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COMPARATIVE PHYTOCHEMICAL ANALYSES OF *ALOE FEROX MILL.* FOUND IN EASTERN AND WESTERN CAPE PROVINCES IN SOUTH AFRICA

By

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DECLARATION

The research reported in this dissertation, except where otherwise indicated, is my original research. This dissertation has not been submitted for any degree or examination at any other university and this thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons. This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then their words have been re-written but the general information attributed to them has been referenced. This dissertation has been submitted in fulfilment of the requirements for a MSc degree at the University of Fort Hare and is deposited at the University's library and no part of it has been commercialised and it may be quoted provided accurate acknowledgement of the source is used.

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1.1. Background of medicinal importance plants in South Africa

Medicinal plants are of such importance that it is estimated that about 80% of the world population residing in the vast rural areas of the developing and under developed countries still rely mainly on medicinal plants. Medicinal plants are the only affordable and accessible source of primary health care for them, especially in the absence of access to modern medical facilities.

As for South Africa, Van Wyk *et al* (1997) states that out of 30 000 species of higher plants approximately 3 000 species are attributed with medicinal properties. Plants were once a primary source of all the medicines of the world and they still continue to provide mankind with new remedies. Kinghorn and Balandrin (1993) also stated that natural products and their derivatives represent more than 50% of all drugs in clinical use in the world, with higher plants contributing no less than 25% percent of that total. Within South Africa there is no doubt that a growing interest in natural and traditional medicines as a source of new commercial products, as well as the contribution indigenous plants make to traditional medicine on a large scale.

In Mander (1998) he states that according to Dold and Cocks (2002) stated that there are 27 million indigenous medicine consumers in South Africa with a large supporting industry. The use and trade of plants for medicine is therefore no longer confined to traditional healers but has entered both the informal and formal entrepreneurial sectors of the South African economy (Cunningham, 1988; Dauskardt, 1990; Dauskardt, 1991; Kearns, 1994 ; Mander, 1997; Cocks and Dold, 2000) resulting in an increase in number of herbal gatherers and traders (Dold and Cocks, 2002).

Street and Prinsloo (2013) stated that medicinal plants are now universally recognised as the basis for a number of critical human health, social, and economic support systems and benefits and that there has been a major resurgence in interest in traditionally used medicinal plants with a number of international and local initiatives actively exploring the botanical resources of southern Africa with the intention to screen indigenous plants for pharmacologically active compounds (Geldenhuys and Mitchell, 2006 ; Gurib-Fakim *et al*., 2010; Rybicki *et al*., 2012).

Studies to determine the chemical profile and composition of medicinal plants reveal the complexity and variety of compounds all contributing to the various uses of plants in treating

numerous aliments including life threatening diseases such as HIV-AIDS, cancer, and diabetes (Street and Prinsloo, 2013).

Spectroscopic methods coupled with good extraction techniques like chromatography, have contributed to the success of natural product chemistry over the past 50 years. A sound isolation strategy has helped in the isolation and characterization of many bioactive molecules. Nowadays, bioassay-guided fractionation of medicinal plants is a feature of routine in the attempt to isolate bioactive components from natural sources. These techniques are not only being restricted to plant sources but they are also being applied to microbial and even fungal sources of metabolites (Gurib-Fakim, 2006).

However, a pharmacological effect observed in vitro or in animal models, for both safety and efficacy needs to be reconfirmed by clinical studies and the information obtained from the preclinical studies can form the basis for further clinical trials (Lipsky and Sharp, 2001; Bleicher *et al.* 2003; Dove, 2003; Kenakin, 2003; Knowles and Gromo 2003 and Verkman 2004). However, before screening, it should be considered that different plants would have different combinations of these secondary products that would often be taxonomically distinct in individual plants resulting in unique medicinal properties (Wink, 1999).

1.2. Statement of the problem

Several people are speculating or assuming that *Aloe ferox* growing in the Eastern Cape is quite different from the one found in the Western Cape in terms of their their secondary metabolites and genetically, they belong to the same species. It therefore becomes difficult for people to know which *Aloe ferox* plants are collected from the Eastern Cape or the Western Cape. This is affecting the quality assurance of the various products prepared from *Aloe ferox*.

Eastern Cape areas are influenced by different topographical features. The area is separated by a great escarpment which easily divides the region into a northern and southern area. The northern areas generally have a high altitude and little water, which result in semi-arid conditions that characterize regions such as the Karoo. To the south, the climate is quite different since a number of rivers trickle down from the mountains and provide an ample supply of water, this and these well-watered areas feature wetland fauna. Generally speaking, the climate at the coast experiences more wind and higher levels of humidity. The humidity increases as you get closer

you get to KwaZulu Natal and decreases as it gets closer to the Western Cape. Along the Eastern Cape, especially along coastal areas, the conditions in summer are hot and humid with plenty of rain. In general, the Western Cape Province enjoys a typically Mediterranean climate; the summers are dry and warm with a low rainfall which alternate with relatively mild and wet winters.

Both climatic conditions and agronomic practices can influence the phytochemical contents of plants (Ahuja *et al*., 2010) thus a better understanding of the effects of climatic factors is needed to increase the predictability of the content of desired compounds. Globally, there is evidence for altered phenology in plants due to changing climatic conditions (Parmesan and Yohe, 2003). These climatic factors can therefore affect the growth patterns of plants that grow naturally in the wild or plants that are cultivated.

1.3. Objectives of the study

This study is designed to compare the phytochemical compounds using thin layer chromatographic techniques. These two provinces fall on different altitudes in the country and experience different and unique climatic conditions/weather conditions and the vegetation types found in each area are evidence that their type of vegetation is different.

1.3.1. Specific objectives of the study

1. To collect *Aloe ferox* growing in Eastern Cape and Western Cape.
2. To analyse and compare the *Aloe ferox* from the two areas for their phytochemicals such as phenols, flavonoids, flavonols, proanthocyanidins, tannins, alkaloids and saponins.
3. To carry out thin layer chromatography of both the dried and fresh *Aloe ferox* from both regions using different mobile and stationary phases.

1.4. Description of the study areas

The first study area is in Alice which falls under the Nkonkobe municipality within the Amathole District of the Eastern Cape Province, South Africa. The Eastern Cape province falls within the latitudes 30º00´ to 34º15´S and longitudes 22º45´ to 30º15´E (Grierson and Afolayan, 1999). The Amathole district is at the heart of the Eastern Cape Province and about 1.7 million people live there (Afolayan, 2003). Most of the population is black people followed by whites then coloured people. Alice is home to the famous University of Fort Hare where the *Aloe ferox* growing in Eastern Cape was harvested for this study.

Figure 1.1 Map of the Amathole District Municipaliy of the Eastern Cape (Source: ecdc.co.za).

Figure 1.2: Map where Albertinia is located around the Western Cape Province (Source: sa-venues.com).

The second study area is Albertinia which is a settlement in Eden District Municipality in the Western Cape Province of South Africa. It is 50 km west of Mossel Bay. It was laid out in 1900 on the farm Grootfontein and became a municipality in 1920. The name is derived from the surname of Johannes Rudolph Albertyn (1847-1920), the first Dutch Reformed minister to serve the community. Geographically, it falls within the latitudes 34º12´S 21º35´E/ 34.200ºS 21.583ºE (*en.wikipedia.org/wiki/Albertinia, _Western_Cape*).

1.5. Significance of the study

The significance of this study will be to examine and prove whether *Aloe ferox* growing in the Eastern Cape and Western Cape has a different phytochemical composition as some people think or assume. These will determine which one should be preferably used to cure or prevent certain ailments. The study would serve as a base line for future research that can be done to investigate *Aloe ferox* species found in different areas across the country up to Kwazulu-Natal to the Free State. It will open up interest on the comparative study of all its compounds found in the inner leaf gel and the bitter sap which also contains a variety of medicinal compounds.

The study will promote more use of *Aloe ferox* as one of the best medicinal plants in South Africa which has been used as a medicinal plant since and it will also provide retailers and users of medicinal plant products with information they need to know about the content of

phytochemicals that can be found in the same species of a plant across different areas. This will also create a system whereby they can buy or sell raw plants from one area and use it to make their products. This study will also be an addition to knowledge of the effect of environmental factors or different geographical areas on the synthesis of secondary plant metabolites.

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CHAPTER 2

LITERATURE REVIEW

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2.1. Botany of *Aloe ferox*

2.1.1. Description

Plate 1: *Aloe ferox* (Source: kimpexenterprises.com)

Aloe ferox is a succulent shrub up to 3-5 m tall with solitary stem up to 30 cm in diameter. It has a tall single stem with old dry leaves persistent (Coates, 1977; Newton, 2004). The leaves are dull green to glaucos and in hot dry periods the leaves have a reddish tinge. The upper surface may be copiously spiny to spineless and the lower surface is copiously spiny to spineless except for a few spines along the median line near the apex (Jeppe, 1969; Coates, 1977). Young plants are always more spiny than mature ones. The leaves are (50-60 cm) long are a dense rosette spreading or recurved, stipules absent, petiole absent, blade laceolate-oblong, long-acuminate, margin with sharp reddish (brown) teeth 6 mm and 1-2 cm apart, the yellow exudate from the leaves dries red (Van Wyk and Gericke, 2000; Newton, 2004). The inflorescence is a branched panicle with 5-8 erect large elongated racemes that are 50-60 cm long (Jeppe, 1969; Van Wyk and Smith, 2005). The flowers are bisexual with a 3-merous pedicel with 1-1.5 cm long lobes, golden-orange to bright scarlet, orange-red being the most frequent form (Jeppe, 1969; Van Wyk *et al*., 1997). The inner segments of the perianth are tipped dark brown to black, which is one of the chief distinguishing characters of this aloe and the name "ferox" means "fierce", thus, strongly fortified with thorns or spines" (Jeppe, 1969; Coates , 1977).

2.1.2. Origin

Aloe ferox is indigenous to South Africa. It is widely distributed from Swellendam and Mossel Bay in the South-West across the across drier parts of the Southern and Eastern Cape to the Transkei, Southern Lesotho southernmost parts of Natal (Reynolds, 1950; Jeppe, 1969; Van Wyk and Smith, 1996; Glen and Hardy, 2000). It is also found in the south eastern corner of the Free State and southern Lesotho (Pooley, 1994).

2.1.3. Habitat and distribution

Due to its wide distribution range, *Aloe ferox* occurs in a broad range of habitats. Plants grow in the open hills slopes or amongst bushes in flat rocky places, and on rocky slopes along river valleys and on mountainsides and due to its wide range, the plants vary considerably (Coates, 1977). In the South Western Cape it grows in grassy fynbos and in the Southern and Eastern Cape it may also be found on the edges of the karoo (Pooley, 1994 and Glen and Hardy, 2000).

2.1.4. Taxonomy

Kingdom: Plantae

Division : Monocotyledon

Class : Equisetopsida

Order : Asparagales

Family : Xanthorrhoeaceae

Genus : *Aloe*

Species : *ferox*

(http://www.sanbi.org/biodiversity/reddata)

2.1.5. Growth and development

Aloes are succulent and warm-climate plants, temperature and water play an important role in establishing them. According to Jeppe (1969) the plant flowers from May to August along the coastal belt in warmer areas and from September to November in colder areas. A broad range of temperature for germination can be attributed to the wide distribution of this species in South Africa (Van der Bank *et al*., 1995; Shackleton and Gambiza, 2007). Van Staden *et al*. (2009) they found that temperature had a vital influence on early developmental stages of the seedlings. *Aloe ferox* seedlings subjected to different temperatures under controlled conditions showed variations in growth. Alternating temperatures (30/15 °C) favours shoot and root growth and increasing the number of leaves. Their study also demonstrated that the seeds of *A. ferox* require a temperature of 21.5 °C for optimum germination and treating *A. ferox* seeds with smoke–water (1:500 v/v) may help in improving percentage germination and alternating temperatures (30/15 °C) and high irrigation frequencies (three times weekly) would be useful to raise seedlings of *A. ferox*. Sexual maturity has been estimated to occur at 4–6 years when plants are approximately 1 m tall (Newton and Vaughan, 1996); however, growth rates have never been determined empirically. Holland and Fuggle (1982) indirectly estimated the age of tall (5–6 m), mature individuals to be 150 years, which is at odds with the 4–6 years required for 1 m growth. Growth rate is clearly variable between sites and is strongly related to plant size, as is common with other plant species (Shackleton, 2002 and Emanuel *et al*., 2005). Other aspects are the response of growth to harvesting, rainfall variability, and other potential stress or damage factors such as fire, browsing and scale insect infestation (Shackleton and Gambizi, 2007).

2.1.6. Cultivation

Aloe ferox can be planted in spring, 1, 4 to 5 m from each other. When the seedlings have three or four leaves, or are about 3 cm tall, they can be planted into 1 kg bags containing a well-drained mixture of sand and compost and after two years they can be planted out into an open ground. About 15 to 18 cm long root suckers or rhizome cuttings can be planted in such a way that a two-third portion of the planting material is underneath the soil (Herbert, 2006).

2.1.7. Harvesting

The exudate is often collected by cutting the leaves transversely close to the stem and putting them in such a way that the exudate drains into pots, tubes or vessels or simple canvas placed over a depression. It can also be obtained by squeezing the leaves by warm or cold water retting. In South Africa, *Aloe ferox* is usually tapped during the rainy season because that is when exudate is more abundant. Tapping is also carried during other periods of the year except for the driest months. Teams of tappers are usually assigned Aloe populations or particular areas where they can harvest leaves. In the Eastern Cape, tappers operate as independent entrepreneurs. Payment for the right to harvest the leaves on a particular farm is made in the form of an agreement to a portion of the harvest that goes to the farmer (O´Brien, 2005). This exudate is then boiled by the tappers in a drum to remove water. Once cooled, the exudate solidifies into a dark brown, amorphous, glass-like lump and is sold as a laxative product (O´Brien, 2005).The *Aloe ferox* bitter exudate product is known as Cape Aloes.

2.1.8. Leaf processing after harvesting

O' Brien (2005) says harvested leaves have dried exudate and dirt on the surface so washing is therefore the first step in the gel processing procedure. *Aloe vera* leaves ideally need to be washed within two to four hours after harvesting. In the *Aloe ferox* industry, this does not happen as the leaves are first tapped, a procedure that takes about six hours (Newton and Vaughan, 1996). If the leaves are too muddy when delivered to a gel factory they are pre-washed in a basin of deionised water. In some cases, the pre-wash step involves scouring the leaf rind with soft brushes (McAnalley, 1990). Washed leaves can either be sold by the factory or further processed for gel (O'Brien, 2005). Washed leaves are trimmed, meaning the sides; tip and base of the leaf are cut away. These trimmed pieces are collected and used to produce whole leaf powder. Filleting is either manually or mechanically and hand filleting produces the best quality gel but nowadays mechanical filleting is used and the gel fillets can be sold as an end product, but are often processed to make liquid gel or gel powder. Preservatives area added and the fillets are transported under refrigeration to prevent bacterial degradation and loss of quality (O'Brien, 2005). To get pure gel the rind is carefully removed using a sharp knife and the gel fillets are soaked in water to ensure that any remaining bitters are washed off. The next step in gel processing is to remove cellular material from the gel. Gel fillets are chopped into small chips

and de-pulped using sieves (Walter *et al*., 2004) in O'Brien, 2005). The fillets can also be liquidised as in the fruit juice industry and filtered to remove cellular material and only liquid gel will remain (O'Brien, 2005). The gel in this crude form is sold as a commercial product, but may also be mixed with activated charcoal, is filter pressed, stabilized (preserved) and dried. Treatment with activated charcoal ensures that any anthraquinone compounds in the gel are removed. In the *A.vera* industry usually 0.05 % w/v charcoal is added to 2000 L gel (Walter *et al*., 2004). After filtration, the filtrate is decolorized and free of fine charcoal particles (Qui *et al*., 1999).

For export purposes, dried Aloe gel is favoured. Gel is mostly dried by spraying or by freeze drying (Walter *et al*., 2004). Two other patented processes to obtain gel are commonly used in the aloe industry. The first is a method to extract gel polysaccharides by precipitation (McAnalley, 1990). The other is by treating pulp with sodium citrate that results in the releasing of polysaccharides from calcium. Water is added and the mixture is heated, filtered, and the liquid fraction, which contains the calcium free polysaccharides is known as aloe 'jelly' (O'Brien, 2005).

2.1.9. Chemical properties

Aloe ferox has three usable parts: the green epidermis which contains fibre, the yellow, aloin rich bitter juice under the skin and the white inner flesh, rich in minerals, inorganic minerals, vitamins, amino acids, alkaloids, saccharides, polysaccharides, enzymes and lipids. The main components of Aloe are aloin, aloe-emodin and aloeresin (Viljoen, 2008). According to Tschirch (1917) Cape aloes contain 'cape-aloin' or ferox aloin' which he thought differed from barb-aloin, the ordinary aloin of commerce. Aschan (1903) proposed the formula $C_{16}H_{10}O_5$ to cape-aloin and stated that Cape aloes contain emodin $C_{15}H_{10}0_5$ and a resin. The resin is an ester of paracumaric acid with a specific resino-tannol.

One of the main biologically active constituents of Aloe extracts is aloin or barbaloin (10-glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)- 9(10H) - anthracenone) (1, aloe-emodin) which is found in nature as a mixture of two diastereomers, aloin A (1) (10S)-10-glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)-9 (10H)-anthracenone) and aloin B (2) (10R)-10- glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)-9 (10H)-anthracenone (Fanali *et al*., 2010;

Speranza, *et al*., 2005; Afolayan *et al*, 2004). A study on the chemical composition of volatile constituents from the leaves of *A. ferox* ideational ntifed 21 compounds, representing more than 99.99 % of the essential oil (Magwa et al., 2006). The compounds identified and their quantities in the A. ferox concentrations of GC-MS identified compounds from lyophilized *Aloe ferox* leaf gel (LGE) and 95% ethanol leaf gel extract (ELGE). LGE and ELGE were a total of all the compounds identified, the groups of compounds best described for their health benefits were the phenolic acids/polyphenols, sterols, fatty acids, and indoles (Du Toit *et al*, 2007). Apart from these, various alkanes, pyrimidines, alkaloids, organic acids, aldehydes, dicarboxylic acids, ketones, and a few alcohols were also identified (Du Toit *et al*, 2007).

2.1.10. Uses of *Aloe ferox*

Horticultural

Aloe ferox is a decorative or ornamental plant used in rural homesteads and by horticulturists working in gardens and also used by landscapers who work for the public sector or private people. The leaves are burnt to repel insects and the stockades of *A. ferox* which are planted round cattle kraals form a characteristic and attractive feature of rural areas (Coates, 1977).

Food

The use of *A. ferox* leaves in commercially manufactured preserves and condiments follows a centuries-old tradition of use in the Western Cape (Watt and Breyer-Brandwijk, 1962). Grace *et al*., 2011 reported that the peeled leaf mesophyll of *Aloe ferox* is used to make jam in South Africa and the sweet floral nectar of *Aloe ferox* and *Aloe secundiflora* are favored as a snack food (Watt and Breyer-Brandwijk, 1962; Maundu *et al*., 2009). In times of drought and famine, farmers have used the leaves of *Aloe ferox* to make fodder for their stock and the gel has been used in South Africa to make a jam that tastes like melon jam, it is also gaining importance as a refreshing and nutritive in food and drinks (Coates, 1977; Kleinschimdt, 2004). An important breakthrough for the South African aloe industry is the fact that the American Food and Drug Administration has permitted the use of *Aloe ferox* as a direct food additive for human consumption as a natural flavouring substance (Food and drug administration, 2002).

Cosmetic industry

Apart from its medicinal applications due to the gels richness in minerals, amino acids, vitamins, and trace elements, it is extensively used in the cosmetic industry too (Drewes *et al*., 2006). The

gel from the core of the leaves has a similar use as the gel from the leaves of *Aloe vera* and is used to treats skin afflictions, burns wound, abrasions, irritations and is applied as a poultice or used as a general refrigerant. It is further used as a hair wash to promote hair growth and to fight dandruff and also as a cosmetic that can improve skin complexion and smoothness (McAnalley, 2009). Grace *et al*., (2011) stated the efficacy of *Aloe ferox*, *Aloe arborscens* and *Aloe vera* in wound healing and antimicrobial properties of the leaves (Jia, et al., 2008 and Steenkamp and Stewart, 2007). The emollient properties of the leaves were described in therapy of rashes, and the leaf exudates for skin irritations (Van Wyk *et al*., 1997). According to Chen *et al*. (2012) commercial preparations of the gel have been reported to heal certain chronic leg ulcers and improve some cases of eczema in addition to providing significant relief in acute sunburn (Van Wyk and Gericke, 2000). Aloe gel can be added to cosmetic products such as cleansers, moisturisers, shampoos, suntan lotions, and sunburn creams and aloesin shows promise as a pigmentation-altering agent for cosmetic or therapeutic applications (Jones et al., 2002; Yagi and Takeo, 2003).

Digestive ailments

Chen *et al.* (2012) stated that *Aloe ferox* is used for treatment of digestive ailments. Concentrated preparations of the leaf exudate ("drug aloe") is taken as laxative effects are attributed to the presence of anthraquinones and in particular, which are aloe-emodin and used for the same indication to treat cattle in Lesotho (Watt and Breyer-Brandwijk, 1962; British Pharamacopeia, 1993; Maliehe, 1997; Grace et al., 2008). The polysaccharide rich mesophyll ("aloe gel") is taken as a source of fiber (Steenkamp and Stewart, 2007)

2.1.10.5. Medicinal uses

Infections and parasites

Aloe ferox is applied topically to sores caused by viral infections such as warts, herpes and shingles (Van Wyk *et al*., 1997) and the leaf pulp (mesophyll) may be applied directly to the skin, without preparation, to treat ringworms (Reynolds, 1950) or to dress open wounds (Morton, 1961).

Chen *et al.* (2010) reported that the crude extract of *A. ferox* was investigated for its *in vitro* anthelintic activity on the egg and larvae of the nematode parasite *Haemonchus contortus.* The extracts exhibited 100 % egg hatch inhibition at 20 mg/ml and larval development (Maphosa, *et al*., 2010). *Aloe ferox* is used to treat numerous infections particularly sexually transmitted infections and internal parasites in the Eastern Cape Province of South Africa (Kambizi *et al*., 2004; Grace *et al*., 2008; Kambizi and Afolayan, 2008). Aloe emodin and aloin A showed inhibitory activity against all the test organisms (*Bacillus cereus, B. subtilis, S. aureus, S. epidermidis, E. coli, Shigella sonnei*) while chrysophanol only inhibited *B. subtilis, S. epidermidis and E. coli* (Kambizi *et al*., 2004). Chen *et al.* (2012) also reported unspecified antifungal activity of *A. ferox* 'juice' against *Trichophyton* spp. causing athlete's foot and thrush and the acetone extract of *Aloe ferox* was found to be fungicidal (10 mg/ml) against five fungi using the agar dilution method (Afolayan *et al*., 2002). Kambizi *et al*. (2007) demonstrated the antiviral effects of this plant on herpes simplex virus type 1 *in vitro*.

The claimed therapeutic applications of anthroquinones and its derivatives rely on their purgative action, anti-inflammatory activity, antiprotozoal action and antioxidant activity (Choi and Chung, 2003). *Aloe ferox* is one of the most frequently used plants in South Africa for the treatment of sexually transmitted infections (Kambizi et al, 2004). Kambizi et al. (2004) found that three compounds, Aloe-emodin, aloin A and chrysophanol isolated from Aloe ferox were active against various bacterial strains. *Aloe ferox* is one of the most frequent and common plants used by the community for the treatment of sexually transmitted diseases.

Sensory system

Chen *et al*. (2012) and Grace *et al*. (2008) stated that the leaf exudate of *Aloe ferox* in southern Africa is used to relieve arthritis and sinusitis, conjunctivitis, ophthalmia and other eye ailments by topical application of the leaf sap as eye drops (Smith, 1888; Watt and Breyer- Brandwijk, 1962; Palmer, 1985; Van Wyk and Gericke, 2000; Crouch *et al*., 2006).

Anti-diabetic activity

Extracts of *Aloe* gum were investigated to increase glucose tolerance in both normal and diabetic rats (Al-Awadi and Gumaa, 1987). A study was done to determine the effects of ethanol extracts of *A. ferox and A. greatheadii* leaf gel in a streptozotocin (STZ)-induced type 2 diabetes rat

model. It was observed that *Aloe ferox* and *Aloe greatheadii* supplementation resulted in moderately increased serum insulin, accompanied by slight corrections in ALP and HDL-C, without any change to end-point plasma glucose values. *A. greatheadii* and, to a lesser extent, *A. ferox*, resulted in a clinically relevant improved diabetic state (indicated by moderate to high effect sizes), suggesting that these *Aloe* species may show potential for treating diabetes (Loots *et al*., 2011).

Inflammation

Aloe ferox has long been used to treat inflammation associated with injuries (Smith, 1888). Speranza *et al*. (2005) found that Aloeresin 1 (1 µmol/cm^2) isolated from Cape aloe reduces the *in vivo* oedematous response by 39%. Mwale and Masika (2010) evaluated the anti-inflammatory activity of *A. ferox* whole leaf aqueous extract and found that high doses (400 mg/kg), *Aloe ferox* exhibited anti-inflammatory and analgesic activities,

Antioxidant activity

Jia and Farrow (2003) identified and purified 7-hydroxychromones, such as aloesin from an aloe extract from whole leaf processing and they found that they suppress free radical generation and the production of reactive oxygen species (ROS) thereby preventing and treating ROS-mediated conditions and other oxidative processes. Loots *et at*. (2007) confirmed the anti-oxidant activity of *Aloe ferox* using oxygen radical absorbance capacity (ORAC) and ferric reducing anti-oxidant power (FRAP) analyses. The majority of the phenolic and alkaloids identified in *A. ferox* are known to possess anti-oxidant activity and may contribute to the ORAC and FRAP values of these extracts. The phytochemical composition of *A. ferox* may show promise in alleviating symptoms associated with/ or in the prevention of cardiovascular disease, cancer and neurodegeneration, and diabetes (Loots *et al*., 2007). Wintola and Afolayan (2011) reported high scavenging activity of *Aloe ferox* in ethanol, acetone and methanol extracts, but low in aqueous medium, indicating their antioxidant potential and although the contents of most phytochemicals evaluated were not very high but they synergistically boost the antioxidant activity of the whole leaf extracts.

Anti-cancer activity

Aloe ferox is used as an anti-cancer agent (Soeda, 1969; Capasso *et al*., 1998; Pecere, *et al*., 2000; Van Wyk *et al*., 2009). According to Chen *et al.* (2012), Aloe emodin has been reported to have selective activity against neuroectodermal tumors, with practically no effect on normal cells (Pecere, *et al*., 2003) and Kametani *et al*. (2007) isolated 10 compounds from dichloromethane extract of Cape aloe and found that they have growth-inhibiting effect on Ehrlich ascites tumor cells (EATC) as investigated using trypan method.

Muscular-skeletal diseases

Aloe ferox is known for the treatment of arthritis and rheaumatism (Powell, 1868; Hocking, 1997; Van Wyk *et* al., 1997).

Blood and circulatory system

Powell (1868) cited *A. ferox* as a medicine for ailments of the spleen and Amusan *et al*. (2002) says A*loe ferox* treats arteriosclerosis, hypertension and stress among reported circulatory ailments.

2.1.11. A*loe ferox* in international trade

The commercial trade in Aloe-derived natural products is based mainly on two materials obtained from the leaves of certain Aloe species: leaf exudate, used in laxatives, and leaf mesophyll, used in products applied topically for skin ailments or taken internally for digestive complaints and general wellbeing. The industries based on Aloe leaf exudate and mesophyll differ markedly in geographical focus, supply chain and species used.

Leaf exudate is principally wild-harvested from populations of A. ferox on communal or privately-owned land in South Africa and, in East Africa. Grace (2011) states that the main importers of leaf exudate are countries in Europe and Asia (Newton and Vaughan, 1996; Oldfield, 2004; Melin, 2009) and the leaf mesophyll from predominantly wild-harvested A. ferox is processed in South Africa (Newton and Vaughan, 1996), A. macroclada Baker in Madagascar and several species in Tanzania (Sachedina and Bodeker, 1999) are used in expanding local industries that seem to mirror the use of A. vera in cosmetics, toiletries and non-scheduled

remedies. Processed derivatives of the leaf mesophyll are sold under names such as gel fillet, concentrated- and crude gel, decolourised- and/or pasteurised gel, freeze- or spray-dried powdered gel (Waller et al., 2004) similar products are made using whole leaves (Grace, 2011). Grace (2011) states that in South Africa, the rural industry supported by wild-harvested A. ferox was estimated to be worth R4 million (US$569000) per annum in the late 1990s (Newton and Vaughan, 1996), and then increased from R12 to R15 million (NUS$2 million) per annum a decade later (Shackleton and Gambiza, 2007; Melin, 2009) and production is necessarily confined to the species' natural distribution in the Western and Eastern Cape Provinces; it was historically centred in small towns east of Cape Town and extended to the Karoo and Eastern Cape Province, where it has recently become the subject of rural development initiatives (Newton and Vaughan, 1996; Melin, 2009).

Annual reports in South Africa indicated that the export of *A. ferox* was 4549 tonnes between 1981 and 1994 with the highest amounts exported to Germany, Japan, Argentina and Italy (Chen et al, 2012). The harvesting and processing has been historically centred in the Eastern Cape and Western Cape where *A. ferox* occurs most abundantly (Melin, 2009). Industrial processing of the plant gel started in the early 1990s when an aloe factory was established in Albertinia, Western Cape (Newton and Vaughan, 1996). The socio-economic benefits of the *A. ferox* industry in South Africa are spreading widely from the poorest people whose only source of income is derived from Aloe tapping, to itinerant agricultural workers and other part-time aloe tappers, their families and communities (Newton and Vaughan, 1996). In the Western Cape, the potential annual income for a full-time tapper was estimated to be R10000 (US$1400) in 1992 but, due to a complex debt cycle and lack of empowerment, this was seldom realised (Newton and Vaughan, 1996). In towns where *A. ferox* industry are located, leaf exudate has been used for obtaining credit and offsetting debt in local stores, or to purchase food (Newton and Vaughan, 1996). Grace (2011) again stated that historical records show that the trade in leaf exudate from Aloe spp. is characteristically vulnerable to fluctuations in demand, driven by export markets and economic conditions, and in supply, influenced by factors such as drought and plant pests, despite the storability of dried exudate preparation (Newton and Vaughan, 1996; Sachedina and Bodeker, 1999).

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CHAPTER 3

COMPARATIVE PHYTOCHEMICAL ANALYSES OF *ALOE FEROX MILL.* FOUND IN EASTERN CAPE CAPE AND WESTERN CAPE, SOUTH AFRICA.

CHAPTER 3

Comparative phytochemical analyses of *Aloe ferox* Mill. found in Eastern Cape and Western Cape, South Africa.

Abstract

The leaf material from the Eastern Cape and Western Cape Provinces in South Africa were collected from the wild and processed in the lab for a series of phytochemical experiments. The following phytochemicals were analysed, phenols, flavonoids, flavonols, proanthocyanidins, tannins, alkaloids and tannins using current methods. The results were analysed using Minitab statistical program. The results showed a variation in the quantity and composition of phytochemical in the leaves analyzed. From the result of this study, it was been observed that phenols were higher in Eastern Cape *Aloe ferox* extracts except in water extract of fresh plant of Western Cape which shows a significant difference. Flavonoids were higher in E.C extracts but the difference was not much in the water extracts. Flavonol contents were similar in water extracts of fresh plant from both areas. The flavonols contents of dried plants was higher in E.C extracts and the difference was not much in acetone extracts of dry plant but it was higher in W.C. Dichloromethane extracts dry plant. Proanthocyanidin contents of fresh plant from W.C were higher than the one from E.C. Dry plant proanthocyanidin contents were similar, acetone extracts of E.C. *A. ferox* were higher than that of W.C. Dry plant extract had a higher content of proanthocyanidins compared to fresh plant extract with E.C. dichloromethane extracts showing a higher content than W.C. Tannins were found to be higher in fresh plant water extract from W.C. with a high difference between each extract. Acetone showed more tannins in fresh plant and dry plant from E.C. Like in water extracts, DCM extracts of both fresh and dry plant material from W.C. showed higher contents of tannins compared to that from E.C. Alkaloid contents of water extracts were significantly higher in *A. ferox* from from W.C. compared to that of E.C. As in tannins the contents of alkaloids in acetone extracts were higher in plant from E.C. with a significant difference. Again as in tannins, the dichloromethane extracts from dry and fresh plant material from W.C. showed higher content of alkaloids than the plant from E.C. Saponin contents were higher in *A. ferox* from the E.C. in all the extracts with a significantly high difference.

3.1. Introduction

Plants produce secondary metabolites that are unique to specific genera or species. Some of the metabolites may not play a role in the plants primary metabolic requirements but rather assist plant species to survive and fight problems around them in their interactions with the environment (Harborne, 1993). As stationary autotrophs, plants have to cope with a number of challenges, including engineering their own pollination and seed dispersal, local fluctuations in the supply of the simple nutrients that they require to synthesize their food and the co-existence of herbivores and pathogens in their immediate environment. Plants have therefore developed secondary biochemical pathways that allow them to synthesize several chemicals, often in response to specific environmental stimuli, such as herbivore-induced damage, pathogen attacks, or nutrient depravation (Baldwin *et al*., 2001; Reymond *et al*., 2007). Some of the roles of secondary metabolites include protective roles as antioxidant, UV light-absorbing, antiproliferative agents and defend the plant against microorganisms such as bacteria, fungi, and viruses (Wink, 1987). More complex roles of these metabolites include dictating or modifying the plant's relationship with more complex organisms (Harborne, 1993, Wink, 2003; Tahara, 2007). By acting as a feeding deterrent this explains why phytochemicals are bitter and/or toxic to potential herbivores often leading to direct interactions with the herbivore's central and peripheral nervous systems (Rattan, 2010).

Synthesis and accumulation of secondary metabolites in plants is regulated in space and time (Wink and Schimmer, 1999) and is affected by abiotic environmental factors, such as light intensity, soil minerals, osmotic stresses (drought and salinity), and seasonality (Waterman and Mole, 1994). Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "secondary metabolities" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids (Harborne, 1973; Okwu, 2004).
Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds (Costa *et al*., 1999). Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions (King and Young, 1999). According to Wintola and Afolayan (2011) the percentage

compositions of total phenols, flavonoids, flavanols, proanthocyanidins, tannins, alkaloids and saponins found in the whole leaf extracts of *Aloe ferox* varied significantly in acetone, ethanol and methanol and water extracts. Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals (American Cancer Society, 2000). These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity (Stevenson and Hurst, 2007) modulation of detoxification enzymes (Tan *et al.* 2010), stimulation of the immune system, decrease of platelet aggregation (Beretz *et al*,. 1982), modulation of hormone metabolism and anticancer property (Gary and Shyam, 2009). There are more than a thousand known phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases (Rao, 2003). Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc.

Phenolics

Saxena *et al*. (2013) stated that phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom. The three most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols. Phenolics are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group. Phenol (C6H5OH) is considered the simplest class of this group of natural compounds. Phenolic compounds are a large and complex group of chemical constituents found in plants. They are plant secondary metabolites, and they have an important role as defence compounds. Flavonoids are the largest group of plant phenols and the most studied (Dai and Mumper, 2010).

Phenolic acids

Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids. Phenolic polymers, commonly known as tannins, are compounds of high molecular weight that are divided into two classes: hydrolyzable and condensed tannins. The term "phenolic acids", in general, designates phenols that possess one carboxylic acid functional group. Naturally occurring phenolic acids contain two distinctive carbon frameworks:

the hydroxycinnamic and hydroxybenzoic structures. Hydroxycinnamic acid compounds are produced as simple esters with glucose or hydroxy carboxylic acids. Plant phenolic compounds are different in molecular structure, and are characterized by hydroxylated aromatic rings (Balasundram *et al*., 2006). These compounds have been studied mainly for their properties against oxidative damage leading to various degenerative diseases, such as cardiovascular diseases, inflammation and cancer (Saxena *et al*., 2013).

Flavonoids

Flavonoids are polyphenolic compounds that are ubiquitous in nature. More than 4,000 flavonoids have been recognised, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks (Pridham, 1960). Flavonoids are ubiquitous among vascular plants and occur as aglycones, glucosides and methylated derivatives. Harborne and Baxter (1999) report that more than 4000 flavonoids have been described so far within the parts of plants normally consumed by humans and approximately 650 flavones and 1030 flavanols are known (Saxena *et al*., 2013).

Tannins

Saxena *et al.* (2013) reported that from a chemical point of view it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers. It might be said that the tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids and minerals, etc (Vansoest, 1994; Schofield and Mbugua, 2001). They are divided into four major groups: Gallotannins, ellagitannins, complex tannins, and condensed tannins (Mangan, 1988; McLeod, 1974; Mole and Waterman, 1987).

Alkaloids

Alkaloids are natural product that contains heterocyclic nitrogen atoms. The name of alkaloids derives from the "alkaline" and it was used to describe any nitrogen-containing base (Mueller-Harvey and McAllan, 1992). Alkaloids are naturally synthesised by a large number of organisms, including animals, plants, bacteria and fungi molar concentration (Mishra, 1989). They are so numerous and contain a variety of molecular structure that their rational classification is difficult. However, the best approach to the problem is to group them into families, depending on the type of heterocyclic ring system present in the molecule (Krishan *et al*., 1983) Saxena *et al*. (2013).

Saponins

Saponins that have one sugar molecule attached at the C-3 position are called monodesmoside saponins, and those that have a minimum of two sugars, one attached to the C-3 and one at C-22, are called bidesmoside saponins (Laziszity *et a.l*, 1998).

3.2. Materials and methods

Fresh matured whole leaves of *Aloe ferox* were collected in 2012 around the University of Fort Hare in Alice, for the Eastern Cape, while the Western Cape Aloe was obtained from Organic Aloe factory owners. The leaves were washed in distilled water, spines were removed and the whole leaf was cut into thin pieces suitable for extraction. The cut leaves were divided into two parts, was dried in an oven at 45° C for 48 hours while the remaining half was used fresh.

A B

Fig 3.1: *Aloe ferox* species used for the study, A is the *Aloe ferox* from W.C and B is the *Aloe ferox*

3.3. Preparation of extracts

Fresh and oven dried portions of *Aloe ferox* were weighed and separately extracted with acetone, dichloromethane and water on a shaker machine for 48 hours at room temperature. Fresh plant

was 200 g each and the dried sample was 100 g each. The extracts were decanted, filtered with Buchner funnel and Whatman No. 1 filter paper and concentrated to dryness using a rotary evaporator to yield the crude extracts that were used for the analysis. The water extracts were freeze dried using a Vir Tis Benchtop freeze dryer.

3.4. Phytochemical analysis

3.4.1. Determination of total phenolics

Total phenolic was determined as described by Wolfe *et al*. (2003). 5 ml of Folin-Ciocalteau reagent diluted with 5 ml distilled water (1:9 v/v) was mixed with 5 ml of aloe extract and 4 ml (7 g/l) of Na_2CO_3. The tubes were vortexed for 15 seconds and then left to stand for 30 minutes at 40^0 C for color development. The absorbance was read at 765 nm using the AJI-CO3 UV-IS spectrophotometer. The samples were evaluated at final concentrations of 0.1 mg/ml. Total phenolic content was expressed as mg/ml of tannic acid equivalent using the equation of the calibration curve Y= 0.121 x, R^2= 0.9365, where x is the absorbance and Y is the tannic acid equivalent (mg/ml).

3.4.2 Determination of total flavonoids

Flavonoid content was determined as described by the method of Ordonez *et al*. (2006) using quercetin as standard. 0.5 ml of 2 % $AlCl_3$ in ethanol was added to 0.5 ml solution of extract then incubated at room temperature for 1 h and the absorbance measured at 420 nm. The result was expressed as mg/g using the equation: Y = 0.0255 x, R = 0.9812; where x is the absorbance and Y the quercetin equivalent. All determinations were in triplicates.

3.4.3. Determination of total flavonols

Total flavonols was determined by following the method described by Kumaran and Karunakan (2007) where 2.0 ml of aloe extract was mixed with 2.0 ml of $ALCl_3$ in ethanol, then 3.0 ml of (50 %) sodium acetate solution was added to the mixture. This was then incubated at 20 °C for 150 min and the absorbance read at 440 nm. Total flavonol content was calculated as quercetin (mg/g) equivalent from the calibration curve using the equation: Y = 0.0255x, R2 = 0.9812, where x is the absorbance and Y the quercetin equivalent in mg/ml.

3.4.4 Determination of total proanthocyanidins

The method described by Oyedemi *et al*. (2010) was used to determine total proanthocyanidins in the aloe extracts where 5 ml of 1 mg/ml of the sample extract was added to 3 ml of vannilin-methanol (4% v/v) and 1.5 hydrochloric acid was added and vortexed. The solution was allowed to stand for 15 minutes at room temperature and the absorbance was read at 500 nm. Total proanthocyanidin contents were expressed as catechin equivalents (mg/g) using the following equation based on the calibration curve: $Y = 0.5825\ x$, $R^2 = 0.9277$, where x is the absorbance and Y the catechin equivalent in mg/ml.

3.4.5. Determination of total alkaloids

Alkaloids were determined quantitatively according to the method of Harborne (2005). Two hundred mililitre of 20 % acetic acid (40 ml) in 160 ml ethanol was added to 5 g of sample, covered and allowed to stand for 4 hours, filtered and then concentrated in water bath to one-quarter of the original volume. Concentrated NH_4OH was then added dropwise until precipitation was complete. The solution was allowed to settle and filtered. The residue on the filter paper which is the crude alkaloid was weighed and the alkaloid content was determined using the formula: % of alkaloid = (final weight of the sample/initial weight of the extract) x 100.

3.4.6. Determination of total saponins

Saponins were determined quantitatively using the method of Obadoni and Ochuko (2001). 20 g of each sample was added to 200 ml of 20.5 % ethanol in (80 ml distilled water), heated for 4 hours in a water bath at 55°C. The mixture was then filtered and re-extracted as with another 100 ml of aqueous ethanol. The combined extracts were reduced to approximately 40 ml over the water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously to extract twice. The ether layer was discarded while the aqueous layer was retained and to which 60 ml n-butanol was added. It was then heated on a water bath and after evaporation the samples were dried in the oven at 40°C to a constant weight. The saponin content was calculated using the expression: % saponin = (final weight of samples/ initial weight of extracts) x 100.

3.4.7. Determination of total tannins

Tannins were determined according to the method described by AOAC (1990) where 0.20 g of each sample was added to 20 ml of 50 % methanol, covered, shaken thoroughly and placed in a water bath at 80°C for 1 hour to ensure uniform mixing. The extract was filtered into a 100 ml volumetric flask, followed by the addition of 20 ml distilled water, 2.5 ml Folin-Ciocalteu reagent and 10 ml of 17 % aqueous Na_2CO_3 and was thoroughly mixed. The mixture was made up to 100 ml distilled water. The mixture was left undisturbed for 20 minutes. A bluish-green coloration also developed at different concentrations ranges of 2, 4, 6, 8 and 10 ppm of tannic acid at the end of the reaction mixture. The absorbance of the tannic acid standard solutions as well as the sample was read after color development at 760 nm. Results were expressed as mg/g of tannic acid equivalent using the calibration curve: $Y = 0.1427 x + 0.2735$, $R^2 = 0.9927$ where x is the absorbance and Y is the tannic acid equivalent.

3.5. Statistical analysis

The results were expressed as mean ± standard deviation (SD) of three replicates where applicable and the data were subjected to one way analysis of variance (ANOVA) using the Minitab program Version 12 for Windows. P values < 0.05 were regarded as significant.

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CHAPTER 4

PHYTOCHEMICAL RESULTS

CHAPTER 4

Phytochemical analysis of the leaf extracts of *Aloe ferox* from Eastern Cape (E.C) and Western Cape (W.C) revealed the presence of phenols, flavonoids, flavanols, proanthocyanidins, tannins, alkaloids and saponins in different proportions in both areas. Figures 4.2 to 4.22 show the comparative phytochemical contents of the comparative *Aloe ferox* growing in Eastern Cape (E.C) and Western Cape (W.C) both in fresh and dry plant extract samples. Values are means of triphate determination. Means with the same letter (s) are not significantly different.

4.1. Total Phenolic contents

Phenol concentrations was higher in *A. ferox* E.C from than the one from W.C for all extracts in the respective solvents used except in dichloromethane (DCM) extract of dry plant which has lower concentration than W.C. (fig 3.1-3.3) .The trend observed was phenol in aqueous extract of plant > acetone extract of fresh plant > acetone extract of dry plant > acetone extract of fresh plant > DCM extract of fresh plant > DCM extract dry plant. Phenols were higher in DCM extract of dry plant from W.C and lowest in DCM extract of dry plant and acetone and highest in aqueous extract of dry plant and acetone extract of fresh plant. Differences of phenol concentrations were significantly different ($P < 0.05$) in all the extracts of aloe from both provinces.

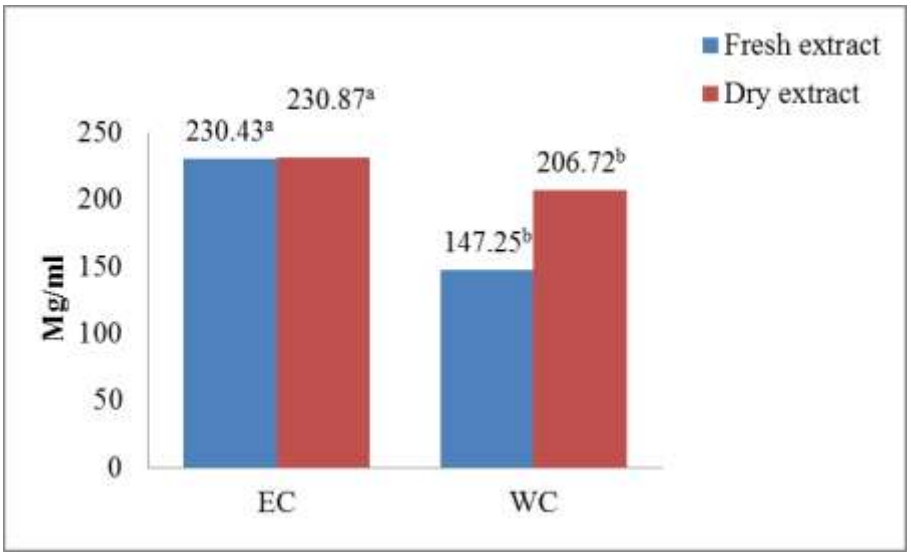

Fig 4.1 : Comparative phenol contents of water extracts of *Aloe ferox.*

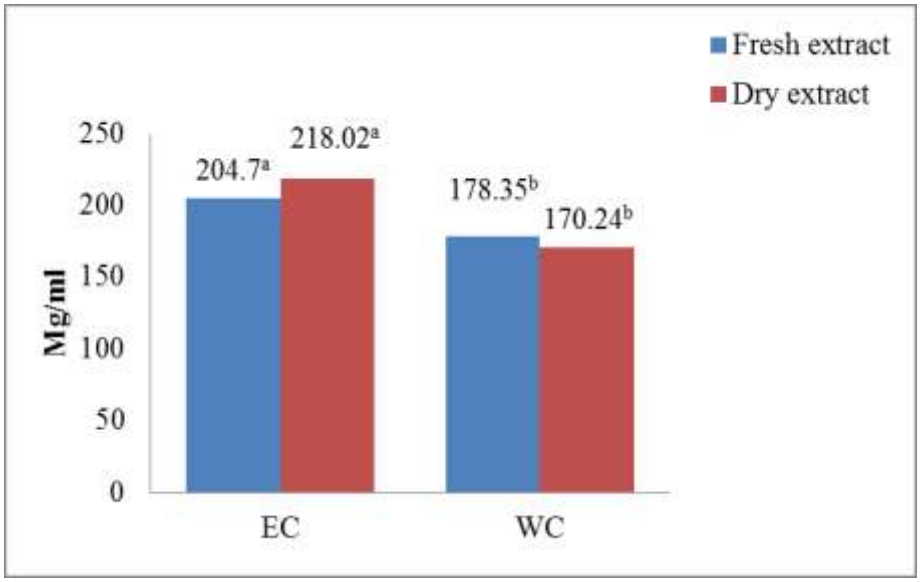

Fig 4.2: Comparative phenol contents of acetone extracts of *Aloe ferox*.

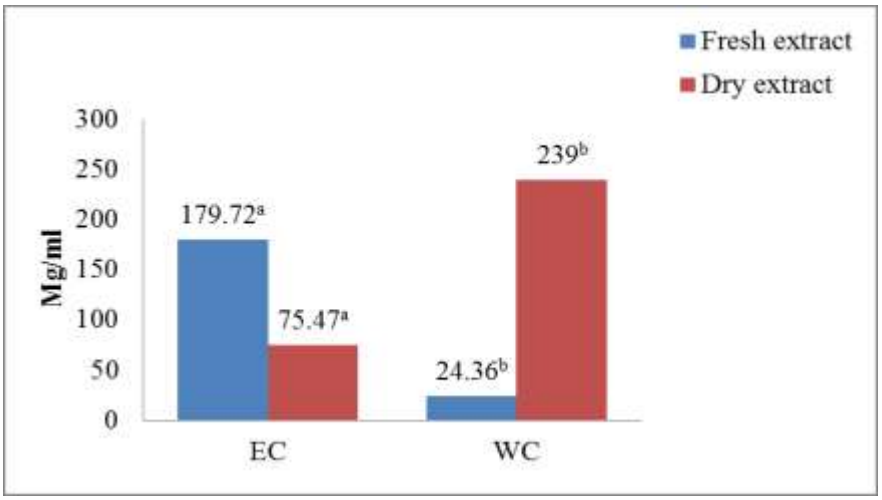

Fig 4.3: Comparative phenol contents of dichloromethane extracts of *Aloe ferox*.

4.2. Total Flavonoid contents

Fig 4.4 to 4.6 show the levels of flavonoid concentrations in *Aloe ferox* from E.C and W.C. Flavonoid concentrations was higher in E.C. Aloe ferox extracts compared to aloe from W.C. The concentrations in different solvents follow this order acetone extract of dry > DCM of dry plant > DCM in fresh plant > aqueous in fresh plant > aqueous of plant dry > acetone of fresh plant. In Aloe ferox from W.C. the concentration of flavonoids was in in the order flavonoids in extract of DCM of fresh plant > acetone extract of dry plant > aqueous extract of fresh plant >

DCM extract of dry plant > aqueous extract of dry plant > acetone extract of fresh plant. The concentrations of flavonoids in the two areas were significantly different except in the aqueous extracts of fresh plant.

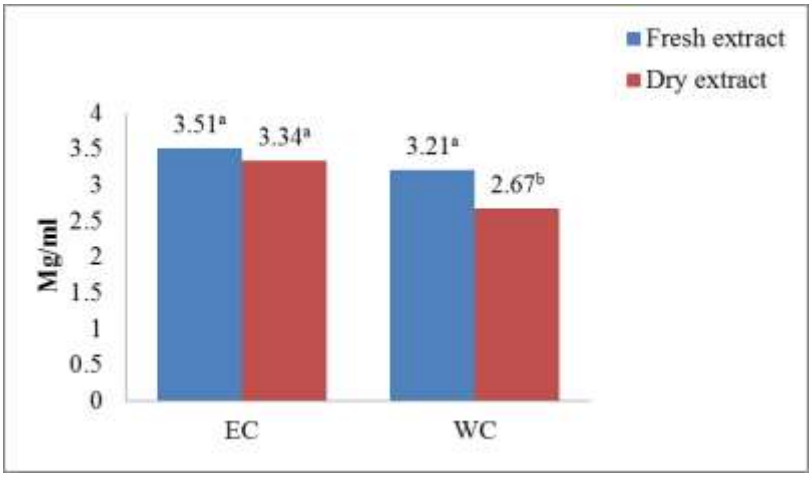

Fig 4.4: Comparative flavonoid contents of water extracts of *Aloe ferox*.

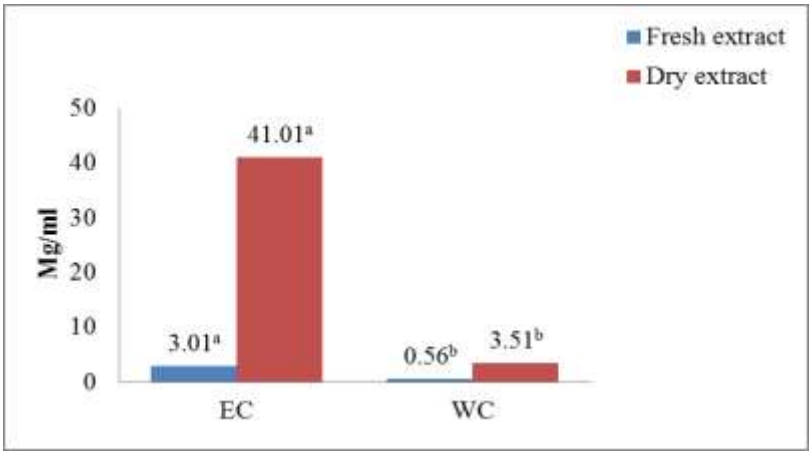

Fig 4.5: Comparative flavonoid contents of acetone extracts of *Aloe ferox*.

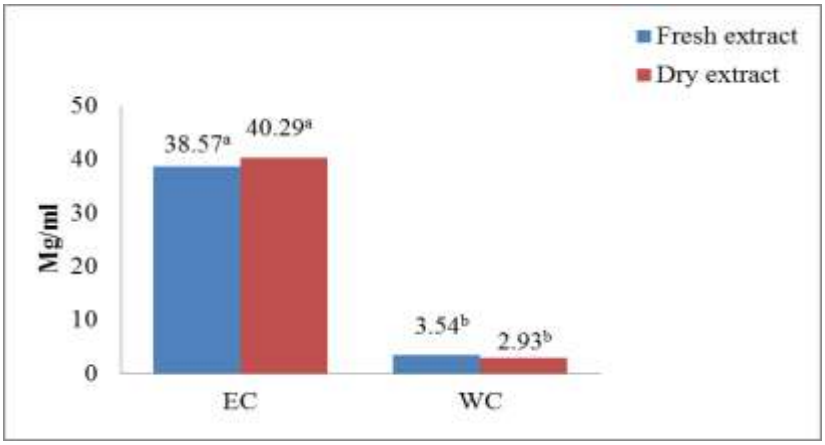

Fig 4.6: Comparative flavonoid contents of dichloromethane extracts of *Aloe ferox*.

4.3. Total Flavonol contents

Flavonol contents are shown in fig 4.7 to 4.9 and followed the trend as in phenols and flavonoids. It was higher in the E.C *Aloe ferox* than that of W.C in all extracts except aqueous extract of the fresh plant and DCM extract in dry plant which were lower than that of W.C. Flavonol content in aqueous extracts of fresh plant and dry plant acetone extract were not significantly different for the two areas. However in other extracts, the difference was significant. The concentration trend between the extracts from E.C and W.C was different, acetone extract in fresh plant had most flavonols for both E.C and W.C dichloromethane extract of dry plant from E.C had lowest concentration of flavonol whilst fresh plant in dichloromethane from W.C had lowest concentration of flavonols.

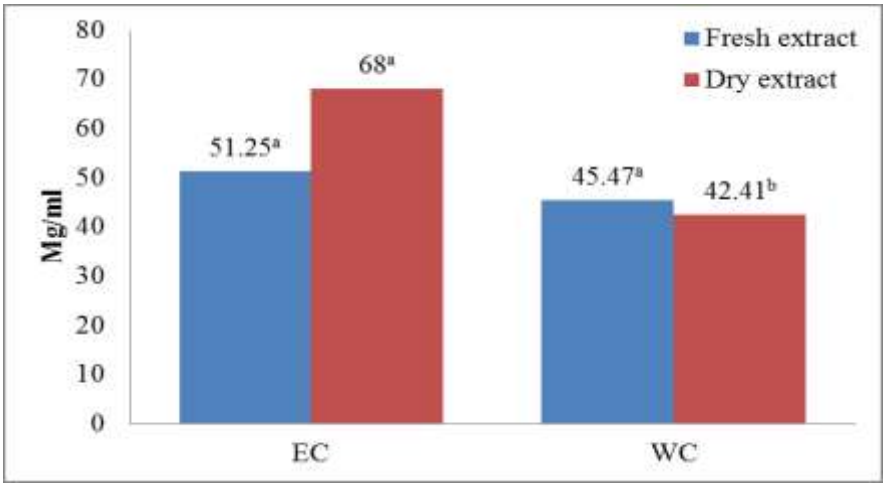

Fig 4.7: Comparative flavonol contents of water extracts of *Aloe ferox*.

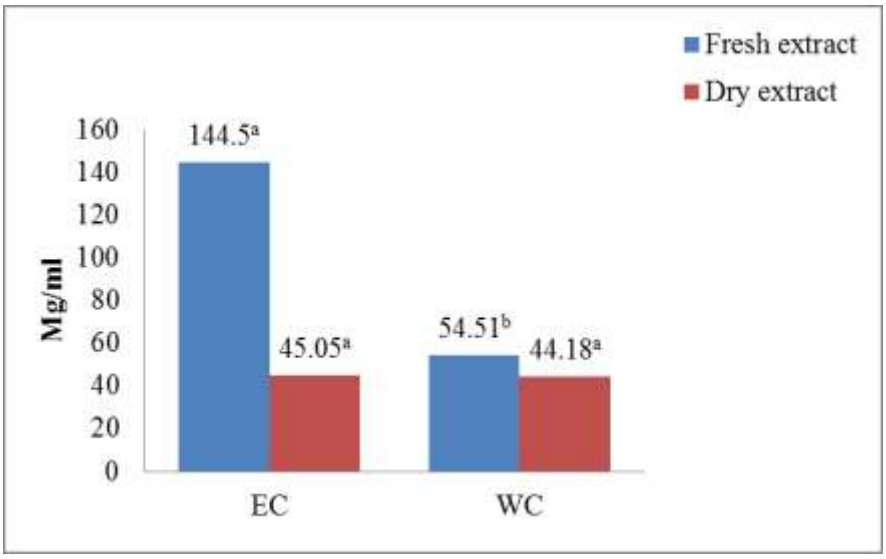

Fig 4.8: Comparative flavonol contents of acetone extracts of *Aloe ferox*.

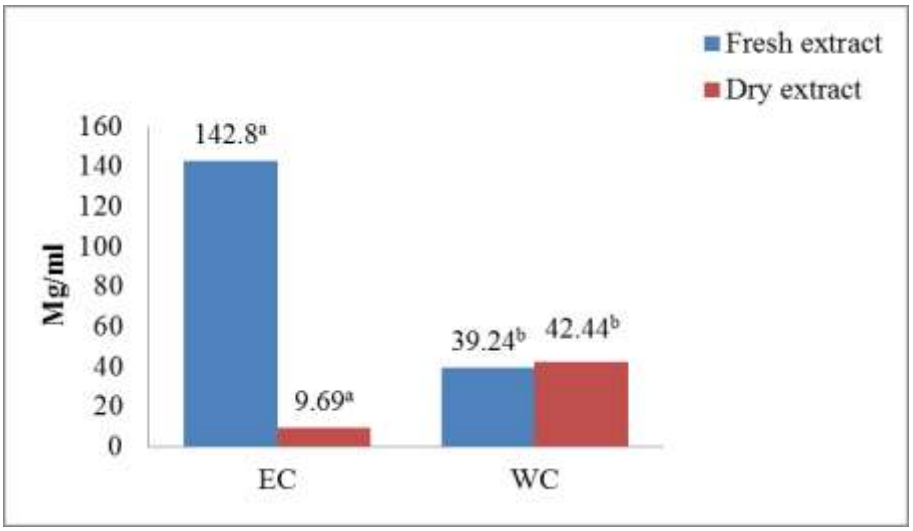

Fig 4.9: Comparative flavonol contents of dichloromethane extracts of *Aloe ferox*.

4.4. Total Proanthocyanidin contents

Proanthocyanidin contents of both fresh and dry samples were higher in E.C *Aloe ferox* compared to the W.C except for the aqueous extract of fresh plant which was lower than that of W.C. (fig 4.10-4.12). Dichloromethane extracts in dry plant had the highest concentration of proanthocyanidins in both E.C and W.C though fresh plant extract of dichloromethane of W.C had very low content. Acetone extracts of dry plant also had high content of proanthocyanidins

for both E.C and W.C. There is significant difference of proanthocyanidins concentrations between the two areas ($P < 0.05$).

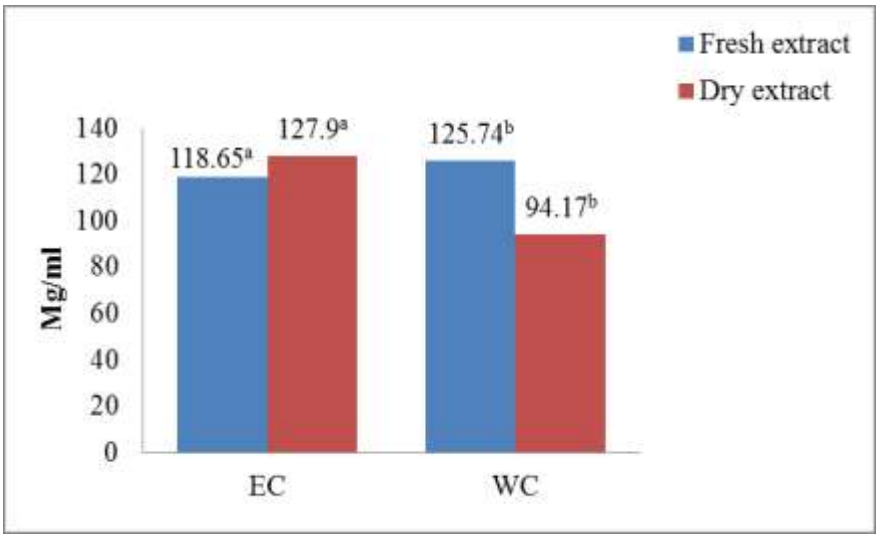

Fig 4.10: Comparative proanthocyanidin contents of water extracts of *Aloe ferox*.

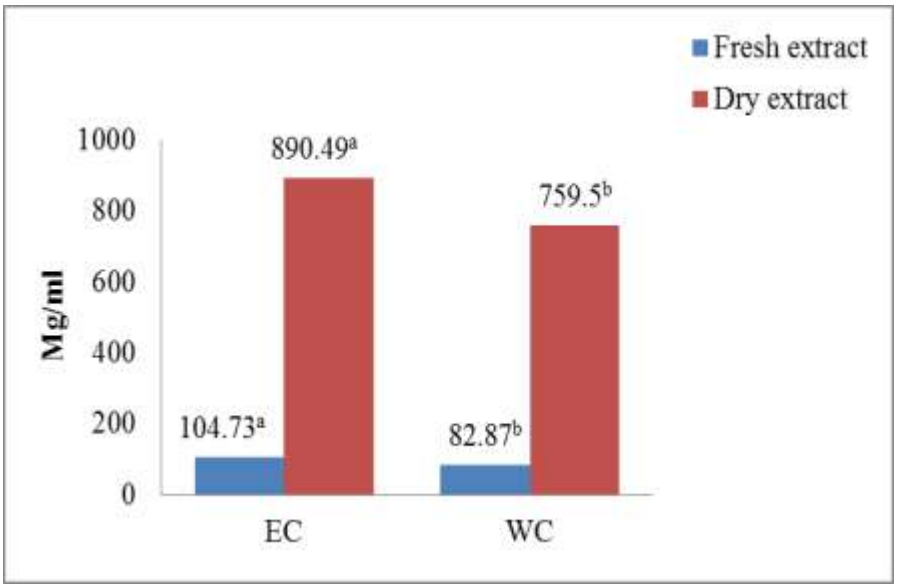

Fig 4.11: Comparative proanthocyanidin contents of acetone extracts of *Aloe ferox*.

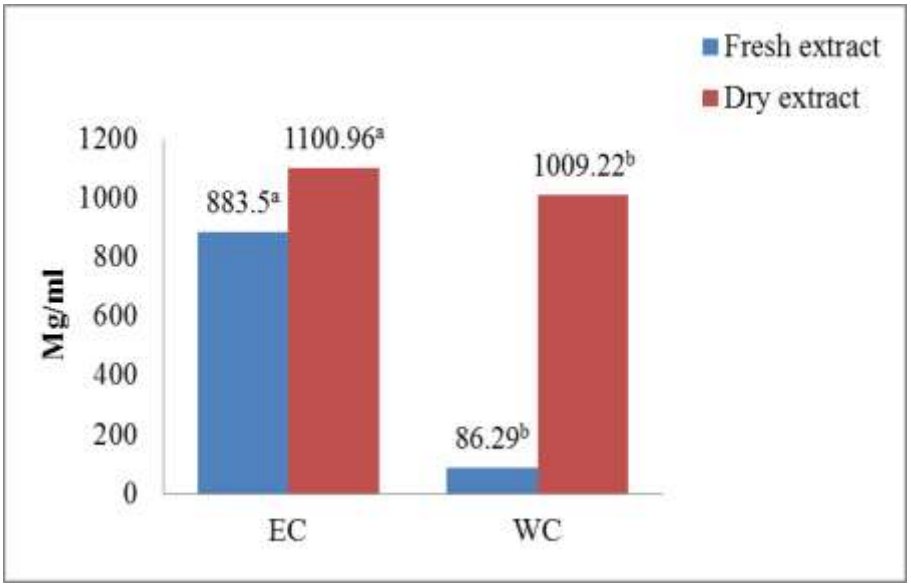

Fig 4.12: Comparative proanthocyanidin contents of dichloromethane extracts of *Aloe ferox*.

4.5. Total Alkaloid contents

Alkaloid percentage concentrations in Western Cape *A. ferox* were found to be higher than in E.C. (fig 4.13-4.15). The trend is as follows, aqueous extract of fresh leaves > DCM extract of dry leaves > DCM extract of fresh leaves > aqueous extract of dry leaves. Alkaloids in the samples from E.C. were higher only in acetone extracts of dry and fresh leaf samples. The content of this phytochemical between the two areas was significantly different (fig 4.13-4.15).

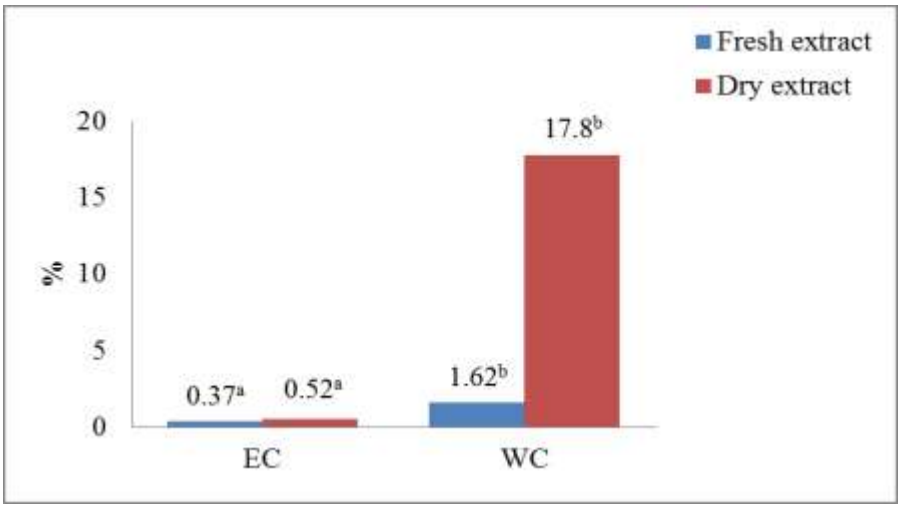

Fig 4.13: Comparative alkaloid contents of water extracts of *Aloe ferox*.

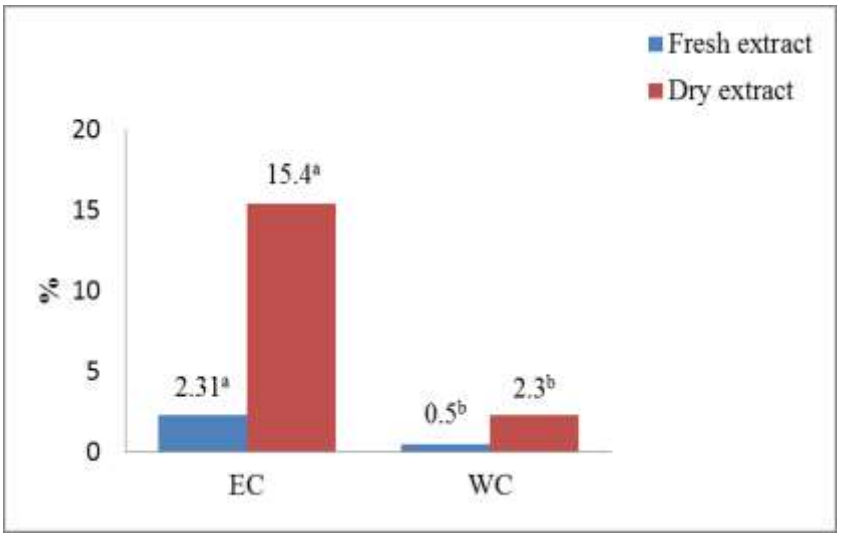

Fig 4.14: Comparative alkaloid contents of acetone extracts of *Aloe ferox*.

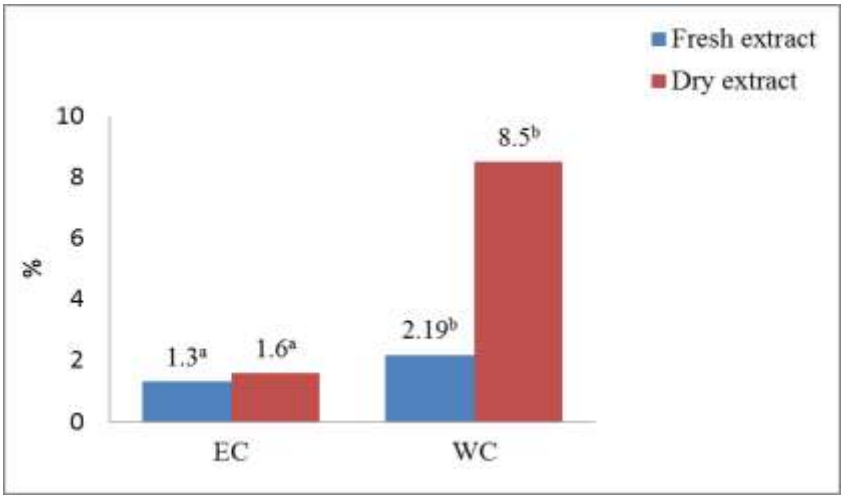

Fig 4.15: Comparative alkaloid contents of dichloromethane extracts of *Aloe ferox*.

4.6. Total Saponin contents

Saponin percentage concentrations are shown in fig 4.16-4.18. It was found to be higher in all extracts of dry and fresh leaves in acetone, dichloromethane and water solvents in Eastern Cape *A. ferox* compared to that of Western Cape. The trend in the E.C extracts for saponin content was in the order; aqueous in dry plant > DCM in fresh plant > acetone in dry plant > DCM in dry plant > acetone fresh plant > aqueous in fresh plant. For W.C the trend was aqueous in dry plant > acetone in dry plant > DCM in fresh plant >DCM in dry plant > acetone in fresh plant > aqueous extract in fresh plant.

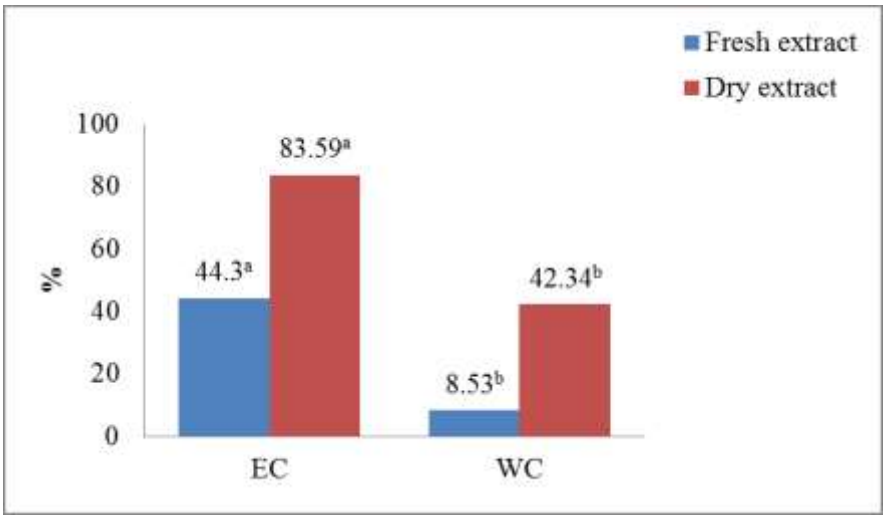

Fig 4.16: Comparative saponin contents of water extracts of *Aloe ferox*.

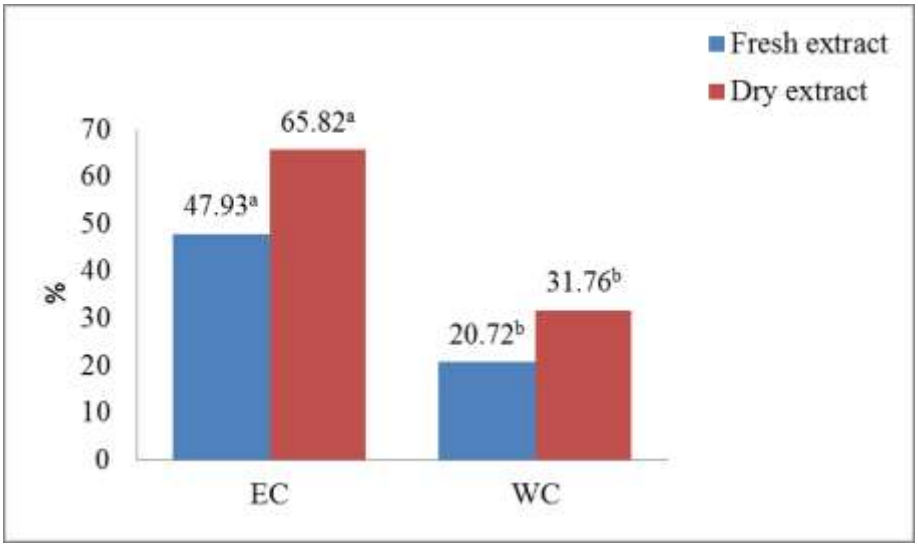

Fig 4.17: Comparative saponin contents of acetone extracts of *Aloe ferox*.

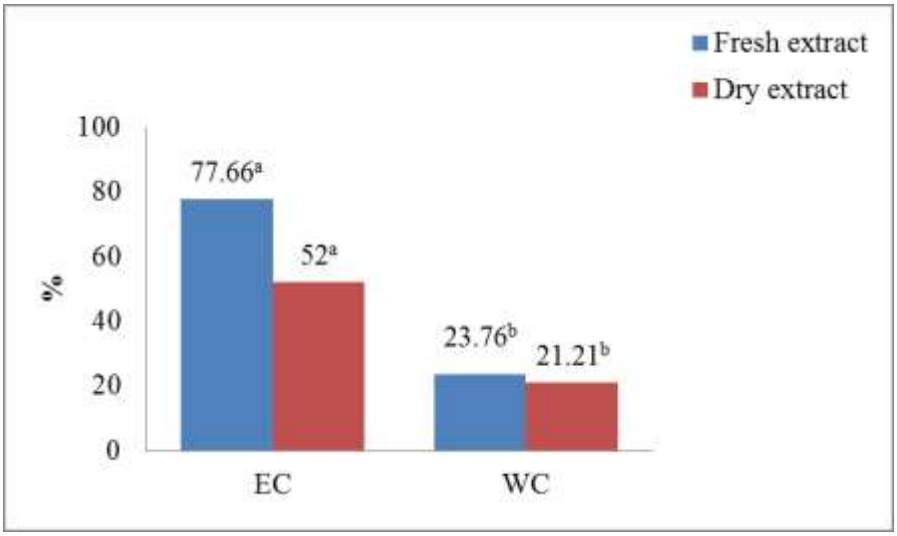

Fig 4.18: Comparative saponin contents dichloromethane extracts of *Aloe ferox.*

4.7. Total Tannin contents

The tannin contents of leaf extracts of *A. ferox* from the two regions differed significantly. More tannins was found in W.C extracts compared to those of E.C. The order of concentration is as follows, aqueous of fresh leaves > acetone extract of fresh leaves > aqueous extract of dry leaves > DCM and aqueous extracts of dry leaves. There was a lower content of tannins in *A. ferox from* E.C in the above solvents but high only in acetone extracts of fresh and dry leaves.

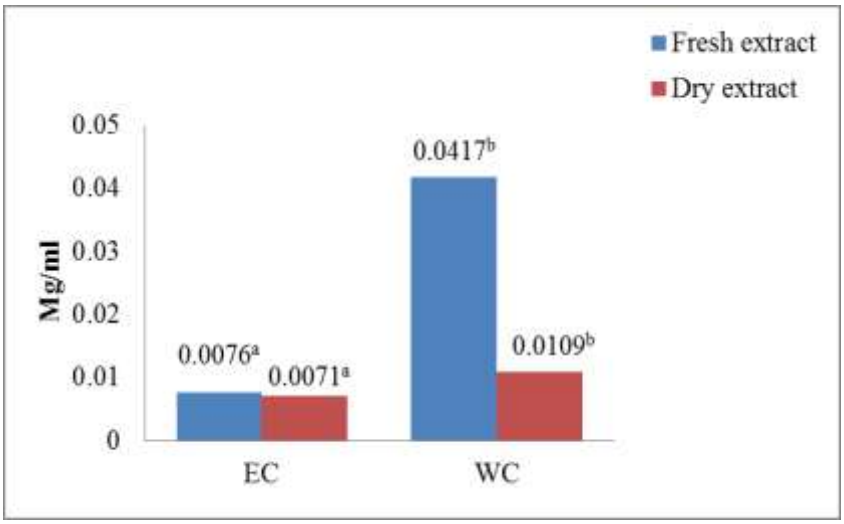

Fig 3.19: Comparative tannin contents of water extracts *Aloe ferox*.

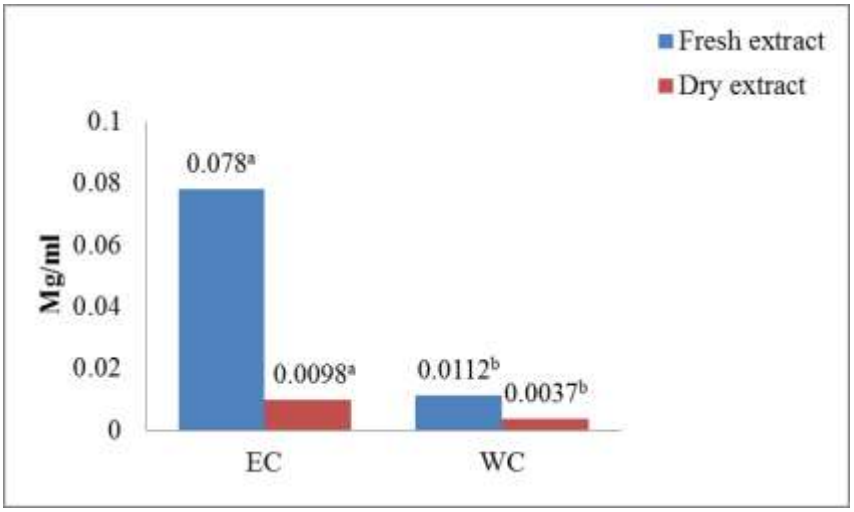

Fig 4.20: Comparative tannin contents of acetone extracts of *Aloe ferox.*

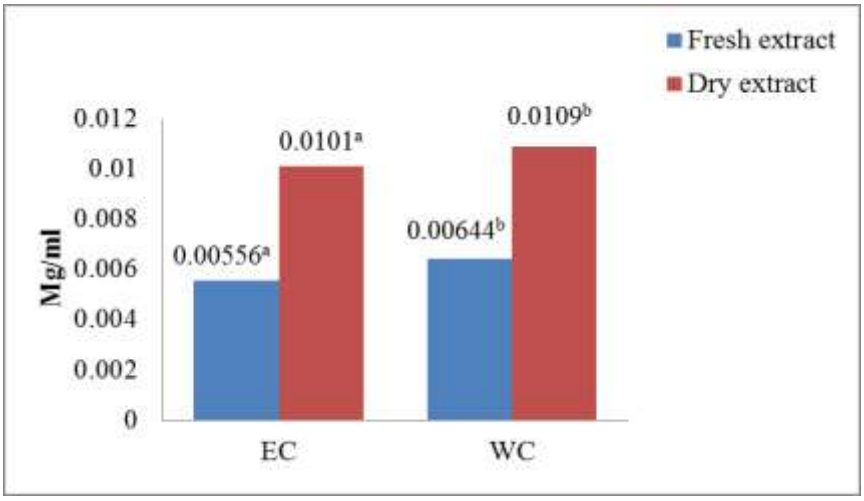

Fig 4.21: Comparative tannin conetents of dichloromethane extracts of *Aloe ferox*.

4.8. Discussion

The variations in phytochemicals contents of *A. ferox* from Eastern Cape and Western Cape *Aloe ferox* are distinct and are possibly caused by environmental factors. There is wide variety of factors which can regulate or affect biogenesis of secondary metabolites in plants, two of these factors are biotic and abiotic effects. Abiotic effects include all physical factors governing habitat, such as light intensity, or UV–vis radiation, water availability (which leads to drought influence), temperature and freezing temperatures, and soil composition or minerals (Goldstein and Brown, 1990). These two areas of South Africa are geographically distinct and experience

unique weather. The Eastern Cape has warm humid climate with most rainfall in summers and the Western Cape has warm, dry, windy summers and wet winters. These different weather conditions could account for the plants to undergo metabolic reactions or processes at different rates. *Aloe ferox* from both areas were harvested from the wild on soils that was not treated with any kind of fertilizer so all the growth conditions were natural.

Biotic effects include sophisticated interactions with plant biochemistry and plant physiology (Briskin, 2000). Synthesis of phenolic derivatives (i.e., flavonols and catechin is upregulated when shoots are overexposed to light which, in leaves results in accumulation of phenolic compounds (Anan and Nakagawa, 1974; Ashihara *et al*., 2008).

Phenols in *Aloe ferox* from both E.C and W.C were high which means the plant received enough light or sunshine from the summers though the E.C have higher phenols high in most extracts. The phenolic content of the E.C aloe could be attributed to the nature of the leaves which are darker in colour than the Aloe from W.C (fig 3.1). In addition, the high phenolic content may be as a response of oxidative pressure produced from excess light energy and as a physiological response to quench reactive chemical species (Close and McArthur, 2002).

Phenolic compounds have antioxidant properties which can protect against degenerative diseases such as cancer, diabetes and cardiovascular disease by scavenging free radicals that are responsible for initiating such diseases (Harborne and Williams, 2000; Rhodes and Price, 1997). Due to their redox properties that make them act as reducing agents the presence of the hydroxyl group in them acts as a hydrogen donor (Oyedemi *et al*., 2010, Ozgen *et al*., 2010). Therefore *Aloe ferox* from both provinces can serve as a good source of phenols. Since the concentration of phenols was more in dry plant than fresh plant of *Aloe ferox* the plant can be a good source of medicine.

The flavonoids and flavanols are high in dry plant extracts and in overall, overally flavonoids were higher in *A. ferox* from E.C. Flavonoids confer UV protection to plant tissues, and their accumulation due to UV-exposure is also well documented. The dried leaves can be a good source of medicine and its dried gel can be used to make cosmetic products that can help cure some skin ailments since the pharmacological effect of flavonoids and flavonols correlates with their antioxidant activities (Shi *et al*., 2006). They also possess biological activities such as anti-

inflammatory, antimicrobial, anti-angionic, analgesic, anti-allergic and cytostatic properties (Hodek *et al*., 2002).

The accumulation of plant flavonoids is enhanced in response to increased light exposure, especially ultraviolet-B rays (Stewart *et al*., 2000) Flavonoids, which are low molecular weight phenolics, are active antioxidants *in vitro* (Nakayama 1994, Rice- Evans e*t al*., 1997, Gardner *et al*., 1998, Sanchezmoreno *et al*., 1998). Similarly, to phenols, the distribution pattern depends on the degree of accessibility to light and previous illumination because formation of the higher oxidized flavonoids is accelerated by light. In leafy vegetables and fruits, flavonols are almost exclusively present as glycosides. Flavonol glycosides are located mainly in the leaves, flowers, and outer parts of plants such as skin and peel and decrease in concentration towards the central core (Ku¨hnau, 1976; Hertog, 1992; Crozier, 1997).

Proanthocyanidins were more in dry leaf extracts for both E.C and W.C. The high concentration in can guarantee *Aloe ferox* as a good source to make medicine for those people who practise licensed ethnopharmacy. The traditional healers have been using *Aloe ferox* to cure sick people decades back. They are a group of condensed flavan-3-ols, such as procyanidins, prodelphinidins and propelargonidins, that can be also be found in many plants, most notably apples, maritime pine bark, cinnamon, aronia fruit, cocoa beans, grape seed and grape skin (*ORAC,* 2007). Common antioxidants currently used are vitamin C and vitamin E and studies show that proanthocyanidins antioxidant capabilities are 20 times more powerful than vitamin C and 50 times more potent than vitamin E (Shi *et al*., 2003). These compounds may have anticarcinogenic, cardiovascular, gastroprotective, anti-ulcerogenic, and cholesterol-lowering properties. They also fight urinary infections (Prior and Gu, 2005; Dykes and Rooney, 2006). Their higher antioxidant capability compared to other phenolic compounds could be due to the fact that they are found to be higher in concentration as discovered in acetone and dichloromethane extracts of this study. High molecular weight condensed and hydrolysable tannins have also been shown to act as strong antioxidants *in vitro*. There are found to be 15–30 times more effective at quenching peroxyl radicals than simple phenolics (Hagerman *et al*., 1998). These tannins are characterised by a high degree of polymerisation and/or many phenolic hydroxyl groups, both of which contribute to antioxidant quenching efficacy (Hodnick *et al*., 1988, Ariga and Hamano, 1990).

Tannins were found to be more in fresh plant extracts like proanthocyanidins in samples from W.C followed by that of E.C *Aloe ferox*. However, hand tannins were the lowest in concentration out of all the phytochemicals studied. Tannins are known for their astringent properties that act on mucuos membranes (Egunyomi *et al*., 2009) and they also have anti-microbial, help to regenerate the skin, anti-inflammatory, diuretic, treat wounds, varicose veins and haemorroids (Okwu, 2004; Nguyi, 1988). *Aloe ferox* can therefore be used to make good cosmetics like skin ointments to help people with skin diseases and wounds because of the tannin content. The amount of tannin extracted depends on the method of preservation and on the maturity of the leaf (Hagerman, 1988). Hagerman states (1988) that tannin can be extracted better with aqueous acetone than aqueous or acidic methanol and extraction and analysis of fresh leaves would minimize changes to the tannins. Hagerman's findings justify why fresh plant extracts had more content of tannins than dried plant extracts and why fresh and dry plant extracts acetone extracts of *Aloe ferox* had more content of tannins. Lindroth *et al.* (2002) found that for condensed tannins, which varied in concentrations was again due primarily to genotype, with less variance attributable to time and nutrient availability. Interestingly, the temporal decline was exhibited only in low-nutrient and not in high-nutrient, trees. Given, that condensed tannins are considered to be 'static' metabolites (Reichardt *et al*., 1991).

The W.C *Aloe ferox* had high content alkaloids compared to E.C. The alkaloid content was found to be highest in its fresh and dry water extracts. The *Aloe* found in this province can serve as a good source of medicine. Eastern Cape *Aloe ferox* also showed a significant amount of alkaloids when the dry plant was extracted in acetone. E.C sample has little alkaloid but it possesses a good amount of other phytochemicals like phenols and proanthocyanidins and saponins. Alkaloids were less than 20 % in all the extracts of samples from both the Eastern Cape and Western Cape. This shows that *Aloe ferox* in general is not rich in alkaloid compounds. Alkaloids are a source of good analgesics (Kam and Liew, 2002) and has potential use in the elimination and reduction of human cancer lines (Nobori *et al*., 1994). It also possess anti-malaria, antiseptic, and bactericidal activities (Madhavi and Salunkhe, 1995). Saponins in samples from for E.C *Aloe ferox* were found to be twice or thrice higher in other extracts compared to W.C and so the traditional healers can depend on it to help fight many ailments. They are an important group of secondary metabolites in plants which on the basis of the aglycon

nature, are divided into two main groups: triterpenoid saponins (based on C30 aglycon) and the steroid saponins (based on C27 aglycon) (Sadeghi *et al*., 2013). The steroid saponins from plants are in turn divided in three groups: cholestane, furostane and spirostane (Challinor and De Voss, 2013; Lacaille-Dubois and Wagner, 2000; Vincken *et al*., 2007). Recently saponins have attracted an increasing attention in the field of medicine because of their anti-inflammatory, antispasmodic, antifungal and antitumor effects (Lanzotti et al., 2013; Jiang and Liu, 2011; Rao and Gurfinkel, 2000).

Drought conditions were discovered to decrease the content of saponins in *Chenopodium quinoa* from 0.46% dry weight (dw) in plants growing under low water deficit conditions to 0.38% in high water deficit plants (Soliz-Guerrero *et al*., 2002).The lower content of saponins samples in W.C could be due to less precipitation or dry summers causing less water in the soil which might reduce their synthesis. Although *Aloe ferox* and *Chenopodium quinoa* are species that can withstand drought conditions, when less water is available in the soil which may reduce the production of saponins significantly.

4.9 Conclusion

The phytochemical contents of Eastern Cape and Western Cape *Aloe ferox* are remarkedly different in most of the extracts. E.C. *Aloe ferox* possesses a higher content of all the analysed phytochemicals except tannins and alkaloids which are higher in W.C *Aloe ferox.* Though less in other phytochemical contents, W.C. *Aloe ferox* also possesses a good quantity of these compounds that could offer many health benefits. This suggests that there are factors responsible for the differences on phytochemical contents between the two areas.

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CHAPTER 5

THIN LAYER CHROMATOGRAPHY PROFILING OF *ALOE FEROX* FROM EASTERN CAPE AND WESTERN CAPE.

CHAPTER 5

THIN LAYER CHROMATOGRAPHY PROFILING OF *Aloe ferox* FROM EASTERN CAPE AND WESTERN CAPE.

Abstract

Aloe ferox samples collected from Eastern Cape and Western Cape were collected and prepared for thin layer chromatography analyses. The plant materials were divided into fresh mass. The dried mass and dry mass which was dried in an oven at 45°C for 48 hours. Three types of solvents were used for extraction namely acetone, dichloromethane and water. The extracts were filtered and concentrated to dryness in a Rotavapor while the water extracts were freeze dried. The final masses recovered or crude yields of the extracts were weighed and calculations to make the stock solution of 10 mg/ml were done. The respective stock solutions were put in labeled bottles and kept to run hexane/acetone/methanol (40:10:4) mls mobile phase. Three silica TLC plates were each spotted with the stock solutions of both the fresh and dry plant extracts from the two areas. One was viewed under normal light, one was sprayed with 1 ml sulphuric acid in a solution of 0.1 g vanillin in 28 ml methanol for color development and the last one was viewed under UV light. The results showed a good separation of compounds in acetone and dichloromethane extracts and it is especially more in dry plant material. The water extracts from the aloe from both areas did not show presence of any compounds. Eastern Cape aloe showed a good presence of phytochemicals than aloe from Western Cape for most of the extracts though dichloromethance solvent showed a good separation for extracts from both areas. Fresh plant material extracts from both areas did not show a good separation like the dry plant material. The results showed the different content of phytochemicals found in *Aloe ferox* from the two provinces which could be due to various environmental factors like climate. The comparative results can be further confirmed by using other advanced techniques like HPLC, HPTLC or GC MS.

5.1. Introduction

5.1.1. Theoretical principle of T.L.C

Thin layer chromatography is an adsorption chromatography (Hahn-Deinstrop, 2000) in which samples are separated based on the interaction between a thin layer of adsorbent and a selected solvent. Thin layer chromatography is mainly used for the separation of low molecular weight compounds. On the basis of experimental technique, it is common practice to distinguish between elution chromatography, displacement chromatography and frontal analysis. TLC belongs to elution chromatography (Stahl, 1965).

The basic procedure of TLC has largely remained unchanged over the last fifty years. It involves the use of a thin, even sorbent layer usually about 0.10 to 0.25 mm thick, applied to a firm backing of glass, aluminium and plastic sheet to act as a support. Of the three, glass has always proved the most popular; although aluminium and plastic offer the advantage that they are flexible and are more easily to cut to any size with minimal disruption to the sorbent layer. Numerous sorbents have been used; some are more successful than others, including silica gels, cellulose, aluminium oxide, polyamides and chemically bonded silica gels.

Chromatography can be considered from two view points. One is diagnostic or qualitative and the other is preparative. The object of the first is to determine the number of components in a system and if possible to learn what they are without isolating them. The second viewpoint involves the separation of a mixture into its components in such a way that reasonable amounts can be isolated and studied. The third aspect is quantitative (analytical) and it concerns about how much of each components is present (Bobbit, 1964).

5.2. Materials and methods

5.2.1. Plant material

Mature leaves of *Aloe ferox* were collected from the field around the University of Fort Hare, Alice Eastern Cape and leaves from the Western Cape were supplied by a company called House of Aloes (Pty) Ltd that also harvests the plant from the wild around Albertinia.

5.2.2. Extraction

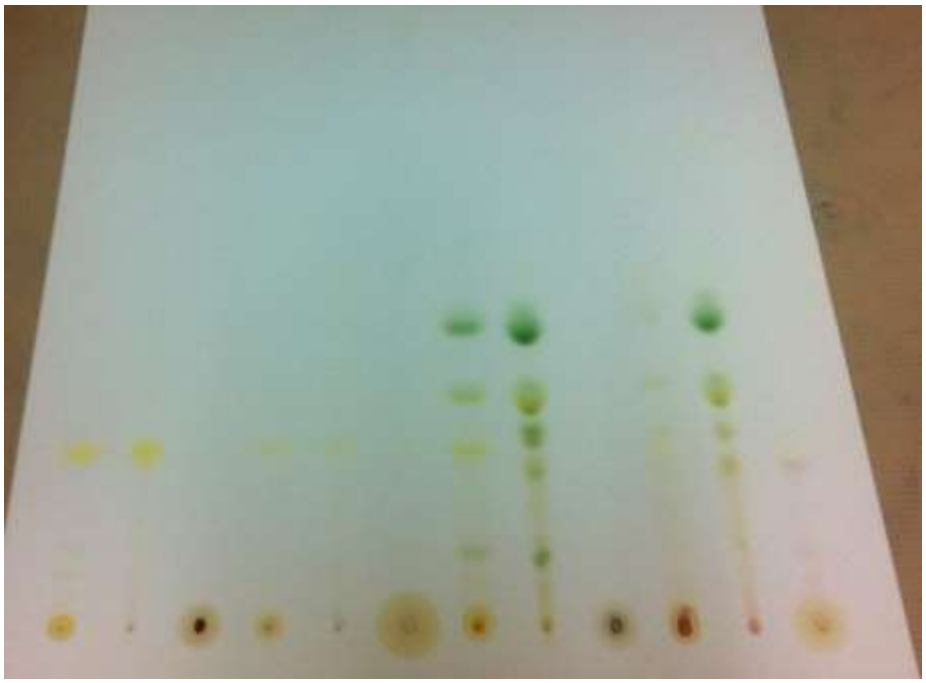
The leaves were first washed in tap water then rinsed with distilled water. Fresh leaves were cut into small pieces and half of the material was oven dried at 45ºC. Fresh pieces of leaves weighing 225 g were soaked in 1025 ml acetone and dichloromethane and 150g of fresh leaves 900 ml distilled water. Dried leaves weighing 80 g were soaked in 800 ml acetone and dichloromethane and 40 g in 700 ml distilled water. All the extracts were allowed to shake on an orbital shaker (Stuart Scientific orbital shaker, UK) for 48 hours at room temperature (23 to 25ºC). Each extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The water extracts were freeze dried using a Vir Tis Benchtop freeze dryer. Extracts of acetone and dichloromethane were concentrated in a rotary evaporator and later reconstituted in their respective solvents of extraction to 10 mg/ml which served as the stock solution.

5.2.3. T.L.C Profiling of the Extracts

The extracts were assayed by thin-layer chromatography (TLC). Macherey-Nagel TLC plates coated with 0.22 mm silica gel 60 with fluorescent indicator UV_{254} were used. The solvent system used was hexane/acetone/methanol (40:10:4 v/v) mls which was prepared on Sigma glass tanks and allowed to dissolve for a few seconds so that a good separation can occur. *Aloe ferox* extracts from Eastern Cape and Western Cape were spotted on a single TLC plate which was divided into two parts. The first part representing fresh whole leaf extracts of the two areas and the second part representing dry leaf extracts of the two areas. The dissolved extracts were spotted at different points on a plate, maintaining the same distance from one edge to another. The marked spots were labelled A, D and W representing acetone, dichloromethane and water respectively. Total of the 12 extracts for both fresh and dry plant material extracts from Eastern Cape and Western Cape were spotted. Aliquots of the extracts were loaded with a syringe on each of the TLC plates. The solvent system was allowed to travel a predetermined distance of 15 cm from the origin. Upon separation of the compounds the TLC plates were air dried in a fume cupboard. Two TLC plates were run on the mobile phase and one of the plates was sprayed with a mixture of 1 ml sulphuric acid in a solution of 0.1 g vanillin in 28 ml methanol for color development. After spraying it was air-dried again whilst the other TLC was visualized under UV light at 360 nm in a dark room for further detection of the chemical compounds.

5.3. Results

5.3.1 Non-sprayed TLC plate



A	D	W	A	D	W	A	D	W	A	D	W
1	2	3	4	5	6	7	8	9	10	11	12
E.C	E.C	E.C	W.C	W.C	W.C	E.C	E.C	E.C	W.C	W.C	W.C

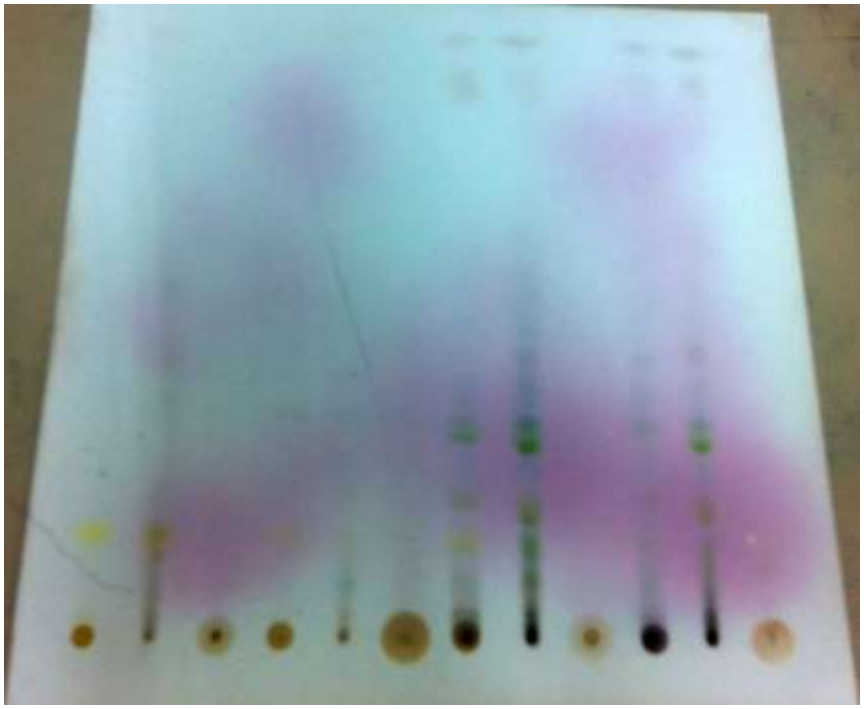
Plate 1: Non-sprayed TLC plate of E.C. and W.C. fresh and dry extracts with 12 spots.

The first 6 spots on the plate from 1-6 represent the fresh extracts of Eastern Cape and Western Cape *Aloe ferox*. The first three on the left are spots of E.C. extracts of acetone, dichloromethane and water of fresh plant material and the other three on the left are spots of W.C. extracts of acetone, dichloromethane and water. The other six spots on the plate from 7-12 represent extracts of dry plant in acetone, dichloromethane and water. The first three (7-9) spots are for E.C aloe and the last three (10-12) represent W.C Aloe.

Western Cape fresh plant in acetone extract represented by no 4 in the figure did not show separation compared to no 1. Extracts of fresh plant material in the non-sprayed TLC plate were not showing a good separation compared to extracts of dry plant. The water extracts of fresh plant material hardly showing any separation even in the TLC plate viewed under UV light with

water extracts of the plant from both areas show no separation at all. Acetone extracts of dry plant material showed a better separation than the fresh plant and no 7 which represents E.C. extract showed a better separation than W.C. extract which is represented by no 10 in all the TLC plates. Dichloromethane extracts of dry plant material which is represented by spot no 8 for E.C. and spot no 11 for W.C. showed a good separation in all the three TLC plates with separation more visible in the sprayed TLC plates. Number 8 had a better separation or variety of isolated compounds to no 11.

5.3.2. Sprayed TLC plate



A	D	W	A	D	W	A	D	W	A	D	W
1	2	3	4	5	6	7	8	9	10	11	12
E.C	E.C	E.C	W.C	W.C	W.C	E.C	E.C	E.C	W.C	W.C	W.C

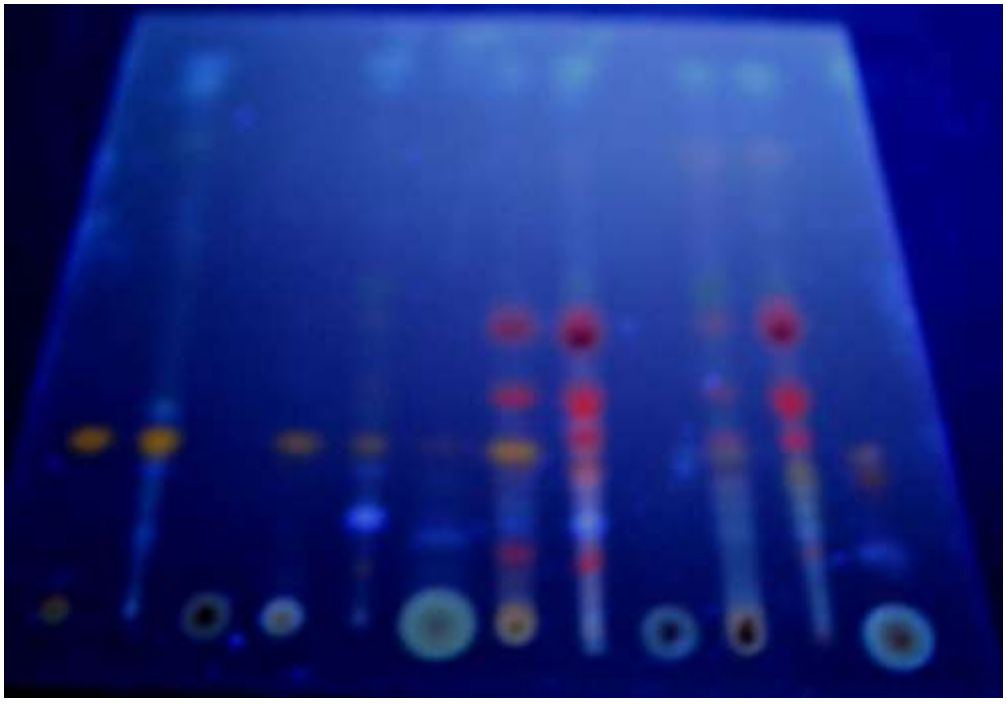
Plate 2: TLC plate of E.C. and W.C. extracts sprayed with 1 ml sulphuric acid in a solution of 0.1 g vanillin in 28 ml methanol solution.

The first 6 spots on the plate represent the fresh extracts of Eastern Cape and Western Cape *Aloe ferox*. The first three (1-3) on the left are spots of E.C. extracts of acetone, dichloromethane and

water of fresh plant material and the other three (4-6) are spots of W.C. extracts of acetone, dichloromethane and water. The other six spots on the plate from 7-12 represent extracts of dry plant in acetone, dichloromethane and water. The first three (7-9) spots are for E.C aloe and the last three (10-12) represent W.C Aloe.

The separation of the compounds in the sprayed TLC plate was showing a well separation of the compounds in the respective solvents but the water extracts of both dry and fresh plant were not showing a well separation as in the non-sprayed TLC plate. For the dry extracts, no 7 which is E.C acetone extract has a better separation than no 10 which for W.C. It was observed that extracts from E.C shows a better separation for the dichloromethane extracts compared extracts from W.C. For the water extracts the separation is quite similar.

5.3.3. TLC plate under UV light



A	D	W	A	D	W	A	D	W	A	D	W
1	2	3	4	5	6	7	8	9	10	11	12
E.C	E.C	E.C	W.C	W.C	W.C	E.C	E.C	E.C	W.C	W.C	W.C

Plate 3: TLC plate E.C and W.C. fresh and dry extracts under UV light (356 nm).

The separation of the compounds of the respective extracts from the three solvents under UV light shows better than the sprayed and the non-sprayed TLC plates (plate 1). For the fresh plant material, acetone extract of E.C showed a better separation than extracts from W.C but the separation on both spots is similar with a yellow compound visible on both spots. Dichloromethane extracts of both areas had a similar separation but W.C extract showed a better presence of the compounds since the visibility is better. The water extracts of the fresh plant material had the least separation compared to the other two solvents with W.C showing a little separation whilst sample from E.C, did not show any separation at all.

For the dry plant material extracts, the separation was much better than the fresh plant extracts. Acetone extracts of E.C and W.C have a similar separation but no 7 was better than no 10. The following colors which represent certain compounds were visible from the bottom, brink red, violet, yellow, reddish color and bluish color at the top of the plate. Dichloromethane dry plant extracts showed a better separation than the acetone extracts as the spots or distinct colors were clearly visible and the E.C extract showed a better separation than the one from W.C. The colors observed at the bottom of the spots were similar to the colors found in the acetone extracts though they appear much better compared to the acetone extracts. The water extract of sample from E.C did not show any separation at all whilst the W.C water extract had a few compounds that are visible at the bottom part of the spot which is violet and orange color. In some of the dry plant extracts, green color which represents the chlorophyll pigment is visible just a little further up the TLC plate i.e in no 8, 10 and 11. Similarly, the bluish color found before at the end of the TLC plate is appearing on almost all the respective extracts except the water extracts. Comparing all the extract spots, the separation rate for the respective extracts from E.C and W.C are similar since the individual colors for each extracts are aligned on the same distance. This means the R_f values for the extracts would be quite similar or almost the same.

5.4. Discussion

Fresh plant extracts of *Aloe ferox* from both areas did not show a good separation of compounds in all the TLC plates but dichloromethane extracts had a better separation than acetone and water extracts. This could be because of the potential of dichloromethane solvent to extract. According to Lowery and Richardson (1977), acetone and dichloromethane are polar aprotic solvents that tend to have a large moment (separation of partial positive and partial negative charges within

the same molecule) and solvate positively charged species via their negative dipole This property of dichloromethane and acetone could account for why they had a better separation of compounds than water. Acetone solvent has a good separation of compounds as shown in dry plant material which could be because of its higher dielectric constant than water which makes it able to break bonds between molecules better than water. Acetone is a polar aprotic solvent which can separate partial positive and negative charges as mentioned above but dichloromethane was better in separation than acetone in all the extracts. Water solvent have the lowest results in separation. From the work done by Loots *et al*. (2007) on phytochemical content, antioxidant capacity and possible health benefits of *Aloe ferox* the results showed that dry plant mass of ethanol leaf gel extracts and lyophilized *A. ferox* gel have a more content of total polyphenols, total flavonoids and total non-flavonoids than the fresh or wet plant mass. The polyphenols were found to be higher in dry plant material than flavonoids and non-flavonoids. Loots *et al*. (2007) also states that they have antioxidant properties and play a role in the prevention of various diseases associated with oxidative stress such as cancer, cardiovascular disease, neurodegeneration (Scalbert and Williamson, 2000), and diabetes (Vogler and Ernst, 1999). Polyphenols constitute a large class of molecules containing a number of phenolic hydroxyl groups attached to ring structures allowing for their antioxidant activities. These compounds are multifunctional and can act as reducing agents, hydrogen-donating antioxidants, and singlet oxygen quenchers (Rice-Evans, 2004). Zhimin and Howard (2012), state that less hydrophilic flavonoids can be resolved in silica TLC plates and a good approximation of flavonoid structure can be obtained by the relative mobility, appearance of spots under UV light and use of various reagents (Rowland et al., 1895). Other solvent systems such as 15 % acetic acid/water (15:85) and TBA (t-BuOH) /acetic acid/water (3:1:1) can be routinely used for the preliminary analyses of flavonids (Rowland *et al*., 1985). The chromatographic plates were viewed at 366 nm and the spots identified after spraying with diphelyboric acid-ethanolamine complex in methanol, dried and viewed under UV light showed most flavonoids but significantly 3’4’-dihydroxyflavones or flavonols are orange and the 4’-hydroxy equivalents are yellow-green. This work done by Rowland (1985) and the results obtained serve as proof that the colors of spots (orange, red or brink red, yellow and violet) which showed well on the TLC plate viewed under UV light in acetone and dichloromethane extracts on spots no 7 and 8 which represent Eastern Cape *A. ferox* represent a separation or isolation of different classes of flavonoids. From

the book written by Jayapraksha *et al*. (1998). anthocyanins can be well separated in cellulose TLC with (HCL/formic acid/water) and the intensely colored spots (violet for the trihydroxylated, brink red for dihydroxy and orange for the mono) do not even require spraying and then R_f increases as the degree of glycosylation increases (Andersen and Francis, 1985). The mobile phase (hexane/acetone/methanol) (40:4:5) used in this experiment was able to separate the different compounds found in *Aloe ferox* extracts from the two areas. This work that was done by Andersen and Francis also gives evidence to the presence of the above group of flavonoids or anthocyanins present in the extracts of *Aloe ferox* from the Eastern Cape and Western Cape though the quantity in content of the flavonoids is not the same as proved in the phytochemical experiments and also the separation or visibility of these respective spots on the TLC plates between the two areas as viewed under UV light was not the same.

It is possible that the results of this phytochemical screening were affected by the fact that the *Aloe ferox* grows in these two areas which have different environmental factors as mentioned in the phytochemical analyses. Brokini and Ayodele (2012) stated that environmental factors have been identified as responsible for changes and determination of the secondary metabolites in a plant (Waterman and Mole, 1989). Similarly, Bhakta and Ganjewala (2009) reported ontogenic variation in secondary metabolites such as phenolics, anthocyanins and proanthocyanidins in *Lantana camara.* Also Fonseca *et al*. (2006) confirmed the fluctuation of secondary metabolite contents in medicinal plants with changing environment. The bluish pigment visible on most of the extracts except in water extracts should be a certain compound found in *Aloe ferox* in which its presence is not likely to be affected by any environmental factors mentioned here.

5.5 Conclusion

The Thin layer chromatographic profiles of *Aloe ferox* from the Eastern Cape and Western Cape exhibited a variation in the presence of phytochemical compounds extracted with three types of solvents and the results serve as proof that Aloe plant growing in E.C. and W.C. did not have the same quantity or quality of phytochemical compounds in them. Since several authors have reported how different climatic conditions could affect the phytochemical contents of plants and as stated also in the problem statement, it is quite apparent that TLC results prove that the Eastern Cape and Western Cape experience unique or different type of climate that can affect the growth and distribution of plants found in each area.

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CHAPTER 6

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

CHAPTER 6

6.1. General discussion

The results obtained on the study revealed of *Aloe ferox* growing in E.C. and W.C. showed that there are variations in its phytochemicals. The presence of these phytochemicals supported the curative claim of number of diseases like diabetes, inflammation and cancer cured by *Aloe ferox*. The second chapter which is literature review of *Aloe ferox* shows that a lot of work has been done to study the growth, habitat, ecology, chemical properties, its economic potential within South Africa and internationally and most importantly its importance as a medicinal plant. Its value as a medicinal plant is very high and important to the economy of South Africa as it has been stated that it has a wide market in other countries outside Africa and it is exported it to places like America and Asia brings millions of rands into the market exchange business and has played a huge role in developing the economy of this country. It has also developed the lives of many people who work as Aloe tappers or harvesters for business sectors manufacture *Aloe ferox* products such as cosmetics, food or medicine, also in the agricultural sector that uses the leaves to treat cattle infections. Tappers who harvest and sell the leaves and leaf exudate in crude form to other local people or business people generate income that helps them sustain their families. The *Aloe ferox* industry started in the Western Cape but had broadened a lot in the Eastern Cape Province. *Aloe ferox* was first exported to Europe in 1761 and today is considered South Africa's main wild-harvested commercially traded species (Chen *et al*., 2012). From the traditional use, it has been reported that the plant has possible side effects that can be toxic if over dosage occurs or use by pregnant or under age people. It's famous traditional uses include use as laxative (Crouch *et al*., 2006). The leaf is traditionally used for skin and hair treatments (Watt and Breyer-Brandwijk, 1962).Cut leaves are applied directly to burns, insect bites, sores, and sunburn (Crouch *et al*., 2006). Leaf and stem decoctions are also used as emetics, whilst leaves and roots are boiled in water and taken for hypertension and stress (Pujol, 1990). Traditional preparations are also used for arthritis, conjunctivitis, toothaches, sinusitis, and stomach pains (Crouch and Symmonds, 2006).

The results show the presence of phenols, flavonoids and flavanols. Flavonoids appeared higher in dried *Aloe ferox* extracts and so it can still be of good use as a medicine even if used in fresh form. Alkaloids, saponins, proanthocyanidins and tannins were also found to be more in dry plant extracts. Proanthocyaanidins were the highest in content out of all the other phytochemicals

and this validates the use of *Aloe ferox* from both provinces as a good source of antioxidants. Antioxidants have several medicinal values as discussed in chapter three and four.

6.2. General conclusion and recommendations.

The TLC results confirmed of the phytochemical results as they also showed that dry plants extracts have a variety of phytochemical compounds as showing in the TLC plates especially the one viewed under UV light. This opens for questions as to why dry plant extracts have more phytochemicals extracted more than fresh plant. This observation supported the results of the work done by Loots *et al*. (2007). The studies by Wintola and Afolayan (2011) and Loots *et al*. (2007) credit *Aloe ferox* as a succulent plant that has potent antioxidant properties that are useful in the fighting of radical related diseases like diabetes and thereby give credence to its traditional usage in South Africa and other places.

A more detailed and comprehensive comparative study of this plant can further be done up to the genetic level of the plant growing in the two areas. The results of this study supported the use of *Aloe ferox* for traditional medicine in the two provinces. Finally not much work has been done on the medicinal properties of *Aloe ferox* and its activities using critical analysis like in *Aloe vera*, this calls for medicinal plant researchers to do further studies on this plant so as to elucidate its medicinal properties like many other medicinal plants found in literature.

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Appendices

APPENDIX 1: LIST OF ABBREVIATIONS

$AlCl_3$: Aluminium chloride

ANOVA: Analysis of variance

AOAC: Association of analytical communities

DCM: Dichloromethane

EC: Eastern Cape

GCMS: Gass chromatography mass spectroscopy

HCL: Hydrochloric acid

HPLC: High pressure liquid chromatography

$Na_2CO_{3:}$ Sodium carbonate

NH_4OH: Ammonium hydroxide

SD: Standard deviation

TLC: Thin layer chromatography

WHO: World Health Organisation

WC: Western Cape

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