PHARMACOGNOSTIC AND SOME BIOLOGICAL STUDIES ON *MAYTENUS SENEGALENSIS* (LAM.) EXELL (CELASTRACEAE)

BY

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AHMADU BELLO UNIVERSITY

ZARIA, NIGERIA

JANUARY, 2014

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE

STUDIESAHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE AWARD
OF A
MASTER DEGREE IN PHARMACOGNOSY

DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT,

FACULTY OF PHARMACEUTICAL SCIENCES

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ZARIA, NIGERIA

JANUARY, 2014

DECLARATION

I declare that the work in this thesis entitled Pharmacognostic and some Biological Studies on *Maytenus senegalensis* (Lam.) Exell (Celastraceae) has been carried out by me in the Department of Pharmacognosy and Drug Development. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

_____ _____

Murjanatu Abubakar Ahmed Date

CERTIFICATION

This thesis entitled PHARMACOGNOSTIC AND SOME BIOLOGICAL STUDIES ON *MAYTENUS SENEGALENSIS* (LAM.) EXELL (CELASTRACEAE) by MURJANATU ABUBAKAR AHMED meets the regulations governing the award of the degree of Master in pharmacognosy of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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AKNOWLEDGEMENT

All praises for the omnipotent al-mighty Allah who blessed me to accomplish my MSc. research work. I pray to Allah that this investigation be of use in the relief of ailments (pain and inflammation) in the future.

On the completion of this work, which is certainly not a single man's task, I would like to pay my sincere gratitude not only to my supervisors but also to other staff members of the department and my family. I am deeply indebted to my late and beloved father Alh. Ahmadu Abubakar for the immense support, love and care that he had shown me all my life. Without him, this work will not have been accomplished. I am highly obliged to my learned and able supervisors: Prof. K.Y. Musa and Dr. G. Ibrahim whose prime supervision has helped accomplished this work. My sincere gratitude goes to Dr. Aliyu Musa of Pharmchem. and Dr. Magaji of pharmacology for their encouragement, assistance and guidance. I appreciate and express my sincere thanks to Mal. Musa Muhammad of the herbarium section of Department of Biological Sciences for his help in identifying the plant used for this research. I also wish to thank our laboratory staff especially Malam Mustafa Abba, Alh. Adamu Muhammad, Mal. Kabir, Mal. Kamilu, and Mal. Isah for their co-operation during my laboratory work. Any sense of gratitude will not be complete without giving words of thanks to our able Head of Department Prof. M.S. Abubakar. I will like to thank the laboratory and animal house staff of the department pharmacology and therapeutics for their assistance and cooperation. It is my pleasure to thankfully acknowledge Dr. Dan Malam, Dr. Ahmad and Dr. Katsayal for their encouragement. Words are confined to express my gratitude to my beloved husband, family, friends and colleagues for their love and support.

DEDICATION

This work is dedicated to my beloved Dad, Alhaji Ahmed Abubakar.

ABSTRACT

Maytenus senegalensis (Celastraceae) is commonly known as thorny staff tree or confettii tree, is of high traditional use among the 'Hausa' community of Northern Nigeria. Hence the present investigation was to establish the pharmacognostic parameters for the leaves and stem bark of the plant, including macroscopic and microscopical, physicochemical constants as well as analgesic and anti-inflamatory studies. Macroscopic features of the leaves include alternate arrangement, glabrous surface, serrated margin with a characteristic taste and odor. The stem bark has grey, rough and firm outer surface, pink and smooth inner surface, the bark is channeled. Microspically, the leaves have stomata that is anomocytic type on both upper (SN= 80.50-70.00-59.50, SI= 10.81-12.72-14.63) and lower epidermis (SN= 101.78-88.50-75.23, SI= 10.65-12.53-14.41), the epidermal cells are polygonal in shape and trichomes are absent. The vein islet number was determined to be 23.46-20.40-17.34 and the vein let termination number was 17.94-15.60-13.26. Transverse sections of the leaves revealed a dorsiventral type having a singled layered epidermis, palisade cell beneath the upper epidermis, with some vascular bundles at the center covered by a bundle carp, and some spongy mesophyll. Transverse sections of stem bark revealed the presence of cork cells, vascular bundles, and tracheids. Longitudinal sections revealed the presence of epidermal cells and some collenchymas cells in the leaves. Some sclereids and parenchyma cells with some calcium oxalates encrusted in them were seen in the transverse sections of the stem bark. Chemo-microscopical study revealed the presence of cellulose cell wall, lignified fibers, starch grains, calcium oxalates, fixed oil and fats.

Other determinations include: moisture content (leaves- 9.33 % w/w ± 0.01, stem bark- 6.33 % w/w ± 0.02), total ash (leaf- 7.83.00 % w/w ± 0.004, stem bark- 15.17 % w/w ± 0.01), acid-insoluble ash (leaf- 01.67% w/w ± 0.01, stem bark- 01.00% w/w ± 0.01), alcohol-soluble extractive values (leaf- 12.00% w/w ± 0.10, stem bark- 13.25% w/w ± 0.10), water-soluble extractive values (leaf- 13.25%w/w ± 0.04, stem bark- 14.25% w/w ± 0.04). Thin layer chromatography of n-butanol fraction I and II when developed in n-Butanol: Acetic acid: Water (8:1:1) revealed the presence of spots under ultraviolet light, when sprayed with 10% sulphuric acid and ferric chloride. In ethyl acetate: methanol and water (10:1.65:1.35) fraction I and fraction II revealed the presence of spots when spread with 10% sulphuric acid, while with ferric chloride. In pharmacological evaluation, median lethal dose, Lorke (1983) of the methanolic leaves extract in both mice and rats via intraperitoneal route was 1264.91 mg/kg, while for the methanolic stem bark extract in mice it was 118.32 mg/kg and in rats it was found to be 177.48 mg/kg. The methanolic leaves extract significantly (p<0.001) inhibited acetic acid-induced writhes in mice, Koster *et al.* (1959) at all doses administered (75, 150, and 300 mg/kg) in a dose dependent manner and significantly (p<0.01) inhibited formalin induced pain in rats (Dubuisson and Dennis, 1977; Tjölsen *et al*., 1992) at the lowest and highest dose administered in phase I (central) and II (peripheral) respectively. The methanolic extracts produced moderate anti-inflammatory effect significantly (p<0.05) at all doses tested. Various pharamacognostic

characters analyzed in this study will be useful in the identification and standardization of *M. senegalensis*, the biological evaluation provides scientific basis for the ethno medical use of the plant in the amelioration of pain and inflammation.

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CHAPTER ONE

1.0 Introduction

1.1 *Maytenus senegalensis*

Maytenus senegalensis (Lam.) Exell belongs to the family Celastraceae, and is commonly known as confetti tree in English and by the Hausa people of Northern Nigeria as 'Namijin Tsada', 'Kunkushewa', 'Kambun-shafu', 'Kyalbuwa', 'Mangaladi' 'Kurunkushewa', or 'Kurunkushiya'. It is widely available in tropical Africa. It has a long history of usage by the 'Hausa' community in traditional medicine and known to exhibit a wide range of biological activities such as anti-inflammatory, analgesic, antibacterial activities, etc. These activities were attributed to the presence of variable phytoconstituents found in the plant (Burkill, 1985).

1.2 Quality Control of Herbal Drugs

Herbal medicines are increasingly receiving more attention than modern synthetic drugs because they are considered to be relatively safe, and having minimal side effects. Generally, herbal formulations consist of fresh or dried plant parts. Correct knowledge of crude drugs is very vital to ensure safety and efficacy of herbal products (Modi *et al*., 2010). Pharmacognostic analysis is a simple and reliable tool by which complete information of crude drug can be obtained (Trease and Evans, 2002). Adulteration and mis-identification of herbal drug can cause serious health problems to consumers. The first step in quality control of medicinal plants is ensuring the authenticity of the desired species for the intended use. The quality control can be conducted via a variety of techniques, namely macro- and micro-scopic identification and chemical analysis. Micro-anatomy sometimes

provides information that gross anatomy does not. Hence the importance of the description of microscopic botanical aspects in order to help determine definitively the proper species of plant material being collected, harvested or processed, when it is in a whole state and/or powdered. (Serrano, *et al.*, 2010)

Microscopical descriptions can include the characterization of the histological structures, cells and cell contents visible only via light microscopy (LM) and scanning electron microscopy (SEM). The observation of cellular-level morphology or anatomy is a major aid for the authentication of drugs. These characters are especially important for identification of broken or powdered drugs, because in these cases most of the morphological diagnostic features are lost. (Serrano, *et al.*, 2010)

The powdered crude drugs can be identified based on the form, the presence or absence of different cell types, e.g. parenchyma, collenchymas, fibers, stone cells, vessels, trichomes, secretory cells; epidermal cells based on their cytomorphological characters. Cell inclusion characteristics are of considerable value in the identification of some unorganized crude drugs, e.g. ergastic contents as aleurone grains, cluster crystals and prisms of calcium oxalate, silica and starch granules (Jackson and Snowdow, 1968). For some well-known medicinal plants, data for the quality control were available in official pharmacopoeias. However, for most of the plants used worldwide, such criteria are not found in these books (Mukherjee, 2007). For convenience, herbal medicines can be arranged into morphological groups as bark, root, leaf, seed, etc. Some of them constitute more than one morphological part, for example, whole herb and commercial root, which may consist of both rhizome and root. When herbal drug used is the whole plant or any part or parts, it is fairly easy to identify the drug botanically, but when the drug is reduced to

powder, a greater degree of expertise is required on part of pharmacognosists. (Serrano, *et al.,* 2010)

Inadequate information regarding some species such as *Artemisia annua* L. (Asteraceae), *Digitalis thapsi* L. (Scrophulareaceae), *Frangula azorica* V. Grubow (Rhamnaceae), *Guiera senegalensis* J. F. Gmel. (Combretaceae), *Hypericum androsaemum* L. (Clusiaceae), *Hypericum foliosum* Aiton. (Clusiaceae), *Hypoxis hemerocallidea* Fisch. & C.A. Mey (Hypoxidaceae), *Jateorhiza palmata* (Lam.) Miers (Menispermaceae), *Maytenus heterophylla* Robson (Celastraceae) and *Maytenus senegalensis* (Lam.) Exell (Celastraceae) is a major obstacle to the establishment of quality control and certification of these medicinal plants. (Serrano, *et al.,* 2010)

Recently chromatographic finger print technique as a more meaningful quality control method of herbal drugs has been attracting scientists attention because the finger print technique emphasizes on the integral characterization of samples with a quantitative degree of reliability and focus on identifying and assessing the stability of the plants (Sirikatitham, *et al*., 2007). Chromatographic finger print is a method that is used to show chemical information of medicine (herbal products) with chromatograms, spectrograms and other graphs by analytical techniques (Gan, and Ye, 2006). Variable chromatographic techniques involving finger prints include thin layer chromatography (TLC), gas chromatography, high performance liquid chromatography (HPLC), etc. (Vierling *et al*., 2003).

1.3 Analgesics

Pain is officially defined as an unpleasant sensory and emotional experience associated with potential or actual tissue damage. Pain causes other effects such as: sinking

sensation, apprehension, sweating, nausea, etc. Currently available analgesics such as opiates and NSAIDs are well known with side effects. Opiate analgesics such as morphine, has strong addictive properties and many of side effects. Some common side effects associated with opiate analgesics are: Nausea, Vomiting, Drowsiness, Dry mouth, Meiosis (contraction of the pupil), Orthostatic hypotension (blood pressure lowers upon standing), Urinary retention and constipation.

Some opiate's less common side effects and adverse reactions are: confusion, hallucinations, delirium hives, etc. Most severe side effects are: respiratory depression. (Carol, 2013)

1.4 Inflammation

The term inflammation is derived from the Latin word – *Inflammare* (burn). Any form of injury to the human body can stimulate a series of chemical changes in the injured area. Earlier, it was believed that, inflammation was contemplated as a single disease caused by disturbances of body fluids. According to the modern concept, inflammation is a healthy process resulting from some disturbances or diseases. The cardinal signs of inflammation are heat, redness, swelling, pain and loss of function (Anilkumar, 2010).

Inflammation usually involves three phases viz: acute transient phase, delayed sub acute phase and chronic proliferate phase. In the first phase, inflammatory exudates develop due to enhanced vascular permeability and leads to local edema. It is followed by the migration of leukocytes and phagocytes from blood to vascular tissues which are the second phase, in the third phase; tissue degradation is followed by fibrosis. At a basic level, the acute inflammatory response triggered by infection or tissue injury involves the coordinated delivery of blood components (plasma and leukocytes) to the site of infection

or injury. This response has been characterized best for microbial infections (particularly bacterial infections (Barton, 2008).

A successful acute inflammatory response results in the elimination of the infectious agents followed by a resolution and repair phase, which is mediated mainly by tissue-resident and recruited macrophages (Serhan and Savill, 2005). If the acute inflammatory response fails to eliminate the pathogen, the inflammatory process persists and acquires new characteristics. The neutrophil infiltrate is replaced with macrophages, and in the case of infection also with T cells. If the combined effect of these cells is still insufficient, a chronic inflammatory state ensues, involving the formation of granulomas and tertiary lymphoid tissues (Drayton et al., 2006). In addition to persistent pathogens, chronic inflammation can result from other causes of tissue damage such as autoimmune responses or undegradable foreign bodies (Anilkumar, 2010).

1.5 Research Questions

- Does the plant extract contain phytochemical constituents with analgesic and anti-inflammatory activities?

1.6 Justification

- The plant has been used by the Hausa community for a long time as an analgesic and anti-inflammatory drug without being standardized. This present the need to standardize the drug.

- To provide scientific basis for the use of the plant as an analgesic and anti-inflammatory drug.

- To develop less costly and readily accessible analgesic and anti-inflammatory drug with minimal side effect.

1.7 Specific Objectives

- To evaluate pharmacognostic characters for the plant *Maytenus senegalensis* so as to provide parameters for identifying the plant.

- To evaluate phytochemical constituents of the plant extracts (fractions) by chemical and chromatographic studies.

- To study analgesic and anti-inflammatory activities of the crude extract.

1.8 Hypothesis

- The plant *Maytenus senegalensis* contains various phytochemical constituents that have analgesic and anti-inflammatory activities.

CHAPTER TWO

2.0 Literature Review

2.1 The Family Celastraceae:

The Celastraceae family comprises approximately 50 genera and 800 species. Most members of the family are shrub to small trees, although members of some genera such as *Bhesa, Koona,* and *Lapophetalum,* reach up to 50 m high and have buttressed trunk. Most Celastraceae are erect but members of some genera such as *Celastrus, Euonymus, Maytenus,* etc are scandent. Most Hippocrateoideae and Salacioideae are lianas. Scandent taxa may climb with the aid of persistent sharp, downward, and pointed bud scales (Ding Hou, 1955), while others use adventitious roots (Troll, 1943), members of Hippocrateoideae and Salacioideae do so by thigmorpism and deciduous scales on dimorphic branches (Halle, 1962).

2.1.2 Botanical Description of the Family

Typical growth forms of the family of the plant include: annual or perennial herbs with erect or prostrate stems e.g Stackhousioideae, Suffrutices e.g. *Gymnosporia spp*., rhizomatous shrubs e.g. *Paxistima,* ericoid shrubs e.g. *Empleuridium* and epiphytic shrub e.g. *Quetzalia standleyi* (Kubitzki, 2010).

Most of the genera are unarmed although, in *Acanthothamus* and *Canotia,* stems terminate in sharp points occur. Thorns occur in *Gloveria, Gymnosporia, Moya and Putterlickia*. Leaves are often fasciculate on short shoot, frequently on the thorns themselves. The leaves on thorns are opposite even when the rest of the plant has alternate phyllotaxy (Kubitzki, 2010).

- **Leaves** -simple, generally alternate, opposite or sub opposite. May be whorled e.g. *Brexia illicifolia* and *Euonymus spp.,* Subverticillate or scattered. Generally petiolate, may be sessile (*Bhesa* is unique with geniculate petioles at the laminar end). They are generally laminar, sometimes needle-like or scales (Kubitzki, 2010).
- **Vegetative anatomy**: consists inconspicuous growth rings.
- **Vessels:** solitary or radial (multiples of 2-4), generally simple perforation plates, rarely sclariform, uni-biseriate or uniseriate rays, thin to thick walled fibers, phloem generally as conspicuous concentric bands, occasionally as isolated strands.
- **Stems** – Cork usually at the sub epidermis of *Euonymus* and *Microtropis,* phloem and xylem are in closed bundles separated by narrow rays. Laticiferous containing gutta-percha occur at the interface of the cortex and phloem (Drennan *et al.*, 1987).

Floral Morphology

- **Flowers-** Actinomorphic, rarely ± zygomorphic. Perianth is generally 4-5 merous, rarely 3 or 6.
- **Nectiferous disk-** Intrastaminal, extrastaminal or may have stamen on disk. Disk is annular, pulvinate or copular. Fleshy or membranous. Margin may be entire, lobed or angular.
- **Stamens-** 4-5 in *Stackhousioideae,* 3 in *Dicarpellum,* In *Hippocrateoideae,* 5 in *Campylostemon* and *Salacia spp*, 2 in *Nicobariodendron*, and numerous in *Plagioteron* (Loesener, 1942).
- **Anthers-** Generally 2 celled, sometimes 1 celled. Dehisce longitudinally (Celastroideae), obliquely (glyptopetalum) or transversely (Hippocrateoideae and Salacioideae). The Direction of dehiscence may be introrse, latrorse or extrorse.

- **Ovaries-** 2-5 locular, placentation is exile. Ovule may be erect, exile or pendulous, simple styles and simple stigma.
- **Testa-** 5-12 cells thick, lignified and unspecialized. Often palisade exotesta is present.
- **Tegimen-** 3-5 cells thick with ribbon-like fibers present on outer layer. Exotegmic seed.
- **Germination-** hypogeal and epigeal (de Vogel, 1980).
- **Dispersal-** wind, birds, animals, and ants (Benson and Mc Dougall, 1995) or Small and large mammals (Kollmann *et al*., 1998).
- **Fruits:** Drupes, berries and nuts. Sepals 4-5, usually free. Petals are 4-5, free.
- **Pollination:** Nectariferous disk attracts bees, beetles, flies, wasps and ants (Sebsebe, 1985) and the flowers produce a sweet smell that attracts insects (Mattfeld, 1942).
- **Reproductive system:** Bisexual or unisexual. Monoecious and dioecious.

2.1.2 Some Important Genera of the Family

These include: *Allocassine*, *Anthodon*, *Apatophyllum*, *Apodostigma*, *Arnicratea*, *Baequaertia*, *Bhesa*, *Brassiantha*, *Brexiella*, *Campylostemon*, *Cassine*, *Catha*, *Celastrus*, *Cheiloclinium*, *Crossopetalum*, *Cuervea*, *Denhamia*, *Dicarpellum*, *Elachyptera*, *Elaeodendron*, *Empleuridium*, *Euonymus*, *Evonymopsis*, *Fraunhofera*, *Glyptopetalum*, *Goniodiscus*, *Gyminda*, *Hartogiella*, *Hartogiopsis*, *Hedraianthera*, *Helictonema*, *Herya*, *Hexaspora*, *Hippocratea*, *Hylenaea*, *Hypsophila*, *Kokoona*, *Loeseneriella*, *Lophopetalum*, *Maurocenia*, *Maytenus*, *Menepetalum*, *Microtropis*, *Monimopetalum*, *Mortonia*, *Moya*,

Myginda, *Nicobariodendron*, *Orthodphenia*, *Paxistima*, *Peripterygia*, *Peritassa*, *Perottetia*, *Platypterocarpus*, *Plenckia*, *Pleurostylia*, *Polycardia*, *Pottingeria*(?), *Prionostemma*, *Pristimera*, *Psammomoya*, *Pseudosalacia*, *Ptelidium*, *Pterocelastrus*, *Putterlickia*, *Quetzalia*, *Reissantia*, *Rzedowskia*, *Salacia*, *Salacighia*, *Salaciopsis*, *Salvadoropsis*, *Sarawakodendron*, *Schaefferia*, *Semialarium*, *Simicratea*, *Simirestis*, *Tetrasiphon*, *Thyrsosalacia*, *Tontelea*, *Torralbasia*, *Tripterygium*, *Tristemonanthus*, *Viposia*, *Wimmeria*, *Xylonymus*, and *Zinowiewia*. (Watson, and Dallwitz, 1992).

2.1.3 Phytochemical Constituents in the Family

- **Dulcitol-** has been reported to accumulate in leaves and bark. It is a highly characteristic biochemical feature of Celastraceae (Hegnauer and Ding Hou, 1962)
- **Alkaloids-** Cathinone and cathine have been reported in young leaves and twigs of *Catha edulis*. They act as stimulants to the central nervous system (Szendrei, 1981). Maytansine and maytein, have been shown to have antitumor activity (Sebsebe1985 and Raintree Nitrition, Inc. 2000).
- **Triterpenes and its derivatives-** celastrol, pristemerin triterpenoid quinonemethides.
- **Gutta-percha-** E-1,4-polyisoprene (Acher and van Wyk, 1998).

2.1.4 **Geographical distribution-** They may be sub-tropical, tropical (most), temperate (fewer) and are found in dry wood lands, forests and swamps, from sea level to 3350m elevation.

2.1.5 Economic Importance

- Leaves and twigs of *Catha edulis* are chewed or drunk as tea for their stimulant property (Zelger *et al*., 1981)
- Plants extracts have been used in traditional medicine (Kubitzki, 2004)
- Arils of *Bhesa* are edible (Ding Huo, 1962)
- Various species of Catha, Maytenus and Celastrus are cultivated as ornamentals (Huxley *et al*., 1992)
- Woods of *Maytenus spp*. and *Siphodon spp*. are a very good source of timber (Kubitzki, 2004)
- Woods of *Euonymus europaeus* and *E. hians* are used as turney (Chant, 1993)
- Several lianas such as *Loeseneriella apocynoides* are used in binding and basket weaving (Muhwezi, 1999)
- Bark of *Kokoona* and *Lophoptalum* have been used to start fire even in wet conditions (Ding Huo, 1962)
- Bark extracts of some species have been used in dart and arrow poison (Ding Huo, 1962)

2.2 ***Maytenus senegalensis*** **(Lam.) Exell** is distributed worldwide and has a long history of being used in traditional medicine. In African traditional medicine, root of this plant is used in the treatment of cancer; in Asia it is used as an insect repellant while the South American people use it for treating gastro intestinal disorders. The plant is one of the

most frequently used species of the *Maytenus* Molina genus in the African traditional medicine (along with *M. acuminata*, *M. buchananii*, *M. mossambicensis*, *M. obscura*, *M. peduncularis*, *M. putterlickioides*, *M. serrata* and *M. undata*). (Neuwinger, 2000).

SYNONYMS: *Gymnosporia senegalensis; Catha senegalensis* (Lam.) G. Don; *Celastrus senegalensis* Lam. (Hutchings *et al*., 1996)

Common names: red spike thorn; confetti tree; thorny staff tree.

Local name (Hausa): "Namijin Tsada"

2.2.1 **Taxonomy**: Viridiplantae

Phylum: Streptophyta. Order: Celastrales. Family: Celastraceae. Genus: *Maytenus*. Species: *Maytenus senegalensis*

2.2.2 Botanical Descriptions of *Maytenus*:

- **Trees or shrub-** erect, rarely scandent, glabrous, rarely pubescent.
- **Leaves-** alternate, entire or serrate.
- **Inflourescence-** bisexual or unisexual. Rarely polygamous or dioecious, 4(5) merous.
- **Disk- fleshy-** Annular, 4(5) angled or lobed, Intrastaminal.
- **Anthers-** longitudinal and Introrse.
- **Ovary-** 2-3 locular, ovules erect, 1-2 per locule.
- **Capsule -** spheroid or obovoid shaped, Carcarious. Longitudinally dehiscent.
- **Seed-** 1-6. Ellipsoid or obovoid. Albuminous, aril basal, enveloping seed (Kubitzki, 2010)..

2.2.3 Phytoconstituents of the Genus

The genus has been reported to contain various phytoconstituents such as flavonoids, phenolic glucosides, triterpenes. Pentacyclic triterpenes- 3-oxofriedelane and 3ß-hydroxyfriedelane are said to be the chemotaxonomic marker of the genus. Compounds isolated from the *Maytenus* genus include the ansa macrolide, maytansine, and related macrolides such as normaytansine, maytanprine and maytanbutine. (Hutchings, *et al.*, 1996) Other isolated compounds include spermidine alkaloids (celacinnine and celallocinine) and nicotin sesquiterpene alkaloids (maytoline and maytolidine) as well as catechin, procyanidins and phenoldienone triterpenoids. (Hedberg, *et al.*, 1982)

2.2.4 Reported Pharmacological Activities of the Genus

- *In vivo* antiplasmodial activity against resistant strains of *P. berghei* was reported (Muregi *et al.*, 2007)
- *In vitro* antiplasmodial activity against a chloroquine-sensitive strain of *P. falciparum* was reported. (Clarkson *et al.*, 2004)

  The *in vitro* activity against two strains of *P. falciparum* (chloroquine and pyrimethamine sensitive 3D7 strain, chloroquine-resistant and pyrimethamine-sensitive Dd2 strain) has also been given. (El Tahir *et al.*, 1999)

- *In vitro* anti-leishmanial activity against promastigotes of *Leishmania major* reference vaccine strain (5AKSH) has been reported. (El Tahir *et al*., 1998)
- Anti-leishmanial activity against resistant strains of *P. falciparum* has been reported. (Matu, and van Staden, 2003)

- Anti-bacterial activity against *Bacillus subtilus*, *Micrococcus luteus* and *Staphylococcus aureus* has been reported. (Khalid *et al*., 2007)
- Maytenonic acid-isolated root-bark has a proven antibacterial activity against *B. subtilus*, *Escherichia coli*, *Klebsiella pneumoniae* and *S. aureus*. (Lindsey *et al*., 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Identification of Samples

The plant material was collected in July, 2011 from Ahmadu Bello University Dam, Samaru, Zaria, Nigeria. It was identified by the taxonomist, Malam Musa Muhammad of the herbarium section, Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State.

3.2 Preparations of Plant Samples

The plant's leaves and stem bark collected were air dried under shade for two weeks, the stem bark and leaves were pulverized into fine powders separately. The powders were stored in an airtight container until further needed.

3.3 Macroscopical Studies of the Leaf and Stem Bark of *Maytenus senegalensis*

Fresh samples were used for this analysis. All distinguishable macroscopical features of the leaves and stem bark such as shape, color, odor, e.t.c, were observed, noted and described with the appropriate terms as described in quality control methods for medicinal plants (WHO, 1998)

3.4 Microscopical Studies

Cleared fresh samples of the leaves and stem bark were prepared, mounted separately and observed under a compound microscope under magnification (×400). The

feature observed, such as stomata, epidermal cells, etc, were analyzed, drawn, and labeled (Evans, 2002) and (WHO, 1998).

3.4.1 Anatomical Studies of the Leaf and Stem Bark of *Maytenus senegalensis*

Transverse and longitudinal sections of the leaves and stem bark were studied separately. Thin sections were placed in a test-tube containing 70% chloral hydrate and boiled for a few minutes until they become transparent. Cleared sections were mounted and viewed under a compound microscope, magnification (x400). Features observed were drawn and labeled.

3.4.2 Chemo-microscopical Studies of Leaf and Stem Bark of *M. senegalensis*

Powdered materials of the leaves and stem were used for this study in order to detect the presence of cell wall material and ergastic substances. This is done according the methods of (WHO, 1998).

(a) Test for Starch

A small amount of the cleared leaves and stem bark powders were stained with N/50 iodine on glass slide. A drop of glycerol was added and the samples were observed under a compound microscope (x400). Appearance/absence of blue-black coloration indicated the presence or absence of starch grains (WHO, 1998).

(b) Test for Tannins

A small amount of the cleared leaves and stem bark powders were mounted separately in 5% ferric chloride; one drop of glycerol was then added, the mounted sample

was analyzed under microscope. Appearance of bluish or greenish-black coloration indicated the presence of tannins (WHO, 1998).

(c) Test for Lignin

Cleared small amount of leaves and stem bark powders were placed separately on slides and a drop of phloroglucinol was added followed by a drop of conc. Hydrochloric acid. Appearance of red coloration indicated the presence of lignin (WHO, 1998).

(d) Test for Gums and Mucilage

Small amount of cleared leaves and stem bark powders were placed separately on slides and a drop of ruthenium red was added. Appearance of pink coloration indicated the presence of gums and mucilage (WHO, 1998).

(e) Test for Oils

Small amount of the cleared leaves and stem bark powders were separately placed on a slide and a drop of Sudan (IV) reagent was added. Appearance of pinkish color indicated the presence of oils (WHO, 1998).

(f) Test for Cellulose

Small amount of the cleared leaves and stem bark powders were separately placed on a slide and a drop of N/50 iodine was added, followed by a drop of 66% sulphuric acid. The appearance of bluish or violet coloration indicated the presence of cellulose (WHO, 1998).

(g) Test for Calcium Oxalates and Calcium Carbonates

Small amount of the cleared leaves and stem bark powder were separately placed on a slide this was viewed under microscope and some crystals were observed which dissolved in sulphuric acid without effervescence and later formed some needle-shaped crystals. Thus, indicating the presence of calcium oxalates. Crystals that dissolve in sulphuric acid with effervescence or in acetic acid indicate the presence of calcium carbonate (WHO, 1998).

3.4.3 Determinations of Some Microscopical Physical Constants

Four physical constants were determined for the leaf. These are: stomatal number, stomatal index, vein islet number, vein-let termination number. The determinations were carried out five times using surface preparations and cut sections.

(a) Stomatal Number

This is the average number of stomata per mm^2 of the epidermis (upper and lower) of leaves of plant species.

A cleared surface section of the upper and lower epidermis of the leaf was mounted using glycerol, this was analyzed under camera Lucida, the size, type and the dimension of the stomata and epidermal cells per square mm was counted (5×).

(b) Stomatal Index

It is the percentage proportion of the ultimate divisions of the epidermis of leaves which have been converted into stomata.

The stomata and epidermal cells per squared mm were counted with the aid of camera lucida and the stomatal index was calculated as follows:

$$\left[\text{S.I} = \frac{S}{E+S} \times 100\right]$$

Where, S.I= stomatal index, S= total number of stomata per mm^2, E= total number of epidermal cells per mm^2 (WHO, 1998).

(c) Vein-islet Number

It is the number of vein-islet per square mm of the leaf surface midway between midrib and margin.

Thin section of the leaf was cleared and mounted in dilute glycerol. Vein islet per four contagious mm^2 in the central part of the lamina of the leaf midway between the mid rib and margin was analyzed under camera Lucida and calculated (Evans, 2002).

(d) Vein let Termination Number

It is the number of vein let termination per square mm of the leaf surface midway between midrib and the margin.

This was also achieved with the aid of camera Lucida. The portion where veins terminate are noted, traced and counted (Evans, 2002).

3.5 Determinations of Some Physicochemical Constants of the Leaf and Stem Bark of *Maytenus senegalensis*

Five physical constants were determined for the plant material. These include: moisture content; ash value; acid insoluble ash value; alcohol extractive value and water extractive value (WHO, 1998). Three different determinations were carried out for each of the parameter and average was taken as shown in the appendix one.

(a) Moisture Content

It is the quantity of moisture contained in a plant material. Moisture content of *Maytenus senegalensis* was determined by loss on drying method. 3.0g each of the leaves powder and stem bark powder were accurately weighed and placed in some clean, dried evaporating dishes of known weights. They were placed in an oven and heated at a temperature of 105^0C. After 30 mins the weight of the drug and the dishes were determined and returned to oven. The weight was taken subsequently after every 30 mins until the weight become constant. The weight of water loss on drying was computed following the formula below:

$$\left[\%\text{Moisture content} = \frac{wt.of\ moisture}{original\ \ wt.\ of\ sample} x\ 100\right]$$

(b) Total Ash Value

Total ash is the ash remaining after ignition of medicinal plant materials. Powdered plant material (2.0g) was accurately weighed and placed in a crucible of known weight. The material was in an even layer and ignited and the heat was gradually increased until it is carbon white. It was allowed to cool in a desiccator and weighed. The ash was again heated

and weighed. The heating and weighing continue until a constant weighed is obtained. The ash value was then calculated using the formula below:

$$\left[\ \%\ \text{Ash value}\ =\ \frac{wt.of\ residual\ ash}{wt.of\ original\ sample} \times 100\ \right]$$

(c) Acid-insoluble Ash Value

This is the residue that remains after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter.

Conc. Hydrochloric acid (25ml) was added to the crucible containing the ash; it was then covered with a watch-glass and gently boiled for 5 mins. The watch glass was rinsed with a 5ml of hot water, which was added to the crucible. It was filtered with ash less filter-paper, the filter paper and the filtrate are placed back into the crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in desiccator for 30 mins, and then weighed without delay. The acid-insoluble ash value was then calculated using the formula below:

$$\left[\%\ \text{Acid} - \text{insoluble ash value}\ =\ \frac{wt.of\ residual\ \ ash}{wt.of\ \ original\ \ \ sample} \times 100\right]$$

(d) Alcohol Extractive Value

The powdered plant material (4.0g) was accurately weighed in a conical flask. It was macerated with 100ml of 95% ethanol for 6 hours, shaken frequently with mechanical shaker and later allowed to stand for 18 hours. It was filtered and 25ml of the filtrate was transferred into an evaporating dish of known weight and evaporated to dryness on a water

bath. Dried at 105^0C for 6 hrs and weighed after it was cool. The alcohol extractive value was calculated using the following formula:

$$\left[\%\text{Alcohol soluble ext. value} = \frac{wt\ of\ residue\ \ in\ 25ml\ extract}{initial\ \ wt\ of\ sample} \times 100\right]$$

(e) Water Soluble Extractive Value

The procedure for water soluble extractive value is the same with that of alcohol soluble extractive value. The solvent for extraction here is water.

3.6 Phytochemical Analysis on Methanolic Extracts of Leaf and Stem Bark of *M. senegalensis*

3.6.1 Extraction of the Powdered Leaf and Stem Bark of *M. senegalensis*

Each of the leaves and stem bark powder (300g) were extracted with aqueous methanol (70:30 v/v) using maceration method for 4 days. The extract was concentrated using water bath at the temperature of 45^0C.

3.6.2 Phytochemical Screening on Methanolic Extracts of Leaf and Stem Bark of *M. senegalensis*

(a) Test for Alkaloids

About 0.5g of each extract was stirred with 5ml of 1% aqueous hydrochloric acid on a water bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated with Dragendorff's reagent. Turbidity or precipitate with either of these reagents may be due to the presence of alkaloids in the extracts (Harbone, 1973).

(b) Test for Saponins

The extract (0.5g) was shaken with water in a test tube followed by warming on a water bath. Frothing which persists on warming was taken as preliminary evidence for the presence of Saponins (Wall *et al*., 1954).

(c) Test for Tannins

(i) Ferric Chloride Test

The extract (0.5g) was stirred with 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins (Evans, 2002).

(ii) Bromine Water Test

Few drops of bromine water was added to the extracts in test tubes, formation of a buff colored precipitate indicates the presence of condensed tannins while none color reaction indicates the presence of hydrolysable tannins (Evans, 2002).

(d) Test for Anthraquinones

To the plant extracts (5g) in separate test tubes, 10ml of benzene was added and was shaken for 5 minutes. The extract was then filtered and shaken with 5ml of 10% ammonia solution. Formation of a bright color in the upper part of the aqueous layer indicates the presence of free anthraquinones (Evans, 2002).

(e) Test for Cardiac Glycosides

(i) Keller-Keliani Test

Small quantities of the extracts (0.5g) each were dissolved in 2ml of glacial acetic acid containing a drop of ferric chloride solution; 1ml of conc. Sulphuric acid was carefully added to form a lower layer. A brown ring formed at the interface indicates the presence of desoxy sugar. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer, indicates the presence of cardenolides (Evans, 2002).

(ii) Salkowski's Test

Five milliliter of extracts each were mixed in 2 ml of chloroform and filtered. Concentrated sulphuric acid (3ml) was carefully added to the filtrate to form a layer. A reddish brown coloration at the interface indicates presence of steroids/Terpenes (Sofowora, 2008).

(f) Test for Carbohydrate

(i) Molisch Test

The plant extracts (0.5g) each were dissolved in distilled water in separate test tubes and filtered. Four drops of Molisch reagent and 4 drops of conc. Sulphuric acid were added to the filtrate without mixing. Appearance of purple ring at the interface as a result of interaction between Molisch reagent and 5-hydroxymethylfurfural produced by dehydration of saccharides indicated the presence of carbohydrates (Silver *et al.*, 1998).

(ii) Fehling's Test

The plant extracts (0.5g) was dissolved in distilled water in a test tube. The test tube was then placed on a water bath to heat. Equal volumes of Fehling's solution A and B were added drop by drop into the test tube. Appearance of brick-red precipitation of cuprous oxide indicates the presence of carbohydrate (Evans, 2002).

(g) Test for Flavonoids

(i) Shinoda Test

The plant extracts (0.5g) each was dissolved in 2ml of methanol and 4 drops of concentrated hydrochloric acid was added followed by some chips of magnesium metal. Appearance of orange color indicates flavones, red-crimson indicates flavonols and pink-magneta indicates flavononones (Mahran *et al.,* 1980).

(ii) Sodium Hydroxide Test

The plant extracts reconstituted was in water and filtered, (5ml) each was shaken with 10% sodium hydroxide. A yellow solution which becomes colorless on addition of dilute hydrochloric acid indicates the presence of flavonoids (Silva *et al*., 1998).

3.6.3 Fractionation of *M. senegalensis* Methanol Extracts

The methanolic extract was defatted by portioning with water and ether. The aqueous fraction was partitioned with n-butanol, this n-butanol fraction was further portioned with 1% KOH, and the n-butanol fraction was collected and stored as n-butanol fraction I. The KOH fraction was further neutralized with HCl followed by addition of n-

butanol, the n butanol fraction was collected and stored as n-butanol fraction II. (Woo *et al*., 1980)

3.6.4 Thin Layer Chromatography of n-Butanol Fractions of *M. Senegalensis*

Silica gel pre-coated plates were used. Plates were spotted with methanolic extracts of *M. senegalensis*. Spots were allowed to dry and chromatograms were developed in the following solvent systems: n-btanol: acetic acid: water (8:1:1) and ethyl acetate, methanol, water (10:1.65:1.35). Three sets of plates were developed. The chromatograms were sprayed with 10% sulphuric acid, ferric chloride and Dragendorff (Gibbons and Gray., 1998).

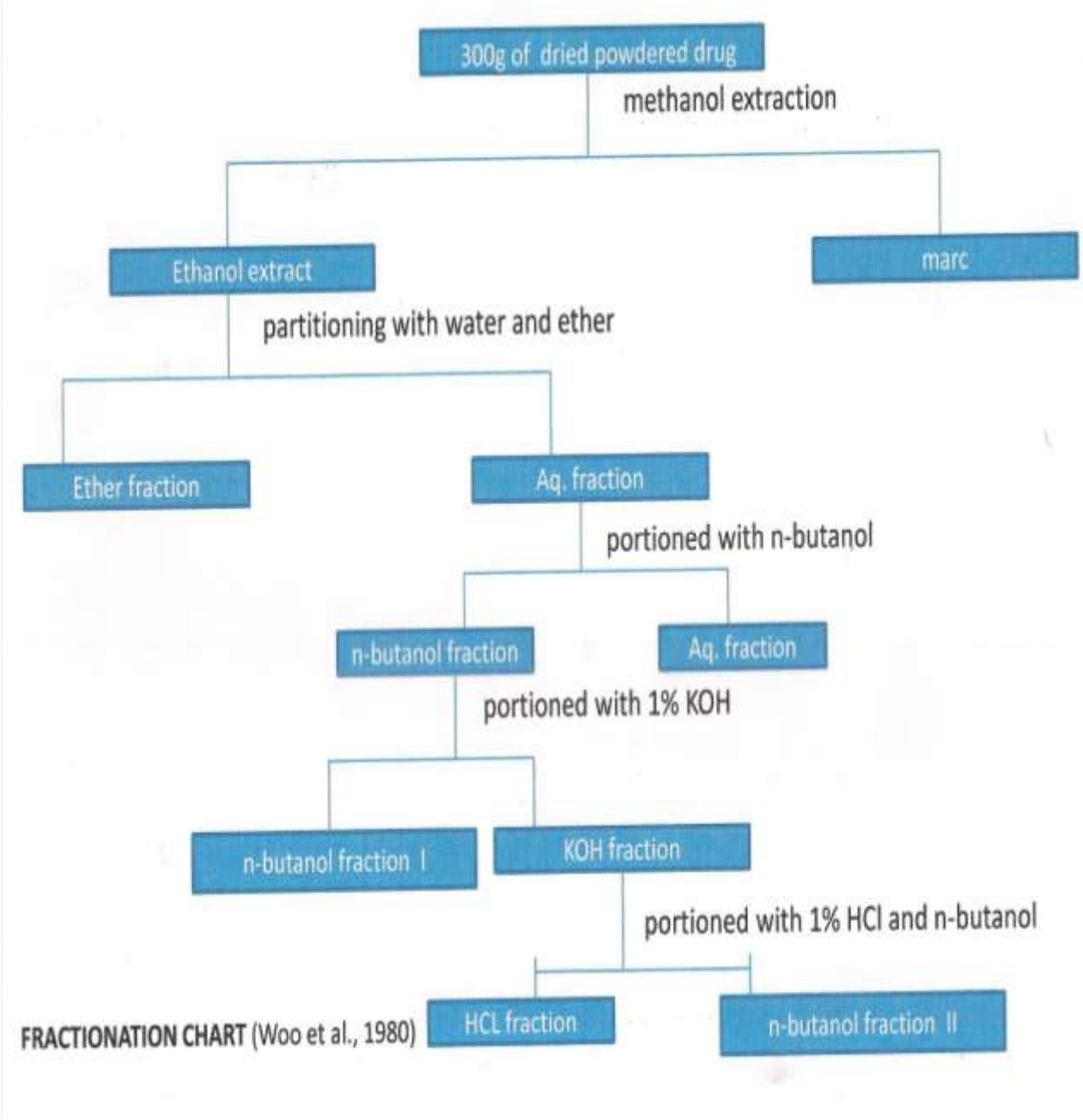


Fig 1: Fractionation Chart

3.70 Biological Studies on Leaf and Stem Bark Methanolic Extracts of *M. senegalensis*

Leaf and stem bark extracts were tested for anti-inflammatory activity, and only the leaf crude extract was tested for analgesic activity.

(a) Animals

Male Wistar rats (130-200g) and Swiss albino mice of either sex were obtained from the Animal House Facility of the University of Jos. They were housed at the animal house facility of the Department of Pharmacology and therapeutics, Ahmadu Bello University Zaria. They were kept in cages and then kept under room conditions of temperature and light. The animals were fed on laboratory animal feed. Food was withdrawn during the experimental hours.

(b) Drugs

The following chemicals and drugs were used: carragenan (Sigma Aldrich), acetic acid, and piroxicam (Pfizer, Nigeria).

3.7.1 Determination of Median Lethal Dose (LD_{50}) on the Leaf and Stem Bark Methanolic Extracts of *M. Senegalensis*

Determination of LD_{50} was conducted following the method of Lorke (1983) and using the intraperitoneal route. In the initial phase, mice/rats were divided into 3 groups of three and treated with the methanolic extract of the plant at doses of 10, 100 and 1000 mg/ kg body weight. The animals were then observed for 24 hours for signs of toxicity and death. In the final phase, mice/rats were divided into 4 groups of one mouse each and

treated with the stem bark methanolic extract at doses of 140, 225, 370 and 600 mg / kg, and leaf methanolic extract at the doses of 600, 1000, 1600 and 2900 mg/kg *i.p*. The final LD_{50} was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e. the geometric mean of the consecutive doses which 0% and 100% survival rates were recorded.

3.7.2 Test for Anti-nociceptive Activity of Leaf Methanolic Extract of *M. senegalensis*

(a) Acetic acid induced writhing in mice

The Acetic acid induced writhing test in mice as described by Koster *et al*. (1959) was employed. Mice were divided into 5 groups of 6 mice each. The first group was given 1 ml/kg of Normal saline (*i.p.)* and served as control, groups 2, 3 and 4 received 75, 150 and 300 mg extract/kg body weight (*i.p.)* group five was treated with piroxicam (10 mg/kg) *i.p*. respectively. Thirty minutes later, mice in all the groups were treated with Acetic acid (0.6%v/v), 1ml per 100g body weight (*i.p.*). Five minutes after acetic acid injection, mice were placed in individual cages and the number of abdominal contractions was counted for each mouse for a period of 10 minutes. Percentage inhibition of writhing was calculated using the formula:

(b) Formalin induced pain in rats.

The rats were divided into five groups each containing 6 rats. Group 1 was administered with normal saline (1ml/kg, *i.p.*), group 2, 3 and 4 with methanolic leaves extract (75, 150 and 300 mg/kg, *i.p*) and group five with piroxicam (10 mg/kg, *i.p*). Thirty minutes after this treatment; 50 µl of a freshly prepared 2.5% solution of formalin was injected subcutaneously under the plantar surface of the left hind paw of each rat. The rats

were placed individually in an observation chamber and monitored for one hour. The severity of pain response was recorded for each rat based on the following scale: (0) rat walked or stood firmly on the injected paw; (1) the injected paw was favoured or partially elevated; (2) the injected paw was clearly lifted off the floor; (3) the rat licked, chewed or shook the injected paw. Anti-nociceptive effect was determined in two phases. The early phase (phase 1) was recorded during the first 5minutes, while the late phase (phase 2) was recorded during the last 45 minutes with a 10 minutes lag period in-between both phases. (Dubuisson and Dennis, 1977; TJölsen *et al.*, 1992).

3.7.3 Test for Anti-inflammatory activity of the Leaf and Stem Bark Methanolic Extracts of *M. senegalensis*

(a) Effect of Leaf and Stem Bark Methanolic Extracts on Carrageenan Induced Paw Oedema in Rats

The rats were divided into five groups of six rats each. Acute inflammation was induced by injecting 0.1 ml of (1%) carrageenan suspension in normal saline into plantar surface of rat hind paw (Winter *et al*., 1962). The leaf methanolic extract (75, 150 and 300 mg/kg), the stem bark methanolic extract (12.5, 25, and 50 mg/kg) normal saline (1ml/kg) and piroxicam (10 mg/kg) as reference agent were administered 30 minutes before carrageenan injection. The paw volume was measured at 0, 1, 2, 3, 4 and 5 hours using a vernier caliper to determine the diameter of oedema. The difference between the readings at time 0 hr and different time intervals was taken as the index of oedema (Winter *et al*., 1962).

Statistical Analysis

Results were expressed as mean ± Standard Error of Mean (SEM). The data was statistically analyzed using the one-way ANOVA to determine whether results in a particular group were significantly different from those in the corresponding control groups. Results were statistically significant when *p* value is less than 0.05 ($p<0.05$) as described by Duncan *et al.,* (1977). *p* value was obtained using post hoc test.

CHAPTER FOUR

4.0 Results

4.1 Plant Identification

The plant collected fitted the description of the plants in the literature. The specimen voucher number obtained was 900144, deposited at the Department of Biological Sciences, Ahmadu Bello University, Zaria for future reference.



Plate I: The Plant *M. Senegalensis* in Its Natural Habitat

4.2 Preparations of Plant Sample

About 400g powder each of the leaves and stem bark of *M. senegalensis* was obtained after preparation of the plant material. This was what was used throughout this research.

4.3 Macroscopical Evaluations on the Leaf and Stem Bark of *M. senegalensis*

Some of the macroscopical features evaluated for the leaves and stem bark are: shape, size, texture etc. The leaf was pale green in color and petiolate; it has alternate arrangement, glabrous surface, serrate margin, acute apex, symmetrical base, with a characteristic odor and taste. The stem bark has grey and rough outer surface with a short fracture, inner surface is pink and soft, with a fibrous fracture. It has a characteristic taste and odor. The dried bark is channeled shaped. Results are given on tables 4.1 and 4.2 respectively.

Table 4.1 Macroscopical Evaluation on the Leaf of *M. senegalensis*

Evaluative parameters	**Results**
Arrangement	Alternate
Shape	Ovate
Size	12.5-22cm in length
Color	Pale green (lower surface), green (upper surface)
Surface	Glabrous
Margin	Serrate
Apex	Acute
Base	Symmetrical
Attachment	Petiolate
Venation	Parallel
Odor	Characteristic odor
Taste	Characteristic Taste



Plate II: The Leaves of *M. Senegalensis*

Table 4.2 Macroscopical Evaluation on the Stem Bark of *M. senegalensis*

Evaluative parameters	**Results**
Color (outer surface)	Grey
Color (inner surface)	Pink
Fracture	Short (0uter), fibrous (inner)
Shape	Channeled
Texture (outer surface)	Rough
Texture (inner surface)	Smooth
Odor	Characteristic odor
Taste	Characteristic taste

4.4 Microscopical Features of the Leaf and Stem Bark of *M. senegalensis*

The surface peel of leaf shows the presence of stomata and epidermal cells, trichomes are absent on the epidermal surface. The stomata are anomocytic type and are present on both upper and lower epidermis with diameter of 48μ, but it is more frequent on the lower epidermis. The epidermal cells are straight walled, polygonal (5 – 6 sided) cells. The mean values of the stomatal number, stomatal index, vein islet and vein let termination numbers are given in table 4.3 and the photomicrograph stomata are shown in plate III and IV.

The transverse sections of the leaf is dorsiventral showing single layered upper and lower epidermis with a layer of compact elongated palisade cell below the upper epidermis, an anomocytic type of stoma and arc shaped vascular bundles that are surrounded by a bundle carp with some spongy mesophyll in the lamina (plate. V). The longitudinal sections of the leaf showed the presence of single layered epidermis and some collenchymas (plate. VI).

Transverse section of the stem bark reveals the presence of some cork cells (plates. VII) and the longitudinal section showed the presence of some long fitted sclereids, and parenchyma cells (Plate VIII).

Table 4.3: Microscopical Physical Constants of *M. senegalensis*

Leaf's constants	**Values**	
	Upper epidermis	**Lower epidermis**
Stomatal Number	80.50-70.00-59.50	101.78-88.50-75.23
Stomatal Index	10.81-12.72-14.63	10.65-12.53-14.41
Vein-Islet Number	23.46-20.40-17.34	
Vein let Termination Number	17.94-15.60-13.26	

*n=5

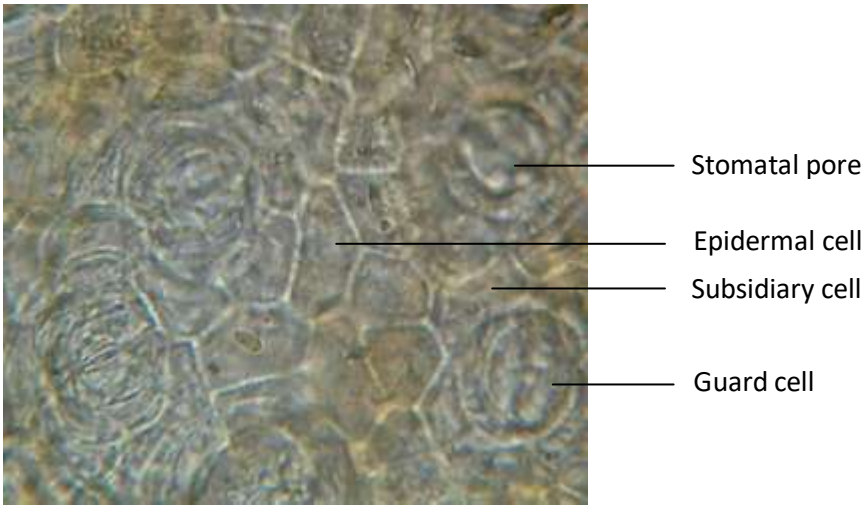


Plate III: Photomicrograph of the Lower Epidermis of the Leaf of *M. senegalensis* (×100)

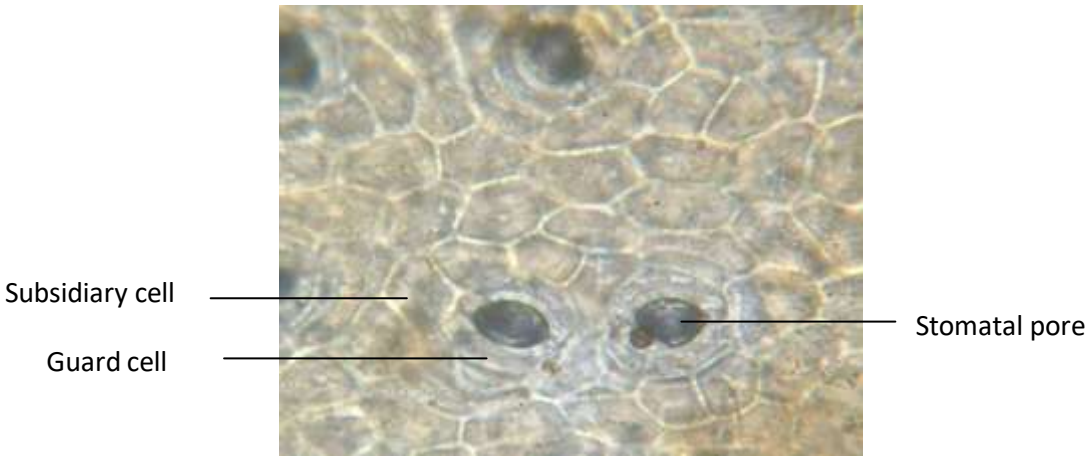


Plate IV: Photomicrograph of the Upper Epidermis of the Leaf of *M. senegalensis* (×100)

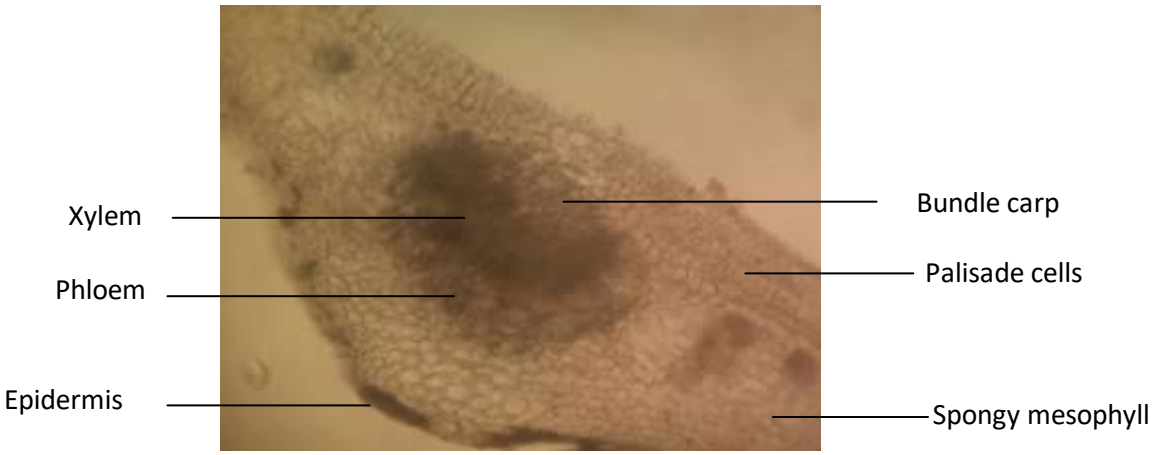


Plate V: Photomicrograph of the Transverse Section of the Leaf of *M. Senegalensis* (×100)

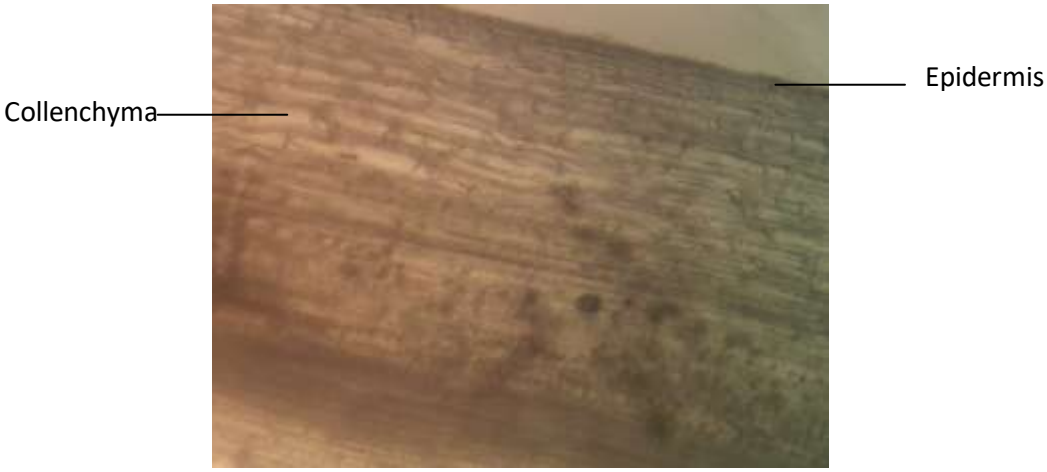


Plate VI: Photomicrograph Showing Longitudinal Section of the Leaf of *M. senegalensis* (×100)

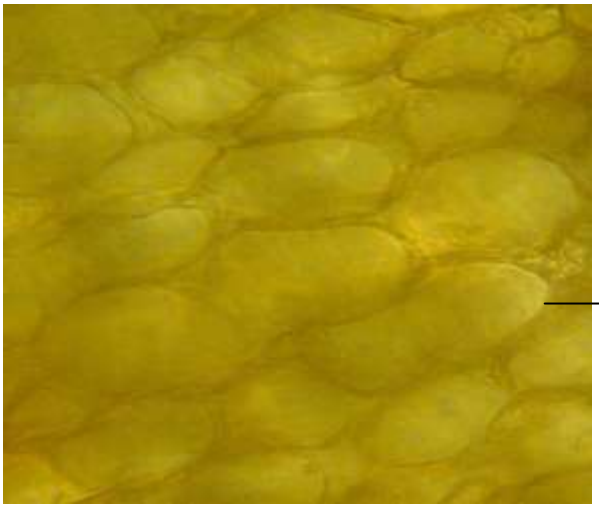


Plate VII: Photomicrograph Showing the Transverse Section of Stem Bark of *M. senegalensis* (×100)

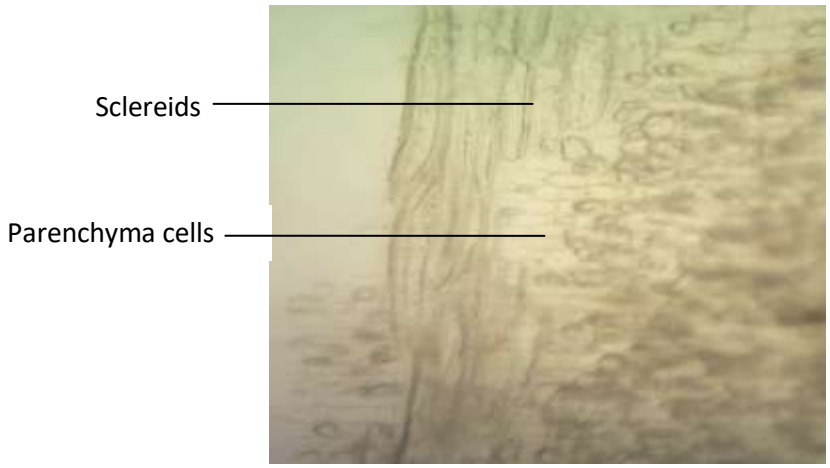


Plate VIII: Photomicrograph of the Longitudinal Section of Stem Bark of *M. Senegalensis* (×100)

4.5 Chemo-microscopical Evaluations of the Leaf and Stem Bark of *M. senegalensis*

Histo-chemical color reaction was carried out using powdered drug and various cell wall materials such as lignin, cellulose, mucilages were tested positive and cell inclusions such as starch grains, calcium oxalates, fixed oil and fats were all present while protein, calcium carbonate and silica were absent. Results of the chemo-microscopical study is shown in table 4.4.

Table 4.4: Chemo-microscopical Characters of Leaf and Stem Bark of *M. senegalensis*

Constituents/reagents	Observations	Inference
Cellulose N/50 iodine + HCl + 66% H2SO4	Violet coloration observed on outer wall of some cells and fibers	Cellulose is confirmed to be present
Lignin	Some fibers stained pink	Lignified fibers are present
Starch N/50 iodine	Some grains in parenchyma cells stained blue	Starch grains are present
Protein Millions reagent	No reaction	Protein is absent
Calcium oxalate crystals Conc. H_2SO_4	clustered crystals dissolved without effervescence	Calcium oxalate crystals are present
Hydroquinone A drop of KOH	No reaction was observed	Hydroquinones are absent
Tannins 1 drop of $FeCl_3$	Green coloration was observed in some parts of the parenchyma cells	Tannins are present
Fixed oil and fat A drop of sudan IV	Red stains were observed in some parts of the cytoplasm of the parenchyma cells	Fixed oil and fats are present
Gums and mucilage Red stains	Red stains were observed in some parts of the cell	Gums and mucilages are present

+ Means present
-Means absent

4.6 Determinations of Physico-chemical Constants of the Powdered Leaf and Stem Bark of *M. senegalensis*

Physico- chemical values such as moisture content, total ash, acid insoluble ash, extractive values in water and alcohol are calculated in terms of air dried weight samples of the leaves and stem bark of *M. senegalensis.* The moisture content was found to be 9.33% ± 0.01 and 6.33% ± 0.02 , ash values 7.83 ± 0.00 and 15.17% ± 0.01, acid insoluble ash values 1.67% ± 0.01 and 1.00% ± 0.01, alcohol extractive value 12.00% ± 0.01 and 13.25 ± 0.10 and water-soluble extractive value 13.25 ± 0.04 and 14.25 ± 0.04 for leaf and stem bark respectively. The result have been summarized in table 4.5

Table 4.5: Physico-Chemical Parameters of the Powdered Leaf and Stem Bark of *M. senegalensis*

Evaluative parameters	Values %w/w ± SEM	
	Leaves	**Stem bark**
Moisture Content	09.33 ± 0.01	06.33 ± 0.02
Total Ash Value	7.83 ± 0.00	15.17 ± 0.01
Acid Insoluble Ash	01.67 ± 0.01	01.00 ± 0.01
Alcohol Extractive Value	12.00 ± 0.10	13.25 ± 0.10
Water-soluble Extractive Value	13.25 ± 0.04	14.25 ± 0.04

*Each value is a mean of three determinations ± SEM

4.7 Phytochemical Analysis on Leaf and Stem Bark Methanolic Extracts of *M. senegalensis*

Phytochemical screening of methanolic extracts of leaf and stem bark of the plant showed the presence of alkaloid, saponins, tannins, cardiac glycosides, carbohydrates, and flavonoids while anthraquinones were found to be absent. The result is shown in table 4.6.

Table 4.6: Result of Preliminary Phytochemical Screening on Leaf and Stem Bark Methanolic Extracts of *M. senegalensis*

Phyto-constituents	Tests	Observations	Inference
Alkaloids	Dragendorff's reagent	Orange-red Precipitate	Present
	Mayer's reagent	Cream coloured Precipitate	Present
Saponins	Frothing test	Frothing persisted on warming	Present
Tannins	Ferric chloride test	Green Precipitate	Present
	Bromine water test	Buff coloured precipitate	Present
Anthraquinones	Borntrager's test	No reaction	Absent
Cardiac glycosides	Keller-Kiliani test	Brown ring at interface	Present
	Salkowski's test	Reddish brown color at interface	Present
	Lieberman-buchard's test	Color changes from violet to blue to green	Present
	Molisch's test	Purple to violet coloration	Present
Carbohydrates			
	Fehling's solution	Brick red Precipitate	Present
	Shinoda test	Pink color	Present
Flavonoids	Sodium hydroxide (Na_2OH) test	Yellow color formed	Present

4.8 Chromatographic Analysis of the n-Butanol Fractions of *M. senegalensis*

Good separation of the fractions was obtained with n-butanol-acetic acid-water (B:A:W) (8:1:1) and ethyl acetate-methanol-water (EtAC:MeOH:H_2O) (10:1.65:1.35). With the first solvent system, five spots were observed under ultraviolet light in the n-butanol fraction II and four spots were observed in the n-butanol fraction I, on spraying with ferric chloride, three spots were obtained in the n-butanol fraction II and one spot was seen in the n-butanol fraction I.

With the second solvent system, five spots were obtained in n- butanol fraction II on spraying with ferric chloride and two spots were resolved in the n-butanol fraction I. The color of the spots ranges from light brown, green, to blue. The result of chromatographic analysis is shown in table 4.7

Table 4.7: Chromatographic Analysis of n-Butanol Fractions of Leaves Extract of *M. senegalensis*

Solvent system- n-Butanol: Acetic acid: Water (8:1:1)

Spraying reagents	I		II	
	R_f	Color	R_f	Color
Under Ultraviolet light	0.49	Blue	0.35	Blue
	0.57	Blue	0.49	Blue
	0.64	Blue	0.57	Blue
	0.66	Blue	0.64	Blue
			0.66	Blue
1% Sulfuric acid (H_2SO_4)	0.60	Light brown	0.49	Light brown
			0.57	Light brown
			0.66	Light brown
Ferric chloride (Fe_2Cl_3)				
	0.60	Green	0.49	Green
			0.57	Green
			0.66	Green

Solvent system- Ethylacetate: Methanol: Water (10:1.65:1.35)

1% Sulfuric acid (H_2SO_4)	0.27	Brown	0.27	Brown
	0.38	Brown	0.38	Brown
	0.42	Blue	0.42	Blue
	0.45	Brown	0.45	Brown
	0.58	Brown	0.58	Brown
	0.68	Pink		
Ferric chloride (Fe_2Cl_3)				
	0.41	Green	0.28	Brown
	0.45	Green	0.39	Green
			0.41	Green
			0.45	Green
			0.55	Green

Chromatogram of the n-Butanol Fraction of the Methanolic Leaf Extract of *M. senegalensis* Developed in Butaniol Acetic Acid and Water Solvent System (8:1:1).



Plate IX: Ultraviolet light

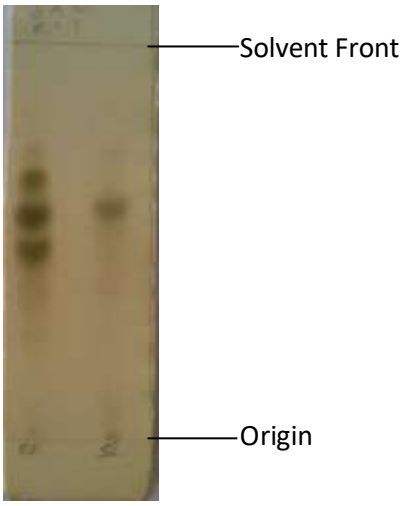


Plate X: sprayed with ferric chloride



Plate XI: sprayed with sulphuric acid

Chromatogram of the n-Butanol Fraction of the Methanolic Leaf Extract of *M. senegalensis* Developed in Acetic acid, Methanol and Water Solvent System (10:1.65:1.35)

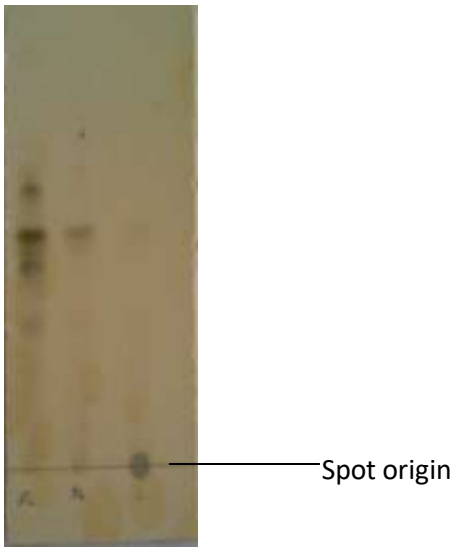


Plate XII: sprayed ferric chloride

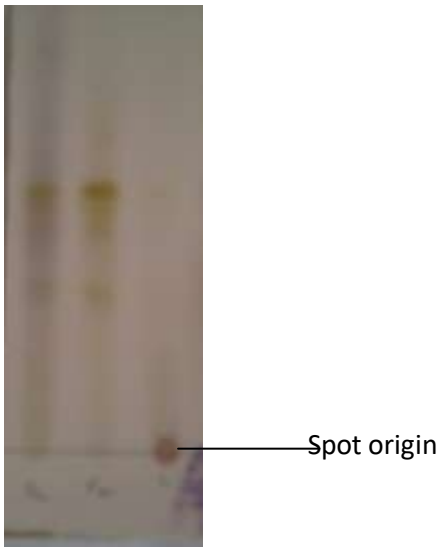


Plate XIII: sprayed sulfuric acid

4.9 Biological Studies on Leaf and Stem Bark Methanolic Extracts of *M. senegalensis*

4.9.1 Determination of Median Lethal Dose (LD_{50}) on the Leaf and Stem Bark Methanolic Extracts of *M. Senegalensis*

The intraperitoneal LD_{50} of the leaves extract for both rats and mice was found to be 1264.91 while that of the stem bark extract was found to be 177.48 and 118.32 mg/kg in rats and mice respectively. The animals presented with weakness and respiratory distress prior to death. Results are shown on table 4.8 and 4.9

Table 4.8: Determination of Median Lethal Dose (LD_{50}) of Methanolic Extracts of *M. senegalensis* on Rats

Dose (mg/kg)		Results	
		MSLME	**MSSBME**
First phase			
10		0/3	0/3
100		0/3	0/3
1000		1/3	2/3
Second phase			
MSLME	**MSSMBE**		
600	140	0/1	1/1
1000	225	0/1	1/1
1600	370	1/1	1/1
2900	600	1/1	1/1

MSLME- *M. senegalensis* leaf methanolic extract,
MSSBME- *M. senegalensis* Stem bark methanolic extract
LD_{50} (MSMLE) = 1264.91 mg/kg, LD_{50} (MSSBME) = 177.48 mg/kg

Table 4.9: Determination of Median Lethal Dose (LD_{50}) of Methanolic Extracts of *M. senegalensis* on Mice

Dose (mg/kg)		Results	
		MSLME	**MSSBME**
First phase			
10		0/3	0/3
100		0/3	0/3
1000		1/3	2/3
Second phase			
MSLME	**MSSMBE**		
600	140	0/1	0/1
1000	225	0/1	1/1
1600	370	1/1	1/1
2900	600	1/1	1/1

MSLME- *Maytenus senegalensis* leaf methanolic extract,
MSSBME- *Maytenus senegalensis* Stem bark methanolic extract
LD_{50} (MSLME) = 1264.91 mg/kg, LD_{50} (MSSMBE) = 118.32 mg/kg

4.9.2 Test for Anti-nociceptive Activity of Leaf Methanolic Extract of *M. senegalensis*

The effect of methanolic leaf extract of *M. senegalensis* on acetic acid induced writhes in mice and formalin induced pain in rats is presented in fig. I and II. The extract at doses of 150 and 300 mg/kg significantly ($P < 0.001$) reduced the number of acetic-acid-induced abdominal constrictions in a dose dependent manner. The highest inhibition of abdominal constriction ($P < 0.001$) observed at 300 mg/kg was higher than that of Piroxicam ($P < 0.001$), the standard non-steroidal analgesic and anti-inflammatory drug used (fig. 1). In the first phase of formalin induced pain, the extract was able to significantly ($p < 0.01$) reduce pain at all doses (75, 150 and 300mg/kg) administered, although, not in a dose dependent manner. Higher inhibition was seen at the lowest dose (75mg/kg) administered. In the second phase, the extract at the dose of 300 mg/kg significantly ($p < 0.01$) inhibit formalin induced pain. Inhibition by the extract was higher than that observed with piroxicam (10mg/kg) (Fig. 2).

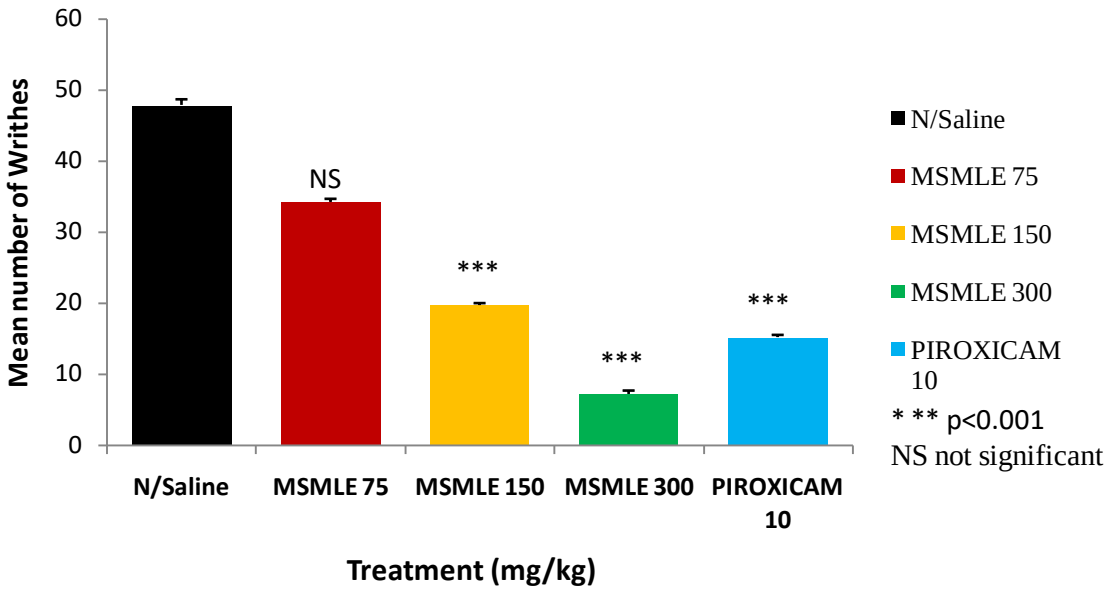


Fig 2: Effect of leaf methanolic extract on acetic acid induced writhes in mice.

Data expressed as meant $\pm$ SEM for 6 animals/group, significantly different from the control at P<0.001.

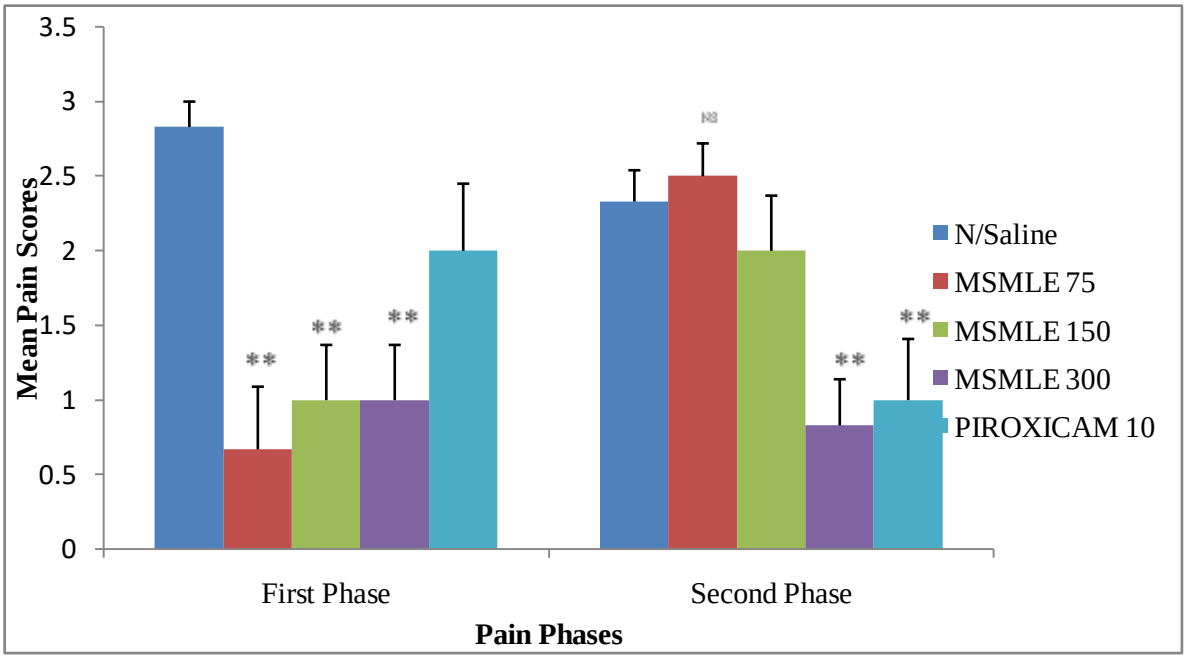


Fig 3: Effect of leaf methanolic extract on formalin induced pain in rats.

Data expressed as meant ± SEM for 6 animals/group, significantly different from the control at P<0.01

4.9.3 Test for Anti-inflammatory activity of the Leaf and Stem Bark Methanolic Extracts of *M. senegalensis*

In the normal saline treated animals, sub plantar injection of 1% carrageenan suspension produced a local oedema reaching its maximum at 3 hour. The leaf extract at all doses (75, 150 and 300 mg/ kg) was able to significantly ($p < 0.05$) show anti-inflammatory effect (at the peak of carrageenan-induced oedema), although, not in a dose dependent manner (Fig. 3). The stem bark extract at all doses (12.5, 25, 50 mg/kg) also showed significant ($p < 0.05$) inhibition (at the peak of carrageenan-induced oedema) (Fig. 4). Piroxicam (10 mg/kg), showed higher inhibition of induced oedema compared to the extracts used.

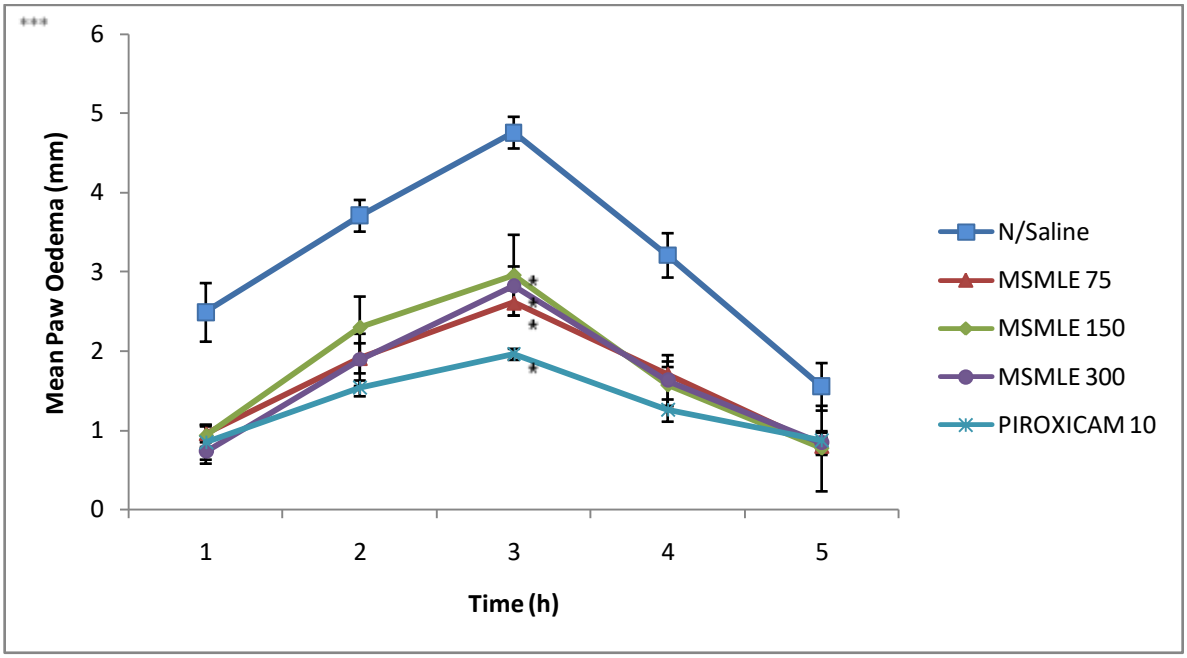


Fig. 4: Effect of leaf methanolic extract on carrageenan induced paw oedema in rats

Data expressed as meant $\pm$ SEM for 6 animals/group, significantly different from the control at $P<0.05$

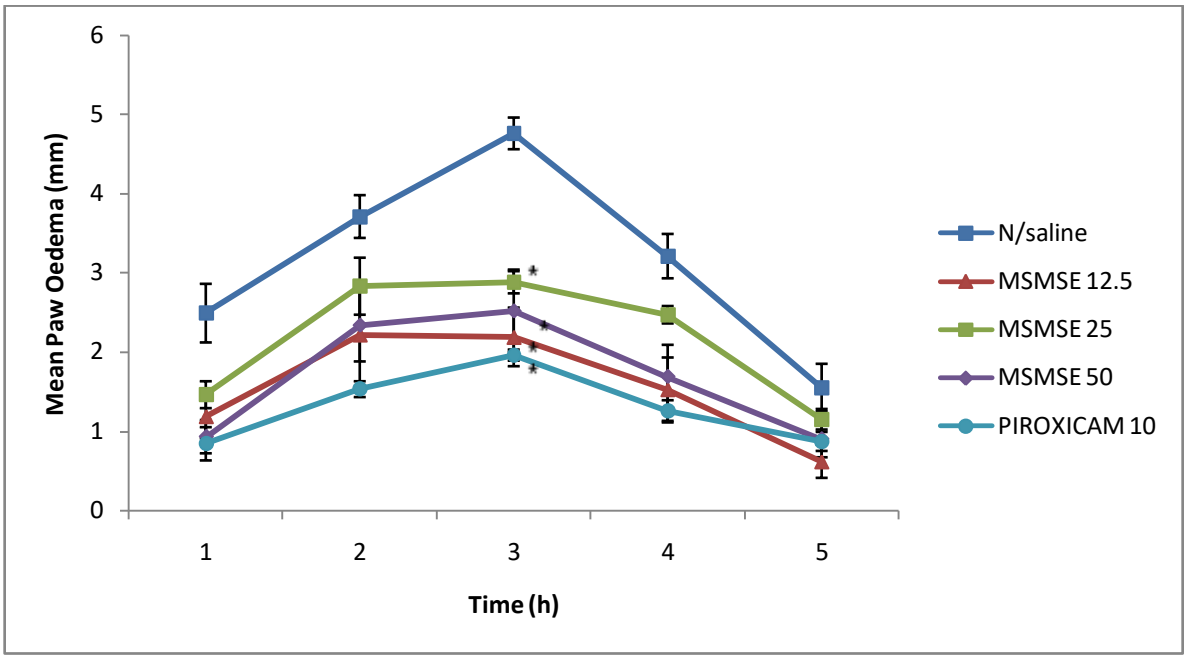


Fig 5: Effect of stem bark methanolic extract on carrageenan induced paw oedema in rats.

Data expressed as mean ± SEM for 6 animals/group, significantly different from the control at $P<0.05$

CHAPTER FIVE

5.0 Discussion

The pharmacognostic studies done, showed leaves are with spikes, a feature that protects the plant from predators. The presence of these spikes serves as a distinguishing feature of the plant. The common name of the plant (red spike thorn) is attributed to the presence of these spikes. Da Silva *et al.* (2010) reported some organoleptic features of the leaves to be petiolated, alternate or fascicled with pale green lamina features that were similarly observed in this research.

The presence of anomocytic type of stomata which are more frequent on the lower surface of the plant as observed in this research has contradicted the findings by Da Silva *et al*. (2010) who reported the presence of paracytic stomata. Type of stomata can be used as a character of identification. Dorsiventral leaves as observed in this research contradict the findings by Da Silva *et al*. (2010) who reported the leaves to be isobilateral. The dorsiventral leaf is an important distinguishing feature. Dorsiventral leaves are a characteristic of dicotyledonous plants therefore, can be used as an identification tool. In the present study, prisms of calcium oxalate crystals were found either scattered or clustered in parenchymatous and epidermal cells in both the leaves and the stem bark. Calcium oxalate crystals in higher plants are most common in form of minerals and are usually formed from environmentally derived calcium and biologically synthesized oxalates and are deposited inside intra-vacuolar membrane chambers of specialized cells in any organ or tissue (Nakata, 2003) and (Webb., 1999). Francheschi and Horner (1980) reported that differentiation of cell into a crystal idioblast is surely under genetic control. Therefore, the shape of the crystals may also be governed genetically. Functions of calcium

oxalates can be related to ionic balance and osmo regulation, storing form of calcium oxalates, mechanical support and protection against foraging animals (Kalaskar *et al*., 2012).

Chemomicroscopical features are unique to a particular plant and are employed in standardization. Allied substances when mixed with original drugs as adulterants can only be detected by chemomicroscopical analysis. Leaves physical parameters determined in quantitative microscopy are relatively constants for plants and can be used in distinguishing members of closely related species.

The result of the moisture content of the leaves and stem bark was determined to be 9.33% and 6.33% respectively. Moisture of the leaves and stem bark is not high, meaning less chance of microbial growth. The general requirement of moisture content in crude drugs should not exceed 14% (A.P., 1986), at this value of moisture content, drugs can be stored for a long period of time without fear of microbial degradation.

Total ash determined for the leaves and the stem bark are 7.83% and 15.17% respectively, this result implies that both the leaves and the stem bark have low content of organic and inorganic matter with the stem bark having comparatively more total ash content. The accepted range of total ash is 22% (B.P., 1980) this signifies that total ash for both leaves and stem bark are within the accepted limit. This parameter is particularly important in determination of purity of crude drugs. The result of acid insoluble ash values for the leaves and stem bark are 1.67% and 1% respectively. This result is also within the range of the accepted limit of the acid insoluble ash value. Total ash determines presence or absence of foreign organic matter (Musa *et al*., 2009).

The alcohol and water soluble extractive values determined for leaves were 12% and 13.25% respectively while for the stem bark the alcohol soluble extractive value was 13.25% and the water soluble extractive value was 14.25%. Alcohol and water soluble extractive values suggest total solvent soluble phytochemical constituents (Sirivastava and Sharad, 2006). Therefore, from the results both the leaves and the stem bark have high water soluble extractive value, meaning water may be a better extractive solvent for both the leaves and the stem bark of *Maytenus senegalensis*. Evans (2002) reported quantitative evaluation as an important parameter in setting standard for crude drugs. To the best of my knowledge physicochemical determinations on *Maytenus senegalensis* is reported for the first time in this work.

Preliminary phytochemical screening using crude methanolic extracts showed the presence of alkaloids, cardiac glycosides, saponins, tannins and carbohydrate in both leaves and stem bark. Flavonoids may be present in the leaves extract. Result of chromatographic analysis on the n-butanol fractions confirms the presence of phenolic compounds in the leaves fraction together with other phytochemical constituents. These phytochemical constituents present in both leaves and stem bark extracts make the plant useful in treating various ailments and a potential source of useful drugs. Da Silva *et al*. (2010) reported the isolation of ansa macrolide maytansine and other related macrolides such as normaytansine, maytan butane and maytan prine from different species of this genus. Maytein and maytansine are major macrolide alkaloids that have been known to have anti cancer activity and have been documented. These phytochemicals, may have acted singly or synergistically to bring about the pharmacological action of the plant.

Pharmacological activities of plants have been attributed to the presence of variable phytochemical constituents in the plants. These phytochemical constituents may have acted singly or synergistically to bring about pharmacological actions of plants (Magaji *et al*., 2008). The analgesic and anti-inflammatory activities of flavonoids, steroids, saponins, alkaloids and tannins have been reported by Das *et al.* (1989), Ahmediani *et al*. (2000) reported analgesic and anti-inflammatory activities of flavonoids and tannins, and Rajnarayana *et al*. (2000) reported the activity of flavonoids such as quercetin against acute inflammation. Middleton (1998) suggested certain flavonoids possess potent inhibitory effect against a variety of enzymes such as protein kinase C, protein tyrosine kinases, etc. Alkaloids, essential oils, and saponins were also reported to exhibit analgesic effects (Reanmongkol *et al*., 2005; de Araujo *et al*., 2005; Choi *et al*., 20005).

The methanolic leaves and stem bark extracts were found to have median lethal dose (LD_{50}) of 1264.91mg/kg and 177.48mg/kg in rats respectively while in mice, only the LD_{50} of the stem bark differs which was determined to be 118.32mg/kg. The LD_{50} of the leaves extract of 1264.9mg/kg indicated the leaves extract to be slightly toxic, while that of the stem bark extract of 177.48mg/kg and 118.32mg/kg indicated it to be moderately toxic to the experimental models. This is supported by Matsumma (1975) who classified chemicals based on their LD_{50} and suggested LD_{50} range 50-500mg/kg to be moderately toxic and LD_{50} range 500-5000mg/kg to be slightly toxic. This range by matsumma, makes it possible to prescribe appropriate dosage of drugs.

The inhibitory effect of leaf methanolic extract on the acetic acid induced writhing in mice in a dose dependent manner with the highest dose (300mg/kg) showing highest inhibitory effect compared to the standard used; piroxicam (10ml/kg) was clearly seen in

this research. Acetic acid-induced writhing method is very sensitive and can detect analgesic effect of a substance at a dose that cannot be detected by other methods such a tail flick test (Collier *et al.,* 1968). The method, have been reported to be used in evaluation of peripheral analgesic effect (Gene *et al.*, 1998). Bentley *et al* (1983) suggested the abdominal constriction to partly involve peritoneal receptors. Therefore, the extract may have interfered with these receptors to bring about analgesia. Acetic acid-induced writhing is associated with prostaglandins (Sutharson *et al.*, 2007). Increase level of prostanoids (Deradt *et al.*, 1980) also lipoxygenase products (Dhara *et al.*, 2000) and sympathetic nervous system mediators (Barsato *et al.*, 2000) have also been suggested to be the cause for analgesia. Analgesic effect of the leaves extract may be due to its ability to have effect on the visceral receptors that are sensitive to analgesic acid or inhibit the production of algogenic substances and /or inhibit the transmission of painful substances at the central level. However, this model may not indicate the mechanism of action of the extract due to some agents such as histamines (Naik *et al.*, 2000) and myorelaxant (Koyama *et al.*, 1997), these agents are able to reduce pain caused by acetic acid.

Formalin-induced pain in rats is biphasic. Drugs that are capable of inhibiting both phases are said to exhibit central activity (acting primarily on the central nervous system) while those that are capable of inhibiting only the late phase are said to exhibit peripheral activity (Chant et al., 1995). The first phase may be as a result of stimulation of nociceptors in the paw, or stimulation of opioid receptors as suggested by Gaertner *et al.*, (1999). Therefore, the first phase reflects centrally mediated pain. The last phase may be as a result of inflammation with the release of serotonin, histamine, bradykinin, prostaglandins and sensitization of central nociceptive neurons (Tjölsen *et al.*, 1992). The ability of the leaf

methanolic extract tested to suppress both phases at the doses administered, 75mg/kg and 300mg/kg in this research suggests the extract to have both central and peripheral activities. Although, greater activity was observed at the second phase even when compared with the standard, piroxicam (10mg/kg).

The leaf extract at all doses (75, 150 and 300 mg/ kg) administered was able to significantly ($p < 0.05$) show anti-inflammatory effect (at the peak of carrageenan-induced oedema) having lesser activity when compared to piroxicam (10 mg/kg), the standard anti-inflammatory agent used. The stem bark extract at all doses (12.5, 25, 50 mg/kg) also showed significant ($p < 0.05$) inhibition (at the peak of carrageenan-induced oedema) having lesser activity compared to piroxicam (10 mg/kg). Magaji *et al.* (2008) reported the carrageenan-induced paw oedema to be the standard experimental model for acute inflammation. The probable mechanism of carrageenan is biphasic. Brook and Day (1991) attributed the first phase to the release of histamine, serotonin, and kinins in the first hour, while the second phase was due to the release of prostaglandins and lysosome enzymes in the second and the third hour. Therefore the ability of both the leaf and stem bark methanolic extracts to inhibit inflammation at the third hour suggests the extracts to have significant effect against acute inflammation (Magaji *et al*., 2008).

CHAPTER SIX

6.0 Summary

In summary, the leaves appeared to be alternately arranged with glabrous surface, serrated margin and a characteristic taste and odor. The stomata is anomocytic type, stomatal number determined was: upper epidermis (SN= 80.50-70.00-59.50, SI= 10.81-12.72-14.63) and lower epidermis (SN= 101.78-88.50-75.23, SI= 10.65-12.53-14.41), the epidermal cells are polygonal in shape and trichomes are absent. The vein islet number was determined to be 23.46-20.40-17.34 and the vein let termination number was 17.94-15.60-13.26. Transverse sections of the leaf is dorsiventral, longitudinal sections revealed the presence of epidermal cells and some collenchymas cells in the leaves. The stem bark has grey, rough and firm outer surface, pink and smooth inner surface, the bark is channeled. Transverse sections of stem bark revealed the presence of cork cells, sclereids, parenchyma cells with some calcium oxalates encrusted in them. Chemo-microscopical study revealed the presence of cellulose cell wall, lignified fibers, starch grains, calcium oxalates, fixed oil and fats. For physicochemical determinations, moisture content was (leaf- 9.33 % w/w ± 0.01, stem bark- 6.33 % w/w ± 0.02), total ash (leaf- 7.83.00 % w/w ± 0.004, stem bark- 15.17 % w/w ± 0.01), acid-insoluble ash (leaf- 01.67% w/w ± 0.01, stem bark- 01.00% w/w ± 0.01), alcohol-soluble extractive values (leaf- 12.00% w/w ± 0.10, stem bark- 13.25% w/w ± 0.10), water-soluble extractive values (leaf- 13.25%w/w ± 0.04, stem bark- 14.25% w/w ± 0.04). Chromatograms developed revealed a number of spots when observed under ultraviolet light and when spread with sulfuric acid and ferric chloride. The median lethal dose determined for the methanolic leaf extract in both mice and rats via intraperitoneal route was 1264.91 mg/kg, while for the methanolic stem bark extract in mice it was 118.32

mg/kg and in rats it was found to be 177.48 mg/kg. The methanolic leaves extract significantly (p<0.001) inhibited acetic acid-induced writhes in mice at all doses administered in a dose dependent manner and significantly (p<0.01) inhibited formalin induced pain in rats at the lowest and highest dose administered in phase I and II respectively. The methanolic extracts (leaves and stem bark) produced moderate anti-inflammatory effect significantly (p<0.05) at all doses tested. Various pharamacognostic characters analyzed in this study will be useful in the identification and standardization of *M. senegalensis*, the biological evaluation provides scientific basis for the ethno medical use of the plant in the amelioration of pain and inflammation.

6.1 Conclusion and Recommendation

Standardization is essential measure for quality, purity and identification. Macromorphology and microscopy along with the quantitative analytical microscopy is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. Physiochemical and chemical analysis of the leaves and stem bark confirm the quality and purity of plant and its identification. This work can be use as a guide to identifying and differentiating *M. senegalensis* from the members of its family, it has also been established that the methanolic leaf and stem bark extracts do possess significant anti-nociceptive and anti-inflammatory effects in laboratory animals at the doses investigated. The results support the traditional use of this plant in some painful and inflammatory conditions and suggest the presence of biologically active components which may be worthy of further investigation and elucidation.

Further studies should be carried out reveal and isolate the exact phyto-constituent(s) that may be responsible for the analgesic and anti-inflammatory activities of *M. senegalensis* leaf and stem bark extracts.

REFERENCES

Acher, R. H., van Wyk, A. E., (1998). *A taxonomic revision of Allocassine N. Robson (Celatraceae). South African Journal of Botany*. 64:189-191.

Adeshina, G.O., Onaolapo, J.A., Ehinmidu, J.O. and Odama, L.E. (2007): Phytochemical and Toxicologic Activity of Leaf Extracts of *Alchornea cordifolia* (Schum and Thonn) Muell. Arg. *Nigerian Journal of Pharmacy. Res*. 6 (1): 19-24.

African Pharmacopoeia, (1986). Determination ash values and extractives. *First edition OAV/STRG Scientific publication* No. 3 Lagos Nigeria, pp 78, 142.

Ahmadiani, A. J., Hosseiny, S., Semnanian. M., Javan, F., Saeedi, M., Kamalinejad, Saremi, S., (2000). Antinociceptive and anti-inflammatory effects of *Elaeagnus* fruit extract. *Journal of Ethnopharmacology*. 72:287–292

Anilkumar, M., (2010). *Ethnomedicinal plants as anti-inflammatory and analgesic agents, Ethnomedicine*: A Source of Complementary Therapeutics, 267-293.

Barsato, M. L. C., Grael, C. F. F., Souza, G. E. P. and Lopes, N. P. (2000). Analgesic activity of the lignans from Lychnophora ericoides. *Journal of Phytochemistry*, 55:809-813.

Barton, G. M., (2008). A calculated response: control of inflammation by the innate immune system. *Journal of Clinical Investigation*; 118: 413–420

Benson, D., McDougall, L., (1995). *Ecology of Sydney plant species part 3: Dicotyledon families- Combretaceae to Euphobiaceae*. Cunninghamia. 4:217-424.

Bentley, G. A., Newton, S.H., Starr, J., (1983). Studies on the anti-nociceptive action of agonist drugs and their interaction with opiod mechanisms. *British Journal of Pharmacol*ogy, 32: 295-310.

British Pharmacopoeia (1980). Ash value, Acid Insoluble Ash, Water Soluble Extractive and Alkohol Soluble Extractive, Vol. II, Appendix xii, Majesty's Stationary Office, London, pp. 1276 – 1277.

Brooks, P. M. and Day, R. O., (1991). Non steroidal anti-inflammatory drugs difference and similarities. *New England Journal of Medicine,* 324: 1716-1725.

Burkill, H. M., (1985). *The useful plants of west tropical Africa*. Vol. 1. Royal Botanical Gardens Kew, United kindom. Pp 325.

Carol E., (2013). Facts about analgesics (pain killers). Available at : http://arhtritis.about.com/od/analgesics/a/factsanalgesics.htm

Chan, Y. F., Tsai, H. Y., Wu, T. S., (1995). Anti-inflammatory and analgesic activity of extracts from roots of *Angelica pubescens*. *Planta medica*, 61: 2-8.

Chant, S. R., (1993). *Celastraceae. In: Heywood, V. H. (ed.) Flowering plants of the world*. New York: Oxford University Press.

Choi, J., Jung, H., Lee, K., Park, H., (2005). Antinociceptive and Anti-inflammatory effects of saponin and sapogenin obtained from the stem of *Akebia quinata*. *Journal of Medicinal Food*. 8 (1) 78-85.

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., (2004). *In vitro* antiplasmodial activity of medicinal plants native to or naturalized in *South African Journal of Ethnopharmacology*, 92:177-91.

Collier, H. O. J., Dinneen, L. G., Johnson, C. A., and Schneider, C., (1968). The Abdominal constriction response and its suppression by analgesic drugs in the mouse, *British Journal of Pharmacology*, **32:** 295 – 310.

da Silva, G., Serrano, R ., Silva, O., (2011). *Maytenus Heterophylla* and *Maytenus senegalensis*. Two Traditional Herbal Medicines. *Journal of Natural Science, Biology and Medicine*. Vol.2, Issue 1, p.59-65.

Das, P. C., Das, A., Mandal, S., (1989). Antimicrobial and anti-inflammatory activities of the seed kernel of *Mangifera indica*. *Fitoterapia*, 60: 235-240.

de Araujo, P. F., Coelho-de-Souza, A. N., Morais, S. M., Ferreira, S. C., Leal- Cardoso, J. H., (2005). Antinociceptive effects of the essential oil of *Alpinia zerumbet* on mice. *Phytomedicine*, 12: 482-686.

de Vogel, E. F., (1980). *Seedlings of Dicotyledons: structure, development, types.* Description of 150 woody Malesian taxa. Wageningen: Centre for Agricultural Publishing and Documentation, pp. 130-135.

Deradt, R., Jougney, S., Delevalcee, F., Falhout, M., (1980). Release of prostaglandin E and Fnin an algogenic reaction and its inhibition. *European Journal of Pharmacol*ogy, 51: 17-24.

Dhara, A. K., Suba, V., Sen, T., Pal, S., Nag Chaudhuri, A. K., (2000). Preliminary studies on the anti-inflammatory and analgesic activity of the methanolic fraction of the root extract of *Tragia involucrate*. *Journal of Ethnopharmacol*ogy, **72:** 265-268.

Ding Hou, (1955). A review of the genus Celatrus (Celastraceae). *Annals of Missouri Botanical Garden*, 42:215- 302.

Ding Hou, (1962). Celastraceae I. In: van Steenis, C.G.G.J. (ed.) *Flora Malesiana* I, 6:227-291. Ley den: Flora Malesiana Foundation.

Drayton, D. L., Liao, S., Mounzer, R. H., Ruddle, N. H., (2006). Lymphoid organ development: from ontogeny to neogenesis. *Nature Immunology*; 7: 344–353.

Drennan, P. M., Drewes, S. E., van Dtaden, J., Mac Ray, S., Dickens, C. W. S., (1987). An anatomical, phytochemical and ultrastructural characterization of the elastic threads of *Maytenus acuminata*. *South African Journal of Botany,* 53:17-24.

Dubuisson, D. and Dennis, S. R., (1977). The formalin test: A quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain, 4: 161-174.*

Duncan, R. C; Knapp, R. G and Miller, M. C.(1977).Test of Hypotheses in population Means, In: *Introductory Biostatistics for Health Sciences*, John Wiley and Son, Inc. N. Y. pp. 71 – 76.

El Tahir, A., Satti, G. M. H., Khalid, S. A., (1999). Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Maytenus senegalensis* (Lam.) Exell *Journa of Ethnopharmacol* ; 64:227-33

.

El Tahir, A., Ibrahim, A. M., Satti, G. M., Theander, T. G., Kharazmi, A., Khalid, S. A., (1998) The potential antileishmanial activity of some Sudanese medicinal plants. *Phytotherapy Research*; 12:576-9.

Evans, W. C., (2002). *Trease and Evans Pharmacognosy*, 15th edition. W.B. Saunders Company Ltd., London. Pp. 191-393. .

Franceschi, V. R., Horner, H. T., (1980). Calcium oxalate crystals in plants. *Botanical Review*; 46:361-427.

Gaertner, M., Muller, L., Roos, J. F., Cani, G., Santos, A. R., Niero, R., Calixto, J. B., Yunes, R. A., Delle Monache F., Cechinel Filho V., (1999). Analgesic triterpenes from *Sebastiania* schottiana roots. *Phytomedicine*, 6: 41- 44.

Gan, F. and Ye, R., (2006). New approach on similarity analysis of chromatographic finger print of herbal medicine. *Journal of Chromatography* A, 1104: 100-105.

Gene, R. M., Segura, L., Adzet, T., Marin, E., Inglesias, J., (1998). *Heterotheca inuloides*: Anti-inflammatory and analgesic effects. *Journal of Ethnopharmacol*ogy, 60: 157-162.

Gibbon, S. and Gray, A. I., (1998). Isolation by planar chromatography *In:* Cannell R.J.P (ed), Methods in Biotechnology. *Natural Product Isolation*. Human Press, New Jersey, Pp 209-245.

Halle, (1962). *Monographie de Hippocrateaceae d'Afique occidentale*. Mem. Inst. Franc. Afri. Noire 64:1-245.

Harborne, J. B., (1973). *Phytochemical Method:A Guide to Modern Techniques of Plant Analysis*, Chapman and Hall, Ltd, London, p. 279.

Hedberg, I., Hedberg, O., Madati, P. J., Mshigeni, K. E., Mshiu, E. N., Samuelsson, G., (1982). Inventory of plants used In traditional medicine in Tanzania I. Plants of the families Acanthaceae-Cucurbitaceae. *Journal of Ethnopharmacology*, 6:29-60.

Hutchings, A., Scott, A., Lewis, G., Cunningham, A., (1996). *Zulu medicinal plants: an inventory*. Pine town: University of Natal Press, Scottsville, South Africa, pp. 195-196.

Huxley, A., Griffiths, M., Levy, M., (1992). *The new royal horticultural society dictionary of gardening. London*: MacMillan Press. ISBN-978-0-333-47494-5

Jackson, B. P., Snowdow, (1968). *Powdered Vegetable Drugs* – An Atlas of Microscopy for use in the Identification and Authentication of some Plant Materials employed as Medicinal Agents. J. & A. Churchill Ltd., London.

Kalaskar, M.G*., Saner, S.Y., Pawar, M.V., Rokade, D.L. and Surana, S.J., (2012). Pharmacognostical Investigation and Physicochemical Analysis of *Celastrus paniculatus* Willd. Leaves. *Asian Pacific Journal of Tropical Biomedicine* S1232-S1236.

Khalid, S. A., Friedrichsen, G. M., Christensen, S. B., El Tahir, A., Satti G. M., (2007) Isolation and characterization of pristimerin as the antiplasmodial and antileishmanial agent of *Maytenus senegalensis* (Lam.) Exell Arkivoc; 9:129-34.

Kolmann, J., Coomes, D. A., White, S. M., (1998). *Consistencies in post –dispersal seed predation of temperate fleshy-fruited species among season, years and sites*. *Functional Ecology*, 12:683-690.

Koster, R., Anderson, M., De Beer, E.J. (1959). Acetic acid for analgesic screening. *Federation proceedings, 18:412.*

Koyama, K., Imaizumi, T., Akiba, M., Kinoshita, K., Takahashi, K., Suzuki, A., Yano, S., Horie, S., Watanabe, K., and Naoi, Y., (1997). Antinociceptive components of Ganoderma lucidum. *Planta Medica*, 63: 224-227.

Kubitzki, K., (2004). Celastraceae. – In: The Families And Genera of Vascular Plants VI. Flowering plants. Dicotyledons. Celastrales, Oxalidales, Rosales, Cornales, Ericales Springer, Berlin, pp. 31-60 .

Lindsey, K. L., Budesinsky, M., Kohout, L,, and van Staden, J., (2006). Antibacterial activity of maytenoic acid isolated from the root-bark of *Maytenus senegalensis. South African Journal of Botany*; 72: 473–477.

Loesener, T.H., 1942. Celastraceae. In: A. EngIer/H. Harms & J. Mattfe1d (editions), *Die naturlichen Pflanzenfamilien*. Duncker & Humblot, Berlin. 2, 20b: *Pp.198-231*.

Lorke, D. (1983). A new approach to acute toxicity testing. *Archives of toxicology, 54:275-287.*

Magaji M. G., Anuka J. A., Abdu-Aguye I., Yaro A. H., Hussaini I. M. (2008). Preliminary studies on anti-inflammatory and analgesic activities of *Securinega virosa* (Euphorbiaceae) in experimental animal models *Journal of Medicinal Plants Research* 2(2), pp. 3.

Matsumma, F., (1975). Toxicology *of insecticides plenum press*, York and London pp 24-2.

Mattfeld, J. (1942). *Stackhousiaceae*. In: Engler, A., Harms, H., Mattfeld, J. editions Die naturlichen Pflanzenfamilien 20b. Berlin: Duncker and Humblot, pp. 240-254.

Matu, E. N., van Staden, J., (2003). Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *Journal of Ethnopharmacology*; 87:35-41.

Middleton, E. Jr., (1998). Effect of plant flavonoids on immune and inflammatory cell function. *Advances in Experimental and Medical Biology*. 439; 175-182.

Modi, D. C., Patel, J. K., Shah, B. N. and Nayak, B. S, (2010). Pharmacognostic Studies of the Seed of *Syzygium cumini* Linn. *An International Journal of Pharmaceutical Sciences*; pp. 1.

Muhwezi, O., (1999). The use of *Loeseneriella apocynoides* around Bwindi Impenetrable National park, southwest Uganda. Pp. 523-527.

Mukherjee, P. K., (2007). *Quality Control of Herbal Drugs*: An Approach to Evaluation of Botanicals. Business Horizons. New Delhi, India pp 21-82.

Muregi, F. W., Ishih, A., Suzuki, T., Kino, H., Amano, T., Mkoji, G. M., (2007). *in vivo* antimalarial activity of aqueous extracts from Kenyan medicinal plants and their chloroquine (CQ) potentiation effects against a blood-induced CQ-resistant rodent parasite in mice. *Phytotherapy Research* 21, 337-43.

Musa, M., Aliyu, A. B., Yaro, A. H., Magaji M. G., Hassan, H. S., and Abdullahi, M. I., (2009). Preliminary phytochemical, analgesic and anti-inflammatory studies of the methanol extract of *Anisopus mannii* in rodents. *African Journal of pharmacy and pharmacology*. 3 (8). Pp. 374-378.

Naik, D. G., Mujumdar, A. M., Wagole, R. J., Kulkarni, D. K. and Kumbhojkar, M. S., (2000). Pharmacological studies of Sterculia foetida leaves. *Pharmacological Biology, 1(38): 13-17.*

Nakata, P. A., (*2003*). Advances in our understanding of calcium oxalate crystal formation and function in plants. *Plant Science; 164: 901-9.*

Neuwinger, H. D., (2000). *African traditional medicine*: A dictionary of plant use and applications. Stuttgart,Germany: Medpharm Scientific Publishers, pp. 589.

Rajnarayana, K., Reddy, M. S., Chaluvadi, M. R., Krishna, D. R., (2001). Bioflavanoids Classification, Pharmacological, Biochemical effects and Therapeutic potential. *Indian Journal of Pharmacology*, 33:2-16.

Reanmongkol, W., Subhadhirasakul, S., Thienmontree, S., Thanyapanit, K., Kalnaowakul, J., Sengsui, S., (2005). Antinociceptive activity of the alkaloid extract from *Kopsia macrophylla* leaves in mice. Songklanakarin. *Journal of Science and Technology. 27(Supplement 2), pp. 509-516.*

Sebsebe, D*., (1985). The genus Maytenus (Celastraceae) in NE tropical Africa and tropical Arabia.-Symbolae Botanicae upsaliensis, 25:1-98*

Serhan, C. N., Savill, J., (2005). Resolution of inflammation: the beginning programs the end. *Nature Immunology; 6: 1191–1197.*

Serrano, R., da Silva, G. and Silva, O. (2010). Application of Light and Scanning Electron Microscopy in the Identification of Herbal Medicines. iMed.UL, pp.1.

Silver, L. G., and Kinghorn, D. A., (1998). *Special problem with the extraction of plants: Natural Products Isolation (Cannel, R. J. P. Ed.).* Humana Press Inc. 999, Revew drive Suite 208. Totowa.New Jessey, USA 072412, pp. 343-364.

Sirikatitham, A., Chuchom, T. and Itharat, A., (2007). Development of the chromatographic fingerprint analysis of dioscorealides and dioscoreanone from *Dioscorea membranacea* Pierre Songklanakarin *journal of Science Technology* pp.101-107.

Sofowora, E.A. (2008). Medicinal Plants and Traditional Medicine in Africa. 3rd edn. Pp 202.

Srivastava, T. and Sharad, A., (2006). Pharmacognostic evaluation of *Curcuma haritha* Linn. *Journal of Science and Industrial Research;* 65: 916-920.

Sutharson, L., Lila, K. N., Presanna, K.K., Shila, E. B., Rajan, V. J., (2007). Antiinflammtory and Antinociceptive Activities of methanolic extract of the leaves of *Fraxinus floribunda* Wallich. *African Journal of Biotechnology* 6 (5) 582-585.

Szenrei, K., (1981). *The chemistry of Khat. Bulletin on Narcotics*. 32:5-36.

Tjölsen, A., Berge, O., Hunskaar, S., Rosland, J. H., Hole, K., (1992). *The formalin test: an evaluation of the method*. Pain, 52:5-17.

Troll, W., (1943). *Vergleichende Morphologie der hoheren Pflanzen 3*. Berlin:Borntraeger.

Vierling, W., Brand, N., Gaedeke, F., Sensch, K.H., Schneider, E. and Scholz, M. (2003). Investigation of the pharmaceutical and pharmacological equivalence of different Hawthorn extracts. Phytomedicine., pp. 8-16.

Wall, M.E., Krider, M.M., Krewson, C.F., Eddy, C.R., William J.J., Cordell, D.S., and Gentry, H.S. (1954). 'Steroidal sapogenins XIII. Supplementary table of data for steroidal spogenins VII'. Pp. 17.

Watson, L., and Dallwitz, M. J., (1992). *The Families of Flowering Plants: Descriptions, Illustrations, Identification*, and Information Retrieval. Version: 14th December 2000. http://biodiversity.uno.edu/delta/'.

Webb, M. A., (1999). *Cell-mediated crystallization of calcium oxalate in plants*. *Plant Cell;11:751-761.*

World Health Organisation, Geneva (1998). *Quality Control Methods for Medicinal Plant Materials*. pp.10-21, 28-30.

Winter, C. A., Riselay, E. A., and Nuss, G. W., (1962). Carrageenan-induced oedema in the hind paw of the rats as an assay for anti – inflammatory drugs. *Proceedins of the Society for Experimental Biology and Medicine. 111: 544-547.*

Woo., S. W., Shin, H. Y., and Kang, K. S., (1980). Chemistry and pharmacology of flavones-c-glycoside from *Zizyphus* seeds. *The Korean Journal of Pharmacognosy, II* (3.4) 141-148.

Zelger, J. L., Schorno, H. X., Carlini, E. A. (1981). Behavioural effects of cathinone, an amine obtained from *Catha edulis* Forks.: Comparisons with amphetamine, norpseudoephedrine, apomorphine and nomifensine. *Bulletin on Narcotics*, 32: 67-82.

APPENDIX A

Acute toxicity evaluation on:

1. Rats

First step:

Doses in mg/kg body weight

Doses	10	100	1000
MSMLE	0/3*	0/3	1/3
MSMSBE	0/3	0/3	2/3

Where '*'= number of animals that died/number of animals used.

Second step

Doses in mg/kg body weight

Doses 1.	600	1000	1,600	2,900
MSMLE	0/1	0/1	1/1	1/1

Doses 2.	140	225	370	600
MSMSBE	0/1	1/1	1/1	1/1

LD_{50} = √lowest lethal dose × highest non-lethal dose

Lowest lethal dose = 1600mg/kg

Highest non-lethal dose = 1000mg/kg

LD_{50} = √1600 × 1000
√1600000
1264.91mg/kg

LD_{50} for MSMSBE= √lowest lethal dose × highest non-lethal dose

Lowest lethal dose = 225mg/kg

Highest non-lethal dose = 140mg/kg

LD_{50} = √225 × 140

√31500

177.48mg/kg

2. Mice

First step:

Doses in mg/kg body weight

Doses	10	100	1000
MSMLE	0/3*	0/3	1/3
MSMSBE	0/3	0/3	3/3

Where '*'= number of animals that died/number of animals used.

Second step

Doses in mg/kg body weight

Doses 1.	600	1000	1,600	2,900
MSMLE	0/1	0/1	1/1	1/1

Doses 2.	140	225	370	600
MSMSBE	1/1	1/1	1/1	1/1

LD_{50} for MSMLE= √lowest lethal dose × highest non-lethal dose

Lowest lethal dose = 1600mg/kg

Highest non-lethal dose = 1000mg/kg

LD_{50} = √1600 × 1000

√1600000

1264.91mg/kg

LD_{50} for MSMSBE= √lowest lethal dose × highest non-lethal dose

Lowest lethal dose = 140mg/kg

Highest non-lethal dose = 100mg/kg

LD_{50} = √100 × 140

√14000

118.32mg/kg

APPENDIX B

a. Analgesic studies:

1. acetic acid-induced writhing in mice

Doses	step 1: mean no. of writhing	step 2: mean no of writhing
75mg/kg	34.17	00.45
150mg/kg		19.67
0.28		
300mg/kg		07.17
0.48		
Piroxicam 10mg/kg		15.17
0.31		
Normal saline		47.83
0.79		

2. formalin-induced pain in rats

Phase 1

Doses	75mg/kg	150mg/kg	300mg/kg	piroxicam
normal saline				
Mean	0.67	1.00	1.00	2.00
2.83				
± SEM	0.42	0.37	0.37	0.45
0.17				

Phase 2

Doses	75mg/kg	150mg/kg	300mg/kg	piroxicam
normal saline				
Mean	2.5	2.00	0.83	1.00
2.33				
± SEM	0.22	0.37	0.31	0.41
0.21				

b. Anti-inflammatory studies

1. MSMLE

Group A - 75mg/kg

Hours	**1**	**2**	**3**	**4**	**5**
Paw (cm)	0.96	0.91	2.61	1.71	0.8
± SEM	0.11	0.19	0.16	0.09	0.11

Group B – 150mg/kg

Hours	**1**	**2**	**3**	**4**	**5**
Paw (cm)	0.93	2.30	2.96	1.57	0.77
± SEM	0.14	0.39	0.51	0.30	0.54

Group C – 300mg/kg

Hours	**1**	**2**	**3**	**4**	**5**
Paw (cm)	0.73	1.89	2.82	1.63	0.84
± SEM	0.15	0.33	0.25	0.32	0.13

Group D – Piroxicam (10mg/kg)

Hours	**1**	**2**	**3**	**4**	**5**
Paw (cm)	0.84	1.53	1.96	1.25	0.87
± SEM	0.21	0.10	0.07	0.14	0.12

Group E- Normal saline

Hours	**1**	**2**	**3**	**4**	**5**
Paw (cm)	2.49	3.71	4.76	3.21	1.55
± SEM	0.37	0.20	0.20	0.28	0.30

2. MSMSBE

Group A - 12.5mg/kg

Hours	**1**	**2**	**3**	**4**	**5**
Paw (cm)	1.19	2.22	2.19	1.53	0.62
± SEM	0.28	0.66	0.37	0.40	0.21

Group B – 25mg/kg

Hours	**1**	**2**	**3**	**4**	**5**
Paw (cm)	1.46	2.83	2.88	2.47	1.15
± SEM	0.17	0.36	0.14	0.11	0.13

Group C – 50mg/kg

Hours	**1**	**2**	**3**	**4**	**5**
Paw (cm)	0.93	2.34	2.52	1.69	0.90
± SEM	0.21	0.46	0.52	0.40	0.23

APPENDIX C

Result of moisture content

LEAVES

a. Weight of evaporating dish (W_1) = 28.87
Initial weight of dish + drug (W_2) = 30.87
Initial weight of drug (W_3) = 2.00
Final weight of evaporating dish + drug (W_4) = 30.69
Loss in weight (W) $= W_2 - W_4$ = 30.87 – 30.69
= 0.18g

b. W_1 = 50.51
W_2 = 52.51
W_3 = 2.00
W_4 = 52.34
W = 0.17g

c. W_1 = 51.20
W_2 = 54.20
W_3 = 3.00
W_4 = 53.99
W = 0.21g

Average loss in weight $= \frac{0.18 + 0.17 + 0.21}{3}$
$= 0.1867 \pm 0.01$

% moisture content $= \frac{0.1867}{2} \times 100$
$= 9.33\% \pm 0.01$

STEM BARK

a. Weight of evaporating dish (W_1) = 98.04
Initial weight of dish + drug (W_2) = 100.04
Initial weight of drug (W_3) = 2.00
Final weight of evaporating dish + drug (W_4) = 99.94
Loss in weight (W) $= W_2 - W_4$ = 100.04 – 99.94
= 0.10g

b. W_1 = 30.74
W_2 = 32.74
W_3 = 2.00
W_4 = 32.62
W = 0.12g

c. W_1 = 50.35
W_2 = 52.35
W_3 = 2.00
W_4 = 52.19
W = 0.16g

Average loss in weight = $\frac{0.10 + 0.12 + 0.16}{3}$
= 0.1267 ± 0.02

% moisture content = $\frac{0.1267}{2} \times 100$
= 6.33% ± 0.02

Results of Ash value

LEAVES

a. Weight of crucible (W_1) = 55.70
Initial weight of crucible + drug (W_2) = 57.70
Initial weight of powdered drug (W_3) = 2.00
Final weight of crucible + drug (W_4) = 55.86
Loss in weight (W) = $W_2 - W_4$
= 55.86 – 55.70
= 0.16g

b. W_1 = 53.82
W_2 = 55.82
W_3 = 2.00
W_4 =53.98
W = 0.16g

c. W_1 = 25.88
W_2 = 27.88
W_3 = 2.00
W_4 = 26.03

W = 0.15g

Average total ash = $\frac{0.16 + 0.16 + 0.15}{3}$

= 0.1567 ± 0.004

% total ash = $\frac{0.1567}{2} \times 100$

= 7.83 ± 0.004

STEM BARK

a. Weight of crucible (W_1) = 51.05
Initial weight of crucible + drug (W_2) = 53.05
Initial weight of powdered drug (W_3) = 2.00
Final weight of crucible + drug (W_4) = 51.33
Loss in weight (W) = $W_2 - W_4$
= 51.33 – 51.05
= 0.28g

b. W_1 = 50.83
W_2 = 52.83
W_3 = 2.00
W_4 = 51.13
W = 0.30g

c. W_1 = 25.88
W_2 = 27.88
W_3 = 2.00
W_4 = 26.21
W = 0.33g

Average total ash = $\frac{0.28 + 0.30 + 0.33}{3}$

= 0.3033 ± 0.004

% total ash = $\frac{0.3033}{2} \times 100$

= 15.17% ± 0.01

Results of insoluble ash

Leaves

a. Weight of crucible (W_1) = 51.05
Initial weight of crucible + residue (W_2) = 51.08
Initial weight of powdered drug (W_3) = 2.00
Weight of residue (W) = $W_2 - W_1$
= 51.08 – 51.05
= 0.03g

b. W_1 = 55.57
W_2 = 55.59
W_3 = 2.00
W = 0.02g

c. W_1 = 53.64
W_2 = 53.69
W_3 = 2.00
W = 0.05g

Average acid-insoluble ash = $\frac{0.03 + 0.02 + 0.05}{3}$

= 0.0333 ± 0.01

% acid-insoluble ash = $\frac{0.0333}{2} \times 100$

= 1.67% ± 0.01

STEM BARK

a. Weight of crucible (W_1) = 51.05
Initial weight of crucible + residue (W_2) = 51.08
Initial weight of powdered drug (W_3) = 2.00
Weight of residue (W) = $W_2 - W_1$
= 51.08 – 51.05
= 0.03g

b. W_1 = 50.83
W_2 = 50.85
W_3 = 2.00
W = 0.02g

c. W_1 = 51.01
W_2 = 51.03
W_3 = 2.00

W = 0.02g

Average acid-insoluble ash = $\frac{0.02+ 0.02 + 0.02}{3}$

= 0.02 ± 0.01

% acid-insoluble ash = $\frac{0.02}{2} \times 100$

= 1.00% ± 0.01

Results of alcohol extractive values

LEAVES

a. Weight of empty dish (W_1) = 50.51
Initial weight of dish + extract (W_2) = 50.64
Initial weight of powdered drug (W_3) = 4.00
Weight of residue (W) = $W_2 - W_1$
= 50.64 – 50.51
= 0.13g

0.13g in 20ml

$\frac{0.13}{20} \times 100$

= 0.65g

b. W_1 = 51.75
W_2 = 51.85
W_3 = 4.00
W = 0.10g

0.10g in 20ml

$\frac{0.10}{20} \times 100$

= 0.5g

c. W_1 = 50.51
W_2 = 50.57
W_3 = 4.00
W = 0.06g

0.06g in 20ml

$\frac{0.06}{20} \times 100$

= 0.30g

Average alcohol extractive value = $\frac{0.3 + 0.65 + 0.5}{3}$

= 0.48 ± 0.1

% alcohol-soluble extractive value = $\frac{0.48}{4} \times 100$

= 12.0% ± 0.1

STEM BARK

a. Weight of empty dish (W_1) = 30.73
Initial weight of dish + extract (W_2) = 30.80
Initial weight of powdered drug (W_3) = 4.0
Weight of residue (W) = $W_2 - W_1$
= 30.80 – 30.73
= 0.07g

0.07g in 20ml

$\frac{0.07}{20} \times 100$

= 0.35g

b. W_1 = 28.87
W_2 = 29.01
W_3 = 4.00
W = 0.14g
0.14g in 20ml

$\frac{0.14}{20} \times 100$

= 0.7g

c. W_1 = 30.74
W_2 = 30.85
W_3 = 4.00
W = 0.11g
0.11g in 20ml

$\frac{0.11}{20} \times 100$

= 0.55g

Average alcohol extractive value = $\frac{0.35 + 0.7 + 0.55}{3}$

= 0.53 ± 0.1

% alcohol-soluble extractive value = $\frac{0.53}{} \times 100$

4

= 13.25% ± 0.1

Results of water-soluble extractive values

LEAVES

a. Weight of empty dish (W_1) = 28.88
Initial weight of dish + extract (W_2) = 28.97
Initial weight of powdered drug (W_3) = 4.0
Weight of residue (W) = $W_2 - W_1$
= 28.97 – 38.88
= 0.09g

0.09g in 20ml

$\frac{0.09}{20} \times 100$

= 0.45g

b. W_1 = 37.07
W_2 = 37.19
W_3 = 4.00
W = 0.12g

0.12g in 20ml

$\frac{0.12}{20} \times 100$

= 0.6g

c. W_1 = 35.58
W_2 = 35.69
W_3 = 4.00
W = 0.11g

0.11g in 20ml

$\frac{0.11}{20} \times 100$

= 0.55g

Average water-soluble extractive value = $\frac{0.45+ 0.6 + 0.55}{3}$

= 0.53 ± 0.04

% water-soluble extractive value = $\frac{0.53}{4} \times 100$

= 13.25% ± 0.04

Stem bark

a. Weight of empty dish (W_1) = 35.03
Initial weight of dish + extract (W_2) = 35.14
Initial weight of powdered drug (W_3) = 4.00
Weight of residue (W) = $W_2 - W_1$
= 35.03 – 35.14
= 0.11g

0.11g in 20ml

$\frac{0.11}{20} \times 100$

= 0.55g

b. W_1 = 28.87
W_2 = 28.97
W_3 = 4.00
W = 0.10g

0.10g in 20ml

$\frac{0.10}{20} \times 100$

= 0.50g

c. W_1 = 30.74
W_2 = 30.87
W_3 = 4.00
W = 0.13g

0.13g in 20ml

$\frac{0.13}{20} \times 100$

= 0.65g

Average water-soluble extractive value = $\frac{0.55+ 0.50 + 0.65}{3}$

= 0.57 ± 0.04

% water-soluble extractive value = $\frac{0.53}{4} \times 100$

= 14.25% ± 0.04