IN VITRO ASSESSMENT OF THE ANTIMICROBIAL PROPERTIES OF THE EXTRACTS OF SOME LOCAL MEDICINAL PLANTS ON AGENTS OF GASTROENTERITIS.

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (MEDICAL MICROBIOLOGY) OF THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, AHMADU BELLO UNIVERSITY, ZARIA.

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DECLARATION

This is to certify that this thesis is written by me and that it is a record of my research work carried out in the department of Microbiology, Faculty of Science, Ahmadu Bello University Zaria under the supervision of Dr. M. Galadima and Dr. S. Ibrahim. The works of other authors referred to have been duly acknowledged and referred to by means of references. I hereby declare that no part of this thesis has been submitted elsewhere for a degree.

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CERTIFICATION

THIS THESIS ENTITLED IN VITRO ASSESSMENT OF THE ANTIMICROBIAL PROPERTIES OF THE EXTRACTS OF SOME LOCAL MEDICINAL PLANTS ON AGENTS OF GASTROENTERITIS BY MUHAMMAD H. UMMU-KULTHUME MEETS THE REGULATIONS GOVERNING THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY OF THE AHMADU BELLO UNIVERSITY ZARIA, AND IS APPROVED FOR ITS CONTRIBUTION TO KNOWLEDGE AND LITERARY PRESENTATION.

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DEDICATION

To

Imamah, Ruhullah, and Mahadi.

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ABSTRACT

An ethnobotannical survey of four communities around Ahmadu Bello Univeristy Zaria was carried out to identify a number of plants (42) used in the traditional herbal treatment of gastroenteritis. These plants are either taken as infusions, decoctions or concoct ions.(of a combination of two or more plant parts).

Crude extracts of parts of ten plants with the highest frequency of use were investigated for antibacterial activity. Preliminary agar dilution test showed that extracts of six out of the ten plants tested (viz: Bridelia ferrugenia, Khaya senega1ensis, Piliostigma thonningii Psidium guajava, Terminalia avicennioedes and Terminalia macroptera) possessed significant antibacterial activities against all four strains of bacteria tested. Extracts of Occimum grattissimum, Parkia c1appertoniana, and Guerra senega 1ensis were inactive on the test organisms while Slerocarya birrea was inhibitory to Pseudomonas aeruginosa only.

The minimum inhibitory concentration range was within 0.96-10.4mg/ml with the extracts of B. ferrugenia showing the highest inhibition with the lowest MIC value of 0.96mg/ml for both S. flexnerii and S. typhi . MIC values of 1.6mg/ml was obtained for P. aeruginosa and 1.9mg/ml for E. coli. The highest MIC range was shown by the crude extracts of P. guajava which gave a value of 10mg/ml for E. coli, S. flexneri i and S. typhi and 2.8mg/ml for P. aerugi nosa.

Five of these active plants showed bactericidal activity against some or all of the test organisms. The bactericidal activities was confirmed by the tube dilution method with the minimum bactericidal concentration (MBC) in the range of 13-28mg/ml. However only B. ferrugenia and P. thonningii were bactericidal to all the test organisms.

Death curves of the organisms in the crude extracts suggested that the bactericidal activities occured within the first hour after treatment and by the 10th hour all the test organisms were killed in the test extracts.

The biologically active principles were isolated by a solvent extraction method using di-ethylether and aqueous sodium hydroxide. All the active components of the 3 test extracts were found to be located in the fraction containing acidic compounds thus indicating that they are acidic compounds in nature.

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1 CHAPTER ONE

INTRODUCTION

At present in every country in the developing world, official health departments are suffering from a malaise which is caused by two problems; insufficient medical services to reach the populace, and an increasing specialization in western medicine with less and less focus on the whole patient let alone his or her mileu. While the industries processing refined, synthetic or semi-synthetic medicaments were in their infancies in developing countries, the production and use of infusions and tinctures (Galenicals) of some plant and animal parts of known medicinal value have supported the practice of medical treatment (Marquise, 1984).

The emergence of resistant strains of organisms due to the abuse of antimicrobials, the spread of multiresistant strains from person to person and in the environment, and also due to the genetic versatility of microbes have rendered some hitherto effective antimicrobial agents useless (Shears, 1993).

In spite of the innovations in drug industry, infectious diseases still account for 7% of all deaths in the United States (Mitscher & Raghav-rao 1984).

Due to the problems of drug resistance and the cost of drugs, scientists especially in Africa and other developing countries are conducting researches into local plants which are used in traditional medicine. The use of plants as charms and for curing diseases has been known and practised in Nigeria for many centuries

and it is a well known fact that in Nigeria about 65% of the population when sick consult traditional doctors (Mume, 1991).

According to the WHO (1979), about 80% of the inhabitants of the world rely chiefly on traditional medicines for their primary health care needs and the major part of traditional therapy involves the use of plant extracts or their active principles (Farnsworth et al 1985).

In addition, the total world annual turn over of the pharmaceutical industry utilizing medicinal plants has been recently estimated by Elliot and Brimacombe (1986) to be at least U.S $20,000 millions, and more than two thirds of the plant species used are still collected from the wild mainly in the tropical countries and rain forests. This implies that plant based drugs which for centuries had remained man's prime therapeutic agents still occupy that prominent position in the treatment of a large number of ailments.

In the developed countries a lot of researches have been carried out and are still being carried out on the chemistry, mode and degree of efficacy of the extractives from a large number of medicinal plants. Thus, the standard attained in mordern medical practice is therefore as a result of continued scientific study and investigation which has provided a wealth of information about the nature and physiological activity of the many compounds extracted.

In Nigeria, we still have a long way to go as research in herbal medicine is besieged by problems such as:

(a) Lack of funds to carry out these researches.

(b) Lack of co-operation between the traditional herbalists and researchers in modern medicine.

(c) Indiscriminate bush burning.

(d) The attitude of some modern/Orthodox medical practitioners and their desire for medical monopoly (Mume, 1991).

Nevertheless, the government is still committed to medicinal plant research as the Nigerian policy includes the promotion and support of research into herbal and traditional remedies which are widely considered to be effective. Also, the establishment of the national institute for pharmaceutical research and drug development, Abuja, is a great step forward in promoting medicinal plant research. Other bodies, such as the Nigerian association for Lady pharmacists are also not left out in the struggle and this has led to their recent launching of an arboretum a "Medicinal plant cultivation project". This project is hoped to enhance research and development of medicinal plants, the conservation of human environment, and the management of natural resources.

For several but obvious reasons, physicians and patients are becoming more interested in traditional herbal drugs and an increasing preference for natural substances in health care. The toxicity of potent synthetics is more and more obvious and by relying exclusively on these preparations, patient pay a heavy price in adverse reactions. The overuse of antibiotics has also contributed to a profoundly dangerous medical threat to mordern health care and this has made it necessary for major prescriptions

to be reviewed and approved by personnel attuned to the problems of antibiotic abuse and aware of the alternatives for treatment. Thirdly, much of the mordern pharmaceutical industry is petroleum based, as oil becomes more and more expensive, other sources of remedies become more attractive. It is in this light that the validation of locally used herbs claimed by herbalists to treat various illnesses is necessary since it is noted that the ability of a single medicinal plant to cure various illnesses as claimed by these traditional medical practitioners is questionable and in most cases is only effective against one or two illness (Sofowora 1982). The verification of such claims by choosing these plants and testing for their pharmacological and antimicrobial properties and the documentation of results from such researches will among other things provide knowledge for alternative sources of drugs and information for further researches into the traditional herbal practice.

Studies to investigate the therapeutic values of traditionally used herbs and plant parts are many and varied (Obaseiki - Ebor 1985, Odama et al. 1986, Babalola 1988, Ikenebomeh and Metiriri 1988, Addae-Mensah et al. 1989, Awosika 1990, Emele et al 1991, Kela and Kufeji 1991). Thus as a result of researches into the active components contained in some plants extracts, considerable information has surfaced into the world of chemotherapy. For example, Bryophyllum pinnanum S.kurtz (Crassulaceae) commonly used as an antitussive and antibacterial agent in the healing of septic wounds has been found to be bactericidal to a wide spectrum of

gram positive and gram negative bacteria such as Bacillus subtilis, Staphylococcus aureus Streptococcus pyogenes, Streptococcus faecalis, Eschericia coli, Proteus spp, Kliebsiella spp Shigella spp, and Pseudomonas aeruginosa (Obaseiki-ebor 1985). These organisms included clinical isolates possessing multiple antibiotic resistance. The crude extracts of the sap and bark of Pynanthus angolensis which is reputed for the traditional treatment of stomach upsets in the Yoruba speaking areas of Nigeria were investigated for antibacterial activity.

The agar diffusion test indicated that both the sap and bark extracts exhibited more than 50% of the antibacterial activity of 6.5% solution of streptomycin against five reference strains of bacteria, namely E. coli, P. aeruginosa, B. subtilis, B. cereus, and S. aureus, and the death curves of the test organisms suggested that the bactericidal activities followed a first order kinetic law (Babalola, 1988). Ceiba pentandra is reputed for the treatment of wound infections in traditional medicines and it has been shown to have antimicrobial properties against S. aureus, S. pyogenes E. coli, and P. aeruginosa (Odama et al. 1986).

There are certainly problems associated with herbal treatment such as lack of official guarantee on purity of these products, effectivity of the preparations, and the risk of toxicity which may be as harmful as the synthetic drugs due to uncontrolled administration of unsuitable herbs. However, the advantages in the use of local herbs which include the opportunity to use a wide

range of essential elements provided by nature in the form of useful plants outweigh the disadvantages (Sofowora 1994). These natural affinity as described by Sofowora (1994), allows total assimilation of all those elements developed by the growth organs of the plant that are indispensable to life. This is encouraged in mordern orthodox medicine by the recommendation of things like fruits, vegetables, cereals in daily diet rather than prescribing pharmaceutial products especially in the treatment of deficiency ailments in the young. Most viral attacks to the immune system is said to be resistible by natural immuno defensive materials in the body. These are enhanced by sources such as garlic, onions, (rich in sulphur) lettuce, ginger, club moss, guava, yoghourt, honey, and vitamin C (Adewale, 1993). It is the general belief that these natural products are more acceptable to the body than the synthetic drugs(Sofowora, 1982).

Awosika, (1993) has pointed out that local medicinal plants, when selected carefully and taken under 'Medical' guidance are safe and are uncomplicated methods of curing ailments

Active ingredients occur in small quantities in plants and are very much diluted when a decoction is prepared (Sofowora 1982) and this perhaps makes the need for accurate dosage unnecessary. The development of resistance to traditional medicine has not been reported (Sofowora, 1982).

Finally, in the light of our present economic predicament that is plaguing all section especially health care, continued efforts are needed to search for and identify those plants that could be

useful medicinally to the society at large. It is in response to this challenge that this present work was designed. The objectives included.

a) Carrying out an ethnobotannical survey of some villages around Ahmadu Bello University, Zaria, so as to find out which particular plants are used traditionally for the treatment of gastroenteritis.

b) The selection of the most frequently used plants and studying the antimicrobial activities of their crude extracts.

c) The determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the active plants.

d) The isolation of the active fraction(s) from the crude extract of the most active plants.

e) The determination of the antimicrobial activity of the isolated fraction(s).

CHAPTER TWO

LITERATURE REVIEW

The antimicrobial properties of natural products has long been demonstrated from plants and records of the use of such plants known to produce healing agents for the treatment of diseases abound.

2.1.1 TRADITIONAL REMEDIES FOR GASTROENTERITIS

In Nigeria and French Guinea, a decoction of Starchytarpheta anguistifolia, an undershrub with natron is given for dysentery in humans and for a similar condition in horses (Dalziel, 1955). Tamarind is the partially dried ripe fruit of Tamarindus indica (linn) that has been deprived of the auter layer of pericarp and sometimes preserved with sugar. The plant material is rich in citric and tartaric acids and it has long had a reputation of usefulness in the treatment of dysentery and fevers (Taylor, 1981).

A cold infusion of the bark of Sclerocarya birrea along with natron is employed in the treatment of chronic dysentery and as a vermifuge or a purging vehicle for other vermifuges (Dalziel, 1955), while a cold infusion of the plant has been used as a remedy for gonorrhoea and as an antidiarrhoeal (Irvine, 1961). Procyanidin, a fractionation product of the bark extract was found to produce inhibition in the peristaltic reflex induced in guinea pig colon (Ghani et al. 1988). Also reported to be effective in the treatment of gastroenteritis in the western parts of Nigeria and parts of Togo is the roots of Walteria indica (linn) (Kerrharo and Bouquet, 1972). It has also been found to be a common remedy for

infants having teething problems or as a mouth wash for a few weeks to strengthen the child's resistance against fevers. An analysis of the bark shows that it contains mucilage, tannins, sugar, but no alkaloid (Dalziel, 1937). Dysentery and diarrhoea which may be due to nervous origin gives no response to mordern treatment but is found to be cured by drinking aqueous extracts of the leaves of Cissamphos Ovariensis (Adegoke 1980). Another widely used herb Potentilla Tormentilla, a member of the rose family found growing in common pasteures, open woods and in dry or damp but particularly acid soils throughout Nigeria is found to be effective against intestinal infections producing symptoms of diarrhoea, or cholera and pestilence and as an antidote to the consumption of poisons (Awosika, 1990). A wine glassful of the decoction taken two or three times daily is found to exhibit the usual effect of checking any gut wall infection or inflammation relieving enteritis, gastritis, and even food poisoning (Awosika, 1990). The herb 'tormentil is almost entirely made up of the condensed tannins which are perfectly safe for the body and functions as an astringent and is employed in traditional medicine in the treatment of severe burns. The presence of a bitter component in P. tormentilla also accounts for its traditional use as a remedy in the treatment of liver diseases (Awosika, 1990).

Dried powdered bark of Khaya senegalensis has been used for the treatment of gastroenteritis along with the dried stem bark of Acacia albida and the seeds of Oryza sativa in some parts of

Northern Nigeria (Abdulrahman, 1991). An infusion of the three has the effect of enhancing the absorption of sodium and water in the gut while also possessing some antibacterial agents. An analysis of K. seneglensis bark has shown it to contain 9.2% tannins, 10.4% mineral matter and oxalates, 2% Saccharase, and 0.4 - 1% calcedrin which is reported to have a strong antiprotozoal action (Irvine, 1961; Olanrewaju et al. 1985). Acacia albida stem bark is also reported to contain 28% tannins, which have astringent as well as antibacterial properties (Irvine, 1961). The plant bark is also reported to contain about 1% sodium and pottasium salts (Dalziel 1955), which may give a rationale for its use as a source of some electrolytes, thus annuling the effect of loss of some electrolytes - during diarrhoea.

Another widely known and confirmed remedy for the treatment of stomach troubles especially dysentery in most parts of northern Nigeria is a combination of the leaves of Acacia nilotica, Psidium guajava, and Adansonia digitata. A filtered hot water extract of the combined leaves is taken orally 4 times daily in large quantities according to the severity of the disease (Abdulrahman, 1991).

An Evaluation of A. nilotica stem bark has shown it to contain tannins 18-23% and the plant bark also produces gum which forms an almost colourless mucilage when mixed with water (Dalziel, 1955). P. guajava dried bark contains some tannins while a higher level of vitamin C and tannin are found in the leaves and fruit (Watt and Bryer Branddwijk 1962). An antimicrobial investigation carried out

on the hot water extracts of the leaves have shown it to have a complete inhibitory effect on the growth of S. typhimurium, S. aureus, Vibrio choleriae, E. coli and P. aeruginosa (Akpulu, 1991). A. digitata pulp contains pectin, pectic and malic acids, pottasium acid tartrate, glucose, and reducing sugars with mucilagenous matter (Hutchinson, 1966). The plant leaves are also reported to contain sodium chloride (common salt) and tannins which therefore result in their astringent properties which might explain its use in the treatment of dysentery (Hutchinson and Dalziel, 1965).

Carica papaya, found in most parts of Nigeria and which has been extensively studied has been and is still being used in traditional/herbal medicine. The leaves are found to contain carpain, 0.4% and the Japanese have reported it to be effective in the treatment of Amoebic dysentery (To and Kyu, 1934).

2.1.3 TRADITIONAL REMEDIES FOR GONORRHOEA AND VENERAL DISEASES

The plant Phyllanthus muellerianus have been applied widely in traditional medicine in Nigeria. It is widely distributed in the French Sudan, the British Congo, and tropical Africa. The fruits and leaves are used in "Zande" medicine (Sudan) for curing gonorrhoea, while in Ghana, the root is cooked with Maiz meal and used in the treatment of severe dysentery (Yangs 1991), while Dalziel (1955) has reported the use of a leaf concoction of the plant for curing urethral infections. Another remedy which has been widely used for the treatment of gonorrhoea and syphilis is the stembark and leaves of Cassia occidentalis (Oliver, 1959). A decoction of the stembark and leaves with potash has been used for

treating gonorrhoea and syphilis while the stem bark only is a remedy for rheumatism and dysentery (Dalziel, 1955; Oliver, 1959) Other plants used as remedies for gonorrhoea, syphilis or such related diseases include Zanthoxylum species, roots of Cocos micetera, Euphorbia hirta and Cajanus cajan (Pigeon Pea) (Seaforth et al. 1985).

2.1.2 TRADITIONAL REMEDIES FOR HELMINTHIC INFECTIONS

Annona senegalensis is one of the medicinal plants reported to possess anthelmintic activity and is largely used in Nigeria for its medicinal value (Deshpande et al. 1978). The petroleum ether and chloroform extracts of the leaves have been found to possess anthelmintic activity when tested invitro against hookworms (Deshpande et al. 1978), Cassia species are small shrubs of the family leguminosiae, some of the important ones include Cassia senna, Cassia aniculata, Cassia fistula, Cassia horrida, Cassia occidentalis and Cassia alata. The plant has been used as an anthelmitic (Watt and Brandwijk 1962) while Ikenebomeh and Metiriri (1988) have reported its use in the treatment of skin diseases in surinam, Tanzania, and Zaire. Extracts of the leaves were inhibitory to Trichophyton mentagrophytes, Trichophyton tonsurans, Microsporon ouduinii, Microsporon ferrugenia, and Aspergillus niger. However, B. subtilis, E. freundii and E. coli were more sensitive to a higher dilution of the extract. The stem and root barks of Holarrhena antidysenterica are also used in infusions for diarrhoea and intestinal worms (Ahuja, 1988).

2.1.4 TRADITIONAL REMEDIES FOR WOUNDS AND SORES.

Bryophyllum pinnanum leaves are commonly used by Nigerians of Edo-tribe as an antitussive and antibacterial agents in the healing of septic wounds (Obaseiki-Ebor, 1985). The whole plant has been documented to contain sapponins, flavonoids, and tannins and also to have diuretic, antipyretic, carminative antiseptic and analgesic properties (Adesina 1983). Extracts of the leaves at 5% weight per volume was bactericidal to a wide spectrum of gram positive and gram negative bacteria namely B. Subtilis S. pyogenes, S. faecalis E. coli, proteus spp, and S. typhi (Boaky-yiadom, 1977; Obaseiki - Ebor 1986). Diospyrus species are forest trees that are commonly used in many Nigerian communities as chewing sticks, (Odelola, 1988). Certain Diospyrus species have also been used as herbal medicines in some communities in the southern part of Nigeria; where an infusion of the barks and leaves are used as antiseptic washes for sores and wounds (Irvine, 1961) while the boiled leaves are applied as poultices. However, certain Diospyrus species have also been used as herbal remedies in the treatment of leprosy. The invitro activity of Diospyrus against a wide range of bacteria have been reported (Odelola and Okorosobo (1988) where extracts from the leaves and seeds of D. montana have shown some antibacterial activity against B. Subtilis and corynebacterium pyogenis (Gontain et al. 1973). Paris and Prista, (1985) have also reported that a 100% infusion of the bark of D. canniculata was bacteriostatic against Staphylococccus spp. While the cold water extract of D. baterii was found to be active against some clinical isolates of P.

aeruginosa even when such isolates where resistant to either gentamycin or ampicillin (Odelola et al. 1988). A decoction of the unripe fruits of Hibiscus esculentus together with the leaves of Occimum grattissimum and laxative agents have been used successfuly to treat old bone injuries and strongly running eruptions (or salt flux) (Hepper, 1976). Vapour of the oils from the plant was also reported to kill protozoa (El-Said et al. 1969; Sofowora, 1970). The condensed Tannins which are present in most plants induce the formation of protective scab over wounds, thereby sealing the flesh temporarily (Awosika, 1990).

Ethyl gallate and gallic acids has been isolated and characterized as the major active principle in Mimosa hamata (wild). Ethyl gallate has also been found in Acacia spp, Bombax malabaricium Phynchosis phaseoloides and in the pannicles of Mangifera indica (Hussain et al. 1982). The compound has been found to be strongly active against S. aureus and E. coli, slightly active against C. albicans thus confirming its broad spectrum of antimicrobial properties. This activity compares well with that of 0.0033% of streptomycin (Hussain et al. 1982).

The ethanolic extract of Occimum grattissimum has both antimicrobial and anthelmintic properties and its volatile oils have been formulated into skin cream for clinical trials by dermatologists (Sofowora, 1982). The antibacterial activities of four Nigerian plants were investigated by Kela and Kufeji (1991). These included Gnidia krausiana, Metracarpum scaber, Moringa Oleifera

and Securidaca longependuculata. A 500mg ethanolic extract of M. Oleifera caused a total inhibition of S. aureus, and B. Subtilis, while the chloroform and ethanol extracts of M. Scaber gave a total inhibition of S. aureus. However, G. kraussiana and S. longependuculata were not active on all the five bacteria species tested.

2.1.5 TRADITIONAL REMEDIES FOR FEVERS

Many plants used in traditional medicine for curing malaria and severe fevers have been the subject of various antimicrobial and pharmacological screening. The activity of the crude extract and of various purified fractions of Azadiracta indica have been tested against B subtilis S. aureus, E. coli, S. Sonnei, and P. aeruginosa. All the organisms tested were susceptible except P. aeruginosa which was totally resistant (Omogbai and Ali, 1985). In another study, a popular remedy which is used in the management of fevers in many parts of Nigeria which is a compounded drug containing the bark and leaves of A. Indica, leaves of Cymbopogan citratus, P. guajava and C. papaya was inhibitory to S. aureus and Klebsiella pneumoniae (Akpulu 1991) while a hot water extract of the bark of A. indica was the most active against tested strains of the organisms with a minimum inhibitory concentration of 3mg/ml for Vibrio choleriae, 4mg/ml for S. aureus and 10mg/ml for E. coli respectively. The fruits and roots of Kigelia africana boiled along with the stem and tassel of plantain (Musa sapientum) is used medicinally in western Nigeria as a cure for post partum haemorrhage, while a decoction of the leaves and stem bark for

drinking and bathing serves as a cure for Malaria fever (Gbile, 1986) while a decoction of the fruit and stem bark is employed in the treatment of wounds in Malawi (Msonthi 1985). The Ethanolic extract of Occimum grattissimum has both antimicrobial and anthelmintic properties the crude extract has been proved to act with actual aromatic ingredients, and its most important use is in the treatment of a malignant bilious fever connected with jaundice which is prevalent near the river volta (Schum and thonning 1976).

The plant Artemisia annua have been shown to contain a rapidly acting schizontocide "Qinghaosu", a sesquiterpene lactone (Peters 1980). It has been used to treat a case of severe falciparum malaria and has been reported as among the most promising antimalarials since it clears malaria parasites more rapidly than other drugs in both mild and severe malaria. It has a rapid onset of action, and it has no observed side effects (Duysi et al. 1993). The compound has also been shown to have some therapeutic effect against Schistosoma japonicum infections in experimental animals (Cheng 1980). Morinda lucida a vegetable plant widely distributed in West Africa and mostly in the Western part of Nigeria has been found to have some medicinal properties in the treatment of fevers (Obih 1985). The plant extract has been found to produce a degree of suppression of Plasmodium berghei- berghei parasitemia (Obih et al. 1985).

Khaya species are plants well known in West Africa to possess antimalarial activities. The bitter stem and root barks of the plant are potential sources of antimalarial drugs (Adesina, 1983).

A decoction of the fresh bark macerated in cold water or even the dry pulverized bark mixed with salt is taken in small dozes as a fever remedy and as a stomachic (Dalziel 1955) while the bark of Boswelia dalzielli is boiled and used for washing in feverish and rheumatic conditions. Another plant which is partly used in the management of fevers in Nigeria is Eniantha clorantha whose bark is an ingredient in a concoction employed in the western part of Nigeria for the treatment of fevers, wounds and ulcers under the traditional health care system. The alcoholic extract of the plant bark have been shown to be inhibitory to the growth of S. aureus, Streptococcus group c, Corynebacterium spp and E. coli (Idika et al. 1991). In the same study, it was observed that only one of four different fractions obtained contained the inhibitory substance which was identified as an alkaloid.

2.1.6 ANTIMICROBIAL AND PHARMACOLOGICAL PROPERTIES FROM FOOD PLANTS

Some antimicrobial and pharmacologic properties have also been demonstrated in plants used as common beverages. Some of these plants have been shown to possess bacteriostatic, bactericidal, antifungal or antihaemolytic activity. In a study to assess the antibacterial activity of some selected tea-leaves and coffee powders by Opara and Ansah (1993), results indicated that a 20% weight per volume (w/v) of the Tea and Coffee extracts showed an antibacterial activities against S. aureus, P. aeruginosa, Proteus spp and V. choleriae. A bactericidal test on the organisms showed that all the organisms were killed by the tea extracts within 3

hours of exposure (Opara et al. 1993). Thymus vulgaris a sub-shrub formerly indegenous to the mediterranean countries but now cultivated worldwide for culinary purposes have been reportedly used as a stimulant, a carminative, and condiment (Chopra et al. 1960). A yellowish-red volatile oil obtained from the plant has a powerful germicidal action and is used in many pharmaceuticals as gargles and mouthwashes. The oil causes mental excitement and serves as a diffusible stimulant in cases of collapse (Gathercol 1936). Thymol is also an antiseptic, a parasiticide, and a deodorant. The oil is also anthelmintic and is especially useful in the treatment of hookworm infections (Gathercol 1936). The latex from Carica papaya (common Paw-paw) contains papain and chymopain commercial papain is usually prepared from the dried latex of the fruit and is used in the foods, brewing, tanning and wool industries (Seaforth 1985). Its leaves contain Vitamin C, Vitamin E, glycosides carposides and an alcaloid carpain which has been shown to have antibacterial properties and is therefore used as a detergent (Seaforth 1975). Infective hepatitis, a variety of joundice has no permanent mordern treatment, but an oral administration of the aqueous extract of the tuber of Zingebar officinale (common ginger) effects a permanent cure within a week in patients suffering from the disease (Adegoke, 1988).

2.1.7 ANTIDIABETIC AGENTS FROM SOME MEDICINAL PLANTS

Another area in which some common plants and herbs have found usefulness is in the management of diabetes. The Fruit Carica papaya (which is found in most parts of Nigeria) when taken ripe

without the external change in colour is believed to stimulate the secretion of insulin by the pancrease to the required standard, thus controlling the onset of diabetes. Bridelia ferrugenia is also widely employed in most parts of West Africa (Iwu, 1983; Okunji et al. 1988; Addae-Mensah 1989). A hot water extract of the dried leafy parts taken orally have been used to treat diabetes and hypertension (Iwu, 1983), while Banquet (1974) have reported that a hot water extract of the root, stem bark and leaves of the plant is used as an aphrodisiac by males in Ivory Coast.

2.1.8 SOME ANTI-CANCER AGENTS FROM SOME MEDICINAL PLANTS

A number of euphorbia species are used in traditional medicine for the treatment of tumours (Abo et al. 1986). The leaves, stembark, and roots of Euphorbia hirta have a long history of use for the treatment of cancers and warts (Abo et al. 1986) the socculent emphorbia species are known to produce the toxic diterpenes tigliane, daphnane, and ingenane which exhibits, inflammatory and tumour promoting activities however, the most activity has been attributed to the inflammatory esters at relatively high doses (Abo et al. 1988). Thus the search for alkaloids with anticancer activity has revealed a number of compounds which can retard the development of tumours. The common ornamental plant, Vinca rosea, (the periwinkel) produces such alkaloidal compounds (Walter and Nowacki 1978). Other plants such as Tylophora crebiflora, Ochrosia elliptica, and Camphothotheca acuminata have yielded still other substances of this type. Some steroid alkaloids have an importance to the pharmaceutical

industry. While the price for some synthetic steroids is prohibitive, certain plants accumulate compounds which after a simple chemical modification become steriod hormones (Walter et al. 1978).

2.1.9 PLANTS USED IN THE MANAGEMENT OF SICKLE CELL ANAEMIA.

The value of a plant extract in the management of sickle cell anaemia was first reported by Sofowora and Isaacs (1971). The aqeous extracts of Zanthoxylum xanthoxyloides was found to possess this activity. The aqueous extract of Z. gilleti has a higher anti-sickling potency than Z. xanthoxyloides (Adesanya and Sofowora 1983). The active principle has been identified as benzoic acid derivatives (Adesanya et al, 1983).

In another study, an alcoholic extract of the root of Fagara xanthoxyloides have been used in relieving sickle-cell anaemia (Adegoke, 1980). Extracts of the root which was found to be rich in reserpine was evaluated for anti-sickling activity invitro. The crude extract in concentrations of 2-8 mg/ml markedly inhibited deoxygenation induced sickling of red blood cells. At the highest concentration of 8.0 mg/ml, only about 10% of the cells sickled compared to 70-90% of the controls, while reserpine in concentrations of 0.05-1 ug/ml similarly inhibited deoxygenation induced sickling of redblood cells; though not as markedly as the crude extract (Obianwu et al, 1986). From these reports, the need to develop these drugs and to find others for the management of sickle-cell anaemia cannot be overemphasized since drugs of these kinds are of special relevance to Nigeria where the problem of

sickle cell anaemia is far from being solved.

It is impossible to list all Nigerian medicinal plants on which some biological studies have been carried out. Though a lot more needs to be done, the published studies contain some very interesting findings. These plants and their derived products have been recorded in pharmacopoeas and some of which have provided templates for synthetic modification. This is usually done in the anticipation that more useful drugs would be produced. Thus, many tinctures, infusions and essential oils from medicinal plants such as castor oil, coconut oil, cotton seed oil, orange peel oil (citrus sinensis), nutmeg (Myristitica fragrance) and physostigma venesum are in present official monographs and are still being used (Akubue, 1986).

2.2 THE ACTIVE PRINCIPLES OF HEALING PLANTS.

The medicinal value of drug plants is usually due to the presence of a chemical substance (the active principle) in the plant tissue which produces the physiologic effect. Many of the active principles are highly complex and their exact chemical nature occasionally is still unknown. Others have been isolated, purified and even synthesized or simulated. They most commonly fall into one of six categories.

(a) Alkaloids:- These are a diverse group of alkaline compounds with marked physiological activity. Their ring structure is usually complex and always contain Nitrogen (Sanff,1978). The alkaloids include morphine (first isolated in 1805), cocaine, nicotine, quinine, and more than 5,000 others.

More than 90 percent of the known alkaloids are produced primarily in higher plants but are also found in lower organisms and in some animals that have signi-ficant pharmacological activity (Walter and Nowacki, 1978).

(b) Glycosides:- These are compounds which, when hydrolysed yield a component of one or several sugars (i.e glycone) and a non sugar component (aglycone). There are eleven types of glycosides classified according to their aglycone moeity. However, among the most important in mordern medicines are the cardiac glycosides (Sanff, 1978). Digitalis, one of the most widely prescribed drugs of plant origin owes it activity to the cardiac glycosides.

(c) Essential oils:- These usually have various chemical constituents, usually tarpene derivatives or aromatic compounds. They often contain alcohols, ketones, aldehydes, phenols, ethers, esters, and other compounds as well as sometimes nitrogen and sulphur. Many are highly germicidal, this property being due to their volatility and ability to penetrate into cell protoplasm, but they usually are too insoluble in water to be important in medicines as antiseptics. However, they are valuable as carminatives, in caughdrops, mouth washes, gargles, sprays and healing ointments.

(d) Gums and resins:- These are polymers and oxidation products of essential oils. Both are employed as purgatives and in ointments.

(e) Fatty oils or Lipids:- are esters of fatty acids, they are vastly used in emulsions and as purgatives.

(f) Antibiotic Substances:- These are various complex organic compounds usually from moulds, actinomycetes, and bacteria. They are capable in small amounts of inhibiting life process of micro-organisms. Thus, healing plants may act in a great variety of ways, many for example, Carminatives exert a soothing action. Others act on the nervous system, while others have neuromuscular or muscular activity. The antimicrobial substances from plants however heal by killing or inhibiting pathogenic microorganisms especially bacteria. Medicinal plants or medicinally useful plants have been classified into three categories. viz:-

1. Plants that are employed directly in therapy.
2. Plants that constitute the raw materials for galenicals.
3. Plants that constitute the raw materials for industrial processes and which are employed either for the extraction and purification of their active principle or which serve as starting materials or intermediates for synthetic preparations (WHO 1979).

It has been suggested by UNIDO that "developing countries (like Nigeria) may have a manifold benefit by cultivating medicinal plants systematically as "cash crops", they are a major component in rural development, the plant resources can supply locally with raw material for primary health care, and either the crude drug or semi-processed products can be exported" (Franz, 1993).

2.3 THE PRACTICE OF ETHNOMEDICINE IN NIGERIA.

Ethnomedicine has been defined as the use of plants by members of an indegenous culture for which there is no organised medical system or formal training by the prescriber, while traditional medicine refers to the use of plants in the treatment of or amelioration of diseases within an organized indegenous system. The use of medicinal plants has always been part of human culture and this has transcended all social, economic, religious and other barriers. Nigerian patients irrespective of their status, ethnic group or religion are the regular clients of traditional herbalists (Akinniyi et al. 1986).

Although there is a need for accurate statistical data, there has been some degree of success in herbal therapy as many of these medicinal plants have not only been found to be the only effective cure for a few diseases which are essentially tropical or Nigerian, but they are also an alternative for the treatment of diseases like infective hepatitis, sickle-cell diseases, osteomyelitis, hydrocoel, etc.

2.4 SCREENING OF TRADITIONAL MEDICINAL PLANTS.

The research on Nigerian medicinal plants which was initially dominated by organic chemists has progressed in a more salutary manner. Recently important and useful contributions are being made by pharmaceutical chemists who are sharing the field equally with pure chemists. Major research on possible medicinal use has been focussed on the isolation, characterization, and testing of antimicrobial antiparasitic, and the antiviral properties of these

compounds. Pharmacologically active compounds studied from some of these plants include:- (a) Anti-diabetic agents from extracts of Discorea dumetorum, and Bridelia ferrugenia. (b) Anti-sickling agents from Fagara species

(c) Anti-hepatotoxic agents from Garcinia cola (d) Spasmolytics from Jatropha tetraptera and Abrus precatorious, (e) Anti-convulsant agents from Calliandra portemricensis and fruit extract of Tetrapleura tetraptera (f) Anti-ulcer agents from Pyrenacantha staudtii and (g) Antibacterial agents from amongst others Eupatorium odoratum, and Diospyrus spp (Akubue, 1986).

Although there are limited reports on the toxicity of these plants, the WHO (1990) has pointed out that research into medicinal plants should also focuss on potentials for adverse side effects from herbal remedies since the medical profession in general and the general public in particular needs to be alerted to any harzards. Since herbal preparations may be marketed with the mandatory clinical trials to evaluate efficacy and safety which applies to the pharmaceutical drugs (Gale, 1992).

2.5 GASTROENTERITIS

Gastroenteritis can be defined as an inflammation of the stomach and intestine. The condition is aften a manifestation of infection by viruses, bacteria, or food poisoning toxins. It usually manifests as diarrhoea, dysentery and or vomitting (Fry, 1979).

However, diarrhoeas and dysentery are gastrointestinal disorders and are terms used to indicate alterations in the normal

pattern of defecation, but because of the wide range of bowel habits among healthy individuals, these terms are difficult to define precisely.

However, 'Diarrhoea' can be defined as the passage of severe watery unformed stools as a result of rapid movement of the faecal contents through the large intestines; there may be mucus present but blood is not usual (Fry, 1979).

Dysentery:- is a clinical manifestation of any infection of the intestinal tract causing severe diarrhoes with blood and mucous. The condition may be due to various pathogenic agents namely bacteria (Bacillary dysentery) or protozoa (Amoebic dysentery) (Thomas, 1988).

2.5.1 CONCEPT OF DIARRHOEA AND DYSENTERY AND ITS MANAGEMENT IN ORTHODOX MEDICINE.

1.a) Diarrhoea:- Diarrhoea can result from any condition in which the segmenting contractions become hypoactive, allowing the feces to be moved along the gastro intestinal tract very rapidly which may result from injury of the lining of the intestine or excessive secretion of water and electrolytes in the intestines stimulated by bacterial toxins or it may be of nervous origin (Willocks 1949). In general, microorganisms are among the more frequent causative agents of acute diarrhoeas. The causative organisms include the Salmonella spp., Shigella spp, pathogenic Escherichia coli, Clostridia, and certain viruses. Microsporidium spp has been recently recognized as an important diarrhoeal agent in

patients with HIV infection (WHO 1994).

i.b) Treatment:- Treatment is dependent on the cause of diarrhoea and the general status of the therapy is to correct the physiologic changes induced by diarrhoea. It is essential that the following secondary or complicating features are treated (i) Fluid imbalance, (ii) Mineral imbalance e.g hypocalcemia, hypokalemia, hyponatremia, and hypomagnesemia (iii) Nutritional disturbances for example hypomagnesemia, and other deficiences. Antibiotics are indicated in Staphylococcal enterocolitis amebiasis and severe Shigella or Salmonelia diarrhoea (Jawetze 1972; Fry 1979 and Islam 1993). Kaolin and Pectin are frequently prescribed for their absorbent qualities to give some consistency to the stool (Grollman 1965).

iia) Dysentery: Caused by bacteria is known as bacillary dysentery. Symptoms are Diarrhoea often with blood and mucous. Cramps, fever, malaise, prostration, clouded sensorium, pus in stool may be manifest while protozoa and shigella spp are usually present in the stool.

iib) Treatment: The restoration of circulating blood volume and renal perfusion are life saving in severe cases. The current antimicrobial agent of choice is ampicillin 100mg/kg daily, orally in four devided doses for 5-7 days. Tetracyclin chloramphenicol, or cotrimoxazole may also be effective. Treatment of amoebic dysentery is achieved by luminal amoebicides such as di-iodohydroxyquine and diloxanide furate.

Tissue amoebicides by dehydroemetine and emetine, chloroquine or metronidazole and Tinidazole which is effective against the organisms in the bowel lumen, bowel wall and tissues (Marcus et al. 1978, Pahrson et al. 1984).

2.5.2(a) CONCEPT OF DIARRHOEA AND DYSENTERY INTRADITIONAL MEDICINE

Traditional healears hold the belief that diarrhoea may be caused by the "dirtiness of the intestinal system" probably due to what the patient had eaten. They also associate dysentery with hookworm infection. Unlike orthodix medicine where laboratory examination is consulted to know the causative organisms responsible for the disease, examination in traditional medicine is symptomatic, that is, the manifestation of bloody stool and mucus confirms dysentery while profuse and frequent watery stool with offensive odour confirms diarrhoea. Though sometimes the eyes and mouth are examined for symptoms (Isah, 1993).

2.5.2(b) TREATMENT

Treatment often is in the form of decoctions of leaves, stem barks, root barks, fruits, or a concoction of the ingredients. The mixture is most often taken orally in large quantities at short intervals or taken about three to four times daily until symptoms dissapear (Isah, 1993).

CHAPTER THREE

3.1 MATERIALS AND METHODS

Ethnomedical Survey: Four villages were selected for this study: Jama'a, Hayi, Koraye, and Samaru. They are all located within a two mile radius from the main campus of Ahmadu Bello University Zaria. Information was obtained from two categories of people, the users and the herbalists. The study was conducted with the help of an indegene from each of these villages. Personal visits were made to each of these persons at their homes or place of work while others were interviewed at the market place.

The information was obtained from responses given to questions prepared as a questionnaire where persons were either interviewed verbally (for the illiterate subjects) or requested to fill in the questionnaire (for the literate subjects). The informations thus obtained from different herbalists were compared (Appendix I).

3.2 COLLECTION OF PLANT MATERIAL

The plants used in this study (section 4) were found growing wildly around the vilages under survey. Relevant specimens collected with the help of an indegene or a herbalist were brought to the herbarium of the department of biological sciences Ahmadu Bello University Zaria where they were identified.

3.3 PREPARATION OF CRUDE EXTRACTS

Relevant part of each plant material (Appendix iii) used in the study was dried in an oven at 45°C for one week. It was then pounded in a mortar with a pestle to a coarse powder and then ground into a fine powder using a blender-Mill (National Mx-391). Ten grams of each powdered material was weighed into 100 mls of cold distilled water in a flask and shaken vigorously. The flask was then left at room temperature for twenty four hours after which the content was shaken again and left to settle. The liquid portion was then filtered through a sterile gauze and the filterate dispensed into sterile tubes and centrifuged.

The supernatant was then filtered through Whatman paper No 1 (W&R Balston Ltd, England) to obtain the final filterate. The volume was then made to 100ml with distilled water. The pH of the extract was then taken and a portion (1ml) was taken and dried in an oven (at 60°C) to a constant weight in order to determine the amount of dry matter in the extract.

3.4 TEST ORGANISMS

The test bacteria species were all clinical isolates obtained from the microbiology laboratory of the Ahmadu Bello University teaching hospital Zaria, and the department of Microbiology, A.B.U. Zaria. The organisms viz: *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Shigella flexnerii* were all maintained on nutrient agar slants and were subcultured in sterile Mueller hinton broth (Oxoid) and incubated at 37°C for 24 hours prior to each antimicrobial testing.

3.5 PRELIMINARY TEST FOR ANTIBACTERIAL ACTIVITY.

The susceptibility test was carried out using the agar dilution technique (Sutter et al. 1979; Williams 1983) where 3.8 grammes of Muellar hinton agar was weighed into a flask containing 100mls of the extract and autoclaved. It was then left to cool down to 45°C and dispensed into Petri-dishes and allowed to set. A control experiment without extract was also prepared by dissolving 3.8 grammes of Mueller hinton agar in 100mls of distilled water. They were autoclaved and also dispensed into Petri dishes after cooling to about 45°C. Aliquot (0.1ml) of a 10^{-3} dilution of a twenty four hour broth culture of each test organism was inoculated into each Muellar hinton extract agar plate and spread with a sterilized bent glass rod. Control experiment was also set up by inoculating Muellar hinton agar plates without extract with the standardized inoculum. All the plates were then incubated at 37°C for 18-24 hours. Susceptibility was determined as the complete absence of growth on plate.

3.6 DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC).

For this purpose, a progressively decreasing concentrations of the extract were prepared by adding known volumes of distilled water to known volumes of the crude extract for example, 10 mls of distilled water added to 90mls of the extract to give a final volume of 100mls (i.e 90% v/v). A concentration range was made from the crude extract by diluting up to six times for all the test extracts. Muellar hinton agar plates were prepared as in section

3.5 above using appropriate dilutions of the extract. The minimum inhibitory concentration was taken as the least concentration i.e the highest dilution that was able to completely inhibit the growth of the organisms. A control was also set up using plain Muellar hinton agar for comparison and to ensure viability.

3.7 DETERMINATION OF THE MINIMUM BACTERICIDAL CONCENTRATION (MBC)

5mls of the crude extract was diluted two-fold serially in 5mls of sterile Muellar hinton broth contained in 15 x 150mm test tubes. As a control, 5ml of sterile water was used in place of the test extract. To each ofthe dilutions was added 0.1ml of a young broth culture of the test organisms. All the tubes were incubated at 37°C for 18-24 hours. At the end of the incubation period, the tubes were examined for growth. About 0.2ml of the broth mixture from the first three tubes containing no visible growth was sub-cultured into 20 mls of sterile molten Muellar-hinton agar in bottles equilibrated at 45°C. The bottles were agitated to mix properly and the contents were then poured into sterile Petri-dishes and allowed to set. The plates were then incubated at 37°C for 18-24 hours after which they were examined for growth. Plates showing no growth were further incubated for another 24 hours. Plates containing less than six colonies were taken as the minimum batericidal concentration (MBC) (Odama et al. 1986).

3.8 MEASUREMENT OF THE RATE OF KILLING

Determination of the rate of killing of the extracts were carried out by the methods of Babalola et al.(1988) and El-Nakeeb and Yousef (1970). In this, 0.1ml of a 10^{-4} Diluted broth culture of the

test organisms were inoculated into 5mls of the extract. The bottles were vigorously shaken to distribute any flocculents that might be present and then left at room temperature (Babalola, 1988). The number of bacteria surviving were then determined by mixing 0.1ml of the above mixture with 20 mls of sterile molten agar at 45 C in sterile bottles. The contents were then poured into sterile Petri-dishes and allowed to set (El-Nakeeb and Yousef, 1970; Aggag and Yousef; 1972). This procedure was repeated at two hourly intervals for 24 hours. A control test in which 5ml of sterile water was used in place of the test extract was similarly set up. The plates were then incubated at 37°C for 24-48 hours and the resulting colonies were counted.

3.9 ISOLATION OF THE ANTIMICROBIAL COMPONENT

The isolation process was carried out by a process described by Abdel-Monem and Henkel (1978). Ten (10) mls of each aqueous extract was acidified with 0.2mls of 0.5m Hcl in a separatory funnel. The mixture was thoroughly mixed and 10mls of di-ethylether (G.P.R. A grade) was then added and remixed. The mixture was allowed to separate, with the ether layer formed above and the aqueous layer below. The aqueous layer was re extracted twice with the ether. The 3 ether fractions were combined and the volume recorded while the aqueous layer was kept for subsequent extractions (Appendix IV).

3:9: 2 ISOLATION OF POLAR-BASIC AND POLAR NEUTRAL COMPOUNDS

The aqueous layer from step 3.9.1 above was neutralized with 0.2mls of 0.5m NaoH and then basified with another 0.2ml of the

aqueous base. The two were mixed and further extracted twice with 10mls portion of ether as above. The ether extracts (containing basic compounds) were combined and stored in screw capped bottles. The remaining aqueous layer (containing polar neutral compounds) was then neutralized with 0.4mls of 0.5m Hcl and stored in another bottle.

3:9:3 ISOLATION OF NON POLAR NEUTRAL COMPOUNDS

The combined ether fractions from step 3:9:1 above was extracted with 10mls of 0.1m NaoH in a separatory funnel and the aqueous fraction was then washed twice with 5 mls each of the base. The two washings(ether layers) were combined with the first ether layer and the total volume noted.

3:9:4 ISOLATION OF ACIDIC COMPOUNDS

The aqueous fraction from step 3:9:3 above was further neutralized with 1ml of 0.5 m Hcl and then acidified with another 1ml of the acid. The mixture was further extracted with the ether and washed twice as in the previous steps. The ether fractions were combined and volume noted (this contained the acidic compounds) while the aqueous layer was discarded. The four extracted fractions were then kept for subsequent test after evaporating the ether fractions to half of the original volume.

3:9:5 DETERMINATION OF THE ANTIBACTERIAL ACTIVITY OF THE FRACTIONS

An agar dilution method was employed. Using a sterile bent glass spreader, 0.1ml of a 24 hour subculture of the test organisms were each uniformly spread over the surface of a sterile nutrient

agar plate and allowed to dry. They were each labelled and four equally spaced wells were cut in the agar using a sterile cork borer 8mm in diameter. Aliquotes (0.2mls) of each of the fractions was pippetted into appropriate wells. The Petridishes were then incubated at 37^{0}C and the zones of inhibition were measured after 24 hour.

CHAPTER FOUR

RESULTS

4.1 FINDINGS OF SURVEY

A survey of the villages selected showed that parts of a total number of 42 plants were used in the treatment of gastroenteritis. (Appendix II). Ten plants (Table II) with the highest frequency were selected for further investigation (Appendix III).

4.1.2 RESULT OF THE PRELIMINARY TEST OF THE ANTIMICROBIAL ACTIONS OF THE CRUDE EXTRACTS.

Preliminary agar dilution tests indicated that the crude extracts of six out of the ten plants tested possessed significant antibacterial activities against all the test organisms. The active plants parts included barks of B. ferrugenia, K. Senegalensis, T. avicennioedes, T. macroptera and the leaves of P. thonningi and P. guajava. The concentrations of the crude extracts were as follows: 10mg/ml for P. guajava, 13mg/ml for T. macroptera, 16mg/ml for K. senegalensis, 18mg/ml for T. avecinnioedes, 26mg/ml for B. ferrugenia and 29mg/ml for P. thonningii. However, extracts of O. grattissimum, P. clappertoniana and G. senegalensis were not active on all the strains of bacteria tested even when a higher concentration of these aqueous extracts was used.

TABLE I RESULT OF THE PRELIMINARY TEST OF THE ANTIMICROBIAL ACTIONS OF THE CRUDE EXTRACTS.

	ORGANISM				
PLANT	CONCENTRATION (Mg/ML)	E. coli	S.flexnerii	S. typhi	P. aeruginosa
Bridelia ferrugenia	26	++++	++++	++++	++++
Guerra Senegalensis	26	-	-	-	-
Khaya senegalensis	16	++++	++++	++++	++++
Occimum grattissimum	28	-	-	-	-
Parkia clappertoniana	17	-	-	-	-
Piliostigma thonningii	29	++++	++++	++++	++++
Psidium guajava	10	++++	++++	++++	++++
Sclerocarya birrea	22	+	+	+	+
Terminalia avicennioedes	18	++++	++++	++++	++++
Terminalia macroptera	13	++++	++++	++++	++++
Control		-	-	-	-

KEY:

- = No inhibition- (Abundant growth of bacteria.)

++++ = Complete inhibition (i.e Absence of growth)

+ = Very little inhibition.

In the case of S. birrea, the aqueous extract only very slightly inhibited the growth of E. coli, S. typhi, and S. flexneri, while in contrast, P. aeruginosa showed a susceptibility to a 16mg/ml concentration of the extract (while the other organisms were insensitive to a similar concentration).The details are presented in Table 1.

TABLE 2: MINIMUM INHIBITORY CONCENTRATIONS (MIC) IN Mg/Ml OF THE CRUDE EXTRACTS DETERMINED FOR EACH ORGANISM.

	ORGANISM			
PLANT	E. coli	S. flexnerii	S. tyhi	P. aeruginosa
B. ferrugenia	1.9	0.96	0.96	1.6
K. senegalensis	3	3	9	10.4
P. guajava	10	10	10	2.8
P. thonningii	4	4	7.25	1.6
S. birrea	-	-	-	6.4
T. avecinnioedes	9	5.4	4.5	3.4
T. macroptera	4	2	2	2.6
Control	-	-	-	-

KEY-: (-) No inhibition - i.e Abundant growth of bacteria

Table 2 shows the minimum inhibitory concentration (MIC) of the extracts that exhibited an activity against the test organisms. The range is within 0.96 - 10.4 mg/ml.

Extracts of B. ferrugenia had an MIC value of 0.96 mg/ml for both S. flexnerii and S. typhi while P. aeruginosa and E. coli had MICs of 1.6 mg/ml and 1.9 mg/ml respectively. Extracts of P. guajava had MIC value of 10mg/ml for E. coli, S. flexnerii, and S. typhi while P. aeruginosa was more susceptible to the extract at a lower MIC value of 2.8 mg/ml. In contrast, the MIC value of the extract of K. senegalensis for P. aeruginosa was the highest with a value of 10.4 mg/ml while E. coli and S. flexnerii had MIC value of 3mg/ml and S. typhi 9 mg/ml respectively. The MIC of the extract

of S. birrea for P. aeruginosa (The only susceptible organism) was 6.4mg/ml.

The bark extracts of the two Terminalia spp showed a differing value. T. macroptera extract appeared to be more active having shown the lesser MIC values for the test organism as follows: E. coli 4mg/ml. P. aerugenosa 2.6 mg/ml, S. flexnerii 2 mg/ml and S. typhi 1.5 mg/ml. However, the bark extracts of T. avicennioedes gave an MIC value of 9mg/ml for E. coli, 3.4 mg/ml for Pseudomonas, 5.4mg/ml for S. flexnerii and 4.5mg/ml for S. typhi respectively.

The result of the test for minimum bactericidal concentration (Activity) is presented in Table 3.

TABLE 3: MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) IN Mg/Ml OF THE CRUDE EXTRACTS DETERMINED FOR EACH ORGANISM.

	ORGANISM			
PLANT	E. coli	S. flexnerii	S. typhi	P. aeruginosa
B. ferrugenia	13	13	13	26
K. senegalensis	16	16	16	-
P. guajava	-	-	22	-
P. thonningii	14.5	29	14.5	8
S. birrea	-	-	-	-
T. avecinnioedes	28	-	28	8.5
T. macroptera	13	-	13	-
Control	-	-	-	-

KEY:(-) Non bactericidal i.e Abundant growth of bacteria.

The least minimum bactericidal values were shown by the extracts of B. ferrugenia and T. macroptera each with 13mg/ml for E. coli, S. flexnerii and S. typhi respectively, while the concentration needed to kill pseudomonas was much higher (26mg/ml). Pseudomonas was not killed by the extracts of T. macroptera. In contrast, the concentration of extracts of P. thonningii needed to kill P. aeruginosa was half (8mg/ml) of that needed to kill E. coli and S. typhi (14.5mg/ml) and less than one third of that needed to kill Shigella flexnerii (29mg/ml). Extracts of K. senegalensis had the same MBC value for E. coli, S. flexnerii, and S. typhi (i.e 16mg/ml). P. aeruginosa was resistant to the extract. However, S. typhi was the only organism that was killed by extracts of P. guajava. The highest MBC value was shown by extracts of T. avecinnioedes on E. coli and S. typhi with a value of 28mg/ml. The extract had an MBC of 8.5mg/ml for P. aeruginosa while S. flexnerii was completely resistant.

4.5 RESULT OF THE RATE OF BACTERICIDAL ACTIVITY OF THE EXTRACTS

The death curves of the four bacteria in the test extracts showed that the bactericidal action of the test extracts commenced within the first hour of treatment (Fig 1). Starting with an inoculum size of about 10^5 organisms/ml, by the second hour, more than half of the organisms had been killed in extracts of B. ferrugenia as compared to the controls. P. aeruginosa appeared to be more susceptible to the extracts of B. ferrugenia as all the cells were killed within the third hour of exposure. While a four hour treatment with extracts of B. ferrugenis was required to

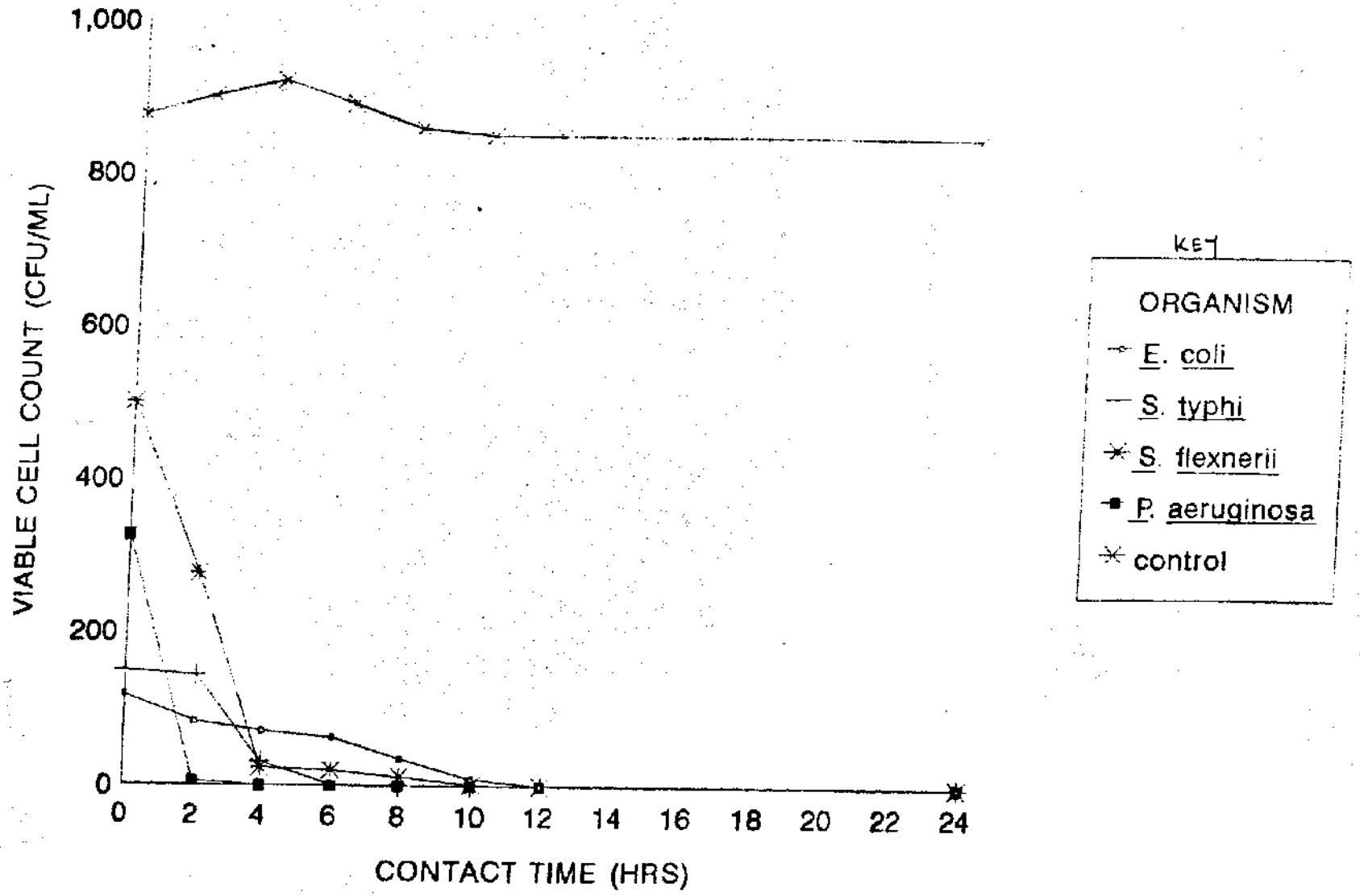


Fig 1: RATE OF BACTERICIDAL ACTIVITY OF CRUDE EXTRACT OF B. ferrugenia (26mg/ml) ON THE FOUR TEST ORGANISMS

produce the same effect on S. typhi.

In the case of E. coli and S. flexnerii no survivors were recovered after a 10hour contact.

The extinction time of E. coli and S. typhi appeared to be reduced by one hour when the test organisms were treated with 5mls of the extract of K. senegalensis (Fig.2) relative to S. flexnerii while P. aeruginosa was not killed by this extract (Table 3). A99.99% killing of all four test organisms was achieved with the extracts of P. thonningii (Fig.3) within 2 hours of treatment. The kinetics of death of E. coli, S. flexnerii and S. typhi were almost identical with the cells drastically reduced by about 90% within the first hour, followed by a complete killing within the second hour (Fig 3). There appeared to be a slightly lesser inhibition of P. aeruginosa during the first hour as a higher number of organisms were recovered, followed by a rapid bactericidal action which resulted in more than 99.99% killing by the end of the third hour.

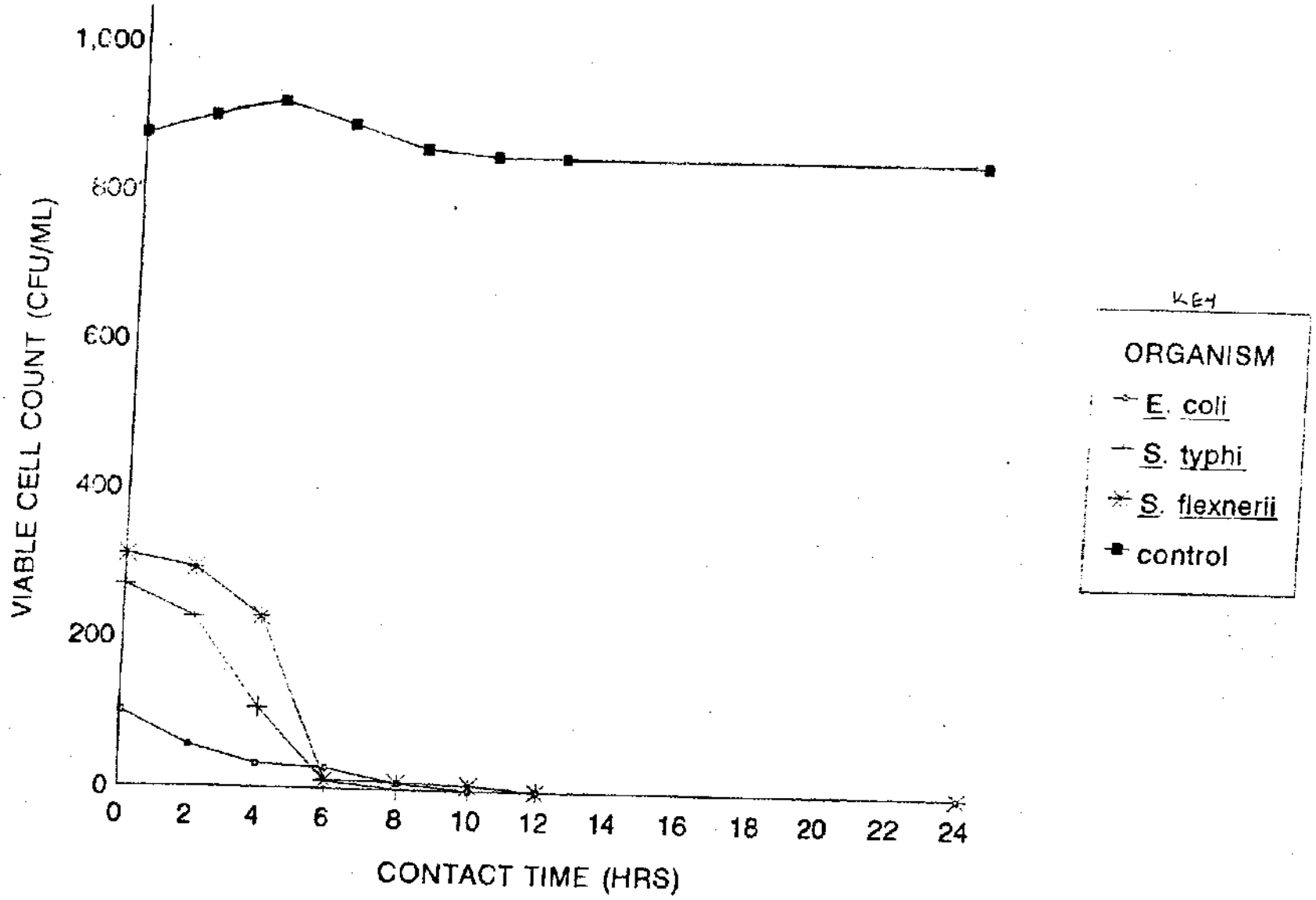


Fig 2: RATE OF BACTERICIDAL ACTIVITY OF CRUDE EXTRACT OF K. senegalensis (16mg/ml) ON THE FOUR TEST ORGANISMS

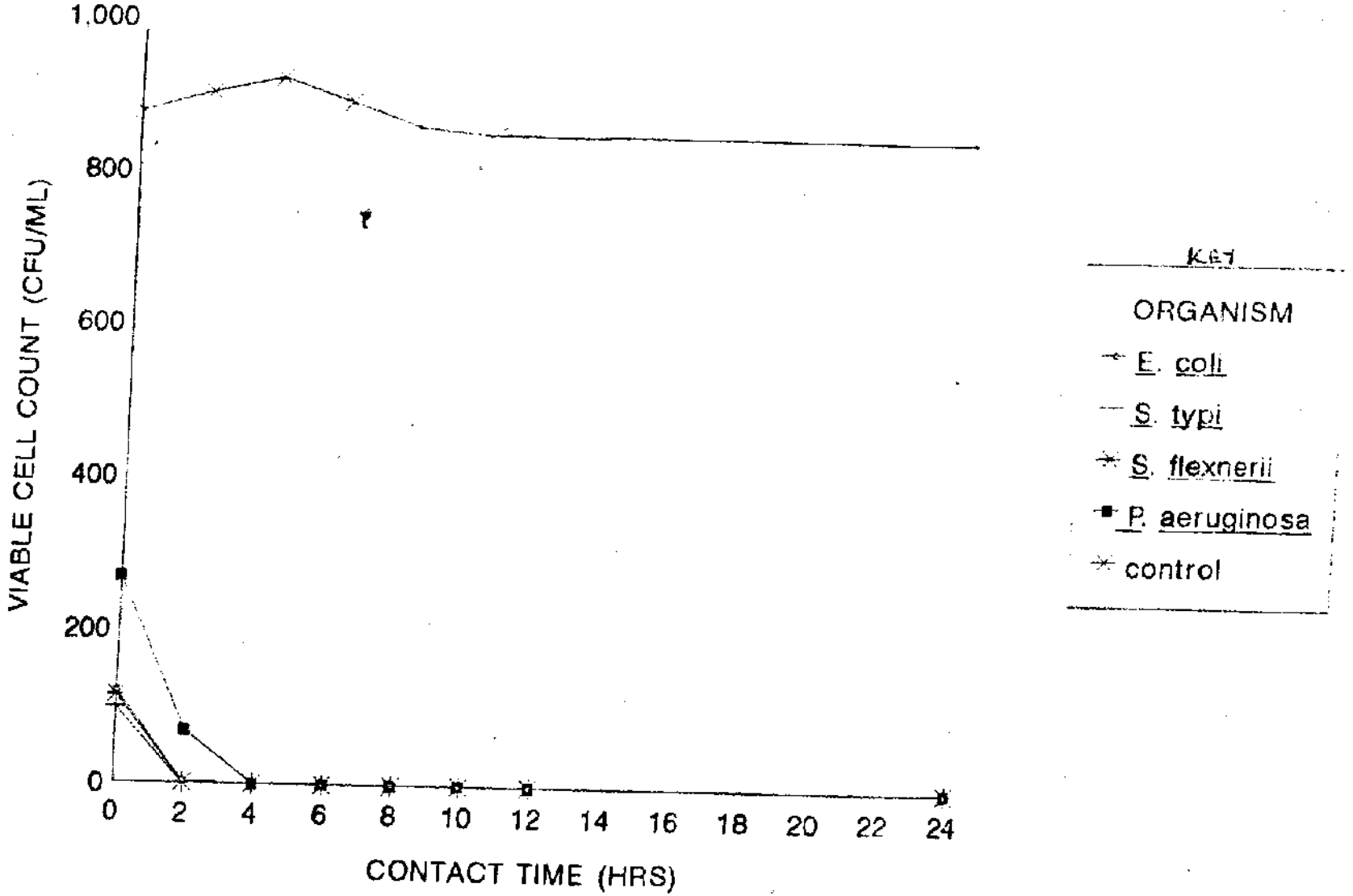


Fig 3: RATE OF BACTERICIDAL ACTIVITY OF CRUDE EXTRACT OF P. thonningii (29mg/ml) ON THE FOUR TEST ORGANISMS

TABLE 4: ANTIMICROBIAL ACTIVITY OF THE FRACTIONS OF PLANT EXTRACTS

		ZONES OF INHIBITION (mm) OF FRACTIONS			
PLANT	ORGANISM	* EBC	PNC	NNC	EAC
B. ferrugenia	E. Coli	-	-	-	8
	S. flexnerii	-	-	-	12
	S. typhi	-	-	-	6
	P. aeruginosa	-	-	-	14
K. senegalensis	E. Coli	4	-	-	16
	S. flexnerii	4	-	-	14
	S. typhi	-	-	-	-
	P. aeruginosa	4	-	-	14
P. thonningii	E. Coli				14
	S. flexnerii				14
	S. typhi				10
	P. aeruginosa				8
CONTROL		-	-	-	-

* EBC = BASIC COMPOUNDS
PNC = POLAR NEUTRAL COMPOUNDS
NNC = NON-POLAR NEUTRAL COMPOUNDS
EAC = ACIDIC COMPOUNDS

Table 4 represents the result of the agar diffusion test of the four fractions for each of the three exracts studied. The test indicated that fraction four from the extract of B. ferrugenia has a considerable amount of antimicrobial activity. The inhibition zones on P. aeruginosa and S. flexnerii were almost the same (14mm & 12mm respectively. E. coli had an inhibition zone of 8mm while S. typhi had the inhibition zones of 6mm (Plate 1).

The active principle in the aqueous extracts of K. senegalensis also appear to be nonpolar and acidic in nature. Fraction 4 (containing acidic compounds) caused an inhibition of

growth of the four test organisms although not to the same degree. P. aeruginosa and S. flexnerii had inhibition zones each of 14mm while E. coli had the largest zone of inhibition of 16mm. However S. typhi was not inhibited since there was no zones of inhibition for all the four wells (Plate 2) . The fraction containing basic compounds produced 4mm zones of inhibition on E. coli S. flexnerii and P. aerugenosa. Only the acidic fraction from the extract of P. thonningii produced zones of inhibition for all the test organisms. (14mm diameter of inhibition in E. coli, 14mm in S. fexnerii, 10mm is S. typhi and 8mm in P. aeruginosa). It may be safe to conclude that the active compound in this plant is an acidic substance since the other fractions containing Basic, polar and non polar neutral compounds were all inactive as there was no zones of inhibition around the wells for all the bacteria tested (Plate 3).

PLATE 1

(A) - Zones of inhibition (12mm) produced by the EAC fraction of Bridelia ferrugenia on Shigella flexnerii.

(B) - Zones of inhibition (8mm) produced by the EAC fraction of Bridelia ferrugenia on Eschericia coli.

(C) - Zones of inhibition (14mm) produced by the EAC fraction of Bridelia ferrugenia on Pseudomonas aeruginosa.

(D)- Zones of inhibition (6mm) produced by the EAC fraction of Bridelia ferrugenia on Salmonella typhi.

PLATE 11

(A) - Zones of inhibition (44mm,4mm) produced by the EAC and EBC fractions of Khaya senegalensis on Shigella flexnerii.

(B) - Zones of inhibition (4mm,16mm) produced by the EAC and EBC fractions of Khaya senegalensis on Eschericia coli.

(C) - Zones of inhibition (4mm,14mm) produced by the EAC and EBC fractions of Khaya senegalensis on Pseudomonas aeruginosa

(D) None inhibition by all the fractions of Khaya Senegalensis on Salmonella typhi.

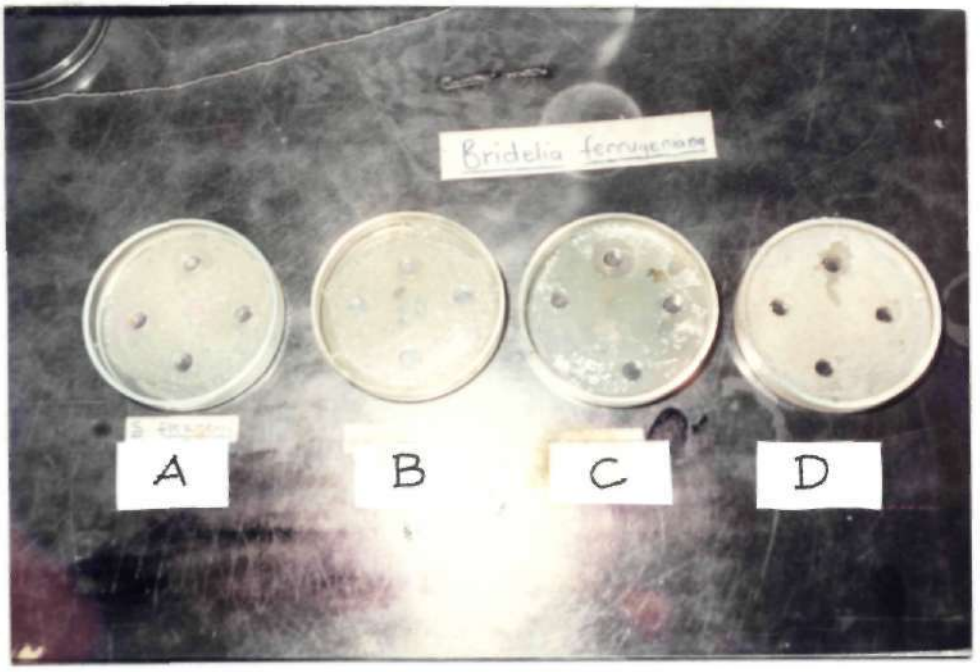


Plate I: Zones of inhibition produced by EAC fraction of the extract of B. ferrugenia on the four test organisms.



Plate II: Zones of inhibition produced by EAC and EBC fraction of the extract of K. senegalensis.

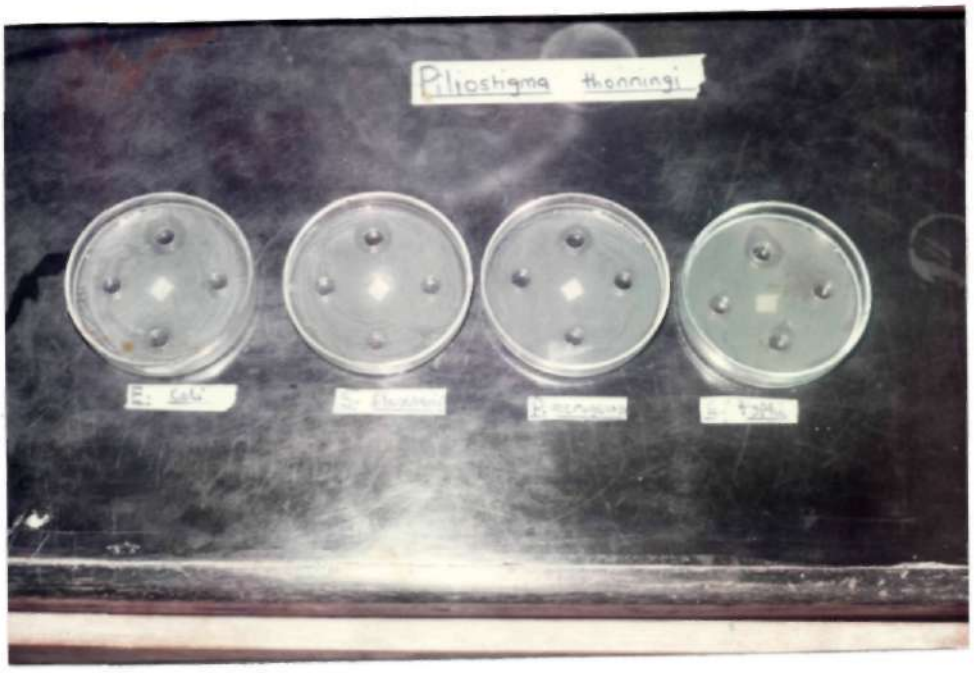


PLATE III: Zone of inhibition produced by the EAC fraction of *P. thonningii*

CHAPTER FIVE

DISCUSSION

Herbalists in Nigeria, most particularly in the rural areas use a variety of herbal remedies for treating various types of ailments. Although these practitioners may be ignorant of the detected pharmacological and toxicological values of their medications, years of practice and committed observation have taught them some principles of dosage and therapeutic assessment. In this study efforts have been made to study the possible antimicrobial properties of 10 plants locally used for the treatment of diarrhoea and dysentery under the traditional health care system by some communities in Zaria. The aqueous extracts were tested on three gram negative bacteria mostly encountered in gastroenteritis and also on Pseudomonas aeruginosa. Preliminary agar dilution tests showed that the aqueous extracts of six of these plants have some appreciable antibacterial activity against the four test organisms (Table I). The extracts at concentrations ranging from 10-29mg/ml showed total inhibition of all the four test organisms. The susceptibility of the four gram negative enteric organisms to the active extracts at these concentrations is highly significant as these organisms were clinical isolates and it has been reported that as a consequence of the widespread use of antibiotics during the past decade, resistant enterobacteria have become common in human and animal wastes and even in river water contaminated with infected faeces (Mutanda et al. 1983). It is noteworthy that in any crude extraction method, only a very small

part of the active materials actually dissolve in any solvent. In this study, cold distilled water proved to be a good solvent since even the minimum inhibitory concentration value for most of the active plants was fairly low even for Pseudomonas aeruginosa which have been reported to be resistant to most antimicrobial agents especially to the penicillins and aminoglycosides (Weinstein et al. 1971, Ayliffe et al. 1972; Jawetz 1972). The minimum inhibitory concentration (MIC) of the active extracts against the susceptible organisms was within the ranges of 0.96 to 10mg/ml. The extract of B. ferrugenia had the lowest range of 0.96mg/ml for S. typhi and S. flexnerii to 1.9mg/ml for E. coli. This observation therefore highlights the fact that the extract of this local plant which has also been reported to be employed in the Eastern part of the country for the treatment of diabetics (Akubue, 1986) and as a molluscicide (Iwu and Okunji, 1988) could also find certain usefullness in future antibacterial preparations formulated for the treatment of a wide range of diseases for which these organisms have been implicated such as bacillary dysentery, Salmonella septicemia, Enteric fevers and in necrotic lesions of the skin (ecthyma gangrenosum) caused by Pseudomonas aeruginosa (Thomas, 1988).

The results for the bactericidal test (Table 3) show that the active plants with fairly low MIC values for all the test organisms also have an irreversible inhibitory effect on the organisms. However, K. senegalensis could only inhibit P. aeruginosa at a concentration of 8.5 mg/ml but could not kill it even at the higher

concentration used. The extract of the powdered bark of T. macroptera and T. avicceniodes were both bactericidal for E. coli and S. typhi. While the extract of T. aviccenioedes was bactericidal to P. aeruginosa at a concentration of 8.5mg/ml, S. flexnerii was completely resistant to killing even at a concentration of 28mg/ml. (Table 3).

The broad antibacterial activity of the hot water extract of the leaves of P. guajava had earlier been reported by Akpulu, (1991). Thus, the fact that the bactericidal activity of the cold water extract of P. guajava against S. typhimurium as shown in this test further supports its use by the traditional healers for treating dysentery, abdominal pains, and in infusions for relieving fevers. It is note worthy that the bactericidal activity of extracts of B. ferrugenia and C. citratus against the test organisms were pronounced at half the concentration of the crude extract, while extracts of K. senegalensis, T. macroptera and T. avicenniodes were bactericidal for S. typhi and E. coli only at the undiluted form. However, the fairly high minimum bactericidal values are probably to be expected from this initial crude extraction when it is considered that the active principle most likely constituted only a proportion of the crude extractives, hence the absolute minimum bactericidal concentration will be expected to be much lower than that quoted in this investigation. The bactericidal effect may also have been affected by the presence of nutrient media in the diluted extracts (Aggag and Yousef, 1972). The kinetics of the bactericidal activities of the crude extracts

have been investigated using all four gram negative organisms. Extracts of B. ferrugenia, and P. thonningii were bactericidal to all four test organisms while extract of K. senegalensis was bactericidal to E. coli, S. flexnerii and S. typhi only. P. aeruginosa was not affected., although growth of P. aeruginosa was inhibited by a 10.4mg/ml concentration of the extract in an earlier experiment (Table 1). The bactericidal time, however for the four test organisms in the crude extracts varied. While all the test organisms were essentially destroyed within one to three hours in extracts of P. thonningii, an exposure time of about eight hours was required to eliminate E. coli and S. flexnerii in extract of B. ferrugenia while the extinction time of S. typhi was reduced by one hour in the same extract. P. aeruginosa was found to be more sensitive to the extract of B. ferrugenia as the extract resulted in a 99,99% destruction of the organisms within 3 hours. This sensitivity pattern was also shown by the test organisms in extracts of K. senegalensis as all the cells were killed within 8 hours but in this extract, there appeared to be a slowly but progressively increasing killing pattern within the first 3 hours followed by a rapid killing within the next two hours for S. typhi and S. flexnerii (Fig 2).

The difference in the rate at which the test organisms were killed in the extracts probably is to be expected as the bacteria species differ in their susceplibility to various bactericides (Brozel and Cloete, 1991) and for any compound to be effective, it must penetrate the cell envelope and attain a sufficiently high

concentration at the target site in order to exert their antimicrobial action (Brozel and Cloete 1991).

Thus, the resistance of P. aeruginosa to complete killing by the extract of K. senegalensis may be due to a mechanism of adaptation of the cell envelope or to plasmid transfer encoding a bactericide degrading enzyme (Brozel and Cloete 1993).

The next step of the research was aimed at trying to find out if any active principle/s could be isolated from the crude extracts and if these fractions could retain their antimicrobial effect after undergoing the extensive extraction procedure. Results, (Table 4) indicate that extracts of the three plants studied actually do have active principles which retained the antibacterial activity even after subjecting the crude extracts to the extraction processes. The antibacterial activity of the crude extract of B. ferrugenia appears to be more potent than that of the isolated fraction, since the crude extract which has the highest bacteriostatic (Table 2) and bactericidal effect (Fig 1) has a fairly low potency when the active fraction is extracted from it. A possible explanation may be that there may be more than one active compound which are synergistic to each other and the separation process might have reduced the potency of these active principles (Plate 1).

The active principles in the extract of K. senegalensis and P. thonningii were also located in the fraction containing acidic compounds. Thus it can be concluded that the active principles are acidic in nature (Plates 2 and 3).

The question of sterility is one of the problems often encountered in the drug industry either in economic terms or in the consideration of heat liability of the drugs. It is therefore worth noting that the active principle of the extracts of B. ferrugenia, K. senegalensis and P. thonningii were not destroyed by autoclaving at 121°C for 15 minutes, a property which is rare among many antimicrobial agents of biological origin (Babalola, 1988). The antimicrobial action of the various crude extracts and their fractions therefore justify the use of these plants in treating gastrointestinal disorders. The efficacy of the traditional method for treating gastrointestinal disorders have been confirmed by Mehta and subramaniam (1986). The starches and proteins in cereals when broken down in the gut slowly release glucose, amino-acids, and short chain peptides all of which enhance the absorption of sodium and water. Another way in which these medicinal herbs are effective against diarrhoea may be by a direct action on the smooth muscle (Sofowora et al. 1969).

Different microbilogical studies have indicated that vegetable tannins have broad spectrum of activity against both gram positive and gram negative organisms. However, the compounds were more effective against the gram positive bacteria (Abdulrahman 1986). Thus, the fact that tannins possess ample antiseptic and anti-inflammatory properties, and are capable of precippitating microbial proteins and rendering them less resistant to proteolytic enzymes (Harbone, 1964), justifies the use of vegetable tannins in traditional medicine for the treatment of some microbial infections

of the gastrointestinal tract.

5.2 CONCLUSION

From the findings in this study it seems reasonable to conclude that the extractives from the active plants investigated viz. *B. ferrugenia*, *K. senegalensis*, *P. thonningii*, *P. guajava*, *T. avicennioedes*, and *T. macroptera* clearly demonstrate the antibacterial potential of the crude extracts and the active fractions isolated. The observed activity against the test strains of bacteria which represent some of the gram negative enteric organisms is significant since gram negative organisms and spore forming bacterias are less susceptible to antibacterial agents.

Although the results obtained in this study have justified the use of these plants for the traditional herbal treatment of dysentery and diarrhoea and the fact that the plants have been used since time immemorial as antidiarrhoeals and in the treatment of dysentery apparently with no reported adverse toxic effects, further studies is required to:-

1. Assess the broad spectrum antimicrobial activity and possibly the mode of antibacterial action of these extracts.
2. Further purify and analyse chemically the active compounds of these extracts.
3. Assess the synergistic or antagonistic influence of the components of the inactive fractions on the potency of the active principles; The effect of other components of the concoctions that contain these leaves also needs to be investigated.

4. Investigate the possibility of improving the extracts for varying chemotherapeutic purposes and for combined therapy using two or more of the active principles.

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APPENDIX I QUESTIONNAIRE

Amongst the questions asked in the questionnaire were

a) Name: .....

b) Age .....

c) Have you ever suffered from gastroenteritis before?....

If yes, state form. i.e was it.

Watery Stool only? or stool with mucus?.....

d) Did you take any herbal remedy for it?.....

e) If yes, state what was the remedy? .....

.....

f) Do you know the name/s of the constituents? state.....

.....

g) Where/How is it obtained?.....

.....

h) Why did you take the medicine? and was it effective? state.....

i) How often did you take the remedy? (i.e dosage).....

.....

j) What do you think was the cause of the gastroenteritis?

.....

.....

APPENDIX II: LIST OF PLANTS WHOSE PARTS ARE COMMONLY USED SINGLY OR AS PART OF PREPARATIONS USED AS REMEDY FOR GASTROENTERITIS IN SAMARU HAYI & KORAYE VILLAGES

PLANT	HAUSA LOCAL NAME	PART EMPLOYED	(%) FREQUENCY
Acacia arabica	Bagaruwa, Barbarta	Leaves, Bark	2
Adansonia digitata	Kuka	Leaves	1
Anogeissus schimperii	Marke	Barks	1
Azadirachta indica	dogon yaro	Leaves	3
Balanites aegyptica	Aduwa	Leaves	1
Boswellia dalzielli	Hannu	Barks	3
Bridelia ferrugenia	Kirni, kizni	Barks	5
Butyrospermum paradoxum	Kadanya	Barks	1
Carica papaya	Gwanda	Leaves	2
Caviisa edulis	Ci zaki	Leaves	1
Ceiba petandra	Rimi	Leaves, Barks	1
ceratotheca sesamoides	Kalkashi	Leaves	1
Citrus aurantifolia	Lemun tsami	Leaves	3
Cymbopogan citratus	Lemon grass	Leaves	1
Detarium senegalense	Taura	Leaves, Barks	1

PLANT	HAUSA LOCAL NAME	PART EMPLOYED	(%) FREQUENCY
Dichrostachys glomerata	Dundu	Leaves	1
Eugenia aromaticum	Kanun fari	fruits/seeds	1
Erythropleum ivorense	Gwaska,	Bark	1
Ficus ingens	Kawuri	Barks	2
Ficus platyphylla	Ganji	Barks	1
Guerra senegalensis	Sabara	Leaves	10
Isoberlina dalzielli	Doka	Barks	1
Irvingia grandifolia	Goron biri	Barks	1
Khaya senegalensis	Madaci	Barks	8
Lamnea aacida	Faru	Barks	1
Masonia altissima	Leaves	Leaves	1
Musa sapientum	Ayaba	Leaves	1
Occimum grattissimum	Dodoya	Leaves, fruits	13
Parkia biglobosa	Dorowa	Barks	6

PLANT	HAUSA LOCAL NAME	PART EMPLOYED	(%) FREQUENCY
Phoenix dactylifera	Dabino	Fruits	1
Piliostigma thonningii	Kalgo	Leaves	3
Prossopis africana	Kirya	Barks	1
Pseudocèdrela kotschyi	Tuna	Barks	5
Psidium guajava	Gwoba, Gwaiba	Leaves(fruits)	20
pterocarpus erinaceus	Madobiya	Barks	1
Sclerocarya birrea	Danya	Barks, roots	2
Solanum incanum	Ganta	Leaves, fruits	1
Spondias monbin	Tsada	Leaves	2
Terminalia avicennioedes	Baushe	Barks, Leaves	4
Terminalia macroptera	Kwandari	Barks	2
Vitellaria paradoxum	Kadanya	Barks	1
Zea maize	Masara	Seeds	2
	Taro	Root, Leaves	1

* This is the number of times the plant is mentioned in the survey.

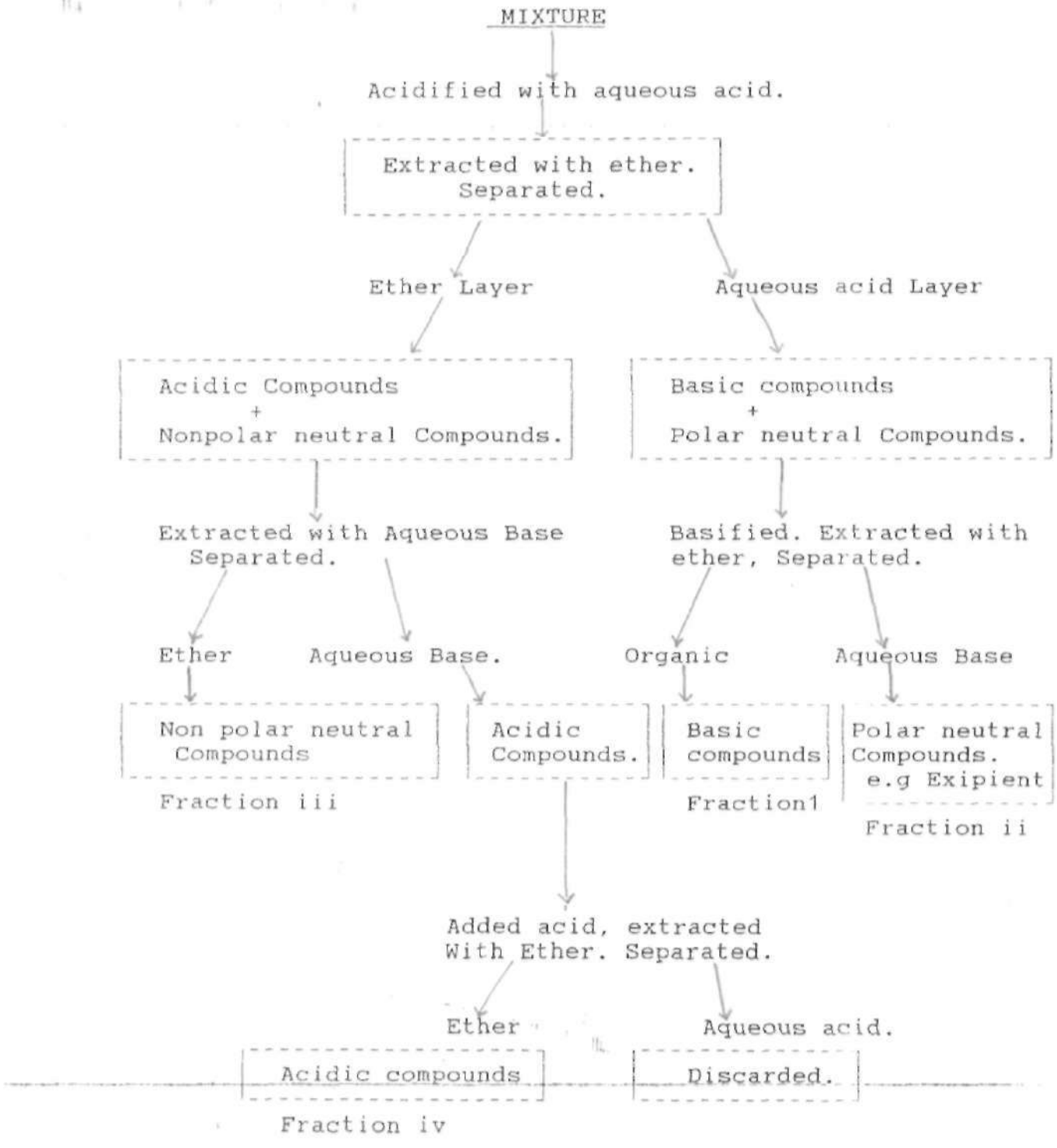
() Used only in very minor cases.

APPENDIX III. LIST OF PLANTS USED IN THE STUDY AND RELEVANT PARTS USED.

PLANT	PART USED	CONCENTRATION (mg/ml)	PH
Bridelia ferrugenia	Bark	32	3.8
Khaya senegalensis	Bark	16	4.2
Guerra senegalensis	Leaves	26	6.3
Occimum grattissimum	Leaves	28	5.9
Parkia clappertoniana	Bark	17	5.2
Piliostigma thonningii	Leaves	29	2.8
Psidium guajava	Leaves	10	4.6
Sclerocarya birrea	Barks	22	4.6
Terminalia avecennioedes	Bark	18	4.9
Terminalia macroptera	Bark	13	4.6

APPENDIX IV

SCHEME FOR THE SEPARATION OF DRUG SUBSTANCES FROM THE ACTIVE EXTRACTS



APPENDIX V: BACTERIA COUNT AFTER TREATING TEST ORGANISMS WITH PLANT EXTRACTS

		TIME OF CONTACT (IN HOURS)						
PLANT	ORGANISM	0	2	4	6	8	10	24
B. ferrugenia	E. coli	117	83	71	63	35	9	0
	S. typhi	149	143	31	2	0	0	0
	S. flexnerii	500	270	23	20	12	2	0
	P. aeruginosa	326	6	0	0	0	0	0
K. Senegalensis	E. coli	108	56	32	28	9	0	0
	S. typhi	269	228	107	10	1	0	0
	S. flexnerii	310	293	229	12	10	7	0
P. thonningii	E. coli	118	3	0	0	0	0	0
	S. typhi	297	0	0	0	0	0	0
	S. flexnerii	113	1	0	0	0	0	0
	P. aeruginosa	268	68	0	0	0	0	0
CONTROL		-	-	-	-	-	-	-

KEY:

- = CONTROL (Abundant growth of bacteria).

APPENDIX V: BACTERIA COUNT AFTER TREATING TEST ORGANISMS WITH PLANT EXTRACTS

		TIME OF CONTACT (IN HOURS)						
PLANT	ORGANISM	0	2	4	6	8	10	24
B. ferrugenia	E. coli	117	83	71	63	35	9	0
	S. typhi	149	143	31	2	0	0	0
	S. flexnerii	500	270	23	20	12	2	0
	P. aeruginosa	326	6	0	0	0	0	0
K. Senegalensis	E. coli	108	56	32	28	9	0	0
	S. typhi	269	228	107	10	1	0	0
	S. flexnerii	310	293	229	12	10	7	0
P. thonningii	E. coli	118	3	0	0	0	0	0
	S. typhi	297	0	0	0	0	0	0
	S. flexnerii	113	1	0	0	0	0	0
	P. aeruginosa	268	68	0	0	0	0	0
CONTROL		-	-	-	-	-	-	-

KEY:

- = CONTROL (Abundant growth of bacteria).