The antimicrobial and antifungal efficacy of indigenous plant extracts against *Streptococcus mutans*, *Escherichia coli* and *Candida albicans*

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A mini-thesis submitted in partial fulfillment of the requirements for the degree of Magister Scientiae in the Department of Paediatric Dentistry, Faculty of Dentistry, University of the Western Cape.

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Keywords

- Antibacterial.
- Antifungal.
- *Streptococcus mutans*.
- *Escherichia coli*.
- *Candida albicans*.
- Indigenous plants.
- *Tamarindus Indica*.
- *Hibiscus sabdariffa*.
- *Adansonia digitata*.
- *Moringa oleifera*.
- Traditional medicine.
ABSTRACT

The antimicrobial and antifungal efficacy of indigenous plant extracts against *Streptococcus mutans*, *Escherichia coli* and *Candida albicans*.

**Aim:**
To determine the antimicrobial and antifungal efficacy of indigenous plant extracts, *Tamarindus Indica* (*T. indica*), *Hibiscus sabdariffa* (*H. sabdariffa*), *Adansonia digitata* (*A. digitata*) and *Moringa oleifera* (*M. oleifera*) against *Streptococcus mutans* (*S. mutans*), *Escherichia coli* (*E. coli*) and *Candida albicans* (*C. albicans*).

**Objectives:**
The objectives of this study were to:

1. Measure the zones of growth inhibition by *T. indica*, *A. digitata*, *M. oleifera* and *H. sabdariffa* extracts against *S. mutans*, *E. coli* and *C. albicans*.
2. Compare the size of inhibition zones of different bacteria or fungus, *S. mutans*, *E. coli* and *C. albicans*, around the same plant extract.
3. Compare the size of inhibition zones for the same bacteria in different plant extracts *T. indica*, *A. digitata*, *H. sabdariffa* and *M. oleifera*.
Methodology

The antimicrobial and antifungal effect of the ethanolic extracts of *T. indica, H. sabdariffa, A. digitata* and *M. oleifera* was performed using the disc diffusion method against *S. mutans, E. coli* and *C. albicans*. The antibacterial and antifungal activity of the plants was determined by measuring the diameter of the inhibition zones.

Results and conclusion:

The results showed that *T. indica* and *H. sabdariffa* ethanolic extracts have an antibacterial effect against *S. mutans* and *E. coli*. However, *H. sabdariffi* showed a significantly higher antibacterial effect against *E. coli* and *S. mutans*, with a range of 14.50mm to 12.01mm and 16.41 mm to 14.39 mm compared to *T. indica*, with a range of 11.41 mm to 7.04mm and 6.88mm to 10.40mm, respectively. Furthermore, the statistical multiple pairwise test (Conover-Iman procedure/Two-tailed test) computed that the effect of *H. sabdariffi* is significantly (critical value >7.229) greater for the Gram positive *S. mutans* than the Gram negative *E. coli*. On the other hand, *T. indica* showed a similar antibacterial effect against *S. mutans* and *E. coli*, respectively.

In contrast, *M. oleifera* and *A. digitata* ethanolic plant extracts showed no antibacterial effect against *E. coli* and *S. mutans*. All the indigenous plants tested, *T. indica, H. sabdariffa, M. oleifera* and *A. digitata* had no antifungal activity on *C. albicans*. 
DECLARATION

I declare that “The antimicrobial and antifungal efficacy of indigenous plant extracts against Streptococcus mutans, Escherichia coli and Candida albicans” is my own work, that it has not been submitted before for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged as complete references.

Balsam Elashi

November 2014

Signed:

[Signature]

UNIVERSITY of the WESTERN CAPE
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Dedication

I dedicate this humble work to my family, my amazing mother Nihal Mirza, my dad Ahmed Elashi, my sisters Yasmine, Ghada and Sarah and my young brother Yassin. I also dedicate this work to all my friends that I have met in South Africa. They were there for me in my ups and downs, they motivated me, encouraged me and made me laugh like crazy. My friends made me acknowledge that as human, we are all diverse and different, we come from different backgrounds, believe in different religions, have special cultures and food. The only thing that will unite us is the love and respect for each other. Thank you, you have made my stay here in Cape Town, an extraordinary experience I will never forget or regret. I express my gratitude to Onengiye Oraene, Bibi Ndzelén, Patrick Lunga and Teboho Nchaba.
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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW
Introduction and literature review

1. History of Herbal medicine

The ‘traditional’ use of herbal medicine implies substantial historical use, and this is certainly true for many products that are available as ‘traditional herbal medicines’ (Tyler, 2000). Herbalism is as old as humankind. Every culture on earth has developed a herbal tradition. Since the beginning of creation, people have relied primarily on plants for nourishment. Through trial and error they discovered that some plants are good as food, others are poisonous, and yet others produce bodily changes such as increased perspiration, bowel movement, urination, relief of pain, hallucination, and healing (Tyler, 2000).

On every part of the globe where humans have lived, a body of herbal knowledge has developed. Native Africans discovered the herb pygeum (*Prunus africana*) which is beneficial for the prostate gland (Tyler, 2000). The Australian Aborigines discovered Tea Tree oil—from the leaves of the Melaleuca tree used by British soldiers during World War II as an antiseptic for wounds (Tyler, 2000). The natives of the South Pacific discovered Noni (*Morinda citrifolia*) which is recommended for many health benefits including the stimulation of the immune system (immuno-stimulant) (Tyler, 2000). The South Pacific Natives discovered Kava *Kava* (*Piper methysticum*) which helps to promote relaxation without dulling the senses (Tyler, 2000). These observations were passed down verbally from one generation to the next over millennia, with each generation adding to and refining the original body of knowledge (Tyler, 2000). Every
culture in the world has developed a body of herbal knowledge as part of its tradition in this manner (Tyler, 2000).

The first written record of herbal medicine dates back five thousand years ago (Tyler, 2000). It was written by the Sumerians who were the settlers of ancient Mesopotamia (Iraq in the present day). Sumerian prescriptions for healing using herbs were written on tablets of clay. In addition, in 3500BC to 30BC the sophisticated civilisation of the ancient Egyptians, organised and recorded an amazing array of medical practices (Tyler, 2000). These included linking illnesses to effective treatments, complicated surgeries, including that of the eye, and prescriptions for herbal preparations (Tyler, 2000).

Around that time, herbal medicine was also developing in China (Shaw, 1998). In fact, herbal medicine books were written in China which contained over 300 herbs including Ma huang, or Chinese ephedra (Shaw, 1998). Ma huang is still widely used today and is the herb from which Western scientists have derived the drug ephedrine (Shaw, 1998).

Furthermore, in the second century BC, Indian herbalists started developing their own herbal medicine system, which was called the Ayurveda (Morgan, 2002). It included diet and herbal remedies, while emphasising the body, mind and spirit in disease prevention and treatment (Morgan, 2002). The Indians have contributed significantly to the knowledge of herbal medicine (Morgan, 2002).
The Greeks and Romans derived much of their herbal knowledge from these early civilisations (Tyler, 2000). Ancient Greece was greatly influenced by Babylonia (or Mesopotamia), Egypt, and somewhat by India and China. The Greek physician Hippocrates (460 - 377 BC), who is often referred to as the "Father of Modern Medicine" was a herbalist. The axiom: “Let your Foods be your medicines, and your medicines your food," was written by Hippocrates (Tyler, 2000).

Furthermore, Europe gained its herbal knowledge in the middle ages from monks who studied the plants and translated the Arabic work on herbalism (Tyler, 2000). Moreover, after the discovery of the American continent, the European settlers had great respect for the herbal wisdom of the American Indians and relied heavily upon their herbal knowledge (Tyler, 2000).

The fascination of the European and North American continents with the traditional herbal medicine of the natives was short-lived as the scientific culture acquired by these nations, replaced herbal medicine with “modern medicine” (Nakato et al, 2010). These scientists claim that the use of herbs as a drug is unscientific (Nakato et al, 2010).

However, during the latter part of the twentieth century, herbal medicine has regained its popularity again, especially in the Western continents and Europe (Tyler, 2000), where herbal products have been incorporated into so-called ‘alternative medicine’. Recently, the World Health Organisation (2003) estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care (WHO, 2005). The increased interest in alternative medicine is due to the current interest in finding alternative drugs to solve the problem of the
increase in drug resistance (Rios and Recio, 2005). In addition, the increase in self-care and the adoption of a healthy lifestyle has resulted in enormous growth in popularity of traditional healing modalities, including the use of herbal remedies, particularly in the USA (Rios and Recio, 2005). Consumers have reported positive attitudes towards these products, in large part because they believe them to be of ‘natural’ rather than ‘synthetic’ origin. They believe that such products are more likely to be safer than drugs. They are considered to be part of a healthy lifestyle, and they can help to avoid unnecessary contact with conventional ‘Western’ medicine. The consumers of herbal medicine state that centuries of use in traditional settings can be used as testimony that a particular herbal ingredient is effective and safe (Angel et al, 1998).

Unlike the Western world, the Africans maintained their ancestral teachings, cultures, beliefs and life styles. Currently, many communities and cultures in Africa consume a variety of foods and beverages indigenous to their environments, which exhibit medicinal value (Nakato et al, 2010). Plants form an essential part in the health of the people of Africa and of their everyday diet (Nakato et al, 2010). Supplementary foods to boost the health status of the individual and herbal remedies are used as prophylaxis, to prevent and cure diseases (Nakato et al, 2010). Approximately 80% of the African populations receive their health care and health education from healers that practice traditional medicine (Nakato et al, 2010). This high demand for traditional medicine in Africa is not limited to the rich or the poor population (Mander et al, 2006). This is clearly illustrated when comparing the countries in Africa. South Africa is categorised as being one of the developed countries in Africa with Ethiopia being one of the less developed countries (Mander et al, 2006). The consumption patterns of traditional medicine for both African countries does not differ significantly, 72% and 68% respectively (Mander et al,
The use of herbal medicine is mostly attributed to the belief of the power of nature, vitalism, spiritualism, and animism in their culture (Angel et al, 1998).

One such community in Africa is Sudan which is unique for its blend of tradition and culture in everyday life. The Sudanese have special customs, foods and beverages (Afolabi and Popoola, 2005; Shahat et al, 2006; Yagoub et al, 2008). The indigenous plants that are most commonly consumed in Sudan that have been shown to exhibit some medicinal and antibacterial properties are: *Tamarindus Indica* (*T. indica*), *Hibiscus sabdariffa* (*H. sabdaraffi*) and *Adansonia digitata* (*A. digitata*) (Afolabi and Popoola, 2005; Shahat et al, 2006; Yagoub et al, 2008). In addition, *Moringa oleifera* (*M. oleifera*) has also shown some antibacterial potential (John et al, 2013; Rao et al, 2010).

The potential for *M. oleifera* to be massively cultivated in African soil makes it suitable for further investigation.

2. *Tamarindus indica*, *(Leguminosae)*

The unique composition of *T. indica* has allowed it to be widely used in traditional medicine in the African region. *T. indica* belongs to the third largest family of flowering plants, which is the *Dicotyledonous* family, *Leguminosae* Sub Family, *Caesalpiniaeae* (Lewis et al, 2005). The use of *T. indica* fruit pulp in the preparation of beverages and in food is very popular in different regions (Ferrara, 2005). Several countries that are under-developed and have populations suffering from malnutrition require affordable sources of nutrition (Amubode and Fetuga, 1983; Conway and Toenniessen, 1999). *T. indica* also has a high level of basic protein (Ishola et al, 1990), is rich in carbohydrates and contains trace amounts of Vitamin A and iron (Khanzada et
In addition, *T. indica* has a high mineral content which includes potassium, magnesium, phosphorous and calcium (Khanzada *et al.*, 2008). The high phosphate and calcium may aid in the remineralisation of teeth (Oshiro *et al.*, 2007). However, the high acidic pH of *T. indica* may counteract the remineralisation effect and may cause dental erosion (Abukakar *et al.*, 2008; Jarvinem *et al.*, 1991).

The use of *T. indica* is not only limited to being a nutritious source. It is also used for the treatment of several diseases such as, gastro intestinal disorders, gonococci, dysentery, and jaundice (Ferrara, 2005). Pharmacological investigations also reported that *T. indica* is a hypoglycaemic (Pousset, 1989), lowers cholesterol levels, (Khanzada *et al.*, 2008) has a cytotoxic effect (Kobayashi *et al.*, 1996), and possesses anti-inflammatory properties (Khanzada *et al.*, 2008). In addition, *T. indica* exhibited antifungal and antibacterial properties (Pousset, 1989).

The antibacterial potential of *T. indica* is due to the presence of active phytochemicals which include flavonoids, tannins, alkaloids and other aromatic compounds detected in the plant (Yagoub *et al.*, 2008). Phytochemicals are compounds in indigenous plants which are responsible for the antibacterial and antifungal properties of the plants (Yagoub *et al.*, 2008; Chadare *et al.*, 2009; Akindahusini *et al.*, 2003; Batista *et al.*, 1994; Kazmi *et al.*, 1994; Rao *et al.*, 2011).

The antimicrobial phytochemicals used in traditional medicine are generally extracted using water. Water extracts of *T. indica* were found to be effective against *E. coli* bacteria, confirming the antimicrobial findings of the water plant extracts (Aida *et al.*, 2001; Osman *et al.*, 2004;
Predrag et al, 2005; Shahat et al, 2006; Yagoub et al, 2008). However, water was found to be an inferior solvent to extract the total antibacterial potential of T. indica (Yagoub et al, 2008).

The phytochemicals of T. indica extracted by using ethanol was more effective against E. coli than that extracted using water, ether and petroleum (Yagoub et al, 2008). Ethanolic extracts of T. indica have been shown to have superior antibacterial potential compared to other solvents, because ethanol allows more of the active antimicrobial phytochemicals to dissolve (Yagoub et al, 2008). The ethanolic extracts using micro-broth have an antibacterial effect against E. coli (Dabur et al, 2007). Furthermore, extracts of T. indica have also been documented as having antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Vibrio cholera, Trichophyton rubrum and Aspergillus niger (Ray et al, 1973).

The oral health benefits of T. indica have also been reported where the leaves of the plant were boiled and the extract effectively healed oral lesions (Tapsoba et al, 2006). In addition, the decoction of the bark of the T. indica tree was also claimed to relieve tooth ache (Tapsoba et al, 2006). The anticariogenic potential of T. indica was confirmed in 2012, by Islam et al (2012) who showed that T. indica bark extracts can inhibit streptococcal growth and may be a promising anticariogenic dental product. In addition, the efficiency of T. indica in a toothpaste formulation was proven to show stability (Islam et al, 2012). Islam et al (2012) also recommended that further studies be performed to ensure the stability of the herbal extracts in commercial dental products.
3. *Moringa oleifera (M. oleifera)*

Another indigenous plant that is widely used in African communities is *M. oleifera*. *M. oleifera* belongs to the family *Moringaceae*, widely cultivated in the tropical areas of Africa (Fahey *et al.*, 2005). It has been shown to be an excellent nutrient source and has also illustrated many medicinal properties (Nikkon *et al.*, 2003; Fahey *et al.*, 2005; Chuang *et al.*, 2007). The medicinal, nutritional and therapeutic potential of the plant has become part of the belief and culture of the native people (Fahey *et al.*, 2005). Various parts of this plant such as the roots, leaves, fruit, bark, immature pods and flowers are used for health and dietary purposes (Fahey *et al.*, 2005). It is rich in calcium, potassium, iron, Vitamin C and A and its high protein content rivals that of eggs and milk (Fahey *et al.*, 2005). *M. oleifera* has been recommended as the solution to malnourishment in Africa (Fahey *et al.*, 2005). The medicinal benefits of *M. oleifera* include the following: anti-trypanosomal, anti-diabetic, anti-cholesterol, hypotensive, hypoglycemic, antipyretic, anti-inflammatory, anti-ulcer, anti-spasmodic, anti-epileptic, anti-inflammatory antibiosis, hepatoprotective and even reduction of *Schistosome cercariae* titer (Fahey *et al.*, 2005). However, the literature recommends that more controlled studies are required to validate the medicinal properties of *M. oleifera* (Fahey *et al.*, 2005). The literature has also discussed the plant’s potential as an anti-oxidant, anti-cancer, anti-fungal (Chuang *et al.*, 2007; Nikkon *et al.*, 2003) and its anti-bacterial effect (Nikkon *et al.*, 2003; Fahey *et al.*, 2005).

The antimicrobial properties of *M. oleifera* leaf extracts were as a result of its active phytochemicals which include, Sitosterol, Niazin A, Stigma sterol, Kaempferol and Quercetin (Rao *et al.*, 2011). Studies have shown that the leaves of *M. oleifera* have an antibacterial potential against several organisms which include, *E. coli, S. aureus, B. subtilis* and *Klebsiella*
aerogenes, Streptococcus mutans (MTCC 890 and MTCC 497), Streptococcus salivarius, Lactobacillus fermentum, Streptococcus mitis, Streptococcus anginosus, Lactobacillus acidophilus, Streptococcus gordonii, Lactobacillus acidophilus and S. aureus (MTCC 96) (John et al, 2013; Rao et al, 2010). However, the antibacterial activity of the M. oleifera leaf was greater against Gram-positive species than against Gram-negative strains according to Peixoto et al (2011).

Furthermore, John et al (2013) and Rao et al (2010) recommend that M. oleifera be used as an anti-cariogenic agent, referring to their findings which demonstrated the antibacterial potential of M. oleifera ethanolic leaf extracts against the cariogenic S. mutans.

4. **Adansonia digitata (A. digitata)**

Similar to M. oleifera, A. digitata is also well known for its high nutritious content. Due to the high nutritious content, A. digitata has been described as a super fruit. The indigenous plant A. digitata is the most widespread Adansonia plant species on the African continent found in the hot, dry Savannahs of sub-Saharan Africa (Gebauer et al, 2002). It also grows in populated areas (Gebauer et al, 2002; Fao, 1988). The pulp, seeds, leaves and bark of A. digitata are used as food and for many medicinal purposes in several regions in Africa (Diop et al, 2005). The pulp of A. digitata is a very rich source of carbohydrates and contains a high amount of protein and essential amino acids (Osman et al, 2004). The fruit pulp and the seeds are also good sources of calcium, potassium and magnesium (Osman et al, 2004). The pulp is acidic, due to the presence of the organic acids citric, tartaric, succinic, malic, and ascorbic or Vitamin C, with pH 3.3 (Nour et al, 1980). The high acidic pH of the fruit may result in dental erosion, if consumed in excess.
(Jarvinen et al., 1991). Erosion of the teeth may not be spared even in the presence of the positive remineralisation effect of the high calcium and phosphate in A. digitata (Oshiro et al., 2007).

The benefits of A. digitata are not only limited to the nutritious benefits (Wickens and Lowe, 2008). A. digitata also has a range of medicinal properties such as anti-oxidant and anti-inflammatory, hepato-protective, anti-diarrheal and anti-malarial properties (Wickens and Lowe, 2008; Brady, 2011; Blomhoff et al., 2010; Al-Qarawi et al., 2003; Ramadan et al., 1993).

The bioactive phytochemicals in the A. digitata which include, tannins, flavonoids, triterpenoids and phenolic compounds (Chadare et al., 2009) contribute to A. digitata having some antiviral (Vimalanathan and Hudson, 2009; Anani et al., 2000) and antibacterial properties (Afolabi and Popoola, 2005) against several bacterial species such as Bacillus sp. Salmonella sp. Streptococcus sp. and E. coli. (Afolabi and Popoola, 2005; Yagoub et al., 2008). The antibacterial activity of the A. digitata plant extracts was found to be weak in water, chloroform and methanol (Singh and Bhat, 2003; Yagoub et al., 2008; Seukep et al., 2013). However, ethanol extracts have shown to have a superior antibacterial effect compared to the latter solvents (Yagoub et al., 2008). The difference in the antibacterial activity between the different plant extracts is explained by the difference of active phytochemicals dissolved in the different extraction solvents (Yagoub et al., 2008). Thus, an optimum extraction method and extraction solvent is necessary to extract active antibacterial metabolites, allowing the use of A. digitata as an efficient drug against infectious bacterial agents (Yusha’u et al., 2010).
Infectious bacterial agents can cause inflammation of tissues (Sibibe and Williams, 2002). Oil extracted from *A. digitata* seeds is used for inflamed gingiva (Sibibe and Williams, 2002). In Tanzania, surveys had reported that the bark of the Baobab tree is boiled and used as a mouthwash for toothache in Tanzania (Wickens, 1979).

*A. digitata* has many health benefits, thus described as a super fruit. The incorporation of the fruit in the diet in the form of beverages or as a food ingredient is desirable (Osman *et al*, 2004).

5. *Hibiscus sabdariffa* (*H. sabdariffa*)

The calyces of the *H. sabdariffa* flower are used to prepare hot and cold beverages in tropical and subtropical countries. Recently, *H. sabdariffa* has gained popularity in the local soft drink industry (Hirunpanich *et al*, 2005; Jo Lin *et al*, 2007). In addition, *H. sabdariffa* extracts are marketed as supplements due to their implied potential health benefits (Ali *et al*, 2005).

*H. sabdariffa* is claimed to treat kidney and urinary bladder stones, has a hypocholesterolemia, diuretic, antispasmodic, mild laxative and uricosuric effect (Chen *et al*, 2003). It can be used in cases of cadmium poisoning (Asagba, *et al*, 2007), treating fertility disorders (Amin and Hamza, 2006), is anti-hypertensive (El-Saadany, *et al*, 1991), and also possesses anti-atherosclerotic effects (Hirunpanich *et al*, 2005). *H. sabdariffa* contains substances against inflammation and mutagenicity (Chen *et al*, 2003; Farombi and Fakoya, 2005) and is also an immune-modulator because of its ethanolic content (Falceve, *et al*, 2008). In addition, *H. sabdariffa* has good anti-oxidant ability and anti-cancer properties due to its phenolic, quercetin, anthocyanins, ascorbic
acid, steroid glycosides and protocatechuic acid content (Suboh, et al, 2004; Wang et al, 2000). The coloring matter of the calyces is considered to be lethal to Mycobacterium tuberculosis (Lewis et al, 2006). It is also used as an antifungal (Edema et al, 2012) and antibacterial treatment (Asagba, et al, 2007).

H. sabdariffa contains secondary metabolites such as tannins, alkaloids (Akindahunsi et al, 2003), flavonoids (Batista et al, 1994) and phenols (Kazmi et al, 1994) that are responsible for the H. sabdariffa antimicrobial properties as suggested by in vitro studies (Wong et al, 2010). H. sabdariffa is a wide spectrum antibacterial agent (Wong et al, 2010) which has antibacterial properties against Gram-positive to a greater degree than against Gram-negative bacteria (Alzoreky and Nakahara, 2003; Murugan et al, 2008). Gram-negative bacteria are generally less susceptible to plant extracts than Gram-positive bacteria due to their outer membrane of lipopolysaccharide and lipoprotein, which is resistant towards antibacterial substances (Chopra and Greenwood, 2001; Alzoreky and Nakahara, 2003). H. sabdariffa’s antimicrobial activities are evident and hold great promise as an antimicrobial agent (Fullerton et al, 2011).
The mouth harbors a diverse, abundant and complex microbial community. The dynamic equilibrium between dental plaque bacteria and the innate host defense system is important to prevent oral diseases (Rogers, 2008). The mouth is an integral part of human anatomy and therefore oral health is intimately related to the health of the rest of the body (WHO, 2003). Despite the remarkable achievements in health in recent decades, millions of people worldwide have been excluded from the benefits of socioeconomic development and the scientific advances that have improved health care and quality of life (WHO, 2003).

Dental diseases are the most prevalent chronic diseases worldwide, and a costly burden to health care services (WHO, 2003). The treatment of dental diseases is expensive, accounting for between 5% and 10% of total health care expenditures in industrialised countries (WHO, 2003). In most developing, low-income countries, the prevalence rate of dental caries is high and more than 90% of caries is untreated. An estimated 5 billion people worldwide suffer from dental caries (WHO, 2003). Dental disease is difficult to eradicate because of its multifactorial aetiology. The caries initiation and progression are associated with social, cultural, nutritional, behavioural and biological risk factors (Ismail, 1999).

The three major aetiological factors of ECC are micro-organisms; especially S. mutans, a fermentable carbohydrate substrate, especially sucrose (very cariogenic) and a susceptible tooth (the host) (Ismail et al, 1999). S. mutans accounts for 70-100% of human oral bacterial isolates (Loesche, 1986). S. mutans are supragingival, scharolytic, facultative and adhesive organisms which adhere to the tooth structure and cause dental caries (Loesche, 1986). Intra oral stresses,
such as high and frequent sucrose consumption (Resine and Douglass, 1998; Tanzer, 1979), favor the colonisation of \textit{S. mutans} and promote the carious process and enamel erosion (Resine and Douglass, 1998). Before sucrose enters the cells, less than 10 percent of sucrose is transformed by hexotransferases into glucans and fructans that either diffuse to the surrounding or remain associated with the cell (Ciardi, 1983; Gibbons \textit{et al}, 1968; Hamada \textit{et al}, 1980). The enzymes glucosyltransferase metabolise sucrose, produces the soluble and insoluble glucans with a release of fructose (Marsh and Martin, 1992). \textit{S. mutans} are not particularly good colonisers (Rickard \textit{et al}, 2003) as they are initially unable to bind to the acquired enamel pellicle formed on the tooth surface. However, \textit{S. mutans} receptors (adhesins) are able to bind to glucan allowing it to strongly adhere to the tooth. Hence, sucrose is considered as the chief substrate in the establishment of cariogenic bacterial flora on the tooth surface (Mikkelsen, 1996). \textit{S. mutans} bacteria colonising on the tooth surface, metabolises sugars producing energy and lactic acid which causes demineralisation of the tooth structure (Gibbons, 1972; Chassy, 1983). Decreasing the \textit{S. mutans} bacterial load was found to reduce the risk of dental caries (Fennis-le \textit{et al}, 1998; Gripp \textit{et al}, 2002). The indigenous plants which express antibacterial properties may serve as cost effective anti-cariogenic agents, especially in underprivileged population, such as the African population (Angel and Kassirer, 1998).

7. \textit{Escherichia coli (E. coli)}

The diseases in under-privileged areas such as Africa are not limited to dental caries (de Onis \textit{et al}, 2004). Africa also holds the highest prevalence of malnourishment globally and this is a major problem especially in the Sub-Saharan region (de Onis \textit{et al}, 2004). The malnourished African individual is an easy target for bacterial infections because of their relatively low
immunity due to their malnourished state (Osman et al, 2004). Moreover, the unhygienic environment and lack of sanitation in the rural and disadvantaged areas in Africa further exacerbates the problem and provides an environment that facilitates the growth and establishment of virulent infectious bacteria, thus increasing the risk of infection in African populations at large (Ishii et al, 2008). The *E. coli* strain is among the many growing bacterial strains and is one of the most frequent causes of the many common bacterial infections, including gastroenteritis, urinary tract infection, neonatal meningitis and pneumonia (Ishii et al, 2008). The risk of contracting a disease from eating contaminated food, drinking contaminated water and personal contact with an infected individual in Africa is extremely high (Ishii et al, 2008). Indigenous plants having an antimicrobial activity (Yagoub et al, 2008) against *E. coli* may be used as medicine to treat *E. coli* caused diseases.

In the dental field *E. coli* was found to be one of the major bacteria responsible for the failure of root canal treatment (Onacag et al, 2003). Indigenous plants having an antimicrobial activity (Yagoub et al, 2008) against *E. coli* may be used as an irrigant with mechanical instrumentation. Furthermore, the use of indigenous plants may be favorable because they are biocompatible with the oral tissues (Fahey et al, 2005) and have no repulsive taste, which renders it more appealing for use in children.

Bacteria on the enamel can further extend into the dentine and may eventually reach the pulpal tissue. The main consideration in treating irreversible, necrotic pulp is the elimination of bacteria from the pulpal tissue and root canals (Krause et al, 2007). Mechanical instrumentation with saline irrigation is the main method to decrease bacterial load (Krause et al, 2007). However,
studies have shown that this method is not adequate for eliminating bacteria from infected canals (Siqueira et al, 1999; Pataky et al, 2002). Unsuccessful elimination of the high bacterial counts made the use of a more potent antimicrobial adjunct for irrigation or as a temporary canal medication a necessity in endodontic treatment, thus further reducing bacterial load (Spratt et al, 2001).

8. **Candida albicans (C. albicans)**

Oral candidiasis is the most common oral fungal disease in children (Ghannoum et al, 1990). It is caused by the overgrowth of a yeast-like fungus (Akpan et al, 2002). The chief opportunistic fungal pathogen is *C. albicans*. This opportunistic infection is highly prevalent in patients who are malnourished, immune-compromised and in patients suffering with HIV (Akpan et al, 2002). Oral candidiasis can lead to altered taste, localised pain and discomfort. It can also cause dysphagia from candidal infection extending to the oesophagus resulting in poor nutrition (Akpan et al, 2002). According to the disease’s presentation and symptoms, oral candidal infection is classified as either, acute or chronic. Management of oral candidiasis include: obliging to a good oral hygiene, application of topical antifungals such as Nystatin or Amphoteracin or administration of a systemic drug such as Fluconazole (Akpan et al, 2002).

However, the access to medicine in disadvantaged populations that lack conventional healthcare services may require alternative therapies to assist in reducing oral diseases. In such
communities, alternative medicine is practiced by the traditional healers who use indigenous plants and herbs as drugs for treating illness and disease (Nakato et al., 2010).

9. **Motivation for the study**

African countries are favored with the environmental conditions that allow for the cultivation and growth of many indigenous plants that may have promising medicinal properties. The vision is to enable a stable, cohesive and internationally competitive African Traditional Medicine industry that will provide an affordable medicine to the African consumer, uplift the African economy, create a number of employment opportunities for the African people and eventually improve the community’s quality of life as a whole.

In this study the African indigenous plants were investigated for their antibacterial and antifungal potential. The following plants: *Tamarindus indica, Hibiscus sabdariffa, Moringa oleifera* and *Andonisia digitata*, were included in this study because they are available in many African countries, are not expensive for the unprivileged consumer and to our knowledge the literature is not yet saturated with the antimicrobial effects of these indigenous plants against bacterial and fungal microorganisms.

The plants will be tested against *Streptococcus mutans* which causes dental caries, which is rated to be the most prevalent oral disease; *Escherichia coli*, the bacteria responsible for failures of most root canal treatments; and the oral fungus, *Candida albicans*, responsible for the most common fungal infection in children. Moreover, *Streptococcus mutans* will be a representative
for the effect of the plants against the Gram positive bacteria; *Escherichia coli*, a representative of Gram negative bacterium; and *Candida albicans* a representative of a fungal species
CHAPTER 2
AIMS AND OBJECTIVES
Aims and Objectives

**Aim:**

To determine the antimicrobial and antifungal efficacy of indigenous plant extracts, *T. indicia*, *A. digitata*, *H. sabdariffi* and *M. oleifera*, against *S. mutans*, *E. coli* and *C. albicans*.

**Objectives:**

The objectives of this study were to:

1. Measure the zones of growth inhibition by *T. indicia*, *A. digitata*, *M. oleifera* and *H. sabdariffi* extracts against *S. mutans*, *E. coli* and *C. albicans*.
2. Compare the size of inhibition zones of different bacteria or fungus, *S. mutans*, *E. coli* and *C. albicans*, around the same plant extract.
3. Compare the size of inhibition zones for the same bacteria in different plant extracts: *T. indicia*, *A. digitata*, *H. sabdariffi* and *M. oleifera*. 
CHAPTER 3

METHODOLOGY
Methodology

This study was a laboratory based experiment of an exploratory nature. The standard Kirby-Bauer disk diffusion test was used to assess the antibacterial potential of *T. indica*, *A. digitata*, *H. sabdariffa* and *M. oleifera* against *S. mutans*, *E. coli* and *C. albicans*.

The microorganisms used in this study were obtained from a standard stock culture collection stored at the Oral and Dental Research Institute, University of Western Cape. The National Collections of Type Cultures (NCTC) (Public Health England) of bacterial and fungal strains used were as follows: *S. mutans* (NCTC strain number 10920), *E. coli* (NCTC 11775) and *C. albicans* (NCTC 36801).

**Inoculum preparation**

The inoculum suspensions for *S. mutans*, *E. coli* and *C. albicans* were prepared using a direct colony suspension method. The micro-organisms were cultured in Brain Heart Infusion Agar for 24 hours. The colonies were then transferred directly from the isolated colonies to the Triptone Soy Broth (Oxoid CM 0129) and vortexed on a Vortex mixer (Hedolph, Germany) to prepare a suspension. The suspensions were standardised to match that of a 0.5 McFarland standard (corresponds to approximately $1.5 \times 10^8$ CFU/ml).
**Preparation of the Brain Heart Infusion Agar**

Thirteen grams of brain heart infusion agar powder (Oxoid CM375) were measured using a
Wirsom precision scale and mixed with 250ml of distilled water. Ten agar plates were prepared.
The Agar Nutrient bottles were then sterilised using an autoclave at 121 degrees C for 15
minutes and cooled for 50 minutes. The bottles containing the agar nutrient were placed in the
water bath to cool to 47 degrees. The agar nutrient was poured in petri dishes and left to cool and
solidify at room temperature.

**Preparation of the Plant extract**

*T. indica, A. digitata and H. sabdaraffi, grown in Sudan,* were purchased from the supermarket
in Khartoum, Sudan. *M. oleifera* leaves that were grown in South African soil, were purchased
from the South African market.

The fruit of *T. indica* was chopped into small pieces and sun dried for ten days. The dried plant
was then ground into a coarse powdery substance using an electric coffee blender (Moulinex,
France). Dried *H. sabdaraffi*, was purchased from the supermarket in Khartoum, Sudan. *H.
sabdaraffi* was ground into a coarse powdery substance using an electric coffee blender
(Moulinex, France). The *A. digitata* fruit pulp was ground into a fine powder-like texture using a
mortar and pestle. A powdered form of *M. oleifera* was purchased from the South African
market. The powdered plant materials were stored in clean dry, glass containers and stored until
further use.
Ten grams of the powdered plant material was dissolved in 100 ml of 95% ethanol solution and incubated at 35°C for 24 hours while rotated at 120 rpm in a rotator shaker. The plant mixtures were then filtered through a 0.45 μm membrane filter and placed in empty containers of which the weight was previously determined. The filtrate was then placed in the water bath at 37 degrees and the ethanol was allowed to evaporate to finally obtain the crude extract from the plants. The quantity (grams) of crude extract was determined by subtracting the weight of the container when empty from the weight of the container with the extract. A 40% concentration of ethanol was then added to the crude extracts to standardise the concentration of all extracts to 1000mg/ml.

Ten plates were used to examine each bacterial or fungal sample. Five wells were prepared in each plate. The 4 plant extracts were placed in 4 wells and 40% ethanol was placed in the fifth well as a control. The wells were prepared using the back of a sterile 6 mm (outside diameter) glass pasteur pipette and a drop of agar was then placed at the bottom of each well to prevent the extract from leaking out of the well. The bacterial or fungal suspension was then pipetted onto each agar plate (0.1 ml). The bacterial or fungal suspensions were evenly distributed using a glass rod. Twenty micro-litres (μl) of each reconstituted extract \((T. \ indica, \ A. \ digitata, \ M. \ oleifera \ and \ H. \ sabdariffi)\) of 1000mg/ml concentration was then poured into the holes. Twenty micro-litres (μl) of 40% ethanol were used as a negative control. The extracts were then allowed to diffuse for 15 minutes into the medium before incubation. The plates were incubated at 37 degrees centigrade for 24 hours.

The agar plates were examined after 24 hours. An electronic caliper was used to measure the diameter of the inhibition zones around the wells containing the plant extracts in the different
bacterial and fungal cultured agar plates. Four measurements were taken from 4 standardised points. All measurements were conducted by the same person.
CHAPTER 4

RESULTS
A. digitata and M. oleifera plant extracts did not reveal any antibacterial effect against S. mutans and E. coli or an antifungal effect against C. albicans.

H. sabdariffi and T. indica did not show any antifungal effect against C. albicans. However, the antibacterial effect against S. mutans and E. coli was evident. The average inhibition diameter zone was calculated in each plate per sample. There were 10 samples per group. The groups which showed inhibition zones were as follows; H. sabdariffi against E. coli bacteria (EcoliHib), T. indica against E. coli bacteria (Ecoli Tam), H. sabdariffi against S. mutans (StrpHib) and T. indica against S. mutans bacteria (StrpTam). The diameters of the inhibition zones were measured in millimetres. This information is tabulated in Table 1.
Table 1. The average inhibition zone diameters of four measurements for each of the ten samples per group

<table>
<thead>
<tr>
<th>Number of trials</th>
<th>Strp Hib</th>
<th>Strp Tam</th>
<th>Ecoli Hib</th>
<th>Ecoli Tam</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.85</td>
<td>9.32</td>
<td>13.47</td>
<td>9.14</td>
</tr>
<tr>
<td>2</td>
<td>14.76</td>
<td>9.65</td>
<td>14.50</td>
<td>9.01</td>
</tr>
<tr>
<td>3</td>
<td>14.86</td>
<td>10.40</td>
<td>12.57</td>
<td>7.04</td>
</tr>
<tr>
<td>4</td>
<td>15.01</td>
<td>7.77</td>
<td>8.74</td>
<td>9.70</td>
</tr>
<tr>
<td>5</td>
<td>16.41</td>
<td>8.27</td>
<td>12.13</td>
<td>8.19</td>
</tr>
<tr>
<td>6</td>
<td>16.26</td>
<td>9.73</td>
<td>12.01</td>
<td>9.66</td>
</tr>
<tr>
<td>7</td>
<td>15.72</td>
<td>7.90</td>
<td>13.82</td>
<td>8.89</td>
</tr>
<tr>
<td>8</td>
<td>14.39</td>
<td>6.88</td>
<td>13.71</td>
<td>11.41</td>
</tr>
<tr>
<td>9</td>
<td>15.04</td>
<td>8.27</td>
<td>13.35</td>
<td>10.34</td>
</tr>
<tr>
<td>10</td>
<td>14.40</td>
<td>9.29</td>
<td>12.28</td>
<td>7.47</td>
</tr>
<tr>
<td>MEDIAN</td>
<td>15.025</td>
<td>8.78</td>
<td>12.96</td>
<td>9.075</td>
</tr>
</tbody>
</table>

Strp Hib - *Hibiscus sabdariffa* against *Streptococcus mutans*, Strp Tam – *Tamarindus indica* against *Streptococcus mutans*, Ecoli Hib - *Hibiscus sabdariffa* against *Escherichia coli*, Ecoli Tam - *Tamarindus indica* against *Escherichia coli*. 
Strp Hib - *Hibiscus sabdariffa* against *Streptococcus mutans*, Strp Tam – *Tamarindus indica* against *Streptococcus mutans*, Ecoli Hib - *Hibiscus sabdariffa* against *Escherichia coli*, Ecoli Tam - *Tamarindus indica* against *Escherichia coli*.

**Figure 1.** A Graph representing the average diameter of the inhibition zones of the bacteria against the tested indigenous plants.
The Box and Whisker graph plot (Figure X) represents the results from the statistical analysis of the average inhibition zone diameters of the sample groups (10 samples per group). The red whiskers in the graph represent the range of the samples. The yellow box represents the 25th and 75th percentile range. The red line inside the yellow box represents the median. The green spots represent the outliers in a sample. The graph demonstrates that the data is equally distributed around the median for EcoliHib, EcoliTam and StrpTam. Moreover, the data for StrpHib was positively skewed and for that reason median values were used for the Box and Whisker plot.

In the EcoliHib group the inhibition zone ranged from 12.01mm to 14.50mm with a median of 12.96mm. The inhibition zones of EcoliTam had a median value of 9.075mm ranged from 7.04 mm to 11.41mm. The inhibition zone of StrpHib ranged from 14.39 mm to 16.41 mm with a median of 15.25mm. The inhibition zones of StrpTam ranged from 6.88mm to 10.40mm with a median of 8.78mm.

The graph clearly demonstrates that \textit{H. sabdariffi} has a higher antibacterial effect against \textit{E. coli} and \textit{S. mutans}, with a median of 12.96mm and 15.025mm respectively, compared to the antibacterial effect of \textit{T. indica}, with a median of 9.075mm and 8.78mm, respectively. The graph also shows that there is a difference between the antimicrobial activity of \textit{H. sabdariffi} towards the Gram positive \textit{S. mutans} and the Gram negative \textit{E. coli}. This difference cannot be seen with \textit{T. indica}.
**The Kruskal-Wallis test**

The Kruskal-Wallis test is a non-parametric test used to compare three or more samples to assess whether there are statistical significant differences between the mean values. It is used to test the null hypothesis ($H_0$) that all samples come from the same population or the alternative hypothesis ($H_a$) states that the samples do not come from the same population. If the computed p-value is less than the significance alpha value in a Kruskal Wallis test, it shows that the data are different. Therefore, the $H_0$ hypothesis is rejected and the $H_a$ hypothesis is accepted.

As illustrated in Table 2, the Kruskal Wallis test has shown that the study data computed p-value ($<0.0001$) is less than the significance alpha value of 0.05. Therefore, the null hypothesis $H_0$ was rejected, and the alternative hypothesis $H_a$ is accepted.

**Table 2. Kruskal Wallis test results**

<table>
<thead>
<tr>
<th>K(Observed value)</th>
<th>30.184</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (Critical value)</td>
<td>7.815</td>
</tr>
<tr>
<td>DF</td>
<td>3</td>
</tr>
<tr>
<td>p-value (Tailed)</td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>Alpha</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Multiple Pairwise comparison test

After acknowledging that there is a significant difference between the groups, identified by using the Kruskal-Wallis test, multiple pairwise comparisons using the Conover-Iman procedure/Two-tailed test were used to compare the significant difference between each pair and to indicate the magnitude of the statistical difference. Table 3 shows the p values when comparing 2 groups. If the p value was less than 0.05, there is a significant difference between the two compared groups. Table 4 summarises the means and the significant differences between the groups when compared with each other. In order to establish the p-value refer to the p-value table.

Table 3. P values of the Multiple pairwise comparisons

<table>
<thead>
<tr>
<th></th>
<th>StrpHib</th>
<th>StrpTam</th>
<th>EcoliHib</th>
<th>EcoliTam</th>
</tr>
</thead>
<tbody>
<tr>
<td>StrpHib</td>
<td>1</td>
<td>&lt; 0.0001</td>
<td>0.000</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>StrpTam</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>&lt; 0.0001</td>
<td>0.646</td>
</tr>
<tr>
<td>EcoliHib</td>
<td>0.000</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>EcoliTam</td>
<td>&lt; 0.0001</td>
<td>0.646</td>
<td>&lt; 0.0001</td>
<td>1</td>
</tr>
</tbody>
</table>

Strp Hib -*Hibiscus sabdariffa* against *Streptococcus mutans*, Strp Tam – *Tamarindus indica* against *Streptococcus mutans*, Ecoli Hib - *Hibiscus sabdariffa* against *Escherichia coli*, Ecoli Tam - *Tamarindus indica* against *Escherichia coli*.
Table 4. Means and significant differences (p=0.0001) between groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Count</th>
<th>Mean</th>
<th>Different from Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecoli Hib</td>
<td>10</td>
<td>12.65725</td>
<td>EcoliTam, StrpTam, StrpTam</td>
</tr>
<tr>
<td>EcoliTam</td>
<td>10</td>
<td>9.08375</td>
<td>EcoliHib, StrpHib</td>
</tr>
<tr>
<td>StrpHib</td>
<td>10</td>
<td>15.569</td>
<td>EcoliHib, EcoliTam, StrpTam</td>
</tr>
<tr>
<td>StrpTam</td>
<td>10</td>
<td>8.74625</td>
<td>Ecoli, StrpHib</td>
</tr>
</tbody>
</table>

Strp Hib - *Hibiscus sabdariffa* against *Streptococcus mutans*, Strp Tam – *Tamarindus indica* against *Streptococcus mutans*, Ecoli Hib - *Hibiscus sabdariffa* against *Escherichia coli*, Ecoli Tam - *Tamarindus indica* against *Escherichia coli*. 
Table 5 illustrates the magnitude of the statistical differences between the groups. If the absolute statistical value calculated is more than that of the critical value (7.229), there is a statistical difference between the 2 groups compared, the higher the statistical value, the greater the difference between the compared group pair. The mean inhibition zone of StrpHib (15.569mm) is statistically different when compared to StrpTam (8.74626mm), with an absolute statistical value of 24.800. This shows that *H. sabdarrifi* has a significantly higher antibacterial effect against *S. mutans* compared to *T. indica*. The statistical value between StrpHib (15.569mm) and EcoliHib (12.657mm) showed a statistical value (10.800mm) higher than that of the critical difference (7.229mm). This implies that *H. sabdariffi* has a greater effect against the Gram positive *S. mutans* than the gram negative *E. coli*, although the difference was very close to the critical difference.

There was no statistical difference between StrpTam and EcoliTam, which shows that *T. indica* has a similar antibacterial effect against *S. mutans* and *E. coli*, respectively. EcoliHib (12.65725mm) showed a statistical difference (12.800mm) when compared to EcoliTam (9.0837mm), indicating that *H. sabdarrifi* has a higher antibacterial effect against *E. coli* than *T. indica*.

In conclusion, *H. sabdariffi* showed a statistically greater inhibitory effect against *S. mutans* and *E. coli* compared to *T. indica*.
Table 5. Magnitude of the statistical differences expressed as an absolute statistical value*

<table>
<thead>
<tr>
<th></th>
<th>StrptHib</th>
<th>StrptTam</th>
<th>EcoliHib</th>
<th>EcoliTam</th>
</tr>
</thead>
<tbody>
<tr>
<td>StrptHib</td>
<td>0</td>
<td>24.800</td>
<td>10.800</td>
<td>23.600</td>
</tr>
<tr>
<td>StrptTam</td>
<td>-24.800</td>
<td>0</td>
<td>-14.000</td>
<td>-1.200</td>
</tr>
<tr>
<td>EcoliHib</td>
<td>-10.800</td>
<td>14.000</td>
<td>0</td>
<td>12.800</td>
</tr>
<tr>
<td>EcoliTam</td>
<td>-23.600</td>
<td>1.200</td>
<td>-12.800</td>
<td>0</td>
</tr>
</tbody>
</table>

Strp Hib - *Hibiscus sabdariffa* against *Streptococcus mutans*, Strp Tam – *Tamarindus indica* against *Streptococcus mutans*, Ecoli Hib - *Hibiscus sabdariffa* against *Escherichia coli*, Ecoli Tam - *Tamarindus indica* against *Escherichia coli*.

*The higher the statistical value, the greater the difference between the compared group pair.
CHAPTER 5

DISCUSSION
Discussion

Traditions of yesterday may become medicine for tomorrow. Indigenous plants are widely consumed in Africa and have been used for many generations and are still currently being used to treat various diseases and infections. Additional evidence is required to confirm that the benefits of some indigenous African plants are “fact and not fiction”.

Current evidence has confirmed that the presence of active phytochemicals such as phenolic compounds in indigenous plants are responsible for the antibacterial and antifungal properties of the plants (Batista et al., 1994; Kazmi et al., 1994; Akindahusini et al., 2003; Yagoub et al., 2008; Chadare et al., 2009; Rao et al., 2011). Therefore, this study focused on the antibacterial and antifungal efficacy of indigenous plant extracts that were documented to contain active phytochemicals. The plants investigated were *T. indica* (Tamarindus fruit), *A. digitata* (Baobab fruit), *H. sabdariffi* (Hibiscus flower) and *M. oleifera* (Moringa leaves) against the microorganisms *S. mutans*, *E. coli* and *C. albicans*.

In this study, *T. indica* was extracted using ethanol and showed antibacterial properties against *S. mutans* and *E. coli* in-vitro. This was confirmed by studies which also tested ethanolic extracts of *T. indica* against *E. coli* and *S. mutans* (Darbur et al., 2007; Yagoub et al., 2008). The fact that *T. indica* showed no difference in action between the Gram positive *S. mutans* and Gram negative *E. coli* in this study, is also confirmed by Dabur et al. (2007). This implies that *T. indica* has the same antibacterial properties against both Gram positive and negative microorganisms.
In this study ethanolic extracts of \textit{H. sabdariffi} exhibited antibacterial effects against \textit{S. mutans} and \textit{E. coli} and this result is supported by several studies (Fullerton \textit{et al}, 2011; Al Hashimi \textit{et al}, 2012; Edema \textit{et al}, 2012). Furthermore, the findings of this study revealed that \textit{H. sabdariffi} had a greater effect against Gram positive \textit{S. mutans} than Gram negative \textit{E. coli}. This is probably due to the fact that Gram positive bacteria are more sensitive towards phytochemicals from the plant extracts than Gram negative bacteria (Nair and Chanda, 2006; Murgan \textit{et al}, 2008). The statistical difference of the inhibitory effect between the Gram negative and Gram positive bacteria was not very high. A larger sample size would probably have resulted in a greater significant difference in the effect of \textit{H. sabdariffi} against Gram positive \textit{S. mutans} and Gram negative \textit{E. coli}. Hence, the use of \textit{H. sabdariffi} as a selective drug against Gram positive bacteria is suggested.

It is clear from this study and the supporting literature that \textit{T. indica} and \textit{H. sabdariffi} have an antibacterial effect against \textit{S. mutans} and \textit{E. coli}.

Although, the other studies conducted had different methodologies compared to this study, the objectives which were to test the ethanolic extract of \textit{H. sabdariffi} and \textit{T. indica} against \textit{S. mutans} and \textit{E. coli}, of all the studies compared, were the same (Darbur \textit{et al}, 2007; Yagoub \textit{et al}, 2008; Fullerton \textit{et al}, 2011; Al Hashimi \textit{et al}, 2012; Edema \textit{et al}, 2012). The different methodological procedures utilised, produced a wide range of different antibacterial effects. The primary factors that contributed to the diverse results in these different studies were probably the different extraction procedures, different extraction solvents and different extract concentrations utilised.
The extraction of active phytochemical compounds from medicinal plants was carried out in various ways using procedures such as the cold extraction method (similar to this study), Soxhlet, maceration, heat reflux and microwave-assistance. Certain procedures showed more superior extraction qualities than others (Wang et al, 2013). The ultrasound assisted method of extraction was found to be an excellent method for the extraction of active phytochemical compounds in *H. sabdariffi* (Wang et al, 2013). The more phytochemical compounds extracted, the higher the antibacterial potential of the extract (Yagoub et al, 2008).

Another major factor responsible for the different results between the studies is the use of different extraction solvents such as water, methanol and ethanol (Al Hamshimi et al, 2012; Yagoub et al, 2008). The antibacterial potential of *H. sabdariffi* and *T. indica* extracted using ethanol as an extraction solvent was superior compared to all the other solvents, including water (Yagoub et al, 2008; Al Hashimi et al, 2012). Hence, ethanol is considered to be the best solvent to extract active phytochemicals from *H. sabdariffi* and *T. indica* (Al Hamshimi et al, 2012).

Unfortunately, traditional healers in the outskirts and rural areas using *H. sabdariffi* and *T. indica* extracts are not well equipped with the sophisticated instruments and solvents that enhance the extraction of the phenolic compounds and this may influence the antibacterial efficacy of the plant extracts they use in their practices (Al Hashimi et al, 2012; Wang et al, 2013). In light of the fact that water is the most readily available extraction solvent for the majority of the population, a higher concentration of the plant could possibly be used to achieve effective beneficial concentrations of the phytochemical
A. digitata and M. oleifera did not exhibit any antibacterial properties against S. mutans and E. coli. This may be attributed to the low concentration of A. digitata (1g/ml) seeing that dilution influences the antibacterial potential of the plant extract (Yagoub et al, 2008). Secondly, the concentration and presence of active metabolites in a plant depends on the area where the plant was cultivated (Bruneton et al, 1999). Perhaps this also contributed to the different results achieved in this study where Sudanese grown A. digitata was used compared to the study of Yagoub et al (2008) who used A. digitata grown in India. Sudan and India have different environmental factors such as the climate, chemical nature of the soil in which the plant is grown, harvesting methods and the conditions of drying (Bruneton et al, 1999). These factors could have an effect on the phytochemical composition of the plant and therefore on its antibacterial effect (Rios and Recio, 2005).

In contrast, some studies in the literature revealed that M. oleifera has an antibacterial effect (Rao et al, 2011; John et al, 2013). However, the effect does not seem to be very strong (John et al, 2013). However, the results of this study showed that M. oleifera has no antibacterial potential and this result is supported by Peixoto et al (2011).

The M. oleifera leaves used in this study were grown and harvested in South Africa and exhibited no antibacterial effect. On the other hand, the M. oleifera leaves that were used in the studies that did display positive antibacterial activity against S. mutans, were cultivated, grown and harvested in India (Rao et al, 2011; John et al, 2013). Perhaps the different environmental and soil factors in the two countries influenced the phytochemical composition of the plant
(Bruneton et al, 1999; Rios and Recio, 2005). This may be a crucial factor in understanding why the South African and Indian M. oleifera leaves produced different results.

Moreover, the studies that achieved a positive antibacterial effect of M. oleifera against S. mutans utilised a different methodology compared to this study (J Rao et al, 2011; John et al, 2013). M. oleifera leaves extracted using the Soxhlet apparatus had an antibacterial effect against S. mutans which was superior to that of the cold extraction method that was used in this study (John et al, 2013). Furthermore, M. oleifera extracted by using the cold extraction method also exhibited an antibacterial effect against S. mutans if the M. oleifera was allowed to soak in the ethanol for 2 days (John et al, 2013). However, in the current study, M. oleifera was allowed to soak in the ethanol for 1 day only and no antibacterial effect was found. The limited time in which M. oleifera leaves were allowed to soak in the solvent may have resulted in the minimal extraction of the active metabolites from the leaves. As a result of the low extraction of active metabolites from the M. oleifera in this study, no antibacterial result was achieved.

In the literature, it is evident that M. oleifera has an antibacterial effect against the Gram positive S. mutans (John et al, 2013). However, the antibacterial effect of M. oleifera leaf extracts against the Gram negative bacteria is debatable (Peixoto et al, 2011). Peixoto et al (2011) examined the antibacterial effect of M. oleifera leaves against a number of bacteria including the Gram negative bacteria, E. coli. The latter was found to be resistant to M. oleifera leaves extracted by using ethanol. The results of this study are similar to the study by Peixeto et al (2011) that also found no antibacterial effect of M. oleifera against E. coli bacteria.
The antifungal effect of the ethanolic plant extracts against \textit{C. albicans} was also examined in this study. \textit{T. indica} exhibited no antifungal activity against \textit{C. albicans}. Similar results were achieved by Dabur \textit{et al} (2007). \textit{A. digitata} investigated also revealed no antifungal effect against \textit{C. albicans} and to our knowledge no studies have researched the effect of \textit{A. digitata} against \textit{C. albicans}. In this laboratory study, \textit{C. albicans} displayed a resistance to \textit{M. oleifera} ethanolic extracts. However, Nikkon \textit{et al} (2000) concluded that \textit{M. oleifera} leaf extracts do have a moderate antifungal activity (Nikkon \textit{et al}, 2003). \textit{H. sabdaraffi} revealed an antifungal activity against \textit{C. albicans}. However, Edema \textit{et al} (2012) has shown that ethanol extracts of \textit{H. sabdaraffi} have an evident antifungal effect against \textit{C. albicans}.

The negligible antifungal effect achieved in this study may be attributed to many factors. The low concentration of the plant extracts in the study may have influenced the phytochemical activity of the plant extract against the organism (Yagoub \textit{et al}, 2008). Furthermore, the concentration of the plant extract is relatively low compared to the other studies that produced antifungal activity (Nikkon \textit{et al}, 2003; Edema \textit{et al}, 2012). Moreover, the plant’s weak antifungal property may be attributed to the absence of the specific chemical structure of the plants metabolites that allow it to combine to the specific molecules and receptors that inhibit growth and replication of the fungus (Mahmoud \textit{et al}, 1999).

None of the indigenous plants investigated in this study revealed any antifungal properties. However, \textit{H. sabdaraffi} and \textit{T. indica} showed antibacterial properties against \textit{S. mutans} and \textit{E. coli}, in contrast to \textit{M. oleifera} and \textit{A. digitata}. Therefore, due to the fact that \textit{H. sabdaraffi} and \textit{T.}
*indica* displayed positive antibacterial activity, it can be recommended for use as an antimicrobial agent against *E. coli* and *S. mutans* in different ways.

*S. mutans* is a bacterial strain that health providers have failed to control and is causing a worldwide epidemic, especially in relation to oral health. *S. mutans* is responsible for dental caries which is considered to be the most prevalent chronic disease globally and constitutes a costly burden to health-care services (WHO, 2003). The treatment of dental diseases is expensive, accounting for between 5% and 10% of total health care expenditures in industrialised countries (WHO, 2003). In most developing low-income countries, such as African countries, the prevalence rate of dental caries is high and more than 90% of caries is untreated (WHO, 2003). An estimated 5 billion people worldwide suffer from dental caries (WHO, 2003).

The use of a chemical agent as an adjunct to mechanical tooth brushing is recommended to suppress the cariogenic microflora (Gripp *et al*, 2002). The exclusive use of chemical agents was found to be effective as a short term control of cariogenic microflora (Gripp *et al*, 2002). Short term control refers to cases where the patients are unable to brush their teeth such as those recovering from oral surgery where it is painful for them to brush their teeth (Gripp *et al*, 2002).

There are many antibacterial chemical agents on the market such as chlorhexidine mouth rinses, herbal mouth rinses and essential oils mouth rinses. These mouth rinses can either contain alcohol or not (Charles *et al*, 2012; Nagappan *et al*, 2012).
The most frequently used chemical agent in oral health is chlorhexidine which has been a gold standard with regard to anti-plaque agents formula (Van Leeumen, 2011). Chlorhexidine binds to soft and hard tissues in the mouth, enabling it to act over an extended period after application of a formula (Van Leeumen, 2011). However, chlorhexidine has several side effects, such as staining and the fact that it alters patients’ taste. The side effects limit the use of chlorhexidine as a long term chemical plaque control measure (Flotra et al, 1971).

Due to the side effects associated with chlorhexidine, researchers have investigated other alternative mouth rinses such as herbal mouth rinses which include riphala, tulsi patra, jyestiamadh, neem, clove oil, pudina and ajwain, used currently and essential oil preparations which include thymol (0.064 %), eucalyptol (0.092 %), menthol (0.042 %), and methylsalicylate (0.060 %) (Ranjan et al, 2011; Charles et al, 2012).

Herbal mouth rinses were found to have antimicrobial effects with minimal side effects on the oral tissues in comparison with synthetic drugs (Ranjan et al, 2011). However, the literature reports that chlorhexidine has a superior antibacterial effect against *S. mutans* compared to Herbal mouth rinses (Nagappan et al, 2012). When integrating the literature regarding the superiority of chlorhexidine in comparison to herbal mouth rinses, it was found that few studies are documented in the literature on the antimicrobial effects of herbal products against oral pathogens. Therefore, additional long-term *in vivo* studies with a larger sample size and *in vitro* investigations on the efficacy of herbal extracts on oral microbial flora should be conducted before a definite conclusion can be reached (Nagappan et al, 2012).
Although herbal mouth rinses were found to be less effective than the chlorhexidine gluconate rinse, the herbal rinse was found to be more effective than the essential oil rinse in inhibiting the growth of oral bacteria *in vitro* (Haffajee *et al*, 2008).

In this study the ethanolic extracts of the fruit of *T. indica* and calyces of *H. sabdariffa* showed an antibacterial potential against *S. mutans* (Yagoub *et al*, 2008; Al Hashimi *et al*, 2012). These plant extracts can therefore be used as a mouth-wash and as an adjunct to mechanical brushing in order to suppress the cariogenic microflora. However, the frequent use of *T. indica* may cause dental erosion due to its acidic pH (Abukakar *et al*, 2008). Therefore, further *in vivo* and *in vitro* studies need to be conducted in order to ensure the safe use of the herbal extracts in commercial dental products.

Another concern is the use of ethanol as the extraction solvent, as the ethanolic extracts of *T. indica* and *H. sabdariffa* were found to have superior antibacterial properties compared to other extracts prepared, using different solvents (Yagoub *et al*, 2008; Al Hashimi *et al*, 2012). The argument against adding ethanol to the mouth-rinse is threefold: 1) the well-known carcinogenic potential of ethanol (Elmore 1995); 2) the tissue irritating properties, which preclude its use in radiation or chemotherapy damaged epithelial surfaces and 3) in immune-compromised individuals whom are more prone to mucocitis, alcohol can further complicate the condition by causing irritation of oral mucosa. Alcohol or ethanol containing mouth-rinses are also contra-indicated in alcoholic patients as it can increase the intensity of the side effects of alcohol abuse (Eldridge 1998). Lemos-Junior and Villoria (2008) also reported that mouth rinses, which
contained 26.9% ethanol, could be lethal for children weighing less than 11 kg if 141 grams to 282 grams were ingested. Furthermore, the availability of ethanol in underprivileged areas for preparation of ethanolic plant extracts that can be used as mouth-wash is of great concern. More investigations are recommended in order to assess if it is possible that by increasing the concentration of *T. indica* and *H. sabdariffa* in water extracts, an antibacterial effect can be reached as significant as that noticed in their ethanolic counterparts. This will then allow for its’ use and preparation in the broader population and therefore will be a more convenient alternative chemical plaque control measure for the disadvantaged populations of Africa.

The ethanolic extracts of *T. indica* and *H. sabdariffa* also exhibited an antibacterial effect against *E. coli* which is one of the primary microorganisms responsible for the failure of root canal treatment (Krause *et al.*, 2007). Perhaps *T. indica* and *H. sabdariffa* can be used as an adjunct to mechanical instrumentation in order to disinfect the canal and thus increase the success of root canal treatment. However, further research should be performed to determine the antibacterial efficacy in *in-vivo* conditions.

The burden of disease in Africa is not limited to dental caries (de Onis *et al.*, 2004). Africa also holds the highest prevalence of malnutrition globally and this is a major problem, especially in the Sub-Saharan region (de Onis *et al.*, 2004). The malnourished African individual is an easy target for bacterial infections as a result of a relatively low immunity (Osman *et al.*, 2004).
Moreover, the unhygienic environment and lack of sanitation in the rural and disadvantaged areas in Africa further exacerbates the problem and provides an environment that facilitates the growth and establishment of virulent infectious bacteria, thus increasing the risk of infection in African populations at large (Ishii et al, 2008). The *E. coli* strain is among the many growing bacterial strains and is one of the most frequent causes of the many common bacterial infections, including gastroenteritis, urinary tract infection, neonatal meningitis and pneumonia (Ishii et al, 2008). The risk of contracting a disease from ingesting contaminated food, drinking contaminated water and personal contact with an infected individual in Africa is extremely high (Ishii et al, 2008). Perhaps *E. coli* can be treated by administering drugs synthesised from indigenous plants such as *T. indica* and *H. sabdariffa* which have shown antibacterial activity against *E. coli*.

In view of the fact that the prevalence of underweight, malnourished children is forecasted to increase from 24.0% to 26.8%, an inexpensive and effective solution needs to be found to eliminate malnutrition in the African population (de Onis et al, 2004). Seeing that the African soil is suitable for the growth of indigenous plants such as *M. oleifera*, *A. digitata* and *T. indica*, which are highly nutritious sources and are affordable by the underprivileged consumer (Osman et al, 2004; Fahey et al, 2005; Ishola et al, 1990) increased consumption of these plants may prevent many from the scourge of malnutrition. African governments need to promote the cultivation of plants such as *M. oleifera*, *A. digitata* and *T. indica* which may provide a simple answer for the malnutrition scourge in Africa.
The high prevalence of diseases in African countries reflects the poor quality of life of the African individual (http://www.worldbank.org/en/region/afr/overview). Africa has failed to strive economically and provide better health and wealth to its population. Although Africa is a resource-rich continent, many African countries are poor (Frank et al, 1979). In March 2013, Africa was identified as the world’s poorest inhabited continent (http://www.worldbank.org/en/region/afr/overview). The super-powers and their allies in Europe, North America and East Asia inadvertently benefit from the natural resources and poverty of countries such as Africa (Frank et al, 1979).

The sad reality is that we live in a capitalistic world where pharmaceutical companies are more interested in their own wealth than the health of the masses (Frank et al, 1979). Africa is a continent which has been exploited by this capitalism (Frank et al, 1979). The root of the problem lies with the inability of the African governments to provide basic healthcare to the African consumer. This has subsequently allowed giant pharmaceutical interest groups such as multi-national foreign companies to fill the healthcare gap at a high cost. The price of good health has become very expensive and unaffordable for the underprivileged African consumer (Frank et al, 1979).

Some economists believe that the poorer regions must reduce their trading ties with the developed world in order to prosper (Frank et al, 1979). Africans need to improve the economy of their countries with the resources they have available (Frank et al, 1979). Traditional medicine has been used for many generations in the African continent. Centuries of use of indigenous medicine in traditional settings bear testimony to its effectiveness and safety (Angel et al, 1998).
Moreover, the plants and herbs used to prepare the herbal remedies that have shown medicinal properties such as *T. indica* and *H. sabdariffa* can easily be cultivated in African soil. The vision is to allow the growth of local traditional medicine industries in Africa, which will result in an enhancement of the African economy, provision of jobs and promotion of affordable drugs to the African consumer.

Moreover, in the West, there has been an increased preference towards herbal products, mostly due to the belief that ‘natural’ products are safer than those which are of ‘synthetic’ origin (Angel *et al.*, 1998). Due to the fact that there is an increase in demand of herbal medicine, the local African traditional medical products are anticipated to expand beyond African borders.

However, this is more easily said than done. African countries need to focus their research efforts on new herbal drugs (Mander, *et al.* 1997). Research also needs to be directed at improving the current harvesting, production, processing, storage and treatment technology (Mander *et al.*, 1997). Research campaigns are required to address the questionable sustainability of the current herbal trade to make it more profitable and tempting to the private sector, given the economic, social and cultural benefits that this type of trade will provide to the African countries. A sustainable industry needs to generate cooperation between all current and/or potential role players from the rural harvesters and traders to the research foundations and healers (Mander *et al.*, 1997).

Another major barrier to the expansion of a herbal industry is obtaining legislation for herbal products (WHO, 2005). The increased popularity of the herbal market over the last 15 years
(WHO, 2005) has necessitated the provision of policies and laws on herbal medicines for the safety of its consumers (WHO, 2005). The regulation of herbal medicines ensures the safety, efficacy and quality of herbal medicinal products marketed (WHO, 2005). Legislation for internal laws in national policies is required to govern the herbal trade within the country and thereby control the quality of imported herbal products (WHO, 2005). The WHO (2005) global survey found that of the 141 countries that responded, 65% apply internal laws and regulations and 34% do not have any rules governing the herbal trade. Moreover, the WHO (2005) report also stated that 60% of countries have no national policy for herbal drugs, whereas 38% of countries do have a national policy.

The above mentioned percentages are dispersed among two extremes in the African and Western and European continents respectively. In the African continent, traditional medicine is widely consumed yet there is a lack of internal and national laws or regulations to govern traditional medicine (WHO, 2005). There is no regulatory authority or expert committees for traditional medicine in African countries (WHO, 2005). A regulatory body is still in the process of being developed. Most African governments lack general consensus on the regulation of traditional medicine (WHO, 2005). Lack of regulation of traditional herbal medicine is seen in countries such as Angola, Ethiopia and Kenya. However, credit must be given to some countries such as South Africa that are seeking to finalise and implement national and internal policies (WHO, 2005). Therefore, the safety of the African consumer cannot be guaranteed due to the lack of legislation. Furthermore, the lack of national quality assurance of herbal medicine in African countries has resulted in the lack of interest of investors in this market, especially those who
want to export to developed countries, where quality and scientific evidence of the plant efficacy is crucial (WHO, 2005).

On the other extreme, in the West, the food and drug act does not recognise traditional medicine practices and herbal medicines are categorised under natural health products (WHO, 2005). The authorities in the West still do not approve of alternative medicine (WHO, 2005). Registration of Herbal products is difficult, especially when it comes to polyhedral remedies, which include approximately 40–50 ingredients for the preparation of most traditional remedies. This hampers progress of traditional medicine in the West (http://anh-europe.org/news/european-medicines-agency-releases-second-report-on-eu-herbal-directive).

The Asian continent has taken the best of both worlds. Traditional medicine is widely used in Asia (WHO, 2005). It has provided a well-established favorable proactive market for traditional medicine with National policy laws and regulations for the safety of its consumers (WHO, 2005). Moreover, the herbal trade in the Asian continent has contributed to the enhancement of the Asian economy (WHO, 2005).

Although many barriers and obstacles are perceived for the herbal trade worldwide, the Asian continent has provided a good example and hope for traditional medicine as a promising alternative in the future (WHO, 2005). However, more research is required to further analyse the real value of the antibacterial potential of the plant extracts including *T. indica, H. sabdariffa, M. oeleifera and A. digitata*. There is great controversy in the literature regarding the antibacterial and antifungal efficacy of the plant extracts. The different results achieved in the documented
studies create controversy mainly as a result of the different methodologies performed in the different studies. Therefore, a standardised methodology is essential in order to enhance the reliability of comparison and contrast between the studies. This will hopefully add more credibility to the evidence base of the beneficial effects of indigenous plants and definite conclusions regarding this controversy can be reached. The extraction method and the extraction solvent and its concentration should be standardised. In addition, plant factors such as the soil composition in which the plant has been cultivated should also be taken into consideration. Moreover, research in this field should continue until the active metabolites in the plant are identified. We also recommend that further studies be performed to investigate the mechanism of interaction of the plants with other medicinal plants and antibiotics. In addition, the pharmacokinetic action of the plant extracts should be of high priority.
CHAPTER 6

CONCLUSION AND

RECOMMENDATIONS
Conclusion

*T. indica* and *H. sabdariffa* ethanolic extracts exhibited antibacterial activity against *S. mutans* and *E. coli*. In contrast, *M. oleifera* and *A. digitata* ethanolic plant extracts had no antibacterial effect against *E. coli* and *S. mutans*.

The indigenous plants *T. indica*, *H. sabdariffa*, *M. oleifera* and *A. digitata* displayed no antifungal activity against *C. albicans*.

The current literature contains conflicting information regarding the antibacterial and antifungal potential of the indigenous plants, *T. indica*, *H. sabdariffa*, *M. oleifera* and *A. digitata*. This is probably due to the fact that the different studies utilised different types of methodological processes for the extraction of the active phytochemicals.
Recommendations

*T. indica* and *H. sabdariffa* ethanolic extracts can be incorporated into a mouth rinse preparation which can be used as an adjunct to tooth brushing in order to reduce the bacterial load of the cariogenic *S. mutans* bacteria.

*T. indica* and *H. sabdariffa* ethanolic plant extracts can also be utilised as an irrigant during root canal treatment in order to eliminate *E. coli* which is one of the primary microorganisms responsible for root canal failure.

The effectiveness of *T. indica* and *H. sabdariffa* ethanolic plant extracts against *E. coli* also means that it can be recommended for treatment of diseases caused by *E. coli*, such as intestinal diarrhoea. A consumable ethanolic extract of *T. indica* or *H. sabdariffa* may be used to treat intestinal diseases caused by *E. coli*. Furthermore, the active phytochemicals can be extracted and concentrated in tablets and pills to act as an effective drug against *E. coli* intestinal diseases.

However, the preparation of *T. indica* and *H. sabdariffa* antibacterial products needs to be investigated further in order to produce an antibacterial agent that is safe and efficient for commercial use.
Ethanol is recommended as the solvent to be used for extraction of the phytochemicals as it has shown to be the most efficient in dissolving the active phytochemicals in *T. indica* and *H. sabdariffa*. However, in light of the fact that water is the most easily available extraction solvent for the majority of the underprivileged African population, additional research studies are recommended to assess whether increasing the concentration of *T. indica* and *H. sabdariffa* water extracts will result in a significant antibacterial effect similar to their ethanolic counterparts.

Additional research is required to further assess the real value of the antibacterial potential of the plant extracts. There is a great debate in the literature regarding the antibacterial and antifungal efficacy of the plant extracts. The different results achieved in the studies are creating this controversy, mainly due to the different methodologies performed in the different studies.

A standardised method is necessary to enhance the reliability of comparison and contrast between the studies. Therefore, the extraction method and the extraction solvent and its concentration should be standardised.

Research in the field of beneficial effects of indigenous plants should continue until the active metabolites in the plant are identified. Studies should also be conducted to investigate the mechanism of interaction of the plants with other medicinal plants and antibiotics.
Limitations

The limitations of this study were that:–

• There was a lack of standardisation in the process of growth, cultivation and drying of the plant samples used.

• A relatively low concentration of 1g/ml of the plant extract was used.

• Only one concentration of plant extract was used

• The indigenous plants were allowed to dissolve in ethanol for only one day before being tested which did not allow for a higher concentration of phytochemical from being extracted

• A small sample size was included.
REFERENCES


Amubode, F.O., Fetuga, B.L., 1983. Proximate composition and chemical assay of the methionine, lysine and tryptophan concentrations of some forest tree seeds. Food Chemistry, 12:


Brady, O., 2011. The characterisation and bioactivity determination of *Adansonia digitata* L. fruit pulp, for commercial product development. Thesis of bachelor of Science in Nutraceuticals for Health and Nutrition, Dublin Institute of Technology, Cathal Brugha Street, 117.


Fahey, J.W., 2005. Trees for Life Journal 1:5 The electronic version of this article is the complete one and can be found online at:
<http://www.tfljournal.org/article.php/20051201124931586>


