

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

**BALSAM ELASHI**

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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**SUPERVISOR**

**DR FATHIMA PEERBHAY**

**CO-SUPERVISOR**

**DR NICOLAAS J BASSON**

## 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. indicia*, *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. indicia*, *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. sabdariffa* 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. sabdariffa* 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:



The image shows a handwritten signature in black ink over a horizontal dotted line. The signature is cursive and appears to read 'Balsam Elashi'. In the background, there is a faint watermark of a classical building with columns and the text 'UNIVERSITY of the WESTERN CAPE'.

# Acknowledgements

A Special word of thanks to my supervisors, Dr. Fathima Peerbhay and Dr. Nicky Basson for their guidance, motivation and effort in assisting me with this study. They were my academic tutors and my family in Cape Town, South Africa.



# 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 Ndzelen, Patrick Iunga and Teboho Nchaba.*



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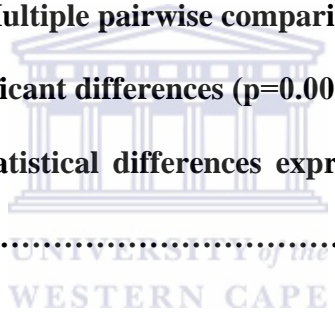
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# **CHAPTER 1**

## **INTRODUCTION AND LITERATURE**

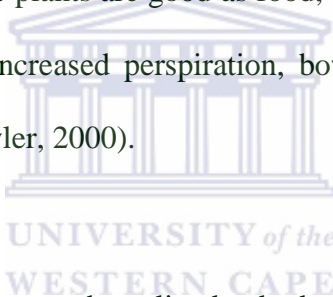
### **REVIEW**



# 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).

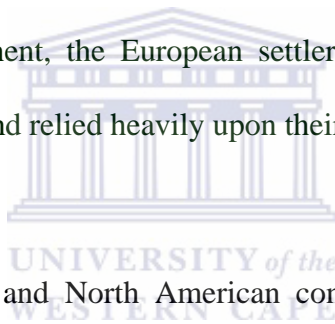


Around that time, herbal medicine was also developing in China (Shaw, 1998). In fact, herbal medicine books were written in China which contained over 300 herbs including Ma huang, or Chinese ephedra (Shaw, 1998). Ma huang is still widely used today and is the herb from which Western scientists have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

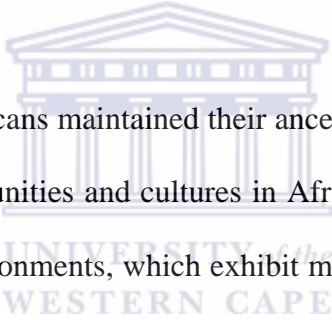
Furthermore, Europe gained its herbal knowledge in the middle ages from monks who studied the plants and translated the Arabic work on herbalism (Tyler, 2000). Moreover, after the discovery of the American continent, the European settlers had great respect for the herbal wisdom of the American Indians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

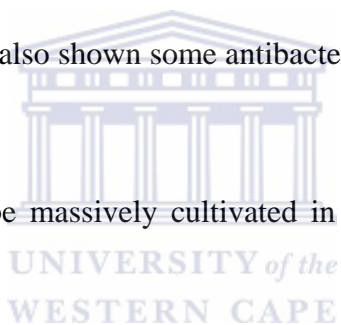


Unlike the Western world, the Africans maintained their ancestral teachings, cultures, beliefs and life styles. Currently, many communities and cultures in Africa consume a variety of foods and beverages indigenous to their environments, which exhibit medicinal value (Nakato *et al*, 2010). Plants form 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*,

2006). 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.



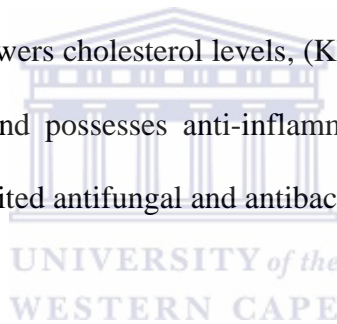
## **2. *Tamarindus indica*, (Leguminosae)**

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, *Caesalpiniaceae* (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 (Khazada *et*



*al*, 2008). In addition, *T. indica* has a high mineral content which includes potassium, magnesium, phosphorous and calcium (Khazada *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, (Khazada *et al*, 2008) has a cytotoxic effect (Kobayashi *et al*, 1996), and possesses anti-inflammatory properties (Khazada *et al*, 2008). In addition, *T. indica* exhibited antifungal and antibacterial properties (Pousset, 1989).

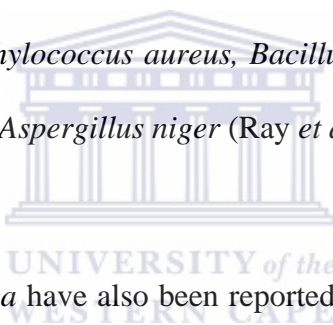


The antibacterial potential of *T. indica* is due to the presence of active phytochemicals which include flavonoids, tannins, alkaloids and other aromatic compounds detected in the plant (Yagoub *et al*, 2008). Phytochemicals are compounds in indigenous plants which are responsible for the antibacterial 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 with 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, anti-pyretic, anti-inflammatory, anti-ulcer, anti-spasmodic, anti-epileptic, anti-inflammatory anti-biosis, hepatoprotective and even reduction of *Schistosoma 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).

## 6. *Streptococcus mutans* (*S. mutans*) and dental decay

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, sacharolytic, facultative and adhesive organisms which adhere to the tooth structure and cause dental caries (Loesche, 1986). Intra oral stresses,

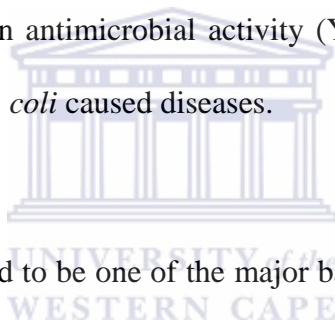


such as high and frequent sucrose consumption (Resine and Douglass, 1998; Tanzer, 1979), favor the colonisation of *S. mutans* and promote the carious process and enamel erosion (Resine and Douglass, 1998). Before sucrose enters the cells, less than 10 percent of sucrose is transformed by hexotransferases into glucans and fructans that either diffuse to the surrounding or remain associated with the cell (Ciardi, 1983; Gibbons *et al*, 1968; Hamada *et al*, 1980). The enzymes glucosyltransferase metabolise sucrose, produces the soluble and insoluble glucans with a release of fructose (Marsh and Martin, 1992). *S. mutans* are not particularly good colonisers (Rickard *et al*, 2003) as they are initially unable to bind to the acquired enamel pellicle formed on the tooth surface. However, *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). *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 *S. mutans* bacterial load was found to reduce the risk of dental caries (Fennis-le *et al*, 1998; Gripp *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. *Escherichia coli* (*E. coli*)**

The diseases in under-privileged areas such as Africa are not limited to dental caries (de Onis *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 *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)

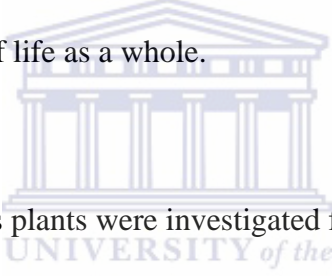
Oral candidiasis is the most common oral fungal disease in children (Ghannoum *et al*, 1990). It is caused by the over growth of a yeast-like fungus (Akpan *et al*, 2002). The chief opportunistic fungal pathogen is *C. albicans*. This opportunistic infection is highly prevalent in patients who are malnourished, 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 Amphotericin 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**



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# 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. indicia*, *A. digitata*, *H. sabdariffi* 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**

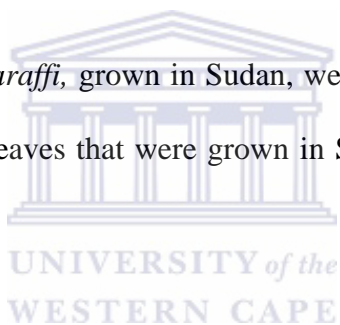
The inoculum suspensions for *S. mutans*, *E. coli* and *C. albicans* were prepared using a direct colony suspension method. The micro-organisms were cultured in Brain Heart Infusion Agar for 24 hours. The colonies were then transferred directly from the isolated colonies to the Triptone Soy Broth (Oxoid 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**

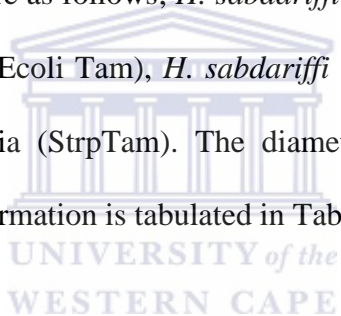
## **RESULTS**



# 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*.

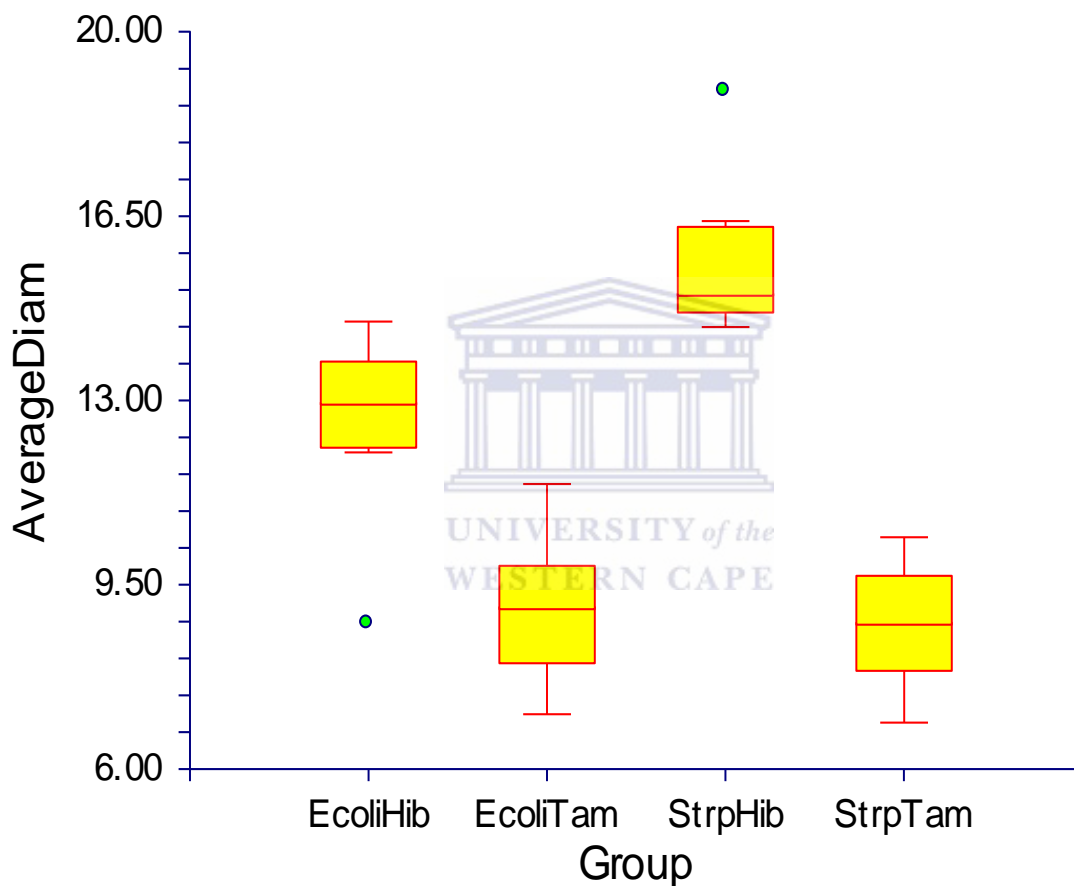
*H. sabdariffi* and *T. indica* did not show any antifungal effect against *C. albicans*. However, the antibacterial effect against *S. mutans* and *E. coli* was evident. The average inhibition diameter zone was calculated in each plate per sample. There were 10 samples per group. The groups which showed 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**

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

**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 25<sup>th</sup> and 75<sup>th</sup> 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.

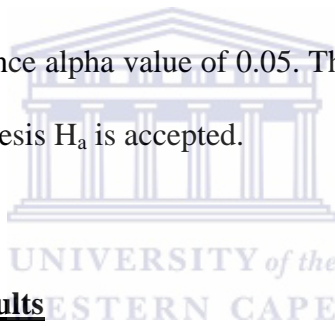
In the EcoliHib group the inhibition zone ranged from 12.01mm to 14.50mm with a median of 12.96mm. The inhibition zones of EcoliTam had a median value of 9.075mm ranged from 7.04 mm to 11.41mm. The inhibition zone of StrptHib ranged from 14.39 mm to 16.41 mm with a median of 15.25mm. The inhibition zones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

The graph clearly demonstrates that *H. sabdariffi* has a higher antibacterial effect against *E. coli* and *S. mutans*, with a median of 12.96mm and 15.025mm respectively, compared to the antibacterial effect of *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 *H. sabdariffi* towards the Gram positive *S. mutans* and the Gram negative *E. coli*. This difference cannot be seen with *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**

K(Observed value)	30.184
K (Critical value)	7.815
DF	3
p-value (Tailed)	< 0.0001
Alpha	0.05

### **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**

	StrpHib	StrpTam	EcoliHib	EcoliTam
StrpHib	1	< 0.0001	0.000	<0.0001
StrpTam	< 0.0001	1	< 0.0001	0.646
EcoliHib	0.000	< 0.0001	1	< 0.0001
EcoliTam	< 0.0001	0.646	< 0.0001	1

**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**

Group	Count	Mean	Different from Groups
Ecoli Hib	10	12.65725	EcoliTam,StrpTam,StrpTam
EcoliTam	10	9.08375	EcoliHib,StrpHib
StrpHib	10	15.569	EcoliHib,EcoliTam,StrpTam
StrpTam	10	8.74625	Ecoli,StrpHib

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*.

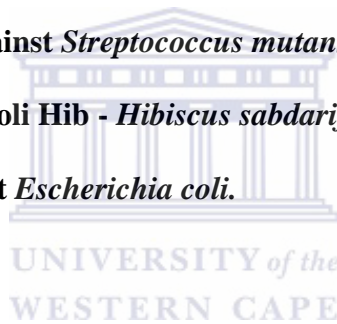
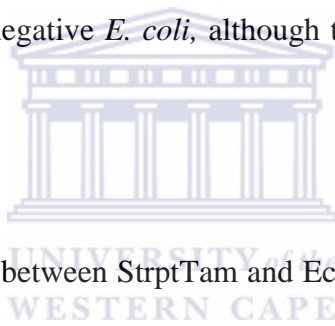


Table 5 illustrates the magnitude of the statistical differences between the groups. If the absolute statistical value calculated is more than that of the critical value (7.229), there is a statistical difference between the 2 groups compared, the higher the statistical value, the greater the difference 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. sabdariffi* 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 (7.2229mm). 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.



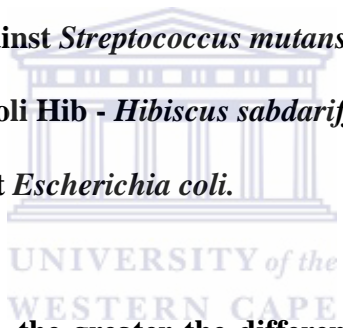
There was no statistical difference between StrpTam and EcoliTam, which shows that *T. indica* has a similar antibacterial effect against *S. mutans* and *E. coli*, respectively. EcoliHib (12.65725mm) showed a statistical difference (12.800mm) when compared to EcoliTam (9.0837mm), indicating that *H.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

	StrptHib	StrptTam	EcoliHib	EcoliTam
StrptHib	0	24.800	10.800	23.600
StrptTam	-24.800	0	-14.000	-1.200
EcoliHib	-10.800	14.000	0	12.800
EcoliTam	-23.600	1.200	-12.800	0

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**



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## Discussion

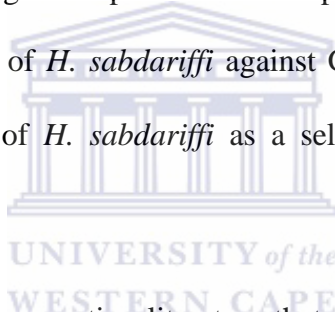
Traditions of yesterday may become medicine for tomorrow. Indigenous plants are widely consumed in Africa and have been used for many generations and are still currently being used to treat various diseases and infections. Additional evidence is required to confirm that the benefits of some indigenous 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. sabdariffa* (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 *H. sabdariffa* exhibited antibacterial effects against *S. mutans* and *E. coli* and this result is supported by several studies (Fullerton *et al*, 2011; Al Hashimi *et al*, 2012; Edema *et al*, 2012). Furthermore, the findings of this study revealed that *H. sabdariffa* had a greater effect against Gram positive *S. mutans* than Gram negative *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 *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 *H. sabdariffa* against Gram positive *S. mutans* and Gram negative *E. coli*. Hence, the use of *H. sabdariffa* as a selective drug against Gram positive bacteria is suggested.



It is clear from this study and the supporting literature that *T. indica* and *H. sabdariffa* have an antibacterial effect against *S. mutans* and *E. coli*.

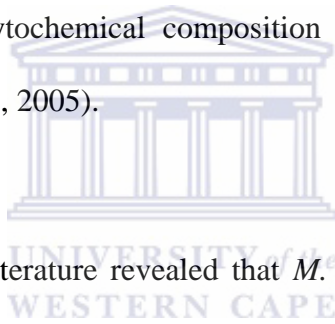
Although, the other studies conducted had different methodologies compared to this study, the objectives which were to test the ethanolic extract of *H. sabdariffa* and *T. indica* against *S. mutans* and *E. coli*, of all the studies compared, were the same (Darbur *et al*, 2007; Yagoub *et al*, 2008; Fullerton *et al*, 2011; Al Hashimi *et al*, 2012; Edema *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. sabdariffa* (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.sabdariffa* 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. sabdariffa* and *T. indica* ( Al Hamshimi *et al*, 2012).

Unfortunately, traditional healers in the outskirts and rural areas using *H. sabdariffa* 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 Peixoto *et al* (2011) that also found no antibacterial effect of *M. oleifera* against *E. coli* bacteria.

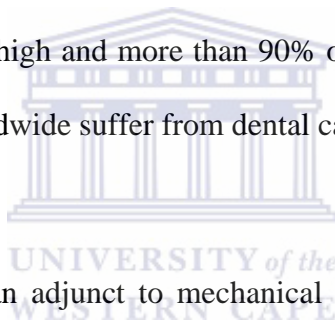
The antifungal effect of the ethanolic plant extracts against *C. albicans* was also examined in this study. *T. indica* exhibited no antifungal activity against *C. albicans*. Similar results were achieved by Dabur *et al* (2007). *A. digitata* investigated also revealed no antifungal effect against *C. albicans* and to our knowledge no studies have researched the effect of *A. digitata* against *C. albicans*. In this laboratory study, *C. albicans* displayed a resistance to *M. oleifera* ethanolic extracts. However, Nikkon *et al* (2000) concluded that *M. oleifera* leaf extracts do have a moderate antifungal activity (Nikkon *et al*, 2003). *H. sabdaraffi* revealed an antifungal activity against *C. albicans*. However, Edema *et al* (2012) has shown that ethanol extracts of *H. sabdaraffi* have an evident antifungal effect against *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 *et al*, 2008). Furthermore, the concentration of the plant extract is relatively low compared to the other studies that produced antifungal activity (Nikkon *et al*, 2003; Edema *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 *et al*, 1999).

None of the indigenous plants investigated in this study revealed any antifungal properties. However, *H. sabdaraffi* and *T. indica* showed antibacterial properties against *S. mutans* and *E. coli*, in contrast to *M. oleifera* and *A. digitata*. Therefore, due to the fact that *H. sabdaraffi* and *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).

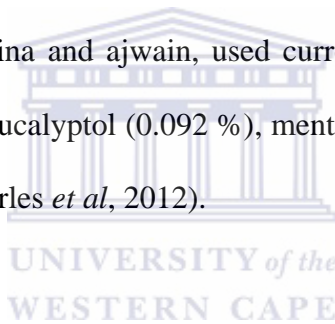


The use of a chemical agent as an adjunct to mechanical tooth brushing is recommended to suppress the cariogenic microflora (Gripp *et al*, 2002). The exclusive use of chemical agents was found to be effective as a short term control of cariogenic microflora (Gripp *et al*, 2002). Short term control 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).

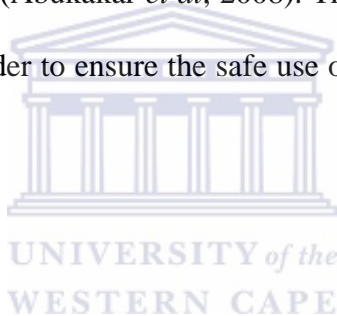
Due to the side effects associated with chlorhexidine, researchers have investigated other alternative mouth rinses such as herbal mouth rinses which include riphala, tulsi patra, jyestiamadh, neem, clove oil, pudina and ajwain, used currently and essential oil preparations which include thymol (0.064 %), 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 contraindicated 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

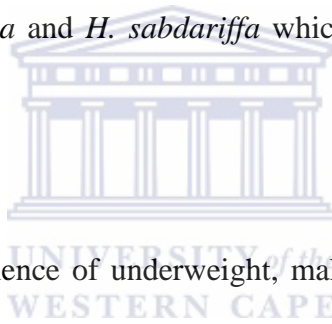


contained 26.9 % ethanol, could be lethal for children weighing less than 11 kg if 141 grams to 282 grams were ingested. Furthermore, the availability of ethanol in underprivileged areas for preparation of ethanolic plant extracts that can be used as mouth-wash is of great concern. More investigations 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.

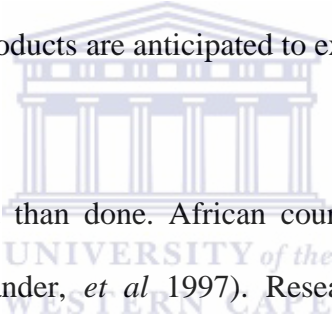
The high prevalence of diseases in African countries reflects the poor quality of life of the African individual (<http://www.worldbank.org/en/region/afr/overview>). Africa has failed to strive economically and provide better health and wealth to its population. Although Africa is a resource-rich continent, 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.

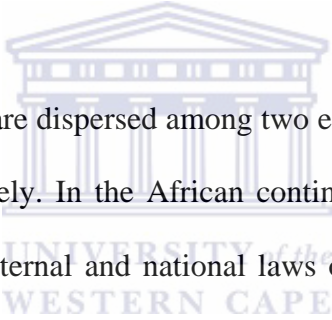
Moreover, in the West, there has been an increased preference towards herbal products, mostly due to the belief that 'natural' products are safer than those which are of 'synthetic' origin (Angel *et al*, 1998). Due to the fact that there is an increase in demand of herbal medicine, the local African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

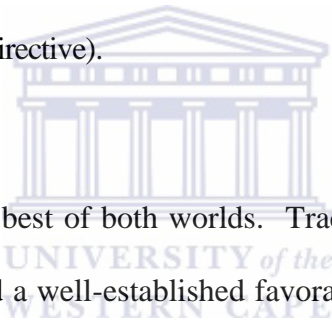
(WHO, 2005) has necessitated the provision of policies and laws on herbal medicines for the safety of its consumers (WHO, 2005). The regulation of herbal medicines ensures the safety, efficacy and quality of herbal medicinal products marketed (WHO, 2005). Legislation for internal laws in national policies 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).

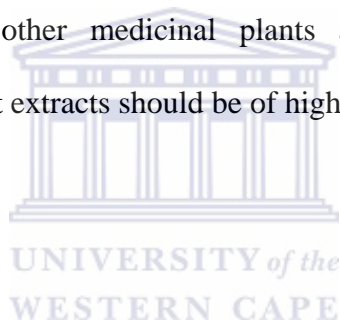
On the other extreme, in the West, the food and drug act does not recognise traditional medicine practices and herbal medicines are categorised under natural health products (WHO, 2005). The authorities in the West still do not approve of alternative medicine (WHO, 2005). Registration of Herbal products is difficult, especially when it comes to polyherbal 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**



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## 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*.

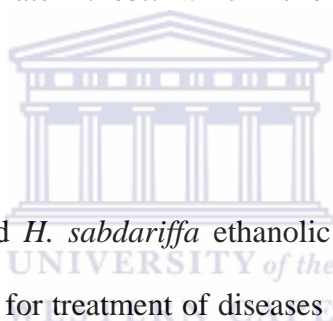
The indigenous plants *T. indica*, *H. sabdariffa*, *M. oleifera* and *A. digitata* displayed no antifungal activity against *C. albicans*.

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

Abubaker, M.G., Ukwuani, A.N., Shehu, R.A., 2008. Phytochemical screening and antimicrobial of *Tamerindus Indica* Pulp extract. *Asian Journal of Biochemistry*, 3 (3), 134-138.

Aida, P., Rosa, V., Blamea, F., Tomas, A., Salvador, C., 2001. Paraguayan plants used in traditional medicine short communication, *J Ethnopharmacol*, 16, 93-98.

Akindahunsi, A.A., Olaleye, M.T., 2003. Toxicological investigation of aqueous methanolic extract of *Hibiscus sabdariffa* L. *J Ethno-pharmacol*, 89, 161–164.

Akpan, A., Morgan, R., 2002. Oral candidiasis. *Postgrad Med J*, 78, 455–459.

Alfolabi, O., Popoola, T., 2005. The effects of Baobab pulp powder on the microflora involved in temp fermentation. *European Res Tech*, 220, 187-190.

Al-Hashimi, A., 2012. Full Length Research Paper Antioxidant and antibacterial activities of *Hibiscus sabdariffa* L. extracts. *African Journal of Food Science*, 6(21), 506-511.

Ali, B.H., Al Wabel, N., Blunden, G., 2005. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: A review. *Phytotherapy Research: PTRP*, 75–369.

Al-Qarawi, A.A., Al-Damegh, M.A., El-Mougy, S.A., 2003. Hepatoprotective influence of *Adansonia digitata* Pulp. *J. Herbs Spices Med. Plants*, 10, 1-6.

Alzoreky, N.S., Nakahara, K., 2003. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *International Journal of Food Microbiology*, 80, 223-230.

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:

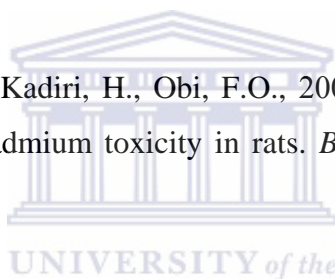
67-72. Anon, 1976. *Tamarindus indica L.* In The Wealth of India (Raw Materials Series), 144-122.

Amin, A., Hamza, A.A., 2006. Effects of roselle and ginger on cisplatin-induced reproductive toxicity in rats. *Asian Journal of Andrology*, 8, 595–607.

Anani, K., Hudson, J.B., de Souza, C., Akpagana, K., Tower, G.H.N., Amason, J.T., Gbeassor, M., 2000. Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. *Pharm Biol*, 38, 40-45.

Angel, M., Kassirer, J.P., 1998. Alternative medicine: The risks of untested and unregulated remedies. *The New England Journal of Medicine*, 339, 839–841.

Asagba, S. O., Adaikpoh, M. A., Kadiri, H., Obi, F.O., 2007. Influence of aqueous extract of *Hibiscus sabdariffa L.* petal on cadmium toxicity in rats. *Biological Trace Element Research*, 115, 47–57.



Batista, O., Duarte, A., Nascimento, J., Simones, M.F., 1994. Structure and antimicrobial activity of diterpenes from the roots of *Plectranthus hereroensis*. *J Nat Prod*, 57, 858–861.

Blomhoff, R., Carlsen, M., Halvorsen, B., Holte, K., Bohn, S., Dragland, S., Sampson, L., Willey, C., Senoo, H., Umesono, Y., Sanada, C., Barikmo, I., Berhe, N., Willett, W., Phillips, K., Jacobs, D., 2010. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutr J*, 9, 3.

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.

Bruneton, J., 1999. *Plantes Médicinales, Pharmacognosie, Phytochimie*. Paris: Tec et Doc.

Chadare, F.J., Linnemann, A.R., Hounhouigan, J.D., Nout, M.J.R., Van Boekel M.A.J.S., 2009. Baobab Food Products: A Review on their Composition and Nutritional Value. *Critic Rev. Food Sc. Nutr*, 49, 254-274.

Chassy, B., 1983. Sucrose metabolism and glucosyltransferase activity in oral streptococci. In R. J. Doyle and J. E. Ciardi (ed.) *Glucosyltransferases, glucans, sucrose and dental caries (a special supplement to Chemical Senses)*. Information Retrieval Limited, Washington, D.C., 3-10.

Charles, C.A., Amini, P., Gallob, J., Shang, H., McGuire, J.A., Costa, R., 2012. Antiplaque and antigingivitis efficacy of an alcohol-free essential oil containing mouth rinse: a 2-week clinical trial. *American Journal of Dentistr.*, 25(4), 195-198.

Chuang, P.H., Lee, C.W., Chou, J.Y., Murugan, M., Shieh, B.J., Chen, H.M., 2007. Anti-fungal activity of crude extracts and essential oils. *Bioresources Technology*, 1, 232-6.

Chen, C. C., Hsu, J. D., Wang, S. F., Chiang, H. C., Yang, M. Y., Kao, E. S, Ho, Y. C., Wang, C. J., 2003. *Hibiscus sabdariffa* extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. *Journal of Agricultural and Food Chemistry*, 51, 5465–5472.

Chopra, I. and Greenwood, D., 2001. Antibacterial agents: basis of action. In: *Encyclopedia of Life Sciences*. Nature Publishing Group, John Wiley and Sons, Limited.

Ciardi, J., 1983. Purification and properties of glucosyltransferases of *Streptococcus mutans*: a review. Doyle and J. E. Ciardi, ed. *Glucosyltransferases, glucans, sucrose and dental caries (a special supplement to Chemical Senses)*. Information Retrieval Limited, Washington, D.C. 51-64.

Conway, G., Toenniessen. G., 1999. Feeding the world in the twenty-first century. *Nature*, 402 (suppl), 55-68.

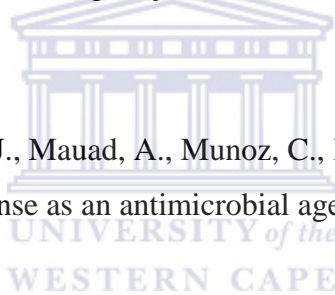
Dabur, R., Gupta, A., Mandal, T.K., Singh, D.D., *et al*, 2007. *Moringa oleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. *Afr. J. Trad. CAM*, 4 (3), 313 – 318.

de Onis, M., Blössner, M., Borghi, E., Frongillo, E.A., Morris, R., 2004. Global Database on Child Growth and Malnutrition; Estimates of global prevalence of childhood underweight in 1990 and 2015. *Journal of American Medical Association*, 291, 2600-6.

Diop, A.G., Sakho, M., Dornier, M., Cissé, M., Reynes, M., 2005. Le baobab africain (*Adansonia digitata* L.): principales caractéristiques et utilisations. *Fruits*, 61, 55-69.

Edema, M.O., Alaga, T.O., 2012. Comparative evaluation of bioactive compounds in the *Hibiscus sabdariffa* and *Suzgium samarangense* juice extracts. *African Crop Science Journal*, 20(3), 179 – 187.

Eldridge, K., Finnie, S., Stephens, J., Mauad, A., Munoz, C., Kettering, J., 1998. Efficacy of an alcohol-free chlorhexidine mouthrinse as an antimicrobial agent. *The Journal of Prosthetic Dentistry*, 80( 6), 685–690.



Elmore, J., Orwitz, R., 1995. Oral cancer and mouthwash use: evaluation of the epidemiologic evidence. *Otolaryngol Head Neck Surg*, 113(3), 253–61.

El-Saadany, S.S., Sitohy, M Z., Labib, S.M., El-Massry, R.A., 1991. Biochemical dynamics and hypocholesterolemic action of *Hibiscus sabdariffa* (*Karkade*). *Nahrung*, 35, 490–567.

European Medicines Agency releases second report on EU herbal Directive. Available from: <http://anh-europe.org/news/european-medicines-agency-releases-second-report-on-eu-herbal-directive> [Accessed: 13.5.14].



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>

Falceve, T.O., Pal, A., Bawankule, D.U., Khanuja, S.P., 2008. Immunomodulatory effect of extracts of *Hibiscus sabdariffa* L. (family *Malvaceae*) in a mouse model. *Phytotherapy*, 22, 664–672.

Fao. 1988. Traditional food plants. Food and Agriculture Organisation of the United Nations, Rome. 24, 63-67.

Farombi, E.O., Fakoya, A., 2005. Free radical scavenging and antigenotoxic activities of natural phenolic compounds in dried flowers of *Hibiscus sabdariffa* L. *Molecular Nutrition and Food Research*, 49, 1118–1120.

Fennis-le, Y.L., Verdonshot, E.H., Burgersdijk, R.C., Konig, K.G., van't Hof, M.A., 1998. Effect of 6 monthly applications of chlorhexidine varnish on the incidence of occlusal caries in permanent molars: A 3 year old study. *Journal of Dentistry*, 26, 233-8.

Ferrara, L. 2005. Antioxidant activity of *Tamarindus indica* L. *Ingredienti alimentary*.;4(6):13–15.

Flotra, L., Gjermo, P., Rolla, G., Waerhaug, J., 1971. Side effects of chlorhexidine mouthwashes. *Scandinavian Journal of Dental Research*, 79, 119-125.

Frank, A.G., 1979. *Dependent Accumulation and Underdevelopment*, New York: Monthly Review Press.; Köhler, G. and Tausch, A., (2001), *Global Keynesianism: unequal exchange and global exploitation*, Nova Publishers; Amin, S. (1976), *Unequal Development: An Essay on the Social Formations of Peripheral Capitalism*, New York: Monthly Review Press.

Fullerton, M., Khatiwada, J., Johnson, J.U., Davis, S., Williams, L.L., 2011. Determination of Antimicrobial Activity of Sorrel (*Hibiscus sabdariffa*) on *Escherichia coli* O157:H7 Isolated from Food, Veterinary, and Clinical Samples. *J Med Food*, 14(9), 950- 956.

Gebauer, J., El-Siddig, K., Ebert, G., 2002. Baobab (*Adansonia digitata L.*): A review on a Multipurpose Tree with Promising Future in the Sudan. *Gartenbauwissenschaft*, 67(4), 155–160.

Ghannoum, M.A. and Radwan, S.S., 1990. *Candida* adherence to epithelial cells. Boca Raton, FL: CRC Press.

Gibbons, R.J., Nygaard, M., 1968. Synthesis of insoluble dextran and its significance in the formation of gelatinous deposits by plaque-forming streptococci. *Arch Oral Biology*, 13, 1249-1262.

Gibbons, R.J., 1972. Presence of an invertase-like enzyme and a sucrose permeation system of *Streptococcus mutans*. *Caries Res*, 6, 122-131.

Grenier, D., Mayrand, D., McBride, B., 1989. Further studies on the degradation of immunoglobulins by black-pigmented *Bacteriodes*. *Oral Microbiol Immunol*, 4, 12–18.

Gripp V.C., Schlagenhaut, U., 2002. Prevention of early mutans streptococci transmission in infants by the professional tooth cleaning and chlorhexidine varnish treatment of the mother. *Caries Research*, 36, 366-72.

Haffajee. A.D., Yaskell, T., Socransky, S.S., 2008. Antimicrobial effectiveness of a herbal mouthrinse compared with an essential oil and a chlorhexidine mouthrinse. *Journal of American Dental Association*, 139(5), 606-11.

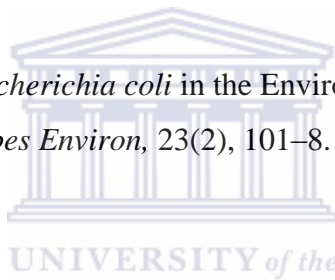
Hamada, S., Slade, H.D., 1980. Biology, immunology and cariogenicity of *Streptococcus mutans*. *Microbiology Research*, 44, 331-384.

Hirunpanich, V., Utaipat, A., Morales, N.P., Bunyapraphatsara, N., Sato, H., Herunsalee, A., Suthisisang, C., 2005. Antioxidant effects of aqueous extracts from dried calyx of *Hibiscus sabdariffa* L. (Roselle) *in vitro* using rat low-density lipoprotein (LDL). *Biological and Pharmaceutical Bulletin*, 28, 477–481.

Ishola, M.M., Agabaji, E.B., Agbaji, A.S., 1990. A chemical study of *Tamarindus indica* (*Tsamia*) fruits grown in Nigeria. *Journal of Science Food Agriculture*, 51, 141-143.

Islam, T.H., Bin Azad, A.H., Akter, S., Datta, S., 2012. Antimicrobial Activity Of Medicinal Plants on *Streptococcus Mutans*, A Causing Agent Of Dental Caries. *International Journal of Engineering Research & Technology (IJERT)*, 1(10), 2278-0181.

Ishii, S., Sadowsky, M.J., 2008. *Escherichia coli* in the Environment: Implications for Water Quality and Human Health. *Microbes Environ*, 23(2), 101–8. [doi:10.1264/jsme2.23.101](https://doi.org/10.1264/jsme2.23.101). [PMID 21558695](https://pubmed.ncbi.nlm.nih.gov/21558695/).



Ismail, A.I., Sohn, W., 1999. A systematic review of clinical diagnostic criteria of early childhood caries. *J Public Health Dent*, 59, 171-191.

Jarvinen, V.K., Rytomaa, I.I., Heinoen, O.P., 1991. Risk Factors in Dental Erosion. *Dent Res*, 70(6), 942-947.

John, R.N., Gala, V.C., Sawan, C.S., 2013. Inhibitory effects of plant extracts of multi species dental biofilm formation *in vitro*. *International Journal of Pharma and Bio Sciences*, 4(2), (B) 487 - 495.

Jo Lin, H.H., Chen, J.H., Kuo, W.H., Wang, C., 2007. Chemopreventive properties of *Hibiscus sabdariffa* L. on human gastric carcinoma cell through apoptosis induction and JNK/p38 MAPK signaling activation. *Chemico-Biological Interactions*. *Journal of Medical and Health Science*, 3, 2: 42 - 44.

Kanbe, T., Li, R-K., Wadsworth, E., *et al* 1991. Evidence for expression of the C3d receptor of *Candida albicans* *in-vitro* and *in-vivo* obtained by immunofluorescence and immunoelectron microscopy. *Infect Immun*, 59, 1832

Kazmi, M.H., Malik, A. Hameed, S., Akhtar, N., Ali, S.N., 1994. An anthraquinone derivative from *Cassia italica*. *Phytochemistry*, 36, 761–763.

Khanzada, S.K., Shaikh, W., Sofia, S., Kazi, T.G., *et al* 2008. Chemical constituents of *Tamarindus Indica L.* Medicinal plant in Sindh. *Pak. J. Bot*, 40(6), 2553-2559.

Kobayashi, A., Adenan, M.L., Kajiyama, S.I., Kanzaki, H., Kawazu, K., 1996. A cytotoxic principle of *Tamarindus indica*, di-n-butyl malate and the structure-activity relationship of its analogues. *Journal of Biosciences*, 51(3-4), 233-242.

Krause, T.A., Liewehr, F.R., Hahn, C.L., 2007. The antimicrobial effect of MTAD, sodium hypochlorite, doxycycline, and citric acid on *Enterococcus faecalis*. *J Endod*, 33, 28-30.

Lemos-Junior, C.A. and Villoria, G.E.M., 2008. Reviewed evidence about the safety of the daily use of alcohol-based mouthwashes. *Braz Oral Res*, 22 (1), 24-31.

Lewis, G.B., Schrire, B., Mackinder, B., Lock, M., 2005. Legumes of the World. Royal Botanical Gardens, Kew.

Lewis, K., Ausubel, F.M., 2006. Prospects of plant derived antibacterials. *Nat Biotechnol*, 24, 1504–1507.

Loesche, W.J., 1986. Role of *Streptococcus mutans* in Human Dental Decay. *American Society for Microbiology, Microbiological Reviews*, 50( 4), 353-380.

Mahmoud, A., Ghannoum., Rice, L.B., 1999. Antifungal agents: Mode of action, Mechanism of Resistance and correlation of these mechanisms with the bacterial resistance. *Clinical microbiology reviews*, 12 (4), 501-517.

Mander, M., 1997. Medicinal Plant Marketing and Strategies for Sustaining the Plant Supply in the Bushbuckridge Area and Mpumalanga Province. South Africa. Pretoria: Department of Water Affairs and Forestry.

Marsh, P. and Martin, M., 1992. *Oral Microbiology*. 2<sup>nd</sup> edition. London: Chapman and Hall

Mikkelsen, L., 1996. Effect of sucrose intake on numbers of bacteria in plaque expressing extracellular carbohydrates metabolising enzymes. *Caries Research*, 30, 65-70.

Morgan, K., 2002. Medicine of the Gods: Basic Principles of Ayurvedic Medicine Available from: <<http://www.compulink.co.uk/~mandrake/ayurveda.htm>> [accessed: 3.3.14].

Murugan, P., Rajesh, A., Athiperumalsami, T., Mohan, V.R., 2008. Screening of certain ethnomedicinal plants for antibacterial activity. *Ethnobotanical Leaflets*, 12, 433-438.

Nagappan, N., John, J., 2012. Antimicrobial Efficacy of Herbal and Chlorhexidine Mouth rinse - A systematic review. *Journal of Dental and Medical Sciences*, V2 (4), 05-10.

Nair, R. and Chanda, S., 2006. Activity of some medicinal plants against certain pathogenic bacterial strains. *Indian Journal Pharmacology*, 38, 142-144.

Nakato, E., Joel-Lewis., Kiwanuka, J-L, Lewis, R.G., 2010. Available from: <[http://www.blackherbals.com/pan\\_african\\_indigenous\\_herbal\\_medicine\\_technology\\_transfer](http://www.blackherbals.com/pan_african_indigenous_herbal_medicine_technology_transfer)> [accessed: August 2013].

Nour, A.A., Magboul, B.I., Kheiri, N.H., 1980. Chemical composition of baobab fruit (*Adansonia digitata L.*). *Tropical Sciences*, 22, 383-388.

Nikkon, F., Saud, Z.A., Rahman, H.M., Haque, E.M., 2003. In vitro antimicrobial activity of the compound isolated from chloroform extract of *Moringa oleifera Lam.* *Pakistan Journal of Biological Sciences*, 6 (22), 1888-1890.

Onacag, O., Hosgor, M., Hilmioglu, S., Zekioglu, O., Eronat, C., Burhanoglu, D., 2003. Comparison of antibacterial and toxic effects of various root canal irrigants. *Int Endod J*, 36, 423-432.

Oshiro, M., Yamaguchi, K., Takamizawa, T., Inage, H., *et al.* 2007. Effect of CPP-ACP paste on tooth mineralisation: an FE-SEM study. *J Oral Sci*, 49(2), 115-20.

Osman, M.A., 2004. Chemical and Nutrient Analysis of Baobab (*Adansonia digitata*) Fruit and Seed Protein Solubility. *Plant Foods for Human Nutrition*, 59, 29–33.

Pataky, L., Ivanyi, I., Grigar, A., Azekas, A., 2002. Antimicrobial efficacy of various root canal preparation techniques: an *in vitro* comparative study. *J Endod*, 28, 603-605.

Peixoto, J.R., Silva, G.C., Costa, R.A., Fontenelle, J.L., *et al* 2011. In vitro antibacterial effect of aqueous and ethanolic *Moringa* leaf extracts. *Asian Pacific Journal of Tropical Medicine*, 201-204.

Pousset, J.L., 1989. Plantes médicinales africaine, Utilisations Pratiques. Ellipses (Ed.), Paris. 95.

Predrag, L.S., Hui, C., Uri, A., Hasswan and Arien, B., 2005. The effect of aqueous extracts prepared from leaves of *Pistacia lentiscus* in experimental liver disease. *J Ethnopharmacol*, 100, 198-204

Rao, P.K.D., Rao, B., Kiran, C., Nadh, M.R., Madhavi, Y., Rao, T.R., 2010. *In vitro* antibacterial activity of *Moringa oleifera* against dental plaque bacteria. Available online through [www.jpronline.info](http://www.jpronline.info).

Ray, P.G., Majumdar, S.K., 1967. Antimicrobial Activity of Some Indian Plants. *Economic Botany*, 30(4), 317-320.

Ramadan, A., Harraz, F.M., El-Mougy, S.A., 1993. Anti-inflammatory, analgesic and antipyretic effects of the fruit pulp of *Adansonia digitata*. *Fitoterapia*, 65, 418-422.

Resine, S., Douglass, J.M., 1998. Psychological and behavioral issues in early childhood caries. *Community Dental Oral Epidemiology*, 26, 32-4.

Rickard, A.H., Gilbert, P., High, N.J., Kolenbrander, P.E., Handley, P.S. (2003). Bacterial coaggregation: an integral process in the development of multi-species biofilms. *Trends Microbiology*, 11 (2), 94-100.

Ríos, J.L. and Recio, M.C., 2005. Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacol*, 2, 100(1-2), 80-4.

Rogers, A.H., 2008. *Molecular Oral Microbiology*. Norfolk: Caister Academic Press.

Roselle. *Hibiscus sabdariffa* L. Available from: < [www.hort.purdue.edu/newcrop/morton/roselle.html](http://www.hort.purdue.edu/newcrop/morton/roselle.html) > [accessed June 2013].



Seukep, J.A., Fankam, G.A., Djeuss, D.A., Voukeng, I.K., *et al* 2013. Antibacterial activities of the methanol extracts of seven Cameroonian dietary plants against bacteria expressing MDR phenotypes. *SpringerPlus* 2013, 2:363. Available from: <<http://www.springerplus.com/content/2/1/363>>

Shahat, A.A., 2006. Procyanidins from *Adansonia digita*. *Pharm Biol.* 44:445-450.

Shaw, D. 1998. Risks or remedies? Safety aspects of herbal remedies. *Journal of Royal Society of Medicine*, 91, 294–296.

Sibibe, M. and Williams, J.T. 2002. Baobab-*Adansonia digitata*. Fruits for the future 4, international Centre for Underutilised Crops, South Hampton, Uk, 96p.

Singh. B., Bhat, T.K., 2003. Potential therapeutic applications of some anti-nutritional plant secondary metabolites. *Journ. Agric. Food Chem*, 51, 5579- 5597.

Siqueira J.F., Lima, K.C., Magalhaes, F.A., Lopes, H.P., Uzeda, M., 1999. Mechanical reduction of the bacterial population in the root canal by three instrumentation techniques. *J Endod*, 25, 332-335.

Spratt, D.A., Pratten, J., Wilson, M., Gulabivala, K., 2001. An in vitro evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. *Int Endod J*, 34, 300-307

Suboh, S.M., Bילו, Y.Y., Aburjai, T.A., 2004. Protective effects of selected medicinal plants against protein degradation, lipid peroxidation and deformability loss of oxidatively stressed human erythrocytes. *Phytotherapy Research: PTR*, 18, 276–280.

Tanzer, J.M., 1979. Essential dependence of smooth surface caries on, and augmentation of fissure caries by, sucrose and *Streptococcus mutans* infection. *Infection.Immunity*, 25, 526-531.

Tapsoba, H., Deschamps, J., 2006. Use of medicinal plants for the treatment of oral diseases in Burkina Faso. *Journal of Ethnopharmacology*, 104 ( 1–2), 68–78.

Tyler, V.E., 2000. Herbal medicine: From the past to the future. *Public Health Nutrition*, 3, 447–452.

Vimalanathan, S., Hudson, J.B., 2009. Multiple inflammatory and antiviral activities in *Adansonia digitata* (Baobab) leaves, fruits and seeds. *J Med Plants Res*, 3, 576-582.

Van Leeuwen, M.P.C., Slot, D.E., Van Der Weijden., 2011. Essential oils compared to chlorhexidine with respect to plaque and parameters of gingival inflammation: A systematic Review. *Journal of Periodontology*, 82(2), 174-194.

Walsh, S.E., Maillard, J.Y., Russel, A.D., Catrenich, C.E., Charbonneau, A.L., Bartolo, R.G., 2003. Activity and mechanism of action of selected biocidal agents on Gram -positive and -negative bacteria. *J Appl Microbio*, 94, 240–247.



Wang, C.J., Wang, J.M., Lin, W.L., Chu, C.Y., Chou, F.P., Tseng, T.H., 2000. Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide- induced hepatic toxicity in rats. *Food and Chemical Toxicology*, 38, 6–411

Wang, J., Zhao, T.I., Tian, Y., Chun-Lin, Yan., Guo C., 2013. Ultrasound-Assisted Extraction of Total Phenolic Compounds from *Inula helenium*. *The Scientific World Journal*. Available from: <<http://dx.doi.org/10.1155/2013/157527>> [accessed: 10/10/2013]

WHO Report 2003 [http://www.who.int/oral\\_health/publications/en/orh\\_fact\\_sheet.pdf](http://www.who.int/oral_health/publications/en/orh_fact_sheet.pdf)

World Oral Health report 2003.

Available from: <[http://www.who.int/oral\\_health/media/en/orh\\_report03\\_en.pdf](http://www.who.int/oral_health/media/en/orh_report03_en.pdf)>

[accessed: 10.9.13].

World bank report. Available from: <<http://www.worldbank.org/en/region/afr/overview>>

[accessed: 13.10.14].

World Health Organisation National policy on traditional medicine and regulation of herbal medicines Geneva 2005. Available from:

<<http://apps.who.int/medicinedocs/pdf/s7916e/s7916e.pdf>>

[accessed: 20.9.13]

Wickens, G.E., Lowe, P., 2008. The Baobabs: Pachycauls of Africa, Madagascar and Australia, Springer.

Wickens, G.E., 1979. The uses of the baobab (*Adansonia digitata* L.) in Africa. In: G. Kinkel, ed. Taxonomic aspects of African economic botany. AETFAT.

Wong, S.K., Lim, Y.Y., Chan, E.W.C., 2010. Evaluation of Antioxidant, Anti-tyrosinase and Antibacterial Activities of Selected *Hibiscus* Species *Ethnobotanical Leaflets*, 14, 781-96.

Yagoub, S.O., 2008. Antimicrobial activity of *Tamarindus Indica* and *Adansonia digita* extracts against *E.coli* isolated from urine and water specimens. *Research Journal of Microbiology*, 3(3), 193-197.

Yusha'u, M., Hamza, M.M., Abdullahi, N., 2010. Antibacterial activity of *Adansonia digitata* stem bark extracts on some clinical bacterial isolates. *International Journal of Biomedical and Health Sciences*, 6(3), 129-135.

