ANTIMICROBIAL ACTIVITY OF SELECTED LOCAL CHEWING STICKS (*ZANTHOXYLUM ZANTHOXYLOIDES* AND *PROSOPIS AFRICANA*)

BY

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PG/M.TECH/99/2000/469

FEBRUARY, 2004

Federal University of Technology
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Dept of Microbiology

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF TECHNOLOGY (M.TECH) DEGREE IN PHARMACEUTICAL MICROBIOLOGY

FEBRUARY, 2004

DEDICATION

This work is dedicated to the Lord Jesus Christ.

CERTIFICATION

This Thesis "Antimicrobial Activity of Selected Chewing Sticks" was examined and found to meet the regulations governing the award of the degree of Master of Technology (Pharmaceutical Microbiology) of the Federal University of Technology Minna, and is approved for its contribution to knowledge and literary presentation.

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DECLARATION

I hereby declare that this thesis is an original work of mine and has never been presented elsewhere for the award of any degree. References made to published literature have been duly acknowledged.

Moing

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Glory be to Lord God Almighty for the grace and the ability bestowed upon me to carry out this research. Honour and adoration be unto our Lord Jesus Christ.

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Omotoso Modupe

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ABSTRACT

Stem and leaves of *Zanthoxylum zanthoxyloids* and *Prosopis africana* possessed phytochemical components namely saponins, polyphenol, cardiac glycosides, tannins and alkaloids. The antimicrobial activity of the crude extracts and the thin layer chromatography fractions was examined and compared with the standard antiobiotics, (Tetracyline, Ampiciline, erythromycin and ciprofloxacin). The crude extract of both ethanol and methanol of all plant parts at 2000µg/ml concentration showed significant antimicrobial activity against the organism isolated from dental carries (*Staphylococcus aureus, bacillus, sp., Streptococcus sp.*, and C*andida albican*). The TLC fraction of the plant parts of *Zanthoxylum zanthoxyloids* at 2000µg/ml conc., showed significant antimicrobial activity against the tested organism while the *Prosopis. africana* fractions does not show any antimicrobial activity.

CHAPTER ONE

1.0 INTRODUCTION

Plant cells undergo complex metabolic reactions that result in the production of chemical energy, nutrients and new structural components which ranges in size from a tiny mass to a giant tree, (Dutta 1979) Besides their role as primary producers of food for man and other animals, plants also assume a special place among the elements in the environment, for their ability to serve as medicinal sources (Sofowora and Hardman 1974; Dutta,1979; Akinsanya, 1988).

Medicinal plants are plants that contain substances in one or more of their organs (root, stem, bark, branches or leaves and flowers) which can be used for therapeutics purpose act as precursors for the synthesis of drugs (Sofowora, 1982). As a result the term crude drug of natural or biological origin is used by pharmacists and pharmacologists to describe whose plant or its parts which have medicinal properties (Abdul, 1990).

Plants have been used for prevention and treatment of disease for the past two decades (WHO, 1977). It has been estimated that as many as 72-90% of the world rural populace rely on herbal traditional medicines, and it is neither possible nor desirable to completely replace this herbal medicine during this century, consequently there is a growing interest in herbal medicine and its traditional role (Smith, 1972; WHO, 1977). The therapeutic properties of medicinal plants are conditioned by the presence in their organs an active secondary metabolites (WHO, 1977).

These metabolites accumulate either as organic metabolites, process by products or mostly at the site of infection in response to microbial attack. The secondary metabolites most commonly found in medicinal plants are compound such as alkaloids anthranoids, anthraquenoids, Cardia\glycosides, phlobatannis, polyphenol, saponins and tannnis, which are biologically active in relation to causative agents of various diseases, (WHO, 1977, Sofowora 1982.

Gbodi *et al* (1994) worked on 14 C – phenol and observed that 14 C – phenol disappeared from the blood of pento – barbital anaesthetized rats and its excretion into bile were also evaluated for 5hr after intravenous administration of 25mg phenol. It was observed that the bile contained 10.7% of the administered dose and the major biliary metabolite was phenol glucuronide. They also concluded from the result of the enterohepatic circulation studies that only 2% of the phenol and its metabolites entered the enterohepatic circulation and that the higher activity of the phenol in the kidneys of the rat was more than other tissues; tends to confirm the importance of the renal system in the elimination of phenol in rats.

Fadeyi and Akpan (1989) reported the antibacterial activities of leaf extract of *Eugenia uniforal* L, against *Staphylococcus aureues, Shigella dysentriae, Escherichia coli* and *Bacillus substilis*. Plants extracts have been used in folk medicine for the treatment of different ailments since antiquity (Okanla and Oyewole, 1990). *Cassia* species are known for their use as laxative/purgative, they are also used as anti–flammatory, antifungal and antibacterial agents (Oliver, 1959) in the treatment of various skin diseases like *eczema* sp., ring worms (Ogunti *et al*, 1991) and prurities. It has been

demonstrated recently by (Benjamin *et al*; 1981) that *Cassia alate* has wounds healing property. The plant has been found to be useful in the treatment of eruptive and pastular skin conditions by simply rubbing the crushed fresh leaves. Many synthetic medicine are modification of herbal drugs that have been included in the standard pharmacopea, today 47% of all drugs used are of botanical origin (Dutta, 1979), 25% of all prescriptions dispensed in public pharmacies in United State of America from 1979 contained drugs extracted from higher plants. (Akinsanya, 1988; Fransworth 1980, Mourice, 1993).

Plant in the traditional medicine is known to be highly efficacious in the treatment of diseases, but this has not been without problems. The very common and complex limitations is in the area of hygiene, both the traditional practitioners clinic and his mode of preparation are often very unhygienic when compared to the conventional modern medical practice (Dutta 1977; Akinsanya, 1988; Abdul, 1990). Also in the area of dosage the actual dosage of drug required to take care of a particular ailment is in most cases lacking by the traditional medical practitioner (Dutta, 1977; Lewis, 1977).

Lack of scientific proof, is another limitation as most claims made by the traditional medical practitioners themselves are baseless as the active materials or agents in the plants and their mechanism of action are unknown.(Lewis 1977). In the areas of diagnoses often given by the practitioners for example a diagnoses of abdominal pain could mean indigestion, an ulcer or cancer of the stomach: such impression is due to the fact that the physiology of part of the body is not known to the herbal practitioner. As a result he tends to treat the symptoms rather than the disease and this can lead to further complications

(Lewis, 1977). It has been estimated that ~~some~~ about 90% of the world's people used herbal medicines as the basis for their health care (Barbara and Jim 1987).Chewing sticks are important non – timber forest product widely used for dental cleaning in topical West Africa. They are generally cut to 15 -20cm long and 0.5 – 1.25cm diameter for use and are different from disposable tooth-picks in that they can be re-used for a period lasting up to one month (Akande *et al.* 1996). They are also chewed and used for both brushing and tooth picking. When chewing sticks are used; extracts will inevitably be released but their efficacy for improving oral hygiene is little more than speculated.

It has been speculated that chewing sticks extracts could contain compounds capable of inhibiting the activities of tooth-decay bacteria while their specific gravity (SG) and calcium contents may improve abrasive properties (Akande *et al,* 1996).

Akande and Hayashi (1998) also stated that to characterize the antibiotic values of specific chewing sticks, extracts of *Garcinia mannii* (*Cutfiferae;* split stem and branches with bark removed) *Masularia Accuminata* (*Ruiaceae*; root split stem with bark retained) *Zanthoxylum gelletii* root split stem with bark retained). *Terminalia glausecens* (combretaceae; root split stem with bark retained), *Azadiracta indica* (*meliaceae*; young stem branches with bark retained, *Angeisus leiocarpus* (combretaceae: stem with bark retained) and *Pseudocedrela kotschyi* meliaceae; stem with bark retained) were subjected to bioassay against *Staphylococcus auricularis* and *Staphylococcus aureus;* and their result shows that at 4.0g/L extract concentration and 30h incubation, all the species tested except

*Zanthoxylum gilleti*__showed some antibiotic activity against *Staphylococcus aureus*; and that it was only *Terminalia glaucescens* and *Garcinia manni* extract that produced appreciable antibiotic activity against *Staphylococcus auricularis* at 4.0g/L concentration at 30h and 72-h incubation.

Akande & Hayashi (1998) now concluded that not all chewing sticks species produced antibiotic effect on *Staphylococcus aureus* and *Staphylococcus auricularis*. *Staphylococcus aureus* is a clinical recognized human bacteria pathogen and several authors have used this organism for evaluating anti-microbiol activity. Ebena *et al* (1991) used *Staphylococcus aureus* as target bacterium to examine the pharmaceutical potential of phytochemical components of medicinal plants such as *Garcinia kola* (root), *Borretia ocymoides* (leave) *Cola nitida* (bark) and *citrus aurantifolia* (root).

Odebiyi and Sofowora (1979) carried out an investigation on the anti microbial activity of some chewing sticks and found that all possessed antimicrobial activity against oral microbial flora but to varying degrees when tested by the cup plate agar method. They reported that zones of inhibitions of *Zanthoxlyum zanthoxyliodes* extract against oral microbial flora on nutrients agar and blood agar are 11.0 and 11.5mm respectively. While that of *Terminalia glaucescens* extract was 8.5mm.

The advantage of the chewing sticks over the conventional toothpaste and brush is that chewing sticks provide mechanical stimulation of the gum, and destroy microbes present in the mouth, a feature which is absent in the common toothpaste in the market, (Odebiyi and Sofowora, 1979). Chewing sticks also remove food

particles from between the teeth and in crevices, as does the toothpaste and brush method.

The antimicrobial activity of *Zanthoxylum zanthoxyloides* (*Fagara zanthoxyloides* lamb) has been shown to be due to benzoic acid derivatives which is active at a pH of about 5 and alkaloids which are active at pH of 7.5. In this root (*Zanthoxylum Zanthoxyloides* root) there exist therefore, compound which will be active during heavy tooth decay (high- pH- alkaline) as well as after a drink of lime juice (low pH acidic) (Odebiyi and Sofowora, 1979).

In another study by Oyeleke and Adebayo (1998) *Anogeinssus leocerrus* and *Axonopus compressus* were examined for their anti-microbial activities on mouth flora *Lactobacillus species, Pseudomonas species, Staphylococcus aureus, Streptococcus mutants* and *Candida albicans* isolated from randomly selected individuals. The investigators observed that the extracts of the two plants had significant anti-microbial activity against the tested organism using agar diffusion method. Besides, the photochemical screening of the chewing stick extracts showed that they contain active ingredients such as; alkaloids, anthraquinone, polyphenol and tannins.

1.1 The Objective of the Study

(1) To identify the photochemical components of *Zanthoxylum zanthoxyloides* and *Prosopis africana* used as chewing sticks.

(2) To study the effect of crude extracts of the chewing sticks on microbial isolates of dental carries.

(3) To study the effect of fractionated (TLC) extracts of the chewing sticks on microbial isolates.

(4) To compare the antimicrobial activity of the extracts of aqueous, acetone and methanolic extracts with those of standard antibiotics.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. **Zanthoxylum** also known as (Fagara Inn)

"Yellow wood" is a large genus, widely spread in the tropics and is generally regarded as fagara inn. In some species the whole and main branches have large conical thorn, composed of layers of woody bark with a sharp prickle at the apex, that are used in the known thorn carving. Similar thorns are found in some species of *Erythrina* and *Bombax*. Most of these species of *Zanthoxylum* have glands spaced along the margin of the leaflet and these sometimes develop into small rounded teeth. The very small flower have inconspicuous sepal 4-5 whitish petal an equal number of stamen in the male flower and a fat somewhat unsymmetrical stigma in the female. The inflorescence are pamcles with unusual dotted line aromatic gland and about 6mm across , that spit open along a longitude rib and expose a glossy black. (Keay and Onochie, 1960).

2.1.1 *Zanthoxylum zanthoxyloids*: (*Fagara zanthoxyloides*)

This is a much – branched rather tangled tree with dense dark green foliage and abundant prickle. The local names are fasa kwari-Hausa, orin-ata-Yoruba, ughanghan-Edo.

The thorns compose of alternate darker and lighter layer, smaller thorn extending to the branch let and leaf–stalk but very variable in quantity. The leave, has a common leaf-stalk 3.5–10 cm long; 3-5 pair of leaflet by 2-3.5 broad, elliptic to elongated elliptic or slightly obvate, rounded or notched or very shortly accumulate at the apex , broadly cuneats dark green , glossy glabrous, rather leathery ;

with a prominent mid –rib and rather faint thin and irregular lateral nerves looped near the margin and connected by an open network of indistinct veins (Keay and Onochie, 1960).

2.1.2 Importance of *Zanthoxylum* species

(1) *Zanthoxylum zanthoxyloide* and *Zanthoxyluro gilletti* has hydroxyl methybenzoic acid which is an antisickling agent (Isaac-Sodeye *et al*,1975, Adesanya and Sofowora, 1979).

(2) *Zanthoxylum nitudum* and *Z zanthoxyloides* has *nitidine* benzoic phenanthridine alkaloids respectively which is an antileukemic agent (Sofowora, 1982).

(3) *Zanthoxylum* (fagara) root bark is use in the treatment of toothache (Sofowora, 1982)

2.2. *PROSOPIS AFRICANA*

There are about 40 species in the tropical areas. It has been cultivated in northern Nigeria. *Prosopis africana* is a savanna tree which can be distinguish by its dark-rough bark, pale drooping foliage with small pointed leaflets and sausage –shape fruits. The native or local names are kiriya-Hausa, ayan-Yoruba and abwa-Igbo.

The tree is 12-18 cm high, often branching very low down and crooked limbs forming an irregular open crown, but with a clean hole in forest conditions. The bark is blackish, strongly fissure, flaking off in ragged patches leaving light brown scars slash reddish to red-brown. The leaves are hard with a glabrous common stalk 7.5-15cm long, much swollen at the base of each pair; 5-13 pairs of minutely hairy opposite leaflets about 18mm long and 6mm broad pointed

elliptic, with the mid-rib slightly nearer the upper margin and projecting beyond the apex base unsymmetrical. Flower (Dec-May) and is yellowish, fragrant, densely crooked in fat spikes 4-6 cm long excluding the short stalk and about 12mm across including the stamens. The fruits very persistent and is about 10-15cm by 2.5cm thick-walled. The wood is very hard and tough rich red-brown with grew sap wood. (Keay and Onochie, 1960).

2.2.1 Importance of *prosopis africana*

(1) It is used in the treatment in the urinary tract infection (Lewis and Elvin, 1977, Newsome, 1991).

(2) It is used in the treatment of wound infection and skin disease (Newsome, 1991).

2.3. STUDIES OF MEDICINAL PLANTS

2.3.1. HISTORY OF MEDICINAL PLANTS

The early man has used plants to cure diseases and relieve physical suffering. Primitive people in all age have had some knowledge of medicinal plants, derived as a result of trial and error; these primitive attempts were based on speculation and superstition (Albert, 1979). Most savage people have believed that disease was due to the presence of evil spirits in the body and could be driven out only by the use of poisonous or disagreeable substances calculated to make the body an unpleasant place. (Albert, 1979).

One of the earliest record of the use of herbal medicine is that of Chaulmogra oil species of *Hydnocarpus geartin*, which was known to be effective in the treatment of leprosy. The use was recorded in the

pharmacopoeia of the Emperor Shen Nung of China between 2730 and 3000 B.C. It is not known, however, who was the discoverer of this medicinal plant oil nor when it was discovered (Le, Strange, 1977). Similarly, the seed of Opium poppy (*Papaver somniferumli*) and castor oil seed (*Ricinus communis F.L*) were excavated from some ancient Egyptian tombs; which indicated their use as purgative, vehicle for eye drops) in that part of Africa as far back as 1500 B.C (Sofowora, 1982).

Early man could have gained some scientific knowledge by watching the effects produced by various plants when eaten by domestic animals. Even today, some herbalists still find out remedies on domestic animals, especially when testing for toxicity on themselves and relations. (Akpata, 1979, Lambo, 1979).

2.4. STUDIES ON MEDICINAL PLANTS

Investigations carried out on the antimicrobial activity of chewing sticks shows that they all posses antimicrobial activities against oral microfloral, but to varying degrees when tested by cup plate agar method. This indicates that chewing stick, besides providing mechanical stimulation of gums; also destroy microbes present in the month. (Akande and Hayashi, 1998).

Ebena *et al* worked on four medicinal plants, *Garcinia kola* (roots), *Borreria oxymoides* (leaves), *Kola nitida* and *Citrus aurantifolia* (root) and they were found to contain tannins, phlobatannins, polyphenols, hydroxymethyl anthraquinones, saponins, alkaloids, cardiac glycosides, flavanoids and reducing agents the aqueous and alcoholic extracts as well as alkaloids and cardiac

glycosides of the medicinal plant were tested and they were found to inhibit such organisms as *Staphylococcus aureus, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa* and *Neisseria gonorrhea.* (Ebena *et al*, 1991).

The antibacterial screening of *chromolaena odorata* leaf extract possesses some antibacterial activity against *Pseudomonas aeruginosa* and *Streptococcus faecalis*. The minimum inhibitory concentration was large for *Pseudomonas auruginosa* (8.0mg/ml), a Gram – negative organisms, and somewhat less (6.0mg/ml) for *streptococcus faecalis* a Gram – positive organism; basis for their utility in traditional medicine for the treatment of skin infections, since the extract produced antibacterial activities against both Gram – positive and Gram – negative bacteria. (Irobi, 1992).

Phytochemical screening of *Trema grueineenses, Bridela* species revealed that the plant contain Alkaloids, Anthranoids, Anthraquinone, Cardiacglycosides, phenolbatannis, saponins and Tanins (Ijah and Sar 1998).

The crude aqueous and alcohol extracts of the plant were found to be active against *Klebsiella aerogenes* and *Proteus mirabilis* for urinary tract infections. The extracts when used individually was found to have less inhibitory effect on the organisms but when combined and tested on the organism. the inhibitory effect were enhanced. Adesina et al (1980) reported that the crude extract of *Acalypha wilkesians* has also proved to be strongly inhibitory to the growth of *Bacillus subtilis, Escherichia coli, Klebsialla pneumonia* and *Candida albican* .

Akinyanju *et al*, (1986) reported that the hot water extract of leaves of *Acalyphas torta* was shown to have significant inhibitory

effect in vitro on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella* sp, *Salmonella paratyphi, proteus* sp, *Serratia marcesscens*. Both crude and purified ethanolic extracts of the leaves also inhibited the same range of organism as did hot water extract with the exception of *Pseudomonas aeruginosa* and *Acinetobacter* sp.

Adebajo (1980) reported that *A. wilkesiana* leaf extracts when subjected to chemical quantitative tests (e.g. UV/Visible, IR, Spectrophotometer analysis) the UV/Visible Spectrophotometer showed that the acid soluble extract has max 206.8nm and that of the base soluble extract is as 206nm, both of them having the same electronic structure (x) are aromatic.

Ebena *et al* (1991) in their studies about medical plants emphasize the necessity to identify the phytochemical components of local medicinal plant usually employed by herbalist in the treatment of disease, especially now that there are proposal on the integration of traditional medicinal practice in health care programme in Nigeria.

According to Kafaru (1994) several diseases have been treated with herbs. Herbs like *Allium sativum* (Garlic) are known to contain thiol limonoid compounds Rastimamanga *et al* (1992) and Rasoaniavo et al (1994) have studied the possible benefit of combining traditional antimalaria plants remedies with the use of chloroquine. Two bisbenzyliso quenoline alkanoids 7-0 dimethylpheanthine), isolated from *Strychnopsis thouarsii* and *spirospernum, Penduliflorum*, respectively, three novels pavin and benzyl-tetrahydroisoquinoline dimmers from *Hermandia vagronic* and the alkaloids *Strychnobrasiline* and Malagashanine from *Strychnos myrtades* all

potentiate the anti-plasmondial activity of chlorine invitro experimental preparations of these plants are currently use as adjuvants to choloroquine therapy in Madagascar. The highly active antiplasmodia quassinoids isolated from species of *Brucea* and *Simarouba* (used throughout the tropics) in the treatment of malaria) are also lipohilic but the greatest stumbling block with these compounds is their extreme toxicity (O'Neil *et al*, 1987) both in vitro and in vivo.

Quassinoids are known to have potent inhibitory actions upon protein synthesis at the level of the ribosome (Kirby *et al*, 1989). Most of the plant origin all showed antiplasmodial activity against both chloroqiune-sensitive resistant Strains of parasite in invitro test (Ekong *et al*, 1969). It is clear that plants are natural antiseptic acting against viruses as well as bacterial; some of them have traditional properties like antioxidants, which are helpful in wound healing.
Nguji (1988) reported that phenols in some plant tissues are oxidized to coquinones which subsequently form effective cross-links with the serum protein of the skin to arrest-bleeding and effect healings.

Villegas *et al* (1998) reported that petroleum ether extracts of *Heteromopha trifoliate* leaves had antifungal properties against *Cladesporium cuicumerinum*. The ethanol extract of *Monpordica charantia. Alstonia boonie* and *Ocinum bacilicum* have been found to possess antimicrobial activity against *Escherichia coli, Salmonella paratyphi* and *Shigella dysenterae*, (Omeregbe *et al*, 1995). A decoction from the root of East African plant *Ozonea mucronate* is used for various folk medicinal treatment, such as intestinal parasites, dysentery, diarrhoea, gonorrhea, bilharzias and abortion. The hexane

extract of the root bark exhibited antimicrobial properties against *Staphylococcus aureus* and *Bacillus subtilis*.

Plant extracts are usually in their crude form in that they have not been chemically purified. However, some therapeutic agent have been identified in medicinal plants which include alkaloids, tannins, Cardiac glycosides Saponins, philobatannins, and tannis (Sofowora, *et al* 1991).

Alkaloids and Cardiac glycosides have been reported (Ebena *et al* 1991) to inhibit pathogenic bacteria *Staphylococcus aureus, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli, Neisseria gonorrhea* and *B-heamolytic Streptococci.* A chromatographic fractions of *Euphorbia kirta* from Taiwan showed antiulcer activity Liu and Hsu (1988) to certain protocatechnic and gallic acid, inositol, kaempferol, melissic acid, myricetin, guarctin and quecitrin.

Fakunle *et al* (1989) and Abo (1990) reported the chemistry of the *Euphorbias* growing in Nigeria. *Igenol* was reported in the lattices of *Euphorbia* and *Slaeophorbia* species growing in Nigeria by Abo (1990). Pousset (1988) also reported a mixture of the extraction of this plant for oral administration against amoebic dysentery. Anti-ulcer activity as been demonstrated in all extract of the plant (Liu and Hsu, 1988) and the plant has been examined for cytotoxicity by Duez *et al* (1991).

Houghton *et al* (1993) working on the chloroform extracts of *Cryotolepsis sanguinolenia* root purchased from a herbal market in Guinea Bissau have characterized three alkaloid's called hydroxyl cryptolpine and cryptoquinedoline is the second bis' alkaloid to be

isolated by Tackie *et al*, (1993). The hypothermic effect probably explains its use by Ghanaian herbalist for malaria fever but there is no evidence of any plasmodicidal effect by the plant. The antimalaria action of plant has been reinvestigated by Boye (1990) in Ghana.

Benjamin (1980) also showed that the volatile oil obtain from the leaves of *Borrer verticillata* by prolong vacuum have distillation under a nitrogen atmosphere, 'inhibited the growth of Gram-positive and Gram-negative bacteria. He concluded that there is strong evidence in support of the use of this plant in skin disease (often involving *Staphylococcus aureus*) and for some other bacteria infections. The essential oil of *B. verticellata* has been reported to *Schistosoma haemotobium* (the organism that cause billarzia) by treating people at a dose of 100mcg/m.

2.5. ADVANTAGES AND DISADVANTAGES OF MEDICINAL PLANTS

2.5.1 Advantages

(1) Medicinal plant is cheaper than orthodox medicine: the cost of orthodox medicine is increased by modern health technology which in many cases is inappropriate or irrelevant to the immediate needs of people in developing nations.

The bill for many countries still represents a sizable proportion of their total health expenditure, especially in developing countries. While drugs account for 10-30 per cent of the total cost of health care in developed countries (Sofowora, 1982).

(2) Local drugs or medicine is widely acceptable among the citizens of developing countries than orthodox medicine (Sofowora, 1982).

This could be due to the inaccessibility of orthodox medicine, but the major factor is that traditional medicine blends readily into the socio-cultural life of the people in whose culture is deeply rooted. It was the only form of health care available to such people before the advent of orthodox and there are instances today in Ethiopia, Ghana and Nigeria where people that are close to hospital consult traditional healers as first choice.

(3) Traditional medicine is a potential source of new drugs, a source of cheap starting products for the synthesis of known drugs or a cheap source of known drugs such as reserpine from *Rauwolfia species*, *Vinblastine* from *Catharanthus roseus* or recent discovery of a contraceptive in *Zoapathe* (*Montanoa tomentosa cery*). (Hahn *et al.*, 1981). There is also the possibility that some revolutionary new drugs for treating such disease as cancer may be discovered from herbal plants.

(4) Since traditional remedies are mostly compounded from natural product, as such there is greater likelihood of their being accepted by the body, than substances which have been invented in laboratory (Sofowora, 1982).

2.5.2 Disadvantages of Medicinal Plants

(1) Lack of scientific proof of its efficacy.

(2) Incorrect diagnosis by the practitioner, a diagnosis of stomach trouble could mean indigestion, an ulcer of the stomach such impression is due to the fact that the pathology of the part of the

body is not known to the traditional medical practitioner (Sofowora, 1982).

(3) The incantation and occult practices by traditional healers cannot be verified scientifically.

(4) Traditional herbs are aseptically prepared.

2.6. TEETH

Teeth is hard, calcified structures, attached to the upper and lower jaw of vertebrates and a few lower animals and are used primarily for mastication. There are various forms of mammalian teeth namely, incisors, canine, molars and premolars.

2.6.1 Human teeth

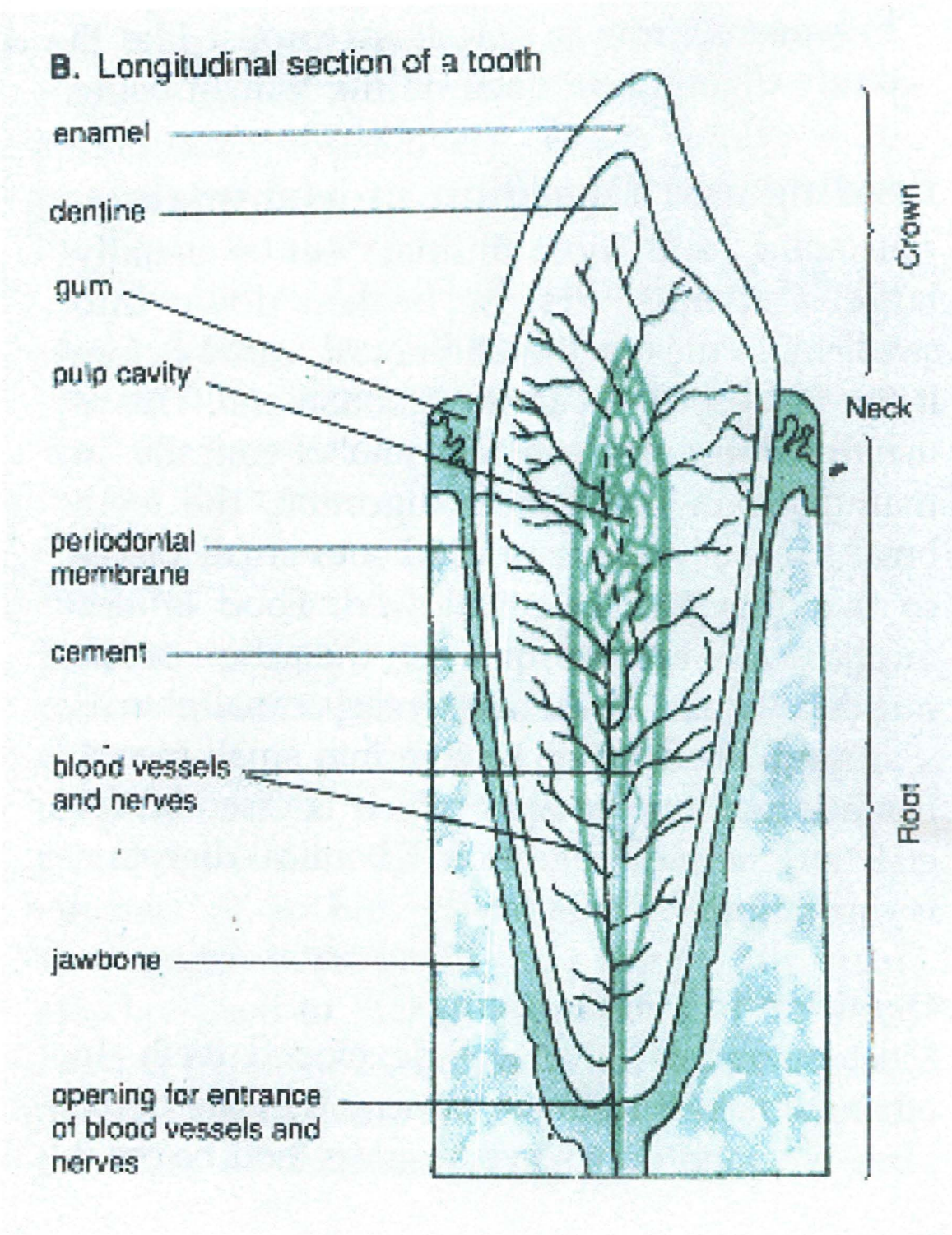
Human teeth also serve major function other than chewing. The teeth are directly involve in the process of speech, acting as a brace against which the tongue presses in the formation of certain sounds. The teeth also affect the dimensions and the expression of the face, the appearance of which may be unpleasantly modified by the loss of neighbouring teeth or by any irregularity tooth growth or colouring. (Zinner and Jablon, 1968).

2.6.2 Tooth structure

Human teeth consist of an external portion, called the crown, and a root that is embedded within the jaw. The outer portion or layer of the crown is composed of calcified tissue known as the enamel, which are the hardest substances in the body. Inside the enamel is the dentine, a bone like substance extending from the inner surface of the enamel into the jaw from the root. Covering the dentine of the root is a thin

layer of a hard tissue called camentum. The roots are held in place by plastic fibres that constitute the periodontal membrane, which extends from the cementum to a thickened layer of bone, known as the lamina dura, within the jaw.

The dentin of the crown encloses the pulpal chamber which penetrates into the root as the root canal. Passing through the root canal opening at the end of the root is blood vessels, nerves and connective tissues, which fill the root canal and the pulpal chamber. See fig 1 below. (Zinner and Jablon, 1968).


B. Longitudinal section of a tooth
enamel
dentine
gum
pulp cavity
periodontal membrane
cement
blood vessels and nerves
jawbone
opening for entrance of blood vessels and nerves
Crown
Neck
Root

2.6.3 Dental caries

Dental caries according to the World Health organization (WHO) (1972) is a localized post eruptive pathological process of external origin, involving softening of the hard tooth tissue and proceeding to the formation of a cavity. Dental caries can also be defined as progressive, irreversible bacteria damage to teeth exposed to the oral environment. The ultimate effect of caries is to break down enamel and dentine and to open a path for bacteria to reach the underlying tissues. This causes infection and inflammation of the pulp and later of the periapical tissues.

A study was undertaken in 1983 to obtain data on tooth loss in two rural areas in Nigeria. In Igbo-Ora, Oyo state and Ganawuri, Plateau state. 308 persons aged 15 years and older were examined for periodontal disease under field conditions using the community periodontal index of treatment needs (CPITN). Despite huge CPITN scores in the practically periodontal carries free population of Men in Igbo-Ora, the average number of teeth lost was less. It was less also among the women with less periodontal disease in Grawuri, among whom the prevalent dental caries seemed to be associated with the loss of few teeth in large proportions of the population, whereas periodontal disease result in the loss of several teeth in only a few individuals. Several periodontal disease has been reported to be prevalent in Nigeria (Shehiam, 1967). However, an increase has been observed in the 1970 and 1980 (Henshaw and Adenubi, 1975; Olojugba and Lenon, 1986).

2.6.4 ETIOLOGY OF DENTAL PLAQUE IN DENTAL CARRIES

Dental caries develop in human or animal mouths only in the presence of three interacting variables. These are Bacteria plaque containing cariogenic bacterial, bacteria substrate, especially sugar and susceptible tooth surfaces. (Silvertone, 1976).

2.7. MICROBIAL ASPECTS

Dental caries is not the result of a reaction at the interface between tooth surface and dental plaque in stagnation area. The formation and properties of dental plaque depends on the micro-organism and their products which forms so large a part of its substances.

2.7.1 Types of bacteria important in Caries

Many bacteria have been isolated from dental caries and have been investigated. The acid-formation bacteria have received the greatest attention and interest has long been centred upon the Lactobacilli. However, it seems probable that Streptococci play a predominant role. Alpha-haemolytic *Streptoccoci* (*S. varidans*) are the most numerous inhabitants of the month. These include *S. mutant, S. salivarius, S. mitor S sanguis* (Kohler *et al.*, 1981).

S. mutans is so called because of its variable morphology affected by the pH of the environment they can be categorized in various ways: by their bacteriological, biochemical activities (such as their ability to ferment different sugars) and serological characteristics. *S. mutans* is strongly acidogenic and under favourable conditions (at low pH and with freely available sugar) stores an intercellular glycogen-like reserve is used and acid production

continues for a time. Drastic reduction in dietary sugar intake leads to elimination of *S. mutans* rapidly decolorizes the plaque but when sugar is freely available again. The organisms proliferate (Kohler *et al.*, 1981).

The ability of *Streptococcus mutans* to initiate smooth surface caries and to form large amount of adherent plaque appears to be related to its ability to polymerise sucrose into high molecular weight (long chain) polysaccharides. These polysaccharides are glucans but are sometimes referred to as dextrans. Glucans help these *Streptococci* to adhere to one another and to the tooth surface. Minute quantities of high molecular weight glucan also cause suspension of *S. mutans* to aggregate: this suggest that *Stretococci* may have specific surface receptor sirtes to which glucan can attach, but which other *Streptococci* lack. *Streptococci mutans* can be stimulated to adhere to the surfaces of extracted teeth which have been treated with minute quantities of glucan; thus the glucan may be important in initiating the attachment of these organisms to the teeth as well as playing a major role in the building up of these masses of plaque. Glucans do not account for the attachment of other micro-organisms notably filamentous to the tooth surface.

Gram-positive rods *lactobacilli*: were at one time regarded as the main cause of dental caries because of their presence in large numbers in the month where caries was active and because of their ability to produce acid (Sharpe *et al*, 1966). *Lactobacillus acidophilus*, which was isolated from dental carries at pH 3.5 is a representative of the homofermentive (lactic acid producing) group though *Lactobacillus casei* is present in large numbers in plaque. *Lactobacilli*

are present only in relatively small number in dental plaque though they may be present in large numbers in the saliva. In general there is evidence that *Lactobacilli* are clinically important in dental caries, though it must be admitted that there so many strains of *Lactobacilli* that the potentialities of these organism for causing caries cannot be said to have been fully tested.

Gram-Negative Bacteria

(i.) ***Neisseria***

These organism are isolated in low numbers from most sites of oral cavity. Along with *Streptococcus sanguls*. They are among the earliest colonizer of a clean tooth surface some strains of *Neisseria* also metabolize lactic acid and this property is quite significant in the dissolution of enamel. (Hendel and Hess, 1989).

(ii.) ***Veillonella***

Veillonella are strictly anaerobic gram-negative cocci that can be separated into two species. *Veillonella parnia* and *Veillonella alcalescens*. On the basic of the breakdown of hydrogen peroxide by the latter. *Veillonella* occur in highest number in dental plague: they glucokinase • and fructokinase ad are unable to metabolize carbohydrate. They can however utilize several intermediary metabolites as lactate as their energy source thus playing an important role in etiology of dental plague and in the ecology of dental caries. (Hendel and Hess; 1989).

Apart from formic acid and lactic acid produced by bacterial. They are capable of dissolving tooth enamel and conversely,

2.7.4 Formation and role of Plague Polysaccharides.

These substances, synthesized by bacterial, play an essential role in the pathogenesis of dental caries. The bacterial polysaccharides are both intracellular and extracellular. The proportion of the different types of polysacchrides and are different dietary sugars available. On a sucrose-rich diet the main extracellular polysaccharides are glucans (dextran-like matriats) fructans formed from fructose are produced in smaller amounts: they are also more soluble and less important in caries. (Zinner *et al.*, 1968).

The cariogenic properties of Streptococcus mutans seem to depend as much on its ability to form extracellular glucans in large amounts as on its ability to produce acid. Acid producing micro-organism that do not produce suitable polysaccharides do not appear to be able to cause carries of smooth surface. (Zinner *et al.*, 1968).

2.8. PREVALENCE OF DENTAL CARIES AMONG NIGERIANS

The DMF (Decay of canons, missing and filled) have been used in most of the caries epidemiological studies carried out in Nigeria. Because of the fact that a missing tooth may not necessary be due to caries, U.S National Institute of Health Office of International research (1967) excluded missing teeth in the computation of caries prevalence in both the decidous and permanent dentitions in Nigeria. The institute adopted the WHO (1962) "df" index (decayed and filled deciduous teeth) for assessing caries experience in the primary teeth because of the difficult in differentiating between a missing tooth lost because of caries and also Sheihan (1967) need "df" index on the Nigeria children and adult in 1964, observed that caries prevalence

was very low in Southern Nigeria about 98% were caries free. A well conducted study (Olujugba and Lenon 1986) in Ondo State, Nigeria found that between the year 1977 and `983, the mean of 5-year old rose from 0.8 to 0.2 and the mean DMFT of 12- years old rose from 0.1 to 2.2. In both age group the higher social class children had substantially more caries than children from social class (Olujugba and Lenon 1986).

2.9. PREVENTION OF DENTAL DISEASE

2.9.1 Antibacterial agents

The use of Antibiotics: Pencillin reliably prevents dental caries in animals and in human its effects is shown as a by-product of its long-term use in the prevention of recurrences of rheumatic fever. (Cawson, 1984).

The use of Chlorhexidine: This is a cationic antiseptic which acts by damaging the cell membrane of a wide variety of bacterial, chlohexidine binds on to enamel surface and plague components. It is slowly released to have an antibacterial effect lasting several hours. This can inhibit bacterial colonization of the teeth and plague formation.

When use as a daily mouth rinse (0.1% or 0.2%) chlorhexidine reduces the amount of plague formed and helps to prevent gingivitis. As long as chlohexidine is used in this way it appears that the salivary flora is decreased by 20 to 50% and most notably the numbers of *Streptococcus mutans* that can be isolated also decline. The main advantages of chlohexidine are its unpleasant flavour and its tendency

to cause brownish straining of the teeth and silicates. This can be removed with polishing. (Cawson, 1984).

2.9.2 Immunization against dental caries

Since dental caries is an infective disease but difficult to prevent, immunization is an attractive possibility. Immunization is through the use of salivary immunoglobulin. In quantitative terms salivary IgA is the main immunoglobulin of saliva but minute amounts of IgG and IgM reach the mouth in the gingival exudates. (Bowen, 1974).

The function of salivary IgA has been suggested as being directly antibacterial in favour of this is the findings that bacteria absorb IgA on to their surface. On the other hand, IgA, unlike other antibodies, cannot fix complement and complement-dependent bacteriolysis does not appear to be possible. Another function is to inhibit the adherence of the oral to epithelial or dental surfaces by coating these surfaces within the mouth, by coating mucosal surfaces, salivary IgA might also function as a blocking antibody and interfere with the absorption of bacterial and other antigens through the mucosa (Cawson, 1984).

Finally it has not been conclusively established that, a few people are almost or entirely free from dental caries, this is as a result of immunity. It is possible to immunize animals against the disease and it seems likely therefore, once the technical problems have been overcome, that the same can be done for man. (Cawson, 1984).

2.9.3 Control of sugar in dental caries

Sugar, particularly sucrose, is the most important cariogenic substrate. Caries is most effective when sugar is taken at frequent intervals or in such a form as to adhere to the teeth and remain in contact with the plague for a long period. Complete sugar restriction is the simplest, safest and most effective way of preventing dental carries. Ideally, sweet eating-this include candy bars and all such confectionery with a high sugar content-should be stopped altogether. For prevention to be effective is to stop sweet between meals, if such things must be eaten, thy can be taken at the end of meal as a desserts (Cawson, 1984).

2.10. GENERAL CHARACTERISTICS OF THE STAPHYLOCOCCI

The genus *staphylococcus* is a common inhabitant of the skin and mucous membranes and it accounts for a considerable portion of human infections (often called "Staph" infections). Its spherical cells are arranged primarily in irregular clusters and occasionally in short chains. As a group, the staphylococci lack spores and flagella, and are only occasionally encapsulated. The most important human pathogens are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus capitis* and *Staphylococcus hominis*, *Staphylococcus aureus* is considered the most serious pathogen although the other species have become increasingly associated with opportunistic infection and can no longer be regarded as harmless commensals.

Staphylococcus aureus:

Staphylococcus aureus grows in large, round opaque colonies at an optimum of 37°C though it can grow anywhere between 10°C and

46°C. It owes its name to the grapelike (Gr. Staphyle) appearance of the clusters. It is facultative anaerobe whose growth is enhance in the presence of O_2 and CO_2. Some strains can digest proteins and lipids, and ferment a variety of sugars. This species is considered the most resistant of all non-spore-forming pathogens with well-developed capacities to withstand high salt (7.5 – 10%) extremes in pH, and high temperatures (up to 60°C for 60 minutes). It also remain viable after months of air drying and resist the effects of many disinfectant and antibiotics. These contribute to the reputation of *Staphylococcus aureus* as a troublesome hospital pathogen. This species produces coagulase, an enzyme that coagulase plasma and blood.

Staphylococcus aureus also produces hyaluronidase or "spreading factor" it promotes invasion, it produces staphylokinase which digest blood clots, a nuclease that digest DNA (DNase) and lipases that help bacteria colonize oily skin surfaces.

Staphylococcus aureus also produces hemolysins (exotoxin) that lysed red blood cells by distrupting the membranes. Other exotoxin includes leukocidin, which damages cell membranes of neutrophils and macrophages, causing them to lyse (Kathleen and Arthur, 1996).

The most common infection of *Staphylococcus aureus* are boil, carbuncle, impetigo, Osteomyelities and abscess.

To identify *staphylococcus*, the primary isolation is achieved by inoculation on sheep or rabbit blood agar or in heavily contaminated specimens, on selected media such as mannitol salt agar. It can be gram stained and observed for irregular clusters of gram +ve cooci.

Other test includes biochemical test like catalase production (Kathleen and Arthur, 1996).

2.10.1 General Characteristics of the Streptococci

The members of this group are known for the arrangement of cooci in long, beadlike chains. The chains are commonly in pairs, their general shape is spherical but can also appear ovoid or rodlike, especially in actively dividing young cultures.

Streplococci are non-spore-forming and non motile (except for an occasional flagellated strains, and they can form capsules and slime layers.

They are facultative anaerobes that ferment a variety of sugars, usually with the production of latic acid streptococci are catalase-negative, but they do have a peroxidase system for inactivating hydrogen peroxide which is why they can survive even in the presence of oxygen.

Their colonies are usually small, nonpigmented and glistening. Most members of the genus are quite sensitive to drying, heat and disinfectants, and seldom develop drug resistance, though pneumococci and enterococci are notable exceptions. (Kathleen and Arthur, 1996).

Despite the large number of streptococcal species, human disease is most often associated with *Streptococci pyogens*, *Streptococci agalactice*, *Streptococci pneumoniae*, *Streptococci matants* and *Enterococcus faecalis* (Kathleen and Arthur, 1996).

To identify the group *streptococci* cultivating a specimen on sheep blood agar plates and occasionally enrichment media. Group A (which is *Streptococci pyogenes*) are by for the most common B - hemolyitc isolates in human lesions.

Group C (*Streptococci equisinnilis*, *Streptococci dysgalactiae*) are also B- hemolytic, A positive bacitracin disc test provides important evidence of Group A.

Group B (*S. agalactiae*) are differentiated from groups A (*S. pyogens*) and group D (*S. feacalis*, *S. bouis*) by the CAMP reaction which is positive for group B and negative for A and D. (Kathleen and Arthur, 1996).

2.10.2 General Characteristics of the Genus *Bacillus*

The genus Bacillus are acrobic, endospore-forming, group –positive, motile, rod-shaped forms. They are catalase positive.

The group is noted for its versatility in degrading complex macromolecules, and it is also a common source of antibiotics. The primary habitat of many species is the soil, spores are continuously dispersed by means of dust into water and onto the bodies of plants and animals.

Despite their ubiquity, the two species with medical importance are *Bacillus anthracis* (causes anthrax) and *Bacillus cereus* (causes one type of food poisoning).

Bacillus species are resistance to heat, drying and chemicals and this accounts for their survival longevity and ecological niche of sporeformers, and is also relevant to their pathogenicity. (Kathleen and Authur, 1996).

2.10.3. *Candida albicans*

Candida albicans is the commonest cause of candidiases. It occurs as normal floral in the oral cavity, genitalia, large intestine skin of 20% humans. Microscopically, *candida albicans* has budding cells of varying size that can be elongated and attached in a pseudomycelium. It is a white- to cream-colored pasty colony with a yeast odor.

Candida albicans causes local infections of the mouth pharynx, vagina, skin, alimentary canal and lungs.

Mucous membranes most frequently involved are the oral cavity and vagina. Thrush is a white, adherent, patchy infection affecting the membranes of the mouth, gums, cheeks or throat, usually in newborn infants and elderly, debilitated patients. (Kathleen and Arthur, 1996).

Candida albicans also causes vulvovagina candidiasis (or yeast infection) has wide spread occurrence in adult women, especially if taking antibiotics or oral contraceptives, diabetic, or pregnant. Other diseases caused by this organisms are cutaneons candidiasis and *candida* blood infection.

Laboratory diagnoses of candidiasis is made if budding yeast cells and pseudohyphae are found in specimens from localized infections. Specimens are cultured on standard media incubated at 30°C.

Identification is complicated by the numerous species of *Candida* and other look-alike yeast, though confirmatory evidence of *Candida albicans* can be obtained by the germ tube test, the presence of chlamydospores. (Kathleen and Arthur, 1996).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. COLLECTION AND PROCESSING OF DENTAL CARIES SAMPLE

Dental caries samples were collected using sterile swab stick from 12 randomly selected diagnosed patients of General Hospital, Minna. The samples were analysed in the laboratory within 24 hours of collection. The caries samples were transferred into tubes containing physiological normal saline, to form a suspension. Prepared nutrient agar plates were inoculated with caries suspension, and the plates were incubated at 37^0 C for 24 hours.

The plates with colony counts of 400 were taken as indicative of infection (Baker and Silverton, 1985 Vandepittes *et al.*, 1991). The colonies were sub-cultured repeatedly on to nutrient agar and incubated at 37^0C for 24 hours for the isolation of pure culture. The pure cultures were maintained on nutrient agar slants for further characterization and identification.

3.2. CHARACTERIZATION AND IDENTIFICATION OF ISOLATES

The isolates were characterized, based on colonial morphology, cultural characteristics and biochemical tests. The biochemical tests carried out included gram stain, production of catalase, urease, coagulase and indole, citrate utilization, methyl red- voges proskauer (MR-VP), starch hydrolysis and fermentation of the following sugars; glucose, lactose, fructose, manitol and sorbitol. The isolates were identified by comparing their characteristics with those of known taxa

using the scheme of Cowan (1974). Some of the biochemical tests carried out are described below.

3.2.1 Gram - Stain

A small portion of each of the isolates was picked with sterile wire loop and emulsified in a drop of distilled water placed on a clean slides and spread uniformly over the surface of the slide to form a dried smear, crystal violet was applied and left on the slide for 1 minute, washed with lugos iodine. The smear was then washed with 70% alcohol until the original visible colour of the initial dyes had disappeared and the smear was rinsed with water. The smear was then counterstained with safranin for 30 seconds after which it was rinsed with water and blot dried with filter paper. The stained smear was examined under the light microscope, using oil immersion objective. Gram-positive organisms retained the original colour of the initial dye and appears dark blue while the gram – negative organisms appeared pink red in colour. Vandepitte *et al* (1991) and Ogbulie *et al* (1998).

3.2.2 Catalase Test

Sterilized glass slides were placed on clean staining racks; and a drop of 3% hydrogen peroxide (H_2O_2) was placed on the centre of each slide. Then a portion of the isolate was aseptically transferred into the slide with a drop of hydrogen peroxide (each isolate for different slides). The evolution (efferverscense) of oxygen gas bubbles indicated a positive reaction. Although some bacterial culture are slow catalase produces and had to be observed for few minutes before

positive results were recorded no gas bubbles were observed in negative reaction. (Ogbulie *et al.*, 1998)

3.2.3 Oxidise Test

Sterile filter papers were aseptically placed on clean petri dishes, 2 to 3 drop of oxidase reagent were added using a piece of sterile wooden stick. The test organisms were smeared on each of the filter paper (Baker and Silver, ton, 1982, Vandepite *et al.*, 1991, Ogbulie *et al.*, 1998) The appearance of a blue colour within 10 seconds indicated a positive reaction while absence of blue purple colour within 10 seconds showed a negative reaction. Vandepitte *et al.*, 1991; Baker and Silverton, 1982; Ogbulie *et al.*, 1998).

3.2.4 Indole Production

To two-day over night culture of the isolates in 1% phosphate peptone broth in test tubes 0.5ml of the kovac's indole reagent was added. The tube was shaken gently and allowed to stand. Development of very deep red colour in the presence of indole, which separates out in the alcohol interphase, indicated positive test while orange or yellow colour showed a negative result.

3.2.5 Methyl red (MR) Test

Bacterial isolates were inoculated into 2ml of sterile glucose phosphate peptone water and inculcated at 37^0C for 48 hours. Using pastures pipette four drops of methyl red reagent was added and mixed thoroughly. The appearance of bright red colour indicated a positive test, while yellow colour indicated a negative test (Ogbulie *et al.*, 1998).

3.2.6 Urease Test

Slants of Christensen's urea agar medium was inoculated with the test organisms in duplicates and incubated at 35^0C for 72hours. The slants were examined after 12hours and 24hours. Urease production was indicated by colour change in the medium from yellow to pink, while the negative result indicated no change in colour i.e. maintained the original yellow colour. (Ogbulie *et al.*, 1998).

3.2.7 Coagulase Test

Using Pasteur pipette, one drop of physiological saline was added to crease free slides and the colony of the test organism was emulsified in each drop to make a thick suspension of the organism. To each suspension, one drop of blood plasma was added and mixed gently.

The appearance of clumping within 10 seconds was an indicative of positive test, while no clumping showed a negative reaction. (Ogbulie *et al.*, 1998).

3.2.8 Critrate Utilization

An overnight culture of each test isolate was inoculated into 3ml of sterile Koser's Citrate medium using a sterile straight wire, and incubated at 37^0C for 4 days. Observing it daily, citrate utilization resulted in an alkaline reaction, which changed the colour of the indicator (bromo – thymol blue) from green to blue.

In addition to the colour change utilization of citrate was indicated by increase turbidity of the medium, thus, a positive result was indicated by (growth) and blue colour while lack of turbidity and greenish colour indicated a negative result. (Ogbulie *et al.*, 1998).

3.2.9. SUGAR FERMENTATION

Six duplicate sterile phenol red fermentation medium containing lactose, sucrose, fructose, glucose, maltose and starch were prepared. Each of the duplicate medium was aseptically inoculated with the test organisms, leaving others as control. Durham tubes were inverted in to each test tube; the inoculated and uninoculated tubes (control) were incubated at 35°C for 24hours. The tubes were later observed for turbidity, colour change and gas formation. (Ogbulie *et al.*, 1998).

3.2.9.1. CONFIRMATORY IDENTIFICATION OF *C. ALBICAN*

The identification of *C. albican* was carried out by the method described by Cheesbrough (2002) in which 0.5mls of human serum was pipette into a small test tube. Using a sterile wire loop, inoculate the serum with a suspected yeast colony from the culture plate. Place the tube in a water bath or incubator at 35 – 37°C for 2 – 3 hours.

Using a pipethe, transfer a drop of the serum yeast culture to a glass slide and cover with a cover glass and examine the preparation using the 10x and 40x objectives with the condenser closed sufficiently to give good contrast, (a drop of lactophenol cotton blue can be added to the preparation to stain the yeast cells.

Look for sprouting yeast cells that is tube-like outgrowths from the cells (known as germ tubes). When sprouting yeast cells are seen – the culture is *Candida albicans*.

When the yeast cells do not show sprouting – the culture as yeast other than *Candida albican* isolated (Cheesbrough, 2002).

3.3. COLLECTION OF MEDICINAL PLANTS (CHEWING STICKS).

The plants namely *Zanthoxylum zanthoxyloides* and *Prosopis africana* were collected in Ibadan and Minna respectively. The traditional names of *Zanthoxylum zanthoxyloides* includes, Orinata (Yoruba), Fasoko richi (Fulani), Fusa Kuwari (Hausa) while *Prosopis africana* traditional names includes: Kiriya (Hausa), Ayan (Yoruba) and Abrua (Igbo). They were identified by Prof. I.Z. Ezenwa of the School of Agric and Agric Technology, Federal University of Technology, Minna. The plants were stored in a cool dry cupboard pending use.

Processing of Medicinal Plants

The leaves and stems of both were washed and dried first in the sun for 3 days and the hot air oven at 80^0C for 24hours.

3.4. PREPARATION OF EXTRACTS

The extraction was done in the conditions in which herbalist generally used the herbs, ethanol as a solvent because of its broad spectrum and relative properties of extraction and methanol solvent because of its selective property of extracting tannins (Iyamabo, 1991).

3.4.1 Aqueous Extraction

Two hundred millitres of distilled water was added to 50g of each of the grounded samples in 500ml capacity conical flask and agitated manually the flask were left overnight in the laboratory cupboard. In each case, the extract was collected by filtering with filter paper (No1 whatmans) and was washed thoroughly with 50mls of distilled water. The solvent was then poured into petri dishes and left open inside the

laboratory cupboard to evaporate to dryness. The dried samples were stored in well corked bottle for further analysis. (Iyamabo, 1991).

3.4.2. Methanolic Extraction

Fifty grames of each of the grounded samples were put into 500ml conical flask and 200ml of 90% methanol was added to the samples. The procedure used for the aqueous extract was followed. A rotary evaporator was used in vacuum at 40^0C to concentrate each of the extract. The extracts recovered were stored in well corked bottles and stored in the refrigerator for further analysis. (Iyamabo, 1991).

3.4.3. Ethanolic Extraction

The procedure was similar to that of methanol extraction except that ethanol was used instead of methanol.

3.5. PRELIMINARY PHYTOCHEMICAL ANALYSIS

A preliminary phytochemical analysis of the plants for the presence of saponins, phenolic compounds or tannins, anthraquinone, glycosides, polyphenol, holobatannis, cardiac glycosidis and anthranoids was performed according to the methods described by Culei (1982) and Sofowora (1984).

3.5.1. Test for Alkaloids

Two grames of each of the grounded plant materials was treated with 10ml of 1% Hydrochloric acid in a water bath for 10 minutes. The filtered solution was then treated with a few drops of dragendorfs reagents:- Development of turbidity or precipitate in the fitterate indicated the presence of alkaloids. (Culei, 1982, Sofowora 1984).

3.5.2. Test for Anthranoids

Two grames of each the grounded plant materials were boiled with 5ml of sodium hydroxide. This was filtered and treated with 5 – 6 drops of acetic acid and 5ml of toluene. This was shakened, allowed to settle and the upper layer transferred to fresh test tube using pipette 0.5ml of sodium hydroxide was added. Development of a red colour indicated the presence of anthranoids. (Culei, 1982; Sofowora, 1984).

3.5.3. Test for Anthraquinone

Five grammes of each extract were added to 10ml of Benzene. This was filtered and 5ml of 10% ammonia solution was added and shaken vigorously, the presence of a pink red or violet colour in the ammonia phase (bottom of the test tube) indicated the presence of anthraquinone, (Culei, 1982; Sofowora, 1984).

3.5.4. Test for Cardiac Glycosides

A small quantity of the extract (water extract) was dissolved in pyridine and a few drops of 2% sodium nitroprusside was added followed by the addition of a few drops of 20% dodium hydroxide. The appearance of deep red colour which faded to a brownish – yellowish indicated the presence of cardiac glucosides (Culei, 1982; Sofowora, 1984).

3.5.5. Test for Phlobatannins

Two millitres of the aqueous plant extracts was boiled with 5ml 1% concentrated hydrocholoric acid (Hcl). Development of a red

For the susceptibility test, nutrient agar (NA) plates were inoculated with 3 hours incubated suspension of mentioned bacteria by sterile wire loop. A sterile cork borer was used to bore five wells into each agar plate. To each of the first set of plates, 2000µg/ml each extract was aseptically introduced into the cups of each plate. The centre well was inoculated with the extractant solvent to act as control. The plates were incubated at 37^0C for 24 hours. (Cruikshank *et al.*, 1977).

3.6.1. ANTIBIOTIC SENSITIVITY TEST

(i) Disc Preparation

Disc diffusion method was used in this test as described by Vandepitte *et al*, (1991). Six milliliter paper discs were prepared using Whartman No. 1 filter paper and impregnated with different concentration of the antibiotics later, sterilized using hot – air oven at 160^0C for 1 hour. The antibiotics and their concentration used were Ampicillin 10µg, Erythromycin 15µg, Tetracycline 30µg, Ciprofloxacin 100µg. Prepared in accordance to the National Committee for Clinical Laboratory Standard. The drugs used in this test were chosen to reflect the range of drugs commonly prescribed for the treatment of dental caries. The antibiotics discs were prepared and stored in the refrigerator at – 20^0C until required

(ii) Sensitivity Test

Cotton wool swabs on wooden applicator sticks were prepared and sterilized by dry heat (Vandepittle *et al*, 1991). Colonies of the test organisms were transferred into tubes containing physiological normal saline, to form a suspension. Prepared nutrient agar plates were inoculated with the test organisms (*Staphylococcus aureus,*

Streptococcus sp, *Bacillus* sp and *Candida albicans* by dipping the sterile swab into the inoculum and removing excess inoculum by pressing and rotating the swab firmly against the side of the tube. The inocula were streaked all over the surface of the medium, three times, rotating the plates through an angle of 60^0C after each application (Vandepitte *et al*, 1991). The inoculated plates were allowed to dry for a few minutes at room temperature with the lid closed. The prepared antibiotics discs stored in refrigerator at 4^0C were kept at room temperature foe one hour before use. The antibiotics discs were later placed on the inoculated plates using a fair of sterile forceps. Each discs was gently pressed down to ensure even contact with the medium. The plates were incubated at 37^0C for 24 hours. At the end of the incubation period, the results were recorded as sensitive or resistance based on the occurrence of zone of inhibition respectively.

3.7. DETERMINATION OF MINIMAL INHIBITORY CONCENTRATIONS OF THE EXTRACTS OF MEDICINAL PLANTS

The Minimal Inhibitory Concentration (MIC) test was done by the method of Cruikshank *et al* (1977). Different concentrations of the extracts were poured into separate plates. The concentrations of the aqueous, methanolic and ethanolic extracts were poured in separate petri plates in duplicates. The concentrations were 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 pipette. Fifteen milliliters (15ml) of sterile nutrient agar was measured using a sterile pipette (25ml) into each of the plates and swirled to ensure uniform mixture of the extracts. The medium was allowed to solidify. A serial dilution of overnight

cultures of the organisms was made and each dilution compared to a mixture of 5g Barium chloride and 100ml distilled water kept in a separate test –tube. The dilution, which had similar or close turbidity to that of the Barium chloride solution was utilized to streaked on the surface of nutrient agar plates containing appropriate concentration of the extract. The inoculated plate were incubated at 37^0 C for 24 hours. At the end of incubation period, the plates were observed for growth or growth inhibition. The lowest concentration that entirely inhibited the growth of the organism was recorded as the minimal inhibitory concentration of the extracts.

3.8. PREPARATIVE THIN LAYER CHROMATOGRAPY FOR SEPARATION OF THE EXTRACTS OF ZANTHOXYLUM ZANTHOXYLOIDES AND PROSOPIS AFRICANA

(i) Preparation of TLC plates

Mini and macro TLC plates used were cleaned with chromic acid, washed with hot water and allowed to dry in order to remove all stains, fungi marks, grease or oil stains and dirt. They were then cleaned on an even laboratory bench. For the mini TLC plates, microscopic slides were used. Two grams of silica gel mixed with 4ml of distilled water was ground together in a mortar until it began to thicken. This slurry was then poured on the slides and spread evenly with a glass spreader. It was allowed to set for 5 minutes and dried at 110^0C in an oven (S – LEEC model oven) for 15 minutes to activate the plates and allowed to cool by exposing to the atmosphere for 30 minutes. Excess silica gel was carefully removed from the slide suing a razor blade.

For the macro TLC plates (20cmx 5cm) were use, 25g of silica gel was mixed with 50ml distilled water in the same procedure as above was used.

(ii) Chromatographic separation of the methanol and ethanol of *Zanthoxylum Zanthoxyloides* and *Prospis africana* extract on preparative macro TLC plates.

A solution of the dried extract was made by dissolving 1g of each extract in 5ml of the solvents. Using a capillary tube, a concentrated band of the solution was made on the TLC plate about 2cm from the base while drying intermittently with a drier to remove the solvent from the band. (About 5.0ml of the extract solution was used). The plate was placed with the spot downwards in a chromatographic jar containing 120ml of hexane (60ml); ethylcatate (40ml); methanol (20ml) as the developing solvent and allowed for 90 minutes. The plates was removed, was allowed to dry and sprayed with vanilline reagent so as to locate the separated bands, which were then circled.

The resultant bands and the residue at the base were cut out and each band mixed with 10ml of the solvent (ethanol or methanol) in a test tube, shaken vigorously, allowed to settle and filtered using filter paper into another test tube. The solvents were evaporated in a water bath and the fractions were collected. (Oloke and Kolawole, 1985).

3.9. DETERMINATION OF THE ANTIMICROBIAL ACTIVITY OF THE FRACTIONS SEPARATED FROM PREPARATIVE MACRO TLC PLATES.

The remaining filtrate obtained from the resultant band and residue was poured into different petri dishes and 5ml of glycerol was added.

The activity of these reconstituted extracts against some selected microorganism were then determined.

The test organisms used were, *Bacillus* sp, *Staphylococcus aureus, Streptococcus* sp and *Candida albicans*. Overnight culture of each of the test organism was prepared and the overnight culture was reinoculated for 3-4 hours. 0.2g of each of the extract was weighed and dissolved in methanol or ethanol solvent. This is to give 2000µg/ml, 20mls of nutrient agar was prepared in universal bottle and the agar were sterilized in the autoclave at 121^0C for 15minutes. The agar were then poured into petri dishes. The disc method was used for the test and surface inoculation of the organisms was done on each of the plate. Each plate was evenly inoculated with a particular organism aid disc dipped into each of the prepared extract was placed on the plate as shown in the pictures (Appendix 10). Each of the plates had a control of standard Ampicillin and Ciprofloxacin disc. (Oloke and Kolawole, 1988).

CHAPTER FOUR

4.0. RESULTS

4.1. PHYTOCHEMICAL SCREENING OF MEDICINAL PLANTS

The results of the phytochemical screening are shown in Table 4. the results showed that the two plants (chewing sticks) showed the presence of Akaloids, cardiac glycosides, tannins and saponin. Both the leaves and the stem of the two chewing sticks show the absence of Anthranoids, anthraquinone and phlobatannis.

Table 4.1. *Phytochemical* components of chewing sticks *(Z. zanthoxyloides* and *P. africana)*

Phytochemical Components	*Z. Zanthoxyloides*		*P. africana*	
	Stem	Leaves	Stem	Leaves
Alkaloids	+	+	+	+
Anthranoids	-	-	-	-
Anthraquinones	-	-	-	-
Cardiac glycosides	+	+	+	+
Tannins	+	+	+	+
Polyphenol	+	+	+	+
Saponins	+	+	+	+
Phlobotannins	-	-	-	-

+: Present, -: Absent

4.2. ANTIMICROBIAL ACTIVITY OF ETHANOL, METHANOL AND AQUEOUS EXTRACTS OF *ZANTHOZYLUM ZANTHOZYLOIDES* AND *PROSOPIS AFRICANA*

Table 4.2 shows the antimicrobial activity of the plant extracts against *Staphylococcus aureus, Streptococcus* sp. *Bacillus* sp and *Candida albicans* isolated from dental caries. The results revealed that all the extracts except the aqueous extract and the extractant solvent exhibited antimicrobial activity on the test organisms. It was observed that ethanol and methanol extracts of the two plants had more activity on *C. albicans* than the rest of the test organisms. It is also clear from the result that *Bacillus* sp. had the least zone of inhibition for both plants.

Generally, extracts of *Zanthoxyloides zanthoxyloides* seem to be more active than those of *Prospis africana.* The result of this test can be seen in Appendix 9.

Table 4.2. Antimicrobial activity of Ethanol, Methanol and Aqueous extracts of *Z. zanthoxyloides* and *P. africana*. (mm).

Organism	Z. zanthoxyloides										P. africana										Average zone
	Ethanol		Methanol		Control Ethanol		Control Methanol		Control Aqueous		Ethanol		Methanol		Control Ethanol		Control Methanol		Control Aqueous		
	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	
S. aureus	12	15	13	12	-	-	-	-	-	-	12	7	9	13	-	-	-	-	-	-	7.75a
Streptococcus sp.	13	13	16	14	-	-	-	-	-	-	15	11	16	11	-	-	-	-	-	-	9.08c
Bacillus sp.	14	9	14	8	-	-	-	-	-	-	9	10	10	8	-	-	-	-	-	-	6.83b
C. albicans	16	17	18	15	-	-	-	-	-	-	15	13	13	16	-	-	-	-	-	-	10.25d
Average zone per colum (mm)	13.75^{bc}	13.50^{bc}	15.25^{c}	12.25^{b}	-	-	-	-	-	-	12.75^{b}	10.25^{a}	12.00^{b}	12.00^{b}	-	-	-	-	-	-	

S: stem, L: leaf.

a, b, c, d = superscript of mean

Mean data in the same row or column carrying different superscript (a or b) are significantly different ($P<0.05$) while mean data carrying the same superscript are not significantly different ($P > 0.05$) Mean data that contain both superscripts (ab) do not differ significantly ($P > 0.05$) from the two extremes.

4.3. COMPARISON OF ZONES OF INHIBITION OF CRUDE EXTRACTS OF *Z. ZANTHOZYLOIDES* AND *P. AFRICANA* WITH STANDARD ANTIBIOTICS.

Table 4.3. shows the results of the comparison of inhibition caused by extracts of medicinal plants with standard antibiotics. The average zone of inhibition of S. aureus on crude extract and the TLC extract is 19.33mm, which is higher than the rest of the test organisms.

The result revealed that *Z. zanthoxyloides* had higher antimicrobial activity (13.75mm) than the Tetracyclin (10.75mm) and erythromycin (11.00mm), but had lower activity than Ampicillin (20mm) and Ciproflaxacin (24.33mm). it was seen from the result that the extracts of *P. africana* had higher activity (11.50mm) than the Tetracyclin (10.75mm) but had almost the same activity as that of Erythromycin (11.00mm). ampicillin (20.00mm) and Ciprofloxacin (24.33mm) had higher antimicrobial activity than the crude extract of *P. africana* (Table 4.3).

Table 4.3. Comparison of zones of inhibition of crude extracts of medicinal plant with standard antibiotics (mm)

Organism	Average (mm) zone of Inhibition of *Z. Zanthoxyloides*	Average zone of inhibition of *P. africana*	TET	AMP	ERT	GP	Average zones Ron $\frac{\Sigma x}{n}$
S. aureus	13	10	18	36	12	27	19.33^{d}
Strept. Sp	14	13	13	30	17	25	18.67^{c}
Bacillus sp	11	9	12	14	15	21	13.67^{b}
C. albicans	17	14	-	-	-	-	5.16^{a}
Average zones	13.75^{b}	11.50^{n}	10.75^{a}	20.00^{c}	11.00^{a}	24.33^{d}	

Amp: Ampicillin, ERT: Erythromycin, TET: Tretracyclin, CIP: Ciprofloxacin

Note: Mean data in the same row or column carrying different superscript (a or b) are significantly different ($P < 0.05$) while mean data carrying the same superscript are not significantly different ($P > 0.05$). Mean data that contain both superscript (ab) do not differ significantly ($P > 0.05$) from the two extremes.

4.4. ZONES OF INHIBITION PRODUCED BY THE FRACTIONS OF *Z. ZANTHOXYLOIDES* AND *PROSOPIS AFRICANA*

Table 4.4 shows the results of the inhibitory effects of the TLC extracts of the *Z. zanthoxyloides* and *P. africana* on the test organisms. Which are *Straphylococcus aureus, Streptococcus* sp., *Bacillus* sp. And *Candida albicans*. The results revealed that all the TLC extracts of *Z. zanthoxyloides* exhibited antimicrobial effects but the TLC fractions of the other plant (*P. africana*) does not show any antimicrobial effect on the organisms, while the base spot of the extract of *P. africana* when tested on the test organisms showed antimicrobial activity. The result also revealed that the antimicrobial activity of TLC fractions of *Z. zanthoxyloids* is greater than the antimicrobial activity of the base spot of *P. africana*. Appendix 10 is showing the picture of zone of inhibition on the two plant extracts.

Table 4.4. Zones of inhibition produced by the fractions of TLC of *Z. zanthozylum* and *P. africana* (mm).

Organisms	*Z. zanthoxyloides*				*P. africana*				Base spot of *P. africana*			
	Ethanol		Methanol		Ethanol		Methanol		Ethanol		Methanol	
	S	L	S	L	S	L	S	L	S	L	S	L
S. aureus	14	18	12	-	-	-	-	-	11	8	9	13
Strept. sp.	16	14	16	-	-	-	-	-	14	12	14	10
Bacillus sp.	14	10	12	-	-	-	-	-	9	8	10	8
C. albicans	16	13	18	-	-	-	-	-	16	15	12	16

S: stem; L. Leaf

4.5. ZONES OF INHIBITION OBTAINED BY THE CRUDE AND TLC FRACTIONS OF THE PLANT *ZANTHOXYLUM ZANTHOXYLOIDES* IN ETHANOL, METHANOL AND AQUEOUS SOLVENT

Table 5.4. shows the results of the inhibitory effect of the crude extract of *zanthoxylum zanthoxyloides* and the TLC fraction of the same plant. The results revealed that both the crude extract and TLC fractions of the plant exhibited antimicrobial effect on the test organisms. The results showed that the TLC fractions of the stem of the plant *Z. zanthoxyloides* in both Ethanol and Methanol is (15.00 and 14.75 respectively) higher than the zone of inhibition exhibited by the crude extract of the same plant. (13.75 and 15.25 respectively). While the same thing is applicable for the leaves extracts of the plant. The results also shows that *Candida albican* shows the highest zone of inhibition than all other organisms (10.67mm) followed by *Streptococcus* sp. While *Bacillus* sp. shows the lowest zones of inhibition (7.92mm) see appendix 10.

Table 4.5. Comprison of zones of inhibition obtained by the crude extract and TLC frictions of the plant *Zanthoxylum zanthoxyloides* in Ethanol, Methanol and Aqueous solvent. (zones sizes measured in milliletres).

Organism	Z. zanthoxyloides										P. africana										Average zone
	CRUDE				Control TLC						CRUDE				Control TLC						
	Ethanol		Methanol		Ethanol		Methanol		Aqueous		Ethanol		Methanol		Ethanol		Methanol		Aqueous		
	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	
S. aureus	12	15	13	12	-	-	-	-	-	-	14	18	13	12	-	-	-	-	-	-	9.08[b]
Streptococcus sp.	13	13	16	14	-	-	-	-	-	-	16	14	16	11	-	-	-	-	-	-	9.42[b]
Bacillus sp.	14	19	14	8	-	-	-	-	-	-	14	10	12	14	-	-	-	-	-	-	7.92[a]
C. albicans	16	17	18	15	-	-	-	-	-	-	16	13	18	15	-	-	-	-	-	-	10.62[c]
Average zone per colum (mm)	13.75[a]	13.50[a]	15.25[b]	12.25[a]	-	-	-	-	-	-	15.00[b]	13.75[a]	14.75[ab]	13.00[a]	-	-	-	-	-	-	

S: stem, L: Leaf

Note: Mean data in the same row or column carrying different superscript (a, b or c) are significantly different ($P < 0.05$) while mean data carrying the same superscript are not significantly different ($P > 0.05$). Mean data that contain both superscript (bc) do not differ significantly ($P > 0.05$) from the two extremes.

4.6. ZONE OF INHIBITION OBTAINED BY THE CRUDE EXTRACT AND TLC FRACTIONS OF PROSOPIS AFRICANA IN ETHANOL, METHANOL AND AQUEOUS SOLVENTS. (ZONE SIZES MEASURED IN MILLIMETERS).

Table 4.6. show the inhibitory activity of the crude extract of *prosopis africana* on the test organisms and the inhibitory effect of the TLC fractions of the same plant. The result reveals that the crude extract of *prosopis africana* has antimicrobial effect on the organisms while the TLC fractions of this plant does not have any antimicrobial activity on the test organisms. The result shows that *C. albicans* has the highest zone of inhibition while *Bacillus* sp has the lowest.

The result also reveals that the stem of the plant has the highest antimicrobial activity than the leaves of the same plant (*Prosopis africana*).

Table 4.6. Zones of inhibition obtained by the crude and TLC fractions of *Prosopis africana* in Ethanol, Methanol and Aqueous solvent

	Crude Extract										TLC fractions								
					Control										Control				
anism	Ethanol		Methanol		Ethanol		Methanol		Aqueous		Ethanol		Methanol		Ethanol		Methanol		Aqu
	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S
ureus	12	7	9	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ptococcus	15	11	16	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
illus	9	10	10	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
lbicans	15	13	13	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: S: stem. L: Leaf.

4.7. MINIMAL INHIBITORY CONCENTRATION (MIC) OF EXTRACTS OF *Z. ZANTHOXYLOIDES* AND *P. AFRICANA*

The results of the minimal inhibitory concentration (MIC) test of the extract of the plants are presented in Table 4.7.

The results showed that MIC value of the extract of the plants *Z. zanthoxyloides* in ethanol for *S. aureus* and *c. albican* was found to be 0.8mg/ml while the MIC for *streptococcus* sp and *Bacillus* sp were 1.6mg/ml and 1.0mg/ml respectively.

The result also reveal that *Prosopis africana* MIC was found to be 0.6mg/ml for *Bacillus* sp. and *C. albicans.*

Table 4.7. Minimum inhibitory concentration (MIC) of extracts of Medicinal Plant (*Z. zanthoxyloides* and *P. africana*) mm

Organisms	*Z. zanthoxyloides*				*P. africana*			
	Methanol		Ethanol		Methanol		Ethanol	
	L	S	L	S	L	S	L	S
S. aureus	0.8	0.4	0.6	0.6	0.8	0.8	0.6	0.8
Strept. sp.	1.2	0.8	0.8	1.6	0.8	1.2	1.0	1.4
Bacillus sp.	0.6	0.8	0.8	1.0	0.6	0.8	1.0	0.6
C. albicans	0.8	0.6	1.0	0.6	0.8	0.8	1.2	0.6

S: Stem, L: Leaf

CHAPTER FIVE

5.0. DISCUSSION CONCLUSION AND RECOMMENDATION

5.1. DISCUSSION

Extraction of bioactive agents from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacological studies leading to syntheses of a more potent drug with reduced toxicity (Ebana *et al.*, 1991). The preliminary phytochemical screening showed that the extracts of *Z. zanthoxyloides* and *Prosopis africana* contain one or more of the following components, Alkaloids, Anthranoids, Anthraquinone, Cardiac glycosides, Tannins, polyphenol, Saponins and phlobatannins. The presence of alkaloids and Cardiac glyiosides in the extracts of *Z. zanthoxyloides* and *P. africana* agrees with the findings of Ebana *et al* (1991) who reported that alkaloids and cardiac glycosides inhibit pathogenic bacteria. The polyphenol found in the extracts of *Z zanthoxyloides* and *P. africaia* were also reported by Nguji, (1988). The investigation stated that phenols in plant tissues are usually oxidezed to co-quinones which the serum subsequently form cross-links with the serum protein of the skin to arrest bleeding and effect healing. The presence of Tannins in the plant extract agrees with the report of Nguji (1988) that tannins are important in herbal medicine and that they are applied in arresting bleeding and heals wound. Tannins and tannic acid own their astrigent action to the fact that they precipitate protein and render them resistant to attack by proteolitic enzymes. Internally they form a fine pellicle of coagulated protein over the lining of the alimentary tract. This now prevents the abstraction of toxic agents and protects the inflamented mucus

membranes. The irritability of the track thus decrease and undue peristalsis consequently dismissed (Nguji, 1988). Some tissues of taniferous plant are also cleaned as an antiscrobutin while infusion of some bark of plants rich in tannins is used as tonic and sometimes provides remedy for dysentery, diorrhoea, cough, fever and veneral diseases (Dalziel, 1995).

The inhibitory activities of the plant extract (*Z. zanthoxyliodes* and *P africana*) on the test organisms indicate that the plants possess active ingredients, which may be water or alcohol soluble.

However, all the extracts of the two plants *Z. zanthoxyloides* and *P. africana* in ethanol and methanol inhibited the growth of the test organisms, while the aqueous extract and the control does not show any antimicrobiol effect, this means that the active substance in the extract is not soluble in water but soluble in other solvents (methanol and ethanol). The results obtained in the present study agree with the finding of Akande and Hayashi (1998) who observed that the extracts of some chewing sticks, *Garcinia mannic, Masularia accuminata, Zanthoxylum gilletti, Terminalia glaucescens, Anogeissus leiocarpis and Pseudocedrela kotschyi* had inhibitory effect on *Staphylococcus aureus* and *Staphylococcus auricularis*.

The antimicrobial effect of the methanolic and ethanolic extracts of the two chewing sticks (*Z. zanthoxylodes* and *P. africana*) varies as revealed by the statistical analyses of the result. The antimionbial activity of the stem of the two plants was higher than that of the leaves of the same plant. This may be due to the fact that the part, which had the highest antimicrobial activity (stem) contain the active components in sufficiently high amounts. A similar observation had

been made by Asoradi (1989) who found that the unripe fruits of *Huntein* plants possessed better antimicrobicl properties than the, leaves or root of the same plant. Generally, the antimicrobal activity of chewing sticks also revealed that *Z. zanthoxyloides* had higher antimicrobal activity then *Prosopis africana* both in methanol and ethanol solvents.

The result revealed that the aqueous extract of both plants did not show any significant activity on the test organism though the herbalist or traditional healers use the extract raw, when plant materials are grounded and soaked in water, plants cells are damaged and a number of phenolases and hydrolases are released as reported by Akpata. (1979). These enzymes might overwhelm the active principle in the aqueous extract and so prevent them from expressing their antimicrobal activity. Another reason may be due to the fact that the active principles are organic molecules and therefore their solubility in water is very slow or low. The extracts of *Z. zanthoxyliodes* had higher antimicrobial activity than Tetracyclin and Erythromycin, which means that there is significant difference between the chewing stick extract (*Z. zanthoxyloides*) and the antibiotics. While Ampicillin and Ciprofloxacin demonstrated greater antimicrobial activity than the crude extract of this plant.

The study also reveal that the crude extract of the plant *Prosopis africana* shows nearly the same antimicrobial activity as that of Tetracyclin and Erythromycin while ampicillin and Ciprofloxacin had greater antimicrobial activity than the crude extract of the plant. Only the TLC extract of *Z. zanthoxyliodes*, showed antimicrobial activity on the test organisms while that of *P. africana* had no activity. The

reason might be that the active ingredients of *P. africana* did not move with the mobile phase (i.e. still in the base spot) or it may be that the active ingredients were not separated. This assumption was supported by the fact that when the spot of application (base spot) of *P. africana* was used on the test organism it showed antimicrobial activity on all the test organisms.

The separation of the methanol and ethanol extracts of stem and leaves of *Z. zanthoxyliodes* into two or three distinct components by thin layer chromatography shows that this plant parts contains more than one active component. This agrees with the finding of Sofowora et al. (1975) that the active ingredient in *Z. zanthoxyliodes* are 2 – hydroxymethyl benzoic acid, P-hydroxy benzoic acid and vanillic acid.

The TLC fraction of *Z. zanthoxyloids* had higher antimicrobial activity than the crude extracts of the same plant. This may be as a result of the fact that the chromatographic procedure purified the extract. On the other hand, the crude extracts may still contain some impurities purification is very important because the presence of some ingredients which reduce the activity of the active component is eliminated, concentrating the active component hence, increasing the activity of the active component.

The Minimal inhibitory concentration (MIC) is the lowest concentration of a drug that prevents growth of a particular micoroganism (Presscort *et al* 1990). Thus the MIC test gives some idea of the effectiveness of a chemothorapeutic agent against a microorganism. The results of the minimal inhibitory concentration

(MIC) test indicated that different concentrations acted as the MIC valves for the respective organism.

It is most probable that some of the organisms are more (sensitive) vulnerable to the action of the antimicrobial substance present in the extracts (which was manifested in their lower MIC valves). The results indicate that the organisms were inhibited by a minimal concentration of between 0.2mg/ml and 1.4mg/ml of extract. Higher MICs were recorded for *Streptococcus* sp *C. albicans* and *Bacillus* sp than for *Staphylococcus aureus*. The high MIC valves for *Streptococcus* sp, *C. albicans* and *Bacillus* sp indicate that these organisms are more resistant to the extracts than *Staphylococcus aureus*. This is probably due to differences in genetic constitution of the organisms. However, this finding seems to show that *Staphylococcus* sp and *C. albicans* may present difficulties in the therapy of dental caries by requiring more drugs at high concentrations.

5.2. CONCLUSION

The results of the study have led to the following conclusions:

(1) The extracts of the two plants (*Z. zanthoxyloides* and *P. africana*) especially ethanol and methanol extracts possessed significant antimicrobial activates against *Streptococcus* sp. *Staphylococcus aureus*, *Bacillus* sp and *Candida albicans*.

(2) The MIC of the ethanol and methanol extracts of the plants were comparable to those of Tetracycline, Ampicillin, Erythromycin and Ciprofloxacin.

(3) Antimicrobial activity of the fractions obtained by TLC of *Z. zanthoxyloids* was compared to that of the crude extract. And the activity of the origin base of the plant Prosopis africana was also compared to the crude extract of the plant.

(4) The results revealed that the plants extracts (i.e. the two chewing sticks) are equally antifungal since their zones of inhibition on *Candida albicans* were high both in the crude form and purified form (TLC).

(5) If the extracts are purified and processed they can be used as toothpaste since they inhibit the growth of bacterial and fungal isolates from dental carries. As a result of the antimicrobial effect of these extract there may be a scientific basis for their utility in traditional medicine basis for the treatment of toothache.

5.3. RECOMMENDATIONS

(1) The chemical structures of the active ingredients present in the test plant parts should be determined.

(2) The stability studies on the isolated antimicrobial constituents to determine practical usability of plants as chemotherapeutic agents is recommended.

(3) Toxicity studies of the active ingredients together with clinic trial to determine their usability as chemotherapeutic agents should be undertaken.

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APPENDIX I

(I) NUTRIENT AGAR

Agar	15g
Peptone	5g
Sodium chloride	5g
Yeast extract	2g
Lab – Lemon Powder	1g
Distilled Water	1 lit
pH	7.4

28g of the agar powder was dissolved in 100ml of distilled water and heated to dissolved completely. The medium was sterilized by autoclaving at 121°C for 15 minutes. It was allowed to cool before dispensing into sterile petri dishes in appropriate volumes.

(II) NUTRIENT BROTH

Lab – Lemco powder	1g
Yeast extract	2g
Peptone	5g
Sodium ahloride	5g
pH	7.4

13g of the agar powder was suspended in 100mls of distilled water and heated to dissolved completely. The broth was pipetted in 100ml amount into test tubes using a sterile 100ml pipette. The test tubes were plugged with cotton wool wrapped in aluminum foil and sterilized by autoclaving at 121°C for 15 minutes.

APPENDIX II

PHYTOCHEMICAL REAGENT

The following reagents were used in phytochemical screening of the medicinal plants. They were prepared as described by Cuilei (1982) and Sofowora (1984).

(i) 1g ferric (iii) Chloride

1g of ferric (iii) chloride was dissolved in 80ml of distilled water. This was diluted to a final volume of 100ml.

(ii) 10% ammonium solution

100ml of ammonia solution was added to 90ml of distilled water.

(iii) 1% Hydrochloric acid

Aqueous hydroch;oric acid solution has 35% hydrogen chloride gas. 30ml of 35% hydrochloric acid was diluted to 100ml with distilled water.

(iv) Dragendoff's reagent

0.85% of bismuth nitrate was dissolved in a mixture of 40ml distilled water and 100ml acetic acid and potassium iodide (8g) dissolved in 200ml of distilled water.

(v) Potassium hydroxide

28g of potassium hydroxide pelletes was dissolved in 100ml of distilled water.

Other reagent used include Benzene, Chloroform, toluene, acetic acid, cons-hydrochloric acid, 1% potassium ferricyande and ethanol (97%).

THIN LAYER

Acetic acid, Glacial acetic acid, silical gel (used as absorbent) chloroform, Hexane, Ethylacetate and methanol were used.

APPENDIX III

EXTRACTS YIELD

The yield of extracts for leaves and stem obtained from aqueous extraction of the plant *Z. zanthoxyloides* was 4.50g – 4.60g of sample whereas the yield was in the range of 2.60g – 3.40g of sample for ethanol and the yield was 4.04g – 3.90g of sample for methanol.

While the yield of extract for leaves and stem obtained from aqueous extraction for the plant *Prosopis africana* was 4.03g – 4.50g of sample and the yield was in the range of 2.40g – 3.60g of sample for ethanol whereas for extracts from methanol ranges from 3.80g – 4.02g of sample.

Name of Plants	Parts of Plants	Solvents	Sample (g)
Z. zanthoxyloides	Leaves	Distilled water	4.50
		Ethanol	2.60
		Methanol	4.04
	Stem	Water	4.60
		Ethanol	3.40
		Methanol	3.90
Prosopis africana	Leave	Water	4.30
		Ethanol	2.40
		Methanol	3.80
	Stem	Water	4.50
		Ethanol	3.60
		Methanol	4.02

APPENDIX IV

A SUMMARY OF THE CULTURAL AND BIOCHEMICAL CHARACTERISTICS OF ISOLATE FROM DENTAL CARIES

Isolate	CULTURAL CHARACTERISTICS	GRAM STAIN REACTION	CATALAE	COXIDASE	INDOLE	MR	VP	UREASE	COAGULAS	CITRATE	STARCH	GALATOSE	SUCROSE	FRUCTOSE	GLUCOSE
S. aureus	Abundant opaque Golden yellow Colony on N. A.	Grampositive cocci	+	-	-	-	+	-	-	+	-	A	A	A	A
Strep. Sp	Thin White spreading Viscous growth on NA	Grampositive cocci	+	-	-	-	+	+	-	-	-	-	+	+	-
Bacillus sp	Big dirty whitish mucoid colonis	Grampositive rod	+	-	-	-	+	-	-	+	+	-	+	+	-
Candida albican	Whitish or cream mycelium colonies growing into the agar fermented odour											+	-	+	-

APPENDIX V

Calculation of the concentration of extracts used 0.2g of the methanol extract + 5ml of methanol.

$\Longrightarrow$ 200, 000µg

for 1ml = $\frac{200,000}{5}$ = 40,000µg

40,000µg in 1ml

Take 1ml of the Mixture (0.2g of each extract + 5ml of each solvent) inside borer of 19ml of agar.

$\Longrightarrow$ 20ml

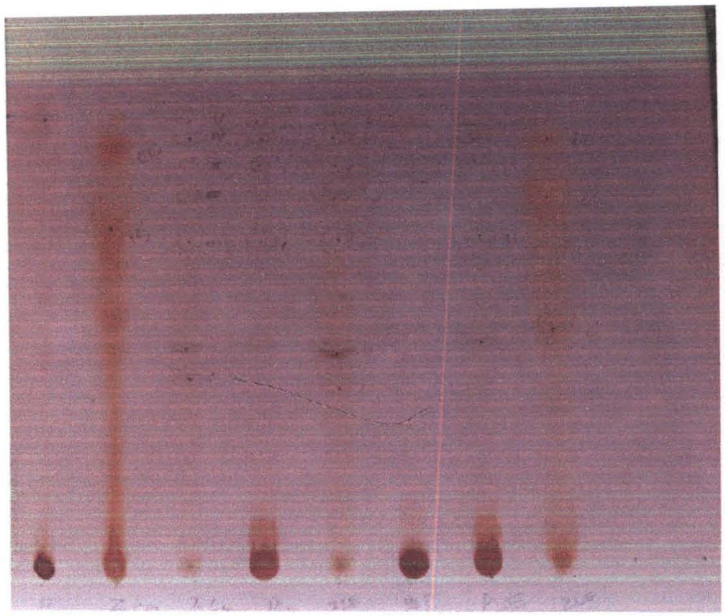
then = $\frac{40,000\mu g}{20ml}$ = 2,000µg/ml

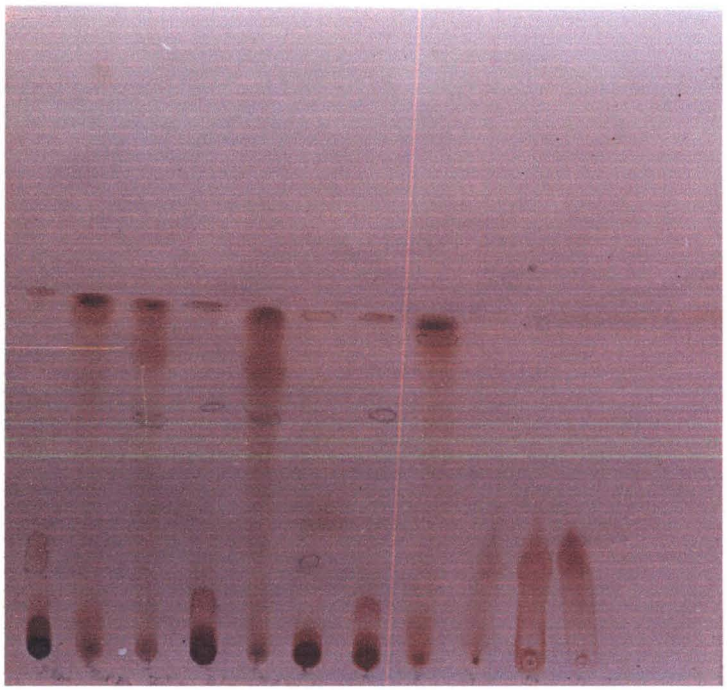
APPENDIX VI

Plant Parts and their number of Fractions on TLC

Plant Parts	Fractions
Zsm	3
ZsE	2
ZLM	2
ZLE	3
Zsw	1
ZLW	1
PLm	2
PLE	2
PSM	1
PSE	1
PLW	1
PSW	1

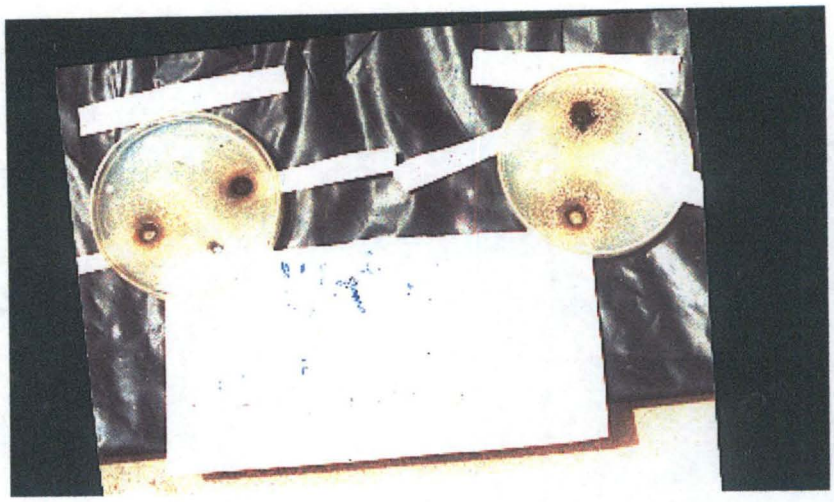
APPENDIX 7

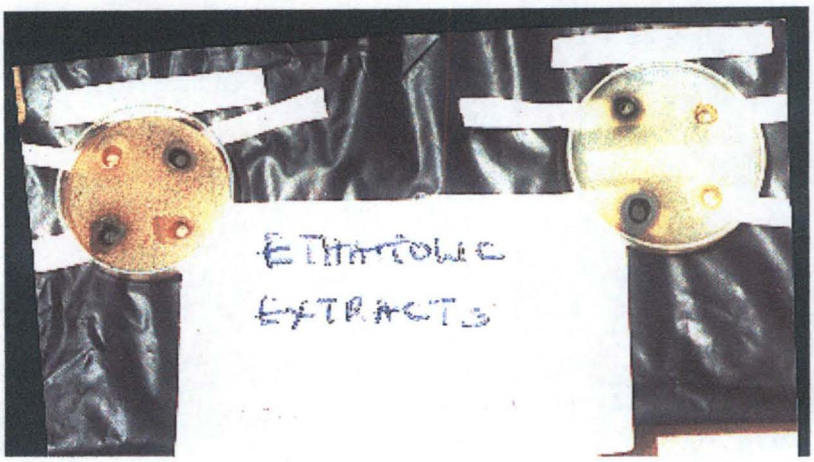




TIN LAYER CHROMATOGRAPHY OF THE EXTRACTS OF ZANTHOXYLUM ZANTHOXYLOIDES AND PROSOPIS AFRICANA

APPENDIX 8





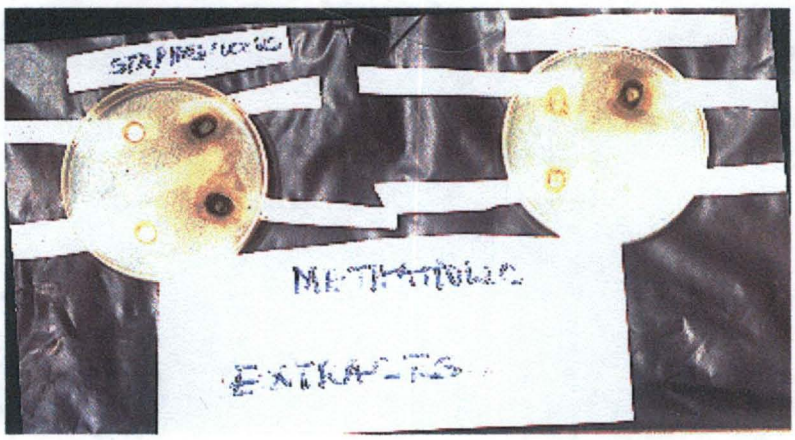
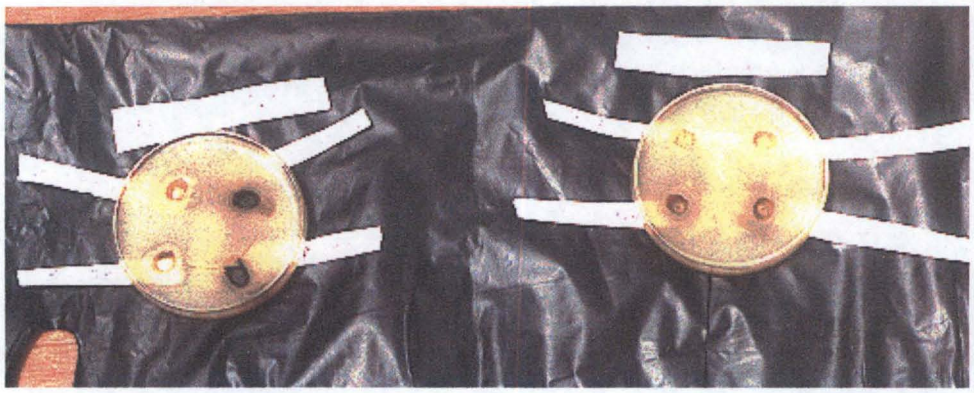


PLATE SAMPLES SHOWING CRUDE EXTRACTS OF PROSOPIS AFRICANA TESTED AGAINST CAUSATIVE AGENT OF DENTAL CARRIES

APPENDIX 9





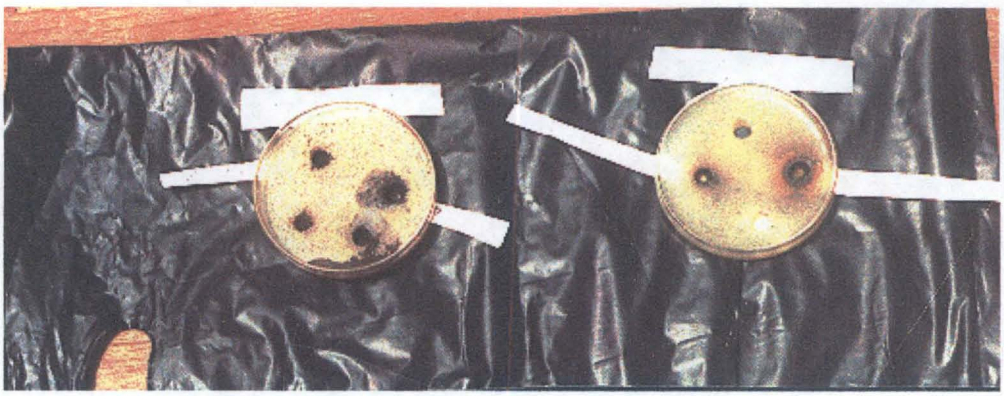


PLATE SAMPLES SHOWING CRUDE EXTRACTS OF ZANTHOXYLUM ZANTHOXYLOIDES AGAINST CAUSATIVE AGENT OF DENTAL CARRIES





SCREENED PLATE SAMPLES SHOWING THE THIN LAYER CHROMATOGRAPHY FRACTIONS OF PROSOPIS AFRICANA AND ZANTHOXYLUM ZANTHOXYLOIDES TESTED AGAINST THE CAUSATIVE AGENT OF DENTAL CARRIES