REGENERATION POTENTIAL OF SELECTED MEDICINAL PLANTS USED TO TREAT HUMAN AND LIVESTOCK DISEASES IN LIMPOPO PROVINCE OF SOUTH AFRICA

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DECLARATION

I, Ojelade Babatunde Solomon declare that this dissertation, for the degree of Masters of Science in Agriculture (Plant production) has never been submitted for any degree at this or any other university. The research work reported is the result of my own original investigation, except where acknowledged and referenced accordingly.

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DEDICATION

This dissertation is dedicated to my lovely amazing wife, Ojelade Oluwatoyin Bukola and my dear son, Ojelade Tumininu Israel for their patience, endless love, support and encouragement during the challenges of life to work hard for the things that I aspire to achieve.

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ABSTRACT

Many high valued tree species of medicinal significance in the Limpopo Province, South Africa exhibit seed dormancy, and also contain aromatic oils which inhibit rooting of their stem cuttings. These plant species are under pressure due to human over-exploitation. The main objective of this study was to investigate effects of rooting hormones on the rooting ability that will help in domesticating some of the selected high valued medicinal plants, *Elaeodendron transvaalense* (bushveld saffron), *Brackenridgea zanguebarica* (yellow peeling plane), and *Warburgia salutaris* (pepper-bark tree). Stem cuttings of these plant species were prepared and treated with various concentrations (500, 1000 and 2000 ppm) of IBA, IAA and NAA in different growth media (Natural soil, farm soil and hygromix) at a nursery house. 180 experimental units were sown and arranged in Randomized Complete Block Design (RCBD), each treatment replicated five times and then monitored for a period of three months. Data were only obtained from *Brackenridgea zanguebarica* as other species dried up two weeks after sprouting. The two variables measured from the experiments were sprouted stems and number of leaves. The data obtained were subjected to analysis of variance and least significant difference (LSD) at 5% probability level was used to compare treatment using STATISTICA software analysis package. The hormone, hormone concentration, growth media and their interactions had effect on sprouted stems and number of leaves produced on *Brackenridgea zanguebarica* cuttings, with no record of rooting ability. IBA (500 ppm and 1000 ppm) and control (without rooting hormone) showed high significant results with natural soil and farm soil in terms of leaf production compared to the hygromix, which is significantly lower from others. IBA at the various concentrations (500, 1000 and 2000 ppm) and the control gave the highest percentage sprouted stem on both natural soil and farm soil as compared with other hormones at the same concentrations on hygromix. Due to non-rooting of the stem cuttings in the current study, further research needs to be conducted for successful rooting of the vegetative part.

Keywords: Medicinal plants, Over-exploitation, Rooting, Growth hormones, Growth media, Vegetative propagation.

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LIST OF ABBREVIATIONS

ANOVA: Analysis of Variance

RCBD: Randomized Complete Block Design

IBA: Indole-3 Butyric Acid

IAA: Indole Acetic Acid

NAA: Naphthalene acetic acid

ppm: parts per million

FS: Farm soil

HM: Hygromix

NS: Natural soil

BNR: Brackenridge Nature Reserve

LSD: Least Significant Difference

SANBI: South African National Biodiversity Institute

IUCN: International Union for Conservation of Nature

LEDET: Limpopo Department of Economic Development, Environment and Tourism

WHO: World Health Organization

ºC: Degree centigrade

%: Per cent

CHAPTER 1: INTRODUCTION

1.1 Background Information

Medicinal plants have important contributions in the primary healthcare system of rural communities (Abbink, 1995). These medicinal plants are used for nutritional purposes, but also have medicinal, ritual or magical values in the eyes of the rural people (Abbink, 1995). Most developing countries have acknowledged the continuous use of traditional medicines, and the need for innovative drug development using medicinal plants (Light *et al.* 2005). Southern Africa boasts of an amazing floral diversity, of which many are endemic to the region (Goldblatt, 1978). Apart from the great range of climatic zone and different types of habitat, South Africa has great cultural diversity (Light *et al.* 2005). Traditional herbal medicine still forms the backbone of rural healthcare in South Africa, despite the provision of western medical care by government health services (McGaw *et al.* 2005). These traditional medicines are customary in regions where western medicines are inaccessible due to unavailability and the comparably high cost (McGaw *et al.* 2005). Mostly, the cultural importance of traditional medicines makes the demand for these herbal remedies quite high (McGaw *et al*. 2005).

In South Africa, a large number of plant species (> 3000 species) are used for medicinal purposes (Dladla, 2001). Among these, over 700 species are traded in large quantities at informal medicinal plant markets (Mander, 1997). It is a “hidden economy’’ which can improve the growth of the country (Williams, 1996; Mander, 1998; McGaw *et al*. 2005). About 27 million South Africans depend on traditional medicine for their primary healthcare needs, and for income generation (Mander *et al.* 2007). Between 35 000 and 70 000 tons of plant parts (roots, stem, bark etc)

were consumed annually in South Africa, and over 700 000 tons of plants materials were illegally harvested from the wild (Spring, 2004).

However, there has been a lack of understanding in terms of the value of domesticating medicinal plants in South Africa (Mander *et al.* 1998). Small-scale farmers, mainly from rural communal lands have not historically focused on farming medicinal plants (Mander *et al.* 1998), which is likely to lead to the extinction of many valued plant species in their natural environments. For example, 86% of the plant parts harvested resulted into death of the entire plants, which has significant implications for the sustainability of supply (Mander, 1998). Supply of these high valued plants is becoming increasingly scarce and inaccessible to the medicinal plant markets due to extinction, and yet very little is being done to cultivate these plants (Mander *et al.* 1998; Tshisikhawe *et al.* 2012). Conservation and documentation of the traditional medicine and/or knowledge of existing medicinal plant species for curing human and animal diseases need to be given a high priority before they are completely eliminated from the wild due to over-exploitation (Joshi *et al*. 2003). Because of the great increase in over-exploitation of these wild species, many agencies have recommended that wild species should be brought into the cultivation system (World Health Organization, 1993; Lambert *et al*. 1997; BAH 2004). Involvement of harvesters and harvesting communities of these medicinal plants in Limpopo Province, in the development of propagation (cultivation) and management methods may kindle their interest in protecting the wild populations from over-exploitation.

There are a number of threatened plant species, such as *Elaeodendron transvaalense* (bushveld saffron), *Brackenridgea zanguebarica* (yellow peeling plane) and *Warburgia salutaris* (pepper-bark tree) that have been over-exploited for traditional medicinal purposes in South Africa, and are commonly traded in the muthi markets around Venda (Raimondo *et al.* 2009, Tshisikhawe, 2002). These species are facing a serious threat of extinction as a result of overharvesting. Bark and rootbark collections from the wild have been the major methods of harvesting, and these practices result in damage and/or death of many trees (Tshisikhawe, 2002; Tshisikhawe *et al.* 2012). Regeneration of these popular medicinal plant species have been adversely affected by seed coat dormancy which results in poor imbibitions and germination. A serious constraint in the nursery is the delay or failure of seed to germinate. Vegetative propagation by cuttings is also difficult due to the presence of aromatic oils, which inhibit rooting. Attempts to domesticate these high valued medicinal plants have yielded little or no positive results.

Therefore, there is urgent need to develop and implement regeneration/cultivation strategies for these exploited medicinal plant species, to ease pressure on their natural habitats, as well as increase the demand for these plant species. The common means of regeneration and propagation of these plants include seed-based and macro-propagation methods. All this knowledge can be articulated in forest conservation and management plans. Regeneration studies need to be carried out to determine the best ecological requirements for the optimum germination of these plant species. This knowledge is needed to help in overcoming the plants' physiological problems, and then encourage local people to domesticate these highly

valued medicinal plants, with a view of arresting the steady disappearance of the plant species from the wild.

For the purpose of this study, selected medicinal plants of the Vhembe District in Limpopo Province, which are highly exploited and threatened by over-harvesting and bad harvesting methods, were considered. Cultivation of these medicinal plant species through vegetative methods might be the best and/or the only solution to restore back these over-exploited plants.

1.2 Objectives of the Study

1.2.1 Main Objective

The main objective of this study was to generate knowledge that will help in domesticating, through vegetative methods, selected high valued medicinal plants of the Vhembe District in the Limpopo Province of South Africa.

1.2.2 Hypotheses

i. Null: Applications of plant hormones will have effect on growth and rooting behavior of the stem cuttings

ii. Alternative: Applications of plant hormones will not have effect on growth and rooting behavior of the stem cuttings

iii. Null: The growth media will have effect on the growth and sprouting behaviour of the stem cuttings

iv. Alternative: The growth media will not have effect on the growth and sprouting behavior of the stem cuttings

1.2.3 Specific Objectives

1. To assess the effect of different hormones on the growth and rooting ability of stem cuttings for each selected plant species.
2. To investigate the effect of different growth media on growth and rooting ability of stem cuttings of the three selected medicinal plant species.

1.3 Structure of the Dissertation

The dissertation starts with background information and study objectives in Chapter 1, and the literature review in chapter 2. Chapter 3 covers both the methodology. Chapter 4 reports on the effects of hormones, hormone concentrations and growth media on rooting performance of stem cuttings of the threatened medicinal plants species. Discussions are reported in chapter 5, while conclusions and recommendation in chapter 6, followed by collation of all the references cited in the dissertation.

CHAPTER 2: LITERATURE REVIEW

2.1 Importance of medicinal plants for curing human and animal diseases

The oldest friends of mankind and livestock are plants because they do not only provide food and shelter but also play a central role in preventing and curing different ailments (Tipu *et al*. 2006). The World Health Organization reported that about 80% of the world population use medicinal plants for curing many diseases (WHO, 2001). People in the developing world rely on medicinal plants as a component of their healthcare (Balick and Cox, 1996). In South Africa, it is estimated that 70-80% of the population consult traditional healers for their healthcare (Kasilo, 2000; UNAIDS, 2006). Also, livestock farmers, due to limited access to modern disease prevention and treatment practices, frequently depend on traditional knowledge for the management of animal health problems, and to improve their productivity (Farooq *et al*. 2008). Such practices and remedies, based on oral transmission from one generation to another, are claimed to be effective (Mathius-Mundy and McCorkle, 1989; McCorkle *et al*. 1996).

According to Kar *et al.* (2004), many plant products possess analgesic and antipyretic properties. They are used as digestive stimulants, anti-diarrheic, antiseptic, anti-inflammatory, anti-parasitic and appetite stimulants in humans and domesticated animals (Charis, 2000). In ethnoveterinary medicine, there seems to be a range of plants or plant extracts suitable for treating almost every parasitic disease of livestock (International Institute of Rural Reconstruction, 1994). For example, seeds of garlic, onion and mint have been used to treat animals that suffer from gastro-intestinal parasitism, whereas extracts from tobacco plants have been used to treat the skin of livestock afflicted with external parasites (Guarrera, 1999).

Tipu *et al*. (2002) concluded that neem fruit in broiler feed had remarkable efficiency in fighting against coccidiosis as compared to salinomycin sodium. Studies (such as Samresh *et al*. 2003) on mouse indicate that the presence of flavonoids in ocimum leaf extracts has a preventive effect on cancer, coronary heart disease and strokes.

Based on the effective use of these medicinal plant species, plant-people relationship needs to be managed for the ultimate sustainable utilization, especially around Limpopo Province, where high valued medicinal plant species are facing a serious threat of extinction.

2.2 Medicinal plants of Limpopo Province: Conservation status and trade

Previous studies on medicinal plants of Limpopo Province indicate that plant materials are still extensively used for medicinal purposes, and will continue to be used considerably in the foreseeable future (Rankoana, 2001; Tshisikhawe, 2002; Tshisikhawe *et al.* 2012). However, harvesting methods (e.g. bark harvest and harvesting entire plants) usually result in detrimental effects on the plants, and depletion of many such plant species (Tshisikhawe, 2002; Tshisikhawe *et al.* 2012). Plant parts that are most sensitive to harvest are the ones that are thus exploited, and the trade of indigenous medicinal plants in the Venda region of the Limpopo Province, is posing a threat to the conservation and preservation of many plant species (Tshisikhawe, 2002). Intense and frequent harvesting of these medicinal plant materials (roots and bark) in the Venda region is a cause for concern.

The Limpopo Province is regarded as one of the poorest provinces in South Africa (Mathebula *et al.* 2017). Unemployment and poverty are thought to be among the

driving forces for informal trade in medicinal plants. This trade plays a critical role in empowering some rural dwellers in the province. It is a lucrative activity which provides considerably good returns (Loundou, 2008). Therefore, initiation of proper plant regeneration programme/project could reduce the over-utilization of medicinal plants, and help in increasing household incomes, encourage sustainable utilization, as well as promote efficient use of medicinal plant materials.

2.3 Endangered medicinal plant species of Limpopo Province

There are a number of endangered medicinal plant species in the Limpopo Province; (*Siphonochilus aethiopicus*, *Warburgia salutaris*, *Securidaca longipedunculata*, *Elaeodendron transvaalense*, *Brackenridgea zanguebarica* etc.) *Siphonochilus aethiopicus* (wild ginger) is in danger of extinction due to over-exploitation despite being afforded legal protection (Crouch *et al*. 2000; SANBI, 2007). Zschocke *et al*. (2000) reported that the main problem faced by this plant species is the destructive harvesting of its underground parts. *Siphonochilus aethiopicus* is already extinct from its natural habitat of KwaZulu-Natal and if care is not taken, this may also happen in the Limpopo Province (Williams *et al*. 2001). *Warburgia salutaris* (Common name: pepper-bark tree, *Mulanga* in Venda) has been listed as an endangered plant species under SANBI Red Data Book due to economic harvesting from Southern African forests (SANBI, 2007; Loundou, 2008). *Encephalartos transvenosus* (Common name: Palm-like cycad, *Tshifhanga* or *Tshitondolo* in Venda) is also listed under the Red Data Book (Victor, 2002, CITES appendices (CITES, 2007). This plant species is unsustainably removed from the wild by collectors, and has resulted in the loss of habitat in some places (SANBI, 2007). Species that were found most frequent in the *"muthi"* trade markets of Venda region, include *Kirkia wilmsii*

(common name: wide pepper tree) and *Securidaca longipendiculata* (Common name: Violet tree, *Mpesu* in Venda), which are considered to be slow-growers (van Wyk *et al*. 1997). *Securidaca longipedunculata* was reported to have a high occurrence in *muthi* shops in Thohoyandou (Tshisikhawe, 2002). Despite legislative protection, increase in utilization of these medicinal plant species threatened their long term sustainability due to harvesting of its roots (Ndou, 2006).

Elaeodendron transvaalense, *Brackenridgea zanguebarica* and *Warburgia salutaris* are some of the commonly traded medicinal plant species in Venda *muthi* shops, which are also facing a serious threat of extinction in the Venda region (Tshisikhawe, 2002; Williams *et al.* 2007). Bark from the stem and the roots of these plant species are preferred and harvested for medicinal purposes. Study shows that the most vulnerable species are the popular and high conservation priority species, slow-growing and slow-reproducing plant species, with specific ecological requirements and limited distribution (Cunningham, 1992). Therefore, cultivation to serve as an alternative means of supply of these medicinal plant species is needed. Cultivation may relieve pressure on the rare and slow-growing species, which can also serve as an important production strategy to support conservation (Cunningham, 1993).

2.4 Biology and ecology of medicinal plant species

Low germination rates and/or specific ecological requirements as well as lack of knowledge about medicinal plant species are major constraints to their cultivation (Vines, 2004). According to Li *et al.* (2000), low germination rate frequently results from fungal infection or mechanical damage of seeds. This can be improved by seed treatments (e.g. soaking), and by ensuring optimum storage conditions (Li *et al*.

2000). In addition, the use of controlled environment may enhance cultivation of these medicinal plants that are difficult to grow on a commercial scale (Canter *et al.* 2005).

Vegetative propagation has been an essential component in most tree improvement programmes by ensuring quick genetic gains through mass multiplication of selected genotypes (Zobel and Talbert 1984; Park *et al.* 1989; Surendran *et al.* 2003). Vegetative propagation is very useful in producing true to type plants, with shorter juvenile period leading to early productivity (Raju and Rao, 2006) and offers a unique opportunity of avoiding the problem of recalcitrant seeds (Gbadamosi and Oni, 2005). Therefore, it is necessary to investigate the optimum ecological requirements with best propagation method for the regeneration of high priority medicinal plant species that were selected for this study. This information is crucial for the recovery of these plant populations and hence sustainable supply of the plant species.

2.5 Significance of seed germplasm

Seeds remain the important starting materials for the propagation of many vital tree species (Akinnifesi e*t al.* 2007). The emergence of the embryo from the seed (seed germination) and the germination process is initiated by a variety of anabolic and catalytic activities (Bewley and Black, 1983). Several reports classify seeds into different groups based on their germination potential and storage behaviour – recalcitrant (intolerant to desiccation), intermediate and orthodox (tolerate desiccation and exhibit dormancy) (Roberts, 1973; Berjak and Pammenter, 2004). Recalcitrant seeds are produced from many tropical and subtropical tree species (Berjak and Pammenter, 2004), but there is still limited knowledge on their germination. Recalcitrant seeds contain high moisture content, ranging between 30%

and 70% at maturity, and germinate rapidly when sown fresh, but are sensitive to desiccation and freezing. This makes them difficult to store and some seeds perish at 26% moisture content (Berjak and Pammenter, 2004; McDonald, 2004). Regardless of the seed class, seed dormancy is still an important factor that affects germination of each group at any stage (Mng'omba *et al.* 2007).

Seed dormancy is referred to as the failure of viable seeds to germinate when conditions are favourable for seed germination. It is more prevalent amongst the orthodox seeds than recalcitrant seeds (Mng'omba *et al.* 2007). The presence of inhibitors in the endosperm can cause chemical dormancy (Hilhorst *et al.* 2006).

Fire plays a major role in regeneration of many plant species (Baskin and Baskin, 1998). Chemical components, such as ethylene, ammonia and nitrogen oxides, and ash, which are known to stimulate seed germination, are released during burning (van Staden *et al.* 2000). However, fire can also cause high seed mortality (Banda *et al.* 2006). Various seed pre-treatment methods, such as water, acid, filing and abrasion are used experimentally to scarify hard seed coats and promote seed germination of selected tree species (Fang *et al.* 2006; Travlos *et al.* 2007; Mng'omba *et al.* 2007). Levels and methods of scarification vary among plant species, and hence must be determined for each species.

Currently, there is hardly any information on successful propagation of indigenous medicinal plant species of Limpopo Province, South Africa. Therefore, this study calls for the investigation to derive best propagation methods for each selected plant species.

2.6 Vegetative propagation

Vegetative propagation techniques are increasingly being applied for the domestication of tropical tree species (Leakey *et al.* 1990) and have been considered as irreplaceable tools for tree domestication and breeding (Aparicio *et al.* 2009). The advantages and implications of vegetative propagation have been widely reported in literature. It represents the first step in the process of domestication of a tree species by offering a unique opportunity of avoiding the problem of recalcitrant seeds predominant in tropical tree species (Leakey and Simmons, 1998). Vegetative propagation facilitates the transfer of genetic potential and non-additive variance of the parent to the new plant (Puri and Khara, 1992). It is also useful in producing true to type plants with shorter juvenile period leading to early productivity (Raju and Rao, 2006). Considerable attention has been given to tree propagation through vegetative means. This technique is emerging as a strong alternative to seed propagation method, and is employed for operational planting in tree species (Buijtenen *et al.* 1975; Armson *et al.* 1980).

Plant cuttings is one of the most extensively used means of vegetative propagation of plants with various advantages such as being economical, and not requiring much space. Cuttings can be made from stem, modified stem, roots or leaves. The most popular of these cuttings is stem cutting which has been used widely to propagate a variety of plants. Stem cuttings can be taken from shoots of the plants with terminal or lateral buds that can develop adventitious roots and then to a complete plant (Hartmann *et al.* 1997). Successful rooting of the cuttings is dependent on factors such as position of the cuttings on the shoots, rooting media, presence and absence of hormone and concentration, as well as environmental factors (Wilson, 1993).

The use of cuttings as propagators in vegetative propagation have shown that the percentage rooting is highly influenced by rooting media and the hormone concentrations (Tchoundjeu *et al.* 2002). Major roles of the auxins include cell elongation, bud formation and root initiation, as well as production of other hormones (Akinyele, 2010; Kumari *et al.* 2010). Auxins have been reported to increase the number of roots per cutting in different plant species (Aminah *et al.* 1995), but very high dosage could result in death of the cutting (de Klerk *et al.* 1997). Therefore, optimum concentration and appropriate sowing media for each selected plant species need to be determined. This is critical for mass propagation and successful integration of the plant species into agro-forestry.

2.7 Rooting hormones and medium

A number of studies have demonstrated the important role that rooting hormones and rooting medium play in the rooting of stem cuttings (Leakey, 1990; Ofori *et al.* 1996).

2.7.1 Types of rooting hormones and their relative importance

In the rooting process of cuttings, rooting hormones are very important (Wiessman-Ben and Tchoundjeu, 2000). Hormones, such as indole butyric acid (IBA), indole acetic acid (IAA) and naphthalene acetic acid (NAA) play an important role in root growth, while hormones like gibberellins are important in stem elongation and bud development of the plants (Wiessman-Ben and Tchoundjeu, 2000). IBA and NAA are two synthetic chemicals that have been found to be reliable in promotion of rooting in cuttings. For general use, the IBA is widely applied, because it can remain non-toxic

within a wide range of concentrations and improves root initiation of cuttings for most plant species (Al-Barazi and Schwabe, 1982; Hartmann *et al.* 1997). However, IBA is toxic to certain cuttings taken from softwood plant species; it causes poor growth, no growth or mortality of the cuttings (Hartmann *et al.* 1997).

Nonetheless, appropriate use is essential because incorrect concentration levels can inhibit rooting, or act as a growth retardant in higher concentrations (Hartmann *et al.* 1990; Wiessman-Ben and Tchoundjeu, 2000). High concentration of auxin antagonizes the endogenous auxin of a plant due to increase of endogenous auxin in the cuttings that led to a level in which the hormonal metabolism and inhibited rizogenesis might have been disturbed (Lebrun *et al.* 1998). So, cuttings of different plant species require different optimum concentration of auxin for best rooting.

Despite the apparent importance of auxins, there have been reports on successful rooting of cutting of several plant species without the application of auxin. Such plant species include*, Nauclea diderrichii* and *Vochsia hondurensis* (Leakey, 1990), *Shorea macrophlla* (Lo, 1985) and *Milicia excelsa* (Ofori *et al.* 1996), which are well supplied with endogenous auxin (Ofori *et al.* 1996).Combinations of different rooting hormones are also reported to be efficient (Smalley *et al.* 1991; Puri and Vermat, 1996).

2.7.2 Rooting medium

There is no ideal or universal rooting medium for cuttings (Hartmann *et al.* 1997), but no propagation method will work if wrong medium of growth is used (Berhe and Negash, 1998). A medium is suitable for propagation based on species, cutting type,

season, propagation system used etc. (Hartmann *et al.* 1990). A perfect medium must be able to provide the cuttings with good aeration, moisture, drainage, physical support, nutrients and be free of disease causing pathogens (Hartmann *et al.* 1990).

It is very important to have a balance between water and air content of the medium, even though the water uptake of cuttings is directly related to the water content of the medium (Grange and Loach, 1983; Loach 1992).Temperature of the rooting medium also influences the rooting of cuttings (Hartmann *et al.* 1997). For example, cuttings of *Syzygium paniculatum* grown on a non-heated substrate (at average temperature of 16ºC) failed to root, whereas when the substrate was heated (at average temperature of 22±0.5ºC), the mean rooting rate exceeded 75% of the best substrates (Lebrun *et al.* 1998).

2.8 Descriptions of selected endangered medicinal plants for propagation

Medicinal plants identified as most used and listed as threatened by SANBI and IUCN were selected and considered for this study. These are:

i. *Elaeodendron transvaalense* (bushveld saffron): *Mukuvhazwivhi* (Venda).
ii. *Brackenridgea zanguebarica* (yellow peeling plane): *Mutavhatsindi* (Venda).
iii. *Warburgia salutaris* (pepper-bark tree): *Mulanga* (Venda).

2.8.1 *Elaeodendron transvaalense,* properties and medicinal use

This is one of the popular medicinal plant species that is facing a serious threat of extinction due to over-exploitation; it is mostly traded in *muthi* markets around Venda (Tshisikhawe and Rooyen, 2013). The species has been classified as vulnerable

(VU) on the IUCN Red Data List categories in South Africa (Raimondo *et al.* 2009). *Elaeodendron transvaalense* does not grow easily from cuttings, also its seed coat needs to be broken before sowing, or else it may take up to a year to germinate (Dlamini, 2007).

Elaeodendron transvaalense is used for a variety of diseases and is being referred to as "*Mukuvhazwivhi*" i.e. "sin-washer" by traditional healers. Its medicinal use includes treatment of stomach disorder, ulcers, venereal diseases (STIs), fungal infections, piles and haemorrhoids in humans and domestic animals, and dysmenorrheal (Mabogo, 1990; van Wyk *et al.* 1997; Tshisikhawe, 2002; Steenkamp, 2003; Bessong *et al.* 2005). Root and stem bark of *E. transvaalense* is mostly used to prepare decoctions and infusions, which can be taken orally 3-4 times a day (Mabogo, 1990; Tshikalange *et al.* 2008). Traditional healers also prescribe it for people suffering from HIV/AIDS (Bessong *et al*. 2005).

Other species belonging to the same family (Celastraceae) as *E. transvaalense* have also been used in the Amazonian region against cancer, rheumatism and inflammation (Nakagawa *et al.* 2004). Species of this family contain biological active metabolites with antimicrobial and cytotoxic activities (Sansores-Peraza *et al.* 2000). Tshikalange and Hussein (2010) concluded that *E. transvaalense* extract, and isolated compounds, contain low toxicity, which support the traditional use of the plant in the treatment of various ailments.

2.8.2 *Brackenridgea zanguebarica* taxonomy, distribution range and medicinal use

The genus *Brackenridgea* belongs to the family Ochnaceae and consists of approximately twelve (12) species distributed from Africa to the Fiji Islands (Issa *et al.* 2013). *Brackenridgea zanguebarica,* the only member of the *Brackenridgea* genus that occurs in South Africa, is a tree species of high medicinal value. Its distribution is restricted and limited, and can only be found in Venda region at Thengwe (a subpopulation in South Africa), and has been classified as a Critically Endangered (CR) plant species according to SANBI Red List categories due to uncontrolled bark harvesting (Raimondo *et al.* 2009). *Brackenridgea zanguebarica* has been in use for many years by the Venda people of South Africa, especially for ritual and medicinal purposes, which has been recorded by some researchers (Todd *et al.* 2004; Tshisikhawe and van Rooyen, 2013). These medicinal and ritual uses include; protection against witchcraft, treatment against wounds, worms, amenorrhea, swollen ankles and aching hands. Moller *et al.* (2006) reported that *B. zanguebarica* has a wide range of biological activity against eukaryotic cells, bacteria and viruses.

Bark of *B. zanguebarica* has an outer grey and inner orange part, which are frequently used as a yellow dye for mats. The powdered yellow bark, as well as its yellow-coloured powdered roots is used for the treatment of wounds (Kokwaro, 1976). Decoction of the bark is applied as eyewash for the treatment of conjunctivitis, and the boiled bark is used in preparations of porridge against jaundice (Neuwinger, 2000). According to Chhabara *et al*. (1990), the plant species is effective in the treatment of anaemia in East Africa. A Combination of the root powder and other

herbal drugs is also well known for the treatment of swollen ankles, amenorrhea, mental illness, and against worms (Arnold and Gulumian, 1984).

There are secondary plant metabolites which have been determined with various phenolic substances in the plant species. These substances include brackenin (Drewes, 1983), Calodenin B (Drewes, 1983; 1987), isochamaejasmin (Drewes, 1987), a derivative of isoswertisin and different flavonoids (Bombardelli *et al.* 1974). Moller *et al*. (2006) determined antimicrobial, antiviral, anti-tumour and antibacterial activities of the *B. zanguebarica* extracts, with their ability to inhibit cell growth in different human cell lines. Also, Moller *et al.* (2006) reported that bark extract of *B. zanguebarica* has high antibacterial activity against Gram-positive strains, when compared with the antibacterial activity of some other extracts of European medicinal plants, used for the treatment and/or prevention of skin lesions (Weseler *et al.* 2002; Weseler, 2004). The *B. zanguebarica* bark extract is effective in low dosages against Gram-positive cocci (Moller *et al.* 2006). Therefore, for further use in modern phytotherapy of skin lesions and disorders, bark extract (or bark powder) application can provide a promising perspective. So, this plant species of high medicinal values needs to be cultivated for sustainable supplies.

Survival of *B. zanguebarica* is attributed to the cultural beliefs of the VhaVenda people during collection (Tshisikhawe and van Rooyen, 2012). This medicinal plant species is found within the Vhatavhatsindi clan, the sole custodian of the plant species, who believe that it can only be collected by a dedicated person from the clan (Tshisikhawe and van Rooyen, 2012). And the collection can only be done by a naked person during the dark (Mabogo, 1990), that an unauthorized collector can

become sterile by touching the plant (Tshisikhawe and van Rooyen, 2012). These beliefs are still adhered to by people from the area, but illegal collections are common. Large amounts of this medicinal plant material are still exploited by traditional healers and medicinal plant traders for the purpose of making money (Tshisikhawe and van Rooyen, 2012). Accessibility to Brackenridge Nature Reserve (BNR), Thengwe was previously controlled solely by the local tribal authority (Tshisikhawe and van Rooyen, 2012), but now in conjunction with Limpopo Department of Economic Development, Environment and Tourism (LEDET). Despite this, there is a still high level of illegal harvesting from the Brackenridge Nature Reserve. Sample pictures of bark harvest taken during visit to the reserve is as shown in Figure 1

2.8.2.3 Ring-barking of *Brackenridgea zanguebarica*

Figure 1: Showing bark harvest of *Brackenridgea zanguebarica*

Increase in demand of this medicinal plant species, together with the effect of over-harvesting and harvesting methods call for proper regeneration protocol which is required in order to reduce its harvesting pressure.

2.8.3 *Warburgia salutaris,* medicinal use and conservation status

Warburgia salutaris belongs to the family Canellaceae. It is an important, over-exploited African medicinal plant species, which is highly threatened with extinction in the wild. *Warburgia salutaris* is listed as an endangered species on both IUCN and SANBI Red Data List (Raimondo *et al.* 2009; IUCN, 2012). *Warburgia salutaris* is very hard to find in the wild, and is on the verge of becoming extinct (Xaba and McVay, 2010). The threats to this plant species include over-harvesting, indigenous timber clearing, human living requirements and poor germination of seed (Xaba and McVay, 2010).

Warburgia salutaris is one of the most sought-after medicinal plants in the South African traditional health care sector (Williams, 1996; Mander, 1997). The stem-bark of *W. salutaris* is generally considered as a solution or remedy for various kinds of human diseases (Gelfand *et al.* 1985). It is highly valued for the treatment of headaches, inflammation of the gums and throat infections, venereal diseases, abdominal pains, rheumatism, chest complaints, malarial fevers, skin diseases and stomach ulcers (Gelfand *et al.* 1985; Jansen and Mendes, 1990; Hutchings and van Staden, 1994). The bark can be taken orally with cold water or smoked for the treatment of common cold and other chest complaints (Watt and Breyer-Brandwijk, 1962). The root-bark of the plant is also reported as a cold remedy and for the treatment of mouth sores and angina (Jansen and Mendes, 1990). Among other

diseases in East Africa, there has been a documentation of the use of the *W. salutaris* leaves and bark for the treatment of malaria (Kokwaro, 1976).

Due to extensive utilization of *W. salutaris* throughout southern and East Africa, there is a severe decline in number of the plant species, even within protected reserves (Scott-Shaw *et al.* 1998). Global conservation status assessment listed *W. salutaris* as a taxon in danger of extinction (Scott-Shaw *et al.* 1998).

2.9 Effects of bark harvesting

There is a variable effect on the misuse of medicinal plant species depending on parts harvested. It is more damaging in terms of survival when bark or roots are harvested (Davenport and Ndangalasi, 2002; Geldenhuys, 2004; Vermeulen, 2006). The term 'bark' is generally considered to include all tissues outside the vascular cambium, regardless of their composition (Junikka, 1994). Complexity of the bark tissues is the mixture of dead tissue, the rhytidome (the dead outer part of the bark) that serves as a physical barrier and protects tree against herbivore, insects, fire and fungi attack, and live tissues, the phloem (the inner bark, also called non-collapsed secondary phloem) which contains open and non-collapsed sieve elements, where elaborated sap is transported from leaves to roots (Trockenbrodt, 1990). When there is a simple wound on the bark of a tree, physiological functioning of the tree and continuous development of new vascular tissues can be disrupted (Aloni, 1987).

Throughout Africa, few studies have assessed the ability of bark to regenerate following harvesting. In Cameroon, it was indicated that *P. Africana* achieves complete bark re-growth after ring-barking (Cunningham and Mbenkum, 1993)

whereas, in Nigeria, Fasola and Egunyomi (2005) indicated that *Adansonia digitata, Gliricidia sepium, Newbouldia laevis* and *Theobroma cacao* have relatively slow re-growth. In South Africa, *P. africana, Ocotea bullata* and *W. salutaris* show good re-growth; whereas the bark of *Rapanea melanophloeos* shows no re-growth (Vermeulen, 2006; Geldenhuys *et al.* 2007). These results show clearly that the ability of a tree bark to regenerate after harvesting is species-specific. Indeed, It was observed at BNR that *B. zanguebarica* also re-grow its bark after harvesting but physiological effects and to what extent this re-growth can occur need to be examined to promote sustainable management of the plant species.

Medicinal plants form the basis of medicines used by most people in developing countries (Pandey and Singh, 2016). Therefore, the loss of these medicinal plant species with a reduced supply would have a direct impact on human health and wellbeing (Bodeker, 1995). Intense removal of the bark around the tree stem usually results into death of the trees, thereafter the species become rare over time. Therefore, due to the continuous high demand of these medicinal plant species, it is important to develop a sustainable way of propagating the tree species for continuous future use and supplies. To promote sustainable use of this medicinal plant species through improved harvesting techniques, cultivation and monitoring of the plant species call for an urgent need for developing appropriate conservation strategies.

CHAPTER 3: METHODOLOGY

3.1 Experimental site

This study was conducted at the University of Venda, Thohoyandou, Limpopo Province, South Africa. The Venda region is located in the northern part of the Limpopo Province (23° 45' - 25° 15' S; 29° 50' - 31° 30' E) within the Soutpansberg region and is an area that is characterized by its great floristic diversity (van Wyk and Smith, 2001). The climate of the region is favourable for many South African tree species (Mostert *et al*. 2008). In Thohoyandou, average daily temperature ranges between 25°C and 40°C in summer and between 22°C and 26°C in winter. The area has a wet and hot summer with a mean temperature of 30°C, and a dry and cool winter with a mean temperature of 18°C (Luseba and Van der Merwe, 2006). Rainfall is highly seasonal with 95% occurring between October and March (M'marete, 2003), often with a mid-season dry spell during critical periods of growth (FAO, 2009).

The area is characterized by two vegetation types within the savanna biome; mixed Lowveld Bushveld and Mopani Bushveld (Low and Rebelo, 1998). They harbor conditions suitable for many plant species e.g. Thohoyandou Botanical Garden where Warburgia salutaris was collected. The Vhembe District is comprised of three ethnic groups: Vhavenda, Pedi and Tsonga, with Vhavenda people being the main ethnic group, followed by Tsonga and lastly Pedi. These ethnic groups form an integral part of the traditional medicine practice in the Vhembe District.

3.2 Collection of vegetative materials for each selected plant species

Stem cuttings of about 3-5 cm in length with 2-3 nodes for each selected medicinal plant species were collected from the mother plant of each plant species in their natural stands. *Brackenridgea zanguebarica* was collected at Brackenridge Nature Reserve, Thengwe, *Warburgia salutaris* was collected at Thohoyandou Botanical Garden, Thohoyandou while the *Elaeodendron transvaalense* was collected at Njelele village, all at Vhembe District, Limpopo Province of South Africa. Before collection of the vegetative materials (stem cuttings), proper preparations of the hormones concentration for immediate handling of the materials on return to the University of Venda were made. The stem cuttings were sprayed with water and stored in moist polythene bags while transporting to the study site.

3.3 Experimental design

This was a factorial experiment consisting of three factors at different levels shown in the Table 1. Different rooting hormones, indole-acetic acid (IAA), indole-butyric acid (IBA) and naphthalene acetic acid (NAA) at different concentrations (500 ppm, 1000 ppm and 2000 ppm) as well as different growth media (farm soil, natural soil and hygromix) were used to assess the rooting ability of the cuttings.

Table 1: Experimental design for the study

	Factors	Levels
1	Rooting Hormones	3 levels: (IBA, IAA, NAA)
2	Concentration	4 levels: 500 ppm, 1000 ppm, 2000 ppm and 0 ppm (control)
3	Growth media	3 levels: Farm soil, Natural soil and Hygromix

Randomized Complete Block Design (RCBD) was used in the design of the experiment to investigate the effects of the different rooting hormones across different concentrations and growth media on the growth and rooting ability of stem cuttings for each selected medicinal plant species.

One hundred and eighty stem cuttings of about 3-5 cm in length for each of the selected plant species were collected and 135 of the cuttings were treated using three different rooting hormones (IBA, IAA and NAA) at 45 stem cuttings for each rooting hormone and the remaining 45 cuttings served as control. Each of the 45 stem cuttings for different hormones were divided into three for the different concentrations as given above to give 15 stem cuttings per rooting hormones, which were then sub divided across different concentration for each rooting hormone to give5 replications per each treatment. One hundred and eighty planting bags, filled with different growth media were used for the planting of the treated cuttings, and sub-divided based on the different concentration as mentioned above.

Quick dip method was used whereby 2.5 cm of the basal end of the stem cuttings, cut below a bud and a slanting cut above a bud on the apical end of each cutting, were immersed in the solutions for 10 seconds (Gbadamosi and Oni, 2005). Each cutting was then planted in the planting bags filled with different growth media immediately.

The 180 experimental units sown were then arranged in a Randomized Completely Block Design (RCBD), and then monitored for a period of three months.

The different stages of the experiment are shown in figure 2.

Figure 2:Pictures of different stages of the experiment *(A – Vegetative parts collection; B – measurement and preparation of the hormone concentrations; C – Cutting and application of the hormones on the vegetative parts; D – Planting of the cuttings; E – Sample emergence on hygromix; F – Sample emergence on farm soil; G – Display of the experiment*).

3.4 Data collection

Due to non-rooting of the plant cuttings, the two parameters observed for this experiment were sprouted stems and number ofleaves produced per cutting and were collected at the end of the experiment (after 3 months). This was determined by counting the sprouted stem and leaves produced per experimental unit. Survival percentage of the cuttings was determined by the expression below, given by Djavanshir and Pourbeik (1976);

$$\text{Survival percentage} = \frac{\text{Number of sprouted stem}}{\text{Total number of planted stems}} \times 100.$$

3.5 Data analysis

The data obtained from the present investigation were subjected to analysis of variance (ANOVA). The ANOVA on the effect of each treatment was carried out on the data collected for the different parameters, and least significant difference (LSD) at 5% probability level was used to compare the significantly different means using STATISTICA Software package. Survival percentage was calculated for those successfully sprouted cuttings after the three months of initial planting.

CHAPTER 4: EXPERIMENTAL RESULTS

4.1 Effect of growth hormones and growth media on leaf production of *B. zanguebarica* cuttings

The analysis of variance of the effect of hormone, hormone concentration and growth media on *Brackenridgea zanguebarica* cuttings is shown in Table 2. Hormone, hormone concentration, growth media and the interaction of hormone and growth media had highly significant effect (P<0.05) on number of leaves produced from the *B. zanguebarica* cuttings.

Table 2 ANOVA of the effect of hormones, hormone concentration and growth media on number of leaves of *B. zanguebarica* cuttings.

Treatments	DF	MS	F	P
Hormone (H)	2	307.617	12.6476	0.000009**
Concentration (C)	3	572.348	23.5319	0.000000**
Growth Media (GM)	2	429.217	17.6471	0.000000**
H x C	6	42.320	1.7400	0.115792
H x GM	4	91.733	3.7716	0.006000*
C x GM	6	21.698	0.8921	0.502481
H x C x GM	12	20.504	0.8430	0.606188
Error	144	24.322		

The superscripts indicate significant difference, DF = Degree of freedom; MS = Mean square.

The effect of different hormones on number of leaves of *B. zanguebarica* cuttings is shown in Figure 3. Application of IBA to *B. zanguebarica* cuttings led to highest number of leaves when compared to IAA and NAA at the end of the experiment. However, there was no significant difference in the number of leaves produced between IAA and NAA.

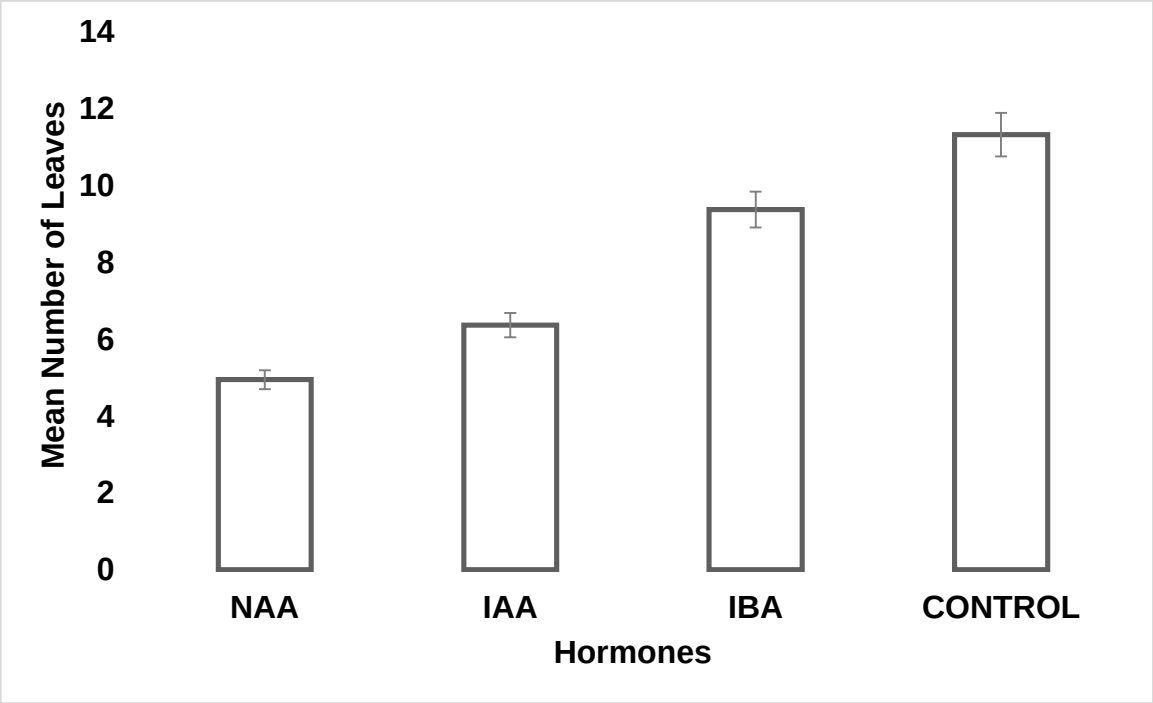


Figure 3: Effect of different hormones on leaf growth of *B. zanguebarica* stem cuttings

Figure 4 shows the effect of hormone concentration on leaf number. The number of leaves produced from the stem cuttings of *B. zanguebarica* decreased with an increase in hormone concentration. Significant difference was observed in the number of leaves produced by the control and concentrations used in the study.

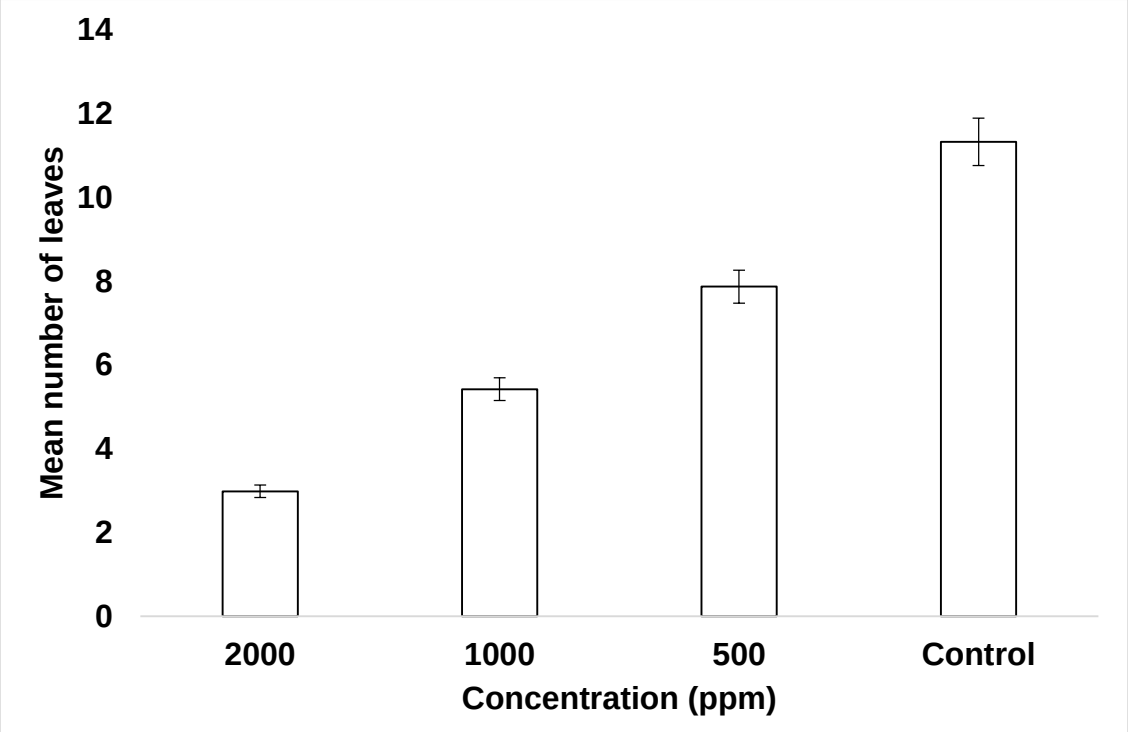


Figure 4: Effect of hormones concentration on leaf growth of *B. zanguebarica* stem cuttings

The growth media affected the number of leaves produced by *B. zanguebarica* stem cuttings (Figure 5). Natural and farm soil produced significantly greater number of leaves compared with hygromix.

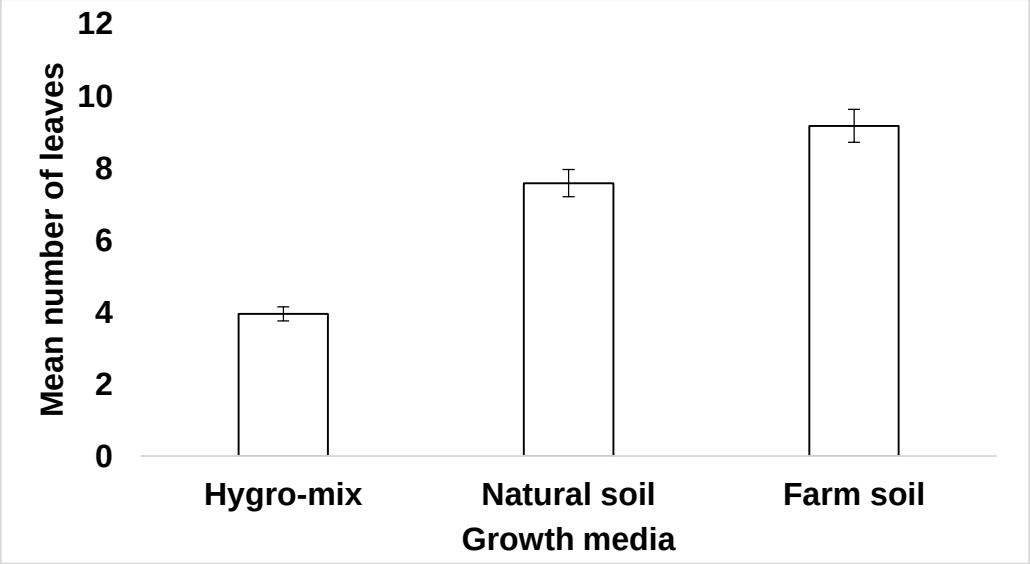
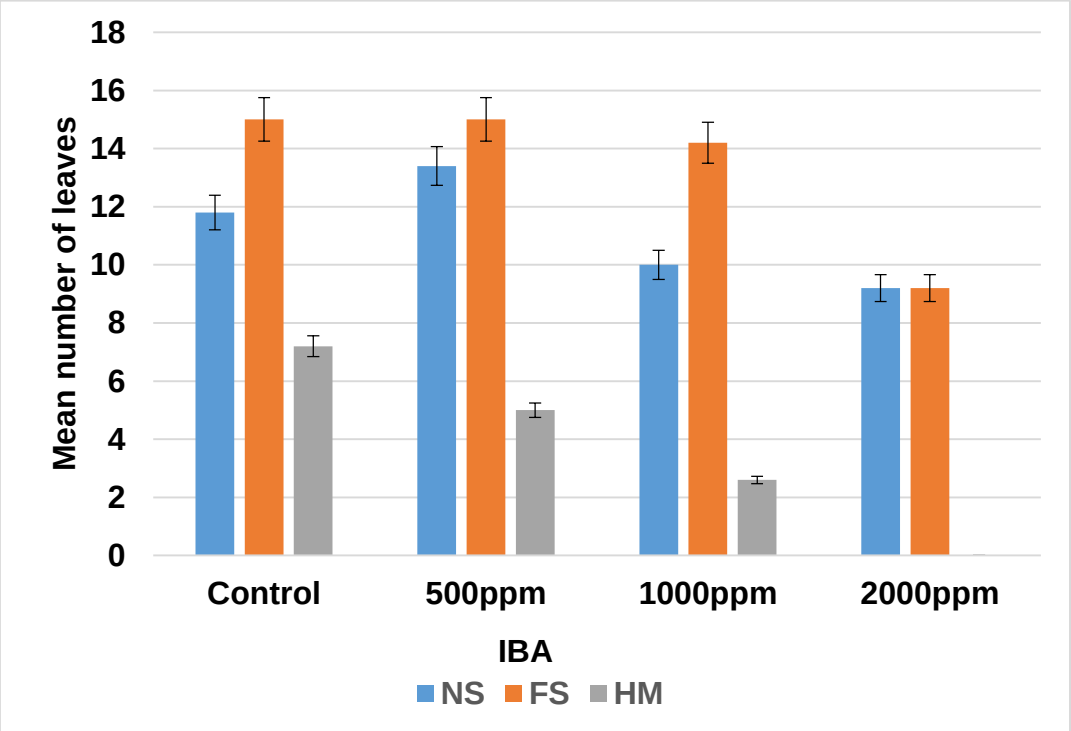


Figure 5: Effect of different hormones on leaf growth of *B. zanguebarica* stem cuttings

The responses of the number of leaves of *B. zanguebarica* stem cuttings to different hormones and growth media were shown in Figures 6-8 below.

Figure 6 shows the effect of IBA concentrations and growth media with regards to leaf number of *B. zanguebarica* cuttings. The applications of IBA at concentrations 500 ppm and 1000 ppm on natural soil and farm soil performed better and were not significantly different from control (i.e. without IBA), whereas when IBA 2000 ppm applied on natural and farm soil led to a significantly lower number of leaves compared to the control.

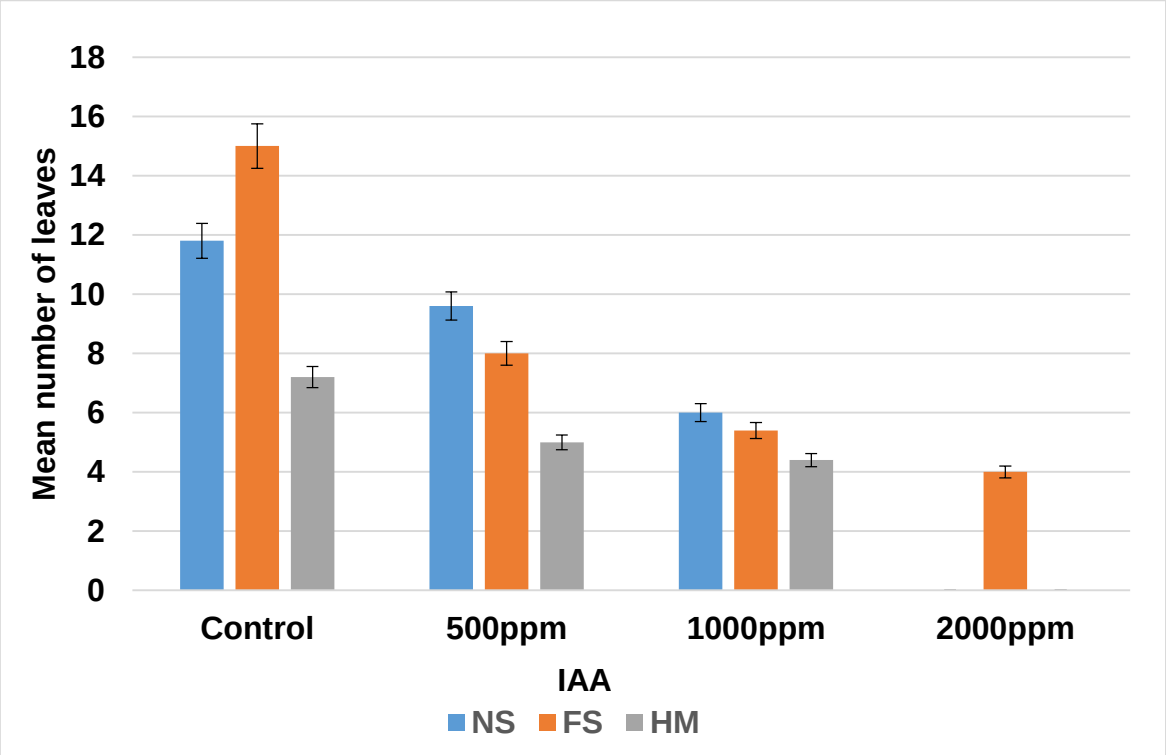
Furthermore, the figure shows that the applications of IBA 500 ppm and IBA 1000 ppm on hygromix gave the lowest number of leaves, and were significantly different from the control in terms of leaf production.



NS = Natural soil, FS = Farm soil, HM= Hygromix

Figure 6: Effect of various IBA concentrations and growth media on leaf production of *B. zanguebarica* cuttings.

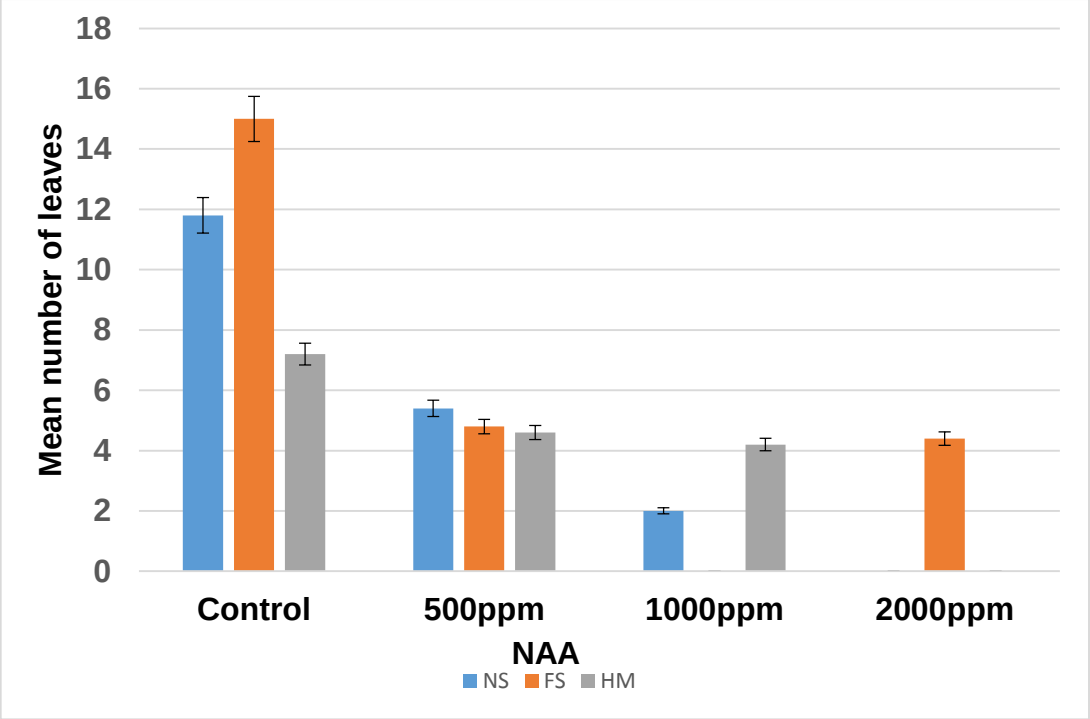
The effect of IAA concentrations and different growth media with regards to leaf production is shown in Figure 7. The applications of IAA 500 ppm, 1000 ppm and 2000 ppm on natural soil, farm soil and hygromix produced a low number of leaves as compared to the control.



NS = Natural soil, FS = Farm soil, HM= Hygromix

Figure 7: Effect of various IAA concentrations and growth media on leaf production of *B. zanguebarica* cuttings.

The effect of NAA concentrations and different growth media on leaf production is shown in Figure 8. The applications of NAA 500 ppm, 1000 ppm and 2000 ppm on natural soil, farm soil and hygromix produced a low number of leaves compared to the control.



NS = Natural soil, FS = Farm soil, HM= Hygromix

Figure 8: Effect of various NAA concentrations and growth media on leaf production of *B. zanguebarica* cuttings.

Interaction effects of hormones and growth media on stem cuttings of *B. zanguebarica* with reference to leaf production is shown in figure 9. This results showed that cuttings planted on both farm soil only and natural soil only are significantly greater than the cuttings planted on hygromix only. The interactions of IBA + farm soil and IBA + natural soil gave higher number of leaf production as compared to IBA + Hygromix. The Interactions of IAA and NAA on each different growth media are not significantly different from cuttings planted on hygromix only in respect of leaf production.

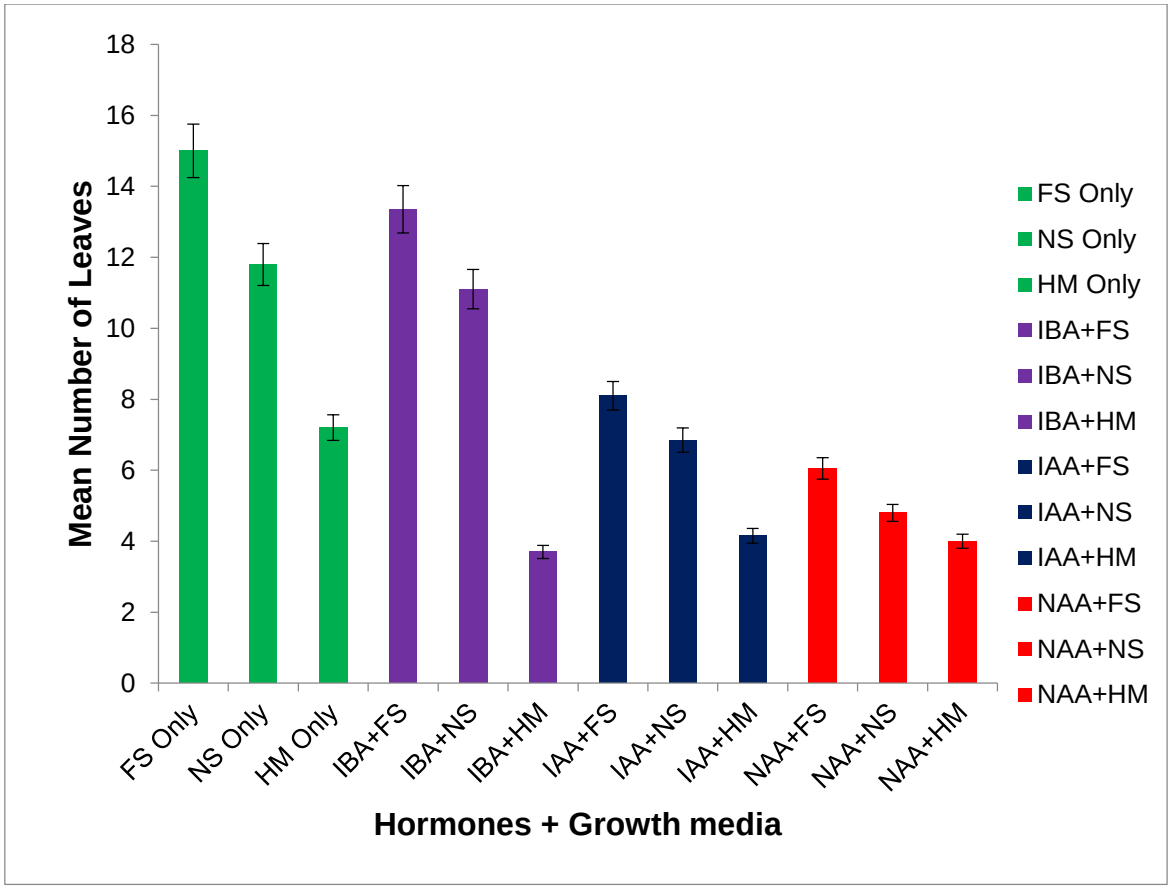


Figure 9: Effect of hormones and growth media interactions on leaf production of *B. zanguebarica* stem cuttings

4.2 Effect of growth hormones and growth media on sprouted *B. zanguebarica* cuttings

The effect of hormone and growth media on percentage sprouted stems of *Brackenridgea zanguebarica* cuttings is shown in Table 3. Hormone and growth media had a significant effect (P<0.05) on sprouted stems of the *B. zanguebarica* cuttings.

Table 3 ANOVA of the effect of hormones and growth media on sprouted stem of *B. zanguebarica* cuttings

Treatments	DF	MS	F	P
Growth media	2	1268.69	11.0623	0.009710*
Hormones	3	1931.26	16.8396	0.002511*
Error	6	114.69		

The superscript (*) indicates significant difference (P<0.05); DF = Degree of freedom; MS = Mean square.

In Figure 10, It can be observed that sprouted stem from *B. zanguebarica* cuttings planted on farm soil (64.97%) and natural soil (63.30%) are significantly greater than cuttings planted on hygromix (33.33%).

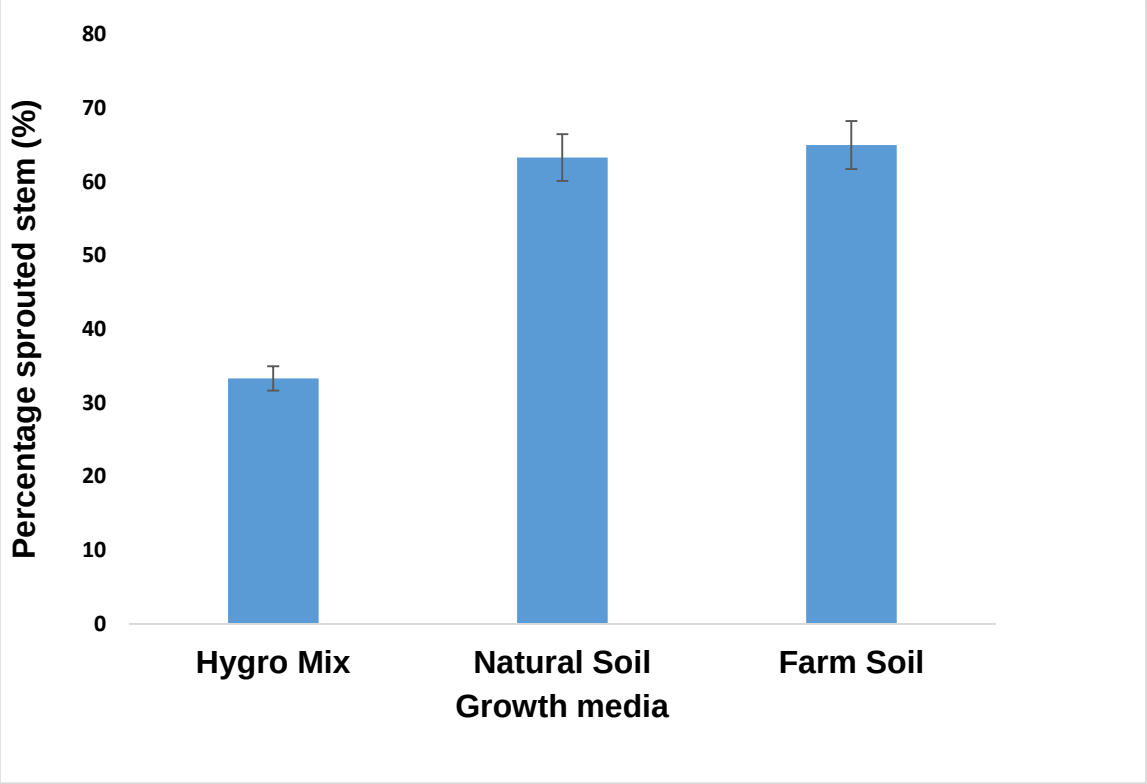


Figure 10 Effect of different growth media on percentage sprouted stems of *B. zanguebarica* cuttings

Effect on *B. zanguebarica* cuttings subjected under different growth hormones is shown in figure 11. IBA (86.7%) gave the highest mean percentage sprouted stem, followed by IAA (59.9%) and NAA (39.9%) as compared to control (28.9%).

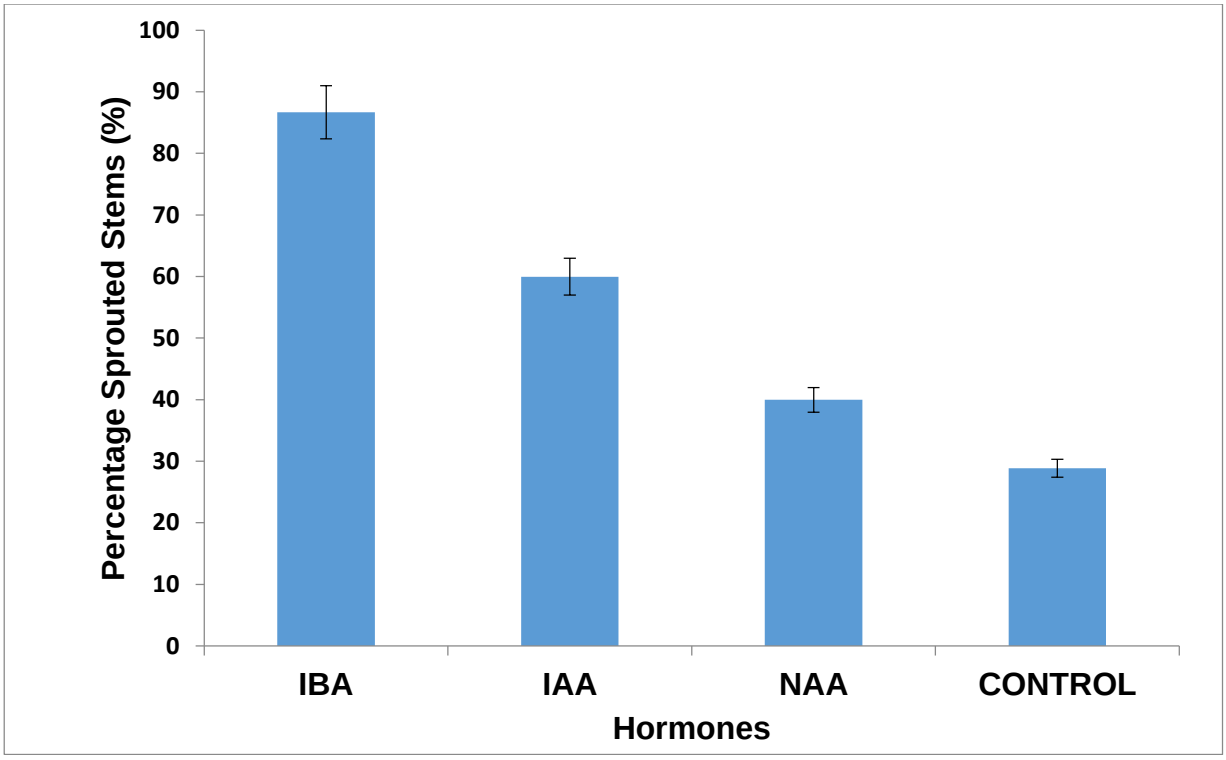


Figure 11: Effect of different hormones on percentage sprouted stems of *B. zanguebarica* cuttings

CHAPTER 5: DISCUSSION

The findings of this present study showed that hormone, hormone concentration, growth media and the interaction of hormone and growth media had significant effect on number of leaves produced from the *B. zanguebarica* cuttings, while only growth media and growth hormones have significant effects on number of sprouted stems.

Cuttings with no hormonal application (control) gave the highest number of leaf production, and are not significantly different from IBA application. The higher the hormone concentration, the lower the number of leaves of *B. zanguebarica* cuttings.

In respect of the leaf production, the cuttings performed well on both farm soil and natural soil only compared to hygromix only. Combinations of growth hormones with growth media decreases the number of leaf production compared with growth media alone. The best combinations were observed using IBA + farm soil and IBA + natural soil.

IBA application to *B. zanguebarica* cuttings gave the highest number of sprouted stem compared with the control which gave the lowest sprouted stem of all the treatments.

Non-rooting of the cuttings on both treatments and control, was observed. Though, reports showed that auxins (IBA, IAA and NAA) were found to be reliable in root promotion as well as improving the root initiation of cuttings of most plant species (Al-Barazi and Schwabe, 1982; Hartmann *et al.* 1997) but in this experiment, the growth hormone regulators did not have any effect on the *Brackenridgea zanguebarica* cuttings with respect to root development. Possibly, endogenous auxin in the cuttings might have led to a level in which hormonal metabolism and root

development are disturbed and inhibited (Lebrun *et al.* 1998). According to Leakey (2004), rooting was described as a complex developmental process that can be affected by internal and external factors. So, physiological conditions of the planting stocks (Mitchell *et al.* 2004) and environmental conditions (Ragonezi *et al.* 2010; Repáč *et al.* 2011) might significantly affect the efficiency of the *B. zanguebarica* cuttings from rooting. Seasonal changes in temperature, rainfall, humidity, and photoperiod might have effect on the growth media; thereby inhibit the rooting ability of the *B. zanguebarica* cuttings.

The results of the current study are comparable to those of Lalit (2014) who reported that cutting of *Acacia catechu* also sprouted profusely after planting and the emerged sprouts (leaves) fell off within 5- 6 months after emergence, probably because stored carbohydrate in the cuttings to enhance the continuous growth of the emerged leaves are quickly used up.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

This study investigated the effect of hormones and growth media on growth and rooting behaviour of *B. zanguebarica* stem cuttings. Hormone, hormone concentrations, growth media and their interactions had significant effect on sprouted stems and number of leaves produced from the *B. zanguebarica* cuttings. Increase in the hormone concentrations reduced the number of leaves produced by *B. zanguebarica* stem cuttings. The *B. zanguebarica* cuttings were observed to perform better on both farm soil and natural soil as compared to hygromix. Concentrations of IAA and NAA at 500 ppm, 1000 ppm and 2000 ppm were significantly different from IBA at 500 ppm, 1000 ppm and 2000 ppm, as the former led to a significantly low number of leaf production compared to IBA at the same concentration. The control samples and IBA application to *B. zanguebarica* cuttings gave the highest percentage sprouted stem and highest means of leaf production compared to IAA and NAA. Nonetheless, no rooting of the cuttings was observed.

6.2 Recommendations

The major finding of this study is the lack of rooting, thereby; it is recommended that further research with possibly alternative means of propagation should be carried out to determine the effect of seasonal changes, environmental conditions and effect of temperature of the growth media on growth and rooting ability of the plant species investigated.

REFERENCES

Abbink, J., 1995. Medicinal and Ritual Plants of the Ethiopian Southwest: An account of recent research. Indigenous Knowledge and Development Monitor, 3(2):6-8.

Akinnifesi, F.K., Sileshi, G., Mkonda, A., Ajayi, O.C., Mhango, J. and Chilanga, T., 2007. Germplasm supply, propagation and nursery management of Miombo fruit trees. In: Akinnifesi FK, Sileshi G, Ajaji O, Tchoundjeu Z, Matakala P (Eds) Indigenous Fruit Trees in the Tropics: Domestication, Utilization and Commercialization, CABI publishing.

Akinyele, A.O., 2010. Effects of growth hormones, rooting media and leaf size on juvenile stem cuttings of *Buchholzia coriacea* Engler. Annals of Forest Research 53(2): 127-133.

Al-Barazi, A. and Schwabe, W.W., 1982. Rooting softwood cuttings of adult *Pistacia vera*. Journal of Horticultural Science, 57(2): 247-252.

Aloni, R., 1987. Differentiation of vascular tissues. Annual Review Plant Physiology, 38: 179–204.

Aminah, H., Dick, J.P., Leakey, R.R.B., Grace, J. and Smith, R.I., 1995. Effect of indole butyric acid (IBA) on stem cuttings of *Shorea leprosula*. Ecological Management, 72: 199-206.

Aparicio, A., Pastorino, M., Martines-Meir, A. and Gallo, L., 2009. Vegetative propagation of patagonian cypress, a vulnerable species from the subantartic forest of South America. Bosque, 30(1): 18-26.

Armson, K.A, Fung, M. and Bunting, W.R., 1980. Operational rooting of Black spruce cuttings. Indian Journal of Forestry, 78:341-343.

Arnold, H.J. and Gulumian, M., 1984. Pharmacopoeia of traditional medicine in Venda. Journal of Ethnopharmacology, 12: 35–74.

BAH, 2004. Herbal medicines today: scientific knowledge and medicinal conditions; Inventory and perspectives. 4th edn. Bundesfachverband der Arzneimittelhersteller (BAH), Bonn, Germany.

Balick, J.M. and Cox, P.A., 1996. In: Plants, People and Culture: the Science of Ethnobotany, Scientific American Library, New York, pp. 228.

Banda, T., Schwartz, M.W. and Caro, T., 2006. Effect of fire on germination of *Pterocarpus angolensis*. Forest Ecology and Management, 233: 116-120.

Baskin, C.C. and Baskin, J.M., 1998. Seed Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press, San Diego, pp: 666.

Berhe, D. and Negash, L., 1998. Asexual propagation of *Juniperus procera* from Ethiopia: a contribution to the conservation of African cedar. Forest Ecology and Management, 4454:179-190.

Berjak, P. and Pammenter, N.W., 2004. Recalcitrant seeds. In: Benech-Arnold RL, Sánchez RA (Eds). Handbook of Seed Physiology, Application to Agriculture, The Haworth Press, Inc, NY, USA, pp. 305-345.

Bessong, P.O., Obi, C.L., Andreola, M.L., Rojas, L.B., Pousegu, L., Igumbor, E.,Meyer, J.J.M., Quideau,S. and Litvak, S., 2005. Evaluation of selected South African medicinal plants for inhibitory properties against human immunodefciency virus type 1 reverse transcriptase and integrase. Journal of Ethnopharmacology, 99: 83-91.

Bewley, J.D. and Black M., 1983. Physiological and Biochemistry of Seeds in Relation to Germination (Vol 1) Development, Germination, and Growth, Springer-Verlag, Berlin, Germany, pp. 306.

Bodeker, G.C., 1995. Introduction. Medicinal plants for Conservation and Healthcare. Institute of Health Sciences, University of Oxford, Oxford, UK, pp. 4.

Bombardelli, E., Bonati, A., Gabetta, B. and Mustich, G., 1974. Plants of Mozambique. IV. Flavonoids of *Brackenridgea zanguebarica.* Phytochemistry, 13: 295–297.

Buijtenen, J.P, Van, J., Toliver, R., Bower and Wendel, M., 1975. Operational rooting of Loblolly and slash pine cuttings. Prog. Rep.Cooper. For. Tree Improvement Program. Texas Forest Service Circular No.111.

Canter, P.H., Thomas, H. and Ernst, E., 2005. Bringing medicinal plants into cultivation: Opportunities and challenges for biotechnology. Trends Biotechnology, 23: 180-185.

Charis, K., 2000. A novel look at a classical approach of plant extracts. Feed Mix (special issue on Nutraceuticals), pp. 19-21.

Chhabara, S.C., Mahunnah, R.L.A. and Mshiu, R.N., 1990. Plants used in traditional medicine in EasternTanzania, III. Angiosperms (Mimosaceae to Papilionaceae). Journal of Ethnopharmacology, 29: 295–323.

CITES, 2007. Convention on International Trade in Endangered Species of Wild Fauna and Flora, Appendices I, II and III.

Crouch, N.R., Lotter, M.C., Krynauw, S. and Pottas-Bircher, C., 2000. *Siphonochilus aethiopicus* (Zingiberaceae), the prized Indungulu of the Zulu, An overview. Herbetia, 55: 115-129.

Cunningham, A.B., 1992. *Imithi isiZulu*: The traditional medicine trade in Natal/KwaZulu. Unpublished MSc Dissertation. University of Natal, South Africa.

Cunningham, A.B., 1993. African medicinal plants: Setting priorities at the interface between conservation and primary healthcare. People and Plants Working Paper. UNESCO, pp. 53.

Cunningham, A.B. and Mbenkum, F.T., 1993. Sustainability of harvesting *Prunus africana* bark in Cameroon: A medicinal plant in international trade. People and Plants Working Paper no.2. UNESCO, Paris.

Davenport, T.R.B. and Ndangalasi, H.J., 2002. An escalating trade in orchid tubers across Tanzania's Southern highlands-assessment, dynamics and conservation implication. Oryx, 37: 5–61.

De Klerk, G.J., TerBruge, J. and Marinova, S., 1997. Effectiveness of indoleacetic acid, indolebutyric acid and naphthalene acetic acid during adventitious root formation in vitro in *Malus* 'Jork 9', Plant Cell Tissue Organ Culture, 49: 39-44.

Djavanshir, K. and Pourbeik, H., 1976. Germination Value - a new formula. Silvae Genetica, 25: 79-83.

Dladla, S. 2001. Muthi Trade Boom: Unemployment finds refuge in traditional world. Sunday World, South Africa, pp: 19.

Dlamini, MD., 2007.www.plantzafrica.com/plantefg/elaeodentrans.htm

Drewes, S.E., 1983. Brackenin, a dimeric dihydrochalcone from *Brackenridgea zanguebarica*. Phytochemistry, 22: 2823–2825.

Drewes, S.E., 1987. Medicinal plants of southern Africa. Part 1. Dimeric chalcone-based pigments from *Brackenridgea.* Journal of Chemical Society Perkin Trans. 1: 2809–2813.

Fang, S., Wang, J., Wei, Z. and Zhu, Z., 2006. Methods to break seed dormancy in *Cyclocarya paliurus* (Batal) Iljinskaja. Scientia Horticulturae, 110(3): 305-309.

FAO, 2009. Climate and Rainfall. http://wwww.fao.org/wairdocs/ilri/x5524e/x5524e03.htm Accessed on 26 February 2016.

Farooq, Z., Iqbal, Z., Mushtaq, S., Muhammad, G., Iqbal, M.Z., and Arshad, M., 2008. Ethnoveterinary practices for the treatment of parasitic diseases in livestock in Cholistan desert (Pakistan). Journal of Ethnopharmacology, 118: 213–219.

Fasola, T.R. and Egunyomi, A., 2005. Nigerian usage of bark in phytomedecine. Ethnobotany Research and Application, 3: 73–77.

Gbadamosi, A.E. and Oni, O., 2005. Macropropagation of an endangered medicinal plant *Enantia chlorantha* Oliv. Journal of Arboriculture, 31: 78-82.

Geldenhuys, C.J., 2004. Bark harvesting for traditional medicine: from illegal resource degradation to participatory management. Scandinavian Journal of Forest Research, 19: 103-115.

Geldenhuys, C.J., Syampungani, S., Meke, G.S. and Vermeulen, J., 2007. Response of different species to bark harvesting for traditional medicine in Southern Africa. In J.J. Bester, A.H.W. Seydack, T. Vorster, I.J.Van der Merwe and S. Dzivhani (eds). Multiple Use Management of NaturalForests and Woodlands: Policy Refinement and Scientific Progress. pp. 55–62. Department of Water Affairs and Forestry, Pretoria, South Africa.

Gelfand, M., Mavi, S., Drummond, R.B. and Ndemera, B., 1985. The Traditional Medical Practitioner in Zimbabwe. Mambo Press, Gweru.

Goldblatt, P., 1978. An analysis of the flora of southern Africa: its characterization, relationships and origins. Annals of the Missouri Botanical Gardens, 65: 369–436.

Grange, R.I. and Loach, K., 1983. The water economy of unrooted leafy cuttings. Journal of Horticultural Science, 58: 9-17.

Guarrera, P.M., 1999. Traditional antihelmintic, antiparasitic and repellent uses of plants in Central Italy. Journal of Ethnopharmacology, 68: 183-192.

Hartmann, H.T., Kester, D.E. and Davies, F.T., J.R. 1990. Plant Propagation: Principles and Practices, 5th Edition, Prentice-Hall International Editions. Englewood Cliffs, New Jersey, USA.

Hartmann, H.T., Kester, D.E., Davies, F.T. JR. and Geneve, L.R., 1997. Plant Propagation: Principles and Practices. 6th Edition. Prentice-Hall International Editions, Englewood Cliffs, New Jersey, USA.

Hilhorst, H.W.M., Bentsink, L. and Koorneef, M., 2006. Dormancy and germination. In: Basra AS (Ed). Handbook of Seed Science and Technology, The Haworth Press, NY, USA, pp. 271-302.

Hutchings, A. and van Staden, J., 1994. Plants used for stress-related ailments in traditional Zulu, Xhosa and Sotho medicine. Part 1: plants used for headaches. Journal of Ethnopharmacology, 43: 89–124.

International Institute of Rural Reconstruction, 1994. Ethnoveterinary medicine in Asia: an information kit on traditional animal health care practices. International Institute of Rural Reconstruction (IIRR), second edition. Silang, Cavite, Philippines.

Issa, H.H., Abe, N., Ali, Z. and Khan, I.A., 2013. Chalcone Oligomers from *Brackenridgea zanguebarica*. Planta Medica, 79(10): PN33.

IUCN, 2012. IUCN red list of threatened species version 2012. Available on: http://www.iucnredlist.org

Jansen, P.C.M. and Mendes, O., 1990. Plantas Medicinais Seu Uso Tradicional em Moçambique. Tomo 3. Imprensa do Partido, Maputo.

Joshi, A.R., Shrestha, S.L. and Joshi, K., 2003. Environmental Management and Sustainable Development at the Crossroad. AnKus, Kathmandu, Nepal.

Junikka, L., 1994. Survey of english macroscopic bark terminology. IAWA Journal, 15: 3–45.

Kar, D.M., Nanda, B.K., Pradhan, D., Sahu, S.K. and Dash, G.K., 2004. Analgesic and antipyretic activity of fruits of *Martynia annua* Linn. Hamdard Medicus, 47(1): 32-35.

Kasilo, O., 2000. Traditional African medicine. WHO, Traditional medicine, better science, policy and services for health development, pp. 86-94.

Kokwaro, J.O., 1976. Medicinal Plants of East Africa. East African Literature Bureau, Nairobi.

Kumari, M., Patade, V.Y., Arif, M. and Ahmed, Z., 2010. Effect of IBA on seed germination, sprouting and rooting in cuttings for mass propagation of *Jatropha curcus* L strain DARL-2, INSI net Publication, Research Journal of Agriculture and Biological Sciences, 6(6): 691-696.

Lalit, T., 2014. Studies on vegetative propagation of *Acacia catechu* Willd. and *Toona ciliata* M. Roem. M.Sc. Thesis, Dr Y.S. Parmar University of Horticulture and Forestry, Solan, India,p. 45.

Lambert, J., Srivastava, J. and Vietmeyer, N., 1997. Medicinal plants. Rescuing a global heritage. Washington DC, World Bank (World Bank Technical Paper 355).

Leakey, R.R.B., 1990. *Nauclea diderrichii*: rooting of stem cuttings, clonal variation in shoot dominance, and branch plagiotropism. Trees, 4: 164-169.

Leakey, R.R.B., Mese´n, J.F., Tchoundjeu, Z., Longman, K.A., Dick, J.McP., Newton, A., Matin, A., Grace, J., Munro, R.C. and Muthoka, P.N., 1990. Low-technology techniques for the vegetative propagation of tropical trees. Commonwealth Forestry Review, 69(3): 247-257.

Leakey, R.R.B. and Simons, A.J., 1998. The domestication and commercialization of indigenous trees in agroforestry for the alleviation of poverty. Agroforestry Systems, 38:165–176.

Lebrun, A., Toussaint, A.N. and Roggemans, J., 1998. Description of *Syzygium paniculatum* Gaertn. 'Verlaine' and its propagation by stem cuttings. Scientia Horticulturae, 75: 103-111.

Li, T.S.C., Bedford, K.E. and Sholberg, P.L., 2000. Improved germination of American ginseng seeds under controlled environments. Horticultural Technology, 10: 131-135.

Light, M.E., Sparg, S.G., Stafford, G.I. and van Staden, J., 2005. Riding the wave: South Africa's contribution to ethnopharmacological research over the last 25 years. Journal of Ethnopharmacology, 100: 127–130.

Loach, K., 1992. Environmental conditions for rooting cutting: importance, measurement and control. Acta Horticulturae, 314: 233-242.

Lo, Y.N., 1985. Root initiation of *Shorea macrophylla* cuttings: effects of node position, growth regulators and misting regime. Forest Ecology and Management, 12: 43-52.

Loundou, P.M., 2008. Medicinal plant and opportunities for sustainable management in the Cape Peninsula, South Africa. Published MSc Dissertation. Stellenbosch University, South Africa.

Low, A.B. and Rebelo, A.G., 1998. Vegetation of South Africa, Lesotho and Swaziland: a companion to the vegetation map of South Africa, Lesotho and Swaziland.

Luseba, D. and van der Merwe, D., 2006. Ethnoveterinary medicine practices among Tsonga speaking people of South Africa. Onderstepoort Journal of Veterinary Research, 73(2): 115–122.

Mabogo, D.E.N., 1990. The ethnobotany of the Vhavenda. Master of Science Dissertation, University of Pretoria, Pretoria.

Mander, M., 1997. The marketing of indigenous medicinal plants in South Africa. A case study in KwaZulu-Natal. INR Investigational Report no. 164. Institute of Natural Resources, University of Natal, South Africa.

Mander, M., 1998. Medicinal plant marketing in Bushbuckridge and Mpumalanga: A market survey and recommended strategies for sustaining the supply of plants in the region. Unpublished report.Darudec and DWAF, South Africa.

Mander, M., Mander, J. and Breen, C., 1998. Domestication and commercialization of nontimber forest products in agroforestry systems. Non-Wood Forest Products, vol. 9. Food and Agriculture Organization of the United Nations.

Mander, M., Ntuli, L., Diederichs, N. and Mavundla, K., 2007. Economics of the traditional medicine trade in South Africa: health care delivery. South African Health Review, (1): 189-196.

Mathebula, J., Molokomme, M., Jonas, S. and Nhemachena, C., 2017. Estimation of household income diversification in South Africa: A case study of three provinces. South African Journal of Science, 113(1-2): 1-9.

Mathias-Mundy, E. and McCorkle, C.M., 1989. Ethnoveterinary Medicine: An Annotated Bibliography. Center for Indigenous Knowledge and Agricultural

and Rural Development (CIKARD). Ames: Iowa State University (Bibliographies in Technology and Social Change, no. 6).

McCorkle, C.M., Mathias, E. and Veen, T.W.S.v., 1996. Ethnoveterinary research and development. Intermediate Technology Publications, London, UK, pp. 338 ISBN 1853393266.

McGaw, L., Jager, A., Grace, O., Fennell, C. and van Staden, J., 2005. Medicinal plants. In: van Niekerk, A. (Ed.), Ethics in Agriculture—An African Perspective. Springer, Dordrecht, The Netherlands, pp. 67–83.

Mitchell, R., Zwolinski, J. and Jones, N., 2004. A review on the effects of donor maturation on rooting and field performance of conifer cuttings. South African Forestry Journal, 201(1):53–63.

M'marete, C.K., 2003. Climate and water resources in the Limpopo Province. In: Nesamvuni AE, Oni SA, Odhiambo JJO and Nthakheni ND (eds.) *Agriculture as the Cornerstone of the Economy in the Limpopo Province*. A study commissioned by the Economic Cluster of the Limpopo Provincial Government under the leadership of the Department of Agriculture. 1-49.

Mng'omba, S.A., du Toit, E.S. and Akinnifesi, F.K., 2007. Germination characteristics of tree Seeds: spotlight on Southern African tree species. Tree and Forestry Science and Biotechnology, 1(1): 81-88.

Moller, M., Suschke, U., Nolkemper, S., Schneele, J., Distl, M., Sporer, F., Reichling, J. and Wink, M., 2006. Antibacterial, antiviral, antiproliferative and apoptosis-inducing properties of *Brackenridgea zanguebarica* (Ochnaceae). Journal of Pharmacy and Pharmacology, 58:1131-1138.

Mostert, T.H., Bredenkamp, G.J., Klopper, H.L., Verwey, C., Mostert, R.E. and Hahn, N., 2008. Major vegetation types of the Soutpansberg Conservancy and the Blouberg Nature Reserve, South Africa. Koedoe, 50(1): 32-48.

Nakagawa, C., Takaishi, Y., Fujimoto, Y., Duque, C., Garzon, C., Sato, M., Okamoto, M., Oshikawa, T. and Ahmed, S.U., 2004. Chemical constituents from the Colombian medicinal plant *Maytenus laevis*. Journal of Natural Products, 67: 1919-1924.

Ndou, A.P., 2006. http://www.plantzafrica.com/plantqrs/securidlong.htm

Neuwinger, H.D., 2000. African traditional medicine - a dictionary of plant use and applications. Medpharm GmbH Scientific Publishers, Stuttgart, Germany.

Ofori, D.A., Newton, A.C., Leakey, R.R.B. and Grace, J., 1996. Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of auxin concentration, leaf area and rooting medium. Forest Ecology and Management, 84: 39-48.

Pandey, A. and Singh, S., 2016. Indigenous Uses of Economically and Commercially important Medicinal Plants Use to Cure Gynecological Diseases by Ethnic People of Bodmalla village, Almora District of Western Himalaya, India. Journal of Biomedical and Pharmaceutical Research, 5(5).

Park, Y.S., Bonga, J.M. and Mullin, T.J., 1989. Clonal Forestry. *In* Mandal AK, GL Gibson eds. Forest Genetics and Tree Breeding. New Delhi, India. CBS Publishers and Distributors. pp. 143-167.

Puri, S. and Khara, A., 1992. Influence of maturity and physiological status of woody cuttings: Limits and promises to ensure successful cloning. Indian Forester, 118(8): 560-572.

Puri, S. and Vermat, R.C., 1996. Vegetative propagation of *Dalbergia sissoo* Roxb.using softwood and hardwood stem cuttings. Journal of Arid Environments, 34(2): 235-245.

Ragonezi, C., Klimaszewska, K., Castro, M.R., Lima, M., de Oliveira, P. and Zavattieri, M.A., 2010. Adventitious rooting of conifers: influence of physical and chemical factors. Trees, 24(6):975–992.

Raimondo, D., Von Staden, L., Foden, W., Victor, J.E., Helme, N.A., Turner, R.C., Kamundi, D.A. and Manyama, P.A., 2009. Red list of 156 South African Plants. Strelitzia 25. South African National Biodiversity Institute, Pretoria.

Rankoana, S.A., 2001. Plant-based medicines of the Dikgale of the Northern Province. South African Journal of Ethnology, 24: 99-104.

Raju, A.J.S. and Rao S.P., 2006. Explosive pollen release and pollination as a function of nectar feeding activity of certain bees in the biodiesel plant, *Pongamia pinnata* (L.) Pierre (Fabaceae), Current Science, 90(7): 960-967.

Repáč, I., Vencurik, J. and Balanda, M., 2011. Testing of microbial additives in the rooting of Norway spruce (*Picea abies* [L.] Karst.) stem cuttings. Journal of Forest Science, 57(12):555–564.

Roberts, E.H., 1973. Predicting the storage life of seeds. Seed Science and Technology, 1: 499-514.

Samresh, D., Srivastava, A., Singh, V. and Sharma, A., 2003. An overview of Ocimum chemistry and pharmacological profile. Hamdard Medicus, 46(4): 43.

SANBI, 2007. IUCN Red Data List Categories. Versions 3.0 (National). www.sanbi.org. Accessed, 07.1 0.2016.

Sansores-Peraza, P., Rosado-Vallado, M., Brito-Loeza, W., Mena-Rejon,G.H. and Quijano, L., 2000. Cassine, an antimicrobial alkaloid from *Senna racemosa*, Fitoterapia, 71: 690-692.

Scott-Shaw, R., Hilton-Taylor, C., Kasseepursad, B. and Church, B., 1998. The conservation status of the pepper bark tree. SABONET News, 3(2): 73–75.

Smalley, T.J., Dirr, M.A. and Armitage, A.M., 1991. Photosynthesis and leaf water, carbohydrate and hormone status during rooting of stem cuttings of *Acer rubrum*. Journal of the American Society for Horticultural Science, 116(6): 1052-1057.

Spring, W., 2004. Introduction of Traditional Medicinal Plants into Cultivation in KwaZulu Natal: a Way to Preserve Medicinal Plants and Educate the Public. www.agriculture.kzntl.gov.za/agriculture/technology.asp.assets/horticulture.asp/medplants/index.asp.

Steenkamp, V., 2003. Traditional herbal remedies used by South African women for gynecological complaints. Journal of Ethnopharmacology, 86:97-108.

Surendran, C., Sehgal, R.N. and Paramathma, M., 2003. Textbook of Forest Tree Breeding, Indian Council of Agricultural Research, New Delhi, India, pp. 247.

Tchoundjeu, Z., Avana, M.L., Leakey, R.R.B., Simons, A.J., Asaah, E., Duguma, B. and Bell, J.M., 2002. Vegetative propagation of *Prunus africana*: effects of rooting medium, auxin concentrations and leaf area. Agroforestry System, 54: 183–192.

Tipu, M.A., Pasha, T.N. and Ali, Z., 2002. Comparative efficacy of salinomycin sodium and Neem fruit (*Azadirachta indica*) as feed additive anticoccidials in broilers. International Journal of Poultry Science, 1(4): 91-93.

Tipu, M.A., Akhtar, M.S., Anjum, M.I. and Raja, M.L., 2006. New Dimension of Medicinal Plants as Animal Feed. Pakistan Veterinary Journal, 26(3): 144-148.

Todd, C.B., Khorommbi, K., Van der Waal, B.C. and Weisser, P.J., 2004. Conservation of woodland and biodiversity. A complementary traditional and western approach towards protecting *Brackenridgea zanguebarica*. In: M.J. Lawes, H.A.C. Eeley, C.M. Shackleton and B.G.S. Geach (eds.), Indigenous Forests and Woodlands in South Africa. Policy, People and Practice (pp. 737-750), University of KwaZulu-Natal Press, Pietermaritzburg.

Travlos, I.J., Economou, G. and Karamanos, 2007. Germination and emergence of the hard seed coated *Tylosema esculentam* (Burch) A. Schreib in response to different pre- sowing seed treatments. Journal of Arid Environments, 68: 501- 507.

Trockenbrodt, M., 1990. Survey and discussion of the terminology used in bark anatomy. IAWA Bulletin, 11: 141–166.

Tshikalange, T. and Hussein, A., 2010. Cytotoxicity activity of isolated compounds from *Elaeodendron transvaalense* ethanol extract. Journal of Medicinal Plants Research, 4: 1695-1697.

Tshikalange, T.E., Meyer, J.J.M., Lall, N., Muñoz, E., Sancho, R., Van De Venter, M. and Oosthuizen, V., 2008. *In vitro* anti-HIV-1 properties of ethnobotanically selected South African plants used in the treatment of sexually transmitted diseases. Journal of Ethnopharmacology, 119: 478-481.

Tshisikhawe, M.P., 2002. Trade of indigenous medicinal plants in the Limpopo province, Venda region; their ethnobotanical importance and sustainable use. M.Sc. Thesis, University of Venda, Thohoyandou.

Tshisikhawe, M.P. and Van Rooyen, M.W., 2012. Population biology of *Brackenridgea zanguebarica* in the presence of harvesting. Journal of Medicinal Plants Research, 6(46): 5748-5756.

Tshisikhawe, M.P., van Rooyen, M.W. and Bhat, R.B., 2012. An evaluation of the extent and threat of bark harvesting of medicinal plant species in the Venda Region, Limpopo Province, South Africa. International Journal of Experimental Botany, 81: 89-100.

Tshisikhawe, M.P. and Van Rooyen, M.W., 2013. Population biology of *Elaeodendron transvaalense* Jacq. in the presence of harvesting. Phyton-International Journal of Experimental Botany, 82: 303-311.

UNAIDS, 2006. Collaborating with traditional healers for HIV prevention and care in sub-Saharan Africa: Suggestions for programme managers and field workers. UNAIDS, Geneva.

van Staden, J., Brown, N.A.C., Jäger, A.K. and Johnson, T.A., 2000. Smoke as a germination cue. Plant Species Biology, 15: 167-178.

van Wyk, B-E, Van Oudtshoorn, R. and Gericke, N., 1997. Medicinal plants of South Africa. Briza Publications. Acardia, Pretoria, South Africa.

van Wyk A.E. and Smith, G.F., 2001. Regions of floristic endemism in southern Africa: A review with emphasis on succulents. Umdas Press, Pretoria.

Vermeulen, W.J., 2006. Sustainable harvesting for medicinal use: matching species to prescriptions. In: J.J. Bester, A.H.W. Seydack, T. Voster, I.J. Van Der Merwe and S. Dzivhani (Eds) Multiple use management of natural forests and woodlands: policy refinements and scientific progress: Symposium on Natural forests and Savanna Woodlands, Symposium IV. http://www2.dwaf.gov.za/webapp/resourcecentre/Documents/

Victor, J.E., 2002. South Africa. In: Southern African Plant red data lists. In: Golding, J.S. (eds). Southern African Botanical Diversity Network Report 14, SABONET, Pretoria. Pp. 93-120.

Vines, G., 2004. Herbal harvests with a future: towards sustainable sources for medicinal plants, Plant life International; www.plantlife.org.uk (accessed on 1 0 October 2016).

Watt, J.M. and Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. Livingstone, Edinburgh.

Weseler, A.R. 2004. Experimental investigations of antimicrobially active aqueous and alcoholic extract preparations of selected teedrogens with local therapeutic application tradition with special consideration of St John's wort (Hypericum perforatum L.). Heidelberg University

Weseler, A.R., Saller, R. and Reichling, J., 2002. Comparative investigation of the antimicrobial activity of PADMA 28 and selected European herbal drugs. Forsch. Komplementa¨rmed. Klass. Naturheilkd, 9: 346–351.

Wiessman-Ben, Z. and Tchoundjeu, Z., 2000. Vegetative tree propagation for arid and semi-arid land.
http://www.cgiar.org/ICRAF/res_dev/prog_5/tr_mat/coursei/t2_nm_ol.htm

Williams, V.L., 1996. The Witwatersrand muthi trade. Veld and Flora, 82(1): 12–14.

Williams, V.L., Balkwill, K. and Witkowski, E.T.F., 2001. A lexion of plants traded in the Witwatersrand *umuthi*shops, South Africa. Bothalia, 31: 71-98.

Williams, V.L., Balkwill, K. and Witkowski, E.T.F., 2007. Size-class prevalence of bulbous and perennial herbs sold in the Johannesburg medicinal plant markets between 1995 and 2001. South African Journal of Botany, 73: 144-155.

Wilson, P.J. 1993. Propagation characteristics of *Eucalyptus globules* Labill. stem cutting in relation to their original position in the parent shoot. Journal of Horticultural Science, 68(5): 715-724.

World Health Organization, 1993. Guidelines on the conservation of medicinal plants. World Health Organization, Geneva. http://www.wwf.org.uk/filelibrary/pdf/guidesonmedplants.pdf

World Health Organization, 2001. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. WHO, Geneva.

Xaba, P. and McVay, 2010. The pepper-bark tree. SANBI, Kirstenbosch National Botanical Garden. Veld and Flora, pp.40–41.

Zobel, B. and Talbert, J., 1984. Applied forest tree improvement. Illinois, USA. Waveland Press. Pp. 505.

Zschocke, D., Rabe, T., Taylor, J.L.S. and Van Staden, J., 2000. Plant part substitution-a way to conserve endangered medicinal plants? Journal of Ethnopharmacology, 71: 281-29.