

**PHARMACOGNOSTIC AND ANTIULCER STUDIES ON THE LEAF OF *LEUCAS
MARTINICENSIS* (JACQ) R.Br. (LAMIACEAE)**

BY

**TABITHA LUBO MUSA
P13PHPD8013**

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

APRIL, 2017

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**TABITHA LUBO MUSA B. Pharm (ABU, 2010)
P13PHPD8013**

**A DISSERTATION SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES
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**DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA**

APRIL, 2017

DECLARATION

I declare that the work in this dissertation entitled “Pharmacognostic and antiulcer studies on the Leaf of *Leucas martinicensis* (Jacq) R.Br. (Lamiaceae) has been carried out by me in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and the list of references.

Tabitha Lubo Musa

Signature

Date

CERTIFICATION

This dissertation titled “**PHARMACOGNOSTIC AND ANTIULCER STUDIES ON THE LEAF OF *LEUCAS MARTINICENSIS* (JACQ) R.Br. (Lamiaceae)**” by Tabitha Lubo, MUSA meets the regulations governing the award of the degree of Master of Science in Pharmacognosy of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

.....

Prof. A. Agunu
Chairman, Supervisory Committee

.....

Date

.....

Dr. A. Ahmed
Member, Supervisory Committee

.....

Date

.....

Dr. G. Ibrahim
Head, Department of Pharmacognosy and
Drug Development

.....

Date

.....

Prof. S.Z. Abubakar
Dean, School of Postgraduate Studies

.....

Date

DEDICATION

This work is dedicated to the Almighty God.

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ABSTRACT

Leucas martinicensis is an annual herb which is used for repellent of mosquito. It has minty odor. It is an erect plant which is usually unbranched, with length of up to 1m, which is finely hairy. The plant are widely used in Northern part of Nigeria and it has been exploited for both its medicinal and economic properties with no scientific justification about its antiulcer activities. It is traditionally used for the management of a number of ailments including the treatment of diarrhea, skin rashes, epilepsy, gastroenteritis, cholera, relieving pain during pregnancy and as anti-malaria. The pharmacognostic studies and antiulcer activities of the leaves of *Leucas martinicensis* (Lamiaceae) was carried out to validate the ethno medicinal claimed of the leaves in treating ulcer. Evaluation of the fresh, powdered leaves and phytochemicals screening were carried out. The acute toxicity and antiulcer properties revealed the leaves potential as source of drug for further utilizations. The microscopical characters and physical constants of the leaf were determined, which revealed the presence of some prominent features like multicellular covering trichomes and diacytic stomata. The physical constants evaluated showed moisture content 8.9% of *Leucas martinicensis*, total ash value of 6.8 %, water soluble ash of 6.0 %, acid insoluble ash of 3.5 %, ethanol extractive value of 16.0 % and water extractive value of 20.3 %. Phytochemical analysis of the leaves extracts revealed the presence of alkaloids, tannins, flavonoids, carbohydrate, cardiac glycosides, saponins, triterpenes and steroids and anthracenes. The median lethal dose (LD₅₀) of the extract was found to be greater than 3000 mg/kg when administered orally in rats and considered practically non-toxic. The extract demonstrated protective activity against ethanol induced gastric ulcer lesions in albino rats. This is evidenced in the significant and increased preventive index with doses (300, 600 and 900 mg/kg) of the extract. The methanol extract was observed to have the best activity compared to organic solvent

fractions. The results provided some pharmacognostic standards for proper identification of the plant and scientific basis for the traditional use of the leaf in the treatment of ulcer.

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ACRONYMS AND ABBREVIATIONS

BH P:	British Herbal Pharmacopoeia
Fig:	Figure
FAA:	Formalin Acetic acid Alcohol
g:	Gram
G.A.A:	Glacial Acetic Acid
HCL:	Hydrochloric acid
H ₂ SO ₄ :	Sulphuric acid
LD ₅₀ :	Median Lethal Dose
OECD	Organization of Economic Co-operation and Development
kg:	Kilogram
ml:	Milliliter
mm:	Millimeter
mg:	Milligram
Vol.:	Volume
w/w:	Weight per Weight
W.H.O:	World Health Organization
%:	Percentage
SEM	Standard error of the mean
LMME :	<i>Leucas martinicensis</i> Methanol Extract
LMEAF :	<i>Leucas martinicensis</i> Ethyl acetate Fraction
LMHF :	<i>Leucas martinicensis</i> Hexane Extract
LMAF:	<i>Leucas martinicensis</i> Aqueous Fraction

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Herbal medicine is recognized as the most common form of alternative medicine (Ogbonnia *et al.*, 2011). The World Health Organization (WHO) estimates that 80 % of the world's population relies on these “alternative” plant-based medicines as their primary medical intervention especially in the developing countries, Even in the developed countries where modern medicines are more popular, interest in the use of natural product is on the increase (Rickert *et al.*, 1999; Kroll and Shaw, 2003; Ogbonnia *et al.*, 2008). The use of herbs in the treatment of illnesses has been very successful over the years and its historic usage has been useful in drug discovery and development. Herbal prescriptions and natural remedies are commonly employed in developing countries for the treatment of various diseases, this practice being an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy (Sofowora, 1989; Zhu *et al.*, 2002). The popularity and availability of the traditional remedies have generated concerns regarding the safety, efficacy and responsibility of practitioners using traditional remedies (Chan, 1995). Herbal remedies are considered safer and less damaging to the human body than synthetic drugs (Alam *et al.*, 2011). However, the lack of standardization has been a major concern regarding the use of herbal medicines (Angell and Kassier, 1998). Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification. The most common error is when one common vernacular name is given to two or more entirely different species (Dineshkumar, 2007).

Generally herbal formulations involve the use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. The process of standardization can be achieved by stepwise pharmacognostic studies (Nivedithadevi and Somasundaram, 2012). Standardization is a system to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic effect (Nasreen and Radha, 2011). Determination of extractive values, ash residues and active components saponin, alkaloids and essential oil content where applicable play a significant role for standardization of the indigenous crude drugs (Hina *et al.*, 2011).

Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles, The quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in modern system of medicine (Satheesh, 2011).

Focus on plants research has increased Worldwide and several studies had showed immense potential of medicinal plants (Dahanurkar *et al.*, 2000). Herbal medicines derived from plants extracts are increasingly being recognized in treating various clinical diseases, with relatively little knowledge of their modes of action (Begum *et al.*, 2008).

It basically deals with authentication, standardization, and study of natural drugs. Most of the researches in pharmacognosy have been done in identifying controversial species of plants, authentication of commonly used traditional medicinal plants through morphological, phytochemical and physicochemical analysis. The importance of pharmacognosy has been widely felt in recent times. Unlike taxonomic classification, pharmacognostic study includes parameters which help in identifying adulteration in dry powder form also.

This is again necessary because once the plant is dried and made into powder form, it loses its morphological identity and is easily prone to adulteration. Pharmacognostic studies ensure plant identity, lay down standardization parameters which will help prevent adulterations. Such studies will help in authentication of the plants and ensure reproducible quality herbal products which will lead to safety and efficacy of natural products (Sumitra, 2014).

Pharmacognostic evaluation includes macroscopic, microscopic, physico-chemical, fluorescence and phytochemical studies of herbal parts or powdered drug. Herbal raw material shows a number of problems when quality and authentication aspects are considered. This is because of the nature of herbal parts, ingredients and different phytochemicals present in plants (WHO, 2011). To ensure quality of herbal medicines, proper control of starting raw material is very important. The physico-chemical evaluation includes qualitative and quantitative tests, assays and instrumentation analysis. Qualitative and quantitative chemical tests include the presence or absence, quantity, values and identification of various phytochemicals like flavonoids, glycosides, saponins, alkaloids etc. (Harborne, 1992; Evans, 2009). Macroscopic identity of medicinal plant materials is based on sensory evaluation parameters like shape, size, colour, texture, odour and taste while microscopy involves comparative microscopic inspection of powdered herbal drug. Further, advances in microscope technology have increased the accuracy and capabilities of microscopy as a means of herbal crude material identification due to the implication of light and scanning electron microscopes (SEM) in herbal drug standardization (Bhutani, 2003).

Peptic ulcer is an excoriated area of the gastric or duodenal mucosa caused by action of the gastric juice. It is a chronic and recurrent disease, and is the most predominant of the gastrointestinal diseases.

Ulcer is basically an inflamed break in the mucus membrane lining the alimentary tract. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance (Sravani *et al.*, 2011). A peptic ulcer in the stomach is called a gastric ulcer. One that occurs in the duodenum is called a duodenal ulcer. People can have both gastric and duodenal ulcers at the same time (Ramakrishnan and Salinas, 2007). The anatomic sites where ulcer occurs commonly are stomach and duodenum, causing gastric and duodenal ulcer, respectively (Rang *et al.*, 2003). Pathophysiology of ulcer is due to an imbalance between aggressive factors (acids, pepsin, *Helicobacter pylori* and non-steroidal anti-inflammatory agents) and local mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins). Integrity of gastroduodenal mucosa is maintained through a homeostatic balance between these aggressive and defensive factors (Raskin *et al.*, 1995). About 19 out of 20 peptic ulcers are duodenal while gastric ulcers found in the stomach wall are less common (Gadekar, 2010). The gastric mucosa is continuously exposed to potentially injurious agents such as acids, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs (Grossman, 2009). These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility (Grossman, 2009). Symptoms of ulcer include epigastric pain of a burning nature (postprandial pain and pain relieved by food or antacids), nausea, vomiting, belching and bloating.

Complications of protracted untreated cases include anemia caused by gastro-intestinal blood loss, weight loss attributed to a reduced appetite caused by fear of pain and vomiting associated with a gastric ulcer or pyloric stenosis and mucosal perforation (Hunt *et al.*, 2006). Current management of peptic ulcer disease involves the use of proton pump inhibitor (PPI), an antibiotic (triple therapy) (Malfertheiner, 2002).

However, reviewed literature showed that plant constituents like Flavonoids, Tannins, Terpenes, Steroids, Saponins, and Alkaloids is responsible for antiulcer activity (Khalid *et al.*, 2010). Recently, there has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Most of the studies focus on newer and better drug therapy. These have been made possible largely by the availability of the proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analog. However, the clinical evaluation of these drugs showed development of popularly known, tolerance and incidence of relapses and side effects which make their efficacy arguable. This has been the rationale for development of new antiulcer drugs, which include herbal drugs (Manonmani *et al.*, 1995).

1.2 Statement of Research Problem

The leaves of *Leucas martinicensis* is used traditionally as anti-ulcer agent without scientific validation. The use of herbs in the treatment of illnesses has been very successful over the years and its historic usage has been useful in drug discovery and development.

1.3 Justification

Phytochemical constituents present in medicinal plants have been used as drug therapy to treat peptic ulcer and which have proved to be clinically effective and relatively less toxic than the existing drugs (Sen *et al.*, 2009). There are no reported data to my knowledge on the distinctive characters for the identification and standardization of *L. martinicensis* leave and leave powder. In addition, there is no known report to our knowledge for the use of *the L. martinicensis* in the treatment of ulcer.

1.4 Aim and Objectives

The aim of this study is:

To provides scientific validation for the use of the leaves of *Leucas martinicensis* as anti-ulcer agent used in North Eastern Nigeria.

The specific objectives are:

1. To produce basic Pharmacopoeial standards for the leaves of *Leucas martinicensis*
2. To establish the anti-ulcer activity of methanol extract and organic solvents fractions of the leaves of *Leucas martinicensis* in indomethacine induced gastric ulcer in rats.
3. To establish the thin layer chromatography of the extract and fractions of the leaves of *Leucas martinicensis*.

1.5 Hypothesis

The leaves of *Leucas martinicensis* contains bioactive constituents that are useful in the management of ulcer.

CHAPTER TWO

2.0 LITERATURE REVIEWS

Plants are indispensable sources of medicine since time immemorial. Studies on natural products are aimed to determine medicinal values of plants by exploration of existing traditional uses scientific knowledge, and discovery of potential chemotherapeutic agents. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs (Balunas and Kinghorn, 2005). Plants of genus *Leucas* (Lamiaceae) have been widely employed by the traditional healers to cure many diseased conditions, which insinuated that this genus has immense potential for the discovery of new drugs or lead molecules. The genus *Leucas* comprises about 80 species (Hedge *et al.*, 1990). The largest species diversity has been found in East Africa.(Ryding,1998). In India, 43 species are available. Plants of genus *Leucas* are generally shrubs, sub-shrubs, annual herbs, or perennial herbs with woody root and/or stem base (Ryding, 1998).

2.1 The Family Lamiaceae

The family Lamiaceae is a large family of about 252 genera and 6700 species worldwide, with 37 genera and 235 species indigenous or naturalized in South Africa. The Lamiaceae are important and many are of great economic importance. They are widely used in many traditional systems of medicines and horticultures (Hedge *et al.*, 1990).

Plants belonging to the family *Lamiaceae* occur almost throughout the world, with the exception of the coldest polar regions. They are particularly well represented in tropical and temperate areas especially those with a seasonal climate, such as the Mediterranean region and in tropical

upland savannas. While some species are characteristic of semi-arid conditions, many others are adapted to wet habitats, in seasonally flooded areas or along riverbanks in forests.

Essential oils are commercially extracted from many species belonging to the *Lamiaceae*.

In general, plants belonging to the *Lamiaceae* family have been exploited for their antiviral, antibacterial, immuno-modulating, antifungal, insecticidal and ornamental properties (Hedge *et al.*, 1990). Examples of the plants in the family include *Leucas glabrata*, *Leucas urticifolia* and *L. cephalotes* etc.

2.2 The Plant (*Leucas martinicensis*)

Leucas martinicensis is an annual herb which is used for repellent of mosquito. It has minty odor (Plate I). It is an erect plant which is usually unbranched, with length of up to 1m, which are finely hairy. It has opposite leaves which are ovate to ovate lanceolate in shape with the margin coarsely serrate- crenate. Inflorescence of several space with many flowered verticals having long thistle- like calyx teeth. The flowers are small and white in colour (Hyde, 2009). The stems are retorse pubescent, having petiole of 0.7 - 1.5 cm. The leaves are reduced to crenate, when crushed the foliage usually emits pleasant odour. The nutlets are dark brown ablong-ovoid and shiny.

Table 2.1: Classification of *Leucas martinicensis*

Sub kingdom	Trachebi-onta
Super division	Spermatophyte
Division	Magnoliohyta
Class	Magnoliophyta
Sub class	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	Leucas

The plant has a local name of “Sarakuwan sauro” in Hausa land of Nigeria and a common name of “mosquito plant” also “white wort” (Wursten , 2009) *Leucas martinicensis* is a native plant to South America and West Indies, may be native or introduced in Africa, it is found habitable in disturbed places and as weed of cultivation (Hyde, 2009). This herb is found in grassy areas, waste land near habitation. Are also found in North America, southern part of Ethiopia and is usually found in the tropical part of the world.

Chemical study carried out on the Plant revealed that the plant contain Volatile oil which is suitable for use in pharmaceuticals, cosmetic and food products examples lotions, creams,

mosquito repellent, soaps, shampoos, rinses, gargles, candies, its antibacterial and antifungal properties have also been reported. It's also used at its ordinary natural state, as mosquito repellent by burning it. The plant is well known for its medicinal value by the communities around Yabello town in Ethiopia. It is taken mainly to prevent diarrhea. (Hyde, 2009).



Plate I: *Leucas martinicensis* in its Natural Habitat (Behind College of Medicine, Gombe State University).

2.3 Reported Ethnomedicinal uses of *Leucas martinicensis*

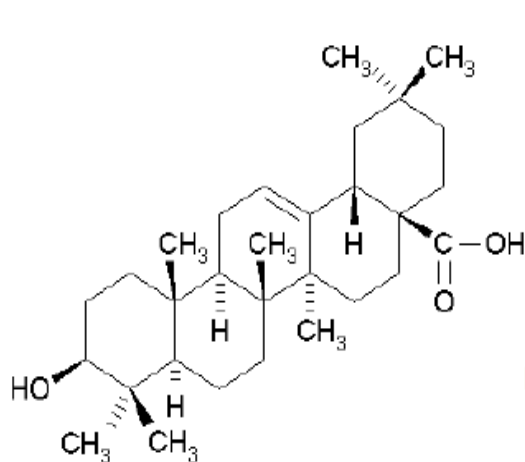
Hot water extract of *L. martinicensis* is used orally for gastroenteritis, cholera (Reddy *et al.*, 1986) and malaria (Valsaraj *et al.*, 1997). The leaves are also used orally for pain during pregnancy. The infusion is used ophthalmically for proptosis (Nene *et al.*, 1968). Aerial parts decoction of the plant is used against kidney disorders, rheumatism and inflammation (Agra *et al.*, 2007). The fresh leaves have also been used to prevent and cure diarrhoea, and also for the treatment of fever. In West Africa, the plant ash is used to repel mosquitoes (Muhammad *et al.*, 2012). Leaves decoction is used for the treatment of skin rashes, epilepsy and convulsion in Nigeria (Qamaruddin *et al.*, 2002).

2.4 Distribution of *Leucas martinicensis*

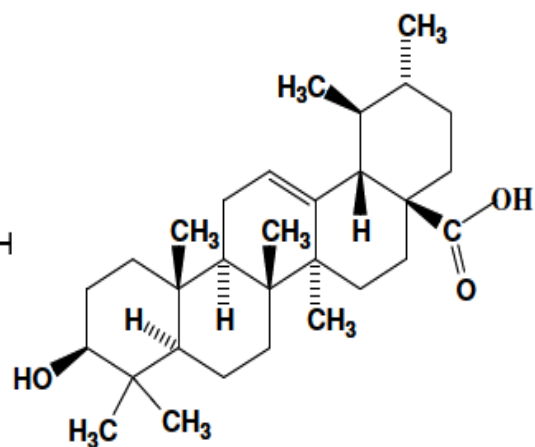
The plant has been reported in Antartica, Australia, Benin, Brazil, Burkina Faso, Cameroon, Canada, China, Congo, Equitorial Guinea, Ethiopia, France, Ghana, Kenya, India, Madagascar, Mali, Malawi, Rwanda, Senegal, Mexico, Niger, Nigeria, Tanzania, Uganda, United states and Zimbabwe.(Ryding, 1998).

2.5 Chemistry of *Leucas* Species

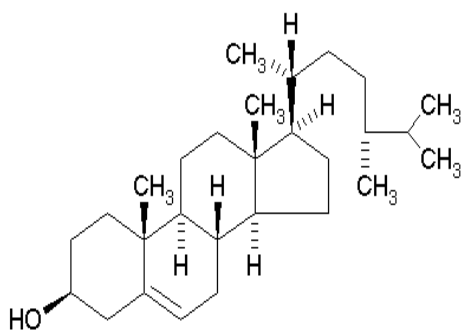
About seven species have so far been chemically investigated out of the 150 species of *Leucas*. A number of secondary metabolites have been isolated and characterized. From a number of *Leucas* species compounds like diterpenes, triterpenes, flavones, alkaloids, glycosides, sitosterols, chromones, sterol, oleanolic acid, ursolic acid, leucolacton, stigmasterol, campesterol, isopimarane, rhamnoglucoside, etc. have been isolated and characterized (Hunt *et al.*,2006).



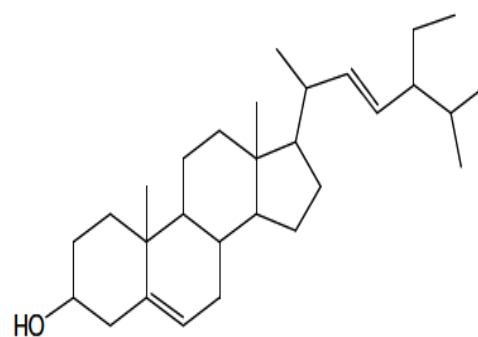
Oleanolic acid



Ursolic acid



Campesterol



Stigmasterol

Fig 2.1: Chemical structures of some compounds isolated from *L. martinicensis* (Adopted from Hunt *et al.*, 2006).

2.6 Chemicals Reported from the Genus *Leucas*

The following are some of the chemicals reported from the Genus *Leucas*.

2.6.1 Phenolic compounds

In the genus *Leucas*, many reports revealed the occurrence of flavonoids in the combined form (Fatima *et al.*, 2008).

2.6.2 Steroids

Sterols structurally comprise of perhydrocyclopenta- (O) phenantherene ring system, which are widely distributed in higher plants (Fatima *et al.*, 2008). Presence of ubiquitous phytosterol such as sitosterol, stigmasterol, campesterol, ursolic acid, and their derivatives have been reported in plants of genus *Leucas* (Al yousuf *et al.*, 1999; Pradhan *et al.*, 1990). A novel steroid “leucisterol” was reported from the methanol extract of whole plant of *Leucas urticifolia* (Fatima *et al.*, 2008) which is similar to stigmasterol, the difference lying in the side chain that is presence of a hydroxyl group at C-20 atom, a double bond at C-22 atom, and S configuration at C-24 atom.

2.6.3 Terpenes

Terpenes constitute one of the largest and structurally diverse class of plant secondary metabolites responsible for flavor, fragrance, and bioactivity of the plants (Humphrey *et al.*, 2006). Plants of genus *Leucas* are found to be rich in terpenes. Report showed the presence of monoterpenes in the essential oil obtained from *Leucas glabrata* by GCMS analysis. It revealed the presence of menthone, pulegone, piperitone, piperitenone, α -thujene, myrcene, α -phellandral, g-terpinene, terpinen-4-ol, nerolidol, carvone, carvacrol, caryophyllene, cumin alcohol, α -

farnesene, menthol, and E-nerolidol in the oil (Vagionas *et al.*, 2007). However, the essential oil fraction from the leaves and flowers of *aspera* were found to contain high amount of afarnesene, α -thujene, and menthol;(Gerige *et al.*, 2007; Mangathayaru *et al.*, 2006). Whereas, high content of β - cubebene, α -pinene, trans-caryophyllene, limonene, and α -terpineolene were reported in the essential oil from *milanjiana* (Moody *et al.*, 2006). The essential oil fraction of *deflexa* leaf was reported to have a high amount of sesquiterpene hydrocarbons, namely, germacrene-D, β -caryophyllene, and α -humulene (Mye-Mba *et al.*,2006). A new type of diterpenes, leucasperones A and B; have been reported from *Leucas aspera* (Sadhu *et al.*, 2006). Also new diterpenes Leucasdins A, B, and C, two protostane-type triterpenes named Leucastrins A and B, and oleanolic acid have been reported from the methanol extract of whole plant of *cephalotes* Spreng (Miyachi *et al.*, 2006).

2.6.4 Glycosides

Two new flavonoid glycosides leufofin A and B were reported from the ethyl acetate fraction of methanolic extract of whole plant *urticifolia* (Noor *et al.*, 2007). A novel phenylethanoid glycoside, 3-O-methyl poliumoside and angoroside C, 2-(3-hydroxy-4-methoxyphenyl)-ethyl-O- α -L-rhamnopyranosyl-(1 \rightarrow 3)-O- α -L-rhamnorhamnopyranosyl-(1 \rightarrow 6)-4-O-E-ferul-oyl- β -Dglucopyranoside, incanoside D, martynoside, and acteoside were reported in the methanolic extract of the whole plant of *indica* (Mostafa *et al.*, 2007). A flavonoid glycoside, baicalin was reported from the fresh flower of *aspera* (Manivanana and Sukuman, 2007).

2.6.5 Fatty acids

Cephalotes and *uriicaefolia* seeds were found to have a high content (28% w/w) of laballanic acid (Aitzetmuller *et al.*, 2006; Sinha *et al.*, 1978).

2.6.6 Miscellaneous

Aspera is widely used in countryside as foods and also for nutritional value. It was reported to have high content (21.3%) of protein (Chen *et al.*, 1979; Prakash *et al.*, 1988). Asperphenamate and alkaloid nicotine have also been reported in *Leucas aspera* (Mangathayaru *et al.*, 2006). Accumulation of heavy metals reported in plants adversely affect the quality, safety, and their medicinal value. Higher concentration of zinc (201 µg/g), iron (809 µg/g), and strontium (133 µg/g), have been reported from *Leucas linifolia* grown in the North Eastern region of India (Rai *et al.*, 2001)

2.7 Reported Pharmacological Activities of the Genus, Leucas

The following were some pharmacological effects of the Genus *Leucas* reported:

2.7.1 Anti-inflammatory activity

The whole plant extract of *Leucas aspara* was reported to have anti-inflammatory activity and caused degranulation of mast cells (Reddy *et al.*, 1986). Significant anti-inflammatory activity of the yellow-colored chromatographic fraction of *Leucas aspera* extract was observed in the chronic and acute models of inflammation in rats. It was observed that the activity was due to the inhibition of histamine and serotonin (Saha *et al.*, 1997). Srinivas *et al.* showed that a dose of 50 mg/kg of *Leucas aspera* dried leaf powder in 2% gum acacia showed significant anti-inflammatory activity, which was found to be better than acetylsalicylic acid in the carrageenan-

induced paw edema model and less active than phenylbutazone, when tested in cotton pellet-induced granuloma in rat model (Srinivas *et al.*, 2000).

2.7.2 Central Nervous System Activity

Methanol and acetone extracts of *Leucas inflata* possess dose-dependent antinociceptive activity, which may be mediated by their central and peripheral actions (Al Yousuf, 1999). In a similar study, ethanolic extract of *the* root showed significant peripheral antinociceptive activity at a dose of 400 mg/kg. Murkherjee *et al.* (1998) reported a yellow-colored fraction from the methanol extract of *lavandulaefolia*, exhibited dose-related effects on general and exploratory behavior and muscle relaxant activity in rats and mice.

Saha *et al.*, (1997) reported that the semisolid mass from the yellow-colored band obtained from methanol extract of *lavandulaefolia* showed significant dose-dependent anti-tussive activity. This effect was comparable to codeine phosphate and suggested that the activity was mediated by the CNS. It was reported that the ethanol extract of aerial part of *lavandulaefolia* significantly reduced the incidence and severity of diarrhea in the castor oil-induced diarrhea in rats (Murkherjee *et al.*, 1998).

2.7.3 Anti-diabetic Activity

The methanol extract of the whole plant was reported to possess a dose-related hypoglycemic activity with similar potency to that of glibenclamide at an oral dose of 400 mg/kg in rats (Saha *et al.*, 1997).

2.7.4 Antimicrobial Activity

Menthone, pulegone, and piperitone-rich essential oil of *Leucas glabrata* produced significant antimicrobial activity against selected Gram positive and negative bacteria and fungi strains at concentrations of 0.45 to 1.14 mg/mL (MIC) (Vagionas *et al.*, 2007).

Significant antimicrobial activity was reported for the alkaloidal fraction and the methanol extract of *aspera* flowers (Mangathayaru *et al.*, 2006). Interestingly, the volatile oil obtained from the leaves of this plant exhibited high sensitivity for *Pseudomonas aeruginosa*, *Haemophilus influenza*, *S. aureus*, and *Candida albicans* but practically no sensitivity against *Bacillus subtilis*, *Proteus. vulgaris*, *Neisseria gonorrhoea*, *Trichoderma vibriae*, and *Aspergillus niger* (Gerige *et al.*, 2007).

2.7.5 Antioxidant Activity

Ethanol extract of *Leucas aspera* root showed significant antioxidant activity using DPPH (IC₅₀ = 7.5 µg/ml) (Rahman *et al.*, 2007).

2.7.6 Hepato-Protective Activity

The chloroform extract of *lavandulaefolia* whole plant, obtained after defatting with petroleum ether, was found to have hepato-protective activity in D (+) galactosamine-intoxicated rat mode (Chandrasekhar *et al.*, 2007). The cold methanol extract of the whole plant of *aspera* was found to exhibit significant hepato-protection in CCl₄ induced liver damage (Mangathayaru *et al.*, 2006).

2.7.7 Cyto-toxicity

Various studies using Brine shrimp lethality assay model showed that the hydroalcoholic extract of *aspera* whole plant exhibited cytotoxicity ($LC_{50} = 1900 \mu\text{g/mL}$) (Krishnaraju *et al.*, 2005) and this activity was more in the root extract ($LC_{50} = 52.8 \mu\text{g/mL}$) (Rahman *et al.*, 2007).

2.7.8 Insecticidal and Repellant Activity

Leucas aspera leaves are used as mosquito repellant and as insecticide (Kirtikar *et al.*, 1990). These claims were established by extensive studies, which indicated that *L. aspera* leaf extract exhibited significant larvicidal activity against first, second, third, and fourth instar larvae of *Culex quinquefasciatus* (Muthukrishnan *et al.*, 1997). *L. aspera* leaf extract (4% solution) showed 90% mortality of the fourth instar larvae (Murugan and Jayabalan, 1999) and 100% mortality after 24 h were recorded for the third instar larvae of *Anopheles stephensi*. (Vinayagam *et al.*, 2008). The petroleum ether extract of the leaves of *aspera* exhibited LC_{50} between 100 to 200 ppm against the fourth instar larvae of *Culex quinquefasciatus*, *Anopheles. stephensi*, and *Aedes aegypti* (Sakthivadivel and Daniel, 2008).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Collection and Identification of *Leucas martinicensis*

Sample of *L. martinicensis* leaf was collected from behind the college of Medicine Gombe State University, Tudun wada Gombe, Gombe State, and were taken to the Herbarium Unit of the Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University Zaria for proper identification and authentication, as compared with Voucher number 7234 deposited in the herbarium.

3.2 Experimental Design

3.2.1 Preparation of *Leucas martinicensis*

Sufficient quantity of the leaves of *L. martinicensis* was obtained by manually picking the leaves from the plants. The leaves were separated, dusted, cleaned and all foreign matter removed. The leaves were then air-dried and comminuted to powder form, stored in an air-tight container for subsequent use.

3.2.2 Evaluation of Pharmacognostic Characters of *Leucas martinicensis*

Detailed pharmacognostic studies of the leaf of *L. martinicensis* were carried out by examining the macroscopical, microscopical and chemomicroscopical characteristics of the leaf in order to establish some Pharmacopoeial Standards, following the procedure outlined by Sofowora, 2008.

3.2.3 Microscopical Examination of *L. martinicensis*

This involves the viewing and identification of some microscopic characters under the microscope.

3.2.4. Micrometric evaluation

This involved measurements of dimensions (length and width) of some diagnostic microscopic characters of the plant; it was carried out by using a binocular microscope with the aid of graticles and stage micrometer (Kokate, 1994).

3.3. Chemo-microscopical Studies of *Leucas martinicensis* powdered leaf

Small amount of the finely ground powdered leaf of the plant was cleared in a test-tube containing 70% chloral hydrate solution. It was boiled on a water-bath for about thirty minutes to remove obscuring materials. The cleared sample was mounted on a microscope slide, using dilute glycerol. Using various detecting reagents, the presence of some cell inclusions and cell wall materials were detected in accordance with Evans (2009) as follows:

3.3.1 Cell wall Materials

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

3.3.1.2 Test for Suberins: Two drops of Sudan red was added to the cleared sample on a slide, cover slip was placed and this was gently heated over hot water bath for 2 minutes. The slide was

then observed under the microscope for red coloration which indicates the presence of suberin in the cell wall.

3.3.1.3 Test for Lignins: Two drops of phloroglucinol was added to the cleared sample and allowed to stand until almost dry. A drop of sulphuric acid was added and cover slip applied and was observed under the microscope the appearance of red coloration on the anatomical section was taken for the presence of lignin.

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

3.3.2 Cell Contents/Cell inclusions

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

3.3.2.2 Test for Calcium oxalates: Powdered sample of the plant was cleared using chloral hydrate solution, which was placed on a glass slide. To the cleared sample, cover slip was applied and this was observed under the microscope. Two drops of hydrochloric acid was then added and also observed under the microscope and dissolution of shining crystals on the sample indicated the presence of calcium oxalates.

3.3.2.3 Test for Tannins: A single drop of ferric chloride was added to the cleared sample and cover slip was placed and this was observed under the microscope. The appearance of greenish black coloration on some cells indicated the presence of tannins.

3.3.2.4 Test for Starch: Two drops of N/50 iodine solution were added to the cleared sample and cover slip was placed and this was observed under the microscope and the appearance of

blue-black coloration in some parenchyma cells of the powdered leaf indicated the presence of starch.

3.4 Determination of Physicochemical Constants of the Powdered Leaf of *Leucas martinicensis*

The methods adopted for the physicochemical examination of the powdered leaf, were described by WHO, (2011).

3.4.1 Determination of Moisture Content

The moisture content was determined by “Loss on drying” method (gravimetric determination). Air-dried leaf (3 g) was weighed using KERN EW Electronic Balance and in a dried and weighed crucible. The crucible was transferred into a hot air sterilizing oven, which was set at 105⁰C. After an hour, the crucible was removed and placed in a desiccator over phosphorous pentoxide and under atmospheric pressure at room temperature. After 30 minutes in the desiccator, the weight of the powder and crucible were quickly determined and the crucible returned to oven. The heating and weighing were repeated until a constant weight was obtained and noted. Three determinations were conducted and the average of these was taken as the moisture content of the drug. The moisture content (loss of weight) was calculated using the following formula:

$$\% \text{ Moisture content} = \frac{\text{Initial Weight of Powder} - \text{Final Weight of Powder}}{\text{Initial Weight of Powder}} \times 100$$

3.4.2 Determination of Total Ash Value

A platinum crucible was heated red hot, cooled in a desiccator and quickly weighed. Exactly 2g of the air-dried leaf powder was weighed into the previously heated crucible. It was ignited by

gradually increasing the heat, until it became white, indicating absence of carbon. It was cooled in a desiccator and weighed. The procedure was repeated three times to obtain average value. The total ash content of the air-dried powder was calculated in percentage, using the following formula:

$$\% \text{ Ash Value} = \frac{\textit{Weight of Residual Ash}}{\textit{Original Weight of Powder}} \times 100$$

3.4.3 Determination of Acid-insoluble Ash

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

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

3.4.4 Determination of Water Soluble Ash

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

$$\% \text{ Water Soluble Ash} = \frac{\text{Weight of Total Ash} - \text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \times 100$$

3.5 Extractive Values of the leaves of *Leucas martinicensis*

Determinations of water and ethanol soluble extractives were determined by using some common methods as follows:

3.5.1 Water Extractive Value of *Leucas martinicensis* leaves

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

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

3.5.2 Ethanol Extractive Value of *Leucas martinicensis* leaves

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

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

3.6 Extraction of the Leaf of *Leucas martinicensis*

The powdered plant material (1 Kg) was extracted using Soxhlet apparatus with aqueous methanol. The filtrate was then concentrated to dryness and stored properly in a dessicator for further use (Fig.3.1).

3.6.1 Fractionation of Extract of *Leucas martinicensis* leaves

The extract was dissolved in distilled water then transferred into a separating funnel and partitioned with 300 ml of Hexane. The hexane portion was collected into a clean and dried conical flask. The aqueous portion was then partitioned twice with 300 ml ethyl acetate. On equilibration, the ethyl acetate portion was collected in a clean and dried conical flask.

The aqueous portion was then evaporated to dryness over water bath, transferred into sample bottles, label appropriately and stored for further use.

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

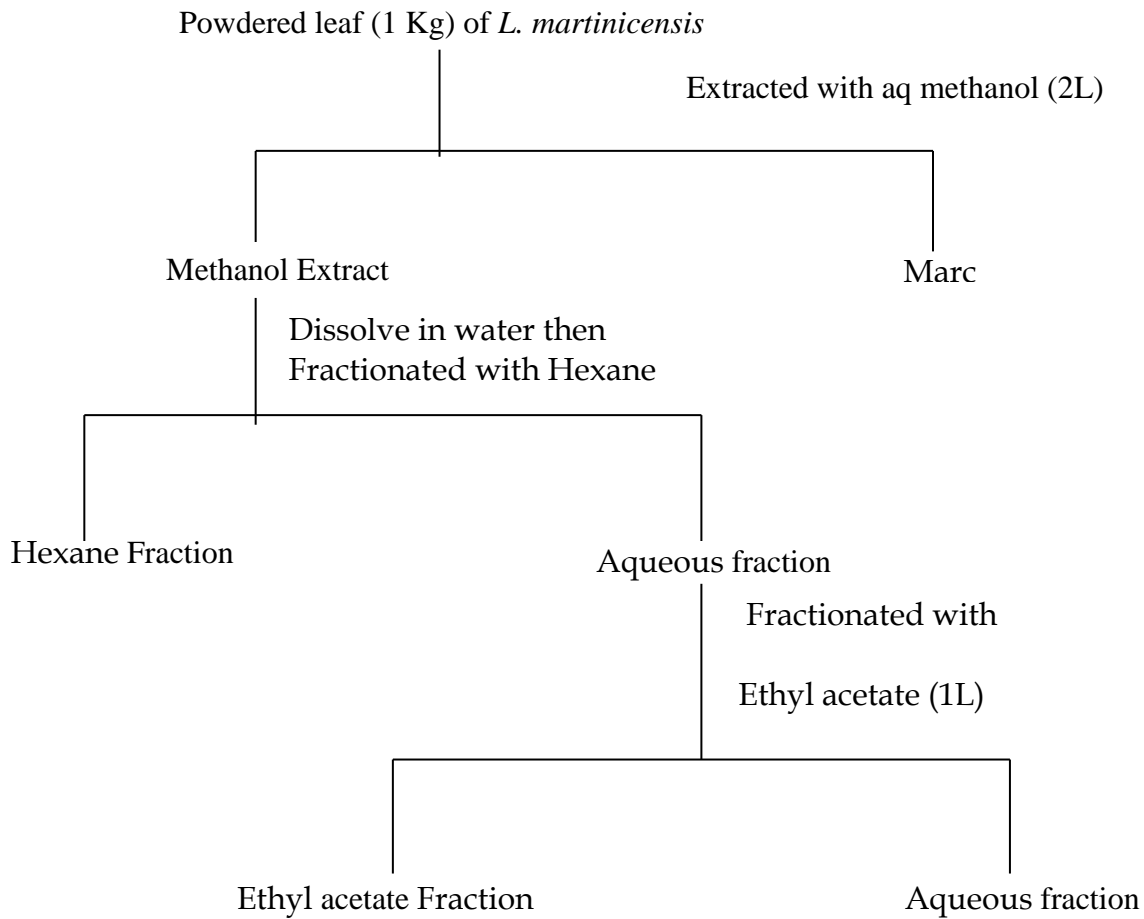


Fig. 3.1: Extraction Chart of *Leucas martinicensis* leaves (Kokate, 1994).

3.7 Phytochemical Screening of the leaf Extract of *Leucas martinicensis*

The leaf extract and fractions (methanol, hexane, ethyl acetate and aqueous) of the plant were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the method described by (Musa, 2005; Sofowora, 2008; Evans, 2009,).

3.7.1 Tests for Saponins

3.7.1.1 Frothing test: About 0.5 g of the extract and fraction (methanol, hexane, ethyl acetate, and aqueous) were separately dissolved in 10 ml of water and shaken vigorously in a test tube for 30 seconds and allowed to stand for 30 minutes. The occurrence of frothing column or honey comb-like of at least 1 cm in height and persisting for at least an hour indicates the presence of saponins (Sofowora, 2008).

3.7.1.2 Haemolysis test: 2 ml of sodium chloride (1.8% solution in distilled water) were added to two test tubes A and B. 2 ml of distilled water were added to test tube A, 2 ml of the extracts were added to test tube B. 5 drops of blood were added to each tube and the tubes were inverted gently to mix the contents. Haemolysis in tube B containing the extracts but not in tube A (i.e. control), indicate the presence of saponins in the extracts (Brain and Turner, 1975). The procedure was repeated for all the organic solvent fractions.

3.7.2 Test for Steroids/ Triterpenes

3.7.2.1 Lieberman-Burchard test: About 1 ml of acetic anhydride was added to 0.5 g of the extract or fraction (hexane, ethyl acetate, and aqueous). Two drops of sulphuric acid were then added gently by the side of the test tube to the solution above and at the junction of the two

liquids a formation of reddish brown or violet brown ring, the upper layer bluish green or violet indicates the presence of sterols and triterpenes (Evans, 2009).

3.7.2.2 Salkowski test: About 2 ml of chloroform and two drops of sulphuric acid were carefully added to about 0.5 g of the extract or fraction (hexane, ethyl acetate, and aqueous) from the side of the test tube to form a lower layer. A reddish brown coloration at the interface indicated the presence of steroidal ring (Sofowora, 2008).

3.7.3 Test for Flavonoids

3.7.3.1 Shinoda test: About 0.5 g of the extract or fractions (hexane, ethyl acetate, and aqueous) was dissolved in 2 ml of 50% methanol. Two drops of magnesium filings and 3 drops of hydrochloric acid were added and the pink coloration indicated the presence of flavonoids (Evans, 2009).

3.7.3.2 Sodium hydroxide test: About 0.5 g of the extract or fractions (hexane, ethyl acetate, and aqueous) was dissolved in water and filtered; 2ml of 10% aqueous sodium hydroxide solution was then added. The solution was observed for the presence of yellow color, a change in color from yellow to colorless on addition of dilute hydrochloric acid was used as an indication for the presence of flavonoids (Evans, 2009).

3.7.4 Tests for Tannins:

3.7.4.1 Ferric chloride test: Two grams (2 g) of the extract and fractions (hexane, ethyl acetate, and aqueous) were dissolved in 10 ml of water each and filtered. Two drops of ferric chloride solution were added to the filtrate. Appearance of blue or green precipitate indicates the presence of phenolic nucleus (Musa, 2005).

3.7.4.2 Lead sub-acetate test: To 2 g of the extract and fractions (hexane, ethyl acetate , and aqueous), two drops of lead sub-acetate solution were added and appearance of whitish-yellow precipitate indicated the presence of tannins (Musa, 2005).

3.7.5 Test for alkaloids:

The extract of the plant was dissolved in 2 N HCl. The mixtures were filtered and the filtrates were divided into 3 equal portions. One portion was treated with few drops of Dragendorff's reagent; one portion was treated with equal amount of Mayer's reagent and the other portion was treated with an equal amount of Wagner's reagent. Appearance of orange, cream and reddish brown precipitates respectively indicated the presence of alkaloids (Salehi-Surmaghi *et al.*, 1992). The procedure was repeated for all the organic solvent fractions.

3.7.6 Test for Cardiac glycosides

3.7.6.1 Keller-killiani test: About 0.5 g of the extract and fractions (hexane, ethyl acetate , and aqueous) was dissolved in glacial acetic acid containing ferric chloride and one drop of sulphuric acid was added to the solution, the appearance of reddish-brown coloration indicated the presence of cardiac glycoside or cardenolides (Sofowora, 2008).

3.7.7 Test for Anthraquinone

3.7.7.1 Bontrager test: About 0.5 g of the extract and fractions (hexane, ethyl acetate , and aqueous) was added to 10 ml of chloroform and shaken. This was then filtered and 5 ml of 10% ammonia solution was added to the Filtrate; stirred and the reaction was observed. The presence of pink or cherish red color indicated the presence of anthracenes derivatives (Evans, 2002).

3.7.7.2 (ii) *Modified Borntrager's test*: About 0.5 g of the extract and fractions of (hexane, ethyl acetate and aqueous) was boiled with 10 ml of aqueous sulphuric acid and filtered hot. After cooling to room temperature, it was shaken with 5 ml chloroform. The chloroform layer was separated and 10% ammonium hydroxide was added to half of its volume. A pink, red or violet coloration in the ammonia phase (lower phase) indication for the presence of combined anthraquinone or anthraquinone derivatives (Evans, 2002).

3.7.8 Test for Cyanogenetic glycoside (Grignard Reaction Test)

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

3.8. Evaluation of the Antiulcer Activity of the *Leucas martinicensis* Leaf Extract

3.8.1 Experimental Animals

Albino Wistar Rats of both sexes weighing 100 - 200 g were obtained from the animal house Veterinary Research Institute Vom, Plateau State. The animals were maintained under standard conditions (12 hours light /12 hours dark cycle, temperature of about $37 \pm 2^{\circ}\text{C}$, 35 - 60% humidity). The rats were fed with standard (grower) mash (Vital Feed, Jos, Nigeria) and water *ad-libitum* (Nwafor, 2000).

3.8.2 Acute Toxicity Study (LD₅₀) of the Plant Extract of *L. martinicensis* leaf

Toxicological evaluation was carried out in experimental animals using OECD method of evaluation to predict toxicity and to provide guidelines for selecting a “safe” dose in animals. It is also used to estimate the degree of toxicity in laboratory animals. The extract was administered orally to three different groups of animals of three per group. The first group of three rats were administered 3,000 mg/kg of the extract, where 24 hours toxicity was recorded for the identification of the toxic dose. The same procedure above was repeated for the other two groups and all the animals were kept for 14 days.

3.8.3 Gastroprotective effect of *Leucas martinicensis* using the Indomethacine induced ulcer model

The modified method of Nwafor, (2000) was adopted in this experiment. Twenty five rats were randomly divided into 5 groups of 5 rats each. The rats were fasted for 48 hours before the commencement of the experiment with the rats having only free access to water (Weisher and Thiemer, 1983). This have been proved to be non-ulcerogenic and sufficient for absolute emptying of stomach as reported (El-Sokkary *et al.*, 1991; Magaji *et al.*, 2007; Okasha *et al.*, 2008; Magaji *et al.*, 2010). Group 1 rats were pretreated with 1 ml of water orally using an orogastric cannula. In the same way, group 2, 3 and 4 were pretreated with methanol extract 300 mg/kg, 600 mg/kg and 900 mg/kg respectively. While 5 was treated with omeprazole 20 mg/kg body weight. After 30 minutes, gastric lesion was induced in all the 5 groups with 20mg/kg indomethacine by orogastric intubation. After an interval of 6 hours the rats were sacrifice by cervical dislocation, their stomachs were removed and open along the greater curvature. Each

stomach was gently rinsed with water to remove the gastric content and blood clots for subsequent ulcer scoring. The same procedure was repeated for the organic solvent fractions.

3.8.4 Measurement of Ulcer Index

The ulceration was examined under a dissecting lens. The ulcerated surface in each stomach was measured with a transparent millimeter scale rule and the result for each group was expressed in mm of mean ulcer \pm SEM (Standard error of mean) (Scepovic and Radmanovic, 1984; Okasha *et al.*, 2008). The percentage inhibition was calculated using the formula; (Kayode *et al.*, 2009).

$$\% \text{ inhibition} = \frac{\text{U.I. (Ulcer control)} - \text{U.I. (Treated)}}{\text{U.I. (Ulcer control)}} \times 100$$

3.9 Thin Layer Chromatographic Profile of the Plant Extract and Fractions of *Leucas martinicensis* leaf

TLC aluminum sheet of 20 by 20cm silica gel 60 F₂₅₄ pre-coated plates using the one way ascending technique was employed for the analysis. The extract and fractions were dissolved in the initial extraction solvent. The plates were cut into size of 5×10cm and spots were applied manually on the plates using capillary tube after which plates were dried and developed in solvent systems for Hexane and ethyl acetate fractions, Hexane : Ethyl acetate (7:3), (8:2), were used. Butanol: Acetic acid: Water (10:1:1), (6:1:1) were used for methanol extracts and aqueous fraction. Developed plates were sprayed using general detecting reagent (*p*-Anisaldehyde/H₂SO₄) and specific detecting reagents (Borntragers, Dragendorf, Ferric chloride, Lieberman-Buchard and aluminium chloride) and heated at 110 °C where applicable. Number of spots, colors and

retardation factors (R_f values) for each of the spots were determined and recorded (Gennaro, 2000; Stahl, 2005).

3.10 Statistical analysis

The results were expressed as mean \pm standard errors of the mean (SEM) for all values. The data were statistically analyzed using one-way ANOVA followed by Dunnett's *post hoc*. All results were presented in tables, figured and plates and considered to be significant when p values are less than 0.05 ($p < 0.05$).

CHAPTER FOUR

4.0 RESULTS

The leaf of *L. martinicensis* to be greenish in colour, with a minty odour, the taste is aromatic and appearance is hairy these are important in the identification of the plant (Table 4.1).

Table 4.1 Pharmacognostic studies of *Leucas martinicensis* leaf

Evaluative features	Characteristics
Colour	Green
Odour	Minty
Taste	Aromatic
Appearance	Hairy

The epidermal cells present in the plant *L. martinicensis* were wavy and the type of stomata was diacytic with multicellular covering trichomes with their size in micrometer as shown in the table below.

Table 4.2: Microscopic Features of the Epidermis of the Leaf of *L. martinicensis*

Characters	Observation	
Epidermal cells	Wavy	(6.34 μm X 3.57 μm)
Stomata	Diacytic stomata	(3.65 μm X 2.87 μm)
Trichome	Multicellular covering Trichomes	(53.3 μm X 6.11 μm)

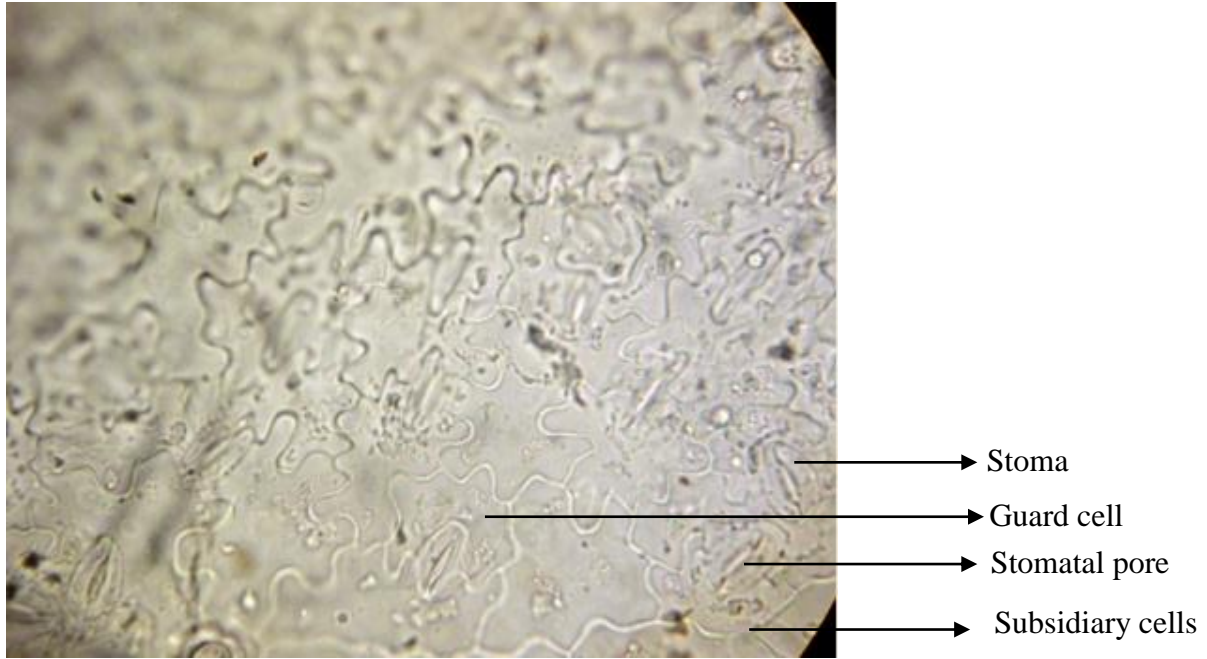


Plate II: Photomicrograph of lower leaf surface of *Leucas martinicensis* (X 100)

The Transverse section of *L. martinicensis* with some important characters which include, trichomes, phloem, xylem and Collenchymas tissues.

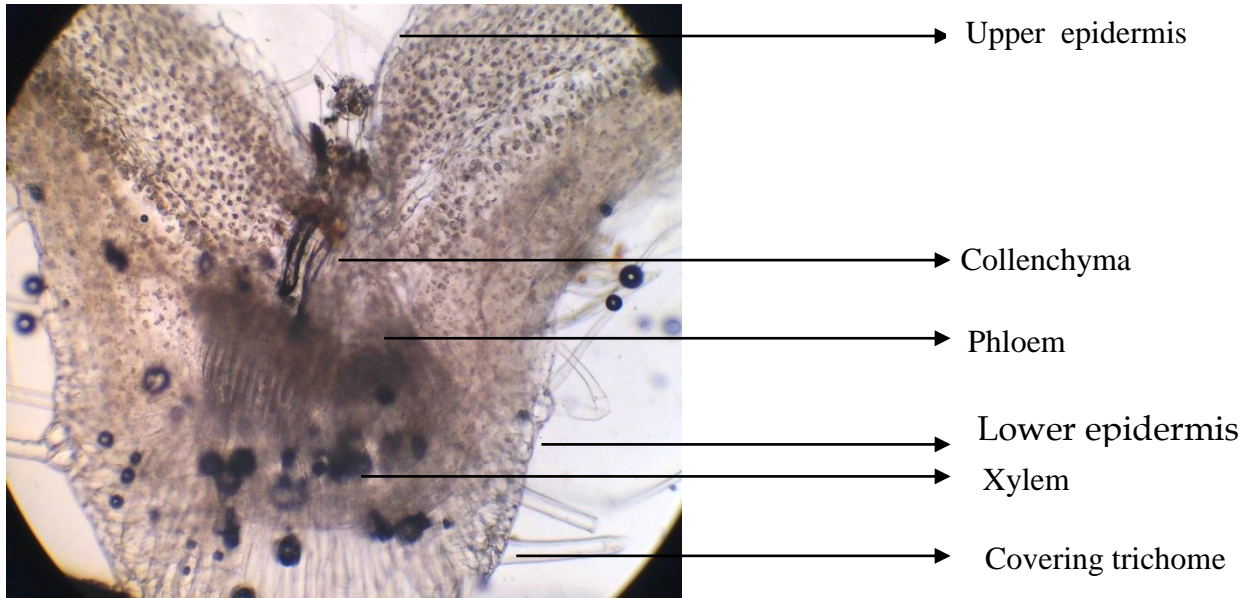


Plate III: The photomicrograph of the transverse section of *Leucas martinicensis* leaf
(×400)

The leaves of *L. martinicensis* were observed to have a stomatal number of 12.47-**14.67**-16.87, stomata index 21.60 – **25.41** – 29.22, palisade ratio 6.61 – **7.78** – 8.95, veinlet termination number 2.13 – **2.5** – 2.88 and vein islet number 6.12 – **7.20** – 8.28 respectively (table 4.3).

Table 4.3: Quantitative Microscopical Values for the Leaf of *L. martinicensis*

Evaluative Parameter	Values*
Stomatal number	12.47 – 14.67 – 16.87
Stomatal index	21.60 – 25.41 – 29.22
Palisade ratio	6.61 – 7.78 – 8.95
Veinlet termination number	2.13 – 2.5 – 2.88
Vein islet number	6.12 – 7.20 – 8.28

*Mean value of 5 counts

The extract and fractions of *L. martinicensis* after conducting preliminary phytochemical screening revealed the presence of some phytochemicals.

Methanol extracts also revealed the presence of saponins, steroids/triterpenes, flavonoids, tannin, alkaloids and cardiac glycosides.

Ethyl acetate fraction (LMEAF) revealed saponins, steroids/triterpenes, flavonoids, alkaloids and cardiac glycosides.

Aqueous fraction (LMAF) contains saponins, flavonoids, tannins and alkaloids and the n-Hexane fraction (LMHF) only steroids/triterpenes present while anthraquinones was absent in the extract and in the organic solvents fractions. (Table 4.4)

Table 4.4: Results of Preliminary Phytochemical Screenings of Extract and Fractions of *Leucas martinicensis* leaf

Phytoconstituents	LMME	LMEAF	LMAF	LMHF
Saponins	+	+	+	-
Steroids/Triterpene	+	+	-	+
Flavonoids	+	+	+	-
Tannins	+	-	+	-
Alkaloids	+	+	+	-
Cardiac glycosides	+	+	-	-
Anthraquinone	-	-	-	-

Key: Present (+), Absent, (-)

Chemo-microscopical examination of the powdered leaf of *L.martinicensis* using appropriate detecting reagents revealed the presence of cellulose cell wall, lignified cell wall, tannins, starch, cutin but mucilage, calcium carbonates and calcium oxalates were absnt.(Table.4.5)

Table 4.5: Results of Chemomicroscopical Features of *Leucas martinicensis* Powdered leaves

Constituents	Detecting reagents	Observation	Inference
Starch	N/50 iodine	Blue-black colour on some grains within the cell.	Starch present
Lignin	Phloroglucinol	Pink/cherry red colour on some lignified cell.	Lignin present
Tannins	5% FeCl ₃	Greenish-black colour in some parenchyma cells.	Tannins present
Calcium oxalate	HCl	Dissolution of shining crystals on the anatomical sections of the leaf.	Crystal present (Prism type)
Calcium carbonate	HCl	No Effervescence in the cell.	CaCO ₃ absent
Cellulose	Chlor-Zinc- Iodine	Blue,violet coloration of the cell wall	Cellulose present
Mucilage	Ruthenium red	Transparent dilated fragments on black background	Mucilage absent
Suberin or Cutin	Sudan red	Orange red colour on cell wall.	Suberin or cutin present

The average moisture contents using loss on drying method was calculated to be $8.9\% \pm 0.59$ and the percentage yield of total ash, acid insoluble and water soluble matter were recorded in percentage values as $6.8\% \pm 0.01$, $3.5\% \pm 0.01$ and $6.0\% \pm 0.003$ respectively. The extractives obtained were $16.0\% \pm 0.33$ and $20.3\% \pm 0.33$ for alcohol and water respectively. (Table 4.6).

Table 4.6: Results of Physicochemical Constants of Powdered leaf of *Leucas martinicensis*

Parameters	Values (%w/w) \pm SEM*
Moisture content	8.9 ± 0.59
Total ash value	6.8 ± 0.01
Acid Insoluble ash	3.5 ± 0.01
Water Soluble ash	6.0 ± 0.003
Ethanol Extractive	16.0 ± 0.33
Water Extractive	20.3 ± 0.33

*Average values of three determinations, SEM; Standard error of mean

1000 g of the powdered leaf of *L. martinicensis* were extracted with methanol using soxhlet apparatus after which it was evaporated. The extract was dissolve in water and fractionated with n-hexane and ethyl acetate and yielded the following results (Table 4.7).

Table 4.7: Yield of extract and organic solvents fractions of *L. martinicensis*

S/n	Crude Extracts	Mass (g)	Percentage Yield (%w/w)
1.	LMME (methanol)	122.73	12.27
2.	LMEAF (ethyl acetate)	3.54	0.36
3.	LMAF (aqueous)	70.35	7.04
4.	LMHF (n-hexane)	24.66	2.47

Key:

LMME = *L.martinicensis* Methanol Extract

LMEAF =*L. martinicensis* Ethyl acetate Fraction

LMHF = *L. martinicensis* Hexane Extract

LMAF = *L.martinicensis* Aqueous Fraction

4.2 Results of Acute toxicity test of the extract of *Leucas martinicensis* leaf

No mortality and no signs of toxicity were found at the dose of 3000 mg/kg body weight of *L. martinicensis* methanol extracts. Therefore, it might be considered that methanol extracts of *L. martinicensis* have LD₅₀ value above 3000 mg/kg. The doses 300, 600 and 900 mg/kg were selected for present study.

4.3 Antiulcer Activity of *Leucas martinicensis* leaf

The antiulcer activity of methanol extract, Ethyl acetate fraction, Hexane fraction and Aqueous fractions were carried out. The doses used were 300, 600 and 900 mg/kg for the extract and each of the fractions using laboratory rats, with indomethacine 20 mg/kg as the inducing agent and Omeprazole 20 mg/kg for positive control.

4.4 Ulcer index of *Leucas martinicensis* leaf extract and organic solvents

The methanol extract of *L. martinicensis* was observed to produce remarkable dose dependent protection on the rats gastric walls against indomethacine induced ulcer. While the control animals showed a mean ulcer index of 10.4 ± 0.97 , animals that received 300 mg/kg of the extract had mean ulcer index of 8.20 ± 2.56 . Although this inhibition was not statistically significant, highest dose of 900 mg/kg significantly ($p < 0.05$) reduced the mean ulcer index to 2.20 ± 0.92 . This effect was comparable to 2.60 ± 0.81 mean ulcer index produced by 20 mg/kg of omeprazole (Fig 4.1). The extract (900 mg/kg) can be said to produce ulcer index of 75% and 79% respectively.

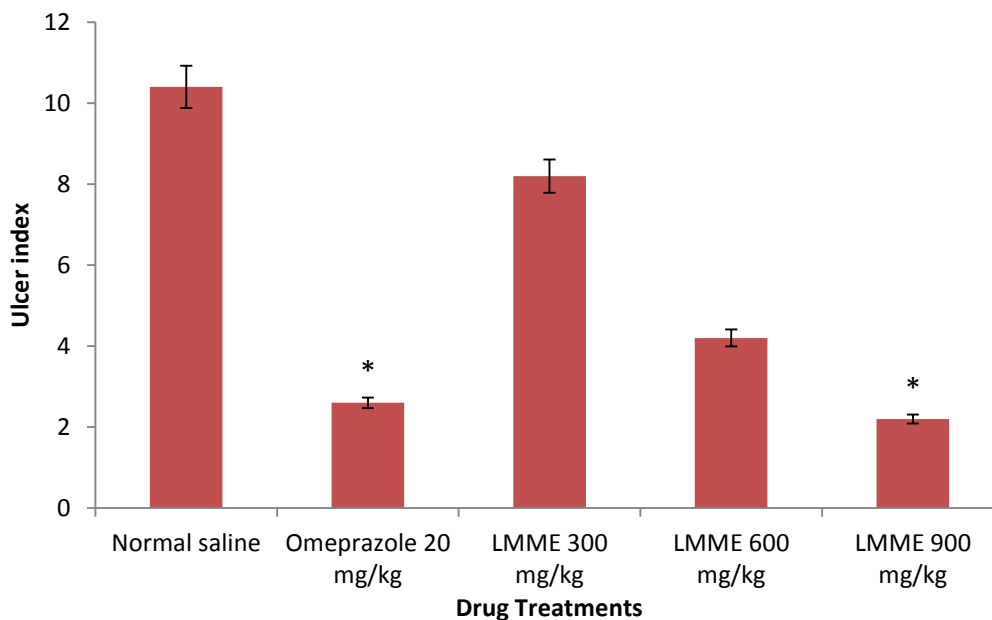


Fig 4.1: Effects of *L. martinicensis* Methanol extracts on Indomethacine induced Gastric Ulcer in rats

Key: LMME, *Leucas martinicensis* methanol extract

The ethyl acetate fraction of *L. martinicensis* was observed to produce dose dependent protection on the rats gastric walls against indomethacine induced ulcer. While the control animals showed a mean ulcer index of 10.4 ± 0.97 , animals that received 300 mg/kg of the fraction had mean ulcer index of 7.60 ± 2.27 . Although this inhibition was not statistically significant even at the highest dose of 900 mg/kg with mean ulcer index of 3.20 ± 1.77 . The omeprazole 20 mg/kg and the fraction (900 mg/kg) can be said to produce ulcer index of 75% and 69% respectively.

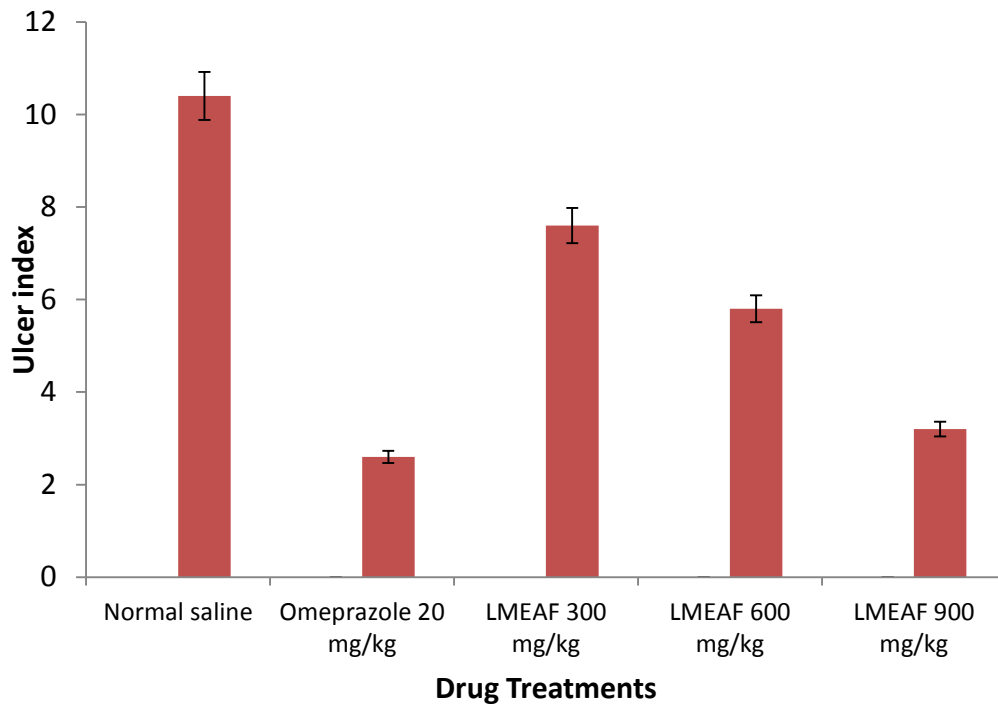


Fig 4.2: Effects of *L. martinicensis* Ethyl acetate fraction on Indomethacine induced Gastric Ulcer in rats

Key: LMEAF, *Leucas martinicensis* ethyl acetate fraction

The aqueous fraction of *L. martinicensis* was observed not to produce dose dependent protection on the rats gastric walls against indomethacine induced ulcer. While the control animals showed a mean ulcer index of 10.4 ± 0.97 , animals that received 300 mg/kg of the fraction had mean ulcer index of 7.00 ± 2.59 . This inhibition was not statistically significant even at the highest dose of 900 mg/kg with mean ulcer index of 7.80 ± 2.58 . The omeprazole 20 mg/kg and the aqueous fraction (900 mg/kg) can be said to produce ulcer index of 75% and 25% respectively.

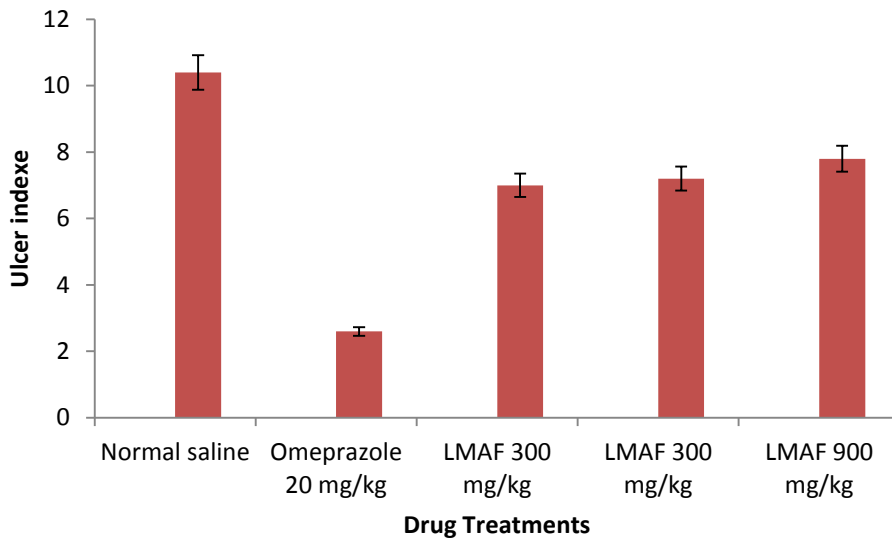


Fig 4.3: Effects of *L. martinicensis* Aqueous fraction on Indomethacine induced Gastric ulcers in rats

Key: LMAF, *Leucas martinicensis* Aqueous fraction

The Hexane fraction of *L. martinicensis* was observed not to produce dose dependent protection on the rats gastric walls against indomethacine induced ulcer. While the control animals showed a mean ulcer index of 10.4 ± 0.97 , animals that received 300 mg/kg of the fraction had mean ulcer index of 6.8 ± 2.67 . This inhibition was not statistically significant even at the highest dose of 900 mg/kg with mean ulcer index of 9.2 ± 2.29 . The omeprazole 20 mg/kg and the Hexane fraction (900 mg/kg) can be said to produce ulcer index of 75% and 25% respectively.

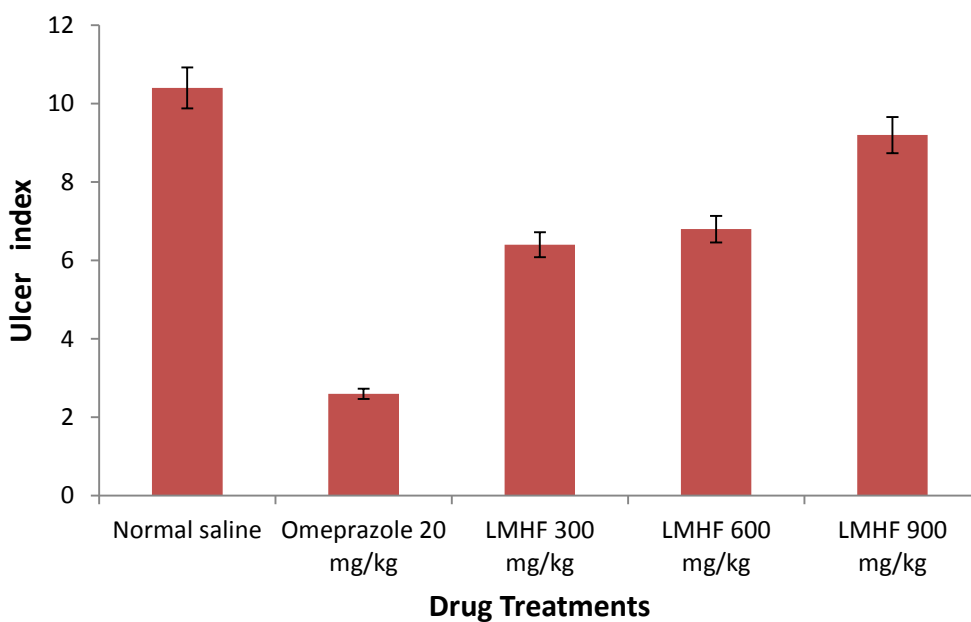


Fig4 .4.4: Effect of *L. martinicensis* Hexane fraction on Indomethacine induced Gastric ulcer in rat.

Key: LMHF, Leucas martinicensis Hexane fraction

4.3 Photomicrographs of Antiulcer Activity of *Leucas martinicensis* leaf

Photomicrographs showing the stomach of Rats after administering Normal Saline ,Omeprazole and different doses of Methanol extracts,Aqueous fraction, Ethyl acetate fraction and Hexane fraction of *L. martinicensis*.

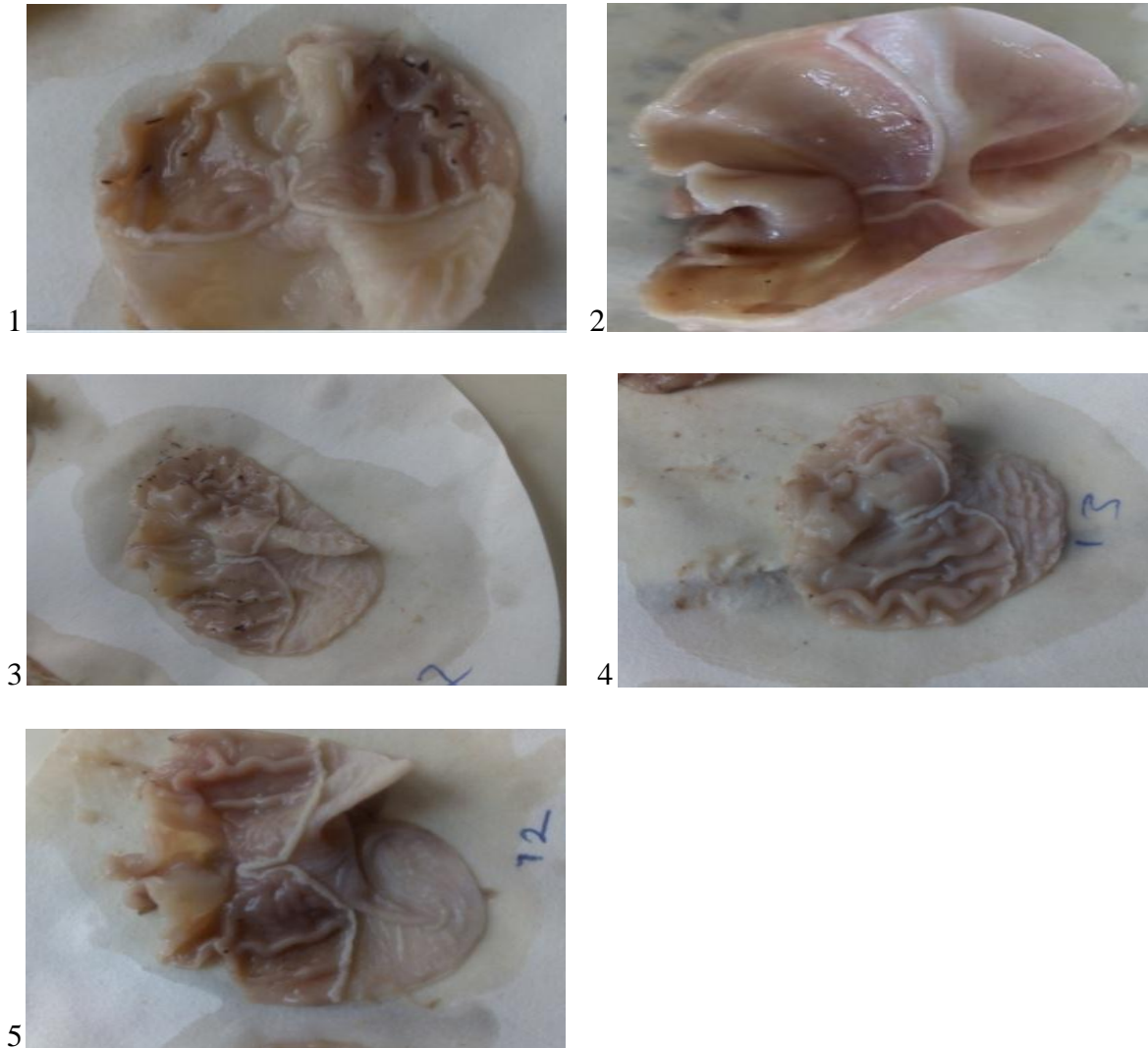


Plate IV: stomach of rats showing ulcer lesions with methanol extract

1. Negative control normal saline
2. Positive control: Omeprazole 20 mg/kg
3. *L. martinicensis* methanol extract 300mg/kg
4. *L. martinicensis* methanol extract 600mg/kg
5. *L. martinicensis* methanol extracts 900mg/kg

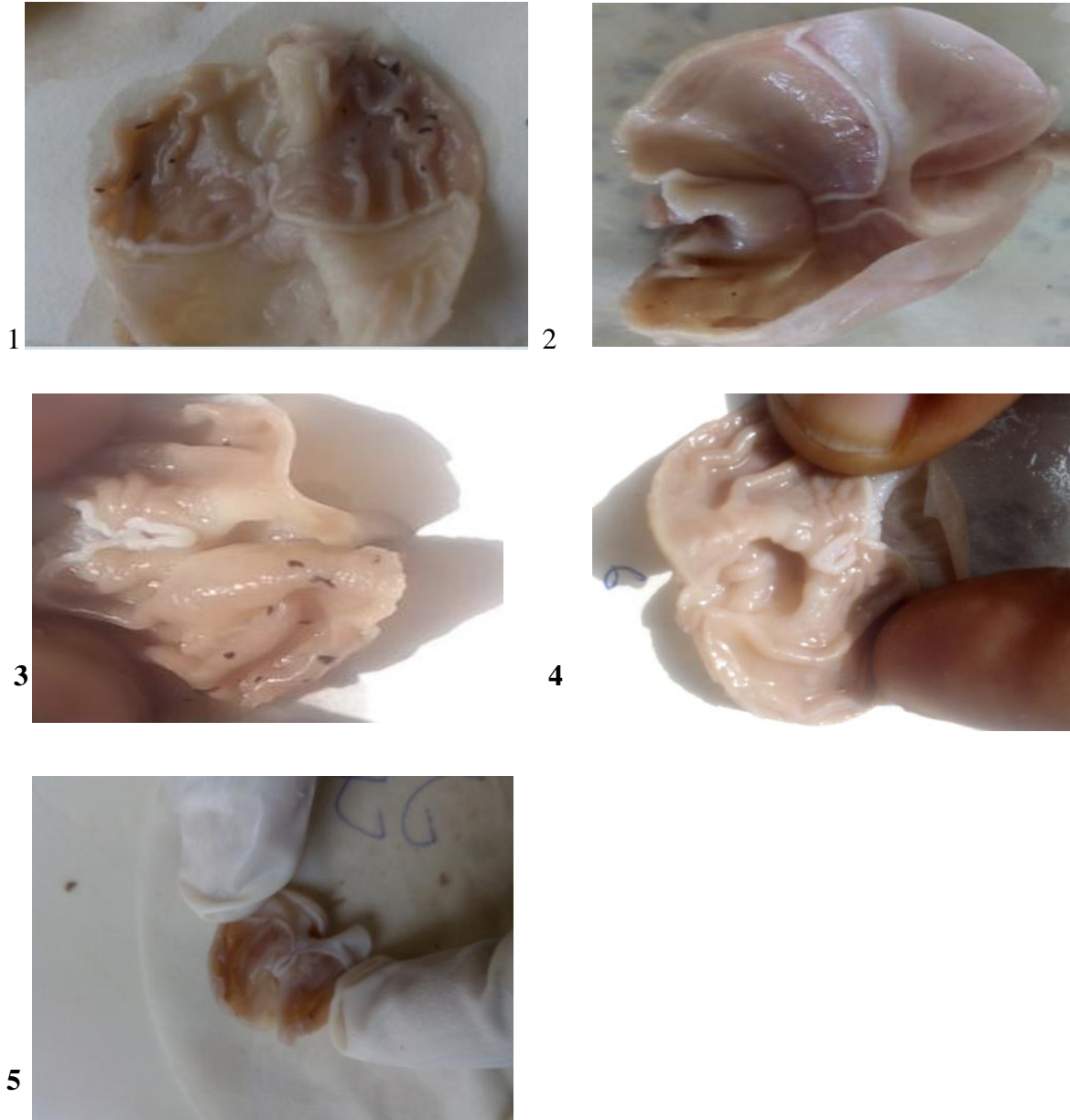


Plate V: stomach of rats showing ulcer lesions

1. Negative control normal saline
2. Positive control: Omeprazole 20 mg/kg
3. *L. martinicensis* ethyl acetate fraction 300 mg/kg
4. *L. martinicensis* ethyl acetate fraction 600 mg/kg
5. *L. martinicensis* ethyl acetate fraction 900 mg/kg

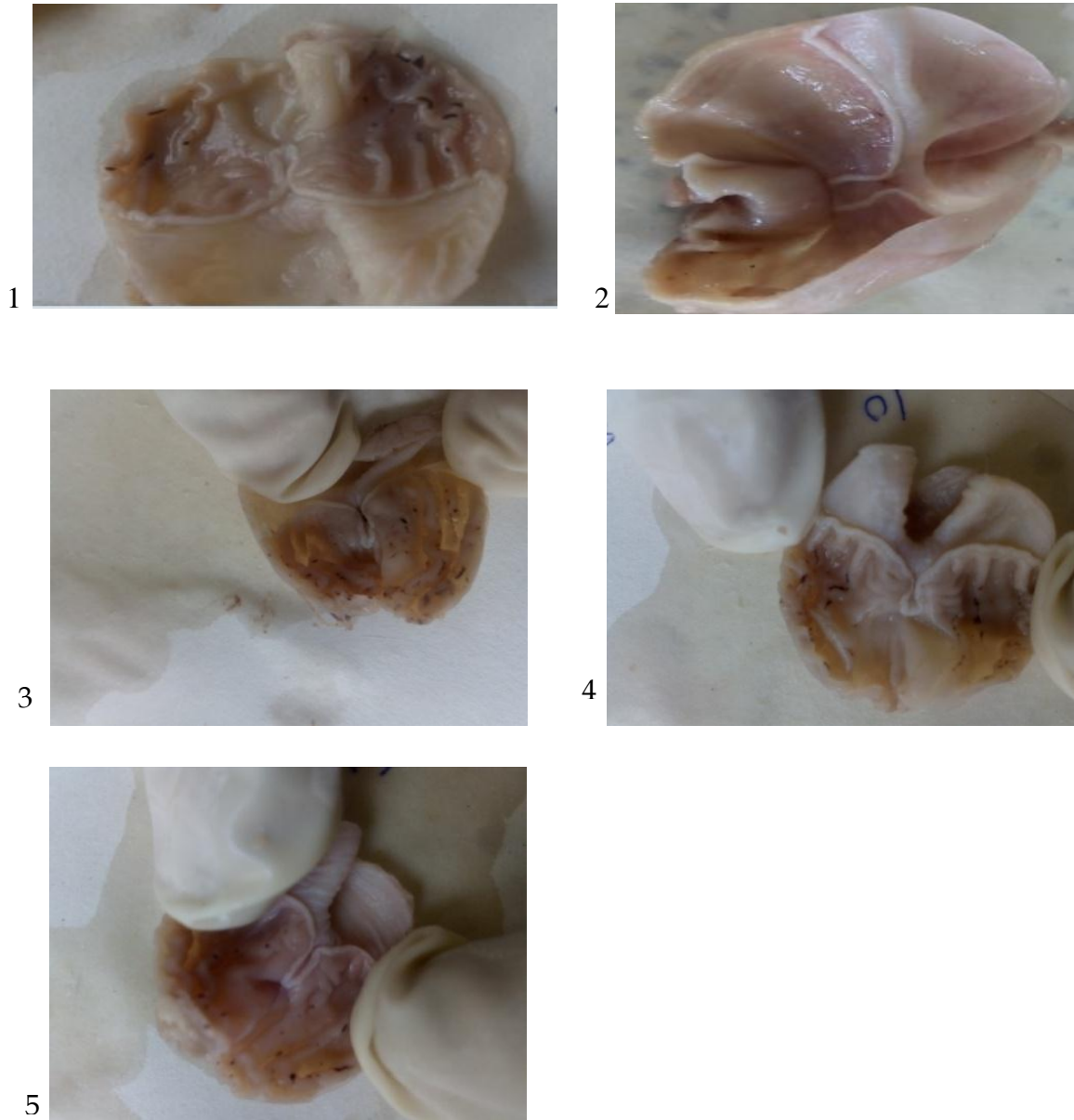


Plate VI: stomach of rats showing ulcer lesions

1. Negative control normal saline
2. Positive control: Omeprazole 20 mg/kg
3. *L.martinicensis* aqueous fraction 300 mg/kg
4. *L.martinicensis* aqueous fraction 600 mg/kg
5. *L.martinicensis* aqueous fraction 900 mg/kg

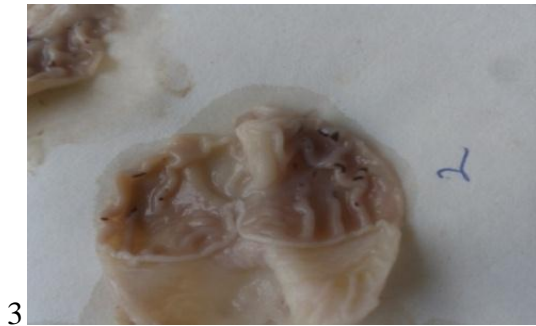


Plate VII: stomach of rats showing ulcer lesions

1. Negative control normal saline
2. Positive control: Omeprazole 20 mg/kg
3. *L.martinicensis* hexane fraction 300 mg/kg
4. *L.martinicensis* hexane fraction 600 mg/kg
5. *L.martinicensis* hexane fraction 900 mg/kg

5.5 Thin Layer Chromatographic Profile of *Leucas martinicensis* leaf extracts

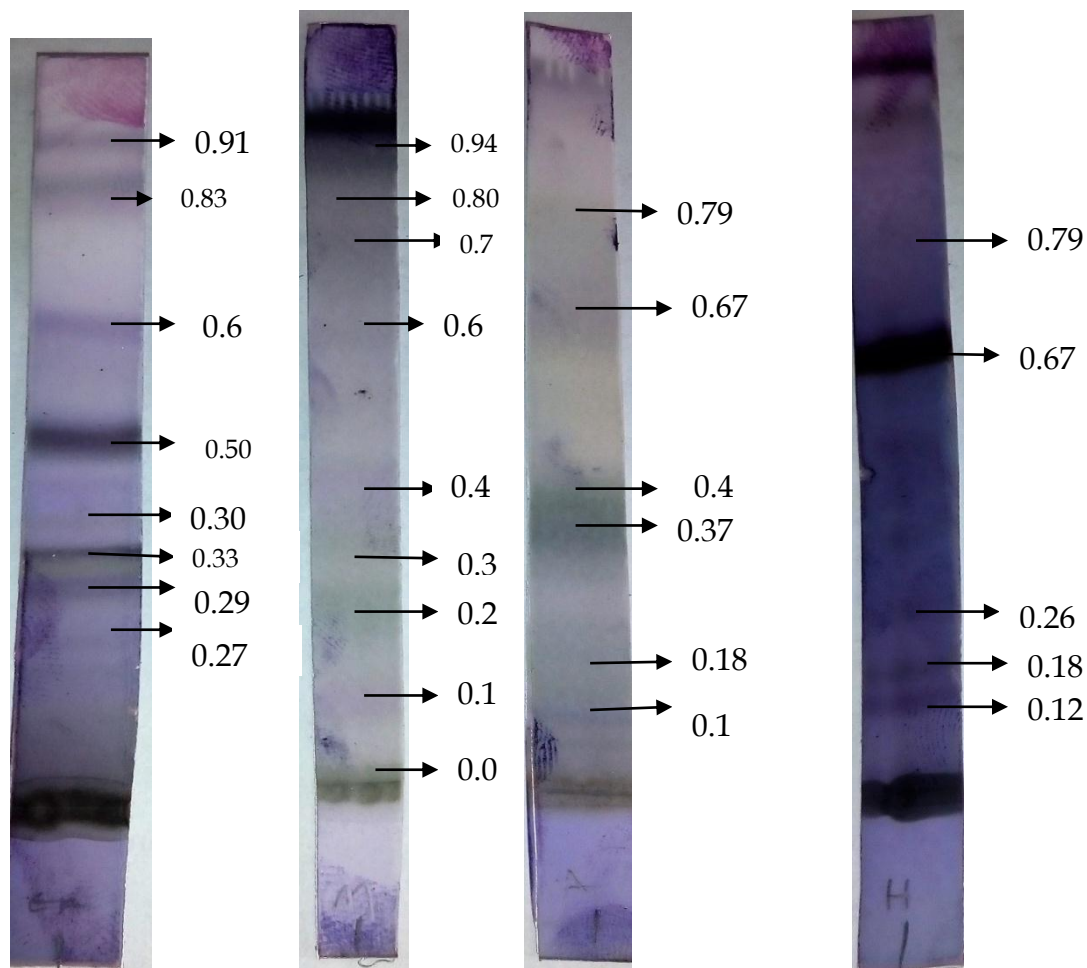


Plate VIII

Plate IX

Plate X

Plate XI

Plate VIII: Chromatogram of Ethyl acetate fraction of *L.martinicensis* leaf on precoated silica plate developed in H: E (7:3) sprayed with *p*-Anisaldehyde/H₂SO₄. Showing about 8 spots with R_f values for I-VIII as 0.27, 0.29, 0.33, 0.36, 0.50, 0.66, 0.83, 0.91, respectively.

Plate IX: Chromatogram of Methanol extracts of *L.martinicensis* leaf on pre-coated silica plate developed in B:A:W (10:1:1) sprayed with *p*-Anisaldehyde/H₂SO₄. Showing about 10 spots with R_f values for I-XI as 0.02, 0.12, 0.23, 0.31, 0.40, 0.62, 0.73, 0.84, 0.88, 0.94, respectively.

Plate X: Chromatogram of Aqueous fraction of *L.martinicensis* leaf on precoated silica plate developed in B:A:W (10:1:1) sprayed with *p*-Anisaldehyde/H₂SO₄. Showing about 6 spots with R_f values for I-VI as 0.13, 0.18, 0.37, 0.43, 0.67, 0.79, respectively.

Plate XI: Chromatogram of Hexane fraction of *L.martinicensis* leaf on precoated silica plate developed in H:E (8:2) sprayed with *p*-Anisaldehyde/H₂SO₄. Showing about 5 spots with R_f values for I-XI as 0.12, 0.18, 0.26, 0.60, 0.76 respectively.

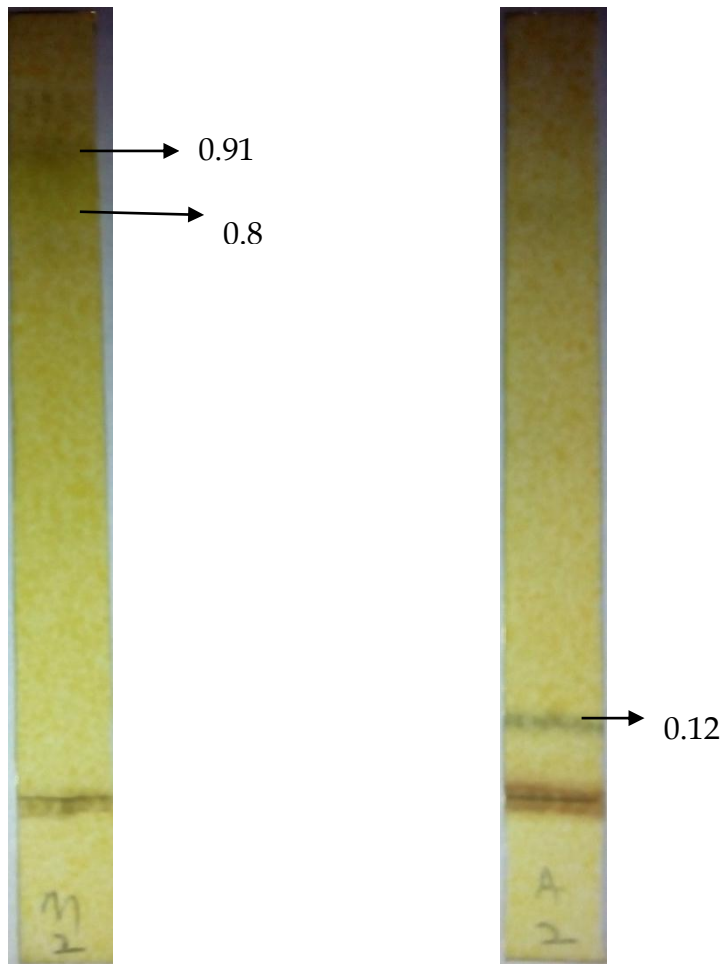


Plate XII

Plate XIII

Plate XII: Chromatogram of Methanol extract on precoated silica plate developed in B: A: W (10:1:1) sprayed with Ferric chloride. Showed about 2 spots with R_f values for I and II as 0.80 and 0.91.

Plate XIII: Chromatogram of Aqueous on precoated silica plate developed in B: A: W (6:1:1) sprayed with Ferric chloride. Showed about one spot with R_f value as 0.12.

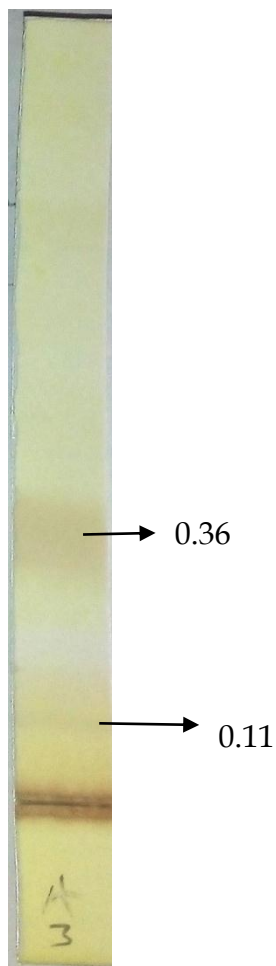


Plate XIV

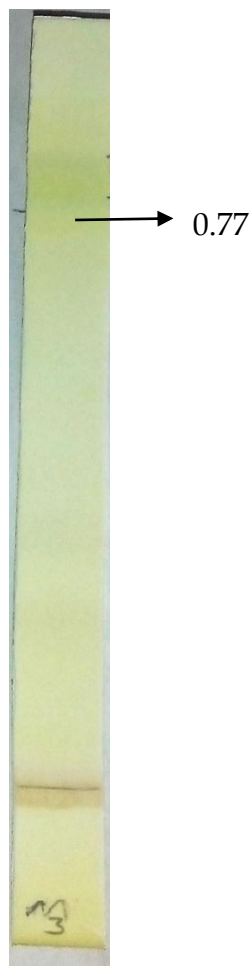


Plate XV

Plate XIV: Chromatogram of Aqueous fraction on precoated silica plate developed in B: A: W (6:1:1) sprayed with Dragendorff spray. Showing 1 spot with R_f values for as 0.11 and 0.36 respectively.

Plate XV: Chromatogram of Methanol extract on precoated silica plate developed in B: A: W (10:1:1) sprayed with Dragendorff spray. Showing about 1 spot with R_f values for as 0.77.

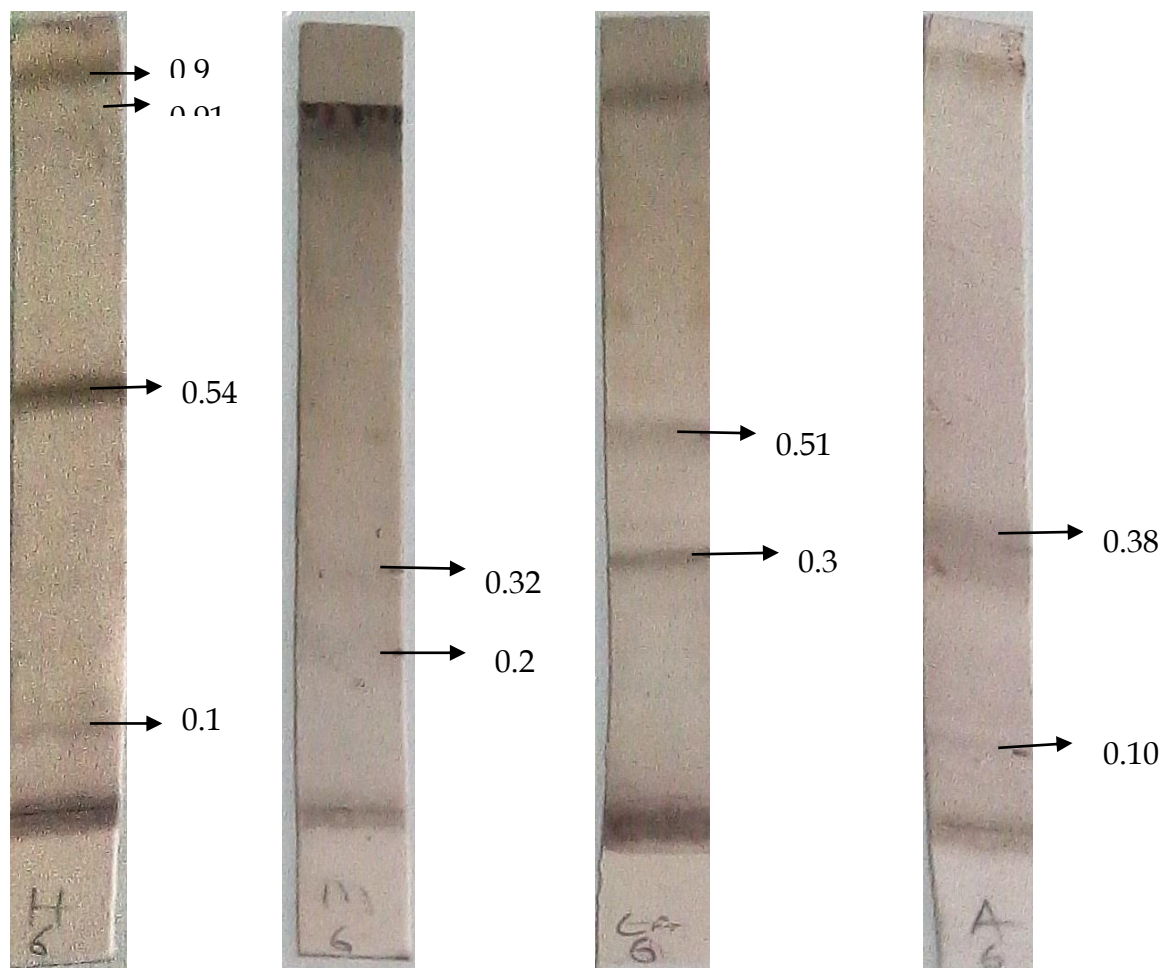


Plate XVI

Plate XVII

Plate XVIII

Plate XIX

Plate XVI: Chromatogram of Hexane fraction on precoated silica plate developed in H: E (8:2) sprayed with Lieberman-Buchard reagent. Showing 4 coloured spots with R_f values for I-II as 0.10, 0.54, 0.91 and 0.94 respectively.

Plate XVII: Chromatogram of Methanol extract on precoated silica plate developed in B: A: W (10:1:1) sprayed with Lieberman-Buchard reagent. Showing 2 spots with R_f values for I and II as 0.21 and 0.32 respectively.

Plate XVIII: Chromatogram of Ethyl acetate fraction on precoated silica plate developed in H: E (7:3) sprayed with Lieberman-Buchard reagent. Showing 2 spots with R_f values for I-II as 0.35, 0.51 respectively.

Plate XIX: Chromatogram of Aqueous fraction on precoated silica plate developed in B: A: W (6:1:1) sprayed with Lieberman-Buchard reagent. Showing 2 spots with R_f values for I-II as 0.10, 0.38 respectively

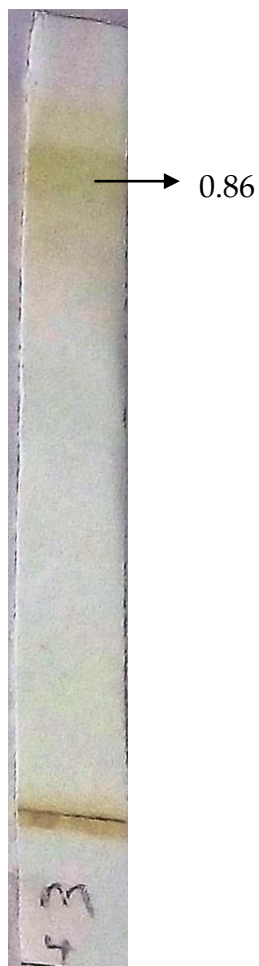


Plate XX

Plate XX: Chromatogram of Methanol extract on precoated silica plate developed in B: A: W (10:1:1) sprayed with Bontragers reagent and heated at 110°C for about 2 minutes. Showed coloured spots with R_f values for I as 0.86.

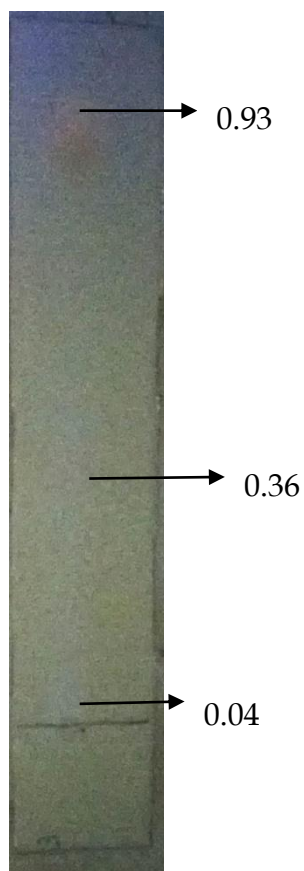


Plate XXI

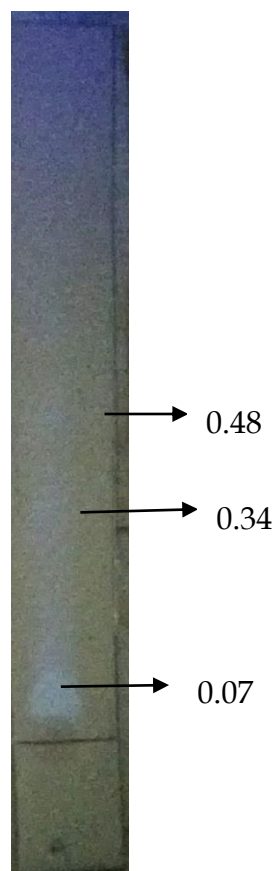


Plate XXII

Plate XXI: Chromatogram of Methanol extract on precoated silica plate developed in B: A: W (10:1:1) sprayed with Aluminium chloride reagent. Showing 2 spots with R_f values for I, II and III as 0.04, 0.36 and 0.93.

Plate XXII: Chromatogram of Aqueous fraction on precoated silica plate developed in B: A: W (6:1:1) sprayed with Aluminium chloride reagent. Showing 3 spots with R_f values for I- III as 0.07, 0.34 and 0.48.

CHAPTER FIVE

5.0 DISCUSSIONS

Microscopic and physicochemical studies are carried out on herbal crude drugs sample in order to establish appropriate data that may be utilized for identification and also to establish the purity and standard of plant sample particularly when supplied in powder form (Ghani, 1990; Kumar *et al.*, 2011). Anatomical features of the internal structures of plant drugs provides an important diagnostic features for the identification of both entire and powdered crude drugs and detection of adulterants in plant materials (Ghani, 1990). Quantitative evaluation is an important parameter in setting standard of crude drugs and the physical constant parameters could be useful in detecting any adulterant in the drug (Musa, 2005). Moisture content (8.9%) is not high which indicated less chances of microbial degradation of the drug during storage. The general requirement of moisture content in crude drug is that, it should not be more than 14% (B. H. P, 1990) and the value obtained in this research work was within the accepted range. Moisture is considered an adulterant because of its added weight as well as the fact that excess moisture is conducive to the promotion of mold and bacterial growth. Low moisture content in a crude drugs suggest better stability against degradation of product (WHO, 1990). The acid insoluble ash values (3.5%) obtained in this study indicated that the plant was in good physiological condition and it contained little extraneous matter such as sand, silica and soil. Total ash value (6.8%) represents both the physiological and non-physiological ash from the plant. The non-physiological ash is an indication of inorganic residues after the plant drug is incinerated. The acid insoluble ash values (3.5%) obtained in this study indicated that the plant was in good physiological condition and it contained little extraneous matter such as sand, silica and soil.

The total ash value is used as criteria to judge the identity and purity of drugs (WHO, 1990, Prasad *et al.*, 2012). Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent.

Physicochemical parameters such as moisture content, total ash content, water soluble ash, acid insoluble ash and extractable matter content serve an important role in standardization and quality control by means of purity and stability of plant drugs (Bharat and Parabia, 2010).

The transverse section of *L. martinicensis* leaf revealed some prominent features like Diacytic Stomata, xylem, phloem; collenchymas cells which help in gaseous exchange, transportation of minerals and water that are needed for the normal functioning of the plants. Anatomical features of the internal structures of plant drugs provides an important diagnostic features for the identification of both entire and powdered crude drugs and detection of adulterants in plant materials (Ghani, 1990). Macroscopical and Microscopical evaluation of crude drugs are important in the identification of right variety and search for adulterant in plant materials (WHO, 2011).

The chemomicroscopical test carried out revealed the presence of cellulose cell wall, lignified tissues, tannins, starch, and cutin but calcium oxalate crystals, calcium carbonates and mucilage were found to be absent. The microscopic structures are most valuable in the identification of powdered drug as their identification is largely based on the form, the presence or absence of certain cell types and cell inclusions (Eggeling *et al.*, 2000).

The compositions of these phytochemicals depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not (Tatiya *et al.*, 2012). This study indicated that the ethanol had low extractive value of (16.0% w/w) compared to

water which had extractive value of (20.3% w/w). Water extractive value was found to be higher than Ethanol extractive value, this may be due to the fact that water is a Universal solvents.

Powdered *L. martinicensis* Leaf were extracted with Methanol this is because methanol have the ability to extracts both polar and non-polar plants constituents also the traditional practitioners uses water for extraction. Then extract was fractionated with n-hexane and ethyl acetate solvents using soxhlet apparatus with the aim of separating its components on the basis of solubility. These could be attributed to the ability of highly polar solvents to attract more of the phytochemical constituents present in a plant material.

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

The information on the presence or absence and the type of phytochemical constituents especially the secondary metabolites are useful taxonomic keys in identifying a particular species and distinguishing it from a related species, thus helping in the delimitation of taxa (Jonathan and Tom, 2008). In addition, certain terpenoids, flavonoids, phenolics, cyanogenic glycosides and non-protein amino acids are illustrated to be of systematic use in particular cases (Jonathan and Tom, 2008).

Acute toxicity studies are done in order to determine the safety margin of drugs and plant products for human use, toxicological evaluation was conducted out in experimental animals using OECD method of evaluation to predict toxicity and to provide guidelines for selecting a “safe” dose in animals and also used to estimate the degree of toxicity. No mortality and no signs of toxicity were found at the dose of 3000 mg/kg body weight of *L.martinicensis* methanol

extracts. Therefore, it might be considered that methanol extracts of *L.martinicensis* have LD₅₀ value above 3000 mg/kg. The doses 300 mg/kg, 600mg/kg and 900 mg/kg were selected for present study.

The antiulcer activities of the methanol extract and fractions (hexane, ethyl acetate and aqueous) of *L. martinicensis* against indomethacine induced ulcers were established in this study. The ulcer inhibitions of the *L. martinicensis* methanol extract at 300, 600 and 900 mg/kg doses were 21%, 59.6% and 79% respectively. The ulcer inhibitions of ethyl acetate fraction 300, 600 and 900 mg/kg doses were 27%, 44.2% and 69.2% respectively. The aqueous fraction at the same doses produced 33%, 31% and 25% inhibitions. The ulcer inhibition of the hexane fraction at the same doses were 39%,35% and 12% respectively. It is remarkable that at 900 mg/kg doses of methanol extracts and ethyl acetate fraction produced a statistically significant ($p < 0.05$) gastro protection than the other fractions but omeprazole has 75% protection, while hexane and aqueous fractions produced no gastro protection when compared with omeprazole. The protection from ulcer was dose dependent.

Methanol extract was observed to have the best activity compared to other fractions. These could be attributed to the presence of flavonoid, saponins, steroids/triterpenes,cardiac glycosides and phenolic compound in the extract. Flavonoids have anti-inflammatory activity and protect the gastric mucosa against a variety of ulcerogenic agents in different mammalian species (Harborne and Williams, 2000). As a result, many studies have examined the antiulcerogenic activities of plants containing flavonoids using either naturally derived or synthetic compounds. Plants containing flavonoids were found to be effective in preventing this kind of lesion, mainly because of their antioxidant properties (Cody *et al.*, 1986, Harborne and Williams, 2000). Tannins are known to ‘tan’ the outermost layer of the mucosa and to render it less permeable and

more resistant to chemical and mechanical injury or irritation (Asuzu, 1990). Several plants containing high amounts of saponins have been shown to possess anti-ulcer activity in several experimental ulcer models (Izzo, 2000). The aetiology of gastric ulceration is multifactorial and non-clearly defined. Pathophysiology of ulcer is due to an imbalance between aggressive factors (acid, pepsin, *H. pylori* and non-steroidal anti-inflammatory agents) and local mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins) (Ode, 2011). To regain the balance, different therapeutic agents including plant extracts are used to inhibit gastric acid secretion or to enhance the mucosal defence mechanisms by increasing mucus production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis (Muralidharan, 2009).

The 900 mg/kg, LMME increased significantly from the negative control ($p < 0.05$), however at 300 and 600 mg/kg it was not significantly different from the negative control ($p > 0.05$). For aqueous fraction and hexane fraction at all the doses tested were not significantly different from the negative control ($p < 0.05$).

Thin layer chromatographic analysis of methanol extracts, n-hexane, ethyl acetate and aqueous fractions from *L. martinicensis* leaf in various developing solvent systems gave different degrees of separations. The chromatogram of hexane and ethyl acetate fractions in hexane: ethyl acetate (8:2) and (7:3) solvent system sprayed with *p*-Anisaldehyde/ H_2SO_4 revealed separation with 5 and 8 spots respectively while the chromatogram of methanol extract and aqueous fraction in butanol : acetic acid : water (10:1:1) revealed separation with 10 and 6 spots respectively. Spots of various colours pink, purple, green and yellow were revealed. The successful separation of bio-molecules by chromatographic technique depends upon suitable solvent system which needs an ideal range of partition coefficient (k) for each target compounds (Ito, 2005).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Pharmacognostic standard for the leaf of *L. martinicensis* was established which include the stomata number, stomatal index, palisade ratio, vein termination number, vein islet number and diacytic stomata was identified as the type of stomata present and covering trichomes was also present. The morphological features showed green colour, minty odour, aromatic taste and hairy appearances. These standards could be used as diagnostic features for proper identification and standardization of *L. martinicensis* in further utilization.

The chemo-microscopical features of powdered leaf of *L. martinicensis* revealed the presence of cellulose cell wall, lignified cell wall, mucilage, tannins, suberin and calcium oxalate crystals which could also be used in standardization of the plant.

The physical parameters (% W/w) of the powdered leaf of *L. martinicensis* were found to be (8.9 %) moisture content, total ash value (6.8 %), acid insoluble ash (3.5 %), water soluble ash (6.0 %), ethanol extractives value (16.0 %) and water extractives value (20.3 %).

Phytochemical analysis of the leaf extract revealed the presence of alkaloids, tannins, flavonoids, cardiac glycosides, saponins and steroids/ triterpenes which could be useful in further isolation and characterization of the active principles.

The median lethal dose (LD₅₀) of the extracts was found to be above 3000 mg/kg when administered orally in rats and considered practically non-toxic.

The extract of *L. martinicensis* leaf showed protective activity against Indomethacine induced gastric ulcer lesions in laboratory rats. This is statistically significant with respect to the

increased preventive index doses (300, 600 and 900 mg/kg) of the extract and fractions. The methanol extract was observed to have the best activity compared to other fractions.

6.2 Conclusion

The present study has established:

Microscopic, chemomicroscopic and physical constants of the leaf of *L. martinicensis* which are important diagnostic features can serve as a basis for the identification and authentication of the plant. The methanol extract was observed to have the highest antiulcer potential compared to other fractions.

The results support the traditional use of the plant in the treatment of ulcer. The presence of pharmacologically active principles such as flavonoids, tannins and saponins may be responsible for the antiulcer activity. This plant may therefore serve as a potential antiulcer agent.

Thin layer chromatographic analysis visualized with specific reagents confirmed the presence of steroids/triterpenes, flavonoids, alkaloids and other phenolic compounds in the extracts.

6.3 Recommendations

- Further work needs to be done to determine, identify, purify and quantify the antiulcer compound within this plant and also to determine its full spectrum of activity.
- Sub-acute and chronic toxicity test should be carried out in order to determine the long-term effects of the extracts.

REFERENCES

- Al- Yousuf, M. H., Bashir, A. K., Blunden, G., Yang, M. H. and Patel, A. V. (1999). . Coumarleucasin and leucasonone from *L. leucas inflata* roots. *Phytochemistry* ;51-95
- Alam, M. B., Hossain, M. S., Chowdhury, N. S., Mazumder, M. E. H. and Haque, M. E. (2011). *In vitro* and *in vivo* antioxidant and toxicity evaluation of different fractions of *Oxalis corniculata* Linn. *Journal of Pharmacology and Toxicology*. 6:337-348.
- Aitzetmuller K., Tsevegsuren, N. and Vosmann, K. (2006). A new allenic fatty acid in *Phlomis (Lamiaceae)* seed oil. *Fet/Lipid.*; 99:74–8.
- Angell, M. and Kassier, J. P. (1998). Alternative medicine – the risk of untested and unregulated remedies. *New England Journal of Medicine*. 339: 839-841.
- Ashutosh, K. (2007). “*Pharmacognosy and Pharmacobiotechnology*”, 2nd edition, *New Age International (P) Ltd*, New Delhi, India. 147, 176.
- Balunas, M. J. and Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life Science*. 78:431–41.
- Bhutani, K. K. (2003). Herbal medicines enigma and a challenge for science and guidelines for new initiatives. *Journal of Natural Products*; 19(1): 3-8.
- Brain, K. R. and Turner, T. D. (1975). *The Practical Evaluation of Phytopharmaceuticals*. Pp 4,
- British Herbal Pharmacopoeia (1990). *British Herbal Medicine*. 1st edition, *Dorset publisher*. *British*.
- Chan, I. (1995). Progress in traditional Chinese Medicine. *Trends Pharmaceutical Science*. 16: 182-187.
- Chandrasekhar, KS., Prasanna, KS. and Joshi, AB (2007). VHEpatoprotective activity of the *Leucas lavandulaefolia* on d (+) galactosamine-induced hepatic injury in rats. *Fitoterapia*. 78:440–2
- Chen SC, Elofson RM and MacTaggart JM. (1979). Carbon-13 nuclear magnetic resonance studies of lipids and starch digestion in intact seeds. *Journal of Agric and Food Chemistry*. 27:435–8.
- Dineshkumar C. (2007). Pharmacognosy can help minimize accidental misuse of herbal medicine. *Current Science*; 3:1356-1358.
- Eggeling, W.J., William, J. and Ivan, R.D. (2000). The Indigenous Trees of the Uganda Protectorate. *The Government Printer Entebbe, Uganda and the Crown Agents for the Colonies, MB, London, SW1*. 130-132.

- El-Sokkary, M., Mansour, M. M., El-Ficky, M. S., Okasha, M. A. M., Bacyoni, M., El-Sayed, A. Z. and Abdel-Mottleb, A. M. (1991). Influence of zinc sulphate on gastric ulceration and secretion in indomethacin-treated male rats. *Journal of Biomedical Science and Therapy*, 7(1): 366-385.
- Evans, W. C. (2009): *Trease and Evans pharmacognosy*. Elseviers 16th edition Pp. 10 – 11.
- Fatima I, Ahmad I, Anis I, Malik A, Afza N and Iqbal L. (2008). New butyrylcholinesterase inhibitory steroid and peroxy acid from *Leucas urticifolia*. *Archives Pharmaceutical Research*. 31:999–1003.
- Gadekar, R., Singour, P. K., Chaurasiya, P. K., Pawar, R. S. and Patil, U. K. (2010). A potential of some medicinal plants as an antiulcer agent. *Pharmacognosy Review*. 4(8): 136 – 140.
- Gerige SJ, Yadav MK, Rao DM and Ramanjeneyulu R. (2007). GC-MS analysis and inhibitory efficacy of *Leucas aspera* L. Leaf volatile oil against selected microbes. *Nigerian Journal of Natural Product and Medicine*. 11:80–3.
- Ghani, A. (1990). *Introduction to Pharmacognosy*. Ahmadu Bello University, press ltd. First edition Pp 187.
- Grossman, M. (2009). Peptic ulcer: A guide for practicing physicians. *American Journal of Pharmacology and Toxicology*. 4, 79, 89 – 93.
- Harborne, J. B. and Williams, C. A. (2000). Advances in flavonoid research, *Phytochemistry*, 55(6): 481-504.
- Harborne, J.B. (1992) *Phytochemical methods*. A guide to modern technique of plant analysis. Chapman and Hill, London, U.K. 279.
- Harborne, J. B., Mabry, T. J. and Mabry, H. (1975). *The Flavonoids*. New York: Academic Press; pp. 1–40.
- Hedge I.C. Labiatae. In: Ali SI, Nasir YJ,(1990) editors. Flora of Pakistan. Karachi: University of Karachi Department of Botany; p. 192.
- Hina, F., Nisar, A. and Mir, A. K. (2011). Physico-chemical, Phytochemical Evaluation and DPPH- Scavenging Antioxidant potential in Medicinal Plants used for herbal formulation in Pakistan. *Pakistan Journal of Botany*. Special Issue 43, 63-67.
- Humphrey, A.J., Beale, M. H. Terpenes. In: Crozier A, Clifford, M. N. and Ashihara, H. (2006). editors. *Plant secondary metabolites: Occurrence, structure and role in the human diet*. Oxford: Blackwell Publishing Company; p. 47.

- Hunt, H. R., Ireneus, T. and Padol, Y. Y. (2006) Peptic Ulcer Disease Today: Nature Clinical Practice. *Gastroenterology and Hepatology* 3(2): 80 – 85.
- Hyde, M. A. and Wurstem, B. (2009). Flora of Zimbabwe Information on *Leucas martinicensis* (<http://www.zimbabweflora.co.zw/speciesdata/specie.php?speciesid149300>). Retrieved 6 May.
- Izzo, F.B., Di Carlo G., Mascolo, N., Autore, G. and Capasso, F. (2000). The plant Kingdom as a source of Antiulcer Remedies. *Phytotherapy Research* 14: 581-591.
- Kayode, A. A. A., Kayode, O. T. and Odetola, A. A. (2009). Antiulcerogenic Activity of two extracts of *Parquetina nigrescens* and their Effects on Mucosal antioxidants defense System on Ethanol-Induced Ulcer in Rats. *Research Journal of Medicinal Plants*, 3, 102-108.
- Khalid M, Gopalakrishna, Dattatrava V, Sharikh K and Suresh D (2010). Gastroprotective and antiulcer activity of mixture of *Symplocos racemosa* bark and *Asarum europaeum* root. *Journal of Pharmacy Research*. 3 (7), 1502-1505
- Kirtikar K. R and Basu B. D. (1990) *Indian medicinal plants*. In: Bidtter E, Caius J. F and Mhaskar KS, editors. Periodical Experts Book Company. Periodical Experts Book Company.
- Kokate, C. K. (2003). *Practical Pharmacognosy*. Vallabh Prakashan, New Delhi, India. pp. 107-127.
- Krishnaraju A. V, Rao T. V, Sundararaju D, Vanisree M, Tsay H. S and Subbaraju G. V (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *International Journal of Applied Science and Engineering*. 3:125–34.
- Kroll, D. J. and Shaw, H. S. (2003). Complementary and alternative medicine (CAM): relevance to laboratory medicine. *Clinical Laboratory Internation*. 27(3):14-16.
- Magaji, R. A, Okasha, M. A. M., Abubakar, M. S. and Fatihu, M. Y. (2007). Anti – Ulcerogenic and Anti – Secretory activity of the N – Butanol portion of *Syzygium aromaticum* in rats. *Nigerian Journal of Pharmaceutical Sciences*, 6 (2): 119 – 126.
- Malfertheiner, P. (2002). Current Concepts in the Management of *Helicobacter pylori* infection- the Maastricht 2000 consensus Report. *Alimentary Pharmacology and Therapeutics*. 16: 167-180.
- Manivannana R. and Sukumar, D. (2007). The RBC membrane stabilization in an *in vitro* method by the drug isolated from *Leucas aspera*. *International Journal of Applied Science and Engineering*. 5:133–8

- Mangathayaru K, Thirumurugan D, Patel P. S, Pratap D. V, David D. J and Karthikeyan J. (2006). Isolation and identification of nicotine from *Leucas aspera* (willd) link. *Indian Journal of Pharmaceutical Science*. 68:88–90.
- Manonmani S, Viswanathan V. P, Subramanian S. and Govindasamy S. (1995). Biochemical studies on the antiulcerogenic activity of cauvery 100, an ayurvedic formulation in experimental ulcers. *Indian Journal of Pharmacology*. 27:101-5.
- Mishra, S. B., Mukerjee, A., and Vijayakumar, M. (2010). Pharmacognostical and Phytochemical Evaluation of Leaves Extract of *Jatropha curcas* Linn. *Journal of Pharmacognosy*. 2:9-14.
- Mishra T. N, Singh R. S., Pandey H. S. and Singh S. (1992). Long-chain compounds from *Leucas aspera*. *Phytochemistry* 31:1809–10.
- Miyaichi, Y., Segawa, A. and Tomimori, T. (2006). Studies on Nepalese crude drugs. XXIX. Chemical constituents of dronapuspi, the whole herb of *Leucas cephalotes* SPRENG. *Chemical Pharmacy Bulletin* (Tokyo). 54:1370–9.
- Moody, J. O, Gundidza M and Wyllie G. (2006). Essential oil composition of *Leucas milaniana* Guerke. *Flavour Fragrant Journal*. 21:872–4.
- Mohanta B, Chakraborty A, Sudarshan M, Dutta R. K and Baruah M. (2003) Elemental profile in some common medicinal plants of India. Its correlation with traditional therapeutic usage. *Journal of Radioanal Nuclear Chemistry*;258:175–9.
- Mostafa M, Nahar N, Mosihuzzaman M, Makhmoor T, Choudhary M. I and Rahman A. U. (2007). Free radical scavenging phenylethanoid glycosides from *Leucas indica* Linn. *Natural Product Research*. 21:354–61.
- Mye-Mba C. E, Menut C, Lamaty G, Zollo P. H, Thouboungang F and Bessiere J. (2006); Aromatic plants of tropical central Africa. XIX: Volatile components from leaves of two Lamiaceae from Cameroon: *Leucas deflexa* hook and *Solenostemon monostachyus* (P. Beauv.) *British Flavour Fragrant Journal*. 9:317–7.
- Murkherjee, S. K. (1998); A Revision of the Labiatae of the Indian Empire. Recds of Botanical Survey of India, *Manager of Publications*. 14(1):205.
- Muthukrishnan, J., Puphlantha, H. and Kasthuribhai, K. (1997). Biological effects of four plants extracts on *Culex quinquefasciatus* Say. Larval stages. *Insect Science Applied*.;17:389–94.
- Musa, K.Y. (2005): Phytochemical and Biological Studies of *Dyschoriste perrottetii* (*Acantheceae*), Ph. D Dissertation, Department of Pharmacognosy and Drug Development, (Ahmadu Bello University, Zaria, Nigeria. Pp. 49.

- Nasreen, S. and Radha, R. (2011). Assessment of Quality of *Withania somnifera* Dunal (Solanaceae) Pharmacognostical and physicochemical profile, *International Journal of Pharmacy and Pharmaceutical Sciences*, 3, Issue 2, 152-155.
- Nene Y. L, Thapliyal P. N and Kumar K. (1968): Screening of some plant extracts for antifungal properties. *Journal of Science and Technology*. 6:226–8.
- NIEHS. (1998). News: herbal health. *Environ Health Perspect*; 106:A590-2. *Web resource*. <http://en.wikipedia.org/wiki/AloeVera>. Retrieved January 5, 2015.
- Nivedithadevi, D. and Somasundaram, R. (2012). Pharmacognostical and Qualitative Phytochemical Studies on the aerial parts of *Tephrosia purpurea* (L), *International Journal of Research in Biological Sciences*, 2, No. 2, 48-53
- Nwafor, P. A., Okwuasaba, F. K., and Binda, L. G. (2000). Antidiarrhoeal and antiulcerogenic effect of methanolic extract of *Asparagus pubescens* root in rats. *Journal of Ethnopharmacology*, 72 (3), 421-427.
- Ode, O. (2011). The Antiulcer Activity of the Methanol Extract of *Cassia singueana* Leaves Using Indomethacin-Induced Gastric Ulcer Model in rats. *Journal of Advanced Scientific Research*, 2 (3), 66.
- OECD, (2006) guideline for testing of chemicals. Acute Oral Toxicity – Fixed Dose Procedure. OECD Guideline- 420. Modified and Adopted 23rd march..
- Ogbonnia, S., Adekunle, A. A., Bosa, M. K. and Enwuru, V. N. (2008). Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. *African Journal of Biotechnology*, 7: 701-5.
- Ogbonnia, S.O., Mbaka, G.O., Anyika, E.N., Emordi, J.E. and Nwakakwa, N. (2011). An evaluation of acute and subchronic toxicities of a Nigerian polyherbal tea remedy. *Pakistan Journal of Nutrient*. 10:1022-8.
- Okasha, M. A., Magaji, R. A, Abubakar, M. S. and Fatihu, M. Y. (2008). Effect of Ethyl acetate portion of *Syzygium aromaticum* Flower Bud Extract on Indomethacin – induced Gastric Ulceration and Gastric Secretion. *European Journal of Scientific Research*, 20 (4): 905 – 913.
- Prakash, D., Jain, R. K. and Misra, P. S. (1988). Amino acid profiles of some under-utilised seeds. *Plant Foods and Human Nutrient*. 38:235–41.
- Rahman M. S, Sadhu S. K and Hasan C. M. (2007) Preliminary antinociceptive, antioxidant and cytotoxic activities of *leucas aspera* root. *Fitoterapia*. 78:552–5.

- Qamaruddin, Parveen N, Khan N. U and Singhal K. C. (2002). *In vitro* antifilarial potential of the flower and stem extracts of leucas cephalotes on cattle filarial parasite setariacervi. *Journal of Natural Remedies*. 2:155–63.
- Ramakrishnan, K, and Salinas, R. C. (2007). Peptic ulcer disease. *American Family Physician*. 76 (7):1005–1012.
- Rang, H. P., Dale, M. M., Ritter, M. and Moore, P. K. (2003). *Pharmacology*, 5th edition. Churchill, Livingstones, Edinburgh; U.K. 797.
- Raskin, J. B., White, R. H., Jackson J. E., Weaver, A. L., Tindall, E. A., Lies, R. B., and Stanton, D. S. (1995). Misoprostol dosage in the prevention of non-steroidal anti-inflammatory drug induced gastric and duodenal ulcers. A comparison of three regimens. *Annal of Internal Medicine*. 123: 344-350.
- Rai V, Kakkar P, Khatoon S, Rawat AK, Mehrotra S. (2001): Heavy metal accumulation in some herbal drugs. *Pharmaceutical Biology*. 39:384–7.
- Reddy, M.K, Viswanathan, S., Sambantham, P. T., Ramachandran, S. and Kameswaran, L. (1986). Effects of *Leucas aspera* on experimental inflammation and mast cell degranulation. *Annal Science of Life*. 5:168–71.
- Rickert, K., Martinez, R. R. and Martinez, T. T. (1999). Pharmacist knowledge of common herbal preparations. *Proc West Pharmacology Society*; 42:1-2.
- Ryding O. (1998). Phylogeny of the *Leucas* Group (Lamiaceae) *Systemic Botany*;23:235–47.
- Salehi-Surmaghi, M. H., Aynehchi, Y., Amin, G. H., and Mahmoodi, Z. (1992).Survey of Iranian Plants for Saponins, Alkaloids, Flavonoids and Tannins IV. *Journal School of Pharmacy* 2:1-11.
- Sadhu, S. K, Okuyama, E., Fujimoto, H. and Ishibashi, M. (2006). Diterpenes from *Leucas aspera* inhibiting prostaglandin-induced contractions. *Journal of Natural Product*. 69:988–94.
- Saha K, Mukherjee P. K, Mandal S. C, Saha B. P and Pal M. (1997). Antiinflammatory evaluation of *Leucas lavandulaefolia* Rees. Extract. *Natural Product and Sciences*. 2:119–22.
- Satheesh, M. N. N., Kumud, U. and Asha, B. (2011); Phytochemical screening and standardization of poly herbal formulation for Dyslipidemia. *Indian journal of physiology and pharmacology*, 3(3).
- Scepovic, Z. and Radmanovic, B. Z. (1984). Interaction between reserpine and non-steroidal anti-inflammatory drugs in producing gastric ulcers in rats. *European Journal of Pharmacology*, 98: 445-448

- Selvamani, P., Dhruvo Jyoti Sen and Jayanta Kumar Gupta (2009). Pharmacognostical standardization of *Commiphora berryi* (Arn) Engl and phytochemical studies on its crude extracts. *African Journal of Pharmacy and Pharmacology*. 3 (2). pp. 37-46.
- Sinha, S, Ansai A. A and Osman S. M. (1978). *Leucas cephalotes* a new seedoil rich in laballic acid. *Chemical and Industrial*. 1:67.
- Sofowora, A. (2008). *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Limited, Ibadan, Nigeria. 3rd Edition. Pp. 200-202.
- Sofowora, E. A. (1989). *Medicinal plants and traditional medicine in Africa*. Ibadan, Nigeria: Spectrum Books. Pp 24.
- Soumendra, D. (2010). Antiulcer effect of Livina, a herbal formulation against ethanol induced acute gastric ulcer in mice. *International Journal for Pharmaceutical Research and Development- Online*, 2(10), 93 – 100.
- Sravani, P., Jayasri, P. S., Ershad K. P. and Nishad K. P. (2011). Review on natural antiulcer agents. *International Journal pharmaceutical and Industrial Research*. 1(1): 67– 70.
- Srinivas, K., Rao, M. E and Rao, S. S. (2000). Anti-inflammatory activity of *Heliotropiu indicum* Linn. and *Leucas aspera* Spreng. in albino rats. *Indian Journal of Pharmacology*. 32:37–8.
- Sumitra, C. (2014). Importance of pharmacognostic study of medicinal plants: An overview, *Journal of Pharmacognosy and Phytochemistry*; 2 (5): 69-73.
- Vagionas K, Ngassapa O, Runyoro D, Graikou K, Gortzi O and Chinou I. (2007). Chemical analysis of edible aromatic plants growing in Tanzania. *Food and Chemistry*. 105:1711–7.
- Verma S and Chauhan N. S. (2007); Indigenous medicinal plants knowledge of Kunihar forest division, district Solan. *Indian Journal of Traditional Knowledge*. 6:494–7.
- World Health Organization, (2011). *Quality Control Methods for Medicinal Plants*. WHO, Geneva, Switzerland, Pp. 9-31.
- Zhu, M., Lew, K. T. and Leung, P. (2002). Protective effects of plant formula on ethanol-induced gastric lesions in rats. *Phytotherapy Research*. 16:276-80.

APPENDIX I

Determination of Ash Value of powdered Leaf of *L. martinicensis*

Description	1	2	3
Constant weight of crucible (g)	39.22	39.40	39.41
Weight of crucible and content (g)	41.22	41.40	41.41
Weight of crucible and Ash (g)	39.54	39.55	39.54
Weight of Ash (g)	0.13	0.15	0.13
Ash Value (%)	6.50	7.50	6.50
Average mean (%)		6.8	

Sample calculation

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

$$\text{Ash Value} = \frac{0.13}{2} \times 100$$

$$= 6.5 \% \text{w/w}$$

APPENDIX II

Determination of Acid insoluble Ash of powdered leaf of *L. martinicensis*

Description	1	2	3
Constant weight of crucible (g)	39.23	39.23	39.23
Weight of crucible and Acid insoluble ash (g)	39.31	39.28	39.31
Weight of Acid insoluble ash (g)	0.08	0.05	0.08
Acid Insoluble Ash Value (%)	4.00	2.50	4.00
Average mean (%)	3.50		

Sample calculation

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

$$\begin{aligned} \text{Acid Insoluble Ash value} &= \frac{0.05}{2} \times 100 \\ &= 2.5 \% \text{ w/w} \end{aligned}$$

APPENDIX III

Determination of water soluble Ash of powdered leaf of *L. martinicensis*

Description	1	2	3
Constant weight of crucible (g)	49.92	49.92	39.90
Weight of crucible and Ash (g)	50.18	50.18	39.63
Weight of Ash (g)	0.26	0.26	0.27
Weight of water Insoluble ash (g)	0.14	0.14	0.15
Weight of water soluble ash (g)	0.12	0.12	0.12
Water soluble Ash Value (%)	6.00	6.00	6.00
Average mean (%)		6.0	

Sample calculation

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

$$\text{Water soluble Ash value} = \frac{(0.32 - 0.14)}{2} \times 100$$

$$= 6.0 \% \text{w/w}$$

APPENDIX IV

Determination of water-soluble extractive value of *L. martinicensis* leaf 4 g of the powder was used in 100 ml of water.

Description	1	2	3
Constant weight of dish (g)	123.07	144.71	64.78
Weight of crucible and content after heating (g)	123.28	144.91	64.99
Water extractive content (g)	0.21	0.20	0.20
water extractive Value (%)	21.00	20.00	20.00
Average mean (%)		20.33	

Sample calculation

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

$$\begin{aligned} \text{Water extractive value} &= \frac{(144.71 - 144.91)}{4} \times 100 \\ &= 21 \% \text{ w/w} \end{aligned}$$

APPENDIX V

Determination of alcohol – soluble extractive value of the leaf of *L. marticinensis* 4 g of the powdered was used in 100 ml of 90% ethanol

Description	1	2	3
Constant weight of dish (g)	64.78	123.07	64.78
Weight of dish and content after heating (g)	64.94	123.23	64.94
Alcohol extractive content (g)	0.16	0.16	0.16
Alcohol extractive Value (%)	16.00	16.00	16.0
Average mean (%)		16.0	

Sample calculation

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

$$\text{Alcohol extractive value} = \frac{(123.23 - 123.07)}{4} \times 100$$

$$= 16.0 \% \text{w/w}$$

APPENDIX VI

Determination of moisture content of powdered *L. martinicensis* leaf 3g of the powdered Plant material was used

Description	1	2	3
Constant weight of crucible (g)	51.62	44.61	46.74
Initial weight of powder (g)	54.62	47.61	49.74
Final weight of powder	54.36	47.38	49.43
Loss in weight (g)	0.26	0.23	0.30
Moisture content (%)	8.67	8.00	10.00
Average mean (%)	8.9		

Sample calculation

$$\% \text{ Moisture content} = \frac{\text{Initial Weight of Powder} - \text{Final Weight of Powder}}{\text{Initial Weight of Powder}} \times 100$$

$$\begin{aligned} \% \text{ Moisture content} &= \frac{(54.62 - 54.36)}{3} \times 100 \\ &= 0.26 \% \text{ w/w} \end{aligned}$$

APPENDIX VII

Effect of *L. martinicensis* Leaf Methanol extract on Indomethacine induced gastric ulcer in Rats

Treatment	Dose	MUI (mm) \pm SEM	UI (%)
Control	1ml	10.4 \pm 0.97	
Omeprazole	20 mg/kg	2.60 \pm 0.81*	75.00
LMME	300 mg/kg	8.20 \pm 2.56	21.00
LMME	600 mg/kg	4.20 \pm 1.74	60.00
LMME	900 mg/kg	2.20 \pm 0.92*	79.00

Key: MUI-mean ulcer index, SEM-standard error mean, UI- ulcer inhibition, (-) - negative, (+)-positive. * p<0 .05 against negative control

LMME = *L. martinicensis* Methanol Extract

APPENDIX VIII

Effect of Ethyl Acetate Fraction of *L. martinicensis* Leaf on Indomethacine induced gastric ulcer in rats

Treatment	Dose	MUI (mm) \pm SEM	UI (%)
Control	1ml	10.4 \pm 0.97	
Omeprazole	20 mg/kg	2.60 \pm 0.81*	75.00
LMEAF	300 mg/kg	7.60 \pm 2.27	27.00
LMEAF	600 mg/kg	5.80 \pm 1.21	44.20
LMEAF	900 mg/kg	3.20 \pm 1.77	69.20

Key: MUI-mean ulcer index, SEM-standard error mean, UI- ulcer inhibition, (-) - negative, (+) - positive.

LMEAF =*L. martinicensis* Ethyl acetate Fraction

APPENDIX IX

Effect of Aqueous Fraction of *L. martinicensis* Leaf on Indomethacine induced gastric ulcer in rats

Treatment	Dose	MUI (mm) \pm SEM	UI (%)
Control	1ml	10.4 \pm 0.97	
Omeprazole	20 mg/kg	2.60 \pm 0.81*	75.00
LMAF	300 mg/kg	7.00 \pm 2.59	33.00
LMAF	600 mg/kg	7.20 \pm 1.59	31.00
LMAF	900 mg/kg	7.80 \pm 2.58	25.00

Key: MUI-mean ulcer index, SEM-standard error mean, UI- ulcer inhibition, (-) - negative, (+) - positive.

LMAF=*L. martinicensis* Aqueous Fraction

APPENDIX X

Effect of Hexane Fraction of *L. martinicensis* Leaf on Indomethacine induced gastric ulcer in rats

Treatment	Dose	MUI (mm) \pm SEM	UI (%)
Control	1ml	10.4 \pm 0.97	
Omeprazole	20 mg/kg	2.60 \pm 0.81*	75.00
LMHF	300 mg/kg	6.4 \pm 3.05	39.00
LMHF	600 mg/kg	6.8 \pm 2.67	35.00
LMHF	900 mg/kg	9.2 \pm 2.29	12.00

Key: MUI- mean ulcer index, SEM-standard error mean, UI- ulcer inhibition, (-) - negative, (+) - positive. LMHF = *L. martinicensis* Hexane Extract

APPENDIX XI

Summary of TLC Profile of ethyl acetate fraction (LMEAF) spray with *p*-Anisaldehyde/H₂SO₄

Extract	Solvent System	No. of Spots	Colour of Spots	R _f values
Ethyl acetate	H:E(7:3)	8	yellow	0.27
			purple	0.29
			yellow	0.33
			green	0.36
			purple	0.50
			purple	0.66
			pink	0.88
			purple	0.91

APPENDIX XII

Summary of TLC Profile of methanol Extract (LMME) spray with *p*-Anisaldehyde/H₂SO₄

Extract	Solvent System	No. of Spots	Colour of Spots	R _f values
Methanol	B:A:W (10:1:1)	10	Yellow	0.02
			Pink	0.12
			Green	0.23
			Green	0.31
			Pink	0.40
			Purple	0.62
			Purple	0.73
			Pink	0.80
			Purple	0.88
			purple	0.94

APPENDIX XIII

Summary of TLC Profile of Aqueous fraction (LMAF) spray with *p*-Anisaldehyde/H₂SO₄

Extract	Solvent System	No. of Spots	Colour of Spots	R _f values
Aqueous	B:A:W (10:1:1)	6	purple	0.13
			pink	0.18
			green	0.37
			purple	0.43
			pink	0.67
			yellow	0.79

APPENDIX XIV

Summary of TLC Profile of Hexane fraction(LMHF) spray with *p*-Anisaldehyde/H₂SO₄

Extract	Solvent System	No. of Spots	Colour of Spots	R _f values
Hexane	H:E (8:2)	5	pink	0.12
			green	0.18
			purple	0.26
			green	0.60
			purple	0.76