PHARMACOGNOSTIC AND ANTIMICROBIAL STUDIES OF THE STEM BARK OF *FICUS THONNINGII* BLUME (MORACEAE)

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By

Nafisah SALIHU (B.Sc. BOTANY, UDUS 2009)

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DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT,
FACULTY OF PHARMACEUTICAL SCIENCES
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

APRIL, 2017

DECLARATION

I declare that the work in this Dissertation entitled "**Pharmacognostic and Antimicrobial Studies on the Stem-bark of *Ficus thonningii* Blume (Moraceae)"** has been carried out by me in the Department of Phamarcongosy and Drug Development. The information derived from the literatures has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

_____ _____

Nafisah Salihu Date

CERTIFICATION

This dissertation entitled "**PHARMACOGNOSTIC AND ANTIMICROBIAL STUDIES ON THE STEM-BARK OF *FICUS THONNINGII* BLUME (MORACEAE)"** written by Nafisah SALIHU meets the regulation governing the award of Master of Science in Pharmacognosy of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to my Parents, Alhaji Salihu Abdullahi Jibril and Haj. Sa'adatu Salihu.

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ABSTRACT

Ficus thonningii Blume is a popular medicinal plant used in Nigeria for the treatment of various ailments including diarrhoea, pneumonia, sore throat and typhoid. Physicochemical, macroscopic, microscopic, phytochemical and antimicrobial evaluations of the stem bark of the plant were carried out. The macroscopic features of the stem bark were examined using sensory organs. The microscopical features of the stem bark were determined by examining its microscopical properties using compound microscope. The powdered stem bark was successively extracted with hexane, ethyl acetate and methanol using cold maceration. Antimicrobial evaluation of the stem-bark of the plant was carried out with all the extracts tested against three Gram-positive bacteria: *Staphylococcus aureus, Streptococcus pyrogenes,* and *Methicillin Resistant Staphylococcus aureus (MRSA)* and five Gram-negative bacteria: *Escherichia coli, Pseudomonas aeroginosa, Proteus mirabilis, Salmonella typhi* and *Klebsiella pneumoniae,* Two Fungi: *C. tropicalis* and *C. albicans*. Agar well Diffusion method was used to screen the extracts. While the minimum inhibitory concentrations (MIC) as well as minimum bactericidal/fungicidal concentrations (MBC/MFC) were determined using broth dilution method. Transverse and longitudinal sections of the bark showed the presence of calcium oxalate crystals (prism type), cork cells, phellogen, fibres, medullary rays, and phloem parenchyma. Physicochemical parameters evaluated were: moisture content (6.56%), ash value (9.50%), water soluble ash (7.50%), acid insoluble ash (2.25%), water extractive value (10.67%) and alcohol extractive value (16.67%). Chemomicroscopically, the study revealed the presence of lignin, cellulose, tannins, calcium oxalate crystals and suberin. Phytochemical screening and thin layer chromatographic fingerprint of the extracts revealed flavonoids, steroids/triterpenes and phenolic compounds.

Antimicrobial evaluation showed that almost all test microorganisms were sensitive to all extracts with zones of inhibition ranging from 20 – 30 mm. Ethyl acetate had the largest zones (best antimicrobial activity), followed by methanol and the least was hexane extract. However, *P. mirabilis, C. albicans* and *MRSA* were not susceptible to the entire extract. The MIC for all the extracts was between 6.25 mg/ml – 25 mg/ml while MBC/MFC was between 12.5 mg/ml – 50 mg/ml. This study therefore showed that, the stem bark extract has broad-spectrum antimicrobial properties against bacteria and fungi. It also confirms scientific reason for the traditional use of *F. thonningii* stem bark in the treatment of microbial infections.

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ACRONYMS AND ABBREVIATIONS

Acronym	Meaning
ABUTH	Ahmadu Bello University Teaching Hospital
$AlCl_3$	Aluminium Chloride
CP	Ciprofloxacin
DE	Diethyl ether
DMSO	Dimethyl Sulfoxide
EA	Ethyl acetate
e.t.c.	Etcetera
et al	and coworkers
$FeCl_3$	Ferric Chloride
HCl	Hydrochloric Acid
H_2S0_4	Tetra Oxosulphate (VI) acid
LD_{50}	Median Lethal Dose
ME	Methanol
ml	milliliter
mm	millimeter
MRSA	*Methicillin Resistant Staphylococcus Aureus*
R_f	Retention Factor
T.S	Transverse Section
T.L.S	Tangential Longitudinal Section

TLC	Thin Layer Chromatography
USDA	United State Department of Agriculture
v/v	Volume by Volume
w/w	Weight by Weight
WHO	World Health Organization
CAM	Complementary and Alternative Medicine

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CHAPTER ONE

1.0 INTRODUCTION

Nature has provided mankind with a complete store house of remedies to cure all ailments of mankind (Yadav and Singh, 2011). The use of plants in the management and treatment of diseases started with life. In more recent years, with considerable research, it has been found that many plants have medicinal values (Sofowora, 2008). The high cost of conventional medicines and their limited availability especially to rural communities in Africa and other developing regions have driven the continued dependence on traditional therapeutics (Dangarembizi *et al*., 2014). About 75-90 % of the world population still relies on plants and plant extracts as a source of primary health care (Benzie and Watchel-Galor, 2011).

Plants use in traditional médicine, also called phytomedicine are plant-derived medicines that contain chemicals, more usually, mixtures of chemical compounds that act individually or in combination on the human body to prevent disorders and to restore or maintain health (Van-Wyk and Wink, 2004). About two (2) decades ago, 3.4 billion people in the developing world were reported to depend on plant based traditional medicines. Owing to poverty, unawareness and unavailability of contemporary health facilities, most people, especially rural people are still compelled to practise traditional medications for the treatment of their day to day illnesses (Khan *et al*., 1993).

1.1 Traditional Medicine

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses (WHO, 2000).

Traditional medicine has been used for thousands of years with great contributions made by practitioners to human health, particularly as primary health care providers at the community level (WHO, 2000).

With these descriptions, various forms of medicines and therapies such as herbal medicine, massage, homeopathy, mud bath, music therapy, wax bath, reflexology, heat therapy, therapeutic fasting and dieting, spinal manipulation, psychotherapy, etc. are elements of traditional medicine. It does show that a large country of the size of Nigeria, with diverse cultures and traditions, should be rich in traditional medicine and should have eminent and respected traditional healers to take care of the teeming population (Adeshina, 2008).

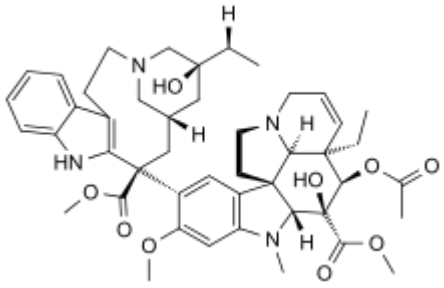
Most Nigerians, especially those living in rural communities don't have access to orthodox medicine and it is estimated that about 80% of the populace still prefer to solve their health problems consulting traditional healers (Adekannbi *et al*., 2014). Where access to orthodox medicine exists, the rising cost of imported medications and other commodities used for medicine has posed big problems. Beside, many rural communities have great faith in traditional medicine, particularly in the inexplicable aspect as they believe that it is the wisdom of their fore-fathers which also recognize their socio-cultural and religious background which orthodox medicine seems to neglect (Todd, 2014). Traditional medicine is the oldest, most tried and tested form of medicine and is as old as man himself (WHO, 2003).

Traditional medicine covers a wide variety of therapies and practices which vary from country to country and region to region. In some countries, it is referred to as "alternative" or "complementary" medicine (CAM) (WHO, 2000). Plants, especially used in traditional medicine can provide biologically active molecules and lead

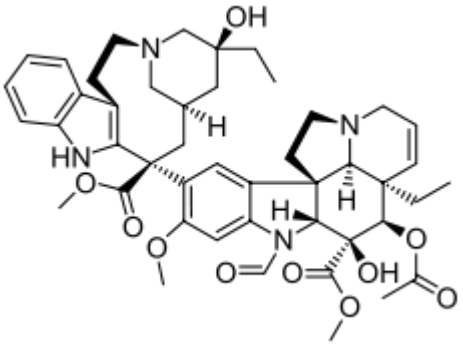
structures for the development of modified derivatives with enhanced activity and /or reduced toxicity (Darsini *et al*., 2015). The small fractions of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants. Some of the useful plant drugs include: vinblastine, vincristine, taxol, artemesinin, digitoxigenin and camptothecin (Fig 1.1). In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, work in both mixture of traditional medicine and single active compounds are very important (Kumar *et al.,* 1997).

1.2 Statement of Research Problem

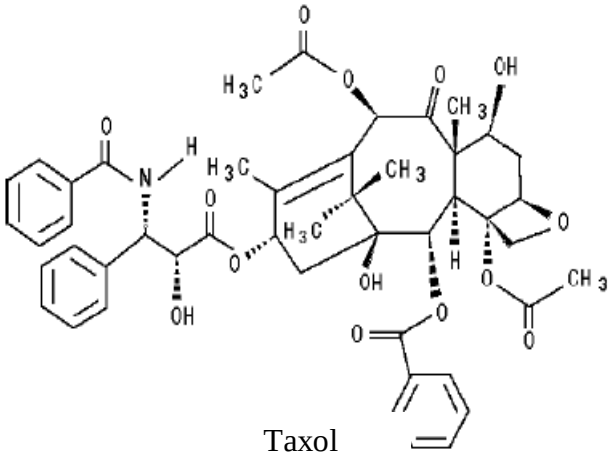
Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. Morbidity and mortality due to bacterial infections continues to be a major problem in many developing countries including Nigeria (Parmar and Rawat, 2012). Infections due to variety of bacterial etiologic agents, such as pathogenic *Escherichia coil, Salmonella spp., Pseudomonas spp., Klebsiella spp* and *Staphylococcus aureus* are most common. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Dangarembizi *et al*., 2014). With the continuous use of antibiotics, microorganisms have become resistant. In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, immunosuppression and allergic reactions (Lopez et *al.,* 2001). This has created immense clinical problem in the treatment of infectious diseases (Parmar and Rawat, 2012).



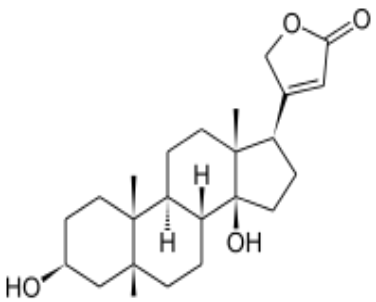
vinblastine



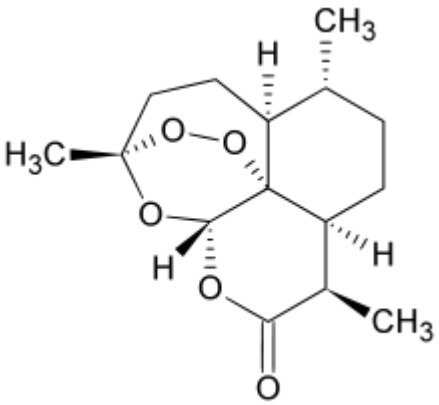
Vincristine



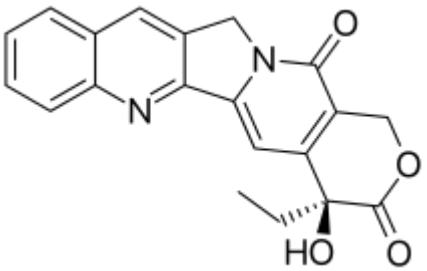
Taxol



Artemisinin



Digitoxigenin



Camptothecin

Fig 1.1 : Chemical structures of some useful drugs obtained from plants (Sofowora, 2008).

1.3 Justification

The use of medicinal plants in traditional medicine has been recognized and widely practiced. According to the World Health Organization (2010), 80% of the world's populations rely on traditional medicines to meet their health regiments. Plant used for traditional medicine contains a wide range of substances that can be used to treat chronic and acute infectious disease.

Despite the claimed ethno medicinal uses of the stem bark of *Ficus thonningii* in the treatment of microbial infections, there is little or no scientific evidence to support the traditional claims and there is no data to standardize the drug for quality control. As a result of this, it becomes extremely important to make an effort towards standardization of the plant as crude drug and also to establish scientific evidence of its traditional use as antibacterial agent.

1.4 Hypothesis

Pharmacognostic studies of *F. thonningii* stem bark are important for its identification and also contains phytochemicals with antibacterial activity.

1.5 Overall Aim

The aim of this research work is to provide scientific bases for the use of *F. thonningii* stem bark for the management of microbial infections as well as the pharmacognostic standards for monograph.

1.5.1 Specific aims

1. To evaluate pharmacognostic characters of the stem bark of *F. thonningii* with a view to providing parameters for the preparation of its monograph.

2. To develop thin layer chromatographic profile of hexane, ethyl acetate and methanol extracts of the stem bark of the plant.
3. To determine the antimicrobial activity of three (3) different extracts of the plant.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of the Family Moraceae

The Moraceae often called the mulberry family or fig family - is a family of flowering plants comprising about 53 genera and over 1,400 species. Most are widespread in tropical and subtropical regions but a few extend into the temperate regions (Singh *et al*, 2008). The uniting character within Moraceae is presence of laticifers and milky sap in all parenchymatous tissues, but generally useful field characters include two carpels sometimes with one reduced, compound inconspicuous flowers, and compound fruits. Plants of the family have alternate or opposite leaves and small, petalless male or female flowers. The fruits of many species are multiple because fruits from different flowers become joined together (Judd *et al.*, 1999). Important genera of the family include *Morus, Artocarpus* and *Ficus* (Venkataraman, 1972).

Some genera produce edible fruits, such as the mulberry (*Morus*), fig (*Ficus carica*), breadfruit and jackfruit (*Artocarpus*), and afon, or African breadfruit (*Treculia*). Others, such as *Antiaris, Ficus,* and *Castilla,* are important for their timber and latex. The latex of the upas tree (*Antiaris toxicaria*) of Java is used as an arrow poison; the latex of the cow tree (*Brosimum utile*) of tropical America is sweet and nutritious. *Ficus*, the largest genus in the mulberry family, contains the banyan (*Ficus benghalensis*) and the India rubber tree (*Ficus elastica*). The bark of the paper mulberry (*Broussonetia papyrifera*) has been used for the manufacture of cloth and paper products. Among the ornamentals in the family are paper mulberry and Osage orange (*Maclura pomifera*) (Judd *et al.*, 1999). Moraceae exhibit an amazing diversity of morphological and life history traits, particularly inflorescence architectures, breeding systems, and pollination syndromes (Berg, 1998). Studies of Moraceae using DNA sequences have reconstructed the

evolutionary history of the family to clarify relationships among genera, to identify the closest relatives of the figs (Datwyler and Weiblen 2004), and to examine biogeography (Zerega *et al*., 2005).

Molecular studies have demonstrated that Moraceae are part of the Rosidae and are closely allied to the Urticalean rosids, including Cannabaceae, Celtidaceae, Urticaceae, Cecropiaceae, and Ulmaceae (Wiegrefe *et al*., 1994; Zavada and Kim, 1996; Sytema *et al*., 2002). The Urticalean rosids differ from most other rosids in the presence of solitary ovules, lactifers, cystoliths, paired inflorescences in leaf axils, and unisexual flowers. Urticaceae plus Cecropiaceae are sister to Moraceae, distinguished from the latter in having lactifers only in the bark, clear latex, and orthotropous ovules (Sytema *et al*., 2002).

2.1.1 Description of the Genus *Ficus*

Ficus is a large genus of angiosperm with approximately 850 species consisting of trees, shrubs, climbers, creepers, epiphytes and hemi-epiphytes in the family Moraceae. Ficus trees are huge growing trees reaching 60 feet tall and 60 – 70 feet wide. They have a dense rounded canopy with dropping branches. Branches bend toward ground forming a canopy. Leaves are evergreen, thick and shiny with a length of 2-5 inches (Frodin, 2004; Berg and Corner 2005; Rønsted *et al*., 2008). Fig species are characterized by their unique inflorescence and distinctive pollination syndrome, which utilizes wasp species belonging to the Agaonidae family for pollination. The specific identification of many of the species can be difficult, but figs as a group are relatively easy to recognize, many have aerial roots and a distinctive shape or habit, and their fruits distinguish them from other plants. The fig fruit is an enclosed inflorescence, sometimes referred to as a syconium, an urn-like structure lined on the inside with the fig's tiny flowers. The unique fig pollination system, involving tiny, highly specific

wasps, known as fig wasps that enter via ostiole sub-closed inflorescences to both pollinate and lay their own eggs, has been a constant source of inspiration and wonder to biologists (Rønsted *et al*., 2008). Finally, there are three vegetative traits that together are unique to figs. All figs possess a white to yellowish latex, some in copious quantities; the twig has paired stipules or a circular stipule scar if the stipules have fallen off; and the lateral veins at the base of the leaf are steep, forming a tighter angle with the midrib than the other lateral veins (Berg and Corner 2005).

2.1.2 Distribution of Ficus

They are native throughout the tropics with a few species extending into the semi-warm temperate zone. It is pretty much generally agreed that figs originated in Asia Minor and have been successful in spreading around the world. The common fig (*F. carica*) is a temperate species native to Southwest Asia and the Mediterranean region (from Afghanistan to Portugal). In Africa, the plants are distributed in South Africa, Mozambique, Zimbabwe, Botswana, Nigeria, and Sudan (Berg, 1992). In Nigeria there are over 45 different species of *Ficus* as reported by Keay *et al.,* (1964).

2.1.3 Taxonomy of Genus *Ficus*

The genus is divided into six subgenera based on preliminary morphology. The monoecious subgenus *Urostigma* is the largest with about 280 species all inclusive, and most of them display distinctive hemiepiphytic habits. *Ficus* includes 23 species of hemiepiphytes and lithophytes which produce aerial and creeping root systems (Rønsted *et al*., 2008)**.**

2.1.4 Ethnomedicinal uses of Ficus

The genus *Ficus* contains plants which are used in traditional herbal medicines for treatment of various ailments. Traditionally, many species of *Ficus* are used for ulcer, diarrhoea, stomach ache, constipation, diabetes, asthma, tuberculoises, pile, hypertension (Inder *et al*., 2010; Kiran *et al*., 2011; Kubmarawa *et al*., 2013; Gbadamasi and Egunyomi 2014; Sunday *et al*., 2015). In South Western Nigeria, leaves of *F. exasperata* are used in treatment of female infertility. Also, young leaves of same plant species are used for treating ulcer, diabetes and fungal disease (Sonibare *et al*. 2006). In Pakistan, *F. religiosa* bark is used for Gonorrhea, and its leaf decoction is used as an analgesic for tooth ache (Kunwar *et al*. 2006). Latex of *F. benghalensis* is also used for treating gonorrhea. Five (5) ripe fruits taken daily of *F. carica* is used for constipation. Decoction of *F. virens* stem bark is used in irregular menstrual cycle. Decoction of *F. foliata* stem bark and leaves are used for treating stomach and chest pain. *F. capensis* paste prepared with palm oil is rubbed on inflamed area or fever (Isah *et al*. 2012). *F. asperifolia* is used for breast cancer (Abbiw, 1990). In Ghana, root, stem bark and seeds of *F. gnaphalocarpa* are used for headache, eye problems, stroke and menstrual pains. In Northern Nigeria, *F. thonningii* leaves are used for stomach upset, *F. abutifolia* leaves for oedema, *F. ingens* stem for diarrhea, *Ficus platyphyll*a stem-bark for stomach trouble and pile (Sani and Aliyu, 2011). Many other *Ficus* species have been used across the globe in various folkloric medicinal applications.

2.1.5 Reported biological Activities of *Ficus* Species

Many biological activities have been reported for various *Ficus* species. Biological activities ranging from antimicrobial, anti-inflammatory, laxative, cardiovascular, respiratory, and antispasmodic, have been associated with plant members of the genus. Scientific investigations involving a number of *Ficus* species proved numerous activities such as antiabortificient of *F. capensis* (Owolabi *et al*., 2009). Anti-

inflammatory, analgesis, lysosomal membrane stabilizing have been reported for *F. benghalensis* (Thakare *et al.,* 2010). The crude ethanol extract of *F. syncomorus* was found to have anticonvulsant and antidiarrhoeal activity (Victor, 2006). *F. carica* fruit latex showed good antimicrobial and antifungal activities (Aref *et al*., 2010). Various pharmacological actions have been described for *F.exasperata* such as antiulcer, antidiabetic, lipid lowering and antifungal (Sonibare *et al*., 2006)

2.1.6 Compounds isolated and identified from some Ficus species

Chemically, the *Ficus* contains mainly glycosides such as saponins, flavonoids, anthraquinones as well as alkaloids and tannins (Ukwubile, 2010). Some bioactive compounds such as arabinose, β-amyrins, β-carotenes, β-sitosterols and xanthotoxol have been reported to exist in the genus (Gilani *et al.,* 2008). Previous phytochemical studies of *Ficus* resulted in the isolation of flavonoids, coumarins, alkaloids, steroids, triterpenes, ceramides and salicylic acids (Kamga *et al*., 2010) (Table 2.1). Chemical structures of some of these compounds are shown in Fig 2.1.

2.2 Description of *Ficus thonningii*

2.2.1 Botanical Description of *Ficus thonningii*

F. thonningii is a multistemmed, evergreen or briefly deciduous tree (Plate 1) with a dense, rounded to spreading crown mainly distributed in upland forests of tropical and sub-tropical Africa (Orwa *et al*., 2009). The tree grows at altitudes of between 1 000–2 500 m and it grows best in light, deep and well drained soils (Hines and Eckman, 1993; Orwa *et al*., 2009).

2.2.1.1 Leaves

Leaves (Plate 2) are simple, glossy, dark green, thin and papery or slightly leathery. They have smooth margin margin smooth, elliptic or obovate shape, sometimes rather elongated or slightly oblanceolate, grouped at ends of twigs, 3-20 x 1.5-10 cm. The

leaves are glabrous, puberulous or pubescent; with 6-12 pairs of upcurving main lateral veins. The leaf base is cuneate or obtuse (sometimes subcordate) while the apex is rounded or obtuse, sometimes shortly and bluntly acuminate. Stipules are about 12 mm long, soon falling off (Orwa *et al*., 2009). Petiole is glabrous about 0.5 – 6cm long, channeles above. Nerves are pinnate more or less prominent, palmate at base with 3 – 5 basal nerves, and 4 – 15 pairs of alternate or sub-opposite fusing nerves. Tertiary venation is finely reticulate (Arbonnier, 2004).

2.2.1.2 Stem

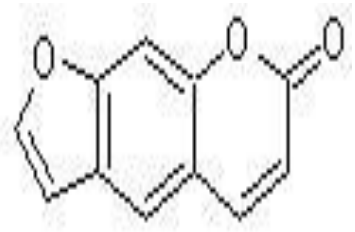
They are thick about 0.2 – 0.7m in diameter, more or less pubescent or glabrous. They appear smooth, pale grey to brown (Plate 3) with a slash exuding abundant latex. Latex is white but sometimes becomes pinkish. Bark on young branches is hairy, with a stipular cap covering the growth tip. Stipules are caduceus or barely persistent about 0.3 – 1.5 cm more or less pubescent, white to brownish in colour (Arbonnier, 2004).

2.2.1.3 Fruits

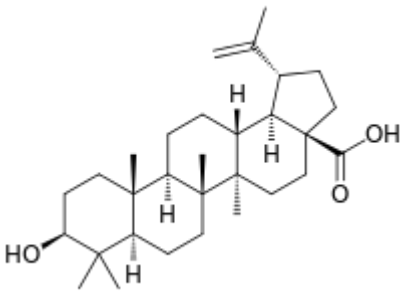
Often called figs, the fruits are solitary or in pairs, beneath or in the axil of young leave (Plate 2). They are sometimes found on old branches having globose or obovoid shape, sessile or pedunculate about 0 – 10mm long, 0.6-1.2cm in diameter. They are glabrous or pubescent, yellow or reddish when ripe. Fruiting is usually around the middle of dry season and at the end of raining season (Arbonnier, 2004).

Table 2.1: Some compounds isolated or identified from some *Ficus spp.*

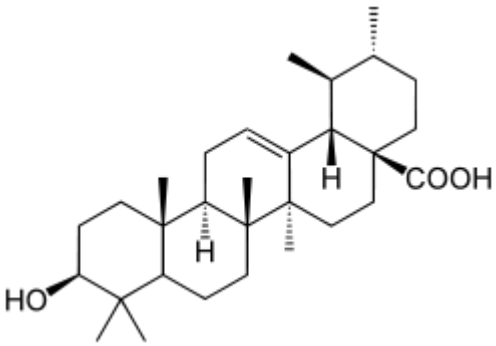
Ficus species	Plant part(s)	Compounds isolated or identified	References
F. carica	Leaf, root and Fruit	Ficusin, raffinose.	Anshul, *et al.* 2012
F. benjamina	Twigs	Benjaminamide	Simon *et al., 2008*
F. polita	Root	Betulinic acid, Ursolic acid, α-amyrin.	Kamga, *et al.*, 2010
F. religiosa	Stem bark and leaf	Lanosterol, stigmasterol, tannic acid.	Joseph and Justin, 2010
F. syncomorus	Leaf	Quercetin	Mohamed *et al.*, 2010



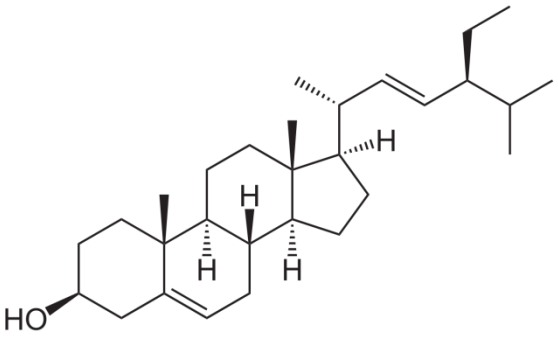
Ficusin



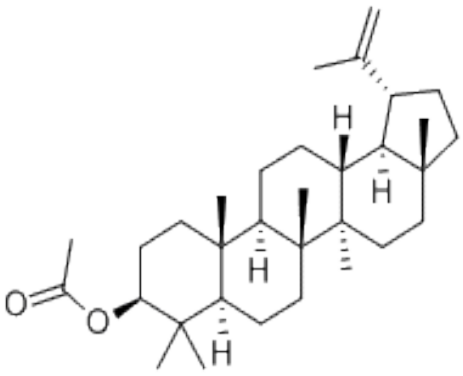
Betullinic acid



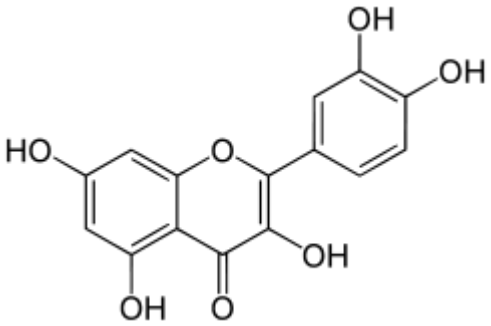
Ursolic



Stigmasterol



Lupeol acetate



Quercetin

Fig 2.1 : Some chemical structures of isolated compounds of *Ficus* species.

Plate 1: *Ficus thonningii* in its natural habitat

Plate 2: *Ficus thonningii* showing leaves and fruits.

Plate 3: The stem bark of *F. thonningii*

2.2.2 Origin and distribution of *F. thonningii*

F. thonningii is a West African species described from Ghana, it ranges from Cape Verde and Senegal to Cameroon as far as Ethiopia, Sao tome and Southern Africa. Although given the uncertainty about delimitation of species it is not feasible to depict an accurate distribution rate.

2.2.3 Synonyms and classification of *F. thonningii*

Urostigma thonningii (Blume), *Ficus microscarpa* Vahl, *F. Schimperi* Miq and *F dekdekena* (Miq)

2.2.4 Scientific Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Rosales

Family: Moraceae

Genus: Ficus

Species: *Ficus thonningii* (Blume)

2.2.5 Common and Local names

It is commonly called Common Wild Fig, *Chediya* in Hausa language of Northern Nigeria and *Roro Odan* in Yoruba language (Orwa *et al.*, 2009)

2.2.6 Ethnomedicinal uses of *F.thonningii*

Although all parts of *F. thonningii* are medicinally useful, people prefer to use the leaves and bark which exude latex because latex has traditionally been associated with potency (Ahur *et al.*, 2010).

2.2.6.1 Leaves

Decoction or macerations of fresh *F. thonningii* leaves, taken orally, have been used for the treatment of diarrhoea, gonorrhoea and diabetes mellitus (Njoronge and Kibunga, 2007). The leaves are also used for treating liver disorders and disease conditions associated with jaundice (Ahur *et al.*, 2010). In Angola, boiled *F. thonningii* leaves are used for treating wounds. Leaf extracts are also used for treating bronchitis and urinary tract infections (Cousins and Huffman, 2002). A decoction of the leaves is used in Mali for treating urinary schistosomiasis (Bah *et al.*, 2006). In Nigeria, a maceration of the leaves is used for treating stomach pains, gastritis, gastric ulcers and colitis (Nwude and Ibrahim, 1980). *F. thonningii* leaves are also used for the treatment of bone movement disorders, ringworm, thrush, scabies and athlete's foot rot (Alawa *et al.*, 2002; Moshi *et al.*, 2009).

2.2.6.2 Stem Bark

Traditionally, the stem bark is pound and the infusion is used for treating influenza, sore throat, colds, arthritis, and rheumatism and to relieve inflammation (Dalziel, 1937, Orwa *et al.*, 2009). In Tanzania the bark is also used to stimulate lactation (Minja, 1994). *F. thonningii* bark decoction is also used in Mali and Senegal, to treat respiratory diseases such as; pneumonia, bronchitis, emphysema (Prelude, 2011). The bark of *F. thonningii* is used for treating diarrhoea, cysts, skin diseases and ulcers in Ethiopia (Teklehaymanot and Gidday, 2007; Prelude, 2011). The bark decoction is also used for fertility enhancement and induction of the menstrual cycle

(Watt and Breyer-Brandwijk, 1962; Mkangare-Minja, 1989) and in Southern Africa it is used as relief for constipation and bowel disorders (Gelfand *et al.*, 1985).

2.2.6.3 Roots

F. thonningii roots are used for the treatment of malaria, fever, hepatitis and dental pains (Prelude, 2011). In Zimbabwe the roots have been reportedly used for preventing miscarriages and for stopping nose-bleeding (Gelfand *et al*., 1985). Additionally, *F. thonningii* roots are used for relieving stomach pains, diarrhoea, pneumonia and chest pains (Njoronge and Kibunga, 2007; Teklehaymanot and Gidday, 2007). In East Africa *F. thonningii* roots are used for treating diseases believed to be caused by evil spirits hence the tree is believed to be sacred (Wondimu *et al.*, 2007).

2.2.6.4 Latex

F. thonningii exudes white, sticky latex that turns pinkish with time (Arbonnier, 2004). This milky latex is used for treating fever, tooth decay and ringworm (Alawa *et al*., 2002; Arbonnier, 2004). The latex is commonly used for treating cataract in the eye (Alawa *et al*., 2002) and is also used as a vermifuge (Mali and Mehta, 2007).

2.3 Chemical Constituents Reported from *F. thonningii*

Research reports on *F. thonningii* have revealed the presence of many metabolites which include carbohydrates, soluble starch, glycoside, steroids, unsaturated steroids, aglycones, tannins, saponins, flavonoids, triterpenes and alkaloids in the plant. All these play important and varied pharmacological actions that contribute to the therapeutic claims of the plant (Ndukwe *et al*., 2007). Literature reports showed the isolation of some compounds from the plant. The presence of flavonoids in various parts (stem bark, leaves and roots) of *F. thonningii* has been reported by (Ndukwe *et al.*, 2007). Greenham *et al.*, (2007) reported the presence of flavone-C-glycosides in

F. thonningii leaves which were further identified as orientin, vitexin and isovitexin. Fongang *et al*. (2014) reported two flavonoids; thonningiol and thonningiisoflavone from the stem and fruit of *F. thonningii*. Isolated terpenoids include teraxarol and lupeol acetate (Ango *et al* 2016), steroids; β-sitosterol and sitosterol glucoside (Fongang *et al.*, 2014). Leaves of *F. thonningii* were reported to contain 6, 10, 14 trimethyl-2-pentadecanone, Phytol, acorenone and β-gurjunene (Ogunwande *et al*., 2008). Other isolated compounds include naringinin, luteolin, lupeol acetate, lupeol hexanoate, β-amyrin acetate, shuterin, taxifolin, garbazol, dihydroquercetin, aromadendrin, thonningiiflavanonol A and thonningiiflavanonol B (Fongang *et al*., 2014; Ango *et al*., 2016). Chemical structures of some compounds mentioned are shown in Fig 2.2.

2.4 Biological activities of *F. thonningii*

2.4.1 Antiprotozoal properties

Traditional healers report the use of *F. thonningii* in the treatment for malaria (Titanji et al., 2008; Chinsembu and Hedimbi 2010). A study carried out to evaluate the antiplasmodial activity of *F. thonningii* against *Plasmodium falciparum*, the protozoan parasite that causes malaria reported no significant antiplasmodial activity (Jansen *et al.*, 2010). *F. thonningii* possibly just possesses good insect repellent properties and hence reduces the contact of the vector with humans, minimising incidence of malaria transmission (Jansen *et al*., 2010; Innocent, 2008).

2.4.2 Anthelmintic properties

The proteolytic compound ficin in *Ficus thonningii* latex justifies its use as an anthelmintic agent. Ficin is a cysteine endopeptidase that is found in the latex of many *Ficus* species and is known to digest living intestinal parasites (Krief *et al*., 2005).

2.4.3 Antioxidant

Flavonoids are good antioxidants which scavenge and reduce free radical formation (*Grassi et al*., 2010). The C - glucosylflavonoids (orientin, vitexin and isovitexin) isolated from *F. thonningii* possess antioxidant properties (Von - Gadow *et al*., 1997).

2.4.4 Analgesic effects

F. thonningii has been reported to possess analgesic properties that are comparable to aspirin in both peripheral and central induced pain. Using the acetic acid induced writhing reflex model in mice, Otimenyin (2004) demonstrated that methanolic extracts of *F. thonningii* (500 mg/kg) administered intraperitoneally had a percentage inhibition (79.7%) comparable to aspirin (80%) showing that *F. thonningii* has analgesic effects that can be useful in the management of peripherally induced pain.

2.4.5 Anti-inflammatory effects

The anti-inflammatory properties of *F. thonningii* have been validated using egg albumin and carageenan induced oedema in rats (Otimenyin, 2004; Coker *et al*., 2009). Phytol, the aliphatic diterpene found in *F. thonningii* has anti-inflammatory effects and has been reported as a potential therapeutic agent for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases such as asthma (Ogunlesi *et al*., 2009).

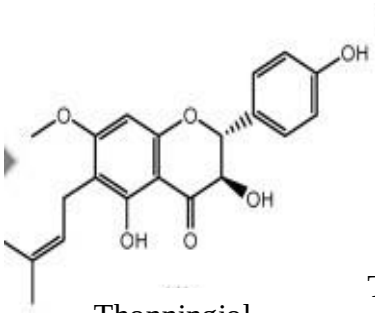
2.4.6 Cardioprotective effects

Ficus thonningii possesses cardio-suppressant and hypotensive properties. Ethanolic stem bark extracts of *F thonningii* were shown to exhibit positive chronotropic and inotropic effects on both electronically driven and spontaneously beating atrial muscle strips (Musabayane *et al*.,

2007). This study also reported an attenuating effect of *F. thonningii* extract (120 mg/kg b.w given for 5 weeks) on mean arterial pressures. The cardioprotective effects of *F. thonningii* could be credited to the presence of resveratrol. Resveratrol has been reported to prevent and slow the progression of various diseases which include cancer and cardiovascular diseases (Baur and Sinclair, 2006).

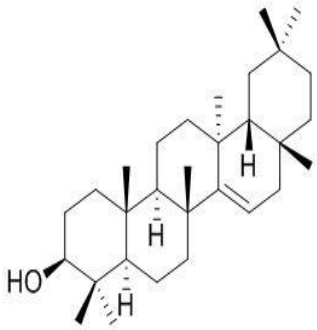
2.4.7 Hypoglycemic effects

Ethanolic extract of *F. thonningii* has been shown to exhibit hypoglycaemic effects in rats (Bwititi and Musabayane, 2007). Oral glucose tolerance tests performed on diabetic and non diabetic rats treated with the stem bark ethanolic extract of *F. thonningii* showed a dose dependant hypoglycaemic effect comparable to that of metformin which was used as a positive control (Musabayane *et al*., 2007).

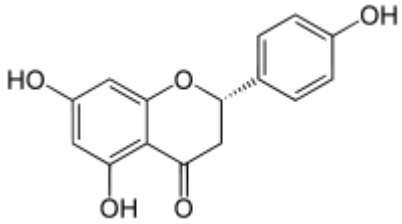


Thonningiol

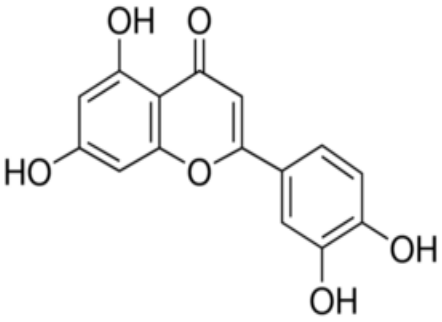
Thonningiiso flavone



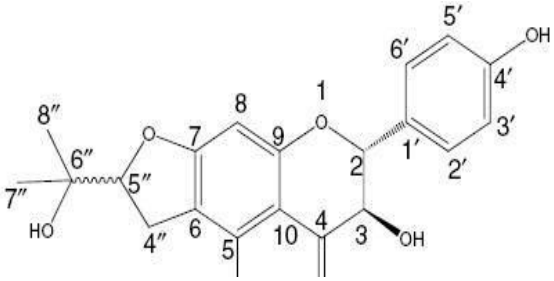
Teraxarol



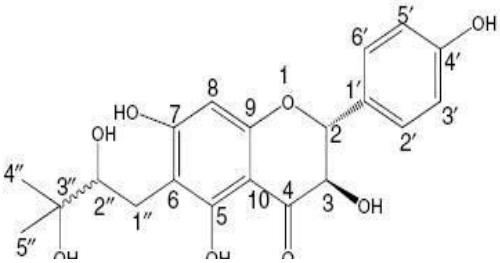
Naringenin



Luteolin



Thonningiiflavanonol A



Thonningiiflavanonol B

Fig. 2.2: Some chemical structures isolated and identified from *F. thonningii* (Fongang *et al*., 2014; Ango *et al*., 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Chemicals, Equipments and Reagents/Solutions

3.1.1 List of Chemicals

Hexane (JHD, AR; Lobal Chem, India), Methanol (Park Scientific Ltd, Northampton, UK),

Ethyl acetate (Qualikems), TLC silica gel 60 F_{254} pre-coated plates (Merk-Germany), Chloroform, Tetraoxosulphate (iv) acid, Anisaldehyde (Sigma-Aldrich, St. Lous, MO, USA) and DMSO

3.1.2 List of reagent

Sodium hypochlorite**,** chloral hydrate, phloroglucinol, glycerol, formalin, acetic acid, hydrochloric acid, methylene blue, safranin, fast green, paraffin wax, sudan red solution, ZnCl2 Solution (BDH Laboratory Chemicals Division, POOLE, England), dragendorff, ferric chloride, aluminium chloride, *p-anisaldehyde*, silica gel and Libermann-Bucchard reagent.

3.1.3 List of Equipment

Compound microscope (Fisher Scientific, UK), Photographic camera, Stage Micrometer and Eye piece graticule (Graticules Ltd, Ton bridge, Kent. England), UV lamp, Water bath (HHS, Mc Donald Scientific International), Mechanical shaker (Stuart Scientific Flask Shaker, Great Britain), Dessicator, TLC tanks (Uni kit® TLC Chromatank® , Microtome (C 740527, Cambridge Instrument Company Ltd, London and Cambridge, England), Shandon Southern Germany), Plant press (local made), Slide dryer (Hospital and Lab. Supply Ltd, London, UK), Microtome (C 740527, Cambridge Instrument Company Ltd, London and Cambridge, England).

3.1.4 List of other materials

Ash less filter paper, Laboratory glass wares (Funnel, Conical flask, Beakers, Measuring cylinder) Disposable syringes 1ml, 5ml, and 10ml, Petri dishes and Cork borer.

3.2 Plant Collection and Identification

The plant material comprising the stem leaves and fruits was collected at Yan karfe, Sabon Gari Local Government, Kaduna State. The plant was identified as *Ficus thonningii* with voucher number 1885 at the herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, by the Taxonomist: Mr. Namadi Sunusi

3.2.1 Preparation of plant material

The stem bark collected was dried at room temperature for about 2 weeks and then reduced to semi powdered form using grinding machine and stored in air – tight container prior to use.

3.3 Pharmacognostic Studies on *F. thonningii stem bark*

Pharmacognostic studies were conducted on the fresh and dried stem-bark of the plant.

3.3.1 Microscopical examination of the stem bark of *F. thonningii*

Transverse section (T.S) and Longitudinal section (L.S) of the fresh stem-bark samples were made and examined under microscope and described using the terms outlined in Dutta (2010). This procedure was carried out according to Feder and Brien (1968):

a) **Fixation;** the fresh stem-bark of *F. thonningii* were picked off directly from the tree and dipped immediately into the fixative, Formalin Acetic Acid (70% ethanol, 40%, formalin and glacial acetic acid at the ratio 45:1:1) and was allowed to stand for 24 hours.

b) **Dehydration;** the sample above was transferred in to 30%, 50%, 70%, 95% and 100% ethanol. The sample was allowed to remain in each graded alcohol for 2 hours each.

c) **Clearing;** the sample was transferred in to chloroform - ethanol (1:3), chloroform: ethanol (1:1), chloroform: ethanol (3:1), and 100% Chloroform. This was also carried out after every 2 hour.

d) **Infiltration and Embedding;** chips of paraffin wax was added slowly in to the sample and this was left to stand for 24 hours before transferring in to an oven at 60^{o}C. After melting, the paraffin containing the sample was transferred in to the embedding box and allowed to solidify. This was then trimmed and mounted on the microtome to get the transverse or cross section of the leaf. The transverse section was then transferred on to slides.

e) **Staining;** the transverse section of sample was dewaxed in xylene, by changing twice for 5 minutes each. This was then hydrated in 95%, 70%, 50%, and 30% ethanol for 2 minutes each. The transverse section was then transferred into safranin and left to stand for 30 minutes before washing with water. It was then transferred in to 0.5% HCl in 70% ethanol shortly before dipping into fast green for 2 minutes and washed with water. The transverse section was then further dehydrated in 30%, 50%, 70%, 95% and 100% ethanol for 2 minutes each and cleared in xylene for another 2 minutes. Gum (Balsam) was sprayed along the transverse section and cover slip was applied.

3.3.2 Micrometric Evaluation

This involved measurements of dimensions (length) of the various diagnostic microscopic characters (Fibres, calcium oxalate crystals and cork cells) of the plant. It was carried out by using a binocular microscope with the aid of graticles (Kokate, 1994).

3.3.3 Chemomicroscopical Studies of Stem bark of *F. thonningii*

These were carried out on the powdered stem bark of the plant. Small amount of the finely ground powdered stem bark of the plant was cleared in a test-tube containing 70% chloral

hydrate solution. It was boiled on a water-bath for thirty minutes to remove obscuring materials. The cleared sample was mounted on a microscope slide, using dilute glycerol. Using various detecting reagents, the presence of some cell inclusions and cell wall materials were examined in accordance with Brain and Turner (1975); Evans (2002) as follows:

3.3.4.1 Cell wall materials

(i) Test for cellulose: Two (2) drops of iodinated zinc chloride were added to the cleared sample on a slide, and this was allowed to stand for 2 minutes. One drop of sulphuric acid was added, cover-slipped and observed under the microscope for blue colour which indicates the presence of cellulose in the cell walls of epidermal cells.

(ii) Test for suberins: Two drops of Sudan red were added to the cleared sample on a slide, cover slip was applied and this was gently heated over hot water bath for 2 minutes. The slide was then observed under the microscope for red colouration which indicate the presence of suberin in the cell wall.

(iii) Test for lignins: Two drops of phloroglucinol was added to the cleared sample and allowed to stand until almost dry. A drop of sulphuric acid was added and cover slip applied and this was observed under the microscope, appearance of red coloration on the anatomical section indicated the presence of lignin.

(iv) Test for gums and mucilage: A drop of ruthenium red was added to the cleared sample on a slide, cover slipped and observed under the microscope. The appearance of pink colouration was positive for gums and mucilage**.**

3.3.4.2 Cell Contents/Cell inclusions

(i) Test for calcium carbonates: The appearance of effervescences on addition of concentrated hydrochloric acid to the powdered sample on the slide showed the presence of calcium carbonate.

(ii) Test for calcium oxalates: To the cleared sample, cover slip was applied and this was observed under the microscope. Two drops of hydrochloric acid was then added and also observed under the microscope, dissolution of shining crystals on the anatomical sections of the stem bark indicated the presence of calcium oxalates.

(iii) Test for tannins: A single drop of ferric chloride was added to the cleared sample and cover slip was applied and this was observed under the microscope. The appearance of greenish black colouration on some cells of the anatomical sections of the stem bark indicates the presence of tannins.

(iv)Test for starch: Two drops of normal iodine solution (N/50) were added to the cleared sample and cover slip was applied and this was observed under the microscope and the appearance of blue-black coloration in some parenchyma cells of the stem bark powder indicated the presence of starch.

3.3.5 Determination of Physico-chemical parameters of the powdered stem-bark of *F. thonningii*

The quantitative physical standard (solvent extractive values, ash values, moisture content) were determined as described in the British Pharmacopoeia (2012).

3.3.5.1 Moisture Content

The loss on drying method was adopted for this procedure. The powdered stem bark of the plant (3g) was weighed into a crucible. It was then heated for 1hour in an oven maintained at 105^0 C, cooled in a dessicator and re-weighed. The procedure was repeated three times until no further loss in weight was obtained and moisture content was determined in percentage as:

$$\text{Moisture Content (\%)} = \frac{Weight\ of\ Water\ Lost}{Original\ \ Weight\ of\ Sample} \times 100$$

3.3.5.2 Ash Values

(i) Total Ash

The total ash of the powdered stem bark was determined by weighing 2 g of the powdered sample in a crucible. This was heated gently at 450^0 C in a furnance. Heating was done until all the carbon was removed. The total ash value was calculated in percentage as:

$$\text{Total Ash Value (\%)} = \frac{Weight\ of\ Residual\ \ Ash}{Weight\ of\ Sample} \times 100$$

(ii) Acid Insoluble Ash

Dilute HCl (25 ml) was added to the ash obtained in (i) above. It was boiled for 5 minutes and the insoluble matter was collected on ashless filter paper. The beaker containing the acid and crucible were washed in hot water and the washings were passed through the filter paper. The washing was continued until the residue became neutral to litmus paper (i.e. was free of acid). The residue and filter paper were dried gently in an oven and ignited in a tarred crucible. It was allowed to cool and weighed. The acid insoluble ash was calculated in percentage as:

$$\text{Acid Insoluble Ash (\%)} = \frac{Weight\ of\ Residual\ \ Ash}{Initial\ \ Weight\ of\ Sample} \times 100$$

(iii) Water Soluble Ash

The water soluble ash value of the powdered stem bark of the plant was determined separately. The ash obtained following method (i) above was used. The procedure used to obtain the acid insoluble ash was the same as in (ii) above except that water was used instead of dilute HCl. The water soluble ash was determined in percentage as:

$$\text{Water ash (\%)} = \frac{Weight\ of\ Initial\ \ ash - Weight\ of\ Residual\ \ Ash}{Initial\ \ Weight\ of\ Sample} \times 100$$

3.3.5.3 Solvent Extractive Values

(i) Alcohol Soluble Extractive Value

Powdered drug was macerated with 100 ml of alcohol in a stoppered flask, shaking was done with the aid of a mechanical shaker during first 6 hr and allowed to stand for 18 hr. It was filtered after 24 hr. Twenty milliliter (20 ml) of the filtrate was evaporated in a tarred evaporating dish at 105°C in an oven and weighed. Alcohol soluble extractive value was calculated (in percentage) with reference to the initial weights of the extract, five determinations were recorded and the average determined.

Alcohol Extractive Value (%) = $\frac{Weight\ of\ residue}{Weight\ of\ Sample} \times 100$

(ii) Water Soluble Extractive Value

The same procedure above was repeated in determining the water soluble extractive value, using chloroform water (1:400) as the solvent. The water soluble extractive value was determined as:

Water Soluble Extractive Value (%) = $\frac{Weight\ of\ Residue}{Weight\ of\ Sample} \times 100$

3.4 Phytochemical analysis of the stem bark of *F. thonningii*

3.4.1 Extraction of the Stem bark of *F. thonningii*

One kilogramme of the air-dried powdered stem bark of *F. thonningii* was sequentially extracted by the cold maceration method, starting from hexane followed by ethyl acetate, and methanol (Fig. 3.1). Each time before extracting with the next solvent, the powdered material was air dried. Each extract was concentrated by evaporating to dryness on a water bath and percentage yield was calculated (Evans, 2002; Sofowora 2008).

Percentage Yield of extracts (%w/w) $= \frac{\text{Weight of total extract}}{\text{weight of powdered mate rial}} \times 100$

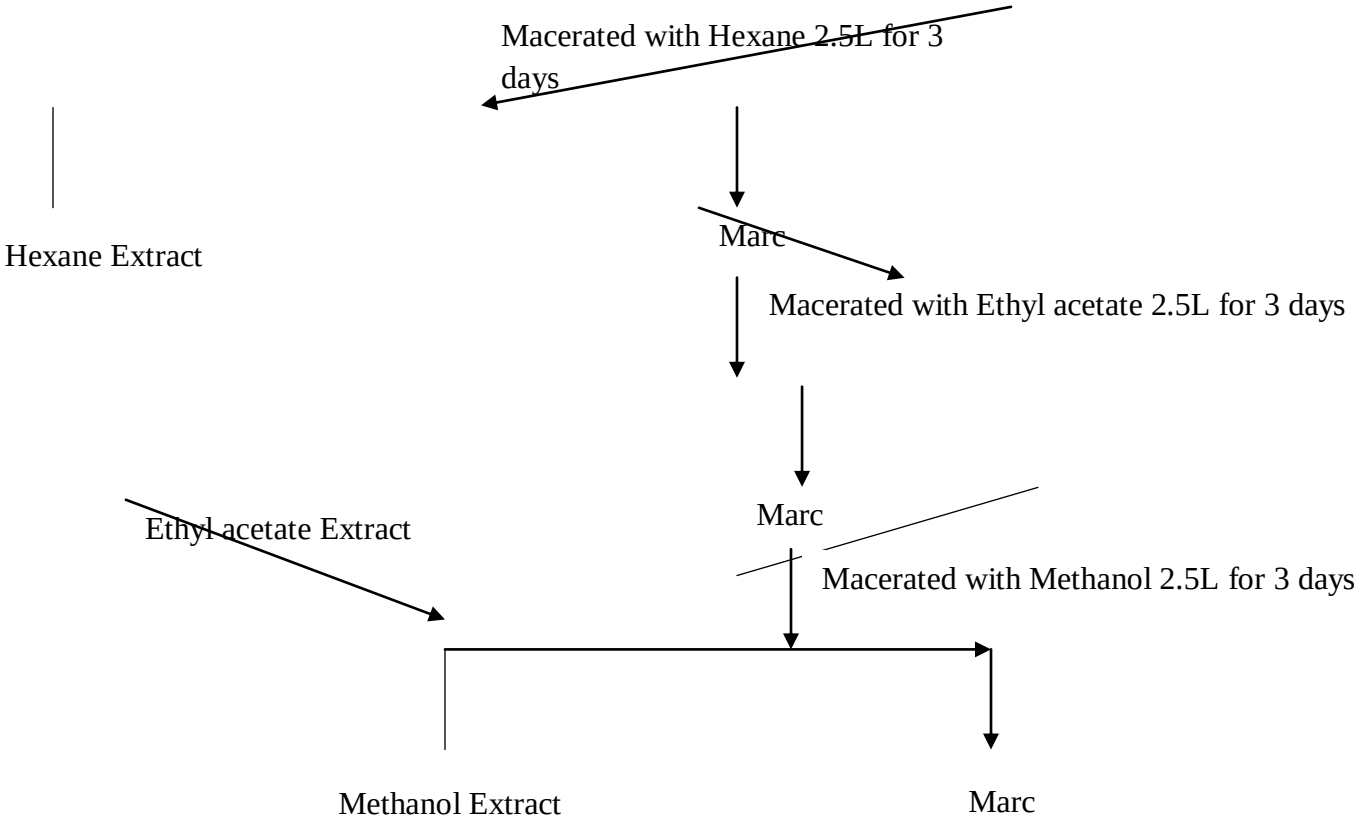


Fig.3.1: Flow Charts for the Extraction of *F. thonningii* Stem Bark (Woo *et al.*, 1980; Kokate, 2003)

3.4.2 Phytochemical Screening of the Stem bark Extracts of *F. thonningii*

The stem bark extracts (hexane, ethyl acetate and methanol) were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the method described by (Evans, 2002, Musa, 2005, Sofowora 2008).

3.4.2.1 Tests for Saponins

(i) Frothing test: Five hundred milligrams (500 mg) of hexane extract was dissolved in ten mililitre (10ml) of water and shaken vigorously in a test tube for thirty (30) seconds and allowed to stand for 30 minutes. The occurrence of frothing column or honey comb-like of at least 1 cm in height and persisting for at least fifteen (15) minute indicated the presence of saponins (Sofowora, 2008). Ethyl acetate and methanol extracts were treated similarly.

(ii) Haemolysis test: Two mililitre (2 ml) of sodium chloride (1.8% solution in distilled water) were added to two test tubes A and B. Two mililitre (2 ml) of distilled water was added to test tube A, 2 ml of the hexane extract was added to test tube B. Five (5) drops of blood were added to each tube and the tubes were inverted gently to mix the contents. Haemolysis (settling of red blood cells) in tube B containing the hexane extract and its absence in tube A (i.e. control), indicated the presence of saponins in the hexane extracts (Brain and Turner, 1975). Ethyl acetate and methanol extracts were treated similarly.

3.4.2.2 Test for Steriods/ Triterpenes

(i) Lieberman-Burchard test: one mililitre (1ml) of acetic anhydride was added to 0.5 g of the hexane extract (dissolved in 2ml of chloroform). Two drops of sulphuric acid were then added gently by the side of the test tube to form lower layer. Blue to blue green colour in the upper layer and a reddish pink or purple colour indicated the presence of triterpene (Evans, 2002). Ethyl acetate and methanol extracts were treated similarly.

(ii) Salkowski test: Two mililitre (2mL) of chloroform and two drops of sulphuric acid were carefully added to 0.5 g of the hexane extract from the side of the test tube to form a lower layer. A reddish brown coloration at the interface indicated the presence of steroidal ring. Ethyl acetate and methanol extracts were treated similarly (Sofowora, 2008).

3.4.2.3 Test for Flavonoids

(i) Shinoda test: Half a gram of hexane extract was dissolved in 2 ml of 50% methanol. One hundred milligrams (100mg) of magnesium fillings and Three (3) drops of hydrochloric acid was added. Appearance of pink colouration indicated the presence of flavonoids (Evans, 2002). Ethyl acetate and methanol extracts were treated similarly.

(ii) Sodium hydroxide test: Half a gram of hexane extract was dissolved in hexane and filtered; 2 ml of 10% aqueous sodium hydroxide solution was then added. The solution was observed for the presence of yellow color. A change in color from yellow to colorless on addition of dilute hydrochloric acid indicated the presence of flavonoids (Evans, 2002). Ethyl acetate and methanol extracts were treated similarly.

3.4.2.4 Tests for Tannins:

(i) Ferric chloride test: Two drops of ferric chloride solution was added to hexane extract (2mL dissolved in hexane). Appearance of blue or green precipitate indicated the presence of phenolic nucleus (Musa, 2005). Ethyl acetate and methanol extracts were treated similarly.

(ii) Lead sub-acetate test: Two drops of lead sub-acetate solution was added to hexane extract (2mL dissolved in hexane) and appearance of whitish-yellow precipitate indicated the presence of tannins (Musa, 2005). Ethyl acetate and methanol extracts were treated similarly.

3.4.2.5 Test for alkaloids: Hexane extract was dissolved in 2N HCl. The mixture was filtered and the filtrate was divided into 3 equal portions. One portion was treated with few drops of Dragendorff's

reagent; one portion was treated with equal amount of Mayer's reagent and the other portion was treated with an equal amount of Wagner's reagent. Appearance of orange, cream and reddish brown precipitates respectively indicated the presence of alkaloids (Salehi-Surmaghi *et al.*, 1992). Ethyl acetate and methanol extracts were treated similarly.

3.4.2.6 Test for Cardiac glycosides

Keller-Killiani test: Half a gram of hexane extract was dissolved in glacial acetic acid containing ferric chloride and 1 mL of sulphuric acid was added to the solution. The appearance of reddish-brown colouration at the interphase of the two liquids, indicated presence of cardiac glycoside or cardenolide (Sofowora, 2008). Ethyl acetate and methanol extracts were treated similarly.

3.4.2.6 Test for Anthracenes

(i) Bontrager test: Half a gram of hexane extract was added to 10 ml of chloroform and shaken. This was then filtered and 5 ml of 10% ammonia solution was added to the Filtrate; stirred and the reaction was observed**.** The presence of pink or cherish red color indicated the presence of anthracenes (Evans, 2002). Ethyl acetate and methanol extract were treated similarly.

(ii) Modified Borntrager's test: Half a gram of hexane extract was boiled with 10 ml of aqueous sulphuric acid and filtered hot. The filtrate after cooling to room temperature was shaken with 5 mL chloroform. The chloroform layer was separated and 10% ammonium hydroxide was added to half of its volume. A pink, red or violet coloration in the ammonia phase (lower phase) indicated the presence of combined anthraquinone or anthraquinone derivatives (Evans, 2002). Ethyl acetate and methanol extracts were treated similarly.

3.5 Thin layer chromatographic profile of the plant extracts of *F. thonningii* stem bark

TLC plate of 20×20 cm coated with silica gel 60 F_{254} on aluminium sheet was used and one way ascending technique was employed for the analysis. The extracts were dissolved in the initial

extraction solvent. The plates were cut into size of 5×10 cm and spots were applied manually on the plates using capillary tube after which plates were dried and developed. Hexane extract was developed in hexane - ethyl acetate (9:1), Ethyl acetate extract was developed in chloroform - ethyl acetate (19:1) and methanol extract was developed in Butanol - Acetic Acid - Water (8:1:1) solvent systems. Developed plates were sprayed using general detecting reagent (*p*-Anisaldehyde/H_2SO_4) and specific detecting reagents (Borntragers, Dragendorffs spray reagent, Ferric chloride, Lieberman-Buchard and aluminium chloride) and heated at 110ºC where applicable. Number of spots, colors and retardation factors (R_f values) for each of the spots were determined and recorded (Gennaro, 2000; Stahl, 2005).

3.6 Antimicrobial studies of *F. thonningii*

3.6.1 Collection of Clinical Isolates

Eight (8) clinical isolates were collected from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH) Zaria, Nigeria.

The clinical isolates used were identified as follows;

Methicillin Resistant Staphylococcus aureus (MRSA), Staphylococcus aureus, Streptococus pyrogens, Escherichia coli, Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa Klebsiella pneumonia, *Candida albicans* and *Candida tropicalis*. All the isolates were checked for purity and maintained in agar slants.

3.6.2 Standardization of organisms

The test organisms were suspended in normal saline and incubated for 6hrs at 37ºC. After incubation, they were standardized according to European Committee for Antimicrobial Susceptibility Testing (EUCAST) by comparing their turbidity to McFarland standard of 0.5.

3.6.3 Preparation of Stock Solution

The extracts (0.5g) each of hexane, ethyl acetate, and methanol of the stem bark of *F. thonningii* were weighed and dissolved in 10mL of DMSO to obtain a concentration of 50 mg/ml as the concentration of the extracts used for the antimicrobial studies. Standard antibiotic (Ciprofloxacin) was used as the positive control drug for bacteria strains and Fluconazole for the fungi (Isonberg, 2005; Cheesbrough, 2006).

3.6.4 Preparation of culture media

Culture media for bacteria was prepared using Muller Hinton Agar (MHA) as follows: The media was prepared according to manufacturer's instruction, sterilized at 121^0C for 15 minutes and poured into sterilized Petri dishes and then allowed to cool and solidify. Culture media for Fungi was prepared using Potato dextrose agar (PDA) as follows:

The media was prepared according to manufacturer's instructions, sterilized at 121^0C for 15 minutes and poured into sterilized Petri dishes and then allowed to cool and solidify (Isonberg, 2005; Cheesbrough, 2006; Sandhyarani *et al*., 2014).

3.6.5 Determination of inhibition

Agar well diffusion method was used to screen all the extracts. The sterilized medium was inoculated with 0.1mL of the standard inoculums (Standardized using McFarland standard 0.5). The inoculum was spread evenly over the surface of the medium with a sterile swab. Using standard cork-borer of 6mm, a well was cut. One tenth milliliter (0.1mL) of the solution of the extract at a concentration of 50 mg/mL was then introduced into each well on the inoculated media. Incubation of the media was done at 37^0C for 24hours, after which the plates were

observed for zones of inhibition. The clear zones were measured with a transparent ruler and the result recorded in millimeters (Isonberg, 2005; Cheesbrough, 2006).

3.6.6 Determination of Minimum Inhibition Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the extracts was carried out on the test organisms. This was done by broth dilution method. Nutrient broth was prepared according to manufacturer's instruction. Ten milliliters of the broth was dispensed into each test tube and sterilized at 121°C for 15 mins, this was allowed to cool. Concentrations of extracts showing inhibition for the organisms when tested during sensitivity test was two-fold serially diluted in the test tube containing nutrient broth. Two fold serial dilutions of the extracts in the sterile broth gave the following concentrations; 50, 25, 12.5, 6.25, and 3.125mg/mL. The initial concentration was obtained by dissolving 0.5g of the extract in 10ml of sterile broth. From the standardized microorganisms in normal saline, 0.1mL was inoculated into the different concentrations of the extract in the nutrient broth. Tube containing nutrient broth only was seeded with a loop full of standardized test organisms to serve as positive control. Another tube containing nutrient broth and the extract was used as a negative control. The inoculated tubes were incubated at 37ºC for 24 hours (bacteria) and 2 – 3 days (fungi). At the end of the incubation period, the test tubes were observed for turbidity (growth). The lowest concentration in the series which showed no turbidity when compared with the control was considered and recorded as MIC (Isonberg, 2005; Cheesbrough, 2006).

3.6.7 Determination of Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC)

The result from the MIC was used to determine the MBC/MFC of the extract. A loop fall from the test tubes that did not show turbidity (clear) was aseptically subcultured on to nutrient agar plates. The plates were incubated at 37ºC for 24hours. At the end of the incubation period, the plates were observed for the presence or absence of growth. The lowest concentration from MIC

tubes that showed no growth on the MHA and PDA plates was regarded as the MBC/MFC (Isonberg, 2005; Cheesbrough, 2006).

3.7 Statistical Analysis

Data were expressed as Mean ± Standard error of mean (S.E.M), which was in accordance with Tom (2004).

CHAPTER FOUR

4.0 RESULTS

4.1 Pharmacognostic Studies on *F.thonningii*

4.1.1 Microscopic Features

The transverse section revealed an outer layer of cork cells which appeared rectangular in lateral view. After this was the phellogen which contained the cortical cells and the sclerenchyma tissue particularly the sclereids which are tightly joined together. The inner part of the stem bark is dominated by the pitted parenchymatous cells and prismatic calcium oxalate crystals (plate 4 and 6). The longitudinal section of the stem bark revealed the presence of pointed ends fibres, medullary rays and phloem parenchyma (plate 5) and the powdered stem bark showed calcium oxalate crystals, cork cells and fibres (plate 7). The micrometric parameters of some of the above mentioned features were measured and fibre were the longest with mean value of 801 µm, cork cells 21.36 µm and calcium oxalate crystals 46.73 µm (Table 4.1).

4.1.2 Chemomicroscopical studies of the powdered Stem Bark of *F. thonningii*

Chemomicroscopical examination of the powdered stem bark of *F. thonningii* revealed the presence of cellulose cell wall, lignified cell wall, tannins, starch, calcium oxalate, suberin or cutin and mucilage

4.1.2.1 Cell wall Materials

(i) Test for Cellulose:

Blue colour was observed on the walls of the plant when treated with Chlor-Zinc-Iodine solution which indicated the presence of cellulose.

(ii) Test for Suberins: Red colour was observed on the walls of some cells when treated with Sudan red and heated for 2 minutes which indicated presence of suberins..

(iii) Test for Lignin

Red stain was observed on the walls of some lignified cells when treated with phloroglucinol and HCl which indicated the presence of lignin.

(iv) Test for Gum and Mucilage

Pink colour was observed on the walls of some cells when treated with ruthenium red, which indicated the presence mucilage**.**

4.1.2.2 Cell Inclusions

(i) Test for Calcium carbonate

Powdered stem bark of the plant when treated with concentrated HCl showed no effervescence in the cell, which indicates the absence of calcium carbonate.

(ii) Test for Calcium oxalates: Powdered stem bark of the plant was observed under the microscope. Two drops of hydrochloric acid on crystals showed dissolution of those crystals on the anatomical sections of the bark which indicates the presence of prismatic calcium oxalate crystals.

(iii) Test for Tannins: Powdered stem bark of the plant when treated with 5% $FeCl_3$ showed greenish-black colouration on some cells which indicated the presence of tannins.

(iv) Test for Starch

Blue-black colouration was observed on some grains within the cells when treated with N/50 iodine solution which indicated the absence of starch granules.

In summary, the stem bark contain cellulose, Lignins, tannins, gums and mucilages, suberin and calcium oxalate crystals as shown in Table 4.2

4.1.3 Determination of Physical Constants of Powdered Stem bark of *F. thonningii*

The average moisture contents using loss on drying method was calculated to be 6.56 % while percentage yields of total ash, water soluble ash and acid insoluble ash were recorded to be 9.50, 7.50 and 2.25% respectively. The extractive values obtained were 16.67 and 10.67% for alcohol and water respectively (Table 4.3).

4.2 Preliminary Phytochemicals Analysis of the Stem Bark of *Ficus thonningii*

4.2.1 Percentage yield of the powdered stem bark of hexane, ethyl acetate and methanol

The yield from the extraction was 16.51g for hexane, 36.75 g for ethyl acetate and 132.28 g for methanol extracts. These percentage yields implied % yields of 1.65, 3.68 and 13.23 respectively for hexane, ethyl acetate and methanol is reported in (Table 4.4).

Table 4.1: Micrometric parameters of Stem bark of *F. thonningii*

Type of cell	**Length in micrometer (µm) ± SEM**
Cork cells	21.36 ± 2.56
Calcium oxalate crystals (Prism)	46.73 ± 3.77
Fibres	801.00 ± 10.0

*****mean for three (3) replication

Table 4.2: Summary of the chemomicroscopical analysis of the powdered stem bark

Constituents tested	Result
Cellulose	Present
Suberin	Present
Lignin	Present
Gums and Mucilages	Present
Calcium oxalate crystals	Present
Calcium Carbonate	Absent
Tannins	Present
Starch	Absent

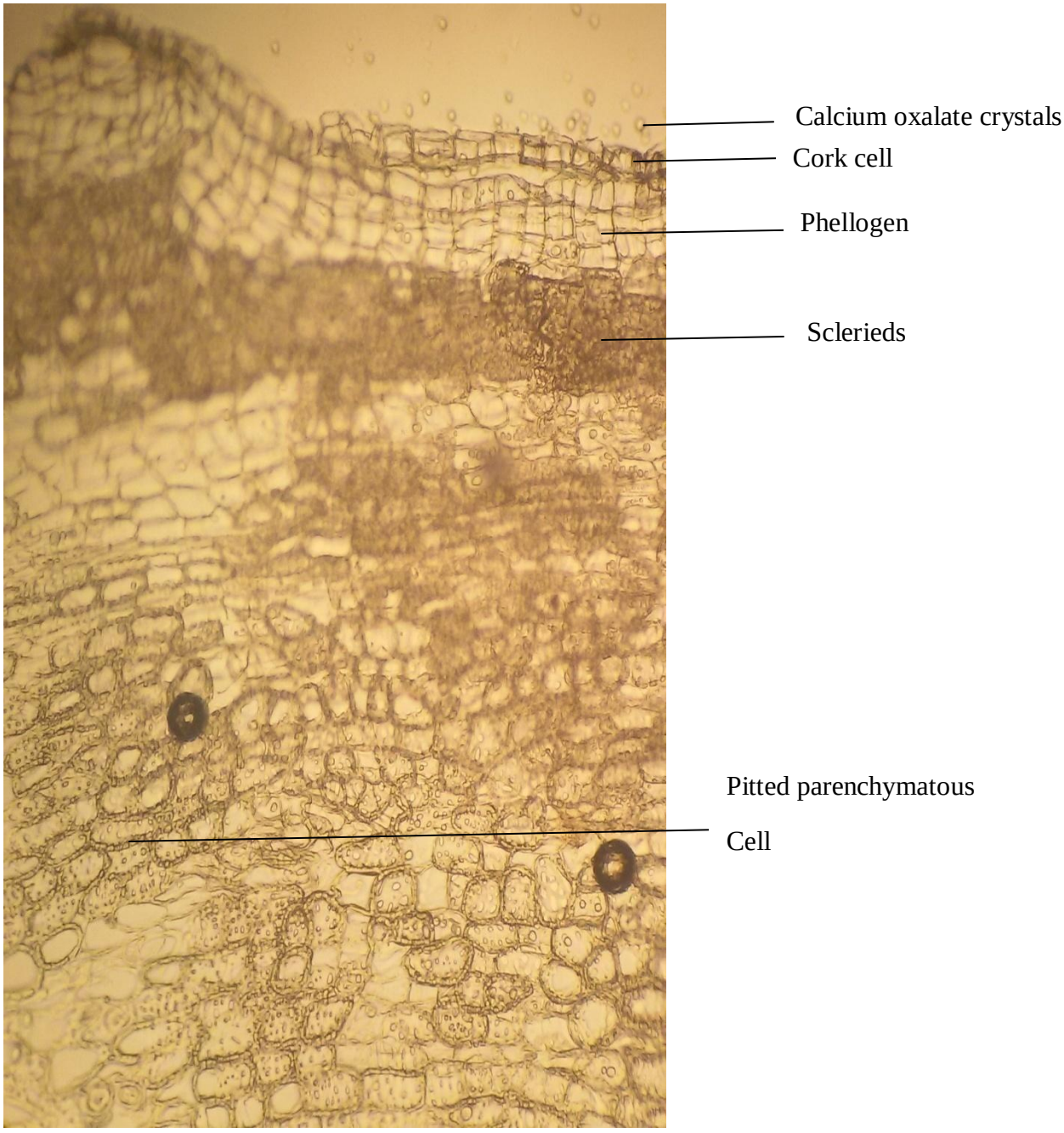


Plate 4: Micrograph of the transverse section of *F. thonningii* showing some cork cells, parenchyma cells, sclereids, phellogen and starch grains (Mag. ×100).

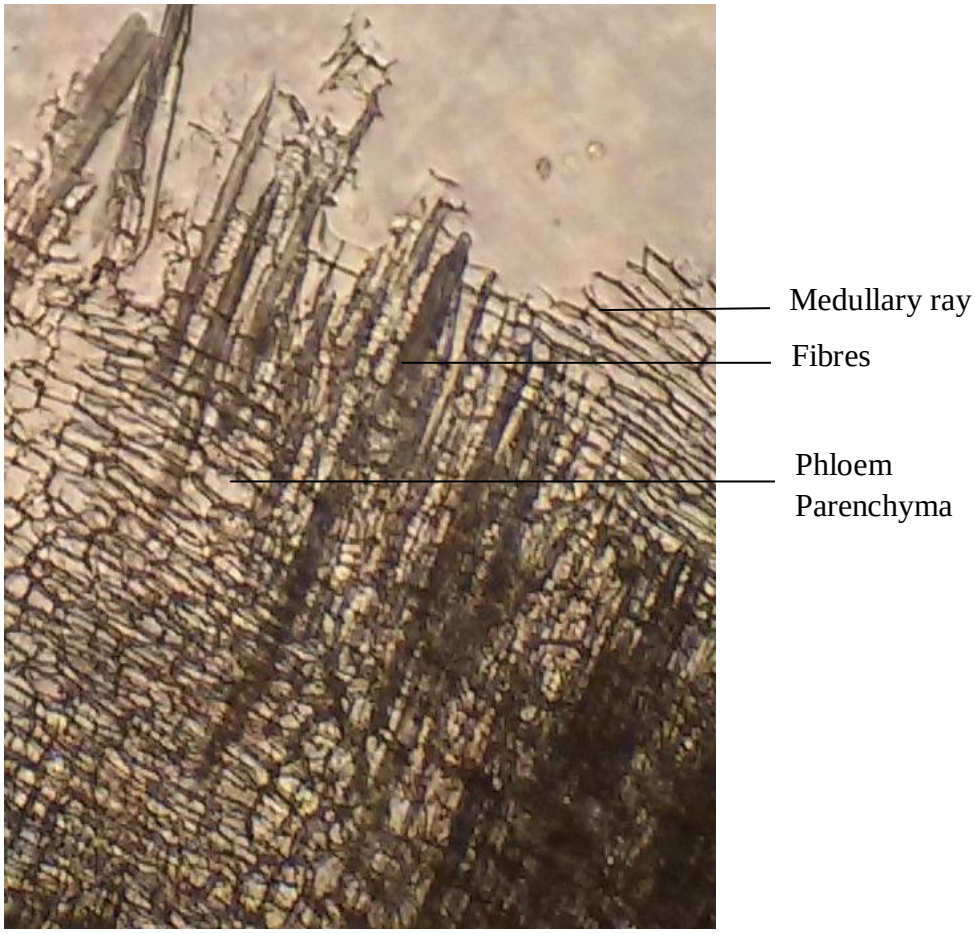


Plate 5: Micrograph of the longitudinal section of *F. thonningii* showing medullary ray, fibres and phloem parenchyma (Mag. ×100)

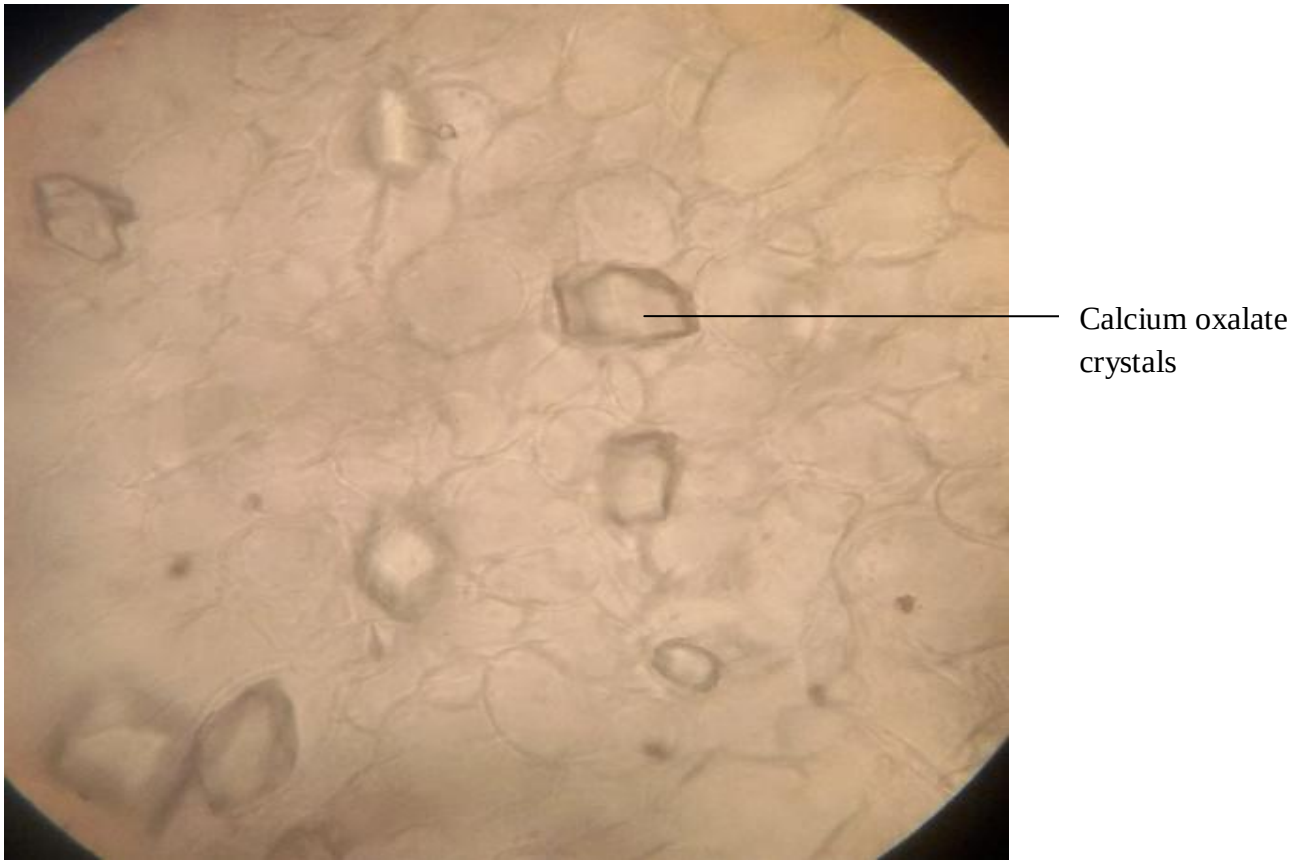


Plate 6: Micrograph of *F. thonningii* transverse section showing calcium oxalate crystals (Mag. ×400)

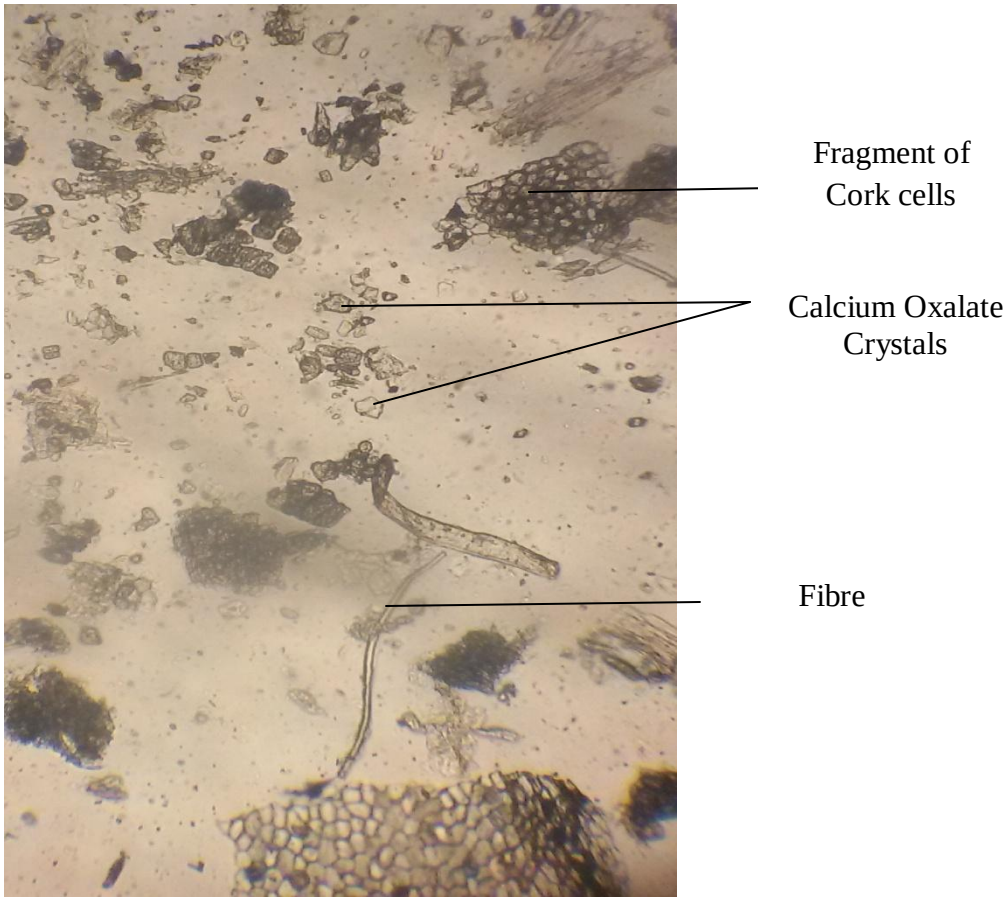


Plate 7: Micrograph of the powdered stem bark of *F. thonningii* showing cork cells, fibres and calcium oxalate crystals. (Mag. ×400)

Table 4.3: Physicochemical Parameters of Stem bark of *F. thonningii*

Parameters	**Values (%w/w) ± SEM***
Moisture Content	6.56 ± 0.00
Total ash value	9.50 ± 0.00
Water soluble ash	7.50 ± 0.06
Acid insoluble ash	2.25 ± 0.00
Alcohol extractives	16.67 ± 0.33
Water extractives	10.67 ± 0.33

***mean for three (3) replication

4.2.2 Phytochemical Screening

Phytochemical screening of hexane, ethyl acetate and methanol extracts using different chemical reagents revealed the presence of cardiac glycoside, carbohydrate, saponins, tannins, anthraquinones, steroid and triterpenes. The results are summarized in Table 4.5.

i) Test for Saponins

(a) Frothing Test

Methanol extract of the plant when dissolved in test tube containing water and shaken vigorously gave persistent froth; whereas hexane and ethyl acetate did not which indicated the presence and absence of saponins respectively.

(b) Haemolysis Test

Haemolysis in the test tube containing methanol extract of the plant was observed when tested with sodium chloride, which indicated the presence of saponins, whereas hexane and ethyl acetate did not which indicates the absence of saponins.

ii) **Test for Steroids/Triterpenes**

(a) Salkowski Test

A reddish-brown colouration at the interface was obtained in the hexane, ethyl acetate and methanol extract of the plant when treated with chloroform and concentrated sulphuric acid which indicated the presence of steroidal ring.

(b) Liberman-buchard-: A reddish-brown ring was obtained in the hexane, ethyl acetate and methanol extract of the plant when treated with chloroform, acetic anhydride, chloroform and concentrated sulphuric acid which indicated the presence of sterols or triterpenes.

iii) Test for Flavonoids

(a) Shinoda Test

A rose red colouration was observed in methanol extract when treated with HCl and pieces of magnesium chips which indicated the presence of flavonoids. Whereas absent in hexane and ethyl acetate extract.

(b) Sodium hydroxide Test

Yellow colour was obtained in methanol extract when tested with 10% NaOH and colour changed from yellow to colourless on addition of dilute HCl which indicated the presence of flavonoid, Whereas absent in hexane and ethyl acetate extract.

iv) Test for tannins

(a) Ferric chloride Test

A blue-black colouration was observed in methanol and ethyl acetate extract when treated with 1% ferric chloride solution, which indicated the presence of tannins, where as it was absent in hexane extract.

(b) Lead sub-acetate Test

Methanol and ethyl acetate when dissolved in 2 ml of ethanol followed by two drops of lead sub-acetate solution indicated a whitish yellow precipitate indicating the presence of tannins but was found absent in the hexane extract.

v) Test for Alkaloids

Creamy coloured precipitates was observed with Mayer's reagent, orange precipitates with Dragendoff's reagent while brownish-red precipitates with Wagner's reagent in ethyl acetate and methanol extracts of the plant. Precipitates observed with the three reagents indicated the presence of alkaloids. Whereas it was absent in hexane extract.

vi) Test for Cardiac glycosides

(a) Keller-kiliani Test

A brown ring at the interphase was obtained in hexane, ethyl acetate and methanol extracts of the plant when tested with glacial acetic acid and 1 drop of sulphuric acid, which indicated the presence of deoxysugar.

(b) Kedde's Test

A purple-blue colour was not observed in hexane, ethyl acetate and methanol extracts of the plant when treated with 1ml of 3, 5- dinitrobenzoic acid and NaOH; this indicates the absent of cardenolides.

vii) Test for Anthraquinones

(a) Borntrager's Test

Pink, rose or red colour was observed at the ammonia (lower layer) when treated with 10 ml of benzene and 5 ml of 10% ammonia solution in methanol extract. This indicated the presence of free anthracenes in methanol extract where as absent in Hexane and ethyl acetate.

(b) Modified Borntrager's Test

Hexane, ethyl acetate and methanol extracts were boiled with 10 ml of aqueous sulphuric acid and filtered hot. The filtrate after cooling to room temperature was shaken with 5 ml benzene, the benzene layer was separated and half of its volume, 10% ammonium hydroxide was added. Pink, red or violet colouration in the ammonia phase (lower phase) was observed in methanol extract and absent in hexane and ethyl acetate extract, which indicated the absence of combined anthraquinone or anthraquinone derivatives.

4.3 Thin Layer Chromatography Studies

The TLC finger prints of extracts of *F. thonningii* were obtained upon subjecting the extracts (n-hexane, ethyl acetate and methanol) to thin layer chromatography on silica gel pre-coated plates.

4.3.1 Thin Layer Chromatography of Crude Hexane Extract

Hexane extract was noted to give ten (10) spots in Hexane - Ethyl acetate (9:1), after sprayed with *p*-Anisaldehyde and heated at 110ºC for about 2 minutes (Plate 7A). TLC of hexane extract developed in Hexane - Ethyl acetate (9:1) sprayed with Lieberman-Buchard reagent, revealed eleven (11) spots of violet, pink and orange-brown colors which are characteristic colors for triterpenes/steroids (Plate 7B). No coloured spots were observed for TLC plates sprayed with Ferric chloride Bontrager`s, Aluminium chloride and Dragendorf`s reagents.

4.3.2 TLC profile of Ethyl acetate extract

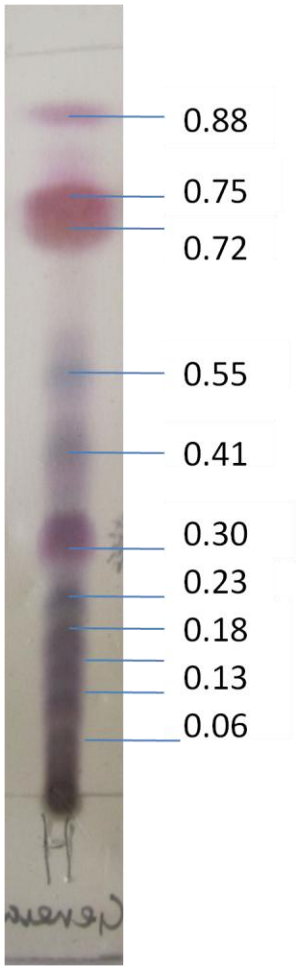
TLC profile of ethyl acetate extract had twelve (12) spots when developed in Chloroform - Ethyl acetate (19:1), sprayed with p-Anisaldehyde and heated at 110ºC for about 2 minutes (Plate 8A). TLC of ethyl acetate extract developed in Chloroform - Ethyl acetate (19:1) sprayed with Lieberman-Buchard reagent, revealed 9 spots (Plate 8B).

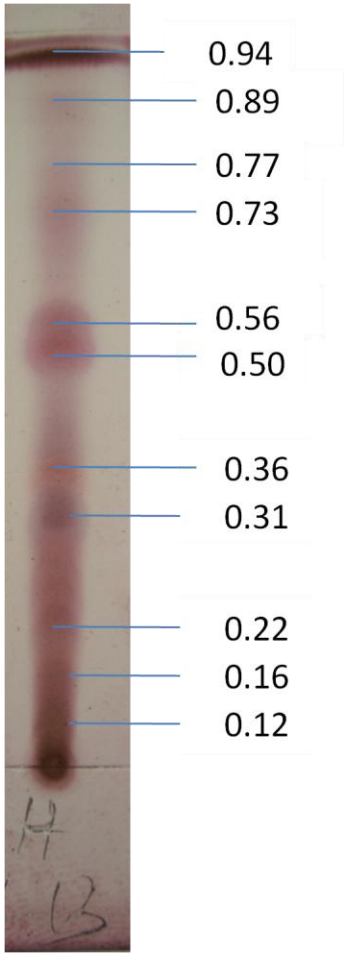
Table 4.4 Percentage yield of extracts from *F. thonningii* stem bark

S/N	Extract	Mass (g)	Colour	Yield (% w/w)
1	Hexane	16.51	greenish and sticky	1.651
2	Ethyl acetate	36.75	yellowish and sticky	3.675
3	Methanol	132.28	dark brown and powdery	13.228

Table 4.5 Phytochemical constituent present in the stem bark extracts of *F. thonningii*

S/n	Phytochemicals	Hexane	Ethyl acetate	Methanol
1	Alkaloids	Absent	Absent	present
2	Cardiac glycosides	Present	Present	Present
3	Saponins	Absent	Absent	Present
4	Tannins	Absent	Present	Present
5	Steroid/Triterpenes	Present	Present	Present
6	Carbohydrate	Present	Present	Present
7	Flavonoids	Absent	Absent	Present
8	Anthraquinones	Absent	Absent	Present

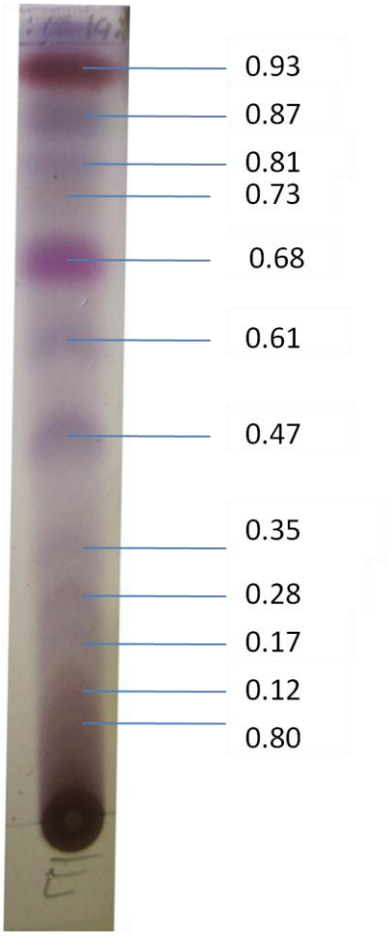




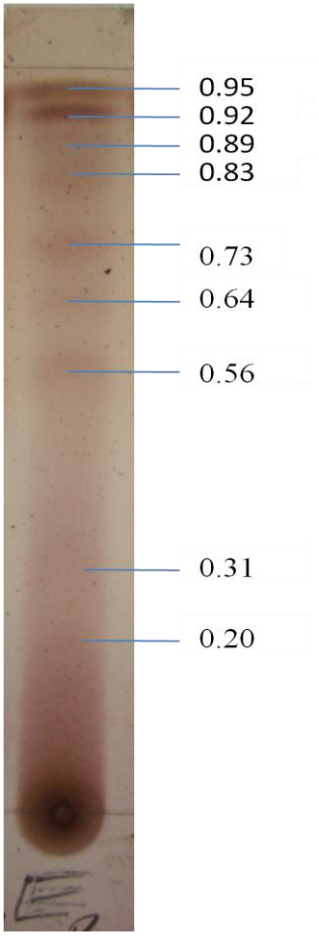
A **B**

Plate 7A: Chromatogram of hexane extract developed in H: E (9:1) sprayed with *p*-Anisaldehyde/H_2SO_4. Showed spots with R_f values.

Plate 7B: Chromatogram of hexane extract in hexane: ethyl acetate (9:1) Sprayed with (LB) Lieberman-Buchard reagent for steroid/triterpenes and R_f values.



A



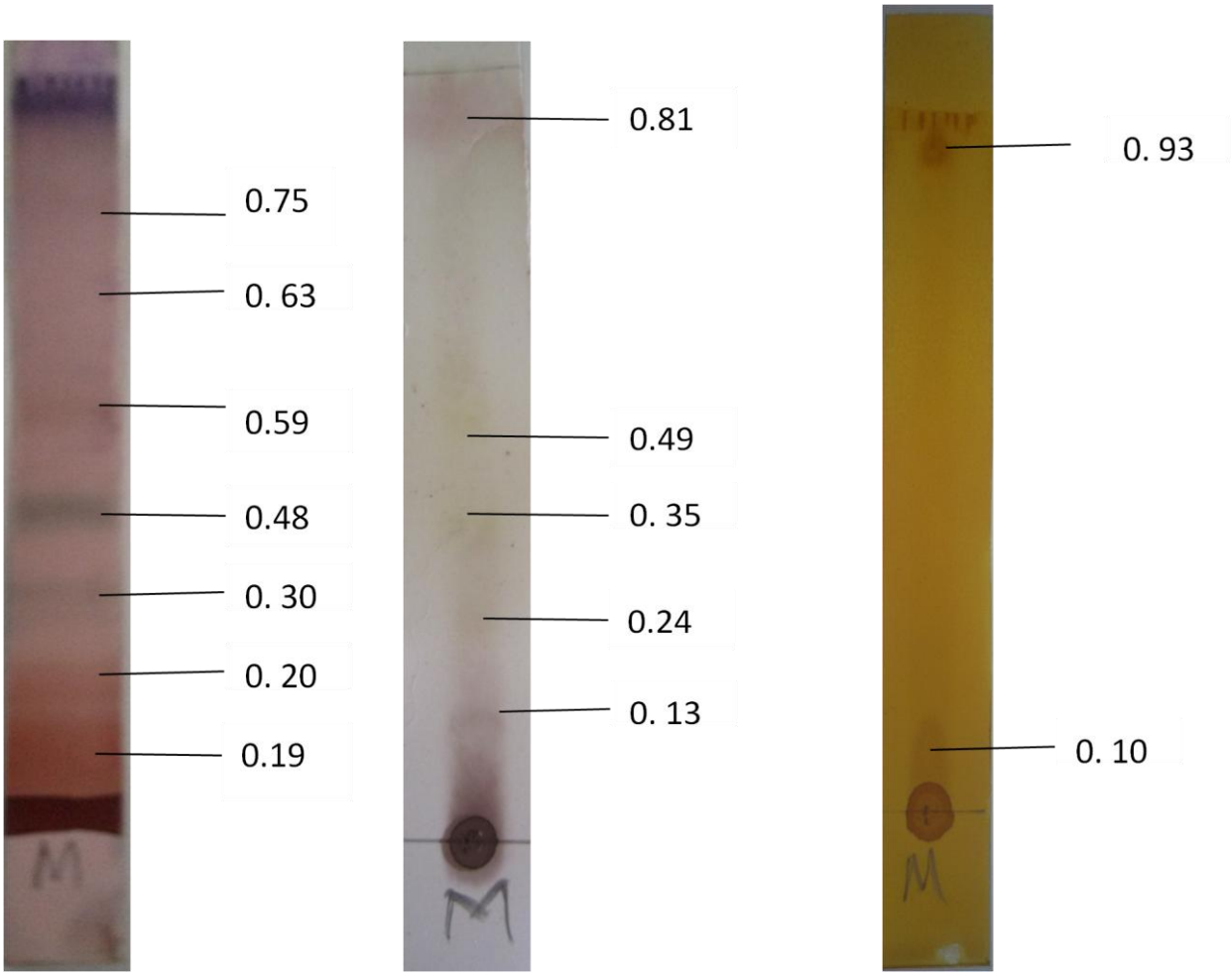
B

Plate 8A: Chromatogram of ethyl acetate extract developed in Chloroform - Ethyl acetate (19:1) sprayed with *p*-Anisaldehyde/H_2SO_4. Showed spots with R_f values.

Plate 8B: Chromatogram of ethyl acetate extract developed in Chloroform - Ethyl acetate (19:1) Lieberman-Buchard reagent for steroid/triterpenes and R_f values.

4.3.3 Thin Layer Chromatography of Methanol Extract

TLC analysis of methanol extract developed in Butanol - Acetic acid - Water (8:1:1) revealed 7, 5, 2, 2, 1 bands/spots when sprayed with p-anisaldehyde in sulphuric acid, Liebermann-Bucchard reagent, Dragendorff reagent, Ferric chloride and Aluminum chloride respectively. Spots were of different colors (Plate 9A, 9B, 9C, 9D and 9E). Chromatogram B obtained with Liebermann-Burchard reagent revealed green spots showing the presence of steroid/triterpenes, chromatogram C showed reddish brown spots (presence of alkaloids), chromatogram D was sprayed with ferric chloride which showed presence of phenolic compounds, chromatogram E was sprayed with aluminum chloride and viewed under UV (256/354nm). The presence of yellow spot indicates presence of flavonoids.



A B C

Plate 9A: Chromatogram of Methanol Extract Developed in Butanol - Acetic Acid - Water (8:1:1) with R_f values.

Plate 9B: Chromatogram of Methanol extract sprayed with Lieberman-Bucchard reagent for steroid/triterpenes and heated at 105ºC for 2 minutes. Showed spots with R_f values.

Plate 9C: Chromatogram of Methanol extract sprayed with dragendorf reagent (alkaloid) which showed coloured spots with R_f value.

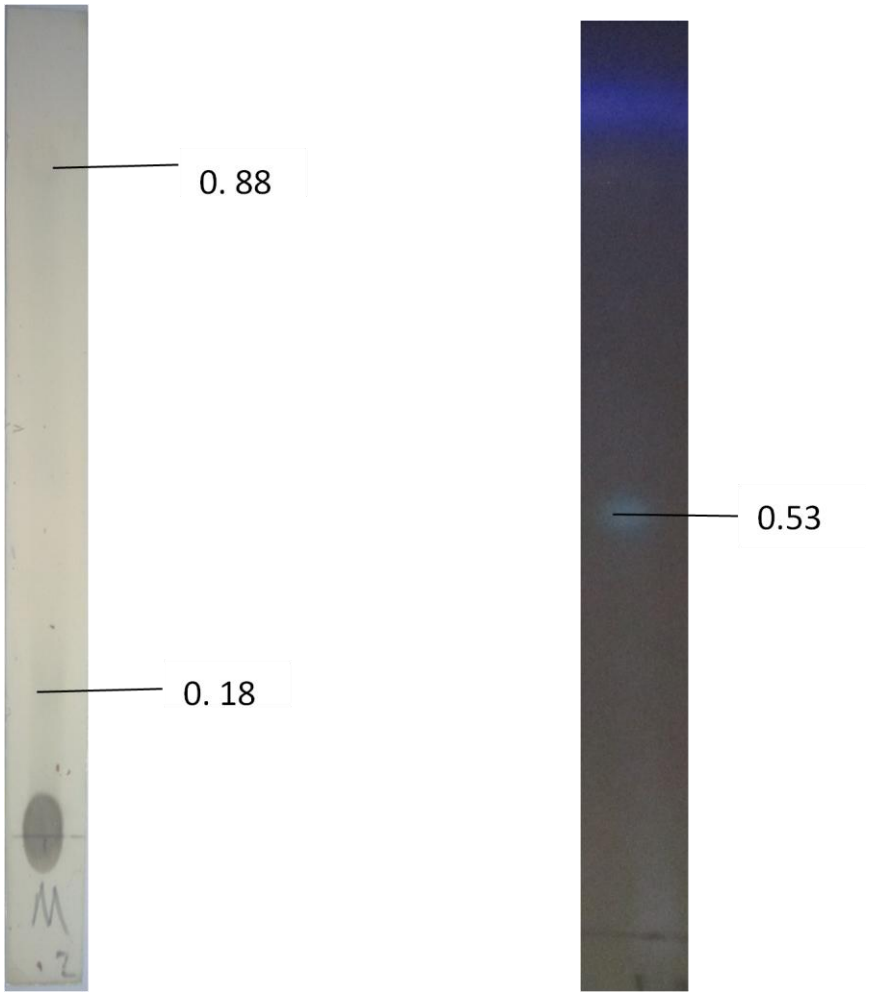


Plate 9D: Chromatogram of Methanol extract sprayed with Ferric chloride reagent (phenolic compounds) showed coloured spots with R_f values.

Plate 9E: Chromatogram of Methanol extract sprayed with Aluminium Chloride reagent (flavonoids) and viewed under UV light. It showed spot with R_f values.

4.4 Evaluation of sensitivities of the stem bark extracts to the micro-organisms

4.4.1 Zones of Inhibitions (mm)

A zone of observable inhibition of growth of each micro-organism served as a criterion for declaring an extract sensitive and was indicated by a clear zone around the well. The hexane, ethyl acetate and methanol extracts showed antimicrobial activities on the test organisms used. The zones of inhibition observed ranged from 20 - 21, 25 - 30 and 22 - 24 mm for hexane, ethyl acetate and methanol respectively and lower than that of the control (Ciprofloxacin) which had 31- 41 mm and Fluconazole 34 – 35. The diameter of zones of inhibition of the extracts against test organisms (in mm) was highest for ethyl acetate extract which has activity against some of the test organisms. Ethyl acetate extract showed remarkable activity against gram negative (-) *E.coli* at a diameter zone of 30m. Hexane extract had minimal effect on the test organisms compared to ethyl acetate and methanol extracts, with diameter zones of inhibition of 20mm for *S. aureus, E. coli, P. aeriginosa* and *K. pneumoniae*. All extracts used had no activity against *C. albicans, MRSA* and *P. mirabilis* (Table 4.6).

4.4.2 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration that is required to inhibit the growth of the test organisms. For hexane extract, no growth was observed at 50, 25 and 12.5 mg/ml. Since 12.5 mg/ml was the lowest concentration that inhibited the growth of the organisms, it was taken as MIC for all the test organisms. MIC of ethyl acetate (EA) extract was observed at 6.25 mg/ml against all pathogens except *P. aeruginosa* and *C. tropicalis* that showed MIC of 12.5 mg/ml, whereas, MIC of methanol extract (ME) was at 12.5mg/ml for all the tested organisms (Table 4.7).

4.4.3 Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The minimum bactericidal concentration (MBC) is the minimum concentration required to kill the organism. HE had MBC of 50 mg/ml because; it was the only concentration that killed all the test organisms. EA showed MBC of 12.5 mg/ml for *S. aureus*, *E. coli*, *S. typhi* and *K. pneumonia* except *S. pyogenes* and *P. aeruginosa* that had MBC's of 25 mg/ml. However, the value obtained for ME against all organisms was 25 mg/ml except *P. aeruginosa* that had MBC of 50 mg/ml. The fungi; *C. tropicalis* had MFC of 50 mg/ml for hexane extract, 25 mg/ml for ethyl acetate and methanol extract. Concentrations that were not able to inhibit the growth of the test organisms were not tested for bacteriacidal effect (Table 4.8).

Table 4.6: Zones of Inhibition of Hexane, Ethyl acetate and Methanol extracts (mm)

Test organism	HE (50mg/ml)	EA (50mg/ml)	ME (50mg/ml)	CP (5mg/ml)	FL (5mg/ml)
MRSA	-	-	-	31	NA
S.aureus	20	28	23	34	NA
S.pyogenes	21	27	24	37	NA
E.coli	20	30	24	39	NA
S.typhi	21	28	23	41	NA
P.mirabilis	-	-	-	-	NA
P.aeriginosa	20	25	22	35	NA
K.Pneumonia	20	29	23	35	NA
C. tropicalis	20	26	24	NA	34
C. albicans	-	-	-	NA	35

Key: HE = hexane extract, EA = ethyl acetate extract, ME = Methanol extract, CP= Ciprofloxacin - = no growth NA = Not Applicable

Table 4.7: Minimum Inhibitory Concentration of Hexane, Ethylacetate and Methanol Extract of *F. thonningii*

Test organism	**HE (mg/ml)**					**EA (mg/ml)**					**ME (mg/ml)**				
	50	25	12.5	6.25	3.12	50	25	12.5	6.25	3.12	50	25	12.5	6.25	3.12
S. aureus	-	-	*	+	++	-	-	-	*	+	-	-	*	+	++
S. pyogenes	-	-	*	+	++	-	-	-	*	+	-	-	*	+	++
E. coli	-	-	*	+	++	-	-	-	*	+	-	-	*	+	++
S. typhi	-	-	*	+	++	-	-	-	*	+	-	-	*	+	++
P. aeriginosa	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++
K. Pneumonia	-	-	*	+	++	-	-	-	*	+	-	-	*	+	++
C. tropicalis	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++

KEY: - = Clear (No growth), *** = MIC**, + =Turbid (Light growth), **++** = (Moderate turbid), **+++** = (High turbidity), HE = hexane extract, EA = ethyl acetate extract, ME = Methanol extract

Table 4.8: Minimum Bacteriacidal/Fungicidal Concentration of Hexane, Ethylacetate and Methanol Extract of *F. thonningii*

Test organism	**HE (mg/ml)**			**EA (mg/ml)**				**ME (mg/ml)**		
	50	25	12.5	50	25	12.5	6.25	50	25	12.5
S.aureus	*	+	++	-	-	*	+	-	*	+
S.pyogenes	*	+	++	-	*	+	++	-	*	+
E.coli	*	+	++	-	-	*	+	-	*	+
S.typhi	*	+	++	-	-	*	+	-	*	+
P.aeriginosa	*	+	++	-	*	+	++	*	+	++
K.Pneumonia	*	+	++	-	-	*	+	-	*	+
C. tropicalis	*	+	++	-	*	+	++	-	*	++

Key: - = Clear (No growth), * = MBC/MFC, + = Scanty colonies growth, ++ = Moderate colonies growth, HE = hexane extract, EA = ethyl acetate extract, ME = Methanol extract.

CHAPTER FIVE

5.0 DISCUSSIONS

The process of standardization can be achieved by stepwise pharmacognostic and phytochemical studies. This study helps in identification and standardization of plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy (Kadam *et al*. 2011).

The transverse section of *F. thonningii* stem bark revealed some prominent features like cork cells, prismatic calcium oxalate crystals, sclereids, parenchymatous cells, medullary rays and fibres. These features were similar to those observed in *F. virens* (Manoj and Sikarwar, 2015). The prismatic calcium oxalate crystals, rectangular cork cells, sclereids and parenchyma cells were common among the two species (*F. thonningii* and *F. virens*). Phellogen, cork layers and fibres have also been reported in stem bark of *F. hispida* (Singh *et al*., 2012). Anatomical features of the internal structures of plant drugs provides an important diagnostic features for the identification of both entire and powdered crude drugs and detection of adulterants in plant materials (Ghani, 1990). Macro and microscopical evaluation of crude drugs are aimed at identification of right variety and search for adulterant in plant materials (WHO, 2002).

Chemo-microscopical studies on stem bark of *F. thonningii* were found to have cellulose cell wall, lignin, calcium oxalate, tannins, starch and mucilage. Similarly, *F. abutilifolia* was reported to contain starch, tannins, mucilages and calcium oxalate crystals (Ukwubile, 2010). The prism type of calcium oxalate crystals found in both *F. thonningii* and *F. virens* were similar. The stem

bark of *F. racemosa* also showed the presence of starch, cellulose and Lignins (Ahmed and Urooj, 2011).

Physicochemical parameters evaluated showed the presence of moisture content (6.56%). It is very essential to control moisture content since higher moisture content in plant material may lead to its deterioration and may therefore result in biodegradation of active constituents. Less moisture is also an indication that the plant material can be kept for some time (Prasad *et al*., 2013). Total ash value represent both the physiological and non-physiological ash from the plant drugs and non-physiological ash is an indication of inorganic residues after the plant drug is incinerated while acid insoluble ash values of these studies indicated that the plant were in good physiological condition and it contained little extraneous matter such as sand, silica and soil. Total ash value is critical to judge the identity and purity of drugs (WHO, 1996). A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing. Acid insoluble ash indicates contamination with silica, for example, earthy materials and sand. Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water soluble salts in the drug. Total ash value content was 9.50 ± 0.00, acid insoluble ash value was 2.25 ± 0.00 and water soluble ash value was 7.50 ± 0.06. The water extractive value was determined to be 10.67 ± 0.33 and this was lower than that of alcohol extractive value that was 16.67 ± 0.33%. This indicated that alcohol is a more suitable solvent for extraction of the bark powder. Alcohol extractives is preferably used in the laboratories because of some reasons; water is a medium that promotes the occurrence of microorganisms as compared to alcohol (Lapornik, *et al.*, 2005)

Phytochemicals are naturally occurring; biologically active, non-nutritive chemical compounds found in plants and act as a natural defense system against various pests. Various phytochemicals have been known to possess medicinal properties and hence widely used in Nigerian systems of

traditional medicine. In this study, various phytochemicals like saponins, alkaloids, tannins, flavonoids, steroid/triterpenes, glycoside and anthraquinones were detected in the stem bark of *F. thonningii* indicating their potential medicinal uses. Previous studies on the phytochemistry of *F. thonningii* have also reported the presence of these phytochemicals (Ndukwe *et al*., 2007) Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example; saponins, flavonoids, tannins and alkaloids have been reported with hypoglycemic and anti- inflammatory activities (Argal *et al*., 2006). The presences of phenolic compounds which are known to have antibacterial activity were revealed in the plant. This therefore, supports the use of the plant in the traditional treatment of cutaneous infections, venereal diseases, and dysentery (Tom *et al.*, 2008). Reports show that saponins possess hypocholesterolemic and antidiabetic properties. The terpenoids have also been shown to decrease blood sugar level in animal studies (Rupasinghe *et al*., 2003). Each plant family, genus, and species produces a characteristic mix of these chemicals, and they can sometimes be used as taxonomic characters in classifying plants (Biology Encyclopaedia, 2015).

Thin layer chromatographic analysis of n-hexane, ethyl acetate and methanol extracts of *F. thonningii* stem bark in different solvent systems at different ratios gave various degrees of separations. The chromatogram of hexane and ethyl acetate extracts Hexane - Ethyl acetate (9:1) visualized with *p*-Anisaldehyde/H_2SO_4 revealed a clear separation with 10 spots each while the chromatogram of methanol extract in Butanol - Acetic acid - Water (8:1:1) revealed a clear separation with seven (7) spots. Spots of various colours blue, grey, green and violet were revealed. The separation of bio-molecules by chromatographic technique depends on suitable solvent system which needs an ideal range of partition coeffiecient for each target compounds (Ito, 2005). The chromatogram of hexane and ethyl acetate extracts were positive to Liebermann-Bucchard reagent which revealed the presence of triterpernoid/steroids. The chromatogram of

methanol extract was positive to Liebermann-Bucchard, ferric chloride and aluminum chloride reagent which revealed the presence of triterpernoid/steroids, phenolic compounds and flavonoids respectively. The yellow fluorescence of aluminum chloride reagent (which was obtained under UV after spraying the plate) was faint which would be as a result of the degree of concentration of the compound in the extract. The presence of these compounds supports the traditional use of the plant in treatment of antimicrobial infections. Thin layer chromatographic analysis is a simple and cheap method for detection of plant active constituents due to its good selectivity and sensitivity of detection providing convincing results (Patra *et al.,* 2012), hence considered a reliable technique for qualitative phytochemical screening of plant active constituents. Phenolic compounds such as tannins and flavonoids possess diverse biological properties such as anti-inflammatory, antibacterial, antiulcer and anti-oxidant activities (Sen and Batra, 2012). In this study, phenolic compounds, steroids/triterpenes and flavonoids were observed to be the most abundant bioactive constituents in *F. thonningii* stem bark.

Research reported indicates that the antimicrobial properties of plants are conferred in them by the presence of secondary metabolites (Edeoga *et al.,* 2005; Enaba *et al.,* 2007; Bishnu *et al.,* 2009). Kitzberger *et al.,* (2006) reported that the antimicrobial activity can be considerate when the diameter zone of inhibition is 9mm or more around the paper disk. From this, it can be explained that all extracts tested strongly against the test organism except *C. albicans*, *MRSA* and *P. mirabilis* which were not susceptible. The antimicrobial activity of some members of the genus *Ficus* has been reported; leaves of *F. abutilifolia* (Ukwubile, 2010), *F. exasperate* (Odunbaku *et al., 2008), F. ingens* (Aliyu *et.al*, 2008), *F. syncomorus* (Njume *et.al*, 2009) etc have been established to exhibit growth inhibitory effects on micro organisms. Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of a

sample that inhibit the growth of microorganisms. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of microorganism to an antimicrobial agent and it monitors the activity of new antimicrobial agents. MIC was 12.5 mg/ml for hexane extract (HE) against all pathogens, MIC of ethyl acetate (EA) extract was observed at 6.25mg/ml against all pathogens except for *P. aeriginosa* and *C. tropicalis* that showed MIC of 12.5 mg/ml. Whereas, MIC of methanol extract (ME) was at 12.5mg/ml for all the test organisms.

The MBC were determined by sub-culturing the test dilution (used in MIC) on to a fresh solid medium and incubated further for 24 hours. The concentrations of the plant extracts that completely killed the bacteria were taken as MBC (Sen and Batra, 2012). Ethyl acetate extract had the lowest MIC value of 6.25mg/ml which means that it was the most effective antimicrobial agent because fewer drug is required to inhibit the growth of the organisms. The result showed that MBC was 2-4 folds higher than MIC values, which means the drug was bacteriocidal at higher concentration probably due to interference by some active principle in the drug. The terpenoids, flavonoids and tannins present in *F. thonningii* are probably responsible for its activity against some microorganisms. Terpenoids disrupt microbial cell membranes and hence give plants bactericidal effects (Cowan, 1999). The MBC/MFCs for ethyl acetate extract was (12.5 mg/ml) for all organisms except *S. pyogene, C. troicalis* and *P. aeriginosa* which had MBC of 25 mg/ml, moreover, the MBCs of methanol extracts was (25 mg/ml) while *P. aeriginosa* had MBC at 50mg/ml and MBC/MFC of hexane extract was 50 mg/ml.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Microscopically, the transverse section of *F. thonningii* showed; rows of rectangular thick walled cork cells (21.36 µm), a band of phellogen, wide continuous band of stone cells or sclereids were also present. The remaining cortical cells contained mass of pitted parenchymatous cells filled with starch grains and prismic calcium oxalate crystals (46.73 µm). The powder showed abundant prism crystals of calcium oxalate crystal scattered, sclereids, fragment of cork cells and elongated fibre with pointed ends (801.00 µm). The tangential longitudinal section showed medullary rays, fibres and phloem parenchymatous cells.

Physicochemical parameters studied showed the moisture content to be (6.56%), total ash value (9.50%), acid insoluble ash (2.25%) and water soluble ash (7.50%). The alcohol and water soluble extractive value investigated using the ground plant powdered material revealed (16.67%) and (10.67%) respectively.

Phytochemical analysis of the stem bark extracts revealed the presence of some secondary metabolites namely alkaloids, tannins, flavonoids, carbohydrate cardiac glycosides, saponins, triterpenes and steroids and anthracenes.

TLC fingerprint for the extract revealed the presence of flavonoids, steroids/triterpenes, alkaloids and phenolic compounds.

The diameter of zone of inhibition of extracts against test microorganisms (in mm) was highest for the ethyl acetate, followed by methanol and the least was hexane extract.

The zones of inhibition for the extracts are in the range of 20 – 30 mm. However, *P. mirabilis, C. albicans* and *MRSA* were not susceptible to the entire extract.

The MIC for all the extracts was between 6.25mg/ml – 25mg/ml while MBC/MFC was between 12.5mg/ml – 50mg/ml for all the extracts.

6.2 Conclusion

The present studies have established:

i) Microscopic, chemomicroscopic and physical constants of the stem bark of *F. thonningii* were determined and are important diagnostic features (starch grains, branchy sclereids, rectangular cork cells, prismic calcium oxalate crystals) which could serve as a basis for the identification and authentication of the plant.

ii) The thin layer chromatographic profile of the stem bark extracts of the plant had shown that it contained chiefly flavonoids, steroids/triterpenes and phenolic compounds.

iii) The ethyl acetate extract was observed to have the best activity compared to other extracts. Minimum Inhibitory Concentration (MIC) value obtained using the Broth dilution method was 6.25mg/ml while MBC/MFC was 12.5mg/ml which supported the traditional use of the plant in treatment of antimicrobial infection.

6.3 Recommendation

- It is recommended that more research should be carried out to establish pharmacognostic profile of the whole plant parts for inclusion into the Pharmacopoeia

- Activity (Bioassay) guided isolation should be carried out especially on the ethyl acetate extract for antimicrobial property so that pure compound with good antimicrobial activity may be isolated and elucidated.

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APPENDICES

APPENDIX I

Determination of some physical parameters of the powdered stem bark of *F. thonningii*

a) **Determination of moisture content of powdered stem bark of *F. thonningii***

3g of the powdered plant material was used

Description	1	2	3
Constant weight of crucible (g)	52.08	45.01	44.74
Weight of crucible + powder before heating(g)	55.08	48.01	47.74
Wt of crucible + content after heating to constant weight (g)	54.88	47.82	47.54
Loss in weight (g)	0.20	0.19	0.20
Loss on drying (%)	6.67	6.33	6.67
Average mean (%)		6.56	

Sample calculation

% Loss on drying= [(wt of crucible and content before heating- wt of crucible and content after heating) ÷ 2g] ×100

= [(55.08 - 54.88) ÷ 3] × 100

=6.67%$^{w}/_{w}$

APPENDIX II

b) **Determination of Ash Value of powdered stem bark of *F. thonningii***

2g of the powdered plant material was used

Description	1	2	3
Constant weight of crucible (g)	39.53	51.20	52.00
Weight of crucible + powder before heating(g)	41.53	53.20	54.00
Wt of crucible + ash (g)	39.72	51.39	52.19
Weight of ash (g)	0.19	0.19	0.19
Ash value (%)	9.50	9.50	9.50
Average mean (%)		9.50	

Sample calculation

$$\text{Ash value} = \frac{\text{weight of Ash}}{\text{Initial weight of drug}} \times 100$$

$$\text{Ash Value} = \frac{0.19 \times 100}{2\ g} = 9.5\ \%w/w$$

APPENDIX III

(c) **Determination of acid insoluble ash of powdered stem bark of *F. thonningii***

2g of the powdered plant material was used

Description	1	2	3
Constant weight of crucible (g)	39.53	38.27	39.32
Weight of crucible + Acid insoluble ash(g)	39.58	38.31	39.3
Weight of acid insoluble ash (g)	0.050	0.045	0.040
Acid insoluble ash value (%)	2.50	2.25	2.00
Average mean (%)		2.25	

Sample calculation

$$\text{Acid Insoluble Ash value} = \frac{\text{weight of acid insoluble ash}}{\text{Initial weight of drug}} \times 100$$

Acid insoluble ash = 0.05/2g x 100 = 2.5 %w/w

APPENDIX IV

(d) **Determination of water soluble ash of powdered stem bark of *F. thonningii***

Description	1	2	3
Constant weight of crucible (g)	51.20	53.40	52.00
Weight of crucible + Ash (g)	51.24	53.42	52.06
Weight of ash (g)	0.19	0.19	0.19
Weight of water insoluble ash (g)	0.04	0.02	0.06
Weight of water soluble ash (g)	0.15	0.17	0.13
Water soluble Ash Value (%)	7.50	8.50	6.50
Average mean (%)		7.5	

Sample calculation

$$\text{Water soluble Ash value} = \frac{\text{Wt of total ash - Wt of Water Insoluble Ash}}{\text{Initial weight of drug}} \times 100$$

$$\text{Water soluble ash Value} = \frac{(0.19 - 0.04) \times 100}{2\ \text{g}} = 7.5\ \%\text{w/w}$$