ANTIMICROBIAL AND ANTI – INFLAMMATORY ACTIVITIES OF EXTRACTS OF *FICUS THONNINGII* BLUME (MORACEAE)

BY

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CERTIFICATION

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DEDICATION

This work is dedicated to the Almighty God who makes all things beautiful in His time.

In blessed memory of my father, Late Chief R.A. Tanimowo, who was a great educationist in his life time, and my foundation supervisor, Late Professor H.A. Odelola.

ABSTRACT

Infectious diseases and the associated inflammation pose a serious health problem worldwide, accounting for about 50% of all deaths in tropical countries. This is further complicated by the frequent development of bacterial resistance to many chemotherapeutic agents. These problems have necessitated the continuous search for new and effective drugs from plant materials. Thus, *Ficus thonningii* Blume (Moraceae), a plant used ethnomedicinally in West Africa for the treatment of some microbial infections, was studied for its antimicrobial and anti-inflammatory activities.

Dried leaves and stem bark of *Ficus thonningii* were screened for secondary metabolites. Successive gradient extraction was carried out on the pulverised plant parts using hexane, chloroform and methanol with Soxhlet apparatus. Antimicrobial activity of the extracts on Gram-positive (10) and Gram-negative (11) bacteria, and fungal (12) isolates was evaluated using agar-diffusion method. Antibiogram of the microorganisms was determined using established antibiotics. Bioassay-guided fractionation of crude extracts using column chromatography was done. Minimum Inhibitory Concentrations (MIC) and minimum bactericidal concentrations of the crude extracts, fractions and isolated compound were determined by agar-dilution. Bactericidal kinetics of the methanol leaf extract against *Staphylococcus aureus* and *Escherichia coli* at 2.5-10.0 mg/mL were determined. Structure elucidation of the bioactive compound was carried out using ^{1}H-NMR, ^{13}C-NMR, DEPT 135, COSY, UV and GC-MS spectroscopy. *In vivo* anti-inflammatory activity of leaf extract was evaluated using carrageenan-induced rat paw oedema with acetylsalicylic acid as the reference drug. Acute oral toxicity, haematological and histopathological evaluations were carried out to determine the safety profile of methanol leaf extract in rats. Statistical analysis was carried out using Student's t-test at $p = 0.05$.

Alkaloids, flavonoids, terpenoids and cardiac glycosides were detected in the plant extracts. Antimicrobial assay of crude extracts and fractions showed a broad spectrum activity on sensitive and multidrug-resistant strains with the leaf and stem bark extracts having similar antimicrobial activity. Hexane leaf extract and bioactive fractions gave MIC range of 78-625 µg/mL and 20-625 µg/mL respectively while methanol leaf extract and bioactive fractions

gave 156-625 µg/mL and 39-625 µg/mL. Structure elucidation of the bioactive compound isolated from hexane leaf fraction revealed a triterpenoid with MIC range of 20-156 µg/mL

(Gram-positive bacteria), 39-156 µg/mL (Gram-negative bacteria) and 10-78 µg/mL (fungi), while that of gentamicin and tioconazole were 5-30 µg/mL and 10-20 µg/mL respectively. Methanol leaf extract showed bactericidal activity in a concentration-dependent manner on the microorganisms, with a 100% bactericidal action at 10 mg/mL on *Staphylococcus aureus* and 84% on *Escherichia coli* within 4 hours. The anti-inflammatory activity of methanol leaf extract was 57.5% while that of acetylsalicylic acid was 93.2%. Acute oral toxicity of methanol leaf extract showed an LD_{50}> 5g/Kg. Significant increases were observed in the red blood cell count and mean corpuscular haemoglobin value, while histopathological evaluation revealed no significant tissue pathological changes in the major organs.

Extracts of *Ficus thonningii* leaves contain antimicrobial and anti-inflammatory agents. These could be useful in the development of safe chemotherapeutic agents for the treatment of relevant microbial infections and inflammation-prone diseases.

Keywords: *Ficus thonningii*, Antimicrobials, Anti-inflammatory agents, Triterpenoid

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LIST OF ABBREVIATIONS

%	-	Percentage
^{o}C	-	Degree Centigrade
$^{13}CNMR$		Carbon -13 Nuclear Magnetic Resonance
^{1}H NMR		Proton Nuclear Magnetic Resonance
CFU		Colony Forming Unit
DMSO		Dimethylsulphoxide
HPTLC		High Performance Thin Layer Chromatography
LD		Lethal Dose
MBC		Minimum Bactericidal Concentration
MDR		Multi-Drug Resistant
MHB		Mueller Hinton Broth
MIC		Minimum Inhibitory Concentration
Min		Minute
mL		Millilitre
NCIB		National Collection of Industrial Bacteria
NCTC		National Collection of Typed Culture
NIIMB		National Collection of Industrial and Marine Bacteria
NMR		Nuclear Magnetic Resonance
TLC		Thin Layer Chromatography
TM		Traditional Medicine
UV		Ultraviolet
WHO		World Health Organisation

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CHAPTER ONE

INTRODUCTION

The use of medicinal plants has been a part of human culture. It is an aspect of traditional medicine which has, and still enjoys wide acceptability among the people of the developing countries of the world today. This could partly be due to the limited availability of western medicine in such countries, and the fact that the use of plants in traditional medicine blends readily into the socio-cultural life of the people in whose culture it is deeply rooted.

Traditional medicine (TM) has existed for ages, and relies largely on experience handed down from one generation to another. It has been described as the total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing, or eliminating physical, mental, or social diseases and which may rely exclusively on past experiences and observation handed from one generation to another, verbally or in writing (Sofowora, 1993).

The use of traditional medicine in Africa, Asia, and Latin America to meet primary health care needs is very common. In Africa, up to 80% of the population use TM for primary health care (World Health Organization, 2003). Chinese medicine for instance, is one of the oldest surviving traditions that has been practised in the Chinese communities for the maintenance of good health and treatment of diseases and is recently being practised worldwide by other ethnic groups. In China, about 60% of healthcare is provided by western medicine and 40% by Chinese traditional medicine (Chan, 2005). Approximately 25% of modern drugs used in the United States of America (USA) have been derived from plants (Farnsworth and Morris, 1976). In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. According to the National Health Experts, 2000 different plants are used as medicinal preparations for both internal and external use in India (Devi *et al.*, 2009).

The World Health Organization has reported infectious diseases as the first cause of death worldwide with more than 50% of the death occurring in tropical countries (WHO, 1996). In developing countries, treatment of such diseases is complicated not only because of the occurrence of resistant microorganisms to the commonly used antimicrobials, but because of the low income of the population which reduce their accessibilities to appropriate drugs (Kuete *et al.*, 2009). It may not be surprising to have such statistics in developing nations, but it is remarkable to note that infectious disease mortality rates are increasing in developed countries such as the United States (US). Death from infectious disease ranked fifth in 1981 and has become the third leading cause of death in 1992. It is estimated that infectious disease is the underlying cause of death in 8% of the deaths occurring in the US and the increase has been attributed to increase in respiratory tract infections and HIV/AIDS (Pinner *et al.*, 1996).

In spite of the influence of orthodox medicine, about 80% of the rural population in Nigeria depend on herbal medical care for their health needs (Inyang, 2004). A wide variety of medicinal plants can be found in Nigeria, but unlike in China, India, and Vietnam where traditional medicine has been researched, developed and integrated with the formal health care system, many of the plants utilised in TM in Nigeria need to be subjected to scientific study to validate their uses. Even though large numbers of plants are constantly being screened world wide for their pharmacological value, it is estimated that only about 1% of Nigerian medicinal plants has been subjected to scientific evaluation for potential chemotherapeutic value (Inyang, 2004).

The importance of plants in human life cannot be overemphasized; be it for medicinal, nutritional, ornamental or commercial purposes, their use has found wide application in almost all aspects of day to day life of man. Plant-derived medicines have made large contributions to human health and well being. Plants have provided a good source of anti-infective agents in the fight against microbial infections. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infections.

Some Nigerian plants of proven medicinal properties include *Zanthoxylum zanthoxyloides* which has anti-sickling properties; *Ocimum gratissimum* which has

been shown to have antimicrobial and antihelminthic properties; *Piper guiniense* (West African black pepper) whose constituents have been shown to have antimicrobial, anticonvulsant, antihypertensive, sedative, tranquilizing and insecticidal properties; *Azadirachta indica* which has been shown to have antipyretic and anti-inflammatory effects and *Ageratum conyzoides* which has antimicrobial properties. *Rauwolfa vomitoria* has been shown to possess hypotensive and sedative properties and is used by herbalists to sedate psychotic patients. *Physostigma venenosum* (Calabar bean) which contains alkaloids is used for the treatment of malaria, diabetes, and sickle cell anaemia while *Garcinia kola* has antimicrobial activities (Sofowora, 1984). Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value which are yet to be discovered.

The WHO Traditional Medical Programme has provided the evidence that ethno botanical information can lead to valuable drug discovery (Farnsworth *et al.*, 1995). The first generation of plant drugs were usually simple botanicals employed in more or less their crude form. Several effective medicines used in their natural state such as opium, belladonna, cinchona and aloe were selected as therapeutic agents based on empirical evidence of their clinical application by traditional societies from different parts of the world. Research on studies of medicinal plants has enabled the screening of such plants for bioactive agents leading to the development of medicinal plants into acceptable dosage forms and such plants serving as new drug leads.

It has been estimated that 14-28% of higher plant species are used as medicines and that 74% of pharmacologically active plant-derived components were discovered after following up on ethno-medicinal use of the plants (Ncube *et al.*,2008). It is reasonable to consider that a survey of ethno-medical uses of a plant may provide useful clues for drug discovery. The criteria considered in such ethno-medical research include the frequency of citation of the association between plant and disease by certain authors and the consistency of use of the particular plant for a given disease over time. Once a plant is deemed strongly associated with use for a particular illness, and there are reports of amelioration of symptoms of the disease following the use of a specific herb, proof of concept studies could seek confirmation of the traditionally-presumed pharmacological action with focus on drug discovery (Paavilainen, 2005).

Several active compounds have been discovered from plants on the basis of ethno botanical information and used directly as patented drugs e.g. Artemisinin, discovered from *Artemisia annua* is used as a potent antimalarial compound against Plasmodium strains resistant to known antimalarials (Klayman, 1993); and Taxol obtained from *Taxus breviflora* as an antitumor drug (Samuelsson, 1992). Many pharmaceutical drugs currently being used by physicians have a long history of use as herbal remedies and examples include opium, digitalis, aspirin® and quinine. Quinine was isolated from *Cinchona officinalis* and was used as a lead compound to develop chloroquine and mefloquine. Digoxin was isolated from *Digitalis purpurea* (Foxglove) and is used to treat heart diseases. Ephedrine was isolated from *Ephedra sinica*, a Chinese herbal plant and used as a lead compound to synthesize drugs like salbutamol and salmetrol which are used in the treatment of asthma. The isoquinolone alkaloid emetine obtained from *Cephaelis ipecacuanha* is used as an amoebicidal drug and for treatment of abscesses (Iwu *et al.,* 1999).

Naturally occurring antimicrobial agents can be derived from plants, animal tissues, or micro-organisms. Medicinal plants are potential antimicrobial crude drugs and also a source for natural compounds that can serve as new anti-infective agents (Newman *et al.,* 2003). Several studies have been carried out worldwide to investigate the antimicrobial potentials of medicinal plants. Reports from such studies revealed varying degrees of antimicrobial activity that could be antibacterial, antiviral or antifungal. Reports on studies on Nigerian medicinal plants have emphasized the antimicrobial activities of individual plants or plant parts. For example, *Psidium guajava* was found useful for treating diarrhoea and abdominal pains (Iwu, 1993). From a survey carried out on 84 medicinal plants used locally in the treatment of various diseases in Bauchi state, Nigeria, 75 of the plants exhibited antimicrobial activity against one or more of the test organisms used for the study (Adamu *et al.,* 2005). A study of the Ghanaian medicinal plants screened for antimicrobial activity was reported by Konning *et al.* (2004). Devi *et al.* (2009) also reported the antimicrobial activity of some selected medicinal plants from Southeast Coast of India.

Rationale for the study

There is a need to find new substances with potent antimicrobial properties as there are new and re-emerging infections. With the continuous use of antibiotics, micro-organisms have become resistant. There is also the emergence of new infectious diseases and antimicrobial resistance among pathogenic bacteria is on the increase thus making the existing conventional drugs obsolete in the treatment of infectious diseases.

In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, allergic reactions, immune-suppression and depletion of beneficial gut and mucosal micro-organisms (Lopez *et al.*, 2001) creating an immense clinical problem in the treatment of infectious diseases.

Most of the current antibiotics have considerable limitations in terms of antimicrobial spectrum (Assob *et al.,* 2011).

Infections are usually accompanied by inflammation (Lansky *et al*., 2008) and due to the inherent problems associated with the current non-steroidal as well as steroidal anti-inflammatory agents there is need to search for alternative agents with low or no side effects.

The ethnomedical uses of *F. thonningii* suggest that the plant may not only be useful for its antimicrobial properties but also for its anti-inflammatory activities. Thus, there is a need to investigate both the anti-microbial and anti-inflammatory properties of the plant.

Moreover, there is rapid rate of plant specie extinction and the implication of this is that the multitude of potentially useful phytochemical compounds which could be synthesized chemically is at risk of being lost irretrievably (Boris, 1996).

Thus, the research into phytopharmaceuticals is a matter of urgency and of utmost importance. Medicinal plants can furnish new chemotherapeutic agents which will serve to complement the existing drugs and there is a need to carry out toxicity tests in order to assess the potential toxicity of a phytochemical substance as it may be used in

clinical practice and to find out probable consequence if it is abused. Female rats were used for the toxicity tests as literature on the effect of *Ficus thonningii* on haematological and tissue pathological changes in female laboratory animals is scanty. Medicinal plant based antimicrobials represent a vast untapped source of pharmaceuticals and thus, there is need to screen local medicinal plants for possible antimicrobial properties.

The discovery of new drugs from plants is a very complex process in which the successful strategies for investigation of medicinal agent from plants include the following processes among others:

a. Screening of plant extracts for biological activity
b. Bioassay guided fractionation of active plant principle
c. Isolation and purification of pure constituents
d. Determination of the structure of pure compounds

The current study is an evaluation of the antimicrobial and anti-inflammatory activities of *Ficus thonningii* Blume (Moraceae). The plant of study, *Ficus thonningii* Blume is one of the indigenous plants of West Africa used in Nigeria for ethno-medical purposes some of which suggest its antimicrobial and anti-inflammatory potentials. The leaves and fruits of plant are used to treat bronchitis and urinary tract infections (Iwu, 1993). The bark is used for the treatment of influenza (Kokwaro, 1976) and also, the bark has been found useful in the treatment of wounds, sore throats, diarrhoea and cold (Watt and Breyer, 1962). In Nigeria, the fresh leaf of *F. thonningii* is used to treat lumbago (an inflammatory condition), and the burnt leaves are rubbed on dislocated limbs to enhance healing and reduce inflammation (Bhat *et al.*, 1990).

This study, which consists of evaluation of plant parts of *Ficus thonningii* Blume for antimicrobial and anti-inflammatory properties, and the isolation of the antimicrobial constituents of the plant is based on some of the folkloric uses which include treatment of bronchitis, urinary tract infections, diarrhoea, colds, sore throat wounds, and lumbago most of which may be of microbial origin.

Some infections accompanied by inflammation include bacterial gingivitis, bronchitis, vulvo-vaginitis, infected wounds, stye, carbuncle, boil, thrush (usually of *Candida* origin), bullous or postular impetigo and some other soft tissue infections. Inflammation is a defensive mechanism exhibited by the body in response to harmful stimuli, pathogens, damaged cells or irritants (Ferrero *et al.*, 2007). The invasion and multiplication within the body tissues by various bacteria, fungi, viruses and protozoa in many instances cause damage by the release of toxins that directly destroy the host cells.

Due to the adverse effects associated with anti-inflammatory agents such as severe gastric disorders and liver dysfunction, there is continuous search for alternative agents with low or no gastrointestinal side effects. Several herbal medicines constitute a potentially important avenue for the development of novel therapeutic agents for inflammation which are safe, relatively inexpensive, highly tolerated and convenient for many patients (Kaneria *et al.*, 2007). The ethnomedical uses of *F. thonningii* suggest that the plant may not only be useful for its antimicrobial properties but also for its anti-inflammatory activities. Thus, there is a need to investigate both the anti-microbial and anti-inflammatory properties of the plant.

Aims and Objectives

Aims and objectives of this study are:

a. To obtain and screen the crude extracts of the leaf and stem bark of *F. thonningii* for phytochemical compounds and antimicrobial property;
b. To conduct a bio-assay guided fractionation on the active crude extracts;
c. To screen the crude extract of the leaf for anti-inflammatory activity;
d. To conduct acute toxicity profile of the extract using animal models;
e. To isolate pure compounds with antimicrobial properties from the fractions obtained;
f. To evaluate the antimicrobial activity of the isolated compound(s).
g. To ascertain the antimicrobial and anti-inflammatory activities of the extracts of *F. thonningii* Blume

CHAPTER TWO

LITERATURE REVIEW

2.1: Medicinal plants as therapeutic agents

Medicinal plants have been found to be of important therapeutic aid for various ailments and diseases. It is estimated that an amount of 20,000 species from several families are useful for these purposes (Penso, 1982). Infectious diseases are the world's leading cause of premature deaths and account for approximately one-half of all deaths in tropical countries (WHO, 1996; Iwu *et al.*, 1999; Kuete *et al.*, 2009). It is estimated that infectious disease is the underlying cause of death in 8% of the deaths in the United States (Pinner *et al.*, 1996).

Natural products are a source of new phytochemicals that can be used to treat infectious diseases or can be used as lead compounds (Newman *et al.*, 2003). Plant-derived medicines have made large contributions to human health as shown in Table 2.1. There are numerous examples of drugs derived from plants. Emetine, which is an isoquinolone alkaloid, is obtained from the underground part of *Cephaelis ipecacunanha* and has been used for many years as an amoebicidal drug. Quinine is another important drug of plant origin with a long history of use. The alkaloid occurs naturally in the bark of Cinchona tree and has been useful in the treatment of malaria. Widely prescribed drugs for antimalarial drug combinations such as chloroquine and mefloquine are analogues of quinine (Iwu *et al.*, 1999). Some plants have made important contributions in the areas beyond anti-infectives, such as in cancer therapies. Examples include the anti-leukaemic alkaloids, vincristine and vinblastine, obtained from *Catharanthus roseus* syn *Vinca roseus* known as the Madagascan periwinkle (Nelson, 1982). A well-known benzylisoquinolone alkaloid, papaverine, has been shown to have a potent inhibitory effect on the replication of several viruses including HIV, measles and cytomegalovirus (Turano *et al.*, 1989). Table 2.2 shows some compounds derived from plants used in modern medicine.

Table 2.1: Common Nigerian medicinal plants

Plant name (Family)	Local names	Parts used	Medicinal use
Abrus precatorius (Leguminosae)	Ojuologbo, idonzaka, crab's eye	Roots, leaves, seeds	Cold, convulsion, cough, rheumatism, conjunctivitis, antimicrobial, ulcer, anaemia, antidote to poison
Abelmoschus esculentus (Malvaceae)	Ila, kubewa, Okra,	Fruits, seeds	Fevers, dysentery, catarrhal infections, emollient, antispasmodic, gonorrhoea
Allium sativum (Liliaceae)	Tafanamu	Rhizomes	Respiratory infection
Acalypha wilkesiana (Euphorbiaceae)	Jiwene, Jiwinini, Copper leaf	Leaves, twigs	Skin rashes, antimicrobial, constipation, flatulence
Ageratum conyzoides (Compositae)	Imiesu, ulaujula, goat weed	Wholeplant, leaves,roots	Wounds, ulcer, craw-craw, digestive disturbance, diarrhoea, emetic, skin diseases, antipyretic, gonorrhoea, sleeping sickness, eye wash
Bidens pilosa (Compositae)	Abereoloko, Spanish needles	Flowers,leaves,whole plant	Rheumatism, antipyretic, anaesthetic, ease labour, cough, diarrhoea, abdominal disorders
Amaranthus spinosus	Teteelegun, inineogwu	Whole plant	Abdominal pain, astringent, diarrhoea, eczema
Jatropha curcas (Euphorbiaceae)	Botuje, lapalapa, zugu, olulu-idu Physic nut	Seed, leaves, stem, roots, sap	Ringworm, eczema, scabies, fever, guinea worm, herpes, rectal enema, blacktongue, whitlow, impotence, irregular menses, convulsion, smallpox.
Garcinia kola (Guttiferae)	Orogbo, adi, akuilu Bitter kola	Seeds, root, stembark, fruits.	Antimicrobial, dysentery, tumours, bronchitis, cough, fever, toothache, throat and respiratory ailments, liver disorders, headache, evacuant, anti-cancer
Blighia sapida (Sapindaceae)	Isin, okpulla, gwanja kusa, akee apple	Leaves, bark, fruit	Malaria, migraine, dysentery, ease labour, hypoglycaemic agent.
Momordica charantia (Cucurbitaceae)	ejinrin were, alo-ose, kakayi African cucumber, bitter gourd, balsam pear	Whole plant, seeds, fruit root	Diabetes, pile, convulsion, jaundice, sore, nervous disorders, diabetic recipe, emetic, night-blindness, aphrodisiac, dysmenorrhoea, antihelminthic, antimicrobial
Allamanda cathartica (Apocynaceae)	Ododo-alamanda, yellow allamanda, angel's trumpet, buttercup	Roots	Antimicrobial, malaria, dysentery, cathartic
Morinda lucida (Ribiaceae)	Oruwo, eruwo, eze, ogu, njisi, brimstone-tree	Leaves, stem, bark, root bark	Malaria, diabetes, heart diseases, purgative, emetic, diuretic, jaundice, flatulence, anticancer
Ocimum gratissimum (Labiatae)	Efinrinnla, efinrinaja, oromoba, Daidoya, Nchanwu, Tea bush, balsam, basil	Leaves, whole plant	Cough, diarrhea, convulsions, fever, cold, bronchitis, colic, insectrepellant, antimicrobial, anthelmintic, hypertension, diabetes, pile
Piper guineense (Piperaceae)	Iyere, Ataiyere, Ozeza, Masooroo, Climbing black pepper	Fruits, leaves, seeds	Herbal recipe ingredient, rheumatism, antipyretic, anti-emetic, stomach ache, mental illness, anthelmintic, carminative, impotence, antimicrobial, hypertension

Table 2.2: Compounds in modern medicine from ethnomedicinal leads

COMPOUND	SOURCE	INDICATION
Emetine	*Cephalis ipecacuanha*	Emetic
Atropine	*Atropa belladonna*	Anticholinergic
Vincristine, Vinblastine	*Catharanthus roseus*	Anticancer
Reserpine, Serpentine	*Rauwolfia serpentine*	Hypotensive, Sedative
Viscine, Viscinose	*Adhatoda zeylanica*	Bronchodilator, Stimulant
Solasidine	*Solanum khasianum*	Steroidal hormone
Quinine	*Cinchona officinalis*	Antimalarial
Guggulu	*Commiphora mukul*	Gout, Rheumatism
Morphine	*Papaver somniferum*	Sedative
Picrorhizin	*Picrorhiza kurroa*	Tonic, Stomachic
Digitoxin	*Digitalis purpurea*	Heart disease
Podophyllotoxin	*Podophyllum hexandrum*	Anticancer
Ephedrine	*Ephedra sinica*	Bronchodilator
Physostigmine	*Physostigma venesosum*	Glaucoma
Noscapine	*Papaver somniferum*	Antitussive
Papain	*Carica papaya*	Attenuates
Quinidine	*Cinchona pubescens*	Cardiac arrhythmia
Benzoin	*Styrax tonkinensis*	Oral disinfectant
Cocaine	*Papaver somniferum*	Analgesic,Antitussive
Eugenol	*Syzygium aromaticum*	Toothache
Caffein	*Camellia sinensis*	Stimulant
Colchicine	*Colchicum autumnale*	Gout
Hyosyamine	*Hyosyamus niger*	Anticholinergic

Plants, in reaction to environmental changes, danger and infection produce a wide range of diverse chemicals and secondary metabolites which are not essential for their primary metabolism but such complex molecules may have therapeutic potentials to cure some human ailments and as such used as medicines. Most of the compounds that have beneficial medicinal effects are secondary metabolites such as tannins, alkaloids, steroids, and phenolic compounds which are synthesized and deposited in specific parts or in all parts of the plant (Edeoga *et al.*, 2005).

2.2. Major groups of antimicrobial compounds from plants

Plants have limitless ability to synthesize aromatic secondary metabolites, most of which are phenols, or their oxygen substituted derivatives. These groups of compounds include phenols, phenolic acids, flavones, flavonoids, flavonols, quinones, tannins and coumarins. They exhibit antimicrobial effect and serve as plant defence mechanisms against pathogenic micro-organisms.

2.2.1. Phenolic compounds

Simple phenols and phenolic acid are bioactive phytochemicals which possess a single substituted phenolic ring. Phenolic toxicity to micro-organisms is related to the site and number of hydroxyl groups present in the phenolic compound (Scalbert, 1991). Cinnamic and caffeic acids isolated from thyme and tea has been found to be active on bacteria, fungi and viruses. Pyrogallol and catechol are hydroxylated phenols possessing activities against micro-organisms (Zhao *et al.*, 1999).

2.2.2. Quinones

Quinones are characteristically highly reactive, colored compounds with two ketone substitutions in aromatic ring. They occur as di-ketone (quinones) or di-phenol (hydroquinone). They exhibit activity against micro-organisms by forming irreversible complex with nucleophilic amino acid in their proteins.

2.2.3. Flavonoids

Flavones, flavonoids, and flavonols are phenolic compounds with one carbonyl group in their structure. They are synthesized by plants in response to microbial infection (Dixon *et al.*, 1983). In-vitro, they have been found to be effective against a wide array of micro-organisms (Bennet and Wallsgrove, 1994). The antimicrobial activity

of cajanin, (methoxy-isoflavone) isolated from *Ficus ovata* has been reported (Kuete *et al.*, 2009).

2.2.4. Tannins

Tannins are polymeric phenolic substances possessing astringent property, soluble in water, alcohol and acetone, and react with proteins to form precipitates (Basri and Fan, 2005). They are found in almost every plant part, in the bark, leaves, root, wood and fruits. Tannins may be hydrolysable or condensed. Hydrolisable tannins are based on gallic acid while the more numerous condensed tannins are derived from flavonoids monomers. A wide range of anti-infective actions have been assigned to tannins with the mode of action related to their ability to inactivate microbial enzymes, cell envelope and transport proteins. Tannins have been reported to be toxic to bacteria, yeasts and filamentous fungi (Scalbert, 1991). They have been used traditionally for protection against inflamed surfaces of the mouth and treatment of wounds, catarrh, diarrhoea and haemorrhoids (Ogunleye and Ibitoye, 2003).

2.2.5. Coumarins

Coumarins are phenolic substances made of fused benzene and α-pyrone rings. They possess a characteristic odor and have been shown to have antimicrobial activities against bacteria, fungi and viruses (O'Kennedy and Thorne 1997; Kuete *et al.*, 2009).

2.2.6. Terpenes and Terpenoids

Fragrance of plant is carried by essential oil fractions which are highly enriched in isoprene structure based compounds. These compounds are called terpenes but when the compound contains an additional element such as oxygen they are called terpenoids.

Terpenes or terpenoids are active against bacteria, fungi, viruses and protozoa. Essential oils possess strong antimicrobial properties and it has been reported that 60% of the essential oil derivaties inhibited fungi while 30% inhibited bacteria (Chaurasia and Vyas, 1997).

2.2.7. Alkaloids

Alkaloids are natural plant compounds which are heterocyclic nitrogenous compounds and are bases. They can be easily isolated from plants because of their

basic nature. Generally, alkaloids are extremely toxic though they have a marked therapeutic effect in minute quantities. They are used medicinally in small quantities and some have shown antimicrobial properties (Fakeye *et al*., 2000).

2.2.8. Xanthones

Xanthones are yellow phenolic pigments which have similar characteristic colour reactions and chromatographic mobility to flavonoids. They have been shown to have a wide range of biological activity such as antibacterial, antiplasmodial, antidiabetic and antihypertensive.

Apart from the major phytochemical groups, antimicrobial properties of polyamines, isothyonates, glycosides (Murakami *et al.*, 1993), and thiosulfinates (Tada *et al.*,1998) have been reported.

2.2.9. Enzymes

Enzymes are bioactive compounds that are found in medicinal plants and some have shown anti-microbial properties, e.g. papain, a proteolytic enzyme obtained from the milky sap of *Carica papaya* with bacteriostatic properties (Cowan, 1999).

2.3. Techniques for Evaluating the Antimicrobial Properties of Medicinal Plant Products

2.3.1 Selection of plant material

Plants are collected either randomly or by following leads supplied by herbal medical practitioners in geographical areas where the plants are found. To avoid the use of random criteria, plants should be selected from an ethno-pharmacological perspective. The selected plants should be well described and identified. The location of plant, the season, date and time of plant collection should be specified.

Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Plants which are used in the dry form or as an aqueous extract by traditional healers are usually air-dried to a constant weight before extraction (Baris *et al.*, 2006). In other studies, plants are dried in the oven at about 40^0 C for 72 hr (Salie *et al.*, 1996).

2.3.2 Choice of solvent

The nature of solvent, as well as solvent concentration and polarity will affect the quantity and secondary metabolite composition of an extract. Properties of a good solvent in plant extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, the preservative action, and inability of the solvent to cause the extract to dissociate or form complexes (Hughes, 2002). As the end product in extraction will contain traces of residual solvent, so the solvent should be non-toxic and should not interfere with the bioassay (Ncube *et al.*, 2008).

The choice of solvent will depend on the compounds targeted for extraction. Initial screening of plants for possible antimicrobial activities usually begins by using the crude or alcohol extractions, and this can be followed by various organic solvent extractions. Though, water is a universal solvent for extracting plant products with antimicrobial activity, plant extracts from organic solvents have been found to give more consistent antimicrobial activity (Parekh *et al*., 2005).

Chloroform has been found to be the best solvent for the extraction of non-polar biologically active compounds (Harmala *et al*., 1992). The most commonly used solvents for preliminary investigations of antimicrobial activity in plants are methanol, ethanol and water (Lourens *et al*., 2004; Parekh *et al*., 2006; Rojas *et al*., 2006). Other solvents used are dichloro-methane (Dilika and Meyer 1996), acetone (Lourens *et al*., 2004), and hexane (Masoko and Eloff, 2006).

Though there is diverse use of solvents for extraction, it is necessary to focus on a standardized method of extraction and solvent system in order to minimize the variability in the antimicrobial efficacy reports.

2.3.3 Methods of extraction

Extraction methods vary and are usually dependent on the length of time for extraction, the solvent used, pH of the solvent, the particle size of plant tissues and the solvent-to-sample ratio. The plant material is ground to finer size to increase the surface area for extraction thereby increasing the rate of extraction. Eloff (1998),

showed that 5 min extractions of very fine particles of diameter 10µm gave higher quantities of extract than 24 hr in a shaking machine with less finely ground material.

The extraction method that has been widely used by researchers is plant tissue homogenization in solvent (Parekh *et al.,* 2005). Dried or wet, fresh plant parts are ground in a blender to fine particles, put in a certain quantity of solvent and shaken vigorously for 5-10 minutes or left for 24hr after which the extract is filtered. The filtrate may then be dried under reduced pressure and re-dissolved in the solvent to determine the concentration (Taylor *et al.*, 1996).

Another common method is serial exhaustive extraction. Successive extraction of material is carried out with solvents of increasing polarity, from a non-polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound is extracted (Green, 2004). Soxhlet extraction of dried plant material using organic solvent has also been used (Kianbakht and Jahaniani, 2003). This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds.

2.4. Methods for Evaluating the Antimicrobial Efficacy of Plant Extract

The antimicrobial susceptibility tests (AST) are classified into two broad groups namely; diffusion and dilution tests. Diffusion tests include agar-well diffusion, agar disc-diffusion, poison food technique and bioautography. Dilution tests include agar dilution, broth microdilution and broth macrodilution techniques (Tenover *et al.*, 1995).

2.4.1. Agar-disc diffusion assay

The agar disc diffusion technique has been widely used to assay plant extract for antimicrobial activity. Sterilized filter paper discs of 6mm diameter are saturated with filter sterilized plant extract of desired concentration. The impregnated discs are placed on the surface of a suitable solid agar medium like Mueller Hinton agar, Trypton soy agar or Nutrient agar. The media must have been pre-inoculated with test organism. The standard inoculum size is 1 X 10^8 Cfu/mL of bacteria for inoculating diffusion plates. Paper discs can be impregnated with plant extracts before placing them on the inoculated plates (Lourens *et al.,* 2004). Plates are then incubated for 24 hr at 37^o C (bacteria) and 2-5 days at 25^o C (fungi).

After incubation, the zone diameter of inhibition is measured in millimetre.

2.4.2. Agar-well diffusion assay

A standardized concentration of inoculum with fixed volume is spread evenly on the surface of gelled agar plate. Holes of 6 mm-8 mm diameter are punched aseptically with a sterile cork borer (20 mm apart from each other). A fixed volume of plant extract is placed in the bored agar well and incubated at optimum temperature and duration depending on the test organism. Agar-diffusion tests are often used as qualitative methods to determine whether a bacterium is resistant or susceptible. However, agar-diffusion method can be used for determination of MIC values provided the necessary reference curves for conversion of inhibition zones into MIC values are available. The agar-diffusion methods are influenced by factors such as agar depth, diffusion rate of the antimicrobial agent and growth rate of the specific bacteria.

2.4.3. Broth-microdilution

The broth microdilution method is a useful technique for determining the Minimum Inhibitory Concentrations (MIC) of large numbers of test samples. It has the advantage of showing increased sensitivity for small quantities of extract when compared with diffusion techniques. It has also been used to distinguish between bacteriostatic and bactericidal effects of agents. This is useful in quantitative determination of MIC for a wide variety of microorganisms with reproducible results (Langfield *et al.*, 2004).

In the micro-titre plate method, a stock solution of the extract is first obtained in solvent (usually the solvent used for extraction) or in dimethyl sulfoxide (DMSO) (Salie *et al.*, 1996). Two-fold serial dilutions are made to obtain a concentration range. The inoculum size for the procedure is usually 1 X 10^6 Cfu/mL. An equal volume of microbial culture is added to the wells containing the stock solution of graded concentrations and incubation is carried out at 37^{o}C for 24 hr (Lourens *et al.*, 2004). After incubation, plates are examined for changes in turbidity as an indicator of growth. The first well which appears clear is taken as the MIC of extract. Some researchers use dyes as indicators (Umesh *et al.*, 2005) or spectrophotometry to determine presence of growth (Devienne and Raddi, 2002).

Extracts having activities with MIC below 8 mg/mL are considered to possess some antimicrobial activity (Fabry *et al.*, 1998). Natural products with MIC values below 1 mg/mL are considered noteworthy for further study and possible isolation of active constituents (Rios and Recio, 2005).

2.4.4. Bioautography

This is a variation of the agar diffusion method where the analyte is adsorbed onto a Thin Layer Chromatographic (TLC) plate.

This method is used for preliminary phytochemical screening, bioassay guided fractionation and for detecting active components (Schomourlo *et al.*, 2004).

2.4.4.1. Contact bioautography

The TLC plate loaded with the plant extract is placed on an inoculated agar layer and left for some time for diffusion to occur. The chromatogram is removed and the agar layer incubated. The zones of inhibition are observed on the agar surfaces where the spots of the sample are stuck to the agar.

2.4.4.2. Direct bioautography

A known concentration of plant extract is applied to silica 60 gel plates and developed with an appropriate solvent system to separate the phytochemicals. A suspension of the test bacteria is sprayed onto the TLC plate. The bioautogram is incubated at 25^{o} C for 48 hr in humid conditions. Tetrazollum salts are sprayed onto the bioautogram and re-incubated at 25^{o} C for 24 hr (Meyer and Dilika, 1996) or at 37^{o} C for 3-4 hr (Dilika *et al.*, 1996). Clear white zones, against a purple background on the TLC plate is an indication that the plant extract has antimicrobial activity.

2.5. Bactericidal Kinetics

The kill kinetics is used to monitor the antimicrobial activity of plant extracts and antibiotics. This assay shows the rate and extent of bacterial killing and provides a more accurate description than MIC. Time-kill studies provide descriptive information on the relationship between bacteriostatic and/or bactericidal activity in relation to the concentration of test substance over time (Tam *et al.*, 2005).

2.6. Assessment of a new antimicrobial agent

Antimicrobial agents are therapeutic agents that are either microbiocidal or microbiostatic in activity. Among the antimicrobial agents are the antibacterial drugs, antiviral agents, antifungal agents and antiparasitic agents while antibiotics are used to treat bacterial infections. New clinically useful discovery antibiotics are being sought and this has culminated in the discovery of more than a thousand antibiotics (Mitscher *et al.*, 1972). Some of the major antimicrobial agents have considerable drawbacks in terms of limited spectrum of action or serious side effects. Thus, there is a continuous search for safe antimicrobial agents effective against clinical infections caused by Gram-negative organisms, fungi, viruses or mycobacteria.

Several stages are involved in determining the likely therapeutic usefulness of a new antibiotic (Hugo and Russell, 1983).

The stages described are as follow:

(a) Primary and Secondary screening

(b) Toxicity testing

(c) Pharmacokinetics

(d) Clinical trials

2.6.1. Primary and Secondary Screening

Basic tests are designed to measure the performance of the antibiotics. The activity of the antibiotic is tested *in-vitro* and possibly *in-vivo* against a small number of the most important test organisms. During the primary stage, a lot of compounds tested can be rejected.

During the secondary screening, the selected compounds are subjected to much more intensive laboratory investigations of their biological properties.

2.6.2. Evaluation of antimicrobial activity

2.6.2.1 Choice of organisms

For antimicrobial screening, a broad spectrum of microorganisms should be used. These should be representatives of Gram-positive and Gram-negative bacterial isolates, moulds and yeasts.

For preliminary or primary screening, representatives of the Gram positive bacteria (*Staphylococcus aureus*), Gram negative bacteria (*Escherichia coli* or *Pseudomonas aeruginosa*) and fungi such as *Candida albicans* are usually used (Mitscher *et al.*,1972).

S. aureus is a bacterium that causes illness ranging from minor skin infections and abscesses to life threatening disease such as pneumonia, meningitis, osteomyelitis, enterocolitis and septicaemia. It causes boils and internal abscesses, and is a frequent cause of sepsis in wounds and burns (Daltrey *et al*., 1981). *S. aureus* can cause food poisoning. Tetracyclines are used for long-term treatment of staphylococci infection.

Bacillus cereus and *Bacillus subtilis* are spore formers, and are usually used for screening for antimicrobial activity of agents. Penicillin is satisfactory for treatment of *B. subtilis* infection although some strains are resistant to penicillin. Doxycycline, erythromycin or ciprofloxacin may be effective alternatives to penicillin (Brooks *et al.*, 2002)**.**

Pseudomonas aeruginosa (*P. aeruginosa*), is a Gram-negative organism which occurs as a secondary invader of infected or traumatised tissues (Stewart, 1974). Also known as an opportunistic pathogen, it rarely causes disease in healthy persons but infects those already sick or who have weakened immune system. It is the causative organism for urinary tract infections, respiratory system infections, bacteraemia and a variety of systemic infections, soft tissue infections, including wound infections particularly in patients with severe burns and in cancer and AIDS patients who are immune-suppressed (Revathi *et al*., 1998). It is a strong agent of nosocomial diseases and highly resistant to the commonly used antibiotics. Polymyxin B, polymyxin E and gentamicin have been found to be of great use in the treatment of infections caused by *P. aeruginosa*. Infections can be treated with combinations of ceftazidime, ciprofloxacin, tobramycin and imipenem. Most antibiotics are administered intravenously or orally for two to six weeks.

Escherichia coli (*E. coli*) is a coliform present in large numbers in the faeces of man and other animals. Its presence in stagnant or flowing water e.g. in wells and stream is

a common indicator for faecal pollution. It is usually implicated in urinary tract infections (Chakupurakal *et al.,* 2010) and postoperative wound infection (Segupta et. al., 1978). It may play the role of a secondary invader in peritonitis, appendicitis and cholecystisis. Most strains of *E. coli* are harmless however, some strains, such as *E. coli 0157*:H7 can cause severe food-borne disease and are referred to as entero-haemorrhagic *E. coli*. Gentamicin, cephalosporin and penicillinase-resistant penicillins are of great use in the treatment of infections caused by *E. coli*.

Candida albicans (*C. albicans*), a yeast-like dimorphic fungus and the dermatophytes such as *Microsporum* species and *Trichophyton* species are pathogenic microbes which may be used for antimicrobial screening. *C. albicans* is an opportunistic fungus that is responsible for a variety of human diseases ranging from superficial skin lesions to disseminate infection and is the most prevalent specie in candidiasis (Cruz *et al.,* 2007). *C. albicans* is normally present in the mouth, intestine and vagina and responsible for infections where there is a disturbance of local conditions or impairment of the defence mechanism (Rogers, 1990). *C. albicans* is typically present in the oral cavity in a non-pathogenic state in about one-half of healthy individuals (Dangi *et al.,* 2010). Under favourable conditions, the organism has the ability to transform into pathogenic hyphae form. Oral candidiasis is one of the most common oral infections seen in individuals with human immunodeficiency virus (HIV) infection or acquired immune deficiency syndrome (AIDS) (Greenspan, 1994). *C. albicans* is the cause of thrush, giving rise to vaginal irritation and discharge in the vagina. Tioconazole, fluconazole, ketoconazole, clotrimazole, itraconazole, amphotericin B and nystatin have been found to be effective in the treatment of candidiasis (Dangi *et al.,* 2010). Fluconazole has systemic effects that may be beneficial to other fungal infections. The widespread use of fluconazole has led to the development of azole-resistant *C. albicans* (Tumbarello *et al.,* 1997).

There have been reports of rising incidences of candidaemia (*Candida* in the blood) all over the world in the past two decades (Hsueh *et al.,* 2003) and crude mortality rates have remained high (30-50%) despite advances in medical care (Tortorano *et al.,* 2006). Candida infections can spread from the blood stream to other parts of the body such as the eyes, liver, kidney, and brain (invasive candidaemia). In the U.S., Candida infection is the 4th most common disease of bloodstream nosocomial infection i.e.

hospital-acquired infection (Ernestin, 2012). Fluconazole, amphotericin B, echinocandin group, or voriconazole can be used in the treatment of candidaemia.

Among the pathogenic fungi, dermatophytes have the ability to invade keratinized tissues of humans and anmals causing the disease known as dermatophytosis which is the commonest human contagious disease (Esquenazi *et al.*, 2004). The most prevalent pathogenic fungus in the world is *Trichophyton rubrum* and it presents 80% of clinical isolates in Brazil (Chan, 2002). The dermatophytes infect the keratinized surface of the body like the skin, nails and hair producing infection known as ringworm. *C. albicans* may also cause infections of the nails though such infection is not as serious as that caused by the dermatophytes. *Trichophyton* species attack the skin, hair and nails while the *Microsporum* species attack the skin and hair but not the nails.

The organisms used in carrying out antimicrobial screening should consist predominantly of fresh clinical isolates from many sources and multiple isolates of one strain should be avoided. The degree of activity of test compound *in-vitro* is more interpretable if it is compared with that of a relevant established antimicrobial agent, tested in parallel against the same microorganisms.

2.6.2.2 Choice of culture medium

The use of simple media is usually the best, as there is less batch to batch variation and is less likely to contain competing or interfering substances. The same routine test media once activity has been established is advisable. The antibacterial activity of a new compound can be measured with reference to another by comparing the size of zones of activity produced when the compounds are allowed to diffuse from wells into seeded culture plates.

2.7. Toxicity testing

In drug development, toxicity testing has three main purposes.

1. To demonstrate toxic effects and the circumstances of their occurrence
2. To suggest the likely mechanisms of toxicity

Toxicity testing is carried out in animals in order to assess the potential toxicity of a substance as it may be used in clinical practice and to find out the most probable consequence if it is abused. The drug with an acceptable therapeutic index passes on to the next stage but clinical trials in man cannot be carried out until the toxicity profile of the substance is established.

2.7.1. Acute oral toxicity test

Acute toxicity tests can provide preliminary information on the toxic nature of a material for which no other toxicological test is available. Information from such tests can be used to deal with cases of accidental ingestion of a large amount of the material. It can also be used in determining the possible target organs that should be scrutinized and special tests that should be conducted in repeated dose toxicity tests. The test is also useful in selecting doses for short-term and sub-chronic toxicity tests when no other toxicology information is available (Bruce, 1985).

In most acute toxicity tests, each animal is administered with relatively high single dose of the test substance. The animals are observed for one or two weeks for signs of treatment-related effects and thereafter necropsied. Some acute toxicity tests (such as LD_{50} test) are designed to determine the mean lethal dose of the test substance. The median lethal dose (LD_{50}) is defined as the dose of a test substance that is lethal to 50% of the animals in a dose group. The LD_{50} test may not be a good representative for other toxic observations.

The main focus of the acute toxicity test should be on observing the symptoms and recovery of the test animals rather than on determining the median lethal dose (LD_{50}) of the substance. Most often, study is carried out with only one sex of the animal model and generally, the female is assumed to be more sensitive to the acute toxic effects of chemicals than the male (Gad and Chengelis, 1988).

2.7.1.1 Acute Limit test

The test compound should be administered orally by gavage to animals ($n \geq 5$) at a dose of 5 gm/kg body weight. The animals must have been fasted overnight for rats and fasted for 4 hr for mice. The test animals should be observed closely up to 14 days and symptoms of toxicity and recovery should be noted. Gross and

histopathological examination of the test animals at the end of the study may help to identify toxic effects on target organs. If no animal dies at this dose, there is no need to test higher doses.

The acute toxicity of a compound can then be expressed as being greater than 5 g/kg or mL/kg body weight which is the practical upper limit for the amount of test material that can be administered to several animals and the results evaluated.

2.7.2 Dose probing test

Dose probing acute toxicity protocol may be of value when there is no preliminary information about the test substance. In this test, one animal per each of 3 widely spaced dosages should be used and the animals should be observed over a sufficient period of time following the administration of the doses. Subsequent toxicity studies may be based on the results of the dose probing study (Gad and Chengelis, 1988).

2.7.3 Up and down tests

The "up and down" procedure involves dosing the test animals one at a time. The first animal is given one dose, and another animal is given a higher dose one or two days later if the first animal survives (a lower dose if the first animal dies). This process is continued until the approximate LD_{50} has been determined. Each animal should be observed for at least seven days after dosing so that delayed deaths can be recorded. This method usually requires only 6 or 8 test animals as compared with the 40-50 that may be used in the classical LD_{50} test (Muller and Clay, 1982; Bruce, 1985).

2.7.4 Pyramiding test

This study involves a minimum number of animals. Two animals are given successively increasing doses of the test substance on alternate days until an acutely toxic dose is reached. The test is often used to assess acute toxicity in non-rodents.

2.8 Inflammation and inflammatory reaction

Inflammation, a defensive mechanism exhibited by the body, is a biochemical and cellular process that occurs in vascularised tissues in response to harmful stimuli, pathogens, damaged cells or irritants (Ferrero *et al.*, 2007). It is usually characterised by five cardinal signs which are pain, swelling, redness, fever, and loss of function. The swelling is usually due to the increased permeability of the endothelial cells of

the blood vessels. There is an increase in the movement of plasma and blood cells from the blood into the tissues surrounding the injury. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation can be caused by toxins, chemical irritants, foreign bodies, burns, infection by pathogens, physical injury, trauma, frost bite, ionising radiation, and immune reactions due to hypersensitivity.

Inflammation generally occurs in three phases namely:

1. An increase in capillary permeability with hyperaemia and oedema. The increase in vascular permeability results in exudation of fluids from the blood into the interstitial space
2. Cellular filtration. This involves the infiltration of leucocytes from the blood into the tissue
3. Proliferation of fibroblasts and synthesis of collagen fibres and muco-polysaccharides, forming new conjuctive tissues (granuloma formation and tissue repair

Chemical mediators in inflammatory processes have been identified as histamine, serotonin, prostaglandins, leucotrienes, bradykinin, lipoxins, cytokines, nitric oxide, vitexin and growth factors. These substances are referred to as local hormones as they are normally produced or released in response to local stimulus and their actions are normally localized in the site in which they are released (Saxena *et al.*, 1982). Mediators of inflammation can also originate from the plasma e.g complement proteins and kinins. The production of active mediators is triggered by microbial products or by host proteins, such as proteins of the complement, kinins and coagulation system that are activated by microbes and damaged tissues.

2.8.1 Biological methods of evaluating anti-inflammatory agents

The anti-inflammatory activity of plant extracts and bioactive compounds can be evaluated using suitable animal models. The assessment of the anti-inflammatory activity of a test substance can be done by administering it topically, orally, intraperitonially or subcutaneously, and measuring the increase or decrease in the oedematous material (Knehl and Egan, 1980). The major responses measured in bioassay in most inflammatory models are superficial swells and gross appearance at

the inflammatory site (Phillips, 1981). There are other parameters that are measured and they include temperature changes by radiometry, vasodilation by visual estimation and biochemical analysis of exuded fluid.

When evaluating medicaments for anti-inflammatory activity, the best method is testing the agent as a specific inhibitor of mediators of inflammation. Different irritants may produce their inflammatory response by similar or different mediators and therefore, evaluating inflammation with several irritants will make it possible to know the kind of inhibitory effect expected with reference to its mode of inducing inflammation.

2.8.1.1 Inflammatory Irritants

In experimental evaluation of inflammation, induction of inflammation is done either by physical, chemical, biological or ultraviolet irradiation means and all of these are called irritants. The inflammatory characteristic of each irritant is produced by a variety of mechanisms which are characteristic of that irritant. For example, inflammation caused by carrageenan evokes inflammation by formation of kinnin-like mediators (Atkinson, 1971), while that caused by kaolin involves the participation of kinnins and prostaglandins as mediators (Gemmel *et al*., 1979).

2.8.1.2 Chemical irritants

These are chemical substances that have the ability to cause inflammation on experimental animals when applied. Such agents include carrageenan (1%), croton oil, mustard oil, yeast (8%), dextran (3%), filipin (0.5%) and castor oil (Atkinson 1971; Gemmel *et al*., 1979).

2.8.1.3 Physical irritants

These involve the use of physical methods that are sufficient to cause pain and stress which can be used to induce inflammation like using thread or rope to tie one of the hind legs of rat to cause inflammation.

2.8.1.4 Microbiological irritants

These are microorganisms that can cause inflammatory lesion when introduced into experimental animals. Examples include *Staphylococcus aureus*, *Streptococcus*

species, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Mycobacterium* species (Grigorchuk and Sarin, 1974).

2.8.1.5 Ultraviolet irritants

A depilated (hairless) area on the experimental animal is exposed to UV radiations at wavelengths between 296.7 - 313.1 nm. Discolouration of the depilated skin is accepted as inflammatory characteristic. The animal should be kept at a distance of 37 cm from UV lamp and exposure to radiation is usually for about 10 minutes (Blazso *et al.*, 1997).

2.8.1.6 Inflammatory models

Several pharmacological tests have been devised to measure anti-inflammatory activity, most being based on experiments with inflammation in rats and some of these are outlined below.

a. Inflammation of the hind paw. The inflammation may either be carragenan-, kaolin-, or yeast-induced (Winter *et al.*, 1962).
b. Adjuvant arthritis, where injection of *Mycobacterium butyricum* in one hind paw produces inflammation in the other paws and granulation in the ears.
c. Graft-versus-host reaction in chicks, which is normally used to test immune reactions.
d. Histamine-induced paw oedema in rats (Amann *et al.*, 1995). In this test, swelling occurs primarily due to the action of histamine. The rats are challenged by a subcutaneous injection of 0.1 mL of 0.1% solution of histamine into the sub-plantar side of the left hind paw.
e. Acetic acid-induced vascular permeability (Whittle, 1964). The test is used to evaluate the inhibitory activity of drugs against increased vascular permeability induced by acetic acid.
f. Xylene-induced ear oedema (Junping *et al.*, 2005). The application of xylene induces neurogenous oedema which is partially associated with substance P, an undecapeptide. Release of substance P from sensory neurons causes vasodilatation and plasma extravasations which can cause the swelling of ear in mice.
g. Arachidonic acid-induced ear oedema (Romay *et al.*, 1998).

Among the several methods used in screening anti-inflammatory drugs, one of the most commonly employed techniques is based on the ability of such agents to inhibit the oedema produced in the hind paw of the rat after injection of a phlogistic agent. Examples of such irritants include egg albumin, brewer's yeast, formaldehyde, dextran, albumin, kaolin and sulphated polysaccharides such as carrageenan. Subcutaneous injection of carrageenan into the rat paw produces inflammation from plasma extravasation, increased tissue water and plasma protein exudation along with neutrophil extravasation (Chatpaliwar *et al.*, 2002). Inflammation induced by carrageenan is a biphasic event which is characterised by release of histamine and serotonin followed by the release of prostaglandins, protease and lysozymes, producing oedema (Vinegar *et al.*, 1965; Crunkhon and Meacock, 1971). The first phase begins immediately after injection of carrageenan and diminishes in two hours. The second phase begins at the end of first phase and remains through the third hour up to five hours.

The carageenan-induced rat paw oedema is the most commonly utilised in anti-inflammatory activity tests due to the ease at which experiment is carried out on experimental animals (Mascolo *et al.*, 1987). The main advantage of this method of evaluation is in the visualization of the inflammation reaction. Several methods of measurements of the inflammation are employed which include the simple plethysinograph, electronic method, weighing, the use of thread or calliper to measure paw conference or diameter and biochemical analysis of exuded fluid (Phillips, 1981).

2.8.2 Drugs used in inflammatory conditions

Anti-inflammatory agents or drugs can be used to reduce inflammation and pain. The drugs used in inflammatory conditions generally include steroidal anti-inflammatory drugs, non-steroidal anti-inflammatory drugs (NSAIDs) and the miscellaneous group.

2.8.2.1 Steroidal anti-inflammatory drugs

These are anti-inflammatory drugs with the basic steroid backbone and examples are hydrocortisone and corticosterone. As a result of their similarity to cortisol, the anti-inflammatory steroids exert important metabolic and endocrine effects in addition to anti-inflammatory action. The corticosteroids are known to suppress both the cellular and hormonal responses with the degree of inflammation being proportional to the

concentration of corticosteroids at the site of inflammation, thus, their anti-inflammatory action is localised. In preventing inflammation, the corticosteroids block the permeability of the capillary endothelium, reducing the leakage of fluid and the transport of proteins into the area of injury. Corticosteroids also decrease the number of circulating lymphocytes particularly T lymphocytes (Brooks *et al.*,1986).

However, the clinical use of corticosteroids is limited because the amount of anti-inflammatory steroid required for maximum depression in rheumatoid arthritis is so high as to result in many side effects and even some life threatening complications. Anti-inflammatory steroids lower the host resistance to microbial infection causing an increased incidence of unusual fungal, viral and protozoan infections in patients receiving suppressive doses of anti-inflammatory steroids (Brooks *et al.*, 1986). Glucocorticoids are potent anti-inflammatory agents that are often marketed as topical formulations, such as inhalers for asthma and nasal sprays for rhinitis.

2.8.2.2 Non-steroidal anti-inflammatory drugs (NSAIDs)

These are drugs with analgesic and antipyretic effects which in higher doses have anti-inflammatory effects. As analgesics, NSAIDs are non-narcotic. These drugs ease discomfort by blocking the pathway of an enzyme that forms prostaglandins (hormones that cause pain and swelling), thereby lessening the pain in different parts of the body. Prostaglandins which are produced by the enzyme cyclooxygenase (COX) promote inflammation, pain and fever; support the blood clotting function of platelets; and protect the lining of the stomach from the damaging effects of acid. Non-steroidal anti-inflammatory drugs block the COX enzymes and reduce prostaglandins throughout the body, thus reducing the ongoing inflammation, pain and fever. Since the prostaglandins that protect the stomach and support platelets and blood clotting are reduced, NSAIDs can cause ulcers in the stomach and promote bleeding. NSAIDs relieve pain, stiffness, swelling and inflammation; they do not cure the diseases that are responsible for these problems.

The most prominent members of the group of NSAIDs are aspirin, ibuprofen and naproxen. NSAIDs are usually indicated for the treatment of acute or chronic conditions where pain and inflammation are present. They are generally indicated for the symptomatic relief of the following conditions: rheumatoid arthritis, osteoarthritis,

acute gout, dysmenorrhoea, mestatic bone pain, headache and migraine, postoperative pain, mild to moderate pain due to inflammation and tissue injury, pyrexia, ileus and renal colic (Simone, 2006). Aspirin, the only NSAID able to irreversibly inhibit COX-1, is also indicated for inhibition of platelet aggregation.

NSAIDs are a broad group of drugs which are classified on the basis of their chemical structure and examples include the propionic acid derivatives (ibuprofen, naproxen, ketoprofen, fenoprofen, oxaprozin); acetic acid derivatives (indomethacin, sulindac, diclofenac); enolic acid derivatives (piroxicam, meloxicam, tenoxicam, isoxicam); and fenamic acid derivatives (mefenamic acid, meclofenamic acid, flufenamic acid).

2.8.2.3 Miscellaneous group

There are other chemical compounds which are primarily used for other clinical conditions but have been found useful as anti-inflammatory drugs e.g. chloroquine, penicillamine and gold compounds

2.8.2.4 Adverse effects of anti-inflammatory drugs

The adverse effects of these drugs have become increasingly prevalent. The use of glucocorticoid drugs may impair many healthy anabolic processes. The adverse effects include immunosuppresion, hyperglycemia, steroid-induced osteoporosis, weight gain, increased skin fragility, muscle break down, anovulation, irregularity of menstrual periods, growth failure, cataract, glaucoma, adrenal insufficiency and negative calcium balance due to reduced intestinal calcium absorption.

The two main adverse effects of NSAIDs are the gastrointestinal (GIT) effects and renal effects. These effects are dose-dependent, and in many cases severe enough to pose the risk of upper gastrointestinal bleeding, ulcer perforation and death. An estimated 10-20% of patients on NSAID experience dyspepsia (Green, 2001). Common GIT adverse drug reactions (ADRs) include nausea, vomiting, dyspepsia, gastric ulceration with bleeding and diarrhoea (Simone, 2006). Studies have shown that over 50% of patients taking NSAIDs have sustained damage to their small intestine (Higuchi *et al.,* 2009). There are some differences in the ability of individual agents to cause GIT adverse drug reactions. Piroxicam, indomethacin and ketoprofen

appear to have the highest prevalence of gastric ADRs while ibuprofen and diclofenac appear to have lower rates.

NSAIDs (excluding low-dose aspirin) are associated with doubled risk of symptomatic heart failure in patients without a history of cardiac disease. In patients with history of cardiac disease, use of NSAIDs was associated with more than ten-fold increase in heart failure (Kearney *et al.*, 2006).

NSAIDs are also associated with a relatively high incidence of renal adverse drug reactions. Common ADRs associated with renal function include hypertension, salt and fluid retention. In very rare cases, NSAIDs may cause more severe renal conditions such as acute renal failure, nephritic syndrome, interstitial nephritis and acute tubular necrosis. Other common adverse drug reactions include; headache, dizziness and raised liver enzymes. Uncommon ADRs include; bronchospasm, hyperkalaemia, rash, rapid swelling of the face or body (Simone, 2006). The adverse effects of known anti-inflammatory drugs have necessitated the search for plant sources of anti-inflammatory agents.

2.8.3 Anti-inflammatory agents of plant origin

Natural products have been an important source of therapeutically effective medicines and have played significant role in human health in the prevention and treatment of inflammatory disorders. Some plants have been found to possess anti-inflammatory properties. Historically, anti-inflammatory drugs had their origin in the serendipitous discovery of certain plants and their extracts being applied for the relief of pain, fever and inflammation. Such herbs include hyssop, harpagophytum, *Arnica Montana*, slippery elum, feverfew (*Chrysanthemum parthenium*), holy basil, boswella, saw palmetto and neem which produces neem oil which is used in inflammatory skin conditions, joint pains and muscle aches. The resin from guggul tree (*Commiphora mukul*) is found to possess anti-inflammatory and cholesterol lowering effects comparable to commercial drugs such as ibuprofen and found useful in reducing pains due to rheumatism and fibromyalgia (Briggs, 1970). Other herbs include frankincense and indigo plant which is used in Chinese medicine.

The root of Licorice (*Glycyrrhiza glabra*) contains glycyrrhizin which binds to steroid receptors in the body mimicking the effects of steroids to reduce inflammation (Ran *et al.*, 2006). In Chinese medicine, a decoction of licorice is used to treat throat inflammation. Glycyrrhizin ointment is employed clinically for anti-inflammatory skin diseases. White willow (*Salix alba*) contains salicin which metabolises to salicylic acid. Sodium salicylate was first used as an antipyretic and treatment of rheumatoid fever in 1875. Indian Echinacea (*Andrographis paniculata*) contains andrographolide which has significant anti-inflammatory action with low toxicity (Suebsasana *et al.*, 2009). Tumeric (*Curcuma longa*) also known as Indian saffron contains curcumin which has ability to switch off the signalling molecules that produce inflammation (Bansal and Chhibber, 2010). Curcumin is a low molecular weight polyphenol derived from the rhizomes of turmeric.

Colchicine is an alkaloid derived from *Colchicum autumnale* (Liliaceae) and has been used for centuries for the treatment of gout. Colchicine acts as an anti-prostaglandin agent. The alkaloids of *Ephedra* species also possess anti-inflammatory activity (Sener and Bingol, 1988). Quercetin, a flavonoid found in abundance in onions (*Allium cepa*), apples, broccoli and berries has demonstrated anti-inflammatory activity (Sanchez *et al.*, 2002). Cineole, a monoterpene oxide present in many essential oils like eucalyptus, sage, rosemary, psidium is considered useful for the treatment of bronchitis, sinusitis, and rheumatism. The anti-inflammatory activity of cineole has been verified (Santos *et al.*, 2000).

Eating a well balanced, varied diet that is low in fats and carbohydrates can serve as a means of controlling inflammation. Anti-inflammatory foods include fruits and vegetables, oily fish (have high levels of omega-3 fatty acids), seeds, nuts and spices such as ginger. A diet high in vegetables and low in refined carbohydrates and fatty acids may enhance creation of prostaglandins.

Microorganisms can attack and proliferate inside the body bringing about infection which can cause injury by harming the various tissues. When the body is injured, the process of inflammation is set in motion by the body's immune system to maintain the body's health. Repair of injured tissues occurs as a sequence of events, which includes inflammation, proliferation and migration of different cell types (Sidhu *et al.*,

1999). By creating more fluid in the area, inflammation sets the healing process in motion while providing protection from further harm. The problem of infection and inflammation can be acute or chronic in nature. Some health conditions like lupus and multiple sclerosis may expose the body to infections by compromising its defences. These autoimmune diseases can cause widespread infection and inflammation. Treatment of infection includes managing both the pathogen and its resulting symptoms. Administering a drug which helps to get rid of the infection and reduce inflammation helps maximise the body's chance for recovery.

2.9. Medicinal plants used as antimicrobial agents

Plant materials remain an important resource to combat infectious diseases especially since infectious diseases account for approximately one-half of all deaths in tropical countries (Iwu *et al.*, 1999; Namita and Mukesh, 2012). Generally, plant-derived medicines have made large contributions to human health and well being. Table 2.3 shows a list of plants with established antimicrobial activity and the constituent responsible for the activity.

Table 2.3: Plants with antimicrobial activity

Plant Name (Family)	Parts used	Constituents	Uses
Carica papaya (Caricaceae)	Leaves, fruit, seed, latex, root	Chymopapain, papain	Amoebicide, antibacterial (Rawat *et al.*, 2012)
Mangifera indica (Anacardiaceae)	Leaves, stembark	Mangiferin	Antiviral, antifungal, antimicrobial (Rawat *et al.*, 2012)
Mimosa pudica (Mimosaceae)	Leaves, root	Mimosine	Antidiarrhoeal,sore gum,antibacterial (Rawat *et al.*, 2012)
Morinda citrifolia (Rubiaceae)	Fruit, leaves, root	Lignans,flavonoids,iridoids	Urinarytract infections,antibacterial,antiviral,antifungal (Ivan, 1998)
Ricinus communis (Euphorbiaceae)	Seeds, leaves	Ricinolein	Antibacterial
Allium cepa (Alliaceae)	Rhizome	Quercetin	Wounds, blisters and boils,sorethroat,intestinal infections antibacterial,antiviral,antiparasitic,antifungal,vibrocidal (Sharma and Kurma, 2009)
Allium sativum (Liliaceae)	Rhizome	Allicin	Cold,cough,chest infections,antiseptic,disinfectant,antifungal,antibacterial,mouthwash, (Jones and Goebel, 2001)
Curcuma longa (Zingaberaceae)	Rhizome	Curcuminoids	Antifungal,antibacterial,antiviral,wound healing,antimycobacterial (Chainani-Wu, 2003)
Glycyrrhiza glabra (Fabaceae)	Whole herb	Glycyrrhizinic acid,glycyrrhetinic acid	Mouthulcers,viral hepatitis,tuberculosis, inhibits helicobacter pylori (Rawat *et al.*, 2012)
Garcinia kola (Guttiferae)	Seed, root, fruit, stembark	Benzophenone,flavones	Bronchitis, throat and respiratory infections,cough, antiviral,toothache, (Iwu, 1993)
Aframomum melegueta (Zingiberaceae)	Fruit	Gingerol,shagol,paradol	Measles,leprosy,antifungal,antihelminthic,antimicrobial (Iwu, 1993)
Cryptolepsis sanguinolenta (Periplocaceae)	Leaf, root, stem bark	Indoquinoline alkaloid	Urinary tract infection, candidiasis, antibacterial, antifungal (Sawer, 1995; Silva, 1996)

Plant Name(Family)	Part used	Constituents	Uses
Araliopsis tabouensis (Rutaceae)	Stem bark	Alkaloids	Sexually transmitted diseases (Irvine, 1961)
Nanclea latifolia (Rubiaceae)	Stem bark	Indole quinolizidine alkaloids,glycoalkaloids,saponins	Toothache, dental caries, septic mouth,dysentery,antibacterial,antifungal (Iwu, 1993; Lamidi, 1995)

2.10. The Genus Ficus

The use of foods and medicinal plants to maintain and improve health is nearly as old as humanity. Among such, is the fig which has been cultivated for over 11,000 years possibly predating cereal grains (Kislev *et al.*,, 2006). Many species of Ficus are used as food and for medicinal purposes in Ayurvedic and Traditional Chinese Medicine (Kapoor, 1990).

The genus Ficus is well documented for its biological activities such as anticancer (Chiang et. al, 2005), anti-microbial (Al-Fatimi *et al.*,. 2007; Maregeresi *et al.*,. 2008), antioxidant (Al-Fatimi *et al.*, 2007; Manian *et al.*, 2008), anti-diarrhoeal (Mandal and Kumar, 2002), antiplasmodial (Muregi *et al.*, 2003), antipyretic (Rao *et al.*, 2002), antiulcer (Galati *et al.*, 2001) and gastroprotective (Rao *et al.*, 2008).

Ficus thonningii Blume (family Moraceae) is one of the indigenous plants of West Africa used in Nigeria for ethno-medical purposes. The plant is widely distributed in upland forests, open grasslands, riverines, and rocky areas. It is found in the Savannahs. It is propagated by cutting and seed dispersals by birds and animals (Ndukwe *et al.*, 2007). *Ficus* is a genus of about 800 species. Plants in the genus are all woody, ranging from trees and shrubs to climbers. The genus *Ficus* is one of about 40 genera of the mulberry family, Moraceae, with the typical copular inflorescence called a syconium (Woodland, 1997).

The plant belongs to the Kingdom Plantae and Sub-kingdom Tracheobionta. The local names in Nigeria include Chediya (Hausa), Bisheki (Fulani), Odan (Yoruba) and Akinda (Tiv). Other native names include Indian laurel fig (French), Jammeiz al ‘abiad (Arabic), Mrubapoli (Swahili), umBombe (Zulu) and in English, it is generally known as bark cloth fig, common wild fig, strangler and Chinese banyan (Kayonga and Habiyaremye, 1997).

The male and female flowers are enclosed in the syconium. The syconia are produced on leafless stems, usually in large clusters. Among the famous species of *Ficus* is the common Fig (*Ficus carica)* which has been widely cultivated from ancient times for its fruit which is referred to as figs. The fruit of most other species are edible and are extremely important food resources for wildlife.

Other species include *F. religiosa (*Sacred Fig tree), *F .benghalensis (*Banyan Fig), *F. benjamina (*Weeping Fig), *F. pumila (*Creeping Fig), *F. elastica (*Indian rubber tree), *F. leprieurii , F. elegans, F. eribotryoides, F. exasperata (*Sand paper leaf), *F. asperfolia, F. glumosa, F. lyrata (*Fiddle leaf Fig), *F. praticola, F. tessellate, F. lutea, and F. palmeri* (Rock Fig), *F. lutea, F. racemosa, F, pachyrrachis, F. salicifolia, F. aurantiacea, F. glomerata, F. maxima, F. obtusifolia, F. padifolia, F.pungens, F. reflexa, F, inspida, F. scabra, F. septica, F. subcuneata, F. thunbergii, F. toxicaria, F. microcarpa, F. hispida, F. ruficaulis, F. hirta, F. formosana, F. nymphaeifolia and F. beecheyana* (Lansky *et al.*, 2008).

Fig trees have characteristic aerial roots and distinct fruits. There are three vegetative traits that are unique to figs. All figs possess a white to yellowish sap (latex), some in copious quantities; the twig has paired stipules and the lateral veins at the base of the leaves are steep. All *Ficus* species possess latex-like material within their vasculatures, affording protection and self-healing from physical assaults. The skin of the fruit is thin and tender when fresh and the fleshy wall is whitish, pale yellow, pink, rose or purple depending on the species. The fig is juicy and sweet when ripe, gummy with latex before ripening. Seeds vary in size and number from 30 to 1600 per fruit and the leaves are described as hand –shaped (Lansky *et al.*, 2008).

2.10.1 *Ficus carica*

F. carica is a tree of medium height that grows up to 15-30 feet high. The young fruit is rich in latex, but when mature, no latex is found and the fleshy axis contains much sugar. Figs contain about 50% of sugars (chiefly glucose), appreciable quantities of Vitamins A and C, smaller amounts of Vitamins B and D, and enzymes (protease, lipase and diastase). Figs can be eaten fresh or dried and are used in jam making.

Figs are utilized in official preparations in the British Pharmacopoeia and British Pharmaceutical Codex (e.g. Compound Fig Elixir). The plant is used traditionally to treat gastrointestinal, respiratory, inflammatory and cardiovascular disorders (Ponelope, 1997). Phytochemical studies of the plant revealed the presence of arabinose, beta amyrins, beta carotenes, glycosides, beta sitosterols, and xanthotoxol (Duke, 1992).

2.10.2 *Ficus leprieurii* (MIQ)

It is a shrubby epiphyte, strangler or tree that grows up to 24 m in height. It has two subspecies, *Natalensis* of dry forest and woodland, often in rocky places from East Cameroon across the Congo basin to South Africa, and sub specie *Leprieurii* (Miq) of the evergreen forest and damp situation in the Savannah, from Senegal to West Cameroon to Zaire and East Africa. The distribution is across Africa in Sudan, Gabon, Central African Republic, Zaire, Nigeria, Ghana, Angola and Zambia (Berg, 1990). The tree is sometimes grown in the regions as a village shade tree and also propagated by stakes for a living hedge.

The bark contains white latex which is used to make bird lime. The latex has some analgesic properties. It is used in Senegal against tooth trouble, especially caries. The bark is used in Tanganyika as a galactogogue and as influenza medicine. The most important value of the bark lies in its fibre. It gives one of the best bark clothes and the tree is commonly grown in East Africa for this purpose. The roots are used in Senegal to soothe lumbago, backache and arthritis. In Tanganyika, the root (Spp *Natalensis)* is used with *Sporobolus indicum* as an antidote to snake bite and used alone for colic.

In Sierra Leone, beaten leaves (Spp *Leprieurii)* are tied over an internal injury when there is no external bleeding. In Zaire, the plant is used in treating skin diseases and as anti-syphilitic. The fruits are small, and are described as being scarcely edible but not poisonous.

2.10.3 *Ficus eriobotryoides*

It is an epiphyte strangler, or tree up to 20 m tall with wider spreading crown; of the humid forest zone across the region from Guinea to Fernando Po and on to Zaire and East Africa.. Bark-slash yields copious amount of white latex. The fruit is edible, sometimes filled with a dark brown liquid and birds feed on them.

2.10.4 *Ficus exasperata* Vahl

It is a tree about 20m high which grows in some parts of the evergreen forest (Berg, 1989). It is widely distributed across the African region from South Senegal to

Fernando Po and widely spread over Tropical Africa to Ethiopia and Southwards to Mozambique and Angola. It is also found in Yemen, India, and Ceylon.

The leaves contain sap and both the upper and lower surfaces are coarse and carry a high quantity of calcium silicate. The bark contains no latex, but on slashing exudes a viscid clear non-milky sap. The fruit is edible and eaten as a snack. Green pigeons are fond of the fruit. The tree has been grown as avenue shade tree (Burkhill, 1997). The leaves and stem bark of *F. exasperata* have been found to contain alkaloids, flavonoids, tannins, saponins, cyanogenic glycosides (Ijeh and Ukweni, 2007).

2.10.5. *Ficus ovata* Vahl

This plant is found in the Savannah woodland, forest edges, riverside forest and secondary forest, up to an altitude of 2100 m is distributed in the subtropical Africa. It grows across the region of Senegal to West Cameroon and extends across Africa to Sudan, East Africa and Angola. It is also known as elephant tree, and is used for street ornament in Dakar, Senegal (Berhaut, 1979). It is commonly grown as a shade and avenue tree in towns.

The bark contains a copious quantity of sticky white latex. It is used as a bird lime. The inner bark is fibrous and an inferior bark cloth is prepared from it. It is sweet to taste and is chewed as kola. Traditionally, the decoction of the stem bark and leaves of the plant is used for the treatment of infectious diseases, gastrointestinal infections, diarrhoea, and as anti-poison. The decoction of the leaves of *F.ovata, Cassia occidentalis,* and *Setaria megaphylla* is used to facilitate birth delivery (Berhaut, 1979). The antimicrobial potentials of the methanolic extract, fractions and compounds from the stem bark of *Ficus ovata* have been reported (Kuete *et al.,* 2009).

2.10.6 *Ficus polita* Vahl

F. polita is an edible plant growing in lowland rainforest and gallery forest (West and Central Africa), coastal and dry forest (East and Southern Africa) (Kuete *et al.*, 2011). It is an epiphyte, often 20-30m from the ground and later becoming independent or a tree up to 20m tall. It is common across the region of Senegal, Southern Nigeria, Uganda and South Africa. The fibre from the bark is used to make bark cloth by the Fulani tribe of Northern Nigeria. An infusion of the flower is taken by Fulani people

to treat diarrhoea during feverish hepatitis. The edible fruits are chewed for dyspepsia, while leaves, bark and root are used in the treatment of infectious diseases, abdominal pains and diarrhoea (Etkin and Ross, 1982).

2.10.7. *Ficus pumila* Linn

It is a lowly semi woody creeper, prostrate or climbing shrub, native of East Asia and introduced into many tropical countries now present in West Africa. The plant is ornamental. The plant contains latex which has proteolytic activity. It contains an enzyme named ficin which is able to digest parasites in the human intestine. The leaf is said to be used in China to treat dysentery, haematoria and carbuncles. Plant sap is used in the treatment of skin diseases. Fruits and leaves in poultice are used for cancer. Sequisterpenoid glycosides, pumilasides A, B and C have been isolated from the fruits of *F. pumila* (Kitajima *et al.*, 2000).

2.10.8. *Ficus thonningii* Blume

It is a dicot plant that can grow as a shrub, a sub shrub or a tree. Specie is variable in size and habit. It can be a free standing tree, a strangler, or a rock fig. It is a medium sized, low branching tree which can grow up to 40 ft high. The tree grows perennially. It is an evergreen tree with a rounded to spreading and dense crown. *F. thonningii* is widely distributed in the tropics and sub tropics. It can be found in West Tropical Africa in Benin, Cote D'Ivoire, Ghana, Niger, Mali, Nigeria, Senegal, Sierra Leone, Togo, in Southern Africa in Botswana, Swaziland, South Africa, in North East Tropical Africa in Ethiopia.

The whole plant exudes copious milky latex. The leaves are simple, glossy, dark green, thin and papery. Leaf margin is smooth or obovate sometimes elongated. Figs are in leaf axils enclosing small flowers. Leaves can be up to 5-20cm long by 2.5-10cm broad, elliptic to ovate, sometimes rather elongated or slightly oblanceolate; rounded or acute at the apex or very shortly acuminate with blunt tip. The stalk is usually slender of about 1-7.5 cm.

Ficus is readily distinguished by the highly characteristic fruits and has often been recognized by the milky juice, the prominent stipulate that leaves a scar on falling and minute unisexual flowers often arrayed on variously shaped receptacles. The flowers

of *Ficus thonningii* are unisexual, pollinated by small wasps, which develop in some of the flowers and live symbiotically inside the synconium. The plant produces fruit between September and October; February and April which can appear solitary or in pairs in the leaf axil and densely crowded along the branchlets (Keay *et al.*, 1964).

Seed dispersal is achieved by bats. In Southern Africa, flowering and fruiting are observed for most of the year with the peak period in October. Trees are commonly planted using 20-50 cm long cuttings from which most of the leaves have been removed. Rooted cuttings are planted in the nursery and kept moist; but inserting cuttings directly in the field is also feasible. Seedlings raised in the nursery are also used. These species grow easily from traucheons that are left in the shade for a few days to dry before planting. River sands are usually placed at the bottom of the planting hole, to prevent the bottom of the traucheon from rotting. It grows quickly into a fair-sized tree but it is sensitive to cold winds. In colder regions, young plants must be protected for the first 2-3 years.*Ficus thonningii* requires wide spacing because of its spreading crown. It should be protected from browsing at the initial stages of establishment. It is tolerant to pruning and lopping.

F. thonningii is used as a means of forage. In the dry season, primary attention is diverted to forage that remain green and succulent, and are competed for by other animals like sheep, goats and cattle. It also plays significant role in animal production primarily by providing animals with feed resources rich in protein, energy, vitamins and minerals at a time when food is scarce or of low quality.

The use of browse as a sole feed for goats has been reported. *Ficus thonningii* has been proved to be used as a standing feed reserve for rabbits so that they can survive critical period of feed scarcity during dry season without weight losses (Jokthan *et al.*, 2003). The ripe fruits are eaten by bats, bulbuls, parrots, pigeons and starlings. Dropped fruits are eaten by baboons, bushpigs, monkeys, porcupines and warthogs. Fruits are edible and are used in the production of alcohol (Watt and Breyer, 1962). Leaves and twigs are eaten by giraffe, bushbuck, elephant and nyala.

The plant is of economic importance. The branches are used for fire wood. The plant is used in the production of fibre. The wood is used for making domestic implements

and ornaments. Fibres from the bark are used in making mats. Considerable amount of useful latex and rubber are produced from *Ficus* trees.

It is planted as a live fence with the intention of using the leaves as mulch or green manure, as well as for shade and fodder for livestock. Leaf litter helps in the improvement of the nutrient status and water holding capacity of the soil. It is very useful due to its high ability to store water and conserve soil (Hines and Eckman, 1993). *Ficus thonningii* is often planted close to each other to help control erosion. It also offers protection from the scorching sun in market centres, school yards and recreational areas.

In Uganda, the tree is inter-cropped with coffee and bananas. This tree has an aggressive root system and should not be planted in a small garden or near buildings and swimming pools but it makes a successful container plant and an ideal shade tree in a large garden or park. Tree is also used for ceremonial and sacred purposes.

2.10.9. Ethno-pharmacological and cultural uses of *Ficus species*

The WHO Traditional Medical Programme has provided evidence that ethno-medical information can lead to valuable drug discovery (Farnsworth *et al.*, 1995). Studies have been carried out and appreciable claims on the ethno-medicinal uses of *Ficus* species have been demonstrated. The leaves and fruits of *F. thonningii* are used to treat bronchitis and urinary tract infections (Iwu, 1993). The bark is used for the treatment of influenza. The bark is boiled or powdered and soaked in water and the infusion drunk (Kokwaro, 1976). The bark of *F. thonningii* has been found useful in the treatment of colds, sore throats, diarrhoea, wounds, itching and to stimulate lactation (Watt and Breyer, 1962). An infusion or decoction of the bark is used for treating sore throat and cold in Guinea.

The ground bark mixed with gum powder is used in Liberia as dressing for cuts and wounds. The bark is used medicinally as an ingredient in the cure for poison in the Kotiola region of Cote D'Ivoire (Dalziel, 1993). In Nigeria, the fresh leaves of *F. thonningii* is ground with potash and applied to affected parts to treat lumbago while the leaves are burned in hot ash to decolorize, and rubbed on dislocated limbs (Bhat *et al.*, 1990). The bark is also used as medicine for painful joint. In Tanzania, the root is

an indigenous galactogogue (Brenan and Greenway, 1949). The latex is used for wound fever while an infusion of the roots and fibre is taken orally to help prevent abortion. Powdered root is taken in porridge to stop nose bleed and the milky latex is dropped into the eye to treat cataracts (Kayonga and Habiyaremye, 1987). *Ficus thonningii* stem-bark extract has been observed to be useful in counteracting the renal and cardiovascular complication resulting from diabetes mellitus (Musabayne *et al.,* 2007).

In Borno state of Nigeria, medication is made from the shoot and aerial roots for cough, gonorrhoea and to counter debility (Akinniyi and Sultanbawa, 1986). The aqueous methanol extract of leaves of *F. thonningii* showed a dose-related reduction in intestinal motility suggesting the probable reason for its anti diarrhoeal effect. Magnesium sulphate-induced diarrhoea was not inhibited by the extract (Onwkeame and Udoh, 2000). In Ethiopia, crushed fresh root is used to treat stomach disorder.

The ethanol leaf extract of *F. thonningii* inhibited egg-induced oedema, pain induced by hot plate and acetic acid induced writhing in animal models (Otimeyin *et al.,* 2004). The effect on the haematological values and tissue pathology is very scanty in literature. Musabayane *et al.* (2007) reported that the body weight of rats given 250-500 mg/kg of *Ficus thonningii* increased progressively but the changes were not significant while, the total leukocyte count and platelet values were significantly increased in male rats. The probable sex influence in the observation was not well elucidated. The haematological and tissue pathological change in female laboratory animals is scanty in literature.

The latex from the bark, young branches, fruits in different stages of ripening, tree bark, leaves, twigs and shoots of *F. carica* and *F. sycomorus* is used for the treatment tumors and diseases associated with inflammation. Usually, fig tree products for cancer and other tumors are used externally, even when the tumor or swelling was internal and often combined with other ingredients such as blue flag, barley and fenugreek (Lansky *et al.,* 2008).

Heated leaves of *F. pungens* are applied externally for body pains in Papua New Guinea (Nyman *et al.,* 1998). In Oman, the leaf of *F. salicifolia* in a formula is used

on bruised fingers and toes (Ghazanfar and Al-Sabahi 1993). Fresh latex of *F. toxicaria* is applied directly to tooth cavity to soothe toothache in Indonesia (Mahyar *et al.,* 1991) while the fresh leaf is taken orally in Indonesia to treat gastroenteritis (Grosvenor *et al.,* 1995). A decoction of the fresh leaf of *F. thunbergii* is taken orally in Japan to treat lumbago and rheumatism (Kitajima *et al*., 1994)

The root of *F. leprieurii* is used to soothe lumbago, backache, and arthritis. In Sierra Leone, the beaten leaves of the plant are tied over an injury when there is no external bleeding. In Zaire, the plant is used as an anti-syphilitic and for skin conditions such as ringworm (Burkill, 1997).

In Democratic Republic of Congo, drops of macerated leaves of *F. exasperata* were used in the treatment of eye disease. The juice obtained from leaves of plant with lemon is drunk for cold and cough. The juice or decoction of leaves is used as an enema for intestinal pains and colic and as an antidote to poison (Burkill, 1997). The sap from the plant is used to arrest bleeding in Ghana (Abbiw, 1990). The liquid in which the bark has been boiled is given to cows in Ghana post parturition to hasten expulsion after birth. The bark macerate is used by traditional attendants in Congo to ease child birth (Bouquet, 1969) and is applied to leprous sores in upper Ivory Coast. The fruit is used in Northern Nigeria as a cough medicine and treatment of venereal diseases (Iwu, 1993). In African traditional medicine, the leaf extract has been used to treat wounds, rheumatism, arthritis, intestinal pains and colic (Irvine, 1961). The root is useful in the management of asthma, dyspnoea, and venereal diseases (Chabra *et al.,* 1990). Ayinde *et al.* (2007) reported a dose-related reduction in mean arterial blood pressure with the methanolic extract of plant.

The green fruits of *F. hispida* are boiled and given to nursing mothers as a galactogue for better milk. Paste of the stem bark of *F. religiosa* is mixed with lime and prescribed as a cure for swelling glands in the armpit while diluted latex is applied as a cure for skin diseases. The powdered bark of *F. religiosa* is a good absorbent for inflammatory swellings and burns. Leaves and tender shoots have purgative properties and are used for wounds and skin diseases. Fruits are laxative and digestive. Dried pulverized fruit taken in water is used as a cure for asthma. The latex is good for neuralgia, inflammation and haemorrhages. In Northern Nigeria, the

Hausa people use the leaves of *F. sycomorus* to treat diarrhoea. The bark is used as an astringent and laxative (Ahmadu *et al.,* 2007).

The bark of *F. benghalensis* is tonic and demulscent. The milky juice is aphrodisiac and the root astringent. The bark leaves and unripe fruits of *F. glomerata* are used in dysentery. The tonic of the fruit is a laxative and used in the treatment of pile, paralysis and cough. The latex from *F. glomerata* is used in India for treating ophthalmia (Singh *et al.,* 1996). The leaves of *F. maxima* are used by the Lancandon Maya to treat snake bite (Kashanipour *et al.,* 2004). The bark of *F. microcarpa* is used to cure liver disorders, ulcers, and leprosy. In Ecuador, leaf infusion is used to treat internal inflammation. In Brazil, it is used as an antihelminthic, antirheumatic, anti anaemic and antipyretic (Diaz *et al.,* 1997).

Decoctions from the leaves of *F. abutifolia* are used in promoting fertility in humans and the milky latex is used to remove skin warts. The fruit of *F. palmata* is demulcent, emollient, laxative and poultice. The sap is used in the treatment of warts. The latex *of F. benghalensis* is used in India for the treatment of warts (Reddy *et al.,* 1989). Also, the fresh latex from *F. carica* is used in Iran for the treatment of warts (Zagari, 1992) and that of *F. salicifolia* is used externally in Oman for the same purpose (Ghazanfar and Al-Sabahi, 1993).

The latex of *F. carica* has been reported to have antihelminthic activity and can be used as a vermifuge (de Amorin *et al.,* 1999). Its leaves are used as ancient poultice for boils and ulcers. The fresh latex is used in India for boils and eruptions (Sebastian and Bhandari, 1984) and also in the treatment of wounds, ulcers and skin ailments to reduce inflammation (Zagari, 1992). In Japan, the fruit of *F. carica* is used in the treatment of cancer (Takeuchi *et al.,* 1978).

The decoction of the *F. elastica* is used to wash parts of the skin afflicted with dermatitis. The decoction of aerial rootlets is used for wounds, cuts and sores. The bark is astringent and used for styptics for wounds.The decoctions of *Ficus dekdekena* root and *Ficus exasperata* stem bark are used in the treatment of gonorrhoea. In Senegal, the macerated leaf of *Ficus dekdekena* is used to treat tuberculosis (Berhaut, 1979).

Ficus chlamydocarpa and *Ficus cordata* are used traditionally in Cameroon to treat filariasis, diarrhoea and tuberculosis. A decoction from the root bark of *Ficus chlamydocarpa* and *Ficus cordata* is used in the treatment of oral infections (Khabe, 2007). The latex of *F. inspida* and *F. maxima* is used for the treatment of rheumatism (Duke and Vasquez, 1994). A decoction from the dried leaves and stem of *F. maxima* is used to treat gingivitis in Honduras, so also is the sap from the plant (Lentz *et al.,* 1998). *F. benjamina* is used traditionally as a stomachic, hypotensive and anti-dysentery agent (Trivedi *et al.,* 1969).

F. polita is an edible plant whose fruits are chewed for dyspepsia and the leaf, root and bark used in the treatment of abdominal pains, diarrhoea and infectious diseases (Etkin and Ross, 1982). Table 2.4 shows the uses of some *Ficus* species in ayurvedic medicine while Table 2.5 shows the ethno-medical uses of *Ficus* species suggestive of anti-neoplastic and anti-inflammatory actions.

Table 2.4: Uses of Ficus spp. in Ayurvedic medicine (Kapoor, 1990)

SPECIES	PLANT MATERIAL	USE
F. benghalensis	Infusion of leaves and buds	Diarrhoea and dysentery
F. benghalensis	Latex, seeds, fruits	External treatment for pains, bruises, rheumatism, lumbago, sores, ulcers
F. benghalensis	Infusion of bark	To lower blood sugar in diabetes, to treat dysentery, gonorrhoea, and as a "powerful tonic" in "seminal weakness"
F. racemosa	Fruits	Aphthae, menorrhagia, hemoptysis
F. racemosa	Fruits, boiled and strained	Gargle for sore throat
F. racemosa	Ground leaves mixed with honey	"Bilious affections"
F. racemosa	Latex (milky juice)	Diarrhoea, haemorrhoids
F. racemosa	Bark powder	Diabetes
F. racemosa	Roots	Dysentery
F. religiosa	Bark water decoction or infusion	Cooling, gonorrhoea, ulcers, skin diseases, scabies, hiccup, vomiting
F. racemosa	Latex boiled with milk	Aphrodisiac
F. racemosa	Oil infused with root bark	External treatment for eczema, leprosy

Table 2.5a: Contemporary ethnomedical uses of *Ficus* spp. suggestive of anti-neoplastic and anti- inflammatory actions

FICUS SPECIES	PLACE	PLANT PART	USES	REFERENCES
F. carica	Japan	Fruit	Cancer	Takeuchi *et al.*, (1978)
F. carica	Iran	Fresh latex	Warts, mixed with egg yolk or vegetable oil to heal wounds and ulcers	Zagari (1992)
F. salicifolia	Oman	Latex	Warts, external	Ghazanfar and Al-Sabahi (1993)
F. carica	Iran	Fruit	Bronchitis, pleurisy, cystitis, nephritis(oral)	Zagari (1992)
F. carica	India	Fresh latex	Boils and eruptions, external	Sebastian and Bhandari (1984)
F. glomerata	India	Latex	Ophthalmia, external	Singh *et al.*, (1996)
F. maxima	Honduras	Dried leaf + stem	Gingivitis, decoction, oral	Lentz *et al.*, (1998)
F. pungens	Papua New Guinea	Leaf	Body pains, heated leaves applied externally	Nyman *et al.*, (1998)
F. thonningii	Nigeria	Leaf	Lumbago, dislocated limbs	Bhat, *et al.* (1990)
F. racemosa	India	Fresh leaf	Pneumonia	Sebastian and Bhandari (1984)
F. religiosa	Bangladesh	Dried fruit	Tuberculosis, haemorrhoids	Khanom *et al.* (2000)

Table 2.5b : Contemporary ethnomedical uses of *Ficus* spp. suggestive of anti-neoplastic and anti- inflammatory actions

FICUS SPECIES	PLACE	PLANT PART	USES	REFERENCES
F. thunbergii	Japan	Fresh leaves	Lumbago, rheumatism, decoction, oral	Kitajima *et al.*, (1994)
F. toxicaria	Indonesia	Fresh leaves	Toothache, direct to cavity	Mahyar *et al.*, (1991)
F. toxicaria	Indonesia	Leaf	Gastroenteritis, oral	Grosvenor *et al.*, (1995)
F. insipida, F. maxima	Peru	Latex	Rheumatism, external	Duke and Vasquez (1994)

2.10.10. Chemical constituents of *Ficus* species

Onwkaeme and Udoh (2000) identified the presence of starch grains, lignin, calcium oxalate crystals and suberin in *Ficus thonningii*. Tannins, flavonoids, saponins, and anthraquinone glycosides were also detected in samples of the plant. An estimate of the composition of *F. thonningii* showed that the composition of the dry matter was 54.32%, while that of the crude protein was 18.50%. The composition of the nitrogen-free extract was 42.40%, ether extract was 5.54% while the ash value was 17.43%. *F. thonningii* has been found to be a good source of protein and fat and an excellent source of calcium, iron, copper, and zinc.

The antihelminthic activity of the latex of *Ficus* species has been attributed to the action of proteolytic enzyme known as ficin. Sequisterpenoid glycosides, pumilasides A, B and C have been isolated from the fruits of *F. pumila* (Kitajima *et al.*, 2000). The wood of *F. glomerata* possesses tannin and wax. The leaves and stem bark of *F. exasperata* have been found to contain alkaloids, flavonoids, tannins, saponins, cyanogenic glycosides (Ijeh and Ukweni, 2007). Sphingolipids have been isolated from the dried stem bark of *F. exasperata* and *F. natalensis*. The latex of *F. elastica* contains a bitter substance, albuminoid. The wax contains carotic acid. *F. religiosa* contains arabinose, mannose, glucose, flacourtin, steroid, ramnoside, sitosterol and glucopyranoside. Root bark contains tannin, cochotone. Beta-sitosterol, stigmasterol, psoralene, amyrins, hesperidins and flavones have been isolated from the roots of *F. hirta* (Li *et al.*, 2006). From the stem and fruits of *F. indica* were isolated flavonoids, kaempferol, quercetin, eriodicytol and terpenoids (Eun *et al.*, 2003). Steroids, terpenes, carbohydrates, sugars and tannins have been identified in *F. sycomorus* (Ahmadu *et al.*, 2007).

From the stem bark of *F. benghalensis* has been isolated beta-amyrin, beta-sitosterol, lupene-3-one, lupeol acetate, palmitic acid and palmitoyl glycerol (Subramanian and Misra, 1978). Root and bark of plant has also been found to contain glycosides, triterpenes, ketones and beta-sitosterol.

The fresh fruit of *F. carica* possesses 1.3% proteins, 0.6% minerals, 17.1% carbohydrates, 0.06% calcium, 0.03% phosphorus, iron, carotene, nicotinic acid, riboflavin, and ascorbic acid. The milk contains resin, sugar and protein.

Phytochemical studies of the plant revealed the presence of arabinose, beta amyrins, beta carotenes, glycosides, beta sitosterols, and xanthotoxol (Duke, 1992).

Beta amyrin, alpinum isoflavone, genistein, laburnetin, luteol and beta sitosterol have been isolated from the root bark of *F. chlamydocarpa*, and from the stem bark of *F. cordata*, catechin and epiafzelechin have been isolated (Kuete *et al.*, 2008) (Fig 3.1)

From the stem bark of *F. ovata* (Kuete *et al.*, 2009) has been isolated taraxeryl acetate, betulinic acid, oleanoic acid, 2-hydroxyisoprunetin, cajanin, and protocatechuic acid and from *F. carica*, chemical compounds such as marmesin, umbelliferone, lupeol, baurenol and 24-methylenecycloartanol have been isolated (Fig. 3.2-3.3)

From the leaves, bark and root of *F. benjamina* has been isolated cinnamic acid, lactose, naringenin, quercetin, caffeic acid and stigmasterol (Hassan *et al.*, 2003). Phytochemical investigation of *F. polita* revealed the presence of cerebroside named politamide, betulinic acid, stigmasterol, lupeol and sitosterol (Kuete *et al.*, 2011). Eight chemical compounds have been also isolated from the root of *F. polita*. They are euphol-3-O–cinnamate, lupeol, taraxar-14-ene, ursolic acid, β-sitosterol, betulinic acid, sitosterol-3-O-β-D-glucopyranoside and 3,5,4'-trihydroxy-stibene-3,5-O-β-D-diglucopyranoside (Kuete *et al.*, 2011), (Fig 3.4). Several chemical compounds have been isolated from *F. hirta*, *F. formosana* and *F. septica* with their structures presented in Fig 3.5-3.7.

Fig.3.1: Chemical Structures of Compounds isolated from *F. chlamydocarpa*(1-6) *and F. cordata*(7-8)

Fig. 3.2: Chemical Structures of Compounds isolated from *F. ovata*

Fig. 3.3: Chemical Structures of Compounds isolated from *F. carica*

1. euphol-3-O-cinnamate;
2. lupeol;
3. taraxar-14-ene;
4. ursolic acid
5. Beta-sitosterol,
6. betulinic acid;
7. sitosterol-3-o-Beta-D-glucopyranoside;
8. (e)-3,5,4'-trihydroxy-stilbene-3,5-O-beta-D-diglucopyranoside.

Fig. 3.4: Chemical Structures of Compounds isolated from *F. polita*

Fig. 3.5: Chemical Structures of Compounds isolated from *F. hirta*

Fig. 3.6: Chemical Structures of Compounds isolated from *F. formosana*

Fig. 3.7: Chemical Structures of Compounds isolated from *F. septica*

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals

The chemicals and reagents used in this study included: Hexane (Sigma-Aldrich chemicals, Germany), Chloroform and methanol (Riedel-de Haen chemicals, Germany), Ethyl acetate and Acetone (Fischer Scientific, U.K.), Vanillin, Sulphuric acid, Acetonitrile, Formic acid, Iodonitrotetrazolium, Iodine crystals, Ammonia, Hydrochloric acid, Ferric chloride, Dragendorff's reagent, Wagner's reagent, Sodium hydroxide, Benedict's solution, Carageenan (Sigma laboratories, UK), Aspirin® (ICI Chemicals, UK.), Normal saline, Tween 80 (Raymond Lamb Chemicals, UK), Ampicillin hydrochloride (Abbott Laboratories, U.K.), Gentamicin sulphate (Nicholas Laboratories Limited, U.K.), Ciprofloxacin hydrochloride, Tioconazole (Pfizer Inc. New York, USA).

3.1.2 Culture media

The culture media used in the study were Nutrient Agar and Nutrient Broth (Oxoid Laboratories, U.K.), Mueller Hinton agar (Lab M, U.K.), Mueller Hinton broth, and Saboraud Dextrose Agar, (International Diagnostics Group Plc, UK), Tryptone Soy Broth (Oxoid Laboratories, UK). The culture media were prepared according to manufacturer's instructions as stated in Appendix 1. All media were sterilized in an autoclave at 121^0C for 15 minutes.

3.1.3 Equipment

Apparatus used for the study included: Soxhlet apparatus (A.G Ltd, England), Water bath (Electrothermal, England), Incubator (Gallenkamp, UK), Hot air oven (Gallenkamp, UK), Colony counter (Gallenkamp, No. 5282, UK), Rotary Evaporator (Ratavac, Germany), Glass plate silica gel $60F_{254}$ (Merck, Germany), Precoated TLC sheets (Macherey-Nagal, Germany), Ultra Violet lamp (Uvitec, UK), Camag HPTLC apparatus (Muttez, Switzerland), UV Spectrophotometer, HPLC apparatus (Spherisorb Waters, MA, USA).

3.1.4 Microorganisms

Table 3.1: List of Microorganisms used

Microorganism	Description	Source
Bacillus cereus	NCIB 3329	Univ of Wolv, UK
Bacillus subtilis I	L. S. C	DPM, UI
Bacillus subtilis II	L. S. C	DVMP, UI
Bacillus subtilis III	NCIB 3318	Univ of Wolv, UK
Escherichia coli I	L. S. C	DPM, UI
Escherichia coli II	UCH 2311	UCH, Ibadan (urine isolate)
Escherichia coli III	W1485	Univ of Wolv UK
Klebsiella aerogenes I	UCH 041	UCH, Ibad (urethral discharge)
Klebsiella aerogenes II	NCTC 418	Univ of Wolv, UK
Pseudomonas aeruginosa I	L. S. C	DPM, UI
Pseudomonas aeruginosa II	UCH 1591	UCH, Ibadan (wound swab)
Pseudomonas aeruginosa III	NCIB 8295	Univ of Wolv, UK
Staphylococcus aureus I	L. S. C	DPM, UI
Staphylococcus aureus II	UCH 5590	UCH, Ibadan (wound swab)
Staphylococcus aureus III	NCIB 6571	Univ of Wolv, UK
Staphylococcus aureus IV	UCH 5467	UCH, Ibadan (throat swab)
Salmonella typhi I	W1497	Cardiff, UK
Salmonella typhi II	UCH clinical isolate	UCH, Ibadan (stool)
Enterococcus faecalis I	NCIB 775	Univ of Wolv, UK
Streptococcus pyogenes	NCIMB 50117	Univ of Wolv, UK
Proteus vulgaris	NCIB 67	Univ of Wolv, UK
Aspergillus niger I	L. S. C	DPM, UI
Aspergillus niger II	L. S. C	DVMP, UI
Aspergillus niger III	NCTC 772	Univ of Wolv, UK
Candida albicans I	L. S. C	DPM, UI
Candida albicans II	L. S. C	DVMP, UI
Candida albicans III	Q176	Univ of Wolv, UK
Penicillium chrysogenum I	L. S. C	DPM, UI
Penicillium chrysogenum II	NCIB 67	Univ of Wolv, UK
Rhizopus nigricans	L. S. C	DVMP, UI
Trichophyton rubrum	L. S. C	DVMP, UI
Microsporium canis	L. S. C	DVMP, UI
Trichophyton mentagrophyte	L. S. C	DVMP, UI

KEY:

DPM, UI -Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria
DVMP, UI -Department of Veterinary Microbiology and Parasitology, University of Ibadan
UCH -University College Hospital, Ibadan, Nigeria
Uni Wolv, UK -University of Wolverhampton, Wolverhampton, United Kingdom
L. S. C -Laboratory Stock Culture
NCTC -National Collection of Typed Culture
NCIB -National Collection of Industrial Bacteria
NCIMB -National Collection of Industrial and Marine Bacteria

3.2 METHODS

3.2.1 Plant collection and preparation

The leaves and stem bark of *Ficus thonningii* were collected from Olodo village in Ibadan and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan. Herbarium sample was deposited at FRIN with voucher number FRIN 1106898. The plant materials were dried in the sun, pulverised and weighed. The whole tree leaves and stem bark are shown in Plates 3.1 to 3.3.

3.2.2 Phytochemical screening

The powdered plant parts were screened for the presence of secondary metabolites using standard procedures (Sofowora, 1993) as follows:

3.2.2.1 Alkaloids

One gram each of the dried powdered leaves and stem bark was heated with 5 mL of 0.1N HCL. Each filtrate was divided into two portions. To the first portion was added five drops of Dragendorff's reagent while Wagner's reagent was added to the second portion drop-wise. Changes in any portions were noted.

3.2.2.2 Cardiac glycosides (Keller Killiani test)

One gram of the powdered sample was heated for 5 minutes with 10 mL of 80% v/v ethanol and then filtered. To the filtrate was added an equal volume of water and a few drops of lead acetate. The filtrate was then extracted with chloroform and the chloroform extract was evaporated and the residue collected. To the residue was added 3 mL of ferric chloride reagent (0.3 mL of 10%v/v ferric chloride in 50 mL glacial acetic acid) in a test tube. 2 mL of concentrated sulphuric acid was carefully poured down the tube and the colour of the interface was noted.

3.2.2.3 Terpenoids (Salkowski test)

To 0.5 g of the sample was added 2 mL of chloroform. Concentrated sulphuric acid (3 mL) was added carefully to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

3.2.2.4 Anthraquinones (Bontrager's test)

To 1 g of the powdered sample was added 3 mL of 0.1N HCL and the mixture was heated and filtered. The filtrate was extracted with an equal volume of chloroform. To the chloroform layer an equal volume of 10 % v/v of ammonia solution was added. The colour changes were noted.

3.2.2.5 Saponins

To 1 g of the powdered sample was added 20 mL of water which was then boiled and filtered. Three 5 mL portions of the filtrate were treated as follows:

(i) The first 5 mL portion was shaken in a test tube. The presence or absence of frothing was noted.

(ii) To another 5 mL portion, 2.5 mL dilute HCL was added and boiled. The presence or absence of white particles was noted.

(iii) To the last 5 mL, an equal volume of Benedict's solution was added and boiled on a water bath. Any change in the mixture was noted.

3.2.2.6 Tannins

To 1 g of the powdered sample, 20 mL of water was added, boiled and filtered. The filtrate was adjusted to 20 mL with water.

(i) One millilitre of the adjusted solution was made up to 5 mL with water, and a few drops of 0.1 % w/v ferric chloride solution were added.

(ii) Two drops of bromine water were added to another 1 mL of the adjusted solution.

Colour changes or formation of precipitate was noted.

3.2.2.7 Flavonoids

The powdered sample weighing 5 g was extracted with 10 mL of methanol and filtered. To the filtrate was added small quantity of magnesium powder and three drops of concentrated hydrochloric acid. The colour change was noted.

Plate 3.1: ***Ficus thonningii*** **Blume (Moraceae) Tree**

Plate 3.2: *Ficus thonningii* leaves

Plate 3.3: *Ficus thonnigii* stem bark and root

3.2.3 Extraction of the plant parts

Gradient extraction of 5 kg each of powdered leaves and powdered stem bark was successively carried out separately with solvents of increasing polarity: hexane, chloroform and methanol using a Soxhlet apparatus. The solvents were dried under pressure and each dried extract was weighed and stored at -4 ^{0}C for further analysis.

3.2.4 Preparation of culture media

Agar media and broth were prepared according to manufacturers` specifications. (Appendix 2)

3.2.5 Preparation of microbial cultures

The identities of the microbial isolates were confirmed before use by culturing on specific media followed by Gram staining and biochemical tests (Stewart, 1974). The microbial cultures were maintained on nutrient agar slants at 4 ^{0}C in the laboratory and sub-cultured in appropriate fresh medium prior to every antimicrobial test. Subcultures of bacteria from nutrient agar slopes were inoculated into 5 mL of nutrient broth and incubated at 37 ^{0}C for 18 hours. For mould and yeast cultures, the spores were inoculated into tryptone soy broth and the tubes were incubated at room temperature and checked for growth at 24 hours to 5 days.

3.2.6. Identification and characterization of microbial isolates

3.2.6.1. Selective plating

Clinical isolates of *S. aureus* were inoculated into sterile broth tubes and incubated for 18 hours at 37 ^{0}C. One millilitre of freshly collected human blood was mixed with 19 mL of molten sterile salt agar at 45 ^{0}C. The mixture was poured and allowed to set. The surface was dried and streaked with the 18 hr broth culture of the organism and incubated at 24 hrs at 37 ^{0}C. The plates were observed for isolated, round golden yellow colonies with surrounding haemolysis.

For *Pseudomonas aeruginosa*, subculturing was carried out on cetrimide agar plate (nutrient agar containing 0.03 % cetrimide) and the plate was incubated at 30 ^{0}C for 48 hrs. The plates were observed for bluish- green colonies.

For *Escherichia coli*, inoculum from nutrient broth was subcultured into 5 mL MacConkey broth containing sterile Durham tube and incubated at 37 ^{0}C for 24 hrs. Presence of acid and gas was an indication of enterobacteriaceae. For confirmatory test for *E. coli*, 0.1 mL of culture showing acid and gas was added to 5 mL of MacConkey broth and tryptone water containing sterile Durham tube. Incubation was carried out at 44 ^{0}C for 48 hrs. Presence of acid and gas in the MacConkey broth was confirmatory for *E. coli*. Kovac's reagent (0.5ml) was added to the culture medium and shaken. A red coloration showed the production of indole by *E. coli*.

For *Salmonella typhi*, 0.1 mL of inoculum was subcultured into 10 mL selenite F broth. The inoculated broth was incubated at 37 ^{0}C for 24 hrs. Content of the tube was inoculated into a plate containing brilliant green agar and another plate containing bismuth sulphite agar for 24 hrs. The presence of small opaque pink or white colonies on the brilliant green agar and black or green colonies on the bismuth sulphite agar indicated the presence of *Salmonella typhi*. Confirmatory test was carried out by subculturing the colonies on triple sugar iron (TSI) agar for 18 hrs at 37 ^{0}C. The slope culture was stabbed with a straight inoculating wire while an inoculating loop was used to streak the agar medium surface with the test organism. Fermentation of glucose alone would show as a yellow color in the butt of the medium, fermentation of sucrose and lactose would cause both butt and slant to be yellow. Production of hydrogen sulphide produced blackening.

3.2.6.2. Motility test

A small amount of culture from an 18 hr agar slope is emulsified in a drop of broth and placed in a hollow-ground slide. A little immersion oil is placed round the edge of the depression in the slide. A small loopful of culture is transferred to a clean dry cover-slip on the bench. The cavity slide was inverted over the cover-slip so that the drop is in the centre of the cavity. The slide is gently pressed down so that the oil seals the cover-slip in position. The slide was inverted quickly with the drop of culture in the form of a hanging drop. Preparation is examined immediately under the microscope with x40 objective lens for bacterial motility.

3.2.6.3. Gram staining of isolates

A sterile wire-loop was used to transfer a speck of colony of microbial culture to a loopful of water on a clean glass slide. This was emulsified to form a thick milky suspension which was spread over an area on the slide and dried. The slide was heat fixed and stained with crystal violet for 30 sec, Gram iodine was added as a mordant for 60 sec. Ethanol (95%) was used for decolorisation for 45 sec and the film was counterstained with carbol fuchsin for 30 sec. The slide was air-dried and viewed microscope using x100 objective lens, with immersion-oil. The organisms were observed for purple coloration for Gram positive bacteria and pink coloration for Gram negative bacteria.

3.2.6.4. Biochemical tests

3.2.6.4.1. Oxidase test

A few drops of freshly prepared oxidase reagent (N,N,N,N-tetramethyl-p-phenylenediamine hydrochloride) were placed on a piece of Whatman filter paper with a glass rod. The impregnated paper was smeared with colonies of each organism from nutrient agar plates. A change of color to purple within 10 seconds was regarded as positive.

3.2.6.4.2. Citrate utilisation test

Koser's citrate broth was inoculated with a suspension of the organism and examined daily for 7 days for turbidity and a change in colour from green to blue was an indication of utilisation of the medium as a sole carbon source, for a positive test.

3.2.6.4.3. Catalase test

Colonies of the organism were transferred to a glass slide and drops of hydrogen peroxide (3% aq. solution) were added. Production of gas bubbles indicates a positive reaction.

3.2.6.4.4. Indole test

The test organism was used to inoculate 3 mL of 2 % w/v sterilised peptone broth and was incubated at 44 ^{0}C for 2-7 days. 0.5 mL of Kovacs' reagent was added with gentle shaking. Formation of a red color in the surface layer within 10 min indicates indole production while absence of the coloration indicates a negative result.

3.2.6.4.5. Coagulase test

Undiluted plasma (0.5 mL) was mixed with an equal volume of an 18 hr broth culture and incubated at 37 ^{0}C for 6 hrs. Tube was examined hourly for coagulum, as a positive test.

3.2.7 Determination of the antibiogram of microbial isolates

The agar-diffusion technique was used to determine the antimicrobial susceptibility patterns of the clinical isolates of the microorganisms used. Seeded plates were prepared by inoculating 20 mL of Mueller-Hinton agar with 0.1 mL of 10^{-2} dilution of inoculum (standardized at 10^{6} cfu/mL) . The inoculated plates were air-dried for 30 min and antibiotic discs were placed on the surface of the agar medium aseptically using flamed forceps. The discs were pressed down gently to ensure maximum contact. The plates were incubated at 37 ^{0}C for 24 hrs and the diameters of zones of growth inhibition measured. The antibiotic discs contained the following; ceftazidime (CAZ) 30 µg, cefuroxime (CRX) 30 µg, gentamicin (GEN) 10 µg, ceftriaxone (CTR) 30 µg, erythromycin (ERY) 30 µg, cloxacillin (CXC) 5 µg, ofloxacin (OFL) 5 µg, Augmentin® (AUG) 30µg, ciprofloxacin (CPR) 5 µg, nitrofurantoin (NIT) 30 µg and ampicillin (AMP) 10µg (Oxoid, USA).

3.2.8. Evaluation of antimicrobial activities of extracts and fractions

The antimicrobial activities of extracts and fractions were determined using standard procedures (Hugo and Russell, 1983). The agar-cup diffusion and agar-disc diffusion methods were used for the initial screening of the crude extracts.

3.2.8.1. Agar- cup diffusion assay

A volume of 0.1 mL from a 10^{-2} dilution (equivalent to 1.5 x 10^{6} cfu/mL) of each bacterium and *Candida albicans* incubated for 24 hrs was used to prepare seeded plate cultures. Similar plate culture was prepared in respect of each mould and incubated for 24 hrs to 5 days. Equidistant wells were cut in the culture medium using a sterile cork-borer of 6 mm diameter. Each well was filled with graded concentrations (3.125-50.00 mg/mL) of the extracts. Following a pre-incubation diffusion period of 1hr at room temperature, the plate cultures were incubated. For bacteria and *Candida albicans*, incubation was at 37 ^{o}C and 28 ^{o}C for 24 hrs to 5 days for moulds. Ciprofloxacin hydrochloride, gentamicin sulphate and ampicillin

were used as positive control drugs for bacteria, and tioconazole for fungi. Dimethyl sulfoxide (DMSO) served as the negative control.

The above procedures were carried out for the re-constituted fractions and experiments were carried out in triplicates.

3.2.8.2. Agar-disc diffusion assay

Whatman filter paper disc (6mm diameter) was inoculated with 10 µl of different concentrations of extract (3.125-50 mg/mL). The discs were placed at equidistant points on the agar medium plates of bacteria and fungi. Gentamicin sulphate, ampicillin and ciprofloxacin hydrochloride were used as control drugs for the bacterial isolates and tioconazole for the yeast and moulds. DMSO was used as a negative control. Incubation was done as described above for the agar-cup diffusion method. The results from this method were found to be more reproducible and reliable, hence its use to determine the subsequent antimicrobial activity of the chromatographic fractions.

3.2.8.3. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The microdilution assay was carried out to determine the MIC and MBC of the extracts and fractions (Kuete *et al.*, 2008). The crude extracts, fractions and isolated compound were dissolved in DMSO and the solution obtained was added to sterile Mueller Hinton Broth (MHB) to obtain a stock concentration of 625 µg/mL which was then serially diluted two-fold to obtain concentration ranges of 5-625 µg/mL. Each concentration in 100 µL volume was put into the wells of the microtitre plates containing 95 µL of MHB and 5 µL of inoculum standardized at 1.5×10^6 cfu/ mL by adjusting the optical density to 0.1 at 600 nm SHIMADZU UV-120-01 spectrophotometer (Kuete *et al.*, 2008). Wells containing 195 µL of MHB and 5 µL of standard inoculum served as fertility control. Gentamicin served as the control drug for bacteria and tioconazole as control drug for fungi. Each plate was covered with a sterile plate sealer, agitated to mix the contents of the wells using a plate shaker and incubation was carried out at 37 ^{0}C for 24 hours (bacteria and *Candida albicans*) and 28 ^{0}C for 24 hours to 5 days (moulds). The assay was repeated three times. The MIC of samples was detected following addition of 40 µL of 0.2 mg/mL of *p*-iodonitrotetrazolium chloride to contents of wells and incubated at 37 ^{0}C for

30 minutes. A colour change from yellow to pink indicated the presence of viable bacteria. The lowest sample concentration that prevented this change and exhibited complete inhibition of bacterial growth was taken as the MIC.

Inoculations from wells showing no visible growth were streaked on Mueller Hinton agar plate and incubated at 37 ^{0}C for 24 hrs. Lowest concentration showing no bacterial growth was taken as the Minimum Bactericidal Concentration (MBC).

3.2.8.4. Determination of kinetics of bactericidal activity

To determine the bactericidal activity of the crude extract, two multi-drug resistant (MDR) strains of microorganisms (*S. aureus* and *E. coli)* were used. A 0.1mL of the log phase cells of the culture was inoculated into 4.9 mL of Mueller Hinton broth mixed with plant extract at varying concentrations of 2.5, 5.0, and 10.0 mg/mL to produce an initial viable count of approximately 10^7 cfu/mL. Samples were taken at intervals of 0, 30, 60, 90, 120 and 240 min and diluted for viable count estimations by spread plate method. 0.1 mL sample was plated on dried Mueller Hinton agar plates and incubated at 37 ^{0}C. Mueller Hinton broth seeded with the test inoculum without the extract served as a fertility control. All plates were allowed to stand for 30 min before incubation at 37 ^{0}C for 24-48 hrs and the number of colonies counted at each time interval. The procedure was carried out in triplicates to ensure reproducibility.

3.2.9. Acute toxicity test

3.2.9.1. Acute oral toxicity test

Twenty mice were divided into five groups of four each. To each of four groups was given 0.1 g/kg, 0.2 g/kg, 0.4 g/kg and 1.0 g/kg body weight respectively of the extracts dissolved in 2.5 % v/v propylene glycol. The fifth group was given an equivalent volume of 2.5 % v/v propylene glycol as control. For the Acute Limit toxicity test, 5.0 g/ kg body weight of extract was administered orally by gavage to five mice. The animals were observed individually at least once during the first 30 minutes after dosing and then periodically during the first 24 hours and daily thereafter, for a total of 14 days.

All the other animals were observed for symptoms of toxicity and mortality for 21 days. Feed and water were administered *ad libitum*. The animals were sacrificed after 21 days.

3.2.9.2. Determination of haematological parameters

The blood samples were collected before administering the extract, mid-way in the experiment (10 days) after administration and then after 21 days.

The haemoglobin concentration was done using the cyanomethaemoglobin method (Schalm *et al.*, 1975).

Packed cell volume (PCV) was done by the conventional method of filling the capillary tube with blood as described by Schalm *et al.*, (1975) and read with a microhaematocrit reader. Erythrocyte count was determined using the haemocytometer method (Coles, 1986).

Total leucocytes and leucocyte differential count were also determined. Erythrocyte indices were determined from values obtained from red blood cell count, haemoglobin concentration and packed cell volume values.

3.2.9.3. Histopathology

The liver, kidney, uterus, ovary, spleen and lungs of the animals were harvested and fixed in 10% buffered formalin in labelled bottles. Tissues were processed routinely and embedded in paraffin wax. Sections were cut, stained with haematoxylin and eosin and examined under light microscope.

3.2.9.4. Determination of weight gain

Body weights of the rats were taken prior to the commencement of the study, midway into the study and lastly before being slaughtered on day 21 and examined for tissue changes.

3.2.9.5. Statistical analysis

Student's t-test and paired t-test were applied for determining the statistical significance between the control and tested groups. The level of significance was set at 0.05.

3.2.10. Evaluation of anti-inflammatory activity of methanol leaf extract

Fifteen female albino rats divided into 3 groups of 5 animals per group were used in this study. The inhibition of carageenan-induced oedema on the sub-plantar region of the paw of the rats was used to measure the anti-inflammatory activity of the extract (Bamgbose and Naomesi, 1981). The albino rats were fasted for 12 hours overnight prior to tests.

A dose of 100 mg/kg body weight of the extracts in 40 % v/v Tween 80 was administered orally to each group of five female albino rats by means of a cannula. The same dose of aspirin (acetylsalicylic acid) suspended in 40 % v/v Tween 80 and 0.5 mL of the vehicle i.e 40 % v/v Tween 80, were used as positive and negative controls respectively on groups of five rats each.

The extracts and controls were given to the rats an hour before injecting the sub-plantar region of the left hind paw of each rat with 0.1 mL of 1% w/v carageenan solution in normal saline. Increase in linear paw circumference, as measured by a micrometer screw guage, was taken as an index of increase in paw volume which is a measure of the oedema (Awe *et al.*, 1997).

Inhibitory activity was calculated according to the formula

$$\text{Percentage inhibition} = \frac{(D_t - D_o)_{control} - (D_t - D_o)_{test}}{(D_t - D_o)_{control}}$$

Where:

D_t = linear paw circumference 4 hours after carageenan injection.

D_o = linear paw circumference at 0 hour (just before carageenan injection.

$(D_t - D_o)_{control}$ = values obtained for 0.5 mL of 40 % v/v Tween 80

$(D_t - D_o)_{test}$ = values obtained for each extract.

3.2.11. Chromatographic separation

3.2.11.1. Fractionation of crude extracts by column chromatography

5 g of the crude extract was subjected to column chromatography and eluted with hexane-ethyl acetate (80:20, 70:30, 60:40, 50:50.), ethyl acetate (100%) and methanol (100%) gradients.

Slurry of silica gel 70-230 mesh (600g) was made with the eluting solvent and packed into the glass column. The tap was opened to allow excess solvent to run off. 5 g of the hexane leaf extract was dissolved in the eluting solvent and packed on top of the silica gel slurry with a pipette. As soon as the cake began to form on the column, glass wool fibre was placed on top of the extract and the eluting solvent was added. Collection of the eluent was done with 50 mL and 100 mL conical flasks. Further elution was done with increasing concentration gradients.

For the methanol leaf crude extract, elution was carried out using dichloromethane-ethyl acetate (80:20, 70:30), ethyl acetate (100%), ethyl acetate-methanol (50:50) and methanol (100%) gradients.

For the fractionation of hexane stem bark crude extract, elution was done with hexane-dichloromethane gradients (60:40, 50:50), ethyl acetate (100%), ethyl acetate-methanol (50:50), and finally with 100% methanol.

Elution of methanol stem bark was carried out with dichchloromethane-ethyl acetate (80:20), ethyl acetate (100%), methanol (100%).

Fractions collected were monitored with spotting on Thin Layer Chromatographic (TLC) plates and viewed under the visible U.V light (254 nm). Plates were also placed in iodine chroma-tanks to view the spots. A spray of 0.5% vanillin and 10% sulphuric acid was used on the plates, and the plates were dried in hot air oven at 110 ^{0}C for 1 hour and colour changes observed. On the basis of analytic TLC, fractions were pooled together and antimicrobial assay was carried out to determine the active fractions. The active fractions were submitted to further separation and purification on a silica gel column chromatography and on high performance thin layer chromatography. The active fractions in hexane leaf extract were HLF 04, HLF 07 and HLF 11 while MLF 01, MLF 06, MLF 07 and MLF 11 were the active fractions in methanol leaf extract.

The retention factor (R_f) of each spot was calculated using the formula:

R_f = Distance travelled by spot / Distance travelled by solvent

3.2.11.2. High Performance Thin Layer Chromatography (HPTLC)

One mg/mL solution of hexane leaf fractions was made with ethyl acetate while a 1 mg/mL solution of methanol leaf fractions was made with methanol. The solutions were placed separately in the HPTLC sample applicator and a sample volume of 2 µL was applied as a broad band unto a silica-gel glass plate $60F_{254}$.

For the hexane leaf fractions, 14 mL of hexane was mixed with 6 mL of ethyl acetate and 15 mL of the solvent mixture was poured into the developing chamber of the HPTLC as the mobile phase. For the methanol leaf fractions 14 mL of hexane was mixed with 4 mL of chloroform and 2 mL of acetone, 15 mL of the solvent was poured into the developing chamber.

The sample-injected silica-gel glass plate was placed in the developing chamber and the saturation time was 15 minutes. Pre and post-drying of plate was done for 5 minutes. Iodine crystals placed in iodine chroma-tank were used as derivatising agent. Also, the plate was sprayed with a mixture of 0.5% vanillin in 10% sulphuric acid.

Chromatograms of both the underivatised and derivatised plates were monitored at 254 nm and 366 nm illumination.

3.2.12. Spectroscopic techniques

3.2.12.1. Ultraviolet spectrometry

Ultraviolet (UV) spectra of compounds were determined using EV 100 UV- visible spectrophotometer. 0.108 g of hexane extract was dissolved in 5 mL of ethyl acetate while 0.10 g of methanol extract was dissolved in 5 mL methanol. Each solution was placed in glass cell in the spectrophotometer and absorption measured. Peaks and valleys were expressed in nanometer.

3.2.12.2. High Performance Liquid Chromatography (HPLC)

This technique can be used to separate and identify compounds. The normal phase HPLC was used for the hexane fractions. 400 mL of the mobile phase was prepared with hexane, chloroform and acetone in the ratio 7:2:1. The UV absorbance was adjusted to 254_{nm} while the flow rate was adjusted to 2.0. 20 µL of 1 mg/mL of sample was injected into the HPLC variable and detector Varian 2050 machine. The peaks and retention times were recorded.

On the other hand, the Reverse Phase HPLC which comprised of a non polar stationary phase and an aqueous mobile phase was used for the separation and identification of compounds in methanol leaf extract and fractions. Gradient elution was used to achieve this effect thus reducing the polarity and surface tension of the aqueous phase during the course of the analysis.

Dried crude extract and fractions were reconstituted in 1 mL methanol prior to HPLC analysis. Separation was obtained using a C18 reverse phase (RP) ODS-2 analytical column (4.6 mm by 250 mm, 5.0 µm Spherisorb Waters, MA, USA) at a flow rate of 1 mL/min on a Perkin Elmer's 200 over a period of 30 minutes. Injection volume was 50 µL and the column was set to 25 ^{0}C. The wavelength was 280 nm.

3.2.12.3. Nuclear Magnetic Resonance (NMR)

NMR spectra were recorded on a Bruker Avance 300 at 300MHz (1H) and Bruker Avance 600 MHz (^{1}H) and 150 MHz (^{13}C) with the residual solvent peaks as internal references. The structures of the compounds were confirmed by comparing with reference data from available literature.

CHAPTER FOUR

RESULTS

4.1. Phytochemical Screening

The phytochemical screening of the leaves and stem bark of the *Ficus thonningii* revealed the presence of alkaloids, tannins, saponins, terpenoids, flavonoids and cardiac glycosides (Table 4.1). The formation of brown upper acetic layer with Keller-Killiani test confirmed the presence of cardiac glycosides in both the leaves and stem bark of the plant. The formation of a reddish brown colour at the interface of chloroform and sulphuric acid layer in both leaves and stem bark confirmed the presence of terpenoids. The presence of saponins was confirmed with foaming and formation of white particles on hydrolysis with dilute hydrochloric acid. More foaming was however observed in the stem bark than in the leaves. The formation of deep blue colour with ferric chloride test in both the leaves and stem bark confirmed the presence of hydrolysable tannins. There was no rose pink colouration with Bontrager's test indicating the absence of anthraquinones.

4.2 Extraction

The macroscopical characteristics and the percentage yields of the crude extracts of plant after successive gradient extraction with hexane, chloroform and methanol are presented in Table 4.2. Succesive extraction of 5 kg of leaves yielded 240.5 g (4.81%) to hexane, 362.5 g (7.25%) to chloroform and 357.0 g (7.14%) to methanol while 5.0 kg of stem bark yielded 191.0 g (3.82%) to hexane, 232.0 g (4.64%) to chloroform and 316.0 g (6.32%) to methanol. The highest yield was obtained from chloroform and the lowest from hexane with the leaves of plant while with the stem bark the lowest yield was obtained from hexane and the highest yield from methanol.

Table 4.1: Phytochemical screening of leaf and stem bark of *F. thonningii*

Metabolites Tested	Name of test	Leaf	Stem bark	Inference
Alkaloids	Draggendorf's reagent	Reddish brown precipitate	Reddish brown precipitate	Alkaloids present
Cardenolides	Keller Killiani Test	Brown ring and green coloration in the acetic layer	Brown ring and green coloration in the acetic layer	Presence of Cardenolides
Terpenoids	Salkowski test	A reddish brown coloration of interface	A reddish brown coloration of interface	Presence of terpenoids
Anthraquinones	Bontrager test	No rose pink coloration in the aqeous layer	No rose pink coloration in the aqeous layer	Absence of anthraquinones
Saponins	Frothing test	Foaming	More foaming	Presence of saponins
Tannins	Ferric chloride test	Deep blue color	Deep blue color	Hydrolisable tannins present
Flavonoids	Magnesium chloride test	Red coloration	Red coloration	Presence of Flavonoids

Table 4.2: Extraction yield and characteristics of leaf and stem bark of *Ficus thonningii*

Extracting solvent	Morphological part	Macroscopical characteristics	Yield (g)	Percentage Yield
Hexane	Leaf	Green gummy mass	240.5	4.81
Chloroform	Leaf	Dark green mass	362.5	7.25
Methanol	Leaf	Dark green cake	357.0	7.14
Hexane	Stem bark	Brown gummy mass	191.0	3.82
Chloroform	Stem bark	Dark brown mass	232.0	4.64
Methanol	Stem bark	Dark brown mass	316.0	6.32

Table 4.3: Identification and characterization of microbial isolates used for susceptibility test

Organism	Gram reaction	Microscopical characteristic	Catalase test	Oxidase test	Citrate test	Indole test	Coagulase test
S. aureus	+	Spheres in pairs, clusters	+	-	+	-	+
E. coli	-	Motile rods	+	-	-	+	-
P. aeruginosa	-	Motile rods	+	+	+	-	-
S. typhi	-	Motile rods	+	-	+	-	-
K. aerogenes	-	Non-motile rods	+	-	+	-	-
B. subtilis	+	Motile rods	+	+	+	-	-

KEY:

- : Negative

+ : Positive

S. aureus: *Staphylococcus aureus*

E. coli: *Escherichia coli*

P. aeruginosa: *Pseudomonas aeruginosa*

S. typhi: *Salmonella typhi*

K. aerogenes: *Klebsiella aerogenes*

B. subtilis: *Bacillus subtilis*

Table 4.4: Antimicrobial sensitivity patterns of Gram positive isolates

Clinical isolate	Gen 10µg	Ctr 30µg	Ery 30µg	Cxc 5µg	Ofl 5µg	Aug 30µg	Caz 30µg	Crx 30µg
B. cereus NCIB 3329	S	R	R	R	S	R	R	R
B. subtilis I	S	S	R	R	S	R	R	R
B. subtilis II	S	R	R	R	S	R	R	R
B. subtilis III NCIB 3318	S	S	R	R	S	R	R	R
S. aureus I	S	S	R	I	S	R	S	S
S. aureus II	S	S	R	R	S	R	S	S
S.aureus III NCIB 6571	S	S	R	S	S	S	S	S
S. aureus IV	S	S	R	R	S	R	S	S
E. faecalis NCIB 775	S	S	R	R	R	S	R	R
S. pyogenes NCIMB 500117	R	S	R	S	S	R	R	S

Key:

B. cereus: *Bacillus cereus* *B. subtilis*: *Bacillus subtilis*,

S. aureus: *Staphylococcus aureus* *E. faecalis*: *Enterococcus faecalis*

S. pyogenes: *Streptococcus pyogenes* S: Sensitive,

I: Intermediate, R: Resistant

Gen: Gentamicin Ctr: Ceftriaxone,

Ery: Erythromycin Cxc: Cloxacillin,

Ofl: Ofloxacin Aug: Augmentin®

Caz: Ceftazidime Crx: Cefuroxime

Table 4.5: Antimicrobial sensitivity patterns of Gram negative isolates

Clinical isolate	Gen 10µg	Cpr 5µg	Ofl 5µg	Aug 30µg	Nit 30µg	Amp 10µg	Caz 30µg	Crx 30µg
S. typhi I	R	R	R	R	R	R	R	R
S. typhi II	S	R	R	S	R	R	R	R
P. vulgaris	S	S	S	R	S	R	S	R
K. aerogenes I	S	S	S	R	I	R	S	R
K. aerogenes II NCTC 418	S	S	S	R	S	R	S	R
E. coli I	S	S	S	R	S	R	S	S
E. coli II	S	S	S	R	S	R	R	S
E. coli III	S	S	S	R	S	R	R	S
P. aeruginosa I	S	S	S	R	R	R	S	R
P. aeruginosa II	S	S	R	R	R	R	S	S
P. aeruginosa III NCIB 8295	S	S	S	R	R	R	S	S

Key:

S.typhi: *Samonella typhi*

P.vulgaris: Proteus vulgaris

K. aerogenes: *Klebsiella aerogenes*

E.coli: *Escherichia coli*

P. aeruginosa: Pseudomonas aeruginosa

S: Sensitive

I: Intermediate

R: Resistant

Gen: Gentamicin

Cpr: Ciprofloxacin

Ofl: Ofloxacin

Aug: Augmentin®

Nit: Nitrofurantoin

Amp: Ampicillin

Caz: Ceftazidime

Crx: Cefuroxime

4.3. Antimicrobial sensitivity patterns of microbial isolates and antimicrobial assay of extracts

The antimicrobial sensitivity pattern showed that 8 out of 10 (80%) of Gram-positive bacteria (Table 4.4) and 9 out of 11 (81%) of Gram-negative bacteria (Table 4.5) were found to be resistant to 3 or more classes of the antibiotics and are therefore multidrug resistant (MDR) strains. All the Gram-positive organisms tested were resistant to erythromycin while all the Gram-negative organisms were resistant to ampicillin. For the Gram-positive organisms, 8 (80%) were resistant to amoxicillin-clavullanic acid (Augmentin®), 7 (70%) were resistant to cloxacillin and 6 (60%) were resistant to ceftazidime. The highest sensitivity was to gentamicin (80%). For the Gram-negative organisms, 10 (91%) were resistant to Augmentin® and 6 (55%) were resistant to cefuroxime. The highest sensitivity was to ciprofloxacin (82%). No isolate was found to be sensitive to all the antibiotics while *S. typhi* I was resistant to all.

For the preliminary antimicrobial screening, all the crude extracts showed remarkable activities in varying degrees against the microorganisms tested except the dermatophytes (Table 4.6). The zones of growth inhibition exhibited by the crude extracts compared favourably with the standard drugs gentamicin and tioconazole used as controls.

From the result of the antimicrobial screening (Tables 4.7-4.9 and Fig. 4.1-4.6), the hexane leaf crude extract showed the highest activity against the test microorganisms with zones of growth inhibition ranging from 10 mm to 18 mm while the chloroform crude extract showed the least activity with the zone of inhibition ranging from 8 mm to 15 mm. The antimicrobial activity demonstrated by the crude extracts of leaf was very similar to that of the stem bark. The hexane and methanol crude extracts of the leaf and stem bark had appreciable activities on both Gram positive and Gram negative organisms with pronounced activity on *E. coli, K. aerogenes* and *S. aureus*. The antimicrobial activity of the extracts on *B. subtilis* was the lowest.

For the fungi, the highest antimicrobial activity was observed on *P. chrysogenum* though there was moderate activity on *C. albicans, A. niger* and *Rhizopus*

nigricans. There was no antimicrobial activity observed on the dermatophytes used in this study namely *Trichophyton rubrum*, *Trichophyton mentagrophyte* and *Microsporum canis*,

Table 4.6: Preliminary screening of crude extracts (12.5 mg/mL) of *F. thonningii* for antimicrobial activity using agar-cup diffusion technique

Diameter of zones of inhibition (mm)							
Extract and Drug	***B. subtilis***	***S. aureus***	***E. coli***	***P.aerugi nosa***	***C. albicans***	***T. rubrum***	***M. canis***
HLE	14	18	16	16	15	-	-
CLE	12	14	14	12	-	-	-
MLE	13	18	16	15	14	-	-
HSE	13	14	15	16	10	-	-
CSE	13	13	14	12	-	-	-
MSE	13	17	14	11	12	-	-
Gent 10µg/mL	14	18	18	13	NT	NT	NT
Tio 40µg/mL	NT	NT	NT	NT	22	14	-

Key:

NT: Not tested

\- No activity

HLE: Hexane Leaf Extract

CLE: Chloroform Leaf Extract

MLE: Methanol Leaf Extract

HSE: Hexane Stem bark Extract

CSE: Chloroform Stem bark Extract

MSE: Methanol Stem bark Extract

B.subtilis: *Bacillus subtilis*

S.aureus: *Staphylococcus aureus*

E.coli: *Escherichia coli*

P.aeruginosa: *Pseudomonas aeruginosa*

C.albicans: *Candida albicans*

T.rubrum: *Trichophyton rubrum*

M.canis: *Microsporum canis*

Gent: Gentamicin

Tio: Tioconazole

Table 4.7: Antimicrobial activity of crude extracts (6.25mg/mL) of *Ficus thonningii* on Gram negative bacteria

Diameter of zones of inhibition (mm) of crude extracts (mean±SEM)											
Extract and Drug	***Escherichia. coli***	***Escherichia. coli* II**	***Escherichia. coli* III**	***Klebsiella. aerogenes* I**	***Klebsiella. aerogenes* II**	***Pseudomonas. aeruginosa* I**	***Pseudomonas. aeruginosa* II**	***Pseudomonas. aeruginosa* III**	***Salmonella. typhi I***	***Salmonella. typhi II***	***Proteus. vulgaris***
HLE	14±0.58	15±0.58	13±0.58	18±0.67	14±0.00	14±0.00	13±0.58	12±1.00	10±0.00	12±0.58	12±1.15
CLE	13±1.00	13±1.15	12±1.00	13±1.00	12±0.58	10±0.58	8±0.58	10±1.00	-	12±1.00	-
MLE	12±0.58	13±1.00	12±0.00	13±1.00	14±1.73	11±1.00	10±0.00	10±1.15	-	11±1.20	11±1.73
HSE	13±1.00	14±1.15	13±0.58	16±1.15	14±0.00	13±1.00	13±1.15	12±0.58	11±0.00	11±1.00	11±0.58
CSE	12±1.00	14±0.00	11±1.15	11±1.00	12±0.00	11±0.58	8±1.00	11±0.00	8±0.00	11±1.73	12±1.00
MSE	12±1.15	14±0.58	11±0.00	12±0.58	12±1.15	10±0.00	10±0.00	11±0.58	8±0.58	-	-
Amp (10µg)	10±1.00	9±1.15	9±1.00	-	-	8±0.00		I0±1.00	-	-	-
Gent (10µg)	18±0.58	19±0.00	18±0.00	14±0.68	-	13±1.00	14±1.00	13±1.15	-	12±0.00	-
Cipro (1µg)	18±0.00	20±1.73	19±0.00	17±1.15	17±1.15	18±0.00	16±1.00	18±0.00	-	17±0.58	14±0.00

Key:

HLE: Hexane Leaf Extract HSE: Hexane Stem bark Extract

CSE: Chloroform Stem bark Extract Gent: Gentamycin

CLE: Chloroform Leaf Extract MLE: Methanol Leaf Extract

MSE: Methanol Stem bark Extract Cipro: Ciprofloxacin

- : No inhibiton NT: Not tested SEM: Standard error of mean

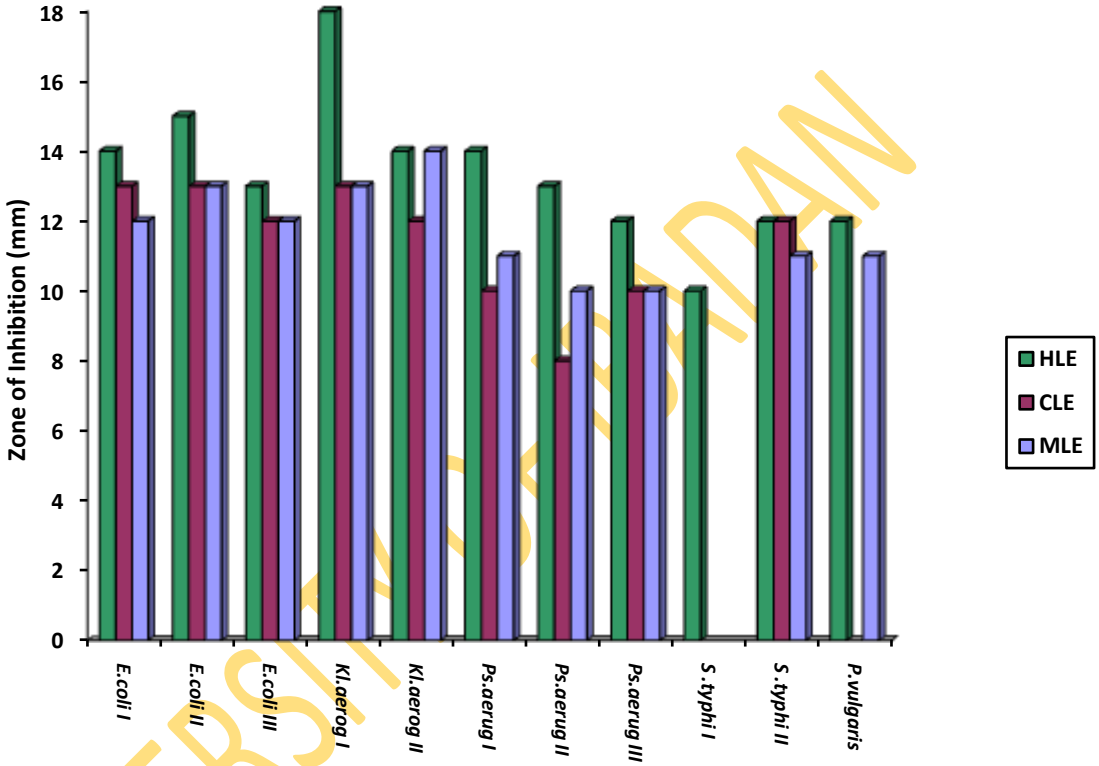


Fig. 4.1: Antimicrobial activity of *F. thonningii* leaf extract (6.25 mg/mL) on Gram negative bacteria

Key:

E. coli: *Escherichia coli*

Kl. aerog: *Klebsiella aerogenes*

Ps. aerug: *Pseudomonas aeruginosa*

S. typhi: *Salmonella typhi*

P. vulgaris: *Proteus vulgaris*

HLE: Hexane leaf extract

CLE: Chloroform leaf extract

MLE: Methanol leaf extract

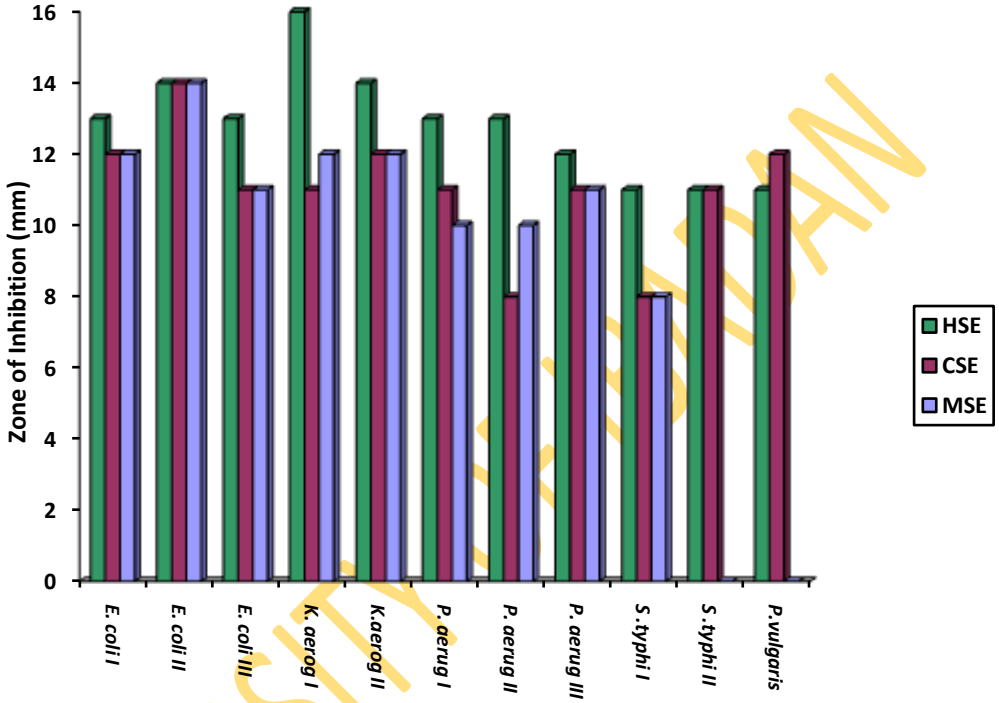


Fig. 4.2: Antimicrobial activity of *F. thonningii* stem bark extract (6.25mg/mL) on Gram negative bacteria

Key:

E. coli: *Escherichia coli*

K. aerog: *Klebsiella aerogenes*

P. aerug: *Pseudomonas aeruginosa*

S. typhi: *Salmonella typhi*

P. vulgaris: *Proteus vulgaris*

HSE: Hexane stem bark extract

CSE: Chloroform stem bark extract

MSE: Methanol stem bark extract

Table 4.8: Antimicrobial activity of crude extracts (6.25mg/mL) of *Ficus thonningii* on Gram positive bacteria

Diameter of zones of inhibition (mm) of crude extracts (mean±SEM)										
Extract and Drug	***Bacillus cereus***	***Bacillus subtilis* I**	***Bacillus subtilis* II**	***Bacillus subtilis* III**	***Staphylococcus aureus* I**	***Staphylococcus aureus* II**	***Staphylococcus aureus* III**	***Staphylococcus aureus* IV**	***Enterococcus faecalis***	***Streptococcus pyogenes***
HLE	14±0.00	12±0.58	14±1.15	12±1.15	14±0.58	16±1.15	12±0.00	14±0.00	12±1.00	12±0.00
CLE	10±1.16	10±0.00	11±1.00	10±0.00	12±0.58	14±1.15	12±1.15	12±1.00	10±0.00	12±0.00
MLE	10±1.53	10±0.00	12±1.00	12±1.15	14±0.58	14±1.00	12±1.67	12±0.00	10±0.58	12±0.58
HSE	12±0.58	10±1.15	14±0.00	10±1.00	11±1.15	14±0.00	14±0.58	12±1.00	12±0.00	10±0.58
CSE	11±1.00	12±1.15	12±0.00	10±2.00	11±0.58	13±1.00	11±0.00	10±0.00	10±1.53	10±0.00
MSE	11±0.00	10±1.00	10±0.00	11±1.00	16±1.15	13±0.58	10±0.58	13±0.58	-	12±2.00
Amp icillin(10µg)	11±0.00	-	9±0.00	10±0.00	11±1.15	-	12±1.00	11±1.00	-	-
Gentamicin(10µg)	14±1.00	14±0.00	16±0.58	14±1.00	18±1.00	-	18±0.58	17±0.00	-	-
Ciprofloxacin (1µg)	16±0.58	NT	17±0.58	18±0.00	20±0.00	20±0.00	19±1.15	20±0.58	18±1.00	17±1.00

Key

HLE: Hexane Leaf Extract CLE: Chloroform Leaf Extract

MLE: Methanol Leaf Extract HSE: Hexane Stem bark Extract

CSE: Chloroform Stem bark Extract MSE: Methanol Stem bark Extract

-: No inhibition NT: Not tested SEM: Standard error of mean

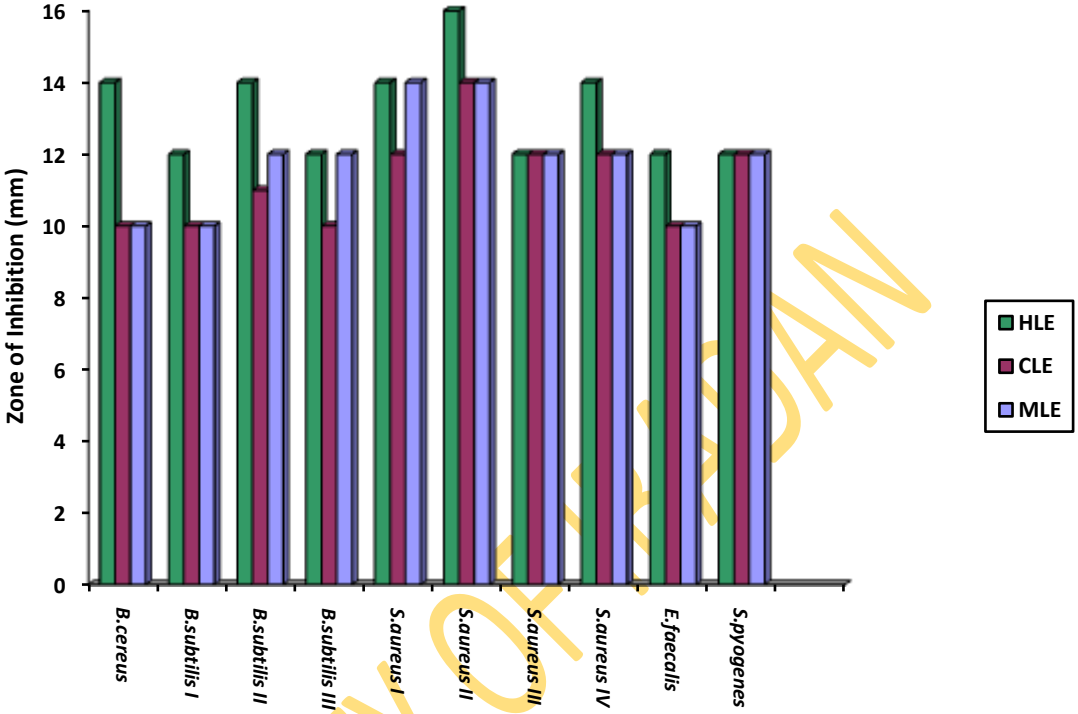


Fig. 4.3: Antimicrobial activity of *F.thonningii* leaf extract (6.25 mg/mL) on Gram positive bacteria

Key:

B. cereus: *Bacillus cereus*

B. subtilis: *Bacillus subtilis*

S. aureus: *Staphylococcus aureus*

E. faecalis: *Enterococcus faecalis*

S. pyogenes: *Streptococcus pyogenes*

HLE: Hexane leaf extract

CLE: Chloroform leaf extract

MLE: Methanol leaf extract

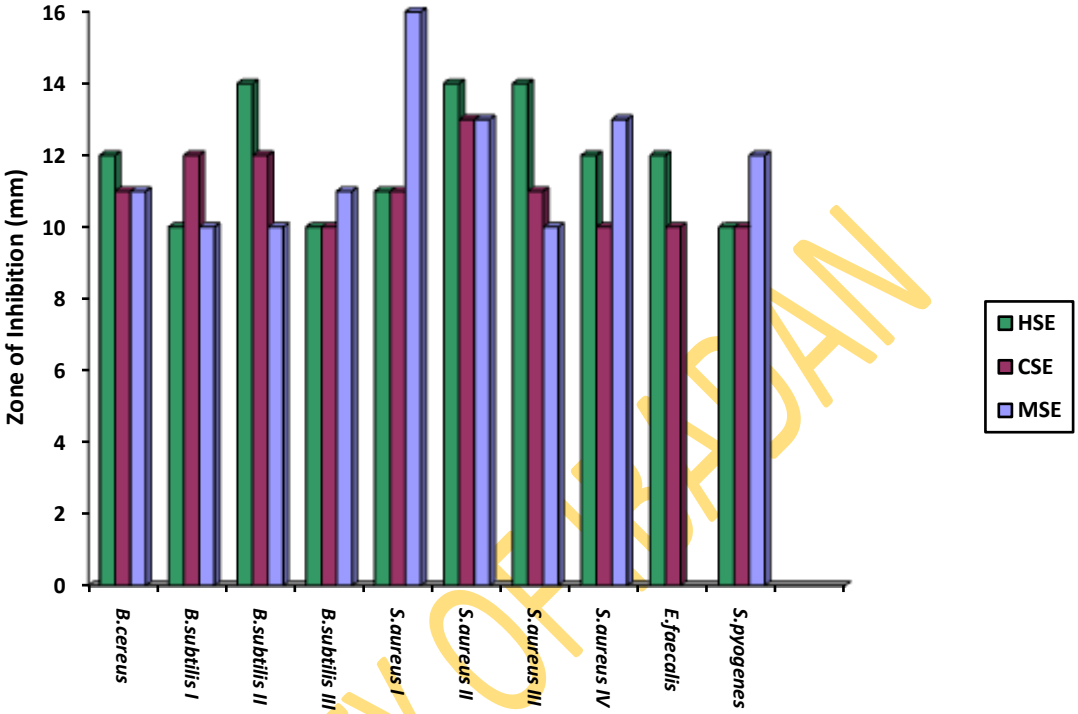


Fig. 4.4: Antimicrobial activity of *F. thonningii* stem bark extract (6.25 mg/mL) on Gram positive bacteria

Key:

B. cereus: *Bacillus cereus*

B. subtilis: *Bacillus subtilis*

S. aureus: *Staphylococcus aureus*

E. faecalis: *Enterococcus faecalis*

S. pyogenes: *Streptococcus pyogenes*

HSE: Hexane stem bark extract

CSE: Chloroform stem bark extract

MSE: Methanol stem bark extract

Table 4.9: Antimicrobial activity of crude extracts (6.25mg/mL) of *Ficus thonningii* on Fungi

Diameter of zones of inhibition (mm) of crude extracts (mean±SEM)												
Extract and Drug	***Aspergillus niger* I**	***Aspergillus niger* II**	***Aspergillus niger* III**	***Candida albicans* I**	***Candida albicans* II**	***Candida albicans* III**	***Penicillium chrysogenum* I**	***Penicillium chrysogenum* II**	***Rhizopus nigricans***	***Trichophyton rubrum***	***Microsporium canis***	***Trichophyton mentagrophyt***
HLE	10±0.58	12±0.00	12±1.15	13±2.33	12±0.00	10±0.00	18±1.15	12±0.00	10±1.76	-	-	-
CLE	-	9±00	-	-	8±1.00	10±0.00	15±1.00	12±0.58	9±0.58	-	-	-
MLE	10±0.00	11±0.58	10±0.00	10±1.15	-	10±1.53	12±1.00	10±0.00	11±1.15	-	-	-
HSE	10±1.15	13±1.15	10±1.00	-	10±0.00	10±0.00	14±0.58	12±0.58	10±2.00	-	-	-
CSE	-	9±0.00	-	-	10±0.00	-	14±1.00	-	-	-	-	-
MSE	10±0.00	11±0.58	-	10±0.00	10±2.65	10±0.00	14±0.00	12±2.65	10±0.00	-	-	-
Amp (10µg)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Gent (10µg)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Cipro (1µg)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Tioc (40µg)	24±1.00	25±0.00	25±0.00	22±1.15	22±1.00	-	24±0.00	24±0.00	22±0.00	14±0.00	-	18±1.00

Key:

HLE : Hexane Leaf Extract CLE : Chloroform Leaf Extract

MLE : Methanol Leaf Extract HSE : Hexane Stem bark Extract

MSE : Methanol Stem bark Extract CSE : Chloroform Stem bark Extract

- : No inhibition NT : Not tested

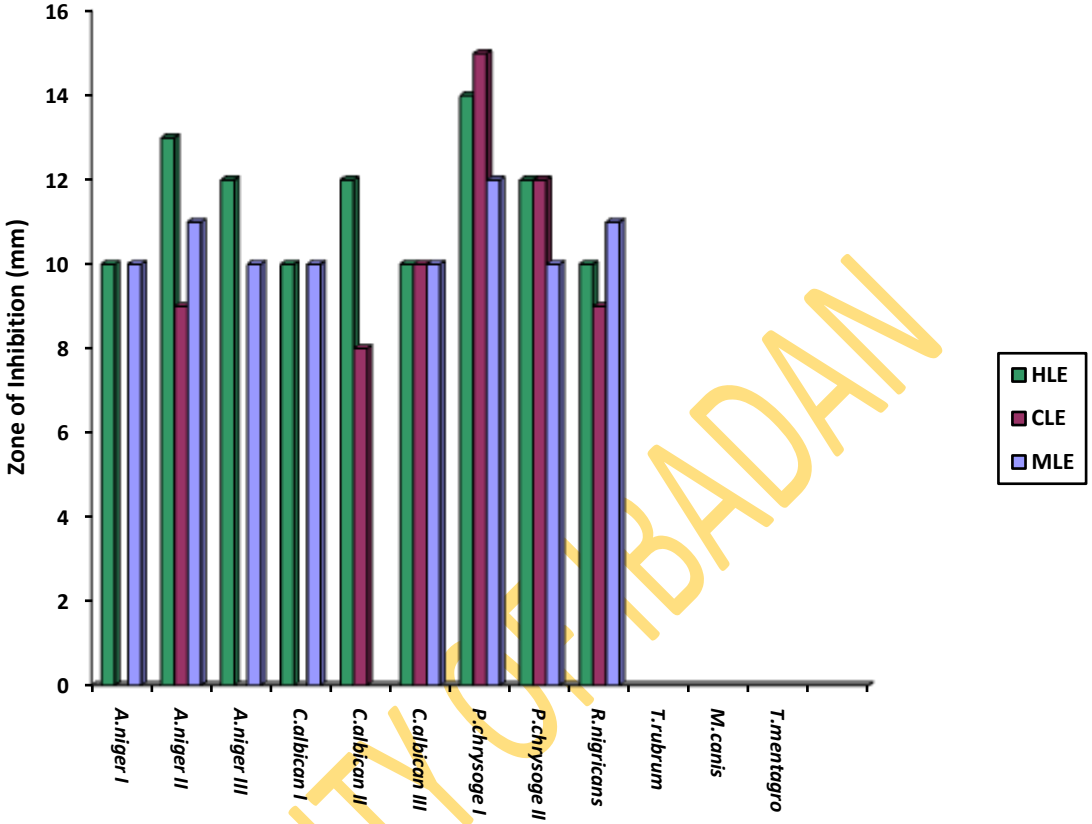


Fig 4.5: Antifungal activity of *F. thonningii* leaf extract (6.25 mg/mL) on fungal isolates

Key:

C. albicans: *Candida albicans*

A. niger: *Aspergillus niger*

P. chrysoge: *Penicillium chrysogenum*

R. nigricans: *Rhizopus nigricans*

T. rubrum: *Trichophyton rubrum*

M. canis: *Microsporium canis*

T. mentagro: *Trichophyton mentagrophyte*

HLE: Hexane leaf extract

CLE: Chloroform leaf extract

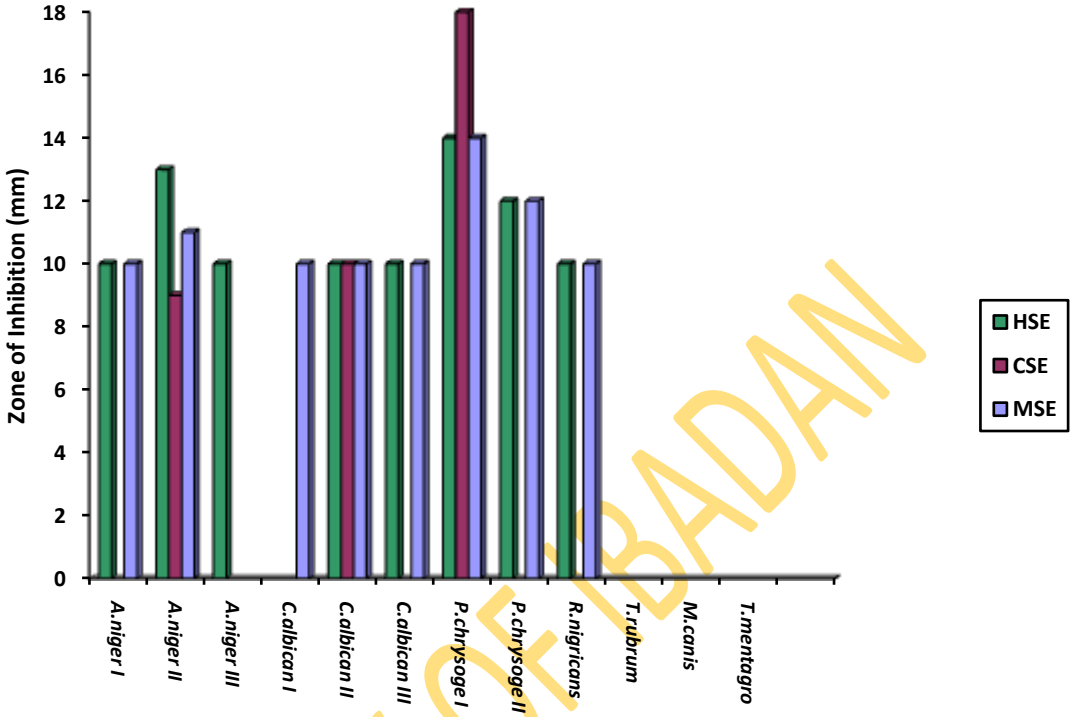


Fig 4.6: Antifungal activity of *F. thonningii* stem bark extract (6.25 mg/mL) on fungal isolates

Key:

C. albicans: *Candida albicans*

A. niger: *Aspergillus niger*

P. chrysoge: *Penicillium chrysogenum*

R. nigricans: *Rhizopus nigricans*

T. rubrum: *Trichophyton rubrum*

M. canis: *Microsporium canis*

T. mentagro: *Trichophyton mentagrophyte*

HLE: Hexane stem bark extract

CLE: Chloroform stem bark extract

MLE: Methanol stem bark extract

4.4 Antimicrobial activity of bioactive fractions of *F thonningii*

From the column chromatography of crude extracts of *F. thonningii*, the identical fractions pooled together on the basis of analytic TLC were monitored for antimicrobial activity using *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa*, and *C. albicans*. From the hexane fractions at 0.625 mg/mL, HLF 04, 07, 11 and HSF 02, 03 showed antimicrobial activity while from methanol fractions (0.625 mg/mL), MLF 01, 06, 07, 11, and MSF 01 and 06 showed antimicrobial activity. The antimicrobial activity of fractions and standard antibiotics are seen in Table 4.10.

4.5 Antimicrobial activity of isolated compound (EC.HL02)

The compound isolated from hexane leaf fraction (EC.HL02), which was tested for antimicrobial activity on *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa* and *C. albicans* showed appreciable antimicrobial activity against all the microorganisms with most pronounced activity on *S. aureus* with a zone of inhibition diameter of 24.0 mm and least activity on *B. cereus* (15.0 mm) as shown in Table 4.11.

Table 4.10: Antimicrobial activity of bioactive fractions from leaf and stem bark of *F. thonningii*

Diameter of zones of inhibition (mm) of crude extracts (mean±SEM)						
Fractions and Drug	**Conc. mg/ml**	***Staphylococcus aureus***	***Bacillus cereus***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Candida albicans***
HLF 04	0.625	18	13	17	14	10
HLF 07	0.625	21	11	19	15	12
HLF 11	0.625	20	11	15	12	11
MLF01	0.625	17	11	15	10	11
MLF06	0.625	18	10	17	14	11
MLF07	0.625	17	11	14	13	10
MLF11	0.625	19	12	15	13	11
HSF 02	0.625	20	12	15	11	10
HSF 03	0.625	21	10	17	13	10
MSF 01	0.625	15	12	14	14	10
MSF 06	0.625	15	10	13	11	10
Gent 10µg		18	14	19	14	NT
Tioco 40µg		NT	NT	NT	NT	24

Key:

HLF: Hexane leaf fraction MLF: Methanol leaf fraction

HSF: Hexane stem bark fraction MSF: Methanol stem bark fraction

Gent: Gentamycin Tioco: Tioconazole

NT: Not tested SEM: Standard error of mean

Table 4.11 Antimicrobial activity of isolated compound EC.HL02 (0.625mg/mL) from hexane leaf fraction

Diameter of zones of inhibition (mm)					
Compound and Drug	***Staphylococcus aureus***	***Bacillus cereus***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Candida albicans***
EC.HL02	24	15	21	17	17
Gent 10µg	18	14	18	14	NT
Tioc 40µg	NT	NT	NT	NT	24

Key:

EC.HL02: Code for isolated compound Gent: Gentamycin

Tioc: Tioconazole NT: Not tested

Table 4.12: The Minimum Inhibitory Concentration (MIC) in µg/mL of hexane crude extract, fractions and EC.HL02 against bacterial and fungal isolates

Extract and Drug	Minimum Inhibitory Concentration (µg/mL)											
	S. aureus	***S. pyogenes***	***B. cereus***	***B. subtilis***	***E. feacalis***	***S. typhi***	***K. aerogenes***	***E. coli***	***P. aeruginosa***	***P. vulgaris***	***C. albicans***	***A. niger***
HL Crude	156	312	625	625	312	>625	312	156	312	>625	312	78
HLF 04	78	156	312	312	156	312	156	78	156	156	156	39
07	39	78	156	312	156	312	78	39	156	312	78	20
11	39	78	312	312	78	625	156	156	156	625	156	39
EC.HL02	20	39	78	156	39	156	39	39	78	78	78	10
Gentamicin	5	5	5	5	20	10	30	5	10	30	NT	NT
Tioconazole	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	20	10

Key:

HL: Hexane leaf

HLF: Hexane leaf fraction

EC.HL02: Code for isolated compound

NT: Not Tested

S.aureus: *Staphylococcus aureus*

S.pyogenes: *Streptococcus pyogenes*

B.cereus: *Bacillus cereus*

B.subtilis: *Bacillus subtilis*

E.feacalis: *Enterococcus faecalis*

S.typhi: *Salmonella typhi*

K.aerogenes: *Klebsiella aerogenes*

E.coli: *Escherichia coli*

P.aeruginosa: *Pseudomonas aeruginosa*

P.vulgaris: *Proteus vulgaris*

C.albicans: *Candida albicans*

A.niger: *Aspergillus niger*

Table 4.13: Minimum Inhibitory Concentration (MIC) µg/mL of methanol crude extract and fractions against bacterial and fungal isolates

Extract and Drug	Minimum Inhibitory Concentration (µg/mL)											
	S.aureus	***S.pyogenes***	***B.cereus***	***B.subtilis***	***E.feacalis***	***S.typhi***	***K.aerogenes***	***E.coli***	***P.aeruginosa***	***P.vulgaris***	***C.albicans***	***A.niger***
MLF 01	156	312	625	312	78	312	156	156	312	625	156	78
MLF 06	78	156	625	625	78	156	156	78	156	312	156	39
MLF 07	156	156	312	625	156	312	156	156	156	625	312	78
MLF11	78	156	312	312	156	156	312	156	156	625	156	39
Gentamicin	5	5	5	5	20	10	30	5	10	30	NT	NT
Tioconazole	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	20	10

Key:

ML: Methanol leaf

MLF: Methanol leaf fraction

S.aureus: *Staphylococcus aureus,*

S.pyogenes: *Streptococcus pyogenes,*

B.cereus: *Bacillus cereus,*

B.subtilis: *Bacillus subtilis,*

E.feacalis: *Enterococcus faecalis,*

S.typhi: *Salmonella typhi,*

K.aerogenes: *Klebsiella aerogenes,*

E.coli: *Escherichia coli,*

P.aeruginosa: *Pseudomonas aeruginosa*

P.vulgaris: *Proteus vulgaris*

C.albicans: *Candida albicans,*

A.niger: *Aspergillus niger*

NT: Not Tested

Table 4.14: Minimum Bactericidal Concentration (MBC) µg/mL of hexane crude extract, fractions and EC.HL02 against bacterial and fungal isolates

Extract and Drug	Minimum Bactericidal Concentration (µg/mL)											
	S. aureus	***S. pyogenes***	***B. cereus***	***B. subtilis***	***E. feacalis***	***S. typhi***	***K. aerogenes***	***E. coli***	***P. aeruginosa***	***P. vulgaris***	***C. albicans***	***A. niger***
HL Crude	312	312	>625	>625	625	NT	625	312	625	NT	625	156
HLF 04	78	156	625	625	312	625	312	78	312	312	312	78
HLF 07	39	78	312	625	312	625	78	78	312	625	156	39
HLF 11	78	156	312	312	156	>625	312	156	312	>625	312	78
EC.HL02	39	78	156	156	78	312	78	39	156	156	78	20
Gentamicin	10	10	10	10	20	20	40	10	20	40	NT	NT
Tioconazole	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	40	20

Key:

HL: Hexane leaf

HLF: Hexane leaf fraction

EC.HL02: Code for isolated compound

NT: Not tested

S.aureus: *Staphylococcus aureus*

S.pyogenes: *Streptococcus pyogenes*

B.cereus: *Bacillus cereus*

B.subtilis: *Bacillus subtilis*

E.feacalis: *Enterococcus faecalis*

S.typhi: *Salmonella typhi*

K.aerogenes: *Klebsiella aerogenes*

E.coli: *Escherichia coli,*

P.aeruginosa: *Pseudomonas aeruginosa*

P.vulgaris: *Proteus vulgaris*

C.albicans: *Candida albicans*

A.niger: *Aspergillus niger*

Table 4.15: Minimum Bactericidal Concentration (MBC) µg/mL of methanol crude extract and fractions against bacterial and fungal isolates

Extract and Drug	Minimum Bactericidal Concentration (µg/mL)											
	S. aureus	***S. pyogenes***	***B. cereus***	***B. subtilis***	***E. faecalis***	***S. typhi***	***K. aerogenes***	***E.coli***	***P. aeruginosa***	***P. vulgaris***	***C. albicans***	***A. niger***
ML Crude	625	625	>625	NT	625	>625	312	625	625	NT	625	312
MLF 01	156	312	>625	625	156	625	312	312	312	>625	312	156
MLF 06	156	625	156	>625	78	312	156	156	156	625	312	78
MLF 07	156	312	625	>625	312	312	312	312	312	>625	312	156
MLF 11	156	156	625	625	312	312	625	312	625	>625	156	78
Gentamicin	10	10	10	10	40	20	40	10	20	40	NT	NT
Tioconazole	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	40	20

KEY:

ML: Methanol leaf MLF: Methanol leaf fraction

S.aureus: *Staphylococcus aureus* *S.pyogenes*: *Streptococcus pyogenes,*

B.cereus: *Bacillus cereus* *B.subtilis*: *Bacillus subtilis*

E.feacalis: *Enterococcus faecalis* *S.typhi*: *Salmonella typhi*

K.aerogenes: *Klebsiella aerogenes* *E.coli*: *Escherichia coli*

P.aeruginosa: *Pseudomonas aeruginosa* *P.vulgaris*: *Proteus vulgaris*

C.albicans: *Candida albicans* *A.niger*: *Aspergillus niger*

NT: Not Tested

4.6. Bactericidal Kinetics

The bactericidal action of the extract can be seen from the graphs of the log viable count against time (Figs. 4.7 and 4.8). An initial delay of onset of visible action was observed between 0 and 30 mins but thereafter, the killing kinetics progressed over a period of 4 hrs. The bactericidal action of the extract was concentration dependent with the highest rate and extent of killing observed with 10 mg/mL of extract. The extract at 10 mg mL was able to reduce the bacterial population of *S. aureus* from 2.0×10^7 cfu/mL to 2.0×10^3 cfu/mL in 90 mins (> 50% kill). The extract at 5 mg/mL and 2.5 mg/mL reduced the bacterial population to 8.0×10^4 cfu/mL and 6.0×10^5 cfu/mL respectively. The rate and extent of kill observed for *E. coli* was slower and lower than that of *S. aureus.* A total kill was observed for *S. aureus* with 10 mg/mL of methanol extract in 4 hrs.

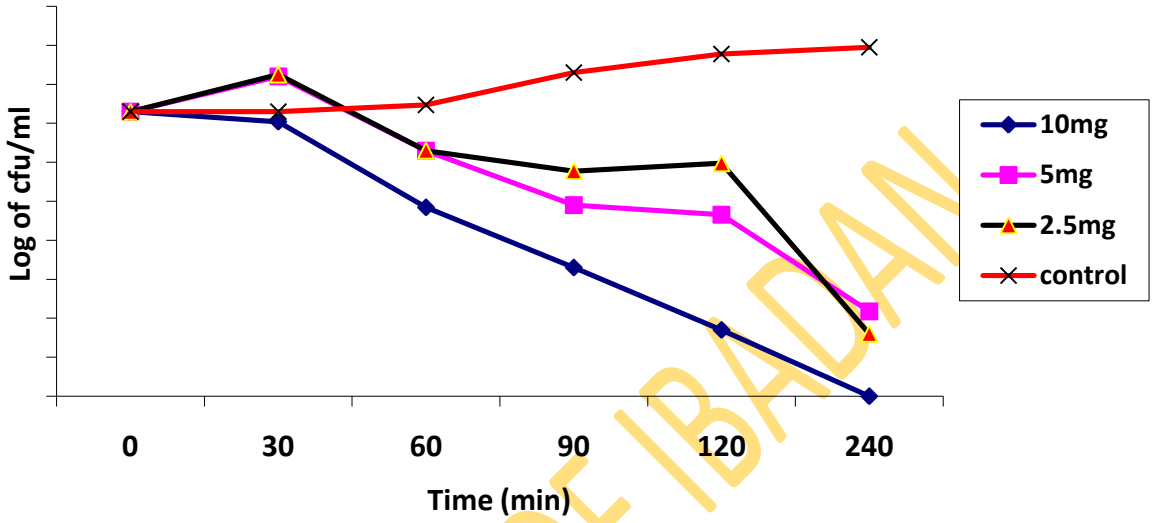


Fig. 4.7: Bactericidal kinetics of methanol leaf extract of *Ficus thonningii* on *Staphylococcus aureus*

Key:

cfu: Colony forming unit

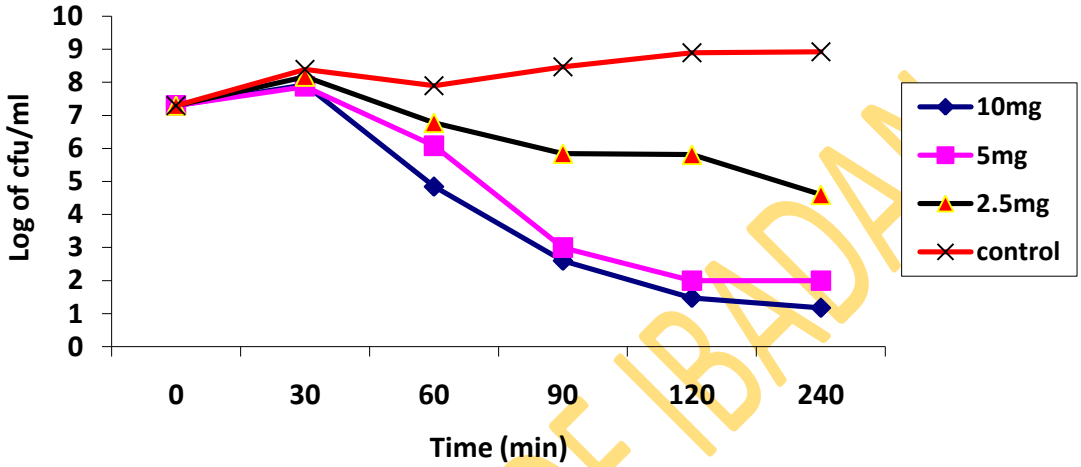


Fig. 4.8: Bactericidal kinetics of methanol leaf extract of *Ficus thonningii* on *Escherichia coli*

Key:

cfu: Colony forming unit

4.7. Acute toxicity test

There was no death of animals in the control and treated groups. In the visual observation, there was no change in the skin, fur and eyes of the animals, also, no change was observed in the motility, respiratory and behavioural patterns of the animals. There were no tremors, convulsions, salivation, diarrhoea and lethargy in any of the test and control animals.

4.8. Haematological studies

The results of the extracts on the haematological parameters such as packed cell volume (PCV), mean corpuscular volume (MCV), red blood cell count, differential white blood cell count and mean corpuscular haemoglobin (MCH) are shown in Tables 4.18 - 4.23. Significant changes were observed only in the animals given 100 mg/kg and 200 mg/kg extract on days 10 and 21 ($p < 0.05$) for the mean corpuscular haemoglobin, MCH and red blood cell count for 100 mg/kg ($p < 0.05$).

4.9. Effect of extract on body weight of mice

The effects of the extracts on body weight are shown in Table 4.24. A significant increase ($p < 0.05$) in body weight was observed with an increase in the amount of extract administered to the test animals. The average body weight of animals on 100 mg/kg increased from 134 ± g to 140 ± g. Animals on 200 mg/kg had an increased body weight from 138 ± g to 146 ± g while animals on 400 mg/kg had an increased body weight from 134 ± g to 148 ±g. The average body weight increase for the control animals was from 135 ± g to 137 ± g.

Table 4.16: Effect of *Ficus thonningii* methanol leaf extract on Packed Cell Volume (PCV) of mice

Extract per kg body weight	Packed Cell Volume (mean±SEM)			P VALUE
	DAY 0	DAY 10	AT DAY 21	
CONTROL	33	34 ± 1.414	43 ± 2.160	> 0.05
100mg	35	36 ± 1.414	36 ± 1.414	
200mg	37	38 ± 1.633	38 ± 1.414	
400mg	35	38 ± 1.225	42 ± 1.414	

Key:

SEM: Standard error of mean

Table 4.17: Effect of *Ficus thonningii* methanol leaf extract on Red Blood Cell (RBC) count of mice

Extract per kg body weight	Red Blood Cell count (mean±SEM)			P VALUE
	Day 0	DAY 10	AT DAY 21	
CONTROL	11.6	11.6 ± 0.7789	11.6 ± 0.7789	> 0.05
100mg	10.46	10.46 ± 0.6020	10.46 ± 0.6225	< 0.05
200mg	12.44	12.44 ± 0.2062	12.50 ± 0.1826	> 0.05
400mg	12.25	12.25 ± 0.3873	12.25 ± 0.3317	> 0.05

Key:

SEM: Standard error of mean

Table 4.18: Effect of *Ficus thonningii* methanol leaf extract on White Blood Cell (WBC) count of mice

Extract per kg body weight	White Blood Cell (mean±SEM)			P value
	Day 0	DAY 10	DAY 21	
CONTROL	26, 400	26, 400 ± 1718.5	26, 500 ± 2254.6	> 0.05
100mg	28, 200	28, 200 ± 1657.3	28, 700 ± 568.82	> 0.05
200mg	26, 800	28, 700 ± 1137.2	30, 600 ± 1089.3	> 0.05
400mg	26, 800	29, 200 ± 2717.8	31, 800 ± 1143.8	> 0.05

Key:

SEM: Standard error of mean

Table 4.19: Effect of *Ficus thonningii* methanol leaf extract on Mean Corpuscular Volume (MCV) of mice

Extract per kg body weight	Mean Corpuscular Volume (mean±SEM)			P value
	Day 0	DAY 10	AT DAY 21	
CONTROL	28	28 ± 1.826	28 ± 1.826	> 0.05
100mg	30	30 ± 2.082	30 ± 2.082	> 0.05
200mg	28	28 ± 3.651	29 ± 4.084	> 0.05
400mg	28	29 ± 3.961	29 ± 2.646	> 0.05

Key:

SEM: Standard error of mean

Table 4.20: Effect of *Ficus thonningii* methanol leaf extract on Mean Corpuscular Haemoglobin (MCH) of mice

Extract per kg body weight	Mean Corpuscular Haemoglobin (mean±SEM)			P value
	Day 0	DAY 10	DAY 21	
CONTROL	13	13 ± 1.826	13 ± 1.826	> 0.05
100mg	10	10 ± 0.8165	10 ± 0.8165	< 0.05
200mg	10	10 ± 0.8539	10 ± 1.708	< 0.05
400mg	10	11 ± 1.155	11 ± 0.8165	> 0.05

Key:

SEM: Standard error of mean

Table 4.21: Lymphocyte and Neutrophil counts ratio of mice

Extract per kg body weight	Lymphocyte and Neutrophil counts					
	DAY 0		DAY 10		DAY 21	
	LYM	NEU	LYM	NEU	LYM	NEU
CONTROL	20, 064	6, 336	16, 368	10, 032	18, 550	7, 950
100mg	21, 996	6, 204	19, 740	8, 460	17, 220	11, 480
200mg	16, 616	10, 184	22, 386	6, 314	23, 868	6, 732
400mg	20, 100	6, 700	20, 440	8, 760	21, 624	10, 176

Table 4.22: The effect of *Ficus thonningii* methanol leaf extract on body weight changes of mice

Extract per kg body weight	Body weight (mean±SEM)			P value
	Day 0	DAY 10	DAY 21	
Control	135.0g	136.0g ± 6.976	137.0g ± 6.583	>0.05
100mg/kg	134.0g	137.0g ± 4.967	140.0g ± 4.243	>0.05
200mg/kg	138.0g	142.0g ± 2.944	146.0g ± 2.449	<0.05
400mg/kg	134.0g	140.0g ± 3.742	148.0g ± 4.690	<0.05

Key:

SEM: Standard error of mean

4.10. Histopathology of experimental rats

There were no significant tissue pathological changes in the uterus, lungs, liver, spleen, kidney and ovary of the mice used for the acute toxicity test.

The uterine cells showed that the glandular and endometrial lining epithelia cells are cuboidal (Plate 4.1). No lesions were observed in the liver, kidney and spleen sections of the animals treated with the extract and propylene glycol (Plates 4.3- 4.5 and Plates 4.8 - 4.9). The ovary section of mice treated with propylene glycol showed numerous follicles at various stages of maturation (Plate 4.6).

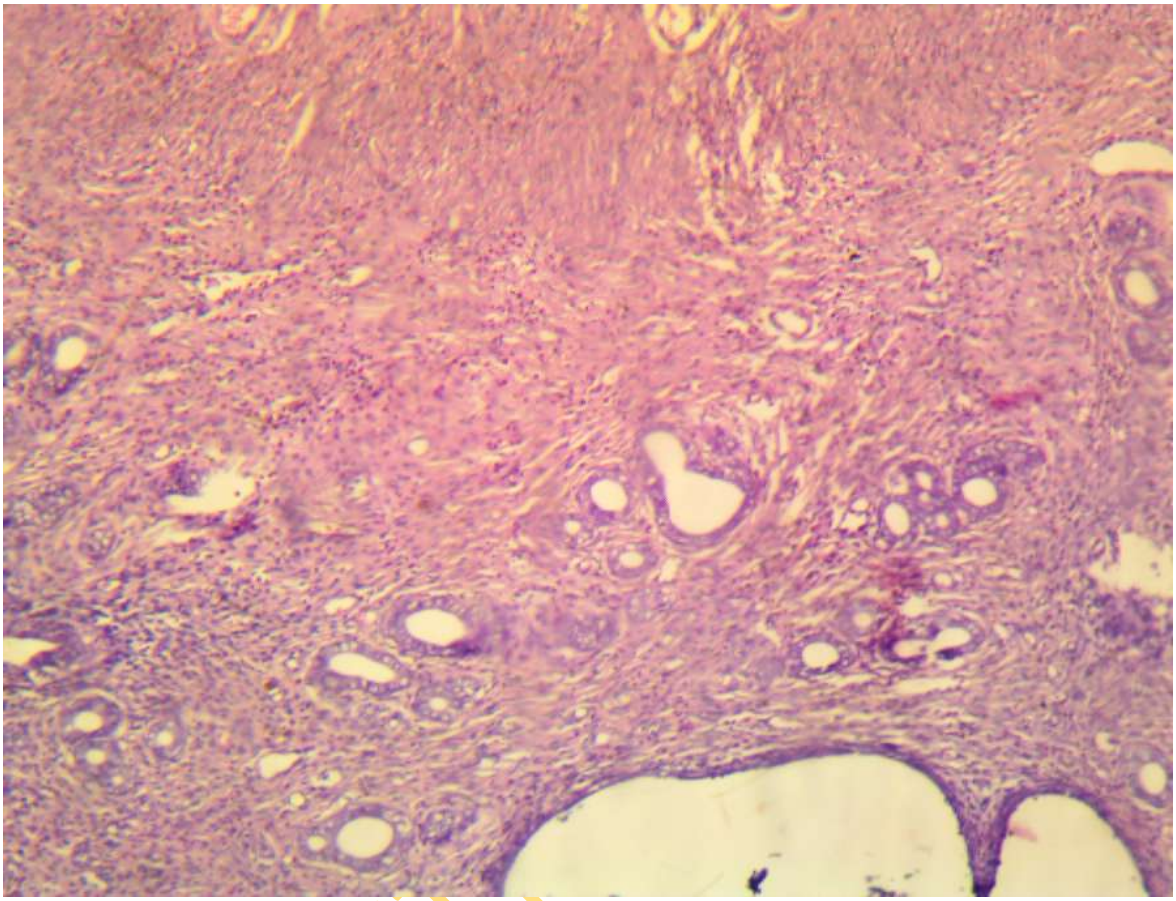


Plate 4.1: Histopathology showing section of uterine body of rat treated with 400 mg/kg methanol leaf extract of *Ficus thonningii*

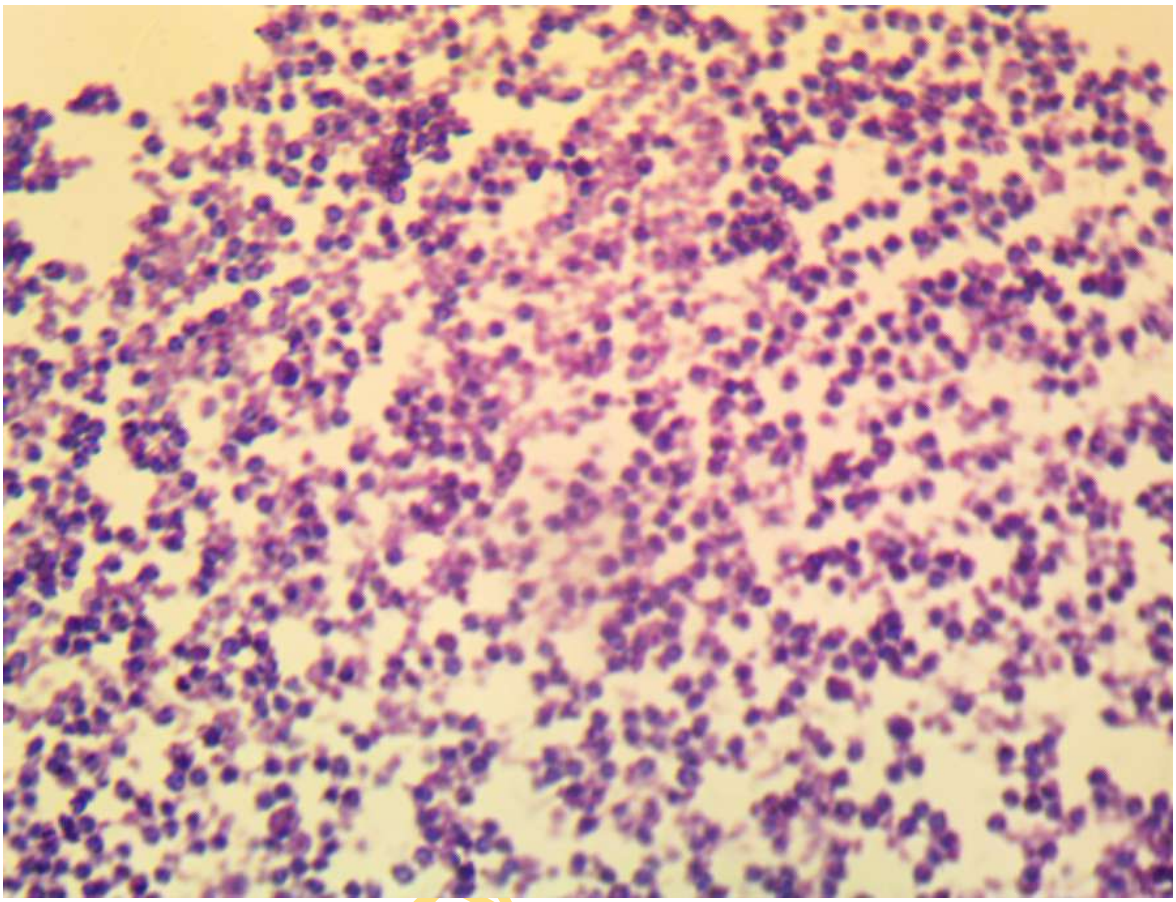


Plate 4.2: Histopathology of lung section of rat showing the cell types in the bronchioles

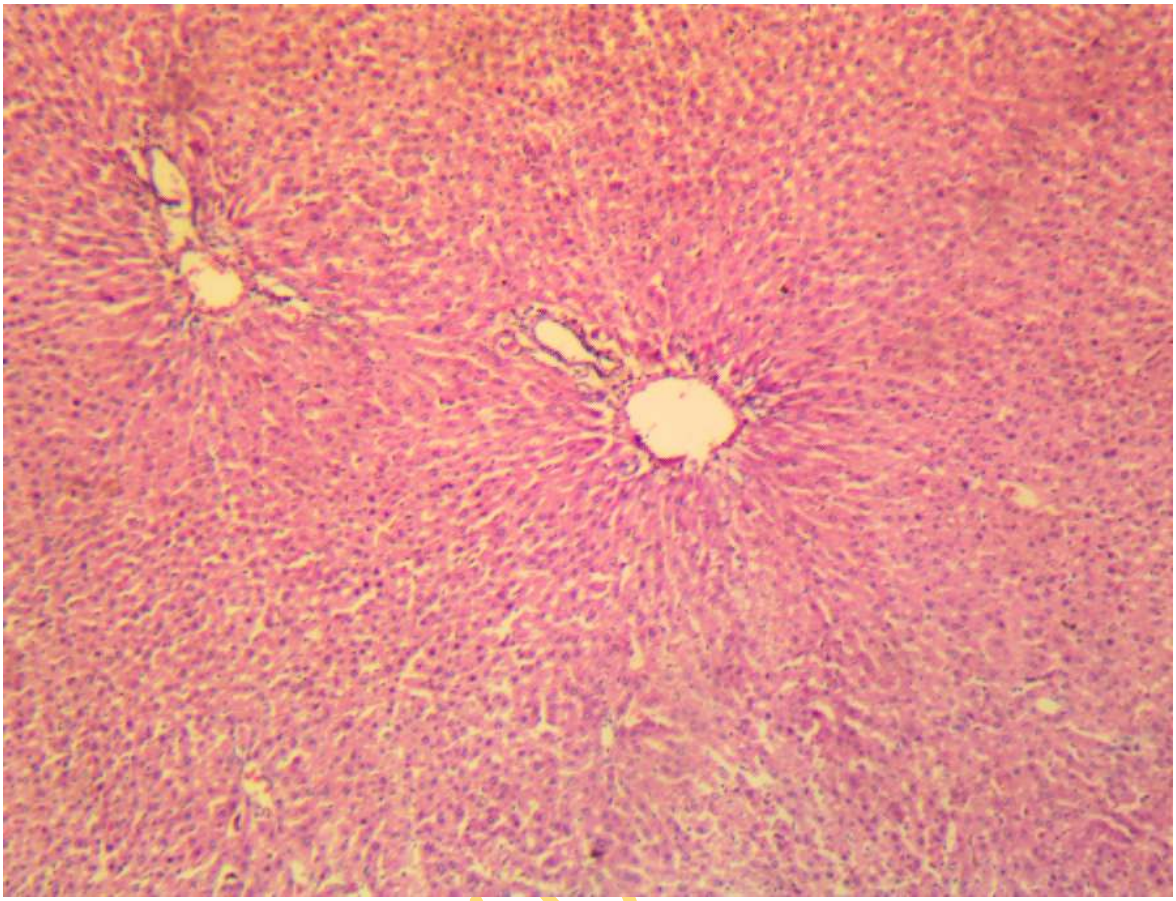


Plate 4.3: Histopathology of liver section of rat treated with propylene glycol (Control)

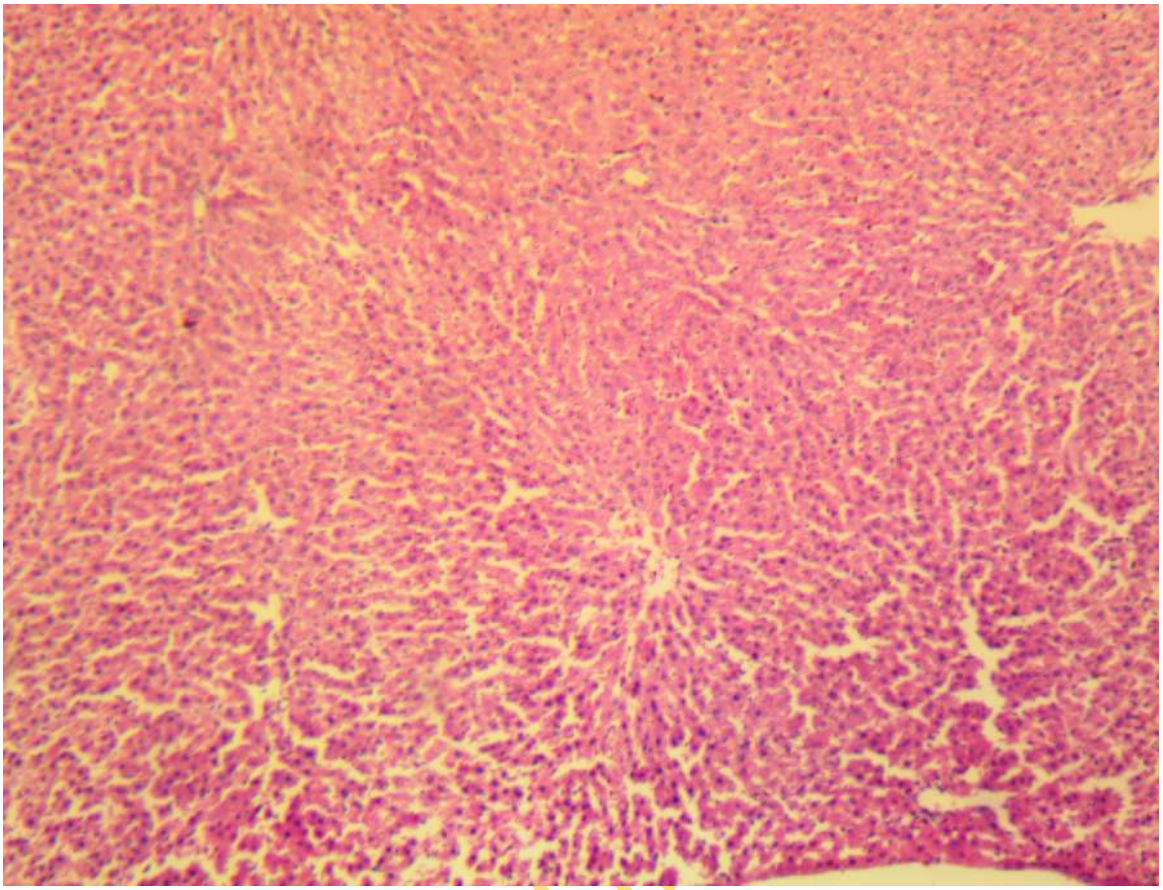


Plate 4.4: Histopathology of liver section of rat treated with 400 mg/kg methanol leaf extract

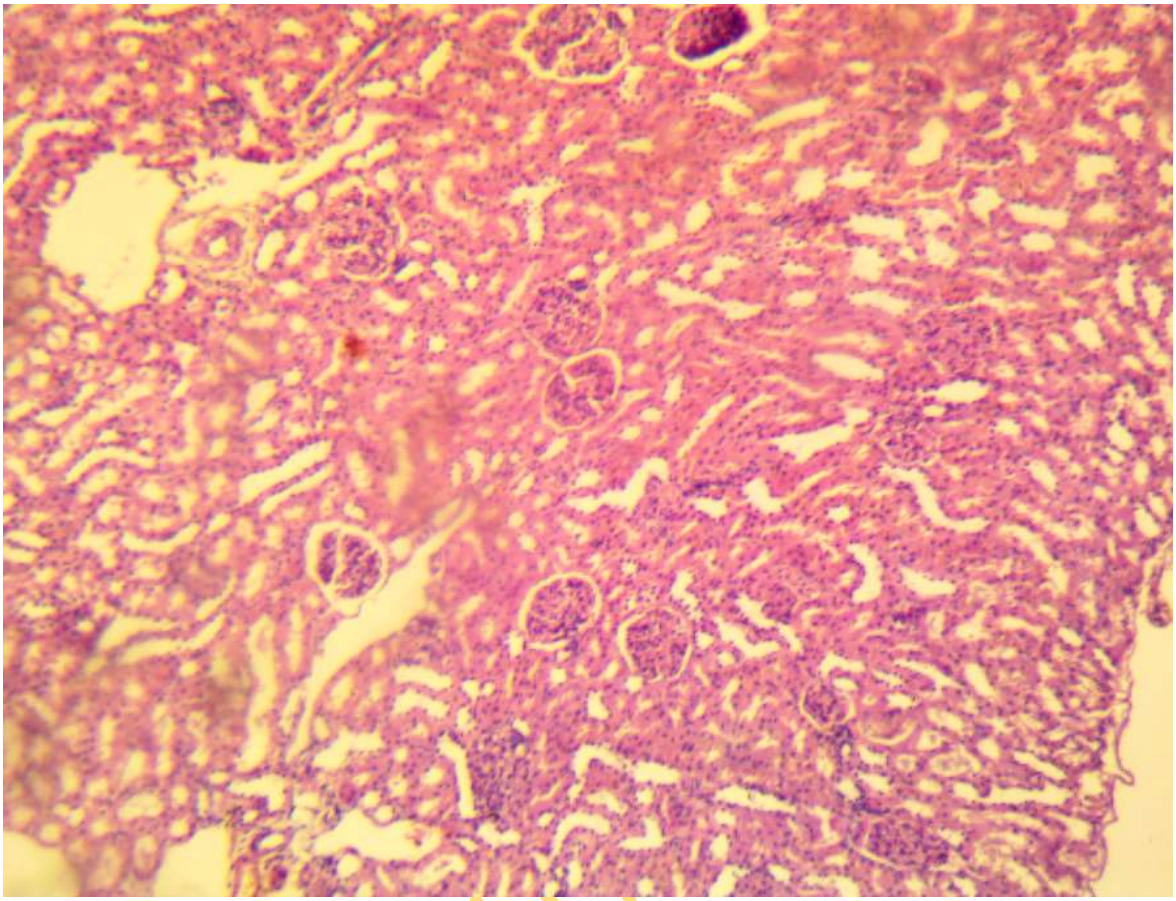


Plate 4.5: Histopathology of kidney section of rat treated with 400 mg/kg methanol leaf extract

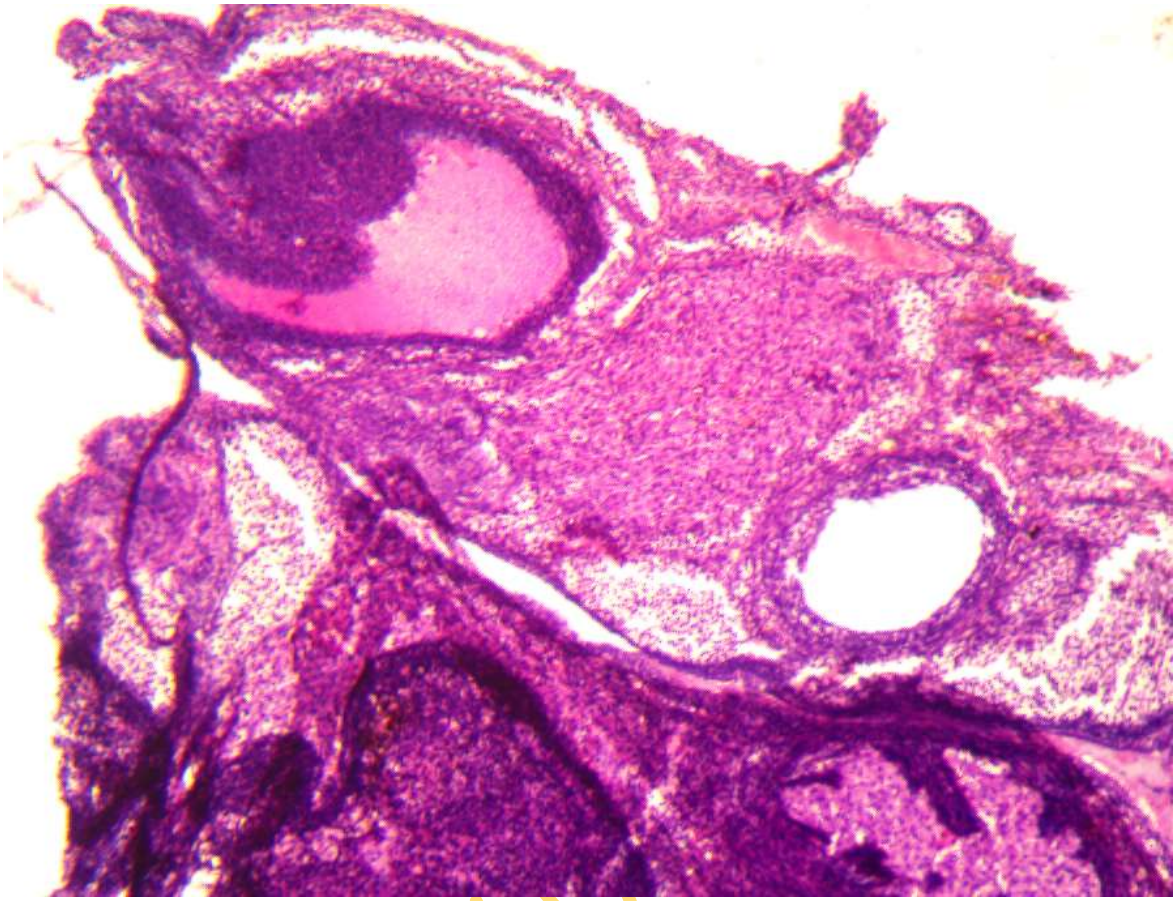


Plate 4.6: Histopathology of ovary section of rat treated with propylene glycol showing numerous follicles at various stages of maturation (Control)

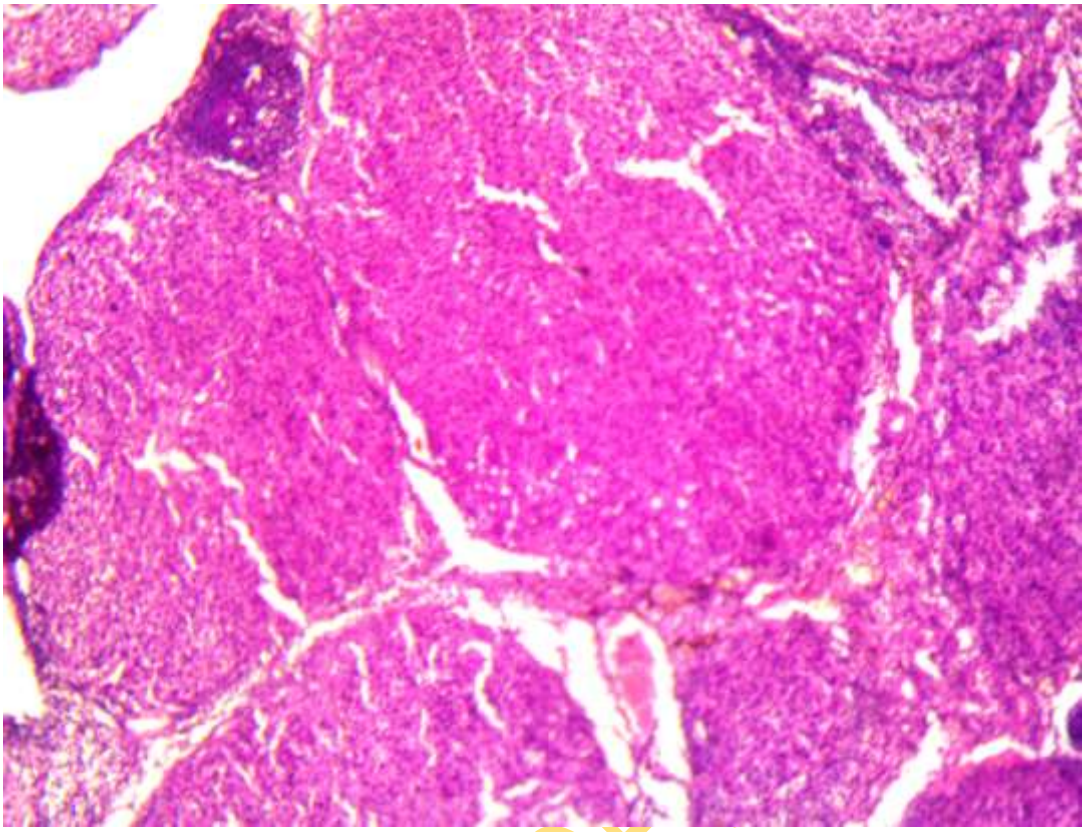


Plate 4.7: Histopathology of ovary section of rat treated with 400 mg/kg methanol leaf extract

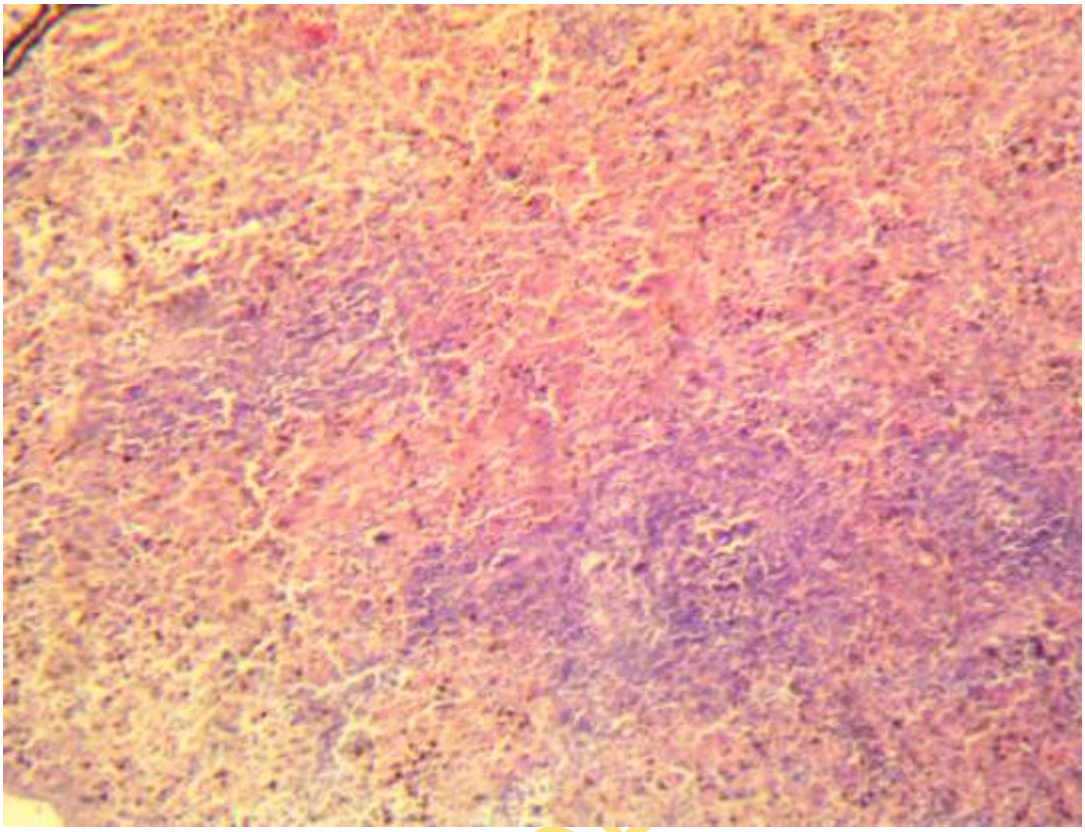


Plate 4.8: Histopathology of spleen of rat treated with propylene glycol (Control)

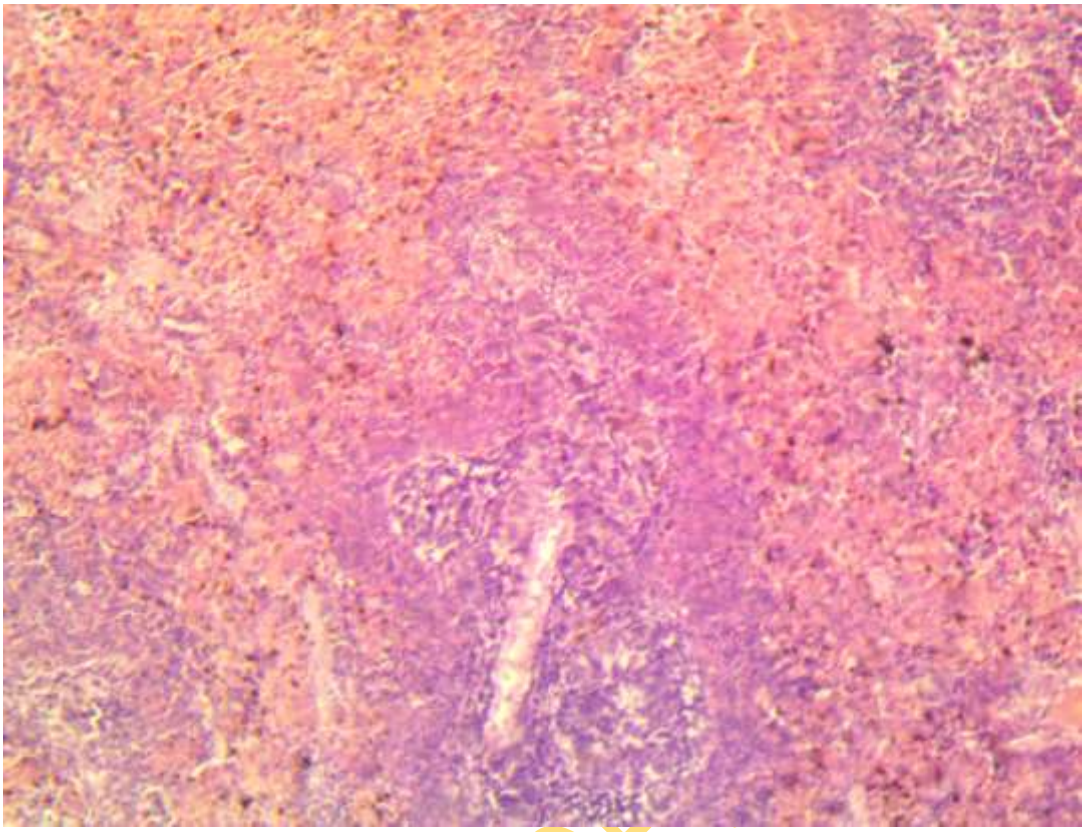


Plate 4.9: Histopathology of spleen of rat treated with 400 mg/kg methanol leaf extract

4.11 Anti-inflammatory evaluation of crude leaf extracts of *F. thonningii* on female rats

The result for the anti-inflammatory evaluation can be seen in Table 4.16 and Fig. 4.9 which shows the anti-inflammatory activity of extract when compared with aspirin. The methanol leaf extract had appreciable anti-inflammatory activity (57.5%) when compared with standard reference drug aspirin (93.2%) by the end of the first 4 hours post induction of inflammation with carageenan in female rats. The two other extracts (chloroform and hexane extracts) were not as active as the methanol extract with the hexane extract producing the least inhibitory activity of 1.4%.

Table 4.23: Anti-inflammatory activity evaluations of the crude leaf extracts of *Ficus thonningii* on female rats

Extract and Drug Control	Do (cm)	Dt (cm) Circumference of paw at time (hr)				Dt – Do at time (hr)					% Inhibition at time 4hr
	0	1	2	3	4	0	1	2	3	4	
Hexane 100mg/kg	2.53	2.71	2.88	3.06	3.25	0.00	0.18	0.35	0.53	0.72	1.36
Chloroform 100mg/kg	2.55	3.14	3.28	3.24	3.20	0.00	0.59	0.73	0.69	0.65	10.95
Methanol 100mg/kg	2.55	3.01	3.12	3.06	2.86	0.00	0.46	0.57	0.51	0.31	57.53
Aspirin® 100mg/kg	2.65	3.02	3.21	2.87	2.70	0.00	0.37	0.56	0.22	0.05	93.15
Tween 80	2.45	2.57	2.74	2.90	3.18	0.00	0.12	0.29	0.45	0.73	-

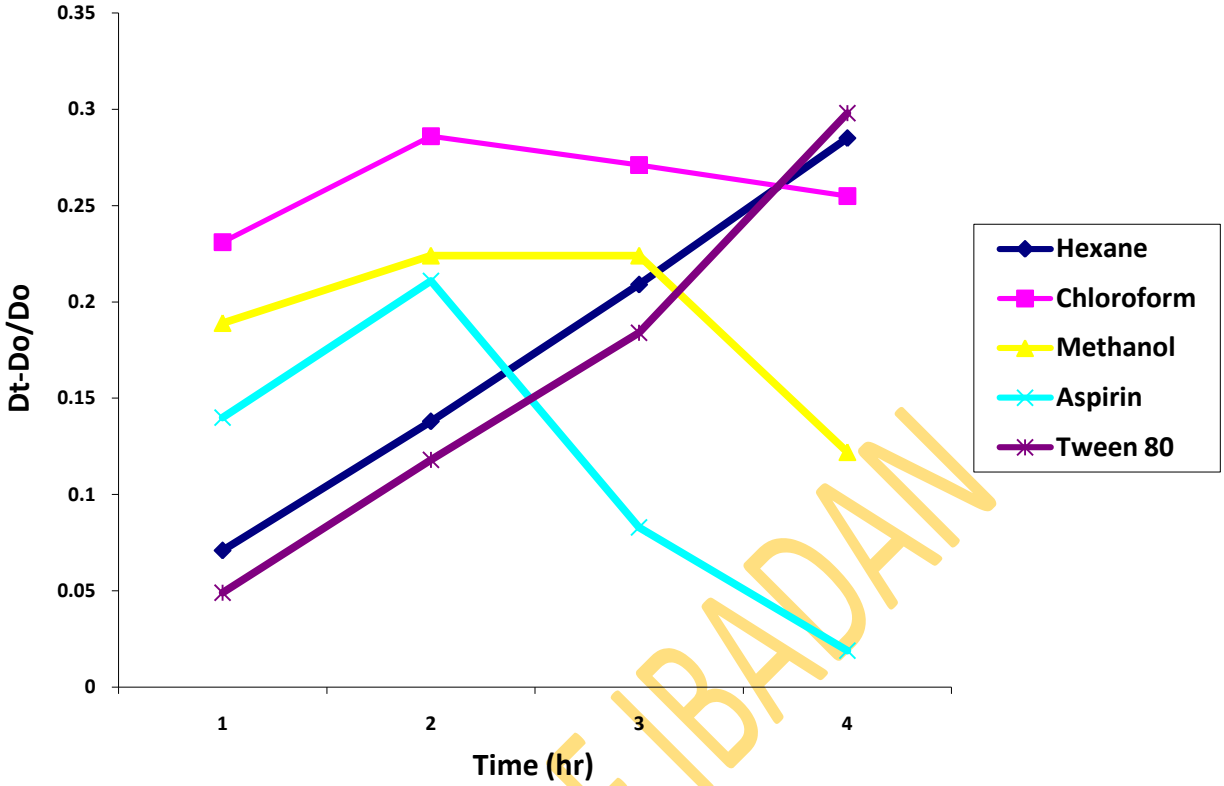


Fig. 4.9: Comparison of the effects of crude leaf extracts of *F. thonningii* on carrageenan-induced rat paw oedema

4.12. Chromatographic analysis

In the preliminary separation of compounds by spotting on pre-coated analytical TLC plates, hexane-ethyl acetate (80:20) produced the best separation in hexane leaf crude extract and was used as the starting eluting solvent for subsequent column separation of extract. For the methanol leaf crude extract, separation was best achieved in dichloromethane- ethyl acetate (80:20), for hexane stem bark, hexane-dichloromethane (60:40) and for methanol stem bark, dichloromethane-ethyl acetate (80:20).

From the column chromatographic separation of hexane leaf crude extract, 68 fractions of 50 mL of each were collected and on the basis of analytic TLC, identical fractions were pooled together to yield 11 fractions (HLF 1-11). Sixty two fractions were collected from the elution of methanol leaf crude extract and the similar fractions pooled together yielded 11 fractions (MLF 1-11). Thirty eight fractions were collected from the elution of hexane stem bark crude extract, pooled together to afford 6 fractions (HSF 1-6). The thirty fractions obtained from methanol stem bark were bulked into 6 fractions (MSF 1-6). Tables 4.25 - 4.27 summarize the results and observation of the column separation.

On the TLC plate, HLF 01 was observed as a yellow spot, had orange fluorescence under UV illumination and showed a yellow colour with iodine vapour. The spot moved with the solvent front and had a high R_f value of 0.97. HLF 02 occurred as four bands with the first band having the same characteristics as HLF1. The 2^{nd} band was colourless under daylight but had a pink colour under UV and also turned yellow with iodine vapour. The 3^{rd} and 4^{th} bands showed light green colouration under daylight and turned light brown under UV and had a yellow colour in iodine vapour. The R_f values were 0.84 and 0.76 respectively.

Resolution of HLF 03-HLF 05 was in hexane-ethyl acetate 70:30. HLF 03 occurred as three bands with all having faint brown colour under daylight and under UV and yellow colour in iodine vapour. HLF 04 was a single band and was observed as light green under daylight. It had a brown colour under UV and yellow colour in iodine vapour. HLF 05 had two bands with R_f values of 0.4 and 0.32. Resolution of HLF 06 was in hexane-ethyl acetate 60:40. The spot was light green under daylight and light brown under UV illumination with R_f value of 0.32. HLF 07 and HLF 08 were observed as colourless spots in daylight but had yellow tints under UV. R_f values were 0.24 and 0.19 respectively. Fractions 9-11 were observed as yellow

spots in daylight and as golden yellow spots under UV. The summary of the results are seen in Table 4.24. HLF 04, HLF 07 and HLF 11 had appreciable antimicrobial activity and were subjected to further separation and purification. HLF 07 yielded white crystals coded as EC.HLO2. The compound was subjected to spectroscopic analysis for structural elucidation.

For the methanol leaf fractions (MLF), resolution of fractions 1-3 was in dichloromethane-ethyl acetate mixture (80:20). The resolution of fractions 4-8 was in dichloromethane-ethyl acetate (70:30) while that of fractions 9-11 was in methanol. The characteristic colours in daylight, under UV and in iodine vapour with corresponding R_f values are presented in Table 4.25.

The spots from hexane stem bark fractions varied from faint yellow colour to golden yellow colour in daylight and all had pink fluorescence under UV illumination and characteristic yellow colour in iodine vapour. The R_f values were similar ranging from 0.95 to 0.96 (Table 4.26). The spots from methanol stem bark fractions were very faint in colour in daylight but had pink fluorescence under UV and yellow colour in iodine vapour. The R_f values are seen in Table 4.27. Yields and % yields of all fractions are shown in Tables 4.28 - 4.31.

The HPTLC finger printing and the corresponding chromatograms are presented in Fig. 4.10 and Fig. 4.22. Fractions which had antimicrobial activities were subjected to further separation and purification.

4.13 Isolation and purification of EC.HLO2

Compound EC.HLO2 was isolated from the hexane leaf fraction (HLF 07) by column chromatography. Further purification of this fraction by chromatography using a smaller column, and eluting with Hex-EtOAc (70:30) resulted in isolation of EC.HLO2 as white solid. This was purified by recrystallisation in Hex-EtOAc (60:40) to produce white crystals (38mg).

4.14 Characterization of EC.HLO2

UV: (MeOH) λmax 227nm at log ε 3.68 (Fig. 4.45)

^{1}H NMR: ($CDCl_3$, 500MH_z): δ (ppm) 0.89 - 2.35 (CH_3s and CH_2s); δ 5.2 - 5.3 (olefinic CH); δ 9.8 (OH).

^{13}C NMR: ($CDCl_3$, 125MH_z): δ (ppm) 14.1, 21.1, 22.7, 24.8, 25.3, 27.9, 31.9, 34.4 (CH_3s and CH2s); 127.9-130.2 (CH=CH); 179.6 (COOH).

Compound EC.HL02 was isolated from HLF07 as white crystalline solid. It was subjected to UV, ^{1}H NMR, ^{13}C NMR and GC-MS in order to elucidate the structure. The UV spectrum gave λmax 227nm at log ε 3.68 (Fig.4.45) which showed the presence of carbon-carbon double bond typical of olefins. The ^{1}HNMR spectrum showed signals due to olefinic protons at δ 5.2-5.3 ppm characteristic of 12-oleanene or 12-ursalene skeletons. Another proton singlet was seen at δ 9.8 ppm (Fig.4.26 and Fig.4.28). ^{13}CNMR spectrum showed a carbon signal at δ 179.6 ppm. No other oxygenated carbon or proton signals were seen in both ^{1}HNMR and ^{13}CNMR spectra. Multiple olefinic signals were seen between δ 5.2 - 5.3 ppm in the ^{1}HNMR and δ 127.9 - 130.2 ppm. Both the ^{1}HNMR and ^{13}CNMR spectra and the DEPT spectrum (Fig. 4.31) revealed the presence of many methylene groups. The ^{1}H-^{13}C COSY (HMQC) spectra showed the carbon atoms to which the protons are attached.

Table 4.24: Fractionation of hexane extract of leaf of *Ficus thonningii*

Fraction	Color observed under			R_f Value
	Daylight	Ultraviolet light	Iodine Vapor	
Hexane: Ethyl acetate (80:20)				
HLF 1	Yellow	Orange	Yellow	0.97
HLF 2A	Yellow	Orange	Yellow	0.97
B	Colorless	Pink	Yellow	0.94
C	Light green	Light brown	Yellow	0.83
D	Light green	Light brown	Yellow	0.76
Hexane: Ethyl acetate (70:30)				
HLF 3A	Faint brown	Faint brown	Yellow	0.63
B	Faint brown	Faint brown	Yellow	0.59
C	Faint brown	Faint brown	Yellow	0.52
HLF 4	Light green	Brown	Yellow	0.40
HLF 5A	Colorless	Light brown	Yellow	0.40
B	Light green	Light brown	Yellow	0.32
Hexane: Ethyl acetate(60:40)				
HLF 6	Light green	Light brown	Yellow	0.32
Hexane: Ethyl acetate (50:50)				
HLF 7	Colorless	Yellow	Yellow	0.24
HLF 8	Colorless	Yellow	Yellow	0.19
HLF 9	Faint yellow	Yellow	Yellow	0.12
HLF 10	Yellow	Golden yellow	Yellow	0.094
HLF 11	Yellow	Golden yellow	Yellow	0.94
HLF 12	Yellow	Golden yellow	Yellow	0.078

Key:

HLF: Hexane leaf fraction $R_{f:}$ Retention factor

Table 4.25: Fractionation of methanol extract of leaf of *Ficus thonningii*

Fraction	Color observed under			R_f Value
	Daylight	Ultraviolet light	Iodine Vapor	
Dichloromethane - ethyl acetate (70 : 30)				
MLF 1	Greenish yellow	Yellow	Yellow	0.97
MLF 2	Greenish yellow	Yellow	Yellow	0.97
Ethyl acetate (100%)				
MLF 3A	Faint green yellow	Yellow	Yellow	0.97
B	Faint green yellow	Yellow	Yellow	0.78
C	Faint green yellow	Yellow	Yellow	0.68
D	Faint green yellow	Yellow	Yellow	0.43
E	Faint green yellow	Yellow	Yellow	0.08
Ethyl acetate - methanol (50:50)				
MLF 4-8- Colorless	Transparent liq invisible			
Methanol (100%)				
MLF 9	Not visible			
MLF 10	Light yellow	Yellow	Yellow	0.74
MLF 11	Light yellow	Yellow	Yellow	0.74
MLF 12	Not visible			

Key:

MLF: Methanol leaf fraction

R_f: Retention factor

Table 4.26: Fractionation of hexane extract of stem bark of *Ficus thonningii*

Fraction	Color observed under			R_f Value
	Daylight	Ultraviolet light	Iodine Vapor	
HSF 1	Faint yellow	Pink	Yellow	0.95
HSF 2	Colorless	Pink	Yellow	0.96
HSF 3	Golden yellow	Pink	Yellow	0.95
HSF 4	Golden yellow	Pink	Yellow	0.95
HSF 5	Colorless	Pink	Yellow	0.96
HSF 6	Faint yellow	Pink	Yellow	0.96

Key:

HSF: Hexane stem bark fraction

R_f: Retention factor

Table 4.27: Fractionation of methanol extract of stem bark of *Ficus thonningii*

Fraction	Color observed under			R_f Value
	Daylight	Ultraviolet light	Iodine Vapor	
MSF 1	Faint	Pink	Yellow	0.95
MSF 2	Faint	Pink	Yellow	0.95
MSF 3A	Faint	Pink	Yellow	0.95
B	Faint	Pink	Yellow	0.80
MSF 4	Faint	Pink	Yellow	0.80
MSF 5	Faint	Pink	Yellow	0.78
MSF 6	Invisible	Invisible	Yellow	---

Key:

MSF: Methanol stem bark fraction

R_f: Retention factor

Table 4.28: Yield of fractions from 5.0 g of Hexane leaf extract of *Ficus thonningii*

Fraction	Yield (g)	Percentage Yield (%)
HLF 1	0.70	14.00
HLF 2	0.62	12.40
HLF 3	0.61	12.20
HLF 4	0.06	1.20
HLF 5	0.10	2.00
HLF 6	0.09	1.80
HLF 7	0.04	0.08
HLF 8	0.35	7.00
HLF 9	0.07	1.40
HLF 10	0.06	1.20
HLF 11	0.04	0.80
HLF 12	0.04	0 80

Key:

HLF: Hexane leaf fraction

Table 4.29: Yield of fractions from 3.0 g of Hexane stem bark extract of *Ficus thonningii*

Fraction	Yield (g)	Percentage Yield (%)
HSF 1	0.55	18.30
HSF 2	0.12	4.00
HSF 3	1.47	49.00
HSF 4	0.14	4.70
HSF 5	0.12	4.00
HSF 6	0.08	2.70

Key:

HSF: Hexane stem bark fraction

Table 4.30: Yield of fractions from 5.0 g of Methanol leaf extract of *Ficus thonningii*

Fraction	Yield (g)	Percentage Yield (%)
MLF 1	0.01	0.20
MLF 2	0.01	0.20
MLF 3	0.02	0.40
MLF 4	0.01	0.20
MLF 5	0.03	0.60
MLF 6	0.01	0.20
MLF 7	0.01	0.20

Key:

MLF: Methanol leaf fraction

Table 4.31: Yield of fractions from 4.06 g Methanol stem bark extract of *Ficus thonningii*

Fraction	Yield (g)	Percentage Yield (%)
MSF 1	0.03	0.74
MSF 2	0.49	12.10
MSF 3	0.28	6.90
MSF 4	0.29	7.10
MSF 5	0.21	5.20
MSF 6	1.32	32.50

Key:

MSF: Methanol stem bark fraction

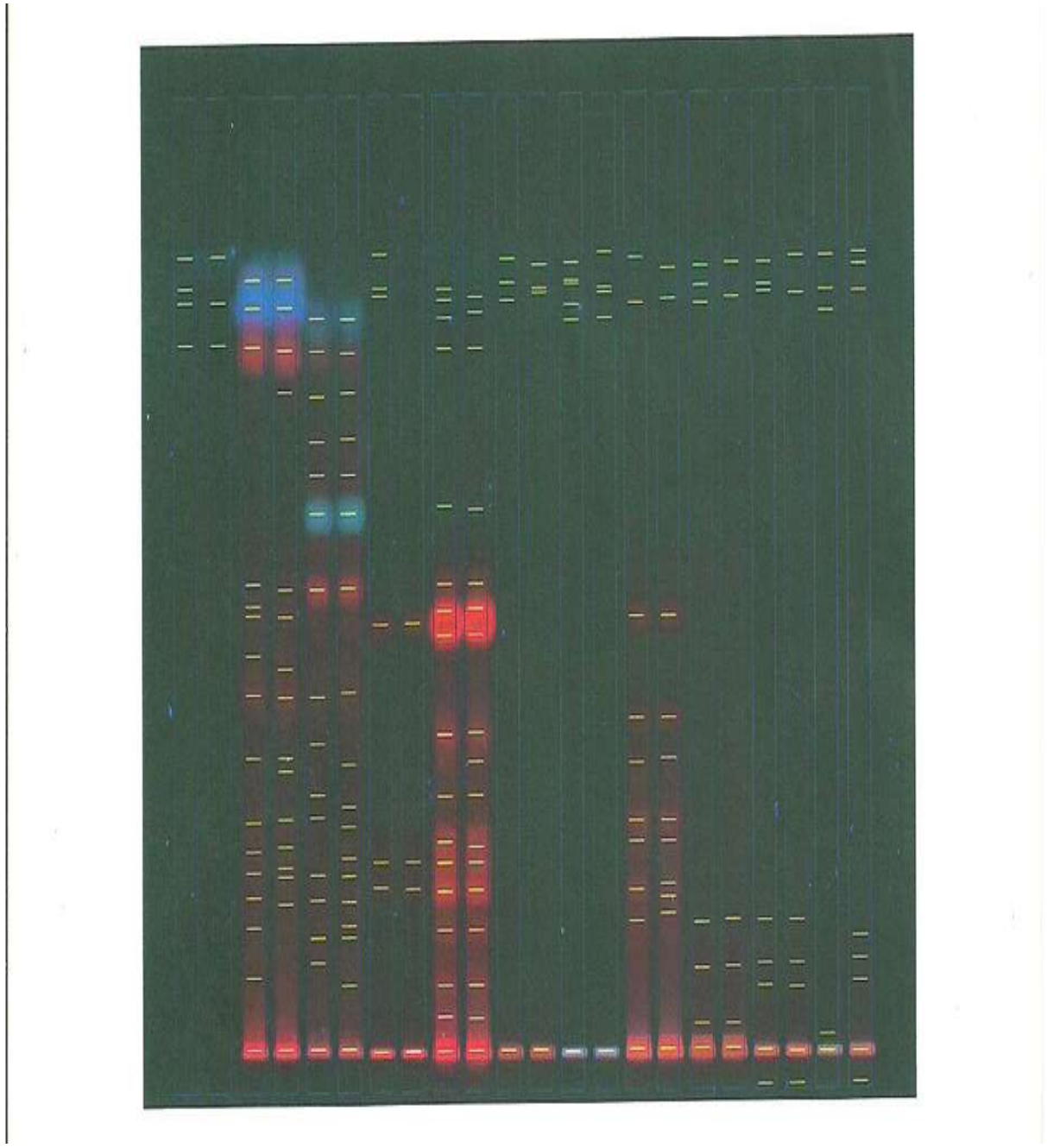
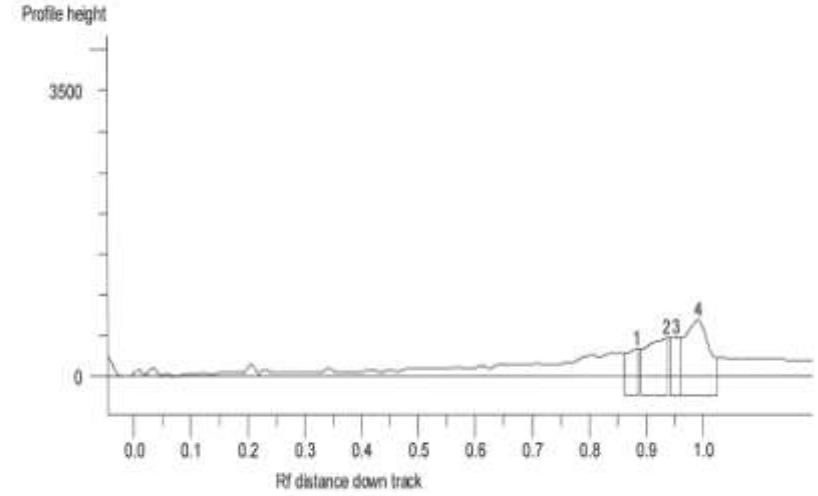


Figure 4.10: TLC/HPTLC image document data of *Ficus thonningii* Hexane leaf fractions derivatised at 366nm illumination

Integration results

Track 1



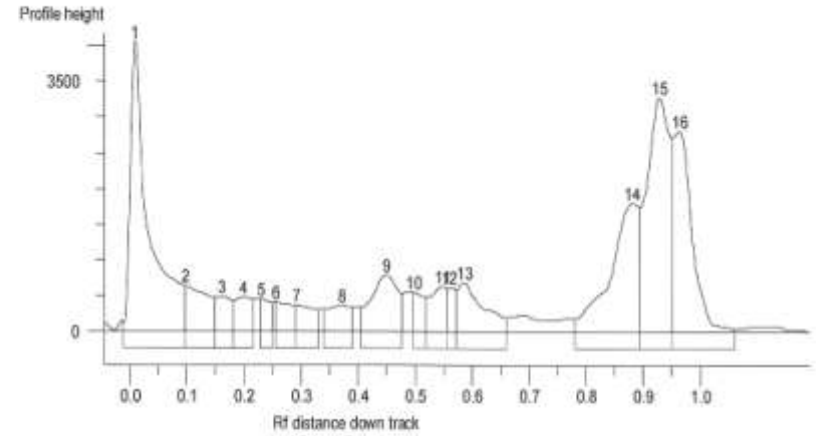
Track 1

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.862	277.5	0.885	337.1	17.10	0.886	335.8	4026.2	10.98	
2	0.890	338.1	0.937	469.0	23.79	0.939	468.5	10586.5	28.87	
3	0.942	468.4	0.953	479.0	24.30	0.961	467.1	4724.8	12.88	
4	0.961	467.1	0.993	686.0	34.81	1.000	228.9	17332.8	47.27	

Total Height 1971.08 Total Area : 36670.3

Fig 4.11: Integration results for Hexane leaf fraction 01 from *Ficus thonningii*

Track 3

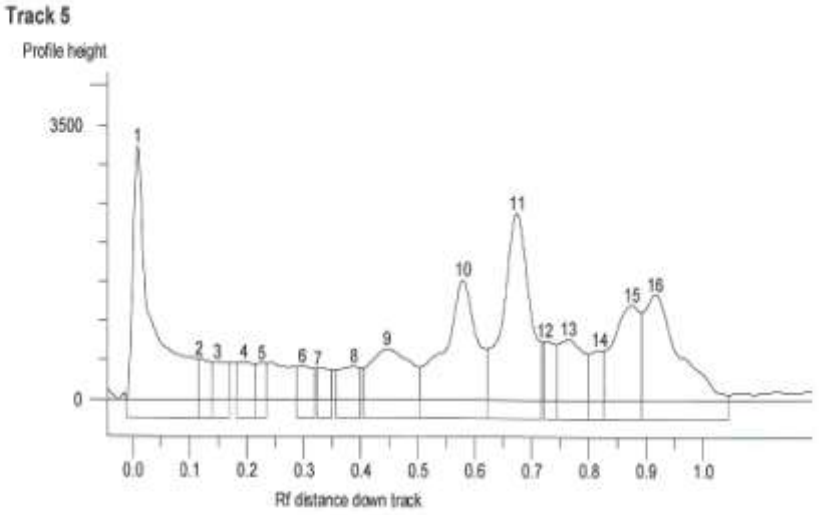


Track 3

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	112.5	0.009	4055.7	22.00	0.097	642.7	81797.1	20.27	
2	0.097	642.7	0.099	642.7	3.49	0.149	483.3	15560.2	3.86	
3	0.149	483.3	0.162	493.7	2.68	0.182	436.6	8534.9	2.12	
4	0.182	436.6	0.199	483.6	2.62	0.216	459.1	8375.0	2.08	
5	0.229	455.2	0.230	456.3	2.48	0.251	409.1	5212.9	1.29	
6	0.255	409.9	0.257	410.5	2.23	0.290	371.7	7439.6	1.84	
7	0.290	371.7	0.292	372.2	2.02	0.331	313.2	7533.0	1.87	
8	0.340	316.8	0.372	368.0	2.00	0.390	344.3	9378.3	2.32	
9	0.405	340.5	0.450	786.4	4.27	0.478	539.6	23161.7	5.74	
10	0.496	545.2	0.498	547.1	2.97	0.519	487.2	6227.8	1.54	
11	0.519	487.2	0.548	626.9	3.40	0.556	612.9	11237.0	2.79	
12	0.556	612.9	0.559	616.9	3.35	0.572	580.3	5481.3	1.36	
13	0.572	580.3	0.587	684.5	3.71	0.660	193.8	19286.7	4.78	
14	0.781	181.6	0.881	1799.1	9.76	0.894	1743.3	54736.8	13.57	
15	0.894	1743.3	0.929	3279.3	17.79	0.952	2714.0	81832.9	20.28	
16	0.952	2714.0	0.965	2812.2	15.25	1.000	46.7	57672.0	14.29	

Total Height 18435.4 Total Area : 403467

Fig 4.12: Integration results for Hexane leaf fraction 02 from *Ficus thonningii*

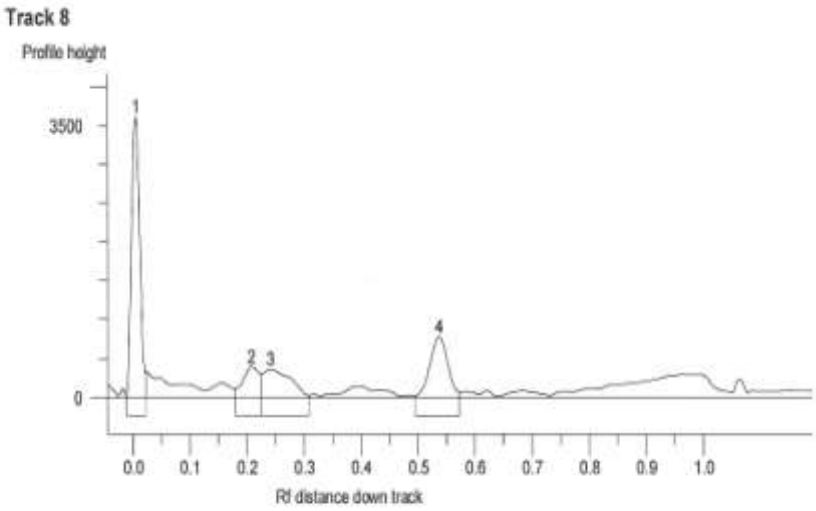


Track 5

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	28.8	0.009	3234.2	20.52	0.115	517.1	67206.0	17.12	
2	0.115	517.1	0.117	518.6	3.29	0.139	473.0	6483.6	1.65	
3	0.139	473.0	0.149	478.4	3.04	0.169	468.9	7594.6	1.93	
4	0.182	469.3	0.195	483.1	3.07	0.214	455.9	8019.0	2.04	
5	0.214	455.9	0.227	467.9	2.97	0.234	457.4	5086.2	1.30	
6	0.288	418.6	0.297	426.8	2.71	0.320	396.5	7070.5	1.80	
7	0.323	397.4	0.325	398.1	2.53	0.349	386.2	5517.5	1.41	
8	0.357	385.4	0.388	430.8	2.73	0.400	413.2	9425.1	2.40	
9	0.405	412.5	0.446	649.0	4.12	0.504	422.9	28547.2	7.27	
10	0.504	422.9	0.580	1534.9	9.74	0.623	653.6	53134.0	13.53	
11	0.623	653.6	0.675	2394.7	15.20	0.717	757.2	68496.8	17.45	
12	0.721	756.2	0.723	758.5	4.81	0.743	711.3	8859.2	2.26	
13	0.743	711.3	0.764	778.4	4.94	0.799	592.3	21129.5	5.38	
14	0.799	592.3	0.820	640.8	4.07	0.827	636.2	9360.8	2.38	
15	0.827	636.2	0.875	1217.8	7.73	0.892	1120.9	34530.8	8.80	
16	0.892	1120.9	0.916	1346.5	8.54	1.000	74.5	52151.5	13.28	

Total Height 15758.7 Total Area : 392612

Fig 4.13: Integration results for Hexane leaf fraction 03 from *Ficus thonningii*

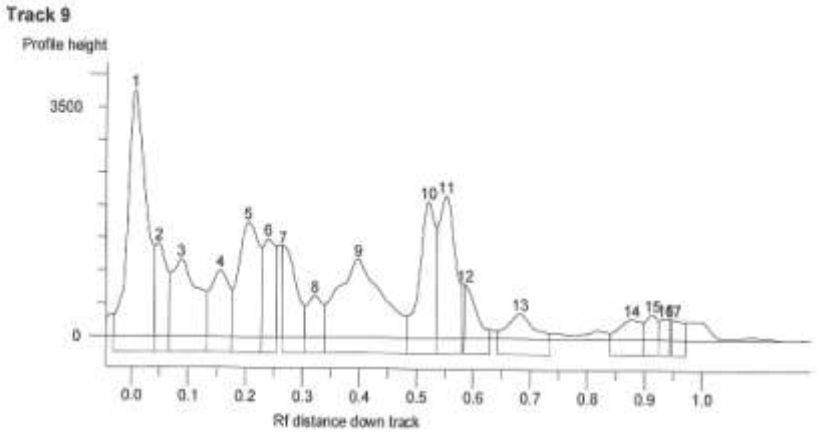


Track 8

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	0.0	0.006	3612.2	70.17	0.022	300.8	32452.7	49.60	
2	0.180	121.6	0.208	393.7	7.65	0.225	301.3	6827.8	10.44	
3	0.225	301.3	0.242	363.4	7.06	0.309	34.1	11009.3	16.83	
4	0.494	24.6	0.537	778.4	15.12	0.572	71.2	15140.6	23.14	

Total Height: 5147.74 Total Area : 65430.3

Fig. 4.14 Integration results for Hexane leaf fraction 04 from *Ficus thonningii*

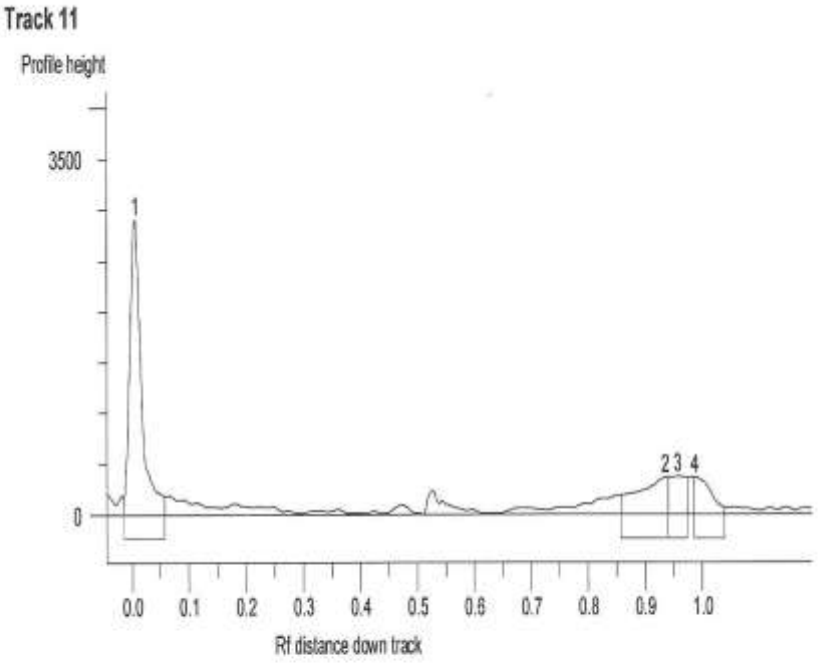


Track 9

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	330.3	0.006	3761.8	18.19	0.041	1368.1	74795.3	18.37	
2	0.041	1368.1	0.048	1426.0	6.90	0.067	972.0	17562.9	4.31	
3	0.067	972.0	0.087	1177.5	5.70	0.132	680.8	31954.5	7.85	
4	0.132	680.8	0.156	1016.1	4.91	0.177	726.1	20554.3	5.05	
5	0.177	726.1	0.204	1751.3	8.47	0.229	1366.2	38625.1	9.49	
6	0.229	1366.2	0.240	1496.3	7.24	0.255	1393.2	20112.8	4.94	
7	0.264	1389.5	0.266	1390.8	6.73	0.305	477.8	21299.1	5.23	
8	0.305	477.8	0.322	637.2	3.08	0.340	499.0	10667.0	2.62	
9	0.340	499.0	0.398	1193.4	5.77	0.483	340.7	56910.0	13.98	
10	0.483	340.7	0.520	2090.3	10.11	0.535	1706.8	34041.1	8.36	
11	0.535	1706.8	0.552	2175.2	10.52	0.580	823.1	39454.1	9.69	
12	0.584	821.8	0.586	822.1	3.98	0.628	148.8	10680.6	2.62	
13	0.643	127.5	0.682	392.9	1.90	0.734	95.3	10753.7	2.64	
14	0.840	109.4	0.877	314.3	1.52	0.898	271.7	7521.3	1.85	
15	0.898	271.7	0.914	392.2	1.90	0.926	304.0	5118.6	1.26	
16	0.926	304.0	0.937	324.7	1.57	0.944	312.7	3160.1	0.78	
17	0.948	312.6	0.950	313.3	1.52	0.972	272.6	3854.2	0.95	

Total Height 20675.2 Total Area : 407065

Fig. 4.15: Integration results for Hexane leaf fraction 05 from *Ficus thonningii*

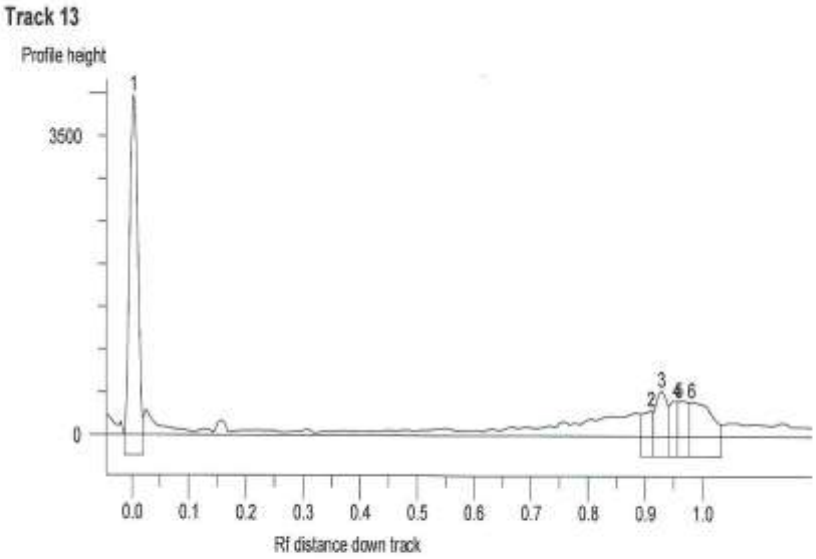


Track 11

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	172.8	0.006	2910.5	72.38	0.058	189.4	37146.9	59.56	
2	0.857	193.2	0.935	365.3	9.08	0.941	363.9	11771.2	18.87	
3	0.941	363.9	0.957	375.1	9.33	0.974	368.0	6665.7	10.69	
4	0.985	370.2	0.987	370.5	9.21	1.000	70.4	6787.8	10.88	

Total Height 4021.4 Total Area : 62371.6

Fig 4.16: Integration results for Hexane leaf fraction 06 from *Ficus thonningii*

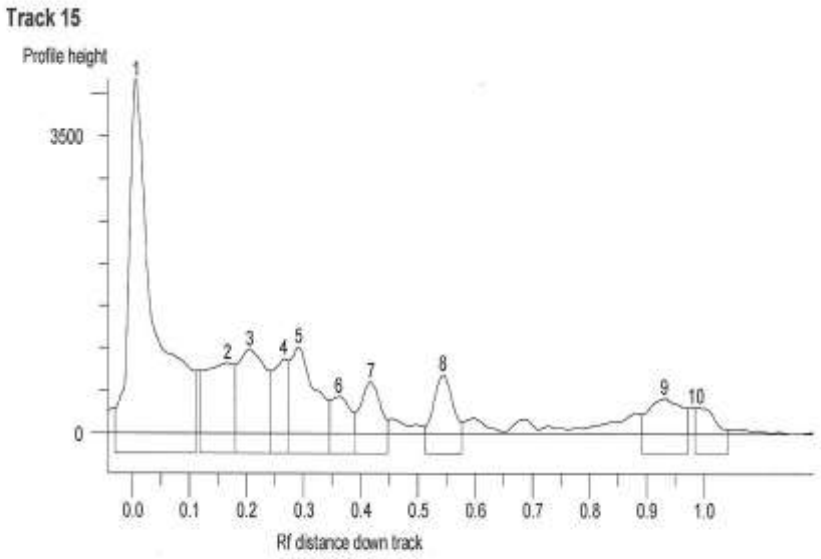


Track 13

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	0.0	0.004	3988.4	65.79	0.020	198.2	35150.6	56.42	
2	0.892	272.7	0.911	309.9	5.11	0.914	289.4	3450.0	5.54	
3	0.914	289.4	0.929	530.2	8.75	0.941	358.7	6200.1	9.95	
4	0.941	358.7	0.955	416.1	6.86	0.957	415.6	3608.5	5.79	
5	0.957	415.6	0.959	415.7	6.86	0.978	400.4	4503.4	7.23	
6	0.978	400.4	0.981	402.2	6.63	1.000	144.3	9392.4	15.07	

Total Height 6062.38 Total Area : 62305.1

Fig 4.17: Integration results for Hexane leaf fraction 07 from *Ficus thonningii*

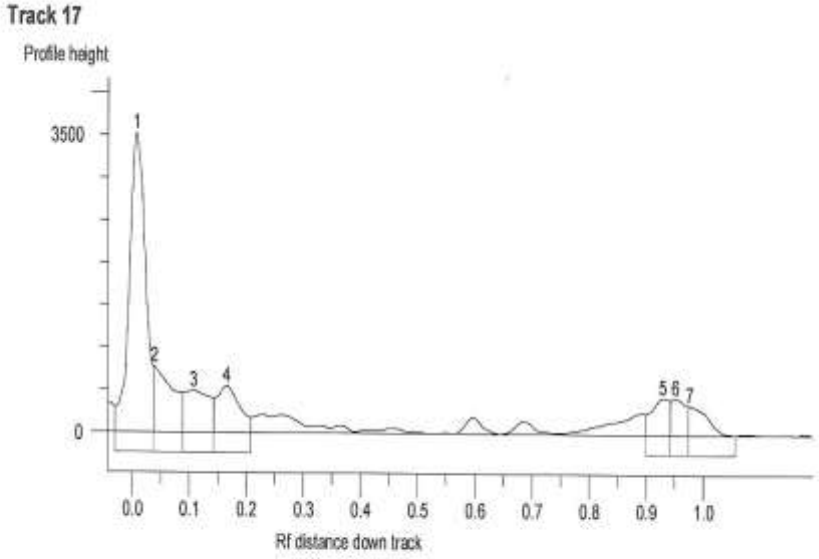


Track 15

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	298.6	0.007	4167.9	40.25	0.111	733.5	110638.2	42.14	
2	0.119	738.8	0.167	830.9	8.02	0.180	812.3	25932.7	9.88	
3	0.180	812.3	0.206	987.7	9.54	0.243	735.0	29661.6	11.30	
4	0.243	735.0	0.265	879.2	8.49	0.273	860.6	12997.6	4.95	
5	0.273	860.6	0.291	1014.9	9.80	0.345	392.3	27026.7	10.29	
6	0.345	392.3	0.362	437.4	4.22	0.390	249.8	8930.3	3.40	
7	0.390	249.8	0.417	605.8	5.85	0.447	166.8	12492.1	4.76	
8	0.512	88.3	0.544	696.2	6.72	0.577	139.6	13591.8	5.18	
9	0.892	232.3	0.931	417.8	4.03	0.972	309.1	14879.8	5.67	
10	0.985	318.2	0.987	318.2	3.07	1.000	52.5	6409.6	2.44	

Total Height 10356 Total Area : 262560

Fig 4.18: Integration results for Hexane leaf fraction 08 from *Ficus thonningii*

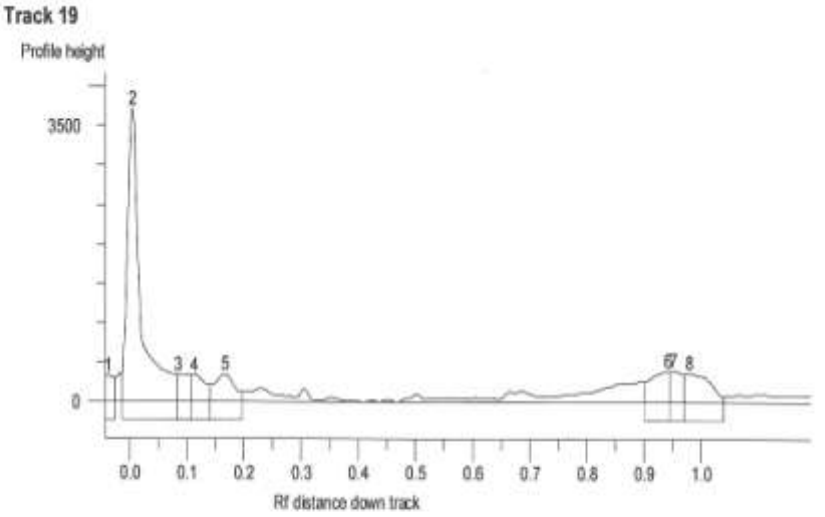


Track 17

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	300.8	0.007	3523.3	53.76	0.037	774.6	61548.9	48.38	
2	0.037	774.6	0.039	780.0	11.90	0.089	464.7	16328.4	12.83	
3	0.089	464.7	0.108	491.7	7.50	0.143	406.7	13309.1	10.46	
4	0.143	406.7	0.165	549.4	8.38	0.208	180.8	13643.6	10.72	
5	0.900	252.7	0.929	434.6	6.63	0.942	414.7	8424.1	6.62	
6	0.942	414.7	0.952	428.0	6.53	0.974	345.7	6766.1	5.32	
7	0.974	345.7	0.976	346.4	5.29	1.000	5.4	7204.2	5.66	

Total Height 6553.33 Total Area : 127224

Fig 4.19: Integration results for Hexane leaf fraction 09 from *Ficus thonningii*

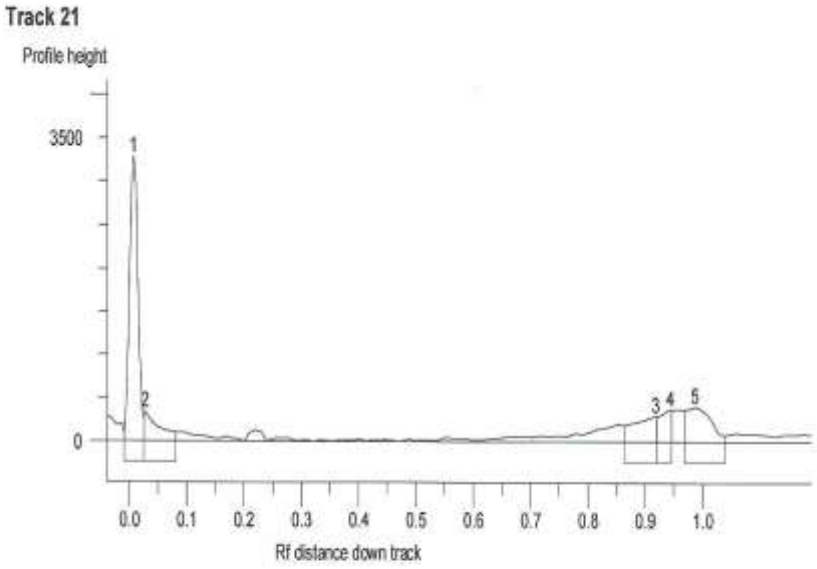


Track 19

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	315.7	-0.037	334.5	5.33	-0.026	299.0	3042.0	3.06	
2	0.000	333.2	0.006	3720.1	59.28	0.083	346.9	55308.1	55.70	
3	0.083	346.9	0.085	347.3	5.53	0.108	330.6	4429.6	4.46	
4	0.108	330.6	0.113	338.7	5.40	0.141	216.5	5135.6	5.17	
5	0.141	216.5	0.167	353.1	5.63	0.197	121.4	7560.6	7.61	
6	0.902	272.6	0.942	401.5	6.40	0.946	398.3	8357.9	8.42	
7	0.946	398.3	0.952	402.0	6.41	0.970	364.2	5045.9	5.08	
8	0.970	364.2	0.980	378.2	6.03	1.000	80.9	10414.2	10.49	

Total Height 6275.54 Total Area : 99294

Fig. 4.20: Integration result for Hexane leaf fraction 10 from *Ficus thonningii*



Track 21

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	97.8	0.007	3285.0	69.68	0.026	287.5	30443.1	51.21	
2	0.026	287.5	0.028	344.3	7.30	0.081	111.2	5527.8	9.30	
3	0.863	198.5	0.919	302.2	6.41	0.921	298.8	7626.5	12.83	
4	0.921	298.8	0.945	379.3	8.04	0.946	378.1	4749.8	7.99	
5	0.969	377.5	0.987	403.8	8.56	1.000	81.4	11104.9	18.68	

Total Height 4714.69 Total Area : 59452.1

Fig. 4.21: Integration result for Hexane leaf fraction 11 from *Ficus thonningii*

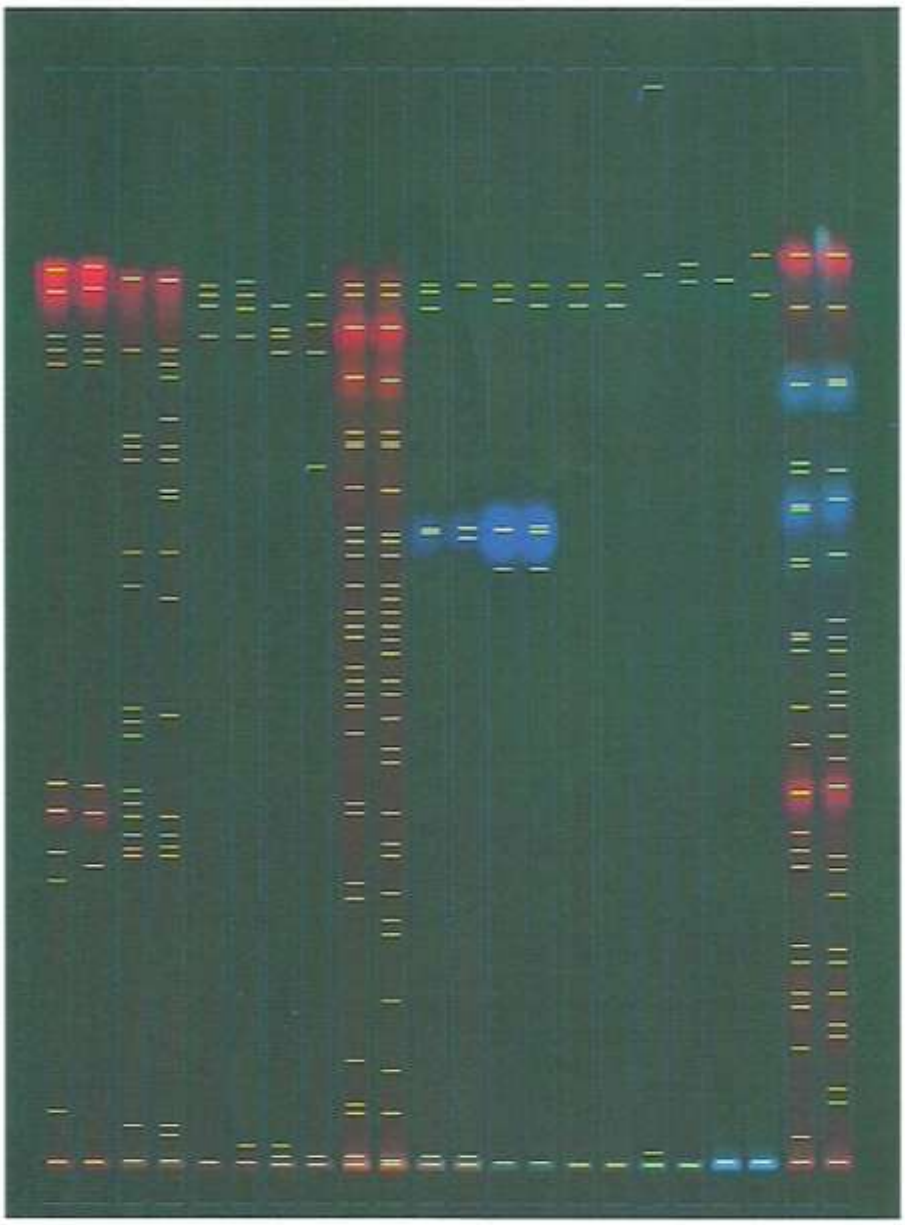


Fig. 4.22: TLC/HPTLCimage document data of *Ficus thonningii* methanol leaf fractions derv-

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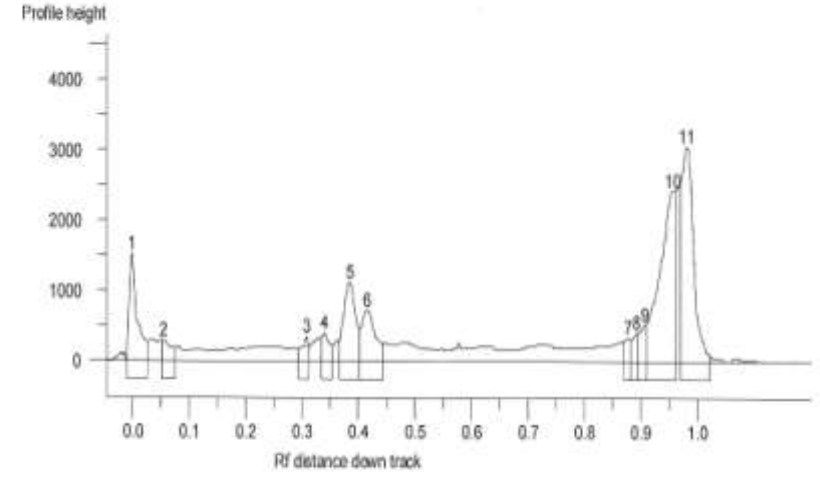
ed at 366nm illumination

Integration results

Track 1

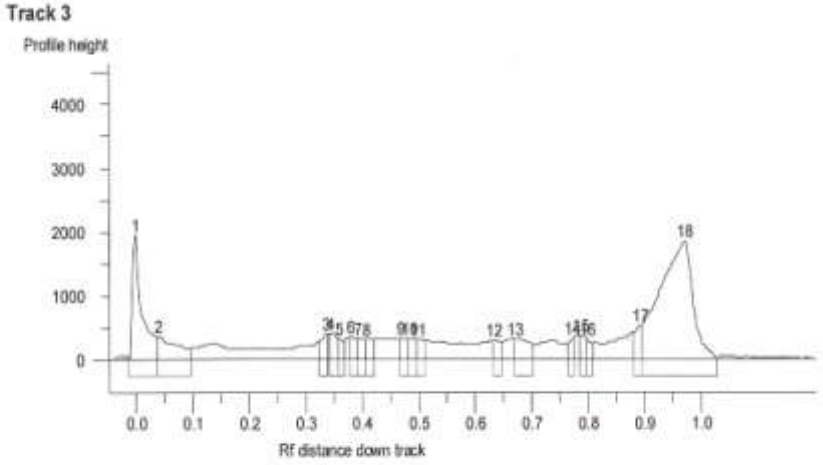


Track 1

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	0.0	-0.002	1525.5	13.59	0.026	289.2	13176.4	9.60	
2	0.053	297.1	0.055	302.5	2.69	0.075	185.0	2819.1	2.05	
3	0.293	168.7	0.308	360.6	3.21	0.312	187.9	2300.5	1.68	
4	0.335	325.8	0.340	413.6	3.68	0.353	227.1	3199.7	2.33	
5	0.367	303.9	0.385	1121.8	9.99	0.402	467.5	14472.8	10.55	
6	0.402	467.5	0.415	728.0	6.48	0.444	245.4	10839.9	7.90	
7	0.870	289.7	0.878	343.0	3.05	0.882	316.3	1961.6	1.43	
8	0.882	316.3	0.893	409.6	3.65	0.895	409.4	2514.8	1.83	
9	0.895	409.4	0.908	529.2	4.71	0.910	529.0	3756.0	2.74	
10	0.910	529.0	0.957	2430.0	21.65	0.962	2425.8	41163.3	29.99	
11	0.970	2507.7	0.981	3062.7	27.28	1.000	88.5	41038.0	29.90	

Total Height 11226.3 Total Area : 137242

Fig. 4.23: Integration result for methanol leaf fraction 01 from *Ficus thonningii*

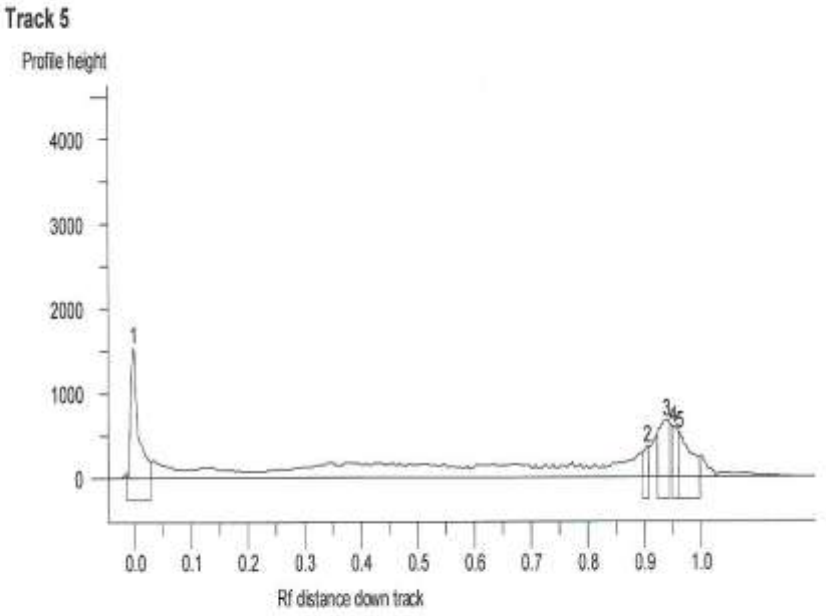


Track 3

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	23.9	0.000	1958.7	20.68	0.038	358.6	20448.1	15.30	
2	0.038	358.6	0.039	367.5	3.88	0.098	185.1	7980.3	5.97	
3	0.323	282.6	0.336	402.1	4.25	0.338	402.0	2761.0	2.07	
4	0.342	409.4	0.344	413.3	4.36	0.357	319.5	3087.3	2.31	
5	0.357	319.5	0.359	337.7	3.57	0.368	280.4	1841.1	1.38	
6	0.378	341.1	0.380	357.0	3.77	0.391	324.0	2398.5	1.80	
7	0.391	324.0	0.393	324.2	3.42	0.406	310.0	2519.6	1.89	
8	0.406	310.0	0.408	310.2	3.28	0.421	309.0	2476.7	1.85	
9	0.466	334.1	0.468	334.7	3.53	0.481	325.2	2642.6	1.98	
10	0.481	325.2	0.483	325.7	3.44	0.496	309.5	2548.9	1.91	
11	0.496	309.5	0.498	309.8	3.27	0.511	294.6	2428.1	1.82	
12	0.632	300.9	0.633	301.1	3.18	0.647	276.9	2323.9	1.74	
13	0.669	314.5	0.671	321.0	3.39	0.701	223.1	4958.4	3.71	
14	0.763	233.1	0.773	328.2	3.46	0.774	328.0	1717.3	1.29	
15	0.786	376.3	0.788	377.9	3.99	0.797	305.3	2155.6	1.61	
16	0.797	305.3	0.799	318.8	3.37	0.808	256.2	1678.9	1.26	
17	0.880	427.3	0.893	534.8	5.65	0.895	534.7	3830.4	2.87	
18	0.895	534.7	0.972	1848.7	19.52	1.000	0.1	65814.5	49.26	

Total Height 9471.24 Total Area : 133611

Fig. 4.24: Integration result for methanol leaf fraction 02 from *Ficus thonningii*

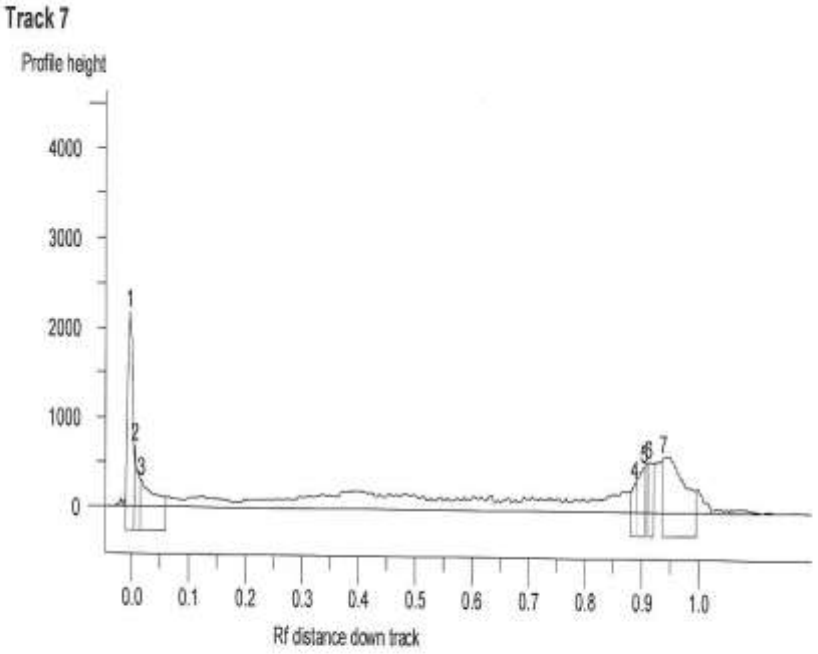


Track 5

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	2.5	-0.002	1545.9	41.88	0.028	192.1	12642.8	39.12	
2	0.897	274.2	0.906	359.7	9.75	0.908	359.6	1989.0	6.15	
3	0.923	486.7	0.940	669.6	18.14	0.946	625.1	7481.9	23.15	
4	0.949	586.2	0.951	590.3	15.99	0.961	521.6	3474.3	10.75	
5	0.961	521.6	0.962	525.7	14.24	0.998	234.8	6732.2	20.83	

Total Height 3691.25 Total Area : 32320.2

Fig 4.25: Integration result for methanol leaf fraction 03 from *Ficus thonningii*

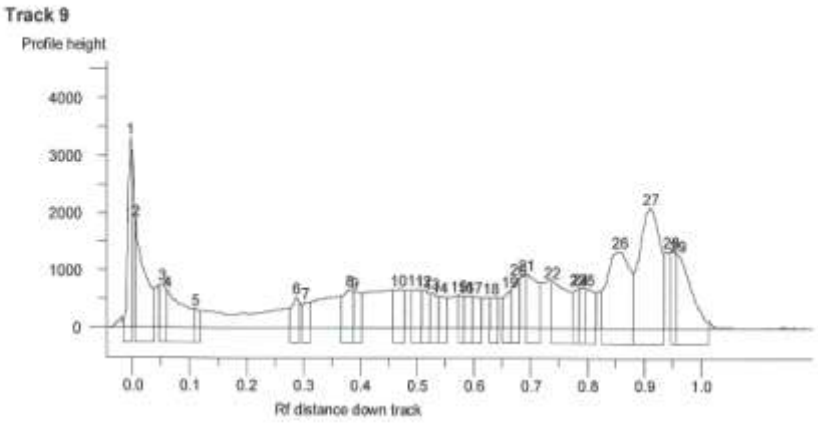


Track 7

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	33.2	-0.004	2181.3	42.03	0.004	584.0	11302.7	27.74	
2	0.004	584.0	0.006	682.2	13.14	0.015	299.4	2701.4	6.63	
3	0.015	299.4	0.017	301.3	5.80	0.060	120.5	4103.2	10.07	
4	0.880	233.1	0.888	337.5	6.50	0.891	334.9	1769.8	4.34	
5	0.891	334.9	0.904	512.1	9.87	0.906	512.0	3561.9	8.74	
6	0.910	550.7	0.912	559.5	10.78	0.921	552.4	3334.0	8.18	
7	0.936	575.2	0.938	616.1	11.87	0.996	263.7	13977.0	34.30	

Total Height 5189.92 Total Area : 40750

Fig.4.26: Integration result for methanol leaf fraction 04 from *Ficus thonningii*

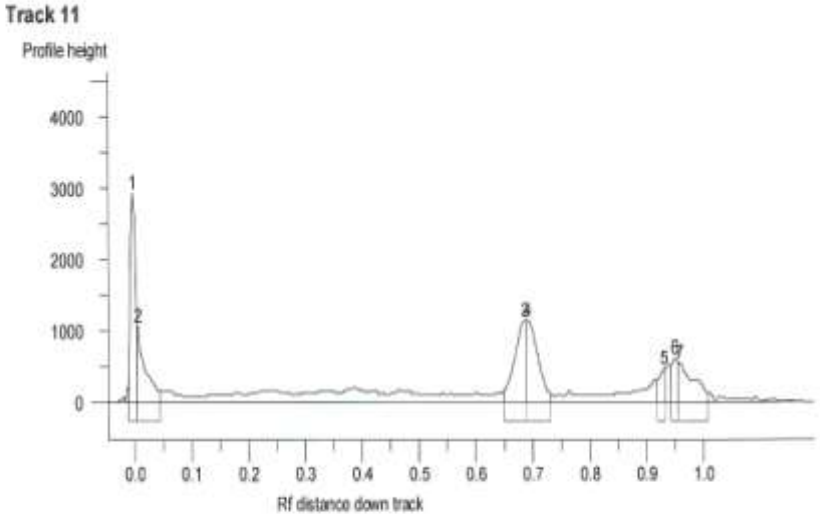


Track 9

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	54.2	-0.004	3322.9	12.69	-0.002	3065.0	12156.1	4.43	
2	0.004	1828.8	0.006	1885.2	7.20	0.037	684.3	19256.6	7.02	
3	0.047	728.3	0.052	768.7	2.94	0.060	644.9	5130.2	1.87	
4	0.060	644.9	0.062	646.4	2.47	0.108	334.5	11327.0	4.13	
5	0.108	334.5	0.110	336.3	1.28	0.120	296.7	1880.7	0.69	
6	0.277	339.1	0.288	536.2	2.05	0.293	422.3	3964.1	1.45	
7	0.297	400.9	0.305	448.5	1.71	0.312	443.0	3449.6	1.26	
8	0.366	546.4	0.381	661.8	2.53	0.389	632.4	7428.9	2.71	
9	0.389	632.4	0.391	636.2	2.43	0.404	615.2	4986.4	1.82	
10	0.458	655.5	0.467	678.2	2.59	0.479	653.4	7309.5	2.66	
11	0.490	655.6	0.497	683.5	2.61	0.508	655.6	6663.3	2.43	
12	0.508	655.6	0.510	664.1	2.54	0.523	605.4	5103.1	1.86	
13	0.523	605.4	0.525	612.5	2.34	0.538	551.5	4648.2	1.69	
14	0.538	551.5	0.540	561.0	2.14	0.553	538.3	4403.7	1.61	
15	0.572	571.7	0.574	572.2	2.19	0.583	562.2	3398.8	1.24	
16	0.583	562.2	0.585	568.7	2.17	0.598	547.3	4458.6	1.63	
17	0.598	547.3	0.600	554.8	2.12	0.613	533.7	4360.8	1.59	
18	0.628	536.2	0.630	544.7	2.08	0.643	529.6	4283.4	1.56	
19	0.650	534.2	0.664	658.8	2.52	0.665	658.6	4823.0	1.76	
20	0.665	658.6	0.679	871.9	3.33	0.680	871.7	6270.9	2.29	
21	0.692	921.6	0.693	944.4	3.61	0.716	794.4	11276.5	4.11	
22	0.736	820.9	0.738	822.4	3.14	0.774	633.4	14368.0	5.24	
23	0.774	633.4	0.783	697.1	2.66	0.785	696.9	4012.4	1.46	
24	0.785	696.9	0.787	706.3	2.70	0.796	697.0	4214.0	1.54	
25	0.796	697.0	0.798	702.2	2.68	0.815	631.4	6687.1	2.44	
26	0.824	650.8	0.858	1346.6	5.14	0.880	978.3	33070.8	12.06	
27	0.880	978.3	0.912	2106.3	8.05	0.935	1311.0	47217.5	17.21	
28	0.946	1347.5	0.948	1352.3	5.17	0.957	1283.0	8008.1	2.92	
29	0.957	1283.0	0.959	1289.5	4.93	1.000	125.5	20160.7	7.35	

Total Height 26179.6 Total Area : 274318

Fig. 4.27: Integration result for methanol leaf fraction 05 from *Ficus thonningii*

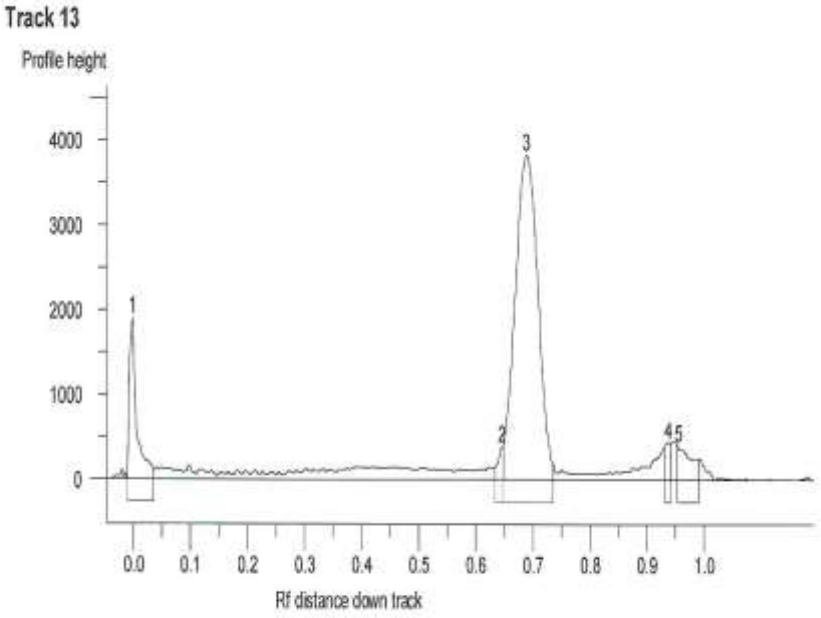


Track 11

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	198.3	-0.004	2940.2	36.97	0.004	1034.7	16466.8	23.18	
2	0.004	1034.7	0.006	1068.6	13.44	0.045	172.7	9775.8	13.76	
3	0.649	149.9	0.687	1143.1	14.37	0.688	1142.0	13831.0	19.47	
4	0.688	1142.0	0.690	1145.9	14.41	0.731	115.9	14547.9	20.48	
5	0.918	312.8	0.931	474.6	5.97	0.933	471.5	3151.8	4.44	
6	0.942	514.5	0.950	624.4	7.85	0.955	539.0	3997.8	5.63	
7	0.955	539.0	0.957	556.9	7.00	1.000	121.0	9262.2	13.04	

Total Height 7953.61 Total Area : 71033.3

Fig 4.28: Integration result for methanol leaf fraction 06 from *Ficus thonningii*

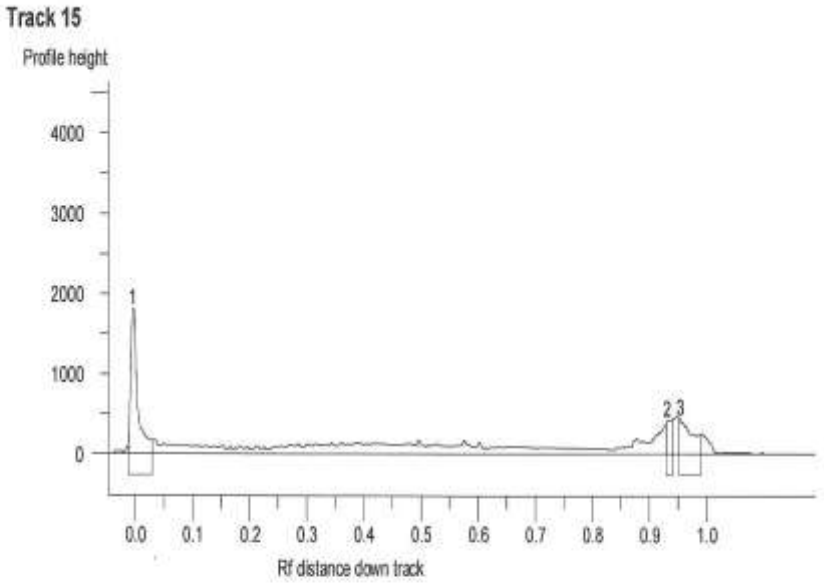


Track 13

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	0.0	-0.002	1904.0	27.26	0.035	117.8	15125.0	12.51	
2	0.633	146.0	0.646	393.4	5.63	0.648	380.0	2146.8	1.77	
3	0.648	380.0	0.689	3832.5	54.88	0.736	176.9	95082.7	78.61	
4	0.931	375.8	0.939	445.5	6.38	0.942	443.8	2604.7	2.15	
5	0.953	403.2	0.955	408.5	5.85	0.991	240.9	5989.3	4.95	

Total Height 6983.82 Total Area : 120949

Fig. 4.29: Integration result for methanol leaf fraction 07 from *Ficus thonningii*

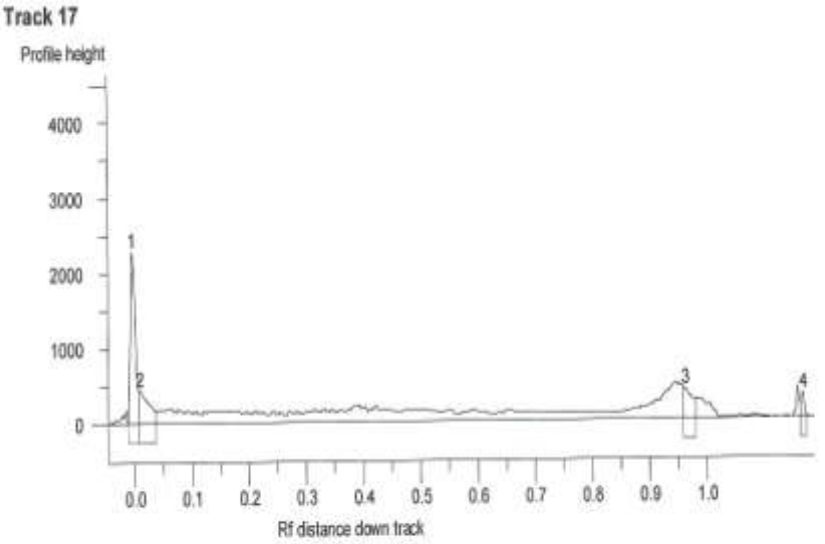


Track 15

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	48.1	-0.004	1805.8	67.46	0.030	174.2	13881.3	61.81	
2	0.929	363.8	0.931	425.2	15.88	0.941	423.6	2516.4	11.20	
3	0.952	431.1	0.954	446.1	16.66	0.989	236.1	6060.6	26.99	

Total Height 2677.09 Total Area : 22458.3

Fig. 4.30: Integration result for methanol leaf fraction 08 from *Ficus thonningii*

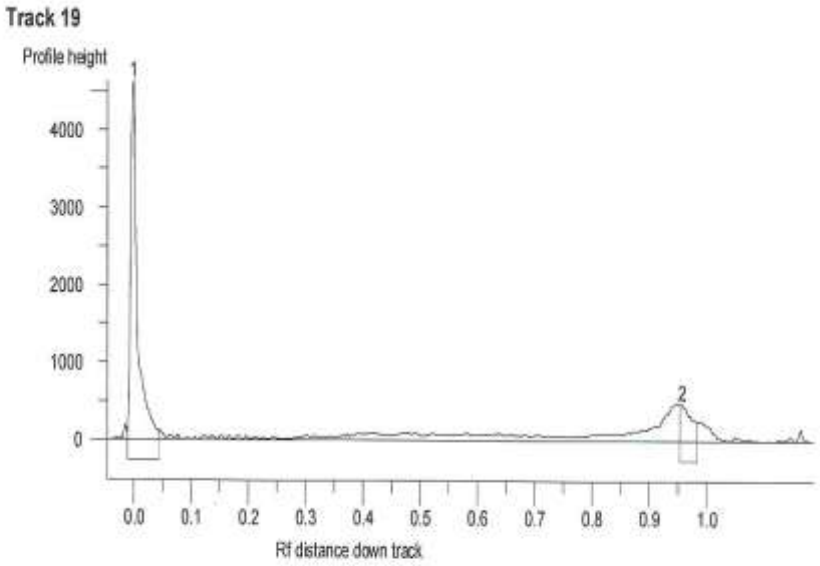


Track 17

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	0.0	-0.004	2290.0	65.82	0.007	433.4	12410.6	55.60	
2	0.007	433.4	0.009	441.7	12.70	0.037	178.6	4842.4	21.69	
3	0.959	400.3	0.963	408.5	11.74	0.981	259.9	3860.3	17.29	
4	1.163	178.0	1.169	338.9	9.74	1.000	27.4	1209.5	5.42	

Total Height 3478.99 Total Area : 22322.8

Fig. 4.31: Integration result for methanol leaf fraction 09 from *Ficus thonningii*

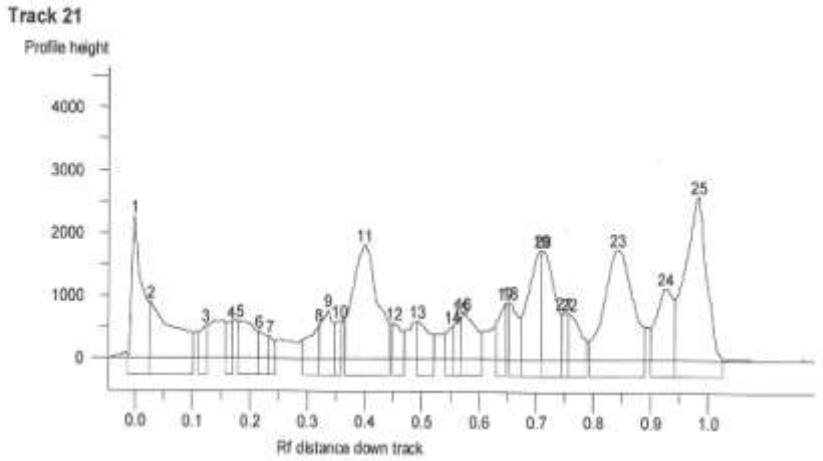


Track 19

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	103.5	0.000	4632.8	90.53	0.045	101.1	34221.4	85.51	
2	0.954	481.8	0.957	484.3	9.47	0.983	256.5	5801.0	14.49	

Total Height 5117.16 Total Area : 40022.4

Fig. 4.32: Integration result for methanol leaf fraction 10 from *Ficus thonningii*



Track 21

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.000	47.4	0.000	2261.0	9.13	0.026	886.3	24799.1	6.39	
2	0.026	886.3	0.028	887.0	3.58	0.102	395.3	22784.9	5.87	
3	0.111	408.8	0.124	525.1	2.12	0.126	524.8	3775.9	0.97	
4	0.157	568.2	0.169	582.4	2.35	0.170	582.1	4027.2	1.04	
5	0.180	575.3	0.183	600.7	2.43	0.215	431.1	10296.8	2.65	
6	0.215	431.1	0.217	434.5	1.75	0.233	351.5	3929.6	1.01	
7	0.233	351.5	0.235	363.5	1.47	0.244	287.7	1930.4	0.50	
8	0.293	298.1	0.320	541.7	2.19	0.322	535.5	6222.7	1.60	
9	0.322	535.5	0.337	773.1	3.12	0.348	550.0	9028.3	2.33	
10	0.348	550.0	0.357	599.6	2.42	0.359	593.3	3504.4	0.90	
11	0.367	665.8	0.400	1817.8	7.34	0.444	544.8	48786.4	12.57	
12	0.448	540.9	0.452	577.3	2.33	0.470	453.7	6108.8	1.57	
13	0.491	583.2	0.493	597.9	2.41	0.522	392.9	8387.2	2.16	
14	0.541	406.9	0.554	520.2	2.10	0.556	513.9	3791.4	0.98	
15	0.556	513.9	0.567	675.5	2.73	0.569	671.2	4238.0	1.09	
16	0.569	671.2	0.572	729.6	2.95	0.606	445.7	11844.5	3.05	
17	0.630	526.6	0.646	895.6	3.62	0.648	894.3	7426.6	1.91	
18	0.652	905.0	0.654	905.4	3.66	0.674	662.9	9385.5	2.42	
19	0.674	662.9	0.707	1739.3	7.02	0.709	1736.3	23786.9	6.13	
20	0.709	1736.3	0.711	1743.0	7.04	0.744	722.4	24580.3	6.33	
21	0.744	722.4	0.748	747.5	3.02	0.756	728.0	4401.2	1.13	
22	0.756	728.0	0.757	729.0	2.94	0.789	301.7	9868.1	2.54	
23	0.793	301.5	0.843	1756.9	7.09	0.889	542.6	53868.3	13.88	
24	0.900	519.2	0.926	1145.4	4.62	0.943	978.1	20901.2	5.39	
25	0.943	978.1	0.983	2618.0	10.57	1.000	26.6	60387.9	15.56	

Total Height 24767.1 Total Area : 388061

Fig. 4.33: Integration result for methanol leaf fraction 11 from *Ficus thonningii*

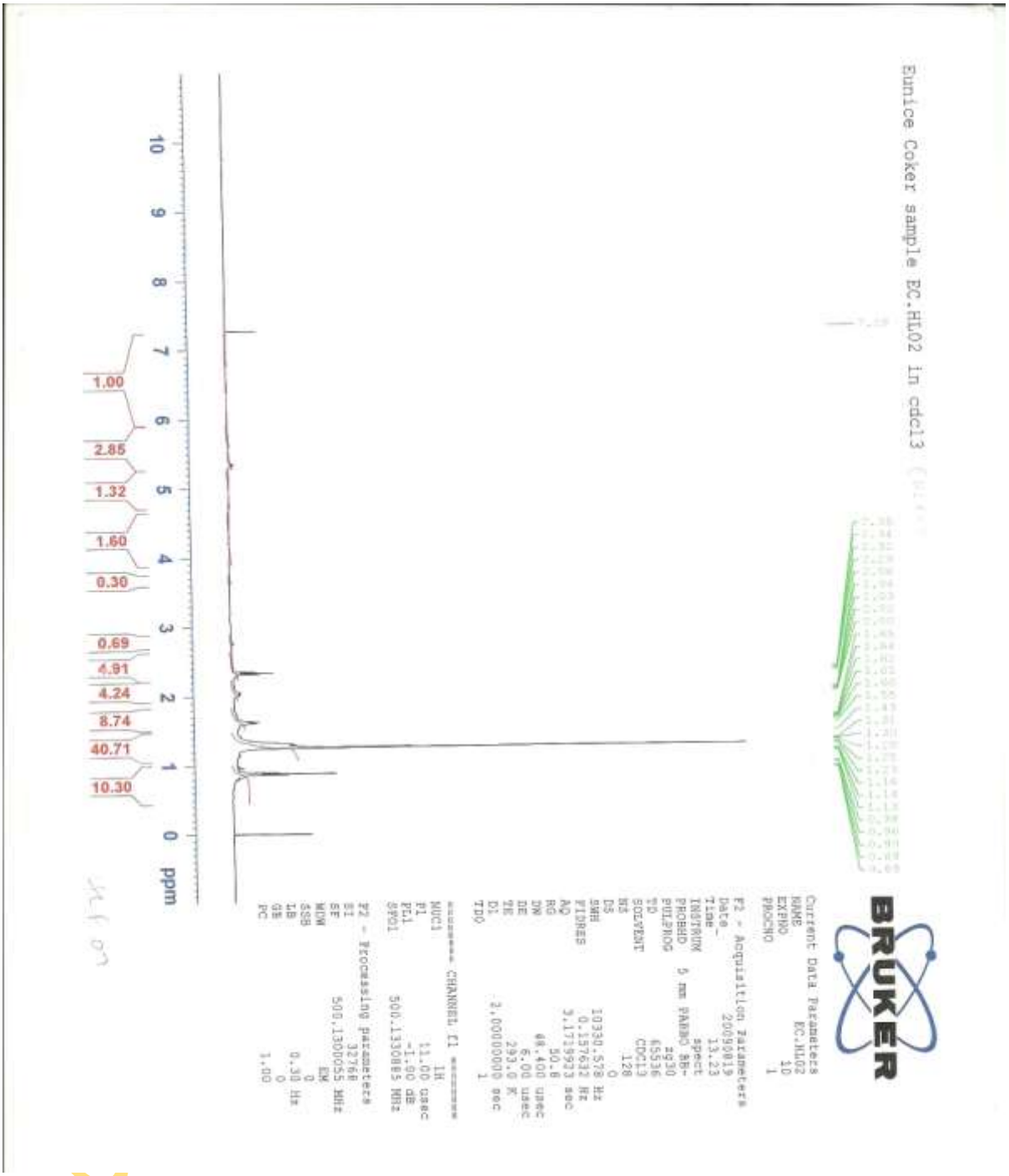


Fig. 4.34: ^{1}HNMR spectrum of purified bioactive compound (EC.HL02) from hexane leaf fraction 07 in $CDCl_3$

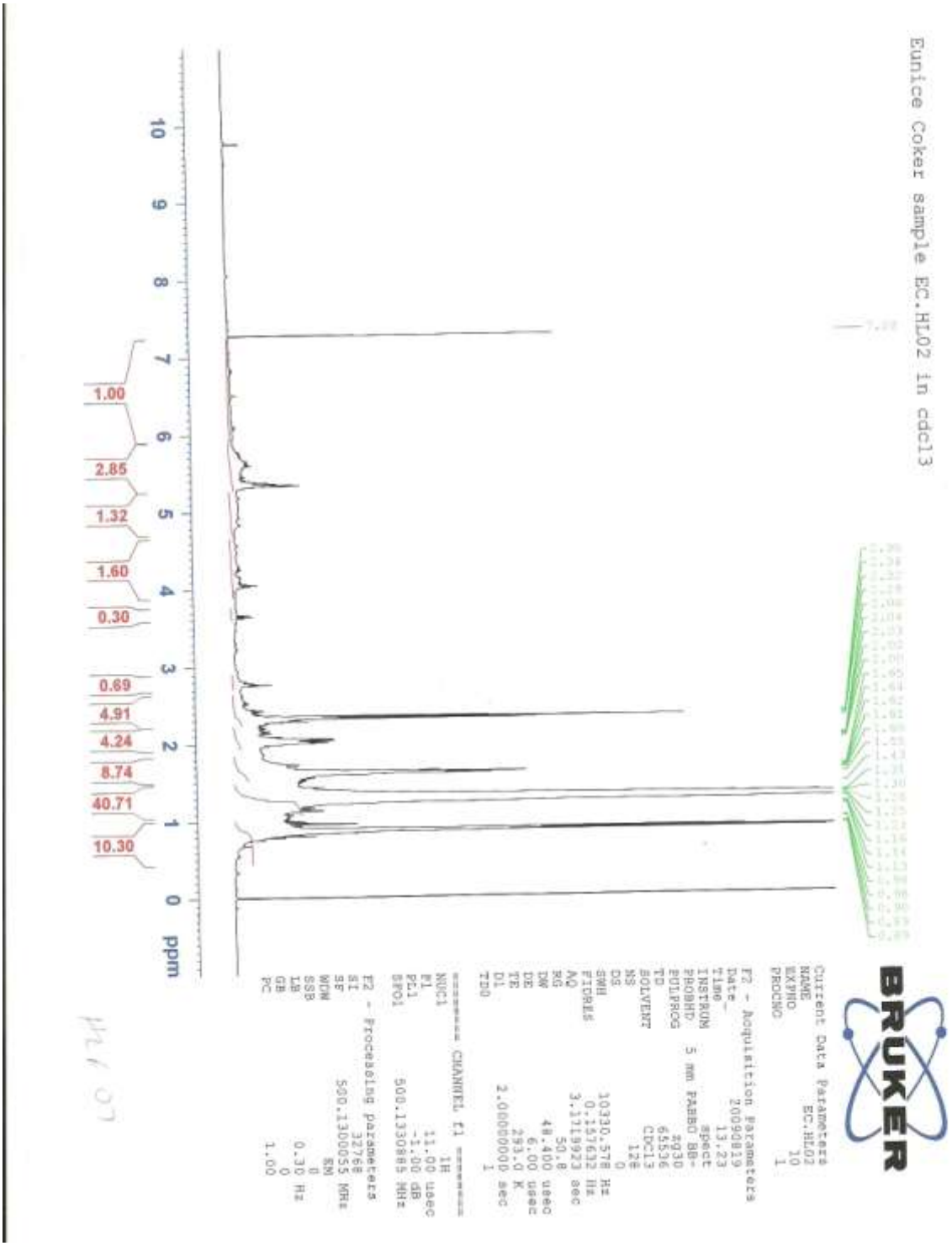


Fig. 4.35: ^{1}HNMR Spectrum of purified bioactive compound (EC.HL02) from hexane leaf fraction 07 in $CDCl_3$

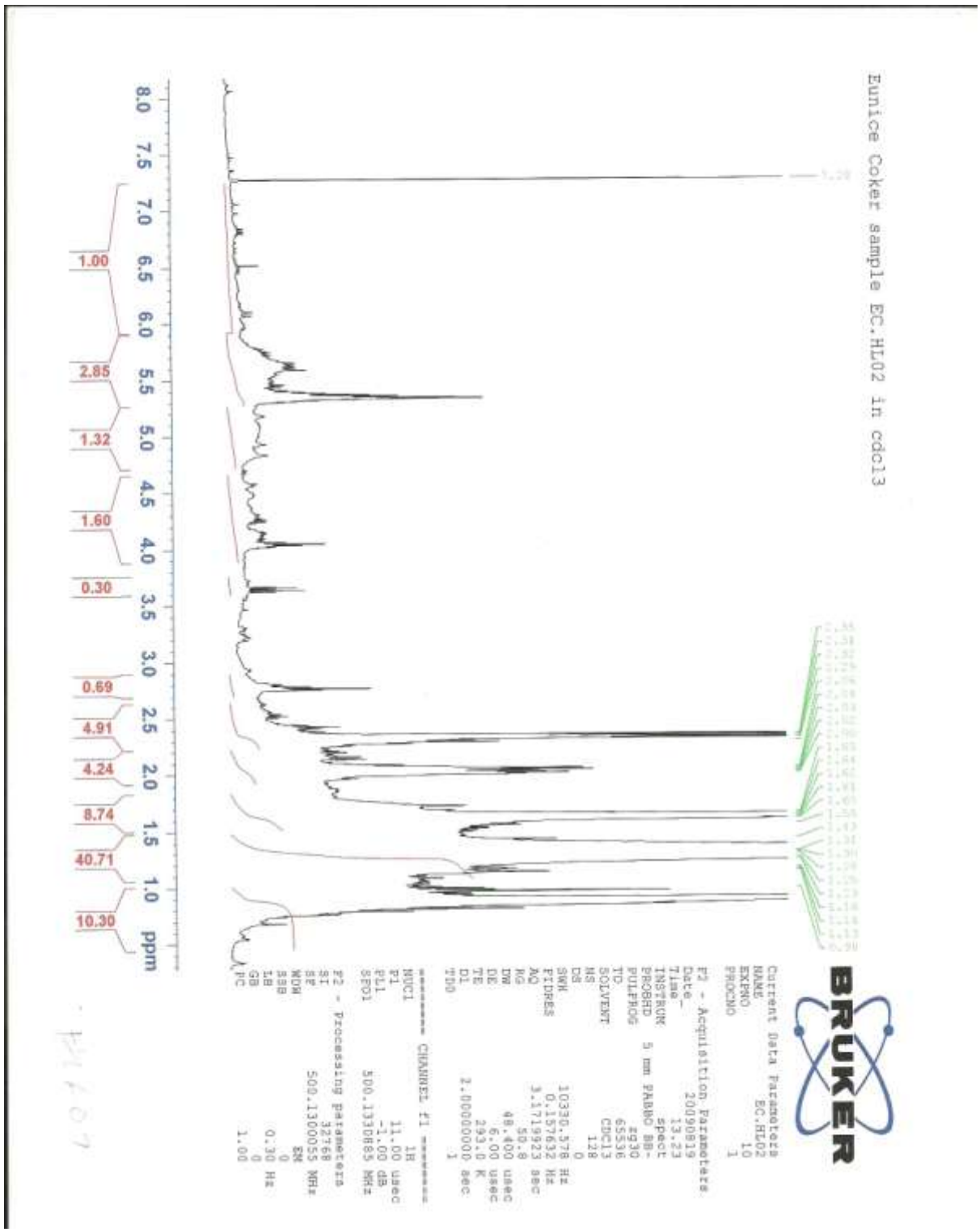


Fig. 4.36: ^{1}HNMR Spectrum of purified bioactive compound (EC.HL02) in $CDCl_3$

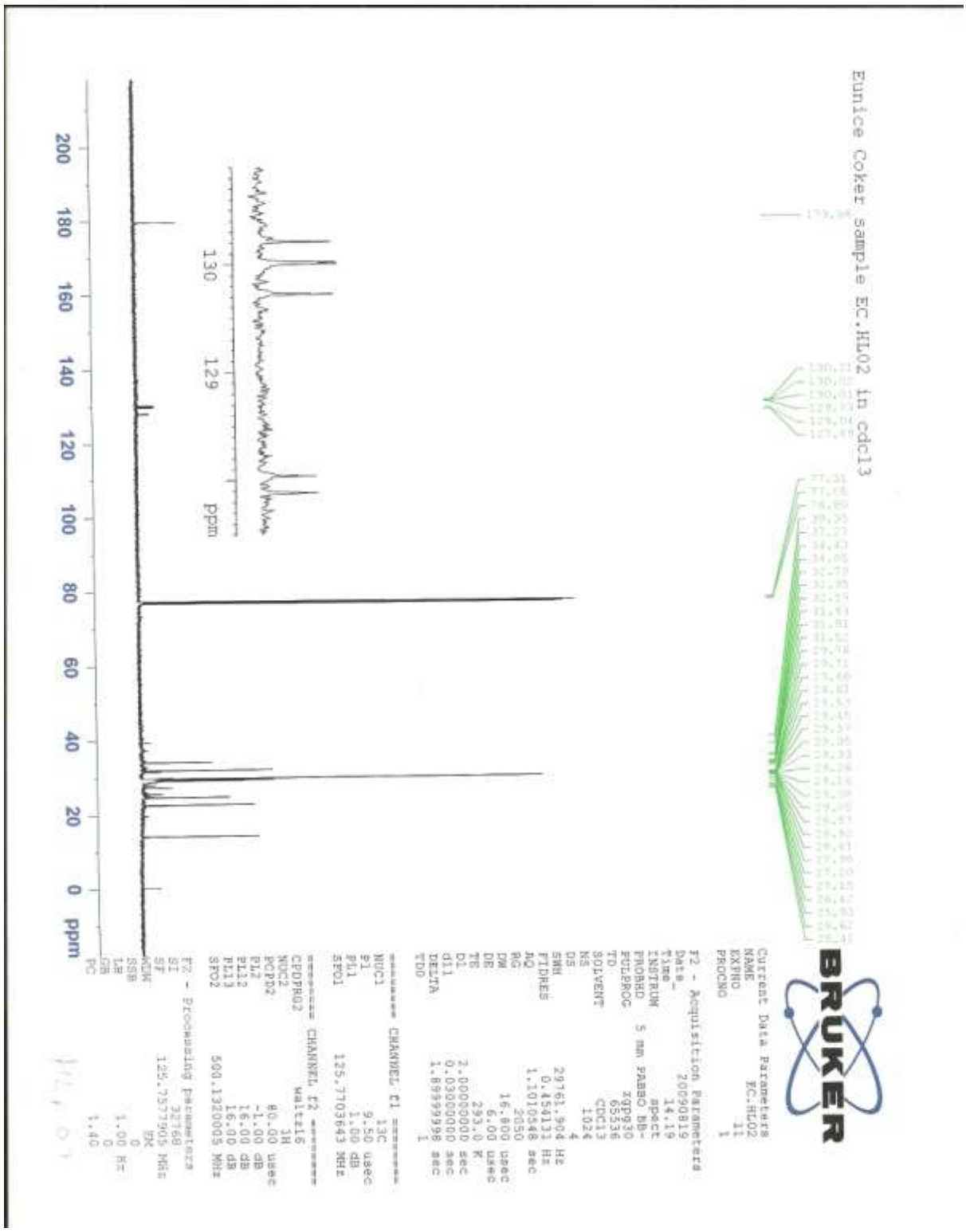


Fig. 4.37: ^{13}CNMR spectrum of purified bioactive compound (EC.HL02) from hexane leaf fraction 07 in $CDCl_3$

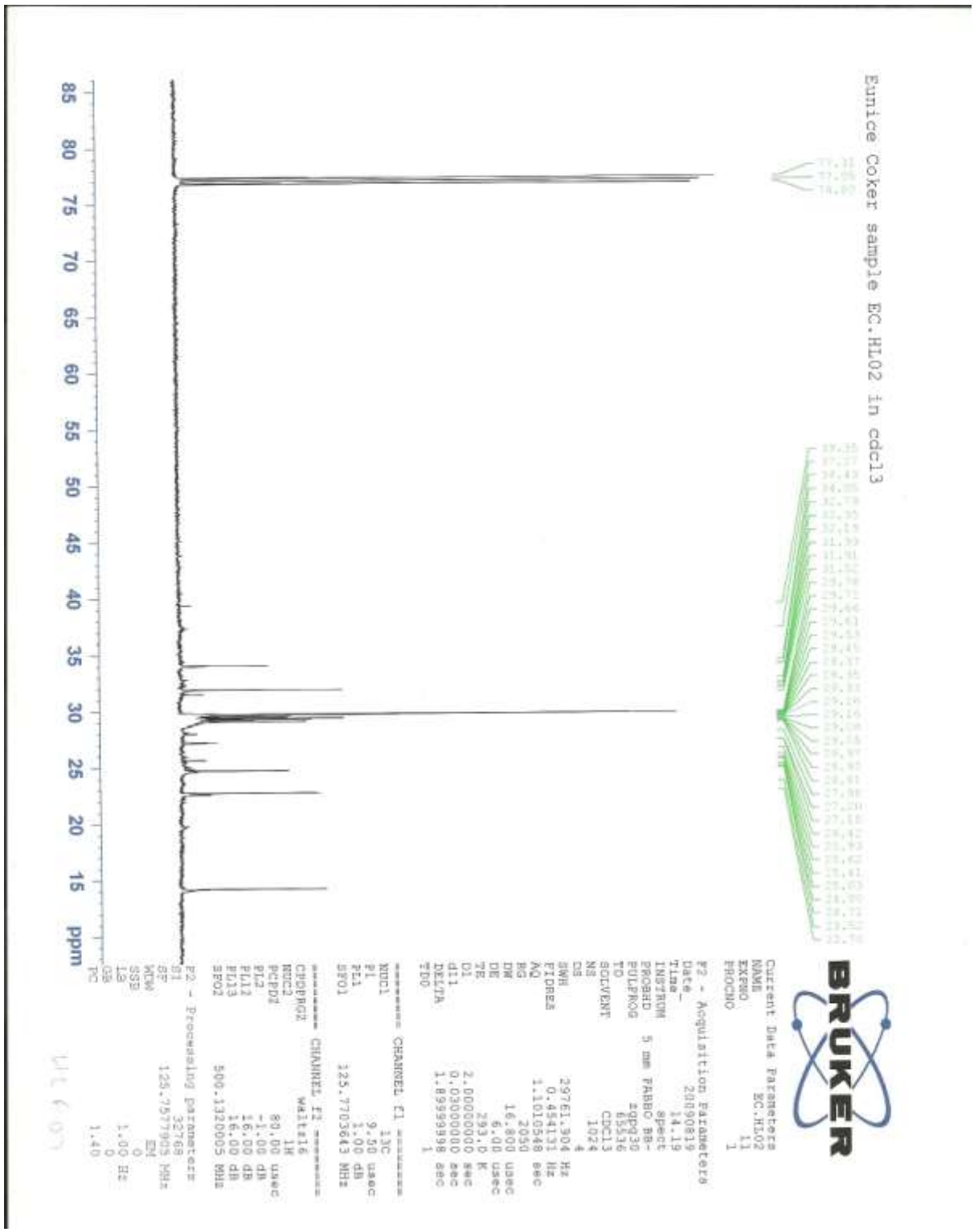


Fig. 4.38: ^{13}CNMR spectrum of purified bioactive compound (EC.HL02) from hexane leaf fraction 07 in $CDCl_3$

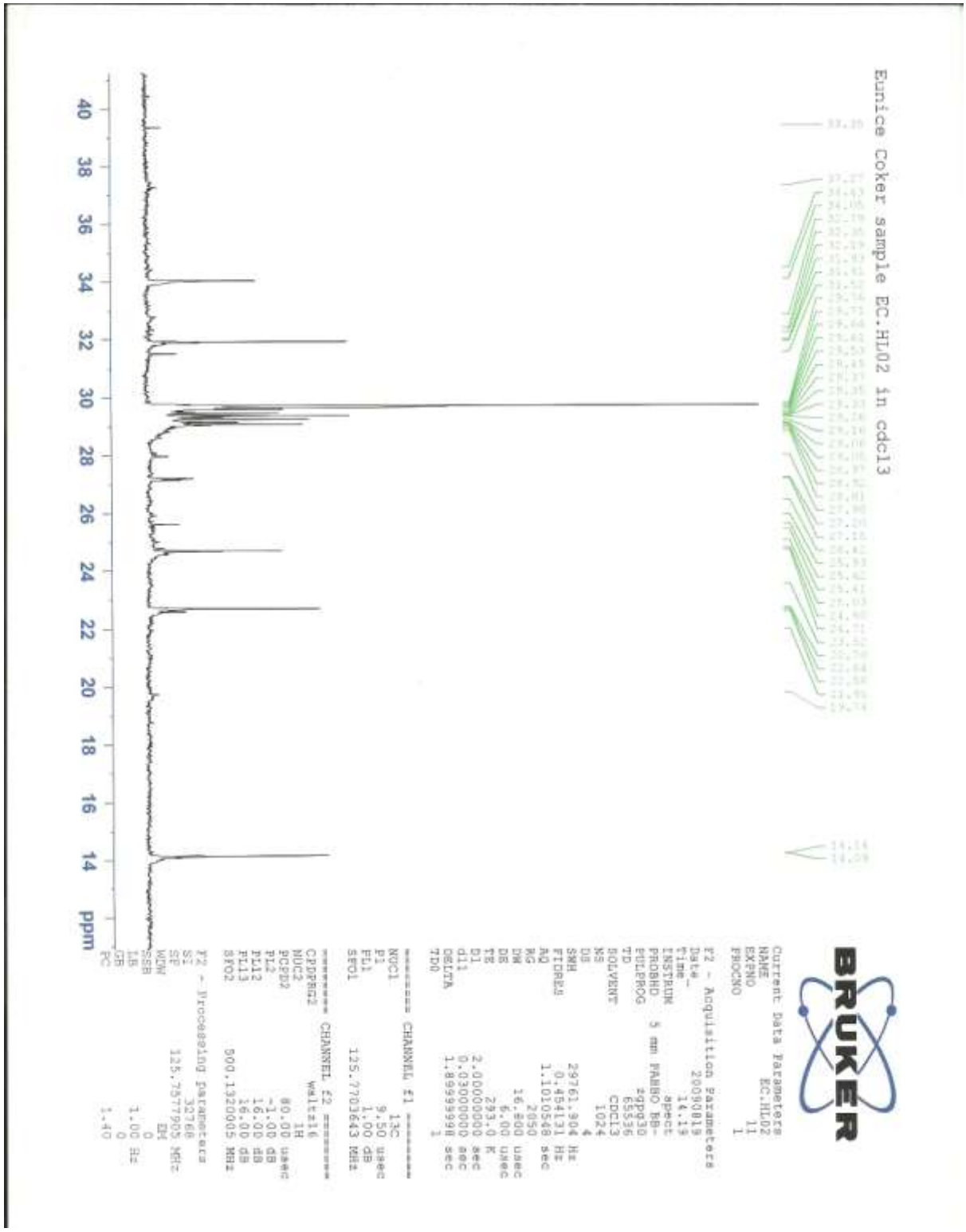


Fig. 4.39: Expanded ^{13}CNMR spectrum of purified bioactive compound (EC.HL02) from hexane leaf fraction 07 in $CDCl_3$

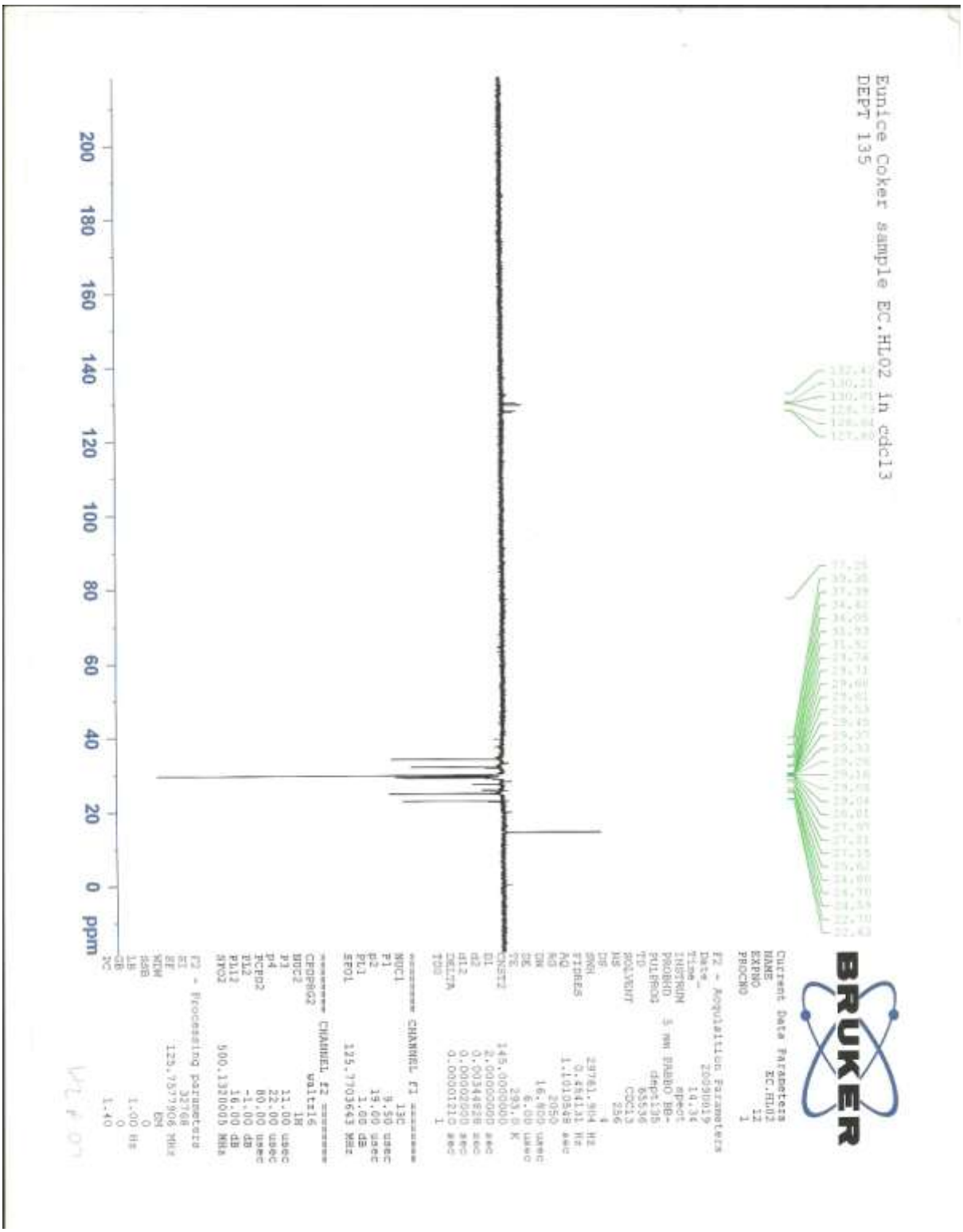


Fig. 4.40: DEPT spectrum of purified bioactive compound (EC.HL02) from hexane leaf fraction 07 in $CDCl_3$

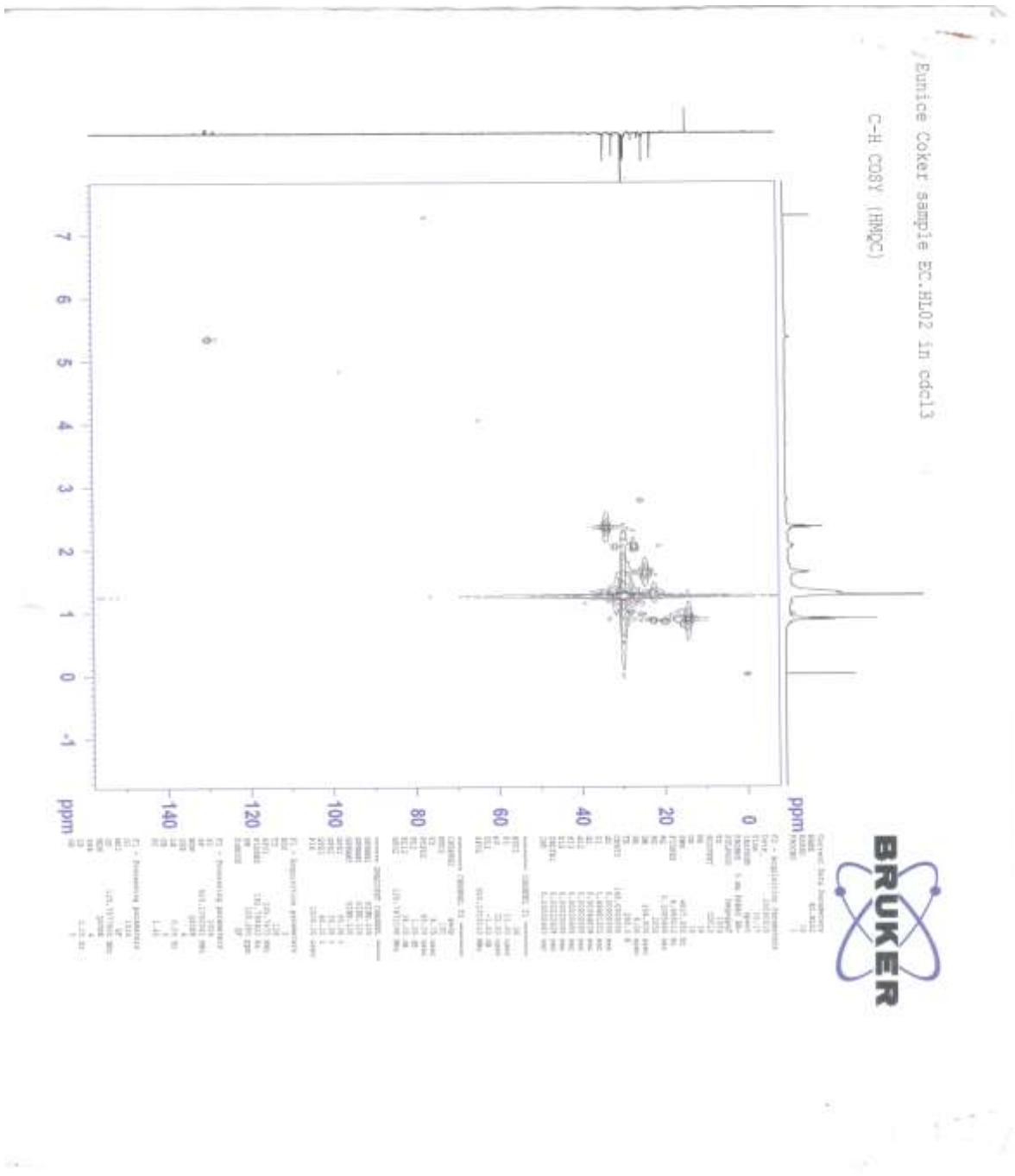


Fig. 4.41: C-H COSY (HMQC) of purified bioactive compound (EC.HL02) from hexane leaf fraction 07 in $CDCl_3$

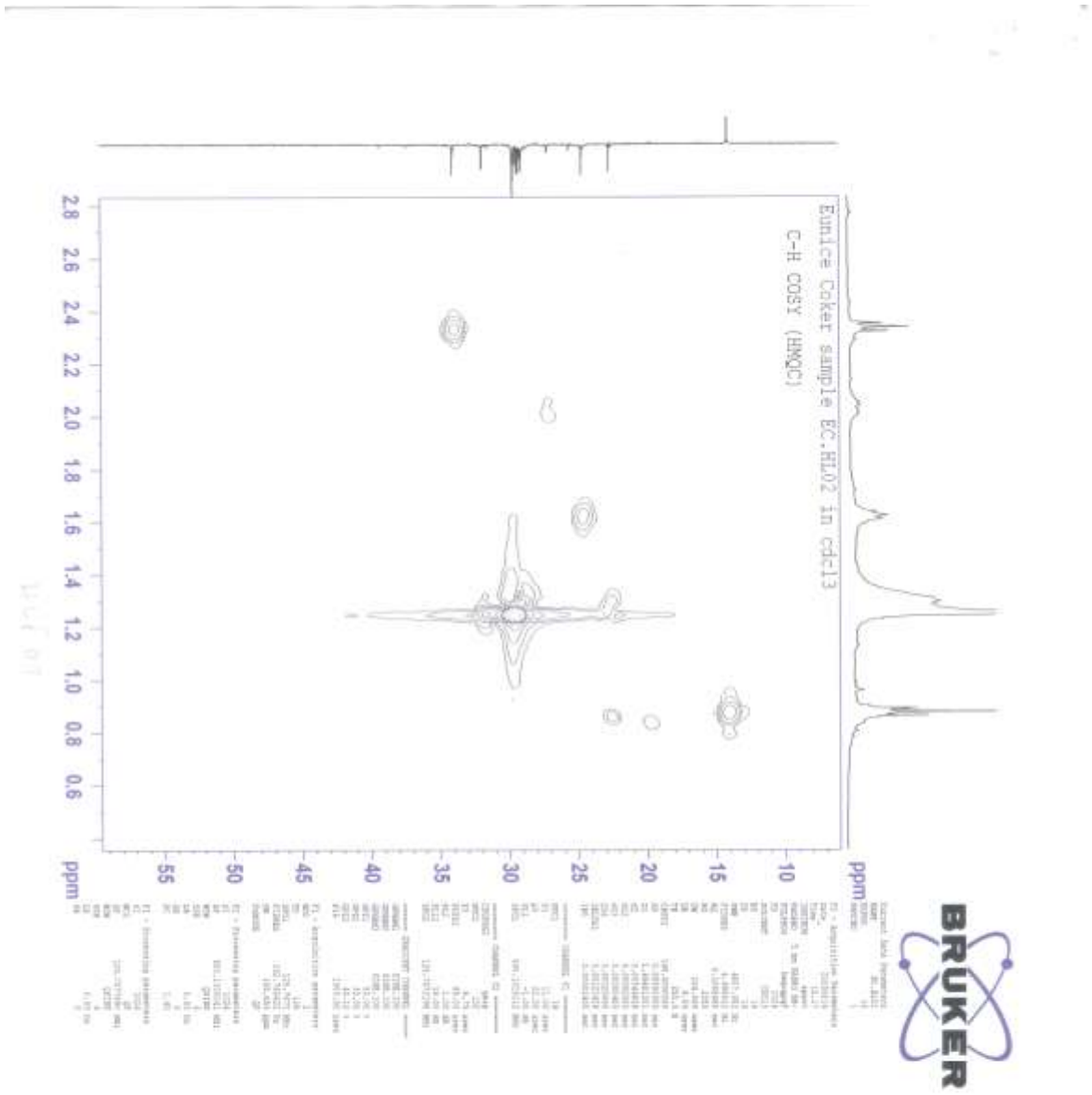


Fig. 4.42: Expanded C-H COSY (HMQC) of purified bioactive compound (EC.HL02) from hexane leaf fraction 07 in $CDCl_3$

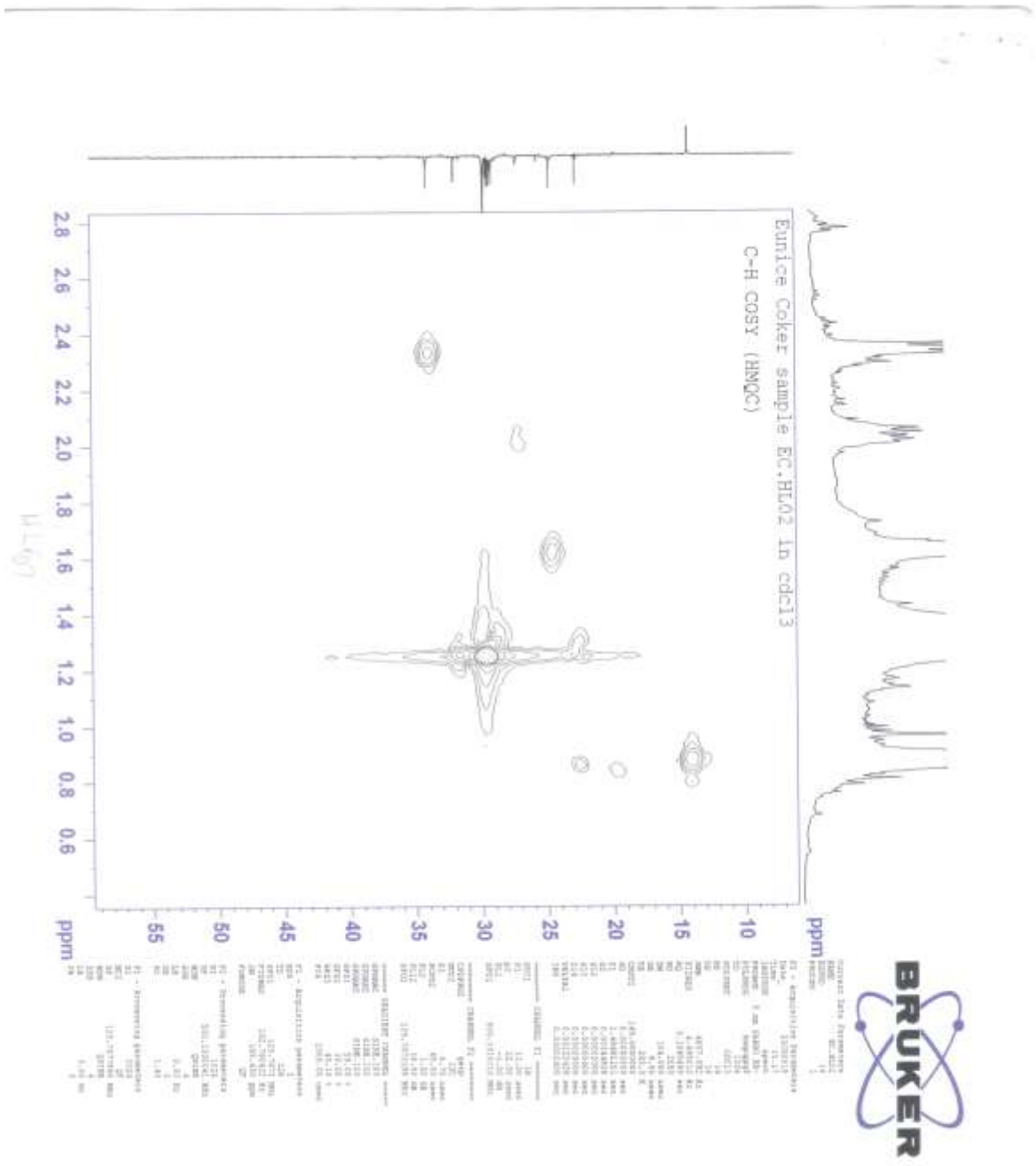


Fig. 4.43: HMQC spectrum of purified bioactive compound (EC.HL02) from hexane leaf fraction 07 in $CDCl_3$

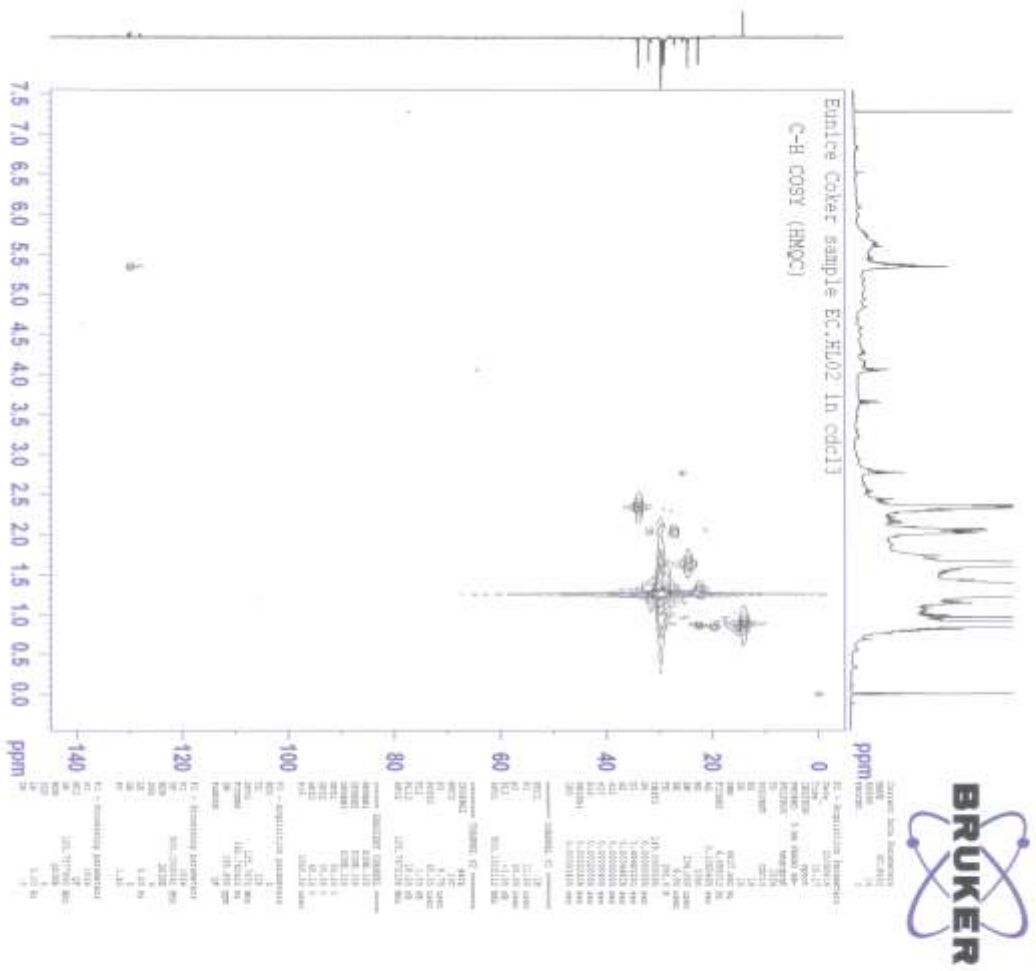


Fig. 4.44: HMQC spectrum of purified bioactive compound (EC.HL02) from hexane leaf fraction 07 in $CDCl_3$

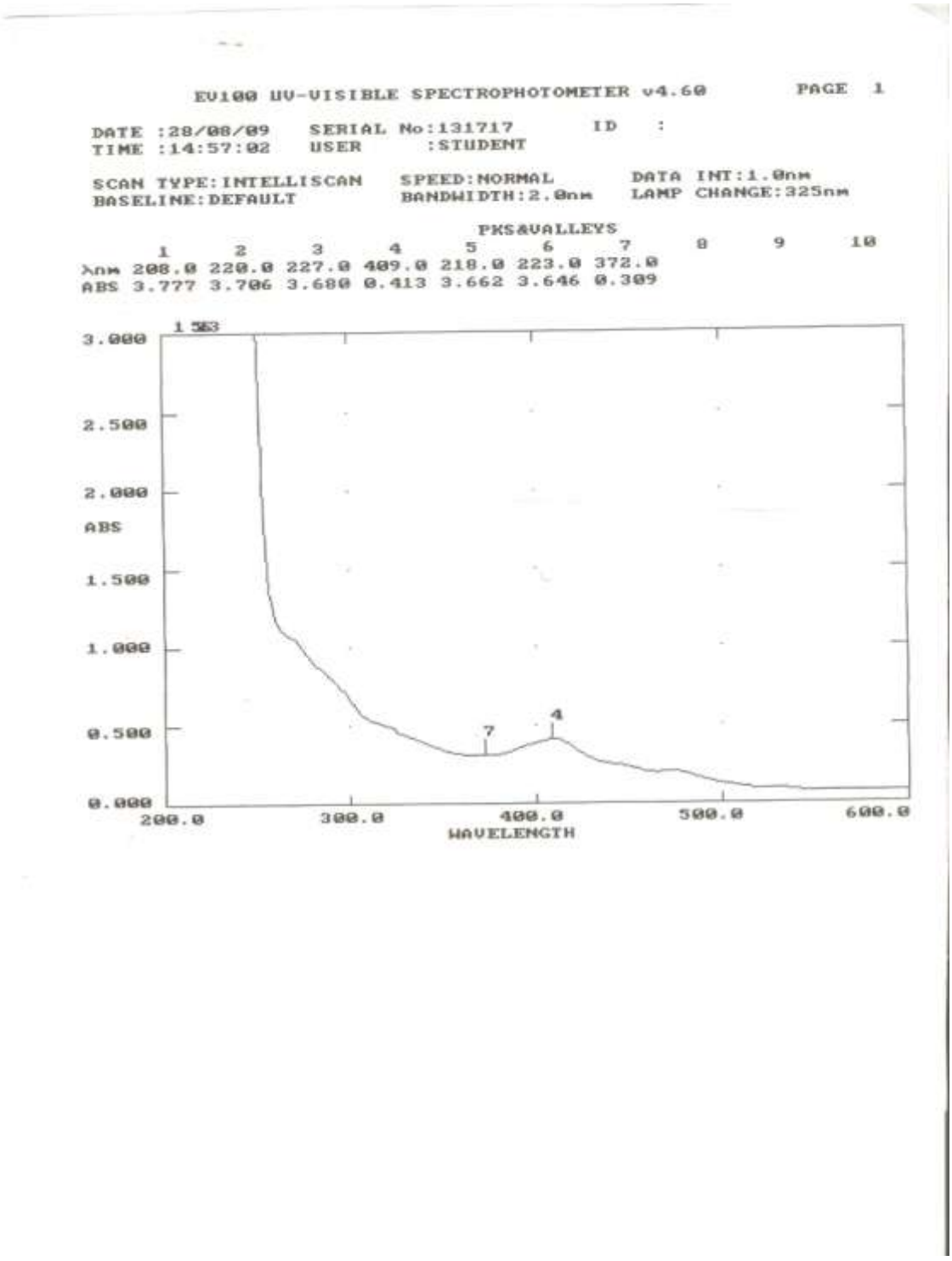


Fig. 4.45: Ultra Violet absorption spectrum of purified bioactive compound (EC.HLO2) from *Ficus thonningii*

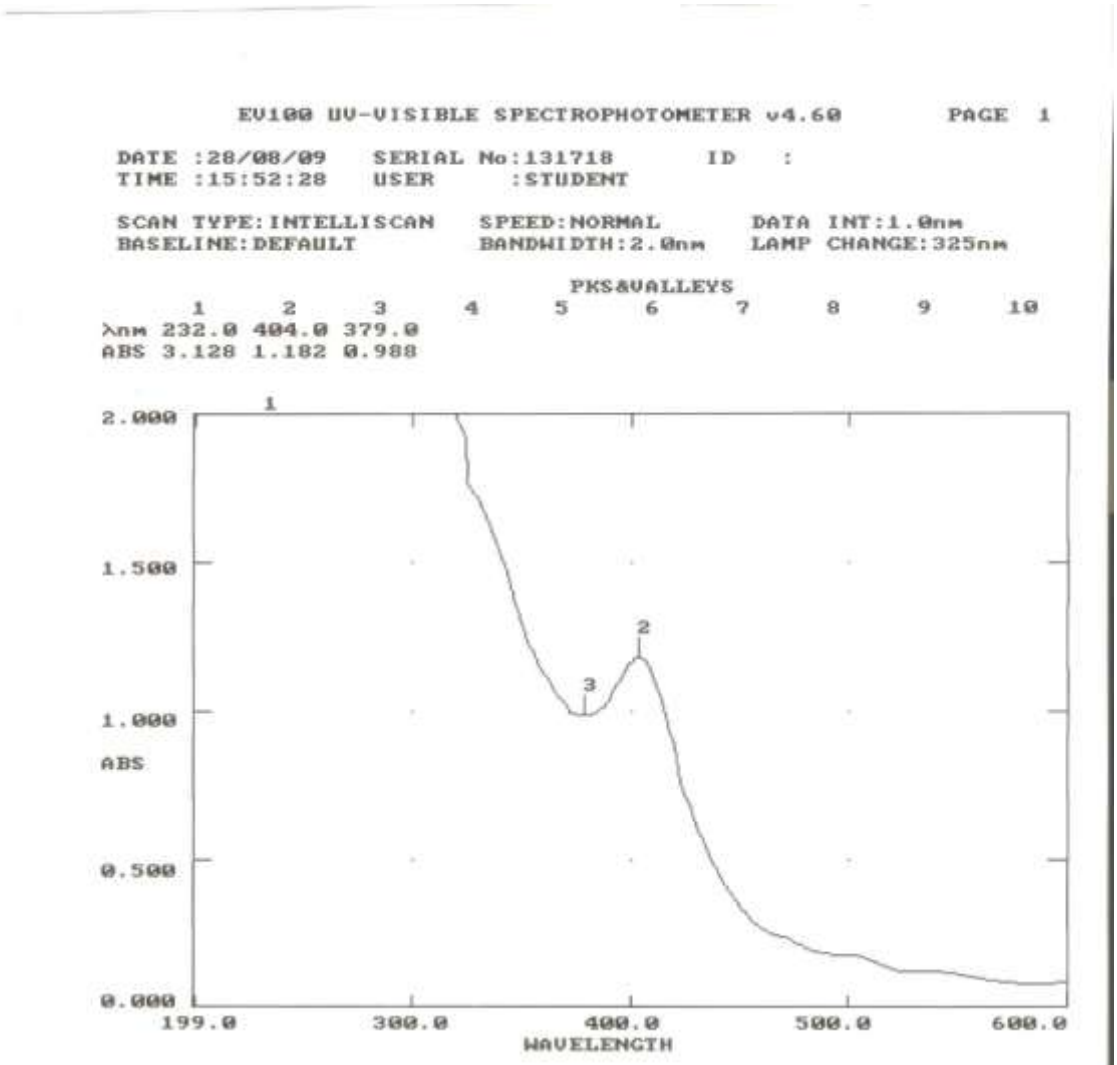


Fig. 4.46: Ultra Violet absorption spectrum of methanol leaf fraction from *Ficus thonningii*

PROPOSED STRUCTURES FOR EC.HL02

CHAPTER FIVE
DISCUSSION AND CONCLUSION

The plant kingdom has many species of plants containing substances of medicinal value which are yet to be discovered. Paavilainen (2005) suggested that once the use of a plant is strongly associated with a particular illness, and there are reports of amelioration of symptoms of the disease following the use of a specific herb, proof of concept studies could seek confirmation of the traditionally-presumed pharmacological action with focus on drug discovery. Hence, the choice of *Ficus thonningii* Blume (Moraceae), a plant used culturally in ethno-medicine in the treatment of various diseases associated with microbial infections such as bronchitis, urinary tract infections, diarrhoea and wounds for this study.

Succesive extraction with hexane, chloroform and methanol was to facilitate the phytochemical analysis and bioassay-guided isolation of the antimicrobial constituents of the plant extract. There were higher yields in the chloroform and methanol extracts than in hexane extract. Less polar compounds are extracted by hexane while the moderately polar compounds are extracted by chloroform or ethyl acetate and the most polar compounds are extracted by methanol. The higher yields obtained in the chloroform leaf extract, methanol leaf extract, and methanol stem bark extract were indications that polar constituents were abundant in the plant. Though, the traditional practitioners make use of water primarily as a solvent, the extraction of the plant materials carried out in this study was done using the organic solvents hexane, chloroform and methanol because these solvents are easily evaporated and permit an easier estimation of extract concentration which is difficult to obtain with water as solvent.

The phytochemical screening carried out confirmed the presence of tannins, flavonoids, saponins, and cardiac glycosides as reported by Onwkaeme and Udoh (2000). The presence of alkaloids and terpenoids that had not been reported was also revealed by the screening. The pharmacological activities of most medicinal plants have been found to be directly related to the types of secondary metabolites they contain (Edeoga *et al.*, 2005).

Antimicrobial susceptibility test is often used to dictate specific management for individual patients and it assists in selecting the appropriate targeted antibiotic therapy in order to optimize clinical outcomes for such patients. The antibiogram of the bacterial isolates used for the study showed that 80% of the Gram-positive bacteria and 81% of Gram-negative bacteria were resistant to 3 or more of the commonly used antibiotics in the treatment of microbial infections. Organisms that showed resistance to at least three or more antibiotics of different structural classes were considered as being multidrug-resistant as described by Sahm *et al.* (2001).

With the continous use of antibiotics, microorganisms have become resistant thus rendering the existing conventional drugs obsolete in the treatment of infectious diseases. Amongst the Gram-negative bacteria, highest percentages of resistance towards standard antibiotics were found for ampicillin (100%), amoxicillin-clavulanate (Augmentin®) (91%) and cefuroxime (55%). For the Gram-positive organisms, the highest resistance were found for erythromycin (100%), amoxicillin-clavulanate (80%), cloxacillin (70%) and ceftazidime (60%). No isolate was found to be sensitive to all the antibiotics. The highest sensitivity recorded for Gram-negative bacteria was to ciprofloxacin (82%) and for Gram-positive bacteria (80%) to gentamicin. All the *E. coli* and *K. aerogenes* strains were found to be sensitive to nitrofurantoin which is one of the first line drugs in the treatment of UTI. The antibiotic resistance pattern of bacterial pathogens isolated from UTI patients in Nepal has shown multidrug-resistance amongst UTI pathogens (Baral *et al.,* 2012) with Gram-negative organisms showing resistance to amoxicillin (57.7%), co-trimoxazole (53.6%), norfloxacin (36.4%) and Gram-positive organisms showing resistance to cephalexin (33.4%), cloxacillin (33.4%) and co-trimoxazole (33.4%). *F. thonningii* crude extracts and fractions showed broad spectrum antimicrobial activity against a wide range of multidrug-resistant Gram-negative and Gram-positive organisms and fungi.

S. typhi I that was resistant to the standard antibiotics showed slight sensitivity to hexane leaf, hexane stem bark, chloroform stem bark and methanol stem bark crude extracts of plant. Also, *S. typhi* II which showed multidrug-resistance (75%) was sensitive to all the crude extracts of plant except methanol stem bark extract. Gautam *et al.,* (2002) reported that 70% of *Salmonella typhi* isolated in India in 1992 were

multi drug resistant. The report by Akinyemi *et al.* (2005), showed that the number of MDR *Salmonella* isolated from the period of 1979 to 2005 was between 70-80%, an indication that multi drug resistant *Salmonella* are prevalent in Nigeria as in other parts of Africa, Asia, North and Central America. *Salmonella* resistance to fluoroquinolones and third generation cephalosporins has been reported among typhoidal and non-typhoidal Salmonella strains worldwide (Crump *et al.*, 2008). Drug resistance among *Salmonella* species may be on the increase especially in developing countries where there is indiscriminate use of antibiotics and measures must be taken to control the spread of resistant *Salmonella*.

All the crude extracts had some inhibitory effects against MDR *P. aeruginosa* used for the study, with zones of inhibition ranging from 8-14 mm. All the *Bacillus* species (spore formers) which were multidrug-resistant (68.8%) were sensitive to all the crude extracts of F. *thonningii* with highest sensitivity being observed in hexane leaf crude extract.

In the preliminary antimicrobial screening of the crude extracts against test microorganisms, the hexane and methanol extracts were the most active and hence fractionation by chromatographic methods was carried out with the two extracts. The hexane and methanol extracts of the leaf and stem bark of *F thonningii* demonstrated good antimicrobial activity on Gram-positive and Gram-negative bacteria and also moderate activity on the moulds and yeast. The antimicrobial activity of leaf extracts was very similar to that of stem bark extracts with a slightly higher activity in the leaf extracts, thus, the leaf extracts were used for further studies.

The plant extracts had antimicrobial activity on the sensitive and multidrug-resistant strains of the isolates used. The plant extracts had good inhibitory effect against the clinical wound isolates of *S. aureus* (*S.aureus* II) and *P. aeruginosa* (*P.aeruginosa* II) and the clinical isolates of *E. coli* (*E. coli* II) and *K. aerogenes* (*K. aerogenes* I) from urine and urethral discharge respectively. The plant extracts also had some inhibitory effects against some organisms which were resistant to the control drugs used. The clinical isolate of *Salmonella typhi* that was resistant to gentamicin and ciprofloxacin was sensitive to the plant extracts. Ampicillin resistant strains of *S.*

aureus, *E. faecalis*, *S. pyogenes* and gentamicin resistant strain of *S. pyogenes* were sensitive to most of the extracts.

Fractionation of the extracts did not lead to loss of antimicrobial activity as the fractions obtained from the leaf and stem bark of plant exhibited improved antimicrobial activity to the crude extracts. The fractions showed good antimicrobial activity on Gram-positive bacteria (*S. aureus* and *B. cereus*), Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and moderate activity was demonstrated against fungal yeast *Candida albicans*. The MIC and MBC values of the bioactive fractions were much lower than that of the crude extracts. Hexane leaf fractions showed MIC range of 39-625 µg/mL on bacteria while hexane leaf crude extract showed MIC range of 156 to >625 µg/mL. HLF07 showed MIC of 20 µg/mL on *A. niger* as compared with 78µg/mL for hexane leaf crude extract. The MBC of HLF07 on *A. niger* was 20 µg/mL while that of hexane leaf crude extract was 78 µg/mL. The antimicrobial activity of *Ficus ovata* has been reported (Kuete *et al*., 2009). The MIC for the crude extract of stem bark on bacterial and fungal isolates ranged from 156-625 µg/mL while that of the fractions ranged from 39-625 µg/mL. The lowest MIC value (156 µg/mL) observed with the crude extract of *Ficus ovata* was on *Streptococcus faecalis*, *Candida albicans* and *Microsporum audouinii* (Kuete *et al*., 2009). Leaf extract of *Ficus racemosa* was found to be active on Gram-negative and Gram-positive bacteria, namely *E. coli*, *P. aeruginosa*, *Bacillus pumilis* and *S. aureus* (Mandal *et al*., 2000). Anti-bacterial, anti-fungal and anti-mycobacterial activities of *Ficus chlamydocarpa* and *Ficus ovata* have also been reported (Kuete *et al*., 2008). The ethanol extract of the stem bark of *Ficus exasperata* has been reported to have a broad spectrum of activity against Gram-positive and Gram-negative bacteria as well as the fungus *Candida albicans* with the highest susceptibility in *S. aureus* (Amponsah *et al*., 2013). The chloroform and ethanol fractions of *Ficus exasperata* showed considerable activity against Gram-positive and Gram-negative organisms with the chloroform fraction being most active against *P. aeruginosa* and *S. aureus* (MIC of 1000 µg/mL for both organisms)

Further purification of hexane leaf fraction (HLF07) yielded a white crystalline compound (EC.HL02), a triterpenoid which showed an improved activity to the bioactive fractions against all bacterial and fungal isolates used. The MIC value of

EC.HL02 for *A. niger* was comparable to that of tioconazole. For the bacterial isolates, the activity was most pronounced against *S.aureus* while the least activity was on *B. subtilis* and *S. typhi*. Terpenoids have been isolated from the stem bark of *Ficus ovata*, namely 3-friedelanone, taraxeryl acetate, betulinic acid and oleanoic acid. The MIC of the isolated compounds ranged from 10-312 µg/mL on tested microorganisms (Kuete *et al*., 2009).

The bioactive fractions of *F. thonningii* and the isolated compound showed good inhibitory activity against all the strains of organisms used that are associated with wound infection with highest inhibitory activity against *S. aureus* and *K. aerogenes*. Wound infections are known to be most common in developing countries due to poor hygienic conditions and wound colonization is most frequently polymicrobial (Bowler, 1998), involving numerous microorganisms that are potentially pathogenic. It has been reported that aerobic pathogens such as *S. aureus*, *P. aeruginosa* and beta-hemolytic *Streptococci* are the primary causes of delayed healing and infection in both acute and chronic wounds (Daltrey *et al.*, 1981). Also, *S. aureus*, *P. aeruginosa*, *E. coli*, *Klebsiella* spp, *Enteroccocus* spp and *Candida* spp have been implicated in burn wound infections (Revathi *et al*., 1998). *S. aureus* has been reported as the commonest organism isolated from surgical and non surgical wounds (Segupta *et al*., 1978) and the organism has been implicated in acute suppurative infections and superficial infections. The crude extracts, fractions and isolated triterpenoid would be useful in the treatment of wound infections which is reflected in the folkloric uses of the plant.

The bioactive fractions had good inhibitory activities against all the strains of *E. coli* used for this study. *Pseudomonas aeruginosa*, a causative organism for urinary tract infections, respiratory system infections, bacteremia, systemic and soft tissue infections was sensitive to the crude extracts and fractions of *F. thonningii*. Urinary tract infection (UTI) is a common disease caused by bacteria which has contributed to frequent cause of morbidity in both out-patients and hospitalized patients (Wagenlehner and Naber, 2005). Enterobacteriaceae are predominantly the causative organisms of UTI, followed by Gram-positive cocci (Zhanel *et al.*, 2000). *E. coli* has been found as a common uropathogen world wide and antimicrobial therapy of UTI caused by *E. coli* is often impaired due to the resistance of the organism to

antimicrobial agents commonly used in treating the infection (Chakupurakal *et al.*, 2010). The high prevalence of multidrug resistance in bacterial uropathogens has been reported (Zhanel *et al.*, 2000; Baral *et al.*, 2012) with resistance patterns alarmingly higher for amoxicillin, fluoroquinolones, co-trimoxazole, and third-generation cephalosporins (Baral *et al.*, 2012). The antimicrobial activities demonstrated by the extracts and fractions of plant make it a potential crude drug in effective treatment of urinary tract infections. This validates the ethnomedicinal use of the leaves and fruits of *F. thonningii* in the treatment of urinary tract infections (Iwu, 1993).

E. coli, an organism which is frequently implicated in urinary tract infection is also implicated in severe food borne diseases. Morbidity and mortality due to diarrhoea are major problems in developing countries, especially amongst children and infections due to a variety of bacterial etiologic agents such as pathogenic *E. coli*, *Salmonella* species, *Klebsiella* species, *Shigella* species, *Vibrio cholerae* and *S. aureus* are most common (Mukesh *et al*., 2012). The bioactive fractions and isolated triterpenoid of *F. thonningii* had appreciable inhibitory effects on *E. coli*, *S. typhi*, *K. aerogenes*, *E. faecalis* and *S. aureus* which have shown multi-drug resistance to standard antibiotics. That makes the plant a potentially useful crude drug in reducing deaths due to diarrhoea and other gastrointestinal diseases. Anti-diarrhoeal activity of the leaf extract of *Ficus hispida* in rats has been reported (Mandal and Kumar, 2002). The broad-spectrum antimicrobial activity of the extracts, isolated fractions and the isolated compound of *F. thonningii* give credence to some of the folkloric uses of the plant in the treatment of wounds, urinary tract infections, sorethroat, diarrhoea, and bronchitis which are of microbial origin.

Crude extracts of the plant demonstrated some antimicrobial activity on the moulds and yeast used in the study but there was no inhibition against the dermatophytes, *Trichophyton* and *Microsporum* species used. Hexane leaf extract was active against all the strains of *A. niger*, *C. albicans*, *P. chrysogenum and R. nigricans* tested, and the highest inhibitory activity was against *P. chrysogenum*. The chloroform leaf and stem bark extracts exhibited poor inhibition on the fungal isolates. *Aspergillus* species have emerged as an important cause of life-threatening infections in immune-compromised patients such as patients with advanced HIV

infection, prolonged neutropenia and patients who have undergone hematopoietic stem cell transplantation (Walsh *et al.*, 2008). Invasive aspergillosis caused by *Aspergillus* species (*A. fumigatus*, *A. niger*, *A. flavus* and *A. terreus*) include infections of the lower respiratory tract, sinuses and skin (Barnes and Marr, 2006). Hexane leaf and stem bark extracts, methanol leaf and stem bark extracts inhibited the three strains of *A. niger*. The plant extracts, fractions and the isolated triterpenoid also inhibited *Candida albicans*, an opportunistic dimorphic fungus responsible for a variety of human diseases ranging from superficial skin lesions to disseminate infection. Infections caused by *Candida albicans* are frequently seen in persons with HIV or AIDS and there have been reports of rising incidences of candidemia all over the world (Hsueh *et al.*, 2003). Hexane leaf and methanol stem bark extracts inhibited the three strains of *C. albicans* screened, thus making the plant a potential crude drug in the treatment of infections caused by *Aspergillus niger* and *Candida albicans*. Antifungal activities of *Ficus chlamydocarpa*, *Ficus cordata* and *Ficus ovata* have also been reported (Kuete *et al.*, 2008; Kuete *et al.*, 2009). The MIC range of the methanolic stem bark extract, fractions and isolated compounds of *Ficus ovata* on *Candida albicans* and *Microsporum audouinii* was 10 - 625 µg/mL. The isolated compounds from *Ficus elastica*; emodin, sucrose, morin and rutin showed antibacterial activity against *B. cereus* and *P. aeruginosa* but no antifungal activity was observed against *Aspergillus ochraceous*, *Sacchromyces cerevisae*, *Sacchromyces lipolytica* and *Candida lipolytica*, the fungal species tested (Hassan *et al*., 2003).

The presence of alkaloids, tannins, flavonoids and terpenoids in the plant parts of *F. thonningii* could account for its antimicrobial activity (Bruneton, 1999; Cowan, 1999; Kuete *et al.*, 2007). This activity might be due to the ability of flavonoids to complex with bacterial cell wall and the ability of terpenoids to cause membrane distruption (Cowan, 1999; Arvind *et al.*,. 2004). Some alkaloids have demonstrated antimicrobial properties (Fakeye *et al.*, 2000; Kuete *et al.*, 2007), tannin, a polyphenolic compound posseses astringent and antibacterial properties while flavonoids have been found to be effective against a wide array of microorganisms (Scalbert, 1991; Bennet and Wallsgrove, 1994; Eloff, 1998).

Kill kinetics have been used to demonstrate the bactericidal activity of antimicrobial agents on target organisms to depict better killing synergism in methicillin sensitive *S. aureus* (Okemo *et al*, 2001). The kill kinetics of methanol leaf extract on selected MDR strains of *S. aureus* and *E. coli* showed a bactericidal kinetics that was concentration dependent. The rate and extent of killing at 10.0 mg/mL was higher than that of 5.0 and 2.5 mg/mL. Though, a bactericidal action is usually considered favourable, a total kill is not essential for many purposes. If there is reduction in the number of viable organisms upon the administration of an antibiotic, or the organisms are prevented from further multiplication, the body defence mechanisms together with further doses of the antibiotic at regular intervals can get rid of the remaining microorganisms. A bacteriostatic or bactericidal agent may be used in the treatment of mild infections in patients with normal immunological response, but for more serious infections involving less susceptible organisms, a bactericidal agent will be more effective in adequate treatment. However, in immunologically compromised patients such as AIDS patients and those on long term chemotherapy, treatment with a bactericidal agent may be essential and thus, *F. thonningii* may be useful in the treatment of infections in patients with low immune response. There has been no literature report on the kill kinetics of other species of *Ficus.*

Purification of the hexane leaf fraction (HLF 07) resulted in the isolation of a white crystalline compound which was subjected to spectroscopical analysis and structural elucidation. The ^{1}H NMR and ^{13}C NMR spectra revealed a triterpenoid-like structure as shown by the presence of many methylenes and some methyl groups in the aliphatic region of the spectra. The ^{1}H NMR spectrum further showed signals due to olefinic protons at δ 5.2-5.3 ppm characteristic of 12-oleanene or 12-ursalene skeletons. Another proton singlet was seen at δ 9.8 ppm which could be due to a carboxylic acid proton. This was confirmed by a carbon signal at δ 179.6 ppm in the ^{13}C NMR spectrum typical of a free carboxylic acid. No other oxygenated carbon or proton signals were seen in both ^{1}H NMR and ^{13}C NMR spectra which show the absence of hydroxyl or oxymethylene groups in the compound. This may be due to dehydration of the compound during the process of isolation or the compound could be a new secondary metabolite of oleanane or

ursane skeleton whose structure is yet to be determined. The presence of multiple olefinic signals between δ 5.2 - 5.3 ppm in the ^{1}H NMR and δ 127.9 - 130.2 ppm in ^{13}C NMR spectra suggest that EC.HLO2 may be a mixture of two compounds composed of oleanane and ursane skeletons. Both the ^{1}H NMR and ^{13}C NMR spectra and the DEPT spectrum revealed the presence of many methylene groups which further supported a triterpenoid skeleton. The ^{1}H-^{13}C COSY (HMQC) spectra showed the carbon atoms to which the protons are attached and this further confirmed the proposed structures. The combined spectra suggested that compound EC.HL02 is a triterpenoid and the two proposed structures are that of olean-12-en-28-oic acid and ursol-12-en-28-oic acid.

Many triterpenoids of oleanane and ursane skeletons are known to possess numerous biological and pharmacological activities which include antimicrobial, antiviral, anti- inflammatory, cytotoxic and cardiovascular activities (Connoly and Hill, 2008; Vechia *et al.,* 2009). Oleanoic acid, betulinic acid, taraxeryl acetate and 3-friedelanone are triterpenoids which have been isolated from the stem bark of *Ficus ovata* (Kuete *et al.,* 2009). The antimicrobial activities of oleanoic acid, betulinic acid and 3-friedelanone have been reported (Kuete *et al.,* 2007). Betulinic, ursolic and oleanolic acids are the main triterpenes present in *Eriope blanchetii* (Harley, 1976) and are known to possess antimicrobial activities. β -amyrin is another terpenoid that has been isolated from the root bark of *Ficus chlamydocarpa* and the stem bark of *Ficus cordata* (Kuete *et al.,* 2008).

The antimicrobial activity of the triterpenoid (EC.HL02) could be considered significant when compared with gentamicin and tioconazole. The isolated triterpenoid seems to be potent on *A. niger* with the same MIC as for tioconazole. Infections caused by *Aspergillus* species could lead to morbidity and mortality in immune-compromised patients (Denning, 1998; Marr *et al.,* 2000). Invasive aspergillosis is an important cause of opportunistic respiratory and disseminated infection in immunocompromised patients (Montoya *et al.,* 2003) and *Aspergillus* species also produce a wide range of chronic, saprophytic and allergic conditions (Barnes and Marr, 2006) while the emerging problem of antifungal drug resistance in *Aspergillus* is of great concern and several studies have reported the prevalence of triazole resistance of up to 4.2% among aspergillius isolates. Known antifungal agents, fluconazole and ketoconazole, are inactive against *Aspergillus* and failure of

Amphotericin B (polyene macrolide) against invasive aspergillosis is common (Moore *et al.,* 2000). The antimicrobial potency of the triterpenoid isolated from *F. thonningii* suggests the plant as a potential drug that can be used in fungal infections such as aspergillosis. The triterpenoids isolated from *Ficus ovata* had a MIC range of 10-156 µg/mL on *Microsporum audouinii* and a MIC of 156 µg/mL on *C. albicans* (Kuete *et al*., 2009).

Inflammation is a protective biological response of vascular tissues to harmful stimuli for their removal and to initiate healing process but if left unchecked, can lead to onset of diseases such as rheumatoid arthritis and atherosclerosis (Singh *et al.,* 2008). Most drugs used for the treatment of inflammatory conditions have one limiting side effect or the other and thus, the research into plants with alledged folkloric use as anti-inflammatory and pain relieving agents could prove useful in the discovery of new therapeutic agents with little or no side effects.

The carrageenan-induced rat paw oedema test was preferably used amongst other biological means of anti-inflammatory determination due to the ease at which the experiment can be carried out, its consistency and the ability for the inflammatory reaction to be visualized. The extracts were administered orally to fasted animals to avoid gastrointestinal or systemic interaction.

The methanol leaf extract demonstrated a comparable *in-vivo* anti-inflammatory activity with the standard drug aspirin while the anti-inflammatory activity observed with hexane leaf extract was poor. The anti-inflammatory activity of the ethanol leaf extract of *F. thonningii* has been reported (Otimeyin *et al.,* 2004), thus confirming the anti-inflammatory activity of the plant. Extracts of leaves of *F. racemosa* demonstrated anti-inflammatory activity against carrageenan, serotonin, dextrane and histamine-induced inflammation (Mandal *et al.,* 2000). Ethnomedicinally, fresh leaves of *F. thonningii* have been used to treat lumbago and burnt leaves are rubbed on dislocated limbs which are suggestive of inflammatory conditions (Bhat *et al.,* 1990). The bark is also used as medicine for painful joint. The leaves and fruits of the plant are used to treat bronchitis, a disease condition in which there is an inflammation of the mucous membranes of the bronchi (Albert, 2010), and the bark is used to treat sore throat which is usually

caused by acute pharyngitis (inflammation of the throat). Thus, the anti-inflammatory properties demonstrated by the plant provide the rationale for the folkloric uses of the plant in diseases associated with inflammation such as lumbago, bronchitis, sore throat and wound healing as the process of wound healing also involves inflammation, cell proliferation and collagen lattice formation (Sidhu *et al*., 1999).

The major classes of anti-inflammatory agents from natural sources such as tannins, terpenoids, flavonoids and alkaloids were found present in the leaf and stem bark of *F. thonningii.* Tannins are polymeric phenolic substances which have been used traditionally for protection against inflamed surfaces of the mouth and in the treatment of wounds and haemorrhoids (Ogunleye and Ibitoye, 2003). Plant terpenoids have been reported to be active anti-inflammatory constituents (Chaurasia and Vyas, 1997; Changa *et al*., 2008). Flavonoids have also been reported to be major anti-inflammatory agents (Chi *et al*., 2001) and figs are a good source of flavonoids and polyphenols (Lansky *et al*., 2008). Biochemical investigations have shown that flavonoids can inhibit both cyclooxygenase and lipooxygenase pathways of the arachidonic metabolism (Chi *et al*., 2001). Some alkaloids have been presented with striking anti-inflammatory activity. The presence of these secondary metabolites in the plant could account for the demonstration of its anti-inflammatory activity. Methanol is an excellent extractant for tannins, terpenoids and other polyphenolic compounds (Cowan *et al*., 1999) and thus confirming the appreciable anti-inflammatory activity of the methanol extract of leaf. Due to the severe side effects associated with the current non-steroidal as well as steroidal anti-inflammatory agents, there is continous search especially from natural sources for alternative agents. *Ficus thonningii* could also be a potential source of anti-inflammatory agent which would be useful in the treatment of inflammatory disorders.

As most microbial infections are associated with inflammation, the combined antimicrobial and anti-inflammatory activities of the extracts from the studied plant of study make it a potential useful agent in the treatment of some infections that are associated with inflammation. The leaves of *F. thonningii* are used to treat bronchitis (Iwu, 1993) and the bark used in the treatment of wounds and sorethroat (Watt and Breyer, 1962). Such infections like bronchitis, bacterial gingivitis, sorethroat, stye,

carbuncle, thrush and vulvo-vaginitis that are accompanied with inflammation could be treated with extracts from *F. thonningii*.

The extracts of *F. thonningii* seem to be safe for use using the toxicity parameters as indication. There were no apparent adverse reactions and no death of animals was recorded in the control and treatment groups during the test period indicating an LD_{50} > 5 g/kg. Thus, it can be said that *F. thonningii* is not toxic with regard to the threshold of toxic substances (5g/kg) as previously stipulated by Delongeas *et al.*, (1983). There were no significant gross and histopathological changes in the liver, kidney, lungs, spleen, uterus and ovary of the test animals. No lesions were observed in the liver and kidney cells. There was also no significant histopathological change in the female reproductive tract in the study contrary to the possible testicular toxicity reported in male rats in an earlier study (Aniagu *et al.*, 2008). As one of the oldest known human foods, figs as a fruit have a very high safety profile (Lansky *et al.*, 2008). There was a significant increase in the body weights of animals given 100 mg/kg body weight of extract. Significant increases were observed in the Red Blood Cell count and Mean Corpuscular Haemoglobin value. *F. thonningii* has been shown to be useful as a standing feed reserve for rabbits in the period of feed scarcity during dry season without the animals experiencing weight losses (Jokthan *et al.*, 2003).

Conclusion

The emergence of multi-drug resistance in human pathogenic bacteria as well as the undesirable side effects of some conventionally used antibiotics and the inherent problems associated with steroidal and non-steroidal anti-inflammatory agents have necessitated the search for new antimicrobial and anti-inflammatory drugs of plant origin.

The leaves and stem bark of *Ficus thonningii* Blume have been shown to possess antimicrobial and anti-inflammatory properties. These activities have been found to be more pronounced in the methanol leaf extract. Bactericidal kinetics study showed that the methanol leaf crude extract had a good bactericidal action on the MDR strains of *Staphylococcus aureus* and *Escherichia coli* used. The broad spectrum antimicrobial activity of the plant extracts, fractions and isolated triterpenoid on sensitive and multidrug-resistant (MDR) strains of microorganisms makes it a potential antimicrobial drug which can serve as a source for natural compounds that act as new anti-infectious agents. The anti-inflammatory activity demonstrated by the plant extracts makes it a natural crude source of an alternative anti-inflammatory agent to the current steroidal and non-steroidal drugs with adverse effects. The plant would also be useful in the treatment of microbial infections that are accompanied with inflammation and other inflammatory disorders.

The toxicity study of the methanol leaf extract showed an LD_{50} >5g/kg, suggesting the plant as being safe for consumption and thus could serve as a source of feed for animals.

The results obtained from the study have justified the ethno-pharmacological and cultural uses of *Ficus thonningii* Blume in the treatment of infectious and inflammation based diseases. The plant extracts could serve as a lead for or be developed into standard chemotherapeutic agents that can be used as anti-infectives which are therapeutically effective, safe, relatively inexpensive, highly tolerated and convenient for many patients.

Recommendation

Medicinal plant-based antimicrobials represent a vast untapped source of pharmaceuticals and there is danger of rapid rate of plant species extinction. Thus, there is a need for intense search for more antimicrobials of plant origin that can be used effectively in the treatment of infectious diseases with little or no side effects that are often associated with synthetic antimicrobials. The pharmacological basis for the efficacy, toxicity and clinical data of such phytopharmaceuticals should be established.

Further purification, isolation and characterization of more bioactive compounds from fractions of *F. thonningii* should be carried out, as these could serve as leads to new therapeutically useful drugs.

The extracts of *F. thonningii* can be formulated into topical pharmaceutical preparations that can be used in the treatment of wounds and burns and the associated inflammation.

A safe integration of herbal medicine into conventional medicine is recommended as both the developing and developed nations rely one way or the other on traditional medicines for their primary health care (Farnsworth and Morris, 1976; WHO, 2003; Chan, 2005).

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Appendix

Appendix 1: Methods for preparation of media

The microbial media used were of analytical grade and prepared according to manufacturer's instructions.

Nutrient broth

Formula (gram per litre)

Beef Extract	3.0 g
Peptone	5.0g
Sodium chloride	5.0g
Water	1000 mL

The ingredients were dissolved by heating in water. pH was adjusted to 8.0 - 8.4 with NaOH and boiled for 10 min. It was filtered and adjusted to pH 7.2 - 7.4. Broth was dispensed into test tubes and sterilized at 115 ^{0}C for 20 min.

Tryptone soya broth

Formula (gram per litre)

Pancreatic digest of casein	17.0
Papaic digest of soyabean meal	3.0
Sodium Chloride	5.0
Dibasic Potassium Phosphate	2.5
Dextrose	2.5
Water	1000 mL

pH 7.3 ± 0.2

The ingredients are dissolved in water by heating and distributed in 5mL volumes into test tubes. They are capped and sterilized at 121^{o}C for 15 minutes.

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Nutrient agar

Formula (gram per litre)

Peptone	5.0
Beef Extract	3.0
Sodium Chloride	8.0

Agar No. 2

pH 7.3 ± 0.2

28g of nutrient agar was weighed and dispersed in one litre of deionized water. This was allowed to soak for 10 minutes then placed into water bath. It was swirled at intervals till it dissolved completely. The nutrient agar was poured into bottles, capped and sterilized at 121^{o}C for 15 minutes.

Nutrient agar is a ready packed dehydrated culture media, a general purpose agar for the culture of non-fastidious organism. It is stored at 10^{o}C – 25^{o}C away from direct sunlight.

Mueller Hinton agar

Beef extract	2.0 g
Acid hydrlysate of casein	17.5 g
Starch	1.5 g
Agar	17.0 g
Distilled water	1000 mL

The dehydrated medium is dissolved in water by heating. pH is adjusted to 7.2 - 7.4 and transferred into bottles. Sterilization is at 110 ^{0}C for 20 min

Saline agar

Sodium chloride	8.5 g
Agar	20.0 g
Water	1000 mL

The solids are dissolved by steaming and sterilized at 115 ^{0}C for 20 min.

MacConkey agar

Peptone	20.0 g
Sodium chloride	5.0 g
Sodium taurocholate	5.0 g
Water	1000 mL
Agar	20.0 g
Lactose	10.0 g
Neutral red 1% aq soln	10.0 mL

The peptone, sodium chloride and bile salt are dissolved in water by heating and solution is adjusted to pH 8.0, it is then boiled for 20 min, cooled and filtered. Agaris then added and dissolved by boiling and mixture is adjusted to pH 7.4. Lactose and the indicator solution are added and mixed. Sterilization is at 115 ^{0}C for 20 min.

MacConkey broth

Peptone	20.0 g
Sodium chloride	5.0 g
Sodium taurocholate	5.0 g
Water	1000 mL
Bromocresol purple (0.2%)	5.0 mL
Lactose	10.0 g

Peptone, sodium chloride and bile salt are dissolved in water by heating. Solution is adjusted to pH 8.0 and boiled for 2o min, cooled, filtered and adjusted to pH 7.4. The lactose and indicator solution are added and mixed. Broth is distributed into tubes containing inverted Durham tubes. Sterilization is done at 115 ^{0}C for 15 min.

Koser's citrate medium

Sodium chloride	5.0 g
Magnesium sulphate	0.2 g
Ammonium dihydrogen phosphate	1.0 g
Pottasium monohydrogen phosphate	1.0 g
Distilled water	1000 mL
Citric acid	2.0 g

The salts are dissolved in water and the citric acid added to the solution. Solution is adjusted to pH 6.8 with sodium hydroxide. It is then filtered through a sintered glass funnel. The medium should be colorless. Solution is sterilized at 115 ^{0}C for 20 min.

Triple sugar iron agar (TSI)

Beef extract	3.0 g
Yeast extracts	3.0 g
Peptone	20.0 g
Glucose	1.0 g
Lactose	10.0 g
Sucrose	10.0 g
Ferrous sulphate	0.2 g
Sodium chloride	5.0 g
Sdium sulphite	0.3 g
Agar	20.0 g
Distilled water	1000 mL
Phenol red 0.2%	12 mL

The solids are dissolved in water by heating. The indicator solution is added and mixed. Sterilization is done at 115 ^{0}C for 20 min and cooled to form slopes with deep butts.

Peptone water

Peptone	10.0 g
Sodium chloride	5.0 g
Water	1000 mL

The solids are dissolved in water by heating and adjusted to pH 8.0 - 8.4. Solution is boiled for 10 min, filtered and adjusted to pH 7.2 - 7.4

Appendix 2: Bactericidal kinetics values of *Ficus thonningii* extracts *(S.aureus)*

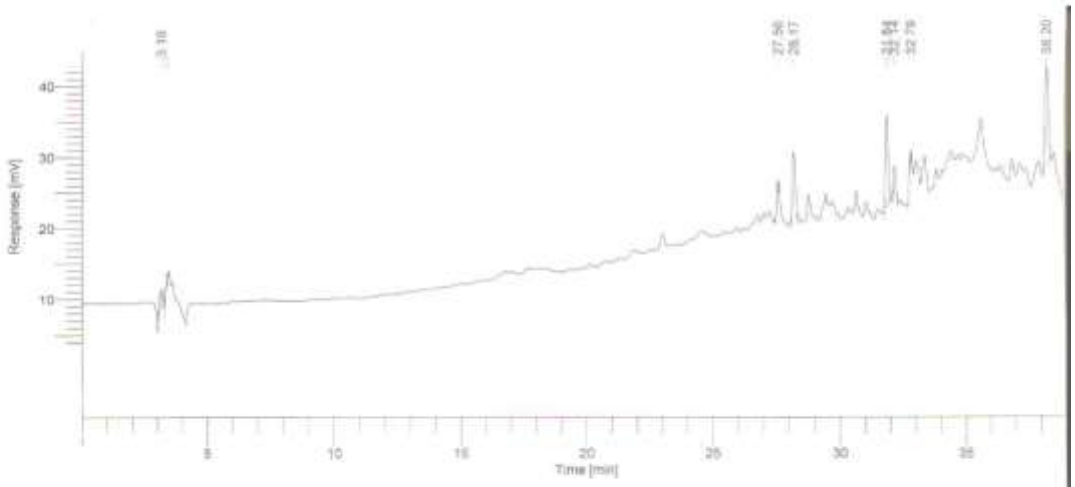
Time (min)	**cfu/mL**	**Log cfu/mL**	**cfu/mL**	**Log cfu/mL**	**cfu/mL**	**Log cfu/mL**	**Control**	**Log cfu/mL**
	10mg		5mg		2.5mg			
0	2.0×10^7	7.301	2.0×10^7	7.301	2.0×10^7	7.301	2.0×10^7	7.301
30	1.1×10^6	7.041	1.6×10^7	8.204	1.8×10^7	8.255	2.0×10^7	7.301
60	7×10^4	4.845	2.0×10^6	6.301	2.0×10^6	6.301	3.0×10^7	7.477
90	2×10^3	3.301	8.0×10^4	4.903	6.0×10^5	5.778	2.0×10^8	8.301
120	5×10^1	1.699	4.5×10^3	4.653	4.5×10^4	5.978	6×10^8	8.778
240	1×10^0	0.000	1.5×10^1	2.176	4×10^1	1.602	9.0×10^8	8.954

Appendix 3: Bactericidal kinetics values of *Ficus thonningii* extracts *(E. coli)*

Time (min)	**cfu/mL**	**Log cfu/mL**	**cfu/mL**	**Log cfu/mL**	**cfu/mL**	**Log cfu/mL**	**Control**	**Log cfu/mL**
	10 mg		5 mg		2.5 mg			
0	2.0×10^7	7.301	2.0×10^7	7.301	2.0×10^7	7.301	2.0×10^7	7.301
30	8.5×10^6	7.929	7.5×10^6	7.875	1.5×10^7	8.176	2.5×10^7	8.398
60	7.0×10^4	4.845	1.2×10^5	6.079	6.0×10^6	6.778	8.0×10^7	7.903
90	4.0×10^2	2.602	1.0×10^3	3.000	7.0×10^5	5.845	3.0×10^8	8.477
120	3.0×10^1	1.477	1.0×10^2	2.000	6.5×10^5	5.812	8.0×10^8	8.903
240	1.5×10^1	1.176	1.0×10^2	2.000	4.0×10^4	4.602	8.5×10^8	8.929

Appendix 4: Values of Dt –Do / Do obtained from the anti-inflammatory activity of the crude leaf extracts of *F. thonningii* on female rats

Extracts	**Hours**				
	0	1	2	3	4
Hexane	0	0.071	0.138	0.209	0.285
Chloroform	0	0.231	0.286	0.271	0.255
Methanol	0	0.180	0.224	0.224	0.122
Aspirin	0	0.140	0.211	0.083	0.019
Tween 80	0	0.049	0.118	0.184	0.298



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	3.178	8086.54	370.38	2.47	2.47	BB	21.8331
2	3.417	4394.90	372.44	1.34	1.34	BB	11.8002
3	27.562	4774.68	874.75	1.46	1.46	BB	5.4584
4	28.172	70730.37	9023.69	21.58	21.58	BB	7.8383
5	31.837	89893.47	12489.75	27.43	27.43	BB	7.1974
6	32.140	22925.53	3804.81	6.99	6.99	BB	6.0254
7	32.789	5820.74	875.00	1.78	1.78	BB	6.6523
8	36.203	121133.19	13768.83	36.96	36.96	BB	8.7976
		327759.41	41579.64	100.00	100.00		

Missing Component Report

Component	Expected Retention (Calibration File)

All components were found

Fig. 4. 47: Reverse Phase HPLC report for methanol leaf fraction 01 from *Ficus thonningii*

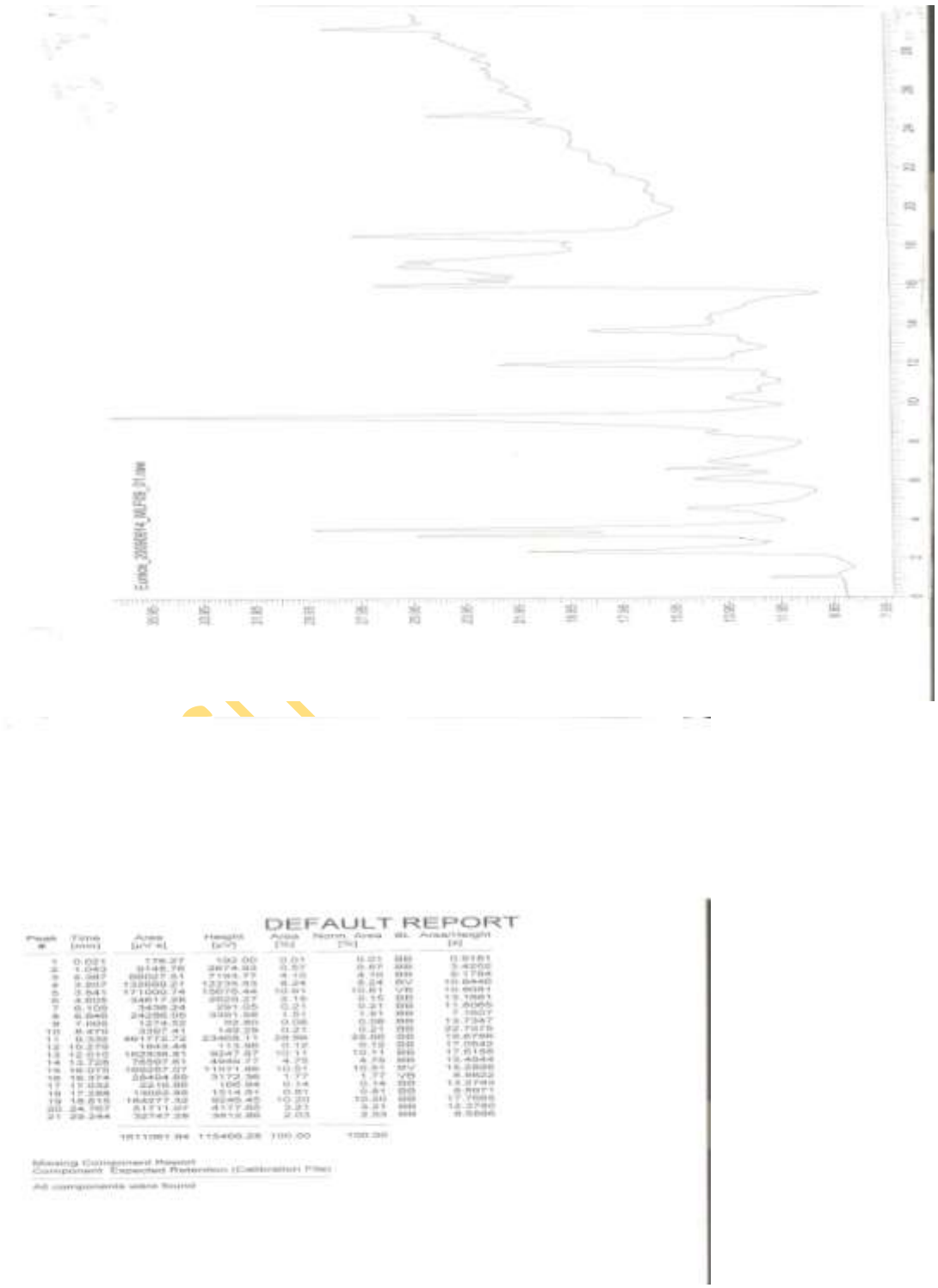
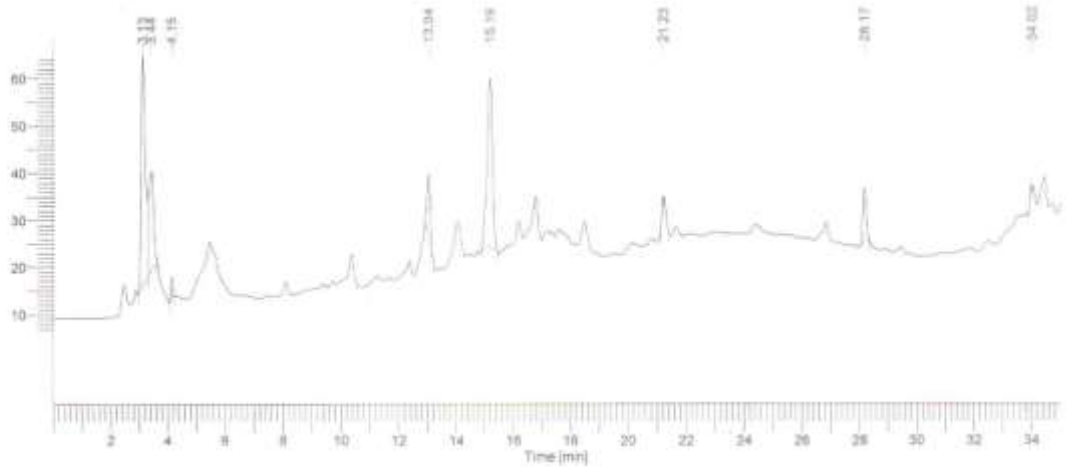


Fig. 4.48: Reverse Phase HPLC report for methanol leaf fraction 09 from *Ficus thonningii*



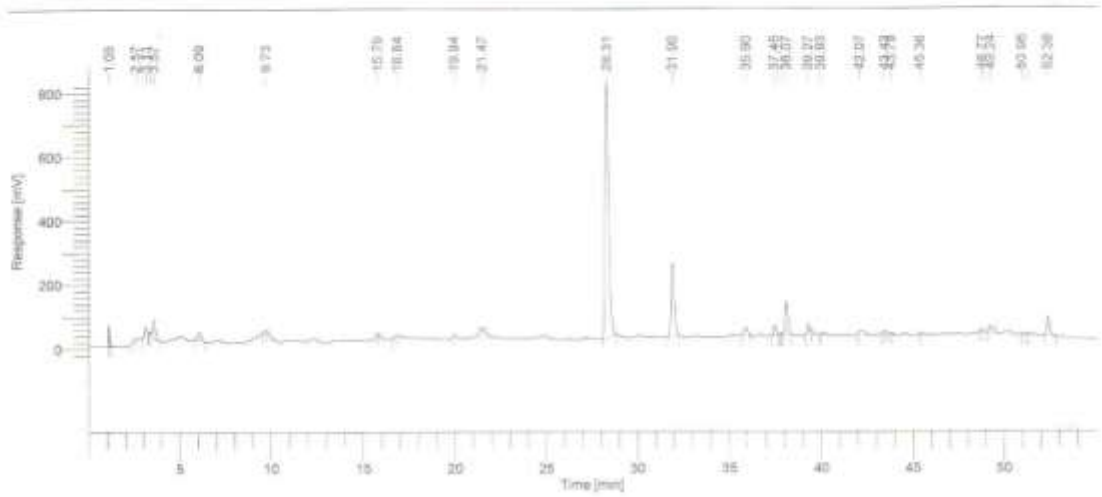
DEFAULT REPORT

eak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
1	3.125	465308.41	48742.27	33.06	33.06	BV	9.5463
2	3.438	252390.09	20933.75	17.93	17.93	VB	12.0566
3	4.153	19869.82	4501.49	1.41	1.41	BB	4.4141
4	13.040	112914.37	13424.05	8.02	8.02	BB	8.4113
5	15.190	453026.33	35635.50	32.18	32.18	BB	12.7128
6	21.232	5842.51	539.07	0.42	0.42	BB	10.8381
7	28.168	92673.18	11409.04	6.58	6.58	BB	8.1228
8	34.018	5566.37	613.48	0.40	0.40	BB	9.0735
		1407591.08	135798.64	100.00	100.00		

lissing Component Report
:omponent Expected Retention (Calibration File)

ll components were found

Fig. 4.49: Reverse Phase HPLC report for methanol leaf fraction 10 from *Ficus thonningii*



DEFAULT REPORT

Peak #	Time [min]	Area [uV*sec]	Height [uV]	Area [%]	BL	Component Name	Adjusted Amount
1	1.094	211472.88	66178.37	1.18	BB		0.2115
2	2.565	29847.54	742.97	0.17	BB		0.0298
3	3.114	207565.76	25463.44	1.16	BB		0.2076
4	3.365	56886.85	8127.22	0.32	BV		0.0569
5	3.569	461853.42	54081.44	2.58	VB		0.4619
6	6.086	267151.59	23223.68	1.49	BB		0.2672
7	9.732	20039.74	828.32	0.11	BB		0.0200
8	15.792	14723.03	731.49	0.08	BB		0.0147
9	16.845	24037.21	695.71	0.13	BB		0.0240
10	19.937	7725.28	452.29	0.04	BB		0.0077
11	21.474	24328.19	1554.49	0.14	BB		0.0243
12	28.314	11007048.60	796595.17	61.48	BB		11.0070
13	31.903	2752836.92	230174.59	15.38	BB		2.7528
14	35.903	346602.15	23914.99	1.94	BB		0.3466
15	37.450	321205.46	30842.31	1.79	BB		0.3212
16	37.804	23759.89	5741.37	0.13	BV		0.0238
17	38.073	1124267.55	104216.34	6.28	VB		1.1243
18	39.267	228836.92	26848.96	1.28	BB		0.2288
19	39.934	2875.90	688.67	0.02	BB		0.0029
20	42.068	20272.41	970.65	0.11	BB		0.0203

Fig. 4.50: Reverse Phase HPLC report for methanol leaf fraction 11 from *Ficus thonningii*