PHYTOCHEMICAL SCREENING AND THIN LAYER CHROMATOGRAPHIC PROFILING OF *ALOE VERA* (L) Burn. f GROWING IN SOUTH AFRICA

ZIMASA BUSISIWE DUBENI

Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE: BOTANY

DEPARTMENT OF BOTANY

FACULTY OF SCIENCE AND AGRICULTURE

UNIVERSITY OF FORT HARE

SUPERVISOR: PROF AJ AFOLAYAN

NOVEMBER 2013

DECLARATION

I, Zimasa Busisiwe Dubeni, declare that this is my own work that has never been submitted for any other degree at this university or any other university.

I also declare that I am fully aware of the University of Fort Hare policy on plagiarism and I have taken every precaution to comply with the regulations of the University.

Again, I declare that I am fully aware of the University of Fort Hare policy on research ethics and have taken every precaution to comply with the regulations. There was no need for ethical clearance for this work.

Signature: _____

ACKNOWLEDGEMENTS

First and foremost, I would like to thank God for giving me the strength and courage to start and finish this journey. Without your guidance, Lord, none of this would have been possible

I would then like to express my deepest gratitude to my advisors Prof. Anthony Jide Afolayan for his constant follow up, guidance, encouragement, dedication and for sharing his expertise with me throughout my study period. You gave me opportunities to participate in this research program, and to work with talented research staff and graduate students in his research centre. I will forever be thankful to God for blessing me with you.

I am grateful to CSIR and DST for funding this research.

I am also grateful to my mother and my family especially my sister for their non-ending support emotionally & financially, continuous encouragement and for believing in me.

My deepest appreciation goes to Sonwabo Dyani, whose support and constant encouragement helped me through the hard times of this program. My deepest appreciation is expressed to him for his love, understanding and inspiration.

My heartfelt appreciation goes to the members of MPED research centre for their unreserved support in carrying out this study.

I would like to take this opportunity to thank the Department of Botany for providing required facilities.

I would also like to extend my sincere appreciation to my fellow colleagues, my seniors Dr Wintola, Dr Gloria, Dr Gita, Dr Wilfred, Dr Michel who gave me enormous valuable discussions, technical support and hands-on help in many aspects of this research program,

and their caring and concern about this dissertation. My fellow colleagues and office mates who helped me in one way or the other.

DEDICATION

This work is dedicated to my parents Lennox Siwowo and Nomthandazo Gladys Dubeni.

ABSTRACT

The chemical profiling, characterization of Aloe products and phytochemical properties of *Aloe vera* were studied. The adulteration of commercial products derived from medicinal plants has been a major muddle for both the society and the pharmaceuticalindustry. Economically motivated adulteration includes the potential for contaminated, sub-potent or counterfeit medication to enter the supply chain at several levels, from the production of raw ingredients through to the point of retail sale. Darwin's theory of evolution states that, species undergo genetic variation with time to adapt to environmental changes. Therefore, the same species growing in widely different habitats may drift from the original genetic makeup as a mechanism of adaptation and that may result in them having different chemical profiles. Therefore this study aimed at investigating the phytochemical properties of *Aloe vera* growing in South Africa. Also, this study aims to utilize Thin Layer Chromatography to profile this plant, as well as use Infra Red spectroscopy to characterize commercial *Aloe vera* products.

A large quantity of *Aloe vera* plant was collected from AloeWay, Iphofolo Game Farm, Polokwane in the Limpopo province of South Africa. The identity of the plant was confirmed from literature and authenticated by Professor DS Grierson of Botany Department, University of Fort Hare, Alice. The plant leaves were divided into two portions. One portion was extracted fresh while the other was cut into pieces and oven dried at 40^0C then and milled to a homogenous powder once dried completely.

The phytochemical composition of the gel and leaf extracts revealed the presence of alkaloids, flavonoids, saponins, tannins and phenols at different concentrations. Results showed that the dry plant material yielded more phytochemicals than the fresh plant material. In particular, it was found that the acetone extract showed much more amounts of

phychemicals than the dichloromethane and aqueous extracts. The percentage compositions of phenols (71.86), flavonols (36.61), proanthocyanidins (82.71), saponins (37.73) and alkaloids (13.29) were significantly high in the acetone extract, followed by the dichloromthane extract with values of 46.85, 37.73, 49.51, 89.0 and 11.11 respectively, while the least composition was found in the aqueous extract. Furthermore, flavonoids were somewhat high in composition in both the aqueous extract of the dried and of the fresh plant material while others were very low. Tannins levels were significantly very low in all the solvent extracts. It was found that the acetone extract showed great amounts of phytochemicals than dichloromethane and aqueous extracts. Since *A. vera* is used in the treatment of different ailments such as skin wounds and abrasions, eczema, constipation, rheumatoid arthritis etc, the medicinal uses of this plant could be associated to such analysed bioactive compounds.

Acetone, hexane, ethanol, water and dichloromethane were used to extract the *Aloe vera* leaf and the best solvent extract was determined. Thin layer chromatography was used to profile the leaf extracts with the aim of documenting the main phytochemicals present in the *Aloe vera* growing in South Africa. The best spraying reagent was determined. Fourier transform infrared spectrophotometer was used to validate the presence of *Aloe vera* ingredients in commercial products.

The yield extraction ability of the solvent was the order: water>ethanol> hexane >dichloromethane and acetone for the dry portion. However, for the plant extracted fresh, the order of yield produced was ethanol>acetone>dichloromethane > and water. The different solvent systems separated the compounds differently. Hexane: acetone: ethanol (20 : 5: 2) and Benzene: ethanol: ammonium (80): ethanol (10): ammonium solvent systems were noted to be the best mobile phase as they gave the best separation compared to other systems.EMW [ethyl acetate (81): methanol (11): water (8)] showed better separation than the other two

separating solvent systems. Vanillin- sulphuric acid spray was seen to be the best spraying reagent as compared to vanillin- phosphoric acid. Fourier transform infrared spectrophotometer validated the presence aloe ingredients in *aloe vera* commercial products.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

whota-Wound Healing, Oral & Topical Activity

Icr- Imprinting Control Region

UV- Ultra violet

SSD- silver sulfadiazine

TNF- Tumor necrosis factor

TGF- Transforming growth factor

IL-1- interleukin-1

PAC- purified acemannan

LPS-lipopolysaccharide

MRSA- methicillin- resistant *Staphylococcus aureus*

DNA- deoxyribonucleic acid

HSV- Herpes simplex virus

PASI- psoriasis and severity index

ROS- Reactive oxygen species

NaOH -Sodium hydroxide

$AlCl_3$- Aluminium Chloride

$FeCl_3$- Ferric chloride Acetic acid,

NH_4OH- Ammonium hydroxide

EtOH - Ethanol

MeOH - Methanol

Na_2CO_3- sodium carbonate

$K_4Fe(CN)_6$- potassium ferricyanide

TCA- trichloroacetic acid,

HCL -Hydrochloric acid

TLC-Thin layer chromatography

dcm- Dichloromethane

BC- Before Christ

AD- Anno Domini

EMW: ethyl- acetate, methanol and water

PFW: n- propanol, formic acid and water

HAE: Hexane, acetone and ethanol

BEA: Benzene, ethanol and ammonium

CHAPTER 1

LITERATURE REVIEW

CHAPTER 1

LITERATURE REVIEW

LITERTURE REVIEW

1.1 *Aloe vera* (L) Burn. f.

Aloe vera (L) Burn. is one of approximately 420 species of the genus *Aloe*, which have been variously classified as belonging to Aspodeleaceae, Liliaceae or Aloaceae families (Dagne *et al*., 2005). Commonly known as *Aloe barbadensis* Miller, its legitimate name according to the international rules of botanical nomenclature, is *Aloe vera* (L) Burn. f. (Grindlay & Reynolds, 1986). The geographical origin of *Aloe vera* is believed to be Sudan, with the plant subsequently introduced to the Mediterranean region and most other warm areas of the world. The most authentic record of *Aloe* as a plant with healing properties is accredited to a Mesopotamian clay tablet dated 2100 BC. However, the first detailed depiction of the plant's medicinal value is found in the Papyrus Ebers, an Egyptian document dated 1550 BC, which sets out multiple *Aloe*-containing preparations for the treatment of external and internal ailments. The *Aloe vera* plant was described in the Greek herbal of Dioscorides (ca 70 AD), and its use promoted for the treatment of wounds, hair loss, genital ulcers and haemorrhoids (Davis, 1997). *Aloe vera* was officially listed as purgative and skin protectant by the US pharmacopoeia in 1820 (Park & Lee, 2006). It was clinically used in the 1930 for the treatment of radiotherapy burns to the skin and mucous membrane (Collins& Collins, 1935; Manderville, 1939). Until today, *Aloe* is an important herbal medicine in many countries, including China, India, the West Indies, South Africa and Japan (Grindlay & Reynolds, 1986).

1.2 Habit

Aloe vera is a succulent perennial herb up to 160 cm tall, with a short stem up to 30 cm long, freely suckering and forming dense groups (Fig 1). Leaves 16-20 in a dense rosette, erect to slightly spreading; stipules absent; petiole absent; blade linear - lancelate, 40- 50 cm x 6-7

cm, apex acuminate , margins slightly pinkish with deltoid, firm teeth 2 mm long, pale, 1-2 cm apart, fleshy, upper surface rather flat, grey green to pale green, lower surface convex; leaf sap yellowish. Inflorescence terminal dense raceme 30-40 cm x 5-6; penduncle simple or sometimes 1-2 branched above the middle, 60-90 cm tall; bracts ovate-acute, deflexed, up to 1 cm long. Flowers bisexual, regular, 3 merous; pedicel c 5 cm; perianth tubular, u to 3 cm long, inflated around the ovary, lobes 6, 3 outer lobes acute, 3 inner lobes obtuse, yellow, orange or red; stamens 6, exserted; ovary superior, 3- celled, 6- grooved, style filiform, stigma head- shaped, exserted. Fruit a capsule, dehiscing loculicadally, many seeded. Seeds are7 mm long, dark brown, winged (Schmelzer & Gurib-Fakim, 2008)

1.3 Natural habitat

Aloe vera grows in a wide range of climatic conditions. It prefers sandy loamy, well-drained soil and can grow naturally in poor soils but thrives better in rich soils. It is tolerant of salinity. Established plants will survive drought quite well even though the root system is relatively shallow. The plant survives at temperature of -3^0C with only slight injury. It grows better in full sun or light shade. It is planted as hedge in house premises and also run wild in the desert conditions and poorest soils Parthipan *et al*., (2011). *Aloe vera* has a habitat variance and is not only in terrestrial habitat but also in rocky habitats as chasmophytes in the Southern Western Ghats of Coimbatore district of Tamilnadu. Parthipan *et al.*, (2011) further observed the species as an epiphyte in the Blue Mountains (2,240 meters above sea level) of Nilgiri District of Southern Western Ghats of Tamil Nadu.

Figure 1: ***Aloe vera***

1.4 Geographic distribution

Aloe vera is only known as a cultivated or naturalized plant. It is generally presumed that the origin is Arabia, Somalia or Sudan and a recently discovered stand of *Aloe vera* in Oman could well prove to be the only population in the world (Afolayan & Adebola, 2006). A Mediterranean origin is often quoted, at present *Aloe vera* is widely distributed throughout tropics and subtropics. It is widely grown as a cash crop in dry regions in the Americas, Asia and Australia. The plant was already used as a drug by the Greeks as early as 400 B.C. and later by Arabian physician. It is probably present as a cultivated plant in all countries of tropical Africa (Schmelzer & Gurib- Fakim 2008). Therefore, *Aloe vera* present in South Africa is cultivated.

1.5 Folk uses

Aloe vera has been widely used in the treatment of wounds since 550 BC (Shelton, 1991). For example the Greek physician Dioscorides reported that *Aloe vera* gel could be used to heal skin infection, chapping and haemorrhoids (Coats & Ahola, 1979; Gunther, 1934). There are many reports about the powers of the plant, including that it was the secrete of Cleopatra's beauty (Grindlay& Reynolds, 1986) which may account for its use in the variety of health and beauty aids and that it was applied to the body of Jesus Christ after his crucification. The species has been widely used in India as a cathartic, stomachic and anthelminthic, in China as a common folk remedy in most of America and the West Indies (Boon & Smith, 2004).

1.6 Main Actions

The active ingredients, whether alone or in concepts, include glycoproteins, anthroquinones, polysaccharides and low molecular-weight compounds such as beta-sito sterol (Choi & Chung, 2003).

1.6.1 Wound healing

Wound healing is associated with various mechanisms and constituents of the healing agent. The omboxane inhibits wound healing and Aloe has been shown to inhibit thromboxane into vitro (Zachary *et al*., 1987).Davis, (1989)conducted a study to prove the whota (Wound Healing, Oral & Topical Activity) of *Aloe vera*. In his findings, he noted a 62.5% reduction in wound diameter in mice receiving 100 mg/kg/day oral *A. vera* and a 50.8% reduction was recorded in animals receiving topical 25% *A. vera*. These data suggest that *A. vera* is effective in the treatment of wounds.

Enzymes in Aloe have also been shown to breakdown damaged tissue which can then be removed by phagocytosis (Bunyapraphatsar *et al*., 1996). A glycoprotein fraction was found to increase proliferation of human keratinocytes and increase the expression of receptors for epidermal grown factor and fibronectin in vitro (Choi *et al*., 2001). The same research team then demonstrated that this glycoprotein enhanced wound healing by increasing cell proliferation invivo. Beta- sitosterol appears to improve wound healing by stimulating angiogenesis and neovascularisation in vivo (Moon *et al*., 1999). Aloe polysaccharides have been shown to eliminate UV-induced immunosuppression (Strickland *et al*., 1994).

Several animal studies support the application of Aloe gel to skin damaged by frostbites as means to maintain circulation and reduce the vasco constrictive effects of thromboxane in the affected dermis (Haggers *et al.*, 1987; Klein & Penneys, 1988; McCauley *et al.*, 1990; Miller & Koltai, 1995). In combination with the pentoxifylline, it acts synergistically to further increase tissue survival (Miller & Koltai,1995). Other studies have found that Aloe gel not

only increased collagen content, but also changes collagen crosslinking, which in-turn increases the breaking strength of scar tissue; making the seal of wound stronger (Chithra *et al* 1998; Heggers *et al* 1996). *Aloe vera* was found to promote healing and decreased inflammation in second- degree burns in vivo (Samboonwong *et a*l., 2000).

Aloe vera prevented delayed hypersensitivity of UV-irritated skin as well as contact hypersensitivity in animal models with allergic reactions (Strickland *et al.,* 1994). Acemannan gel (beta- (1, 4 acetylated manna) has demonstrably improved radiation burns in mice (Roberts &Travis, 1995).

1.7 Use with pharmacological agents

Several topical pharmaceutical antimicrobial agents such as SSD(silver sulfadiazine)inhibit wound construction, thereby slowing the rate of wound healing. An experimental model was used to investigate whether co- administration of *Aloe* could reverse this effect and improve wound healing rate (Miller *et al.,* 2003). *Aloe vera* was found to reverse the delayed wound healing produced by SSD, resulted in the shortest wound half- life and healing time.

Aloe vera (100 and 300 mg/ kg daily for 4 days) blocked the ability of hydrocortisone acetone to suppress wound healing by up to 100% (Davis *et al*., 1997 a). Growth factors in *Aloe vera* were thought to mask sterols and certain amino acids that prevent wound healing. An earlier study identified the sugar mannose -6- phosphate to be one of the chief constituents responsible for wound healing (Davis *et al.,*1994 b)

1.7.1 Ani-oxidants

Studies have shown that several compounds in Aloe gel protect tissues against oxidative damage caused by free radicals (‘t Hart *et a*l., 1990; Singh *et a*l., 2000; Wu *et al.,* 2006; Yagi *et al.,* 2002 and Zhang *et al*., 2006). This is achieved by direct anti-oxidant activity and indirect activity through stimulation of endogenous anti-oxidant systems. Two aloe dihydroisocoumarins have been identified and have demonstrated free scavenging properties (Zhang e*t al.,* 2006; Zhang *et al.,* 2008).

Treatment with aloe gel extract decreased lipid peroxidation and hydroperoxides in diabetic rats to near normal levels (Rajasekaran *et al.,* 2005). The extract also significantly increased superoxide dismutase, catalase, glutathione peroxidase and glutathione -S- transferees in the liver and kidney. In another study, data obtained 3.7 and 10 days after exposure to radiation showed that aloe gel significantly reduced oxidative damage in the liver, lungs, and kidney tissues of irradiated rats (Saada *et al*., 2003).

1.7.2 Immuno- stimulant

It has been suggested that *Aloe* may have immune- stimulating capabilities. Much of the available research has been performed on mice or in vitro and *Aloe* shows antiviral, antitumor and non- specific immune-stimulant activity. An experiment in 1980 demonstrated that mice given *Aloe* extract 2 day before exposure to pathogens were protected against a variety of fungi and bacteria (Brass *et al*.,1981). Later the isolated compound acemannam (beta (1,4)-acelatylatedmannan) was shown to increase the response of lymphocytes to antigens in vitro (Wamble & Helderman, 1988). In mice, acemannan stimulated cytokines, bringing about an immune- attack on implanted sarcoma cells, leading to necrosis and regression of cancer cells (Peng*et al*., 1991). A later trial investigated the effects of acemannan on mouse macrophages (Zhang & Tizard, 1996). Acemannan stimulated macrophage cytokine production (lL-6 and TNF- alpha, no release, surface molecule expression, and cellular morphologic changes.

Similarly, a polysaccharide fraction isolated from *Aloe vera* promoted human keratinocytes secrete TGF- alpha, TGF- beta- 1, IL- 1- beta, IL- 6, lL-8 and TNF, and inhibited the release of NO as compared to control (Chen *et al.,* 2005). The immune enhancing effects of acemannan may be due in part to the compound's ability to promote differentiation of immature dendritic cells (Lee *et al.,* 2001). The cells are crucial for the initiation of primary immune response.

Multiple studies have highlighted the diverse immunomodulatory activities of *Aloe* polysaccharides. Recent research has identified that immunomodulatory activity varies with size of the polysaccharides entity. It appears that *aloe* polysaccharides that are of smaller molecular weight (specifically between 5 and 400 kDa) have the greatest immunological effects possibly because they are better absorbed than MW entities (Im *et al.,* 2005).

The purified polysaccharides fraction (PAC- I, PAC- II and PAC-III) from *A. vera* stimulated peritoneal macrophages and splenic T and B cells, and increased the ability of these cells to secrete TNF- alpha, IL- I- beta, IFN- gamma, IL-2 and IL6 (Lueng *et al.,* 2004). The compound with the highest mannose content and therefore the highest molecular weight (PAC-I), demonstrated the most potential. This finding was again observed by (Lui *et al.,* 2006) whereby PAC-I exhibited potent stimulation of murine macrophages and produced tumouricidal properties and activated macrophages in vitro, thus providing evidence in support of its antitumor properties.

A 99% pure carbohydrate compound (purified acemannan) isolated from *Aloe* demonstrated potent haematopoietic and haematopoietic activity in myelosuppresed mice (Talmadge *et al.,* 2004). Specific manufacturing methods can be applied to enhance the extracts. For example, 1g of extract obtained from leaves subjected to cold and dark treatment contained 400mg of neutral polysaccharide compared with 300mg in leaves not specially treated.

1.7.3 Anti-inflammatory

A number of in vitro and in vivo studies confirm the anti- inflammatory activity of *Aloe vera*. The gel reduces oxidation of arachidonic acid, thereby reducing PG synthesis and inflammation (Davis *et al.,* 1987). It inhibits the production of $PGE_2$by 30% and IL-8 by 20%, but has no effect on thromboxane B2 production in vitro (Langmead *et al.,* 2004). Following burn injury in vivo, *A. vera* was found to inhibit inflammation by reducing leukocytes adhesion and decreasing the pro- inflammatory cytokines TNF- alpha and IL- 6 Duansak *et al* (2003).

One study conducted on rats with croton oil- induced oedema reported a 47% reduction in swelling after the application of topical aloe gel (Davis *et al*., 1987). Another study found aloe gel to reduce vascularity and swelling by 50 % in the inflamed synovial fluid within the pouch. When aloe gel was applied topically there was also an increase in fibroblast cell numbers (Davis *et al.,* 1992). C- glucosylchromone, isolated from aloe gel extracts, is chiefly responsible for the anti-inflammatory effect, with activity comparable to hydrocortisone in experimental models (Hutter *et al.,* 1996). A study of strptozotocin- induced diabetic mice further confirmed the anti- inflammatory activity of *Aloe vera* and identified the isolated constituent gibberellin as also effective (Davis & Maro, 1989). Both compounds inhibited inflammation in a dose-dependent manner. A recent in vitro assessment using human immune cells displaced aloe's potential to reduce bacteria- induced pro- inflammatory cytokine production specifically TNF- alpha and IL-1-beta, by peripheral blood leukocytes stimulated with *Shigellaflexneri* or lipopolysaccharide (LPS) (Habeeb *et al*., 2007a).

1.7.4 Laxative

The Aloe latex contains anthraquinones, which have a stimulated laxative activity. Studies in rats have shown that aloe latex increases intestinal water content, stimulates mucus secretion, and induces intestinal peristalsis (Ishii *et al*., 1994). However, aloe as a laxative is more irritating than other herbs (Reynolds & Dweck, 1999) and long-term use can cause an electrolyte imbalance through depletion of potassium salts. Alternatives are recommended if long- term treatment is required.

1.7.5 Anti-Ulcer

The anti- ulcer of *A. vera* has been proposed to be due to anti- inflammatory, cytoprotective, healing and mucus stimulatory effects. According to an in vivostudy by Eamlmnam *et al*. (2006), *A.vera* promotes gastric ulcer healing In contrast to these results, in another study a stabilised fresh aloe gel preparation prolonged the effect of histamine- stimulated acid secretion but inhibited (Suvtayavat *et al*., 2004).

A number of case reports tell of a positive effect on lung ulcers with to leg ulcers with topical use of aloe gel, including cases that did not respond to standard medical interventions (Zawahry *et al*., 1973). Application of water-based aloe-gel saline soaks, broad- spectrum antibiotics and antifungals allowed a wound, caused by necrotising fasciitis, to heal in 45 days in a 72- years- old woman. Aloe gel and saline- soaked sponges were also used to treat two large seroma cavities caused by deep vein thrombosis in a 48-year-old man (Ardire, 1997).

Although aloe gel is commonly used as a topical agent for wound healing it is also used internally. A small study of six patients with chronic leg ulcers found that ingesting 60 mL aloe juice daily and applying aloe gel directly to the ulcer and surrounding area resulted in less exudate, odour and seepage through the bandaging (Atherton, 1998).

1.7.6 Hypoglycaemic

Gluconnan slows carbohydrate absorption and slows the postprandial insulin response by up to 50% (McCarty, 2002). *A. vera* leaf gel has been investigated as a possible hepato-protective and kidney protective agent in diabetes type 2 using animal models. In one study, the leaf gel and glibenclamide both decreased degenerative kidney changes, serum urea levels and creatinene levels, but only aloe further reduced kidney lipid peroxidation (Bolkent *et al*., 2004). Can *et al*.(2004) tested aloe pulp, aloe gel extract and glibenclamide, finding that all treatments decreased liver tissue damage compared to control animals. Aloe gel extract also increased glutathione levels and decreased non- enzymatic glycosylation, lipid peroxidation, serum alkaline phosphatase and alanine transaminase.

1.7.7 Antimicrobial

A. vera is active against a wide variety of bacteria in vitro, such as *Pseudomonas auroginosa, Klebsiella pneumonia, Strptococcuspyogenes, Staphylococcus aureus and Escherichia coli* (Heck *et al*., 1981). More recent research indicates antimicrobial activity against *Shigellaflexneri,* MRSA, *Enterobacter cloacae* and *Enterococcus bovis* (Habeeb *et al*., 2007a).

1.7.8 Antiviral

In vitro studies suggest that *Aloe vera* has antiviral activity due to its interference with DNA synthesis (Saoo *et al*., 1996). The polysaccharide fractions of aloe gel inhibit the binding of benzopyrene to primary rat hepatocytes and thus prevent the formation of potentially cancer-initiating benzopyrene- DNA adducts in vitro. This was later confirmed by in vivo studies conducted by Kim &Lee (1997). Moreover, in vitro experiments have shown the

anthraquinones in aloe to be virucidal against HSV 1 and 2, vaccine viru, para-influenza virus and vasicular stomatitis virus (Anderson, 2003).

Investigation with the acemannan component has identified antiviral activity, particularly against feline AIDS, HIV type, influenza virus, measles virus and herpes simplex (Kahlon *et al.,* 1991a and b; Sdydiskis *et al.,* 1991).

1.8 Clinical Use

Although *A. vera* products are used for many indications, the chief use I treating skin conditions.

1.8.1 Skin condition

Aloe is used in the treatment of wound, burns, radiation burns, ulcers, frostbites, psoriasis and genital herpes, and is the only traditional wound- healing herbal medicine which has been subjected to a variety of cell culture- based, animal and human- based studies (Krishnan, 2006). The healing properties may be attributed to antimicrobial, immune- stimulating, anti-inflammatory and antithrombaxane activities. Allontoin has also been shown to stimulate macrophage production of IL- 1 and TNF, which are associated with wound healing (Liptak, 1997).

Most human studies have found that topical application of *aloe vera* gel increases wound healing rate and effectively reduces microbial counts; however, there are some negative studies, most likely related to the fact that the composition of *aloe vera* gel varies, even within the same species. Chemical composition depends on source, climate, region, and the processing method used (Choi &Chung, 2003).

Dry coated *aloe vera* gloves were tested by 30 women suffering from dry, cracked hands, with or without contact dermatitis due to occupational exposure; in a contralateral

comparison study carried out byWest & Zhu (2003). Results indicated that *aloe vera* glove significantly reduced dry skin, irritation, wrinkling, dermatitis, redness and improved skin integrity. It would be interesting to see this study repeated using a standard non-aloe fortified glove on the opposing hand.

The effects of aloe gel applied to skin following dermabrassion in humans are more controversial, with some patients responding well Fulton (1990), while others have had severe adverse reactions, including burning sensation and dermatitis (Hunter & Frumkin, 1991). A standard polyethylene oxide gel dressing saturated with stabilised aloe gel was compared to the standard oxide dressing alone in the study by Fulton and appears to exhibit the strongest evidence overall to support the beneficial effects of *aloe vera*. The addition of *aloe vera* produced a significant vasoconstriction and anti- inflammatory affect 24 and 48 hours after application. Overall, wound healing was quicker with *A. vera* and completed by an average of 72 hours before the oxide gel- treatment. In contrast, one study found that topical *aloe vera* gel actually slowed healing after caesaream delivery (Schmidt & Greenspoon, 1991).

1.8.2 Burns

A recent systematic review of the efficacy of *a. vera* for healing of burns considered four controlled clinical trials involving 371 patient. Meta-analysis concluded that topical treatment with *Aloe vera* decreased healing time and was specifically more effective for the first- and second degree rather than the third degree. It was noted that due to variations in the Aloe preparations and outcome measures used in the studies, more specific conclusions and cannot be drawn (Maenthalsong *et al*., 2007).

One study involving 18 outpatients with moderate to deep second degree burns aging from 2 to 12% of total body surface area showed that commercial *aloe vera* ointment was as

affective as SSD in regard to protection against bacterial colonisation and healing time. More specifically, the mean healing time with *aloe vera* treatment was 13 days compared with 16.15 days for SSD (Heck *et al*.,1981).

Results are less encouraging for sunburn protection and healing. A randomised double-blind trial in 20 healthy volunteers evaluated the effect of *aloe vera* cream for both prevention and treatment of sunburn (Puvabanditin & Vongtongsri, 2005). The cream (70% aloe) was applied30 minutes before, immediately after, or both before and after UV irradiation. The cream then continually applied daily for 3 weeks. The results showed that the *aloe vera* cream did not protect against sunburn and was not an effective treatment.

1.8.3 Frost Bite

In combination with other treatments, topical *A. vera* significantly enhances healing and has a beneficial effects of topical *aloe vera* cream in combination with standard treatment, such a rapidly re-warming the affected areas, analgesics, antibiotics and debridement (n= 56) with another group of 98 patients who did not receive *A. vera* treatment. Of those receiving *A. vera* in addition to usual treatment, 67% healed without tissue loss compared with 32.7% in the group control. Additionally, 7.1% of the total group of 56 required amputation compared with 32.7% in the control group. Although encouraging, this study is difficult to interpret because the groups were not well matched and combination therapies differed (Heggers *et al*., 1987).

1.8.4 Radiation- induced dermatitis

A recent reviewed concluded that aloe gel was as mild steroids creams, such as 1% hydrocortisone, in reducing the severity of radiation burn, without the side-effects associated with steroid creams (Muddocks-Jennings *et al*., 2005). In contrast, another review concluded that aloe was ineffective for the prevention or reduction of side-effects to radiation therapy in cancer patients (Richardson *et al.*, 2005). That review analysed 1 past review, 5 published RCTs. It is important to note that various preparations such as cream, juices, gels and fresh aloe had been tested, which makes it difficult to assess the evidence.

1.8.5 Psoriasis

A double-blind placebo-controlled study found topical *aloe vera* extracts 0.5% in a hydrophilic cream can be beneficial in the treatment of psoriasis and PASI (psoriasis and severity index) scores between 4.8 and 16.7 (mean 9.3) participated in the study, which was scheduled for 16 weeks with 12 months of follow-up. Patients were examined weekly and those showing a progressive reductive of lesions, desquamation followed by decreased erythema, infiltration and lowered PASI score were considered healed. By the end of the study, *Aloe vera* extract cream had cured 83.3% of patients compared with the placebo cure rate of 6.6% ($P < 0.001$). PASI scores decreased to a mean of 2.2 (Syed *et al*., 1996a). In contrast, a randomised, double- blind, placbo- controlled trial found no significant benefits with a commercial *aloe vera* gel in 41 patients with stable plaque psoriasis (Paulsen *et al.*,2005). Following a 2- week washout period patients applied either the aloe gel or placebo twice daily for 1 month. Redness and desquamation decreased by 72.5% in the active treatment group as compared to 82.5% in the placebo group. It should be pointed out that 82.5% is an extremely high placebo responder rate. Fifty-five per cent of patients reported local side-effects, mainly drying of the skin on test areas.

1.8.6 Genital Herpes

Two clinical studies have investigated the effects of *Aloe vera* 0.5% topical preparations in genital herpes, producing good results. A double- blind, placebo-controlled study has demonstrated that *aloe vera* extract 0.5% in a hydrophilic cream is more efficacious than placebo in the treatment of initial episodes of genital herpes in men (n= 60, aged 18-40 years). Each patient was provided with a 40 g tube, containing placebo or active preparation of trial medication to their lesions three times daily for 5 consecutive days (maximum 15 topical application per week). The treatment was well tolerated by all patients (Syed, 1997). The other study involving 120 subjects used a preparation containing 0.5% of whole aloe leaf extract in a hydrophilic castor and mineral oil cream base, which was applied three times daily 5 days per week for 2 weeks. Treatment resulted in a shorter mean duration of healing compared with placebo, Aloe cream also increased the overall percentage of healed patients and there were no significant adverse reactions reported (Syed *et al.*, 1996b).

1.8.7 HIV

The acemannan component of *Aloe vera* has been used as adjunctive therapy to antiretroviral therapy in HIV infection. A preliminary clinical trial found that acemannan may enhance the activity of the anti-HIV drug AZT. A dose of 800 mg acemannan daily significantly increased circulating monocytes (macrophages) in 14 HIV patients. Aloe increased the number and activity of the monocytes (McDaniel *et al.*,1990). Subsequently, a randomised, double-blind placebo-controlled study of 63 male subjects with advanced HIV, taking zidovudine and didanosine, investigated the effects of 400 mg of acemannan taken four times daily for 48 weeks. Results showed a decrease in CD4 cell numbers in the acemannan group compared with placebo (Montaner *et al*., 1996).

1.9 Problem Statement

Growing and selling *Aloe vera* and its products is a multi-million dollar industry especially in South Africa. As result of its increasing demand, many commercial products in the market are claimed to have been made from *Aloe vera* yet, many of such products lack *Aloe vera* ingredients. In fact, at the beginning of this research work, we paid visits to a number of *Aloe* product factories during which a couple of them confessed verbally that their products do not contain *Aloe vera*.

1.10 Hypothesis

Some commercial products said to be made from *Aloe vera* plant may not contain *A. vera* active ingredients.

1.11 Aim of Research

The aim of this project was to screen *Aloe vera* for its phytochemical contents and to profile the species using chromatographic techniques. The research work will also involve the screening of selected commercial products with *Aloe* labels in order to confirm if, actually, the products contain *Aloe vera*.

1.12 Specific Objectives

- To collect/purchase *Aloe vera* from Aloeway, Iphofolo Game Farm, Polokwane in the Limpopo province since *Aloe vera* does not grow naturally in South Africa and only available in this farm.

- To carry out Thin Layer Chromatography of both the dried and fresh plants using different mobile and stationary phases.

- To analyse *Aloe vera* for its phytochemicals such as alkaloids, tannins, flavonoids, tarpenes, phenols, saponins, and anthraquinones. Usually, information on the

phytochemicals of a medicinal plant provides good knowledge on its pharmalogical property.

- To document all possible information on its chemical profile for future research on *Aloe vera*.
- To purchase and screen some commercial products that claim to contain *Aloe vera*, and compare their profiles with that of *Aloe vera* in order to validate these claims.

1.13 REFERENCES

Aeolian, A.J., Areola, P.O., 2006. *Aloe vera* (L.) Burm.f. In: Schmelzer, G.H. & Gurib-Fakim, A. (Editors). Prota 11(1): Medicinal plants/Plantes médicinales 1. [CD-ROM] .PROTA, Wageningen, Netherlands.

Anderson, D., 2003. Wound dressing unravelled. In Practice 25.2, 70-83.

Ardire, L., 1997. Necrotyizing fasciitis: case study of a nursing dilemma. Ostomy Wound Management 43.5, 30-40.

Atherton, P., 1998. *Aloe vera*: magic or medicine? Nursing Standard12.41, 49-52.54.

Boon, H., and Smith, M., 2004. The complete natural medicine guide to the 50 most common Medicinal Herbs. Ontario Canada: Robert Rose Ink publishers.

Brossat, J.Y., Ledeaut, J.Y., Ralamboranto, L., Rakotovao, L.H., Solar, S., Gueguen, A., Coulanges, P., 1981. Immunostimulating properties of an extract isolated from Aloe vahombe. 2. Protection in mice by fraction F1 against infections by Listeria monocytogenes, Yersinia pestis, Candida albicans and Plasmodium berghei. Archives de l'Institut Pasteur de Madagascar 48 (1) 1981, 11-34.

Bunyapraphatsara, N., Yongchaiyudha, S., Rungpitarangsi, V., Chokechaijaroenporn, O., 1996. Antidiabetic activity of *Aloe vera* L. juice II. Clinical trial in diabetes mellitus patients in combination with glibenclamide.Phytornedicine 3(3), 245-8.

Can, A., Akev, N., Ozsoy, N., Bolkent, S., Arda, B.P., Yanardag, R., Okyar, A., 2004. Effects of *Aloe vera* Leaf gel and pulp extracts on kidney in type-II diabetic rat model. Indian Journal of Experimental Biology. 42.1, 48-52.

Chen, X. D., Huang, L.Y., Wu, B.Y., 2005.Effect of *aloe vera* polysaccharide on the release of cytokines and nitric oxide in cultured human keratinocytes. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue* 17.5, 296-298.

Chithra, P., Sajithlal, G.B., Chandrakasan, G., 1998. Influence of *Aloe vera* on collagen characteristics in healing dermal wounds in rats. Molecular and Cellular Biochemistry 2, 71-76.

Choi, S., Chung, M.H., 2003. A review on the relationship between *aloe vera* components and their biologic effects. Seminars in Integrative Medicine 1, 53-62.

Choi, S.W., Son, B.W., Son, Y.S., Park, Y.I., Lee, S.K. and Chung, M.H., 2001. The wound-healing effects of a glycoprotein fraction isolated from *aloe vera*. British Journal of Dermatology 4, 535- 545.

Coats, B. C., Ahola, R., 1979. The silent healer: A modern study of *aloe vera*. Rochester, Vermont (USA): Healing Arts Press.

Collins, E., Collins, C., 1935. Roentgen dermatitis treated with fresh whole leaf of *Aloe vera*. American Journal of Roentgenology 33,396–7.

Dagne, E., Bisrat, D., Viljoen, A., Van Wyk, B.E., 2000.Chemistry of *Aloe* species. Current Organic Chemistry 24, 1055-1078.

Davis, R.H., Stewart, G.J., Bregman, P.J., 1992. *Aloe vera* and the inflamed synovial pouch model. Journal of the American Podiatric Medical Association 82 (3) 140-148.

Davis R.H., Maro N.P., 1989.*Aloe vera* and gibberellin: Anti- inflammatory activity in diabetes. Journal of the American Podiatric Medical Association.1, 24- 26.

Davis, R.H., 1997*Aloe vera*: A Scientific Approach. New York: Vantage Press.

Davis, R.H., Di Donato, J.J., Hartman, G.M. and Hass, R.C., 1994. Anti-inflammatory and wound healing activity of a growth substance in *Aloe vera*. Journal of the American Podiatric Medical Association 84, 77-81.

Duansak, D., Somboonwong, J., Patumraj, S., 2003 Effects of *Aloe vera* on leukocytes adhesion and TNF-alpha and IL-6 levels in burn wounded rats. Clin Hemorheol Microcirc. 2003; 29. 3-4:239-246.

Eamlamnam, K., Patumraj, S., Visedopas, N., Thong-Ngam, D., 2006.Effects of *Aloe vera* and sucralfate on gastric microcirculatory changes, cytokine levels and gastric ulcer healing in rats. World Journal of Gastroenterology 12.13: 2034-2039.

Fulton J. E. Jr., The stimulation of post-dermabrassion wound healing with stabilized aloe gel- polyethylene oxide dressing. The Journal of dermatologic surgery and oncology 16.5, 460-467.

Grindlay D, Reynolds T., 1986. The Aloe vera phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. Journal of Ethnopharmacology 16,117–51.

Gunther, R. T., 1934, The Greek herbal of Dioscorides, Oxford University Press.

Habbeb, F., 2007. The inner gel component of *Aloe vera* supresses bacterial- induced pro-inflammatory cytokines from human immune- cells. Methods 42.4: 388-393.

Heck, E., Head, M., Nowak, D., 1981. *Aloe vera* (gel) cream as a topical treatment for burns..7.4, 291-4.

Heggers J.P., Robson, M.C., Manavalen, K., 1987. Experimental and clinical observations on frostbite.Annals of Emergency Medicine16.9, 1056-1062.

http://books.google.co.za/books?id=zCiH3XmzhpIC&printsec=frontcover&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false (Accessed 2012/10/16)

Hunter, J.A., Frumkin, A.,1991. Adverse reactions to vitamin E and *aloe vera* preparations after dermabrassion and chemical peel. Cutis 47.3, 193-196.

Hutter, J.A., Salman, M., Stavinoha, W.B., Satsangi, N.,Williams, R.F., Streeper, R.T.,

Im, S.A., 2005. Identification of optimal molecular size of modified Aloe polysaccharides with maximum immunomodulatory activity. International Immunopharmacology5.2:271-279.

Ishii, Y., Tanizawa, H., Takino, Y., 1994. Studies of *aloe vera*: Mechanism of cathartic effect. Biological & Pharmaceutical Bulletin. 17.5, 651-653.

Kahlon, J.B., Kemp, M.C., Carpenter, R.H., 1991.Inhibition of AIDS virus replication by acemannan in vitro. Gene therapy & molecular biology 3, 127-135.

Kahlon, J.B., Kemp, M.C., Yawei, N., Carpenter, R.H., Shannon, W.M., McAnalley, B.H., 1991. In vitro evaluation of the synergistic antiviral effects of acemannan in combination with azidothymidine and acyclovir. Gene therapy & molecular biology 3.4, 214-223.

Kim, H.S., Lee, B.M., 1997.Inhibition of benzo [a] pyrene- DNA adduct formation *by Aloe barbadensis* Miller. Carcinogenesis 18.4, 771-776.

Klein, A.D., Penneys, N.S., 1988. *Aloe vera*. Journal of the American Academy of Dermatology 18, 714-720.

Krishnan, P., 2006. The scientific study of herbal wound healing therapies: Current state of play. Current Anaesthesia & Critical Care 17.1-2, 21-27.

Langmead, L., Makins, R.J., Rampton, D.S., 2001. Anti- inflammatory effects of *aloe vera* gel in human colorectal mucosa in vitro. Alimentary Pharmacology & Therapeutics 19.5, 521-527.

Lee, K.J , Myung, M.K., Yuna, Y., Kima, Y., Kimb, J.S., Kimb, Y.S., Kimc, K., , Seong Sun Hana, S.S., Lee, C.K., 2001. Acemannan purified from *Aloe vera* induces phenotypic and function maturation of immature dentitic cells. International Immunopharmacology.1.7, 1275-1284.

Leung, M.Y., Liu, C., Zhu, L.F., 2004. Chemical and biological characterization of a polysaccharide biological response modifier from *Aloe vera* L. var. chinensis (Haw.) Berg Glycobiology 14, 501-510.

Liptak, J.M., 1997. An overview of the management of wounds. Australian Veterinary Journal 75.6, 408-413.

Liu, C., 2006. Macrophage activation by polysaccharide biological response modifier isolated from *Aloe vera* L. Var. chinensis (Haw.) Berg. International Immunopharmacology 6.11, 1634-1641.

Maddocks- Jennings, W., Wilkinson, J.M., Shillington, D., 2005.Novel approaches to radiotherapy induced skin reactions: a literature review. Complementary Therapies in Clinical Practice 11.4, 224-231.

Maenthalsong, R., Chalyakunapruk, N., Nirutropon, S., 2007.The efficiency of *Aloe vera* for burns and wound healing: a systematic review: Burns 33, 713-718

Manderville F., 1939. *Aloe vera* in the treatment of radiation ulcers and mucous membranes. Radiology 32,598–9.

McCauley R. L., Heggers J. P., Robson M. C. Frostbite: Methods to minimize tissue loss. Postgrad Med. 1990; 88.8: 67.

McDaniel, H.R., Carpenter, R.H., Kemp, M., 1990 Extended survival and prognostic criteria for acemannan (ACE-M) treated HIV-1 patients. Antiviral Research 1: 117-147.

Miller, M.B., Koltai, P.J., 1995. Treatment of experimental frostbite with pentoxifylline and *aloe vera* cream. Archives of Otolaryngology - Head and Neck Surgery 121.6, 678-680.

Montaner, J.S., Gill, J., Singer, J., Raboud, J., Arseneau, R., McLean, B.D., Schechter, M.T., Ruedy, J., 1996. Double- blind placebo- controlled pilot trial of acemannan in advanced human immunodeficiency virus disease.Journal of Acquired Immune Deficiency Syndromes and Human Retrovirolog 12.2, 153-157.

Moon, E.J, Lee, Y.M, Lee, O.H., Lee, M.J., Lee, S.K., Chung, M.H., Park, Y.I., Sung, C.K., Choi, J.S., Kim, K.W., 1999. A novelangiogenic factor derived from *Aloe vera* gel: beta Sitosterol, a plant sterol. Angiogenesis 3, 117–123.

Park Y, Lee S., 2006.New Perspectives on Aloe. New York: Springer Verlag.

Parthiban, M., Srikrishnan, M.R., Viju, S., 2011. Studies on odour retention and antibacterial efficacy of non-woven fabrics for technical textile applications. International Journal of Pharmarcolgy & Life Sciences (IJPLS) 10, 1137-1141.

Paulsen, E., Koesholm, L., Brandrup, F., 2005. A double- blind, placebo- controlled study of a commercial aloe vera gel in the treatment of slight to moderate psoriasis vulgaris. Journal of the European Academy of Dermatology and Venereology 19.3, 326-331.

Peng, S.Y., Norman, J., Curtin, G., Corrier, D., McDaniel, H.R., Busbee, D., 1991. Decreased mortality in Norman murine sarcoma in mice treated with the immunomodulator, Acemannan. Gene Therapy & Molecular Biology 3, 79–87

Puvabanditin, P., Vongotongsri, R., Efficacy of *aloe vera* cream in prevention and treatment of sunburn and suntan. Journal of the Medical Association of Thailand88 (Suppl 4): S173-S 176.

Reynolds, T., Dweek A.C., 1999. *Aloe vera* leaf gel. A review update. Journal of Ethnopharmacology 68.1-3, 3-37.

Rodriguez- Bigaz, M., Cruz, N.I., Suarez, A., 1988. Comparative evaluation of *aloe vera* in the management of burn wounds in guinea pigs. Plastic and Reconstructive Surgery 81.3, 386-389.

Saada, H.N., Ussama, Z.S., Mahdy, A.M., 2003. Effectiveness of Aloe on the antioxidant of different tissues in irradiated rats. Pharmazie 58 (12), 929- 931.

Saoo, K., Miki, H., Ohmori, M., Winters, W.D., 1996. Antiviral activity of aloe extracts against cytomegalovirus. Phytotherapy Research 10 (4), 348-350.

Schmelzer, G.H., Gurib-Fakim, A., 2008. Plant Resources of Tropical Africa 11 (1): Medicinal plants 1.Netherlands: Wageningen.

Schmidt, J.M., Greenspoon, J.S.,*Aloe vera* dermal wound gel is associated with a delay wound healing. Obstetrics & Gynecology78.1, 115-117.

Shelton R. M.,1991. *Aloe vera*, its chemical and therapeutic properties. International Journal of Dermatology 10, 679- 683.

Singh R. P., Dhanalakshi S., Rao A.R., 2000. Chemomodulatory action of *Aloe vera* on the profiles of enzymes associated with carcinogen metabolism and antioxidant status regulation in mice. Phytomedicine 7.3, 209-219.

Strikland, F.M., Pelley, R.P., Klipke, M.L., 1997. Prevention ultraviolated-induced suppression of contact and delayed hypersensitivity by *Aloe barbadensis* gel extract. Journal of Investigative Dermatology 102.2, 197-204.

Suvitayavat, W., Sumrongkit, C., Thirawarapan, S.S., Bunyapraphatsara, N., 2004. Effects of Aloe preparation on the histamine-induced gastric secretion in rats. Journal of Ethnopharmacology 90 (2-3), 239-47.

Syed, T.A, Cheema, K.M, Ashfaq A, E., 1996. *Aloe vera* extract 0.5% in ahydrophilic cream versus *Aloe vera* gel for the management of genital herpes in males. A placebo-controlled, double-blind, comparative study [letter]. Journal of the European Academy of Dermatology and Venereology 7, 294-295.

Syed, T.A., Afzal, M., Ashfaq Ahmad, S., 1997. Management of genital herpes in men with 0.5% *Aloe vera* extract in a hydrophilic cream: a placebo-controlled double-blind study. Journal of Dermatological Treatment 8, 99-102.

Syed, T.A., Ahmad, S.A., Holt, A.H., 1996. Management of psoriasis with *Aloe vera* extract in a hydrophilic cream: a placebo-controlled, double-blind study. Tropical Medicine & International Health 1, 505-509.

't Hart, L. A, Nibbering, P. H., van den Barselaar, M.T., 1990. Effects of low molecular weight constituents from *Aloe vera* gel on oxidative metabolism and cytotoxic and

bactericidal activities of human neutrophils. International Journal Immunopharmacology 12, 427-434.

Talmadge, J., Chavez, J., Jacobs, L., Munger, C., Chinnah, T., Chow, J.T., Williamson, D., Yates, K., 2004. Fractionation of *Aloe vera* L. inner gel, purification and molecular profiling of activity. International Immunopharmacology 4, 1757–1773.

Weintraub, S.T., 1996. Anti-inflammatory C- glucosyl chromone from *Aloe barbadensis*. Journal of Natural products 59.5, 547-543.

West D.P., Zhu, Y.F.,2003. Evaluation of *aloe vera* gel gloves in the treatment of dry skin associated with occupational exposure. American Journal of Infection Control. 31.1, 40-42.

Womble, D., Helderman, J.H.,1988. Enhancement of allo- responsiveness of human lymphocytes by acemannam (Carrisyn (TM). International Journal of Immunopharmacology 10.8, 967- 974.

Wu, J.H., Xu, C., Shan, C.Y., Tan, R.X., 2006. Antioxidant properties and PC12 cell protective effects of APS-1, a polysaccharide from *Aloe vera* var. *chinensis*. Life Sciences 78, 622–630.

Yagi, A., Kabash, A., Okamurs, N., Haraguchi, H., Moustafa, S.M., Khalifa, T.I., 2002.Antioxidant, free radical scavenging and anti-inflammatory effects of aloesin derivatives in *Aloe vera*. Planta Medica 68, 957–960.

Zachary L. S., Smith D. J Jr., Heggers J. P., 1987; The role of thromboxane in experimental inadvertent intra-arterial drug injections. Journal of Hand Surgery 12.2, 240- 245.

Zawahry M. E., Hegazy M. R., Helal M., 1973. Use of aloe in treating leg ulcers and dermatoses. International Journal of Dermatology 12, 68-73.

Zhang, L., Tizard, I.R., 1996. Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from *Aloe vera* gel. Immunopharmacology 2, 119-128.

Zhang, X.F., 2008. Binding of the bioactive component Aloe dihydroisocoumarin with human serum albumin. Journal of Molecular Structure 1, 87-92.

CHAPTER 2

PHYTOCHEMICAL ANALYSIS OF *ALOE VERA* (L) Burn. f IN DIFFERENT SOLVENTS

CHAPTER 2

PHYTOCHEMICAL ANALYSIS OF *ALOE VERA* (L) Burn. f IN DIFFERENT SOLVENTS

2.1 INTRODUCTION

Plants naturally contain a number of phytochemicals as secondary metabolites. In most cases, the pharmalogical properties of medicinal plants are attributed to these phytochemicals. Some of these chemicals are radical scavenging compounds or antioxidants.

Reactive oxygen species (ROS) which are also called active oxygen species, are various forms of activated oxygen that include free radicals such as superoxide ions (O_2^-), non-free radical species such as hydrogen peroxide (H_2O_2) as well as hydroxyl radicals (OH^-). In living organisms different ROSs can be produced in a number of ways which includes aerobic respiration, upon stimulation of polymorphonuclear leukocytes, macrophages and peroxisomes. The medicinal activities of *Aloe vera* may largely be attributed to the antioxidant property of its phytochemicals. There is little or no information in the literature on the phytochemical constituents of *Aloe vera* that is cultivated in South Africa. This experiment was therefore aimed at investigating the major groups of compounds found in *Aloe vera*.

2.2 MATERIAL AND METHODS

2.2.1 Plant collection and preparation

Aloe vera materials were collected from AloeWay, Iphofolo Game Farm, Polokwane in the Limpopo province of South Africa. The identity of the plant was confirmed from literature (Schemelzer & Gurib-Fakim, 2008) and authenticated by Professor DS Grierson of Botany Department, University of Fort Hare, Alice. The plant leaves were divided into two portions. One portion was extracted fresh while the other was cut into pieces and oven dried at 40^0C and then milled to a homogenous powder once dried completely.

2.2.2 Preparation of extract

The homogenized plant materials were extracted separately in distilled water, acetone and dichloromethane on a shaker for 48 h. Each extract was filtered using a Buchner funnel and Whatman No. 1 filter paper. The water extract was frozen at −40°C and dried for 48 h using a freeze dryer. The other extracts were individually concentrated to dryness under reduced pressure at 45°C (acetone) and 40^0C (dichloromethane) using a rotary evaporator. The extracts were then reconstituted in their respective solvents of extraction to 10 mg/ml which served as stock solutions. The same process was carried out for the fresh portions.

2.2.3 Chemicals and reagents used

The following chemicals were used for the various experiments: Vanillin, Benzene, Sodium hydroxide (NaOH), Aluminium Chloride ($AlCl_3$) Ferric chloride ($FeCl_3$), Acetic acid, Ammonium hydroxide (NH_4OH), Diethyl ether, n- butanol, tannic acid, Ethanol (EtOH), Methanol (MeOH), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), potassium ferricyanide [$K_4Fe(CN)_6$], trichloroacetic acid (TCA), Hydrochloric acid (HCL) were purchased from Gauteng, South Africa. All other chemicals used in this study including the solvents, were of analytical grade.

2.2.4 Determination of flavonoids

The method described by OrdonEz*et al.,* (2006) was used to determine total flavonoids content in aqueous extract of *Aloe vera*. A volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of the sample solution. After 1 h at room temperature, the absorbance was measured at 420 nm. A yellow colour indicated the presence of flavonoids. Plant extracts were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content was calculated

as mg/g of quercetin using the following equation based on the calibration curve: Y = 0.0255x, R2 = 0.9812, where x is the absorbance and Y is the quercetin equivalent.

2.2.5 Determination of total phenols

The total phenolic content of the extract was determined using the method of Wolfe*etal.*(2003) with Folin-Ciocalteu reagent. An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water at a concentration of 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 s and left to stand for 30 min at 40°C for colour development. The absorbance of total phenolics was then measured at 765 nm using the AJI-C03 UV-VIS spectrophotometer. Total phenolic content was expressed as mg/g of tannic acid equivalent using the calibration curve: Y = 0.121 xs, R2 = 0.936512, where x is the absorbance and Y is the tannic acid equivalent.

2.2.6 Determination of total tannins

Tannin determination was done according to the method of Wintola & Afolayan (2011)with some modifications. To 0.20 g of the sample was added 20 ml of 50% methanol. This was shaken thoroughly and placed in a water bath at 80°C for 1 h to ensure uniform mixing. The extract was filtered into a 100-ml volumetric flask, followed by the addition of 20 ml of distilled water, 2.5 ml of Folin-Denis reagent and 10 ml of 17% aq. Na_2CO_3 and was thoroughly mixed. The mixture was made up to 100 ml with distilled water, mixed and allowed to stand for 20 min. The bluish-green colour developed at the end of the reaction mixture of different concentrations ranges from 0 to 10 ppm. The absorbance of the tannic acid standard solutions as well as sample was measured after colour development at 760 nm using the AJI-C03 UV-VIS spectrophotometer. Results were expressed as mg/g of tannic acid equivalent using the calibration curve: Y = 0.0593x – 0.0485, R2 = 0.9826, where x is the absorbance and Y is the tannic acid equivalent.

2.2.7 Determination of total alkaloids

Alkaloids were quantitatively determined according to the method of Harborne(2005). Two hundred ml of 10%acetic acid in ethanol was added to 5 g powdered plant sample, covered and allowed to stand for 4 h. The filtrate was then concentrated on a water bath to one-fourth of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed and the whole solution was allowed to settle. The collected precipitates were washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed. The alkaloid content was determined using the formula: % alkaloid = final weight of the sample/ initial weight of the extract × 100.

2.2.8 Determination of total flavonols

Total flavonol content was determined by adopting the procedure described by Kumaran & Karunakaran(2007). The reaction mixture consisted of 2.0 ml of the sample, 2.0 ml of AlCl3 prepared in ethanol and 3.0 ml of (50 g/l) sodium acetate solution. The absorbance at 440 nm was measured after 2.5 h at 20°C. Total flavonol content was calculated as mg/g of quercetin equivalent from the calibration curve using the equation: Y = 0.0255 xs, R2 = 0.9812, where x isthe absorbance and Y is the quercetin equivalent.

2.2.9 Determination of total proanthocyanidin

Total proanthocyanidin was determined based on the procedure described by Oyedemi*et al* (2010). To 0.5 ml of 1 mg/ml of the extract solution was added 3 ml of vanillin-methanol (4% v/v) and 1.5 ml of hydrochloric acid and vortexed. The mixture was allowed to stand for 15 min at room temperature and the absorbance was measured at 500 nm. Total proanthocyanidin content was evaluated at a concentration of 0.1 mg/ml and expressed as

catechinequivalent (mg/g) using the calibration curve equation: Y = 0.5825x, R2 = 0.9277, where x is the absorbance and Y is the catechin equivalent.

2.2.10 Determination of saponins

Quantitative determination of saponins was done using themethod ofObadoni &Ochuko (2001). The powdered sample (20 g) was added to 100 ml of 20% aqueous ethanol and kept in a shaker for 30 min. The samples were heated over a water bath for 4 h at 55°C. The mixture was then filtered and the residue re-extracted with another 200 ml of 20% aqueous ethanol. The combined extracts were reduced to approximately 40 ml over the water bath at 90^0C. The concentrate was transferred into a 250 ml separating funnel and extracted twice with 20 ml diethyl ether. The ether layer was discarded while the aqueous layer was retained and to which 60 ml n-butanol was added. The n-butanolextracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation, the samples were dried inthe oven at 40°C to a constant weight. The saponin content was calculated using the formula: % saponin = final weightof sample/initial weight of extracts × 100.

2.3 RESULTS AND DISCUSSION

2.3.1 Phytochemical analysis

The curative properties of medicinal plants are believed to be due to the presence of different phyto-constituents such as alkaloids, flavonoids, phenols etc (Lalnundanga and Lalrinkima, 2012). As a result of this, phytochemical compounds are now being recognized as playing an important role in the disease prevention properties of medicinal plants. Such role includes the antioxidant properties. The results of the phytochemical screening of both the fresh and dry

leaves of *Aloe vera* extracted using different solvents, showed varying degrees of phytochemicals (fig:2.1 and 2.2).

2.3.2 Proanthocyanidins

Proanthocyanidins quantities ranged between 31.64 and 82.71 mg/ml of stock solution (fig 2.1 c & 2.2 a). Their opulence was seen in the dry acetone extract and moderately present in the dry DCM and aqueous extracts whereas they were present in small quantities in both the fresh acetone and aqueous extracts. According to Eloff (1998) acetone is able to extract more yields from plants. This is because the solvent is miscible with water, very volatile, and is easily removed from the plant material at lower temperatures. Proanthocyanidin also named condensed tannins, are oligomers and polymers of monomeric flavonoids that are responsible for the plants medicinal used hence its traditional use for various ailments. Beninger and Hosfield (2003) have shown that proanthocyandins have high antioxidant and radical scavenging activity usually greater than vitamins C and E. The high Proanthocyanidin content in the *Aloe vera* leaves maybe responsible for its high therapeutic activities. The presence of proanthocyanidin in *A. vera* in this study contradicts the observation of Prajapati (2011) who reported that proanthocyanidins were absent in this plant. Their observation may be due to the method of extraction or chemicals used in the extraction.

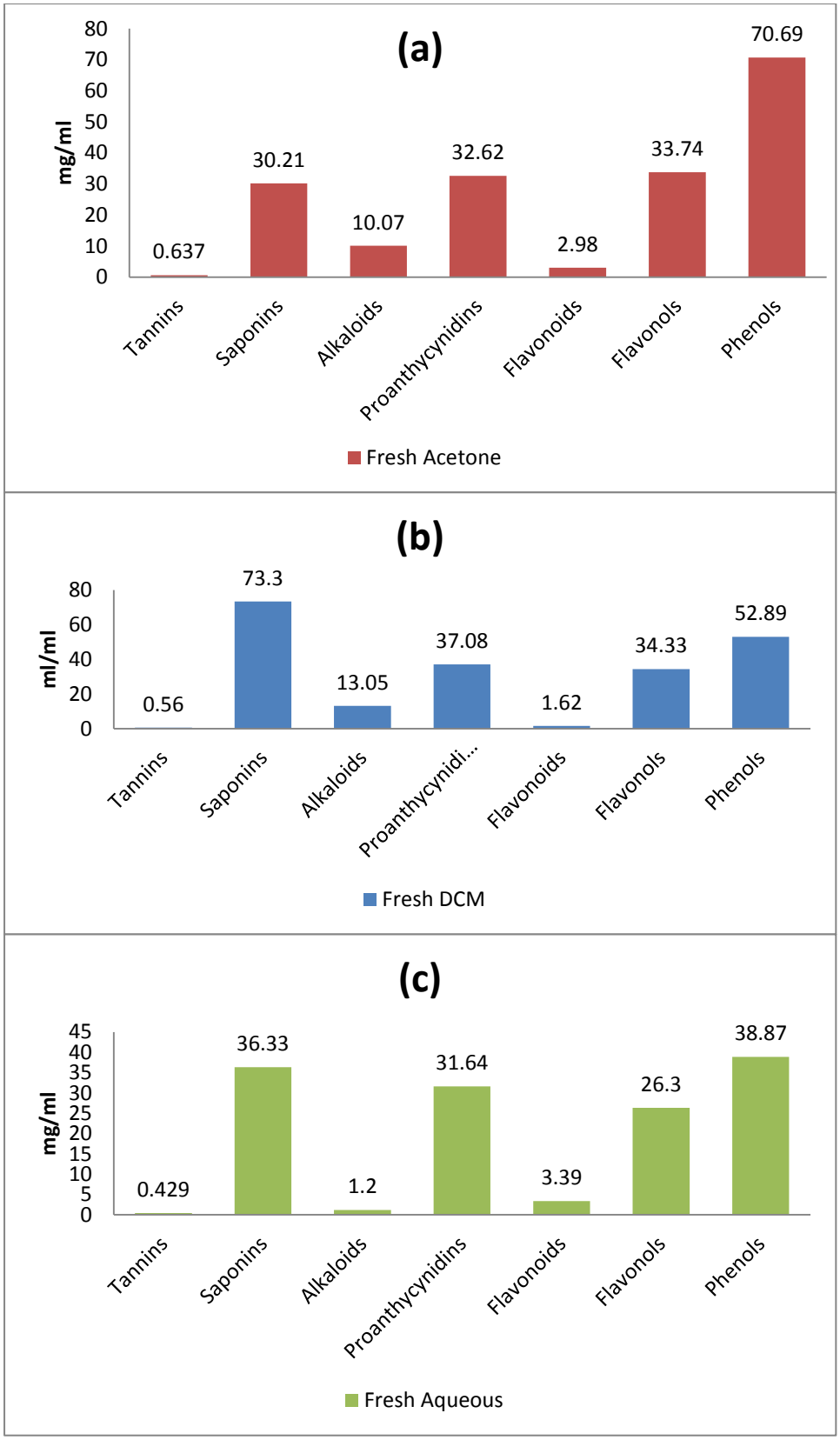

Figure 2.1: Phytochemicals in fresh portions of *A.vera*

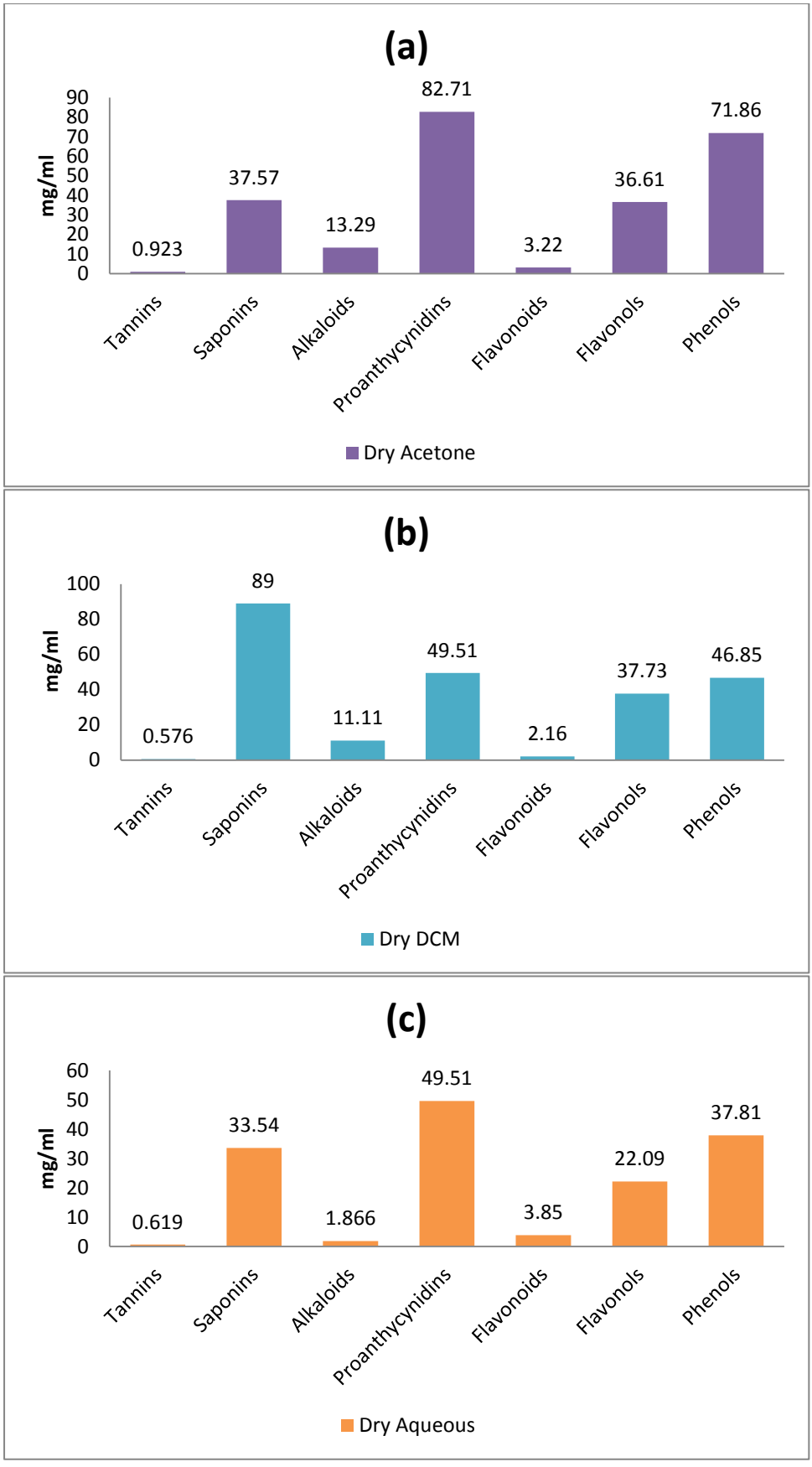

Figure 2.2: Phytochemicals in dry portions of *A.vera*.

2.3.3 Flavonoids

The maximum flavonoid content 3.85 mg/ml was observed in the dry aqueous extract (fig 2.2 c). Minimum contents of flavonoids were seen in in fresh aqueous (3.39 mg/ml) and dry acetone (3.22mg/ml) extracts, whereas trace amounts were noticed to be (2.16 and 1.62 mg/ ml) in dry and fresh DCM extract. Flavonoids are low molecular weight bioactive polyphenols that are widely distributed in the plant kingdom and play a vital role in photosynthesizing cells Fernandezet *et al* (2006). Flavonoids have been reported to possess a wide range of biological activities such as anti-inflammatory, antibacterial, antiviral, antiallergic Waller *et al* (1980). Furthermore flavonoids are powerful antioxidants against free radicals and are described as free-radical scavengers (Mahattanadul, 1995). The flavonoid content was found to be higher in the dried water extract than in the fresh water extract, and that of dried acetone extract on the other hand was estimated to be extremely higher whereas the fresh acetone was found to be slightly smaller than that of the dried. Research undertaken by Hu *et al* (2003) has shown similar results when evaluating the antioxidant potential of various *Aloe vera* extracts. Their findings were in harmony with the current study. The high flavonoid content bears out the capacity of *Aloe vera* gel may have capacity to protect human beings and rats against gastric ulceration due to its anti-inflammatory cytoprotective healing and mucus stimulatory effects.

2.3.4 Flavonols

The flavonol content in the whole leaf extracts in the various solvents was in the order: DCM>acetone > aqueous extracts (fig 2.1 & 2.2). The flavonol content in the extract extracted dry was significantly higher than the one extracted fresh. DCM extract was higher in content than the acetone extract meanwhile the aqueous extract of both the fresh and dry portion showed the slight flavonol contents. Flavonols are phytochemical compounds found

in high concentrations in a variety of plant-based foods and beverages. They have been reported to increase erythrocyte of superoxide dismutase (an antioxidant enzyme found in red blood cells) activity, to decrease the damage of lymphocyte DNA and have an ability to scavenge free radicals Williamson & Manach, (2005). The flavonol content in the *Aloe vera* leaves is considerably high in the dichloromethane extract. This is agreement with Eloff(1998) that referred to dichloromethane as a best extractant for the isolation of phyto-compounds as it yields the highest number of visible compounds than any other extract in a study he conducted.

2.3.5 Phenols

Phenolic compounds are a large group of anti-oxidant compounds found in many food systems and are very common in fruits and vegetables Sun(2002). As shown in Figure1, the Phenols were present in the *Aloe vera* whole leaf. These finding supported Adesuyi (2012) who reported that showed *Aloe vera* possesses significant flavonoids content. In the present study, the flavonoid content was found to be higher in the acetone extracts of both the dry and fresh plant material.

2.3.6 Tannin

Tannin content was generally low in all the solvent extracts of *A. vera* compared to all other phytochemicals (Fig 2.1 & 2.2). Tannin is usually a bitter plant polyphonic compound and astringent that binds and precipitates proteins and other various organic compounds including amino acids and alkaloids. The tannins protein complex can provide persistent antioxidant activity Mohun & Cook (1962). Tannin was found in trace amounts in all solvents extracts, both in dry and fresh plant material. The highest yield of Tannins in *Aloe vera* leaves was observed in the acetone extract of the dry plant material. The yields of other extracts did not differ significantly. Tannins, though in trace amounts have astringent properties, hasten the

healing of wounds and inflamed mucous membrane Okwu & Okwu (2004). The presence of tannins in *Aloe vera* strongly confirmed its use in treating wounds, skin infections, and intestinal inflammations bronchitis in herbal medicine as reported by Khan & Iqbal (2011).

2.3.6 Alkaloids

Alkaloids were present in moderate amounts ranging from 1.2 – 13.29 mg/ml (fig 2.1 c & 2.2 a). The highest content of alkaloids was seen in dry acetone, followed by fresh DCM extract and fresh acetone extract. The fresh and dry aqueous extract showed the least alkaloid content. Alkaloids are the most diverse group of secondary metabolites found in living organisms and have an array of structure types in biosynthetic pathways and pharmalogical activities. Many alkaloids have been used for hundreds of years in medicine and some are still prominent drugs today Pelletier (1983).Among the three solvents extracts maximum yield of alkaloid was observed in the acetone extract. Aqueous extracts however yielded small alkaloid content.

2.3.7 Saponins

Saponins one of the phytochemicals that were found to be present in maximum quantity compared to other phytochemicals investigated in the study. The maximum saponin content was noted in the both the dry and fresh DCM extracts (89 and 73.3 mg/ml) fig 22b & 2.1b). Saponins were found to be present in *Aloe vera* extracts. Levels of saponins were found to be significantly high in dichloromethane extract of the dry plant material and have supported the usefulness of this plant in managing inflammation. Just *et al* (1998) revealed the inhibitory effect of saponins on inflamed cells. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell. However, the dichloromethane extract of the fresh plant material was observed to have high saponin content which was not

very far from that of the dry plant material. There was no significant difference in the saponin content of other extracts investigated in this present study.

2.4 CONCLUSION

The variation in type of phytochemicals present in different solvents as shown in the result of phytochemical screening might be attributed to the ability of the solvents to dissolve into solution specific type of phytochemicals as reported by Yushau (2011). In the current study, the usefulness of *A.vera* for medicinal purposes was verified. The investigation has revealed the presence of many secondary metabolites in its leaves. All the whole leaf extracts of *Aloe vera* gave good indication that it possesses significant amounts of phytochemicals. This partly explained the uses of this plant in herbal medicine. High yields of phytochemicals were obtained in the dry portion of the plant. Acetone and dichloromethane extracts showed higher phytochemical content than the aqueous extracts.

2.4 REFERENCE

Adesuyi A.O., Awosanya O.A., Adaramola, F.B., Omeonu, A.I., 2012. Nutritional and Phytochemical Screening of *Aloe barbadensis*. Current Research Journal of Biological Sciences 4(1), 4-9.

AOAC, 1990 (15thed): Official Methods of Analysis .Association of Official Analytical Chemists. Arlington, V.A.;

Barcroft, A., Myskja, A., 2003. Aloe vera: Nature's Silent Healer. London: BAAM Publishing Ltd;

Beninger, C.W., Hosfield, G.L., 2003. Antioxidant activity extracts, condensed tannin fractions, and pure flavonoids from Phaseolus vulgaris L. seed coat colour genotypes. Journal of Agricultural and Food Chemistry 51, 7879–83.

Bozzi, A., Perrin, C., Austin, S., Vera, F.A., 2007. Quality and authenticity of commercial *Aloe vera* gel powders. Food and Chemical Toxicology 103(1), 22-30.

Bruneton, J., 1995. Pharmacognosy, Phytochemistry, Medicinal Plants. France: Lavoisier Publishing.

Eldridge, J., 1978. Bush medicine in the Exumas and Long Islands, Bahamas, A field study. Economic Botany 29, 307-332.

Eloff, J.N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 60, 1–8.

Fernandez, S.P., Wasowski, C., Loscalzo, L.M., Granger, R.E., Johnston, G.A.R., Paladini, A.C., Marder, M., 2006. Central nervous system depressant action of flavonoid glycosides. European Journal of Pharmacology 539,168-176.

Ghannam, N., Kingston, M., Al-Meshaal, I.A., Tariq, M., Parman, N.S., Woodhouse, N., 1986. The antidiabetic activity of aloes: preliminary clinical and experimental observations. Hormone Research 24, 288-294.

Gordon, M.C., David, J.N., 2001. Natural product drug discovery in the next millennium. Pharmaceutical Biology 39, 8-17.

Gur, D.,Ozalp, M., Sumerkan, B., Kaygusuz, A., Toreci, K., Koksal, I., Over, U., Soyletir, G., 2002. Relevance of antimicrobial resistance in Haemophilus influenzae, Klebsiella pneumoniae, Moraxella catarrhalis and Streptococcus pyogenes: results of a multicentre study in Turkey. International Journal of Antimicrobial Agents 19, 207-211.

Harborne, J.B., 2005 (3rd ed): Phytochemical methods. A guide to modern techniques of plant analysis , New Delhi: Springer Pvt. Ltd.

http://www.assignmentpoint.com/science/medical/report-on-qualitative-and-quantitative-determination-of-antibacterial-properties.html (Accessed 2013/ 10/ 17).

Hu, Y., XU, J., HU, Q., 2003: Evaluation of Antioxidant Potential of *Aloe vera* (*Aloe barbadensis* Miller) Extracts. Journal of Agricultural and Food Chemistry 51 (26), 7788-91.

Hurley, J.C., 1992. Antibiotic Induced Release of Endotoxin: a reappraisal. Clinical Infectious Diseases 15, 840-854.

Just, M.J., Recio, M.C., Giner, R.M., Cueller, M.J., Manez, S., Bilia, A.R., 1998.Anti-inflammatory activity of unusual lupine saponins from Bupleurum Fruticescens. Planta Medica 64, 404-407.

Khan, N.A., Iqbal, S.A, (eds.) (2011) IMPORTANCE OF MEDICINAL PLANTS. 1STed. New Delhi: TilakWasan, pp.8 – 9.

Kumaran, A., Karunakaran, R.J., 2007. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from Journal of Food Science and Technology 40, 344-52.

Lalnundanga, N.L., Lalrinkima, R., 2012. Phytochemical analysis of the methanol extract of root bark of Hiptagebenghalensis (L.). KurzSciVis 12(1): 8-10.

Mahattanadul, S., 1995. Antigastric ulcer properties of *Aloe vera*. Songklanakarin Journal of Science and Technology 18, 49–57.

Mohun, A.F., Cook, I.J.K., 1962. An improved dinitrosalicylic acid method for determining blood and cerebrospinal fluid sugar levels. Journal of Clinical Pathology 15, 169-180.

Obadoni, B.O., Ochuko, P.O., 2001. Phytochemical studies andcomparative efficacy of the extracts of some haemostaticplants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences 8, 203-8.

Okwu, D.E., Okwu, M.E., 2004. Chemical composition of Spondiasmombialinn plants parts. Journal sustainable agriculture environment 6, 140-147.

OrdonEz, A.A., Gomez, J.D., Vattuone, M.A., Isla, M.I., 2006. Antioxidant activities of Sechiumedule (Jacq). Food Chemistry 2006, 97:452-8.

Oyedemi, S.O., Bradley, G., Afolayan, A.J., 2010. In vitro and Invivo antioxidant activities of aqueous extract of StrychonoshenningsiiGilg.African Journal of Pharmacy and Pharmacology 4, 70-8.

Pelletier, S.W., 1983. The nature and definition of an alkaloid, in: Alkaloids: Chemical and Biological Perspective. Vol 1 (S. W. Pelletier. Ed. Wiley, New York, pp 1-31)

Prajapati, M., Patel, P.S., Vyas, P.J., 2011.Phytochemical Analysis of Aloe Vera and Study of Mixing Antibiotic with Aloe Vera and Its Antibacterial Activity. Asian Journal Of Biochemical And Pharmaceutical Research 1, 475-476.

Prior, R.L, Cao, G., 2000. Antioxidant phytochemicals in fruits and vegetables: diet and health implications. Horticulture Science 35, 588-592.

Pugh, N., Ross, S.A., ElSohly, M.A., Pasco, D.S., 2001.Characterisation of aloeride, a new highmolecular-weight polysaccharide from *Aloe vera* with potent immunostimulatory activity. Journal of Agricultural and Food Chemistry 49, 1030-1034.

Rabe, T., Van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology. 56 (1), 81-87.

Schemelzer, G.H., Gurib-Fakim, A., 2008.Plant Resources of Tropical Africa. Medicinal plants .Volume 11.1stedition. Edited by Lemmens RHMJ, Oyen L P A.Netherlands: Wegenigeng; 2008: 82-84.

Sun, J., Chu, Y.F., Wu, X., Liu, R., 2002.Antioxidant and anti-proliferative activities of common fruits. Journal of Agricultural and Food Chemistry 50, 7449-7454.

Waller, J., Klopfenstein, T., Poos, M., 1980. Distiller's feeds as protein sources for growing ruminant's. Journal of Animal Science 5, 51-55.

Williamson, G., Manach, C., 2005. Bioavailability and bio-efficacy of polyphenols in humans. II. Review of 93 intervention studies. The American Journal of Clinical Nutrition 81, 243S-255S.

Wintola, O.A., Afolayan, A.J., 2011. Phytochemical constituents and antioxidant activities of the whole leaf extract of *Aloe ferox* Mill. Pharmacognosy Magazine 7, 325-33.

Wolfe, K., Wu, X., Liu, R.H., 2003. Antioxidant activity of apple peel. Journal of Agricultural and Food Chemistry, 51, 609-14.

Yusha'u, M., Olonitola, S.O., Aliyu, B.S., 2008.SENSITIVITY OF EXTENDED-SPECTRUM β - LACTAMASES PRODUCING ENTEROBACTERIACEAE TO ACALYPHA MACCAFEANA EXTRACTS. Bayero Journal of Pure and Applied Sciences 1(1), 1 – 5.

CHAPTER 3

THE USE OF THIN LAYER CHROMATOGRAPHY AND INFRARED SPECTROSCOPYFOR THE PROFILING AND CHARACTERIZATION OF *ALOE VERA* COMMERCIALS PRODUCTS.

CHAPTER 3

THE USE OF THIN LAYER CHROMATOGRAPHY AND INFRARED SPECTROSCOPY FOR THE PROFILING AND CHARACTERIZATION OF *ALOE VERA* COMMERCIALS PRODUCTS.

3.1 INTRODUCTION

Herbs have been used for medical treatments since the beginning of civilization and some derivatives (eg, aspirin, reserpine, and digitalis) have become mainstays of human pharmacotherapy. Since the beginning of human civilization, herbs have been an integral part of society, valued for both their culinary and medicinal properties. Herbal medicine has made many contributions to commercial drug preparations manufactured today including ephedrine from *Ephedra sinica* (ma-huang), digitoxin from *Digitalis purpurea* (foxglove), salicin (the source of aspirin) from *Salix alba* (willow bark), and reserpine from *Rauwolfiaserpentina* (snakeroot), to name just a few. For cardiovascular diseases, herbal treatments have been used in patients with congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, venous insufficiency, and arrhythmia. However, many herbal remedies used today have not undergone careful scientific assessment, and some have the potential to cause serious toxic effects and major drug-to-drug interactions (Nick *et al*, 2008). With the high prevalence of herbal use around the globe today, be informed about their potential for benefit and harm. Continuing research is necessary to elucidate the pharmacological activities of the many herbal remedies now being used to treat many cardiovascular diseases. (Nick *et al*, 2008).

The growing use of herbal medicines has posed quality problems. Therefore, there is a corresponding need for international guidance on reliable methods for quality control. Identity, purity, and content of herbal materials are recommended test procedures that assist national laboratories engaged in pharmaceutical quality control (WHO, 1998)

Insufficient data exist for most plants to guarantee their quality, efficacy and safety. There are several challenges as standardization of herbal product is considered like controversial identity of various plants, deliberated adulteration of plant material, problems in storage and

transport, which should be considered Thatte (2003). One of the impediments in the acceptance of the herbal products worldwide is the lack of standard quality control profiles Shinde *et al.* (2009). Recommended procedures, IR, High Performance Thin Layer Chromatography or the use of thin-layer chromatography for the qualitative determination of impurities should also prove useful to the pharmaceutical industry and pharmacists working with herbal materials (WHO, 1998).

To my knowledge, there is no information in literature on the profile of *Aloe vera* that grows in South Africa. This study therefore aims at determining the chemical profile of hexane, DCM, acetone, ethanol and aqueous extracts of fresh and dry leaf portions of *Aloe vera* plant using thin layer chromatography technique. Furthermore, techniques such as IR and HPLC will also be used to affirm traces or aloe ingredients in selected *aloe* cosmetics which are said to be made from *Aloe vera*.

3.2 MATERIAL AND METHODS

Plant material used for these experiments were collected, identified and extracted as described in Chapter 2. All the solvents used for extraction and TLC analysis were of L.R. and A.R. grade, respectively. Aluminium based pre-coated TLC plates of 0.2 mm thickness (20 × 20 cm) from (E. Merck, Germany) were used for TLC studies.

Direct extraction

Both fresh and dried samples were directly extracted with hexane, DCM, acetone, ethanol and water. In this method, (20 g) finely ground plant material was weighed, to this 100 ml of each solvent hexane, was added. The beaker was kept in a shaker with gentle swirling at 120 rpm for 48 h. The extract was then filtered using a Buchner funnel and Whatman No. 1 filter paper. The process was repeated for DCM, acetone, water and ethanol. The filtrate extracted

using water was frozen at −40°C and dried for 48 h using a freeze dryer. The other extracts were individually concentrated to dryness under reduced pressure at 56°C (acetone), 68^0C (hexane), 78^0C (ethanol) and 39^0C (dichloromethane) using a rotary evaporator. The residue was weighed and the extracts were then reconstituted in their respective solvent of extraction to 10 mg/ml which served as a stock solution. The same was done for the fresh portion of the plant material.

Determination of the best mobile phase

About 20 µl each extract of solution was applied on the TLC plates using a micro syringe (25 µl, Hamilton). The plates were developed in a glass chamber with the following different solvent systems, thus Ethyl acetate: methanol: water (100: 13.5:10), N-propanol: formic acid: water (90:1:9), Ethyl acetate: methanol: water (81:11:8), Hexane: acetone: ethanol (40:10:4) and Benzene: ethanol: ammonium (80:10:1). Plates were dried and spots were visualized in UV chamber. The chromatograms were observed directly under UV_{254} nm and UV_{365} nm. The plates were further visualized by spraying them with several spraying reagents.

Determination of the best spraying reagent

For the determination of the best spraying reagent, only the DCM extractant was used. Several plates were spotted with DCM extract of the dry plant material. They were individually sprayed with Ethanolic Potassium Hydroxide, Vanillin Phosphoric, Vanillin Sulphuric and acid spray. They were dried at 100°C for 5-10 and were viewed on day light and under UV at 356 nm.

The determination of the presence of *aloe vera* ingredients in the shelf cosmetics using thin layer chromatography was unsuccessful. Therefore, alternatively FT-IR was

utilized to determine the presence of *aloe vera* in the products.

FT-IR spectroscopy

The hexane, ethanol, acetone, DCM of both dry and fresh *Aloe vera* extracts and some shelf cosmetics were characterized using a Fourier transform infrared spectrophotometer separately. Small aliquots of each *aloe* extracts as well as splatter of each of the cosmetics were mixed separately with Nujol- mineral oil. A dab of each mixture was mounted on to the sodium chloride window for observation. Tested samples were scanned for FT-IR spectra measurement at room temperature in the frequency range of 400 to 4,000 cm−1.

3.3 RESULTS AND DISCUSSION

Yields extraction with acetone, DCM, ethanol, hexane and water

Leaf extraction of both the fresh and dry plant material with the five solvents, yielded different amounts of dry extracts and these were used for the TLC profiling (Figure 3.1). The highest yield of plant material was extracted by water from the dry plant portion (2.2 g). Ethanol extracted the highest quantity of material from the fresh plant portion (0.3 g). Acetone was found to have extracted the lowest quantity of material from the dry plant portion (0.5 g), whereas hexane extracted the lowest from the fresh plant portion (0.04g).

The differences in the extract yields from the tested plant materials in the present analysis might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of plants (Hsu *et al.,* 2006). The amount of the compounds that can be extracted from a plant material is mainly affected by the vigor of the extraction procedure, which may probably vary from sample to sample. Amongst other contributing factors, efficiency of the extracting solvent to dissolve endogenous compounds might also be very important (Shelton, 1991; Gur *et al*., 2002).

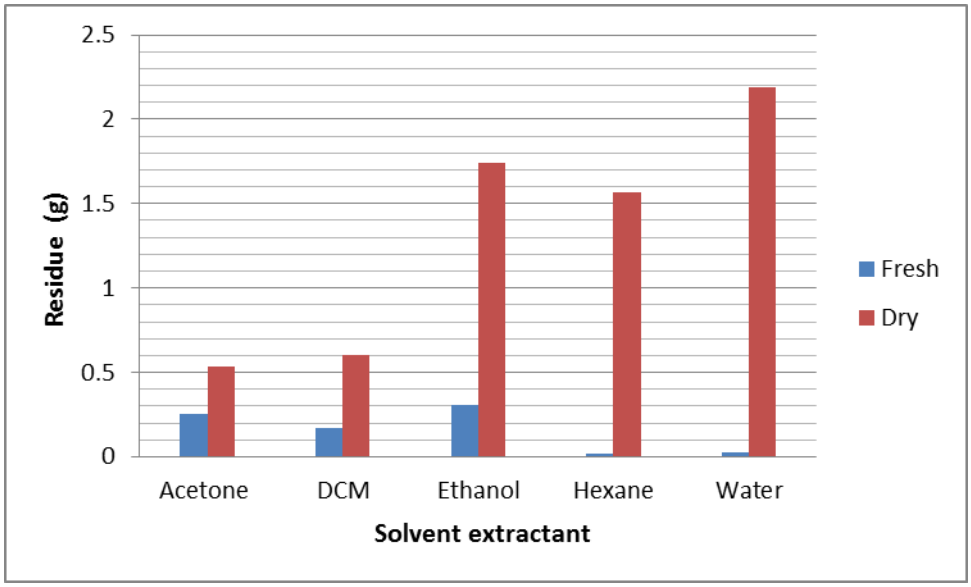

Figure 3.1: Quantity (g) of material extracted from 20g of *Aloe vera* leaf with DCM, Acetone, Ethanol, Hexane and Water.

TLC analysis of plant extracts

The variation in herbal medicines is a result of the presence of many phyto- constituents. Therefore, as far as the quality of a medicinal plant drug is concerned, a systematic consideration of all its phyto-constituents is as important as the quantification of the active constituents present in it. Thin layer chromatographic profiling is a vital parameter of herbal drug standardization for the proper identification of medicinal plants. It documents all the constituents of a sample extract resolved in a given chromatographic system. It provides a semi-quantitative sketch of the chemo profile of the plant extract. The TLC profiles of both the dry and fresh *Aloe vera* samples using different mobile phases (EMW: 50:7:5, PFW: 45:0.5:5, EMW: 40.5:5.5:4, HAE: 20:5:2 and BEA: 40:5:0.5) are shown in figure 3.2 and 3.3.

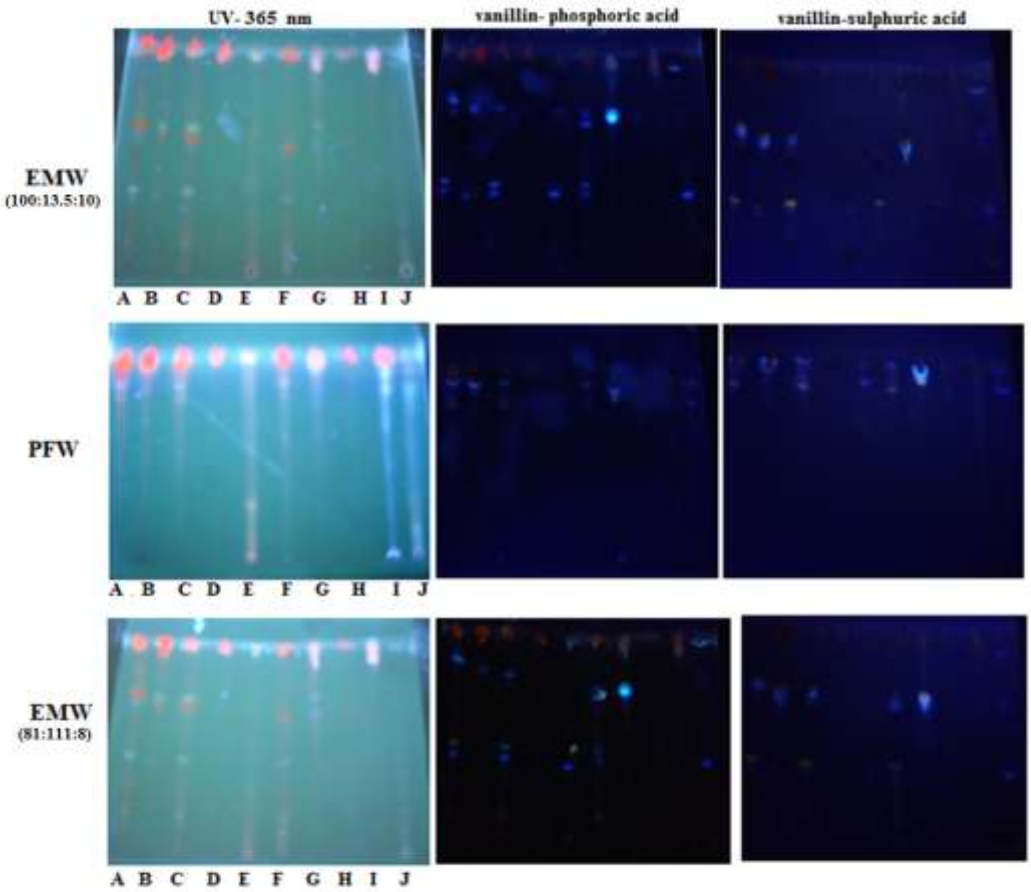

Figure 3.2: TLC profiles of *Aloe vera* extracts viewed under UV at 365 nm.

(A- acetone, B- DCM, C- ethanol, D- hexane E- water, F- acetone, G- DCM, H- ethanol, I- hexane, J- water), A-E: Fresh plant material and F-G: Dry material.

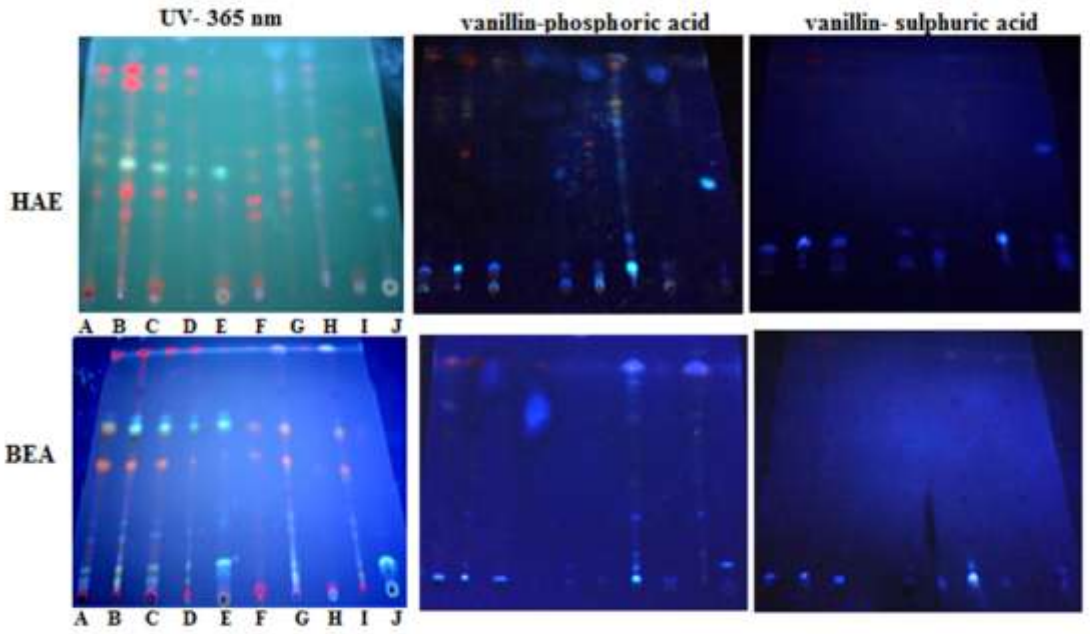

Figure 3-3: TLC profiles of *Aloe vera* extracts viewed under UV at 365 nm.

(A- acetone, B- DCM, C- ethanol, D- hexane E- water, F- acetone, G- DCM, H- ethanol, I- hexane, J- water), A-E: Fresh plant material and F-G: Dry material.

From the direct extraction with the five solvents of both the dry and fresh plant material of *Aloe vera*, water extracted broad range of phytochemicals. Most plant material was extracted from the Dry plant portion. Water extracted the highest yield of plant material at about (2.186). It was followed by Ethanol (1.742 g) and Hexane (1.567 g). DCM (0.600 g) and Acetone (0.536 g) had the lowest yield of plant material.

We determined the best mobile phase combination for the confirmation of the presence of diverse compelling bio molecules established using TLC profiling. The TLC profile of DCM, Hexane, Acetone, Ethanol and water extracts of both the fresh and dry plant material was carried out by different solvent system described above. Amongst various solvent systems, HAE: Hexane: acetone: ethanol (40:10:4 v/v/v) and BEA: Benzene: Ethanol: Acetone (40:5:0.5 v/v/v) showed maximum resolution compared to and EMW, and PFW because

many compounds were distributed, or separated, efficiently (Fig 3-3) .This was visible because many spots were seen after separation. This was similar to the result obtained in a previous study on *Aloe vera* (Waller *et al*,1978; Mokoka, 2007). The construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines. Thus chromatographic fingerprint should be considered to evaluate the quality of herbal medicine globally considering multiple constituents present in the herbal medicines (Patel, 2011).

Results of best spraying reagent

After the development of the TLC plate of all the extracts all the spots were observed under UV light showed bands at 356 nm and prayed with vanillin- sulphuric and vanillin phosphoric acid spray (fig 3.4).

We observed that a combination of vanillin- sulphuric acid spray gave maximum separation after spraying. This was evident by the number of spots revealed after spraying

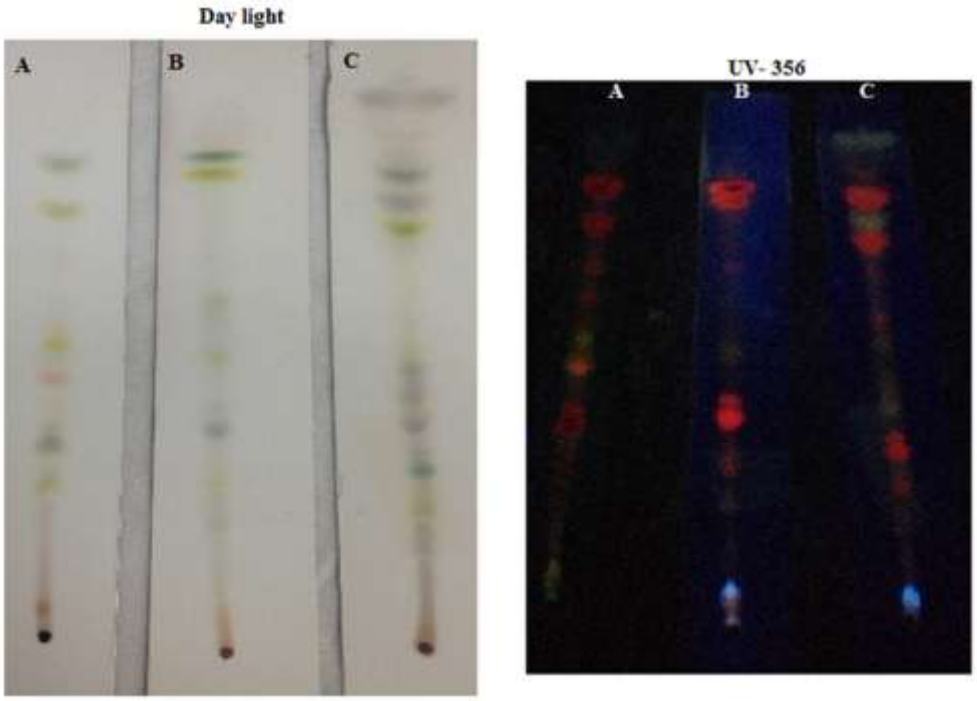

Figure 3.4: TLC profiles of *Aloe vera* DCM extract viewed after spraying on Day light and under UV at 365 nm.

A- (Ethanolic Potassium Hydroxide), B- (Vanillin Phosphoric) and C-(Vanillin Sulphuric) acid spray**.**

Samples are compared with one another with regard to the number, relative position (Rf) color and intensity of the spots in their respective TLC plate (Table 3.1, Fig 3.4).

Table 3.1: Active compounds in DCM fraction.

Plate	No of Spots	Relative positions	Color
A	5	0.85, 0.78, 0.57, 0.51, 0.4, 0.35	Olive green, green, yellow, dark pink
B	3	0.88, 0.85, 0.44,	Olive green, green, blue
C	7	0.97, 0.85, 0.8, 0.74, 0.44, 0.37, 0.25	Periwinkle, Olive green, green

An important factor in quantifying the movement of a compound on a stationary phase e.g., silica with a certain solvent system is the Rf (retention factor value which is defined as the ratio of the distance travelled by the compound from its origin to the movement of the Solvent from the origin Suleiman at al., 2010). Thin layer chromatography using hexane: acetone: ethyl acetate (40:10:4) as a mobile phase was able to separate different compounds having different retention factors (Rf values) in the DCM fraction of the plant extract (Table 3.1, Fig 3.4). The TLC analysis revealed the Rf values ranging from 0.25 to 0.97 with seven

spots observed in plate C whereas six were observed in plate A. The least number of spots was seen in plate B. This suggests that the combination of vanillin- sulphuric acid is the spraying reagent. This was evident by the number of spots revealed after spraying. Rf values are important in identifying a particular chemical substance, in most cases an unknown substance based on known Rf value of a list of substance.

Results of IR analysis of the *Aloe vera* extracts and commercial products.

The Fourier transform infrared spectrophotometer Spectra of the *A. vera* leaf extracts both fresh and dry portions as well commercial products from *Aloe vera* are shown in (figures 3.5-3.14). In all samples adsorption bands occurred between 394.26 cm^{-1} to 3854.18 cm^{-1}. The IR spectrum of all simples is closely related but differs in adsorption regions. Fresh dichloromethane adsorption bands occur at 430.59 cm^{-1}, 567.82 cm^{-1}, 742.53 cm^{-1}, 1051.90 cm^{-1}1159.66 cm^{-1}, 1263.70 cm^{-1}, 1375.08 cm^{-1}, 1458.16 cm^{-1}, 1715.97 cm^{-1}, 2287.39 cm^{-1}, 2723.88 cm^{-1}, 2862.44 cm^{-1}, 2927.23 cm^{-1}, 3744.92 cm^{-1} and 3852.04 cm^{-1}.

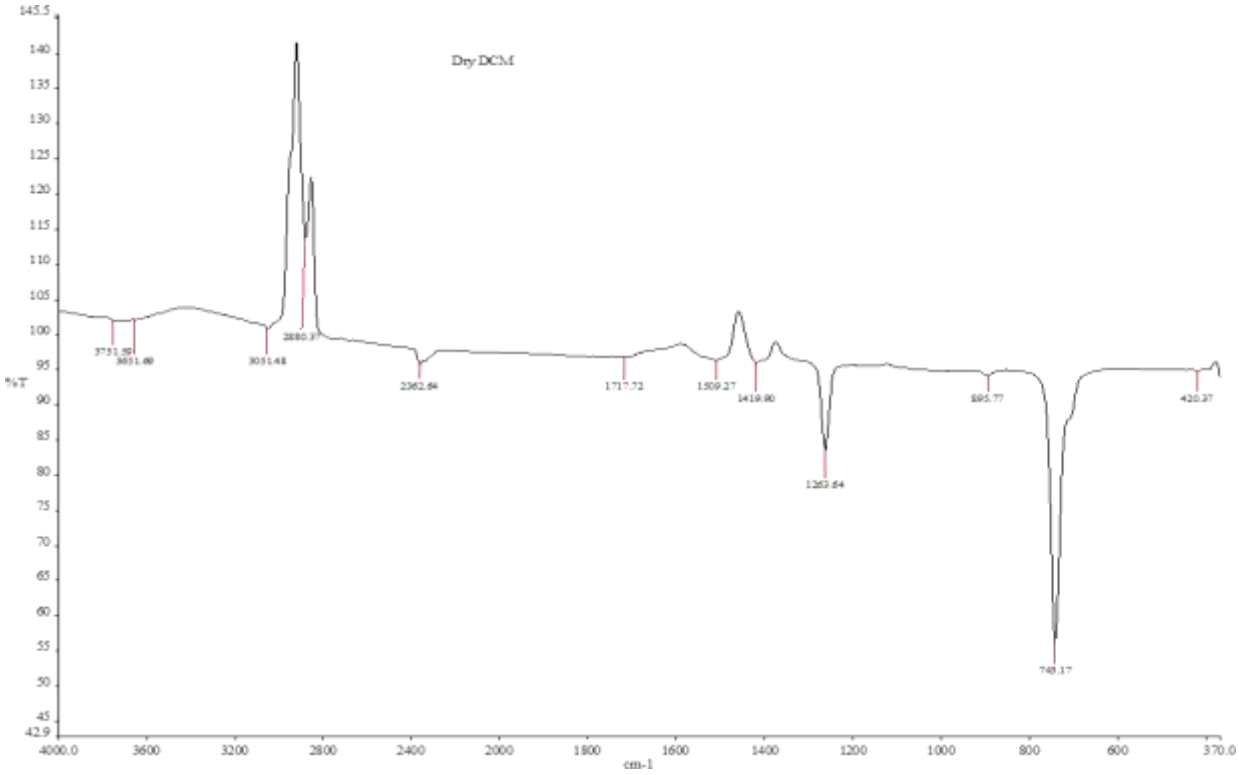

Figure 3.5: Absorbance spectrum of DCM extract of dry *Aloe vera.*

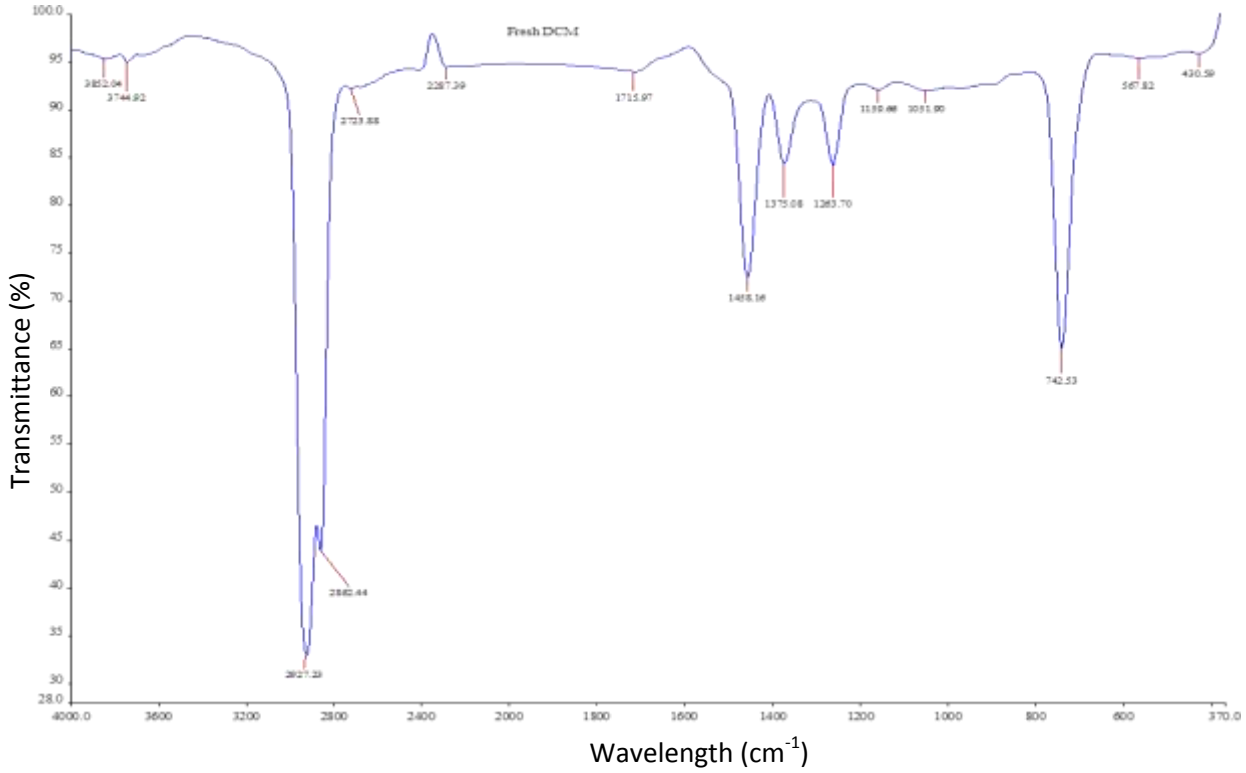

Figure 3.6: Absorbance spectrum of DCM extract of fresh *Aloe vera*.

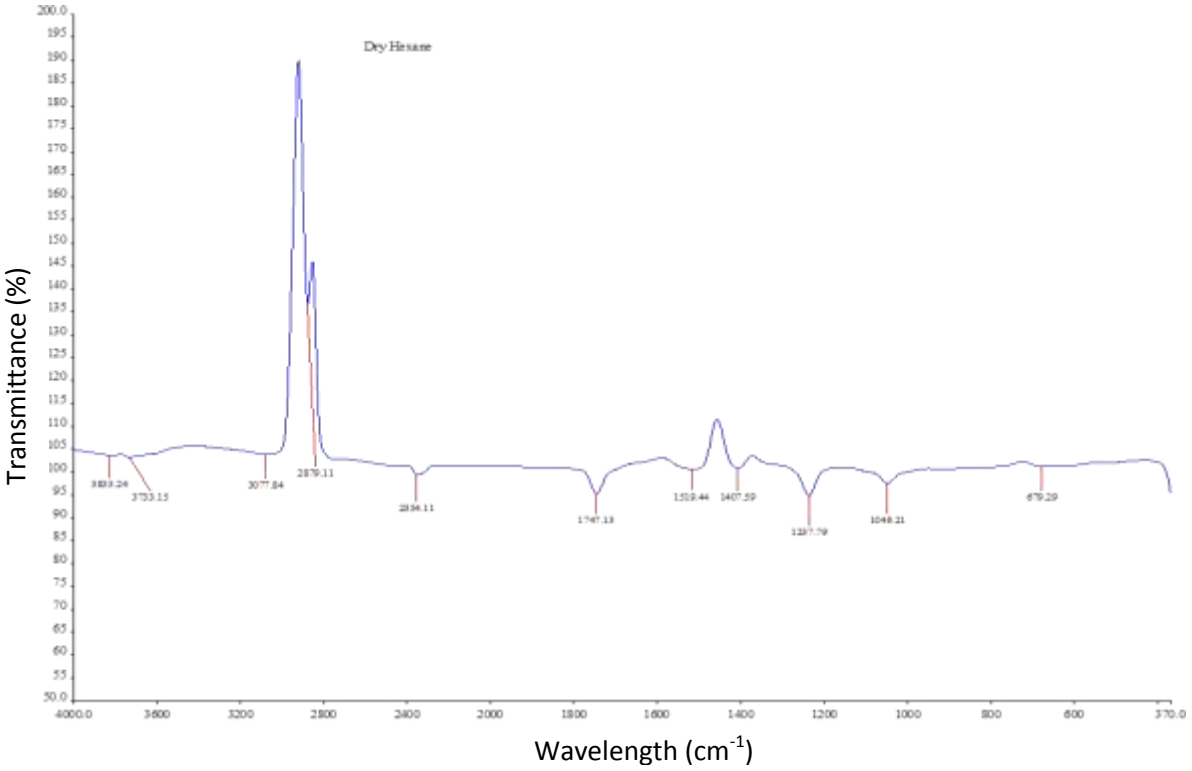

Figure 3.7: Absorbance spectrum of the hexane extract of dry *Aloe vera*.

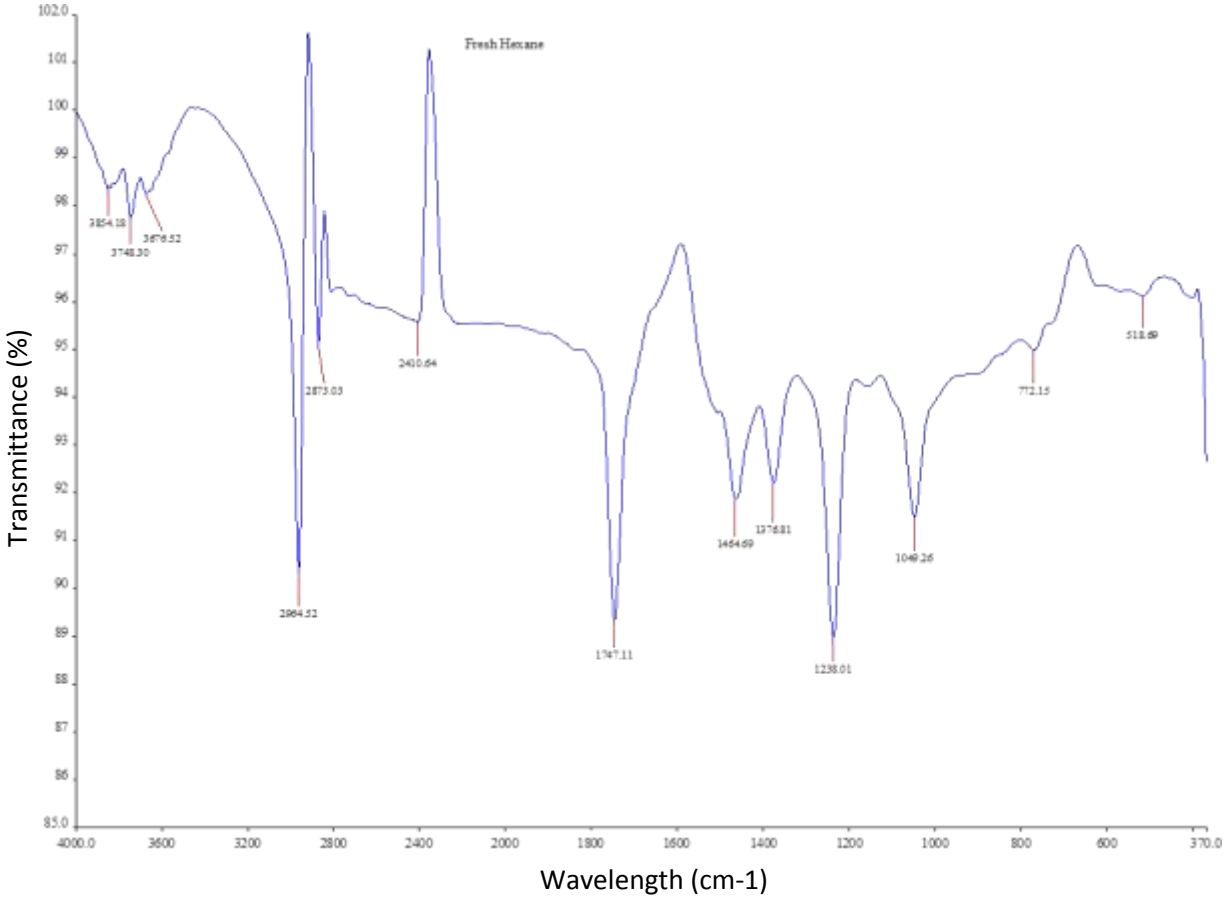

Figure 3.8: Absorbance spectrum of the hexane extract of fresh *Aloe vera*.

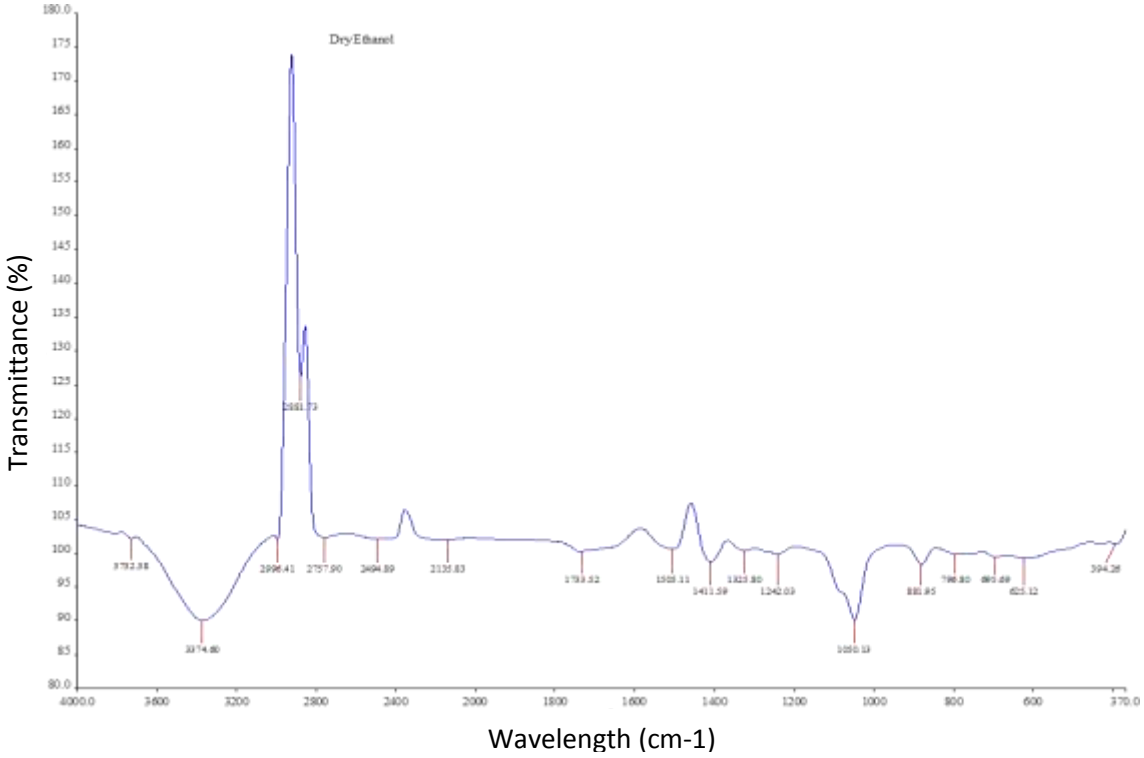

Figure 3.9: Absorbance spectrum of the ethanol extract of fresh dry *Aloe vera.*

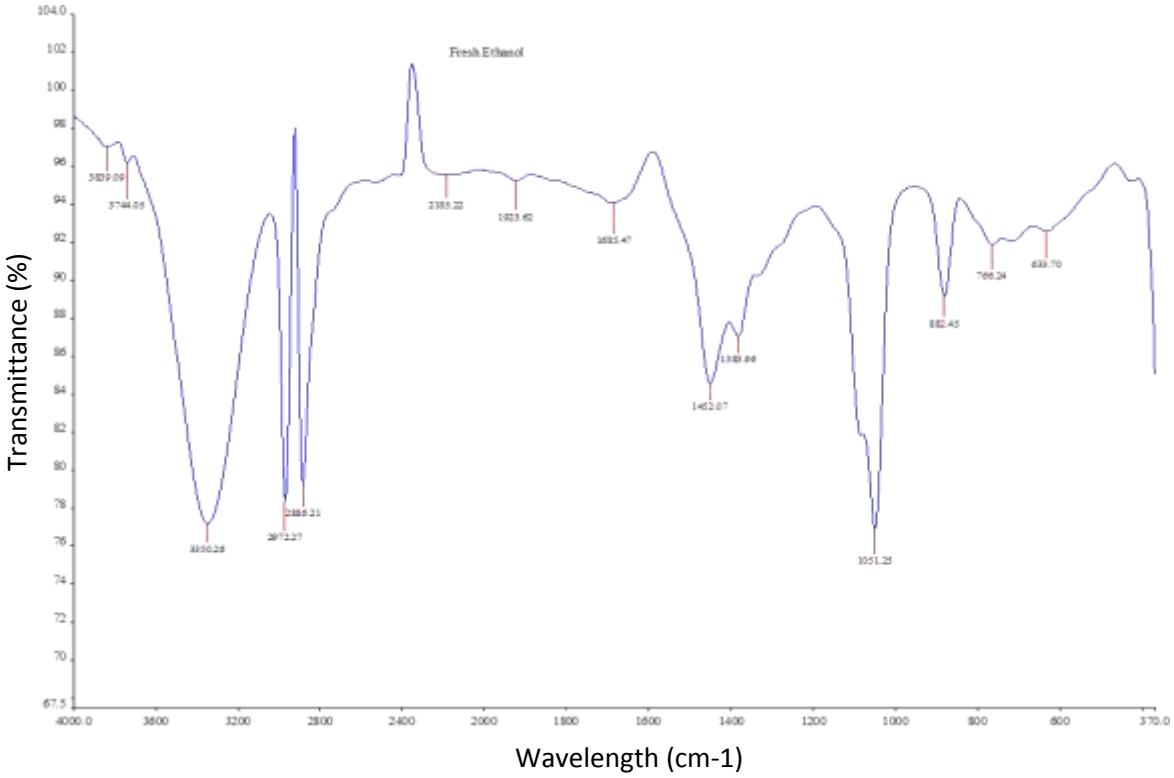

Figure 3.10: Absorbance spectrum of the ethanol extract of fresh *Aloe vera*.

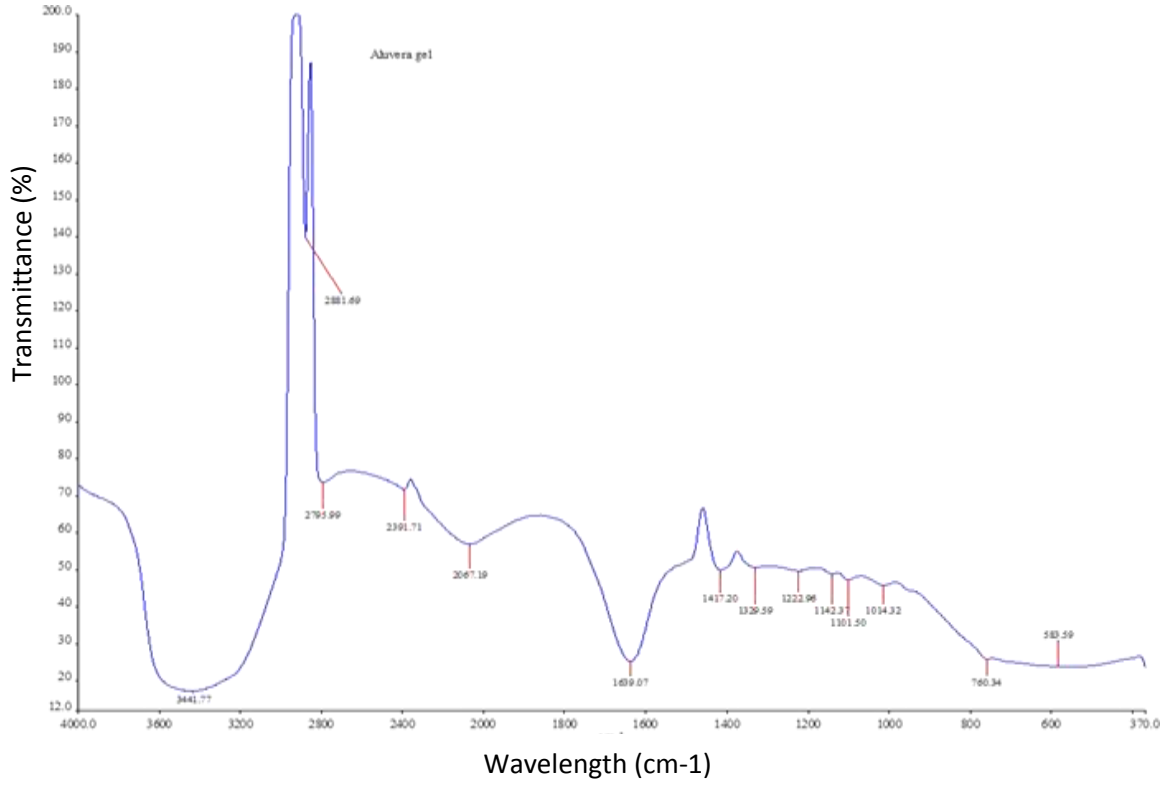

Figure 3.11: Absorbance spectrum of the commercial *Aloe vera* gel.

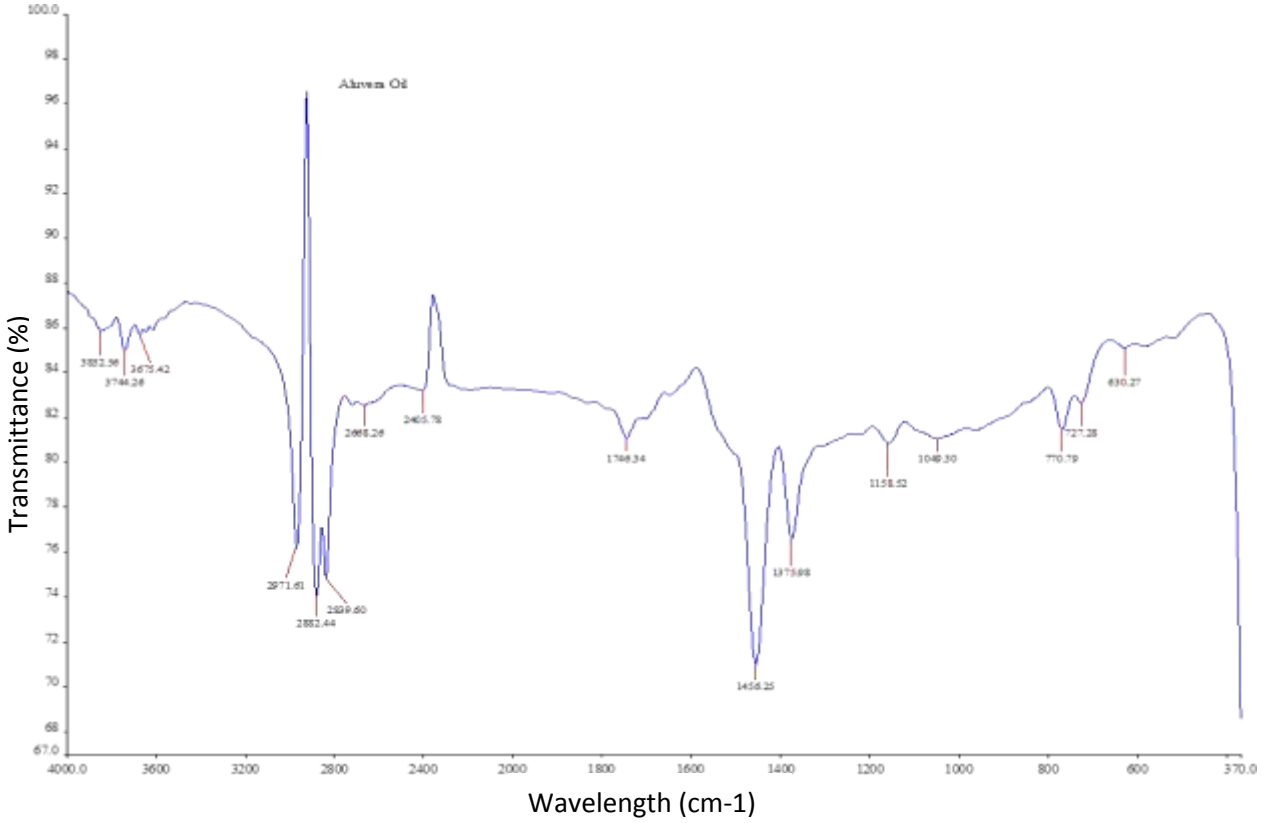

Figure 3.12: Absorbance spectrum of the commercial *Aloe vera* oil.

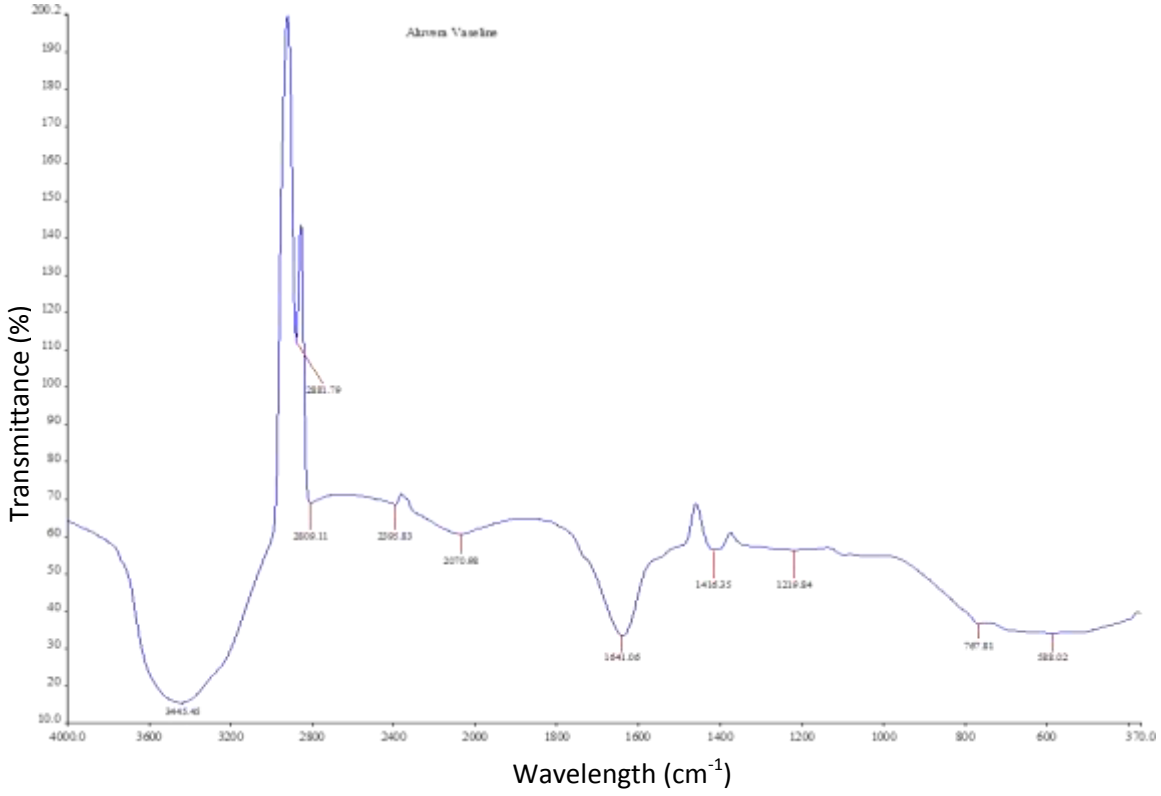

Figure 3.13: Absorbance spectrum of the commercial *Aloe vera* Vaseline.

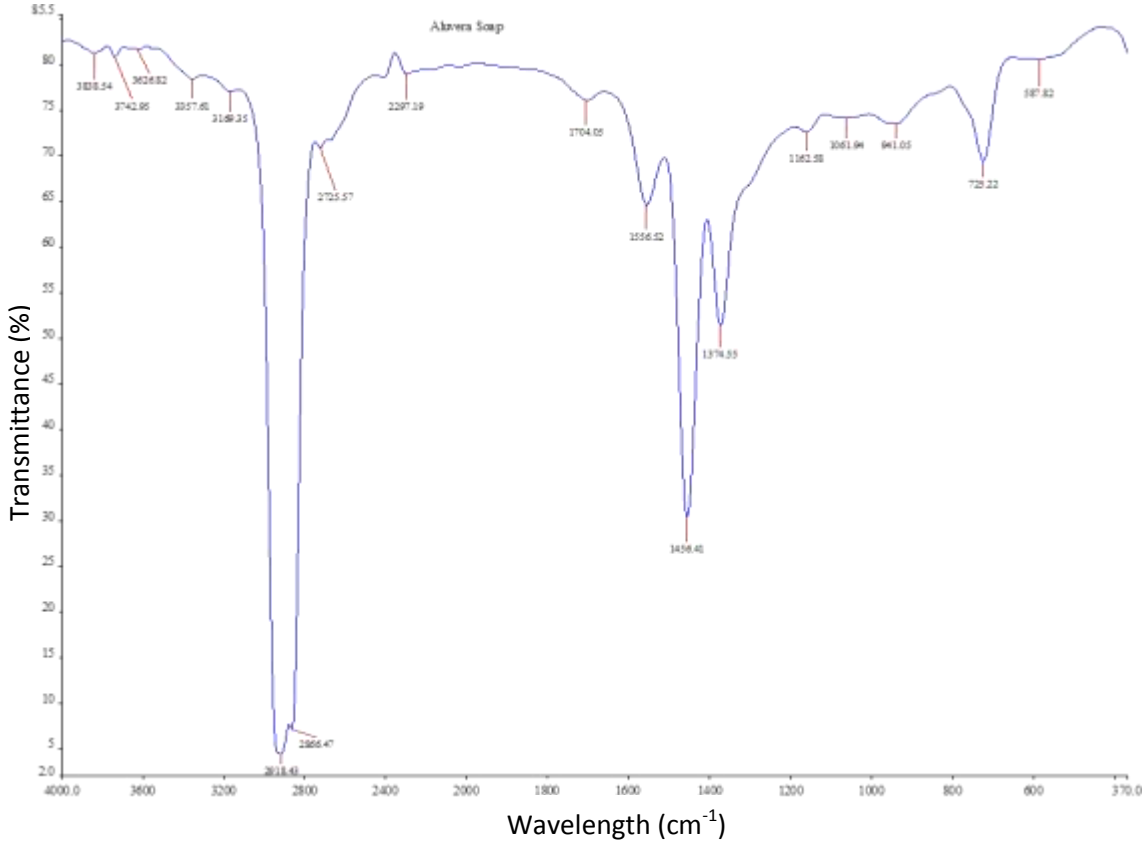

Figure 3.14: Absorbance spectrum of the commercial *Aloe vera* Soap.

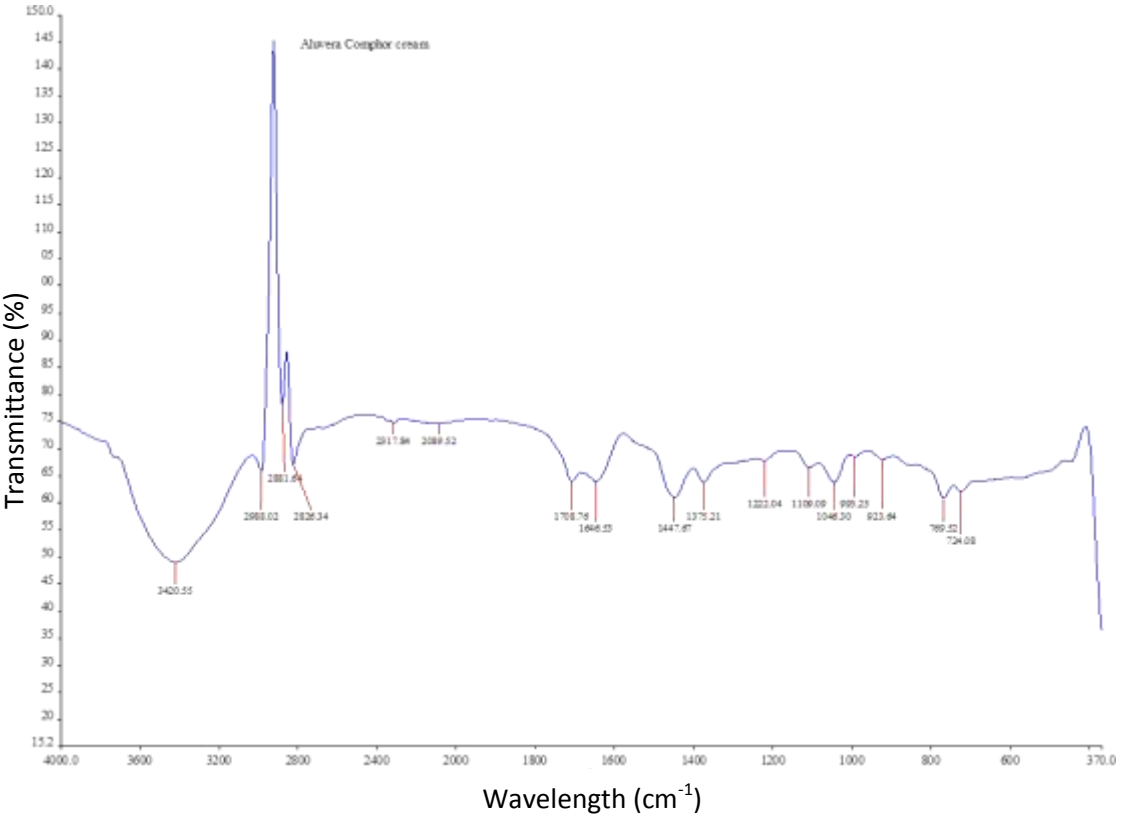

Figure 3.15: Absorbance spectrum of the commercial *Aloe vera* comphor cream.

The absorption band at 420.37 and 430.59$cm^{-1}$is a C-I stretching of the alkyl halide functional group and common in both the dry and fresh extracts of the plant. Alkanes and Alkyls functional group is also represented by the adsorption at 2862.44 and 2880.34 which are strong C-H vibrations. The absorption in the region 1383.96-1325.80 cm^{-1} is due to the symmetric stretching of NO_2 which characterizes aromatic nitro compounds. The absorption band at 33740.60 cm^{-1} is due to the strong vibrations of broad O-H stretch. The commercial products made from *A.vera* also had indistinguishable wavelengths compared to those of the extracts. The absorption band in the region 1160-1100cm^{-1} corresponds to the stretching vibrations of C = S. The strong absorption band at 780 cm^{-1} is due to the C-H out of plane deformation. The absorption in the region 1530-1450cm^{-1}corresponds to the anti-symmetric stretching of N = N-O. The presence of strong to medium intensities bands of the hexane extract were also observed at 1464.69 and 1519.44 cm-1 which is confirmation of a ring aromatic stretch. Narsih *et al* (2012) had similar finding when Identifying Aloin and Saponin and Chemical Composition of Volatile Constituents from *Aloe vera* (L.) peel. The 1464.69 cm^{-1} was visible in both the extracts and commercial products. These findings implicate that the selected commercial products tested did contain amounts/ did have traces of *Aloe vera* in them.

A

B

C

D

E

Figure 3.5: *Aloe vera* commercial products.

3.4 CONCLUSION

The study was set out to explore the concept of identifying the bio- active compounds found in *Aloe vera* that grows in South Africa, the best solvent extractant and best spraying reagent. The study has also sought to know whether the *Aloe vera* growing in South Africa would have the same bio- activity as any other growing in other parts of the world. The general theoretical literature on this subject and specifically in the context of plants, it is believed that plants growing in different parts of the world differ in chemical composition as a result of geographical distribution different geographical origin, seasonal and cultivar variation, technical differences used to isolate and enzyme activity. A whole *Aloe* plant was divided into two portions where one was dried and the other extracted fresh. The two portions were extracted using five solvents with different polarity affinities. Water was seen to be the best solvent extractant for the dried portion of the plant, whereas ethanol was seen to best extract on fresh plant material. In conclusion, the analysis using thin layer chromatography was able to definitively confirm the presence of the bio- active compounds present in *Aloe vera* leaf. Our findings were not far compared to reports on the thin layer chromatographic analysis of *Aloe vera* that grows in other parts of the world. With these findings, we can conclude that, aloe vera growing in South Africa is not entirely different from the one which grows in other parts of the world. However, they may differ due to the different geographical location, weather etc. The FT-IR was very instrumental in the confirmation of *Aloe vera* in the commercial products. The presence of similar functional groups in both the cosmetics and the plant extracts affirmed that the commercial products dis have *aloe vera* in them

3.5 REFERENCES

Barcroft, A., Myskja, A: Aloe vera: Nature's Silent Healer. London: BAAM Publishing Ltd; 2003.

Binu, T., Ramachandran, V.S., Rajendran, A., 2009.Habitat diversity, Morphological and systematic analysis of multipotential species of *Aloe barbadensis* Mill.(Liliaceae) from the Southern Western Ghats of Tamil Nadu, India. Journal of Research in Biology 3, 237 – 241.

Bozzi, A., Perrin, C., Austin, S., Vera, F.A., 2007. Quality and authenticity of commercial *Aloe vera* gel powders. Food Chememistry 103 (1), 22-30.

Bruneton,J., 1995. Pharmacognosy, Phytochemistry, Medicinal Plants. France: Lavoisier Publishing.

Canigueral, S., Vila R., 1994. *Aloe*. British Journal of Phytotherapy3 (2), 67-75.

Chopra, R.N., Nayer, S.L., 1956. Glossary of Indian Medicinal Plants. New Delhi, India: Council of Scientific and industrial Research.

Coats, B.C., Ahola, R., 1979.*Aloe vera* the Silent Healer. A modern Study of *Aloe vera*. Dallas, TX: Bill C. Coats;

Poole, C.F., 2003. Thin-layer chromatography: challenges and opportunities. Journal of Chromatography A, 1000 (2003) 963–984.

Collins, E., and Collins, C. 1935. Roentagen dermatitis treated with fresh whole leaf of *Aloe vera*. American Journal of Roentgenology 33, 396-7.

Cordell, G.A., 1995. Changing strategies in natural products chemistry. A review Cordifolia leaves. Phytotherapy Research 4, 198-200.

Dagne, E., Bistrat, D.,Viljoen, A., Van Wyk, B.A., 2000. Chemistry of *Aloe* species. Current Organic Chemistry 4,1055-78.

Dastur, J.F., 1962. *Aloe Barbadensis* Mill. In: medicinal Plants of India and Pakistan. Bombay: D.B.: Taraporevala Sons and Co. Private Ltd. 16-17

Davis, R.H., 1997. *Aloe vera*: A scientific approach. New York Vantage Press.

Eldridge J., 1978. Bush medicine in the Exumas and Long Islands, Bahamas, A field study. Econonomic Botany 29, 307-332.

Waller, G.R., Mangiafico, S., Ritchey, C.R., 1978.A CHEMICAL INVESTIGATION OF *ALOE BARBADENSIS* MILLER. Proceedings of the Oklahoma Academy of Science 58, 69-76.

Ghannam, N., Kingston, M., Al-Meshaal, I.A., Tariq, M., Parman. N.S., Woodhouse, N., 1986. The antidiabetic activity of aloes: preliminary clinical and experimental observations. Hormone Research 24, 288-294.

Gordon, M.C., David, J.N., 2001. Natural product drug discovery in the next millennium. Pharmaceutical Biology39: 8-17.

Grindlay, D., Reynolds, T., 1986. The *Aloe vera* leaf phenomen: A review of the properties and modern use of the leaf Parenchyma gel. Journal of Ethnoparmacology16: 117-51.

Gunther, R.T., 1934. The Greek Herbal Dioscorides. Oxford: Oxford University Press.

Gur, D., Ozalp, M., Sumerkan, B., Kaygusuz, A., Toreci, K., Koksal, I., Over, U., Soyletir, G., 2002.Relevance of antimicrobial resistance in *Haemophilus influenza, Klebsiella pneumoniae*, *Moraxella catarrhalis* and *Streptococcus pyogenes*: results of a multicentre study in Turkey. International Journal of Antimicrobial Agents 19: 207-211.

Hurley, J.C., 1992. Antibiotic Induced Release of Endotoxin: areappraisal Clinical Infectious Disease 15, 840-854.

Hsu, B., Coupar, I.M., Ng, K. 2006. Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. Food Chemistry 98, 317-328.

Mahattanadul, S., 1995. Antigastric ulcer properties of *Aloe vera*. Songklanakarin Journal of Science and Technology 18, 49–57.

Makoka, T.A., 2007. Isolation and characterization of compounds active against *Cryptococcus neoformans* from Maytenusundata (Thunb.) Blakelock (Celastraceae) leaves. (Unpublished doctoral dissertation).University of Pretoria, Phytomedicine Programme, Department of Paraclinical Sciences Faculty of Veterinary Sciences.

Mandeville, F., 1939. *Aloe vera* in the treatment of radiation ulcers and mucous membranes. Journal of Radiology 32, 598-9.

Morton, J.F., 1977. *Aloe*. In: Major Medicinal Plants Botany, Culture and uses. Springfield IL: Charles C. Thomas. 46 -50.

Nick, H., Mashour, M.D., George, I., Lin, M.D., William, H., Frishman, M.D., 1998. Herbal Medicine for the Treatment of Cardiovascular Disease. Archives of Internal Medicine 158 (20), 2225-2234.

Park, Y., and S. Lee. 2006. New Perspectives on Aloe. New York: Springe Verlag.

Rabe, T., Van Staden., 1997.Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology 56 (1), 81-87.

Shelton, R.W., 1991.*Aloe vera*, its chemical and therapeutic properties. International Journal of Dermatology 30 (10), 679-683.

Suleiman M.M., McGaw, L.J, Naidoo, V., Ellof, J.N., 2010. Evaluation of several tree species for activity against the animal fungal *Aspegillus fumigates*. South African Journal of Botany 76, 64-71.

Patel, S.S., Himesh, S., Verma, S., Mishra, K., Singhai, A.K., 2011. RP- HPLC for the isolation of *C- glycosylflavones* from methalonic extract of *Passiflora nepalensis* Wall, Passifloraceae. International Journal of Drug Discovery And herbal Res Earch 1 (1), 22-24.

CHAPTER 4

GENERAL DISCUSSION AND RECOMMENDATIONS

Chapter 4

8.0 GENERAL DISCUSSION AND RECOMMENDATIONS

GENERAL DISCUSSION

From the study, we concluded that:

- Thin layer chromatography technique can be used to document plants of the same species growing in different parts of the world.
- From the phytochemical analysis conducted, it was noted that *Aloe vera* growing in South Africa does contain phytochemicals such as alkaloids, flavonoids, phenols, saponins, proanthycynodins, tannins and proanthocynidins which was similar to other *Aloes* growing in other parts of the world.
- Also, it was noted that indeed geographic influence may affect the genotype of a plant species over a period of time.
- Commercial products do contain ingredients of *aloe vera* but differ in content at with the plant extract is present.
- Infrared spectroscopy is a suitable tool to use in the affirmation of the presence of plant ingredients in commercial products.

RECOMMENDATIONS

- In this work TLC profiling of the both the fresh and dry portions of *Aloe vera* growing in South Africa was successful and it indeed was similar to that of other countries. However, future studies on profiling *Aloe vera* growing in South Africa can be performed. Profiling could be improved using several approaches. One such approach could be the use of Bioautography, a microbiological screening methods commonly used for the detection of antimicrobial activity of an extract. With this method, several microorganisms and fungi could be used against the *aloe vera* extracts for the testing

of antibacterial and antifungal properties of the plant. This would also contribute towards the profiling of the plant and by comparing it with *aloe vera* growing in other parts of the world; it would prove their similarity even though they grow in different parts of the world. Also, with the aim of profiling *Aloe vera* growing in South Africa GC-MS could also be utilized. Analysed sample structures could be compared with standards to ensure quality.

- An alternative to the use of FT-IR in determining the presence of *aloe vera* in the products, HPLC could also be used to quantify and determine presence of *aloe vera* in the commercial products. This procedure is helpful in determining whether or not there has been substitution or adulteration of the commercial products. This therefore will help to determine, the extent of adulteration in these products.