PHARMACOGNOSTIC AND ANTI INFLAMMATORY STUDIES OF THE STEM BARK OF *FICUS GLUMOSA* DEL. (MORACEAE)

BY

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FACULTY OF PHARMACEUTICAL SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

APRIL, 2017

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P13PHPD8021

A DISSERTATION SUBMITTED TO THE SCHOOL OF POST-GRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A

MASTER OF SCIENCE DEGREE IN PHARMACOGNOSY

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 Anti inflammatory Studies of the Stem bark of *Ficus glumosa* Del. (Moraceae)" has been carried out by me in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and list of references.

Nafisah Nana YAHUZA

Name	**Signature**	**Date**

CERTIFICATION

This dissertation entitled "PHARMACOGNOSTIC AND ANTI INFLAMMATORY STUDIES OF THE STEM BARK OF *FICUS GLUMOSA* DEL. (MORACEAE)" by Nafisah Nana YAHUZA, meets the regulations governing the award of the degree of Master of Science in Pharmacognosy of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my late mother Hajiya Hindatu, may Allah (SWT) make jannatul firdaus your final abode, ameen.

ACKNOWLEDGEMENT

All praises are due to Almighty Allah, the Creator of the heavens and earth, the fountain of knowledge for His mercies, guidance and protection in my life, may His mercy continue to be upon the prophet Muhammed (SAW). My profound gratitude to my supervisors Dr. U. A. Katsayal and Dr .A. A. Ambi for their untiring support and encouragement throughout the course of undertaking this research work and also for their patience in monitoring the progress of the work in the laboratory and the paper work.

My appreciation goes to the love of my life in person of Lt Col M. S. Usman for his understanding, love and unalloyed support, in cash and in kind during the course of the programme. I want to appreciate my lovely kids Khadeejah, Abubakar, Uthman (Arafat), Hindatu (Umaymah) and Aliyu Haidar. May Almighty Allah bless you all abundantly, amen.

My sincere appreciation and profound gratitude to my Father Alhaji Yahuza for his love, prayers, care, encouragement and support. I wish to acknowledge the support of Hajiya Hadiza Yahuza (step mother) and all members of my family for their support in one way or the other in the course of my studies. I would continue to remain grateful and may Almighty Allah bless you all. I appreciate the support of my brothers and sisters, for their caring and encouragement given to me during the course of this programme.

I am also thankful to all academic staff of the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria for their co-operation and academic inputs, and also to the technical staff in persons of Mallam Kabiru Ibrahim and Mallam Kamilu Mahmud for their guide and co-operation in issuing ideas and materials in the

laboratory. I sincerely thank everyone that put an effort to the success of this academic work.

My special appreciation to all my friends and my course mates for their prayers, love, advice and encouragement, few to mention include our able class rep Aliyu Nuhu, Nafisat Salihu, Mariya Saidu, Aisha Barau, Khadijah Imam, Zakir Abdulhamid, Pharm. S. Sani, Pharm. D. Musa, Pharm. M. Troy, Pharm. M. Tabitha, Pharm. M. Sanusi, Pharm. I. Hassanat, Pharm. E. Serah and to those that are not mentioned here due to limited space but, their efforts are duly acknowledged. I really appreciate your company.

Finally, I will not forget Mrs Farhat Khan of Pharmacology Department, for being there all the time, I appreciate, may Almighty Allah reward you abundantly.

ABSTRACT

Ficus glumosa is widely distributed in Northern part of Nigeria; it has been exploited for both its medicinal and economic importance. It is traditionally used for the treatment of number of ailments including gastrointestinal, respiratory, inflammatory, cardiovascular disorders, ulcerative diseases, and cancers. Specifically, the roots are used in treatment of female sterility where as stem bark, have shown to be useful in the management of inflammations and paralysis. Evaluations of the fresh anatomical sections, powder and extract/fractions of the stem bark were carried out to determine the micromorphological, chemomicroscopic, some physicochemical, phytochemical, elemental content, acute toxicity and anti inflammatory properties by using standard procedures. Microscopical evaluation revealed the presence of the cork cells 10.84 µm, calcium oxalate crystals prism type 36.04 µm, fibres 126.96 µm, phloem parenchyma cells, and medullary rays. The physical constants revealed moisture content of 11.1 %, total ash value of 9.1 %, water soluble ash of 6.5 %, acid insoluble ash of 2.0 %, ethanol extractive value of 16.6 % and water extractive value of 12.0 %. Phytochemical analysis of the stem bark extract revealed the presence of alkaloids, tannins, flavonoids, glycosides, saponins, steroids and triterpenes. Elemental analysis of the powdered stem bark revealed the presence of Na, K, Ca, Mg, Cu, Zn, Fe, Ni and Co. The median lethal dose (LD_{50}) of the extract and fractions were found to be greater than 5000 mg/kg when administered orally in rats and considered practically non-toxic. The methanol extract of *F. glumosa* stem bark demonstrated protective activity against carageenan induced inflammation in rats at 500mg/kg with 28% protection at the end of the 3^{th} hour which is comparable to the piroxicam 10mg/kg treated group with 28% protection and the results were statistically significant ($p<0.05$). The ethyl acetate, n-butanol and aqueous fractions also showed protective activity at the dose of 500, 1000 and 1500 mg/kg. The ethylacetate produced significantly ($p<0.05$) sustained, dose dependant reduction of hind paw oedema in rats with upto 26% protection at the end of the 3^{th} hour. The butanol and aqueous fractions produced time related, sustained but non dose depentant significant reduction ($p<0.05$) of carageenan induced inflammation of the rats hind paw. The results provided pharmacognostic profile for proper identification of the plant and scientific basis for the traditional use of the stem bark in the management of inflammation.

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ACRONYMS AND ABBREVIATIONS

AAS:	Atomic Absorption Spectrophotometry
B.H. P:	British Herbal Pharmacopoeia
CAM:	Complimentary or alternative medicine
DNA:	Deoxyribo Nucleic Acid
Fig:	Figure
FAA:	Formalin Acetic acid Alcohol
g:	Gram
G.A.A:	Glacial Acetic Acid
HCL:	Hydrochloric acid
$H_2SO_{4:}$	Sulphuric acid
LD_{50}:	Median Lethal Dose
kg:	Kilogram
ml:	Milliliter
mm:	Millimeter
mg:	Milligram
T L C:	Thin Layer Chromatography
UV:	Ultraviolet Light
Vol.:	Volume
w/v:	Weight per Volume

w/w:	Weight per Weight
W.H.O :	World Health Organization
%:	Percentage
K:	Potassium
Na:	Sodium
Ca:	Calcium
Zn :	Zinc
Fe :	Iron
Cu :	Cobalt
Ni :	Nickel
Cd:	Cadmium
Mg:	Magnesium
Mn:	Manganese
Cr:	Cromium
COX:	Cyclo-oxygenase
SEM:	Standard Error of Mean
ANOVA:	Analysis of Variance
UK:	United Kingdom
USA:	United State of America
NSAIDS:	Non Steroidal Anti inflammatory Drugs

CHAPTER ONE

1.0 INTRODUCTION

Traditional medicine is the sum total of the 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, 2011).

Traditional medicine includes diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness (WHO, 2002). Practices of traditional medicine vary greatly from country to country, and from region to region as they are influenced by factors such as cultures, history, personal attitudes and philosophy (WHO, 2003).In developed countries they are termed Complimentary or Alternative medicine (CAM).

Plants have been used as sources of medicine in virtually all cultures and according to World Health Organization (WHO), herbal medicines serve the primary health needs of about 80% of the world's population, especially for people in the rural areas (Rajadurai *et al*., 2009).

Medicinal plants are those plants that possess medicinal properties to cure various ailments. These plants are untapped rich sources containing biologically active compounds (phytochemicals) vital to human health. The phytochemicals, usually referred

to as secondary metabolites of the plants, have no nutritive value in plants. These are the compound that possess the diverse medicinal properties of the plants. The common phytochemicals include saponins, alkaloids, tannins, terpenoids, volatile or essential oils and flavonoids among others. These compounds are widely distributed in the plant kingdom and are usually found at varying concentrations in different parts of plants. Such medicinal plants are increasingly gaining acceptance even amongst the literates in urban settlements, probably due to increasing resistance of many orthodox drugs used for control of many diseases. Natural products have been an integral part of the ancient traditional medical system, e.g Chinese, Ayurvedic and Egyptian (Sarker and Nahar, 2007).

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. Natural products (secondary metabolites) have been the most successful source of potential drug leads. However, their recent implementation in drug discovery and development efforts have somewhat demonstrated a decline in interest (Mishra *et.al,* 2010). Nevertheless, natural products continue to provide unique structural diversity in comparison to standard combinatorial chemistry, which presents opportunities for discovering mainly novel low molecular weight lead compounds. Since only less than 10% of the world's biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery with the challenge being how to access this natural chemical diversity (Cragg and Newman, 2005).

1.1 Pharmacognostic Evaluation of Medicinal Plants

Pharmacognosy is the study of medicines derived from natural sources, mainly from plants. It basically deals with standardization, authentication and study of natural drugs. Most of theresearch in pharmacognosy has been done in identifying controversial species of plants,authentication of commonly used traditional medicinal plants through morphological,phytochemical and physicochemical analysis. The importance of pharmacognosy has beenwidely felt in recent times. Unlike taxonomic identification, pharmacognostic study includes parameters which help in identifying adulteration in dry powder form also. This is again necessary because once the plant is dried and made into powder form, it loses its morphological identity and easily prone to adulteration. Pharmacognostic studies ensure plant identity, lays down standardization parameters which will help prevent adulterations and ensure reproducible quality of herbal products which will lead to safety and efficacy of natural products(Sumitra, 2014).The field of Pharmacognosy is not limited to special area and is constantly being re-invigorated by input from time to time by new developments in scientific fields and technologies. This is the reason that nowadays Pharmacognosy is a good option for those who like to work at the interface of many diverse but harmonizing branches of science that relate to the natural world (Kinghorn*et al*., 2004; Samuelsson, 2004).

Pharmacognostic evaluation includes macroscopic, microscopic, physico-chemical, fluorescence and phytochemical studies of herbal parts or powdered drug. Herbal raw material shows a number of problems when quality and authentication aspects are considered. This is because of nature of herbal parts, ingredients and different phytochemicals present in plants (WHO, 2002). To ensure quality of herbal medicines,

proper control of starting raw material is very important. The physico-chemical evaluation includes qualitative and quantitative tests, assays and instrumentation analysis. Qualitative and quantitative chemical tests include the presence or absence, quantity, number, values and identification of various phtyochemicals like flavonoids, glycosides, saponins, alkaloids etc (Brain and Turner, 1975; Harborne, 1992; Evans, 2002). Macroscopic identity of medicinal plant materials is based on physicaland sensory evaluation parameters like shape, size, colour, texture, odour and taste while microscopy involves comparative microscopic inspection of powdered herbal drug. Further, advances in microscope technology have increased the accuracy and capabilities of microscopy as a mean of herbal crude material identification due to the application of light and scanning electron microscopes (SEM) in herbal drug standardization (Bhutani, 2003).

1.2 Inflammation

Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases (Katzung, 2004).

Acute inflammation is characterized by unpleasant feelings, with characteristic symptoms such as pain, swelling, and loss of mobility. On the other hand, chronic inflammation is an inflammation that doesn't go away but rather a symptom that something has gone wrong (Gil, 2002). The conventional drugs used to ameliorate this phenomenon are either too expensive or toxic and not commonly available to the rural folks that constitute the major populace of the world (Dharmasiri *etal*., 2003).

Examples of disorders related with inflammation include: Rheumatoid arthritis, asthma, pelvic inflammatory disease, hypersensitivities, acne vulgaris, autoimmune diseases, inflammatory bowel diseases, chronic prostatitis etc

Drugs used in the management of inflammatory conditions are called anti-inflammatory drugs. They are classified into two categories:

Steroidal Anti-inflammatory Drugs: These are synthetic derivatives of cortisol, the major endogenous glucocorticoids and are used extensively in the management of inflammatory disorders and for their immunosuppressive actions. They act on the inflammatory cells and the inflammatory mediators. Some examples include cortisol, prednisolone, triamcinolone, dexamethasone and betamethasone.

Non-steroidal Anti-inflammatory Drugs (NSAIDs) – They alleviate pain by counteracting the cyclooxygenase (COX) enzyme which synthesizes prostaglandins, creating inflammation. Some common examples are piroxicam, aspirin, ibuprofen, naproxen, diclofenac. Long term use of NSAIDs can cause gastric erosions, gastro-intestinal bleeding, exercebation of asthma and can cause kidney damage to mention a few (Abiodun*et al*., 2014).

Owing to the numerous adverse effects associated with the use of these synthetic drugs, attention is now drawn on exploring anti-inflammatory agents of plant origin which are considered to be equally effective with minimal or no side effects. Plants that have contributed to the development of some anti-inflammatory drugs include*Salix alba* (white willow tree) from which salicin is obtained and it is believed to be less toxic than Aspirin. Some metabolites from plants that have been reported to show potential anti-inflammatory activities include the Phenolic compounds, Coumarins, Alkaloids,

Saponins, Flavonoids,Sterols, Terpenoids and Essential oils (Mona *et al*., 2014) and these may provide important sources of anti-inflammatory agents.

1.3 Elemental Analysis Using Atomic Absorption Spectroscopy (AAS)

Therapeutic role of certain medicinal plant materials, particularly have been correlated with the presence of specific elements in their composition (Javed*et al.*, 1987). Medicinal plants are of great importance to the health of individual and communities (Ata,2011). Although the efficacy of medicinal plants for curative purposes is often accounted for in terms of their organic constituents such as essential oils, glycoside, vitamin and minerals (Jabeen *et al.*,2010). Direct correlation of elemental composition of medicinal plants and their curative ability is not yet identified in terms of modern pharmacological concepts, thus the quantitative estimation of various trace elements composition is very important for determining the effectiveness of the medicinal plants in management of various disease conditions and also to probably explain the pharmacological properties (Nazuredeen, 2010). Part of objective of this study is to establish the trace (Zn, Cu, Cd, Cr, Mn and Fe) and Major (Na, K, Ca, C, P and Mg) elemental composition present in the stem bark of this important medicinal plant (*Ficus glumosa*).

1.4Statement of ResearchProblem

In spite of the progress made so far in the area of drug development with respect to inflammatory diseases, such drugs are associated with many side effects and heavy costs, the search for safer and accessible drugs, therefore remains. Prolong use of drugs such as NSAIDs commonly used for inflammatory diseases causes ulcer and intestinal bleeding due to damage to mucosal lining of the intestine.Most medicinal plants used in

management of inflammation and other ailments are not scientifically validated for which *F.glumosa* is included.

The standardization of a crude drug is integral part of establishing its correct identity. Before any crude drug is included in herbal pharmacopoeia, pharmacognostic as well as other standard parameters must be established (Abere *et al*., 2007).This is necessary because once the plant is dried and made into powder form, it loses its morphological identity and easily prone to adulteration. Hence there is the need to establish the pharmacognostic profile of this plant for proper identification to avoid adulteration.

1.5 Justification of the Research

Stem bark of*Ficu*s plant species has been used among traditional healers for the treatment of inflammatory diseases. *Ficus glumosa* has been used traditionally asa source of anti rheumatic agent and many other ailments in Northern part of Nigeria. This study therefore aimed to investigate the anti inflammatory effects of the stem bark of this plantwith a view to support the claim and evaluate its pharmacognostic parameters to serve as reference for proper identification of the plant.

1.6General Aim of the Research

To determine the pharmacognostic parameters and evaluate the anti-inflammatory properties of the extract of the stem bark of *Ficus glumosa.*

1.7 Specific Aims of the Research

i. To evaluate the morphological and physicochemical features of the stem bark of*F. glumosa*

ii. To analyse the elements and phytochemicals present in the stem bark of the plant

iii. To evaluate the anti inflammatory properties of the stem bark of the plant.

1.8Hypothesis of the Research

Ficus glumosa contains constituents with potential anti-inflammatory activities.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of Moraceae Family

The Moraceae family consists of about 50 generaand nearly 1400 species including important groups suchas *Artocarpus, Morus* and *Ficus.*They are usually woody plants with alternate leaves, conspicuous and milky latex. The flowers are unisexual, with or without sepals, 1-4 stamens and one independent ovule in a uniocular ovarium. They are arranged in many kinds of inflorescence, such as racemens in *Scocea* and *synconia* in *Ficus*, and monoecy, androecy and gymnodioecy seem to have evolved multiple times in the history of the family from ancestral dioecy (Datwyler and Weiblem, 2004). Moraceae exhibit an amazing diversity of morphologicaland life history traits, particularly inflorescence architectures,breeding systems, and pollination syndromes (Berg, 1998).Recent studies of Moraceae using DNA sequences havereconstructed the evolutionary history of the family to clarifyrelationships among genera, to identify the closest relatives ofthe figs (Datwyler and Weiblen 2004), and to examine biogeography (Zerega *et al*., 2005).

2.2 Description of the Genus*Ficus*

Ficus is a genus of about 800 species and 2000 varieties of woody trees, shrubs and vines occurring in most tropical and subtropical forests worldwide (Hamed, 2011). Many *Ficus* species consists of numerous varieties, significant genetic diversity, outstanding pharmacologicalactivities which are of remarkable commercial importance(Woodland, 1992).Collectively they are regarded as figs, and produced sap, leaves and flower buds (Ijeh and Ukweni, 2007). Most of them are found growing very well on rocks, bush, and

swamps and on hard surfaces (Abbiw, 1990). They are mainly trees ranging from 21–50 m high (Aluka, 2008). They produce flowers between August and February of each year, and the flowers are borne inside the plant, which is a unique chacteristic among the figs and they are propagated by seeds(Bouquet, 1969). A number of *ficus* speciesare used as food and are utilized for their medicinal properties especially amongst people where thesespecies grow(Lansky *etal.*, 2008).In Africa, the plants are distributed in South Africa, Mozambique, Zimbabwe, Botswana, Nigeria, and Sudan (Berg, 1992). In Nigeriathere are over 45 different species of Ficusasreported by Keay and Onochie 1964.

2.3 Nutritional Properties of *Ficus*

Figs are very nourishing and areused in industrial products in food industries. It is rich in vitamins, mineral elements, water, and fats. Figs are amongst the highest plant sources of calcium and fiber. According to USDA data for the Mission variety, dried figs are richest in fiber, copper, manganese, magnesium, potassium, calcium, and vitamin K, relative to human needs. Many other nutrients are also present in smaller quantities (Vinson, 2009).

2.4 Some reported medicinal properties and uses of the Genus*Ficus*

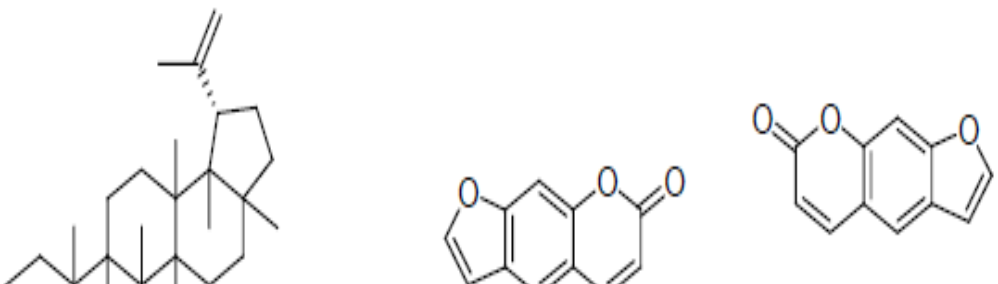
Figs are reported to posses' laxative effects, antioxidant properties and a good source. They are good sources of flavonoids and polyphenols (Vinson, 2009).Figs are also reported to be used in various disorders, such as gastrointestinal, respiratory, inflammatory, cardiovascular disorders, ulcerativediseases, and cancers(Rubnov*et al.*,2005). Specifically, the roots are used in treatment of ringworms whereas the fruits used as antipyretic, purgative, aphrodisiac and have shown to be useful in inflammations and paralysis (Kirtikar and Basu, 1996).

Anti inflammatory activities of some members of the genus has been reported for example,the aqueous methanolic leaves extract of*Ficus retusa* produced significant reduction of paw oedema at 400mg/kg p. o. (Jaya and Sreekanth, 2011), whereas the ethanolic leaves extract of *Ficus arnottiana* produced significant reduction in paw oedema at 200mg/kg and 300mg/kg which was in dose dependant manner (Saha *et al.*,2011). It was also reported that the methanolic leaves extract of *Ficusmicrocarpa* at a dose of 200mg/kg reduced significantly the formation of oedema induced by carageenan in rats (Shripad *et al*., 2012).

2.5 Phytochemical Constituents of the Genus*Ficus*

Chemically, the *Ficus*species have been reported to contain mainly glycosides such as saponins, flavonoids, anthraquinones as well as alkaloids and tannins (Ukwubile, 2010).Also some bioactive compounds such as arabinose, β-amyrins, β-carotines, β-setosterols and xanthotoxol have been reported (Gilani*etal*.,2008).Previousphytochemical studies of *Ficus*resulted in the isolation offlavonoids, coumarins, alkaloids, steroids, triterpenes, ceramidesand salicylic acids(Kamga*etal.*, 2010)

Phytochemical investigation of *F. carica*leaf (Vikas and Vijay, 2010) led to the isolation of some compounds such as: Lupeol (1), Umbeliferone (5), Scopoletin (7) andXanthotoxin (8). Ahmad *etat*., 2011 reported that some compounds were isolated from seeds of *F. banghalesis*which include Psoralen (2) and Bergapten (4).Some of the compounds reportedly isolated from *Ficus* species are shown in Fig. 2.1



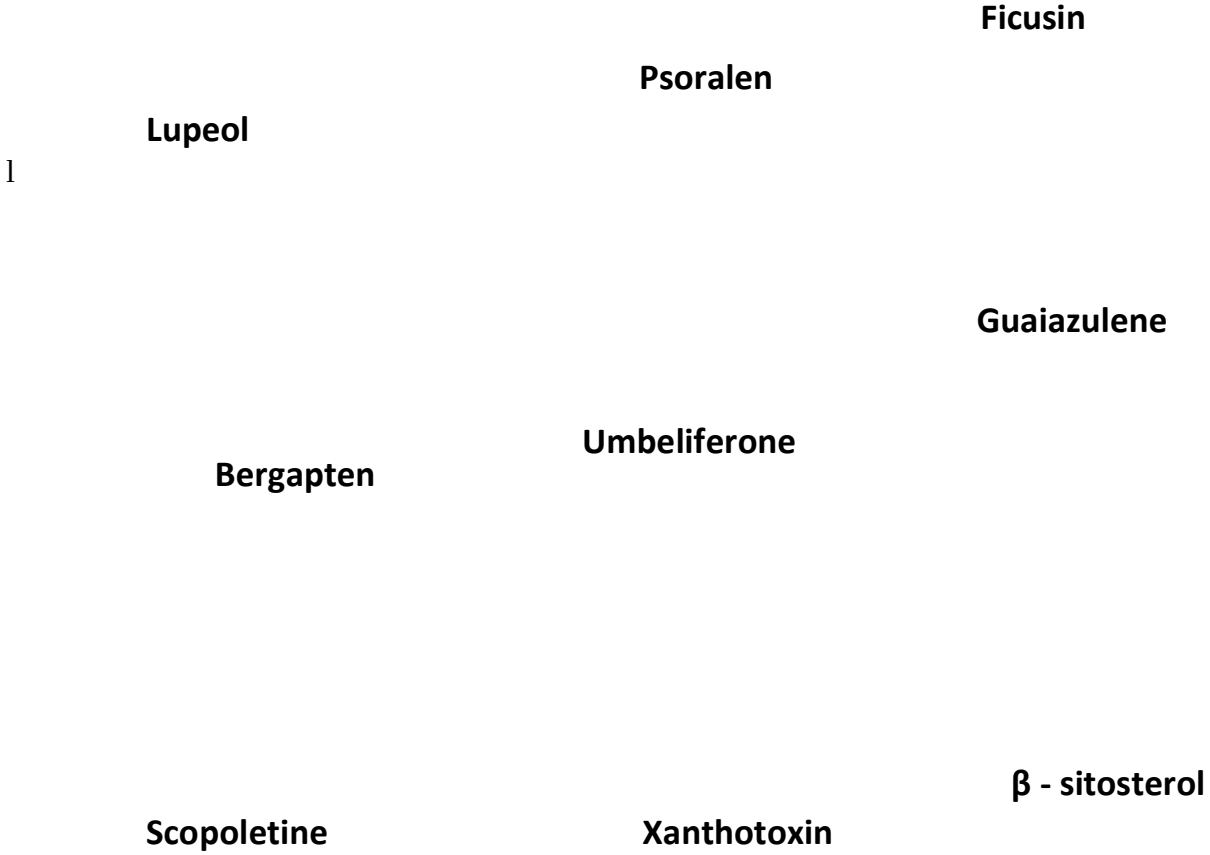


Fig. 2.1: Chemical structures of some compounds isolated from the genus *Ficus*(Vikas and Vijay, 2010, Ahmed *et al*., 2011).

Table 2.1 : Some Chemical compunds isolated from *Ficus* species

Plant species	Compounds	Part of plant	Refences

F. carica	Lupeol	Leaf and fruit	Vikas and Vijay, 2010
	Umbeliferrone	,,	,,
	Scopoletin	,,	,,
	Xanthotoxin	,,	,,
	Ficusin	,,	,,
	Guaiazulene	,,	Saeed *et al*., 2013
F. banghalesis	Bergapten	Seed and leaf	Ahmed *et al.*,2011
	Psoralein	,,	,,
	β sitosterol	,,	,,

2.6 Description of the *Ficus glumosa*

2.6.1 Botanical Description

Ficus glumosa is a small- to medium-sized tree, 5-10 m tall, although it may become a large tree reaching 24 m in height and 2 m in girth. Branches are widely spreading, more or less horizontal, often supported by stilt roots. It is a plant with immense medicinal value that is indigenous to the tropical and subtropical Africa, including Nigeria, with a few species extending into the semi-warm temperate zones in South West Asia and the Mediterranean region. In Nigeria, although it is more distributed in the Southern region,it is easily found in the Northern region of the country, where it is referred to as"*kawuri"* in Hausa language (Burkill,1985).

Local names:Afrikaans (Afrikaanse rotsvy, Afrikaans rostvy); English (African rock fig); Hausa (kawuri); Somali (berde);Tigrigna (chekomte, check lang) (Tanko *et al.*, 2012).

2.6.2 Taxonomic Classification

Kingdom: Plantae

Order: Rosales

Family: Moraceae

Genus: *Ficus*

Species:*Ficus glumosa*

Synonyms: *F. barbarata* Warb, *F. montana* Sim, *F. glumosoides* Hutch, *F. durandiana* Warb*F. kitaba* De Wild, *F. rubicunda* (Miq) Miq, *F. sunderi*Miq, *F. rukwaensis* Warb.

2.6.3 Ethanomedical uses and biological activity of *Ficus glumosa*

Traditional medicinal uses are numerous; In Zimbabwe the latex is diluted with water and used to treat diarrhea. In central Africa and Tanzania the powdered bark mixed with latex is used plug carious teeth; a decoction of the bark is used as mouth wash against toothache, also applied topically against headache, and bark macerate is applied to the eyes to prevent conjunctivitis in newborn. In Senegal and Cote d'iviore the roots and fruits are used in preparations to cure female sterility (Jasen, 2005). In Nigeria the stem bark is used for rheumatism (Abubakar *et al*., 2007).*Ficus glumosa* is used in traditional medicine in Cameroon, Senegal, and East Africa for the treatment of edema, hypertension, diabetes, hemorrhoids, rheumatism, and stomatitis (Orwa *etal.*, 2009). The leaves are also used in these regions for the treatment of skin diseases and diabetes (Madubunyi *et al.*, 2012).

Pharmacological properties of *F.glumosa* extracts on gastrointestinal motility were demonstrated (Tanko *et al.*, 2012). Also the effects of ethanol leaf extract of *Ficus glumosa* on fasting blood glucose and serum lipid profile in diabeticrats were investigated by (Umar *et al*., 2013).

2.6.4 Phytochemical compounds from *Ficus glumosa*

Many compounds were isolated from *F. glumosa*stem bark which include; lupeol, catechine, luteolin, genisten, dongnoside, polystachyol, dongnoside E along with ceramides known to have cytotoxic activity (Frederic *et al.*, 2012).

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Plate I.*Ficus glumosa* in its natural habitatat Goriba village, Giwa Local Government Area, Kaduna State

CHAPTER THREE

3.0 MATERIALS

3.1 List of Solvents

Distilled Water, Ethyl acetate(Qualikems), Hexane(JHD, AR, Lobal Chem. India), Methanol(Merck-Germany), n- Butanol(Lobal Chem, India)

3.1.1 List of Reagents

Acetic acid(Avondale Laboratory, England)**,**Aluminium Chloride**,** Anisaldehyde (Sigma-Aldrich, St.Lous, MO, USA)**,** Chloral hydrate(BDH Laboratory Chemicals Division, POOLE. England),Dragendorff**,** Ferric Chloride**,** Formalin**,** Glycerol(BDH Laboratory Chemicals Division, POOLE. England),Hydrochloric acid**,** Libermann-Bucchard reagent**,** Mayers reagent, Methylene blue**,** Phloroglucinol,Sodium Hypochlorite**,** Sulphuric acid (Sigma-Aldrich, St.Lous, MO, USA)*,* Sudan Red Solution**,** Wagner reagent Xylene**,** Zinc ChlorideSolution.

3.1.2 List of Equipments

Atomic absorption spectrophotometer machine, Compound microscope (Fisher Scientific, UK)Camera Lucida, Crucibles, Mechanical shaker (Stuart Scientific Flask Shaker, Great Britain)Microtome (C 740527, Cambridge Instrument Company Ltd, London and Cambridge, England)Stage Micrometer and Ocular Lens (Graticules Ltd, Ton bridge, Kent. England),Venier caliper Water bath (HHS, Mc Donald Scientific International).

3.1.3 Other Materials

Ashless filter paper, Disposable syringes, Glass slides and covers, Razor blade, TLC Tanks.

3.2 METHODS

3.2.1 Plant Collection, Identification and Preparation

Sample of *Ficus glumosa*leaves, fruits and stem barkwere collected from Goriba village, Giwa Local Government Area of Kaduna State, Nigeria in the month of July, 2015.The plant was identified by Namadi Sunusi of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria and a specimen voucher number (900746) was assigned to its voucher specimen.

Sufficient quantity of the stem barkwas obtained by making longitudinal and transverse incisions through the outer layer of the stem of the plant followed by peeling. The bark was dusted, cleaned and all foreign matter removed, it was then air-died under shade until a constant weight was obtained. It was comminuted to powder form using grinder, then stored in an air-tight container for subsequent use.

3.3 Macroscopic Examination on the Stem bark

Macroscopic observations were conducted on the stem bark which include; colour, odour, taste, surface texture (Brain and Turner, 1975).

3.4 Microscopical Examination of Stem bark

3.4.1 Anatomical Section/Surface Preparations

Anatomical section of the stem bark sample was examined under the microscope and features were described by using the terms according to Dutta, (2003) and Ahlam and Bouran (2011).

Methods

Fixation: the fresh stem bark of was picked off directly from the plant and dipped immediately into the fixative, Formal acetic acid (70% Ethanol, 40% formalin and glacial acetic acid) in the ratio 90:5:5 and was allowed to stand for 24 hours.

Dehydration: the sample above was transferred in to 30%, 50%, 70%, 95% and 100% ethanol. This was carried out in each graded alcohol for 2 hours each.

Clearing: the sample was transferred into Ethanol-Chloroform (75:25), Ethanol-Chloroform (50:50), Chloroform-Ethanol (75:25), and 100% Chloroform. This was also carried out after every 2 hours each.

Infiltration and Embedding: Chips of paraffin wax were added slowly in to the stem bark sample and this was left to stand for 24 hours before transferring in to the oven at 60^{o}C. After melting, the paraffin containing the sample was transferred into 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 stem bark. The transverse section was then transferred on to slides.

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 into 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 placed (Ahlam and Bouran, 2011). This was observed under the microscope and appropriate images were taken and documented.

3.4.2 Chemomicroscopical examinationsof Stem bark

These were carried out on the powdered plant. Small amount of the finely ground powdered stem bark of the plant was cleared in a test-tube containing 70% chloral hydrate solution. They were boiled on a water-bath for about 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 detected in accordance with Brain and Turner (1975); Evans (2009) as follows:

(a) Cell Wall Materials

(i) Test for Cellulose: About 2 drops of iodinated zinc chloride were added to the cleared sample on a slide, and this was allowed to stand for few minutes. One drop of sulphuric acid was added, cover- slipped and observed under the microscope.

(ii) Test for Suberins: Two drops of Sudan red was 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.

(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.

(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.

(b) Cell Contents/Cell Inclusions

(i) Test for Calcium Carbonates: The appearance of effervescence 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.

(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.

3.5 Determination of Physicochemical Constants of the Powdered Stem bark

The methods adopted for the physicochemical examination of the stem bark powder, are as described in W H O (2011).

3.5.1 Determinationof Moisture Content

The moisture content was determined by "Loss on drying" method (gravimetric determination).Air-dried stem bark (3 g) was weighed using (KERN EW Electronic Balance) to a dried and previously weighed crucible. The crucible was transferred into a hot air sterilizing oven, which was set at 105^0C. After an hour, the crucible was removed and placed in a desiccator over phosphorous pentoxide and under atmospheric pressure and at room temperature. After 30 minutes in the desiccator, the weight of the powder and crucible were quickly determined and the crucible returned to oven. The heating and weighing were repeated until a constant weight was obtained and noted. Three determinations were conducted and the average of these was taken as the moisture content of the drugs.The moisture content (loss of weight) was calculated using the following formula:

$$\% \ Moisture\ content = \frac{Initial Weig\ ht of Powder - Final Weig\ ht of Powder}{Initial Weig\ ht of Powder} X\ 100$$

3.5.2 Determination Total Ash Value

The total ash of the powdered stem bark of the *F.kamerunensis* was determined by weighing 2 g of the powdered sample in a crucible. This was heated gently at 450^0 C. Heating was done until all the carbon was removed.It was cooled in a dessicator and weighed. The procedure was repeated three times to obtain average value. The total ash content of the air-dried powder was calculated in percentage, using the following formula:

$$\% \ Ash\ Value = \frac{Weig\ ht\ of\ Residual\ Ash}{Original\ Weig\ ht\ of\ Powder} X\ 100$$

3.5.3 Determination of Acid-insoluble Ash

To the crucible containing the total ash, 25ml of dilute hydrochloric acid was added and covered with a watch glass and boiled gently for 5 minutes. About 5ml of hot water was used to rinse the cover glass into the crucible. Theinsoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. This was then transferred back to the crucible and dried on a hot plate and ignited to a constant weight. The residue was allowed to cool in a dessicator for 30 minutes and quickly weighed. The acid insoluble was calculated as follows:

$$\%\ Acid\ insoluble\ Ash = \frac{Weight\ of\ Residual\ \ Ash}{Original\ \ Weight\ of\ Powder}\ X\ 100$$

3.5.4 Determination of Water Soluble Ash

To the crucible containing the total ash, 25ml of water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered glass crucible. It was then washed with hot water and ignited in a crucible for 15 minutes at 105^{o}C. The weight of the residue was subtracted from the weight of the total ash. The water soluble ash of air dried powder was calculated using the following formula:

$$\%\ Water\ Soluble\ Ash = \frac{Weight\ of\ Total\ \ ASh - Weight\ of\ residual\ \ Ash}{Original\ \ Weight\ of\ Powder}\ X\ 100$$

3.6 Extractive Values

Determinations of water and ethanol soluble extractives were carried out using the followingmethods.

3.6.1 Water Extractives Values

Exactly 4 g of air-dried stem bark powder was weighed into a 250 ml glass stoppered conical flask and 100 ml of chloroform-water (1: 400) was added to macerate the powder for 6 hours with frequent shaking by using mechanical shaker and was allowed to stand for 18 hours. It was then filtered rapidly and 25 ml of filtrate was transferred into a previously dried and weighed evaporating dish and evaporated to dryness on a hot water bath.This was further dried in the oven at 105^0C for 6 hours, cooled in a desiccator for 30 minutes and then weighed without delay. The percentage water extractive value was calculated using the following formula:

$$\%\ Water\ Extractive\ Value = \frac{Weight\ of\ Extract\ \ in\ 25ml\ X\ 4}{original\ \ Weight\ of\ Powder}\ X\ 100$$

3.6.2 Ethanol Extractives Value

Exactly 4 g of air-dried stem bark powder was weighed into a 250 ml glass stoppered conical flask and 100 ml of ethanol was added to macerate the powder for 6 hours with frequent shaking by using mechanical shaker and was allowed to stand for 18 hours. It was then filtered rapidly and 25 ml of filtrate was transferred into a previously dried and weighed evaporating dish and evaporated to dryness on a hot water bath.This was further dried in the oven at 105^0C for 6 hours, cooled in a desiccator for 30 minutes and then weighed without delay. The percentage water extractive value was calculated using the following formula:

$$\%\ Ethanol\ Extractive\ Value = \frac{Weight\ of\ Extract\ \ in\ 25ml\ X\ 4}{original\ \ Weight\ of\ Powder}\ X\ 100$$

3.7.1 Extraction of *Ficus glumosa* Stem bark

The powdered plant material (1 kg) was macerated using 75 % $^{V}/_{V}$ methanol with occasional shaking for 72hrs after which it was filtered and rinsed repeatedly with the solvent.The filtrate was concentrated via rotary evaporator and final evaporation to dryness of the extract was done via the water bath after which it was stored in desiccator for subsequent use. The percentage yield was calculated using the formula:

$$\text{Percentage Yield of extract} = \frac{\text{Weight of total extract}}{\text{weight of powdered material}} \times 100$$

3.7.2 Fractionation of Aqueous Methanolic Extract of *Ficus glumosa* Stem bark

The *F. glumosa* stem bark aqueous methanol extract (70g) was dissolved in distilled water. This was placed in a separating funnel and known volume of n-hexane was added and mixed slightly. The aqueous layer was then collected first into a clean container, followed by n-hexane fraction in a separate container. The aqueous layer was transferred back into the separating funnel and the above process repeated with ethylacetate and butanol to obtain ethylacetate, butanol and aqueous fractions respectively. The individual fractions were concentrated to dryness and kept in a dessicator for further use (Fig 3.1).

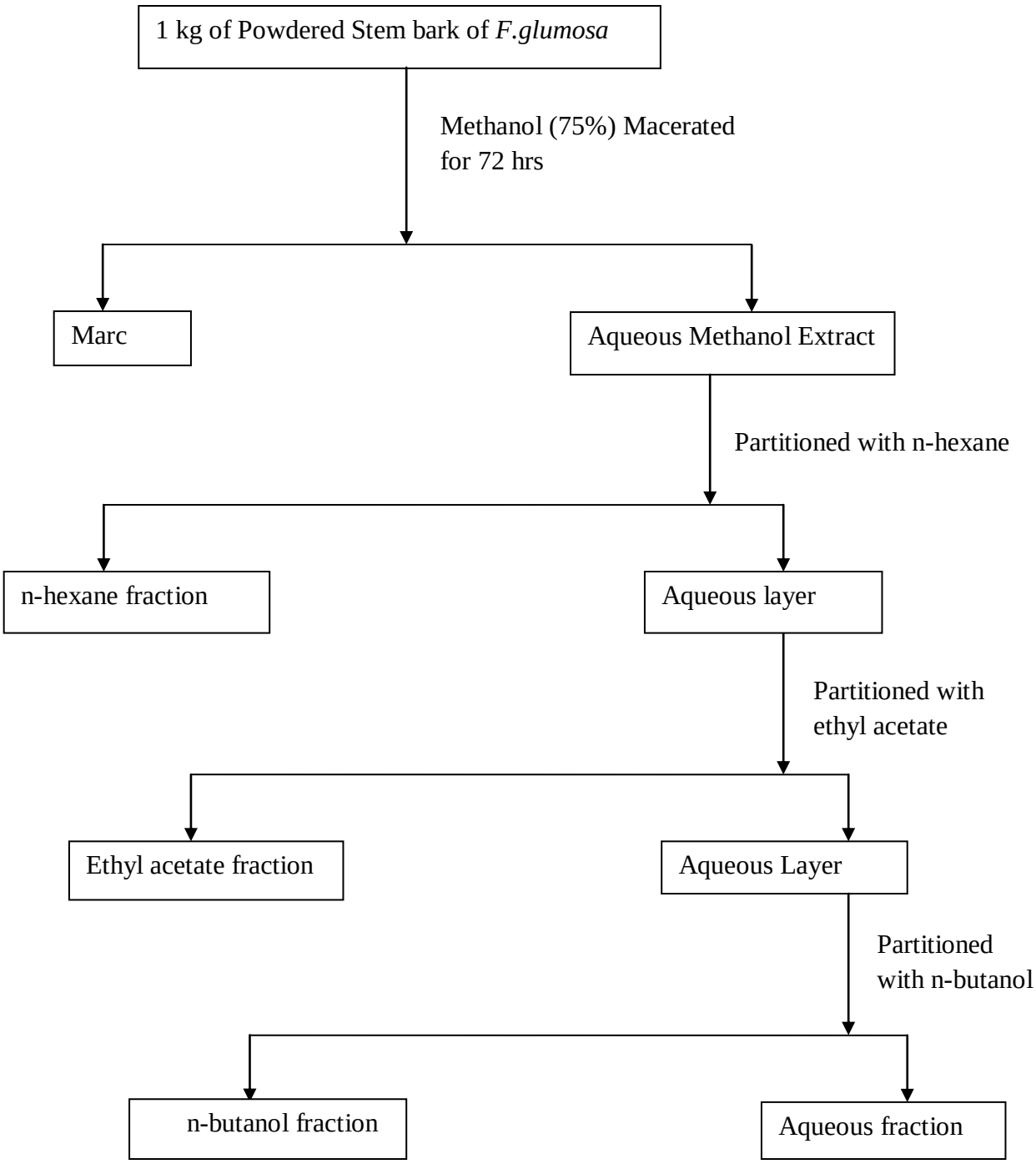

Figure 3.1: Chart showing Extraction and fractionation of stem bark of *F.glumosa*(Kokate, 2003)

3.8Phytochemical Screening of the Stem bark Extract of *F.glumosa*

The stem bark methanol extract was subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the method described by (Evans, 2009, Musa, 2005, Sofowora, 2008).

3.8.1 Tests for Saponins

(i) Frothing Test: About 0.5 g of the extract was dissolved in 10 mL of water and shaken vigorously in a test tube for 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 an hour indicates the presence of saponins (Sofowora, 2008).

(ii) Haemolysis Test: About 2 mL of sodium chloride (1.8% solution in distilled water) was added to two test tubes A and B. 2 mL of distilled water was added to test tube A, 2 mL of the extract was added to test tube B. 5 drops of blood was added to each tube and the tubes were inverted gently to mix the contents. Haemolysis in tube B containing the extract but not in tube A (i.e. control), indicate the presence of saponins in the extracts (Brain and Turner, 1975).

3.8.2 Test for Steriods/ Triterpenes

(i) Lieberman-Burchard Test: About 1 mL of acetic anhydride was added to 0.5 g of the extract. Two drops of sulphuric acid was then added gently by the side of the test tube to the solution above and at the junction of the two liquids a formation of reddish brown or violet brown ring, the upper layer bluish green or violet indicates the presence of sterols and triterpenes (Evans, 2009).

(ii) Salkowski Test: About 2 mL of chloroform and two drops of sulphuric acid was carefully added to about 0.5 g of the 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 (Sofowora, 2008).

3.8.3 Test for Flavonoids

(i) Shinoda Test: About 0.5 g of the extract was dissolved in 2 mL of 50% methanol. Two drops of magnesium fillings and 3 drops of hydrochloric acid were added and the pink coloration indicated the presence of flavonoids (Evans, 2009).

(ii) Sodium Hydroxide Test: About 0.5 g of the extract was dissolved in water and filtered; 2mL 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 was used as an indication for the presence of flavonoids (Evans, 2009).

3.8.4 Tests for Tannins:

(i) Ferric Chloride Test: Two grams (2 g) of the extract was dissolved in 10 mL of water each and filtered. Two drops of ferric chloride solution were added to the filtrate. Appearance of blue or green precipitate indicates the presence of phenolic nucleus (Musa, 2005).

(ii) Lead Sub-acetate Test: To 2 g of the extract, two drops of lead sub-acetate solution were added and appearance of whitish-yellow precipitate indicated the presence of tannins (Musa, 2005).

3.8.5 Test for Alkaloids: The crude extract ofthe plant was dissolved in 2 mL 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).

3.8.6 Test for Cardiac Glycosides

Kellar-killiani Test: About 0.5 g of the extract was dissolved in glacial acetic acid containing ferric chloride and one drop of sulphuric acid was added to the solution, the appearance of reddish-brown coloration indicated the presence of cardiac glycoside or cardinolide (Sofowora, 2008).

3.8.7 Test for Cyanogenetic Glycosides

About 2 g of the fresh material was crushed and mixed with 5 mL of water in a test tube. A prepared damp sodium picrate paper was suspended at the mouth of the test tube by means of a cork. The tube was placed in water bath for one hour. A brick red colouration on the picrate paper was indication for the presence of cyanogenic glycoside (Ashutosh, 2007).

3.8.8 Test for Anthraquinones(Bontrager's Test)

Chloroform 5 mL was added to 0.5g of powdered drug in a dry test tube and shaken for 5 minutes. The mixture was filtered and shaken with an equal volume of 10% ammonium solution.A bright pink color in the upper aqueous layer indicates the presence of anthraquinones (Evans, 2002).

3.9Thin layer chromatography of the various fractions of the extract

Thin layer chromatography for the various fractions of the extract were developed using different solvent systems and the chromatograms detected by day light, using spraying reagents and or with UV- light as shown on the table below.

Table: 3.1Solvent systems used and detecting reagents for TLC analysis of the various fractions

Fraction	Solvent system	Detecting reagent
n-Hexane	Hexane-ethylacetate (7:3)	PA, 20%SA,$FeCl_3$,LB, $AlCl_3$,BT,DG
Ethyl acetate	Hexane-ethylacetate (8:2)	PA, 20%SA,$FeCl_3$,LB, $AlCl_3$,BT,DG
n- Butanol	Butanol-acetic acid:water (6:1:1)	PA, 20%SA,$FeCl_3$,LB, $AlCl_3$,BT,DG

KEYS: PA; *p*-anisaldehyde reagent, LB; Libbermann buchard reagent, BT; Borntragers reagent, DG; Dragendoff's reagent, SA; Sulphuric acid, $FeCl_3$; Ferric chloride, $AlCl_3$; Aluminium chloride.

3.10 Atomic Absorption Spectrophotometry

Dried material was ground to fine powder. About 5g was taken in pre-cleaned crucible and heated in a muffle furnace at 400^{o}C until there was no evolution of smoke. The crucible was then kept in a dessicator and cooled at room temperature. The ash was moistened with concentrated H_2SO_4 (0.5-1.0) and heated on a heating mantle till the fumes of H_2SO_4 ceased. Sulphated ash was then dissolved in 10mL conc. HCl and diluted to 100mL with distilled water and filtered through Whatmann filter paper. Solution was stored in a tightly capped plastic bottle and directly used for Atomic Absorption Spectrophotometry (AAS). A standard solution of requisite elements was prepared and a

calibration curve was drawn. Percentage concentration of elements in pre-dried plant material was calculated (Aftab, 1999). Elements analysed were Na, K, Ca, Mg, Zn, Cu, Mn, Fe, Ni, Co, Cd, and Cr.

3.11 Source and maintenance of experimental Animals

White Wistar albino rats of both sexes weighing 150 - 200 gwere obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were maintained under standard condition. The rats were fed with standard (grower) mash (Vital feed, Jos, Nigeria) and water ad-libitum (Nwafor, 2000).

3.11.1 Determination of Acute Oral Toxicity of Fractions of Aqueous Methanolic Extract of*Ficus glumosa* Stem Bark using OECD guidelines (425)

LD_{50} was conducted using Organization for Economic Cooperation and Development (OECD) guideline (OECD Test Guideline 425). The limit dose test, up and down procedure as revised by Dixon (1965) was adopted to evaluate the acute toxicity of the sample following oral administration in rats. A total of five (5) rats were randomly selected and used for the experiment as outlined by Sani *et al.,* (2009). They were maintained in the laboratory for 7 days to allow for acclimatization to the laboratory conditions. The rats were fasted 24hrs but allowed free access to water prior to dosing on each occasion. A rat was picked, weighed and dosed orally with a limit dose 5000mg/kg body weight of the freshly prepared aqueous methanol extract then food was further withheld for 2hrs. Another animal was given the same dose until all the 5 rats were administered with the same dose of the extract. Observation was done on each animal for every 15 min in the first 4 h after dosing, every 30 min for 6 h and daily for 48 h for the

short-term outcome each time for instant death and then watched for the successive 24 hours for the short-term toxicity outcome and finally for the next 13 days for any delayed toxic effects. The signs of toxicity observed for include increased motor activities, rolling, writhing, depression, behavior pattern, diarrhea, sleep, coma, etc. the time of onset, disappearance and length of recovery period from toxic symptoms if any were systematically recoded. The same procedure was carried out for all theother fractions.

3.11.2 Determination of Anti-inflammatory Properties of Aqueous Methanol Extract and fractionsof *Ficus glumosa* Stem Bark

The fractions as well as the crude extract were subjected to anti-inflammatory activities using the method of Carrageenan-induced paw oedema in rats. Seventy rats were divided into groups of 5 rats each, the rats were fasted for 24 hours before commencement of experiment with only free access to water (Weischer and Thiemer, 1983). Group I receives 1mL/kg normal saline (negative control), group II receives piroxicam 10mg/kg (positive control), while other groups receives extract at doses of 500, 1000 and 1500 mg/kg respectively. After an hour, 0.1ml of sterile saline suspension of 1% carrageenan was injected into the sub-planar surface of the left hind paw of all animals. Paw size was measured using vernier caliper at time 1, 2, 3, 4 and 5 hours after the carrageenan administration (Winter *et al.,* 1963). The difference between the readings at time 0 hour and different time interval was taken as the thickness of oedema. The result was expressed in terms of mean increase in paw thickness at 0-5hrs and anti-inflammatory activity was expressed in terms of percentage inhibition of paw at 5hrs. The inhibitory activity was calculated according to the formula below: (Aiyelero*et al.,* 2009).

$$\% \text{ inhibition} = \frac{T_{0\,(control)} - T_{t(test)} X\ 100}{T_{0\,(control)}}$$

Where T_o is paw size for negative control and T_t is paw size for test group at different time interval.

3.12 Statistical Analysis

The results were expressed as mean ± standard errors of the mean (SEM) for all values. The data was statisticallyanalyzed using repeated measures ANOVA followed by Bonferroni*post hoc*. Results were considered to be significantly when *p* values are less than 0.05 ($p<0.05$).(Okasha *et al*., 2008).

CHAPTER FOUR

4.0 RESULTS

4.1 Pharmacognostic Parameters of *Ficus glumosa* Stem Bark

4.1.1 Macroscopic Examination of the Stem Bark

The dried pieces of the bark possessbrown colour on the outer surface, the pieces have astringenttaste with no particular odour. The surface is soft and produce fibrous end when fractured. Most of the pieces have channelled shapes.

4.1.2 Microscopic Examination of theStem Bark

The transverse section of *F. glumosa* stem bark revealed some prominent features. Generally, it showed the cork, cortex, medullary ray, fibres, calcium oxalate crystals and parenchyma cells.The cork is the outermost part of the stem bark with polygonal thin walled cells; the cortex contains many layers of thin cell walls of polygonal parenchyma cells. The cell also showed abundant calcium oxalate crystals scattered throughout the sections; they are prism-type in shapeandlong lignified fibres. (See plates II & III**)**.

The result for the micrometric evaluation of the diagnostic features such as cork cells, calcium oxalate crystals and fibre are shown in Table 4.1

Table 4.1 Micrometric Evaluation of Some of the Diagnostic Features

Diagnostic Features	Length in µm
Cork cells	9.36 – **10.84** – 12.3
Calcium oxalate crystal	31.8 – **36.04** – 42.4
Fibre	110.6 –**126.96** –143.2

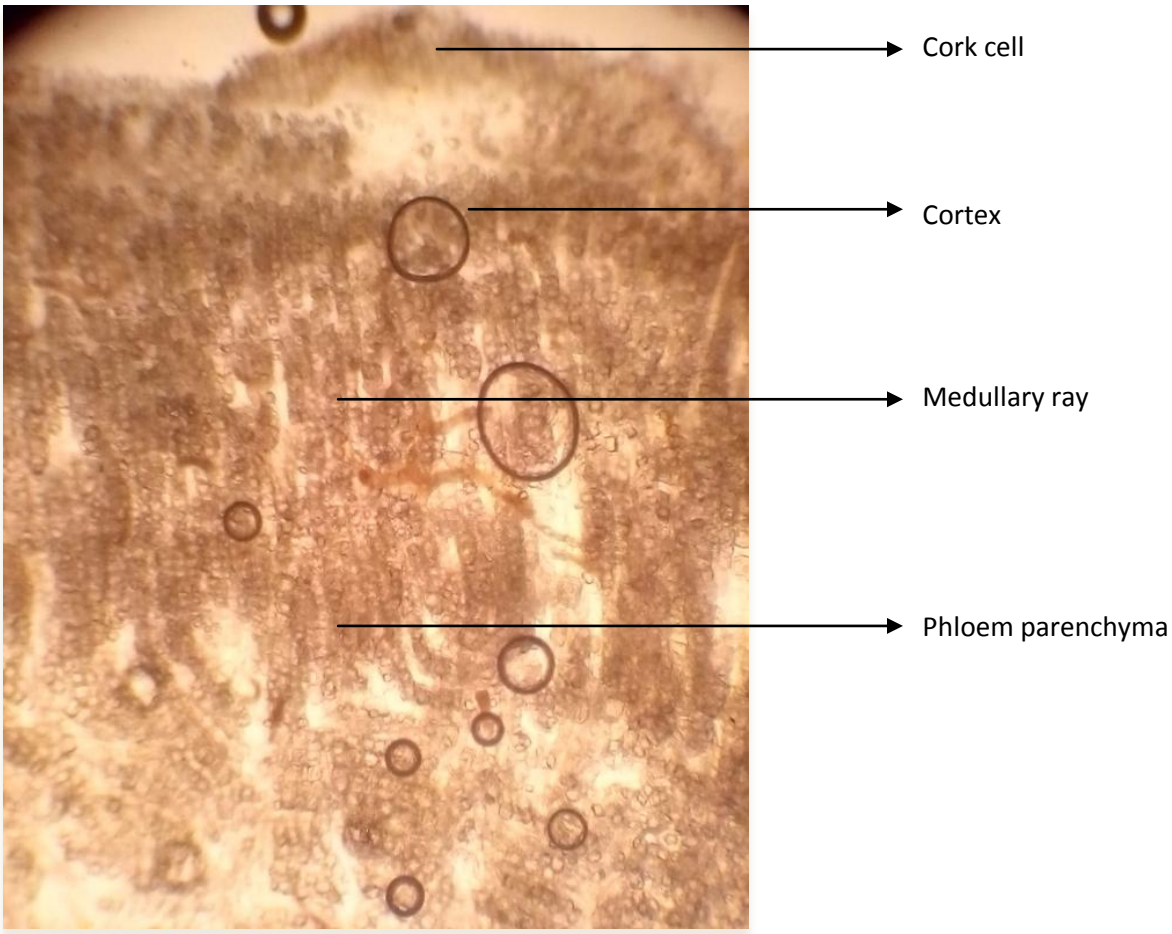

Plate II:Photomicrograph of transverse section of *Ficusglumosa* stem-bark showing some diagnostic features (Mag x100)

Plate III:Photomicrograph of powdered sample of *Ficus glumosa* stem-bark showing some diagnostic features (Mag x100)

4.1.3 Chemomicroscopical Examination of the Powdered Stem Bark

Chemomicroscopical examination of the powdered stem bark of *F. glumosa*revealed the presence of cellulose cell wall, lignified cell wall, tannins, calcium oxalate, suberin and mucilage (Table 4.2).

Table 4.2:Chemomicroscopical features of *Ficus glumosa* powdered stem bark

Constituents	Observation	Inference
Lignin	Red-pink colour on the walls of some lignified cell	Lignin present
Tannins	Greenish-black colour in some parenchyma cells	Tannins present
Calcium oxalate	Dissolution of shining crystals on the anatomical sections of the plant	Calcium oxalate crystal present
Calcium carbonate	No Effervescence in the cell	$CaCO_3$absent
Cellulose	Blue coloration of the cell wall	Cellulose present
Mucilage	Red coloration	Mucilage present
Suberin	Orange red colour on cell wall	Suberin present

4.1.4 Determination of Physical Constants of Powdered Stem bark of *Ficus glumosa*

The result of average moisture contents using loss on drying method was calculated to be 11.1± 0.49 % and the percentage yield of total ash, acid insoluble and water soluble matter were recorded in percentage values as 9.1±0.33%, 2.0±0.50% and 6.5±0.03% respectively. The extractives valueobtained were 16.6±0.33% and 12.0±0.57% for ethanol and water respectively (Table 4.3).

Table 4.3: Physicochemical Constants of *Ficus glumosa* Stem Bark Powder

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Parameters	**Values (%$^{W}/_{W}$) ± SEM***
Moisture content	11.1 ± 0.49
Total ash value	9.1 ± 0.33
Acid Insoluble ash	2.0 ± 0.50
Water Soluble ash	6.5 ± 0.03
Ethanol Extractive	16.6 ± 0.33
Water Extractive	12.0 ± 0.57

*****Average values of three determinations.

4.2 Extraction of the Stem bark of *Ficus glumosa*

The yield from the extraction of 1kg was 108.3g for aqueous methanol and the percentage yield for the fractions using 70g of the extract are hexane 1.5g, ethyl acetate 3.9g, butanol 17.9g and aqueous fraction 20.2g as reported in (Table 4.4).

Table 4.4: Mass and Percentage Yield of the Stem Bark of *Ficus glumosa*

S/N	Extract	Mass (g)	Percentage Yield (%$^{W}/_{W}$)
1.	Aqueous Methanol	108.3	10.83
Fractions			
1.	n-Hexane	1.5	0.15
2.	Ethyl acetate	3.9	0.39
3.	n- Butanol	17.9	1.79
4.	Aqueous	20.2	2.02

4.3 Preliminary Phytochemical Constituents of Aqueous Methanol Extract of *F.glumosa*

The preliminary phytochemical screening of the extract indicated the presence of alkaloids, steroids, terpenoids, tannins, flavonoids and cardiac glycosides. TLC profiles of the fractions were obtained as shown below (Table 4.5).

Table 4.5: Phytochemical Screenings of the various fractions of *Ficus glumosa* Stem BarkAqueous Methanol Extract

Detecting Reagent	Fraction	Observation	Inference
Libermann Buchard	n-Hexane	Dark brown spot	Steroids and triterpenes present
	Ethylacetate	Dark brown spot	Steroids and triterpenes present
	n- Butanol	Dark brown spot	Steroids and triterpenes present
Ferric Chloride	Ethylacetate	Blue- black spot	Phenolics present
	n-Butanol	Blue- black spot	Phenolics present
Aluminium Chloride	Ethylacetate	Yellow spot	Flavonoids present
	n-Butanol	Yellow spot	Flavonoids present

Table 4:6 Summary of TLC Profile of Hexane, Ethyl acetate and Butanol fractions spray with *p*-Anisaldehyde/H_2SO_4

Fractions	Solvent System	No. of Spots	ColourofSpots	R_f values
n-Hexane	H:E (7:3)	6	Purple	0.17
			Pink	0.31
			Pink	0.35
			Pink	0.68
			Pink	0.82 and
Ethylacetate	H:E (8:2)	4	pink	0.93
			Pink	0.36
			Pink	0.48
			Pink	0.70 and
			Purple	0.89
			Brown	0.22
n-Butanol	B:A:W (6:1:1)	4	Brown	0.48
			Brown	0.66 and
			Brown	0.71

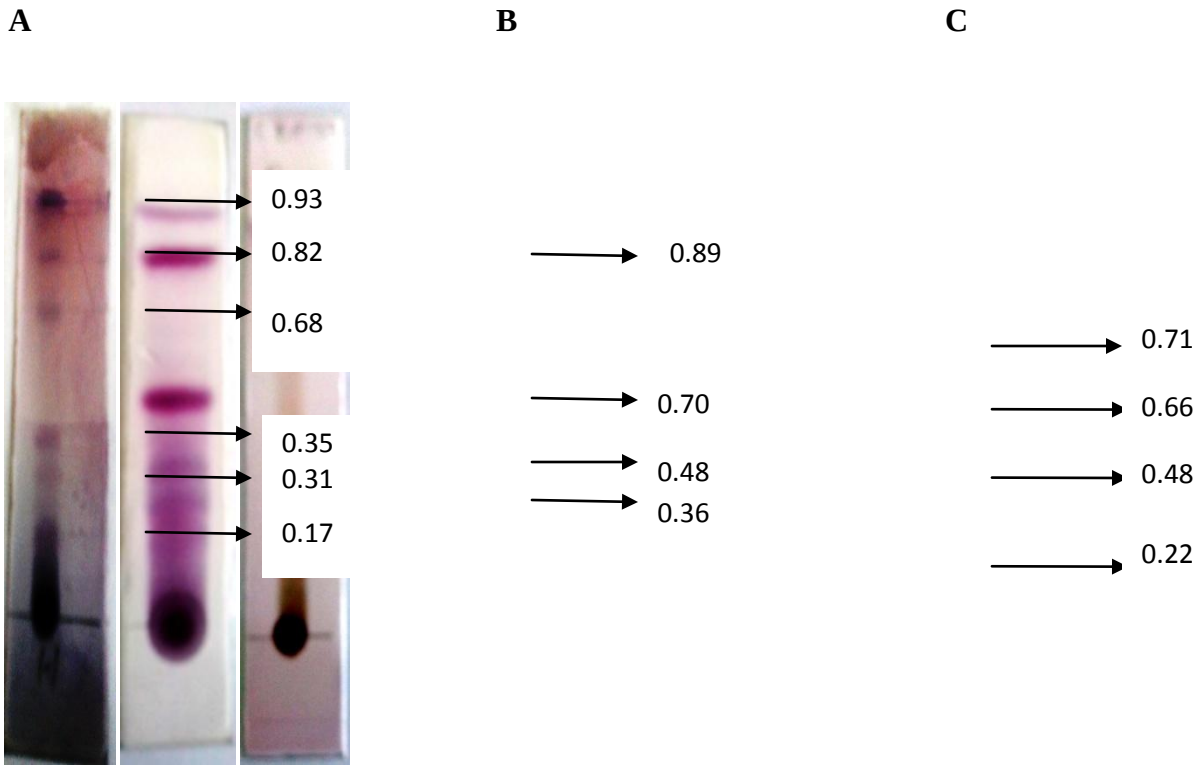

Plate IV: Chromatogram of n- hexane, ethyl-acetate and n- butanol fraction on precoated silica gel plate developed in different solvent system sprayed with *p*-anisaldehyde/H_2SO_4.

A: n-haxane fractiondeveloped in H: E (7:3)

B: Ethyl-acetate fractiondeveloped in H: E (8:2)

C: n- butanol fraction developed in B: A:W (6:1:1)

A B C D E F

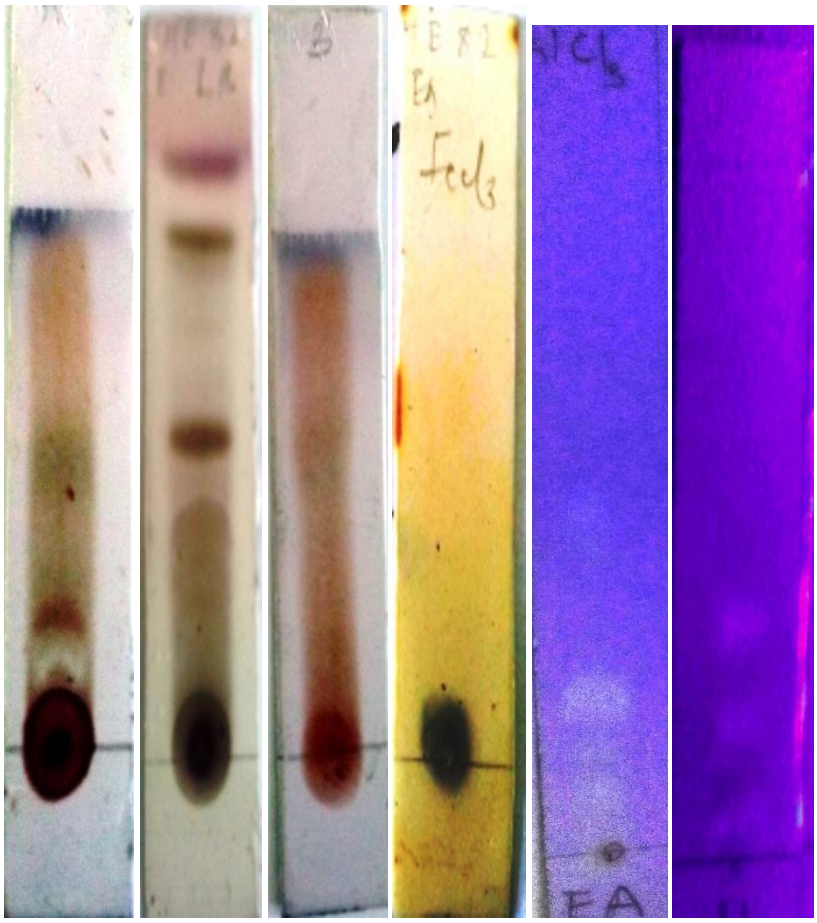

Plate V: Chromatogram of n-hexane, ethyl-acetate and n-butanol fraction sprayed with different reagent on precoated silica gel plate.

A: n-hexane fraction sprayed with Libbermann Buchard reagent developed in H: E (7:3)

B: Ethyl-acetate fraction sprayed with Libbermann Buchard reagent developed in H: E (8:2)

C: n-butanol fraction sprayed with Libbermann Buchard reagent developed in B: A: W (6:1:1)

D: Ethyl-acetate fraction sprayed with Ferric chloride reagent developed in H: E (8:2)

E: Ethyl-acetate fraction sprayed with aluminium chloride reagent developed in H: E (8:2)

F:n-butanol fraction sprayed with aluminium chloride reagent developed in B: A:W (6:1:1)

4.4 Elemental Analysis of the stem bark of *Ficus glumosa*

The results of elemental analysis of the stem bark of *F.glumosa* carried out using Atomic Absorption Spectroscopy revealed the presence and proportion of some essential elements such as K, Na, Ca, Mg, Fe, Zn, Cu, as summarized in (Table 4.7).

Table 4.7: Concentrations of Mineral Content (ppm) of the Stem Bark of *Ficus glumosa*

Elements	Concentration (ppm)
K	52500±0.4
Ca	11016±0.5
Mg	963.3±0.5
Fe	750±0.4
Na	725±0.3
Co	250±0.2
Zn	144.5±0.3
Ni	83.2±0.5
Cu	19.2±0.2

4.5 Determination of Acute Toxicity (LD_{50})of *Ficus glumosa* Stem Bark Extract and Fractions

The Acute toxicity (LD_{50}) of the fractions(butanol, ethyl acetate and aqueous) as well as aqueous methanol extractof *F. glumosa* stem bark were determined using OECD guideline 425 method via oral route and showed that the LD_{50} were greater than 5000 mg /kg. There were no death or any sign of toxicity recorded.

4.6 Anti inflammatory Evaluation of the Aq. Methanol extract of *F. glumosa* stem bark

The aqueous methanol extract of *Ficus glumosa*at the dose of 1000 and 1500 mg/kg were not able to produce significant reduction, where as dose of 500 mg/kg producedsignificant (p<0.05)reductionin the inflammation produced by carageenan. The anti-inflammatory effect of the methanol extract at 500 mg/kg produced 28% protection and was comparable to piroxicam (10 mg/kg)the standard anti-inflammatory agent used with 28% protection at the3th hour.The percentage protection at the 4thand 5thhour were 24% and 22% at the dose of 500 mg/kg. The result was statistically significant as compared with the normal saline treated group at the dose of 500mg/kg with(p<0.05) as presented (Table4.8)

Table 4.8: Effects of aqueous methanol extract of *Ficus glumosa* stem barkon carrageenan induced paw oedema in rats

Treatment	Mean Paw Diameter (mm) and % inhibition in parenthesis					
(mg/kg)	**0 hr**	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5hr**
N/Saline 1ml/kg	2.23±0.02	2.90±0.07	3.11±0.16	3.03±0.05	2.72±0.13	2.59±0.20
MEFG 500	2.00±0.0.3	2.23±0.06* (23)	2.32±0.08* (25)	2.17±0.08* (28)	2.06±0.06* (24)	2.02±0.09* (22)
MEFG 1000	2.22±0.08	2.54±0.08 (12)	2.85±0.04 (8)	2.80±0.03(7)	2.65±0.06 (2)	2.53±0.05 (2)
MEFG 1500	2.30±0.06	2.72±0.11 (6)	2.91±0.07(6)	2.81±0.07 (7)	2.68±0.04 (1)	2.55±0.07 (1)
Piroxicam10	2.00±0.10	2.49±0.05 (14)	2.42±0.06* (22)	2.18±0.05* (28)	2.06±0.06* (24)	2.03±0.02* (21)

Values are presented as Mean± S.E.M * = $P < 0.05$ compared to normal saline control using repeated measures ANOVA followed by Bonferroni's Post hoc multiple comparison test. n = 5.

MEFG = Methanol extract of *Ficus glumosa.*

4.7 Anti inflammatory Evaluation of Ethyl acetate fraction

The Ethylacetate fraction produced time related, sustained reduction of carageenan induced inflammation of rats hind paw compared to negative control, it was in dose dependant manner and statistically significant ($p<0.05$) at all the doses used.Paw size decreases with increase in time, with upto26% protection at the dose of 1500mg/kg at the end of the 3^{th} hourwhere the peak of protection was reached as shown on (Table 4.9).

Table 4.9: Effect of Ethylacetate fraction of aqueous methanol extract of *Ficus glumosa* stem barkon carrageenan induced paw oedema in rats

Treatment	Mean Paw Diameter (mm) and % protection in parenthesis					
(mg/kg)	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
N/Saline 1ml/kg	2.23±0.02	2.90±0.07	3.11±0.16	3.03±0.05	2.72±0.13	2.59±0.20
EFFG 500	2.04±0.05	2.61±0.07* (10)	2.75±0.08* (11)	2.44±0.07* (19)	2.28±0.09* (16)	2.07±0.03* (20)
EFFG 1000	2.00±0.09	2.37±0.05* (18)	2.47±0.05* (20)	2.29±0.05* (24)	2.12±0.03* (22)	2.01±0.03* (22)
EFFG 1500	2.05±0.07	2.31±0.04* (20)	2.48±0.08* (20)	2.24±0.07* (26)	2.15±0.06* (20)	2.05±0.02* (20)
Piroxicam10	2.00±0.10	2.49±0.05 (14)	2.42±0.06* (22)	2.18±0.05* (28)	2.06±0.06* (24)	2.03±0.02* (21)

Values are presented as Mean± S.E.M * = $P < 0.05$ compared to normal saline control using repeated measures ANOVA followed by Bonferroni's Post hoc multiple comparison test. n = 5. EFFG = Ethyl acetate fraction of *Ficus glumosa*.

4.8 Anti inflammatory Evaluation of n-butanol fraction

The butanol fraction at all the doses (500, 1000, and 1500mg/kg) used were statistically significant ($p<0.05$) with 26% protection at the end of the 3th hour for the 500 mg/kgand 25% at doses of 1000 and 1500 mg/kg.The percentage protection, decline after the 3th hour at all the doses used. The oedema paw size reduction increases with increase in time (Table 4.10)

Table 4.10: Effect of Butanol fraction of methanol extract of *Ficus glumosa* stem barkon carrageenan induced paw oedema in rats

Treatment	Mean Paw Diameter (mm) and % protection in parenthesis					
(mg/kg)	0 hr	1 hr	2 hr	3 hr	4 hr	5hr
N/Saline 1ml/kg	2.23±0.02	2.90±0.07	3.11±0.16	3.03±0.05	2.72±0.13	2.59±0.20
BFFG 500	2.02±0.08	2.31±0.03* (20)	2.47±0.03* (20)	2.24±0.05* (26)	2.14±0.04* (21)	2.03±0.02* (21)
BFFG 1000	2.03±0.05	2.31±0.03* (20)	2.42±0.06* (22)	2.26±0.06* (25)	2.16±0.05* (20)	2.05±0.02* (20)
BFFG 1500	1.98±0.05	2.35±0.08* (18)	2.53±0.09* (18)	2.27±0.02* (25)	2.15±0.02* (20)	2.03±0.08* (21)
Piroxicam10	2.00±0.10	2.49±0.05 (14)	2.42±0.06* (22)	2.18±0.05* (28)	2.06±0.06* (24)	2.03±0.02* (21)

Values are presented as Mean± S.E.M * = $P < 0.05$ compared to normal saline Control using repeated measures ANOVA followed by Bonferroni's Post hoc multiple comparison test. n = 5.

BFFG = Butanol fraction of *Ficus glumosa*.

4.9 Anti inflammatory Evaluation Aqueous fraction

The aqueous fraction produced effect best at the dose of 500 mg/kg with 22% protection at the end of the 3^{th} hour and was statistically significant ($p<0.05$) at all doses as compared with negative control but the activity decreased with increase in dose.It also showed that the oedema paw size reduction ofthe fraction increased with increase in time. (Table 4.11)

Table 4.11: Effect of Aqueous fraction of methanol extract of *Ficus glumosa* stem barkon carrageenan induced paw oedema in rats

Treatment	Mean Paw Diameter (mm) and % protection in parenthesis					
(mg/kg)	0 hr 1 hr	2 hr	3 hr	4 hr	5 hr	
N/Saline 1ml/kg	2.23±0.02	2.90±0.07	3.11±0.16	3.03±0.05	2.72±0.13	2.59±0.20
AFFG 500	2.02±0.07	2.44±0.14* (15)	2.48±0.15* (20)	2.36±0.12* (22)	2.20±0.10* (19)	2.15±0.06* (16)
AFFG 1000	2.07±0.05	2.40±0.02* (17)	2.51±0.04* (19)	2.42±0.04* (20)	2.29±0.05* (15)	2.22±0.03* (14)
AFFG 1500	2.00±0.08	2.47±0.05* (14)	2.65±0.10* (14)	2.54±0.08* (16)	2.33±0.05* (14)	2.24±0.04* (13)
Piroxicam10	2.00±0.10	2.49±0.05 (14)	2.42±0.06* (22)	2.18±0.05* (28)	2.06±0.06* (24)	2.03±0.02* (21)

Values are presented as Mean± S.E.M * = $P < 0.05$ compared to normal saline control using repeated measures ANOVA followed by Bonferroni's Post hoc multiple comparison test. n = 5. AFFG = Aqueous fraction of *Ficus glumosa.*

CHAPTERFIVE

5.0 DISCUSSION

Pharmacognostic evaluation of a plant or plant parts is considered to be an important step that provides valuable information in terms of its morphological, microscopical and physico chemical characteristics. The macroscopic, microscopic and physical evaluation of the fresh and dried plant materials revealed some features which are ofimportance and can be applied in establishing the identity of the plant parts in its fresh and dried form. This is important in the study of crude drugs since the first step in the study of crude drug is establishing the correct identity of the plant.

Macroscopically, the bark is brown in colour,inner surface is light-brown, shape is channelled,taste is astringent without any odour, soft surface, and fracture is fibrous.The microscopical features of the drug under study using the transverse section revealed some prominent features like the cork, cortex, calcium oxalate crystals(prism type), long lignified fibres, medullary ray and phloem parenchyma cells.

The 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, 1996). The macroscopic and microscopic features of the plant were in conformity with those of some plant members of the genus *Ficus* such as *Ficus bengalensis* Linn as described by (Vikas and Vijay, 2010).

The chemomicroscopical features of powdered stem bark ofrevealed the presence of cellulose cell wall, lignified tissues, mucilage, tannins, suberin or cutin and calcium oxalate crystals while calcium carbonates was found to be absent.

The physicochemical constants were determined from the stem bark powder of the plant.The moisture content (11.1%), total ash value (9.1%), acid insoluble ash (2.0%), water soluble ash (6.5%), alcohol (Ethanol) extractives value (16.6%) and water extractives value (12.0%).These values are useful as the criteria to judge the identity and purity of crude drug (WH0, 1996). It also indicates presence of various impurities like carbonate, oxalate and silicate in plant materials (Kaneria and Chanda, 2011).

Quantitative evaluation is an important parameter in setting standard of crude drugs and the physical constant parameters could be useful in detecting any adulterant in the drug (Musa,2005).The general requirement of moisture content in crude drug is that, it should not be more than 14% (B. H. P, 1990) and the value obtained in this research work was within the accepted range.Moisture is considered an adulterant because of its added weight as well as the fact that excess moisture is conducive to the promotion of mold and bacterial growth, up to 5% is usually not considered excessive and low moisture content in a crude drugs suggest better stability against microbial and biochemical degradation of herbal products (WHO, 1996).

Total ash value (9.1%) represents both the physiological and non-physiological ash from the plant. The non-physiological ash is an indication of inorganic residues after the plant drug is incinerated. The acid insoluble ash values (2.0%) obtained in this study indicated that the plant was in good physiological condition and it contained little extraneous

matter such as sand, silica and soil. The total ash value is used as criteria to judge the identity and purity of drugs (WHO, 1996,Prasad *et al*., 2012).

Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not (Tatiya *et al.,* 2012).This study indicated that theethanol had high extractive value of (16.6%) compared towater which had extractive value of (12.0%). Ethanol penetrates more easily the cellular membrane to extract the inter-cellular ingredients from the plant materials (Wang, 2010).

Despite that water is a universal solvent and its use primarily as solvent by traditional healers, alcohol is still given preference in terms of choice of solvent when it comes to medicinal plant researches. The choice of solvent in a research involving plants depend on so many factors among which include the diversity of different phytochemicals to be extracted and also what is intended with the extract. Methanol was chosen as the solvent of extraction, this is due to higher activities of methanol extract (Tiwari and Mishra, 2011). The yield obtained from the extraction of (1kg) powdered material of the plant was 108.3g i.e 10.83%. The yield for the various fractions using 70g of the crude extract were, hexane (1.5g i.e 0.15%), ethyl acetate (3.9g i.e 0.39%), and for the n-butanol which is more polar yielded (17.9g i.e 1.79 %) while the aqueous gave (20.2g i.e 2.02%). This shows the richness of the drug in polar compound (Umar, 2010).

Preliminary phytochemical screening gives a brief idea about the qualitative nature of active phytochemical constituents present in plant extracts, which will help the future investigators regarding the selection of the particular extract for further investigation or isolating the active principle (Mishra *et al.,* 2010).

The phytochemical screening of the aqueous methanol extract of the stem bark of the *Ficus glumosa* revealed the presence of steroids, terpenoids, saponins, cardiac glycosides, flavonoids, tannins and alkaloids. This is consistent with some members of the genus as in the leaves of *F. ingens* as reported by (Aiyelero *et al.*, 2009).Plants have different kinds of secondary metabolites such as flavonoids, tannins, saponins and these have been found to have anti-inflammatory activities both in vivo and in vitro (Mona *et al*., 2014). Thin layer chromatographic analysis is a simple and cheap method for detection of active constituents in plants due to its good selectivity and sensitivity, providing convincing results (Patra *et al.,* 2012), this is considered a reliable technique for qualitative phytochemical screening of plants active constituents.The fractions of the crude extract; n-hexane, ethylacetate and n-butanol were found to contain steroids and triterpenes, flavonoids and other phenolic compounds were shown to be present in the ethyl acetate and n-butanol fraction.For aqueous fraction no suitable solvent system could be obtained during the course of this study.

Elemental analysis of stem barkof *F. glumosa* was carried out by Atomic Absorption Spectroscopy and the concentrations of mineral contents in (ppm) of plant sample indicated that Na, K, Ca, Fe, Mg, Cu, Zn, Ni, and Co were present. These elements play a vital role in the formation of secondary metabolites which are responsible for pharmacological actions of medicinal plants (Khan *et al*., 2011). The mineral

contentsshowed that Potassium concentration was higher in the elements analyzed followed by Calcium. Potassium, the major cation of intracellular fluid, is present in small amounts in extracellular fluid, it was found in the range of 52500ppm. With sodium, potassium maintains normal water balance, osmotic equilibrium and acid–base balance.Magnesium is an essential mineral element in biological systems it is present in every cell type in every organisms. In plants, magnesium is necessary for synthesis of chlorophyll and photosynthesis and was found to be 963.3ppm. Calcium is essential in keeping the bones strong and reduces osteoporosis in old age. In this sample it was found to be 11016.8ppm. Sodium was also present at concentration of 725ppm and it is the main monovalent ion of extracellular fluid. Sodium ions constituting 93% of the ions bases found in the blood stream, it is involved inthe production of energy. In plants, sodium is a micronutrient that aids in metabolism, specifically in regeneration of phosphoenolpyruvate and synthesis of chlorophyll. Iron is an essential mineral to prevent anaemia and was found to be 750ppm in this sample. Zinc is an essential mineral element of importance necessary for plants, animals and micro organisms. Zinc is important in metabolic function and for growth in man,deficiency may contribute to growth retardation, hair loss. Concentration of 144.5ppm for zinc was observed. Cobalt and Nickel are required in little amount in human body. Their concentrations in this sample were 250 and 83.2ppm respectively.Cobalt is used to treat different types of cancers while the health benefits of Nickel are optimal growth, healthy skin but required in low quantity. Copper is essential to all living organisms as a trace dietary mineral element, itplays important role in prevention of inflammation it was found at concentration of 19.2ppm. Manganese, chromium and cadmium were absent. Cadmium being absent is

good as it is an element of toxicityparticularly when consumed in powdered form, this may suggest that the plant is safe for consumption.The high concentration of certain metals such as; Mg, K, Ca, and Fe in the plant are essential for proper growth and normal functioning of the plant (Underwood, 1971).

From the above presentation, it is obvious that this plant accumulate essential, important, necessary, useful and helpful elements for plant, man and animals. The presence and concentrations of various elements in plants depend on the composition of soil, water, and fertilizers usedas well as permissibility, selectivity and absorbability of plants for the uptake of these elements (Rajurkar and Damame, 1997).

The claimed anti-inflammatory activity of the plant materialin ethnomedicine could also be due to the contribution of the trace elements present.Copper and zinc supplementation have been reported to produce anti-inflammatory effect due to their ability toform complexes that serve as selective antioxidants (Di-Silvestro and Marten, 1990).

In order to determine the safety margin of drugs and plant products for human use, toxicological evaluation was carried out in experimental animals using OECD guideline method to predict toxicity and to provide guidelines for selecting a ‘‘safe’’ dose in animals and also used to estimate the therapeutic index (LD_{50}/ED_{50}) of drugs (Olson *et al.,* 2000; Rang *et al.,* 2001; Maikai *et al.,* 2008).

In this study, median lethal dose (LD_{50})was carried out orally in rats. The LD_{50} was found to be greater than 5000 mg/kg when administered orally in rats. These studies showed theextracts of *F. glumosa*stem bark is practically non-toxic when administered using the oral route. This is based on the toxicity classification by Loomis and Hayes (1996) which

states that substances with LD_{50} values of 5000 to 15,000 mg/kg body weight is practically non-toxic.

The anti inflammatory study showed that in the normal saline treated rats, sub plantar injection of 1%w/v carrageenan produced a local oedema reaching its maximum at the second hour. The aqueous methanol extract of *F. glumosa*significantly inhibited the progressive increase in paw oedema produced by carrageenan at the dose of 500 mg/kg. The anti-inflammatory effect of the extract at 500 mg/kg was comparable to piroxicam (10 mg/kg), the standard anti-inflammatory agent used, the activity decreased with increase in dose.The extract inhibited both the first and second phases of inflammation, the ethyl acetate fraction produced dose dependant and time related reduction of paw size. The butanol and aqueous fraction produces effect at all the doses used but they were in non dose dependant manner.Activity was not carried out on hexane extract due its low yield.This result indicated that the stem bark has anti-inflammatory activity. It seems the anti inflammatory property is short lived and one may suggest this action is quickly metabolized and removed from the system after reaching its peak in 2 h.The ability of the extract to inhibit carrageenan induced paw oedema suggests it possesses a significant effect against acute inflammation.

According to (Musa *et al.,* 2010), inflammation induced by carrageenan is thought to be biphasic. The early phase is mediated by histamine, serotonin and prostaglandin increased synthesis in the damaged surrounding tissues. The late phase is sustained by prostaglandins produced by tissue macrophages and prostaglandins released and mediated by polymorphonuclear cells, leukotrines and bradykinins. The extract and fractions inhibited both phases of inflammation.The extract produced an inhibitory effect

on carrageenan induced inflammation over a period of 5 hours which is similar to the effect of most non-steroidal anti-inflammatory drugs.

Anti-inflammatory effects of secondary metabolites like flavonoids, tannins, saponins, alkaloids have been reported (Mona *et al*., 2014) and these have been found present in the extract. Hence, the anti-inflammatory effect produced by the extract and fractions may be attributed individually or collectively to the constituents in the extract.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The pharmacognostic standards for stem bark of *F. glumosa* was established in this study and the data could be used as a diagnostic tool for identification and authentication of the plant. The datacould also be used for standardization of crude drugs obtained from it.

Pharmacognostic evaluation of the plant *Ficus glumosa*had shown that, macroscopically, the bark is light brown in colour, shape is channelled, taste is astringent without any characteristic odour, soft surface, and fracture is fibrous.

Microscopic examination of the transverse section of *F. glumosa* stem bark revealed some prominent features like the cork (10.84µm),prism type ofcalcium oxalate crystals(36.04µm), fibres (126.96 µm), phloem parenchyma cells, cortex, and medullary ray.

Chemomicroscopical features of powdered stem bark of *F. glumosa*revealed the presence of cellulose cell wall, lignified cell wall, mucilage, tannins, suberin and calcium oxalate crystals.

The Physical parameters ($\%^{W}/_{W}$) of the powdered stem bark of *F. glumosa*were Moisture content (11.1 %), Total ash value (9.1 %), Acid insoluble ash (2.0 %), Water soluble ash (6.5 %),Ethanol extractives value(16.6 %) andWater extractives value (12.0 %).

Phytochemical analysis of the stem bark extractrevealed the presence of alkaloids, tannins, flavonoids, steroids and triterpenes.Thin layer chromatographic analysis

visualized with specific reagents confirmed the presence of steroids/triterpenes, flavonoids, and other phenolic compounds inthe fractions.

Themedian lethal dose(LD_{50})of the extract and fractionswere found to be greater than 5000 mg/kg when administered orally in rats and considered practically non-toxic.

The elemental concentration of the stem bark powder of *F. glumosa*indicated that potassium, calcium, magnesium, zinc, iron, copper, nickel, cobalt and sodium were present.

The aqueous methanol extract of *F.glumosa* stem bark demonstrated protective activity against carageenan induced oedema in rats as evidenced in the significant reduction of paw oedema produced with dose of dose 500 mg/kg.

6.2 Conclusion

The present studies established:

Morphological features of*F. glumosa* stem barknamely calcium oxalate crystals(36.04 µm) prism type, cork cells (10.84 µm),long lignifiedfibres (126.96 µm), medullary rays and parenchyma cells which could serve useful towards its identification. Physicochemcal values such as ash value9.5%, acid insoluble value 2.0%, water soluble value 6.5%, ethanol extractive value 16.6% and water extractive value 12.0%.

Elemental analysis revealed the concentration in (ppm) of Na, K, Cu, Ca, Zn, Fe, Co, Ni and Mg in the powdered stem bark it can be concluded that it is a source of essential mineral elements.The result obtained from LD_{50} showed the plant has wider margin of safetyusing oral route.

The methanol extract showed activity against inflammation induced by carageenanat 500mg/kg with 28% protection at end of the 3th hour and statistically significant ($p<0.05$). The results support the traditional use of the plant in treatment of inflammation and related inflammatory diseases.

6.3 Recommendations

i. Further studies should be carried out to establish the chronic toxic profile of *Ficus glumosa* stem bark.

ii. More investigations should be carried out to evaluate the activity of the hexane fraction and develop a suitable solvent system for the aqueous fraction.

iii. Isolate the major anti inflammatory principle responsible for the activity.

iv. It is also recommended that more research should be carried out to establish pharmacognostic profile of the whole plant parts for inclusion into the Pharmacopoeia which will assist in its standardization for quality, purity and sample identification.

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APPENDIX A

a). Determination of Ash Value of powdered Stem bark of *F. glumosa*

Description	1	2	3
Constant weight of crucible (g)	39.62	38.6939.23	
Weight of crucible and content (g)	41.62	40.6941.23	
Weight of crucible and Ash (g)	39.81	38.86 39.42	
Weight of Ash (g)	0.19	0.170.19	
Ash Value (%)	9.5	8.59.5	
Average mean (%)		9.1	

Sample calculation

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

$$\text{Ash Value} = \frac{0.19}{2} X\ 100$$

$$= 9.5\ \%^{W}/_{W}$$

APPENDIX B

b) Determination of Acid insoluble Ash of powdered Stem bark of *F. glumosa*

Description 1	2	3	
Constant weight of crucible (g)		39.62	38.6939.23
Weight of crucible and Acid insoluble ash (g)		39.67	38.7139.28
Weight of Acid insoluble ash (g)		0.05	0.020.05
Acid Insoluble Ash Value (%)		2.5	1.02.5
Average mean (%)	2.0		

Sample calculation

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

$$\text{Acid Insoluble Ash value} = \frac{0.05}{2} X\ 100$$

$$= 2.5\ \%^{W}/_{W}$$

APPENDIX C

c). Determination of water soluble Ash of powdered Stem bark of *F. glumosa*

Description	1	2	3
Constant weight of crucible (g)	39.62	38.69	39.23
Weight of crucible and Ash(g)	39.68	38.73	39.29
Weight of Ash (g)	0.19	0.17	0.19
Weight of water Insoluble ash (g)	0.06	0.05	0.06
Weight of water soluble ash (g)	0.13	0.12	0.13
Water soluble Ash Value (%)	6.5	6.0	6.5
Average mean (%)	6.3		

Sample calculation

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

$$\text{Water soluble Ash value} = \frac{(0.19 - 0.06)}{2} X\ 100$$

$$= 6.5\ \%^{W}/_{W}$$

APPENDIX D

d). Determination of water–soluble extractive value of Stem bark of *F. glumosa*

4 g of the powder was used in 100 ml of water.

Description	1	2	3
Constant weight of dish (g)	123.08	84.16	64.86
Weight of crucible and content after heating (g)	123.21	84.28	64.75
Water extractive content (g)	0.13	0.12	0.11
water extractive Value (%)	13.0	12.0	11.0
Average mean (%)	12		

Sample calculation

Water extractive value = $\frac{\text{Wt of dish \& content after heat (g)} - \text{Constant wt.of dish (g) X 4}}{\text{Initial weight of drug}} X\ 100$

Water extractive value = $\frac{(123.21 - 123.08)\ \text{X}\ 4}{4} X\ 100$

= 13 $\%^{W}/_{W}$

APPENDIX E

e). Determination ofalcohol – solubleextractive value of Stem bark of *F. glumosa*

4 g of the powdered wasused in 100 ml of 90% ethanol

Description1	2	3	
Constant weight of dish (g)	49.17	64.76144.67	
Weight of dish and content after heating (g)	49.33	64.93144.84	
Alcohol extractive content (g)	0.160.17	0.17	
Alcohol extractive Value (%)	16.017.0	17.0	
Average mean (%)			16.7

Sample calculation

$$\text{Alcohol extractive value} = \frac{\text{Wt of dish \& } content\ \ after\ \ heat \text{ (g)} - \text{Constant wt.of dish (g) X 4}}{\text{Initial weight of drug}}\ X\ 100$$

$$\text{Alcohol extractive value} = \frac{(49.33 - 49.17)\text{ X } 4}{4}\ X\ 100$$

$$= 16.0\ \%^{W}/_{W}$$

APPENDIX F

f). Determination of moisture content of powdered stem bark of *F. glumosa*

3g of the powdered plant material was used

Description	1	2	3
Constant weight of crucible (g)	49.28	44.32	46.73
Initial weight of powder (g)	52.28	47.32	49.73
Final weight of powder	51.92	47.01	49.40
Loss in weight (g)	0.36	0.31	0.33
Moisture content (%)	12.0	10.3	11.0
Average mean (%)		11	

Sample calculation

$$\% \text{ Moisture content} = \frac{Initial Weig\ ht of Powder - Final Weig\ ht of Powder}{Initial Weig\ ht of Powder} X\ 100$$

$$\% \text{ Moisture content} = \frac{(52.28 - 51.92)}{3} X\ 100$$

$$= 12\ \%^{W}/_{W}$$

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