SCREENING OF SOME NIGERIAN HERBS FOR POTENCY AGAINST SELECTED DISEASE CAUSING MICROORGANISMS

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(Ph.D/SSSE/68/97/98)

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MINNA.

FEBRUARY 2005

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA. NIGERIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) IN MICROBIOLOGY (PHARMACEUTICAL MICROBIOLOGY OPTION)

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CERTIFICATION

This Thesis titled" Screening of some Nigerian herbs for potency against selected disease causing Microorganisms" meets the regulations governing the award of the degree of Doctor of Philosophy in Microbiology (Pharmaceutical Microbiology Option), Federal University of Technology, Minna and is approved for its contribution to knowledge and literary presentation.

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DECLARATION

I declare that this is an original record of work conducted by Augustine Egbukwe Uwabujo in the Department of Microbiology, School of Science and Science Education, Federal University of Technology, Minna. Nigeria

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DEDICATION

This research project IS dedicated to Almighty God for his mercies, protections, provisions and blessings throughout my period for the award of Doctor of Philosophy (Ph.D) in the Department of Microbiology, Federal University of Technology, Minna. Nigeria. Also it is dedicated to my beloved wife Mrs. Maureen Ngozi Uwabujo and my Children Emmanuel and Esther for their understanding and encouragement.

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ABSTRACT

The emergence of incurable diseases, the resurrection of eradicated diseases, the drawbacks of the much cherished antibiotics and the success rate of traditional medicinal preparations have rekindled interest in plant-derived antimicrobial compounds to control human infections. The roots, stems and leaves of the four medicinal plants *Senna alata* (Gungoroko - Nupe), *Calotropis procera* (Ekopo - Nupe), *Schwenkia americana* (Rogwogi - Nupe) and *Merremia tridentata* subspecies *angustifolia* (Esakaregi - Nupe) used by herbalists for the treatment of various microbial infections in Bida, were screened for their antimicrobial activities. Phytochemical analysis of the plants revealed the presence of resins in n-hexane extracts of *Senna alata, Calotropis procera, Schwenkia americana* and *Merremia tridentata* subspecies *angustifolia.* The ethnol extract had alkaloids, tannins, saponins and polyphenols, The water extracts of *Calotropis procera, Schwenkia americana* and *Merremia tridentata* subspecies *angustifolia* contained alkaloids, tannins, saponins and volatile oils. Anthraquinones, cardiacglycosides. polyphenols and phlobatanins were also found in appreciable quantity in ethanol extracts of *Senna alata.* N-hexane extract from *Senna alata* root showed appreciable antibacterial activity against *Bacillus subtilis* and *Escherichia coli* at 2000 us/ml while the root and leaf extracts showed appreciable antifungal activity against *Trichophyton mentagrophyte, Microsporium canis* and *Candida albieans.* The root and stem n-hexane extracts of *Calotropis proeera* at 2000 *llg/ml* showed appreciable activity against *Microsporium canis and Epidermophytonflocossun* respectively. The root and leaf enthanol extracts of *Senna alata* at 2000 *llg/ml* showed appreciable activity against *Epidermophyton flocossun* and *Candida albicans.* The leaf ethanol extract of *Calotropis proeera* at

1000 Ilg/ml showed appreciable activity against *Microsporium canis* and *Epidermophyton jlocossun* while at 2000 Ilg/ml it exhibited appreciable activity against all the test fungal except *Candida albicans.* The leaf ethanol extracts of *Schwenkia americana* and *Merremia tridentata* subspecies *angustifolia* showed appreciable activity against *Candida albicans.* Partially purified extracts (the bioactive freactions) from accelerated gradient chromatography ad flash column chromatography were more active than the crude. While some were bacteriostatic or fungistatic, others were bactericidal or fungicidal. The MIC and MFC (lOOOllg/ml- 2000llg/ml) were low, an indicative of increased activity of the extract. The regression output of the rate of killing of the organism by the extracts revealed high death rate. The effect of temperature on the activity of the extracts showed that the *Senna alata* and *Calotropis procera* can stand fairly high temperature without losing their antimicrobial activities compared with other tested extracts. The antimicrobial activity on different storage time of the extracts showed that *Senna alata* and *Calotropis procera* extracts could be stored for about 30 months without losing their antimicrobial properties. Some of these results obtained in this work confirm the efficacy of the extracts of the plant parts in the treatment of many microbial infections.

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

Life, disease and decay are inseparable. From his first awakening, man had sought to fight and control diseases and pains with assistance, inspiration and guidance from nature. During the thousands of years of early human existence, many plant materials by instinct, intuition or trial and error were used to combat various ailments. Even in the present day, medicinal plants are widely used by all sections of people especially in remote rural areas where modern medical facilities are inadequate either directly as folk remedies, or indirectly in the pharmaceutical preparations of modern medicines to diagnose, cure or prevent diseases such as malaria, jaundice, cough, chronic ulcers, diarrhoea, dysentery, leprosy, piles, skin disease, chronic bronchitis, syphilis typhoid fever, whooping cough, gastro-urinary diseases and sore throat (Sofowora, 1984).

As ideas of different tribes, communities and cultures permeated each other; the use of medicinal plants became widespread, occasionally being intermixed with religion, rituals and magic. Almost all ancient cultures and communities evolved their own plant medica or folklore (Akpulu *et al.,* 1994, AbdulGani and Amin 1997).

The use of traditional remedies has relied exclusively on past experience and observations handed down from generation to generation verbally or in recent times in writing (Sofowora, 1982). In Nigeria there is tremendous application of these local remedies as alternatives to expensive imported drugs. Medicinal properties of plants are normally dependent on the presence of certain phytochemical bases such as alkaloids, tannins, anthraquinones, cardiac

glycosides, saponins, and polyphenols (Harbore, 1973; Trease and Evans, 1978; Sofowora, 1982; Uwabujo and Adebisi 200 I; Moody *et a12003;* Onocha *et et al et al*

A survey by the United Nations Commission for Trade and Development (UNCTAD) has shown that 33% of total drugs produced by the industrialized nations are plant-derived and when microbes are considered, 60% of medicinal products are of natural origin (UNCTAD, 1974). Rigveda mentioned 67 plants having therapeutic effects, Yajurveda listed 81 plants and Atharveda, 200 plants (Nabaehandra and Manjula, 1992).

A medicinal plant, according to Sofowora (1982) is one, which in one or more of its organs contain substances that can be used for therapeutic purposes. It may be in the form of vegetable drugs, which may either be organized (material which possess a cellular structure e.g. leaf, bark, petal, root etc) or unorganized (a cellular structural medicinal agents such as gums, latex etc). It may be a decoction, which may be in cold water or prepared by bringing it to the boil and allowing it to cool for about 15 minutes, or Tisane, which is a tea made by either decoction or infusion (Trease and Evans, 1978).

Studies have shown that medicinal plants and fruits possess antimicrobial activity. Any chemical substance inhibiting the growth or causing the death of microorganisms is known as an antimicrobial agent and it is said to possess antimicrobial activity (Wistreich and Lechtman, 1988). The range of organisms sensitive to an antimicrobial agent is known as the antimicrobial spectrum.

A wide variety of microorganisms are known to be capable of producing substances, which are inhibitory to other organisms Hugo and Russell, (1983). Some Gram negative organisms e.g *Pseudomonas* and *Proteus* have been known to be resistant to some commonly used drugs and even those organisms that are normally susceptible to these marketed drugs are constantly turning resistant e.g Staphylococci. Drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Piddock and Wise, 1989; Singh *et al., 1992;* Mulligen *et al.,* 1993; Davis, 1994; Robin *et al.,* 1998). However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug resistant bacterial and fungal pathogens have further complicated treatment of infectious diseases in immunocompromised AIDS and cancer patients (Rinaldi, 1991; Diamond, 1993).

Various antibiotics and antimicrobial agents are known to be effective against some microorganisms and, according to Varro *et al.* (1981) antibiotics probably represent the greatest single contribution of drugs therapy in the past half century, a period characterized by unprecedented advancements in health care. This group of drugs provide effective control of many human microbial pathogens that previously cause prolonged incapacitation of health without appreciable regard for age, economic status or physical fitness.

Varro *et al.,* (1981) and Hugo and Russell, (1983) showed that antibiotics and synthetic compounds though inhibitory to pathogenic microorganisms of mammals both in tissue cultures and on growing media, have limitations in the treatment of infections. The main problem is lack of selective toxicity possessed by most compounds. Apart from this limitation to the use of antibiotics, these workers further r~ported that microorganisms are capable of establishing resistance towards substances, whicli have a selective lethal or inhibitory action

upon them. According to the authors, resistance is a temporary or permanent ability of an organism and its progeny to remain viable under environmental conditions that should normally destroy or inhibit other cells. Each population of microorganisms contains a range of sensitivities towards antimicrobial agents e.g. some are very sensitive, some are moderately sensitive and others have absolute resistance. Hugo and Russell (1983) also noted that if a population is exposed to unfavourable conditions, a population of exclusively resistant organism will arise by natural selection. Some bacterial species, because of their inherent structural features possess an intrinsic resistance e.g Gram-negative organisms and Myeobacteria.

Marques and Congregado (1979) reported that *Pseudomonas aeruginosa* isolated from the soil showed resitance towards antibiotics and heavy metals. The authors noted that out of the total of 71 strains which were isolated and tested for resistance to 14 drugs (Tobramycin, Fosfomycin, Colistin, Ampicilli, Cephalosporin, Furadantin, Chloramphenicol, Nalidixic acid, Neomycin, Steptomycin, Tetracycline, Sulphadiazine, Kanamycin and Gentamycin) along with twelve heavy metals, 6% of the strains were found to be resistant to 12 drugs and nine heavy metals. Threes strains of *Enterobacter* were studied for their response to Ampicillin and were found to exhibit a basic level of resistance that depended on medium used and high-level mutational resistance at a frequency of 10^{-5} to 10^{-7} (Marques and Congregado, 1979).

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Lyengar, 1985; Chopra *et al,* 1992; Harbone and Baxter, 1995). The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. In recent years,

antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Grosvenor *et al,* 1995; Ratnakar and Murthy, 1995; Silva *et al, 1996;* David 1997; Saxena 1997; Nimri *et al,* 1999; Sexena and Sharma, 1999.). It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants (Hasegawa *et al.,* 1995; Lee *et al.,* 1998). In the present study, four Nigerian medicinal plants were screened against multi-drug resistant bacteria and fungi.

The selection of the medicinal plants is based on their traditional uses. The pathogenic organisms were selected for the study on the basis of the clinical and pharmaceutical importance as well as for their potential to cause contamination of food and drugs.

1.1 JUSTIFICATION OF STUDY

It has been established that a large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of diseases. It has also been established that several hundred genera of plants are used medicinally, mainly as herbal preparations in the indigenous systems of medicine in different countries and are sources of very potent and powerful drugs, which have stood the test of time, and modem chemistry has not been able to replace most of them. The World Health Organisation reported that 80% of the world's populations rely chiefly on traditional medicine and a major part of the traditional therapies involve the use of plant extracts or their active constituents (WHO, 1993). Due to the indiscriminate use of antimicrobial drugs, the microorganisms have developed resistance to many antibiotics. This has created immense clinical problem in the treatment of infectious diseases (Davis, 1994). In addition to this problem, antibiotics are sometimes associated with adverse effects on host, which include hypersensitivities, depletion of beneficial gut and mucosal microorganisms, inmunosuppression and allergic reactions (Idose *et al., 1968).* Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. One approach is to screen local medicinal plants for possible antimicrobial properties (Irobi, 1992; Olorundare *et al.,* 1992; Alade and Irobi, 1993).

Medicinal herbs represent a rich source from which novel antibacterial and antifungal chemotherapeutic agents may be obtained. Plants have been tested against various pathogenic microorganisms because they contain active components, which are effective in controlling microbial diseases of man and animals. However, not much work has been done on some

plant species. Therefore, there is need to expand work to address plant species with little or no work done on them.

The search for sources capable of yielding new antimicrobial agents will continue for the following reasons

(a) To obtain less expensive drugs that possess greater resistance to the beta-lactamases

(b) To produce simpler methods of administration with increased activity and stability; better diffusability into remote areas of the body and slower rates of excretion.

(c) To provide greater selective toxicity.

Thus, from the tremendous successes recorded by herbalists and tradomedical practitioners (Sofowora, 1984) in curing systemic and other microbial infection using plant materials, there is an urgent need for intensive research for clinically effective medicinal plants. It is therefore necessary that man goes back to nature for these antimicrobial agents. A total of 4 plants from 4 families, selected on the basis of medicinal folklore reports and their common use in Nigerian traditional systems of medicine (Watt, 1889; Trimen, 1995; Irvine, 1961; Rahman, 1991; Agoha, 1974) were studied for their antimicrobial properties. These plant species include *Senna alata, Calotropis procera, Schwenkia americana* and *Merremia tridentata* subspecies *angusitifolia*. Claims of effective therapy for the treatment of leprosy, asthma, cough, wound disinfectant, swelling and rheumatic pains, syphilis, skin infection, ringworm, conjunctivitis, dysente~y and gonorrhoea by traditional herbalists in Nigeria have prompted our interest in the scientific investigation of such herbal medications.

The choice of microorganisms for the susceptibility testing was based on their clinical and pharmaceutical importance such as the ability to cause skin infection, typhoid fever, food poisoning, multidrug resistance and sexually transmitted diseases. The organisms are *Bacillus subtilis, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Candida albicans, Trichophyton mentagrophyte, Trichophyton rubrum, Mircosporium canis* and *Epidermophytonjlocossun.*

1.2 OBJECTIVE OF THE STUDY

The objectives are:

1. To screen some herbs for antimicrobial properties with the view of establishing the in-vitro activities
11. To prepare fractions of each effective extract to determine the bioactive fractions
111. To characterize and identify the partially purified bioactive fractions of each herb using thin layer chromatography (TLC).
IV. To determine the spectrum of activity of the partially purified bioactive fractions.
v. To compare the antimicrobial activities of the plant extracts with known antibiotics using minimum inhibitory concentration (MIC) values in order to justify their uses in traditional medicine.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General

Antimicrobial compounds are known to be present in the extracts of different tissues of some medicinal plants (Sofowora, 1982). Protocatechuric acid, thurjic acid and chromgenic acid are some examples of phytoacids capable of inhibiting phytopathogenic fungi (Horowitz 1948). Horowizt, (1948) reported that comavantine, an amino acid isolated from Jack beans has antimicrobial activity against most species of *Neurospora.* In an effort to finding alternative antimicrobial substances for the treatment of infection in the light of growing cases of microbial resistance to antibiotics, one approach has been to screen local herbs for possible antimicrobial activities (Irobi, 1992; Olorundare *et al.,* 1992). Alade and Irobi (1993) indicated that certain medicinal plants used in Nigeria folklore medicinal practice possess antimicrobial properties.

Mamedov *et al* (2005) reported that 2,500 plants are known in Russia and central Asia for the treatment of allergic rashes, skin, irritations, boils, wounds dermatitis and pyoderma Fadeyi and Akpan (1979) have reported the antimicrobial activities of leaf extracts of *Eugenia uniflora* against *Staphylococcus aureus, Shigella dysenteriae, Escherichia* and *Bacillus subtilis.* Mann *et al.* (1997) in their study noted that the extracts of *Calotropis procera* showed strong antimicrobial activity against *Clostridium perfringens, Streptococcus faecalis* and *Salmonella typhi.* Igoli *et al* (2004). Reported in their ethnobotanical survey that over 30 taxa of plants were used in traditional medicine Fleurentin and Pelt (1982); Schopen (1983) working on the ethanol leaf extract of *Calotropis procera_reported* its use as antiseptic for skin infection. Awadh *et al* (2001) reported that the ethanol leaf extracts of *Calotropis*

procera, Chenopodium murale, Pulicaria orientalis, Tribulus terrestris and *Withamia somniferurn* displayed a remarkable antibacterial activity against both Gram positi *ve* and Gram-negative bacteria. In their preliminary studies on the analgesic activity of latex of *Calotropis pro cera,* Dewan *et al* (2000) reported that the analgesic effect of dry latex (DL) was delayed by 1h by naloxone at a dose of 0.5mg/kg i.p. which completely blocked the analgesic effect of morphine (10mg/kg i.p.). Also Ikenebomeh and Matitire (1988) reported the antimicrobial activity of *Senna alata* against some bacteria and fungi. Adedayo *et al.* (1999) reported the antifungal properties of some components of *Senna alata* flower using methanolic crude extract and the partially purified fractions. The authors observed the minimum inhibitory concentration of the methanolic crude extract was low for all the fungal strains except *Aspergillus niger* with 5.0 mg/mL concentration, which the minimum inhibitory concentration of partially purified components (C and F) ranged between 0.112 mg/mL for extract F against *Penicillium* species and 2.5 mg/ml for extract C against *Aspergillus niger, Penicillium* species and *Candida utilis.* The authors also observed that the minimum fungicidal concentration of the crude methanolic extract was higher than 5mg/ml for most of the fungi but the partially purified extract exhibited fungicidal concentration ranging from 2.5 mg/rnl to 5.0 mg/ml.

Barerjee and Sen (1980) found that the water and ethanol extracts of pteridophytes (from 67 ferns and 6 ferns allies), assayed against 3 Gram-positive and 5 Gram-negative bacteria and 3 fungal plant pathogens were active. Okonkwo (2005). reported that plant material from families Annonaceae, Piperaceae and Rutaceae are used for the protection of stored products against pests in Nigeria.

Ebana *et al,* (1991) reported that aqueous and ethanol extracts as well as alkaloids and cardiac glycosides of *Garcina kola, Borreria ocymoides, Kola nitida* and *Citrus aurantifolia* inhibited the growth of *Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa,* ~- haemolytic streptococci, *Escherichia coli* and *Neisseria gonorrhoea.* Similarly, Ebana *et al* (1993) also reported that *Strophantus hipids* and *Secamore afzeli* are used by many people in South Eastern Nigeria for the treatment of various diseases including rheumatism, stomach ache, malarial and veneral diseases. The organisms inhibited included *Neisseria gonorrhoea, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes* and *Proteus mirabilis.*

Jovad *et al,* (2004) reported that single and repeated oral administration of the aqueous extract of *Eucalyptus globulus* leaf at the dose of 150 and 300 mg/kg body weight exhibited a significant, dose-dependent hypoglycemic effect in streptozotocin diabetic rats. The authors also observed that there was no significant changes in blood glucose levels in rats with a normal functioning pancreas after a single or repeated oral administration of *Eucalyptus globulus* leaf extract. Kazuaki and Shinji (1987) reported that *Exostema caribasum* is a tropical plant found in West Indies, Mexico and Costa Rica and the stem bark of this plant is used in folk medicine as substitute for quinine. Quinine is also contained in the bark of trees of the *Cinchona* species, and was reported by Swaminathan and Koehler (1987) as an antimalarial drug of the Peruvian Indians. Alison and James (1993) also reported the antifungal activity of the major glyco-alkaloids, 2-solanine and 2-chaconine against *Ascobolus cranulatus, Alternaria brassicola, Phoma madicaginis* and *Rhizoctonia solani.* The same workers also observed that the methanol extract of fresh hulls of *Tabarnamontana. crocosmas*

flora exhibited antimicrobial activity against some Gram-positive bacteria, fungi and yeasts. Ethanol extracts of the root bark of *Terminelia chippa* showed strong antimicrobial activity against both Gram-positive and Gram-negative bacteria and weak antifungal and anti-yeast activity. In their work, Terries and Andres (1985) indicated that ethanol extracts of the root of *Sassafras randiasa* exhibited antimicorbial activity against a number of microorganisms. Kazuhiro *et al* (1993) indicated that the methanol extract (50 ppm) of the leaves of *Pispanes malanophlosus* had molluscidal activity against the schistosomiasis transmitting snail *Biomphalaria glabrata* and displayed antifungal activity against the plant pathogenic fungus *Cladosporium cucumerium.* It has been reported that cyclopeptides derived from *Zizyphna lotus* have antibacterial and antifungal properties and that a mixture of dried ground leaves and fruits are applied topically in the treatment of boils while the root bark is renowned for antidiabetic activity (Kamel and Rachid, 1993).

Emeruwa (1982) studied the antibacterial substance from *Carica papaya* fruit using the ripe and unripe *Carica papaya* fruits (epicarp, endocarp, seeds and leaves) extract and found that all the extracts except that of leaves produced strong antibacterial activity against *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Shigella jlexugri.* The investigator observed that the minimum inhibitory concentration (M.I.e) of the substance was small (0.2-0.3 mg/ml) for Gram-positive bacteria and large (1.5-4.0 mg/ml) for Gram-negative bacteria. In addition, the researcher noted that the substance was bactericidal and showed the property of a protein. Zavala *et al* (2004). reported that both the aqueous and methanol extracts of *Acalypha aff. Mollis* inhibited castor oil induced diarrhoea in mice but failed to inhibit mg so4 induced diarrhoea. The authors also observed that maximum inhibition for aqueous and methanol extracts was 54.35% and 30.57%

respectively. Ogboli *et al* (2000) reported the curative and prophylactic effects of petroleum ether and ethanolic leaf extracts of *Vernona amygdalina* extracts in the treatment of Schistosomiasis in mice. They found in their result that the non effected, non treated mice (negative control)T showed no sign of infection while the infected but not treated (positive control) were anaemic with severe enteritis, enlarged and conjected kidney, liver and spleen with areas of necrosis. The work of Bonavaenture *et al* (1993) showed that crude extracts of *Hoslundia opposita* possess strong antibacterial activity. Also the crude extract of *Uvaria* species which was screened for in-vitro activity against the multidrug resistance Kl strains of *Plasmodiumfalciparum* showed antimalarial activity (Majunga *et aI1993).*

Idowu *et al* (2003) reported that Eleagine is the main antibacterial compound in the *Chryso phylum albidum* seed cotyledon with wide spectrum of activity and *MIC* (250 -500) j.l9 *lml.* Aqueous extracts of *Podopyllum pettatum* was reported by Bedow (1982) to show antiviral activity against simplex (Type II) influenza A, vassinia viruses and measles virus. Oresanya *et al* (2000) reported that the physical characteristics of edible oil extracted from Guna melon *(Citrullus colocynthis)* when compared with *Citrullus lanatus* and *Citrullus valgaris* types showed appreciable differences (P< 0.01). The investigation of Irobi, (1992) showed the activities of *Chromolaena odorata* methanol leaf extract against *Pseudomonas aeruginosa* and *Streptococcus faecalis.* He noted that the minimum inhibitory concentration was high for *Pseudomonas aeruginosa (8.0mg/ml),* a Gram- negative organism and some what less than (6.0mglml) for *Streptococcus faecalis,* a Gram positive organism. Similarly, Irobi *et ai,* (1992) studied the antifungal activities of crude extracts of *Mitracarpus villous* leaves and inflorescences, using the Agar-diffusion and tube dilution techniques and reported that ethanol extracts produced definite antifungal activities against *Trichophyton rubrum,*

Microsporum gypsum, Candida albicans, Aspergillus niger and *Fusarium solani.* The aqueous extracts and the glycerol vehicle control did not inhibit any of the fungi tested.

Oyewole *et al* (2000) reported the lavicidal properties of aqueous extracts of the leaves, stem and root bark of *Senna didymobtrya* agains the malaria vector under physiological conditions. The authors observed that early larval stages were prone to the lethal effects of the plant extracts prossessed the strongest larvicidal activity. The antimicrobial activity of *Zanthorytum zanthoxyloides* (Fagara) has been shown to be due to benzoic acid derivatives (Odebiyi and Sofowora, 1978). Muyibi *et al* (2000) reported that the aqueous leaf extract of *Senna occidentalis* produced appreciable decreases in the packed cell volum (PCV), haemoglobin (Hb) concentration and total red blood cell count (RBC) ($P < 0.05$) but did not influncethe white blood cell (WBC). They observed similarly that the extract produced histopathological lesions of the liver and kidney. Dalziel, (1956) reported that *Ocimum gratissimum* which is rich in volatile oils (which contain up to 75% of thymol) has some antimicrobial activity. The extracts of the flowering and fruiting parts of *Euphorbia hirta* are used for asthma and respiratory tract inflammations and are sometimes combined with bronchiole sedatives like *Grindelia robusta* in preparation for inhalation (Oliver, 1960).

Ekanem (1978) indicated that *Azadirachta indica* (neem) cures malaria fever. The investigator observed a fall in parasite count in chloroquine sensitive strains of *Plasmodium bervhi* in infected mice when treated with extracts of the leaves. Okpanyi and Ezeukwu (1978) demonstrated that extract of the leaves and bark shows antipyretic effects thus providing some justification for its use in treating malaria fever. The antimicrobial properties of -neem, which shows therapeutic value, have also been extended for preservation of

materials of economic importance. Ibrahim *et al* (1987) studied the storage of yam tubers and showed that yams treated with neem bark water extract, neem bark slurry and neem leaves have been stored for six months. The study further showed that rot in yams treated with neem bark extracts was delayed for three months. The wood ash from neem tree has been shown to suppress rot of yam during storage for twenty-four weeks (Williams and Akano, 1985). Several studies have been conducted using different spices to inhibit the growth of pathogenic organisms. Dhar *et al,* (1998) indicated that neem *Azadirachta indica* seeds and purified fractions are active not only against the parasite stages that cause the clinical infection but also against the stages responsible for continued malaria transmission. The bacteriostatic and bactericidal activities of twenty-two essential oil from Turkish spices and citrus were tested against *Aerobacter aerogenes, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus albus* and *Staphylococus aureus* were recorded by Kivano and Akgul, (1986). The results showed that the essential oils tested varied in their antibacterial activity. Aniser celery, parslus and sage were active against all tested bacteria to varying extent (Kivano and Akgul, 1~86). *Staphylococcus aureus* and *Pseudomonas vulgaris* were the most sensitive organisms while *Pseudomonas aeruginosa* was the most resistant except towards thipne oil.

The antibacterial and antifungal effectiveness of the components of the spices *Aframomum melegueta* have been studied (Oloke and Kolawole, 1987). They reported that the crude extracts of parasols and shogoals and the volatile oil sho:ved considerable fungicidal activities against *Candida albicans, Trichophyton mentagrophytes, Aspergillus niger, Botrydiploidia theobromae* and species of *Cladosporium cladosporiodes.* Poh (2000) reported the antibacterial activity of *Salada pyriformis* using the disc diffusion method against *Proteus*

mirabilis, and *E. coli* at 100 *llg/ml.* Nnaja, (1995) found that the methanol extracts of *Newbouldia laevis* and *Aspilia africana* posses some antimicrobial activity against *Streptococcus faecalis, Clostridium tetani, Clostridium perfringens, Nocardia asteriodes, Serratia marcescens* and *Proteus mirabilis.* He noted that antibacterial activity of the extracts against Gram-positive bacteria suggest that there is a scientific basis for their utility in traditional medicine for the treatment of wounds and other infections. He further observed that high minimum inhibitory concentration MIC *(0.9mg/ml)* was required by the extracts of stem bark of *Newbouldia laevis* to inhibit *Clostridium tetani.* Ramos *et al,* (1996) reported the genotoxic activity of aqueous and ethanol extracts from 13 medicinal plants as folk medicine in Cuba. Their findings showed that only aqueous extracts of one of the plants *(Momordica charantiai* exhibited appreciable increase in the frequency of segregant sectors per colony.

Adekunle (2000) reported the antifungal property of ethanol and water crude extracts of bark of *Brachystegia eurycoma* and leaf of *Richardia brasiliensis using disc diffusion agar method.* They observed that the extracts inhibited *A. favus, A. fumigatus, E. jlocossun, Fusarium solani, Mucor mucedo, M audonii and Trichophyton verrucosum* with greater activity for ethanol extract. In their invitro study of antimicrobial activity of South African plants used for medicinal purposes, Tonia and Johannes (1997) reported that the majority of the antimicrobial activity was present in the methanol rather than aqueous extracts. Iqbal *et al,* (1998) screened some medicinal plants for invitro antimicrobial properties using agar well diffusion method at sample concentration of *200llg/mi* and found that ethanol extracts showed greater activity than their corresponding aqueous hexane extracts.

Catalano *et al,* (1998) reported the biological activity of methanol and water extracts of *Mutisia amminata* against some bacteria and fungi. Ajaiyeoba *et al,* (1998) worked on crude extracts from leaves, stem bark and roots of *Ritchiea capparoides* val'. *longipedicellata* for invitro antifungal activity using the agar tube dilution method and reported that at 400mg/ml, all the four extracts inhibited the growth of six of the test fungi used. Amabeoku *et al, (1998)* reported the antimicrobial and anticonvulsant activities of *Viscum capense* and established that the plant had activity against *Staphylococcus aureus*. Doris *et al,* (1998) reported that *Chromolaena moritziana* is an important plant used in local ethnomedicine as an anticarrhal, a depurative and against *Staphylococcus aureus* responsible for skin infection. Mann and Okwu:e, (1999) reported that the stem bark of *Commiphora Ker-tsinti* could be used in genito-urinary infections and other related diseases in Nigeria. From their results ethanol extract of the stem bark showed invitro activity against *Neisseriae gonorrhoeae, Staphylococcus aureus, Clostridium perfringens* and *Streptococcus faecalis* at a concentration of 100mg/ml.

Ekpendu *et al* (2001) reported that the volatile oil from *Spermacoce, verticillata* showed appreciable antimicrobial activities against Gram negative, Gram- positive and some fungi. Oladosu (2001) on his study on the bioactive guided fractionation of *Parkia biglobosa* on caged laboratory bred snail infected with Schistosma larvae the cercaria reported treament with methanol soluble fraction resulted in a remankable decrease of cercariael infection. that The antifungal activity of the leaf and bulb extracts and the alkaloidal fraction of *Cranium jagus,* a plant commonly used in traditional medicine was studied (Oliver, 1960; Ajayi *et al,* 1999). The investigators found that the ethanol extracts of both the leaf and bulb inhibited the growth of *Candida albicans, Candida neoformans, Aspergillus niger* and *Aspergilus*

fumigatus. Grann and Deniello (1999) revealed the antimicrobial activity of an aqueous extract of three Brazilian medicinal plants using plate count, disk inhibition zone and turbidity techniques. They noted that *Goiaba* leaf extract showed good antimicrobial activity against *Staphylococcus aureus.* Asima and Adelheid (1999) reported the antimicrobial efficacy of steroid alkaloids from the stem bark of *Holarrhena pubescens* against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus faecalis, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.* They found that the crude methanol extract of the plant inhibited all the test organisms.

In their studies Offiah and Chikwendu (1999) reported the anti diarrhoeal effects of the aqueous extract of the leaves of *Ocimum gratissimum.* They noted that the extract inhibited castor oil-induced diarrhoea in rats as judged by a decrease in the number of wet faeces in the extract treated rats. Agarwal *et al.* (2000) reported the antifungal activity of anthraquinone derivatives from *Rheum emodi.* They observed that the extract exhibited activity against *Candida albicans, Cryptococcus neoformans, Trichophyton mentagrophytes* and *Aspegillus fumigatus.* In their own contribution, Lall and Meyer (2000) reported the antibacterial activity of water and acetone extracts of the roots of *Euclea natalensis* in invitro studies. They observed that the water and acetone extracts inhibited the growth of *Bacillus cereus, Bacillus rumilus, Bacillus subtilis, Micrococcus kristinae* and *Staphylococcus aureus* at concentration ranging between 0.1 and 6.0mg/ml. Hernandez *et al.* (2000) in their invitro antimicrobial activity studies of flavonoids in medicinal plants from Argentina reported that five native species from Tafi del valle showed antimicrobial activity against Gram-negative and Gram-positive microorganisms. The crude methanol extracts of *Garcinia atroviridis* exhibited antimicrobial, antioxidnat, antitumour-prornoting and Brime shrimp toxic activities Mackeen

et al, (2000). Iqbal and Arina, (2001) in their invitro antimicrobial .ind phytochemical studies of some Indian medicinal plants against multi-drug resistant human pathogens reported that extracts from twelve plant species exhibited broad spectrum antimicrobial activity against *Staphylococcus aureus, Salmonella paratyphi. Shigella dysenteriae, Escherichia coli, Bacillus subtilis* and *Candida albicans.* Srinivasan *et al,* (2001) worked on some medicinal plants used for treating various prevailing diseases in India and reported that about 22 plant extracts from 15 families exhibited activity against both Gram-positive and Gram-negative bacteria while 8 plant extracts belonging to 7 families exhibited both antibacterial and antifungal activity.

Onwkaeme and Udoh (2000) reported the pharmacognostic antidiarrhoeal portentials of leafs of Ficus thonningii using rats. They observed that in all the doses applied, there was an initial increase in purgation. by the 2h of the test but this was followed by constipation. Aderemi *et al* (2000) reported the pharmacopoeial standards for the fruits of *Senna fistula and Senna podocarpa* and observed that both pods of the two *Senna* species containd free and combined (O and C glycosides) anthraquinones. Akinloye and Olrede (2000) reported the effect of *Amaranthus spinosus* leaf extract on haematology and serum chemistry on rats. The authors observed that the aqueous extract of *Amarathus spinosus* leaf produced no appreciable difference (P<O .05) in the value of haematological parameters such as total red blood cell (RBC),

Agunu *et al* (2003) erported that the methanolic extract of stem bark of *Steganoteania araliacea* when administered (i.p.) in male rat showed relative toxicity in the order: intestine > liver> kidney but no acute toxic effect was seen in the pancreas, spleen and stomach. Gupta

et al (2003) reported that the antioxidant property of the methanol extract of *Acalypha fruticosa* in 1, 1, - dipehny I -2- picryl hydrazyl radical scarvenging assay LC_{so} was 57 1l9/ml while that of the ascorbic acid was 42 ~l9/ml . Rajikimar and Jebanesan (2003) reported that alkaloidal extract from *Murriaya loenigii* showed oviposition activity at 250 - 1000 ppm concentration and ovicidal activity at 25 - 100 ppm concentration against *Culex quinquefasitus* and *Culex tritaeniorhynchus.*

Ram. *et al* (2003) reported that the latex of *Codiaeum variegatum* had high molluscidal activity against freshwater snail *Lymnaea acumin.* Moody *et al* (2003) reported that both the aqueous and ethanolic leaf extracts of *Terminalia catappa .exhibited* highest antisickling acitivity compared with other leaf colours. Yusuf *et al* (2000) reported that intraperitoneal injection of *Aloe vera* ethanolic extract on acute gastric mucosal leisons in rats induced by O.OMHCI and acid output inhibited gastric acid secretion. Onucha *et al* (2003) reported tha the leaf methanol extract of *phyllanthus amarus* and the leaf chloroform extract of *Phyllanthus muellerianus* exhibited antimicrobial properties. The authors observed that the . method extract of *P. amarus* displayed antifungal activities against *Candida albicans* and inhibited the growth of *Staphylococcus aureus, Streptococcus pyrogenas, E. coli* and *Proteus* species exhibiting greater antimicrobal acitvity with 3 micro organisms than the reference compound they noted on the ohtre hand that the chloroform extract of *P. muellerianus* displayed sensitivity higher than *P.amarus* against *Candida albicans* but inhibited only *Staphylococcus aureus and E. coli.*

Srivastava *et al* (2003) reported that different dilutions of aqueous and methanolic extracts of lattices of *Nerium indicum and Euphorbia royleana* administered to the culex

quinquefasciatus mosquito larvae exhibited larvicidal activity. Venkatesh *et al (2003)* reported the antipyretic effect of the haxane, chloroform, ethyl acetate and methanolic root extracts of *Rumex nepalensis* in rats 500mg/kg throuh route but observed that only haxane and methano extracts showed appreciable antipyretic activity. Jaime *et al* (2005) reported the important secondary metabolites and essential oils used in folk and modern medicine within the family Asteracae.Bellini (2005) reported that the horse chetnut *(Aesculus hippocastanum)* used in dermatology and pharmacology works is a common ornamental tree that possessed numerous useful derivatives such as escin, cholesterol -escin complex, glycolic, dry and soft extracts and esculin stored mainly in the seeds and trunk bark.

Mirunalini *et al* (2004) reported the chemopreventive potential of garlic oil against 7, 12 drimethylbenzyl [a] anthracene (DMBA) - induced hamster buccal pouch (HBP) carcinogenesis. Rajendra *et al* (2004) reported an ethnobotanical survey of 43 plant spcies belonging to 42 genera and 24 families in Tamil Nadu, India for a biological secreening programe of the Central Drug Research Institute and a search for plants for ethnomedicals. Sethesh and Pari (2004) reported the antioxidant activity of an aqueous *Boerhavia difJusa* leaf extract (200mg/kg) in rats with alloxan - induced *Diabetes mellitus.* They observed that the administration of the extract for four weeks resulted in a appreciable reduction in plasma thiobarbituric acid reactive substances, lipid peroxidase, ceruloplasmic and tocopherol and a appreciable elevation in plasma of reduced glutathione and vitamin C in the diabetic rats. Beigh *et al* (2004) reported that a total of 25 plants within 19 famlies was indentified for treatment of a variety of animal afflictions in veterinary medicine.

Adebayo *et al* (2005) on their haematological and serum lipid studies of ethanolic extract of *Bougainvillea spectabilis* in rat reported that the extract administered appreciablely reduced (P<O .05) packed cell volume haemoglobin concentration and red blood cell count at the dose of 200mg/kg body weight when compared with controls. Elekwa *et al* (2005) reported the effects of aqueous extyract of *Zanthoxylum macrophylla* roots on membrane stability of human enythroerytes of different genotypes. The corpuscular gragility (MCF), which is the concentration of saline causing 50% haemolysis of the erythrocytes, showed that the aqueousplant extract increase MCF values of the various erythrocytes. Adenji and Adesokan, (2005) reported the effect of repeated administration of aqueous extract of *Enantia chloranta* stem bark on selected enzme activities of white abino rat liver. They observed that the administration of the extract produced appreciable reduction (P<O. 05) in the activities of alkaline phosphtase (ALP) and lactate dehydrogense (LDH) in the liver but noappreciable change in the activities of the serum enzymes (P> 0.05).

2.2 REVIEW ON THE PLANT SPECIES STUDIED.

The four plant species used are *Calotropis procera* R.Br, *Senna alata* Roxb, *Merremia tridentata* subspecies *angustifolia* (Jacq) Ooststr and *Schwenkia americana* L.

2.2.1 Calotropis procera R.Br.

Nupe: Epuko

Yoruba: Bombom

(i) DESCRIPTION: The genus *Calotropis* belongs to the family AscJepiadaceae. *Calotropis procera* is a large erect shrub. The stem is slightly woody and much branched at

the base. The young branches and leaves are covered with a white cottony tomentum. The leaves are nearly sessile, oblong obovate to broadly obovate, apex apiculate base narrowly cordate; lamina 9-15 (-20) x 5.5-10 (-IS) em, thick and fleshy, cymes lateral umbellate, covered with a cottony tomentum, mostly solitary at the modes; peduncle 5-8.5cm long, shortly branched at the tip, pedicel 1.5-4.0cm long. Bracts ovate-Ianceolate, about 4-6 mm long. Calyx lobes ovate-lanceolate, 5-6 x 3-4mm, tip acute corolla pink above and white at the base; tube shorter than the lobes; lobes ovate-Ianceolate, 6-8 x 4-5mm, erect, tips sub-acute. Coronal scales 5, broad, andante to and equally or longer than the stamina column, glabrous, margins thinly ciliate; apex bifid without auricles; basal spur acute, in reved; anthers with thin white membranous tips. Pollinia of long lanceolate, caudicular, pendulous, solitary in each anther-loculus. Corpusculum dark brown, rod shaped, 2-celled. Style apex 4mm long pentagonal. Follicles inflated subglobose to obliquely ovoid, 6-12.5 x 3-7 ern, tips acute. Seed ovate with a tuft of silky hairs (Bailey and Bailey, 1976; Kleinschnudt and Johnson, 1977; Rahman, 1990; Nicholson, 1991).

(ii) MEDICINAL IMPORTANCE

The fresh leaves are used as dry fomentation for swellings and rheumatic pains. The extract of the roots is used in medicine as a tonic and the milky juice is given as a remedy for leprosy (Trimen, 1895). The sap is also applied to fresh wounds as a disinfectant (Rahman, 1990). Watt (1889) cited by Rahman (1990) stated that the root is employed by the pathans in India as a toothbrush and it is said to cure toothache. The roots are soaked in the latex, re l rverized and prepared in the form of candles and burnt while the smoke is being inhaled in India by people with asthma and cough. The dried pulverized root-bark is used in parts of

West Africa is added to soup as a stomachic and for colic, and is also believed to encourage lactation in women (Irvine, 1961). The root-bark in Cote D' Ivore is used for the treatment of leprosy, alone or with *Antiaris toxicaria* and *Cochlospermun tinctorium* (Irvine, 1961). The charred roots and root-bark usually made up into an ointment, are applied to skin eruptions, syphilis and leprosy foul ulcers, camel sores etc (Irvine, 1961).

The pithy stems and leaves are burnt and the smoke inhaled by the Hausas for asthma and cough treatment (Irvine, 1961). The dried powdered stems are added to soup as a stomachic (Irvine, 1961). The leaves are reported to cure headache, eye troubles, swollen legs and wounds made by rusty nails (personal communication). The leaves are used in Senegal to destroy head-lice and may form an ingredient in antihelmintics (Irvine, 1961). The latex is used both in Northern Nigeria and Ghana for the treatment of conjunctivitis and ringworm infection (Irvine, 1961).

2.2.2 Senna a/ala Roxb

Nupe: Gungoroko

Yoruba: Asunwon

(i) DESCRIPTION: The genus *Senna* belongs to the family *Caesalpinaceace. Senna alata* is a shrub of up to 4.5m high; leaves pinnate, leaflets 8-12 pairs, 15cm x 7.5cm, narrowly winged rachis; transverse ridge connecting leaflets; flowers yellow, in stout, dense and erect racemes. The fruits are up to 15cm long, winged along sides and prominent broad crenate wing along the middle and 30-40 seeded (Iruvine 1961).

(ii) MEDICINAL IMPORTANCE

The pulp fresh leaves are or the juice is applied to skin complaints such as herpes, blotch, eczema and mycosis e.g. ringworm (Irvine, 1961). The fresh leaf juice mixed with limejuice is applied for worm treatment (Irvine, 1961). In French Equatorial Africa, the leaves are made into a paste with palm oil, or soaked in lemon juice and act as a purgative and for the treatment of gonorrhoea (Irvine, 1961). A leaf decoction is drunk as a purgative as well as for the treatment of dysentery and gonorrhoea in Cote D' Voire. In some parts of the tropics, the dried leaf is made into an infusion or into pills, and used as a purgative or a strong decoction as an abortifacient or to hasten delivery (Irvine, 1961). The juice of the roots is used in West Africa for tattooing or tribal markings (Irvine, 1961). Also, the root or leaves serve as a good purgative (Kennedy, 1952; Rai & Abdulahi, 1978: Sawyer, 1982), and the leaves, flowers and stems as antifungal agents for eradication of skin infections (Benhamin. 1980: Benjamin & Larnikanra, 1981; Olubadewo *et al.,* 1992). Other biological activities of the genus include antiviral (Babber & Rani, 1981), anticancer (Ogura, 1977), insecticidal (Jaipal *et al., 1983),* bactericidal (Aladesami *et al.,* 1991; Adebayo *et al* 1999) and it is used as a viriliing agent (Bassir & Odebiyi, 1974).

2.2.3 Merremia tridentata subspecies angustifolia (Jacq) Ooststr

Hausa: Yimbururu

Nupe: Esakaregi

(i) DESCRIPTION: The genus *Merremia* belongs to the family Convolvulaceae. *Merremia tridentata* subspecies *angostifolia* is a prostrate or climbing herb. Leaves are very narrow, pointed and lobed at the base, about 3cm long and alternatively arranged on the stem.

Leaf stalks are very short. Flowers are yellow in colour, funnel-like, and about 8mm long. There are five persistence sepals and a corolla with 5 lobes. The 5 stamens are attached to the base of the corolla tube. The style is single and the fruits are very small and rounded (Agoha, 1974).

(ii) MEDICINAL IMPORTANCE

A decoction of the plant together with native carbonate of soda is drunk as a remedy for gonorrhoea (Agoha, 1974). The local users claim that this plant cures Acquired Immune Deficiency Syndrome (AIDS) (personal communication)

2.2.4 Schwenkia americana L.

Hausa: Dandana

Nupe: Rogwogi

Yoruba: Ale odan

(i) DESCRIPTION: The genus *Schwenkia* belongs to the family Solanaceae. *Schwenkia americana* is a common weed, with a thin, erect, herbaceous, much branched stem, and up to 50cm tall. The leaves are oval-shaped, about 4cm long and 2cm broad. Usually, the leaves are rounded at the apex and wedge-shaped at the base. The stalks are sparsely covered with short, soft hairs and about 1.3cm long. The flowers are greenish or purple in colour. Calyx is cylindrical, about 3mm long and with pointed lobes. The Corolla is tubular, cylindrical and about 8mm long, narrow and 5-lobed. The lobes are small, unequal, rounded, and each with a tiny round, greenish, unsymmetrical knob on its edge. The two stamens are at~ached to the corolla tube. It has one style and the flower stalk is very short. The fruits are very small with

round capsules, about 3mm long, and pointed in four places. The fruits contain many tiny seeds.

(ii) MEDICINAL IMPORTANCE

A decoction of the plant is taken or applied locally for rheumatic pains and swelling (Agoha, 1974). A nursing mother, who believes her milk is purging her baby, drinks the decoction together with native carbonate of soda (Agoha, 1974). Agoha (1974) also reported that the whole plant is pounded and used as fish poison while the cooked whole plant cures gonorrhoea. This plant is also claimed by the local users to cure Acquired Immune Deficiency Syndrome (AIDS) (personal communication).

2.2.5 REFERENCE COMPOUNDS

Chloramphenicol was used in this research as a reference compound for the bacteria bioassay while Griseofulvin was used for the fungi (Ajaiyeoba *et al,* 1988; Kaleab and Tesfu, 1996)

(i) CHLORAMPHENICOL.

Chloramphenicol was isolated in 1947 from a culture of *Streptomyces venezuelae* and chemical degradation showed that the structure was D(-) - threo-2- dichloro-acetamido-l-p-nitrophenyl-l,3-propane-diol. The most surprising feature of this relatively simple compound was the presence of an aromatic nitro-group which was previously unknown in nature.

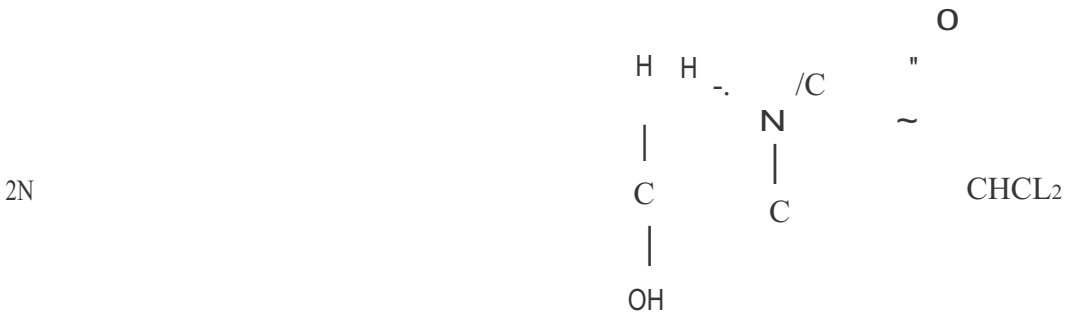

Fig. 1: Structure of Chloramphenicol

Although chloramphenicol was originally obtained by fermentation, a number of syntheses have been derived and is now exclusively prepared synthetically. Numerous analogues of chloramphenicol have been synthesized but these have shown reduced activity or increased toxicity. Chloramphenicol is a broad-spectrum antibiotic, which exerts a bacteriostatic effect. It is bacteriostatic against a whole range of gram-positive and Gram-negative bacteria. This bacteriostatic drug binds to the 50s ribosomal subunit and blocks the peptidyl transferase reaction necessary for protein synthesis. Some bacteria can produce an enzyme, chloramphenicol acetyl transferase that acetylates the hydroxy groups in the side chain of the antibiotic to produce initially, 3-acetoxychloramphenicol and finally 1,3-diacetoxychloramphenicol which lack antibacterial activity (Hugo and Russel 1983). Each 5ml contains chloramphenicol palmitate, equivalent of 125mg chloramphenicol potent, potentially toxic broad spectrum antibiotic against life threatening infections caused by *Haemophilus influenzae, Salmonella typhi and Salmonella paratyphi,* Rickettial infections and chronic infection of urinary tract caused by *Proteus vulgaris* (Hugo and Russel 1983).

(ii) GRISEOFUL VIN

Griseofulvin is a metabolic by - product of *P. griseofulvin.* Griseofulvin was first isolated in 1939 but it was not until 1958 that its antifungal activity was discovered (Hugo and Russel 1983). Many analogues have also been synthesized but none has so far proved superior to Griseofulvin against Dermatophytes.

Fig. 2: Structure of Griseofulvin

Griseofulvin has no antibacterial action but it is very active against the derrnatophytes that affect animals and human hairs, nail and skin such as *Microsporium audouini, Trichophyton rubrum* and *Trichophyton menlagrophytes* (Hugo ancl Russel, 1983). Griseofulvin is administered orally as tablets. It is deposited in the deeper layers of the skin and in hair keratin. It is therefore employed in chemotherapy of fungal infections of these areas.

2.3 REVIEW OF THE MICROORGANISMS USED

The test organism used include bacteria and fungi. The standard bacteria and fungal strains of American Type Culture Collection (ATCC) and clinical strains were obtained from the stock culture of the Department of Microbiology, Biotechnology and Human Virology of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu - Abuja

2.3.1 Bacteria-

(i) *Escherichia coli*

E. coli belongs to the family Enterobacteraceae. There are a variety of *E. coli* bacteria present in nature. They are usually found in the intestines of healthy humans and healthy animals. Even though these bacteria offer properties, there are those strains that are pathogenic

E. coli 0157:H7 is one important strain that is an emerging cause offoodborne illness. Symptoms such as bloody diarrhoea and abdominal cramps may be observed or no such symptom may appear. The elderly and children under five years old are highly susceptible to Haemolytic Uremic Syndrome, a disease in which red blood cells are destroyed and kidneys fails (Kendrick and Wrobel- Woerner, 1997)

The centres for Disease Control and Prevention (CDC) Atlanta approximates that 10,000 to 20,000 people in the United State are annually affected, including 500 deaths (Reed and Kaplan, 1996). The most frequency outbreaks of *E. coli* 0157:H7 are associated with water, raw milk, unpasteurized apple juice, lecttuce, yoghurt and uncooked beef and poultry (Buchanan and Doyle, 1997). Collins (1997) reported that preparation and sanitation methods are key to preventing foodborne illness in the home as in the other areas of food handling.

E.coli is naturally sensitive to any antibiotics ampicillin, cephalosprim, tetracycline, streptomycin, chloramphenicol, kanamycin, gentamycin, trimethoprim, sulphonamides and polymyxins (David, *et al.,* 1997). Many strains however have acquired plasmids conferring resistance to one or more of these drugs.

(ii) *Pseudomonas aeruginosa*

P. aeruginosa is a Gram-negative bacillus, non sporing, non capsulate and usually motile by virtue of one or two polar flagella. *P. aeruginosa* is noted for its environmental versality, ability to cause disease in particular susceptible individuals, and its resistance to antibiotics (Ellen, *et al., 1994).*

The most serious complication of cystic fibrous is respiratory tract infection by the ubiquitous bacterium *P. aeruginosa* Cancer and burn patients also commonly suffer serious infections by *P. aeruginosa,* as do other certain individuals with immune systems deficiencies. Unlike many environmental bacteria, *P. aeruginosa* has a remarkable capacity to cause disease in susceptible hosts. It has the ability to adapt to and thrive in many ecological niches, from water and soil to plant and animal tissues. The bacterium is capable of utilising a wide range of organic compounds as food sources, thus given it an exceptional ability to colonise ecological niches where nutrients are limited. *P. aeruginosa* can produce a number of toxic proteins which do not only cause extensive tissue damage but also interfere with human immune systems defence mechanisms. Analysis of its genome sequence has identify genes involved in sensing and responding to environmental changes. The knowledge of the genome will help to develop new antibacterial drugs to successfully treat infections by *P. aeruginosa* and other related bacteria that are resistant to many antibiotics. (Ellen, *et a!., 1994).*

(iii) *Staphylococcus aureus*

Clinically, the most important genome of the micrococcaceae family is *Staphylococcus.* It can be distinguished from other species of *Staphylococcus* by a positive result in a coagulase test.

The pathogenic effect of *Staphylococcus aureus* are associated with the toxins its produces in the stationary phase of growth. The *Staphylococcus aureus* enterotoxin causes quick onset food poisoning which can lead to cramp and severe vomiting. This microbe also secrete. leukocidin, a toxin which destroys white blood cells and leads to the formation of pus and acne. S. *aureus* causes pneumonia, meningitis, boils, arthritis and osteomyelitis (chronic born infection) (Ellen, *et al* 1994). *Staphylococcus aureus* is penicillin resistant but vancomycin and nafcillin are known to be effective against most strains.

iv) *Klebsiella pneumoniae*

Klebsiella pneumoniae belongs to the family Enterobacteraceae. *Klbsiellae* are distinguished by the presence of a capsular polysaccharisde, of which there are 77 antigenic types (David *et al.,* 1992). Although *Klebsiella pneumoniae* can cause a severe pneumoniae, it is most commonly the cause of hospital acquired urinary tract infections or burn wound infections .: The autoimmune disease, anklyosing spondylitis, is thought to be a possible cause of K *pneumoniae.* The virulence of *Klabsiella* is not well understood but its antiphygocytic capsule plays a role in the long infections by preventing phagocytosis (Ellen *et al.,* 1994). It is thought aerobactin, an iron bending protein present in *Klebsiella* also contribute to virulence.

Klebsiella infection of the urine often responds to trimethoprim, nitrofurantoin, co-arnoxiclav or oral cephalosporins. *Pneumonia* and other serious infections require vigorious treatment with an aminoglycosides or a cephalosporin such as cefataxime (Ellen *et al., 1994)*

(v) *Salmonella typhi*

Salmonella typhi, a typical member of the Enterobacteraceae is facultative, anaerobic Gram-negative bacillus. It is able to grow on a wide range of relatively simple media and distinguished from other member of the family by biochemical characteristics and antigenic structure. *Salmonella typhi* is human specific pathogen causing the systemic fibrile illness typhoid fever (David *et aI., 1992)*

All vertebrates appear capable of habouring salmonellae in their gut, and salmonellae have also been isolated from a wide range of arthropods such as flies, cockroaches, and ticks (David *et al.,* 1992). Most animal infections seem to be symptomless, or to cause a self limiting gastro-enteritis of variable severity. Many serotypes such as S. *typhi* show a wide host range and can be isolated from many animal species

The introduction of chloramphenicol in 1948 transformed the treatment of enteric fever, turning a life threatening illness of several weeks duration and a mortality of more than 20% into a short lasting fibrile illness with a mortality below 2%. The problem of bone marrow toxicity and a wide spread of emergence of chloramphenicol resistance in S. *typhi* in many part of the world prompted the research for alternative agent. Among these, the ampicillins and cotrimoxazole had been shown to be comparable in efficacy with chloramphenicol. The-newer quinolones such as ciprofaloxacin are likely to find a place in the treatment of

infections with strains of salmonellae resistant to chloramphenicol. David *et al. (1992)* reported that heat killed, phenol preserved whole cell vaccine containing a mixture of cultures of S. *typhi,* S. *paratyphi* A and S. *paratyphi* B have been used for many years in countries with high endemic level of typhoid fever and field trials have shown that such preparation confer considerable protection against typhoid fever. Recent trials of oral live attenuated typhoid vaccines have shown encouraging protection rates (David *et al., 1992)*

2.3.2 Fungi

(i) *Candida albicans*

Candida albicans is a dimorphic fungus. Most of the time exists as oval, single yeast cell, which reproduce by budding. *Candida* is a commensal organism found in 40- 80% of normal humans, and is presnt in the mouth, gut, and vagina (Ajayi, *et al 1999).*

There are many species of the genus *Candida* which cause the disease known as candidiasis Pathogenicity starts when a person experience some alterations in cellular immunity e.g. immuno suppression, or AIDS, normal body flora or normal physiology (Ajayi *et al., 1999).* Although *Candida* most frequently infects the skin and mucosal surfaces, it can cause systemic infections manifesting as pneumonia, septicaemia or endocarditis in severely immuno compromised patients (Ajayi *et al.,* 1999). There does not appear to be appreciable difference in pathogenic potential of different *Candida* strains, therefore establishment of infection appear to be determined by host factors and not the organism (Ajayi *et al., 1999).* Fortunately, several drugs are available to treat serious systemic infectious e.g. Itraconazole and fluconazole.

(ii) *Epidermophyton jlocossun*

Epidermophyton jlocossun, the only member of the genus *Epidermophyton* is the common cause of tinea cruris and tinea pedis (Balagee, *et al.,* 1997). The genus Epiderphyton is characterised by the presence of large, club-shaped, multi-segmented, smooth walled macroconidia that are commonly produced either singly or in clusters of two or three from the tip of short conidiophores. Microconidia are not produced. *E. jl.ocossun* grows slowly, and growth appears as an olive-green to khaki colour with the periphery surrounded by a dull orange brown colour.

The organism is susceptible to cold temperatures and for this reason, it is recommended that specimens submitted for dermatophyte culture should not be refrigerated before and after culturing.

(iii) *Trichophyton mentagrophyte*

Microscopically, the genus *trichophyton* is characterised by smooth, club-shaped, thin walled macroconida with eight or ten septa ranging from 4 x 8 um to 8 x 15urn. The macroconidia are born singly at the terminal of hyphae. The species of this genus are widely distributed and are common causes of infections of the feet and nails (Baley and Scott, 1994).

T rubrum is slow growing organism that produces a flat or heap-up colony that is generally white to reddish with a cottony or velvety surface. *T rubrum_produces* fluffy or granular colonies. Macroconida are uncommon in most of the fluffy strains but are more common in the granular strains

There are a number of orthodox drugs (topical and systemic) which are useful for treating tinea infections caused by the species of *Trichophyton.* Cleotrimazone, (topical cream) is used to treat athlete's foot (ringworm of the foot, tinea pedis), joint itch (ringworm of the groin,

tinea cruris) and ring worm of the body, tinea corporis. Terbinafine (tablets) suppressed the biosynthesis of ergosterol by inhabiting the fungal enzyme. It is fungicidal to dermatophytes. Griseofulvin (tablets) inhibits nucleic acid synthesis and cell mitosis by interfering with spindle microtubules (Graser, *et al.,* 1999b).

Some herbs that have proven effective in the fight against ringworm include garlic, ginger, neem tree oil to mention but a few (Irobi, 1992; Olodumare *et al.,* 1992; Alade and Irobi 1993)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Identification of the Plants

The plants used in this study were collected from Bida, Niger State, Nigeria and identified by Mr. T. K. Odewo, The Assistant Chief Forest Superintendent, Forest Herbarium, Forest Research Institute of Nigeria (FRIN), Ibadan using the scheme described by Burkil (1985). The plants with their specific voucher specimen numbers, are *Senna alata* Roxb (FHI 106513) Plate 1, *Calotropis procera* R.Br. (FHI 106511) Plate 2, *Schwenkia americana* L. (FHI 106510) Plate 3, and *Merremia tridentata* subspecies *angustifolia* (Jacq) Ooststr (FHI 106512) Plate 4.

3.2 Drying and Micronization of Plant Parts

The plant materials which included roots, stems and leaves were separately spread thinly on a flat, clean floor and allowed to dry at room temperature for 7 days. Spreading in thin layer was necessary to prevent spoilage by overheating and moisture condensation (Stary *et al,* 1998). The dried plant materials were crushed separately with mortar and pestle into their parts and later blended with electric blender (Binatone model BLG - 400). Micronization was necessary to enhance the penetration of the extracting solvents into the cells, thus facilitating the release of active principles (Iyamabo, 1991).

Plate 1: A twig of Senna *alata* Roxb FHI 106513

Plate 2: A twig of *Calotropis procera* R. Br. FHI 106511

Plate 3: Twigs of *Schwenkia americana* L.
FHI106510

Plate 4: A whole plant of Merremia *tridentata* subspecies *angustifolia* (~acq.)Ooststr FHI 106512

3.3 Extraction

The methods employed in the extraction of the plant parts were cold marceration for the water extracts and soxhlet extraction, for ethanol and n-hexane extracts (Sofowora 1982), which was modification of the method of Odebiyi and Sofowora (1978).

(i) Aqueous extraction

Two hundred millilitres (200ml) of distilled water was added to *SOg* of powdered sample of each plant parts in SOOmlconical flask and allowed to soak for 2 hours. The flask was agitated manually at 30 minutes interval for 3 hours. The mixture was allowed to stand on the bench for 48 hours, after which it was filtered using filter paper (No 1 Whatman, U.K.). The extract was poured into a beaker and placed in a water bath to evaporate the solvent. The dried or semi-solid extracts which weighed between 10 and *13g/S0g* of sample depending on the plant part were put in a sterile container and stored in the refrigerator.

(ii) Ethanol extraction

Fifty grams *(SOg)* of each powdered sample of the plant parts was separately extracted to exhaustion in a soxhlet extractor using 200ml of *95%* ethanol. A rotary evaporator (Buchii Laboratory Technique, Switzerland) was used in vacuo at 40°C to concentrate the extract, evaporate and recover the solvent. The dried or semi-solid extracts which weighed between 9 and *12g/S0g* of sample depending on the plant part were poured into sterile containers and stored in the refrigerator for further characterization.

(iii) N-Hexane extraction

Fifty grams (SOg) of each powdered sample of the plant parts was separately extracted to exhaustion in a soxhlet extractor using 200m 1 of n-hexane. A rotary evaporator (Buchii Laboratory Technique, Switzerland) was used in vacuo at 40°C to concentrate the extract, which weighed between 10 and 14g/S0g of the sample depending on the sterile containers and stored in the refrigerator.

3.4 Preliminary Phytochemical Analysis

The preliminary phytochemical analysis was to test for the presence of biologically active compounds in the plant extracts. The active components include alkaloids, anthraquinones, tannins cardiac glycosides, phlobatannins, polyphenols, saponins and sesquiterpenes. The components were tested for as described below:

(i) Tannins

A O.Sg of each of the extracts was dissolved in 4ml of distilled water to form a solution. To 1ml of the solution, few drops of 10% ferric chloride solution were added and observed. A blue black, blue green or green precipitate indicated the presence of tannins (Trease and Evans, 1978).

(ii) Alkaloids

A 0.5g of each of the extracts was dissolved in 2ml of water to form a solution. To lml of the solution, a few drops of Dragendorff's reagent was added. Development of deep brown precipitate indicates the presence of alkaloids (Cuilei, 1982; Sofowora, 1984).

(iii) Anthranoids

A 0.5g of each of the extracts was added to 2ml of NaOH to form a solution. To 1ml of 5 drops of acetic acid and 5ml of toluene were added, shaken and allowed to settle. The upper layer was transferred to fresh tube containing 0.5111NaOH using a pipette. Development of a red colour indicated the presence of anthranoids (Cuilei, 1982; Sofowora, 1984).

(iv) Anthraquinone glycosides

A 0.5g of each of the extracts was dissolved in 4ml of distilled water. To Iml of the solution in a test tube, 0.2ml of dilute IN H_2S0_4 and 911Il of Benzene were added. Upon separation of Benzene layer into another test tube, 5 drops of dilute ammonium solution were added. Presence of pink or violet colour in the ammonia phase (bottom of the test tube) indicated the presence of anthraquinones (Trease and Evans, 1978).

(v) Cardiac glycosides

A 0.5g of each of the extracts was dissolved in 2ml or Chloroform. To it. few drops of dilute H_2S0_4 were carefully added. Development of a reddish chloroform/sulphuric acid indicated

the presence of a steroidal ring, i.e. aglycone portion of the cardiac glycosides (Cui lei, 1982; Sofowora, 1984).

(vi) Phlobatannins

A 0.5g of each of the extracts was dissolved in 3ml of distilled water in a test tube. To 2ml of it, 5ml of 1% aqueous hydrochloric acid was added. Development of a red precipitate indicated the presence of phlobatannins (Trease and Evans, 1978).

(vii) Polyphenols

A 0.5g of each of the extracts was dissolved in 4111bf distilled water. To 1ml of it, 1ml of ferric chloride and 1ml of 1% potassium ferric cyanide were added and observed. Development of a blue green colour indicated the presence of polyphenols (Cuilei, 1982; Sofowora, 1984).

(viii) Sesequiterpenes

A 0.5g of each of the extracts was added to 2ml of distilled water in a test tube to form a solution. 0.5ml of it was mixed with 0.1 ml of methanol and shaken vigorously. To the tube, OAml of 5% l-hS04 containing 0.5% ferric chloride was added and stirred with a glass rod. The mixture was put to boil in water for 1 minute. Colour changes from colourless to pink indicated the presence of scsquitcrpcncs (Odebiyi and Sofowora, 1978).

(ix) Saponins

A 0.1g of each of the extracts was added to a test tube containing 2ml of distilled water and shaken. Development of frothing which persisted on warming was preliminary evidence of saponins (Trease and Evans, 1978).

(x) Volatile oils

A 0.1g of each of the extracts was dissolved in 2ml of 90% ethanol in a test tube. To the solution, 4 drops of ferric chloride solution were added and the development of a green colour was characteristic of volatile oils (Trease and Evans, 1978).

(xi) Phenols

A 0.5g of each of the extracts was dissolved in 5ml of distilled water in a test tube. To 2ml of the solution, 2ml of ferric chloride solution was added. Formation of deep bluish green solution was an evidence of the presence of phenols (Trease and Evans, 1978)

(xii) Resins

A 0.5g of each of the extracts was dissolved in 5ml of distilled water in a test tube. To 4ml of the extract solution, 4ml of copper in acetic acid solution was added, shaken vigorously and allowed to separate. Dark blue colouration showed the presence of resins.

(xiii) Flavonoids

To O.1g of each of the extracts, a small quantity of zinc chips and 5 drops of concentrated HCl were run down the side of the test tube. A reddish colouration indicated the presence of flavonoids (Trease and Evans, 1978).

3.5 Test Organisms

The fungi and bacteria employed in this research work were obtained from the stock culture of the Department of Microbiology, Biotechnology and Human Virology of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu - Abuja. The bacteria included *Staphylococcus aureus* (ATCC 37_P9), *Pseudomonas aeruginosa* (ATCC 27850), *Escherichia coli* (ATCC 9637), *Salmonella typhi* (Local), *Klebsiella pneumoniae* (Local) and *Bacillus subtilis* (Local). The clinical specimens (Local) before use were characterized based on cell morphology and biochemical tests and identified by comparing their characteristics with those of known taxa as described by Cowan (1974). Each of the bacteria was maintained on Nutrient agar slant at 4°C prior to use for antibacterial susceptibility test. The fungi included *Microsporium canis* ATCC 11622, *Trichophyton mentagrophyte* ATCC 4808, *Trichophyton rubrum* ATCC 28941, *Epidermophyton flocossun* ATCC 10227 and *Candida albicans* ATCC 10231. Each fungus was maintained on Sabourauds dexlrose agar (SDA oxoid) slant at 4°C prior to use for antifungal susceptibility test.

3.6 Standardization of Organisms

(i) Bacteria

The method adopted by Collins *et (//.* (1995) was used. An overnight culture of each of the test bacteria was prepared. A loopful of each of the organisms was inoculated in 5ml of sterile nutrient broth and incubated for 24 hours. From this, double dilutions of each organism was made to obtain approximately 1million cells/ml MacFarland turbidity used for antibacterial test.

(ii) Fungi

The method adopted by Fessia *et al.* (1988) was used. The culture from the slant of each organism maintained at 4°C was used to inoculate the assay medium, Sabouraud's dextrose agar (SDA) in slant with the help of sterile inoculating loop and incubated between 48hours and 14 days at 27±30C for spore development and maturity. To 10111dfSabouraud's dextrose broth containing 0.05% Tween 80, drops of few sterile glass beads were added and shaken gently from cover to base. The spore suspension of each organism was decanted and diluted with sterile distilled water Loobtain approximately 1million cells/ml Macf-arland turbidity.

3.7 Determination of Activity of Plant Extracts

The activity of each of the plant extracts against the organisms used for this research was considered under 2 concentrations. A 0.2g of the extracts was dissolved in 5ml of distilled water to give 40,000~lg/ml of extract. Ilalf a millilitre of this concentration (40,000~lg/ml) was added to 19.5111land Iml (40,000 pg/ml) was added to 19m1 of molten nutrient agar or Sabouraud's dextrose agar (NA or SDA) at 54°C to give the concentration of IOOO~lg/mlupon

mixing and solidification; and 2000 ~lg/ml respectively. J\ loopful of each of the broth culture/spore suspension of the organism was streaked on the NA/SDA plates containing extracts and incubated at for 18 to 24 hours (bacteria) or for 48hours to 7 days (fungi). The following controls were set up.

MSC - Medium sterility control (19ml of agar)

OVC - Organism viability control (Organism + 19m1 of agar)

ESC - Extracts sterility control (1111extract + 19m1 of molten agar)

DC - Diluent control (Solvent for dissolving extracts was also plated out)

3.8 Antifungal Susceptibility Test

The method of Washington and Sutter (1980) was used. Universal bottles having sterile molten SDA at 54°C were inoculated with test extracts from all the plants parts at concentrations 2000 and IOOO~lg/mlof agar respectively and kept in a slanting position at room temperature (27±3°C). Test fungal cultures werc inoculated on the slants. Growth inhibitions were observed after incubation period of between 5 and 7 days for fungistatic action of the extracts while further incubation between 7 and 10 days was observed as the fungicidal action of the extracts. Control agar bottles were provided such as Extract Sterility Control (ESC), Medium Sterility Control (MSC) Organism Viability Control (OVC) and Diluent Control CDC) to compare results. All treatments were duplicated. Griseofulvin *(10~g/ml)* was incorporated into the medium as a reference compound (Ajaiycoba *et of 1998).* All incubations were carried out at room temperature (27±30C).

3.9 Antibacterial Susceptibility Test

The extracts of the roots, stems and leaves of the four plants investigated were each screened for antibacterial activity using the cup plate method of Ronald *ct (l/* (1988). First, the dried sample of the extracts was reconstituted to form IOOO~lg/ml and 2000~lg/ml concentrations with minimum amount of the extracting solvents. Twenty rnillilitre (20m l) of the nutrient agar (NA) was dispensed by means of 20m l syringes into universal bottles and sterilized using the autoclave at 151b, 121 *DC* for 15 minutes. The sterilized medium was kept at 54°C to stabilize and kept in molten state. Plates were labelled in duplicates for the six organisms. Twenty rnillilitres (20ml) of molten nutrient agar were then poured into plates in duplicate and allowed to gel. About 0.2ml of the overnight culture of each of the organisms was inoculated into 5ml of sterile nutrient broth and incubated far 24 hours. The broth was further diluted with sterile distilled water to obtain 10(, *eells/ml* Macf-arland turbidity of each organism used for antibacterial test.

The gelled plates were then dried in the dryer to exclude the condensation of water. A cork borer No.4 was sterilized using 70% ethanol and 3 holes were bored per plate to represent the test extract, diluent control and chloramphenicol (30~Lg/llll) as reference compound. The bases of these holes were thcn scaled using a ICw drops of the molten nutrient agar by means of steriled syringes to prevent the extract from entering the bottom of the agar in the plate to give false positive results. The holes per plate were then filled with drops of the extract, diluent and chloramphenicol using sterilcd syringes. The plates were duplicated and incubated at *37DC* for 24 hours to allow the bacteriostatic action or the extracts to develop. Further incubation for 24 hours at *37DC* was carried out to determine the bactericidal action of the extracts. All plates were incubated in their normal position to prevent spilling or the

extracts and to allow the zone of inhibition to develop. Controls such as Extract Sterility Control (ESC), Medium Sterility Control (MSC) and Organism Viability Control (OVC') were provided to compare results.

3.10 Minimum Fungistatic and Bactcr iostatic Concentration (MFC & MBC)

The method Alade and lrobi (1993) was used. This was determined by incorporating various amounts (1 *OOO~lg/ml - 2000~lg/ml)* of the extracts into sets of universal bottles containing the assay medium and inoculating with the test organisms. Each of the concentrations *(1000~g/ml* or *2000~g/ml)* of extracts was determined by dissolving appropriate amount of the extract in 1ml of the diluents in each plastic vial and the solution added into sterile universal bottles containing 15ml of the assay medium (SDA) and allowed to mix properly and solidified as slants. fourteen days spore suspension of' each fungus was diluted to approximately 1million eells/ml MacFarland turbidity used for antifungal assay. The assay media were inoculated by streaking using a wircloop meant to deliver 0.021111 (20~L1) of the spore suspension. The inoculated assay media containing extract with each of' the f'ungi were incubated at 27±30C for 5 - 7 days. The minimum fungistatic concentration (MFC) of the extracts was regarded as the lowest concentration of each of the extracts that did not permit any turbidity or visible growth of the test fungi.

For bacteria assay, each of the concentrations (1 *OOO~lg/ml* or *2000~lg/ml)* of the extracts was determined by dissolving appropriate amount of extract in Iml of the diluent in each plastic vial and the solution added into sterile universal bottles containing 15ml or the nutrient agar (NA) and allowed to mix properly. Plates were poured and allowed to gel. 24 hours broth culture of each of the bacteria was diluted to approximately 1 million cells/rn] MacFarland turbidity used for antibacterial assay. The assay media were inoculated by streaking using a

wireloop meant to deliver 0.02ml (20~L1) of each or the organisms. The inoculated assay media containing extracts were incubated at 37°C for 24hours. All treatments were duplicated. The minimum bacteriostatic concentration (M13C) or the extracts was regarded as the lowest concentration of each of the extracts that did not permit any turbidity or visible growth of the test bacteria.

3.11 Rate and Extent of Killing of the Fungi and Bacteria by the Extracts

The viable plate count technique described by Adebayo *et at* (1986) was used. The minimum fungistatic concentration (MFC) of each of the original crude extracts against the fungi were prepared on each petri dish by dissolving appropriate amount minimum fungistatic concentration of each extract in 1ml of diluent (in each plastic vial) and adding the solution into sterile universal bottles containing 15ml of assay medium (SDA) and allowed to mix properly. Plates were poured and allowed to gel so as to obtain the original crude extract medium. The corresponding set or media plates or each extract with different time interval as 0, 5, 10, 15, 20, 25 and 30 (mins) were prepared. Forty eight hours - 14 days spore suspension of each of the organisms was diluted to approximately I million cells/rnl Macfarland turbidity. To each of the original, crude extract medium, Irnl 0J spore suspension of each fungus was poured and rocked round. Immediately following this, a sterile loop meant to deliver 0.021111 spore suspension of the fungi was used to inoculate the plate at 0 minute and the subseq uent plates after every 5 min utes from each master crude ex tract rued ium. Treatments were duplicated. The media were incubated at 27±3()C 1'01' 5 - 7 days to determine through plate count the rate of killing ofthe fungi by the extracts.

The III I11in UII1 bacteriostatic conccntrutiou (MI~(.') 0r cucl: 01' the urigill;ti crude cxtructs against the bacteria were prepared on each petri dish by dissolving appropriate amount or

minimum bacteriostatic concentration of each extract in 1ml of the diluent in each plastic vial and the solution added into sterile universal bottles containing 15ml of the assay medium (nutrient agar) and allowed to mix properly. Plates were poured and allowed to gel so as to obtain the original crude extract medium. The corresponding set of media plates of each extract with different time interval as 0, 5, 10, 15, 20, 25 and 30 (minutes) was prepared. 24 hours broth culture of each of the bacteria was diluted to approximately 1million cells/ml Macfarland turbidity. To each of the original crude extract medium, 1ml of the diluted 24hour broth culture of each bacterium was poured and rocked round. Immediately following this, a sterile loop meant to deliver 0.02ml of each of the bacteria was used to inoculate the plate 0 and the subsequent plates after every 5 minutes from each original crude extract medium. Treatments were duplicated. The media were incubated at 37°C for 24 hours to determine through plate count the rate of killing of the bacteria by the extracts.

3.12 Effect of Temperature on Potency of Plant Extracts

(i) Fungi and Bacteria

The freshly prepared extracts produced from roots and leaves of the plants being studied were investigated for their antifungal activity at varying temperature. The temperatures were - 4°C, oDe, 27±30e, 100°C, and 121Dc. 3g of the root and leaf extracts of the plants were put in sterile screw cap bottles and exposed to temperature of - 4De and O'C for 48 hours, 27±3De for 1 week, iOO'C for 1 hour, 121DC for 15 minutes.

2000~lg/ml of each of the extracts was made by dissolving 200mg in 5ml of diluent to give the concentration of 40mg/ml of extract. 1ml of each extract was introduced into 19m1 of molten Sabouraud's dextrose agar (SDA) for fungi, but to 19m1 of nutrient agar (NA) at 54De

in universal bottles and allowed to mix properly. Plates were poured and allowed to gel to give final concentration of 2000!J.g/ml of agar. 2 - 7days spore suspension of each of the fungi was diluted to approximately I million *cells/ml* MacFarland turbidity. Also Iml of 24 hours broth culture of each bacterium was diluted to approximately I million *cells/ml* MacFarland turbidity. The assay media were inoculated by streaking with a wire loop meant to deliver 0.02ml (20!lI) of the fungal spores and incubated at 27±30C for 2 - 7 days for fungi, but at 37°C for 24 hours for bacteria. Treatments were duplicated for validity. Controls as ESC, MSC, OVC and DC were provided for reference. Griseofulvin (1O~Lg/ml)was also used as a reference compound for the fungi while chloramphenicol (30!J.g/ml) was used for bacteria.

3.13 Accelerated Gradient Chromatography (AGC)

Silica gel" which served as a stationary phase, was activated by removmg the moisture content. A 4 g of the leaf ethanol extract of *Schwenkia americana* was dissolved in 5ml of ethanol in a beaker. 109 silica-gel was poured gradually and mixed with the help of a glass rod. The mixture was eventually introduced into a clean mortar and ground with pestle to produce a more homogenous silica-gel extract mixture. A column size 300mm x 60mm was prepared by adding 20g of the silica-gel into the column to serve as the stationary phase, and on top of it was packed the silica-gel sample mixture. Solvent system in order of increasing polarity was prepared (Appendices 3 -8). 100mi of the least polar solvent was let into the reservoir by the pump (FMI LAB PUMP model QO, Quick-fit, England). When the solvent in the reservoir was getting to exhausted, more of the polar solvent was added one after the other with increasing polarity until elution was completed. Fractions ofdifferent colours were collected in 10ml test tubes until column component was finished. Fractions were tested using

thin layer chromalography (TLC) method and developed with hexane:dichloromethane :ethanol(3 :2: 1) solvent system. Similar fractions were pulled together and concentrated using the rotary evaporator (Buchii Laboratory technique, Switzerland) and stored in sample bottles for further work. Other extracts involving AGC were treated in the similar way as shown above to produce fractions for antimicrobial activity.

3.14 Effect of Storage on the Antimicrobial Activity of Plant Extracts

A 3g of each of the extracts in sterile screw cap bottles were kept in an enclosure at room temperatures (27±30C) while another set was kept in the fridge when the extracts were obtained fresh. The antifungal and antibacterial activities after 6 months, 12 months and 30months from the date of production were carried out using the Agar Dilution Streak teclmique of Mitscher *et al,* (1972) for comparison.

(i) Fungi and Bacteria

Two thousand microgram per millilitre (2000flg/ml) of each of root and leaf extracts of *Calotropis procera* and *Senna alata, Schwenkia americana, Merremia tridentata subspecies angustifolia* plants was prepared by dissolving 200mg in 5ml of diluent to give *40mg/ml* of extract. 1ml of each extract was introduced into 19m1 of molten Sabouraud' s dextrose agar (SDA) medium at 54°C in universal bottles and allowed to mix properly. Plates were poured and allowed to gel to give final concentration of 2000~lg/ml of agar. 2 - 7 days spore suspension of each of the fungi was diluted to approximately 1 million cells/ml MacFarland turbidity. The assay media were inoculated by streaking with a wireloop meant to deliver 0.02ml of the fungal spores and incubated at 27±30C for 2 - 7 days. Also for the bacteria,

1ml of each extract was introduced into 19m1 of molten nutrient agar (NA) medium at 54°C in universal bottles and allowed to mix properly. Plates were poured to give final concentratrion of 2000J-lg/ml of agar. 1ml of 24 hours broth culture of each bacterium was diluted to approximately 1 million cells/ml MacFarland turbidity. The nutrient agar media were inoculated by streaking with a wireloop meant to deliver O.02ml of each of the bacteria cells and incubated at 37°C for 24 hours. Treatments were duplicated. Controls as ESC, MSC, OVC and DC were provided for validity. Griseofulvin (l Ouq/ml) was used as a reference compound for fungi, while chloramphenicol (30J-lg/ml) was used for bacteria.

3.15 Flash Column Chromatography (FCC)

Flash column chromatography was employed to separate the components of the water plant extracts, which usually are not ideal for AGe. Chromatographic alumina type H which served as stationary phase was activated by removing moisture content. Forty grams (40g) alumina type H was mixed with leaf water extract 'of *Calotropis procera* in a clean beaker with the help of a glass rod. The mixture was eventually introduced into a clean mortar and ground with pestle to produce alumina-extract mixture. A column size 707mm x 90mm was prepared by adding 60g of the alumina into the column to serve as the stationary phase and on top of it was packed the alumina-extract mixture. Solvent system in order of increasing polarity was prepared (Appendices 1-3). The selection of solvent system for elution was guided by thin layer chromalography (TLC) characteristics. Two hundred millilitres (200ml) of the solvent with increasing polarity was let into the reservoir one after the other using positve pressure generated by the pump (FMI LAB PUMP model QD, Quick fit, England). Positive pressure produced from compressed air was applied manually to force the eluent through. Fractions

which vary in colours but fewer in number compared with AGC were collected in 250ml flat bottom flasks and tested using TLC method and developed using hexane:dichloromethane: methanol (3:2:1) solvent system. Similar fractions were pulled together and concentrated using the rotary evaporator (Buchii Laboratory technique, Switzerland) and stored in sample bottles for further analysis. Other water extracts were treated as described above to produce fractions for antimicrobial activity profile.

CHAPTER FOUR

4.0. RESULTS

4.1 Phytochemical Composition of Medicinal Plant Parts

(a) Phytochemical Composition *of Senna alata* (SAA) using different methods of extraction

N -hexane was used to extract resins while ethanol was used to extract tannins, nthraquinones, cardiacglycoside and phenols which were detected in the root, stem and leaf (Table 1). Saponins was detected in root and leaf extract while, alkaloids was detected on the leaf. Water was used to extract anthraquinones and resins which were detected in the root and leaf extract (Table 1).

Table 1: Phytochemical composition of *Senna a/ala* (SAA) using different methods of extraction.

Phytochemical Components	Method of Extraction								
	N - hexane			Ethanol			Water (aq)		
	R	S	L	R	S	L	R	S	L
Alkaloids					-	+			
Tannins				+	+	+			
Saponins				+	-	+			
Anthraquinones				+	+	+	+	-	+
Cardiac glycosides				+	+	+			
Polyhenols				+	+	+			
Anthranoids									
Phlobatannins				+	+	+			
Sequi terpenes									
Flayonoids				-	-	+			
Volatile oils				-	-	+			
Resins	+	+	+	-	+		-	-	+
Phenols									

R: Root; S: Stem; L: Leaf

+: Presence of active compound in the plant part.

- : Absence of active compound in the plant part.

(b) Phytochemical composition of *Calotropis procera* (CP) using different methods of extraction.

N - hexane was used to extract resins which was detected in the root extract while ethanol was used to extract alkaloids, tannins and saponins which were detected in the root, stem and leaves extracts. Also ethanol was used to extract polyphenols which was detected in the leaf. (Table 2). Water was used to extract alkaloids, tannins, saponins and volatile oils which were detected in the leaf extract while polyhenols and saponins were detected in the root and stem extracts.

Table 2: Phytochemical composition of *Calotropis procera* (CP) using different methods of extraction.

Phytochemical Components	Method of Extraction								
	N - hexane			Ethanol			Water (aq)		
	R	S	L	R	S	L	R	S	L
Alkaloids				+	+	+	-	-	+
Tannins				+	+	+	-	+	+
Saponins				+	+	+	+	+	+
Anthraquinones									
Cardiac glycosides									
Polyhenols						+	+	+	-
Anthranoids									
Phlobatannins									
Sequiterpenes									
Flayonoids									
Volatile oils							-	-	+
Resins	+	-							
Phenols									

R: Root; S: Stem; L: Leaf

+: Presence of active compound in the plant part.

- : Absence of active compound in the plant part.

(c) Phytochemical composition of *Scltwenkia americana* (SA) using different methods of extraction.

N-hexane was used to extract resins which were detected in the root extract. No active ingredient was extracted by n-hexane from the stem or the leaf (Table 3). Ethanol was used to extract alkaloids, tannins, saponins, cardiacglycosides, volatile oils and phenols which were detected in the root, stem and leaf extracts while tannins, polyphenols and volatile oils were detected in the stem and leaf extracts Also detected were cardiacglycosides and phenols from the root extract.

Alkaloids, tannins, saponins, volatile oil and phenols were extracted using water which were detected from the leaf extract while saponins, polyphenols, volatile oils and were detected in the stem extract (Table 3). Also, alkaliods and saponins were detected from the root extract using water.

Table 3: Phytochemical composition of *Schwenkia americana* (SA) using different methods of extraction.

Phytochemical Components	Method of Extraction								
	N - hexane			Ethanol			Water (aq)		
	R	S	L	R	S	L	R	S	L
Alkaloids				+	+	+	+	-	+
Tannins				-	+	+		-	+
Saponins				+	+	+	+	+	+
Anthraquinones									
Cardiac glycosides				+	+				
Polyhenols				-	+	+	-	+	-
Anthranoids									
Phlobatannins									
Sequiterpenes									
Flayonoids									
Volatile oils				-	+	+	-	+	+
Resins	+						-	-	+
Phenols				+	-	+	-	+	-

R: Root; S: Stem; L: Leaf

+: Presence of active compound in the plant part.

- : Absence of active compound in the plant part.

(d) Phytochemical composition of *Merremia tridentata subspecies angustifolia* (MT) using different methods of extraction.

N-hexane was used to extract resins which was detected in the root and leaf extracts. No other active ingredient was detected using n-hcxane from either the root, stem or the leaf extract. Alkaloid, tannins, saponins, cardiacglycosides, polyphenols, volitile oils, resins and phenols were extracted from the root, stem and leaf extracts using ethanol. Phlobatannins and flavonoids were extracted using water and were detected in the leaf extract.

Water was used to extract alkaloids, saponins cardiacglycosides which were detected in the root and leaf extracts while alkaloids, volatile oils and resins were detected in the stem extract (Table 4). Also tannins was detected in the root extract using water.

Table 4: Phytochemical composition of *Merremia tridentata* subspecies *angustifolia* (MT) using different methods of extraction.

Phytochemical Components	Method of Extraction								
	N - hexane			Ethanol			Water (aq)		
	R	S	L	R	S	L	R	S	L
Alkaloids				+	+	+	+	+	+
Tannins				+	+	+	+		
Saponins				+	+	+	+	-	+
Anthraquinones									
Cardiac glycosides				+	+	+	+	-	+
Polyhenols				+	+	+			
Anthranoids									
Phlobatannins						+			
Sequiterpenes									
Flayonoids				-	+	+			
Volatile oils				+	+	+		-	+
Resins	+	-	+	+	+	+	+	+	-
Phenols				+	+	+			

R: Root; S: Stem; L: Leaf

+: Presence of active compound in the plant part.

- : Absence of active compound in the plant part.

4.2 Antibacterial activity of the extracts of *Senna a/ala*

The stem and leaf ethanol extracts of *Senna* alata at 2000~lg/ml showed appreciable activity against *E. coli* and *Bacillus subtilis* having the zone of inhibition of 12.1mm and 10mm for *E. coli* and *Bacillus subtilis* respectively. Also the *Senna alata* root hexane extracts at 2000~g/ml showed appreciable antibacterial activity against *E. coli* and *Bacillus subtilis* having zone of inhibition of l2.2mm and 10.lmm respectively. The stem water extract of *Senna alata* showed appreciable antibacterial activity against *E. coli* having zone of inhibition of 12mm (Table 5). The root water extracts at 2000~g/ml had moderate activity against S. *aureus.* However, the root and the leaf ethanol and water extracts exhibited lower activity against *E. coli.* Also the root and stem water extracts showed low activity against S. *typhi* and *K. pneumoniae.* The root ethanol extract at 2000~lg/ml and root and leaf ethanol extracts at 1000~g/ml showed low activity. The rest organisms were resistant to *Senna alata* extracts. At 2000~g/ml, the stem n-hexane extracts showed moderate activity against *E. coli* having 5.5nim zone of inhibition. The root and leaf water extracts at 2000~g/ml have low activity against S. *typhi* and *K. pneumoniae* while the n-hexane have no activity against S. *typhi.The* root and leaf ethanol extracts atl OOO~g/mlhac! low activity against *Bacillus subtilis.(Table 5).* The mode of action of the root n-hexane, stem ethanol and stem water extracts was bactericidal against *E. coli* while that of root n-hexane and leaf ethanol extracts was bacteristatic and bactericidal repectively against *B. subtilis* at 2000~g/ml (Table 5).

Table 5: Average diameter of zone of inhibition (mm) produced by extracts of *Senna a/ala* against test bacteria

Organism	Extracts (1OOOllg/ml)									Extracts (2000llg/ml)									Control			
	n-hexane			Ethanol			Water			n-hexane			Ethanol			Water			MSC	ESC	OVC	DC
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L				
										*				*			*					
E. coli	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.2	0.0	0.0	1.2	12.1	1.2	1.3	12	1.2	NG	NG	G	NG
S *aureus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	NG	NG	G	NG
P aeruginosa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
										*												
B subtilis	0.0	0.0	0.0	1.1	0.0	1.0	0.0	0.0	0.0	10.I	0.0	0.0	1.2	0.0	10	0.0	0.0	0.0	NG	NG	G	NG
S. *typhi*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	1.1	NG	NG	G	NG
K. pneumoniae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	1.3	NG	NG	G	NG

R: Root; S: Stem and L: Leaf
NG: No growth observed
G: Growth observed

*: Bacteriostatic action of extract
**: Bactericidal action of extract
MSC: Medium sterility control
ESC: Extract sterility control
OVC: Organism viability control
DC: Diluent control

4.3 Antibacterial activity of the extracts of *Ca/otropis procera*

At 1OOO~g/ml, all the extracts had no antibacterial activity against test organisms (Table 6). At *2000jlg/ml,* the stem n-hexane extract showed moderate activity against *E. coli* having average zone of inhibition of 5.5mm. The root and leaf n-hexane extract at 2000jlg/ml showed low activity against S *typhi* having average zone diameter of inhibition of 1.0 and 1.1 respectively while the root and leaf ethanol extracts showed low activity having average zone diameter of inhibition of 1.1 and 1.0 respectively. Also the root and leaf ethanol extracts had low activity against *K. pneumoniac* having average zone diameter of inhibition of 1.0 and 1.1 respectively (Table 6). The water extract at *2000jlg/ml* had no antibacterial activity against test organism.

Table 6: Average diameter of zone of inhibition (mm) produced by extracts of *Ca/otropis procera* against test bacteria

Organism	Extracts (1OOOllg/ml)							Extracts (2000llg/ml)							Control			
	n-hexane			Ethanol			Water			n-hexane			Ethanol			Water			MSC	ESC	OVC	DC
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L				
E. coli	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
S *aureus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
P aeruginosa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
B. subt ilis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
S. *typhi*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	1.0	1.1	0.0	1.0	0.0	0.0	0.0	NG	NG	G	NG
K. pneumoniae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	1.1	0.0	0.0	0.0	NG	NG	G	NG

R: Root, S: Stem and L: Leaf
NG: No growth observed
G: Growth observed.
MSC: Medium sterility control
ESC: Extract sterility control
OVC: Organism viability control
DC: Diluent control

4.4 Antibacterial activity of the extracts of *Scltwenkia americana*

The extracts had no activity at 1000).1g/ml. At *2000).1g/ml,* only leaf n -hexane extract had low activity against S. *typhi* (Table 7).

r

Table 7: Average diameter of zone of inhibition (mm) produced by extracts of *Scltwenkia amaericana* against test bacteria

Organism	Extracts (1OOOJ.lg/ml)						Extracts (2000llg/ml)						Control			
	n-hexane			Ethanol			Water			n-hexane			Ethanol			Water			MSC	ESC	OVC	DC
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L				
E. coli	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
S *aureus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
P aeruginosa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
B. subtilis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
S. *typhi*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
K. pneumoniae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG

R: Root, S: Stem and L: Leaf
NG: No growth observed
G: Growth observed.

MSC: Medium sterility control
ESC: Extract sterility control
OVC: Organism viability control
DC: Diluent control

4.5 Antibacterial activity of the extracts of *Merremia tridentata* subspecies *angustifolia*

At lOOO)lg/ml and 2000)lg/ml, the stem ethanol extract showed low activity against *E. coli* having average zone diameter of inhibition of 1.0mm and 1.2mm respectively (Table 8). The rest extract at 1OOO~lg/mldid not show activity against the test bacteria. At 2000)lg/ml, the leaf n -hexane and stem water extracts showed low activity against S. typhi and *B. subtilis* (Table 8)

Table 8: Average diameter of zone of inhibition (mm) produced by extracts of *Merremia tridentata* subspecies *angustifolia* against test bacteria

Organism	Extracts (1000~g/ml)									Extracts (2000~g/ml)									Control			
	n-hexane			Ethanol			Water			n-hexane			Ethanol			Water			MSC	ESC	OVC	DC
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L				
E. coli	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	NG	NG	G	NG
S *aureus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
P aeruginosa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
B. sub/dis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	NG	NG	G	NG
S. *typhi*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG
K. pneumoniae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NG	NG	G	NG

R: Root, S: Stem and L: Leaf
NG: No growth observed
G: Growth observed.

MSC: Medium sterility control
ESC: Extract sterility control
OVC: Organism viability control
DC: Diluent control

4.6 Antifungal activity of *Selina alata* n-Iiexane extracts.

At 1OOO~g/ml, the root and leaf extracts showed appreciable antifungal activity against *Microsporium canis* (Table 9). The root and stem extracts also showed moderate activity against *Trichophyton rubrum, Trichophyton mentagrophyte* and *Epidermophyton jlocossun.* The leaf extract showed low activity against *Trichophyton mentagrophyte* and *Epidermophyton flocossun* (Table 9). At 2000~g/ml, the root extract showed appreciable activity against *Candida albicans* while the root and leaf extracts also showed appreciable activity against *Trichophyton mcntagrophyte, Microsporium canis* and *Epidermophyton jlocossun.* While the root had moderate activity against *Trichophyton rubrum.* The stem extract moderate activity against *Trichophyton ntentagrophyte, Microsporium canis* and *Epidermophyton flocossun* while the root had moderate activity against *Trichophyton rubrum.* The stem and the leaf extracts had no activity against *Trichophyton rub rum* and *Candida albicans.*

The mode of action of the root and leaf *Senna alata* n-hexane extract against *Microsporium canis* at IOOO~g/ml and 2000~g/ml; and *Epidermophyton jlocossun* and *Candida albicans* at 2000~g/mJ was fungicidal while it was fungistatic against *Trichophyton mentagrophyte* at 2000~g/ml.

4.7 Antifungal activity of *Senna alata* ethanol extracts.

The root and leaf extracts showed low activity against *Trichophyton rubrum, Microsporium canis* and *Epidermophyton jlocossun* at IOOO~lg/ml while the stem showed low activity against *Trichophyton rubrum* and *Microsporium canis* (Table 9). The stem and leaf extracts showed no activity against *Trichophyton mentagrophyte* and *Candida albicans.* At 2000~g/ml, the root extract showed appreciable activity against *Trichophyton mentagrophyte, Epidermophyton jlocossun* and *Candida albicans* while the stem extract showed appreciable activity against *Epidermophyton flocossun.* The leaf extract showed moderate activity against *Epidermophyton flocossun* while the stern and leaf extracts showed no activity against *Candida albicans* (Table 9).

The mode of action of the root *Senna alata* ethanol extract against *Trichophyton mentagrophyte* was fungistatic at IOOO~lg/ml and 2000~lg/ml while it was fungicidal against *Candida albicans.* The mode of action of the stem extract was also fungicidal against *Epidermophyton flocossun* at IOOO~g/11 and 2000~Lg/ml

4.8 Antifungal activity of *Senna alata* water extracts.

At 1000119/01land *2000~l9/ml,* the root and leaf extracts showed appreciable activity against *Epidermophyton flocossun* while at 1000~l9/111lthe leaf extract showed appreciable activity against *Trichophyton mentagrophyte* (Table 9). At 1000119/ml, the leaf extract showed moderate activity against *Trichophyton rubrutn* while the root had moderate activity against *Trichophyton mentagrophyte.* The stem extract showed low activity against *Trichophyton mentagrophyte* and *Epidermophyton flocossun.* The root and stem extracts did not show any activity against *Trichophyton rubrum.* Also the stem and the leaf extracts showed no antifungal activity against *Candida albicans* (Table 9. Also at *2000119/ml,* the root extract showed moderate activity against *Microsporiuin canis* and *Candida albicans.* While the root extract showed appreciable activity against *Trichophyton rubrum,* the leaf extract showed moderate activity. The stem extract showed low activity against *Trichophyton mentagrophyte* and *Epidermophyton flocossun* but no activity against *Trichophyton rub rum, Microsporium canis* and *Candida albicans.*

The mode of action of the leaf *Senna alata* water extract against *Trichophyton mentagrophyte* was fungistatic at 1000~l9/ml and *2000119/111*while the root and leaf was fungicidal against *Epidermophyton flocossun.* The root water extract at *2000119/ml* also was observed to be fungistatic against *Trichophyton mentagrophyte* and *Trichophyton rubrum.*

Table 9: Antifungal activity of extracts of *Senna a/ala*

Organism	Extracts (1000).!g/ml)									Extracts (2000).!g/ml)									Control			
	n-hexane			Ethanol			Water			n-hexane			Ethanol			Water			MSC	ESC	ove	DC
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L				
T rubrum	++	++	-	+	+	+	-	-	++	++	++	-	+	+	+	+++	–	++	+++	+++	-	+++
T mentagrophyte	++	++	+	+++	-	-	++	+	+++	+++	++	+++	+++	-	+++	+++	j-	+++	+++	+++	-	+++
M canis	+++	++	+++	+	+	+	++	-	-	+++	++	+++	+	+	+	++	-	+	+++	+++	-	+++
E.jlocossun	++	++	+	+	+++	+	+++	+	+++	+++	++	+++	+++	+++	++	+++	+	+++	+++	+++	-	+++
C *albicans*	-	-	-	+++	–	-	+	-	-	+++	-	-	+++	-	-	++	-	-	+++	+++	-	+++

R: Root, S: Stem and L: Leaf
+++: Appreciable activity; ++: Moderate activity; +: Lowactivity; -: No activity
*: Fungistatic action of extract
**: Fungicidal action of extract

MSC: Medium sterility control
ESC: Extract sterility control
OVC: Organism viability control
DC: Diluent control

4.9 Antifungal activity of *Ca/otropis procera* n-hexane extracts.

At 1OOO~g/ml,the extracts of the three plant parts tested showed moderate antifungal activity against *Microsporium canis.* Similarly, the stem and the leaf extracts exhibited moderate activity against *Trichophyton mentagrophyte,* The root and stem extracts showed no activity against *Epidermophyton flocossun* and *Candida albicans* while the root and leaf extracts exhibited no activity against *Trichophyton rubrum* (Table 10). At 2000~lg/ml, the stem and the leaf extracts showed appreciable activity against *Trichophyton mentagrophyte* and *Microsporium canis* (Table 10). The stem extract showed moderate activity against *Trichophyton rubrum* and low activity against *Epidermophyton jlocossun* and *Candida albicans.* The root extract had no activity against *Epidermophyton flocossun* and *Candida albicans.* Similarly, the leaf extract exhibited no activity against *Trichophyton rubrum* and *Candida albicans* (Table 10).'

The mode of action of the root, stem and leaf n-hexane extracts against *Trichophyton mentagrophyte,* stem and leaf extracts against *Microsporium canis* and leaf extract against *Epidermophytonjlocossun* was fungicidal at 2000~g/ml.

4.10 Antifungal activity of *Calotropis pro cera* ethanol extracts.

At 1000~g/ml, the root, stem and leaf extracts showed low to moderate activity against all test organisms with the exception of *Candida albicans* on which no activity of the extracts was observed (Table 10. Similarly, the root and stem extracts did not inhibit the growth of *Epidermophyton jlocossun.* At 2000~g/ml, the root, stem and leaf extracts exhibited appreciable activity against *Epidermophyton jlocossun* while showing low to moderate ,-'

activity against other test organisms (Table 10). It was however, observed that root and leaf extracts had no activity against *Candida albicans.*

The mode of action of the root, stem and leaf ethanol extracts at 2000~lg/ml against *Epidermophytonflocossun* was fungicidal.

4.11 Antifungal activity of *Ca/otropisprocera* water extracts.

The leaf extract at 1OOO~lg/ml showed appreciable activity against *Trichophyton mentagrophyte, Microsporium canis* and *Epidermophyton flocossun* (Table 10). The root extract showed low activity against *Microsporium canis* and *Epidermophyton flocossun.* The stem extract was observed to show low activity against *Microsporium canis.* The root, stem and leaf extracts did not show activity against *Trichophyton rubrum* and *Candida albicans.* Similarly, the root and stem extracts did not inhibit the growth of *Trichophyton mentagrophyte* (Table 10). At 2000~g/ml, the leaf extract showed appreciable activity against the test fungi except *Candida albicans* where the extract had no activity. The root and stem extracts showed low activity against *Trichophyton mentagrophyte, Microsporium canis* and *Epidermophyton flocossun.* It was also observed that the root and stem extracts had no activity against *Trichophyton mentagrophyte* and *Candida albicans* (Table 10). The leaf extract did not show activity against *Candida albicans.*

The mode of action of the leaf water extract against *Trichophyton rubrum* at 2000~g/ml was fungistatic while it was fungicidal against *Trichophyton mentagrophyte, Microsporium canis* and *Epidermophytonflocossun.*

Table 10: Antifunzal activity of extracts of *Calotroois procera.*

Organism	Extracts (1000j.!9/ml)									Extracts (2000j.!9/m1)									Control			
	n-hexane			Ethanol			Water			n-hexane			Ethanol			Water			MSC	ESC	ave	DC
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L				
T rubrum	-	+	-	+	+	+	-	-	-	+	++	-	+	+	+	-	-	+++ *	+++	+++	-	+++
										* *	* *	* *						* *				
T mentagrophyte	-	++	++	+	+	+	-	-	+++	+++	+++	+++	++	+	++	+	+	+++	+++	+++	-	+++
											* *	* *						* *				
M canis	++	++	++	+	+	++	+	+	+++	++	+++	+++	++	+	++	+	+	+++	+++	+++	-	+++
												* *	* *	* *	* *			* *				
E.jlocossun	-	-	+	-	-	+	+	-	+++	-	+	+++	+++	+++	+++	+	+	+++	+++	+++	-	+++
C. *albicans*	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+++	+++	-	+++

R: Root, S: Stem and L: Leaf
+++ : Appreciable activity; ++ : Moderate activity; + : Low activity; -: No activity
* : Fungistatic action of extract
** : Fungicidal action of extract

MSC: Medium sterility control
ESC: Extract sterility control
OVC: Organism viability control
DC: Diluent control

4.12 Antifungal activity of *Scltwenkia americana* n-hexane extracts.

At *10001l9/ml,* the root extract showed low activity against *Trichophyton rubrum, Microsporium canis* and *Epidermophyton jlocossun* while the stem extract showed low activity against *Epidermophyton jlocossun* and *Candida albicans* (Table 11). The stem and leaf extracts showed no activity against *Trichophyton rubrum, Trichophyton mentagrophyte* and *Microsporium canis.* However, the leaf extract showed moderate activity against *Epidermophytonjlocossun.* At *20001l9/ml,* the root extracts showed moderate activity against *Trichophyton rubrum, Microsporium canis* and *Epidermophyton jlocossun* while the stem and the leaf extracts showed moderate activity against *Candida albicans* and *Epidermophyton jlocossun* (Table 11). The stem and leaf extracts did not show activity against *Trichophyton rubrum, Trichophyton mentagrophyte* and *Microsporium canis.*

4.13 Antifungal activity *Schwenkia americana* ethanol extracts.

The extracts at *10001l9/ml* did not show activity against the test fungi with the exception of the stem extract which exhibited low activity against *Trichophyton rubrum* (Table 11). Similarly, the root, stem and leaf extracts at *20001l9/ml* showed low activity against *Epidermophytonjlocossun.* It was also observed that the root and stem extracts showed low to moderate activity against *Candida albicans* while the leaf extract exhibited appreciable activity against *Candida albicans* (Table 11). The mode of action of the leaf ethanol extract at *2000ll9/ml* against *Candida albicans* was fungistatic.

4.14 Antifungal activity of *Scltwenkia americana* water extracts.

At *10001l9/ml,* the extracts did not show activity against the test fungi but at 2000llg/ml, the leaf extract showed low activity against *Epidermophyton jlocossun* and *Candida albicans* (Table 11).

Table 11: Antifungal activity of extracts of *Schwenkia americana*

Organism	Extracts (1 OOOIl9/ml)									Extracts (20001l9/ml)									Control			
	n-hexane			Ethanol			Water			n-hexane			Ethanol			Water			MSC	ESC	ove	DC
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L				
T rubrum	+	-	-	-	+	-	-	-	-	++	-	-	-	-	-	-	-	-	+++	+++	-	+++
T mentagrophyte	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	+++	+++	-	+++
M canis	+	-	-	-	-	-	-	-	-	++		-	-	-	-	-	-	-	+++	+++	-	+++
1E.jlocossun	+	+	++	-	-	-	-	-	-	++	++	++	+	+	+	-	-	+	+++	+++	-	+++
															*							
C. *albicans*	-	+	+	-	-	-	-	-	-	-	++	++	+	++	+++	-	-	+	+++	+++	-	+++

R: Root, S: Stem and L: Leaf
+++: Appreciable activity; ++: Moderate activity; +: Low activity; -: No activity
* : Fungistatic action of extract

MSC: Medium sterility control
ESC: Extract sterility control
OVC: Organism viability control
DC:Diluent control

4.19 Antibacterial activity of fractions from *Senna alata* extracts on *Bacillus subtilis*

Senna alata n-hexane root extract produced 6 fractions (FO-F5). The antibacterial activity of the fractions against *Bacillus subtilis* revealed that F4 was appreciably active against the test organism while F5 showed low activity. Other fractions were not active. The phytochemistry of the fractions (Table 15) showed that phenols were the active ingredients responsible for the antibacterial activity of the fraction. The *Senna alata* leaf ethanol extract produced 8 fractions FO - F7 The antibacterial activity of the fractions against *Bacillus subtilis* showed that F2 was appreciably active against the test organism while F3 showed low activity. The rest of the fractions were not active It was observed that tannins were the active substance responsible for the antibacterial activity of the fraction (Table 15).

Table 18: The phytochemical analysis of the fractions from *Senna a/ala* stem ethanol extract

Phytochemical Components	Fractions					
	FO	Fl	F2	F3	FS	F6
Alkaloids						
Tannins		+++				
Saponins						
Anthraquinones						
Cardiac glycosides	+++		+++			
Polyphenols					+++	+1-+
Phlobatannins				+++		
Sesquiterpenes						
Flavonoids						
Volatile oils						
Resins						
Phenols						

+++: Present of active ingredient in appreciable amount in the fraction

-: Absence of active ingredient in the fraction.

4.24 Antifungal activity of fractions from *Senna alata* leaf ethanol extract on *Trichophyton mentagropltyte.*

The *Senna alata* leaf ethanol extract produced 8 fractions (FO - F7). The antifungal activity of the extract against *Trichophyton mentagrophyte* showed that F5 had a moderate activity while F2, F3, f4 and F7 had Jow activity. FO, Fl and *FG* had no activity

Since no fraction showed appreciable activity, during the formulation of drugs combinations of the fractions may be necessary to achieve synergism.

4.25 Antifungal activity of fractions from *Calotropis procera* stem ethanol extract on *Epidermophyton flocossun,*

The *Calotropis procera* stem ethanol extract produced 8 fractions (FO - F7) The antifungal activity of the extract against *Epidermophyton jlocossun* showed that F 1 and F6 exhibited appreciable activity against the test organism while the remaining fractions had moderate activity. The phytochemistry of the fraction showed that alkaloids was the active ingredient in Fl while cardiac glycosides was the active substance in F6 (Table 19)

4.26 Antifungal activity of fractions from *Calotropis procera* leaf ethanol extract on *Epidermophyton flocossun.*

The *Calotropis procera* leaf ethanol extract produced 5 fractions (FO - F4). The antifungal ?cl ivity of the extract against *Epidermophyton jlocossun* revealed that F4 had appreciable activity while F2 showed moderate activity against the organism. Tannins was the active i:.[;redien~in F4 (Table 19)

4.27 Antifungal activity of fractions from *Ca/otropis procera* leaf n-hexane extract on *Trichophyton mentagropliyte, Microsporium canis* and *Epidermophyton flocossun.*

The *Calotropis procera* leaf hexane extract produced 8 fractions (FO - F7). The antifungal activity of the extract against the organisms revealed that F2, F4, F5 and F6 had appreciable activity while the remaining fractions had no activity (Table 26). Flavonoids was identified as the active ingredient in F2, F4 and F6 while resins was the active component in F5 and F6 (Table 20).

Table 20: The phytochemical analysis of fractions from *Ca/otropis procera* leaf n-hexane extract

Phytochemical Components	Fractions			
	F2	F4	F5	F6
Alkaloids				
Tannins				
Saponins				
Anthraquinones				
Cardiac glycosides				
Polyphenols				
Phlobatannins				
Sesquiterpenes				
Flavonoids	+++	+++		+++
Volatile oils			+++	
Resins				+++
Phenols				

+++ : Present of active ingredient in appreciable amount in the fraction

-: Absence of active ingredient in the fraction.

4.28 Antifungal activity of fractions from *Schwenkia americana* leaf ethanol extract on *Candida albicans*

The *Schwenkia americana* leaf ethanol extract produced 4 fractions (FO - F3) The antifungal activity of the extract *against Candida albicans* revealed that only F3 exhibited appreciable activity while the remaining fractions were not active The phytochemistry of the fractions showed that tannins and saponins were the active substances in F3 (Table 21).

Table 21 The phytochemical analysis of the fractions of *Scltwenkia americana* extracts

Phytochemical Components	Fraction
	F3
Alkaloids	
Tannins	+++
Saponins	+++
Anthraquinones	
Cardiac glycosides	
Polyphenols	
Phi o batannins	
Sesquiterpenes	
Flavonoids	
Volatile oils	
Resins	
Phenols	

+++: Present of active ingredient in appreciable amount in the fraction

-: Absence of active ingredient in the fraction.

4.29 Antifungal activity of fractions from *Merremia tridentate* subspecies *angustifolia* leaf ethanol extract on *Candida albicans.*

The *Merremia tridentata* subspecies *angustifolia* leaf ethanol extracts produced 7 fractions (FO-F6) The antifungal activity of the extract against *Candida albicans* revealed that none of the fractions was active against the organism

Since no fraction showed appreciable activity, during the formulation of drugs combination of the fractions may be necessary to achieve synergism.

4.30 Antifungal activity of fractions from *Senna a/ata* water extract on *Epidermophyton flocossun, Trichophyton mentagrophyte* and *Trichophyton rubrum.*

The *Senna alata* root water extract produced 6 fractions (Fa - FS). The antifungal activity of the extract against the organisms showed that F3- FS had appreciable activity on *Epidermophytonjlocossun* while the rest fractions had no activity. The fractions were not active against *Trichophyton rubrum.* Saponins and anthraquinones were the active substances identified in F3; saponins in F4; and saponins and resins in FS (Table 22).

Table 22: The phytochemical analysis of fractions from *Senna a/ala* root water extract

Phytochemical Components	Fractions		
	F3	F4	FS
Alkaloids			
Tannins			
Saponins	+++	+++	+++
Anthraquinones	+++		
Cardiac glycosides			
Polyphenols			
Phlo batannins			
Sesquiterpenes			
Flavonoids			
Volatile oils			
Resins			+++
Phenols			

+++: Present of active ingredient in appreciable amount in the fraction

-: Absence of active ingredient in the fraction.

4.31 Antifungal activity of fractions from *Senna alate* leaf water extract on *Trichophyton ntentagropltyte* and *Epidermopliyton flocossun,*

The *Senna alata* leaf water extract produced 6 fractions (FO - FS) The antifungal activity of the extract against the test organisms revealed that F I, F2, F4 and FS had appreciable activity. FO and F3 had no activity on the test organisms. It was observed that anthraquinones was the active substance in Fl and F2 while resins was found in F4 and FS (Table 23).

Table 23: The phytochemical analysis of fractions from *Senna alata* leaf water extract.

Phytochemical Components	Fractions			
	Fl	F2	F4	FS
Alkaloids				
Tannins				
Saponins				
Anthraquinones	+++	+++		
Cardiac glycosides				
Polyphenols				
Phlobatannins				
Sesquiterpenes				
Flavonoids				
Volatile oils				
Resins			+++	+++
Phenols				

+++ : Present of active ingredient in appreciable amount in the fraction

-: Absence of active ingredient in the fraction.

4.32 Antifungal activity of the fractions from *Calotropis procera* leaf extract on *Trichophyton mentagropliyte, Epidermophyton flocossun, Microsporium canis* and *Trichophyton rubrum.*,

The *Calotropis procera* leaf water extract produced 6 fractions (FO - *FS).* The antifungal activity of the fractions against the test organisms revealed that FO, F2, F3 and F4 showed appreciable activity while Fl and *FS* had no activity against the test organisms. No fraction was active against *Trichophyton rubrum.* The phytochemistry of the fractions showed that alkaloid was the active substance in FO while sapoins was present in F2, F3 and F4 (Table 24).

Table 24: The phytochemical analysis of fractions from *Calotropis procera* leaf water extract.

Phytochemical components	Fractions			
	FO	F2	F3	F4
Alkaloids	+++			
Tannins				
Saponins		+++	+++	+++
Anthraquinones				
Cardiac glycosides				
Polyphenols				
Phlobatannins				
Sesqui terpenes				
Flavonoids				
Volatile oils				
Resins				
Phenols				

+++ : Present of active ingredient in appreciable amount in the fraction

-: Absence of active ingredient in the fraction.

4.33 The rate of killing of the test organisms by the extracts.

The rate of killing otherwise known as x-coefficient of the organisms (Table 25) ranged from - 0.052 for *Schwenkia americana* against *Candida albicans* to 0.108 for *Calotropis procera* against *Trichophyton rubrum.* The negative sign that preceded the x-coefficient (Table 38 and Appendices 9-37) is an indication that for every unit of time, there was a reduction or death of the test organisms. R2 x 100, the coefficient of determination and a measure of goodness of fit gave a more than 95% explanation between time of killing and *CFU/ml* of the organism in a linear expression of Y = a + bx where x-coefficient represents the rate of killing (Chatfield, 1988).

Schwenkia americana root and leaf ethanol extracts showed appreciable activity against *Candida albicans* but low activity against *Epidermophyton flocossun* (Appendix 47).

4.37 The effect of temperature on the antimicrobial activity of *Merremia tridentate* subspecies *angustifolia* extracts at 2000 1l9/ml.

Temperature had effect on the activity of the extracts against *Epidermophyton flocossun* and *Candida albicans* as it was raised to 121°C for 15 minutes (Appendix 48). The activities of n-hexane and water extracts were lost as the temperature was increased to 121°C for 15 minutes (Appendices 48 and 49). Temperature changes led to loss of activity of the root ethanol extract at 121°C against *Candida albicans* while the leaf ethanol showed appreciable activity against *Candida albicans* (Appendix 50).

4.38 Antimicrobial activity on different time of storage of *Senna alata* extracts.

The *Senna alata* root hexane extract showed appreciable activity against *Bacillus subtilis, Candida albicans, Trichophyton mentagrophyte,* and *Microsporium canis* while the leaf extract showed appreciable activity against *Microsporium canis* and *Trichophyton mentagrophyte.* However, the activity of the extracts decreased with increased time of storage against *Epidermophyton flocossun* (Appendix 51). The root and leaf water extracts showed appreciable antimicrobial activity during the time of storage against *Trichophyton mentagrphyte* and *Epidermophyton flocossun* respectively. The extracts lost activity with increased time of storage against the remaining susceptible test organisms (Appendix 52). The leaf ethanol extract showed appreciable activity against *Trichophyton mentagrophyte,*

while the root extract showed appreciable activity against *Candida albicans* and *Trichophyton mentagrophyte* during the time of storage (Appendix 53) However, the extracts showed decrease activity with increased time of storage against the remaining test organisms.

4.39 Antimicrobial activity on different time of storage of *Calotropis procera* extracts.

The leaf n-hexane extract showed appreciable antifungal activity against *Microsporium canis, Trichophyton mentagrophyte* and *Epidermophyton jlocossun* while the root extract showed decreased activity against *Microsporium canis, Salmonella typhi, Trichophyton rub rum* and *Trichophyton mentagrophyte* with increased time of storage (Appendix 54). The leaf water extract showed appreciable antifungal activity against *Microsporium canis, Trichophyton rubrum, Trichophyton mentagrophyte* and *Epidermophyton jlocossun* during the period 01 storage (Appendix 55). Also, the root extract showed decreased antifungal activity against *Microsporium canis, Trichophyton mentagrophyte* and *Epidermophyton jlocossun* with increased time of storage. The root and leaf ethanol extracts showed appreciable activity against *Epidermophyton flocossun* during the time of storage while the root extract showed moderate activity against *Candida albicans* and *Trichophyton mentagrophyte.* The leaf ethanol extract showed moderate activity against *Trichophyton mentagrophyte* during the time of storage. The extracts showed decreased activity against *Microsporium canis, Trichophyton rubrum,* S. *typhi* and *Klebsiella pneumoniae* with increased time of storage (Appendix 56).

4.19 Antibacterial activity of fractions from *Senna alata* extracts on *Bacillus subtilis*

Senna alata n-hexane root extract produced 6 fractions (FO-F5). The antibacterial activity of the fractions against *Bacillus subtilis* revealed that F4 was appreciably active against the test organism while F5 showed low activity. Other fractions were not active. The phytochemistry of the fractions (Table 15) showed that phenols were the active ingredients responsible for the antibacterial activity of the fraction. The *Senna alata* leaf ethanol extract produced 8 fractions FO - F7 The antibacterial activity of the fractions against *Bacillus subtilis* showed that F2 was appreciably active against the test organism while F3 showed low activity. The rest of the fractions were not active It was observed that tannins were the active substance responsible for the antibacterial activity of the fraction (Table 15).

Table 15: The phytochemical analysis of the fractions from *Senna a/ata*

Phytochemical Components	Fractions	
	F2	F4
Alkaloids		
Tannins	+++	
Saponins		
Anthraquinones		
Cardiac glycosides		
Polyphenols		
Phlobatannins		
Sesquiterpenes		
Flavonoids		
Volatile oils		
Resins		+++
Phenols		

+++: Present of active ingredient in appreciable amount in the fraction

-: Absence of active ingredient in the fraction.

4.20 Antifungal activity of fractions from *Senna a/ata* root n-hexane extract on *Candida a/bicans, Microsporium canis,* and *Trichophyton mentagrophyte.*

The *Senna alata* root hexane extract produced 6 fractions (FO - FS). The antifungal activity of the extract against *Candida albicans, Microsporium canis* and *Trichophyton mentagrophyte* showed that F3 was appreciably active against the test organisms while the rest of the fractions had no activity. It was observed that resins was the active ingredient responsible for the antifungal activity of the extract (Table 16).

Table 16: The phytochemical analysis of the fraction from *Senna alata* root n-hexane extract

Phytochemical Components	Fraction F3
Alkaloids	
Tannins	
Saponins	
Anthraquinones	
Cardiac glycosides	
Polyphenols	
Phlobatannins	
Sesquiterpenes	
Flavonoids	
Volatile oils	
Resins	
Phenols	+++

+++: Present of active ingredient in appreciable amount in the fraction

-: Absence of active ingredient in the fraction.

4.21 Antifungal activity of fractions from *Senna a/ata* leaf n-hexane extract on *Microsporium. canis, Trichophyton mentagrophyte* and *Epidermophyton flocossun.*

The *Senna alata* leaf hexane extract produced 9 fractions (FO - F8). The antifungal activity of the fractions against *Microsporium canis, Trichophyton mentagrophyte* and *Epidermophyton flocossun* revealed that F6 and F7 had moderate activity against *Microsporium canis;* F2, F3, F4 showed low activity while FO, FI and F8 had no activity against *Microsporium canis.* FO, FI, F2, F4 and F6 were appreciably active against *Trichophyton mentagrophyte,* F3 and F5 were moderately active while F7 and F8 showed no activity. FO, Fl, F2, F3 and F4 showed appreciable activity against *Epidermophyton jlocossun* while F6 showed moderate to no activity. Tannins was the active ingredient in FO and F6; flavonoids and volatile oils in Fl; phenols in F2 and F4, and resins in F3 (Table 17).

Table 17: The phytochemical analysis of the fractions from *Senna a/ata* leaf n-hexane extract

Phytochemical Components	Fractions					
	FO	Fl	F2	F3	F4	F6
Alkaloids						
Tannins	+++					+++
Saponins						
Anthraquinones						
Cardiac glycosides						
Polyphenols						
Phlobatannins						
Sesquiterpenes						
Flavonoids		+++				
Volatile oils		+++				
Resins				+++		
Phenols			+++		+++	

+++ : Present of active ingredient in appreciable amount in the fraction

Absence of active ingredient in the fraction.

4.22 Antifungal activity of fractions from *Senna alata* root ethanol extract on *Candida albicans* and *Trichophyton mentagropliyte.*

The *Senna alata* root ethanol extract produced 7 fractions (FO - F6). The antifungal activity of the fractions showed that F5 had low activity against *Candida albicans.* Similarly, F2 to F6 showed low activity against *Trichophyton mentagrophyle,*

Since no fraction showed appreciable activity, during the formulation of drugs combination of the fractions may be necessary to achieve synergism

4.23 Antifungal activity of fractions from *Senna alata* stem ethanol extract on *Trichophyton mentagrophyte* and *Epidermophyton flocossun.*

The *Senna alata* stem ethanol extract produced 8 fractions (FO - F7) . The antifungal activity of the extract against *Trichophyton mentagrophyte* and *Epidermophyton jl.ocossun* showed that F6 had appreciable activity against *Trichophyton mentagrophyte* while F 1 - F4 had low activity against the same organism. Fractions FO and F7 showed no activity. FO - F2 and F5 showed appreciable activity against *Epidermophyton jl.ocossun* while F3 and F7 showed moderate activity against the same organism F4 had no activity against *Epidermophyton jl.ocossun.* The phytochemistry of the fractions showed that *Cardiac glycosides* was the active substance in FO and F2; tannins in Fl; polyphenols in F5 and F6 while phlobatannins was the active substance in F3 (Table 18).

4.40 Antimicrobial activity on different time of storage of *Scltwenkia americana* extracts.

The root and leaf n-hexane extracts showed decreased activity against *Microsporium canis, Trichophyton rubrum* and *Epidermophyton flocossun* with increased time of storage (Appendix 57). The root and leaf water extracts showed decreased antifungal activity with increased time of storage against *Trichophyton mentagrophyte, Epidermophyton jlocossun* and *Candida albicans* (Appendix 58). The leaf ethanol extract showed appreciable antifungal activity against *Candida albicans* while the root extract showed decreased activity with increasing time of storage against *Candida albicans* and *Epidermophyton jlocossun* (Appendix 59).

4.41 Antimicrobial activity on different time of storage of Merremia *tridentata* subspecies *angustifolia* extracts.

The root and leaf n-hexane extracts showed decreased activity against *Candida albicans* and *Epidermophyton jlocossun* during the period of storage (Appendix 60). The root and leaf water extract showed decreased antifungal activity against *Epidermophton jlocossun* with increased time of storage (Appendix 61). The leaf ethanol extract showed appreciable activity against *Candida albicans* during the period of storage (Appendix 62). The root extract showed low activity with increased time of storage against *Candida albicans.*

CHAPTER FIVE

5.0 DISCUSSION

The solvents, n-hexane, ethanol and water used for the extraction were choosen due to their differences in their polarity (Rawlins, 1977). For this reason, n-hexane, the least polar solvent was used to extract resins which was detected in the root of *Calotropis procera* and *Schwenkia americana,* leaves of *Merremia tridentata* subspecies *angustifolia* and roots, stems and leaves of *Senna alata.* Ethanol was used to extract polyphenols, saponins, tannins, alkaloids and anthraquinones while water was used to extract alkaloids, anthraquinones, polyphenols, tannins, saponins, volatile oils, resins and phenols. This may be due to polarity difference of the solvents reported by Rawlins in 1977; water been the most polar solvent followed by ethanol and n-hexane the least.

Senna alata n-hexane root extract at 2000)..lg/ml showed appreciable antibacterial activity against *E. coli* with average zone diameter of l2.2mm. It was also observed that the stem and leaf ethanol extracts at 2000 Ilg/ml showed appreciable antibacterial activities against *E. coli* and *Bacillus subtilis* having average zone diameter of 12.lmm and 10.0mm respectively. These observations indicated the attainment of minimum inhibitory concentration of the extracts within the cells of the organisms which was prevented in other test organisms used that were resistant (Table 5). This further suggests the possibility of using the purified n-hexane and ethanol extracts of the plants to treat some enteric diseases. The measurable in vitro activities of *Senna alata* species may be due to anthroquinones (Abo *et al., 1990).*

Akinremi *et al.* (2000); Uwabujo and Adebisi, (2001) also reported the presence of anthraquinones in *Senna* species. Akinremi and other workers observed that the laxative activity of *Senna* species was due to anthraquinones. The low activity of the extracts at *1000flg/ml* may be due to low active substance present hence there was no appreciable inhibition of growth. It was also observed that water extract showed low activities against test bacteria. This observation was made by Rawlins in 1977 which he said was due to enzymatic and microbial degradation of drug constituents.

The extracts at 1000 *fl9/ml* and 2000 fl9/rnl of *Calotropis procera, Shwenkia Americana* and *Merremia tridentate* subspecies and *angustifolia* did not show appreciable activity to any of the test bacteria (Table 6). This may be due partly to little or no active substance in the extracts or resistance from the test bacteria. *Psuedomonas aerogenosa* and S. *aureus* have been reported for their environmental versality, ability to cause disease in particular susceptible individuals and their resistance to antibiotics (Ellen *et al.,* 1994).

The fungal used in these research work were more susceptible to the extracts than the bacteria species. These may be due to the complex nature of their cell envelope and other factors that contribute to resistance to antibacterial agents. The n-hexane extracts of the root and leaf of *Senna alata* showed appreciable activity at 1000 fl9/rnl and 2000 *Jl9/rnl*against *Microsporium canis* while at 2000 *flg/ml* against *Trichophyton mentagrophyte* and *Epidermophyton jlocossun.* The root extracts at 1000 fl9/rnl and 2000 *flg/ml* also showed appreciable activity against *Candida albicans.* Oloke and Kolawole (1987) made similar observation on the extracts of *Aframomium melegueta* against white range of fungi. Broad spectrum of activity of *Senna* species has been reported by Akinrefni in 2000. This also goes point out that when the

extracts is fully purified it will be use for the treatment of scalp, nail and foot mycoses and *Candida* infections.

The appreciable activity of the root ethanol extracts of *Senna alata* observed against *Trichophyton mentagrophyte* and *Microsporium canis* and the leaf extracts against *Microsporium canis* showed that the extracts may be useful in the treatment of infections cause by the organisms. Appreciable activities also observed at 2000 flg/ml, by the root and stem extracts against *Epidermophyton jlocossun* showed a broad spectrum of activity of the extracts. The appreciable activity exhibited by the root and leaf water extracts at 1000 flg/ml and 2000 flg/ml against *Epidermophyton jlocossun* and the leaf extracts against *Trichophyton mentagrophyte* showed that the drugs attainded minimum inhibitory concentration within the cells of the organisms and further suggests the use of the plant drugs in phytomedical chemotherapy.

Inability of *Calotropis pro cera* n-hexane extracts to show activity at 1000 flg/ml while it *exhibited* appreciable activity against *Trichophyton mentagrophyte, Microsporium canis* and *Epidermophyton jlocossun* at 2000 uq/ml confirms the findings of Odebiyi and Sofowora (1979) that active substances are more liberated at higher concentrations. This observation confirms the extracts as broad spectrum antifungal agents which may be use for medication against the ringworm of the nail, foot and scalp. The ethanol extracts which showed appreciable activity at 2000 flg/ml against *Epidermophyton jlocossun* proved that when fully purified could be used to control the ringworm of the groin caused bythe fungus in humans. Th~ appreciable activity shown by *Calotropis procera* w;ater extracts against *Trichophyton mehtagrophyte, Microsporium canis, Epidermophyton flocossun and Candida albicans* is an

indication of broad spectrum of activity of the extracts and could be used to treat mycoses of varying forms.

The ethanol extracts of *Schwenkia americana* and *Merremia tridentata* subspecies *angustifolia* showed appreciable activity against *Candida albicans* at 2000 uq/ml but showed low to moderate activity against the other test fungi. This may be due to the active substance in the extracts that evoked the attainment of minimum inhibitory concentration within the cell of *Candida albicans*. This also goes to suggest that if cream is formulated and a drug for oral administration propounded from purified extracts, the combined therapy could cure *Candida* infection

The minimum bacteriostatic concentration (MBC) and mimmum fungistatic concentration (MFC) carried out on crude extracts that showed appreciable activity against testr organisms revealed that the values ranged from 1000J..lg/ml to 2000J..lg/ml (Tables 13 and 14). These values are on the higher side compared with that of the reference compounds (chloramphenicol and griseofulvin), which were 30J..lg/ml and IOuq/ml respectively against the test bacteria and fungi. The high values may be due to other impure materials in association with the active substance in the extracts. If the crude plant drugs could undergo full purification, their MBC and MFC would compare well with their orthodox counterpart.

The antimicrobial activity of the accelerated gradient chromatography (AGC) and flash column chromatography (FCC) fractions of various solvents used against the test organisms revealed the presence of specific bioactive fractions that were characterized of activity against test organisms in the same way as the controlled refere~ce compounds. The AGC and FCC

tests revealed that no fraction was active against *Candida albicans* and *Trichophyton rubrum* respectively. This are easily subjects of further research work. When a fraction is appreciably active against one or more microorganisms, drugs could be formulated from it to control infections caused by the organism(s). Where there is no fraction that is active, the pharmacist usually combines fractions in various ratios to ascertain if synergism exists among the fractions.

The rate of killing of the susceptible organisms by extracts (Appendices 9 - 38) at specified minimum bacteriostatic and fungistatic concentrations (uq/ml) indicated that the extracts showed measurable killing of the test organisms. It reveals that for every unit of time, there was a reduction or death of the organisms. This is shown as a negative sign that preceded the x coefficient (Appendices 9 - 38). This further agrees with the fact that drugs usually destroy organisms involved in infections through a gradual process by having targets on either cell envelope, DNA, RNA or protein synthesis (Prescott *et al.,* 1999). The coefficient of determination which measures goodness of fit (R^2 x 100) gave a greater than 95% explanation between the two variables, time and *CFU/ml* in the linear expression of Y = a + bx where x coefficient represents the rate of killing (Chatfield, 1988).

The effect of temperature on extracts was quite noticeable. Between _4o C and 121o C, temperature had little or no effect on the extracts of *Senna alata* and *Calotropis procera* compared with *Schwenkia americana* and *Merremia tridentata* subspecies *angustifolia* extracts which lost activity as the temperature was raised to 121o C for 15minutes (Appendic.es 39-50). Temperature helps to control contamination of extracts, that is to say that contaminated extracts can be autoc1aved and still retain their antimicrobial properties as

observed in *Senna alata* and *Calotropis procera* extracts while *Schwenkia americana* and *Merremia tridentata* subspecies *angustifolia* are heat sensitive.

The shelf life of an extract is a function of stability of the active substance in it. The retention ability of antimicrobial activity of *Senna alata* extracts during the period of storage against most test organisms indicated that the active substance did not break down during the period. The active principle has been reported as anthraquinone derivatives by Ghana harbal pharmacopoeia, (1992), which was also identified in this study in the phytochemistry (Table 1). This observation also revealed that if the extract is fully purified, the shelf life could become longer and the drugs formulated from it may also compare well with the orthodox counterpart. Irvine (1961) had reported that the pulped fresh leaves of the juice could be used to treat mycosis. He also reported that the leaf decoction could be used for the treatment of dysentery and gonorrhoae.

The appreciable shelf life of *Colotropis procera* leaf hexane and leaf ethanol extracts was due to the stability of the active substance present. Such an active substance may have been degraded in the root hence the loss of antimicrobial activity with increasing time of storage. This means that the root extracts may not be used for period more than 6 months from the date of extraction to prevent the break down of active substance that contribute to activity of the extract.

The leaf ethanol extract of *Schwenkia Americana* showed appreciable activity during the period storage against *Candida albicans* but lost activity with increasing time of storage while

Merremia tridentate subspecies *angustifolia* retained activity. This may be due to loss of active ingrideints in the extracts (Odebiyi and Sofowora 1979).

5.1 CONCLUSION

some of the extracts of the best test plants have performed creditably well in inhibiting test fungi in this research. It is of interest to note in this research the close relationship between claims of traditional healers and the demonstrated antimicrobial activity. Since the extracts of the plants proved dominantly antifungal, it would appear logical that efforts be made to use them in the treatment of fungal infections. *Senna alata* and *Calotropis procera* are used as folk medicine in the treatment of skin infections. Their broad spectrum antifungal activity against *Trichophyton mentagrophyte, Trichophyton rubrum, Epidermophyton flocossun* and *Microsporium canis* may justify their ethnomedical use. For humans, topical treatment may be satisfactory for mild type of lesions. In the case of more infiamatory or persistent dermatophytoses systemic treatment is necessary. However, it is very important very important to know when using systemic therapy that one should be aware of the potent risk of drug interactions and adverse effects like hepatoxicity or general drug reaction. Topical antifungal agents are far less likely to cause adverse effect. A shortcoming of many antifungals is that they are maily fungistatic and that is one of the reasons while they have to be applied during a long period of time (Graser *et al.,* 1999b).

The shelf life of the extracts of *Senna alata* and *Calotropis procera* appears to be longer than that of *Schwenlda americana* or *Merremia tridentata* subspecies *angustifolia.* The effect of temperature on the extracts of *Schwenlda americana* and *Merremia tridentata* subspecies *angustifolia* was quite noticeable compare with that of of *Senna aZata* and *Calotropis procera* (Appendices 39-50).

To our knowledge, this is the first report of antifungal activity and phytochemistry of *Schwenkia americana* and *Merremia tridentata* subspecies *angustifolia* against *Candida albicans* which may justify their ethnomedical use as antiveneral agent.

5.2 RECOMMENDATION

The results obtained from this research appear to indicate that plants tested have the potential to generate novel metabolites. Researches into the effects of these plants are expected to boost their use in the therapy against diseases. Further studies on the characterization of the bioactive principles of some of the plants used in this work are recommended. These would involve phytopharmaceutical as well as spectra-characterization e.g. infra-red (IR) radiation and nuclear magnetic resonance (NMR) to be able to identify the chemical structures. Since the extracts were typically antifungal, the formulation of antifungal drugs or lotions should be pursued.

The botanical definition including the genus, species and authorities should be supplied and authenticated by indigenous national authorities so as to facilitate the correct identification of the plants. It is advised that the voucher specimen representing each of the plant material which has been processed be appropriately authenticated by a qualified botanist.

The chemical studies should determine the importance of using the extracts which correlate to the way in which the original recipe is utilized as a reference material in all specimens e.g. if the plant part is soaked in ethanol before it is used then the total ethanol extract will be used as a reference specimen while if the plant material is boiled in water, then water extract would

be the appropriate reference materials. However, in each of such cases, the solvent should be removed from the material prior to use. The ultimate goal of the chemical evaluation is to identify and characterize the structure of the pharmacologically active constituents.

If the pnrnary use of the herbal medicine is to combat microbes, then the antimicrobial potential of the preparation should be determined. In such a situation, standard antimicrobial protocols for the specified disease should be used. When the herbal medicine is not intended to be used as an antimicrobial agent, its microbial load should be determined and limit established following the broad World Health Organisation (WHO) guidelines on purity.
Both intensive and extensive pharmacological studies should be conducted using all the fractions including the total crude extracts. The intensive studies should embrace the use of appropriate animal model, tissue and other in vitro techniques for the particular disease for which the herbal medicine is eventually intended to be used. The extensive studies should be necessary so as to identify possible side effects of the herbal medicine.

It has been stated by World Health Organization (WHO) that the most critical assessment of herbal medicine is safety evaluation. Although, Farnsworth (Wambebe, personal communication) indicated that phytotoxicology is very low, nonetheless, from scientific, professional and moral viewpoints, toxicological assessment must be conducted on the herbal medicine intended for either veterinary or human use.

The procedures regarding processing and formulation of herbal medicine should be strictly in accordance with good manufacturing practice (GMP).

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APPENDICES

APPENDIX 1: Solvent system for Flash column and Thin layer chromatographic analysis of leaf water extract of *Calotropis procera*

Weight of Sample used = 6.0g

n-Hexane	Ethyl acetate	Methanol	Water	Volume
100	0	0	0	200ml
50	50	0	0	200ml
0	100	0	0	200ml
0	50	50	0	200ml
0	0	100	0	200ml
0	0	50	50	200ml
0	0	0	100	200m I

Thin Layer chromatography (TLC) solvent system ratio

Hexane	Dichloromethane	Methanol
60	40	20

APPENDIX 2: Solvent system for Flash column and Thin layer chromatographic analysis of root and leaf water extracts of *Senna a/ala*

Weight of Sample used = 6.0g

n-Hexane	Ethyl acetate	Methanol	Water	Volume
100	0	0	0	200ml
50	50	0	0	200ml
0	100	0	0	200m I
0	50	50	0	200ml
0	0	100	0	200ml
0	0	50	50	200ml
0	0	0	100	200ml

Thin Layer chromatography (TLC) solvent system ratio

Tubes (2, 3, 4A, and 4B)	Hexane	Ethylacetate	Methanol
	60	40	20
Tubes (5, 6, and 7)	Hexane	Dichloromethane	Methanol
	60	40	20

APPENDIX 3: Solvent system for Accelerated gradient and Thin layer chromatographic analysis of leaf ethanol extracts of *Schwenkia americana*

Weight of sample used = 3.09

n-Hexane	Ethylacetate	Ethanol	Volume
100	0	0	100mI
90	10	0	100mI
80	20	0	100mi
50	50	0	100mi
0	100	0	100mi
0	90	10	100mi
0	50	50	100mi
0	0	100	100mi

Thin layer chromatography (TLC) solvent system ratio.

Hexane	Dichioromethane	Ethanol
60	40	20

APPENDIX 4: Solvent system for Accelerated gradient and Thin layer chromatographic analysis of leaf n-hexane extract of *Calotropis procera*

Weight of sample used = 5g

n-Hexane	Ethylacetate	Methanol	Volume
100	0	0	200ml
90	10	0	200ml
50	50	0	200ml
0	100	0	200ml
0	90	10	200ml
0	0	100	200ml

Thin layer chromatography (TLC) solvent system ratio.

Hexane	Ethylacetate
60	20

Appendix 5: Solvent system for Accelerated gradient and Thin layer chromatographic analysis of stem and leaf ethanol extracts of *Merremia tridentata* subspecies *angustifolia*

Weight of sample used = 3.09

n-Hexane	Ethylacetate	Methanol	Volume
100	0	0	100mi
90	10	0	100ml
80	20	0	100mi
50	50	0	100ml
0	100	0	100ml
0	90	10	100ml
0	50	50	100mi
0	0	100	100mi

Thin layer chromatography (TLC) solvent system ratio

Hexane	Ethylacetate	Ethanol
60	40	20

Appendix 6: Solvent system for Accelerated gradient and Thin layer chromatographic analysis of stem and leaf ethanol extracts of *Cafotropis pro cera.*

Weight of sample used = 5.09

n-Hexane	Ethylacetate	Methanol	Volume
100	0	0	100mi
90	10	0	100mi
80	20	0	100mi
50	50	0	100mi
	100	0	100mI
	90	10	100mi
	50	50	100mi
	0	100	100mi

Thin layer chromatography (TLC) solvent system ratio

Tubes (1-16)	Hexane	Ethylacetate	Ethanol
	60	40	20

Tubes(17-40)	Ethylacetate	Ethanol
	60	40

Appendix 7: Solvent system for Accelerated gradient and Thin layer chromatographic analysis of root, stem and leaf ethanol extracts of *Senna a/ala*

Weight of sample used = 4.0g

n-Hexane	Ethylacetate	Methanol	Volume
90	10		200ml
50	50		200ml
0	100		200ml
	90	10	200ml
	0	100	200ml

Thin layer chromatography (TLC) solvent system ratio

Ethyl acetate	Hexane
40	60

Appendix 8: Solvent system for Accelerated' gradient and Thin layer chromatographic analysis of root and leaf n-hexane extracts of *Senna alata*

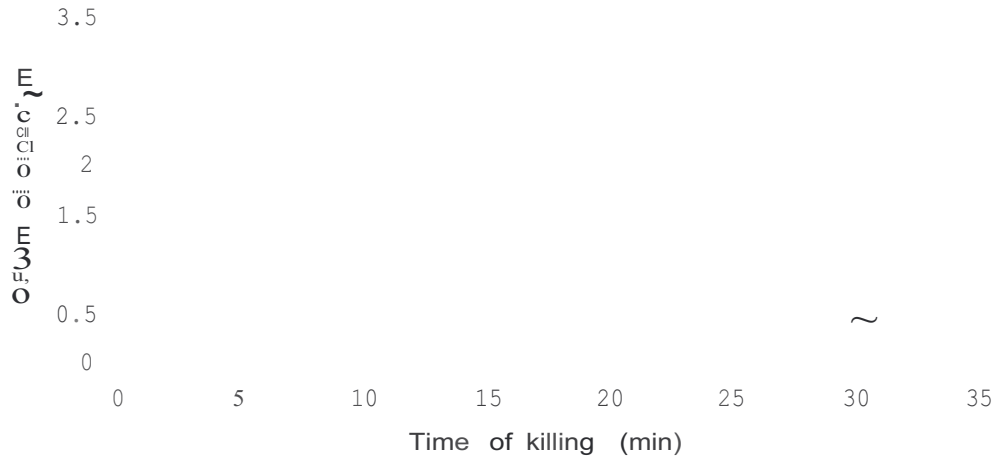
Weight of sample used = 3.59

n-Hexane	Ethylacetate	Methanol	Volume
100	0	0	200ml
90	10	0	200ml
50	50	0	200ml
0	100	0	200ml
0	90	10	200ml
0	0	100	200ml

Thin layer chromatography (TLC) solvent system ratio

Ethyl acetate	Hexane
60	20

Appendix 9: Rate of killing of *Bacillus subtilis* by *Senna alata* root hexane extract over time at 190011g/1111

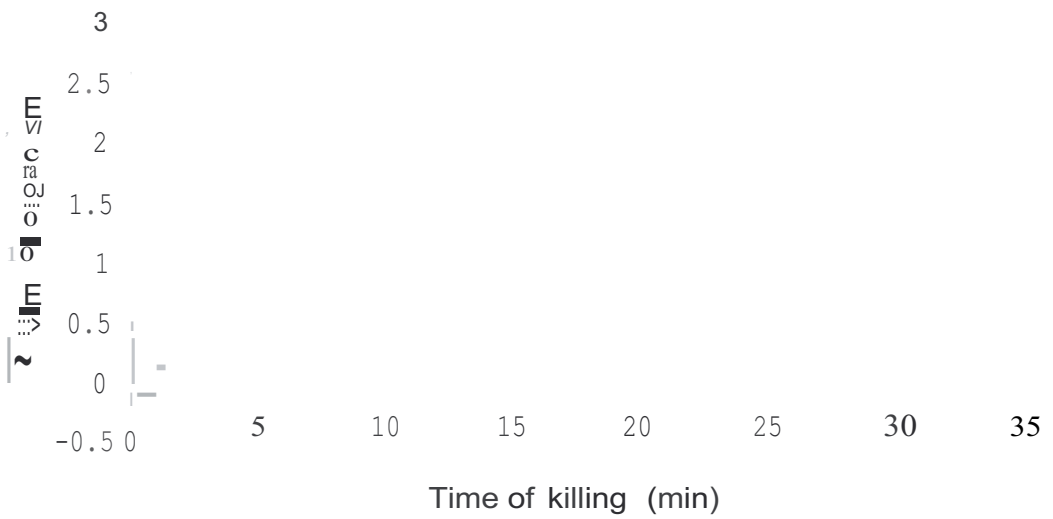


OVC - BS: 3.6X10$_3$
MSC:No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Bacillus subtilis* by *Senna alata* root hexane extract over time at 1900flg/ml.

Constant	3.3125
Std Err of Y Est	0.1744
R Squared	0.9741
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.09
Std Err of Coef.	0.0066

Appendix 10: Rate of killing of *Candida albicans* by *Senna alata* root hexane extract over time at 1900llg/ml

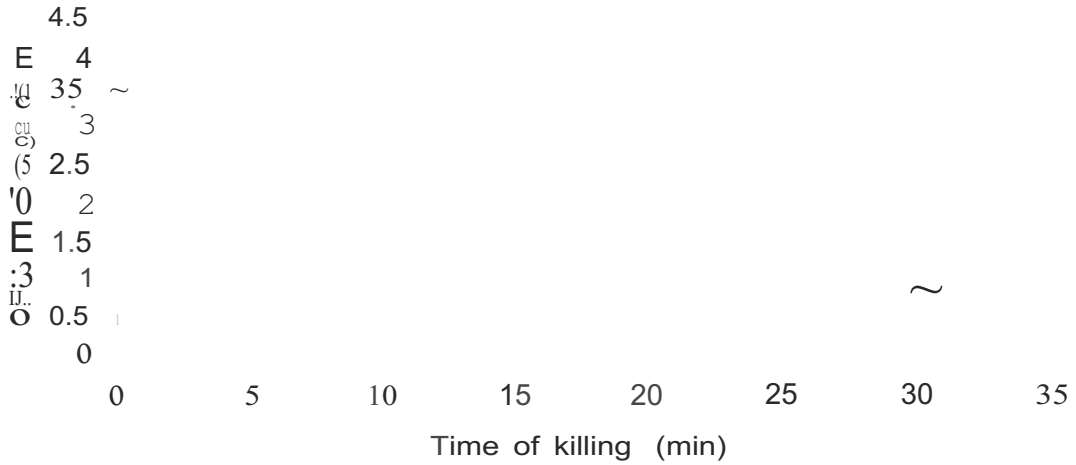


avc - CA: 3.0X10$_3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Candida albicans* by *Senna alata* root hexane extract over time at 1900llg/ml.

Constant	2.3893
Std Err of Y Est	0.1256
R Squared	0.9836
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.082
Std Err of Coef.	0.0047

Appendix 11: Rate of killing of *Microsporium canis* by *Senna alata* root hexane extract over time at *2000j...lg/ml*

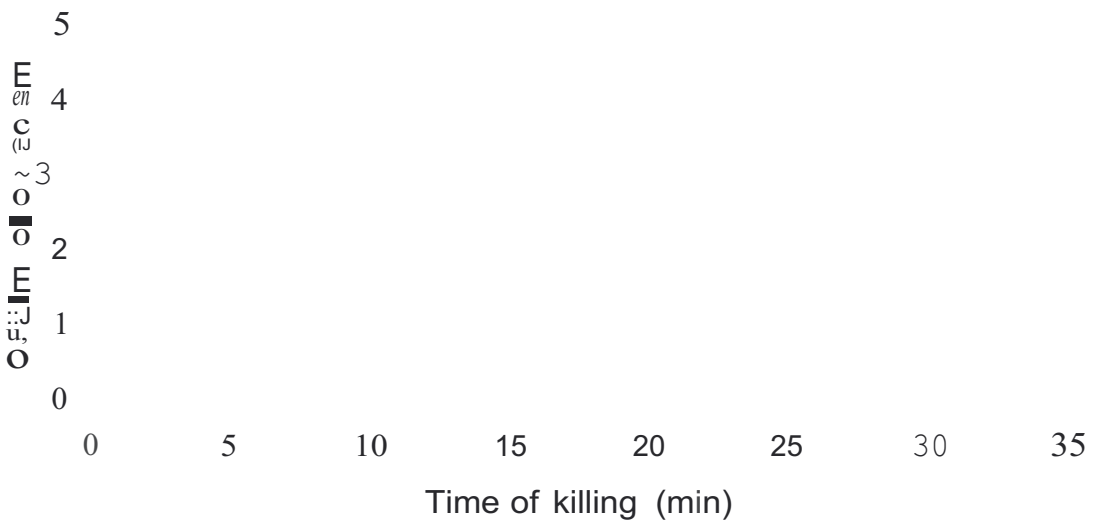


OVC - MC: 4.4x10$_3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Microsporium canis* by *Senna a/ata* root hexane extract at 2000IJg/mi

Constant	3.8339
Std Err of Y Est	0.2197
R Squared	0.9618
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.093
Std Err of Coef.	0.0083

Appendix 12: Rate of killing of *Trichophyton mentagrophyte* by *Senna alata* root ethanol extract over time at *1900~Lg/ml*

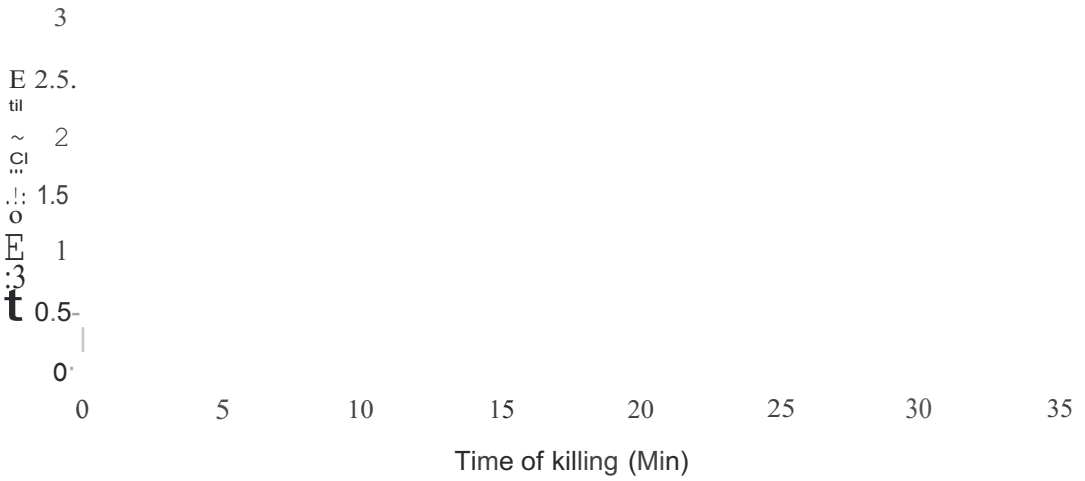


avc - TM: 5.05x10$_3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Trichophyton mentagrophyte* by *Senna a/ata* root ethanol extract at 1900j.Jg/ml

Constant	4.6232
Std Err of Y Est	0.0572
R Squared	0.9977
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.101
Std Err of Coef.	0.0022

Appendix 13: Rate of killing of *Candida albicans* by *Senna alata* root ethanol extract over time at 1900J.lg/ml

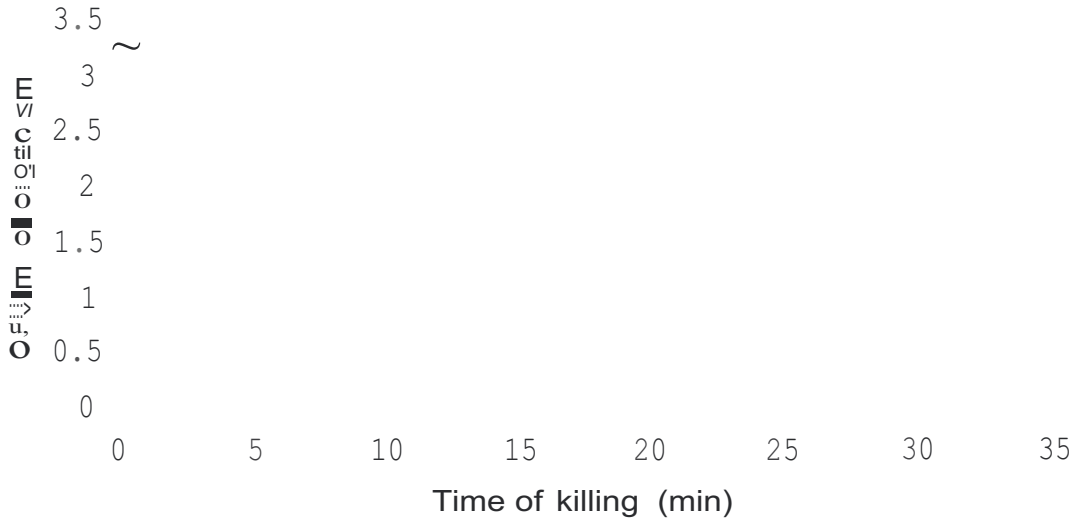


DVC- CA: 3.0X10$_3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Candida albicans* by *Senna alata* root ethanol extract over time at 1900 *Jlg/ml*

Constant	2.551786
Std Err of Y Est	0.076415
R Squared	0.99033
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.06536
Std Err of Coef.	0.002888

Appendix 14: Rate of killing of *Bacillus subtilis* by *Senna a/ala* leaf ethanol extract over time at 1500)lg/ml

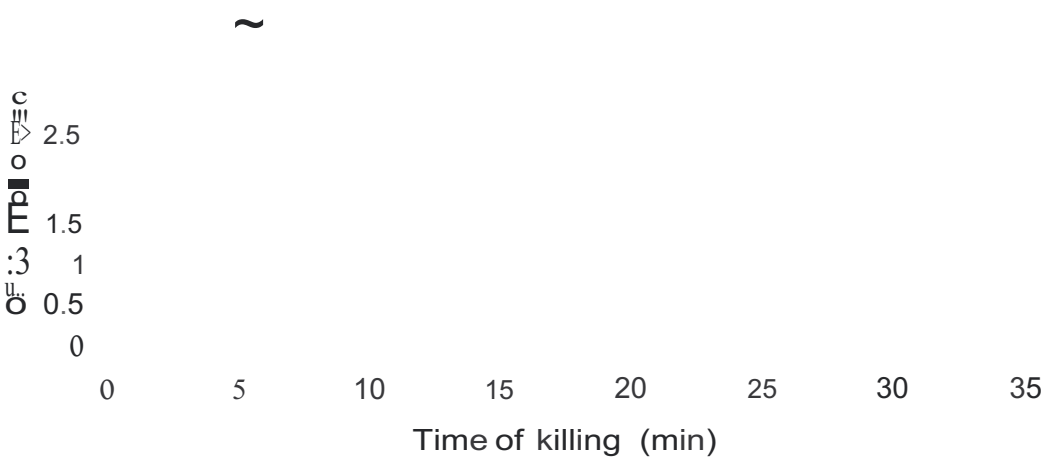


avc - BS: 3.6X10$_3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Bacillus subtilis Senna alata* leaf leaf ethanol extract over time at IS00)lg/ml.

Constant	3.0357
Std Err of Y Est	0.1978
R Squared	0.9656
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.089
Std Err of Coef.	0.0075

Appendix 15: Rate of killing of *Epidermophyton jlocossun* by *Senna alata* root water extract over time at 1OOO~lg/ml

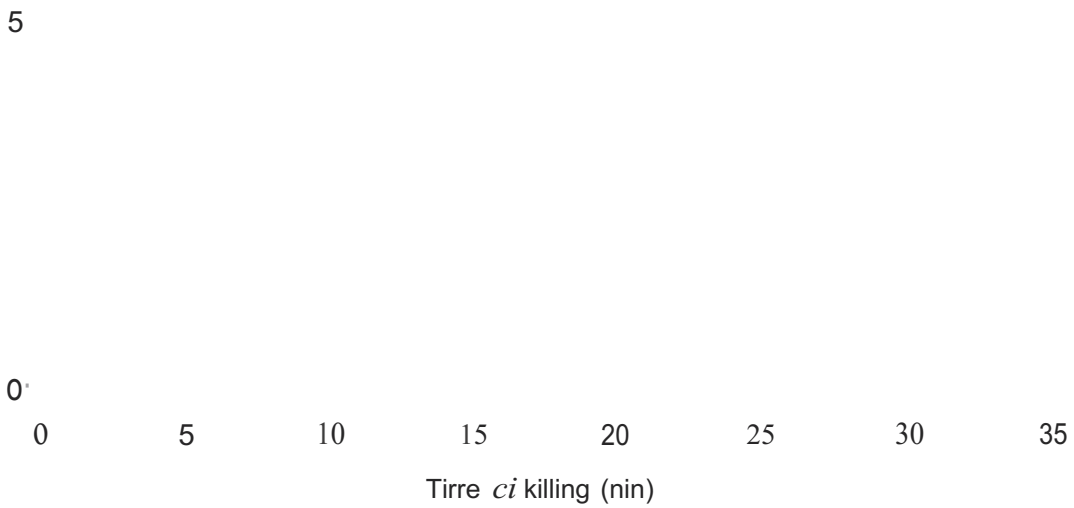


OVC- EF: 3.45X10J
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Epidermophytin f/ocossun* by *Senna a/ata* root water extract at *1000J.lg/ml*

Constant	3.5946
Std Err of Y Est	0.3768
R Squared	0.9023
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.097
Std Err of Coef.	0.0142

Appendix 16: Rate of killing of *Trichophyton rubrum* by *Senna alata* root water extract over time at 1900)..ig/ml

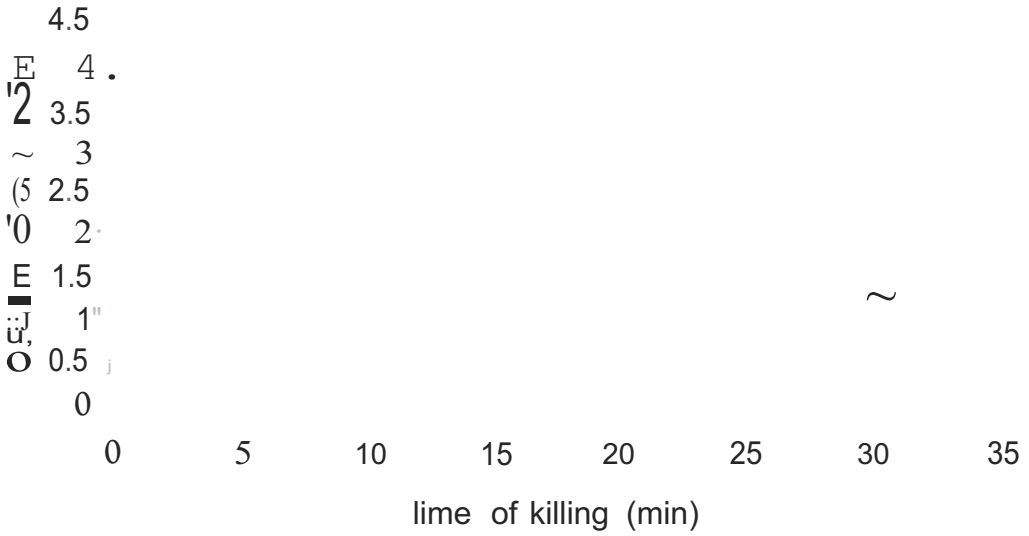


OVC- TR: 4.7x103
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Trichophyton rubrum* by *Senna a/ata* root water extract at *1900llg/mi*

Constant	4.625
Std Err of Y Est	0.2389
R Squared	0.9449
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.084
Std Err of Coef.	0.009

Appendix 17: Rate of killing of *Trichophyton mentagrophyte* by *Senna alata* root water extract over time at *2000llg/mi*

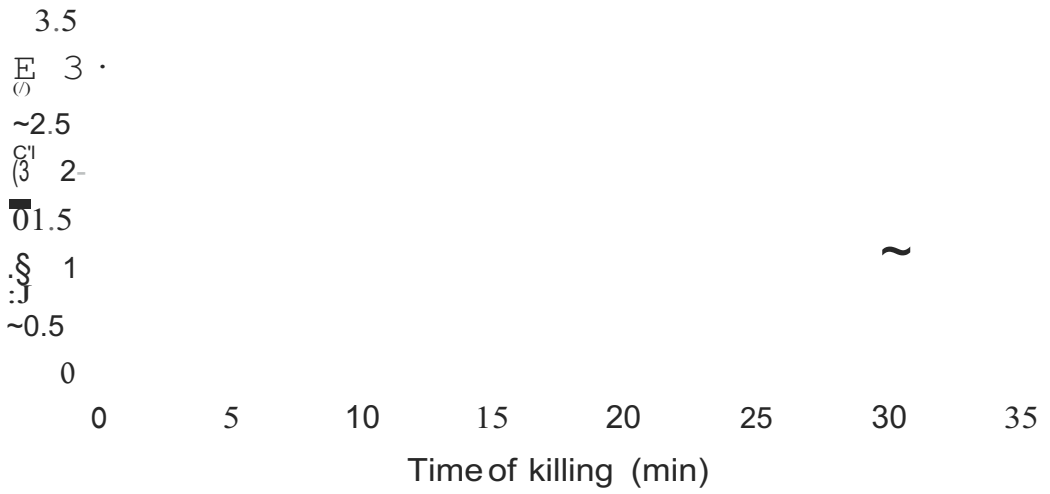


OVC - TM: 5.05x10^3
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Trichophyton mentagrophyte* by *Senna elete* root water extract at 2000l-lg/mi

Constant	4.1143
Std Err of Y Est	0.1773
R Squared	0.9722
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.089
Std Err of Coef.	0.0067

Appendix 18: Rate of killing of *Epidermophytonflocossun* by *Senna alata* stem ethanol extract over time at 200011g/ml

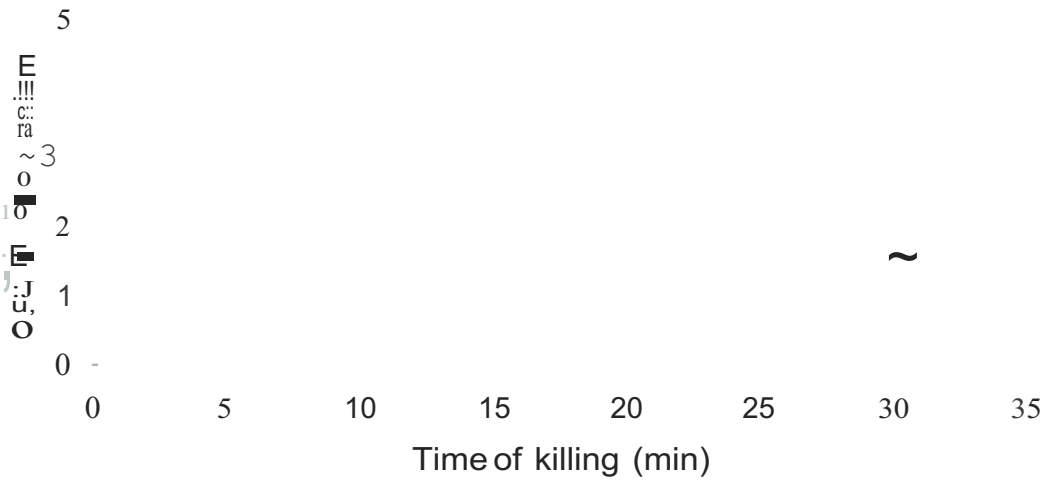


OVC- EF: 3.45X103
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Epidermophytin flocossun* by *Senna alata* stem ethanol extract at *2000)lg/ml*

Constant	3.2107
Std Err of Y Est	0.0982
R Squared	0.9832
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.064
Std Err of Coef.	0.0037

Appendix 19: Rate of killing of *Trichophyton mentagrophyte* by *Senna alata* stem ethanol extract over time at 2000 Ilg/ml



OVC- TM: 5.05x10^3
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Trichophyton mentagrophyte* by *Senna a/ata* stem ethanol extract at 2000jJg/mi

Constant	4.5161
Std Err of Y Est	0.2507
R Squared	0.9487
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.091
Std Err of Coef.	0.0095

Appendix 20: Rate of killing of *Trichophyton mentagrophyte* by *Senna a/ala* leaf ethanol extract over time at 1900 uq/ml

OVC - TM: $5.05x10_3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Trichophyton mentagrophyte* by *Senna a/ata* leaf ethanol extract at 1900J.Jg/ml

Constant	4.2804
Std Err of Y Est	0.1916
R Squared	0.9603
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.08
Std Err of Coef.	0.0072

Appendix 21: Rate of killing of *Trichophyton mentagrophyte* by *Senna alata* leaf water extract over time at 2000)lg/ml

OVC- TM: 5.05x10^3
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Trichophyton mentagrophyte* by *Senna a/ata* leaf water extract at 2000IJg/mi

Constant	4.5089
Std Err of Y Est	0.1956
R Squared	0.9661
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.088
Std Err of Coef.	0.0074

Appendix 22: Rate of killing of *Epidermophytonflocossun* by *Senna alata* leaf water extract over time at 1000 !l9/m1

OVC- EF: 3.45X10$_3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Epidermophytin f1ocossun* by *Senna a/ata* leaf water extract at *1000J.!g/ml*

Constant	3.5946
Std Err of Y Est	0.3768
R Squared	0.9023
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.097
Std Err of Coef.	0.0142

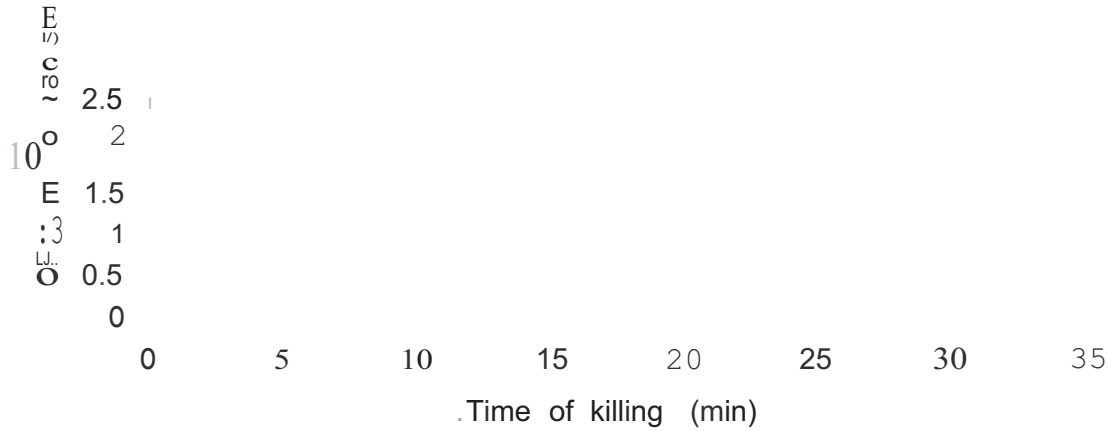
Appendix 23: Rate of killing of *Epidermophytonflocossun* by *Calotropis procera* leaf n-hexane extract over time at 1900 uq/ml

OVC - EF: 3.45x10$_3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Epidermophyton flocossun* by *Ca/otropis procera* leaf hexane extract at *1900)lg/ml*

Constant	3.1714
Std Err of Y Est	0.1146
R Squared	0.9839
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.076
Std Err of Coef.	0.0043

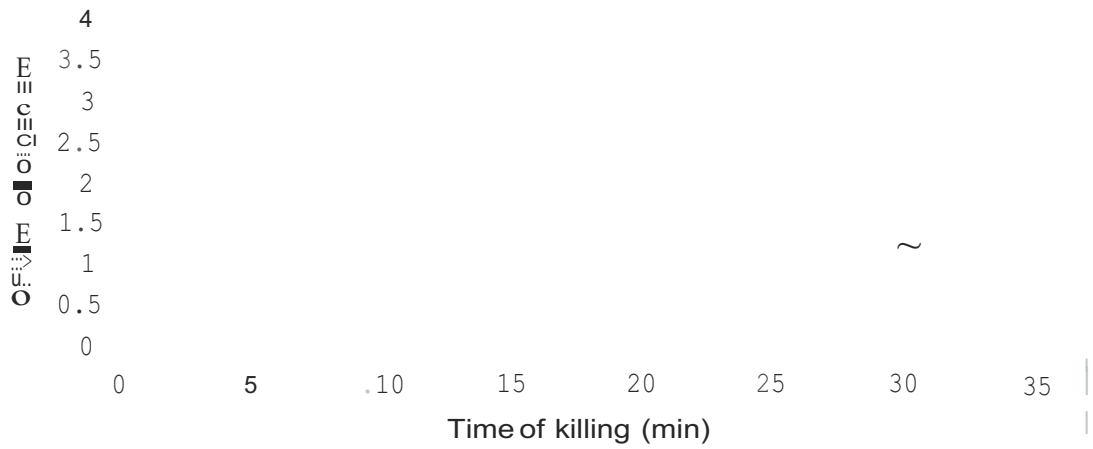
Appendix 24: Rate of killing of *Epidermophylonjlocossun* by *Calotropis procera* leaf ethanol extract over time at 1900llg/ml



VC - EF: 3.45x10^3
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Epidermophyton flocossun* by *Ca/otropis procera* leaf ethanol extract at *1900ug/ml*

Appendix 25: Rate of killing of *Epidermophytonflocossun* by *Calotropis pro cera* leaf water extract over time at 2000 Jlg/ml



avc - EF: 3.45x10$_3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Epidermophyton flocossun* by *Calotropis procera* leaf water extract at 2000ug/ml

Constant	3.4
Std Err of Y Est	0.2432
R Squared	0.9456
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.086
Std Err of Coef.	0.0092

Appendix 26: Rate of killing of *Trichophyton mentagrophyte* by *Calotropis procera* leaf n-hexane extract over time at 1900 flg/ml

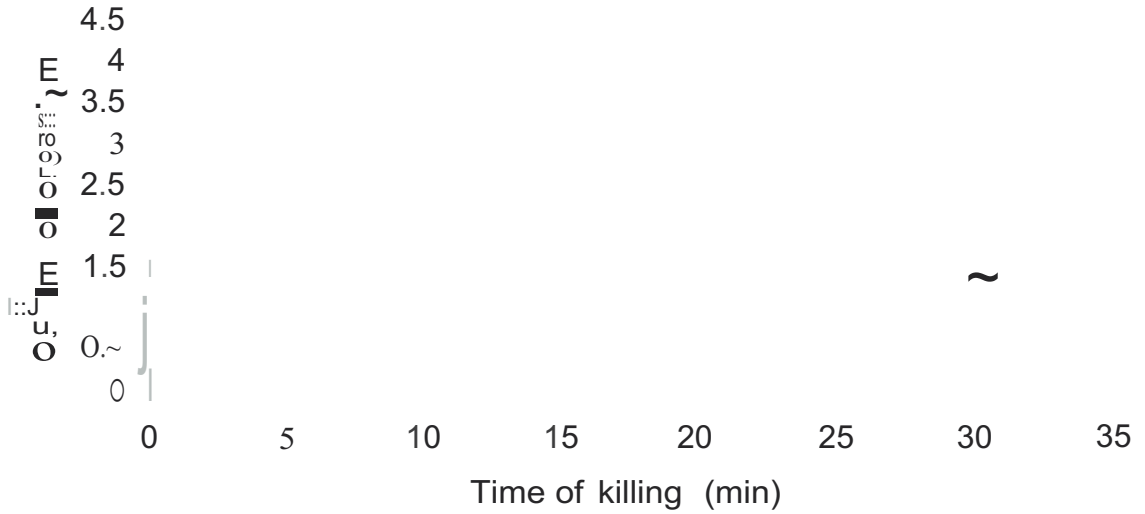


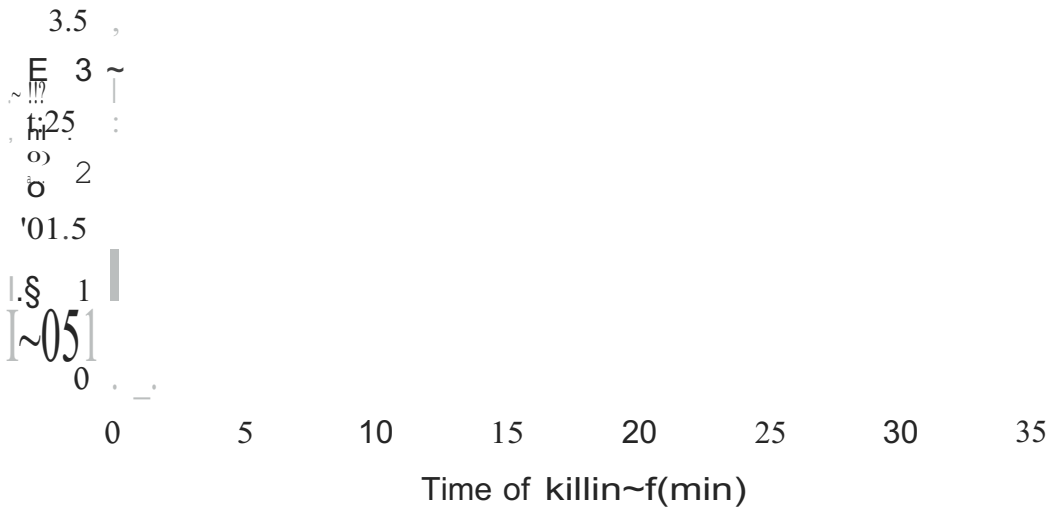
Fig 18: Rate of killing of *Trichophyton mentagrophyte* by *Calotropis procera* leaf hexane extract over time at 1900ug/ml

OVC - TM: 5.05×10^3
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Trichophyton mentagrophyte* by *Ca/otropis procera* leaf hexane extract at *1900J-lg/ml*

Constant	4.5089
Std Err of Y Est	0.1956
R Squared	0.9661
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.088
Std Err of Coef.	0.0074

Appendix 27: Rate of killing of *Epidermophytonjlocossun* by *Calotropisprocera* stem ethanol extract over time at 1900)..I.g/ml

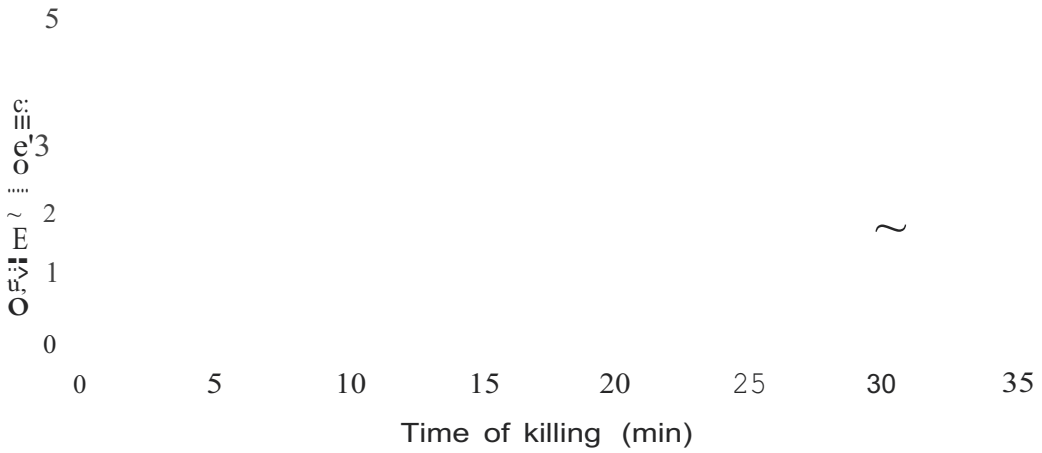


OVC - EF: 3.45X10$_3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Epidermophyton flocossun* by *Calotropis procera* stem ethanol extract at *1900)lg/ml*

Constant	3.3214
Std Err of Y Est	0.1219
R Squared	0.9837
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.08
Std Err of Coef.	0.0046

Apendix 28: Rate of killing of *Trichophyton mentagrophyte* by *Calotropis procera* stem n-hexane extract over time at 2000 Ilg/ml

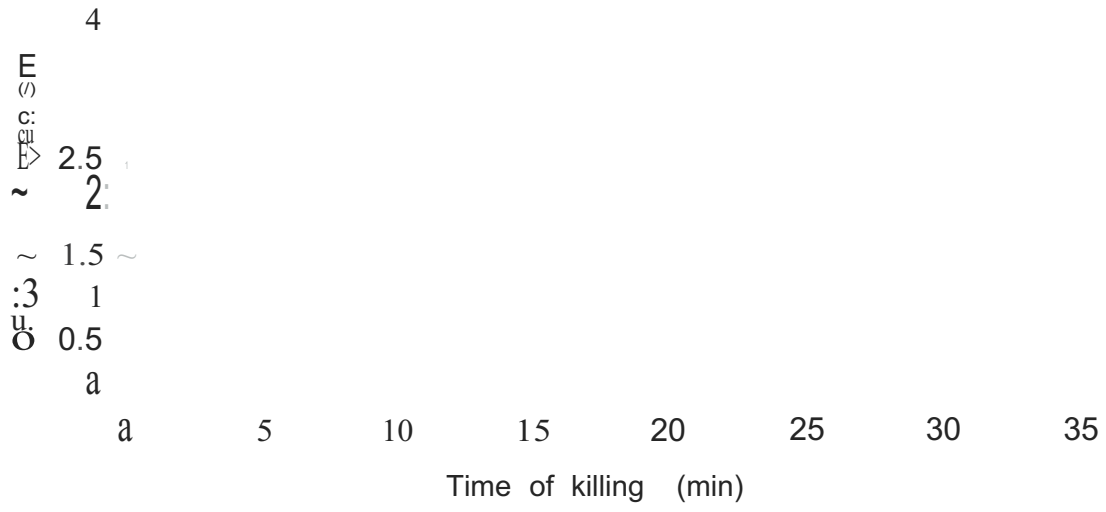


OVC - TM: 5.05x10^3
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Trichophyton mentagrophyte* by *Ca/otropis procera* stem hexane extract at *2000~g/ml*

Constant	4.3946
Std Err of Y Est	0.1788
R Squared	0.9652
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.08
Std Err of Coef.	0.006,8

Appendix 29: Rate of killing of *Microsporium canis* by *Calotropis procera* stem n-hexane extract over time at 2000 jlg/ml

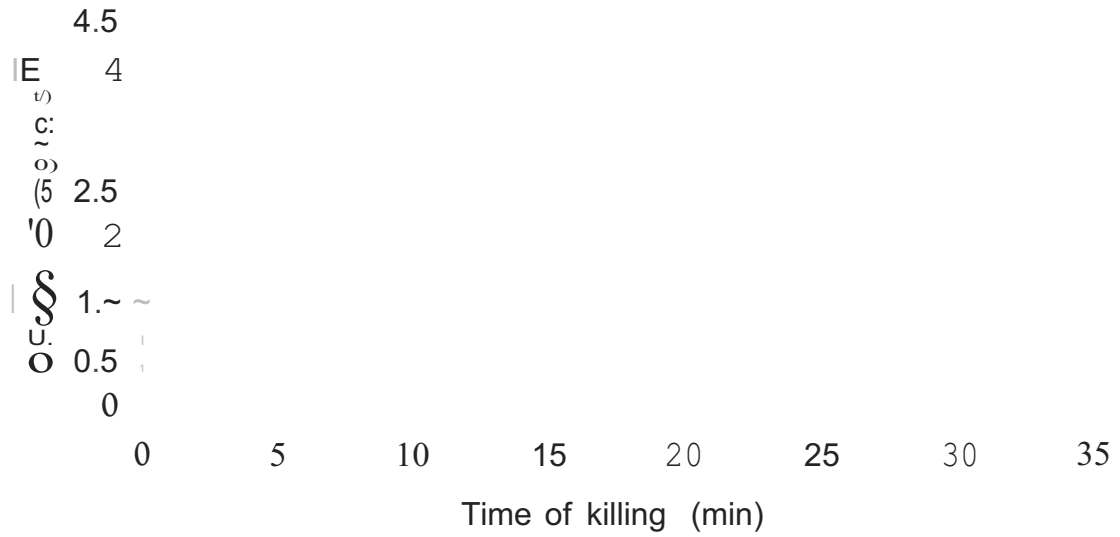


OVC - MC: $4.4x10^3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Microsporium canis* by *Ca/otropis procera* stem hexane extract at 2000J,Jg/ml

Constant	3.7268
Std Err of Y Est	0.2292
R Squared	0.9573
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.092
Std Err of Coef.	0.0087

Appendix 30: Rate of killing of *Microsporiurn canis* by *Calotropis procera* leaf n-hexane extract over time at 2000 1l9/ml

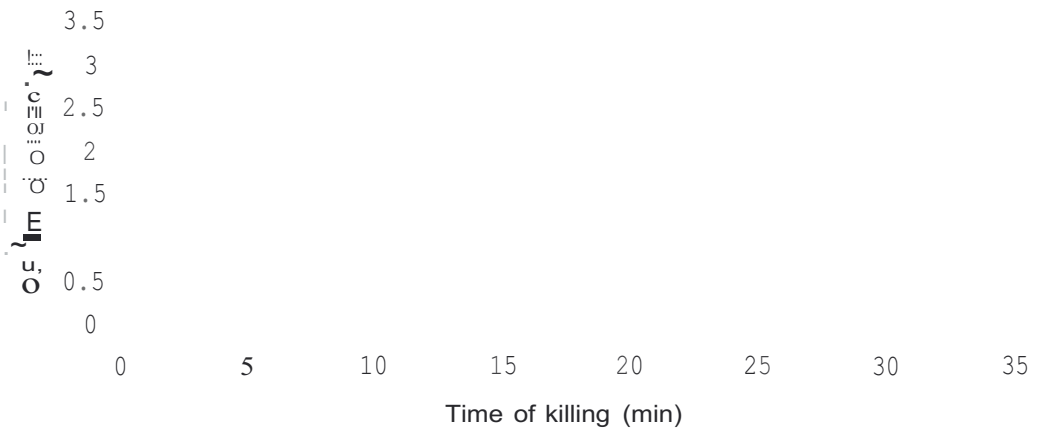


ove - MC: 4.4x10$_3$
MSC: No growth

Diluent control (DC): No growth

Regression Output of rate of killing of *Microsporium canis* by *Caiotropis procera* leaf hexane extract at *200011g/ml*

Appendix 31: Rate of killing of *Microsporium canis* by *Calotropis procera* leaf water extract over time at 1000 1l9/ml

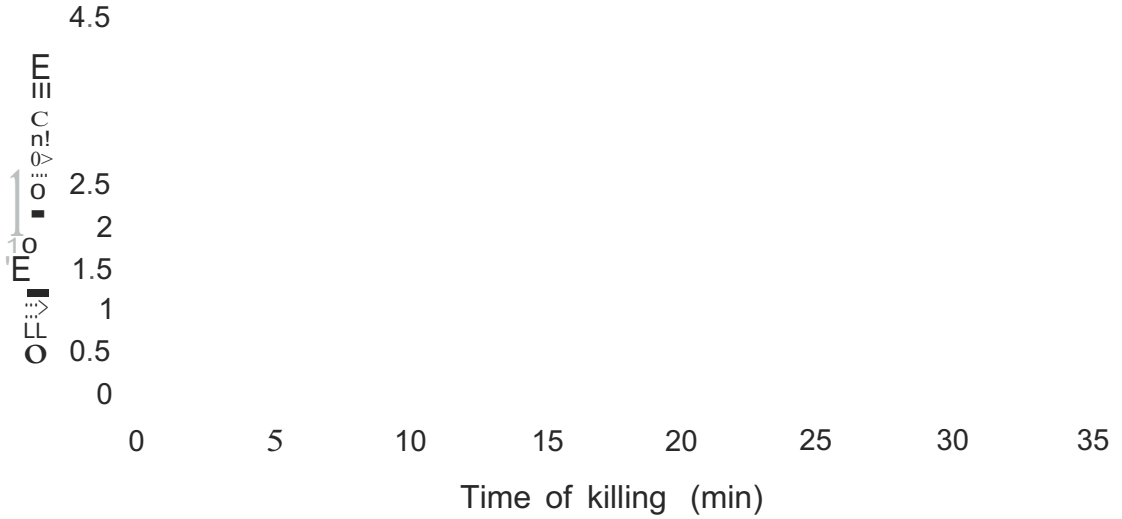


OVC- MC: 4.4x10^3
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Microsporium canis* by *Ca/otropis procera* leaf water extract att *000(.Jg/ml* ..

Constant	3.1429
Std Err of Y Est	0.0746
R Squared	0.988
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.057
Std Err of Coef.	0.0028

Appendix 32: Rate of killing of *Trichophyton mentagrophyte* by *Calotropis procera* leaf water extract over time at 1000 uq/rnl

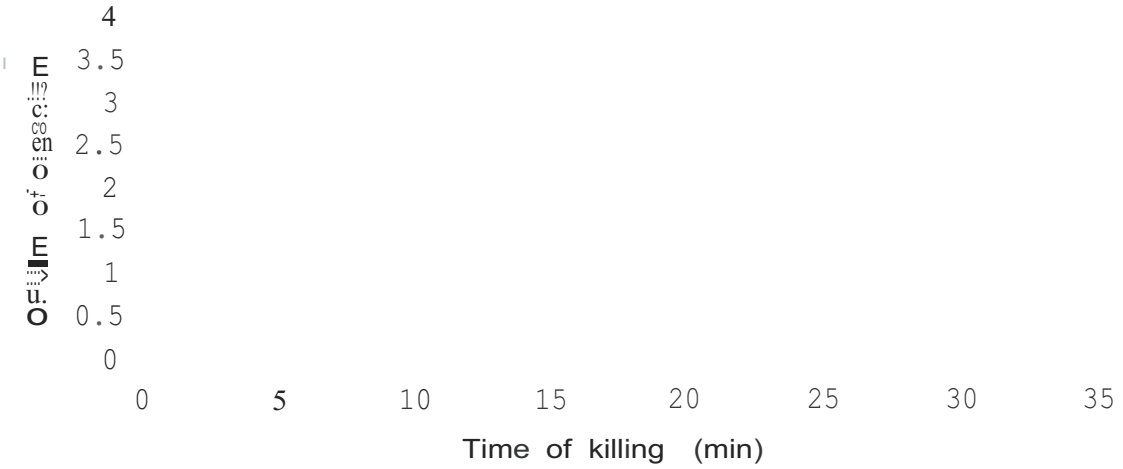


OVC - TM: $5.05x10^3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Trichophyton mentagrophyte* by *Ca/otropis procera* leaf water extract at 1000jJg/mi

Constant	4.1179
Std Err of Y Est	0.1381
R Squared	0.9745
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	£0.072
Std Err of Coef.	0.0052

Appendix 33: Rate of killing *ofTrichophylon rubrum* by *Cafotropis procera* leaf water extract over time at 2000 j.lg/ml

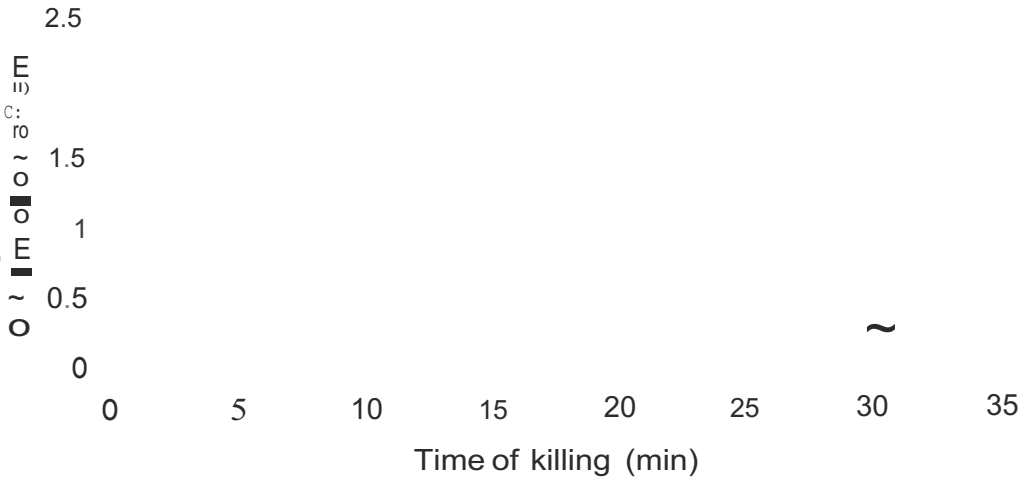


OVC- TR: 4.7x10^3
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Trichophyton rubrum* by *Ca/otropis procera* leaf water extract at 2000IJg/mi

Constant	3.7679
Std Err of Y Est	0.2034
R Squared	0.9752
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.108
Std Err of Coef.	0.0077

Appendix 34: Rate of killing of *Candida albicans* by *Schwenkia americana* root ethanol extract over time at 2000 J-Lg/ml

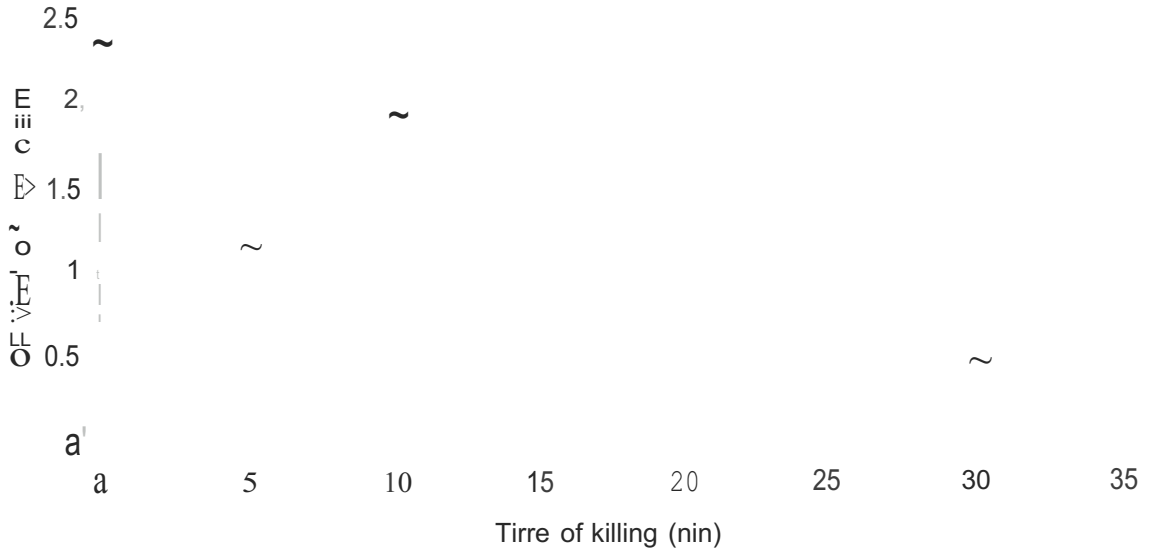


avc - CA: 3.0X10^3
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Candida a/bicans* by *Schwenkia americana* root ethanol extract over time at 2000J.lg/ml.

Constant	2.2661
Std Err of Y Est	0.1095
R Squared	0.9786
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.063
Std En' of Coef.	0.0041

Appendix 35: Rate of killing of *Candida albicans* by *Schwenkia americana* stem ethanol extract over time at 1900 Ilg/ml

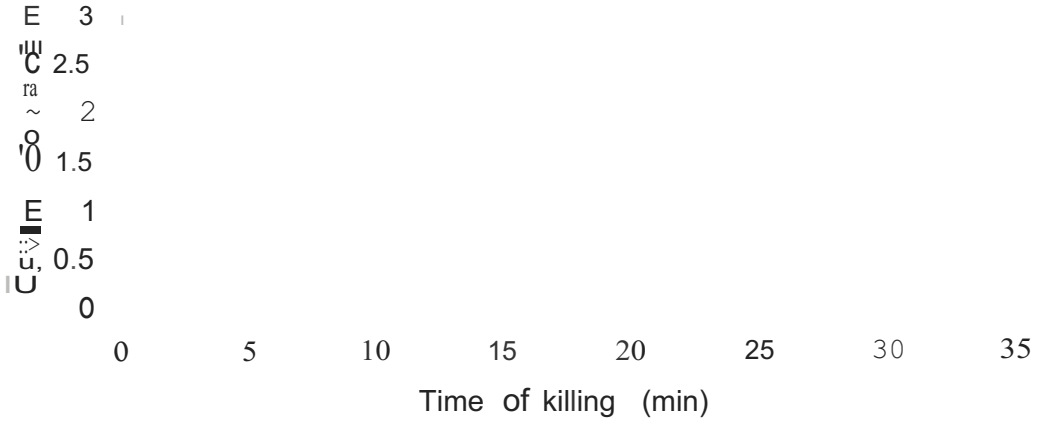


avc - CA: 3.0X103
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Candida albicans* by *Schwenkia americana* stem ethanol extract over time at 1900~g/ml.

Constant	2.0839
Std Err of Y Est	0.3647
R Squared	0.7384
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.052
Std Err of Coef.	0.013,8

Appendix 36: Rate of killing of *Candida albica'ns* by *Schwenkia americana* leaf ethanol extract over time at 1900 ~lg/ml

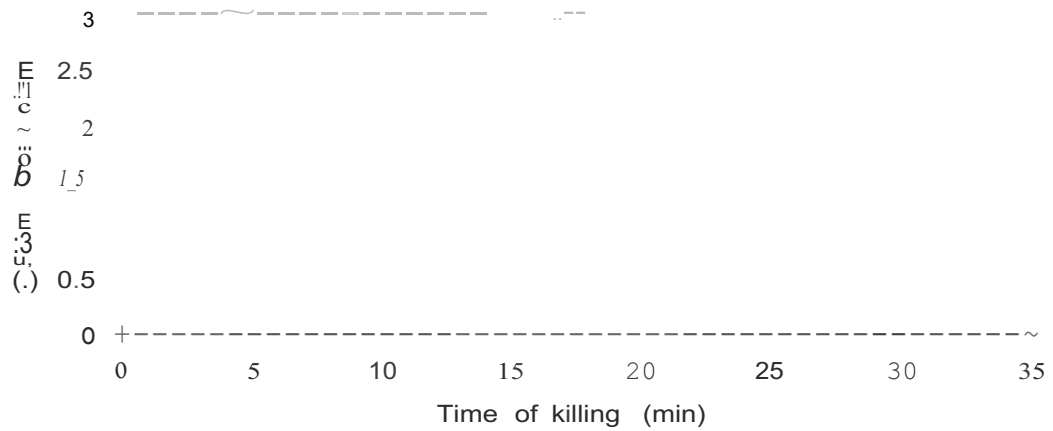


OVC - CA: $3.0X10_3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Candida albicans* by *Schwenkia americana* leaf ethanol extract over time at *1900flg/ml*

Constant	2.414286
Std Err of Y Est	0.087831
R Squared	0,98888
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.07
Std Err of Coef.	0.00332

Appendix 37: Rate of killing of *Candi~a elbicen«* by *Merremia tridentata* subspecies *angustifolia* stem ethanol extract over time at 1900 l1g/1111



avc - CA: 3.0X10$_3$
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Candida albicans* by *Merremia tridentata* subspecies *angustifolia* stem ethanol extract over time at 1900 ug/ml,

Constant	2.539286
Std Err of Y Est	0.082375
R Squared	0.990773
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.07214
Std Err of Coef.	0.003113

Appendix 38: Rate of killing of *Candida albicans* by *Merremia tridentata* subspecies *angustifolia* leaf ethanol extract over time at 1900 uq/rnl

avc - CA: 3.0X103
MSC: No growth
Diluent control (DC): No growth

Regression Output of rate of killing of *Candida albicans* by *Merremia tridentata* subspecies *angustifolia* leaf ethanol extract over time at 1900JIg/ml.

Constant	2.4411
Std Err of Y Est	0.094
R Squared	0.9888
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.075
Std Err of Coef.	0.0036

Appendix 39: The effect of temperature on the, antimicrobial activity of *Senna a/ata* root and leaf n-hexane extracts at *20001-l9/ml.*

Organism	*Senna alata* root n-hexane					*Senna alata* leaf n-hexane				
	-4DC	OC	27±3DC	1OQoC	121°C	-4°C	OOC	27±3OC	100°C	121°C
Ecoli	+++	+++	+++	+++	+++					
S.aureus										
P.aeruginosa										
B.subtilis	+++	+++	+++	+++	+++					
S. *typhi*										
K. pneumoniae										
C. *albicans*	+ ++	+++	+++	+++	+++					
M. *canis*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
T. rubrum	++	++	++	++	++					
T. mentagrophyte	+ ++	+++	+++	+++	+++	+ ++	+++	+++	+++	+++
E *f1ocossun*	+++	+++	+++	+++	+++	+ ++	+++	+++	+++	+++

+++: Appreciable activity
++: Moderate activity
+: Low activity
No activity.

Appendix 40: The effect of temperature on the antimicrobial activity of *Senna alata* root and leaf water extracts at *20001-l9/ml.*

Organism	*Senna alata* root water					*Senna alata* leaf water				
	-4°C	OC	27±3OC	100°C	121°C	-4°C	OC	27±3OC	100°C	121°C
Ecoli	+	+	+	+	+	+	+	+	+	+
S. *aureus*	++	++	++	++	++					
P. *aeruginosa*										
B. *subtilis*										
S. *typhi*	+	+	+	+'	+	+	+	+	+	+
K. pneumoniae	+	+	+	+	+	+	+	+	+	+
C. *albicans*	++	++	++	++	++					
M. *canis*	++	++	++	++	++	+	+	+	+	+
T. rubrum	+ ++	+++	+++	+++	' + + +	++	++	++	++	++
T. mentagrophyte	+ ++	+++	+++	+++	+++	+++	+++	+++	+++	+++
E *f1ocossun*	++	++	+ +.	++	++	++	++	++	++	++

+++: Appreciable activity
++: Moderate activity
+: Low activity
No activity .

Appendix 41: The effect of temperature on the antimicrobial activity of *Senna alata* root and leaf ethanol extracts at *20001-19/ml.*

Organism	*Senna alafa* root ethanol					*Senna alafa* leaf ethanol				
	-40C	DoC	27±30C	1000C	121°C	-40C	DoC	27±30C	1000C	121°C
E. coli	+	+	+	+	+	+	+	+	+	+
S. *aureus*	++	++	++	++						
P. *aeruginosa*										
B. *subfilis*	+	+	+	+	+	+++	+++	+++	+++	+++
S. *typhi*										
K. pneumoniae										
C. *albicans*	+++	+++	+++	+++	+++					
M. *canis*	+	+	+	+	+	+	+	+	+	+
T. rubrum	+	+	+	+	+	+	+	+	+	+
T. menfagrophyte	+++	+++	+++	+++	+++					
E. flocossun	+++	+++	+++	+++	+++	++	++	++	++	++

+++: Appreciable activity
++: Moderate activity
+: Low activity
No activity.

Appendix 42: The effect of temperature on the antimicrobial activity of *Calotropis procera* root and leaf n-hexane extracts at *20001-19/ml.*

Organism	*Calofropis procera* root n-hexane					*Calofropis procera* leaf n-hexane				
			27±30C	1000C	121°C					
E. coli										
S. *aureus*										
P. *aeruginosa*										
B. *subfilis*										
S. *typhi*	+	+	+	+	+	+	+	+	+	+
K. pneumoniae										
C. *albicans*										
M. *canis*	++	++	++	++	++	+++	+++	+++	+++	+++
T. rubrum	+	+	+	+	+					
T. menfagrophyte	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
E. flocossun						+++	+++	+++	+++	+++

+++: Appreciable activity
++: Modeate activity
+: Low activity
-: No activity

Appendix 43: The effect of temperature on the antimicrobial activity of *Calotropis procera* root and leaf water extracts at *2000J.l9/ml.*

Organism	*Calotropis procera root water*					*Calotropis procera leaf water*				
						OOC	*27±3oC*	100oe	121oe	
E. coli										
S. *aureus*										
P. aeruginosa										
B. *subtilis*										
S. *typhi*										
K. pneumoniae										
C. *albicans*										
M. *canis*	+	+	+	+	+	+ ++	+++	+++	+++	+++
T. rubrum						+ ++	+++	+++	+++	+++
T. mentagrophyte	+	+	+	+	+	+ ++	+++	+++	+++	+++
E. f1ocossun	+	+	+	+	+	+ ++	+++	+++	+++	+++

+++: Appreciable activity
++: Modeate activity
+: Low activity
-: No activity

Appendix 44: The effect of temperature on the antimicrobial activity of *Calotropis pro cera* root and leaf ethanol extracts: at *2000J.l9/ml.*

Organism	*Calotropis procera root ethanol*					*Calotropis procera leaf ethanol*				
	-4°e	ooe	27±3oe	100De	121De	-4oe	OC	27±3oe	100oe	121°e
E. coli										
S. *aureus*										
P. *aeruginosa*										
B. *subtilis*										
S. *typhi*	+	+	+	+		+	+	+	+	+
K. pneumoniae	+	+	+	+		+	+	+	+	+
C. *albicans*										
M. *canis*	++	++	++	++		++	++	++	++	+
T. rubrum	+	+	+	+		+	+	+	+	+
T. mentagrophyte	++	++	++	++		++	++	++	++	+
E. f1ocossun	+++	+++	+++	+++	+++	+ ++	+++	+++	+++	+++

+++: Appreciable activity
++: Modeate activity
+: Low activity
- : No activity

Appendix 45: The effect of temperature on the antimicrobial activity of *Schwenkia americana* root and leaf n-hexane extracts at *2000llg/mi.*

Organism	*Schwenkia americana* root n-hexane				*Schwenkia americana* leaf n-hexane			
E. coli								
S. *aureus*								
P. *aeruginosa*								
B. *subtilis*								
S. *typhi*					+	+	+	+
K. pneumoniae								
C. *albicans*					+	+	+	+
M. *canis*	++	++	++	++				
T. rubrum	++	++	++	++				
T. mentagrophyte								
E. flocossun	++	++	++	++	++	++	++	++

+++: Appreciable activity
++: Modeate activity
+: Low activity
-: No activity

Appendix 46: The effect of temperature on the antimicrobial activity of *Schwenkia americana* root and leaf water extracts at 2000llg/ml.

Organism	*Schwenkia americana* root water				*Schwenkia americana* leaf water			
E. coli								
S. *aureus*								
P. *aeruginosa*								
B. *subtilis*								
S. *typhi*								
K. pneumoniae								
C. *albicans*					+	+	+	+
M. *canis*								
T. rubrum								
T. mentagrophyte								
E. flocossun					+	+	+	+

+++: Appreciable activity
++: Modeate activity
+: Low activity
- : No activity

Appendix 47: The effect of temperature on the antimicrobial activity of *Schwenkia americana* root and leaf ethanol extracts at 20001-19/ml.

Organism	*Schwenkia americana* root ethanol					*Schwenkia americana* leaf ethanol				
	OOe									
E. coli										
S. *aureus*										
P. *aeruginosa*										
B. *subtilis*										
S. *typhi*										
K. pneumoniae										
C. *albicans*	+	+	+	+	+	+++	+++	+++	+++	+++
M. *canis*										
T. rubrum										
T. mentagrophyte										
E. flocossun	+	+	+	+	+	+	+	+	+	+

+++: Appreciable activity
++: Modeate activity
+: Low activity
- : No activity

Appendix 48: The effect of temperature on the antimicrobial activity of *Merremia tridentata* subspecies *angustifolia* root and leaf n-hexane extracts at *2000,..,9/ml.*

Organism	*Merremia tridentata* root n-hexane (					*Merremia tridentata* leaf n-hexane				
						OC	27±30e	1000e	1210e	
E coli										
S, *aureus*										
P. *aeruginosa*										
B. *subtilis*										
S. *typhi*						+	+	+	+	+
K. pneumoniae										
e. *albicans*						+	+	+	+	
M. *canis*										
T. rubrum										
T. mentagrophyte										
E. flocossun						+	+	+	+	

+++: Appreciable activity
++: Modeate activity
+: Low activity
-: No activity

Appendix 49: The effect of temperature on the antimicrobial activity of *Merremia tridentata* subspecies *angustifolia* root and leaf water extracts at 2000~lg/ml.

	Merremia tridentata root water					*Merremia tridentata* leaf water				
Organism	-4oe	ooe	27±3oe	1000e	1210e	-4oe	OC	27±30e	1000e	1210e
E. coli										
S. *aureus*										
P. *aeruginosa*										
B. *subtilis*										
S. *typhi*										
K. pneumoniae										
e.albicans										
M. *canis*										
T. rubrum										
T. *mentagrophyte*										
E. flocossun	+	+	+	+		+	+	+	+	

+++: Appreciable activity
++: Modeate activity
+: Low activity
- : No activity

Appendix 50: The effect of .temperature on the antimicrobial activity of *Merremia tridentata* subspecies *angustifolia* root and leaf ethanol extracts at 2000~g/ml.

	Merremia tridentata root ethanol				*Merremia tridentata* leaf ethanol			
Organism					OC	27±3oe	1000e	1210e
E. coli								
S. *aureus*								
P. aeruginosa								
B. *subtilis*								
S. *typhi*								
K. pneumoniae								
e. *albicans*	+	+	+	+	+++	+++	+++	+++
M. *canis*								
T. rubrum								
T. mentagrophyte								
E. flocossun					+	+	+	+

+++: Appreciable activity
++: Modeate activity
+: Low activity
-: No activity

Appendix 51: Antimicrobial activity, on different time of storage of *Senna a/ala* root and leaf n- hexane extracts at 2000J.lg/ml.

	Senna alata root n-hexane			*Senna alata* leaf n-hexane		
Organism	6 months	12 months	30 months	6 months	12 months	30 months
S. *aureus*						
P. *aeruginosa*						
B. *subtilis*	+++	+++	+++			
S. *typhi*						
K. pneumoniae						
C. *albicans*	+++	+++	+++			
M. *canis*	+++	+++	+++	+++	+++	+++
T. rubrum						
T. mentagrophyte	+++	+++	+++	+++	+++	+++
E. flocossun	++	++	+	+++	+	

+++: Appreciable activity
++: Modeate activity
+: Low activity
No activity

Appendix 52: Antimicrobial activity, on different time of storage of *Senna alata* root and leaf water extracts at 2000J.lg/ml.

	Senna alata root water			*Senna alata* leaf water		
Organism	6 months	12 months	30 months	6 months	'12 months	30 months
S. *aureus*	++	++				
P. *aeruginosa*						
B. *subtilis*						
S. *typhi*	+			+		
K. pneumoniae	+			+	+	
C. *albicans*	++	+	+			
M. *canis*	++	++	++			
T. rubrum	+++	+++	++	++		
T. mentagrophyte	+++	+++	+++	+++	++	++
E. flocossun	++	++	+	+++	+++	+++

+++: Appreciable activity
++: Modeate activity
+: Low activity
-: No activity

Appendix 53: Antimicrobial activity, on different.time of storage of *Senna a/ala* root and leaf ethanol extracts at 2000~lg/ml.

	Senna a/ata root ethanol			*Senna a/ata* leaf ethanol		
Organism	6 months	12 months	30 months	6 months	12 months	30 months
S. *aureus*	+	+				
P. *aeruginosa*						
B. *subti/is*	+			+++	+	+
S. *typhi*						
K. pneumoniae						
C. *albicans*	+++	+++	+++			
M. *canis*	+			+		
T. rubrum	+	+		+	+	
T. mentagrophyte	+++	+++	+++			
E. flocossun	+++	++		++		

+++: Appreciable activity
++: Modeate activity
+: Low activity
-: No activity

Appendix 54: Antimicrobial activity, on different time of storage of *Calotropis procera* root and leaf n- hexane extracts at *::iOOO~g/ml.*

	Ca/otroQ_is *Q_rocera* root n-hexane			*Ca/otroels Q_rocera* leaf n-hexane		
Organism	6 months	12 months	30 months	6 months	12 months	30 months
S. *aureus*						
P. aeruginosa						
B. subtilis						
S. *typhi*	+			+		
K. pneumoniae						
C. *albicans*						
M. *canis*	++	++	+	+++	+++	+++
T. rubrum	+	+	4-			
T. mentagrophyte	+++	++		+++	+++	+++
E. flocossun				+++	+++	+++

+++: Appreciable activity
++: Modeate activity
+: Low activity
-: No activity

Appendix 55: Antimicrobial activity, on different time of storage of *Calotropis procera* root and leaf water extracts at *2000J.1g/ml.*

	Calotrogis grocera root water			*Calotrogis I2rocera* leaf water		
Organism	6 months	12 months	'30 months	6 months	12 months	30 months
S. *aureus*						
P. *aeruginosa*						
B. *subtilis*						
S. *typhi*						
K. pneumoniae						
C. *albicans*						
M. *canis*	+	+	+	+++	+++	+++
T. rubrum				+++	+++	+++
T. mentagrophyte	+	+		+++	+++	+++
E. flocossun	+	~+		+++	+++	+++

+++: Appreciable activity
++: Modeate activity
+: Low activity
-: No activity

Appendix 56: Antimicrobial activity, on different time of storage of *Calotropis procera* root and leaf ethanol extracts at *2000J.1g/ml.*

	Calotrol2is I2rocera root ethanol			*Calotrol2is I2rocera* leaf ethanol		
Organism	6 months	12 months	30 months	6 months	12 months	30 months
S. *aureus*						
P. *aeruginosa*						
B. *subtilis*						
S. *typhi*	+			+		
K. pneumoniae	+			+		
C. *albicans*	++	++	++			
M. *canis*		+	+	++	++	+
T. rubrum	+	+		++	++	+
T. mentagrophyte	++	++	++	++	++	++
E. flocossun	+++	+++	+++	+++	+++	+++

+++: Appreciable activity
++: Modeate activity
+: Low activity
- : No activity

Appendix 57: Antimicrobial activity, on different time of storage of *Schwenkia americana* root and leaf n-hexane extracts at *2000l1g/ml.*

	Schwenkia americana root n-hexane			*Schwenkia americana* leaf n-hexane		
Organism	6 months	12 months	30 months	6 months	12 months	30 months
S. *aureus*						
P. *aeruginosa*						
B. *subtilis*						
S. *typhi*				+	+	
K. pneumoniae						
C. *albicans*				++	++	
M. *canis*	++	+	+			
T. rubrum	++	++	+			
T. mentagrophyte						
E. flocossun	++	++	+	++	+	

+++: Appreciable activity
++: Modeate activity
+: Low activity
-: No activity

Appendix 58: Antimicrobial activity, on different time of storage of *Schwenkia americana* root and leaf water extracts at *2000l1g/ml.*

	Schwenkia americana root water			*Schwenkia americana* leaf water		
Organism	6 months	12 months	30 months	6 months	12 months	30 months
S. *aureus*						
P. aeruginosa						
B. *subtilis*						
S. *typhi*						
K. pneumoniae						
C. *albicans*				+	+	
M. *canis*						
T. rubrum						
T. mentagrophyte						
E. flocossun				+	+	

+++: Appreciable activity
++: Modeate activity
+: Low activity
- : No activity

Appendix 59: Antimicrobial activity, on different time of storage of *Schwenkia americana* root and leaf ethanol extracts at *2000f.1g/ml.*

	Schwenkia americana root ethanol			*Schwenkia americana* leaf ethanol		
Organism	6 months	12 months	30 months	6 months	12 months	30 months
S. *aureus*						
P. *aeruginosa*						
B. *subtilis*						
S. *typhi*						
K. pneumoniae						
C. *albicans*	+	+		+++	+++	
M. *canis*						
T. rubrum						
T. mentagrophyte						
E. f1ocossun	+			+		

+++: Appreciable activity
++: Modeate activity
+: Low activity
No activity

Appendix 60: Antimicrobial activity, on different time of storage of *Merremia tridentata* subspecies *angustifolia* root and leaf n-hexane extracts at *2000f.1g/ml.*

	Merremia tridentata root n-hexane			*Merre,ia tridentata* leaf n-hexane		
Organism	6 months	12 months	30 months	6 months	12 months	30 months
S. *aureus*						
P. *aeruginosa*						
B. *subtilis*						
S. *typhi*				+		
K. pneumoniae						
C. *albicans*				+		
M. *canis*						
T. rubrum						
T. mentagrophyte						
E. f1ocossun				+		

+++: Appreciable activity
++: Modeate activity
+: Low activity
-: No activity

Appendix 61: Antimicrobial activity, on different time of storage of *Merremia tridentata* root and leaf water extracts at *2000,...,g/ml.*

	Merremia tridentata root water			*Merre,ia tridentata* leaf water		
Organism	6 months	12 months	30 months	6 months	12 months	30 months
S. *aureus*						
P. *aeruginosa*						
B. *subtilis*						
S. *typhi*						
K. pneumoniae						
C. *albicans*						
M. *canis*						
T. rubrum						
T. mentagrophyte						
E. flocossun	+	+		+		

+++: Appreciable activity
++: Modeate activity
+: Low activity
- : No activity

Appendix 62: Antimicrobial activity, on different time of *stor~remia* *tridentata* subspecies *angustifolia* root and leaf ethanol extracts at *2000llg/mi.*

	Merremia tridentata subspecies *angustifolia* root ethanol			*Merremia tridentata* subspecies *angustifolia* leaf ethanol		
Organism	6 months	12 months	30 months	6 months	12 months	30 months
S. *aureus*						
P. *aeruginosa*						
B. *subtilis*						
S. *typhi*						
K. pneumoniae						
C. *albicans*	+	+	+	+++	+++	+++
M. *canis*						
T. rubrum						
T. mentagrophyte						
E. flocossun				+		

+++: Appreciable activity
++: Modeate activity
+: Low activity
- : No activity

APPENDIX 63: Preparation of McFarland Nephelometer Barium Sulphate

Tune	Barium Chloride 1%(ml)	Sulphuric acid 1%(ml)	Corresponding approx. Density of Bacteria (million/ml)
1.	1.0	9.9	300
2.	0.2	9.8	600
3.	0.3	9.7	900
4.	0.4	9.6	1,200
5.	0.5	9.5	1,500
6.	0.6	9.4	1,800
7.	0.7	9.3	2,100
8.	0.8	9.2	2,400
9.	0.9	9.1	2,700
10.	1.0	9.0	3,000