IN VITRO ASSESSMENT OF EXTRACTS FROM SOME LOCAL MEDICINAL PLANTS ON AGENTS OF UROGENITAL INFECTIONS.

By

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DECLARATION

I hereby declare that this thesis has been prepared by me and that it is a compilation of my own research work. No part of this thesis has previously been submitted for a higher degree or presented in any previous publication.

All quotations are clearly indicated and their sources duly acknowledge by means of references.



BODA Maurice

April 1997.

CERTIFICATION

This thesis titled " *In vitro* Assessment of Extracts from some local Medicinal Plants on Agents of Urogenital Infections " by BODA Maurice meets the regulations governing the Award of the degree of Master of Science in Microbiology of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to my late Father PENKO Joseph, my mum MOUKAM Madeleine, my uncle DEUDJUI Albert, my elder brother NINTCHEU Raphael and to my cousin YITEMBE Gabriel.

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ABSTRACT

Aqueous and methanolic extracts from the following plant parts were screened for their antimicrobial properties: *Abrusprecatorius* (Stem. Seed and Leaf). *Khaya senegalensis* (Stem bark and Leaf). *Jatropha curcas* (Stem bark and Leaf), *Securidaca longipedunculata* (Leaves mixed with Fruits), *Parkin biglobosa* (Stem bark). *Cassia alata* (Leaves). and the whole plant *o(Borreria verticillata* and *Euphorbia hirta.* The micro-organisms on which the extracts were tested were. *Xeisseria gonorrhoeae. Streptococcus pyogenes Staphylococcus aureus, Salmonella spp. Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Candida albicans,* and *a Tricomonas spp.* The result showed thai all the nine test organisms were susceptible to the alcoholic and aqueous extracts of the stem bark of *P. biglobosa.* Extracts from the stem bark of *K. senegalensis* and stem of *Abrus precatorius* also exhibited a broad spectrum antimicrobial activity on seven out of nine test organisms. Extracts (aqueous or methanolic) from *S. longipedunculata. E. hirta, B, verticillata, K. senegalensis* leaves, *A. precatorius* seed, and leaves of C. *alata,* showed a moderate microbicidal activity with effect on at least four test organisms, while extracts from leaves of *J. curcas* and *A. precatorius* were poorly effective. Some extracts were found to be highly inhibitory but not microbicidal to the test organisms. *N, gonorrhoeae, S. pyogenes, C. albicans* and *Tricomonas spp* were more sensitive to the alcoholic extracts than to aqueous extracts, while A, *pneumoniae P. aeruginosa* and *E. coli* showed the reverse. The susceptibility *of Staphylococcus* and *Salmonella* to aqueous and methanolic extracts was indiscriminate, and these two pathogens were recorded as the most sensitive test organisms.

All the test organisms used were resistant to tlje aqueous stem bark extract *of J. curcas*, and the methanolic extract of this plant part was effective only on *Salmonella spp,*

For alcoholic extracts, the lowest M.I.C value (0.625 mg/mL) was obtained with the stem bark extracts *of K. senegalensis* and *P. biglobosa on S. aureus* and *Salmonella spp.*. while the lowest M.M.C value (1.25 mg/mL) was obtained with the same extracts on *S. aureus.* Many extracts were inhibitory or microbicidal at the highest M.I.C. and M.M.C. value which was 40mg/mL. For aqueous extracts the M.I.C. values range from 0.23mg'mL to 56mg'mL, the lowest value was obtained with *K. senegalensis* stem bark extract on *S. aureus* and the highest value with *S. longipedunculata* on *C. albicans.* The lowest M.M.C value (0.75mg mL) was also recorded with *K. senegalensis* stem bark on *S. aureus* and the highest value (46.1 mg/mL) with *P. biglobosa* stem bark on *C. albicans.*

Methanol extract from all the plant parts were also screened lor their possible toxic potential, using brine shrimps (*Anemia salina)* toxicity assay. Four extracts, precisely from the stem and seed of *A. precatorius* and stem bark and leaf of J *curcas* showed a significant toxicity on the shrimps, with LC_{50} values lower than 200ug/mL.

The stem extract of A *precatorius* was the most toxic (LC_{50}=104.0857 u,g/mL) while extracts from the mixture of leaf and fruit of *S. longipedunculata* and leaf of *K. senegalensis* were the least toxic to the shrimps with LC_{50} value greater than 1000 ug'mL.

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LIST OF ABBREVIATIONS

CA :	Chocolate Agar
GTI :	Genital tract infection
HPV :	Human papilloma virus
HSV :	Herpes simplex virus
MHA :	Mueller Hinton Agar
MHB :	Mueller Hinton Broth
MIC :	Minimum Inhibitory Concentration
MMC :	Minimum Microbicidal Concentration
PDA :	Potato Dextrose Agar
PDB :	Potato Dextrose Broth
PID :	Pelvic Inflammatory Diseases
TM :	Trichomonas Medium
UTI :	Urinary Tract Infection

Chapter 1
INTRODUCTION

With modern civilisation and the increasing sexual promiscuity, infections of the genital and urinary organs (urogenital infections) have emerged to be the most common health problem in both developed and developing countries (Theide, 1989; Gene, 1989; Lucas and Gary, 1992; Heine and McGregor, 1993). These diseases are caused by a variety of germs of which the most prevalent include, *Trichomonas vaginalis, Candida albicans, Neisseria gonorrhoeae, Gardnerella vaginalis, Chlamydia trachomatis, Mycoplasma spp and Ureplasma spp* causing mostly genital tract infections (G.T.I.s) and *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* which cause urinary tract infections (U.T.I.s). Organisms such as *Streptococcus spp* and *Staphylococcus aureus* are involved in both genital and urinary tract infections (Cheesbrough, 1985; Zagrebina and Torchinov, 1989). Also included is *salmonella enteritidis*, a common opportunistic contaminant of the urinary tract (Lainy *et. al.*;1993). It has been reported that 25% to 35% of women between 20 and 40 years of age have had at least one U.T.I. and many of these have experienced recurrent infections (Theide, 1989). Urogenital infections are the most common causes of morbidity and occasional mortality with pregnant women considered as the most affected due to their physiological state (Gene, 1989; Lucas and Gary, 1993) and the fact that treatment of these diseases in pregnant women is always delicate (Marilyn *et. al.*, 1983; Hager and Rapp, 1992).

The prevalence of urogenital infections in developing countries vary widely in different populations with high incidence among pregnant women, teenagers with multiple sexual partners, prostitutes and their customers (Thomason and Gelbart, 1989). This high prevalence is also due to poor hygiene and drug abuse through indiscriminate use of antibiotics, which

results in improper treatment of the disease condition (Stanier *et. al.*,1986; Fallon and Slack 1992). One of the most adverse consequences of indiscriminate use of drugs is the spreading of antibiotic resistant strains of micro-organisms.

Although urogenital infections are in most cases not life threatening, failure to apply appropriate therapy can cause severe complications such as renal failure, sterility, ectopic pregnancy, and other complications attached to pelvic inflammatory diseases (Zagrebina and Torchinov, 1989; Fallon and Slack, 1992; Gross 1992). Some pathogens of genital organs are implicated in preterm labour and delivery, low-birth weight and neonatal morbidity and mortality (Carey *et. al.*,1993, Lucas and Gary, 1993)

The toxicity of potent pure or synthetic drugs is obvious (Hans *et. al.*, 1976),and by relying exclusively on these drugs patients pay heavily in adverse effects. Hans *et. al.*,(1976) observed that purifies tannins have a very drastic action on diarrhoea patients being treated with this drug compared to the natural tannins from the plants. Similarly "Digoxin BP" a pure glycoside derived from the plant *Digitalis purpurea* L. and used in England to treat dropsy was found to be toxic to the patient with a safety dose range being too narrow, compared to "Prepared Digitalis" a drug made of crude leaf extracts of the plant *Digitalis purpurea* (Lanchman *et. al.*, 1976). Another example is "Flagyl" (Metronidazole), the drug of choice against vaginal trichomoniasis has been proved to be potentially carcinogenic and embryotoxic, hence contra-indicated during the first trimester of pregnancy (Marilyn *et. al.*, 1983; Hager and Rapp, 1992); more over a prolonged treatment with this drug can damage cells of the Central Nervous System (Alvarez *et. al.*, 1983).

The rapid spread of antibiotic resistant strains among pathogens of the urogenital tract has rendered useless the most common and cheap antibiotics, and the affected population of the third world countries cannot afford the cost of the few reliable modern drugs available. Many patients have thus resolved

to turn to traditional herbalists where the cost of the treatment is affordable. In effect herbal medicine provides a possible source of cheap and effective drugs with little or no side effect to patients (Babalola 1988; Kokwaro, 1993). In China today, herbal medicine forms part of school curriculum and the official health care system (W.H.O., 1989; Sofowora,1993), while in the present Europe and USA organised research institutes are undertaking researches in local medicinal plants, and many drugs in use are of plant origin (Principe, 1989)

In Africa, scientific investigation on medical plants is still in its infancy. The knowledge of herbals medicine is kept secret by families and is passed on to the next generation only orally. Thus, herbals healing is so far been inaccessible and shrouded in mysticism (Sofowora, 1986). A few number of African medicinal plants were however brought to international recognition by some European investigators in the 1930's (Aubreville, 1936; Dalziel, 1937); and today the pharmacopoeial standard of many African medicinal plants has been established (Sofowora, 1993; Adjanohoun *et. al.*;1993). For example a paste prepared from powdered leaf of the plant *Harrisonia abyssinica* Oliv, is used in Tanzania to treat cancerous tumours and skin diseases including leprosy (Uiso, 1979). Uiso found that extracts from the root and leaf possessed antimicrobial activities against *Neisseria gonorrhoeae*, *Trichopyton mentagraphytes* and *Schistosoma mansonii.* Obacunone and harrisonin were isolated as active constituents (Sofowora, 1993). Aqueous extract of the roots of the plant *Cryptolepis sanguinolenta* Schtlr claimed by Ghanaian herbalists to be effective in treating urogenital infections was proved scientifically by work conducted by Boakye-Yeadom (1979) to be effective against *Candida albicans, Neisseria gonorrhoeae,* and *Escherichia coli.*

In view of the emergence of resistance to a good number of modern drugs and the availability of plants of potential medicinal value, it is imperative to establish the scientific basis for use of herbs as drugs. Lots of

these herbs remain unidentified. The need to identify and document their usefulness for curative / preventive purposes cannot be over-emphasises.

The objectives of this work are therefore :

(i) To screen the alcoholic and water extracts from some selected local medicinal plants for their antimicrobial activity against some urogenital pathogens.

(ii) To determine the Minimum Inhibitory Concentration (M.I.C.) and Minimum Microbicidal Concentration (M.M.C.) of the extracts against the selected urogenital pathogens.

(iii) To examine the toxicity of these extracts using brine shrimps (*Artemia salina*) toxicity assay.

The selected plant part for this study include, the stem, leaves, and seeds of *Abrus precatorius*, the whole plants of *Borreria verticillata* and *Euphorbia hirta*, the stem barks and leaves of *Khaya senegalensis* and *Jatropha curcas*, the stem bark of *Parkia biglobosa*, the leaves of *Cassia alata* and the leaves mixed with fruits of *Securidaca longipedunculata*. The selection of the plants and plant parts was based on available information collected from local herbalists, on personal experiences, and the scientific literature.

Chapter 2

LITERATURE REVIEW

2.1 UROGENITAL INFECTIONS.

Urogenital infections are infections pertaining to the urinary and genital organs (Nancy, 1987) . They are caused by micro-organisms of various origins; some belong to the commensal flora of the urogenital tract, which could become pathogenic due to a change in the body physio-chemical condition (Lossick, 1984; Cheesbrough, 1985). Inflammation of the urinary and genital organs occurs in many cases by acquisition through sexual intercourse or contaminated materials, of exogenous infectious agents, and, depending on the affected organ(s) different clinical signs are observed (Cheesbrough, 1985 ;Zagrebina and Torchinov, 1989). In general urogenital infection can be categorised into four major groups: fungal, protozoal, bacterial, and non specific infections (Lossick, 1984) .

2.1.1 - Urogenital Infection of Fungal Origin

The fungus of importance in this case is *Candida albicans* which causes vaginitis and urethritis (in men). This air borne dimorphic and Gram positive budding yeast has also been implicated in skin, nails and mucous membrane infections (Lossick, 1984; Glenns, 1990). *C. albicans* is a normal flora of the vagina, which could become pathogenic when the normal flora is altered as a consequence of use of wide - spectrum antibiotics, or when the host resistance to infection is lowered by disease, use of immunosuppressants and/or immunodepressers, pregnancy, and other pathological conditions such as diabetes mellitus and Acquired Immune Deficiency Syndrome (Lossick,1984; Seaman, 1987; Evans 1992).

The most prominent features of vaginal candidiasis are vulvar pruritus, vulvo-vaginal and cervical erythema, dyspareumia, and perturbation of the menstrual cycle (Evans, 1992). Vaginal discharge is frequent but not

universal (Glenns, 1990). In men the most common clinical picture is balanitis associated with mild pruritus of the glans penis; meatal erythema and dysuria are occasionally noted (Cheesbrough, 1985; Glenns, 1990; Evans, 1992). Absence of treatment leads to systemic candidiosis in immuno-depressed individuals. The systemic form may be localised in kidney, bladder, heart valves (*endocarditis*), meninges, liver, and eyes (*Candida endophthalmitis*) (Evans, 1992). Deep seated systemic candidiosis is difficult to diagnose and many cases are often revealed post-mortem.

Vaginal and urethral candidiasis are diagnosed by microscopy, and / or isolation of *C. albicans* from vaginal or urethral swab, or from urine (Cheesbrough, 1985; Evans, 1992).

Vaginal candidiasis responds well to topical therapy with nystatin. Amphotericin B is normally recommended for urethral candidiasis. Systemic candidiosis is treated with intravenous injection of Amphotericin B combined with Fluconazole (Evans, 1992). Antifungal drugs used are limited by high rate of spontaneous mutation to resistance and the bone marrow toxicity demonstrated with Fluconazole.

2.1.2 **Urogenital infection of protozoal origin.**

Trichomonas vaginalis is the only pathogenic protozoan known to inhabit the urogenital area (Slack, 1992). It is an egg-shaped anaerobic flagellate known to be very unstable in external environment. It dies at temperatures above 40 °C, in the presence of soap and disinfectants. Studies in tissue culture indicate that *T. vaginalis* is a destructive epithelial pathogen, destroying up to 10% of an epithelial cell monolayer within six hours (Alderet and Pearlman, 1984). The organism exists as an active trophozoite always demonstrating a characteristic busy movement that helps to identify it (Honigberg *et. al.*, 1984).

Trichomoniasis develops when the vaginal Trichomonad finds its way into genital and urinary tracts. It is a sexually transmitted pathogen, but extra

sexual contamination is possible through sharing items of personal hygiene (Cheesbrough, 1985). In males *T. vaginalis* colonises the urethra and may cause no clinical manifestation. Trichomonal urethritis in men is often transient; the disease disappears without treatment. Men are said to be protected against *T. vaginalis* infection because of the high zinc concentration of the prostatic fluid which inhibit excessive proliferation of the parasite (Krieger and Frien, 1982). However, in the 20% symptomatic cases the common complaints include dysuria with a scanty mucopurulent urethral discharge and mild pruritus of the external genitalia. It is thus recommended that men with non-specific urethritis unresponsive to usual antimicrobial therapy should be screened for *T. vaginalis* (Krieger, 1981). Epididymitis and balano-prostitis can be observed in some few cases (Heine and McGregor, 1993). Trichomonal cystitis has not been demonstrated in men (Thomason and Gelbart, 1989)

In women, symptoms are not specific to preclude diagnosis; up to 30% of the infected women may be asymptomatic, but these are thought to be infected with the non pathogenic strains of *T. vaginalis* (Krieger, 1981). After four to five days of incubation, symptoms start with profuse vaginal discharge and vulvo-vaginal irritations. The discharge is described as green, frothy, and foul smelling (Thomason and Gelbart 1989). Lower abdominal pain is a common finding as a result of inflammation of the bladder (cystitis) and the cervix (cervicitis). This is always associated with urethritis and colpitis macularis ("strawberry" cervix) which can be demonstrated by colposcopy in as many as 90% of cases (Latif *et. al.*,1987; Wilner *et. al.*,1989). Other symptoms are dysuria, dyspareunia and bartholinitis. Infection of the rectum (proctitis) may also occur (Zagrebina and Torchinov, 1989). *T. vaginalis* has recently been implicated in the pathogenesis of preterm-labour, low birth weight and neonatal morbidity and mortality (Carey *et. al.*,1993), constituting thus one of the major causes of Obstetric and Gynecologic pathology (Heine and McGregor 1993).

Persistent infection by the parasite in women leads to a reversible infertility. The observed infertility is due to the following factors:

(i) The vagina and the cervix are covered with purulent discharge containing *T. vaginalis* sperm cells inhibitory factors, and the free movement of the sperm cells in the cervical canal is hampered.

(ii) In case of endocervicitis there is a pseudo-blockage of the cervical canal and sperm cells cannot cross.

(iii) The dyspareumia associated with the infection always make the infected woman to stop the coitus without receiving the male semen (Otto and Miroslav, 1968; Jeriki-Black and Lush-Baugh, 1988).

Chronic trichomoniasis leads to a change in the urogenital environment, promoting the colonisation by other pathogen such as *Chlamydia trachomatis, Mycoplasma spp, Neisseria gonorrhoeae,* Herpes Simplex Virus and yeast. In men it also leads to urethral stricture in 10% of cases (Latif *et. al.*,1987;Thomason and Gelbart,1989; Greenwood,1992)

The mainstay of therapy of trichomoniasis continues to be Metronidazole (Flagyl). The rate of cure ranges from 80 to 88% (Nix *et. al.*,1995; Krieger, 1981). Many patients cannot withstand the side effects of this drug. Apart from the nausea, cephalalgia and vertigo the most common undesirable effects, Metronidazole diffuses readily across the placenta and has been found to be carcinogenic, taratogenic and embryotoxic. It is thus advised not to be used during the first trimester of pregnancy (Marilyn *et. al.*,1983; Hager and Rapp,1992). Rare cases of Myalgia, peripheral neuropathy and encephalopathy associated with the use of Metronidazole have been reported (Alvarez *et. al.*,1983). Metronidazole resistant strains of *T. vaginalis* have also been reported (Kulda *et. al.*, 1982)

2.1.3 Urogenital infections of bacterial origin.

Three major species of bacteria are implicated in this category; these include *Neisseria gonorrhoea, Treponema pallidium* and *Gardnerella vaginalis.*

Neisseria gonorrhoeae

Neisseria gonorrhoeae is the causative agent of the well known sexually transmitted disease called gonorrhoea. It is a Gram of negative oval cocci occurring in pairs with the opposed surfaces flat or slightly concave (bean-shaped). It grows anaerobically or in carbon dioxide enriched environment. *Neisseria gonorrhoeae* is exclusively a human pathogen which causes purulent infection, and in the pus from inflammatory exudates it is found inside the polymorphonuclear cell (Cheesbrough 1984, Fallon and Slack 1992, Whittington *et. al.*,1995).

The lower urogenital system is the primary focus of gonococcal infection. Clinical manifestations include, acute urethritis accompanied with dysuria in both sexes, vulvitis and bartholinitis. Endocervicitis is observed in 50% of cases with fresh gonorrhoea. This acute stage is characterised by dull low abdominal pains and micro-purulent discharge from the vagina (Fallon and Slack, 1992). If the primary infection is not promptly treated, the gonococci may invade the upper genital organs, and its spread in the uterine mucous will cause endometritis, attack of the fallopian tubes and ovary. The inflammation of these organs (salpingo-oophoritis) is the cause of the irreversible sterility observed with the chronic gonorrhoea in women. In men prostatitis and epidydimitis are the causes of irreversible sterility (Zagrebina and Torchinov, 1989; Schneider, 1995).

The infected mother will transmit the germ to the new-born during child delivery. This affects the eyes of the child, giving rise to *ophthalmia neonatorum* (gonococcal conjunctivitis) (Fallon and Slack, 1992)

Due to the fact that up to 50% of the infected women are asymptomatic in the primary stage of the infection, the disease is discovered in women

almost always at the chronic and complicated stages. Perihepatic inflammation due to peritoneal spread of gonococci has been reported. Also, some auxotype of *Neisseria gonorrhoeae* spread gives rise to disseminated gonococcal infection characterised by painful joints, fever, septic skin lesion (rashes) on the patient extremities (Fallon and Slack, 1992)

The most usual diagnostic method of gonorrhoea is through microscopy and culture. Serodiagnosis is of less value since it is only after three to four weeks that the antibodies against *Neisseria gonorrhoeae* appear in the bloodstream (Zagrebina and Torchinov, 1989). Molecular diagnosis is also being used successfully (Ellen and Sydney, 1990).

Penicillin used to be the drug of choice against gonococcal infection in the 1960's, but the overuse of this drug has resulted in the development of penicillinase producing strains of *Neisseria gonorrhoeae.* Today the susceptibility of *Neisseria gonorrhoeae* isolates to commonly used antibiotics vary so much in times and places that, sensitivity testing remains essential (Cheesbrough, 1985; Whittington *et. al.*;1995).

Treponema pallidium

Treponema pallidium is a tightly coiled helical rod known to be a causative agent to syphilis. It belongs to the family of *Spirochaetaceae* and its infection is acquired by sexual contact with an infected individual. Congenital infection also occurs following transplacental transmission of the spirochaete from the infected mother to the foetus (Cheesbrough, 1985; Cockayne, 1992). The bacterium multiplies at the initial entry site and a painless chancre characteristic of the primary syphilis forms after an incubation period of three weeks. The chancre forms most frequently on the external genitalia but can also occur in the cervix (Zagrebina and Torchinov, 1989).

Untreated, syphilis may be a progressive disease with a secondary, latent and a tertiary stage. After the spontaneous healing of the chancre which marks the end of the primary syphilis, the spirochaetes rapidly enter the

lymphatics and is widely disseminated via the bloodstream, and may lodge in any organ. This is marked by the characteristic highly infectious rashes on the entire body (secondary syphilis). The tertiary syphilis which normally develops decades after the primary infection, is a slowly progressive, destructive inflammatory disease which can affect any organ. The most common forms observed are neurosyphilis, cardiovascular-syphilis and gummatous syphilis (Cockayne, 1992).

Treponemal infections can be diagnosed by direct microscopy of the chancre or skin lesions, but serodiagnosis is the common practice. The growth of *T. pallidium in vitro* is impossible (Cheesbrough, 1985).

A prolonged high dose therapy of procaine penicillin or benzylpenicillin has been the traditional treatment of syphilis. Individuals allergic to penicillin rely on erythromycin, tetracycline or chloramphenicol. These drugs have been demonstrated to fail sometimes and their side effects constitute a limitation for prolonged treatment (Cheesbrough, 1985; Cockayne, 1992).

Gardnerella vaginalis.

G. vaginalis is a Gram variable, anaerobic, non motile pleomorphic coccobacillary bacterium, known to exist as pathogenic and non pathogenic strains. Most infections are thought to be sexually transmitted. The vagina is the normal habitat where it causes an excessive vaginal discharge with "fishy odour", acting probably with other anaerobes such as *Bacteroids* and *Peptococcus* (Lossick 1984, Cheesbrough, 1985; Carey et. al.,1993). The inflammation process is rather rare, hence the term vaginosis is often applied in lieu of vaginitis. The common complaints are dyspareunia and a general daily discomfort. A chronic discomfort leads to bladder bacteraemia, neonatal sepsis and post partum bacteraemia (Cheesbrough 1985).

G. vaginalis is usually diagnosed microbiologically by observing the intra - epethelial cell coccobacilli (clue cells) from a fresh unstained smear of exudates (Gross,1992 ;Carey *et. al.*, 1993). *G. vaginalis* are sensitive to penicillin, vancomycin erythromycin, clindamycin and chloramphenicol. The

drug of choice for treatment is Metronidazole (Cheesbrough, 1985; Carey *et. al.*, 1993)

2.1.4 Non Specific Urogenital Infections

Non specific urogenital infections are caused by a variety of organisms whose normal habitat is not solely the urinary and genital organs, but can be found implicated in diseases of other organs. Species of high medical importance include *Chlamydia trichomatis, Mycoplasma hominis, Ureplasma urealyticum, Escherichia coli, Streptococcus pyogenes* and *Staphylococcus spp.* Also included are some opportunistic organisms such as *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella spp* (Lossick, 1984; Cheesbrough, 1985; Gross, 1992; Lainy *et. al.* 1993; Goullet *et.al.*,1994)

Non gonococcal urethritis in men is usually due to sexually transmitted *Chlamydia trachomatis, Ureplasma spp* and *Streptococcus pyogenes* (Cheesbrough, 1985; Slack, 1992; Smith, 1992). If untreated these organisms can cause prostitis or Epididymitis. Sterility caused by chlamydial infection is commonly due to urethreal stricture and Epididymitis (Smith, 1992). In women these organisms have been found implicated in acute non gonococcal endocervicitis and endometritis, in association with *Staphylococcus* spp (Zagrebina and Torchinov, 1989). Signs and symptoms of chlamydial and streptococcal infections in women are much less pronounced and 60% of women develop a chronic pelvic inflammatory disease (P.I.D.) (Slack, 1992; Smith, 1992). *M. hominis* infection is much common with women than men who often serve as carrier. In women the pathogen causes colpitis with purulent discharge (Lossick, 1984; Smith 1992).

When *E. coli, Streptococcus* Group B species, *S. aureus* find their way in the urogenital tract they cause cystitis, endometritis, and pelvic inflammation. *E. coli* is the commonest and its implication in pyelitis and pyelonephritis is attracting the more, the attention of many investigators (

Zagrebina and Torchinov, 1989; Lucas and Gary, 1993; Schalger *et.al.*, 1995; Johnson *et. al.* 1995).

K. pneumoniae, P. aeruginosa, Proteus spp and *S. enteritidis* have been isolated from patient with P.I.D and pyelonephritis (Cheesbrough, 1985; Slack, 1992; Lainy et. al. 1993; Johnson *et. al.*, 1995).

With the exception of *Chlamydia trichomatis* which is diagnosed only serologically or by microscopy, the diagnosis of non specific urogenital infections is mainly by culture and biochemical characterisation. The treatment is solely based on sensitivity screening. *Chlamydia spp* are sensitive to chloremphenicol, tetracycline, erythromycin and azithromycin (Smith, 1992; Gross, 1992; Welsh *et. al.*, 1996).

Viruses are also implicated in diseases of genital and urinary organs. Genital ulcer and warts in both sexes may be caused by Herpes Simplex Virus (HSV) and Human Papilloma Virus (HPV) respectively. These viruses are acquired by auto inoculation from lesion elsewhere on the body , but most often as a result of intimate sexual contact with an infected individual (Ward and Coleman, 1992; Ogilvie, 1992). In men the glans and the shaft of the penis are the frequent site of infection, while in women the labia, vagina and cervix may be involved.

HSV and HPV infections are easily recognised by the appearance of typical ulcer or lesion. However DNA probe hybridisation technique and serodiagnosis are the commonest methods of diagnosis (Ann *et. al.*, 1995; Russell *et. al.*, 1995).

In most cases warts disappear spontaneously but in cases of persistence often frequent in immuno-suppressed subjects, topical application of salicylic acid, podoplyllin or trichloro acetic acid is recommended (Ward and Coleman, 1992). Genital ulcer are treated with antiviral drugs such as vidarabine and acyclovir; when used early enough and at appropriate dose the therapeutic rate is satisfactory (Olgivie, 1992).

2.2 ORTHODOX APPROACH OF UROGENITAL INFECTIONS: PROBLEMS

In the orthodox approach of urogenital infections, the treatment, like in many other diseases is often applied after a precise diagnosis and antimicrobial sensitivity test (Cheesbrough, 1985; Slack, 1992). *In vivo* resistance to many drug has been reported; there is thus no evidence that a drug that kills or prevents the growth of a pathogen *in vitro*, will be a successful treatment to the patient (Slack, 1992; Greenwood, 1992). The selection of a sensitive drug involves considering the patient's clinical conditions, the site of infection, and any history of drug hypersensitivity (Stewart and Weir, 1992). The history of drug hypersensitivity is still difficult to apply in developing countries where patients and hospital find it difficult to keep medical records. The chosen drug may be too toxic and the side effects makes the patient under prolonged treatment to stop medication before the required time, without the consent of the physician.

The cost of prolonged treatment appears to be one of the causes of failure in treatment. Chronic infections arise often as a result of lack of means to treat the disease at the early stage. This also contributes to the propagation of the pathogen by the infected subject. Combination of drugs is often necessary to render faster and more effective the treatment and this constitutes an extra-load to the patient in terms of cost of treatment.

2.3 MEDICINAL PLANTS: HISTORY AND TERMINOLOGY

2.3.1 History.

The world of plants has been vital to mankind since intelligence began to glimmer. Apart from using plant as food, firewood, in constructing hut and making cloth, man has been able to sort out plants which can be used as poison and plants with medicinal value. One of the oldest records of such is the pharmacopoeia of Emperor Shen Nung which reveals the use of oil from *Chaullmoogra*, a species of *Hydnocarpus* in treating leprosy by Chinese as far back as 3000 BC (Le Strange, 1977). The same book describes the medicinal uses of Indian hemp and opium seed as anaesthetic or as narcotic. These plants are used for the same purpose today (Le Strange, 1977).

The possibilities for healing or harming with which the herbs are endowed touched man's essentially superstitious nature, and spurred him to start the collection and the study of these diverse plants. This formed the basis of botanical knowledge which at the early stages was not different from medicine (Le Strange, 1977; Hexley, 1977). Hippocrates, a distinguished Greek physician who lived in the 4th and 3rd century BC was the first to regard medicine as a science. Most of the remedies recorded in his *Material medica*, and which were used as purgatives, sudorifices, diuretics and remedies for internal infections were derived from plants. At his death he left at least 400 remedies to the world and he is known today as the father of medicine (Le Strange, 1977).

Theophratus, another famous biologist-botanist after Hippocrates (370 BC) produced a number of manuscripts, including the well known *Historia platarium*, in which he teaches how to extract gum myrrh and frankincense from plants (Sofowora,1993). Up till the 17th century in ancient Greece, the

study of medicinal plants was an adjunct of Medicine and Pharmacy, and medicinal plants were recorded in books known as Herbal (Hexley, 1977).

In China and other western pacific ocean regions, the Chinese traditional medicine has had several years of practical experiences. The unity between practitioners of traditional medicine and those of modern medicine, is one of the cardinal principles of health work in this part of the world (Sofowora, 1993). Schools and research institutes for traditional medicine have long been established and investigators from various parts of the world are interested in studying the marriage of traditional and modern medicine in China. In 1989 the World Health Organisation (WHO) published a compilation of herbs used as medicine in China.

In India and other south-east Asian regions, traditional medicine has been integrated into the official health care system, and formalised and indigenous training of the practitioners exist side by side. Kurup (1977) reported that university departments of traditional medicine and medicinal plants have existed in India for many years, and institutes carrying out multi-disciplinary research in medicinal plants abound throughout the region. Medicaments of plant origin are a common sight in drug stores (Kurup, 1977; Sofowora,1993).

In Europe and America, the use of concoction or decoction from plants has long been substituted by pharmaceutical products. In these regions, organised drug research Institutes undertake research on local medicinal plants and many drugs in use today are of plant origin. For example in USA 25% of all prescriptions available in community pharmacies from 1959 - 1973 were drugs extracted from plants (Principle. 1989).

2.3.2 Terminology

During its eighth general programme work meeting on traditional medicine and medicinal plant, the WHO (1991) redefined the terms up till then used in these disciplines.

A medicinal plant is any plant which one or more of its parts contains substances that can be used for therapeutic purpose or which are precursors for synthesis of useful drugs.

Vegetable drug is applied to any part of a medicinal plant (leaf, bark etc) used for therapeutic purpose.

A decoction is a preparation obtained by placing the plant material in cold water, bringing to boil by simmering for 15 to 60 minutes and then allowing the mixture to cool for a further 15 to 30 minutes.

An infusion is obtained by pouring boiling water on a specific quantity of dried plant material and allowing the mixture to stand for 10 to 30 minutes. Cold infusion is obtained by using cold water and allowing to infuse for 18 to 24 hours.

Maceration. This is prepared by placing the plant material in a liquid (water, oil, palm wine etc)and press with the hand or allow to soak for 2 to 7 days with constant stirring. The liquid extract is clarified by filtration.

2.4 STATE OF MEDICINAL PLANTS RESEARCH IN AFRICA.

During the pre-colonial period in Africa, the knowledge of medicinal plant was strictly a family affair. It was only in the 1930's that some African medicinal plants were recorded in books by the colonial masters (Aubreville, 1936 ;Daziel, 1937). Herbal medicine has for long been inaccessible in Africa and shrouded with a lot of mysticism. This explains why medicinal plant research in Africa is just germinating. The study of medicinal plants in Africa has taken a wider scale by the effort of Professor A. Sofowora (Nigeria) and

Professeur E. Adjanohoun (Benin Republic) who contributed remarkably for the compilation of the first African Pharmacopoeia (vol 1. 1985, vol 2. 1986), with the assistance of the Organisation of African Unity, through its Scientific Technical and Research Commission (OAU / STRC) and the *Agence de Cooperation Culturelle et Technique* (Paris) (ACCT) (Adjanohoun *et.al.*,1991, 1993). The African Pharmacopoeia specifies quality standards required for African plants to be used as raw material in drug industries. It also provides more than 100 monographes of African medicinal plants with special reference to the local names (Hausa, Peuhl, Swahili, Yoruba...), the description and habitat, and the local medicinal application (Sofowora 1993)

Still under the auspices of OAU/STRC and ACCT (Paris), the ethnobotanical survey of medicinal plants in twelve Francophone countries and West African Anglophone countries was carried out in 1988-1989 (Adjanohoun *et.al.*,1988,1993). The majority of the publications were on the phytochemical studies and bioassay research, toxicological and antimicrobial screening being less frequent.

However, the screening of African medicinal plants for their antimicrobial activity is a new trend in medicinal plants research in Africa. Susceptibility screening is now done on micro-organisms known to cause specific diseases, unlike general antimicrobial tests using common Gram positive and Gram negative organisms, as it was the case in the past. Examples are, the screening of aqueous extract of *Euphorbia hirta* for its anti-amoebic effects, so as to assess its traditional use against amoebic dysentery (Pousset, 1988); and the testing of antimicrobial properties of aqueous infusion and methanoic extract of the bark of the silk cotton tree against wound infecting microbes (Odama *et. al.*, 1986) .

Research in African medicinal plants has revealed itself important because : i) many of these plants can provide alternatives to imported drugs ; ii) they can serve as a starting point for drug development by pharmaceutical

industries; iii) from the plants in study new substances can be discovered that are useful against diseases for which suitable cures are not yet available. It is with regard to these reasons that the Nigerian government established the National Institute for Pharmaceutical Research and Development (NIPRD) - ABUJA. This step forward give a gleam of hope for the future of health care in Africa.

2.4.1. Examples of African Antimicrobial Plants

A number of African medicinal plants have been found to possess bioactive compounds endowed with anthelminthic, antifungal, antiparasitic, antibacterial antiviral properties (Ekong, 1986; Adesina, 1988).

Anthelminthic plants

The plant *Hagenia abyssinica* Moench, is known in Ethiopia and Kenya as taenicle, while *Mollugo hirta* has been used against hookworms and whipworms. The antihelminthic effect of the aqueous extracts from both plants have been demonstrated in vitro (Abegaz and Dagne ,1979; Abegaz and Tecle ,1980).

Boiled fresh leaves and stem of Abrus precatorius and fresh leaves of *Cassia alata* serve as anti - ascaris in Peuhl population of Adamaoua in Cameroon and Nigeria.

The most interesting example of use of the plant as an anthelminthic has been observed in Ghana and Mali where local herbs were being used in modern hospitals (Dwuma-Badu, 1983). The department of public health of Ghana Medical School, with the collaboration of The Centre for Research into Medical Plants (Mampong-Ghana) started using the root decoctions of *Mitragyna stipulsa* and *Combretum mucronatum* Leufl since 1976 to fight against an epidemic of guinea-worm in Ghanaian villages. Ampofo (1977) reported that within one week 88 patients were treated, with a total extrusion of the worm, and complete healing of the wound took place after topical application of palm oil for two weeks.

Antifungal plants.

The root bark of *Polygala nyikensis is* used in Malawi to treat skin problems of fungal origin. This activity is attributed to the high concentration of xanthones found in the root bark of this plant (Marston *et.al.*, 1993). In southern Cameroon the juice from the fresh leaves of *Cassia alata* is applied on ringworm and eczema. In 1988 Ikenebomeh and Mitiriri reported that extract from *Cassia* species was effective against *Trichophyton spp, Microsporum spp* and *Aspergillus spp*.

Antiparasitic drug from plants.

In addition to the antiamoebic effect of *Euphorbia hirta* reported by Pousset in 1988, several other antiparasitic plant remedies have been reported. For example the antiplasmodium and antitrypanosomal activities of *Azadirachta indica* have been demonstrated both in vitro and clinically Aladanmi *et.al.*, 1988). The antimalarial constituents of the plant were found to be gedunin and nimbolide (limonoid derivatives) (Khalid and Duddock 1989). In the same line, Modupe *et.al.* (1986) and Obineh and Makinde (1986) both reported that *Plasmodium berghei berghei* was responding to treatment with aqueous leaf extracts of Solarium erianthum and *Cymbopogum citratus* (lemon grass) in infected mice. Voncir *et.al.* (1986) worked on *Plasmodium falciparum* and revealed that aqueous extracts of *Acacia polycantha* was highly effective against the parasite.

Antibacterial plant remedies

As a result of research on antibacterial effects of plants, considerable information has surfaced into the world of chemotherapy. For instance, it has been proved that chewing sticks destroy bacteria present in the mouth, and thus prevent dental caries (Odebiyi and Sofowora, 1979). Babalola in 1988 screened the crude extracts of the sap from *Pycnanthus anglolensis* against *E. coli* and *P. aeruginosa* and reported that the effectiveness of the extract against these bacteria justifies the use of the plant in the traditional treatment

of diarrhoea. A recent investigation in the traditional treatment of gastro-enteritis by Ummu-Kulthame (1995) reveals that guava leaves used in the form of decoction is very effective against the four major gastro-enteritis causing bacteria, namely *Salmonella typhi, P. aeruginosa, E. Coli and Shigella spp.*

Antiviral plant remedies

Due to the cost of research in virology, African researchers have ignored the screening of African medicinal plants for their antiviral properties. Nevertheless researchers from overseas are developing bioassay techniques for the antiviral activities of African medicinal plants, and their findings are quite interesting. Over 120 plants with antiviral properties have been demonstrated so far and many of these plants are growing in Africa (Sofowora 1993). Examples are *Diospyros*, Spondias and *Terminalia* species. Some other plants such as *Aloe* and *Zingimbe*r species have been reported to have immunostimulating properties, which explains their uses in traditional treatment of some viral infection (Koumare, 1993).

A plant species, *Ancistrocladus korupensis (Ancistrocladaceae)* described not long ago from Cameroon was found to contain new alkaloids called *Michelamines A* and *B* possessing a wide spectrum of antiviral activity, including anti-HIV-cytopathic activity (Jato *et. al.* ,1993).

2.5 RELATIONSHIP BETWEEN HERBAL AND SYNTHETIC DRUGS.

Researchers on herbal remedies have provided scientific evidences supporting the use of herbs as medicine. The current objective of these researches is to isolate the active components and establish the chemical structure so as to develop synthetic drugs of the same effect. But the synthetic drugs still face the problem of being too pure for consumption. A good example of such is observed with two British drugs used in treating.

dropsy, *Prepared Digitalis* BP and *Digoxin* BP, both sold in the pharmacy (Sofowora 1993). *Prepared Digitalis* BP is a tablet made from powdered leaves of the plant Digitalis purpurea, while *Digoxin* BP is the pure active component, Digoxin, found in the leaf of this plant. *Digitalis* BP is used 1000 times less than *Prepared Digitalis* BP. Despite this low dosage, the side effects are more pronounced with the pure product as compared with the natural *Prepared Digitalis* BP.

In the mixture of plant preparation, some components potentiate the action of the active constituent, while some serve as demulcent, and others take care of side effects (Hasson 1991).

Examples of plant extracts made standard and sold in pharmacies abound in developed countries. In France a preparation of *Euphorbia hirta* known as "Socambe" is commercially available (Sofowora,1993). In Russia an immunostumulant drug called "Ukrain" extracted from the plant *Chemidonium majus* has been proved very good in the treatment of various carcinoma (Nowicky *et. al.* 1991). Also in Senegal, Pousset and co-workers developed an anticough syrup based on leaves of *Guinera senegalensis*, and a laxative capsule based on leaves of *Cassia italia* (Pousset *et. al.* 1986).

2.6 TRADITIONAL APPROACH UROGENITAL INFECTIONS : DIAGNOSIS AND TREATMENT.

Unlike in modern medicine where laboratory analysis and clinical signs constitute the basis of diagnosis, the diagnosis of urogenital infection by the traditional healer is solely based on symptoms. For example, the frequency and the colour of the urine, or the appearance of the discharge. In some cases some herbalists use divination to diagnose and even to know the type of herb to be used as medicine (Hasson, 1991).

The treatment is often made of decoction, maceration, and infusion to be taken for a particular period of time. The combination of herbs is also a

usual practice. The traditional treatments for many venereal diseases are well documented, and many of the documented plants have been proved effective in vitro. Some of the plants that have been found useful in the treatment of this condition are: *Cassia pertersiana (Caesalpiniaceae)* in which decoction of the root bark is used to treat syphilis (Msonthi 1982). Decoction of the root of *Cryptolepis saguinolenta (periplocaceae)*, that of the leaf and root of *Momordica Chananti (Cucurbitaceae)* and Maceration of the leaf of *Mateacarpus scabra (Rubiaceae)* are common traditional remedies for venereal candidiasis urinary disease and pelvic inflammatory disease (George and Pondali, 1949; Tunde, 1986; Cimaga *et.al.*; 1991 and Paulo *et.al.* 1993).

Decoctions from dried whole plants of *Dianthus superbus* (wild oats) coal ashes from silky hair of *Trachycarpus excelsa* (palm tree), or root bark of *Harrisona abyssinica (Simirabaceae)*, have been reported to be good treatment for gonorrhoea (Awosika 1992, Kokwaro ,1993).

2.7 SOME BIOACTIVE PLANT CONSTITUENTS AND THEIR PHARMACOLOGICAL APPLICATIONS.

Alkaloids

Alkaloids form a group of diverse alkaline compounds of vital physiological activity. Their bioactivity is attributed to their complex nitrogen containing ring. More than six thousand alkaloids have been identified. The common ones include, morphine which act as a pain killer, Quinine known to possess antimalarial activity, caffeine known to stimulate cardiac muscle and cocaine used as local anaesthetic (Le Strange, 1977; Sim, 1971 a ;Taylor 1965).

Glycosides

These are compounds complexed to one or more molecules of glucose. A number of glycosides have been found to possess antipyretic and anti-inflammatory activities, e.g. Salicin extracted from the bark of *Salixella spp*, known to act as an antipyretic. Another group, flavonol - glycosides or

flavonoids are used as diaphoretic, diuretic or immunostimulant (Sim, 1971b; Taylor 1965).

Saponins

Saponins are also a group of glycosidic compounds made up of steroid sapogenins and triterpinoid sapogenins. Stigmasterol is a steroid sapogenins which can be converted to human progesterone. b- sistosterol another steroid saponin is known to have anticholesteremic activity. Some saponins are known to be erythrolitic (Guta and Gard ,1966 ; Ribet and Charlot 1964).

Tannins

Tannins form a large group of complex substances found in plants. They are soluble in water and constitute the basic composition in the synthesis of tannic acid and used in coating soluble antigen on particulates such as latex or red blood cells (Stites and Channing, 1987). Tannins are also used as antiseptics, and they also possess the ability to protect proteins from proteolytic enzymes (Taylor, 1965; Trease and Evans, 1989).

Mucilages.

Mucilages are sulphonic acid esters generally considered as decomposition products of cellulose. Pharmacologically, they are used as a suspending medium or as a binding agent in tablets. Mucilages possess demulcent and emollient properties. Physiologically they reduce the coagulation time by acting on prothrombin metabolism. This explains its use in stopping the bleeding of wounds (Kerharo and Adams, 1974).

Volatile and Fixed oils.

Volatile oils from plants are of the family of terpenes and its derivatives. They serve as preservatives in some preparations or as flavouring agents. Terpenes have the ability to stimulate the gastric and the intestinal juice, thus acting as an appetiser. They are also used as carminatives and as astringent (Sofowora, 1993)

Fixed oils are generally used as solvents for intramuscular injections, they also act as a purgative and as an anthelminthic (Sofowora 1993, Okelola *et.al.*; 1986)

2.8 ECONOMIC IMPORTANCE OF PLANTS USED IN THIS STUDY.

2.8.1. *Abrus precatorius* Lin *(Palilonaceae)*

Common names: Indian Liquorice
Idom zakara (Hausa)
Ojuologba (Yoruba)

Traditional uses

Abrus precatorius is widely distributed in Nigeria where it is used in black magic and as abortifacient. In Northern Nigeria and Cameroon, the powder from the seeds is used as poison on arrows. In India, a decoction of its leaves and stem is taken orally by women in labour to facilitate child birth (Nwodo and Botting, 1983). The same preparation is taken against chest pain, cough and flu and in the form of eye drops for eye diseases (Nwodo, 1981; Winiger *et. al.*; 1986). In Côte d'Ivoire boiled fresh leaves and stem are taken as aphrodisiacs and as antihelminthes. The Yorubas in Nigeria use the same decoction in treating malaria (Nwude, 1986; Akubue, 1986)

In Cameroon and south-eastern Nigeria, a decoction of leaves mixed with two or three seeds is used in treating venereal diseases. It is believed two or three seeds swallowed with water can provide sterility for a period of one year to a woman. The seeds can also be used to provoke criminal abortion. The leaves soaked in water for at least three days is given to chickens to improve their fertility (Nwude,1986).

Bioactive constituents

Cool infusion of powdered seed of *A. precatorius* is very poisonous to man. The toxic principle is a glycoprotein called Abrin (Ghosal and Dutta

1971). Caiquo et. al. (1986) demonstrated that Abrin acts by inhibiting protein synthesis through inactivation of the 60s rRNA. Other constituents of the plant are Flavonoids, alkaloids, steroids and sweet components such as glycerhizin highly concentrated in the leaves (Genest *et.al.*, 1971).

2.8.2 *Borreria verticillata* Meyer *(Rubiaceae)*

Common names: Green Borreria, African Borreria.

Traditional uses

Borreria verticillata is a bushy shrub found along the West African coast where it is known as an abortive herb. The Yorubas of western Nigeria and Benin Republic used the paste obtained by pounding the fresh leaves in treating boil and skin diseases (Okelola *et.al.*, 1986;Sofowora,1982). In Senegal the root decoction is used as an antileprotic, antibilarzia, antiparalitic and as a diuretic in both traditional veterinary and human medicine (Sofowora,1993). In Gambia the macerate of the whole plant is used as laxative and as antipyretic for children. (Kokwaro, 1993).

Bioactive constituent and pharmacology .

Analysis of the plant reveal a presence of a volatile oil in its leaves, highly antimicrobial against a variety of Gram positive and Gram negative bacteria, including those involved in skin diseases (Tunde, 1980; Bona, 1986).

The whole plant of *B. verticillata* contains two major alkaloids called *borrevine* and *borrerivine* (Pousset *et. al.*, 1973; Maynart et. al.;1980) . Sesquiterpene has also been demonstrated (Saintly *et. al.*, 1981). Adewunmi (1984) reported that the volatile oil was active against Schistosoma haematobium at a concentration of 100 milligrammes per millilitre.

2.8.3. *Cassia alata.* Linn *(Caesalpinaceae)*

Traditional uses

Cassia alata known by Igbos as Umu ahia is a West African forest shrub growing all year round; it flowers during November and January (Dalziel, 1956). It is well known in south eastern Nigeria for its application in the treatment of skin diseases and as a purgative. Decoction of the leaf is used as a purgative while the juice from the pounded fresh leaves is topically applied on eczema, scabies and ringworm (Irvine, 1961,Bona, 1986). In China decoction of the leaf is used for dysentery and rheumatism (Ikenebomeh *et. al.*, 1988).

Bioactive constituent and pharmacology

Tunde studied the leaf composition and found out that the purging principle is an essential oil. He also found out that the leaves contain glycosides similar to those found in *Cassia acutifolia*, possessing diuretic and anticonvulsant activities.

By comparing the leaf composition of *C.alata* from Nigeria and *C.alata* from Ghana, Ayini (1986) discovered that *C.alata* from Nigeria contains more active ingredients and is thus a potential source of commercial drug if well developed. Abdul-Ghani (1986) revealed that *C. alata* is the source of two modern drug "Vitristin" and "Vinbrastin " used for skin diseases and as a purgative respectively.

2.8.4. *Euphorbia hirta (Euphorbiaceae)*

Common names : Asthma herb

Nonan kurchiya (Hausa).

Traditional uses

Euphorbia hirta is a small annual shrub widely spread in tropical countries. A good number of workers have reported its uses in both traditional and modern medicine. In Nigeria and many other West African countries, a decoction of the plant collected in the flowering and fruiting stages, is

indicated for asthma and other respiratory tract infections (Oliver, 1959; Kokwaro, 1993). In Madagascar and Australia it is prescribed for cough, chronic bronchitis and other pulmonary disorders (Wong-Ting-Fook, 1980). In Northern Nigeria and Cameroon, an infusion of dried whole plant is given to sheep, goats, cattle to increase lactation; also a decoction of the leaves is given to young girls for greater development of the breast (Hargreaves, 1979; Kokwaro, 1993). In Ghana, pounded fresh leaves mixed with water are used as enema for constipation, and also to treat sores and wounds (Sofowora, 1993). In Benue state (Nigeria) juice from fresh leaves is used as an antivenon.

In Angola the plant is widely used against diarrhoea and amoebic dysentery. At Conakry General Hospital , Guinea decoction of *E. hirta* is prescribed for amoebic dysentery (Sofowora, 1993). In Liberia and also in Cameroon, the latex from the fresh plant is used as an eye drop for conjunctivitis and ulcer of the cornea (Sofowora, 1993) . In 1986 Abo and co-workers reported that the stem and root of the plant have long been used for the treatment of cancer and warts.

A preparation of *E. hirta* is now available in France under the commercial name of "Socambe". The drug is said to be antispasmodic, antipyretic and amoebicidal (Sofowora, 1993).

Bioactive constituent

Phytochemical studies of *E. hirta* reveals the presence of gum resin, calcium oxalate crystal, sugar, mucilage and some volatile components. palmitic, oleic, linoleic and melisyl acids are also present in trace (Kerharo and Adams 1974, Sofowora, 1993). The stem and leaves have been found to be rich in saponins and flavonoids of the family quecetin and derivatives. Atallah and Nicholas (1972) reported the presence of alkaloids endowed with antitumor activity in the root and stem.

2.8.5. ***Jatropha curcas.*** **Linn** ***(Euphorbiaceae)***

Conmon names: purging nut

pourgiere (French)

Chini da Zugu (Hausa)

Traditional uses

Jatropha curcas is a sap abundant small tree, grown in different parts of West and Central Africa for its multiple domestic applications (Dalziel, 1956). Its multiple uses in traditional medicine has attracted a number of investigators interested in drug development. In Yoruba land *J. curcas* also known as "Iyalode" is believed to cause a nuisance to witches and wizards, thus its use to prepare sacrifice for those troubled by witches (Elewude, 1986). The Yorubas also use the dried dropped leaves to prepare concoction for children who sweat too much in the night. The child is bathed with the concoction before going to bed. The leaves are also known to be efficient medicine for "kolobo" (black tongue). Individuals suffering from infertility due to return of sperm, drink leaf infusion (Elewude, 1986).

A decoction of the root bark and stem bark mixed with corn flour is indicated against dysentery in Northern Nigeria. The young leaf infusion is drunk for gonorrhoea (Dalziel, 1956; Irvine, 1961). The sap of *J. curcas* is applied on wounds or cuts to stop bleeding. In Côte d'Ivoire , the greenish juice from pounded fresh leaves is applied on carious teeth (Kerharo and Banquet, 1972; Sofowora, 1986). Leaf decoction mixed with lime is drunk against jaundice and intestinal worms.

In Cameroon the Bakweri people use the leaves decoction mixed with beer as diuretic and for rheumatism (Dalziel 1937, Irvine, 1961). The root decoction is used in treating smallpox by bathing. The purging oil containing seed, crushed and mixed with cereal food is indicated for syphilis. The seed oil is also recommended in the treatment of guinea worm (Kerharo and Bouquet, 1950)

Bioactive constituents

Phytochemical study of the plant showed that the sap is rich in tannins, alkaloids, steroids, saponins and triterpenes. The seed is rich in mucilage while the leaf, roof and stem bark have been proved to contain wax and little mucilage (Naengchomnong *et.al.*, 1986; Sinha *et. al.*, 1970)

2.8.6 ***Khaya senegalensis.*** **A Juss** ***(Meliaceae).***

Common names: Dry zone mahogany
Acajou du Senegal (French)
Ma'dachi (Hausa).

Traditional uses

Khaya senegalensis is a savannah tree cultivated in southern Nigeria for its medicinal uses. The stem bark is widely used along the West African coast as febrifuge and as anticonvulsant (Adesina,1983; Adegbola et. al. 1992). In Zaria and environs a decoction of young leaf and/or stem bark is used as douche for vaginal irritation. The stem bark is also used in treatment of diarrhoea, typhoid fever, dysentery, as vermifuge and taenia infestation, and in treatment of syphilis (Olayinka, 1992).

Bioactive constituents and pharmacology

Phytochemical analysis of the plant confirm the presence of anticonvulsant and febrifuge constituent. Extracts from leaf and stem bark possess analgesic, antimicrobial and cardiovascular effects (Olayinka, 1992). Chemical characterisation reveals the presence of glycosides, saponins, tannins terpines and a variety of steroids in the stem, bark, and leaf. The bitter principles of the stem bark were found to be limonoids (Adegbola *et. al.*,1992; Adesina, 1983)

2.8.7. ***Parka biglobosa*** **Benth (*Mimosaceae*)**

Common names: African locust bean

Mimosa poupre (French)

Daudawa (Hausa)

Traditional uses

Parkia biglobosa is a West African savannah tree also found cultivated in tropical America and the West India for its domestic uses. The fruit pulp and pounded seed is used in northern Nigeria as food seasoner ("Daudawa"), While in Sudan and also in Côte d'Ivoire the yellowish pulp is used in preparing fermented liquor (Irvine, 1961).

Decoction of the stem bark of *P. biglobosa* is used to cool down fever by bathing or when hot to steam the mouth and throat for cough and toothache. In Côte d'Ivoire pulp bark mixed with lemon juice is often used for wounds and ulcers (Dalziel, 1956).

In Europe the pulp from the fruit was used as a dietetic flour or as diuretic. It is also prepared with honey as a refreshing emollient drunk by children or patient with fever (Dalziel 1956).

West African herbalists use the plant with 32 other ingredients to prepare the remedy for leprosy (Kerharo and Bouquet, 1950). In Hausa land the decoction of the bark is used for stomach-ache while in Gambia the leaf and root-bark are used in preparing remedy for conjunctivitis. The young fruits are lightly roasted, crushed and applied on burns. Unripe fruits cause severe colic if eaten (Irvine, 1961).

2.8.8. ***Securidaca longipedunculata* Fres *(Polygalaceae)***

Common names: Sanya or uwar magunguna (Hausa)

Traditional uses

Securidaca longipedunculata is a shrub of 2 to 3 metres widely spread in the tropics. In northern Nigeria it is known as an effective cure for venereal diseases and is sold by local herbalists. Msonthi (1986) reported that in Malawi the decoction of leaf is used in the treatment of cough, wounds, gonorrhoea, and bilarzia; while root and stem are boiled and drunk as remedy for rheumatism.

In Hausa land the plant is called "mother of medicine"; the powdered root is used as snuff and it causes violent sneezing. It is also used in small doses as a purgative. Root powder is a dangerous poison if used in large quantity (Irvine, 1961). Infusion of the dry root, leaf and seed is used in southern Rhodesia in treating malaria and in Senegal for sleeping sickness and leprosy (Irvine,1961).

In various regions of French West African countries the root or leaf mixed with fruit are used as taenifuge, vermifuge or with incision and ligature for snake bite. The steam from boiling leaf is also inhaled for snake bite. Decoction of leaf and fruit causes violent vomiting and purging. Dalziel (1956) reported that wearing part of the root is believed to keep away snakes. The sap can blind the eyes (Dalziel, 1956).

The root tips are inserted in the nostrils for catarrh, and also the irritating sap is believed to restore consciousness after fainting (Irvine 1961).

Bioactive constituents

The main constituents are oleanolic acid, saponins, tannins and methyl salicylate (Msonthi, 1986). *S. longipedunculata* saponins cause severe damage to the bone marrow and if in contact with blood act like certain poisons by producing haemolysis (Irvine, 1961).

Chapter 3
MATERIALS AND METHODS

3.1. COLLECTION OF PLANTS

The plant materials screened in this study were collected in October and November from Samaru village (Zaria) and environs; their identification was done by the Department of Biological Sciences Herbarium, Ahmadu Bello University, Zaria. They include the stem, leaves and seeds of *Abrus precatorius*, the stem bark and leaves of *Khaya senegalensis* and *Jatropha curcas*, the leaves mixed with fruit of *Securidaca longipedunculata*, the stem bark of *Parkia biglobosa*, the leaves of *Cassia alata* and the whole plant of *Borreria verticillata* and *Euphorbia hirta* (colour plates 1 to 8).

The relevant plant parts (seed of *A. precatorius* exclusive) were dried in an oven at 45°C- 50°C until constant weight was attained (Abdul-Ghani, 1990). *A. precatorius* seeds were harvested when dried. The plant material was pounded with mortar and pistle and further ground to powdery consistency using a blender mill (Okelola, 1986) and the powdered material was stored in a clean plolythene bag in cool and dry place.

3.2 EXTRACTS PREPARATION

Aqueous Extracts

Fifty grammes of each powdered plant material were mixed with 500mL of distilled water in 1000mL conical flask and boiled by simmering for 30 minutes. When cool to room temperature each preparation was sieved through a four-layer cotton fabric absorbent gauze (from Heml Pharmaceutical C.M.B.H.-Hambug) and the filtrate was allowed to stand for at least two hour after which the supernatant was filtered through Whatman

filter paper N°1 (from W&R Balston Ltd England) (Ummu-Kulthane, 1995). The decoctions (extracts) obtained were used immediately or stored at 4°C.

The concentration of extract was determined as described in Appendix II.

Alcohol Extracts

Twenty five grammes of each powdered plant material was extracted with 300ml of methanol for 24 to 36 hours employing a Soxhlet extractor. The solution of extract obtained was allowed to evaporate till dryness at 40°c and the dry matter was harvested and stored in powdered form (Akinyanju *et. al.*, 1986).

3.3 TEST ORGANISMS AND CULTURE MEDIA

The test organisms used were, *Neisseria gonorrhoeae, Streptococcus pyogenes, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Trichomonas gallinae.*

T. gallinae was isolated in the Department of Microbiology Ahmadu Bello University (ABU), Zaria, from the oral mucosa of an infected pigeon, using a sterile swab and Modified *Trichomonas* Medium (TM) broth from Oxoid (Appendix I). The protozoan was maintained in TM broth at room temperature and was subcultured on fresh broth every 48 hours (Feinberg and Whittington, 1957).

N. gonorrhoeae used was a clinical isolate obtained from the Department of Medical Microbiology ABU-Teaching Hospital (ABUTH), Zaria. The organism was maintained on Chocolate Agar (CA) plate at 37°C in candle jar, and was subcultured every 48 hours (Cheesbrough, 1985). The C.A was prepared from Azide Blood Base Agar (Oxoid).

S. aureus, E. coli, S. typhi, K. pneumoniae and P. aeruginosa were clinical isolates obtained from the Department of Medical Microbiology

ABUTH, Zaria. They were maintained on Nutrient Agar (Biotec) slant at 4°C (Cheesbrough, 1985) and were subcultured on Muller Hinton Broth (MHB) 24 hours before used for antimicrobial screening.

Candida albicans was also obtained from ABUTH, Zaria and was maintained on Potato Dextrose Agar slant (Appendix I) at 4°C. Inoculum was prepared from a 24 hours Potato Dextrose Broth culture (Glenn and Roberts, 1990; Ellen and Sydney, 1990).

S. Pyogenes was obtained from the Department of Microbiology A.B.U. and was maintained at 4°C on CA slant (Cheesbrough, 1985). The organism was subcultured on sterile MHB 24 hours before use.

3.4 PREPARATION OF STANDARD INOCULA FOR SUSCEPTIBILITY TESTING.

For *Trichomonas spp*, 0.1mL of a 24 hours broth culture was diluted with 0.1mL of methanol in order to inhibit the busy moment of trichomonads. Using an haemocytometer the concentration of the cell-suspension of the 24 hours broth culture was determined from the portion mixed with methanol (Ray *et. al.*., 1982), following the standard formula $C=(2 \times count \times 4)/10^{-6}$: 10^{-6} = the volume of the counting chamber in mL, 4=is the number of compartments of the chamber, the total is multiply by 2 because the portion used was diluted twice with methanol, count = the number of cells counted in one compartment, and C = the cell suspension in cell/mL. This concentration was adjusted to 1.6×10^6 cell/mL using TM broth as diluent and used as Inoculum (Tsutomut *et. al.*, 1985).

For other organisms (*N. gonorrhoeae* exclusive) a turbidity standard of Mcfairland N°3 was prepared from a 24 hours broth culture; a10 fold serial dilution of this preparation was carried out by transferring 1mL of broth in 9ml. of sterile normal saline to obtain eight different dilutions. For *N.gonorrhoeae*, the 10 fold dilution was done on a preparation obtained by

suspending a single colony from a 24 hours Chocolate Agar culture in 10mL of sterile normal saline. One hundred microlitres (0.1mL)of each of the various dilution was plated on the corresponding culture medium and incubated at 37°C for 48 hours (in candle jar for *N. gonorrhoeae* and *S. pyogenes*, and aerobically for the rest of micro-organisms). The dilution that yielded a semicofluent growth was selected as standard inoculum (Cheesbrough, 1985). To evaluate the cell suspension of the standard inoculum, the colony count of the plate in the series with an evenly distributed growth and isolated colonies was selected. Following the standard formula

$C=(count \times 10^n)/0.1$ the cell suspension of the selected standard inoculum was determined (Cheesbrough, 1985). C=cell suspension in cell/mL, count = the number of colonies counted and n= the number of dilution steps from the standard inoculum to the dilution that yielded the evenly distributed and isolated colonies (of the plate counted). The various cell suspensions (in cell/mL)obtained for each standard inoculum were: *N.gonorrhoeae* ($1.4x10^5$), *S. pyogenes* ($1.2x10^5$), *S. aureus* ($1.288x10^5$), *S. typhi* ($1.155x10^5$), *E. coli* ($1.12x10^5$).*K. pneumoniae* ($9.8x10^4$), *P. aeruginosa* ($9.4x10^4$), and *C. albicans* ($1.02x10^5$)

3.5 ANTIMICROBIAL SCREENING OF AQUEOUS EXTRACTS

3.5.1 Preliminary screening.

Agar dilution method was used (Cheesbrough, 1985; Odama *et.al.*,1986); the antimicrobial activity of the different extracts was evaluated by comparing the growth of test organism on medium incorporated with the plant extract , with growth on extract-free medium (control)

Test on *Trichomonas*

A screw cap tube was filled with 5mL of extract and was allowed to evaporate at 40°C till dryness (Akinyanju *et. al.*, 1986); the tube with the dry mater was sterilised by autoclaving, and when cool to room temperature was filled aseptically with 5mL of sterile Modified T.M broth (prepared as described by the manufacturer). The tube was inoculated with 0.1mL of the standard inoculum (i.e. $1.6x10^5$ viable Trichomonad cells. This was incubated at 35°C for 42 to 48 hours. Extract-free broth was also prepared inoculated and incubated in the same condition to serve as control. Susceptibility was determined as a complete absence of motile cells on wet mount preparation under the microscope (Krieger *et. al.*., 1982; Meingas *et. al.*., 1982; Lossick *et. al.*., 1986)

Test on *N. gonorrhoeae* and *S. pyogenes*

Three grammes of Azide Blood base Agar (Oxoid) was weighed into a flask containing 100mL of extract. This was dissolved and autoclaved, and when cool to 45°C to 50°C, 5mL of sterile blood was added and heated to obtain the chocolate agar (Cheesbrough, 1985; Ummu-Kulthane,1995). This was dispensed in sterile petri dishes and allowed to set. Extract free C.A was prepared the same way. Both the control (extract-free) and extract containing plates were inoculated with 0.1mL of the standard inoculum and spread evenly with a sterile bent glass rod. The plate were incubated in candle jar

(Cheesbrough, 1985) at 37°C for 48 hours. The susceptibility was determined as total absence of growth after incubation.

Test on *C. albicans*

Dehydrated potato extract obtained from 50mL of aqueous potato extract prepared using Difco formula (Appendix I) was dissolved with 50mL of plant extract. 1g of Dextrose and 0.75g of Agar N°3 (both Difco products) were added to the preparation and sterilised by autoclaving. This constituted the extract containing medium (Odama *et. al.*, 1986). Extract-free Potato Dextrose Agar (PDA) was prepared the same way using distilled water in lieu of plant extract. Extract- free and extract containing medium were dispensed in sterile petri-dishes and allowed to set. The plates were inoculated with 0.1ml standard inoculum and spread evenly. The plates were incubated aerobically at 37°C for 42 to 46 hours (Cheesbrough, 1985).

The susceptibility was determined as prescribed above. The experiment was repeated with higher extract concentration for all the plate with low inhibition, i.e. plate showing one quater growth compared to the control plate.

Test on *S. aureus, E. coli, S. typhi, K. pneumoniae* and *P. aeruginosa*

The susceptibility test of plant extract on these organisms was done on Mueller Hinton Agar (Oxoid).prepared as described by the manufacturer for control plates or using plant extract in lieu of distilled water for extract containing medium. The inoculation,.incubation and susceptibility determinationwas done as described for *C. albicans*.

3.5.2 Determination of Minimum Inhibitory Concentation (MIC).

Due to the fact that all the water extracts were densely coloured. Agar plate dilution method was prefered to the tube dilution method for MIC determination. The determination of the MIC for each extract which showed an inhibitory activity in the preliminary screening was done as described by Cheesbrough (1985) and Ummu-Kulthane (1995).

Six progressively decreasing concentrations of crude extract (i.e 75%, 50%, 25%, 12.5%, 6.25% and 3.125% v/v) were prepared using distilled water as diluent. Each concentration was used to prepare extract containing agar plates (or extract containing T.M. broth for *Trichomonas* (Meindas *et.al.*, 1982)) as described in section 3.5.1. above. Each concentration was challenged with 0.1ml of standard inoculum and incubated accordingly (section 3.5.1 above) for 36 to 48 hours. The MIC was considered to be the lowest extract in which growth of test organism was totally inhibited. Extract free medium was always prepared to serve as positive control.

3.5.3. Determination of Minimum Microbicidal Concentration (MMC).

Trichomonas Gallinae

Extract containing T.M. broth was prepared in tubes at the various dilutions indicated in section 3.5.2 above. Each of the tubes was inoculated with 0.1mL of standard inoculum of *Trichomonas* and incubated at 35°C for 24 hours. 0.5mL of broth from each of the tubes in which the growth of *Trichomonas* has been inhibited after incubation (as shown by the wet mount preparation under the microscope), was transfered into 4.5mL of sterile extract-free T.M. broth. This was further incubated at 35°C for 42 to 48 hours (Krieger *et.al.*, 1982) The Minimum Trichomonocidal Concentration (M.T.C.) was taken as the least concentration of extract which showed no growth of *Trichomonas* after subculture.

N. gonorrhoeae* and *S. pyogenes

Mueller Hinton Broth was used for *S. pyogenes* while a modified form of Mueller Hilton broth was used for *N. gonorrhoeae*. The modification consisted of an addition of 0.1% w/v of sodium thioglycollate in order to reduce the oxygen tension (Cheesbrough, 1985) and addition of 5% v/v sterile blood (Collins and Lynes, 1995).

The different extract concentrations inhibitory to these organisms (as obtained in the MIC determination result, i.e from the neat concentration to MIC) were used to prepare the extract containing broth. The extract containing broths were dispensed as 6mL aliquots in 7.5mL bijou bottles, each bottle was inoculated with 0.1mL of standard inoculum. Extract-free broth (control was also prepared and inoculated the same way). The bottles were tighly capped and incubated for 24 hours after which 0.1mL from each bottle was subcultured on extract free chocolate agar plate. The plates were inoculated at 37°C in cadle jar for 42 to 48 hours and checked for growth (Cheesbrough, 1985; Collins and Lynes,1995). The Minimum bactericidal Concentration (MMC) was considered to be the lowest concentrationwhich showed no growth after subculture.

C. albicans*, *S.aureus*, *K. pneumoniae*, *P aeruginosa* and *S. typhi

Potato Dextrose broth (Appendix I) was used for *C. albicans* while Mueller hinton broth (Oxoid) was used for the bacteria. Extract-containing broth at various concentrations (or dilutions) inhibitory to these organisms (i.e starting from the neat concentration to the MIC) were dispensed in 10mL aliquot in bijou bottles. Each botlle was inoculated with 0.1mL of the standard inoculum and incubated at 37°C for 24 hours, after which 0.1mL from each bottle was plated on the coresponding extract-free agar plate (PDA for Candida and MHA for the bacteria) . The plates were incubated for 42 to 48 hous and checked for growth; the MMC was taken to be the lowest concentration of extract which showed no growth after subculture from tubes to plates (Cheesbrough, 1985).

3.6 ANTIMICROBIAL SCREENING OF METHANOLIC EXTRACTS.

Considering the fact that some active components relatively polar or organic may be present in the plant drugs (Abdul-Ghani, 1990), the antimicrobial screening of methanolic extracts of the various plant parts used were also done. For this exercise the tube method as described by Collins and Lynes (1995) was used. 4g of powdery methanol extract was disolved in 5mL of Dimethylsulphoxide (DMSO) then in 45mL of normal saline (or inactivated cow serum for *Trichomonas*). This was serially diluted to obtain 2 fold decreasing concentration ranging from 80mg/mL to 1.25mg/mL and using normal saline (or inactivated cow serum) as diluent. The various extract concentrations were sterilised by membrane filtration (0.45 pore size), and dispensed in duplicate, 1mL aliquot (3mL for *N.gonorrhoeae*) in sterile 7.5mL test tubes. to each of the tube were added 1mL (or 3mL for *N.gonorrhoeae*) of sterile double strength broth (T.M. broth fo *Trichomonas* , modified MHB for *Netisseria* and *Streptococcus*, P.D.B for *Candida* and MHB for other bacteria). For each concentration one of the tube was inoculated while the other duplicate serves as negative control. A positive control extract- free broth incorporated with 6.25% DMSO (1.25mL in 20mL of broth) was also prepared and inoculated with 0.1mL of standard inoculum. After 24 hours incubation the cultures were checked for turbidity, change of colour (or haemolysis for *Streptococcus*) and compared with the negative control. Wet mount preparation of tubes inoculated with *Tricomonas* was made to confirm absence of growth. The method constituted at the same time the preliminary screening as well as MIC determination. The MIC was considered to be the lowest concentration that did not produce turbidity or change of colour as compared with the negative control. Extracts not inhibitory to the targeted test organisms at the highest concentration (i.e. 40mg/mL) were considered as non-effective.

One hundred microlitres (0.1mL) from all the tubes that showed no growth was subcultured on the correspondig extract free agar plate for determination of MMC as described for aqueous extract in section 3.5.3.

3.7. TOXICITY ASSAY ON BRINE SHRIMPS

3.7.1 Preparation of shrimps

For this exercise, a small plastic tank (Appendix III) made from a soap case of dimension 8cm x 12cm x 3.5cm, provided with a divider and a cover was used. (Amupitan, 1996. Personal communication). In one of the sides of the divided tank filled with natural sea water (from the Atlantic Ocean) were added *Artemia salana*'s eggs. The egg containing the compartment of the tank was covered with aluminium foil, and the other side was illuminated with a table lamp. The preparation was allowed to stay for 48 hours at room temperature for the shrimps to hatch and mature (Heyer *et.al.*, 1982; Solis *et.al.*, 1993). When matured the nauplii (small shrimps) were attracted to the illuminated side of the tank where they were easily harvested with the aid of a Pasteur pipette.

3.7.1 Toxicity screening

With the assumption that non water soluble components present in alcohol extract may be toxic and due to the fact that the waterextract from the various plant parts and studied are locally taken without serious danger in terms of toxidity; only the methanolic extracts were screened for toxidity.

A solution of 1000 µg/mL of extract was prepared by dissolving 20mg of powdered methanol extract of plant and material in 0.5mL of D.M.S.O, then in 19.5mL of sea water. From this solution,five serial double dilution , i.e. 1000, 500, 250, 125 and 62.5µg ml, were prepared using sea water as a diluent (Fatope, 1990; Solis *et. al.*,1993). 5mL of each concentration was distributed in triplicates in test tubes. Three control tubes were also prepared using solely sea water and D.M.S.O.

Into each of the tubes 10 shrimps were added (control tube inclusive). After 24 hours of incubation at room temperature,the number of surviving shrimps were recorded. The data was analysed with Finney computer program to calculate the LC_{50} values (Lethal Concentration for 50% of the shrimps) (Fatope, 1990; Solis *et. al.*, 1993).

COLOUR PLATES



PLATE 1a : *Abrus precatorius*

Leaves and the climbing stem



PLATE 1b : *Abrus precatorius* seeds (beans)



PLATE 2 : *Borreria verticillata*



PLATE 3 : *Cassia alata*



PLATE 4 : *Euphorbia hirta*



PLATE 5 : *Jatropha curcas*



PLATE 6a : *Khaya senegalenses* (leaves and fruit)



PLATE 6b : Khaya senegalenses (whole plant)



PLATE 7 : *Parkia biglobosa*



PLATE 8 : *Securidaca longipedunculata*

Chapter 4

RESULTS

4.1 RESULTS OF THE PRELIMINARY SCREENING OF AQUEOUS EXTRACTS.

The preliminary antimicrobial screening of the various aqueous extracts was expressed as no inhibition (+ + +) for cases where the growth was apparently more than one quater of that of the control, low inhibition (+) for growth equal or less than one quater of that of the control, and complete inhibition (-) for cases where there was no growth at all (Table 1) . The case of low inhibition was observed only with the extract from the mixture of leaf and fruit of *S. longipedunculata* on *K. pneumoniae*, *E. coli* and *C. albicans*. The repeated tests with the higher concentrations (above 28mg/mL) showed a total inhibition of *E. coli* and *C. albicans*, while at 112mg/mL (Table 1) this extract still failed to inhibit the growth of *K. pneumonae*. However at the initial neat concentration used (28mg/ml) *S. longipedunculata* extract was inhibitory *to N. gonorrhoeae*, *S. aureus*, *S. typhi* and *P. aeruginosa*.

From a total of 12 extracts screened, only the stem bark extract of J. curcas did not show antimicrobial activity against any of the test organisms (Table 1). Leaf extract of *A. precatorius* was inhibitory only to S. aureus while the seed of *A. precatorius* and the leaf of *K. senegalensis* where each inhibitory to three out of nine test organisms. In contrast, extract of the stem bark of *P. biglobosa* was inhibitory to all the nine test organisms. Only *C. albicans* was resistant to the antimicrobial effect of *K. senegalensis* stem bark extract, while *C. albicans* and *Trichomonas* spp were resistant to the stem extract of *A. precatorius*. *B. verticillata*, *E. hirta* and *J. curcas* leaf extracts were all effective against 5 test organisms, and

Table 1 : RESULT OF THE PRELIMINARYSCREENING OF AQUEOUS EXTRACTS

TEST ORGANISMS	Highest conc.	*Neisseria*	*Streptococcus*	*Staphylococcus*	*Salmonella*	*Klebsiella*	*Escherichia*	*Pseudomonas*	*Candida*	*Trichomonas*
	mg/mL									
PLANT EXTRACT										
Abrus precatorius, stem	22.22	—	—	—	—	—	—	—	+++	+++
Abrus precatorius, seed	20.3	+++	—	—	+++	+++	+++	—	+++	+++
Abrus precatorius, leaf	24	+++	+++	—	+++	+++	+++	+++	+++	+++
Borreria verticillata,	24	+++	+++	—	—	—	—	—	+++	+++
Cassia alata , leaf	30	+++	—	—	—	+++	+++	—	+++	+++
Euphorbia hirta	24	+++	+++	—	—	—	—	—	+++	+++
Jatropha curcas, Bark	32	+++	+++	+++	+++	+++	+++	+++	+++	+++
Jatropha curcas, leaf	28	+++	+++	—	—	—	—	—	+++	+++
Khaya senegalensis, bark	30	—	—	—	—	—	—	—	+++	—
Khaya senegalensis, leaf	25	+++	+++	—	—	+++	+++	+++	+++	+++
Parkia biglobosa, Bark	34	—	—	—	—	—	—	—	—	—
Securidaca longepedunculata	112	—	+++	—	—	+	—	—	+++	+++
Control		+++	+++	+++	+++	+++	+++	+++	+++	+++

KEY: — = Complete inhibition

\+ = Low inhibition (scanty growth)

\+++ = No inhibition(abundant growth)

the leaf extract of *C. alata* was inhibitory to four out of nine pathogens used.

S. aureus was the most sensitive organism followed by *Pseudomonas* and *Salmonella*, all sensitive to 11, 10, and 9 plant extracts respectively. On the other hand, the most resistant organisms to the various water extracts were found to be *C. albicans* and *Trichomonas gallinae* which were sensitive only to two plant extracts each (Table 1). *N. gonorrhoeae* was the most resistant among the bacteria because it appeared sensitive to only 4 out of 12 extracts tested (Table 1).

4.2 MINIMUM INHIBITORY CONCENTRATIONS OF AQUEOUS EXTRACTS.

It was noted that the sensitivity of *S. pyogenes* to *K. senegalensis* stem bark and *C. alata* leaf, and that of *C. albicans* to *P. biglobosa* stem bark were limited only to the non-diluted extract (neat starting concentration) (Table 2). The values obtained from the screening of *E. coli* and *C. albicans* with higher concentrations of *S. longipedunculata* leaf plus fruit extract were considered as the MIC of this extract to the test organisms. *S. aureus, Pseudomonas* and *Salmonella* were the most sensitive test organisms to the various extracts with MIC values lower than 15mg/mL (Table 2).

The majority of MIC values (65%) obtained were below 10mg/mL and only 8 cases of MIC were above 15mg/mL. The highest MIC value was obtained with *S. longipedunculata* on *C. albicans* (56mg/mL) and the smallest with the stem bark extract of *K. senegalensis* on *S. aureus*. The stem bark extracts of *P. biglobosa*, and *K. senegalensis* as well as the stem extract of *A. precatorius* exhibited a characteristic broad spectrum antimicrobial activity (Table 2). *Klebsiella* and *E. coli* presented almost a similar sensitivity pattern to the various aqueous extracts (Table 2)

4.3 MINIMUM MICROBICIDAL CONCENTRATIONS OF AQUEOUS EXTRACTS.

A good number of extracts failed to kill the micro-organisms tested despite the inhibitory activity (cases indicated with "A" in tables); fourteen such cases were recorded. *K. senegalensis* stem bark extract and the stem extract of *A. precatorius* (two of the broad spectrum inhibitory extracts) were thus microbicidal to 6 pathogens each.(Table 3). The leaf extract of *J. curcas* appeared bactericidal only to *S. aureus* and the leaf extract of *A. precatorius* failed to kill *S. aureus*, the only pathogen which was inhibited by this extract (Table 3). *A. precatorius* leaf extract was thus the least potent extract among the effective ones.

The MIC and MMC values were the same for some extracts (Table 2 and 3) but in most cases the MMC was at least twice as high as the MIC In 16 cases, the MIC values were less than 10mg/mL and 7 of these 16 cases were recorded with *P. biglobosa* stem bark extract on the various bacteria screened. MMC values equivalent to the highest extract concentration used were recorded in the cases of *C. alata* leaf on *Pseudomonas*, *K. senegalensis* stem bark on *Klebsiella* and *P. biglobosa* stem bark on *C. albicans*. The least MMC value was obtained with *K. senegalensis* stem bark extract on *S. aureus*. This particular organism was the most sensitive to the various extracts screened, followed by *Salmonella* and *Pseudomonas*.

The most resistant pathogens to the plant extracts were *C. albicans* which was sensitive only to the stem bark extract of *K. senegalensis*, and *Trichomonas spp* which was sensitive to *K. senegalensis* and *P. biglobosa* stem bark extracts (Table 3). However *P. biglobosa* stem bark, *K. senegalensis* stem bark, *A. precatorius* stem and *B. verticillata* whole

Table 2 : MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF VARIOUS AQUEOUS EXTRACTS ON TEST ORGANISMS (in mg/mL)

TEST ORGANISM	*Neisseria*	*Streptococcus*	Staphylococcus	*Salmonella*	*Klebsiella*	*Escherichia*	*Pseudomonas*	*Candida*	*Trichomonas*
PLANT EXTRACT									
A. precatorius, stem	5.55	5.55	2.78	11.11	11.11	5.55	5.55	ND	ND
A. precatorius, seed	ND	2.54	10.15	ND	ND	ND	2.54	ND	ND
A. precatorius, leaf	ND	ND	12	ND	ND	ND	ND	ND	ND
B. verticillata, whole plant	ND	ND	0.75	0.75	6	24	6	ND	ND
C. alata leaf	ND	30	7.5	3.75	ND	ND	7.5	ND	ND
E. hirta, whole plant	ND	ND	3	6	12	12	6	ND	ND
J. curcas bark	ND	ND	ND	ND	ND	ND	ND	ND	ND
J. curcas, leaf	ND	ND	7	14	7	14	14	ND	ND
K. senegalensis, bark	15	30	0.23	0.47	3.75	3.75	1.8	ND	22.5
K.senegalensis, leaf	ND	ND	6.25	12.5	ND	ND	12.5	ND	ND
P. biglobosa, bark	4.32	2.15	0.53	2.15	2.15	1.08	1.08	34.6	17.3
S. longipeduniclata	3.5	ND	7	3.5	ND	56	3.5	56	ND
Control	+++	+++	+++	+++	+++	+++	+++	+++	+++

KEY : ND = MIC value not determined in these cases

+++ = Perfect growth (Control)

plant extracts maintained a broad spectrum of microbiacidal activity with effect on at least 5 of the 9 organisms tested (Table 3).

4.4 RESULTS OF THE SCREENING OF ALCOHOL EXTRACTS

4.4.1. Preliminary screening and MIC determination

The results of the preliminary screening and the MIC values are presented in Table 4. These values were determined from the same experimental set up.

N. gonorrhoeae did not produce turbidity or haemolysis in blood enriched broth and this posed a problem of obtaining a result of the various extracts for their inhibitory effects on this organism. None of the tubes inoculated with *N. gonorrhoeae* (positive control tube inclusive) yielded turbidity or change of colour; this handicap gave a false impression that all extracts were effective even at the lowest concentration used (0.625mg/ml.) (Table 4). However subculture from all the tubes on chocolate agar plate indicated that only some extracts were actually bactericidal to *N. gonorrhoeae* but the inhibitory effect could not be estimated.

Considering the result of MMC (Table 5) determination for *Neisseria* and the overall result of the MIC obtained with alcoholic extracts, the stem bark extract of *P. biglobosa* was the most effective alcohol extract with the inhibitory effect on all the nine test organisms. *A. precatorius* stem and *K. senegalensis* stem bark extracts also exhibited a broad spectrum antimicrobial activity, with each having effect on 8 pathogens. Extracts from the whole plant of *E. hirta*, leaf and fruit mixture of *S. longipedunculata* were effective against 7 and 6 organisms respectively. *A. precatorius* seed and *C. alata* leaf inhibited four

Table 3 : MINIMUM MICROBIACIDAL CONCENTRATIONS (MMC) OF THE VARIOUS AQUEOUS EXTRACTS ON TEST ORGANISMS (in mg/mL)

TEST ORGANISM	*Neisseria*	*Streptococcus*	Staphylococcus	*Salmonella*	*Klebsiella*	*Escherichia*	*Pseudomonas*	*Candida*	*Trichom*
PLANT EXTRACT									
A. precatorius, stem	5.55	11.11	11.11	22.22	A	11.11	11.11	ND	ND
A. precatorius, seed	ND	10.15	A	ND	ND	ND	20.3	ND	ND
A. precatorius, leaf	ND	ND	A	ND	ND	ND	ND	ND	ND
B. verticillata, whole plant	ND	ND	1.5	12	24	24	24	ND	ND
C. alata leaf	ND	A	7.5	7.5	ND	ND	30	ND	ND
E. hirta, whole plant	ND	ND	6	18	A	A	18	ND	ND
J. curcas bark	ND	ND	ND	ND	ND	ND	ND	ND	ND
J. curcas, leaf	ND	ND	14	A	A	A	A	ND	ND
K. senegalensis.bark	A	A	0.75	22.5	30	15	15	ND	22.5
K.senegalensis, leaf	ND	ND	6.25	12.5	ND	ND	12.5	ND	ND
P. biglobosa, bark	4.32	4.32	2.15	8.65	4.32	4.32	8.65	46.1	17.3
S. longipeduniclata	3.5	ND	14	21	ND	A	7	A	ND
Control	+++	+++	+++	+++	+++	+++	+++	+++	+++

KEY : A = Growth observed after subculture (extract only inhibitory but not microbiacidal

ND = MMC not determined in these cases

+++ = perfect growth (control)

pathogens each, while *B. verticillata, A. precatorius* leaf and *J. curcas* leaf extract were all effective against only two test organisms. The least effective extract was the stem bark extract of *J. curcas* which could inhibit only the growth of *S. typhi* (Table 4).

The most sensitive test organisms to the inhibitory effect of the various alcoholic extracts was found to be *S. aureus* (which was resistant only to the stem bark extract of *J. curcas),* followed by *S. typhi* which was only resistant to the seed and leaf extracts of *A. precatorius* (Table 4). *Trichomonas spp* and *Streptococcus spp,* two major pathogens of the genital tract were found to be sensitive to up to 6 different methanolic extracts, though the sensitivity of *Trichomonas* to *A. precatorius* seed, *C. alata* leaf and *E. hirta* whole plant extracts was limited only to the starting concentration (i.e. 40mg/mL).The highest MIC value was recorded thus with these plant extracts on *Trichomonas.*

In contrast *P. biglobosa* and *K. senegalensis* stem bark extracts inhibit the growth of *S. aureus* and *S. typhi* at the smallest concentration used (0.625mg/mL). *E. coli* and *C. albicans* were the most resistant organisms to alcohol extracts, each with only four cases of susceptibility (Table 4).

4.4.2 MMC of Alcoholic extracts

After subculturing on extract free chocolate agar plate, it was found that the methanolic extracts of *A. precatorius* stem, *E. hirta* whole plant, stem bark and leaf of *K. senegalensis, P. biglobosa* stem bark and *S. longipedunculata* extracts were bactericidal to *N. gonorrhoeae.* For other organisms with observed susceptibility (as confirmed by absence of turbidity or change of colour) a subculture to the corresponding extract-free agar plate revealed that *P. biglobosa* and *K. senegalensis* stem bark extracts showed the best microbicidal activities with effects on 9 and 8

Table : 4 MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF THE VARIOUS ALCOHOLIC EXTRACTS (in mg/mL)

TEST ORGANISM	*Neisseria*	*Streptococcus*	Staphylococcus	*Salmonella*	*Klebsiella*	*Escherichia*	*Pseudomonas*	*Candida*	*Trichomonas*
PLANT EXTRACT									
A. precatorius, stem	N	10	2.5	10	20	20	10	20	+++
A. precatorius, seed	N	20	5	+++	+++	+++	20	+++	40
A. precatorius, leaf	N	+++	5	+++	+++	+++	+++	20	+++
B. verticillata, whole plant	N	+++	1.25	2.5	+++	+++	+++	+++	+++
C. alata leaf	N	+++	2.5	2.5	10	+++	+++	+++	40
E. hirta, whole plant	N	+++	2.5	2.5	10	10	20	+++	40
J. curcas bark	N	+++	+++	5	+++	+++	+++	+++	+++
J. curcas, leaf	N	+++	1.25	10	+++	+++	+++	+++	+++
K. senegalensis,bark	N	1.25	0.625	0.625	1.25	1.25	1.25	+++	20
K.senegalensis, leaf	N	20	5	10	+++	+++	+++	+++	+++
P. biglobosa, bark	N	1.25	0.625	0.625	5	5	5	5	10
S. longipeduniclata	N	10	5	10	+++	+++	+++	20	20
Control	- - -	+++	+++	+++	+++	+++	+++	+++	+++

KEY : - - - = No turbidity or change of colour observed

N = The MIC cannot be determined

+++ = Perfect growth or no inhibition even at the highest concentration ie 40mg/mL

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Table 5 : MINIMUM MICROBIACIDAL CONCENTRATIONS (MCC) OF VARIOUS ALCOHOLIC EXTRACTS (in mg/mL)

TEST ORGANISM	*Neisseria*	*Streptococcus*	Staphylococcus	*Salmonella*	*Klebsiella*	*Escherichia*	*Pseudomonas*	*Candida*	*Trichomonas*
PLANT EXTRACT									
A. precatorius, stem	5	10	5	20	40	20	A	40	ND
A. precatorius, seed	A*	20	5	ND	ND	ND	40	ND	40
A. precatorius, leaf	A*	ND	10	ND	ND	ND	ND	A	ND
B. verticillata, whole plant	A*	ND	10	5	ND	ND	ND	ND	ND
C. alata leaf	A*	ND	5	20	40	ND	ND	ND	40
E. hirta, whole plant	20	ND	2.5	10	40	A	40	ND	40
J. curcas bark	A*	ND	ND	20	ND	ND	ND	ND	ND
J. curcas, leaf	A*	ND	5	20	ND	ND	ND	ND	ND
K. senegalensis, bark	10	ND	1.25	10	10	20	20	ND	20
K.senegalensis, leaf	20	2.5	5	10	ND	ND	ND	ND	ND
P. biglobosa, bark	25	20	1.25	5	10	5	10	20	10
S. longipeduniclata	5	2.5	10	20	ND	ND	ND	40	20
Control	+++	10	+++	+++	+++	+++	+++	+++	+++

KEY : A = Growth observed after subculture (Extract only inhibitory but not microbiacidal)

A* = Growth observed after subculture but case difficult to evaluate the inhibitory effect with tube method

ND = MMC not determined

+++ = Perfect growth (control)

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Table 6 : COMPARISON BETWEEN MMC VALUES OF AQUEOUS EXTRACTS AND MMC VALUES OF ALCOHOLIC EXTRACT.

TEST ORGANISM	*Neisseria*		*Streptococcus*		*Staphylococcus*		*Salmonella*		*Klebsiella*		*Escherichia*		*Pseudomonas*		*Candida*		*Trichomonas*	
PLANT EXTRACT	W	M	W	M	W	M	W	M	W	M	W	M	W	M	W	M	W	M
A. precatorius, stem	5 55	5	11.11	10	11.11	5	22 2	20	A	40	11.11	20	11 11	A	ND	40	ND	ND
A. precatorius, seed	ND	A*	10 15	20	A	5	ND	ND	ND	ND	ND	ND	20 3	40	ND	ND	ND	40
A. precatorius, leaf	ND	A*	ND	ND	A	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	A	ND	ND
B. verticillata, whole plant	ND	A*	ND	ND	1 5	10	12	5	24	ND	24	ND	ND	ND	ND	ND	ND	ND
C. alata leaf	ND	A*	A	ND	7 5	5	7 5	20	ND	40	ND	ND	30	ND	ND	ND	ND	40
E. hirta, whole plant	ND	20	ND	ND	6	2 5	18	10	A	40	A	A	18	40	ND	ND	ND	40
J. curcas bark	ND	A*	ND	ND	ND	ND	ND	20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
J. curcas, leaf	ND	A*	ND	ND	14	5	A	20	A	ND	A	ND	A	ND	ND	ND	ND	ND
K. senegalensis bark	A	10	A	2 5	0 75	1 25	22 5	10	30	10	15	20	15	20	ND	ND	22 5	20
K.senegalensis, leaf	ND	20	ND	20	6 25	5	12	10	ND	ND	ND	ND	12 5	ND	ND	ND	ND	ND
P. biglobosa bark	4 32	2 5	4 32	2 5	2 15	1 25	8 65	5	4 32	10	4 22	5	8 65	10	46	20	17 3	10
S. longipeduniclata	3 5	5	ND	10	14	10	21	20	ND	ND	A	ND	7	ND	A	40	ND	20

KEY : ND = MMC Test not carried out

A = Extract only inhibitory but not microbiacidal

A* = inhibitory effect difficult to evaluate wth tube method

W = aqueous extract

M = Methanolic extract

test organisms respectively (Table 5). Other alcoholic extracts with broad microbicidal effects were *A. precatorius*, the whole plant of *E. hirta*, and the mixture of the leaf and fruit of *S. longipedunculata*, each having effect on at least 6 test organisms.

Some extract still failed to kill the organism tested despite their high inhibitory activity; such were the cases of the stem extract of *A. precatorius* and *Pseudomonas*, *A. precatorius* leaf on *C. albicans* and *E. hirta* whole plant extract on *E. coli* (Table 5). In most cases the MMC value was at least twice the MIC value (Table 4 and 5) and up to 8 cases of MMC value equal to 40mg/mL (the highest concentration used) were recorded. The lowest MMC value (1.25mg/mL) was obtained with *K. senegalensis* and *P. biglobosa* stem bark extracts on *S. aureus* (Table5).

S. aureus and S. typhi remained the most susceptible organisms to the microbicidal effect of alcoholic extracts, while *E. coli* and *C. albicans* were the most resistant (Table 5).

4.5 RESULT OF THE BRINE SHRIMP (*Artemia salina*) TOXICITY TEST.

Only alcoholic extracts were screened for toxicity. The LC_{50} value (i.e. the lethal concentration to 50% of the shrimps) of the majority of the extracts (65%) fell within the tolerance level of toxicity i.e. LC_{50} <200 μg/mL (Meyer *et. al.*, 1982; Fatope *et. al.*,1990) as presented in Table 7. However extracts from the stem bark and leaf of *J. curcas*, and those from the seed and stem of *A. precatorius*, appeared quite toxic to the shrimps with the highest toxicity recorded with the stem extract of *A. precatorius* (one of the most effective antimicrobial extracts)(LC_{50} 104.0857 μg/mL). Two extracts cytocidal to *Neisseria spp.* *K. senegalensis* leaf and *S. longipedunculata* were the least toxic to brine shrimps *Artemia salina* with LC_{50} values greater than

1000μg/mL. The remaining plant extract had LC_{50} values ranging from 224μg/mL to 899.1782μg/mL (Table 7).

Apart from *A. precatorius* stem extract, the other three most toxic drugs (i.e. *A. precatorius* seed and *J. curcas* bark and leaf) were the least antimicrobials obtained.

TABLE 7 : RESULT OF THE BRINE SHRIMP TOXICITY SCREENING OF METHANOLIC EXTRACTS

EXTRACT CONCENTRATION	Number fo dead (from 30 shrimps) after 24 hours incubation						
	1000µg/mL	500µg/mL	250µg/mL	125µg/mL	62 5µg/mL	0µg/mL (control)	LC50 in µg/mL
PLANT EXTRACT							
A precatorius, stem	30	30	30	20	7	0	104 0857
A precatorius, seed	28	25	23	14	2	0	173 0198
A precatorius, leaf	30	30	18	4	0	0	264 4598
B verticillata, whole plant	20	5	0	0	0	0	899 1782
C alata leaf	25	20	16	11	6	0	224 1459
E hirta, whole plant	20	13	5	2	0	0	652 0665
J curcas bark	30	30	26	18	4	0	127 1176
J curcas, leaf	30	30	28	11	0	0	145 5985
K senegalensis, bark	19	5	0	0	0	0	822 7123
K senegalensis, leaf	13	8	4	2	2	0	1558 967
P biglobosa, bark	29	22	7	0	0	0	336 899
S longipeduniclata	6	1	0	0	0	0	>2000

KEY : LC50 : Lethal concentration for 50% of the shrimps (Artemia salina)

Chapter 5
DISCUSSION

From the results obtained, almost all the vegetable drugs screened in this study was effective on at least one of the well known pathogens of the urinary and genital tracts. These observations support their use in the traditional treatment of these diseases. A critical look at the results reveals that some of the drugs notably the aqueous stem bark extract of *Jatropha curcas* and leaf of *Abrus precatorius* were less effective against the test organisms used . This seems contradictory to the claims that these vegetable drugs are useful in treating urogenital infections traditionally. The decoction of stem bark and leaf of *J. curcas* has been reported to be useful in treating gonorrhoea, and decoction from stem and leaf of *A. precatorius* for the treatment of venereal diseases (Dalziel, 1956; Irvine, 1961). However, the healing process *in vivo* does not depend exclusively on the antimicrobial effect of drugs, for example extracts from the stem bark of *J. curcas* are well known to possess diuretic effect (Dalziel, 1937) and the increase flow of urine induced by this plant may help in flushing out the pathogens from the urethra. It has also been reported that, water extract from leaf of *A. precatorius* is immunostimulant (Nwodo, 1981) and can help the host's immune system to fight against the invading pathogens, hence, the failure of its extracts to eliminate test organisms *in vitro* does not preclude the useful application of the leaf decoction of this plant in the treatment of venereal diseases.

Another striking observation from this work is the failure of a good number of extracts with high inhibitory activity to kill the concerned test organisms *in vitro*. Two explanations may be proffered for this observation: i) The active constituents in the plant extract may only stop the cell multiplication without impairing the cell structure; ii) One of the constituents of the extract may chemically alter the culture medium to make the environment unconducive for growth.

By comparing the MIC and MMC of each extract on a particular test organism (Table 2-5), it appears that some of the extracts are microbicidal at the MIC level (i.e. MIC = MMC). Examples are aqueous stem bark extract of *P .biglobosa* on *Trichomonas spp.*, aqueous extract of the leaves of *K. senegalensis* on *Salmonella spp.* and *Pseudomonas spp.*, e.t.c. This suggests that the antimicrobial components in the extract act solely on the cell structure to cause their lysis (Stanier *et. al.*, 1986). On the other hand, extract with low MIC and high MMC on test organisms (e.g. aqueous extract of *B. verticillata* on *Salmonella spp.*) may possess more than one active component, some affecting only the cell multiplication and others possessing cytolytic effect.

Extract with high MIC/MMC will have to be taken in high concentrations to bring about desirable effects while those with low MIC/MMC could be taken at low concentrations.

For the first time, local herbs have been screened against *Trichomonas spp.* The results of this are quite encouraging as from the Trichomonocidal effect of both alcoholic and aqueous extracts from the stem bark of *K. senegalensis and P. biglobosa*, and the alcoholic extract from the seeds of *A. precatorius*, leaf of *C .alata*, fruit plus leaf of *S._longipedunculata* and the whole plant of *E. hirta* (Table 3 and 5). The detected Trichomonocidal effect and the absence of toxicity of the stem barks extracts of *P. biglobosa and K. senegalensis* as well as extracts from the plant *E. hirta* (Dalziel, 1956; Olayinka, 1992; Sofowora, 1993), should be good news to the scientific world. The results of the brine shrimp toxicity test (LC50 > 250µg/ml.) support earlier reports that *E. hirta* and *K. senegalensis* have antispasmodic properties (Meyer *et. al.*, 1992). Based on these observations *K. senegalensis, E. hirta*, and *P. biglobosa* are promising sources of drugs for the treatment of trichomoniasis. Drugs developed from these plants could serve as valuable alternatives to "Flagyl" reported to be potentially neuritic, embryotoxic, and carcinogenic (Hager and Rapp, 1992). The Trichomonocidal effect of the stem bark extracts of *K. senegalensis* correlates

with its use traditionally in Zaria as douche for vaginal irritation. Other plant extracts which showed Trichomonocidal properties such as those of the leaf of *C. alata*, seed of *A. precatorius*, and a mixture of fruits and leaves of *S. longipedunculata*, though reported to be spasmodic and sometimes used as abortifacient or toxic to man at high dosage (Irvine, 1961; Nwodo and Botting, 1983; Ikenebomeh *et. al.*, 1988) could also serve as sources of antitrichomonal drugs in male and non pregnant women.

The effectiveness of both aqueous and methanolic extracts of the stem barks of *K. senegalensis* and *P. biglobosa* on *N. gonorrhoeae, S. pyogenes, S. aureus and E. coli* revealed in this study should be good hope in the management of urogenital infections in Obstetric and Gynaecology pathology.

By comparing the microbiacidal effect of aqueous and alcoholic extracts of the various vegetable drugs on *N. gonorrhoeae, S. pyogenes, C. albicans* and *Trichomonas spp.* (Table 6), it appears that alcohol extracts are more effective on these organisms than the water extracts. This implies that the microbiacidal constituents are more soluble in methanol, or some are heat labile and were destroyed during autoclaving.

In contrast *Pseudomonas spp, Klebsiella spp,* and *E. coli* were more sensitive to the water extracts than to the alcoholic extracts; this indicates that the active components causing the lysis of these pathogens are non organic or less polar and are thus more soluble in water than in alcohol (Table 6).

The effectiveness of alcoholic extracts on *S. aureus* and *S. typhi* was not too different from that of water extracts (Table 2 and 4). This suggests that the plant constituents responsible for the *in vitro* inhibition of these pathogens are soluble in alcohol and in water.

It is noteworthy that *Pseudomonas aeruginosa*, (a stubborn organism of the urinary tract and wound infections) reported to be resistant to the most common antimicrobial agents especially to the penicillins and aminoglycosides (Ayliffe *et .al.*,1972; Cheesbrough, 1985), was sensitive to seven of the twelve aqueous extracts tested and to five methanolic extracts. Its

susceptibility to *S. longipedunculata* and *P. biglobosa* stem bark water extracts were the best with MIC values of 7mg/mL and 8.6mg/mL respectively (Table 6). This is good news for the management of urinary tract, wounds, and post-surgical wound infections (Cheesbrough, 1985). The principal beneficiary of this application remains the patient who will be saved from the numerous side effects and the high cost of Cephalosporins, the present drug of choice for pseudomonal infections(Govan,1992).

The observed resistance of *C. albicans* and *Trichomonas gallinae* to the majority of extracts can be attributed to their eukaryotic nature. One may have expected that water extract from some of the plants used locally in treating worm infestations such as *B. verticillata*, *A. precatorius* and *C. alata* leaf, or plants used as antifungal or against amoebic dysentery such as *C. alata*, and *E. hirta* would have inhibited the growth of these eukaryotic pathogens *in vitro*. Aqueous leaf extract of *C. alata*, a plant well known for its antifungal properties (Ikenebomeh *et. al.*,1988) did not inhibit the growth of *C. albicans in vitro* but the alcoholic extract did (Table 4). It can be concluded that the antifungal constituents present in the plant are either more soluble in alcohol than in water, or they are heat labile and could not withstand the autoclaving temperature. The same applies to *E. hirta* which only the alcoholic extract was found to be Trichomonocidal. Aqueous extract of *B. verticillata* did not inhibit the growth of *N. gonorrhoeae* , though maceration of this plant is reported to be used by local people in Gambia to treat gonorrhoeae (Kokwaro, 1993). If the gonococcocidal principle of this plant is one of its multiple essential oils (Bona,1986), it is expected this oil may have evaporated during the boiling for extraction.

Salmonella, *Klebsiella*, and *E. coli* are known to cause not only urinary tract infection but enteric fever and gastro-enteritis, pulmonary infections, and diarrhoea respectively. These organisms were sensitive to a good number of aqueous and methanolic extracts (Table 1 and 4); these findings will definitely help to develop new drugs for the treatment of cystitis, plyelonephritis,

endocervicitis and other forms of urogenital infections caused by these organisms, as well as a solution to typhoid fever, pneumonia and diarrhoea in children (Zagrebina and Torchinov,1989; Slack,1992; Lainy et. al.,1993; Schalger et. al.,1995)

The observed broad spectrum activity of the stem bark extracts of *K. senegalensis* can be attributed to its high content of tannins, terpenes, and its various steroids (Adegbola *et. al.*,1992). Meanwhile less phytochemical studies have been conducted on the bark of *P. biglobosa* for us to presume which of the constituents are responsible for the observed broad spectrum activity of its stem bark extract. the only phytochemical report on *P. biglobosa* is that of Irvine (1961) who reported that the stem bark of the plant harvested in Côte d'Ivoire was rich in tannins and it is known that some tannins from plants act like broad spectrum antibiotics (Harbone, 1964).

Extracts from the stem of *A. precatorius* (one of the most active drugs with effect on eight out of the nine pathogens tested) was found to be the most toxic drug in this study (Table 2, 3 and 7). In effect it is expected that plants possessing antimalarial property, and spasmodic property will be toxic to brine shrimps *Artemia salina* (Solis *et. al.*, 1993). The high toxicity of this plant as obtained in this study, correlates well with its traditional use as antimalaria, aphrodisiac or its use to facilitate child birth (Nwunde, 1986; and Akubue, 1986). The application of this remedy in the treatment of infections due to the organisms sensitive to it in this work, requires serious care because of its toxic potentials. However, traditional practitioners are well aware of the toxic effect of this plant.

Alcoholic extracts of the leaves mixed with fruits of *S. longipedunculata* appeared to be the least toxic to brine shrimps *Artemia salina*, but cool infusion of leaf and powdered root of this plant were reported by Irvine in 1961 to be toxic and even poisonous to man at higher dose. The result of the toxicity obtained here suggests that, the toxic principles have no

effect on shrimps or they are not soluble in methanol. Other laboratory animals such as mice should be used to check its toxicity.

It will not be wise to declare at this point that plants which extracts failed to be inhibitory or cytocidal to the various test organisms do not contain active principles able to act against these organisms. Such conclusions could only be made if the screening of cool water extracts from these plants sterilised by other means different from autoclaving was conducted. Also, the result of the toxicity with brine shrimps can only serve as a clue of toxicity if it is compared with other toxicity screening methods such as screening using mice, or KB cells (human nasopharyngeal carcinoma cells) because the metabolic pathways in shrimps are not always the same in mammals or human beings (Solis *et. al.*, 1993). The metabolic pathways of purine in *Artemia salina* is markedly different from that of mammalian cells; thus, a compound such as 6-mecaptopurine, very toxic to man will not be toxic to brine shrimps. Another example is cyclo-phosphamide, a compound which requires metabolic activation in the liver for its activity in man, will appear non toxic to brine shrimps (Solis *et. al.*, 1993). However, toxicity screening using brine shrimps remains very useful for all biologically active compounds possessing similar effects in both shrimps and mammals.

CONCLUSION

This study has provided information on alternative drug sources for treating urogenital infections and at the same time considerable proof for the use of medicinal plants in treating various illnesses due to micro-organisms. Considered with interest, informations provided here can serve as starting point for development of pharmaceutical products for the treatment of these diseases. example of plant extracts processed in form tablets, ointments, capsules, or syrup are many, and if the same is done for the plants studied in this work, a gleam of hope will begin to shine in third world countries where there is prevalence of urogenital diseases. more over this work suggest alternatives for 'Flagyl' (Metronidazole) especially to pregnant patients or other individuals who cannot withstand the side effects of Metronidazole. With proper application of these findings trichomoniasis and other urogenital infections will cease to be the major problems of Obstetrics and Gynaecology

Further studies are strongly recommended in the following areas in order to concretise the useful information collected in this work:

i) To investigate the *in vivo* antimicrobial activity of the plant extracts with remarkable *in vitro* antimicrobial effect on the test organisms, using laboratory animals.

ii) To further investigate the toxicity of these plants to mammals such as mice or KB cells (human nasopharyngeal carcinoma cells) in order to set up standards for drug development from these plants.

iii) To find out what the various constituents of the effective extracts are, and study the phenomenon of combination of antagonistic activities of the constituents in order to highlight the usefulness of the application of these plant remedies or drug processed from their crude extracts, in the treatment of urogenital infections.

iv) Finally, to investigate the mode of action of the responsible constituents on the cell structure of the test organisms in order to develop reliable broad spectrum antimicrobial agents from these plants

If the above recommended further studies are carried out it should be possible to develop drugs from the natural crude extracts from these plants for curative purposes, and these will be available to the public at affordable prices. It is strongly recommended that African countries endowed with many plants/herbs should commit a substantial part of their annual budget to medicinal plant research. Some of these plants need to be identified and preserved from extinction. The strive towards this direction which necessitated the setting up of the National Institute of Pharmaceutical Research and Development (Abuja) by the Nigerian Government is highly commendable and should be emulated by other African countries.

REFERENCES

Abdu Ghani (1986) Medicinal Plants and Tradition Medicine Potions: Problems and Prospects of their standarzation. In *State of Medicinal Plant Research in Nigeria* pp125-131 Ed Sofowora A. Nigerian Society of Pharmacognosy. Ibadan University press

Abdu Ghani (1990) Cultivation and Conservation of Plant Drugs. in *Introduction to Pharmacognosy*. 1st Edition Ed. Abdul-Ghani pp 153-168 A.B.U. Press

Abegaz B. and Dagne E. (1979). Comparative Bioassay Studies of some Traditional Anthelminthic Plants : Plant Extract and Modern Drugs. *Sinet: Ethiopian journal of Sciences* 2(1):117

Abegaz B. and Tecle B. (1980) A new Triterpenoid glycoside from the seed of *Glinus lotoides*. *Phytochemistry* 19 : 1553-1554

Abo A. Kio, F. J. Evans and D. Gordon (1986). New anti-leukemic principles from the latex of *Euphorbia kamerunica* Pax.In *State of Medicinal Plant Research in Nigeria* pp 237-242 Ed Sofowora A. Nigerian Society of Pharmacognosy. Ibadan University press

Adesina S. K. (1983). Examination of Khaya senegalensis and *Khaya ivorinsis*. *Fitoterapia* 3:_141-

Adesina S. K. (1988) Drug production from natural product. *Proceeding. 7th International Sympostum of the DRPU.* Medex pub Ltd Lagos (article)

Adegbola O. L. , Adeoye O. A. and Sofowora A.. (1992) Establishment of Pharmacopoeial Standard for Nigerian Medicinal Plant VII. The stem bark of *Khaya senegalensis*. A juss. *A paper presented at 2nd NAAP Scientific Conference* pp 58-66

Adjamohoun (1986) . Medecine Traditionelle et Pharmacopee. *Bulletin de liatson* 1(1) ACCT, Paris

Adjamohoun E. Ahyi A. Ake-Assi, Baniankina J. L., Chibon P. *et.al.* (1988).*Contribution aux etudes Ethnobotanique en Republique Populaire du Congo*. ACCT, Paris

Adjamohoun E. Ahyi A. Ake-Assi, Baniankina J. L., Chibon P. *et.al.* (1991) *Contribution to Ethnobotanical and floristic Study in Western Nigeria* OAU/STRC, Lagos.

Adjamohoun *et.al.* (1993) Traditional Medicine and Pharmacopoeia: *Contribution to Ethnobotanical and Forestic studies of Uganda.* OAU/STRC , Lagos.

Akubue P. Iwe (1986). Nigerian Medicinal Plants: Pharmacology and Toxicology In *State of Medicinal Plant Research in Nigeria* pp 53-66 Ed Sofowora A. pub Nigerian Society of Pharmacognosy. Ibadan University press

Aladesanmi A., J. Awe, S. A. Adesanya and Bray D. H. (1988). Drug Production from Natural Plants *Processing of the 7th International Symposium DRPU.* (Ed. Adesina) Medex pub Ltd Lagos, Nigeria

Alderete and Pearlman (1984). Pathogenic Trichomonas vaginalis, cytotoxic to cell culture monolayers. *Br J vener dis 60:* 99-105.

Alvarez R. S. , D. A. Richardson, Alfred E. B. and Donald R. O.(1983). Central Nervous System Toxicity related to prolonged Metronidazole therapy. *Am J Obst Gynecol 145*(5):640-641

Ampofo O. (1977). Some clinical observations of Treatment of selected diseases by herbal preparation. *Perpestive in Medicinal Plant Research Today.* Drug Research Unit. O.A.U. , Ile Ife. Nigeria. pp 35-45.

Ann Mary H., and Priscilla A. S. (1995). Cloning and Characterisation of Herpes Simplex Virus (HSV) type I Oril : comparison of replication and protein-DNA Complex. Formation by Oril and Oris. *Journal of Virology 69*(3):1377-1388.

Atallah A. M. and Nicolas H. J. (1972). Triterpenoid and steroid of *Euphorbia pululifera, Phytochemistry 2* :1861-0-1861

Aubreville A (1936) *La flore forestier de Cote d'Ivoire* .Vigot & Frère, Paris

Aubreville A (1936) *La flore forestier du Cameroun et d'Afrique Equatorial Francaise,* Vigot & Frère,Paris

Adewunmi C. O (1984) " Natural products as agents in Schistosomiasis Control in Nigeria, A review of Progress" *Int J. Crude drugs Res 22(4)*:161-166

Ayim J. S. K. (1986). Studies on Cassia alata leaves In *State of Medicinal Plant Research in Nigeria* pp 213-217 Ed Sofowora A. pb Nigerian Society of Pharmacognosy. Ibadan University press

Awosika Fola (1992). What you should know about gonorrhoea. *Clinical pharmacyand Herbal Medicine. 8*(6/7):19-20

Babalola G. O. (1988) Antimicrobial Properties of crude extracts of the sap and bark of *Pulnanthus angolensis* (Warb). *Nigerian Journal of Microbiology* 8(1/2): 4-11

Boakye-Yiadom K.(1979). The antimicrobial activity of some West African Medicinal Plants. Quart. *J. Crude drug Research 2*:78-80

Bona A. , Obiorah (1986) Sourcing Pharmaceutical Raw Material From Indigenous Medicinal Plants In *State of Medicinal Plant Research in Nigeria* pp 79-88 Ed Sofowora A. pub Nigerian Society of Pharmacognosy. Ibadan University press

Caiquo A.W.K , John E. Kay and T. Animashau (1986) Toxic principle of *Abrus fructiculosus* seed. In *State of Medicinal Plant Research in Nigeria* pp 368-369 Ed Sofowora A. pub Nigerian Society of Pharmacognosy. Ibadan University press

Carey J. Chris, Sumner J. Y. and C. Cart. (1993) The vaginal Infections and Prematurity Study: An Overview. *Clin Obst Gynecol 36*(4):809-820

Cheesbrough Monica (1985) *Medical laboratory Manual for Tropical Countries. Vol II* Microbiology ELBS pub . Low price edition.

Cimaga K. Pieters L. Clocys M., Berghe D. V. and Lietinck A. J. (1991). Biological activities of Cryptolepine an alkaloid from *Cryptolepsis sanginolenta. Planta Medica 57*(52): A98-99

Collins C. H.,, O. M. Lynes and J. M. Granges (1995) Antimicrobial Susceptibility test. In *Collins and Lynes Microbiological Method* 7th edition C. H. Collins *et.al.* (eds) pp178-205 Pb . Butteword Great Britain. Heineman Ltd-Oxford

Dalziel J.M (1937 *The useful Plants of Tropical Africa* 1st Edition Crown Agens for the colonies, London

Dalziel J.M . (1956) *The Useful Plant of Tropical Africa* 2nd Edition Mallbank, London S. W.

Dwuma-Badu D. (1983) Anti-inffective agents from Traditional Useful Plant from West Africa . In Essein et.al. (eds). Proceeding of VISOMP *5th Symposium on Medicinal Plant. Drug Research Prodution* Ile Ife pp 32-40.

Ekong D.E (1986) Medicinal Plant Research in Nigeria : Prospectus and Retrospectrus. In *State of Medicinal Plant Research in Nigeria* pp 1-12. Sofowora A.(Ed). Nigerian Society of Pharmacognosy.(pub) Ibadan University press

Elewunde (1986) *Jatropha curcas* " Lapalapa Funfun". In *State of Medicinal Plant Research in Nigeria* pp 153-155 Ed Sofowora A. pub Nigerian Society of Pharmacognosy. Ibadan University press

Ellen J. Baron , Sydney M. F. (1990). Cultivation and isolation of viable pathogen: In *Bailey and Scott's Diagnostic Microbiology* 8th edition. The C.V Moshy Company , Toranto, Saint Louis. pp 81-98.

Ellen J. Baron , Sydney M. F. (1990) Aerobic Gram Negative cocci (*Neisseria* and *Brahamella)* In *Bailey and Scott's Diagnostic Microbiology* 8th edition. The C.V Moshy Company , Toranto, Saint Louis. pp353-363.

Evans E.G.V. (1992). Fungi , ringworm, subcutaneous and systemic Mycosis. In *Medical Microbiology* . Greenwood *et. al.* (eds) ELBS pub 14th Edition pp 673-698

Fallon R. F. , R. C. B. Slack (1992) *Neisseria* and *Branhamella.* Meningitis Gonorrhoea , Respiratory Infections In Medical *Microbiology* . Greenwood *et.al.* (eds) ELBS pub 14th Edition pp 293-303

Fatope O.Majek. (1990) *Material and procedures for Brine Shrimps Lethality bioassay.* Unpublished Data

Feinberg J. G. and Whittington M. Joan (1957). A culture media for *Trichomonas Vaginalis* Donné and Species Candida. *J Clin Path. 10:* 327-329

Gakunja D, Mbera S.K Dossaji Sf Gray I. A et.al. (1995). Potent antimalarial activity of the alkaloid Nitidine Isolated from Kenyan Herbal remedy. *Anti Microbial agents and chemotherapy; 39(*12): 2606-2609

Gene N. Neeley (1989). Gonococcal Infection in Women. *Obst Gynecol Clin North Am. 16*.(3):467-478

Georges M. and Pandalai K.M.(1944). Investigation on plant antibiotics IV. Further search for antibiotic substances in Indian Medicinal Plants. *Indian J Med Res. 37*(2):169-181

Ghosal S. and Dutta S. K. (1971). Poisonous principle of *Abrus precatorius* seeds. *Phytochemistry 10:* 195-198

Glenns Robert (1990). Laboratory Methods , Basic Mycology. In *Bailey and Scott's Diagnostic Microbiology 8th* edition. By Ellen J. Baron, Sydney M. and Finegold. pp 681-775. The C.V. Moshy Company St Louis, Toronto

Guoullet P. H., Picard B., Contrepois J., and De Rycke (1994) Correlation between Esterase-Electrophoretic Polymorphism and Virulence-Associated Trait in Extraintestinal invasive strains of *Escherichia coli. Epidemiology and infections* 1994, *112*(1): 51-62

Greenwood David (1992a). Protozoa. In *Medical Microbiology*. 14th Edition (Ed.) David Greenwood et.al. E.L.B.S pub. pp 699-710

Greenwood David (1992b). Antimicrobial Agents. In *Medical Microbiology*. 14th Edition (Ed.) David Greenwood *et. al.* E.L.B.S pub. pp 67-78

Gupta D. R. and Gary S. K. (1966) A chemical examination of *Euphorbia hirta* Linn. Bull. chem-soc Japan 39(11): 2532-2534

Hager David and Robbert Rapp (1992). Metronidazole.

Obst & Gynecol clin North Am 19(3): 497-510

Hans Fliick, Rita J. S., J. M. Rowsom (1976). *Medicinal Plant and their Uses.* Land W. Foldhan & Co Ltd pp 20-25.

Harbone J. B. (1964) *Biochemistry of phenolic compounds* . Academic Press INC New York pp 367-369

Hargreaves B. J. (1979). Harmful and helpful Euphorbiaceae in Malawi. *3rd OAU/STRC Inter- Africa Symposium on African Medicinal Plants and Traditional Pharmacopoeia.* Abidjan-Côte d'Ivoire.

Hasson A. U. (1991). New Approach to Pharmacology. *Clin Phar and Herb Med 7*(6&7): 6-7

Hawser Stephen and Julio Douglas. (1995). Resitance of *Candida albicans* biofilm to Antifungal Agents in vitro. *Antimicrobial Agent and Chemotherapy. 39*(9): 2128-2131

Heine Phillip and James A. McGregor . (1993). *Trichomonas vaginalis*: A re-emerging pathogen *Clin Obstec Gynecol 36*(1): 137-144

Hexlux (1977) *History of Herbal Plant* . by Le StangeRichard. Angnus & Roberton. U.K Ltd

Honigberg B.M. Gupta P.K. Spence N. and Frost J.K (1984). Patogenicity of Trichomonas vaginalis : Cytopathological and Histopathological change of the Cervical Epithelium. *Obstec Gynecol 64*: 179-184

Ikenebomeh M.J. and Metiriri (1988). Antimicrobial effects of extracts from Cassia alata. *Nigerian Journal of Microbiology* 8(1-2): 12-23

Irvine R.F. (1961) *Woody Plant of Ghana* London Oxford university Press.

Jato J., Symond P., Thomas B., Tchoundjou Z. and Mbi C. In *Proceeding of the 5th OAU/STRC Symposium on African Medicine and Medicinal Plants* OAU/STRC, Lagos.

Jeriki-Black J. C. and Lush Bdaugh W.B. (1988). Trichomonas vaginalis and sperm motility inhibitory factors . *Am J. Chem Lab Sci 18*: 484-489

Johnson A.P. . M. Halde, Woodford N. CunneyrJ and Smyth E.G (1995). Urinary Isolates of Apramycin-Resistand Escherichia coli and Klebsiella pneumoniae from Dublin . *Epidemiol Infect, 1995; 114*(1):105-112

Kerharo J. and Bouquet A. (1950). *Plante Médécinale et toxique de la Côte d'Ivoire et Haute Volta* Vigot & Frères, Paris

Kerharo J. and Adams J.G. (1974). La parmacopéé Sénegalaise Vigot & Frères, Paris

Khalid S.A., and Duddedk H. (1989). Isolation and Characteration of an Antimicrobial agents of Neem Tre Azadirachta indica . *Journal of Natural Products*. 52(5): 922-927

Kokwaro J.O. (1993). Medicinal Plants in East Africa. East Africa literature bureau. Nairobi Kenya (article)

Koumare M. (1993) Le SIDA, Origine anciennes et actuelles , Mythe ou Realité, Diverse strategie de la lutte. In OUA/CTRS *Symposium sur la Médécine Traditionelle africaine Plante Médécinales*, Yaoundé , Cameroun

Kreiger N.J.(1981) Urologic aspect of Trichomoniasis. *Investigative Urology 1981; 18*(6): 411-416

Krieger N. J. and Michael F. R. (1982). Zinc sensitivity of Trichomonas vaginalis In vitro study and clinical application. *J. inf Dis 146*(3): 341-345

Kulda J. M. , Jan T. Pavol and Eva K. (1982). Metronidazole resistance of Trichomonas vaginalis as cause of treatment failure in trichomoniasis. *Br. J. vener Dis 48*: 394-399

Kurup. (1977). The science of life. *W.H.O. Health magazine , November 1977* pp 12-15

Lachman L., Lieberman H.A. and Kanig J.L. (1976). *The Theory and practice of Industrial Pharmacy*. By Lachman L. pp 27-28. Lea & Fibiger, Philadelphia, USA.

Lainy R. R. S . Smith F. W. and Douglas J. G. (1993). Salmonella enteritidis : Urinary infection associated with polycystic renal disease. The Journal of infection . 27(1):71-74

Latif A. J., Masso P.R., Marowa E. (1987) Urethreal trichomoniasis in Men. *Sex Trans Dis 14*: 9-14

Le Strange Richard. (1977). *History of Herbal Plants*. Angnus & Roberston Ltd , U.K

Lossick J. G. (1984) . Sexually Transmitted vaginitis. *Urologic Clinics of North America 11*(1): 141-153

Lossick J.G., Mueller M. Gerrel T.E. (1986). In vitro drug susceptibility and dose of Metronidazole required for cure in cases of refractory vaginal trichomoniasis *J. Infect Dis 1986,153*: 948-955

Lucas Michael J., Gari F. Cunnighan (1993) Urinary infection in pregnancy. *Clin Obst Gynecol. 36*(4): 855-868

Marilyn Olson , Robbie R.N., Richard L.S (1983) Metronidazole used in Obstetric and Gynecology: A review . Am. J. Obst Gynecol. 145:865-881

Mayart G., Pousset J. L., Mboup S., and Denis F. (1980). Action antimicrobiène de la borevine, alkaloide isolé du *Borreria verticillata C. R. Soc de Biol 174*(5): 925-928

Meingas S. J.G., Heyworth P.G (1982). Sensitivity of Trichomonas vaginalis to Metronidazole in medium with different concentration of medium of iron and ascorbat J. Parasitol. 68:11631165

Meyer B.N., Ferigni N. R., Putman J. E., Jacobseni L. B., et. al. (1982). Brine Shrimps a convenient General Bioassay for plant constituents *Planta Medica 45*: 31-34

Msonthi Jerome D. (1986). Status of Medicinal plant research and traditional Medicine in Malawi. In *State of Medicinal Plant Research in Nigeria* pp 335-343 Ed Sofowora A. pub Nigerian Society of Pharmacognosy. Ibadan University press

Modupe M., Obih P. Jimoh A.A (1986) Effect of *Solannum erianthum*, aqueous leaf extract on Plasmodium Berghei berghei in mice. In *State of Medicinal Plant Research in Nigeria* pp 371 Ed Sofowora A. pub Nigerian Society of Pharmacognosy. Ibadan University press

Naengchomnong N. , Okanmeto K. T., Clardy J., Niriyachita P. et. al., (1986). Isolation and structure determination of For novel Diterpenes from Jatropha Curcas. *Tetrahedron Lettres 27*(22): 2439-2442

Nancy Roper (1987). *Pocket Medical dictionary* 14th edition Churchill Livingstone London.

Narston A., Hamburger M., Sordat- Diserins *et. al.* (1993). Xanthones from *Polygala nyikensis. Phytochemistry. 33*(4): 809-812

Nix D., Robert T., and Lilos M. (1995). Pharmaco dynamic of Metronidazole, determined by Time Kill Assay For *Trichomonas vaginalis Antimicro Ag Chemother 39*(8): 1848-1852)

Noweiky J. W., Staniszewski A. Bruja S. *et. al.* (1991) Evaluetiont of Thiophosphoric acid derivative From *Chelidonium majus* L. ("Ukrain") as an immunostimulant in parties various carcinomas *Clin Pham & Herbal Med* 7(6-8): 19-20

Nwondo O. F. C. and Botting J.H. 1983) Spasmolytic of activity of *Abrus precatorius. Planta medica 47*: 230-233

Nwunde N. (1986) Veterinary aspect of Medicinal Plant Research in Nigeria. In *State of Medicinal Plant Research in Nigeria* pp 97-112 Ed Sofowora A. pub Nigerian Society of Pharmacognosy. Ibadan University press

OAU/STRC (1985,986) *African pharmacopoeia vol I* and *vol II* OAU/STRC. Lagos Nigeria

Obebigi O.O and A. Sofowora (1979) Antimicrobial alkaloid from Nigeria Chewing Sticks. *Planta medica 36*(3):205

Obineh P.O. Makinde .M (1986) An investigation into antimicrobial activity of *Cymboparpagon citratus* (Lemon grass) on *Plasmodium berghei berghei* in mice. In *State of Medicinal Plant Research in Nigeria* pp 372 Ed Sofowora A. pub Nigerian Society of Pharmacognosy. Ibadan University press

Ogilvie M.M (1992) HerpesVirus In *Medical Microbiology* by D. Greenwood *et. al.* 14th edition pp 481-508

Okelola I.O. Elujoba and A. Sofowora (1986) Establishment of Pharmacopoeial Standard for Nigeria Medicinal Plant III Borreria verticillata Meyer. In *State of Medicinal Plant Research in Nigeria*

pp 203-211 Ed Sofowora A. pub Nigerian Society of Pharmacognosy. Ibadan University press

Okogun J.I , (1986) Chemistry of Nigeria Medicinal Plants. In *State of Medicinal Plant Research in Nigeria* pp 31-52 Ed Sofowora A. pub Nigerian Society of Pharmacognosy. Ibadan University press

Olayinka O. A. (1992) Preliminary Phytochemical screening and microscopical Studies of leaf and bark of *Khaya senegalensis* A. juss. *2th NAAP Scientific conference* pp 59-66

Oliver B (1959) *Medicinal plant in Nigeria* . College of Art Science and Technology Ibadan, (article)

Odama L.E , Shok M. and Olurinola . (1986) *Ceiba pentandra* (silk cotton tree) . Microbiological evaluation of its use traditional wound treatment. In *State of Medicinal Plant Research in Nigeria* pp 253-259 Ed Sofowora A. pb Nigerian Society of Pharmacognosy. Ibadan University press.

Otto J. and M. Petrii. (1968). *Trichomonas vaginalis* and trichomoniasis. *Advances in parasitology* 6:117-178

Owosika Fola (1992) . What you should know about gonorrhoea. *Clin pharm & Herbal Med.* 1992; 8(687) :19-20

Paulo A; Duarte A. and Gomes E.T. (1993). " Antibiotic activity of some alkaloids isolated from Crystolepis sanginolenta ". *International Symposium on phyto-chemistry of plants used in Traditional Medicine.* Book of Abstract. Poster NS 108. Lausunme, September 1993.

Pousset J.L., Kerharo J. Maynar G. et. al. (1973). *La Borrevine_verticillata. Phytochemistry 12* : 1308-10

Pousset J.L. (1986). Perfection of Medicament based on traditional plants in the sahelian pharmacopoeia. In *State of Medicinal Plant Research in Nigeria.* PP. 351-354. Sofowora (Ed). Nig. *pharmacognocy* Soc Pub. Ibadan University Press.

Pousset J.L. (1988) Medicinal plant used as source of raw material for pharmaceutical Industry. Problem and prospect. In drug production from natural source. *Proceeding of 7th international Symposium of DUPU* (Ed Adesina S.K.) Ile Ife, Nigeria PP 37-41.

Principe p.p. (1989) The economic significance of plants and their constituents as Drugs In *Economic and Medicinal Plant Research* (Eds Wayner H, Hikino H and Farnworth N.R.) Academic Press London. Vol 3 PP 1- 17

Ray D.K., Charlefee D.K. and Stendukar J.S. (1982). Comparative Efficiency of GO10213 and some Nitroamidazole Against *Trichomonas vaginalis* and *Trichomonas foetus* in mice infected subcutaneously. An Trop. Med. Parasitol 1982 76 (2): 175-178.

Ren H. John, Rinaleli M and M.A. Pfalle (1995) Resistance of Candida species to Plucomazole *Antimicrobial Agent and Chemotherapy 1995. 39*(1) : 1 - 8

Ribet J. Chartol A. (1964) Les proprietés antidysenterique de *l'Euphorbia hirta. Med Trop Fr* 24(2) : 119-143

Russell Jackie and Botchan Michael (1995). Cis-Acting Component of Human papillomavirus (HPV). DNA Replication linker- stimulation. Analysis of HPV Type II origin. *Journal of Virology*.

Sainty D, Bailleal F., Dalaveau P. and Jacquemin H. (1981). Irrodoid of *Borreria verticillata. Planta Medica 42*: 260 - 264

Schalger T.A., T.S. Whittan and J.O. Hendley (1995). Comparison of expression of virulence by *E. coli* causing cystitis and *E. coli* colonising the periurethra of healthy girls *J. Infect Dis* ; 172(3) : 772-777

Schneider Herman, Alan S. Cross and Robert A Kuschner (1995). Experimental Human Gonococcal Urethritis 250 *Neisseria gonorrhoeae* M511 MKC are ineffective. *J. infect Dis* ; 172 (i):180-185

Seaman E. William (1987). Immuno-modulation in *Basic and Clinical Immunology*. 6th Edition pp228-240 Des D-P State,J.D. Stobo and J.V. Wells. A-Lange Medical Book.

Sinha A.M.K., Mitra C.R. and Bhutnag asc(1970) Chemical Examination of *Jatropha curcas. Indian Journal of Chemistry* 8(11). 1047-49

Sim S.K. (1971a). *Medicinal Plant Alkaloid.* University of Toronto press, Toronto and Buffalo.

Sim S.K. (1971b) *Medicinal plant glycosides*
University of Toronto press, Toronto & Buffalo (Article)

Slack R.B.C. (1992b) Diagnostic procedures in *Medical Microbiology* 14th Edition pp 749-758 (Eds) Greenwood *et. al.* ELBS Pub. Low Priced Edition.

Smith I.W. Chlamydiae . In *Medical Microbiology*. 14th Edition pp.435-446 David Greenwood *et. al.* (Eds). ELBS Pub. Low Priced Edition.

Stites Daniel P., Channy Rodgers (1987). Clinical Laboratory Methods for Detection of Antigens and Antibodies. In *Basic and Clinical Immunology* 6th Edition pp 241-284. (Eds) D.P. Stites, J.D. Stobo and J.V. Wells. A Lange medical book.

Solis Pablo N., Colin w. Wright, Margaret M.A, Mahabir P.O. and J.D. Phillipson (1993). A Micorwell cytotoxicity Assay using Artemi salina (Brine shrimp). *Planta Medica*. *59* : 250-252

Sofowora (1982) *Medicinal Plants and Traditional Medicine in Africa.* Published by John Wiley and Sond. New York, Toronto, Singapore.

Sofowora Abayomi (1993) *Medicinal Plant and Traditional Medicine in Africa* 2nd Edition Spectrum Books Ltd Ibadan, Owerri, Kaduna, Lagos.

Stanier Y. Roger, J.L. Ingrahana, Mark l. Wheelis and P.R. Painter. 1986. *General Microbiology* 5th Edition Macmillan Education Pub. London.

Taylor N (1965) Plant Drugs That Change The World. George Allen and Unwin Ltd. London

Thiedi A. Henry (1989). The prevalence of Urogynecologic disorder.

Obstet. Gynecol. Clinic. North America 16(4); 709-716

Thomason J.L. and Gelbart S.M. (1989). *Trichomonas vaginalis Obstet Gynecol 1989; 74*: 536

Trease and Evans (1975) . *Textbook of Pharmacognosy*. 11th Edition pp475-486 Baillerie Tindrall, London.

Trease G.E. and Evans W.C. 1989. *Pharmacognosy* 13th Edition Balliere Tindrall, London

Tunde Benjamin V. 1980. Analysis of volatile constituents of local plants used for skin diseases. *J. Afric. Med. Plant 3* : 135-139

Tsutomut, Sieki K, Masanobu T. and F. Tatsasho (1985). in vitro Inhibition of Giardia Lambia and Trichomonas vaginalis growth by Bithinol, Dichloropene and Hexachlorophene. *Antimicrobial agent and chemotherapy 1985 27*(1): 65-70

Uiso F.W. (1979). Traditional Medicine Research Unit, *Annual Report*. Muhimbili Medical Centre. University of Dar-es-salaam, Tanzania

Ummu-Kulthane Hassan Muhammad (1995). In *vitro Assessment of Antimicrobial properties of the extract of some local medicinal plants on agents of Gastro-enteritis*. MSc Thesis p.37

Varo E. Edward and Lynn R.B. 1970. *Pharmacognosy* 6th Edition Lea and Fibige Philadephia.

Voncir N.C., E.K. Penyong, I.K. Asharf and Ekwenchi M.M. (1986). Activity of extract of *Acacia polycanthi* on *Plasmodium falciparum* (malaria parasite). *In state of Medicinal Plant Research in Nigeria* pp 370 (Ed A. Sofawora) (Pub: Nig Soc Pharmacogn). Ibadan University Press.

Ward P.V. Coleman (1992). Papovaviruses in *Medical Microbiology* 14th Edition pp 519-526.(Ed)Greenwood *et. al.*ELBS Pub. Low Priced Edition.

Welsh Laura, Charlotte Gaydo and Thomas C. Quinn. 1996 In vitro activities of Azithromycon, Clarithomycin, Erythromycin and Tetracycline against 13 stains of Chlamydia pneumoniae. *Antimicrob Ag chemother 40*(1): 212-214

WHO (1991) Traditional Medicine and Modern health Care. *Progress Report by the Director General.* Doc No *al.4/10*, Maren 1991 - Geneva

WHO (1989) *Medicinal plants in China*. WHO regional office for Western Pacific, Manilla. (Article)

Wittington W., Robert M.C. Hale and K.K. Holmes (1995) Susceptibility of Neisseria gonorrhoeae to glyculciclines. *Antimicrobial Agent and Chemotherapy 39*(8): 1864-1865.

140 Wolner H. P. et. al. Vaginal trichomoniasis 1989. 261:541-576

Wong-Ting-Fook W. T. H. The *Medicinal Plants of Mauritius* ENDA Publication N° 10 Duka. (Article)

Zagrebina V. A. and Torchinov A. M. (1989) *Gynaecology*. Mir publishers Moscow.

APPENDIX I

Cultural Media

1. Trichomonas Medium (T.M.)

The Trichomonas Medium (oxoid) was modified by adding 0.1% (w/v) of sodium-thyoglycolate as reducing agent. Inactivated cow serum was used in lieu of horse serum. The pH was adjusted to 6.0 using concentrated hydrochloric acid. The medium was prepared as directed by the manufacturer.

2. Potato Dextrose Agar

The Potato Dextrose Agar was composed in our laboratory following Difco formula, i.e. for each 1000mL of medium, the composition was as follows:

- 1000mL of aqueous potato extract from 200grammes of potatoes
- Dextrose (Difco) 20 grammes
- Agar No3 (Oxoid) 15 grammes - pH 7.2

3. Potato Dextrose Broth

The Potato Dextrose was also composed in our laboratory following Difco formula:

i.e.

- 1000mL of aqueous potato extract from 2000 grammes of potato
- Dextrose (Difco) 20 grammes

4. Modified blood enriched Mueller Hinton Broth

Mueller Hinton Broth (Oxoid) enriched with 5% (v/v) sterile blood and an additional 0.1% w/v of sodium thioglycolate was used.

APPENDIX II

Determination of aqueous extracts concentrations

10 millimetres of extract was allowed to evaporate till dryness at 40°C in a pre-weighed watch glass. The mass of the glass plus the dry matter was determined by weighing. The concentration of extract was calculated as follows:

$$C = \frac{M - m}{10}$$

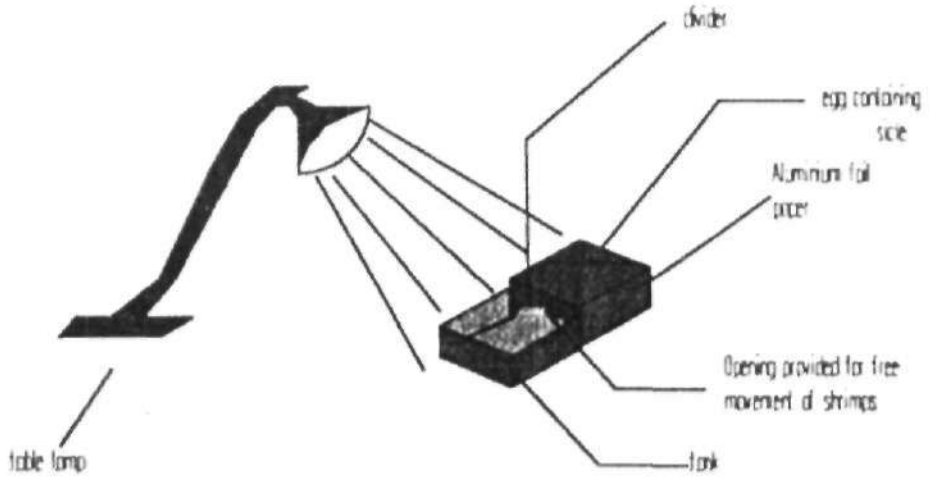
where C = concentration in mg/mL

M = mass of the watch glass plus dry matter (mg)

m = mass of empty glass (in mg)

APPENDIX III

Design of the tank used for toxicity test.



APPENDIX IV

The plants, their common names and the plant part used in this study.

Name of plant	Family	Common names	Plant part used
Abrus precatorius	*Papilonaceae*	Indian Luquorice	1 - stem
		Idom Zakaru (Hausa)	2 - seed
		Ojuologba (Yoruba)	3 - leaf
Cassia alata	*Ceasalpinaceae*	Uma ahia (Igbo)	1 - leaf
Borreria verticillata	*Rubiaceae*	Green Borreria	1 - whole plant
		African Borreria	
Euphorbia hirta	*Euphorbiaceae*	Asthma herb	1 - whole plant
		Nanan Kur Chiya (Hausa)	
Jatropha curcas	*Euphorbiaceae*	pig nut	1 - stem
		chini da zuga (Hausa)	2 - leaf
Khaya senegalensis	*Meliaceae*	Dry zone Mahogany	1 - stem bark
		Madachi (Hausa)	2 - leaf
Parkia biglobosa	*Mimosaceae*	African locust bean	1 - stem bark
		Daudawa (Hausa)	
Securidaca longipedanculata	*Polygalaceae*	Sanya (Hausa)	1 - leaf plus fruit