane,	1235	1.5
Tetradecene	1391	1.6
Tetradecane	1400	10.8
Pentadecane	1500	1.1
Octadecene	1786	1.6
Hexadecane	1600	4.6
octadecane	1800	1.6
Heneicosane	2100	1.2
Hexamethyl Tetracosane	2475	0.9
Fatty acid esters		
Methyl hexadecanoate	1922	2.2
Methyl-9,12-octadecadioenoate	2112	3.2
Methyl-9,12,15-octadecatrienoate	2165	2.3
Miscellaneous compounds		
O-Phthalic acid	2464	14.9

Chapter 5

Antimicrobial activity of *Solanum aculeastrum*

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ANTIMICROBIAL ACTIVITY OF *SOLANUM ACULEASTRUM*

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Antimicrobial Activity of *Solanum aculeastrum*

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Abstract

Solanum aculeastrum Dunal (Solanaceae) is used in traditional medicine to treat various human and animal diseases, specifically stomach disorders and various cancers, in the Eastern Cape, South Africa. The fruit and leaf extracts of this plant were investigated for *in vitro* antimicrobial activity against 10 selected bacterial and 5 fungal strains. The methanolic extracts of both the fruits and the leaves showed appreciable activity against Gram-positive and Gram-negative bacteria ranging from 4.0 to 10.0 mg/ml. Whereas the methanol extracts were the most active material, the water extracts showed the least activity against the bacteria. The methanol extracts were particularly inhibitory to the growth of the fungi with percentage inhibition ranging from 60.26% to 100% and 56.0% to 100% on *Aspergillus flavus* and *Pencillium notatum*, respectively. The acetone extracts were active against *Aspergillus flavus* (100%) and *Penicillium notatum* (64.81%), and the water extract of the fruit significantly inhibited the growth of *P. notatum* (69.89%). The most resistant organisms were *Aspergillus niger, Candida albicans*, and *Fusarium oxysporum.*

Keywords: Antibacterial, antifungal, antimicrobial, medicinal plants, *Solanum aculeastrum.*

Introduction

There has been an increasing incidence of microbial infections in recent years, largely due to the increase in AIDS-related opportunistic fungal pathogens and the emergence of resistance microbial species (Silva et al., 2001; Afolayan et al., 2002). Although, fungal-related diseases may not be as common as other bacterial infections, when they are present, they could be difficult to eradicate, especially in immunosuppressive situations (Bryce, 1992). Mycotic infections have been observed to be the primary cause of mortality in patients with severely impaired immune mechanisms (Kelberg, 1997). Hitherto, natural products from microorganisms have been the primary source of antibiotics, but with the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important because these may serve as promising sources of novel antibiotic prototypes (Meurer-Grimes et al., 1996; Rabe & Van Staden, 1997).

Solanum aculeastrum Dunal (Solanaceae), known as "goat bitter-apple," is a native of Africa and widely distributed in South Africa, mainly in Limpopo, Mpumalanga, KwaZulu-Natal, Western and Eastern Cape Provinces, and also in Swaziland. It is a thorny perennial plant that grows up to 3 m in height with white flowers and lemon-shaped berries that become yellow-green when ripe. The bitter fruits of *S. aculeastrum* are used medicinally in various ways for humans as well as domestic animals (Hutchings et al., 1996). The decoction of the fruits and leaves are taken orally for the treatment of cancer, indigestion, and stomach disorders. The fresh and boiled berries are used as a cure for jigger wounds, gonorrhea, and the treatment of acne (Kokwaro, 1993; Agnew & Agnew, 1994). Previously isolated compounds such as solanculine A, β-solanmarine, and solanmargine from the root bark and berries, respectively, have been reported to have molluscicidal activity (Alphonse et al., 2002). The antimycotic activity of *S. aculeastrum* has not been reported in the literature; yet, the members of the genus *Solanum* are known to be rich in steroidal glycoalkaloids and sesquiterpenoids that have antibacterial and antimycotic properties (Cipollini & Levey, 1997; Nagaoka et al., 2001; Shamim et al., 2004).

The aim of this study was to investigate the antimicrobial activity of *S. aculeastrum* by preliminary bioassay screening of its extracts against 10 selected bacterial

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and five fungal strains. According to Mathekaga and Mayer (1998), *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations.

Materials and Methods

Plant material

The berries and leaves of *S. aculeastrum* were collected from Kayalethu village in the Eastern Cape Province of South Africa. The plant was identified at the Department of Botany, University of Fort Hare, and a voucher specimen (Vedic Med 2005/16) was deposited in the Griffen Herbarium of the university.

Extract preparation

S. aculeastrum fruits were oven-dried at 60°C overnight and the leaves were air-dried at room temperature. Dried plant material (200 g) was shaken separately in acetone, methanol, and water for 48 h on an orbital shaker. Extracts were filtered using a Buchner funnel and Whatman no. 1 filter paper, and each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. The water extracts were freeze-dried. Each extract was resuspended in the respective solvent to yield a 50 mg/ml stock solution (Taylor et al., 1996).

Bioassays

The bacterial cultures used in this study were obtained from the Department of Biochemistry and Microbiology, Rhodes University, South Africa. They consisted of five Gram-positive and five Gram-negative strains (Table 1). Each organism was maintained on nutrient agar plates and was recovered for testing by growth in nutrient broth for 24 h. Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth (Afolayan & Meyer, 1997).

Test organisms were streaked in a radial pattern on sterile nutrient agar plates containing filtered extracts at final concentrations of 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/ml (Meyer & Afolayan, 1995). Plates containing only nutrient agar and another set containing nutrient agar and the respective solvents served as controls. After inoculation, the plates were incubated at 37°C for 24 to 48 h. Each treatment was performed in triplicate, and complete inhibition of bacterial growth was required for an extract to be declared bioactive. Chloramphenicol and streptomycin were used as standard control in the experiment.

Five species of fungi were used for the antimycotic investigation (Table 2). The cultures were maintained on potato dextrose agar (PDA) and were recovered for testing by subculturing on fresh PDA for 3 days. PDA plates were prepared in the usual fashion by autoclaving before the addition of the filtered extracts. Each extract was mixed with the molten agar (at 45°C) to final concentrations of 0.1, 0.5, 1.0, and 5.0 mg/ml, poured into Petri dishes, and left overnight for the solvent to evaporate. Control plates containing only PDA or PDA with the respective solvents served as controls. The prepared plates containing the extracts were inoculated with plugs obtained from the actively growing margins of the recovered fungal cultures and were incubated at 25°C for 5 days. The diameter of fungi growth was measured and expressed as percentage growth inhibition of three

Table 1. Antibacterial activity of the fruits and leaves of *S. aculeastrum*.

		MIC (mg/ml)							
		Acetone		Methanol		Water			
Bacteria–species	Gram +/−	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Chlor[a]	Strept[b]
Bacillus cereus	+	na	4	4	4	na	na	<2	<2
Staphylococcus epidermidis	+	na	na	4	6	na	na	<2	<2
Staphylococcus aureus	+	na	8	6	4	na	na	<2	<2
Micrococcus kristinae	+	na	2	6	6	na	10	<0.2	<2
Streptococcus pyogenes	+	na	6	6	6	na	na	<2	<2
Escherichia coli	−	na	na	6	6	na	na	<2	<2
Salmonella pooni	−	na	6	6	4	na	na	<2	<2
Serratia marcescens	−	na	na	10	8	na	na	<2	<2
Pseudomonas aeruginosa	−	na	na	10	8	na	na	<20	<5
Klebsiella pneumoniae	−	na	na	10	8	na	na	<2	<2

Minimum inhibitory concentration (mg/ml); na, not active.
[a]Chloramphenicol in µg/ml.
[b]Streptomycin sulfate in µg/ml.

Table 2. Antifungal activity of the fruits and leaves of *Solanum aculeastrum*.

	Growth inhibition (%)							
	Fruit extract				Leaf extract			
Concentrations (mg/ml)	*A. flavus*	*A. niger*	*F. oxysporum*	*P. notatum*	*A. flavus*	*A. niger*	*F. oxysporum*	*P. notatum*
Acetone extracts								
5.0	100.00^{c}	35.19^{c}	0.59^{a}	9.6^{a}	100.00^{d}	27.75^{b}	0.00^{a}	64.81^{c}
1.0	42.29^{b}	12.71^{b}	0.00^{a}	4.00^{a}	61.06^{c}	2.220^{a}	0.00^{a}	49.33^{b}
0.5	15.40^{a}	0.71^{a}	0.00^{a}	2.313^{a}	36.86^{b}	2.37^{a}	0.00^{a}	-1.567^{a}
0.1	0.11^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	1.53	>5	>5	>5	0.77	>5	>5	1.17
Methanol extracts								
5.0	100.00^{d}	26.40^{b}	24.64^{c}	60.26^{c}	100.00^{c}	44.92^{a}	1.66^{a}	56.00^{c}
1.0	62.13^{c}	0.95^{a}	-1.40^{b}	15.82^{b}	42.29^{b}	0.900^{a}	0.92^{a}	18.07^{b}
0.5	13.11^{b}	-1.90^{a}	-2.82^{a}	1.52^{a}	15.40^{a}	-0.26^{a}	1.61^{a}	0.79^{a}
0.1	2.53^{ab}	0.00^{a}	0.00^{a}	0.00^{a}	0.11^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	0.88	>5	>5	4.08	1.53	>5	>5	4.37
Water extracts								
5.0	2.08^{a}	12.44^{a}	6.45^{b}	69.89^{d}	3.30^{a}	1.38^{a}	0.52^{a}	-0.097^{a}
1.0	0.82^{a}	3.18^{a}	0.00^{a}	37.35^{c}	2.15^{a}	-0.66^{a}	-0.54^{a}	-0.08^{a}
0.5	2.19^{a}	3.81^{a}	0.00^{a}	26.54^{b}	3.30^{a}	2.05^{a}	-0.54^{a}	-0.17^{a}
0.1	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	>5	>5	>5	2.56	>5	>5	>5	>5

Values are means of percentage growth inhibition of three replicates: values within a column followed by the same superscript of the same species are not significantly different at $p < 0.05$ according to the LSD test. LC_{50} values in mg/ml.

replicates (Afolayan & Meyer, 1997; Barreto et al., 1997; Quiroga et al., 2001). Significance differences within the means of the treatments and the controls were calculated using the LSD statistical test (Steel & Torrie, 1960). LC_{50} (the concentration at which there was 50% inhibition of the growth of the test fungi) was calculated by extrapolation.

Results and Discussion

Antibacterial property

Minimal inhibitory concentration (MIC) values of ethanol, acetone, and water extracts from the fruits and leaves of *S. aculeastrum* against the tested bacteria are given in Table 1. Methanol extracts inhibited the growth of both the Gram-positive and Gram-negative bacterial at MIC ranging between 4.0 and 10.0 mg/ml. There was, however, more inhibition on Gram-positive strains. Whereas the acetone and water extracts of the fruits did not show any activity against any bacterial species, the leaf extracts of the two solvents showed moderate activity against *Micrococcus kristinae*. Acetone extract of the leaves showed activity against Gram-positive bacteria at concentrations of 2.0–8.0 mg/ml except *Staphylococcus epidermidis*, whereas there was no activity against the Gram-negative bacteria at the highest concentration tested with the exception of *Salmonella pooni*. In a similar experiment, moderate activity by *Solanum aculeastrum* was reported against *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *S. faecalis*, and *B. subtilis* (Alphonse et al., 2003).

Antifungal property

The results of the antifungal assay of *S. aculeastrum* extracts are presented in Table 2. The majority of the extracts (33.33%) showed antimycotic activity against the tested organisms at concentrations of 5 mg/ml or lower. Extracts from the leaves were more inhibitory to the growth of the tested fungi. The acetone and methanol extracts from the leaves inhibited the growth of *Aspergillus flavus* and *Penicillium notatum* with inhibitory percentage ranging from 56.0% to 100.0%. Similarly, the growth of *A. niger* was inhibited by methanol extract of the leaves at 5 mg/ml, which was the highest concentration used. However, it is interesting to note that both acetone and methanol extracts of the fruits completely inhibited the growth of *A. flavus* (100%), whereas methanol and water extracts of the fruits suppressed the growth of *P. notatam* with percentage

inhibition ranging from 60.26% to 69.89%, respectively. The susceptibility of *A. flavus* to the extracts of *S. aculeastrum* is noteworthy, as the fungus has recently been implicated in cases of immuno-compromised patients that frequently develop opportunistic and superficial mycosis (Ngane et al., 2000; Portillo et al., 2001; Silva et al., 2001). Acetone, methanol, and the water extracts from the fruits and leaves did not show any activity against *A. niger*, *Fusarium oxysporum*, and *C. albicans*.

The results of this study agreed with Portillo et al. (2001) who showed that *A. niger* is resistant to dichloromethane, aqueous and methanol extracts of 14 plants used for traditional medicine in Paraguay. In this study, the acetone and methanol extracts were found to have broad-spectrum activity against the fungal species tested. Generally, these two extracts were more active than the water extracts. Traditionally, however, plant extracts are prepared with water as infusions, decoction, and poultices; therefore, it would seem unlikely that the traditional healers are able to extract those compounds which that responsible for activity in the acetone and methanol extracts. Work is in progress on the isolation, purification, and structural identification of the bioactive compounds in this plant in order to validate the claims for its use in traditional medicine by the people of the Eastern Cape, South Africa.

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References

Afolayan AJ, Grierson DS, Kambizi L, Madamombe I, Masika P (2002): *In vitro* antifungal activity of some South African medicinal plants. *South African J Bot 68*: 72–76.

Afolayan AJ, Meyer JJM (1997): The antimicrobial activity of 3, 5, 7-trihydroxyflavone isolated from the shoot of *Helichrysum aureonitens*. *J Ethnopharmacol 57*: 177–181.

Agnew ADQ, Agnew S (1994): *Upland Kenya Wild Flowers. A Flora of the Ferns and Herbaceous Flowering Plants of Upland Kenya.* Nairobi, East Africa Natural History Society.

Alphose WW, Sumesh CC, Gerld M, Udo E, Wilson MN (2002). Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum*. *Phytochemistry 59*(1): 79–84.

Alphonse WW, Sumesh CC, Gerald M, Wilson N, Paul KT (2003): Molluscicidal and antimicrobial activity of *Solanum aculeastrum*. *Fitoterapia 74*: 298–301.

Barreto M, Straker CJ, Critchley AT (1997): Short not on the effects of ethanolic extracts of selected South African Sea weeds on the growth of commercially important plant pathogens, *Rhizoctonia solani* Kuhn and *Verticullium* sp. *South African J Bot 63*: 521–523.

Bryce K (1992): *The Fifth Kingdom.* Ontario, Mycologic Publication.

Cipollini ML, Levey DJ (1997): Antifungal activity of *Solanum* fruit glycoalkaloids: Implications for frugivory and seed dispersal. *Ecology 78*: 799–809.

Hutchings A, Scott AH, Lewis G, Cunningham AB (1996): *Zulu Medicinal Plants, an Inventory.* Pietermaritzburg, University of Natal Press.

Kelbergh BJ (1997): Trends in immunotherapy of fungal infections. *Eur J Clin Microbiol Infect Dis 16*: 51–55.

Kokwaro JO (1993): *Medicinal Plants of East Africa.* Nairobi, East African Literature Bureau.

Mathekaga ADM, Meyer JJM (1998). Antibacterial activity of South African *Helichrysum* species. *South African J Bot 64*: 293–295.

Meurer-Grimes B, Mcbeth DL, Hallihan B, Delph S (1996): Antimicrobial activity in medicinal plants of the Scrophulariaceae and Acanthaceae. *Int J Pharmacog 34*: 243–248.

Meyer JJM, Afolayan AJ (1995). Antibacterial activity of *Helichrysum aureonitens* (Asteraceae). *J Ethnopharmacol 47*: 109–111.

Nagaoka T, Goto K, Watanabe A, Sakata Y, Yoshihara T (2001): Sesquiterpenoids in root exudates of *Solanum Aethiopicum*. *Z Naturforsch 56c*: 707–713.

Ngane NA, Biyiti L, Zollo PHA, Bouchet PH (2000): Evaluation of antifungal activity of extracts of two Cameroonian Rutaceae: *Zanthoxylum leprieurii* Gruill et Perr. and *Zanthoxylum xanthoxyloides* Waterm. *J Ethnopharmacol 76*: 347–354.

Portillo A, Vila R, Freixa B, Adzet T, Canigueral S (2001): Antifungal activity of Paraguayan plants used in traditional medicine. *J Ethnopharmacol 76*: 93–98.

Quiroga EN, Sampietro AR, Vattluone MA (2001): Screening antifungal activities of selected medicinal plants. *J Ethnopharmacol 74*: 89–96.

Rabe T, Van Staden J (1997): Antibacterial activity of South African Plants used for medicinal purposes. *J Ethnopharmacol 56*: 81–87.

Shamim S, Ahmed SW, Azhar I (2004): Antifungal activity of *Allium*, *Aloe*, and *Solanum* species. *Pharm Biol 42*: 491–498.

Silva MV, Costa TR, Costa MR, Ferrera EC, Fernandes OFL, Santos SC, Liao LM, Ferri PH, Paula JR, Ferriera HD, Silva MRR (2001): Growth inhibition effect of Brazilian Cerrado plant extracts on *Candida* species. *Pharm Biol 39*: 138–141.

Steel RGD, Torrie JH (1960): *Principles and Procedures of Statistics.* New York, McGraw-Hill.

Taylor RSL, Edel F, Manandhar NP, Towers GHN (1996): Antimicrobial activity of southern Nepalese medicinal plants. *J Ethnopharmacol 50*: 97–102.

CHAPTER 6

IN VITRO ANTITUMOUR ACTIVITY OF *SOLANUM ACULEASTRUM* BERRIES ON THREE CARCINOMA CELLS

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In vitro Antitumour Activity of *Solanum aculeastrum* Berries on Three Carcinoma Cells

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Abstract: *Solanum aculeastrum* is a medicinal plant used by the traditional healers of the Eastern Cape of South Africa, for the treatment of cancers. The antiproliferative activities of this plant were studied *in vitro* using three human tumour cell lines (HeLa, MCF7 and HT29). Methanolic extracts of the fruits had the highest antiproliferative activity with IC_{50} between 17.1 and 41.9 μg mL^{-1} while the activities of their aqueous extracts ranged between 27.9 and 48.5 μg mL^{-1}. The leaf extracts had no anticancer activity under the experimental conditions tested. Overall, the HeLa and MCF7 cell lines were much more sensitive to both extracts than HT29 cells.

Key words: Traditional medicine, *Solanum aculeastrum*, antitumour, anticancer, cytotoxicity

Introduction

Over one million people are diagnosed annually with breast cancer which is one of the primary causes of deaths among women globally (Ferlay *et al*., 2001). The rate of increase of cancer incidence and lack of anticancer drugs has forced scientists to pharmacological and chemical investigations of medicinal plants in search for anticancer agents. The results of the screening of plant extracts for anti proliferative activity have shown that higher plants are a potential source of antioncogenic agents which can compete favourably with chemotherapy and hormonal treatments (Pezzuto, 1997; Wu *et al*., 2002).

Solanum aculeastrum Dunal (Solanaceae) is widely used in traditional medicine for the treatment of human and livestock diseases (Hutchings *et al*., 1996). Both fresh and boiled berries of the plant are used as a cure for jigger wounds and gonorrhea (Agnew and Agnew, 1994). Antimicrobial and antioxidant activity of *Solanum aculeastrum* using crude extracts has been previously reported (Koduru *et al*., 2006a, b). Also, ethnomedical information from the indigenous people of the Eastern Cape Province of South Africa revealed that this plant is used for the treatment of breast cancer (Koduru *et al*., 2006c, d). There is, however, no report on the anticancer property of *S. aculeastrum* in the literature. Yet, species in the genus *Solanum* are known to be rich in steroidal alkaloids and flavonoids which are known to induce apoptosis in tumor cell lines (Papamichael, 2000; Esteves-Souza *et al*., 2002). Indeed, phytochemical investigations of *S. aculeastrum* have revealed the presence of steroidal alkaloids such as solaculine A, solamargine, β-solamarine, solasonine and solasodine (Drewes and Van Staden, 1995; Wanyonyi *et al*., 2002).

In vitro studies have provided evidence that chemotherapeutic agents such as plant extracts may induce apoptotic tumour cell deaths *in vivo*. In this investigation, the *in vitro* cytotoxic properties of the crude extracts of leaves and berries of *S. aculeastrum* was tested against three cancerous cell lines viz. HeLa, HT29 and MCF7 using standard procedures.

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Materials and Methods

Plant Material

The berries and leaves of *S. aculeastrum* were collected from trees naturally occurring in the wild at Kayalethu village in the Eastern Cape Province of South Africa (latitudes 30°00'- 34°15'S and longitudes 22°45'-30°15'E). The plant was identified at the Department of Botany, University of Fort Hare and a voucher specimen (Vedic Med 2005/16) was prepared and deposited in the Griffen Herbarium.

Preparation of Extracts

S. aculeastrum fruits were oven dried at 60°C while the leaves were air dried at room temperature. Three equal portions (200 g) of each dried plant material were shaken separately in acetone, methanol and water for 48 h on an orbital shaker. Extracts were filtered using a Buchner funnel and Whatman No. 1 filter paper and each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. The water extracts were freeze dried.

Human Carcinoma Cell Lines and Culture Medium

HT-29 (colonic adenocarcinoma), HeLa (cervical carcinoma) and MCF7 (breast adenocarcinoma) cells were cultured in 10 cm culture dishes in growth medium [antibiotic-free RPMI 1640 medium (Sigma, Germany) containing 10% heat-inactivated fetal bovine serum (Highveld Biological, South Africa), 25 mM HEPES and 2 mM glutamine] in a humidified 5% CO_2 incubator at 37°C. The cancer cell lines were obtained from the American Type Culture Collection (ATCC), USA.

In vitro Cytotoxic Assays

For the determination of cell viability, cells were seeded into 96-well culture plates (Nunclon) at a density of 6000 cells/well in 200 µL aliquots. Cells were allowed to attach for 24 h in a humidified 5% CO_2 incubator at 37°C. Dried fruit and leaf extracts were solubilized in DMSO before further dilution with growth medium. The final concentration of DMSO in the wells never exceeded 0.25%. Cisplatin was used as positive control at concentrations of 10 and 100 µM. Cells were exposed to the extracts or cisplatin for 48 h. Immediately following the 48 h incubation period, cell numbers were determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay as previously described (Alley *et al*., 1988; Brauns *et al*., 2004). Briefly, cells were incubated with 200 µL MTT (Sigma) (0.5 mg mL in growth medium) for 4 h at 37°C. The formazan product was then dissolved in DMSO and plates were agitated on a shaker for 5 mins, before the absorbance was read at 540 nm on multiwell scanning spectrophotometer (Multiskan MS, Labsystems). The values obtained were used to determine the percentage inhibition of cell growth caused by the extracts (Hagopian *et al*., 1999; Huq *et al*., 2004). Cisplatin was used as a standard control.

Calculations and Statistics

Initial screening for cytotoxicity and log dose-dependent responses were performed in triplicate and quadruplicate, respectively. Results were expressed as percentage growth inhibition of control and treatment values were compared to control values using the Two-sample Students t-test. IC_{50} values for growth inhibition was derived from a nonlinear regression model (curve fit) based on sigmoidal dose response curve (variable) and computed using GraphPadPrism 4 (Graphpad).

Results

Antiproliferative activities of the different extracts of leaves and berries from the *S. aculeastrum* on the growth of three human cancerous cell lines were carried out *in vitro* using tetrazolium assay. Cell proliferation was analyzed at 48 h after cell lines had been cultured with an extracts of 0, 125 and 250 µg mL^{-1} in media while cisplatin was used as positive control. Results of the initial screening

showed that two of the six crude extracts [Fruit Methanol (FM) and Fruit Water (FW)] inhibited the growth of all three tumour cell lines by more than 80% after 48 h exposure ($p<0.001$ for both concentrations tested (Fig. 1). Inhibition by the other four extracts (three extracts of leaves and acetone

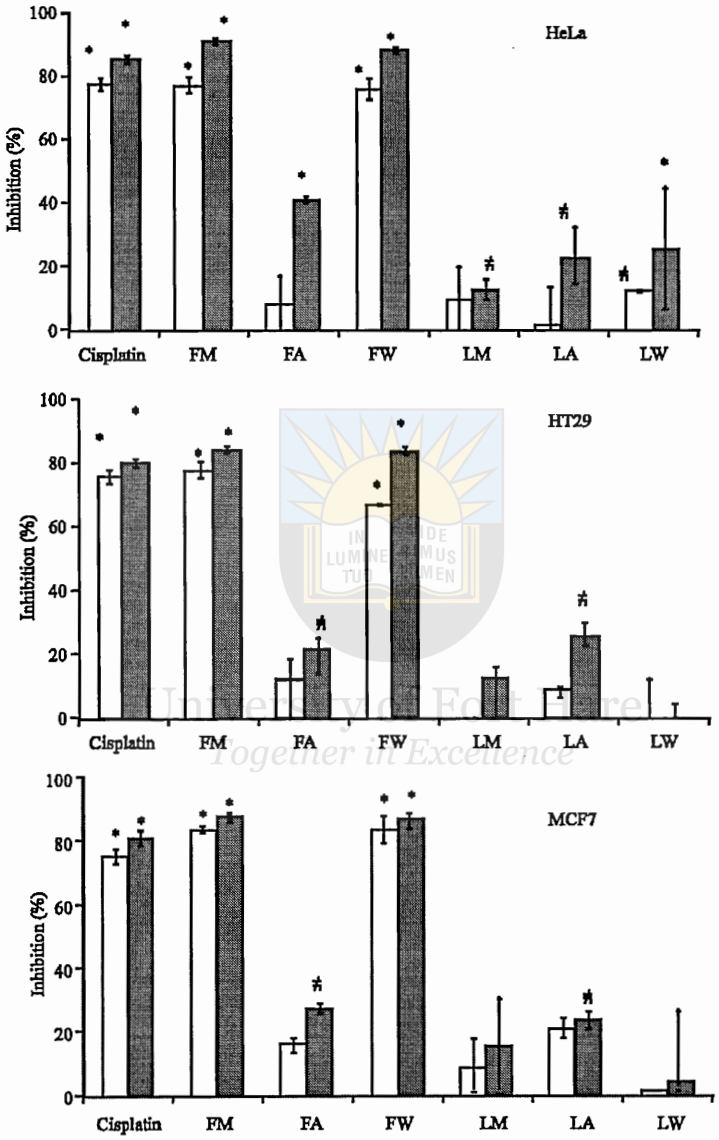

Fig. 1: Initial screening results for six extracts at 125 μg mL^{-1} (clear bars) and 250 μg mL^{-1} (hashed bars) against HeLa, MCF7 and HT29 cells. Cisplatin at 10 μM (clear bars) and 100 μM (hashed bars) was used as a positive control. Error bars represent the standard deviation of triplicate determinations. # $p<0.05$; * $p<0.001$ compared to control (FM = Fruit Methanol; FA = Fruit Acetone; FW = Fruit Water; LM = Leaf Methanol; LA = leaf Acetone; LW = Leaf Water)

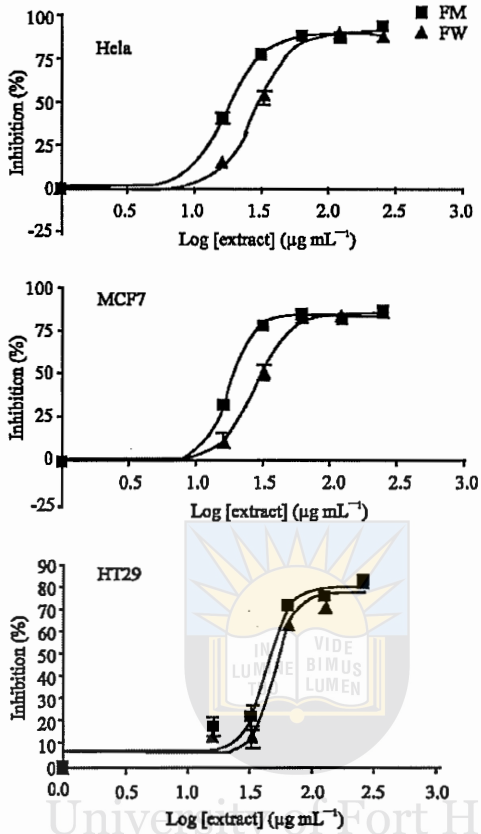

Fig. 2: Log dose-response curves for Fruit Methanol (FM) and Fruit Water (FW) extracts against HeLa, MCF7 and HT29 cells. Error bars represent the standard deviation of quadruplicate determinations

Table 1: IC_{50} values for fruit methanol and fruit water extract for growth inhibition against HeLa, MCF7 and HT29 carcinoma cell lines

	IC50 (µg mL^{-1})	
Cell line	Extract FM	Extract FW
HeLa	17.1	28.4
MCF7	17.8	27.9
HT29	41.9	48.5

extract of berries) were less than 50%, therefore, they were not considered for further testing. The methanol and water extracts of the berries which were further tested against three tumour cell lines at lower concentrations, indicated that IC_{50} values of methanol extract ranged between 17.1 and 41.9 µg mL^{-1} while those of the aqueous extract ranged between 27.9 and 48.5 µg mL^{-1} under experimental conditions (Fig. 2 and Table 1). The methanol extract had a slightly lower IC_{50} than water extract in all the three tumour cell lines tested (Fig. 2 and Table 1). The highest activity was shown on cervical carcinoma (HeLa) and breast adenocarcinoma (MCF7) cell lines and their IC_{50} values for both cell lines were less than 18 µg mL^{-1} (Table 1). This indicates that the extracts were anti cancerous

based on the criterion set by the National Cancer Institute (Geran *et al.*, 1972). The leaf extracts had no anticancer activity under the experimental conditions tested.

Discussion

The methanol and water extracts of *S. aculeastrum* was shown to have inhibitory effect on cancer cell lines in the *in vitro* studies. It was effective in the suppression of proliferation of the three cancerous cell lines, HeLa, MCF7 and HT29 in a dose-dependent pattern. A vast variety of naturally occurring substances have been shown to protect against experimental carcinogenesis (Ju *et al.*, 2004). Thus, it is becoming increasingly evident that certain phytochemicals, particularly those included in our daily diet, many have important cancer chemopreventive properties (Sanaha *et al.*, 1997). In this study, there may be inhibitors and active ingredients in the *S. aculeastrum* extracts, which can induce cytotoxic action against cancer cells and initiate anti proliferative pathway leading to cancer cell death. Traditionally, *S. aculeastrum* is used for the treatment of breast cancer in South Africa. The berries of this plant are boiled in water until they burst into pieces. After filtration, the decoction is administered once a day until signs of relief is observed. This study has confirmed the ethnomedical application of *S. aculeastrum* in the treatment of cancer (Koduru *et al.*, 2006c, d). The active compounds in the plant extracts are not yet known. However, plants belonging to the Solanaceae family have been reported to contain complex glycosides and saponins (Tan *et al.*, 2005), which may be responsible for the observed activity.

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References

Agnew, A.D.Q. and S. Agnew, 1994. Upland Kenya Wild Flowers. A Flora of the Ferns and Herbaceous Flowering Plants of Upland Kenya. East Africa Natural History Society, Nairobi.

Alley, M.C., D.A. Scudiero, A. Monks, M.L. Hursey, M.J. Czerwinski, F.L. Fine, B.J. Abbott, J.G. Mayo, R.H. Shoemaker and M.R. Boyd, 1988. Feasibility of drug screening with panels of human tumour cell lines using a microculture tetrazolium assay. Cancer Res., 48: 589-601.

Brauns, S.C., P. Milne, R.M.V. Naude and an de Venter, 2005. Selected cyclic dipeptides inhibit cancer cell growth and induce apoptosis in HT-29 colon cancer cells. Anticancer Res., 24: 1713-20.

Drewes, F.E. and J. Van Staden, 1995. Aspects of the extraction and purification of solasodine from *Solanum aculeastrum* tissues. Phytochem. Anal., 6: 203-206.

Esteves-Souza, A., T.M. Sarmento da Silva, C.C. Fernandes Alves, M.G. de Carvalho, R. Braz-Filho and A. Echevarria 2002. Cytotoxic activities against enrlich carcinoma and Human K562 luekaemia of alkaloids and flavonoid from two Solanum species. J. Braz. Chem., 13: 838-842.

Ferlay, J., F. Bray, P. Pisani and P.M. Parkin, 2001. *GLOBOCAN 2000:* Cancer Incidence, Mortality and Prevalence Worldwide. IARC Cancer Base No. 5. [1.0] Lyon, France: IARC.

Geran, R.I., N.H. Greenberg, M.M. Machonaled, A.M. Schumacher and B.J. Abbott, 1972. Protocols for screening chemical agents and natural products against animal tumours and other biological systems. Cancer Chemother. Rep., 3: 59-61.

Hagopian, G.S., Mills, G.B. Khokhar, A.R.R.C. Bast and Z.H. Jr. Siddik, 1999. Expression of p53 in Cisplatin-resistant Ovarian Cancer Cell Lines: Modulation with the Novel Platinum Analogue (1R, 2 R-Diaminocyclohexane) (*trans*-diacetato) (dichloro)-platinum (IV). Clin. Cancer Res., 5: 655-663.

Huq, F., H. Daghriri, J.Q. Yu, H.P. Tayyem, Beale and M. Zhang, 2004. Synthesis, characterization, activities, cell uptake and DNA binding of [{*trans*-$PtCl(NH_3)_2$} {μ-$(H_2N(CH_2)_6NH2)$}{*trans*-$PdCl(NH_3)_2$}$(NO_3)Cl$. Euro. J. Med. Chem., 39: 947-958.

Hutchings, A., A.H. Scott, G. Lewis and A.B. Cunningham, 1996. Zulu medicinal plants, an inventory. University of Natal Press, Pietermaritzburg.

Ju, E.M., Lee, S.E., H.J. Hwang and J.H. Kim, 2004. Antioxidant and anticancer activity of extracts from *Betula platyphylla* var. *japonica*. Life Sci., 74: 1013-1026.

Koduru, S., D.S. Grierson and A.J. Afolayan, 2006a. Antimicrobial activity of *Solanum aculeastrum*. Pharm, Biol., 44: 283-286.

Koduru Srinivas, D.S. Grierson, M.A. Aderogba., J.N. Eloff and, A.J. Afolayan, 2006b. Antioxidant activity of *Solanum aculeastrum* (Solanaceae) berries. Intl. J. Pharmacol., 2: 262-264.

Koduru, S., D.S. Grierson and A.J. Afolayan, 2006c. Effect of high temperatures on seed germination of *Solanum aculeastrum*. Asian J. Plant Sci., 5: 353-356.

Koduru, S., D.S. Grierson, M. van de Venter and A.J. Afolayan, 2006d. Validating the anticancer property of *Solanum aculeastrum*. In Proceedings of the South African Association of Botany (SAAB) conference, 16-19 January 2006. NMMU, Port Elizabeth, South Africa.

Papamichael, D., 2000. The use of thymidylate synthase inhibitors in the treatment of advanced colorectal cancer. Current status, Stem Cells, 3: 166-175.

Pezzuto, J.M, 1997. Plant-derived anticancer agents. Biochem. Pharmocol., 53: 121-133.

Sanaha, H.S., G.J. Kelloff, V. Steele, C.V. Rao and B.S. Reddy, 1997. Modulation of apoptosis by sulindac, curcumin, phenyl-ethyl-3-methylcaffeate and 6-phenylhexyl isothiocyanate: Apoptotic index as a biomarker in colon cancer chemoprevention and promotion. Cancer Res., 57: 1301-1305.

Tan, M.L., S.F. Sulaiman, N. Najimuddin, M.R. Samian and T.S. Tengku Muhammad, 2005. Methanolic extract of Pereskia bleo (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma, T47-D cell line. J. Ethnopharmacol., 96: 287-294.

Wanyonyi, A.W., C.C. Sumesh, M. Gerld, E. Udo and M.N. Wilson, 2002. Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum*. Phytochemistry, 59: 79-84.

Wu, J., Y. Wu and B.B. Yang, 2002. Anticancer activity of *Hemsleya amabilis* extract. Life Sci., 71: 2161-2170.

CHAPTER 7

ANTIOXIDANT ACTIVITY OF *SOLANUM ACULEASTRUM* (SOLANACEAE) BERRIES

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Antioxidant Activity of *Solanum aculeastrum* (Solanaceae) berries

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Abstract: The antioxidant activity of the crude methanol, acetone and water extracts of the berries of *Solanum aculeastrum* was examined by 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical-scavenging. The methanol and water extracts had moderate antioxidant activity ranging between 53.1 to 65.5 μg mL^{-1}, while the acetone extract did not demonstrate significant antioxidant activity at the tested concentrations. The higher antioxidant activity of this plant exhibited by the water extract may be due to the presence of substantial amount of polar constituents from the plant material.

Key words: *Solanum aculeastrum*, antioxidant activity, DPPH, Solanaceae

INTRODUCTION

Solanum aculeastrum Dunal (Solanaceae) is a multi-branched shrub, 1-5 m high, heavily armed with large prickles and is wide-spread in South Africa. It grows in areas with high rainfall of more than 700 mm per year and at altitudes from 275 to 1 780 m on gentle to steep slopes and various soil types. *S. aculeastrum* has high medicinal value. Its berries are used as a soap substitute, apparently because of its high saponin content. Local healers use the extremely bitter berries and leaves for the treatment of various diseases in humans and domestic animals (Hutchings *et al.*, 1996). Both mature and immature berries contain the poisonous alkaloid, α-solanine (Hutchings *et al.*, 1996). Other bioactive compounds that have been isolated from this plant include solaculine A (Wanyonyi *et al.*, 2002) from the root bark and solamargine, beta-solamarine, solasonine and solasodine from the fruits (Drewes and Van Staden, 1995; Wanyonyi *et al.*, 2002). The fresh and boiled ripe berries are used as a cure for jigger wounds and gonorrhoea, respectively (Agnew and Agnew, 1994). Recent discussion with traditional healers of the Eastern Cape Province in South Africa revealed that the plant is used for the treatment of cancer, particularly breast cancer.

Many plants in the Solanaceae family accumulate steroidal alkaloids (Tan *et al.*, 2005). These compounds are nitrogen analogues of saponins. They are usually present as glycosides and are known to possess a variety of biological properties, including antifungal (Kusano *et al.*, 1987), molluscicidal (Alzerreca and Hart, 1982; Wanyonyi *et al.*, 2003), teratogenic, embryotoxic (Friedman *et al.*, 1992) and hemolytic (Dewick, 1998) properties. They also exhibit strong antioxidant properties (Badami *et al.*, 2005). Despite the well reported medicinal values of *S. aculeastrum*, its antioxidant potential has not been studied. In the present investigation extracts of berries of this plant were screened for *in vitro* antioxidant activity.

MATERIALS AND METHODS

Collecting of plant material and extracts preparation: The berries of *S. aculeastrum* were collected from shrubs naturally occurring in the wild at Kayalethu village in the Eastern Cape Province of South Africa (latitudes 30°00'- 34°15'S and longitudes 22°45'-30°15'E). The fruits were oven dried at 60°C overnight. Two hundred gram portions of dried plant material were shaken separately in methanol, acetone and water for 48 h on an orbital shaker. Extracts were filtered using a Buchner funnel and Whatman No. 1 filter paper. The methanol and acetone filtrates were concentrated to dryness under reduced pressure at 40°C using a rotary evaporator, while the water extract was freeze dried.

Evaluation of antioxidant activity: Quantitative antioxidant activity was determined spectrophotometrically as described by Mensor *et al.* (2001), with reactions carried out in 96-well microtitre plates. Briefly, different concentrations of the extracts were prepared between 250.0 and 1.0 μg mL^{-1}. Twenty microliter of 0.3 mM DPPH

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in ethanol was added to 50 µL of each concentration of sample tested and allowed to react at room temperature in the dark for 30 min. Blank solutions were prepared with the test solution (50 µL) and 20 µL of ethanol only while the negative control was DPPH solution (20 µL), plus 50 µL ethanol. The decrease in absorbance was measured at 518 nm on a micro plate reader ($VERSA_{max}$). Values obtained were converted to percentage antioxidant activity (AA%) using the formula:

$$AA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{control}\}$$

(Abs_{sample} is the absorbance of the sample, Abs_{blank} is the absorbance of the blank and $Abs_{control}$ is the absorbance of the control). L-ascorbic acid (Vitamin C) was used as a positive control (antioxidant agent).

The EC_{50} value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the linear regression of plots of concentration of the test extracts (µg mL^{-1}) against the mean percentage of the antioxidant activity obtained from three replicate assays.

Statistical analysis: The results were expressed as mean±SEM and the EC_{50} values obtained from the regression plots (SigmaPlots®2001, SPSS Science) showed a good coefficient of determination, with most values being $r^2 \geq 0.913$.

RESULTS AND DISCUSSION

The radical-scavenging activities of the samples were determined from the reduction in the optical density (OD) of DPPH free radical at 518 nm. Hydrogen-donating ability is an index of the primary antioxidants. These antioxidants donate hydrogen to free radicals, leading to non-toxic species and therefore to inhibition of the propagation phase of lipid oxidation (Lugasi *et al.*, 1998). According to Hochstein and Atallah (1998), the antimutagenic activity of antioxidants is due to their ability to scavenge free radicals or induce antioxidative enzymes. The extracts of the berries of *S. aculeastrum* differed in antioxidant activity in quantitative measurement. A low EC_{50} value is an indication of strong antioxidant activity. Methanol and water extracts of berries had moderate antioxidant activity ranging between 53.1 to 65.5 µg mL^{-1} (Table 1), while acetone extract did not demonstrate significant antioxidant activity at the tested concentration. The higher antioxidant activity exhibited by the water extract may be due to the presence of substantial amounts of polar constituents from the plant material.

Table 1: DPPH free radical scavenging activity of berries extracts of *S. aculeastrum*

Extracts	EC50±SEM (µg mL^{-1})
Methanol	65.5±4.90
Acetone	ND
Water	53.10±2.33
L-Ascorbic acid	2.60± 0.08

ND- Not Determined, the sample did not demonstrate considerable antioxidant activity at the highest concentration (250 µg/ml) tested in this experiment.

In *Solanum aculeastrum*, steroidal alkaloid glycosides were isolated from the berries (Wanyonyi *et al.*, 2002). This study emphasizes the antioxidant potential of *S. aculeastrum*. The components responsible for the antioxidative activities of the extracts, however, are unknown. Further research is therefore needed for the isolation and identification of the antioxidative components in the extracts.

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REFERENCES

Agnew, A.D.Q. and S. Agnew, 1994. Upland Kenya Wild Flowers. A Flora of the Ferns and Herbaceous Flowering Plants of Upland Kenya. East Africa Natural History Society, Nairobi.

Alzerreca, A. and G. Hart, 1982. Molluscicidal steroid glycoalkaloids possessing stereoisomeric spirosolane structures. Toxicol. Lett., 12: 151-155.

Badami, S., Prakash Om, S.H. Dongre and B. Suresh, 2005. In vitro antioxidant properties of Solanum pseudocapsicum leaf extracts. Indian Journal of Pharmacology, 37: 251-252.

Dewick, P.M., 1998. Medicinal Natural Products: A Biosynthetic Approach, John Willey and Sons Ltd: West Sussex, England.

Drewes, F.E. and J. Van Staden, 1995. Aspects of the extraction and purification of solasodine from *Solanum aculeastrum* tissues. Phytochemical Analysis, 6: 203-206.

Friedman, M., J.R. Rayburn, J.A. Bantle, 1992. Structural relationships and development toxicity of Solanum alkaloids in the frog embryo teratogenesis assay-Xenopus. J. Agric. Food Chem., 40: 1617-1624.

Hochstein, P. and S.A. Atallah, 1998. The nature of oxidant and antioxidant systems in inhibition of mutation and cancer. Mutation Research, 202: 363-375.

Hutchings, A., A.H. Scott, G. Lewis and A.B. Cunningham, 1996. *Zulu medicinal plants, an inventory.* University of Natal Press, Pietermaritzburg.

Kusano, G., A. Takahashi, K. Sugiyama and S. Nozol, 1987. Antifungal properties of solanum alkaloids. Chem. Pharm. Bull., 35: 4862-4867.

Lugasi. A., E. Dworschák, A. Blázovics and A. Kéry, 1998. Antioxidant and free radical scavenging properties of squeezed juice from black radish (*Raphanus sativus* L., var. niger) root. Phytotherapy Research, 12: 502-506.

Mensor, L.L., F.S. Menezes, G.G. Leitao, A.S. Reis, T.C. Santos, C.S. Coube and S.G. Leitao, 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res., 15: 127-130.

Tan, M.L., S.F. Suleiman, N. Najimuddin, M.R. Samian and T.S. Tengku Muhammad, 2005. methanolic extract of pereskia bleo (kunth) dc. (cactaceae) induces apoptosis in breast carcinoma, t47-d cell line. J. Ethnopharmacol., 96: 287-294.

Wanyonyi, A.W., C.C. Sumesh, M. Gerald, M.N. Wilson and K.T. Paul, 2003. Molluscicidal and antimicrobial activity of *Solanum aculeastrum.* Fitoterapia, 74: 298-301.

Wanyonyi, A.W., C.C. Sumesh, M. Gerld, E. Udo and M.N. Wilson, 2002. Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum.* Phytochemistry, 59: 79-84.

Chapter 8

Anticancer activity of isolated steroid alkaloids from *Solanum aculeastrum*

CHAPTER 8

ANTICANCER ACTIVITY OF ISOLATED STEROID ALKALOIDS FROM *SOLAMUM ACULEATRUM*

* This chapter has been submitted for publication in "***Pharmaceutical Biology***"

Anticancer activity of isolated steroid alkaloids from *Solanum aculeastrum*.

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Abstract

Solanum aculeastrum is a medicinal plant, which has long been used to treat various cancers and many other conditions in the Eastern Cape Province, South Africa. In this study two steroid glycosides were isolated from the berries of this plant, which were identified as tomatidine and solasodine by spectroscopic techniques. Antitumour activity of these compounds was investigated on HeLa, MCF7 and HT29 cancer cell lines. The IC_{50} values confirmed that tomatidine and solasodine had the highest inhibitory effect on HeLa cells and the IC_{50} of the combined compounds was lower than the value for solasodine and unchanged from that of tomatidine. However, the IC_{50} values of the two compounds combined was also lower in HT29 and MCF7 cells than for the individual compounds. Both tomatidine and solasodine inhibited cell growth by blocking the cell cycle in the G_0/G_1 phase after 24 h exposure with an increase from 55.6% to 64.2 and 66.8%, respectively. Using Annexin V-FITC/PI staining by flow cytometry, the compounds showed very low apoptotic indices.

Keywords: Apoptosis, *Solanum aculeastrum*, tomatidine, solasodine, anticancer.

1. Introduction

Solanum aculeastrum Dunal is a medicinal plant, which occurs from tropical Africa down to South Africa (Koduru et al., 2006d). It is a thorny perennial plant widely distributed in South Africa and grows up to 2–5 m height. Local healers use the extremely bitter berries for the treatment of various diseases in humans and domestic animals (Hutchings et al., 1996). The fresh and boiled ripe berries are used to treat jigger wounds and gonorrhoea, respectively (Agnew & Agnew, 1994). Previous studies have reported on antimicrobial and antioxidant activities of this plant using crude extracts of its berries. (Koduru et al., 2006a; 2006c). Earlier discussions with traditional healers of the Eastern Cape Province in South Africa revealed that the plant is also used for the treatment of cancer (Koduru et al., 2006a). This claim was confirmed in our recent bioassay of its crude extracts on three tumour cell lines (Koduru et al., 2006c). In continuation of our research, this study was aimed at isolating the cytotoxic compounds in this plant and to investigate their antitumour activity on cultured carcinoma cell lines.

Induction of apoptosis or programmed cell death is one approach to cancer therapy (Los et al., 2003). Apoptotic cell death is a physiological mechanism that eliminates unwanted cells by triggering the cell's intrinsic 'suicide program' (Kerr et al., 1972). Impairment of the apoptotic mechanism ultimately generates a pathological condition that includes developmental defects such as autoimmune diseases, neurodegeneration or cancerous neoplasia (Reed et al., 2001). Apoptosis is characterized by morphological changes such as membrane blebbing, cell shrinkage, protein fragmentation, chromatin condensation and DNA degradation followed by rapid engulfment of cell debris by neighbouring cells

(Chritop, 2003). It is therefore possible to take advantage of this intrinsic mechanism by manipulating the apoptotic process for therapeutic gains. Another objective of this study was to broaden the understanding of the relationship between *Solanum aculeastrum* and induced apoptosis, which could be beneficial in anticancer therapy.

2. Materials and methods

2.1. Plant material

The berries of *S. aculeastrum* were collected from plants naturally occurring in the wild at Kayalethu village, near the town of Alice, in the Eastern Cape Province of South Africa (latitudes 30°00'- 34°15'S and longitudes 22°45'-30°15'E). The plant was identified at the Department of Botany, University of Fort Hare and a voucher specimen (Vedic Med 2005/16) was prepared and deposited in the Griffen Herbarium of the University.

2.2. Extraction and isolation of tomatidine and solasodine

S. aculeastrum berries were oven dried to constant weight at 60°C and ground to a powder using a blender. Following the method of Weissenberg (2001), 100 g portion of the powder was added to 300 g toluene plus 200 ml water and 100 ml of 32% HCl and refluxed with stirring for 5 h. The reaction mixture was subsequently alkalinised with 40% aq. NaOH (200 ml) and refluxed again with stirring for 2 h. Following phase separation, the upper, pale-yellow toluene layer was siphoned off, and the remaining dark brown aqueous mixture was further extracted three times with 100 ml portions of toluene. The purity of the formed precipitate was ascertained by TLC and NMR spectroscopy.

The procedure gave consistently colourless and crystalline steroid glycosides with 90–95% recovery (Weissenberg, 2001). The white precipitate was separated by preparative TLC using the solvent system $CHCl_3$-EtOAc (9:1) and the compounds were identified using NMR spectroscopy.

2.3. NMR spectroscopy and preparative TLC

^1H and ^{13}C NMR spectra were recorded on a Bruker AMX400 spectrometer at 400 MHz and 100.60 MHz, respectively. Deuterated chloroform was used as the solvent for all the NMR experiments. Chemical shifts are reported in ppm, using TMS as internal references. Preparative TLC-silica gel 60 $F_{254+366}$ was used for preparative thin layer chromatography (Merck) and visualized using sulphuric acid spray (1% H_2SO_4 in MeOH).

2.4. Human carcinoma cell lines and culture medium

HT-29 (colonic adenocarcinoma), HeLa (cervical carcinoma) and MCF-7 (breast adenocarcinoma) cells were cultured in 10 cm culture dishes in growth medium [antibiotic-free RPMI 1640 medium (Sigma, Germany) containing 10% heat-inactivated fetal bovine serum (Highveld Biological, South Africa), 25 mM HEPES and 2 mM glutamine] in a humidified 5% CO_2 incubator at 37°C. All cancer cells were obtained from the American Type Culture Collection (ATCC).

2.5. Cytotoxicity assay

For the determination of cell viability, cells were seeded into 96-well culture plates (Nunclon) at a density of 6000 cells/well in 200 µL aliquots. Cells were allowed to attach

for 24 h in a humidified 5% CO_2 incubator at 37°C. Test compounds were solubilized in DMSO before further dilution with growth medium. The final concentration of DMSO in the wells never exceeded 0.25%. Cisplatin (positive control), tomatidine or solasodine at concentrations ranging between 0.1 and 1000 μM, was added to test wells while no compound was added to the control cells. Cells were exposed to the test compounds for 48 h. Immediately following the 48 h incubation period, cell numbers were determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay as previously described (Alley et al., 1988; Brauns et al., 2004). Briefly, cells were incubated with 200 μL MTT (Sigma) (0.5 mg/mL in growth medium) for 4 h at 37°C. The formazan product was then dissolved in DMSO and plates were agitated on a shaker for 5 minutes, before the absorbance was read at 540 nm on multiwell scanning spectrophotometer (Multiskan MS, Labsystems). The values obtained were used to determine the percentage inhibition of cell growth caused by the compounds (Hagopian et al., 1999; Huq et al., 2004).

2.6 Annexin V-FITC/PI

HeLa cells were seeded into 10 cm dishes (Sarstedt) in growth medium at $1.9x10^6$ cells per plate. After a recovery period of 24 h, 2.5 μM cisplatin, 500 μM tomatidine or 500 μM solasodine, or the two alkaloids combined at 100 μM each, were added to test plates while no compound was added to the control cells. The cells were incubated for a further 24 h before the Annexin V–FITC Apoptosis Detection Kit (Beckman Coulter) was used to distinguish between apoptotic and necrotic cells. The reagents were prepared as per kit instructions. The cells were collected in ice-cold PBSA and centrifuged for 5 minutes at

500 x g at 4°C. The supernatant was discarded and the pellet resuspended in ice-cold 1X binding buffer to $2x10^6$ cells/mL. The tubes were kept on ice. To 100 µL of the cell suspensions, 1 µL of annexin V-FITC solution and 5 µL of dissolved PI were added and mixed gently. The tubes were incubated for 15 minutes on ice and in the dark before 400 µL of ice-cold 1X binding buffer was added. The samples were analysed on a Beckman Coulter FC500 flow cytometer. A minimum of 10 000 events were acquired per sample.

2.7 Cell cycle analysis

The cell suspensions prepared for Annexin V-FITC/PI staining were also used for cell cycle analysis. Cells were fixed and stained using the Coulter®DNA Prep™ Reagents Kit (Beckman-Coulter). The cells were analysed on a Beckman-Coulter FC500 Flow Cytometer. A minimum of 10 000 events were acquired for each sample.

2.8 Calculations and statistics

Cytotoxicity tests were performed in quadruplicate. Results were expressed as percentage growth inhibition of control and treatment values were compared to control values using the Two-sample Students *t*-test. IC_{50} values for growth inhibition was derived from a nonlinear regression model (curvefit) based on sigmoidal dose response curve (variable) and computed using GraphPadPrism 4 (Graphpad).

3. Results

3.1 Tomatidine and Solasodine

The phytochemical analysis of ground berries of *S. aculeastrum* afforded two steroid glycosides, tomatidine and solasodine. The structures of the compounds were established

with the aid of NMR spectroscopic techniques in addition to comparison with data found in the literature (V'azquez et al., 1997; Weissenberg, 2001; Wanyonyi et al., 2002).

3.2 *In vitro* antitumour assay

The comparative growth inhibition results (Figure 1) suggest that, at 10 and 100 μM concentrations; tomatidine and solasodine were both most effective against HeLa cervical cancer cells. There were no significant differences between the results obtained with tomatidine and solasodine on any of the three cell lines. The positive control, cisplatin, which is a well know anticancer agent, consistently performed better than tomatidine and solasodine at the same concentrations ($p<0.001$). By combining the two alkaloids in a 1:1 ratio to obtain final concentrations of 10 or 100 μM, the inhibitory effect was higher than the individual effects, especially at 100 μM. It should be noted that the total concentration of the two alkaloids was 10 or 100 μM (Fig 1). Therefore it appears as if the two compounds had a synergistic effect, at least at the higher concentration of 100 μM (50 μM of each of tomatidine and solasodine).

The IC_{50} values confirmed that tomatidine and solasodine had the greatest inhibitory effect on HeLa cells (Table 1). In HeLa cells, the IC_{50} of the combined alkaloids was lower than the value for solasodine and unchanged from that of tomatidine. The IC_{50} values of the two compounds combined was also lower in HT29 and MCF7 cells than for the individual compounds, again suggesting synergistic effects.

3.3 Cell cycle analysis

To establish whether the alkaloids inhibited cell growth by blocking cells in a certain phase of the cell cycle and/or inducing apoptosis, cellular DNA was stained with PI and cells analysed using flow cytometry. The sub-G_1 phase of the cell cycle was used to calculate the percentage of apoptotic cells (Chang et al., 1998). Table 2 shows that a 24 h exposure to cisplatin, the positive control increased the number of apoptotic cells from 1.7% in control samples to 21.2%. At the same time, there was a delay in the S phase, with an increase from 19.2% to 52.1%. This was expected according to previously published reports (Shapiro & Harper, 1999). Tomatidine and solasodine on their own (500 μM) and in a 1:1 combination (100 μM each) caused an increase in the percentage inhibition of cells in the G_0/G_1 phase but no increase in apoptosis was observed (Table 2).

3.4 Annexin V-FITC assay

To confirm the apoptosis, Annexin V-FITC/PI staining was done and again the results obtained after the flow cytometry, showed very low apoptotic indices for the two compounds on their own (Table 3). In this assay, however, there was a moderate increase in the apoptotic index when the two alkaloids were both present at 100 μM each. The percentages of cells that stained with PI and therefore, underwent necrotic death were higher than the control for all treatments. Cisplatin was the only treatment that gave a higher percentage apoptotic when compared to necrotic cells.

4. Discussion

S. aculeastrum grows widely in South Africa, and is used for the treatment of many infectious illnesses (Koduru et al., 2006a). In recent years it has been used clinically to

treat cancers and has demonstrated the ability to inhibit tumour development (Koduru et al., 2006b). However, the mechanism underlying its anticancer effect is not clear. The results of this study have demonstrated that compounds from *S. aculeastrum* had significant inhibition on tumour cell growth by blocking certain phase of the cell cycle. Previous studies have shown that solasodine inhibits the growth of Hep3B (Chang et al., 1998), HT29, HepG2 (Lee et al., 2004) and human 1547 osteosarcoma cells (Trouillas et al., 2005). The aglycons tomatidine and solasodine were shown to be less active than their respective glycoalkaloids in inhibiting HT29 and HepG2 cell growth (Lee et al., 2004). Contradictory reports were found regarding the induction of apoptosis by solasodine. Chang et al (1998) found that 11 and 22 μM solamargine, but not its aglycon solasodine, was able to induce apoptosis in HepG2 cells even though they had comparable growth inhibitory properties. Trouillas et al (2004) showed DNA fragmentation when human 1547 osteosarcoma cells were treated with 40 μM solasodine for 24 h, suggesting apoptosis induction. Our results on HeLa cells confirmed that of Chang et al (1998), with no evidence of apoptosis, even at the much higher concentration of 500 μM solasodine that was used in this study. Instead, the cell cycle was blocked in the G_0/G_1 phase by both tomatidine and solasodine. It is possible that the contradictory results were obtained because of the different cell types that were used in the different studies and that these compounds are only capable of inducing apoptosis in specific cell types. No previous reports were found in literature about the ability of tomatidine to induce apoptosis; the present study showed no apoptosis induction in HeLa cells despite the block in G_0/G_1 phase. Friedman et al (2005) have shown that specific ratios of α-solanine and α-chaconine exhibit synergistic anticarcinogenic activities. This study

showed for the first time that the two alkaloids, tomatidine and solasodine, have a synergistic effect on growth inhibition. The relatively high percentages of necrotic cells observed with the Annexin V/FITC assay can possibly be explained by the observation made by Friedman et al (2005) that some glycoalkaloids disrupt cell membranes.

Inhibiting tumor growth has been a continuous effort in cancer treatment. A reduction of cell growth and an induction of cell death are two major events to inhibit tumor growth. In this study, we demonstrated that, at low concentrations, some compounds from *S. aculeastrum* caused significant inhibition of growth in HeLa, MCF7 and HT29 cells. The inhibitory effect of these compounds on cell growth implies that they may have a general function in antitumour cell growth. This is not unexpected, since cancer cells have developed the capacity of increased proliferation through a variety of growth signal pathways. This includes elevated external growth factors, increased intracellular matrix signal via integrin (Lukashev & Werb, 1998), and Ras protein mutation-derived constitutive mitogenic signals (Medema & Bos, 1993), resulting in growing neoplasm, that causes destruction and atrophy of the surrounding tissue and adjacent organs. In a specific tumor, one pathway may play a more important role than the others. Tomatidine and solasodine may act on more than one pathway. Nevertheless, different sensitivities of tumor cells to the growth inhibitory effect of treatments of the pure compounds were observed. It is worth mentioning that there are several reports on solasodine and tomatidine derivatives possessing pharmacological effects, including significant cytotoxic activity *in vitro* against a variety of cancer cell lines (Nakamura et al., 1996; Chang et al., 1998; Lee et al., 2004; Trouillas et al., 2005; Koduru et al., 2006c). It thus has a great

advantage over other types of treatments such as chemotherapy and more recently, hormonal treatments. *S. aculeastrum* contains a variety of compounds that may act on different pathways of tumor cell growth and survival. The molecular mechanisms underlying these effects await further investigation.

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References

Agnew ADQ, Agnew S (1994): *Upland Kenya Wild Flowers. A Flora of the Ferns and Herbaceous Flowering Plants of Upland Kenya*. Nairobi, East Africa Natural History Society.

Alley MC, DA Scudiero, A Monks, ML Hursey, MJ Czerwinski, DL Fine, BJ Abbott, JG Mayo, RH Shoemaker, MR Boyd (1988): Feasibility of drug screening with panels of human tumour cell lines using a microculture tetrazolium assay. *Cancer Research 48*: 589-601.

Brauns SC, Milne P, Naude R, M Van de Venter (2005): Selected cyclic dipeptides inhibit cancer cell growth and induce apoptosis in HT-29 colon cancer cells. *Anticancer Research 24*: 1713-1720.

Chang LC, Tsai TR, Wang JJ, Lin CN, KW Kuo (1998): The rhamnose moiety of solamargine plays a crucial role in triggering cell death by apoptosis. *Biochemical and Biophysical Research Communications 242*: 21-25.

Christop B (2003): The Bcl-2 protein family: sensors check point for life or death decision. *Molecular Immunology 39*: 615–647.

Friedman M, Lee KR, Kim HJ, Lee IS, Kozukue N (2005): Anticarcinogenic effects of glycoalkaloids from potatoes against human cervical, liver, lymphoma, and stomach cancer cells. *Journal of Agricultural and Food Chemistry 53*: 6162-6169.

Hagopian GS, GB Mills, AR Khokhar, RC Bast, ZH Jr. Siddik (1999): Expression of *p53* in Cisplatin-resistant Ovarian Cancer Cell Lines: Modulation with the Novel Platinum Analogue (*1R, 2R*-Diaminocyclohexane)(*trans*-diacetato) (dichloro)-platinum (IV). *Clinical Cancer Research 5*: 655-663.

Huq F, H Daghriri, JQ Yu, H Tayyem, P Beale, M Zhang (2004): Synthesis, characterization, activities, cell uptake and DNA binding of [{*trans*-$PtCl(NH_3)_2$}{μ-$(H_2N(CH_2)_6NH2)$}{*trans*-$PdCl(NH_3)_2$]$(NO_3)Cl$. *European Journal of Medicinal Chemistry 39*: 947–958.

Hutchings A, Scott AH, Lewis G, AB Cunningham (1996): *Zulu medicinal plants, an inventory*. Pietermaritzburg, University of Natal Press.

Kerr JFR, Wyllie AH, Currie AR (1972): Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer 26*: 239–257.

Koduru Srinivas, DS Grierson, AJ Afolayan (2006a): Antimicrobial activity of *Solanum aculeastrum*. *Pharmaceutical Biology 44*(4): 283-286.

Koduru Srinivas, DS Grierson, MA Aderogba, JN Eloff, AJ Afolayan (2006b): Antioxidant activity of *Solanum aculeastrum* (Solanaceae) berries. *International Journal of Pharmacology 2*: 262-264.

Koduru Srinivas, DS Grierson, M van de Venter, AJ Afolayan (2006c): *In vitro* antitumour activity of *Solanum aculeastrum* berries on three carcinoma cells. *International Journal of Cancer Research 2*: 387-402.

Koduru Srinivas, DS Grierson, AJ Afolayan (2006d): The Foliar Micro-morphology of *Solanum aculeastrum*, a Medicinal Plant of South Africa. *Pakistan Journal of Biological Sciences 9*(6): 1056-1060.

Lee KR, Kozukue N, Han JS, Park JH, Chang EY, Baek EJ, Chang JS, Friedman M (2004): Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells. *Journal of Agriculture and Food Chemistry 52*(10): 2832-2839.

Los M, CJ Burek, C Strol, K Bnedyk, H Hug, A Mackiewicz (2003): Anticancer drugs of tomorrow: apoptotic pathways as targets for drug design. *Drug Discovery Today 8*: 67–77.

Lukashev ME, Z Werb (1998): ECM signalling: orchestrating cell behaviour and misbehaviour. *Trends in Cell Biology 8*: 437–441.

Medema RH, JL Bos (1993): The role of p21ras in receptor tyrosine kinase signaling. *Critical Review of Oncology 4*: 615–661.

Nakamura T, C Komori, YY Lee, F Hashimoto, S Yahara, T Nohara, A Ejima (1996): Cytotoxic Activities of *Solanum* Steroidal Glycosides. *Biological and Pharmaceutical Bulletin 19*(4): 564-566.

Reed JC (2001): Apoptosis regulatory proteins as targets for drug discovery. *Trends in Molecular Medicine 7*: 314–318.

Shapiro GI, JW Harper (1999): Anticancer drug targets: cell cycle and checkpoint control. *The Journal of Clinical Investigation 104*: 1645-1653.

Trouillas P, Corbiere C, Liagre B, Duroux JL, Beneytout JL (2005): Structure-function relationship for saponin effects on cell cycle arrest and apoptosis in the human 1547 osteosarcoma cells: a molecular modelling approach of natural molecules structurally close to diosgenin. *Bioorganic and Medicinal Chemistry 13*: 1141-1149.

V´azquez Alvaro, Gabriel Gonz´alez, Fernando Ferreira, Patrick Moyna, Lennart Kenne (1997): Glycoalkaloids of *Solanum commersonii* Dun. ex Poir. *Euphytica 95*: 195–201.

Wanyonyi AW, Sumesh CC, Gerld M, Udo E, Wilson MN (2002): Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum*. *Phytochemistry 59*: 79-84.

Weissenberg M 2001. Isolation of solasodine and other steroidal alkaloids and sapogenins by direct hydrolysis-extraction of Solanum plants or glycosides therefrom. *Phytochemistry 58*: 501–508.

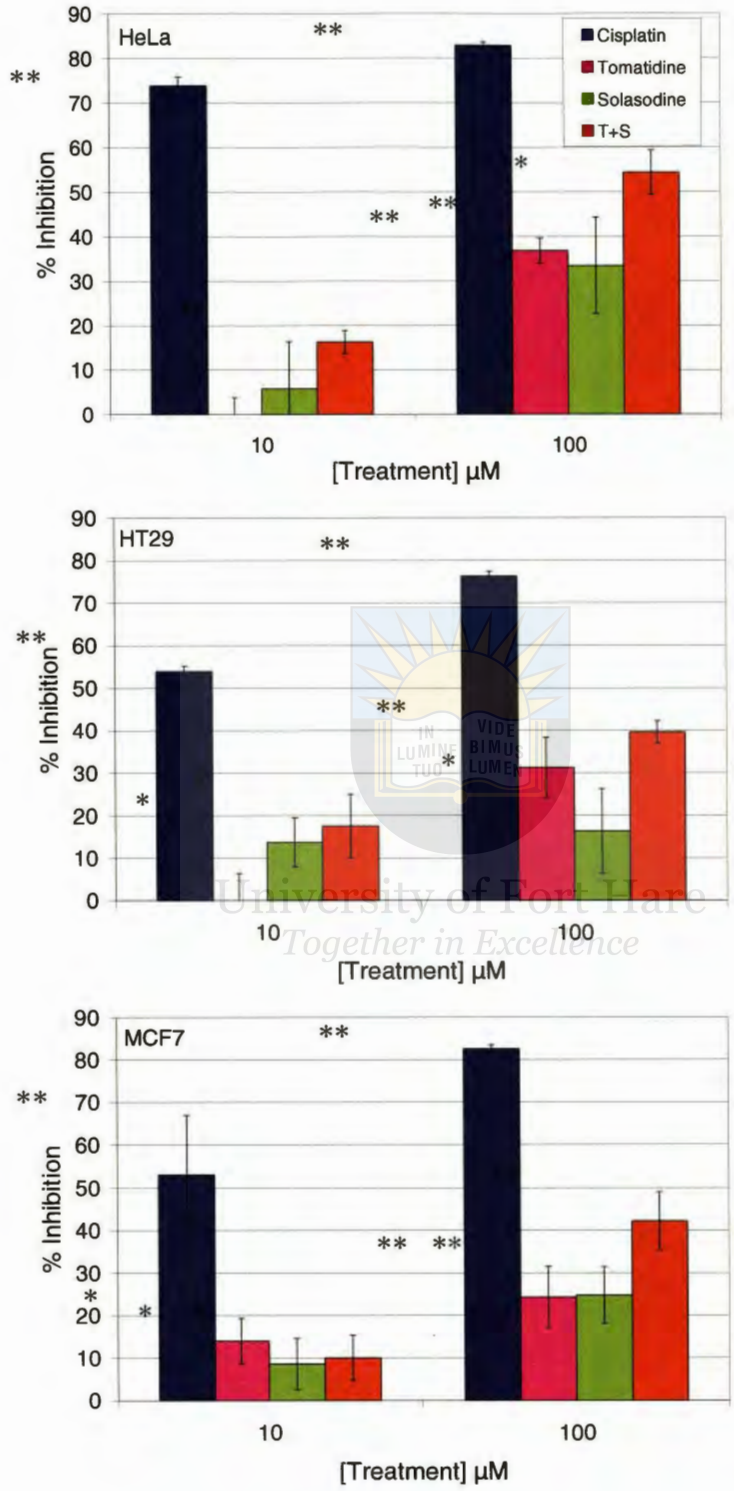

Figure 1: Comparison of the growth inhibitory effects of two concentrations of tomatidine and solasodine alone and in combination (T+S) with that of cisplatin. HeLa, HT29 and MCF7 cells were exposed to 10 or 100 µM of cisplatin, tomatidine, solasodine or the latter two combined for 48 hours. Mean ± SD (n = 4). **p<0.001; *p<0.05 compared to control.

Table 1: IC_{50} values for the test compounds as obtained from log dose response curves using GraphPad Prism. Cells were incubated in the presence of cisplatin, tomatidine, solasodine or a combination of the latter two for 48 hours.

Cell line	IC_{50} (μM)			
	Cisplatin	Tomatidine	Solasodine	Tomatidine + Solasodine
HeLa	2.5	141.7	252.5	149.3
HT29	2.5	309.0	725.5	169.0
MCF7	2.5	350.1	888.0	126.9

Table 2: Results of cell cycle analysis using nuclear PI staining. HeLa Cells were exposed to compounds at the indicated concentrations for 24 hours before cell cycle analysis was performed.

Treatment	Sub G_1 (%)	G_0/G_1 (%)	S (%)	G_2/M (%)
Control	1.7	55.6	19.2	23.5
Cisplatin (2.5 μM)	21.2	12.4	52.1	14.3
Tomatidine (500 μM)	1.7	64.2	18.8	15.3
Solasodine (500 μM)	2.1	66.8	16.6	14.4
Tomatidine + Solasodine (100 μM each)	2.5	59.2	24.4	14.0

Table 3: Apoptosis results from Annexin V-FITC/PI analysis. HeLa Cells were exposed to compounds at the indicated concentrations for 24 hours before the experiment was performed.

Treatment	Apoptotic (%)	Necrotic (%)
Control	1.6	3.8
Cisplatin (2.5 μM)	9.6	7.0
Tomatidine (500 μM)	0.3	6.3
Solasodine (500 μM)	1.2	5.1
Tomatidine+Solasodine (100 μM each)	4.2	6.6

Chapter 9

Effect of high temperatures on seed germination of *Solanum aculeastrum*

CHAPTER 9

EFFECT OF HIGH TEMPERATURES ON SEED GERMINATION OF *SOLANUM ACULEASTRUM*

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Effect of High Temperatures on Seed Germination of *Solanum aculeastrum*

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Abstract: *Solanum aculeastrum* is a medicinal plant that showed low seed germination under laboratory conditions. The objective of this study was to establish whether germination could be improved by the exposure of its seeds to high temperatures such as those registered on surface soils during natural fires. Our results indicated that temperatures of 100 and 120°C applied to green mature seeds of *S. aculeastrum*, for 45 to 60 min may break their innate dormancy, thus stimulating their subsequent germination to more than 85%. Germination in dry seeds, however, showed very low germination when subjected to high temperatures. Where the effect of smoke was tested on the germination of the green mature and dry seeds of *S. aculeastrum*, no stimulation of germination was observed. The ecological implications of these observations are discussed.

Key words: Fire, high temperature, *S. aculeastrum*, seed germination, smoke, Solanaceae

INTRODUCTION

High temperatures, especially when generated by fire, are an ecological factor which plays an important role in the evolution of ecosystems (Trabaud, 1980). It may affect plant regrowth potential and seed germination (Gibson *et al.*, 1990; Canadell *et al.*, 1991). Several authors (Auld, 1986; Tarrega *et al.*, 1992; Bradstock *et al.*, 1992; Harrington and Driver, 1995) observed that after fire, the seed germination rates of many species increased. In Africa, bush fire is a wide-spread feature of the dry savannas and contributes largely to changes in the composition of vegetation communities (Gillon, 1983; Greerling, 1985; Monnier, 1990; Kozlowski, 2000). A number of reasons have been given for the enhancement of seed germination by high temperatures caused by fire. Among these are a reduction of inhibitory substances in soil and litter (Keeley *et al.*, 1985; Gonzalez-Rabanal and Casal, 1995), chemical stimulation from charred wood (Keeley *et al.*, 1985), fracturing of hard seed coats, stimulation of seed embryos, desiccation of seed coats and release of ethylene and ammonia in the plant-derived smoke (Keeley, 1987; Brown, 1993; Brits *et al.*, 1993; Baskin and Baskin, 1998).

Solanum aculeastrum Dunal, a member of the family Solanaceae, is frequently used in herbal medicine. It is a multi-branched shrub, 1-5 m high, heavily armed with large prickles and is wide-spread in South Africa. Local healers use the extremely bitter fruits for the treatment of various diseases in humans and domestic animals (Hutchings *et al.*, 1996). Both mature and immature berries contain the poisonous alkaloid, α-solanine (Hutchings *et al.*, 1996). Other bioactive compounds that have been isolated from this plant include solaculine A (Wanyonyi *et al.*, 2002) from the root bark and solamargine, beta-solamarine, solasonine and solasodine from the fruits (Drewes and Van Staden, 1995; Wanyonyi *et al.*, 2002). The fresh and boiled berries are used as a cure for jigger wounds and gonorrhoea respectively (Agnew and Agnew, 1994). Traditional healers of the Eastern Cape Province in South Africa also use the plant for the treatment of neoplasm, particularly breast cancer.

S. aculeastrum is increasingly being exploited in South Africa due to its widespread medicinal use. Thus, there is an urgent need to conserve this species. Reinforcement of wild plant populations using individuals raised *exsitu* is considered a valid means of reducing the risk of extinction of overexploited plant populations (Bowes, 1999). If the future demand for this plant is to be met, it is imperative that this species be domesticated and commercially cultivated. Techniques for efficient low-cost cultivation practices are determined, to a large extent, by the germinability of the seeds. However, each species has peculiar requirements for seed germination as a result of adaptive radiation into patchy and changing environments (Schütz and Milberg, 1997). Many species respond well to sterile *in vitro* conditions, including a nutrient rich medium and also phytohormone supply (Fay, 1992; Pence, 1999). However, for some species, these techniques are not necessary for successful seed germination and would waste resources if

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used. Appropriate techniques must be selected based on the requirements of each species (Fay, 1994; Benson *et al.*, 2000).

Requirements for seed germination in *S. aculeastrum* have not been investigated. This study was designed to evaluate the *exsitu* requirements for optimal seed germination in *S. aculeastrum*. Specifically, the aim of this project was to investigate the effect of high temperature and smoke on the germination of its seeds under controlled environmental conditions.

MATERIALS AND METHODS

Seed collection: Two types of berries of *S. aculeastrum* were collected from plants naturally growing in the wild at Kayalethu village in the Eastern Cape Province of South Africa (latitudes 30°00'-34°15'S and longitudes 22°45'-30°15'E), the green looking mature berries and the yellow, dry-looking mature fruits. For the purpose of this report, they are referred to as green mature seeds and yellow dry seeds respectively. The plant was identified at the Botany Department, University of Fort Hare and a voucher specimen (Vedic Med 2005/16) was prepared and deposited in the Griffen Herbarium of the University. The seeds were removed from both groups of berries and used immediately for the germination experiments. This work was carried at the Botany Department, University of Fort Hare, during February, 2005 to April, 2005.

Treatment with high temperature: Three replicates of 50 seeds each were exposed to 60°C for 30, 45 or 60 min, respectively. Prior to the treatment, the seeds were placed in Petri dishes containing two discs of Whatman No. 1 filter paper. The filter papers were moistened with distilled water to ensure adequate moisture for seed germination. The petri dishes containing the seeds were then left and observed on the side benches at room temperature. Seeds were considered to have germinated when the radicle had grown 2 mm (Come, 1970; De Villalobos *et al.*, 2002). The number of the germinated seeds were recorded every 24 h over 21 days (De Villalobos *et al.*, 2002). Same treatments were repeated at 80°C, 100°C and 120°C.

Treatment with smoke: Instant smoke (Baxter *et al.*, 1994; Sparg *et al.*, 2005) was obtained from Kirstenbosch, National Botanical Garden, Cape Town, South Africa. Each smoke-impregnated paper was soaked in 50 mL water for 5 min to allow the diffusion of smoke into the water. Seeds were then placed in the smoke-impregnated water for 12, 24 and 48 h, before they were transferred into Petri dishes, containing two discs of Whatman No. 1 filter paper. The filter papers were moistened as needed with distilled water to ensure adequate moisture for seed germination. Three replicates of 50 seeds each were used for each treatment. Seeds were considered to have germinated when the radicle had grown 2 mm. The number of the germinated seeds were recorded every 24 h over 21 days. Untreated seeds were used as control.

Germination percentage was calculated as the proportion of germinated seeds within a replicate. Significance differences within the means of the treatments and the controls were calculated using the LSD statistical test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Significant differences ($p<0.05$) in percentage germination was observed between temperatures and the period of exposure of the seeds of *S. aculeastrum* (Fig. 1). The germination of green mature seeds was significantly higher than the germination of dry seeds when exposed to 100°C and 120°C for 30, 45 and 60 min. In all temperature treatments, the highest germination was recorded in green mature seeds exposed to 100° C for 45 min and 120° C for 45 and 60 min, the germination being one hundred percent (Fig. 1). Higher germination percentages of green mature seeds were also observed when they were exposed to 100°C for 30 and 60 min (81.33 and 85.33%, respectively). Dry seeds showed very minor responses in germination to the increased temperatures except when exposed to 120°C for 60 min (26% compared to the control). There are significant differences between germination percentages of control and green mature seeds at all temperatures (Fig. 1). It was also noted that there was a difference between the germination percentages of the green mature and dry seeds control treatments (3.2 and 0%, respectively), which might indicate that the seeds become dormant on ripening.

Our results indicate generally that high temperatures applied for a few minutes to green mature and yellow, dry seeds of *S. aculseastrum* may break their innate dormancy, allowing their subsequent germination (Fig. 1). The optimum temperatures needed for breaking the innate seed dormancy in *Acacia suaveolens* were 60-80°C for a range of exposure times of 1-360 min. Seeds exposed to 100°C for less than 2 h also broke dormancy (De Villalobos *et al.*, 2002). At temperatures above 100°C, only short exposure times led to successful germination, while longer exposure caused seed death (Auld, 1986). Auld and O'Connel (1991) found that for 35 species of Leguminosae under study, seed dormancy was broken in over half the species by exposure to temperatures over

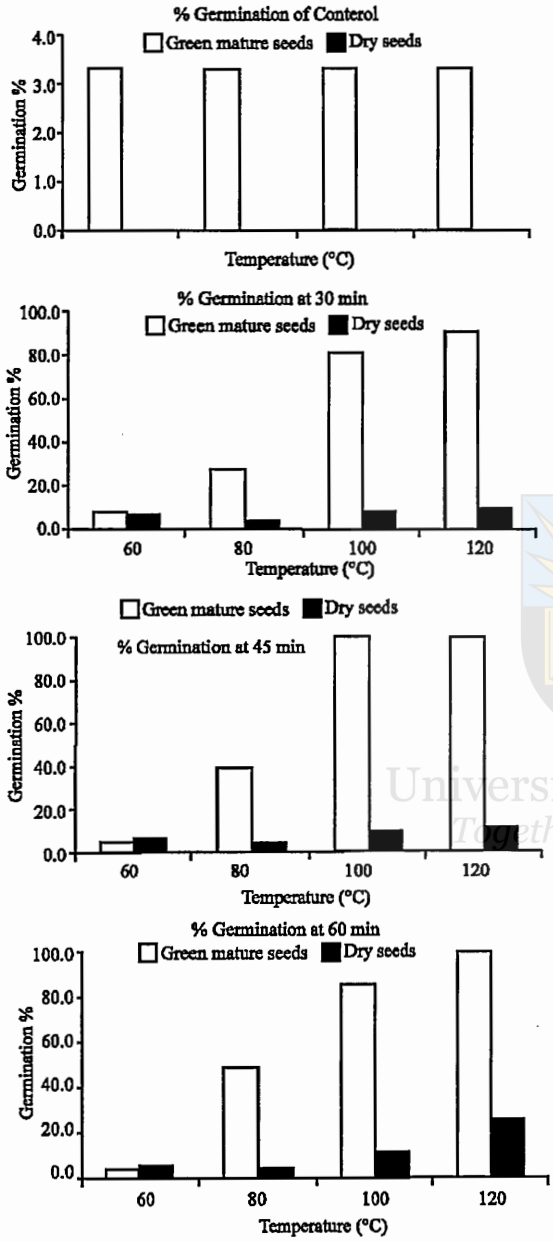

Fig. 1: Effects of the temperature and exposure time on seed germination of *S. aculeastrum*

60°C, whereas dormancy was broken in all those species with exposures above 80°C. Tarrega *et al.* (1992) reported that moderate heat treatments (70°C and 100°C) significantly increased the germination rate of *C. scoparius* and *G. florida* seeds. Where the effect of smoke was tested on the germination of the green mature and yellow-dry seeds of *S. aculeastrum*, no stimulation of germination was observed.

In generally, our laboratory results demonstrated that high temperatures might stimulate the germination of *S. aculeastrum* seeds. This might encourage, under favorable environmental conditions, the post-fire establishment of seedlings. However, plant response to fire depends on the interaction of several environmental and biotic factors. Therefore, more detailed studies on this subject should be conducted in the future.

ACKNOWLEDGMENT

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REFERENCES

Agnew, A.D.Q. and S. Agnew, 1994. Upland Kenya Wild Flowers. A Flora of the Ferns and Herbaceous Flowering Plants of Upland Kenya. East Africa Natural History Society, Nairobi.

Auld, T.D., 1986. Population dynamics of the shrub *Acacia suaveolens* (Sm.) Willd.: Fire and ransition to seedlings. Aust. J. Ecol., 11: 373-385.

Auld, T.D. and M.A. O'Connel, 1991. Predicting patterns of post-fire germination in 35 eastern Australian Fabaceae. Aust. J. Ecol., 16: 53-70.

Baskin, C.C. and J.M. Baskin, 1998. Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press, New York.

Baxter, B.J.M., J. Van Staden, J.E. Grander and N.A.C. Brown, 1994. Plant-derived smoke and smoke extracts stimulate seed germination of the fire-climax grass *Themeda triandra* Forssk. Environ. Exp. Bot., 34: 217-223.

Benson, E.E., J.E. Danaher, I.M. Pimbley, C. Anderson, J.E. Wake, S. Daley and L.K. Adams, 2000. *In vitro* micropropagation of *Primula scotica*: A rare Scottish plant. Biodiversity and Conservation, 9: 711-726.

Bowes, B.G., 1999. A Colour Atlas of Plant Propagation and Conservation.Manson Publishing Ltd, London.

Bradstock, R.A., T.D. Auld, M.E. Ellis and J.S. Cohn, 1992. Soil temperatures during bushfires in semi-arid, mallee shrublands. Aust. J. Ecol., 17: 433-440.

Brits, G.J., F.J. Calitz, N.A.C. Brown and J.C. Manning, 1993. Desiccation as the active principle in heat stimulated seed germination of *Leucospermum* R. Br. (Proteaceae) in fynbos. New Phytologist, 125: 397-403.

Brown, N.A.C., 1993. Promotion of germination of fynbos seeds by plant-derived smoke. New Phytologist, 123: 575-583.

Canadell, J., F. Loret and L. Lo'pez-Soria, 1991. Resprouting vigour of twoo Mediterranean shrub species after experimental fire treatments. Vegetatio, 95: 119-126.

Come, D., 1970. The Hindrances to Growth. Masson, Paris, pp: 162.

De Villalobos, A.E., D.V. Pelaezwz, R.M. Boowz, M.D. Mayorz and O.R. Elia, 2002. Effect of high temperatures on seed germination of *Prosopis caldenia* Burk. J. Arid Environ., 52: 371-378.

Drewes, F.E. and J. Van Staden, 1995. Aspects of the extraction and purification of solasodine from *Solanum aculeastrum* tissues. Phytochem. Anal., 6: 203-206.

Fay, M.F., 1992. Conservation of rare and endangered plants using *in vitro* methods. *In vitro* Cellular Developmental Biology-Plant, 28: 1-4.

Fay, M.F., 1994. In what situations is *in vitro* culture appropriate to plant conservation? Biodiversity and Conservation, 3: 176-183.

Gibson, D.J., D.C. Hartnett and G.L.S. Merril, 1990. Fire temperature heterogeneity in contrasting fire prone habitats: Kansas tallgrass prairie and Florida sandhill. Bulletin of the Torrey Botanical Club, 117: 349-356.

Gillon, D., 1983. The Fire Problem in Tropical Savannas. In: Bouliere, F. (Ed.), Tropical Savannas Ecosystems of the World. Elsevier, Amsterdam, pp: 617-641.

Gonzalez-Rabanal, F. and M. Casal, 1995. Effect of high temperatures and ash on germination of ten species from gorse shrubland. Vegetatio, 116: 123-131.

Greerling, C., 1985. The status of the woody species of Sudan and Sahel zones of West Africa. For. Ecol. Manage., 13: 247-255.

Harrington, G.N. and M.A. Driver, 1995. The effect of fire and ants on the seed-bank of a shrub in a semiarid grassland. Aust. J. Ecol., 20: 538-547.

Hutchings, A., A.H. Scott, G. Lewis and A.B. Cunningham, 1996. Zulu Medicinal Plants, An Inventory. University of Natal Press, Pietermaritzburg.

Keeley, J.E., 1987. Role of fire on seed germination on woody taxa in California chaparral. Ecology, 68: 434-443.

Keeley, J.E., B.A. Morton, A. Pedrosa and P. Trotter, 1985. Role of allelopathy, heat and charred wood in the germination of chaparral herbs and suffrutescents. J. Ecol., 73: 445-458.

Kozlowski, T.T., 2000. Response of woody plants to human induced environmental stresses: Issues, problems and strategies for alleviating stress. Crit. Rev. Plant Sci., 19: 91-170.

Mannier, Y., 1990. Dust and ash. Ministry for the Co-operation and Developpemnet, Paris.

Pence, V., 1999. The application of biotechnology for the conservation of endangered plants. In: Benson, E.E. Ed., 1999. Plant Conservation Biotechnology, Chapter 15, Taylor and Francis, London, pp: 227-241.

Schütz, W. and P. Milberg, 1997. Seed dormancy in *Carex canescens*: Regional differences and ecological consequences. Oikos, 78: 420-428.

Sparg, S.G., M.G. Kulkarni, M.E. Light and J. Van Staden, 2005. Improving seedling vigour of indigenous medicinal plants with smoke. Bioresou. Technol., 96: 1323-1330.

Steel, R.G.D. and J.H. Torrie, 1960. Principles and Procedures of Statistics. New York, McGraw-Hill.

Tarrega, R., L. Calvo and L. Trabaud, 1992. Effect of high temperatures on seed germination of two woody Leguminosae. Vegetatio, 102: 139-147.

Trabaud, L., 1980. Biological and ecologic impact of fires of vegetation on the organization, the structure and the evolution of the vegetation of the garrigues of Bas-Lauguedoc. University of Languedoc, Montpellier.

Wanyonyi, A.W., C.C. Sumesh, M. Gerld, E. Udo and M.N. Wilson, 2002. Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum*. Phytochemistry, 59: 79-84.

Chapter 10

General Discussion and Conclusions

CHAPTER 10

GENERAL DISCUSSION AND CONCLUSIONS

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Cancer is one of the most common diseases of this century and it is spreading fast with increasing incidences (Premalatha and Rajgopal 2005). In South Africa, incidences of cancer are increasing every year in all the ethnic groups (Human and Bajic 2002). In this country, many people still use phytomedicines as an alternative or to supplement modern western drugs for the treatment of diseases, including cancer (Van Wyk *et al.* 1997).

The Eastern Cape Province is particularly known for its richness in plant species (Phillipson, 1987), many of which possess pharmacological properties. Despite this wealth of natural pharmaceuticsals, only a small proportion of the plants have been scientifically investigated (Coetzee, 2000). Traditional healers and herbalists in the province, for many years, have treated cancer patients using various plant species (Koduru *et al.* 2006a, 2006b). Despite the long history of this practice, the knowledge and experience of these herbalists have not been scientifically documented to a significant extent.

Ethnobotanical information

At the beginning of this study, an ethnobotanical survey of plants used for the treatment of cancer was carried out in the Eastern Cape (Chapter 2). Information on the names of plants, the parts used and the methods of preparation was collected through questionnaires which were administered to the herbalists, traditional healers and rural dwellers. Information collected from these people revealed 17 plant species that are used for the treatment of cancer in the province. Some of the healers are well trained to recognize cancers (e.g. the nursing staff). In other instances, patients are diagnosed at a hospital or clinic, and than

present themselves for traditional treatment. Often there were language and cultural barriers making it difficult to fully understand and interpret the interview. *Solanum aculeastrum* was the most frequently and commonly reported plant species. The choice of *S. aculeastrum* for further study was thus, based on the ethnomedical information from indigenous peoples of South Africa who have been using the plant for years against cancer, especially breast, colon and cervical cancers.

The foliar micro-morphology and the isolation of volatile compounds

Scientific interest in plant trichomes is based on their functional importance as well as on the economic usefulness of some trichome-produced products (Valkama *et al*. 2003). Such compounds which showed antitumour activity have been isolated from some members of Solanaceae (Guo and Wagner 1995). Histochemical studies by Afolayan and Meyer (1995) and Ascensao *et al* (1999) have shown that the secretions from most trichomes contain terpenoids and flavonoid aglycones. The structure and distribution of foliar appendages on the leaves of *S. aculeastrum* were also examined by scanning electron microscopy. Both glandular and non-glandular trichomes were observed. These differed from each other in morphology and location on the leaf (Chapter 3). Based on the observation from this study, it is hypothesized that the bioactive therapeutic compounds secreted by *S. aculeastrum* may be produced in the glandular trichomes. The GC-MS analyses of the volatile oils obtained by hydrodistillation of the leaves of the plant yielded 31 volatile compounds, representing 84.5% of the total oil composition. The oil consisted mainly alkanes (17.5%), aldehydes (17%) and aromatic hydrocarbons (15.2%). The hexane fraction of the methanolic extract of the berries of the plant was also subjected to GC-MS analyses, yielding 16 compounds, which accounted for 87.1% of the total volatiles (Chapter 4).

Antibacterial and antifungal activity

The berries and leaf extracts of *S. aculeastrum* were investigated for *in vitro* antimicrobial activity against 10 bacterial and five fungal strains. The methanolic extracts of both the fruits and the leaves showed appreciable activity against Gram-positive and Gram-negative bacteria ranging from 4.0 to10.0 mg/ml. While the methanol extracts were the most active material, the water extracts showed the least activity against the bacteria. The methanol extracts were particularly inhibitory to the growth of the fungi with percentage inhibition ranging from 60.26% to 100% and 56.0% to 100% on *Aspergillus flavus* and *Penicillium notatum* respectively. The acetone extracts were active against *A. flavus* (100%) and *P. notatum* (64.81%) while the water extract of the fruit significantly inhibited the growth of *P. notatum* (69.89%). The most resistant organisms were *Aspergillus niger, Candida albicans* and *Fusarium oxysporum*. The results therefore validated the use of this plant for the treatment of infectious diseases.

Anticancer activity of the crude extracts

The antiproliferative activities of *S. aculeastrum* were studied *in vitro* using three human tumour cell lines (HeLa, MCF7 and HT29). Methanolic extracts of the fruits had the highest antiproliferative activity with IC_{50} between 17.1 and 41.9 µg/ml while the activities of their aqueous extracts ranged between 27.9 and 48.5 µg/ml. The leaf extracts had no anticancer activity under the experimental conditions tested. The methanol and water extracts of *S. aculeastrum* showed inhibitory effect on cancer cell lines in the *in vitro* studies. Again, the findings from these experiments confirmed and validated the use of this plant by the traditional healers for the treatment of cancer.

The antioxidant activity

The antioxidant activity of the crude methanol, acetone and water extracts of the berries of *S. aculeastrum* was examined by DPPH radical-scavenging. The methanol and water extracts had moderate antioxidant activity ranging between 53.1 to 65.5 μg/ml, while the acetone extract did not demonstrate significant antioxidant activity at the tested concentrations. The higher antioxidant activity of this plant exhibited by the water extract may be due to the presence of substantial amounts of polar constituents from the plant material. In *S. aculeastrum*, steroidal alkaloid glycosides were isolated from the berries (Wanyonyi et al., 2002). This study emphasizes the antioxidant potential of *S. aculeastrum*. This observation is particularly significant considering the fact that most cancer diseases are initiated by the activities of free radicals in our bodies (Bagchi and Puri 1998).

The isolation of compounds, their anticancer activities and induction of apoptosis

Two closely related bioactive compounds (solasodine and tomatidine) were isolated from *S. aculeastrum* berries. Their spectral data were compared with those found in the literature (Weissenberg 2001; Wanyonyi et al., 2002). Antitumour activities of these compounds were investigated on HeLa, MCF7 and HT29 cancer cell lines. The IC_{50} values confirmed that these compounds had the greatest inhibitory effect on HeLa cells. The IC_{50} of the combined alkaloids (149.3 μM) was lower than the value for solasodine (252.5 μM) and unchanged from that of tomatidine (141.7 μM). The IC_{50} values of the two compounds combined was also lower in HT29 (169.0 μM) and MCF7 cells (126.9 μM) than for the individual compounds. The alkaloids inhibited cell growth by blocking certain phase of the cell cycle after 24 h exposure with an increase from 19.2% to 52.1%. To confirm the

apoptosis using, Annexin V-FITC/PI staining by flow cytometry showed very low apoptotic indices.

Effects of temperature and smoke on the seed germination

Many high valued medicinal plants are facing the danger of extinction from their populations due to over harvesting by medicinal plant traders. *S. aculeastrum* is one of them. Many conservation projects have been initiated by the researchers on medicinal plants. This study was conducted as a contribution to the conservation of this plant through the study of its germination requirements. *S. aculeastrum* is a medicinal plant that showed low seed germination both on the field and under laboratory conditions. The objective was to establish whether its germination could be improved by the exposure of its seeds to high temperatures such as those registered on surface soils during natural fires. Our results indicated that temperatures of 100 °C and 120 °C applied to green mature seeds of this plant, for 45 to 60 min may break their innate dormancy, thus stimulating their subsequent germination to more than 85%. Germination in dry seeds, however, was low when subjected to high temperatures. Again, exposure of its seeds to smoke did not improve germination. In general, the laboratory results demonstrated that high temperatures might stimulate the germination of *S. aculeastrum* seeds. This might encourage, under favorable environmental conditions, the post-fire establishment of its seedlings.

Conclusions

In this study, solasodine and tomatidine derivatives were observed to be the major constituent of the berries from *S. aculeastrum*. The anticancer properties of these

compounds and the ability to inhibit the growth of the tumour cell lines probably explain the successful use of extracts from this plant by the indigenous people of the Eastern Cape for the treatment of cancer. Ethnomedically directed research such as this, is necessary in order to optimize the search for novel pharmaceuticals that could solve the problems of cancer disease. Recently, a greater emphasis has been placed on research on complementary and alternative medicines used in cancer management. Several studies have been conducted on herbs under a multitude of ethnobotanical grounds. For example, Hartwell (1969a; 1969b; 1969c; 1970a; 1970b; 1971a; 1971b; 1971c; 1971d) has collected data on about 3000 plants, some of which possess anti cancer properties and have subsequently been used as potent anticancer drugs (Pandey 2002). Drug discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer (Newman *et al.* 2000, 2003; Butler 2004). Of all available anticancer drugs between 1940 and 2002, 40% were natural products or natural product-derived with another 8% considered natural product mimics (Newman *et al.* 2003). The results from this study support the traditional use of this plant for the treatment of cancer in the Eastern Cape Province of South Africa, and the whole study has contributed immensely to the search for the cure of cancer among the people of the Easter Cape.

References

Afolayan, A.J. & J.J.M. Meyer. 1995. Morphology and ultrastructure of secreting and non secreting foliar trichomes of *Helichrysum aureonitens* (Asteraceae). *Int J Plant Sci.* 156: 481-487.

Ascensao, L., Mota, L. & M.M. De Castro. 1999. Glandular trichomes on the leaves and flowers of *Plectranthus ornatus*: Morphology, Distribution and Histochemistry. *Annals of Botany* 84: 437-447.

Bagchi, K. & S. Puri. 1998. Free radicals and antioxidants in health and disease. *Eastern Mediterranean Health Journal* 4(2): 350-360.

Butler, M.S. 2004. The role of natural product chemistry in drug discovery. *J Nat Prod.* 67: 2141–2153.

Coetzee, E. 2000. The magic of muti plants. *Farmer's Weekly April* 14:26-29.

Guo, Z. & G.J. Wagner. 1995. Biosynthesis of cembratrienols in cell-free extracts from trichomes of *Nicotiana tabacum*. *Plant Science* 110: 1-10.

Hartwell, J.L. 1971b. Plants used against cancer. A survey. *Lloydia* 34: 204–255.

Hartwell, J.L. 1969a. Plants used against cancer. A survey. *Lloydia* 32: 247–296.

Hartwell, J.L. 1969b. Plants used against cancer. A survey. *Lloydia* 32: 153–205.

Hartwell, J.L. 1969c. Plants used against cancer. A survey. *Lloydia* 32: 78–107.

Hartwell, J.L. 1970a. Plants used against cancer. A survey. *Lloydia* 33: 288–392.

Hartwell, J.L. 1970b. Plants used against cancer. A survey. *Lloydia* 33: 97–194.

Hartwell, J.L. 1971a. Plants used against cancer. A survey. *Lloydia* 34: 386–425.

Hartwell, J.L. 1971c. Plants used against cancer. A survey. *Lloydia* 34: 310–361.

Hartwell, J.L. 1971d. Plants used against cancer. A survey. *Lloydia* 34: 103–150.

Human, S. & V.B. Bajic. 2002. Contribution to Skin Cancer Prevention in South Africa: Modelling the UV Index Utilizing Imprecise Data. *Austrian Journal of Statistics* 31(2,3):169-175.

Koduru Srinivas, D.S. Grierson & A.J. Afolayan. 2006b. Antimicrobial activity of *Solanum aculeastrum*. *Pharmaceutical Biology* 44(4):283-286.

Koduru Srinivas, D.S. Grierson. & A.J. Afolayan. 2006a. Effect of high temperatures on seed germination of *Solanum aculeastrum*. *Asian J Plant Sciences* 5(2): 353-356.

Newman, D.J., G.M. Cragg. & K.M. Snader. 2000. The influence of natural products upon drug discovery. *Natural Product Reports* 17(3): 215–234.

Newman, D.J., G.M. Cragg. & K.M. Snader. 2003. Natural products as sources of new drugs over the period 1981–2002. *Journal of Natural Products* 66(7): 1022–1037.

Pandey, G. 2002. Anticancer herbal drugs of India with special reference to Ayurveda. *New Delhi*: *Sri Satguru Publications*, 18–121.

Phillipson, P.B. 1987. A checklist of vascular plants of the Amatole mountains Eastern Cape Province-Ciskei. *Bothalia* 17(2):237-256.

Premalatha, B. & G. Rajgopal. 2005. Cancer-an ayurvedic perspective. Pharmacological Research 51:19-30.

Valkama, E., Juha-Pekka, S., Julia, K. & P. Kalevi. 2003. Comparative analysis of leaf trichome structure and composition of epicuticular flavonoids in Finnish Birch Species. *Annals of Botany* 91: 643-655.

Van Wyk, B.E., B. Van Oudtshoorn. & N. Gericke. 1997. Medicinal Plants of South Africa, Briza Publications, Pretoria.

Wanyonyi, A.W., C.C. Sumesh, M. Gerald, E. Udo. & M.N. Wilson. 2002. Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum*. *Phytochemistry* 59:79-84.

Weissenberg, M. 2001. Isolation of solasodine and other steroidal alkaloids and sapogenins by direct hydrolsis-ectraction of Solanum plants or glycosides therefrom. Phytochemistry 58: 501-508.

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Lastly, sincere thanks are expressed to the sangomas and herbalists who provided ethnobotanical information.

APPENDICES

APPENDICES

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e        : D:\DATA\VEDIC\LEAFOIL.D
rator    : YINKA
uired    : 18 Oct 2005  00:33      using AcqMethod YINKA
trument :   GC/MS Ins
ple Name: SOLANUM AC.
c Info   : DRY LEAVES
l Number: 1
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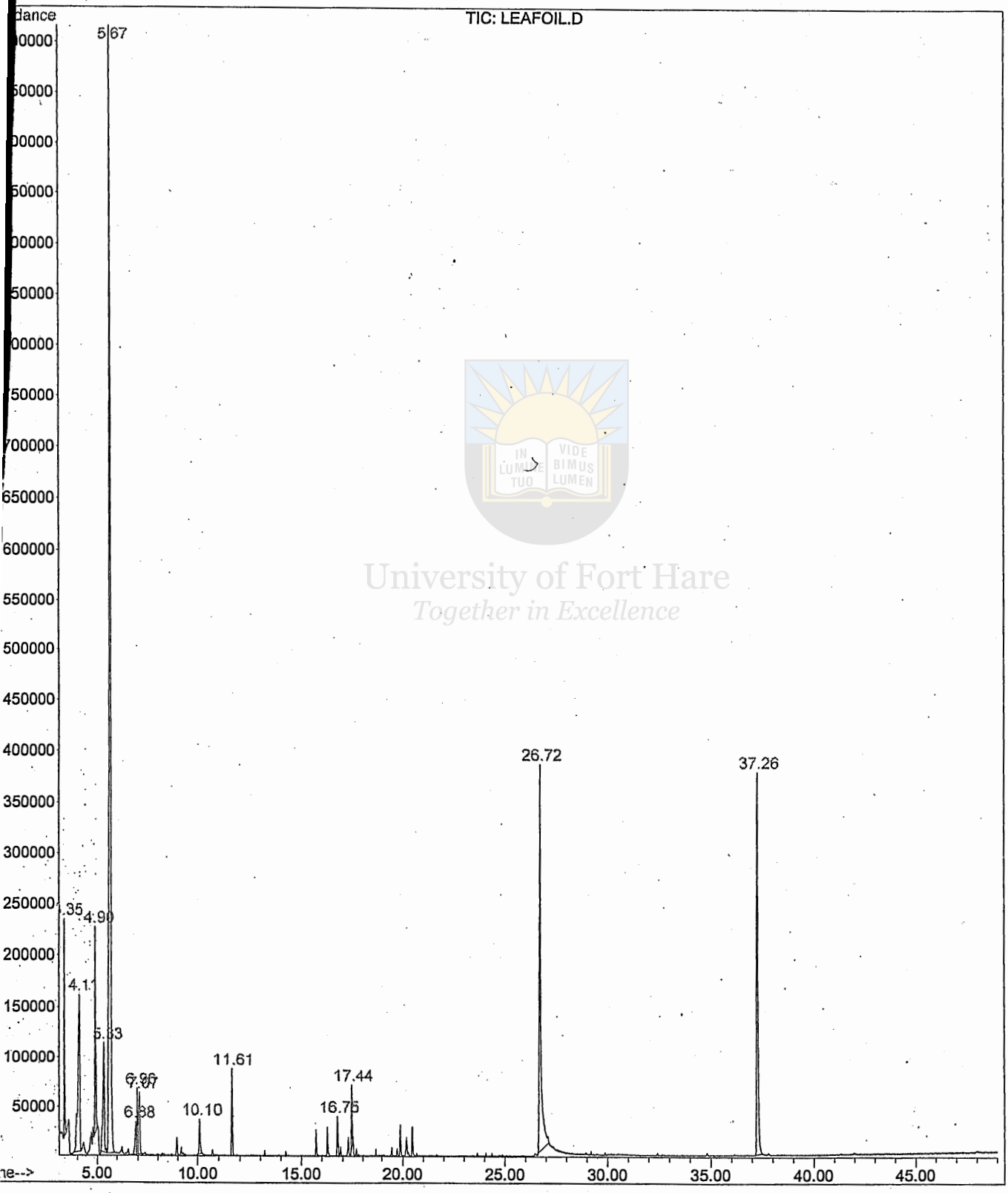
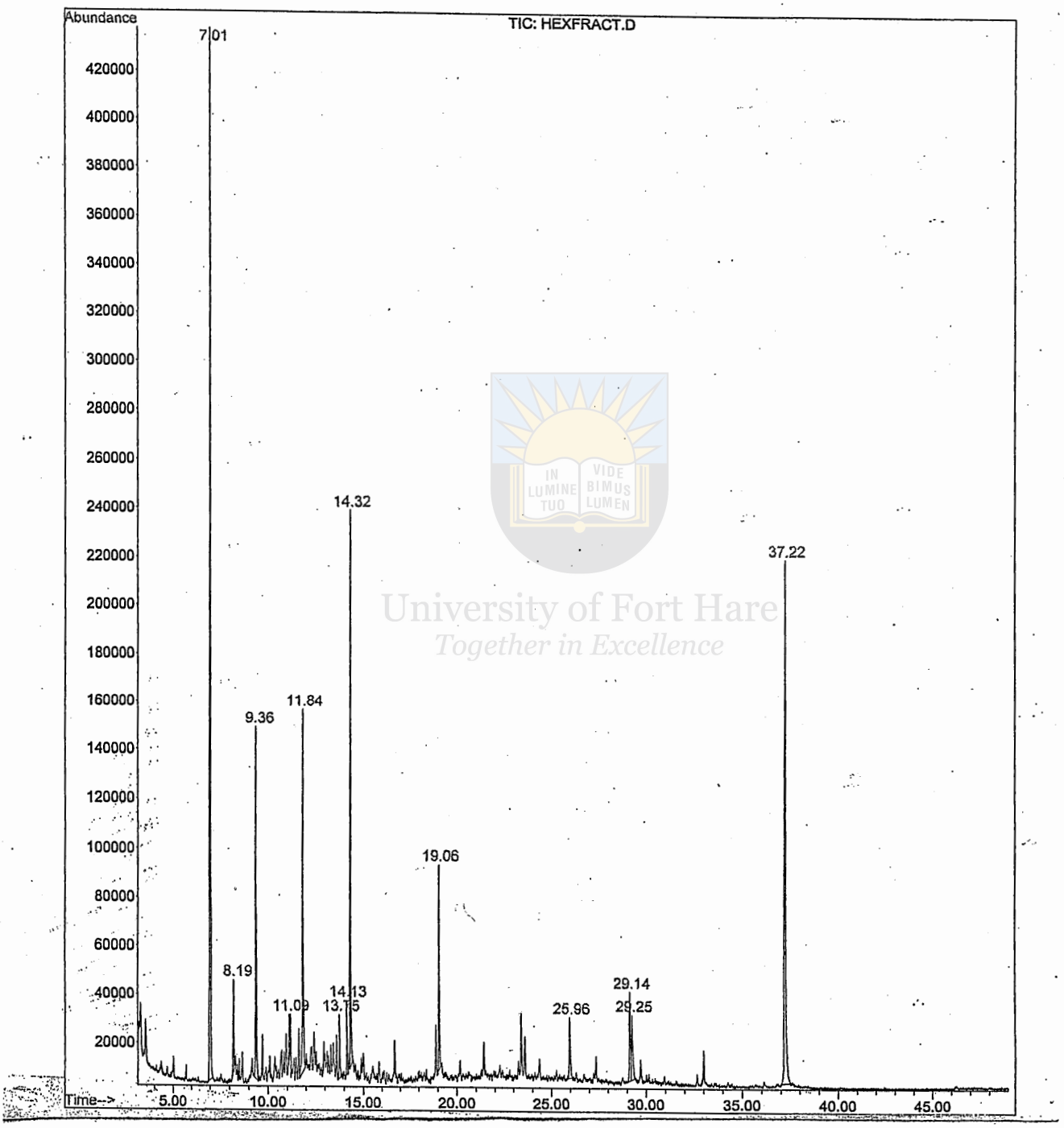


Appendix 1: GC-MS Total Ion peaks of hydrodistillation of leaves of *S. aculeastrum*

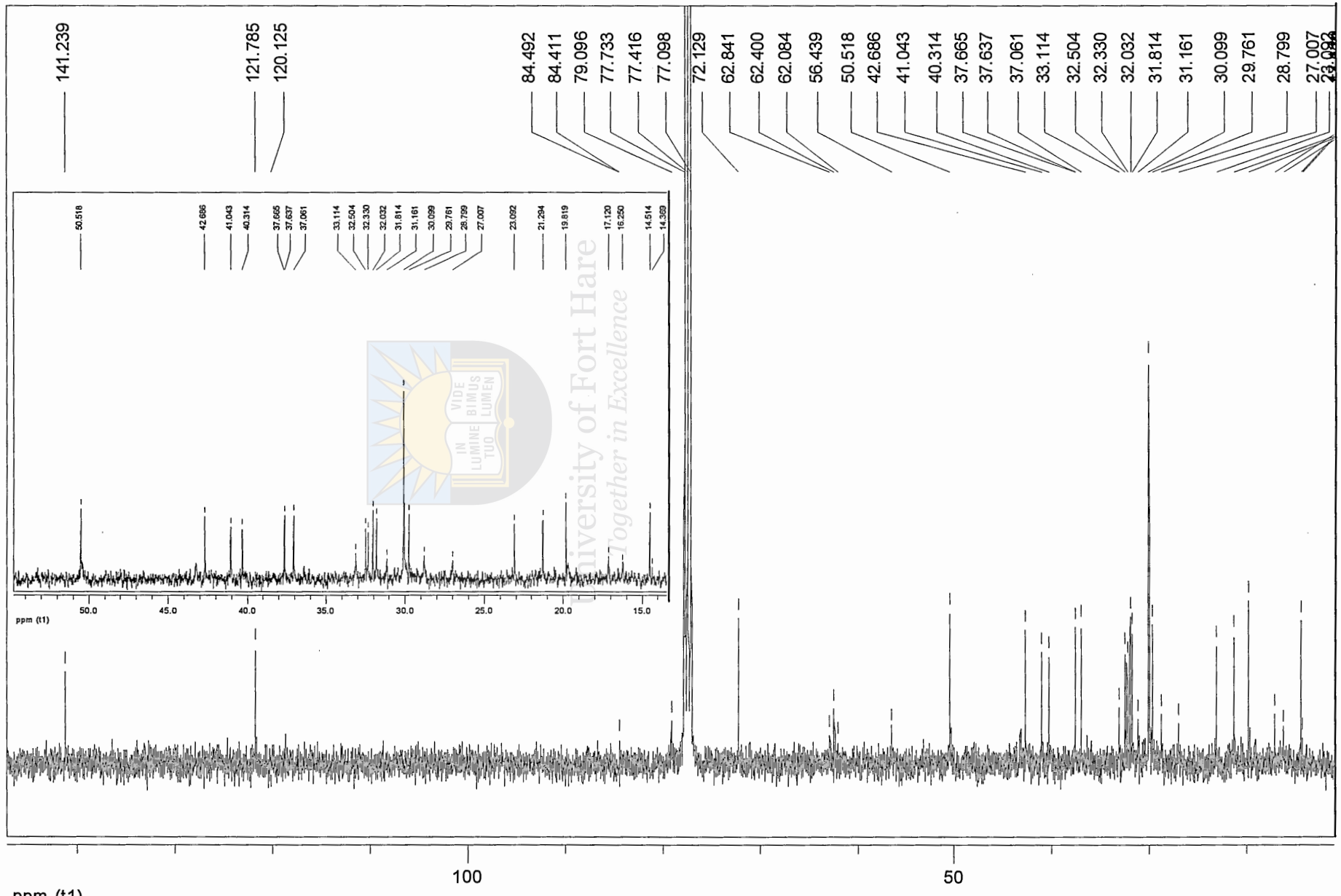
```
File        : D:\DATA\VEDIC\HEXFRACT.D
Operator    : DSG
Acquired    : 13 Oct 2005    3:12      using AcqMethod YINKA
Instrument :
  GC/MS Ins
Sample Name: solanum
Misc Info   : Hexane Fraction
Vial Number: 1
```



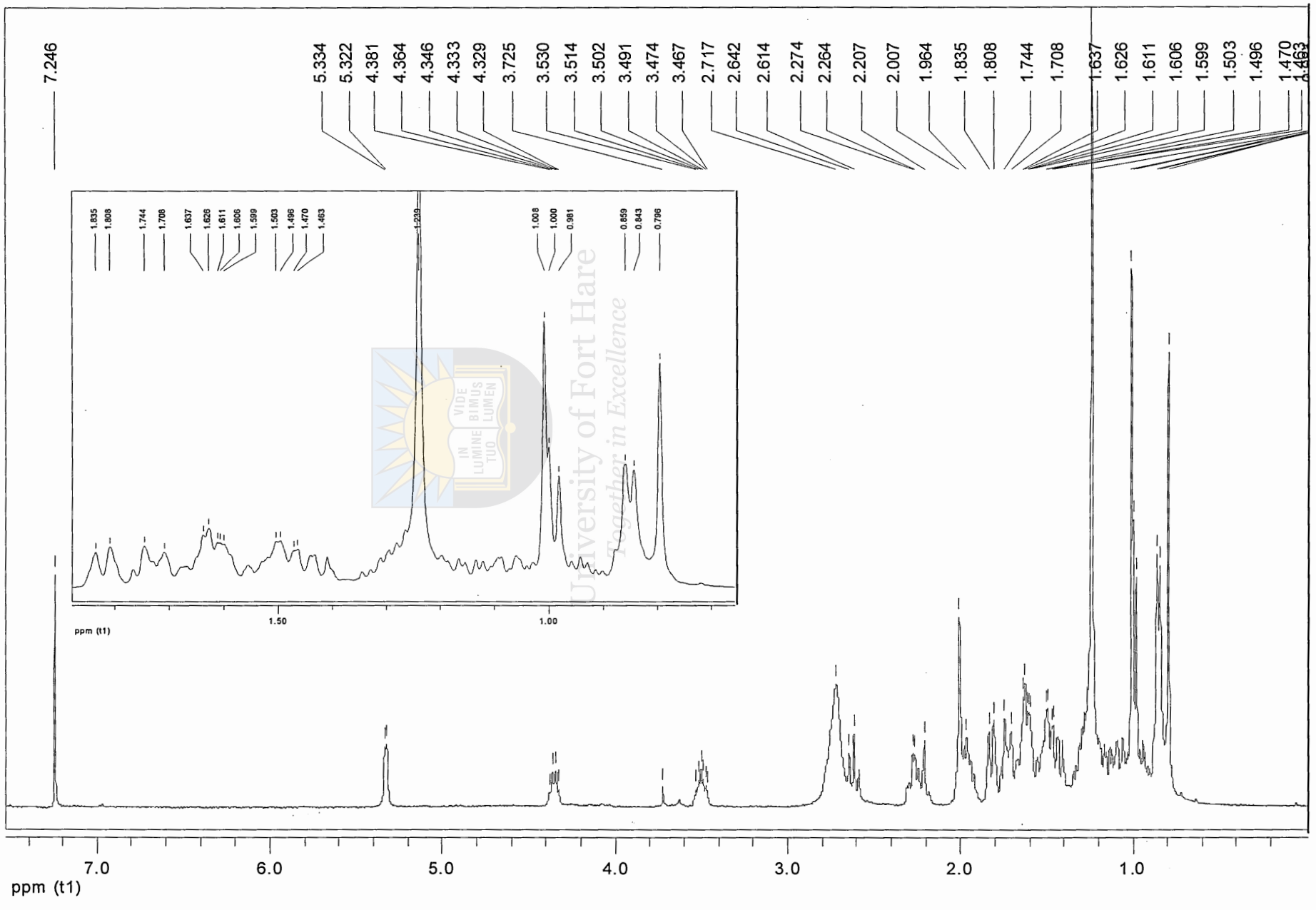
Appendix 2: GC-MS Total Ion peaks of hexane fraction of berries of *S. aculeastrum*

Appendix 3: ^{1}H NMR spectrum of tomatidine from ***Solanum aculeastrum***

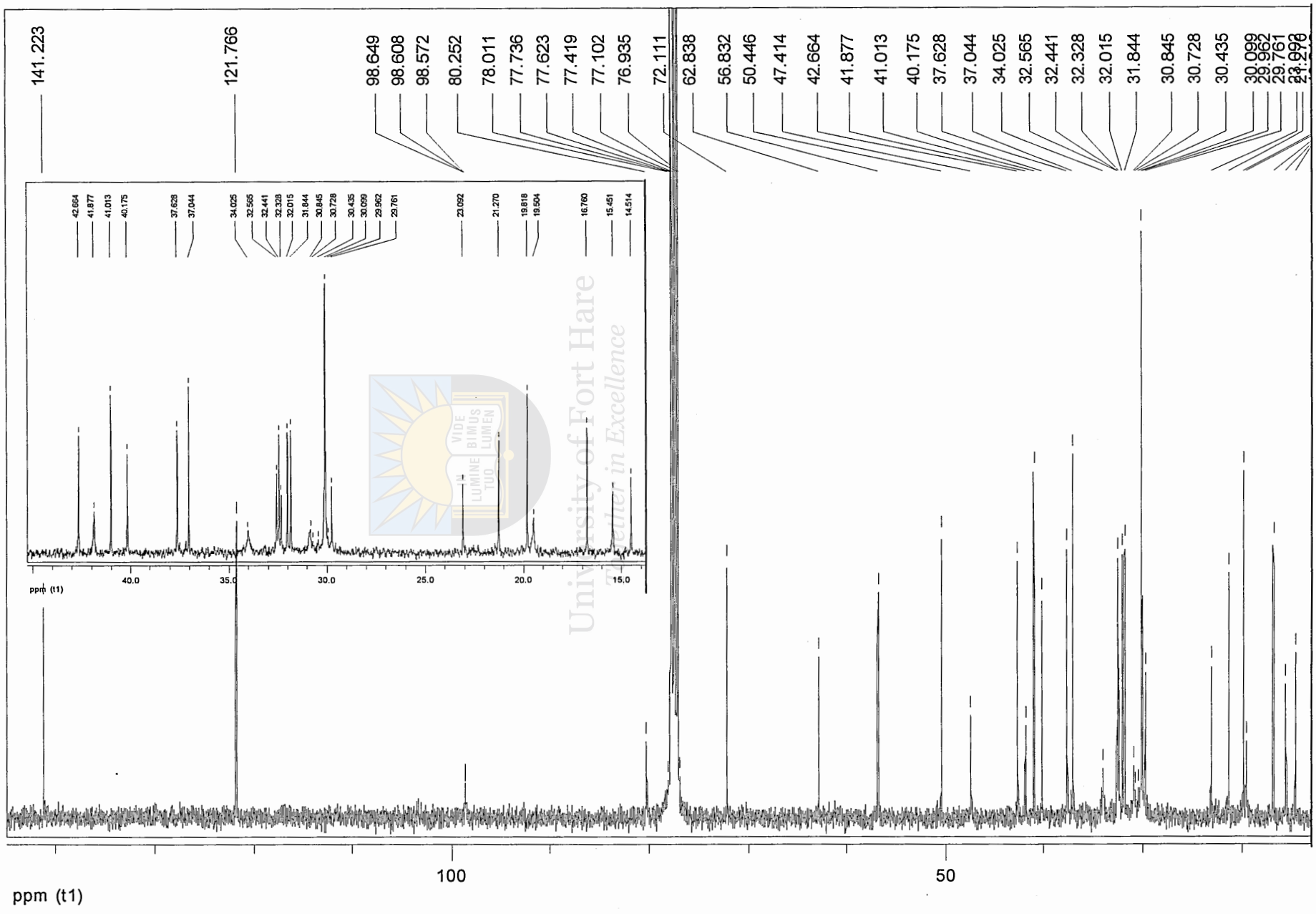
Appendix 4: ^{13}C NMR spectrum of tomatidine from ***Solanum aculeastrum***



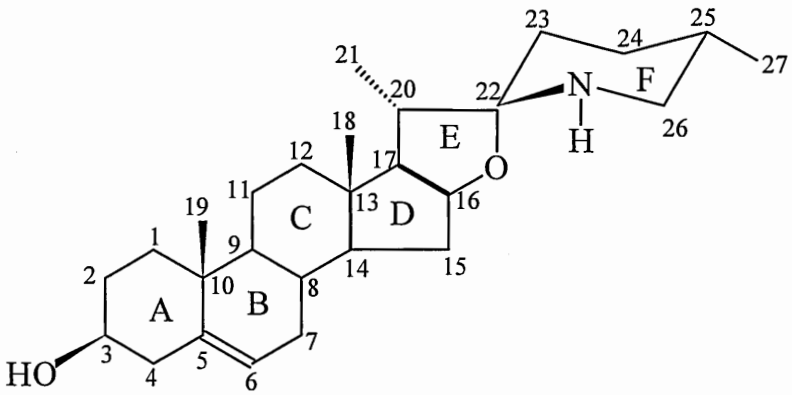
Appendix 5: ^{1}H NMR spectrum of solasodine from ***Solanum aculeastrum***



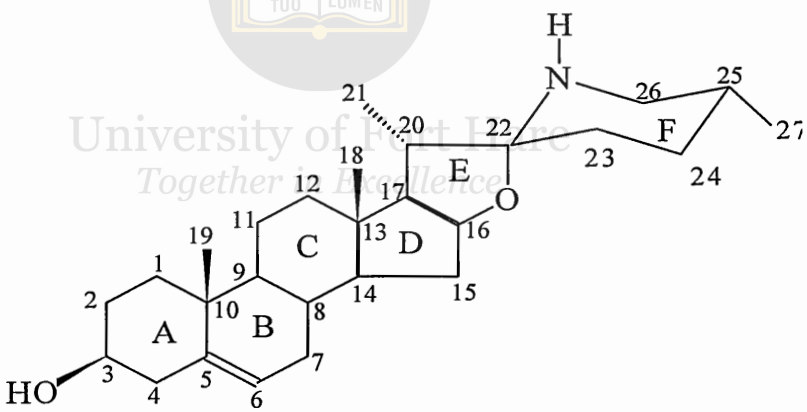
Appendix 6: ^{13}C NMR spectrum of solasodine from ***Solanum aculeastrum***



Tomatidine



Solasodine



Appendix 7: Structures of tomatidine and solasodine

Appendix 8: ^{13}C and ^{1}H spectral data for *S. aculeastrum* glycoalkaloids aglycones.

Carbon No	Tomatidine		DEPT 135	Solasodine	
	^{13}C	^{1}H		^{13}C	^{1}H
1	37.0	1.41-1.49[a]	CH2	37.0	1.40-1.50[a]
2	32.0	2.26	CH2	32.0	2.26
3	72.1	3.65	CH	72.1	3.65
4	38.6	2.49	CH2	38.3	2.58
5	141.2	-	C	141.2	-
6	121.7	6.00	CH	121.7	6.97
7	32.3	2.23	CH2	32.4	2.24
8	31.8	1.51	CH	31.9	1.59
9	50.5	1.06	CH	50.9	1.12
10	37.6	-	C	37.6	-
11	21.2	1.86	CH2	21.3	1.92
12	40.1	2.22-2.28[a]	CH2	40.2	2.00-2.64[a]
13	41.0	-	C	41.0	-
14	56.8	1.41-1.49[a]	CH	56.8	1.40-1.50[a]
15	32.5	1.41-1.49[a]	CH2	32.6	1.40-1.50[a]
16	80.2	5.73	CH	80.2	5.32
17	62.8	2.22-2.28[a]	CH	62.9	2.20
18	16.7	1.02	CH3	16.8	0.99
19	19.5	1.05	CH3	19.5	1.00
20	42.6	2.91	CH	42.7	2.71
21	15.4	1.16	CH3	15.5	1.16
22	98.6	-	C	98.6	-
23	23.1	2.22-2.28[a]	CH2	23.1	2.00-2.64[a]
24	29.7	1.41-1.49[a]	CH2	29.8	1.40-1.50[a]
25	30.8	1.93	CH2	30.9	1.83
26	50.4	2.28(α) 2.88(β)	CH2	50.4	2.27(α) 2.64(β)
27	19.8	1.00	CH3	19.8	0.98

[a]Approximate values