# PHARMACOGNOSTIC AND ANTIULCER EVALUATION OF THE STEM BARK OF *PROSOPIS AFRICANA* (GUILL. & PERR.) TAUB. (MIMOSACEAE)

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DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT,
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JULY, 2014

## Declaration

I declare that the work in this thesis entitled "Pharmacognostic and antiulcer evaluation of the stem bark of *Prosopis africana* (Guill. & Perr.) Taub. (Mimosaceae)" 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 a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

| Johnson Ogbe ABAH | _____ | _____ |
|---|---|---|
| Name of Student | Signature | Date |

## Certification

This thesis entitled "Pharmacognostic and antiulcer evaluation of the stem bark of *Prosopis africana* (Guill. & Perr.) Taub. (Mimosaceae)" by Johnson Ogbe ABAH meets the regulations governing the award of the degree of M.Sc. Pharmacognosy of Ahamdu Bello University, and is approved for its contribution to knowledge and literary presentation.

_____ _____

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Chairman, supervisory committee

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Dean, School of Postgraduate Studies

## Dedication

This thesis is dedicated to my wife Stella and my children, Abigail, Favour and Praise

**Acknowledgement**

All that I am, all my accomplishments and all that I hope to become and accomplish has and will always be dependent on the Grace of God. I therefore, without any reservation, express my most profound gratitude to God Almighty. HE gave me life, strength and wisdom to carry out this work.

"A man without a coach is a man that will definitely end up in reproach". I am however blessed with two great coaches for this work; Prof. M.S. Abubakar and Prof. K.Y Musa to whom I am very grateful. They coached me well. I am heavily indebted to Dr. Ahmed A. and Mr. Mshelia Halilu for their unquantifiable contributions to this work both technical and advisory. I roll out an avalanche of thanks to the Laboratory technical crew who were always there for me at the Pharmacognosy laboratory of Usmanu Danfodiyo University, Sokoto, namely, Mal. Adamu Mansir, Mal. Aminu Mahmood, Mal. Ishyaku Isah, Mal. Mohammed Aliyu, and Mal. Nuhu Mahmood.

"A friend in need is a friend indeed". That is what Mr. Joshua Bulama has been to me in the course of this research work. It took a team of friends to give me that necessary support and encouragement to complete this work. They include Mr. Michael Ugwa, Mrs. Tayo Ohemu, Miss. Murjanatu A. Abubakar, Mal. Abubakar Abba and a host of others who are so important yet too innumerable to mention. May the lord God Almighty reward all of them. I am so grateful.

**Abstract**

*Prosopis africana* (Guill. & Perr.) Taub. (Mimosaceae) is the only known species of its genus found in Africa. Pharmacognostic standardization was carried out on the stem bark powder and its anatomical sections, to determine the micro-morphological characters, along with its quantitative and qualitative profiles. Identified microscopic features in the bark powder include radial bands of rectangular, tangentially elongated, thin-walled cork cells in rows; Secondary phloem composed of phloem fibres in small patches with thin walled parenchyma in between and few druse type of calcium oxalate crystals with unequal axes. Identified also are elongated fibres cells with blunt end and thick walls. Sclerids are isodiametric in shape. Histochemical evaluation revealed the presence of lignin, tannin and hydroxyanthraquinnones. After repeated evaluations, the bark powder was found to have an average of 5.27% Moisture Content, 3.93% Total Ash Value, 1.61% Acid Insoluble Ash Value, 0.33% Water Soluble Ash Value, 22% Water Extractive Value (Hot Maceration).These parameters serve as reference data for the identification and quality control of *P. africana* crude drug. The stem bark was extracted by Soxhlet extraction method using ethylacetate as the solvent. The acute toxicity evaluation of the crude extract shows that it is safe, up to a dose of 3000 mg/kg administered orally in rats. Antiulcer activity of the crude extract was evaluated using ethanol and indomethacin induced gastric ulcer models in rats. Using the ethanol model, doses of the extract at 300, 600 and 900 mg/kg produced significant ($p<0.05$) protective effect in rats with preventive index of 19, 71 and 81% respectively as against 15% with omeprazole (20 mg/kg) and 0% with distilled water. Using the indomethacin model, the extract at 300, 600, and 900 mg/kg produced significant ($p<0.05$) gastroprotective effects of 22, 43 and 56% respectively as against 28% with omeprazole (20 mg/kg) and

0% with distilled water. *Prosopis africana* stem bark extract was found to be significantly ($P<0.05$) protective against ethanol and indomethacin-induced gastric ulcers in the experimental rats. The crude ethylacetate extract was fractionated with petroleum ether, chloroform and methanol to obtain their respective fractions. Friedelin, a pentacyclic triterpenoid was isolated by column chromatography from the chloroform fraction. The structure of the compound was assigned on the basis of spectroscopic data. Friedelin is being reported for the first time as a chemical constituent of *P. africana*.

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# CHAPTER ONE

# INTRODUCTION

## 1.1 Introduction

Many herbs are moving from fringe to main stream use with a good number of people seeking remedies and health approaches free from the side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilizing eco-friendly and bio-friendly plant based products for the prevention and cure of different human diseases. With the present surge of interest in phyto-therapeutics, the availability of genuine plant material is becoming imperative. Therefore, accurate morphological and anatomical standardization of drug plants is very much essential. According to the World Health Organization (WHO, 1988) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.

Prosopis are pod bearing trees or shrubs consisting of 44 reported species which are found in arid and semi-arid regions of the world (Agboola, 2004). *Prosopis africana* (Guill. & Perr.) Taub. is the only tropical African Prosopis species, occurring from Senegal to Ethiopia in the zone between the Sahel and savannah forests.

The fact that *P. africana* has found tremendous use on the local and international scene (Weber, 2008) makes it a good candidate plant for research into its uses, composition and standardization as an attempt to contribute to the health and wellbeing of humanity.

Almost all parts of the tree are used in medicine; the leaves and bark are combined to treat rheumatism (Orwa *et al*., 2009). Orwa *et al*., (2009) also reported that remedies for skin diseases, caries, fevers and eyewashes are obtained from the bark. In Nigeria, the juice expressed from the stem bark of *P. africana* is applied on open wounds as an astringent and to cleanse the wound surface (Orwa *et al*., 2009). In Mali the leaves, bark, twigs and roots are used to treat and relieve bronchitis, dermatitis, tooth decay, dysentery, malaria and stomach cramps (Orwa *et al*., 2009). This tempting and useful profile of *P. africana* makes it necessary to develop quality standards for the plant and its parts so as to provide identification parameters for its proper identification when needed. This research is therefore structured to deal with determining the requisite anatomical features of *P. africana* stem bark so as to provide pharmacognostic data for its correct identification. To accomplish this, therefore, pharmacognostic evaluation tools such as chemomicroscopy and physicochemical analysis are adopted.

The healing profile of any plant is dependent on its chemical composition. This work is structured to isolate the pharmacologically active compounds from the stem bark of the plant. In this research work also, *P. africana* stem bark is evaluated for its antiulcer activity. Peptic ulcer is one of the world's major gastro-intestinal disorders, embracing both gastric and duodenal ulcers, and affecting 10% of the world's population. The most common symptom of peptic ulcers is dyspepsia (Schwartz, 2002). It may be persistent or recurrent and can encompass a variety of problems in the upper abdomen. This includes, epigastric pain or discomfort (this can be either a localized or diffused burning, gnawing or aching in the upper abdomen or as a stabbing pain penetrating through the width of the gut); bloating and a feeling of fullness, hunger and an empty feeling in the stomach, often one to three hours after a meal, mild nausea (vomiting, in

fact may relieve symptom), regurgitation and belching. (Schwartz, 2002). It is therefore of great interest to have a plant that would both serve as food and a potent antiulcer. With the known profile of *P. africana* it is very desirable to investigate its antiulcer property. To achieve this, therefore, the method described by Nwafor *et al* (2000), is adopted in this work, using the indomethacin and ethanol protocols. This work, in its entirety, investigates the standardization parameters for *P. africana* stem bark, its chemical composition and antiulcer properties.

## 1.2 Statement of Research Problem

There are no reported data on the distinctive characters for the identification and standardization of *P. africana* stem bark powder. There is no report of any verification of the use of this plant in the treatment of ulcer.

## 1.3 Justification of Research

To date there are no reported studies on the pharmacognostic data required for its identification as a powdered drug. Isolation of natural compounds from the plant will further enhance the understanding of its application as a medicinal remedy and may serve as a source of vital pharmacologically active natural compounds.

Peptic ulcer is the most common gastro intestinal tract (GIT) disorder in the present day life of the industrialized and civilized world. The prevention or cure of peptic ulcers is one of the most important challenges confronting medicine today, as it is certainly a major illness affecting 8 to 10% of the global population and of these 5% suffer from gastric ulcer (Soumendra, 2010). Despite the enormity of the health and economic implications of gastric ulcer, treatment options are limited.

As a result of the wide spread and daily use of *Prosopis africana*, establishment of its pharmacognostic identification and standardization parameters, antiulcer profile and subsequent isolation of compounds from it, would be an immense contribution to the health and well-being of humanity.

## 1.4 Hypothesis

The stem bark powder of *Prosopis africana* contains bioactive principles that are useful in treatment of ulcers.

## 1.5 Research Question

1. Can the identification and standardization parameters for the stem bark powder of *Prosopis africana* be established?
2. Does the extract from *Prosopis africana* have the potential to heal or prevent gastric ulcer disease?

## 1.6 AIMS

**Specific Aim 1**:

To determine the requisite anatomical features of *Prosopis africana* stem bark so as to provide pharmacognostic and physicochemical information for correct identification of the stem bark

**Specific Aim 2**:

To evaluate the acute toxicity and antiulcer profile of *P. africana* stem bark extract and fractions.

**Specific Aim 3**:

To isolate and characterize a pharmacologically active pure compound from the plant bark extract.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Botanical Description of *Prosopis africana* (Guill. & Perr.) Taub.

*Prosopis africana* belongs to the family Mimosaceae. The common names include, Iron wood, Locust beans (English), Ubwa (Ibo), Kiriya (Hausa), Okpehe (Idoma).

Prosopis are pod bearing trees or shrubs consisting of 44 reported species which are found in arid and semi-arid regions of the world (Agboola, 2004).



Plate I: Trunk view of *P. africana* (Guill. & Perr.) Taub.



Plate II: *P. africana* leaves and fruits

## 2.2 Ethnomedical Uses of *Prosopis africana*

**Food**: In northern and the middle belt areas of Nigeria, the seeds are boiled and made into 'daddawa' (Hausa) 'okpehe' (Idoma), a product used for favouring local dishes (Barminas, 1998). **Fuel**: The wood has a high calorific value of about 1720 joules/kg and produces excellent charcoal and firewood. In Senegal it is preferred for art and craft work, while in Ghana it is used for pestles, mortars, mallets, cudgels, furniture, joinery, sleepers in the construction of railway lines, boat building and axe handles. **Gum or resin:** *P. africana* yields a gum. **Tannin or dyestuff:** The bark and roots contain 14-16% tannin and a colouring matter that gives a reddish tint to leather. **Poison:** Pounded dry fruits are suitable as a fish poison. **Medicine:** Almost all parts of the tree are used in medicine; the leaves in particular for the treatment of headache and toothache as well as various other head ailments. Leaves and bark are combined to treat rheumatism. Remedies for skin diseases, caries, fevers and eyewashes are obtained from the bark. The roots are a diuretic and are used to treat gonorrhoea, tooth and stomach-ache, dysentery and bronchitis. In Mali the leaves, bark, twigs and roots are used to treat and relieve bronchitis, dermatitis, tooth decay, dysentery, malaria and stomach cramps. In Ghana, boiled roots serve as a poultice for sore throat, root decoction for toothache, and bark as a dressing or lotion for wounds or cuts. **Other products:** In Ghana the pod ashes of *P. africana* are a source of potash for soap making (Abbiw, 1990; Orwa *et al*., 2009).

## 2.3 Phytochemical Constituents of *Prosopis africana*

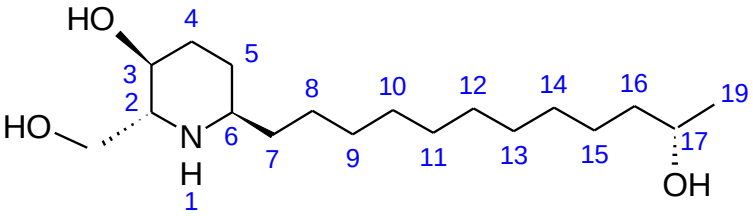
Chemical compounds have been isolated, which contribute to the medicinal properties of Prosopis. The leaves of many species contain quantities of many different free amino

acids and flavonoids (Carman, 1974), with alkaloids and diketones isolated as active ingredients.

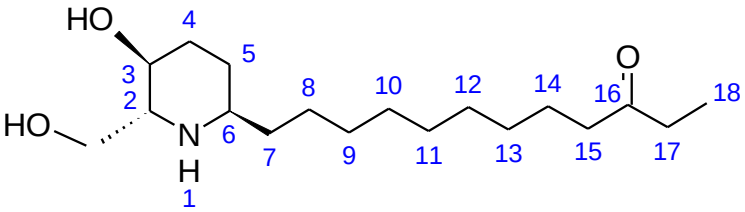
### 2.3.1 Alkaloids

Several 2,6-disubstituted piperidin-3-ol alkaloids have been isolated from the *Prosopis africana,* whose leaves have been used in Africa to treat toothaches (Mathew, 1999).

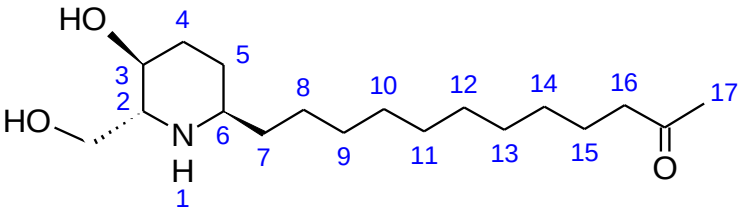
These alkaloids, shown in Figure 1, all have a hydroxyl group in the 3-position, an *n*-$C_{12}$ side chain in the 6-position, and either a methyl or hydroxymethyl group in the 2-position.



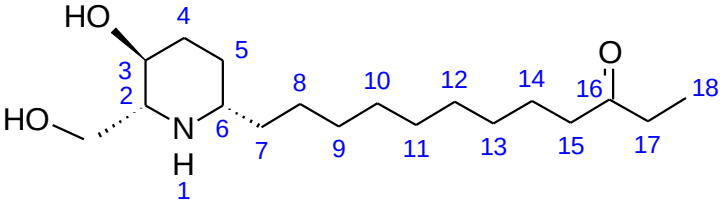

i. Prosopine



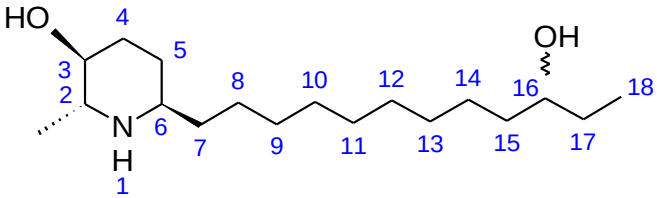

ii. Prosopinine




iii. Prosopinone




iv. (+/-)-Prosophyline




v. Prosafrine

*Figures 1:* Alkaloids of *Prosopis africana* (Mathew, 1999)

The concentration of alkaloids varies between species and within populations but is 0.4-3.6% of leaf dry weight. Concentrations are significantly higher in younger rather than in older leaves (Cates, 1977). Studies on the aqueous and ethanol extract of the root and stem of *Prosopis africana* indicate the presence of alkaloids, tannins and saponins in high concentration in the stem and root, with the former containing a significantly higher ($P<0.05$) quantity of these phytochemicals. Phenols and steroids were also present in the investigated plant parts (Kolapo, 2009).Of these alkaloids, two piperidine alkaloids have been studied (Neuwinger, 1996). These include prosopine which is a weak excitant of the nervous system and prosopinine having a weak sedative effect but also has local anaesthetic effects three times stronger than cocaine. However, their strong irritant actions precludes their use in modern medicine (Pasiecznik, 2001).

### 2.3.2 Flavonoid compounds:

A flavonoid compound 7,3',4'trihydroxy-3-methoxyflavanone was isolated from the ethylacetate extract of *Prosopis africana* (Jamal, 2013). The ESI-MS showed an [M-H]-at m/z 301.1201 which established the molecular formula of the compound as $C_{16}H_{14}O_6$. The $^1H$ NMR spectrum [400MHz, CD3OD,] showed peaks at δ 6.32 (d, J= 2.2Hz, H-8), δ 6.53 (dd, J= 2.2, 8.0Hz, H-6) and 7.69 (d, J= 2.2Hz, H-5) for ring-A and peaks at δ 6.78 (d, J= 8.0Hz, H-5'), 6.81 (dd, J= 2.0, 8.0 Hz, H-6') and 6.93 (d, J= 2.0Hz, H-2') for ring B. The spin system was confirmed from the COSY spectrum (400MHz, CD3OD). The $^{13}C$ NMR data showed the presence of 15 carbons including a carbonyl at δ191.4, one methoxy group at δ 60.1 and quaternary carbons indicating a flavonoid type structure (Jamal, 2013).

The HMBC experiment [400MHz, CD3OD,] played a key role in assigning all protons and carbons as well as the position of the methoxy group in the molecule. The H-2 proton showed 3J correlation to C-4 of ring-C, C-2' and C-6' of ring B. whereas the proton at position 3 showed 2J correlation to C-4 and C-2 of ring C while the methoxy protons showed 3J correlation to C-3, indicating that the methoxy is attached to C-3. In ring A, the proton at position 5 showed 3J correlations to the carbonyl carbon (C-4) of ring C & hydroxylated carbon (C-7) and also showed a 1, 2, 4-substitutions pattern indicating that a hydroxyl group is at the position 7, while H-8 and H-6 showed 3J correlations to each other's carbon. long range 4J 'W' coupling was also observed between H-8 and C-4. Ring-B exhibited an ABX substitution pattern with hydroxyl groups at C-3' and C-4'. The H-2 and H-3 of ring-C showed a large coupling constant (10.5Hz) indicating that they follow the *trans* diaxial configuration. Based on the spectral data and by comparison with literature, the structure was identified as 7,3',4'trihydroxy-3-methoxyflavanone reported for the first time in the plant *Prosopis africana* (Jamal, 2013).

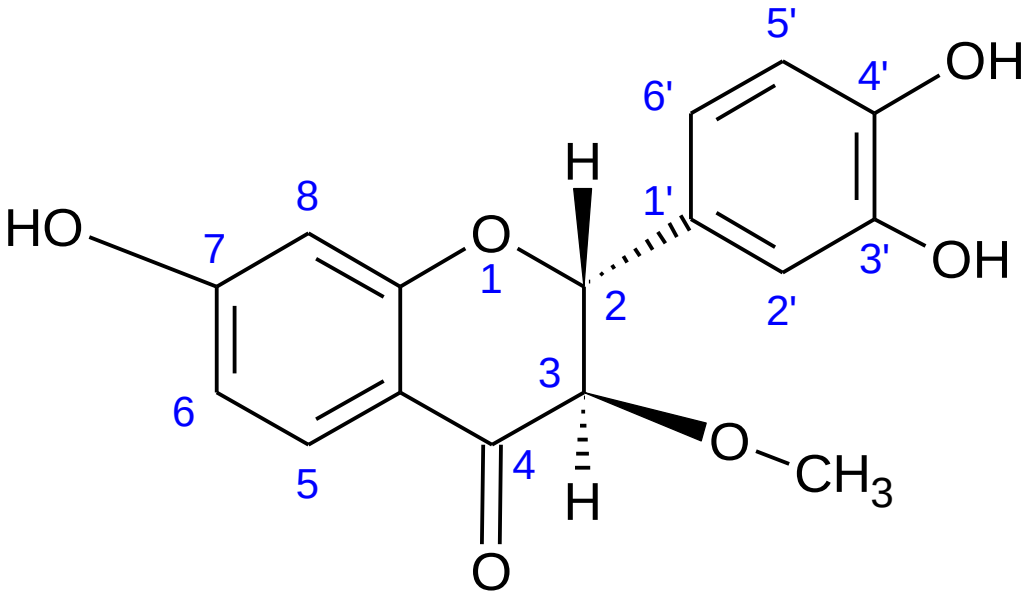



**Figure 2: 7,3',4'trihydroxy-3-methoxyflavanone**

**Activities of 7,3',4'trihydroxy-3-methoxyflavanone**:

It has been shown that 7,3',4'trihydroxy-3-methoxyflavanone has activity against *Mycobacterium aurum* (CIP 104482) with an MIC of 413.63µM as compared to the standard drug (ethambutol at 29.36 µM) as a positive control whereas its activity against *S. aureus* as measured in MIC is 827.81 compared to gentamicin as a standard at 0.31 µM (Jamal, 2013). Flavonoids are known to have anti-mycobacterial activity (Begum, 2008). The lipophilicity of flavanone type compounds could be responsible for the activity of 7,3',4'trihydroxy-3-methoxyflavanone against *S. aureus* by helping in its penetration of the mycobacterial cell wall (Gibbons, 2004).

The compound 7,3',4'trihydroxy-3-methoxyflavanone was observed to exert cytotoxicity against ZR75 (Breast cancer cell) with 40% cell viability against ZR75 cell line with low toxicity against human cell lines as compared to Triton X as a positive control. Other studies have shown significant activity of the plant extracts against lung carcinoma (EL Merzabani, 1979) and against lymphocytic leukemia and other carcinomas (Ahmad, 1989).

### 2.3.3 Tannins:

The original name of *Prosopis* in North America is 'misquitl', from the use of the tree bark as a tanning agent, with the bark containing 14-16% catechol tannins (Doat, 1978). Tannins are poly-phenols present in plants, foods and beverages, and are of great economic and ecological interest (Karamać, 2009). They are water soluble and with molecular weights ranging between 500 and 3000 Daltons. They also form complexes with water-insoluble proteins, alkaloids and gelatin. They are responsible for the

astringent taste of many fruits and vegetables, causing precipitation of salivary glycol-proteins and reducing oral lubrication (Okuda, 2011).

Many plant species producing tannins are used in folk medicine for different purposes. The tannin drug applications are mainly related to their astringent properties. They exert internal anti-diarrheal and antiseptic effects by waterproofing the outer layers of more exposed mucous membranes. Precipitating proteins, tannins provide antimicrobial and antifungal effects. Tannins are also haemostatic, and can serve as an antidote in poisoning cases (Neyres-Zinia, 2012). In the process of healing wounds, burns and inflammations, tannins help by forming a protective layer (tannin-protein/tannin-polysaccharide complex), over injured epithelial tissues permitting the healing process below to occur naturally (Neyres-Zinia, 2012). Studies show that many tannins act as radical scavengers, intercepting active free radicals (Neyres-Zinia, 2012). Various degenerative diseases such as cancer, multiple sclerosis, atherosclerosis and the aging process itself are associated with high concentrations of intercellular free radicals.

**Tannins and Peptic Antiulcer Activity**

Peptic ulcer is one of the world's major gastro-intestinal disorders, embracing both gastric and duodenal ulcers, and affecting 10% of the world population (Zapata-Colindres, 2006).

Tannins are used in medicine primarily because of their astringent properties. They react with the proteins of the tissue layers. Tannins precipitate micro proteins at the site of the peptic ulcer, forming a protective pellicle that prevents absorption of toxic

substances, and promotes resistance to the action of proteolytic enzymes, an associated activity against *Helicobacter pylori* (Vasconcelos, 2008). Murakami, (1991) showed that ellagic acid is a potent competitive inhibitor of gastric $H^+$, $K^+$-ATPase, and proposed that ellagic acid may compete with ATP at the ATP hydrolysis site, thus markedly inhibiting acid secretion, and stress-induced gastric lesions (Murakami, 1991). Enzyme inhibition was also evident for tannic acid (Murakami, 1992).

Khennouf & Kim, (2003) examined the gastro-protective effects of 70% acetone leaf extracts of *Quercus suber* and *Quercus coccifera* (Fabaceae), as well as tannins purified from these extracts, in mice and rabbits using an ethanol-induced gastric ulcer model. Both extracts, as well as the purified tannins prevented the formation of stomach lesions and strongly inhibited lipid peroxidation in rabbit brain homogenate. The authors suggest that the gastro-protective effects are related to the anti-lipoperoxidant properties (Khennouf & Kim, 2003). The authors also concluded that the protection afforded by these substances was very high, and might be due to the inhibition of acid secretion.

Purified tannins were tested against *Helicobacter pylori* (Funatogawa, 2004) and these include twenty hydrolysable tannins, 3 catechin and 6 proanthocyanidins. All of the hydrolysable tannins tested demonstrated promising antibacterial activity against *Helicobacter pylori* (Funatogawa, 2004).

In several experimental models of gastric ulcer, purified tannins have been shown to be involved with gastrointestinal tract anti-inflammatory actions, promotion of tissue

repair, acid secretion inhibition, and to present both antioxidant and anti-*Helicobacter pylori* activity (Funatogawa, 2004).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Collection and Preparation of Plant Material

Stem barks, fresh mature leaves and pods were collected from fully-grown plants in fields at the outskirts of Zuru town in Kebbi State, Nigeria. The identity and authenticity of the plant was established by comparing its morphological characters with the available literature (Vautier, 2007) and by a taxonomist of the Herbarium Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen numbered PCG/UDUS/Mim/004 is kept in the herbarium of the same department.

The stem bark was shade dried for seven days and powdered using pestle and mortar to obtain a coarse powder. The powder was then stored in appropriate containers until required for use.

## 3.2 Specific Aim 1: Pharmacognostic Evaluation of *P. africana* Stem Bark

### 3.2.1 Macroscopic evaluation of the stem bark

The macroscopic and organoleptic properties were evaluated for the stem bark using standard methods (WHO, 1998).

#### *3.2.1.1 Colour*

The untreated powder was examined with the naked eye under bright daylight.

### *3.2.1.2Texture and fracture characteristics*

The dried bark was examined by touching. The bark was also fractured to obtain information on the fracture pattern and plane.

### *3.2.1.3Odour and taste*

The odour and taste of *Prosopis africana* bark was analysed using the procedure prescribed in Quality Control Methods for Medicinal Plant Material by WHO, (1998).

## 3.2.2 Microscopic examination of the stem bark powder

The equipment used for the microscopic examination include Kyowa Microscope Model number XSZ-21, Electronic eyepiece (YJ EYE model 10-130), Bunsen burner, slides and cover-glasses.

### *3.2.2.1Determination of microscopic features*

The procedure described in WHO, (1998) was adopted. A small quantity of the stem bark powder was cleared using chloral hydrate solution with little heating and mounted with a few drops of dilute glycerol and observed under the microscope for characteristic microscopic features.

This process was repeated using difference slides and the representative microscopical features were recorded using photomicrography facility provided with the microscope.

*3.2.2.2 Chemomicroscopical examination of cell wall materials and the cell contents of P. africana*

Small amount of Cleared stem bark powder was subjected to staining with chemical reagents to identify its characteristic microscopic cell wall materials and cell contents. The slides were properly stained with drops of specific reagents for each corresponding diagnostic characters (cellulose, mucilage, phloem, starch grains, aleurone grains, calcium oxalate crystals, cork cells and sclerids) and were observed under the microscope for the presence of such diagnostic characters. The representative microscopical features were recorded using photomicrography facility provided with the microscope. The above procedure was repeated with different slides (WHO, 1998). The various tests are as described below:

***Cellulose test*:** Two drops of iodinated zinc chloride solution were added to the powder mounted on the slide and allowed to stand for 1 minute. The excess reagent was removed with a strip of filter paper and 1 drop of 66% sulphuric acid was then added.

***Lignified cell wall test:*** The powder on the slide was moistened with a small volume of phloroglucinol and allowed to stand for about 2 minutes until almost dry. Then 1 drop of conc. hydrochloric acid was added and the cover glass applied.

***Calcium carbonate test:*** A few drops of hydrochloric acid was added to a mounted and cleared bark powder and observed for slow dissolution of crystals with effervescence which will indicate the presence of calcium carbonate.

***Subrerized cell wall test:*** A drop of Sudan Red solution was added to a mounted and cleared powder and allowed to stand for a few minutes and observed for orange-red or red stain.

***Test for Aleurone Grains:*** A drop of 1% picric acid in water was added to a mounted and cleared stem bark powder and observed for yellow colouration.

***Test for Tannin:*** A drop of ferric chloride was added to a mounted and cleared bark powder and observed for bluish black or green black colouration.

***Test for Starch:*** The stem bark powder was mounted in N/50 iodine and observed for a bluish colouration.

***Test for Hydroxyanthraquinones:*** A drop of potassium hydroxide (55 g/l) was added to the mounted powder and observed for red stain.

### 3.2.3 Physicochemical analysis

The methods adopted for the phytochemical examination of the stem bark powder, are as described in WHO, (1998).

#### *3.2.3.1Determination of Moisture Content*

The moisture content was determined by "Loss on Drying" method (gravimetric determination). Air-dried stem bark powder (3 g) was weighed using KERN EW Electronic Balance and placed in a previously dried and weighed crucible. The crucible was transferred into a hot air sterilizing cabinet (model GRX-9053A), which was set at

$105^0$C. After an hour, the crucible was removed and placed in a desiccator over phosphorus pentoxide and under atmospheric pressure and at room temperature. After 30 minutes in the desiccator, the weight of the powder and crucible were quickly determined and the crucible returned to the oven. The heating and weighing process were repeated until two consecutive constant weights were achieved. The moisture content (loss of weight) was calculated using the following formula:

$$\%\ Moisture\ content = \frac{Initial\ Weight\ of\ powder - Final\ weight\ of\ Powder}{Initial\ Weight\ of\ Powder}\ X\ 100$$

*3.2.3.2 Determination of Total Ash Value*

A Nikel crucible was heated red hot, cooled in a desiccator and quickly weighed. Exactly 3g of the air-dried stem bark powder was weighed into the previously heated crucible. The powder was spread in an even layer and ignited by gradually increasing the heat on an electric hot plate until the powder was almost white, indicating absence of carbon. The crucible with ash was cooled in a desiccator for 30 minutes and weighed without delay. This procedure was repeated three times to obtain average value. The total ash content of the air-dried powder was calculated in percentage, using the following formula:

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

*3.2.3.3 Determination of Acid-insoluble Ash*

To the crucible containing the total ash, was added 25ml of 2N hydrochloric acid and covered with a watch glass. This was boiled for 5 minutes on a hot water bath. The

watch glass was rinsed with 5ml of hot water and added to the crucible. The mixture was filtered through a 125mm Dia Ashless Whatman® Filter paper (No. 41). The residue in the filter paper was repeatedly washed with hot water. The filter paper containing the residue was transferred to the original crucible, dried on hot plate and allowed to be heated to ignition to a constant weight. The heated crucible with residue was placed in a desiccator for 30 minutes to cool and quickly weighed. The acid insoluble value was calculated as follows:

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

*3.2.3.4 Determination of Water Soluble Ash*

To the nickel crucible containing the total ash, was added 25ml of water and boiled on a water bath for 5 minutes. The mixture was filtered through a 125mm Dia Ashless Whatman® Filter paper (No. 41). The residue in the filter paper was repeatedly washed with hot water. The filter paper containing the residue was transferred to the original crucible, dried on hot plate and allowed to be heated to ignition to a constant weight. The weight of this residue was subtracted from the weight of the total ash. The water soluble ash content of the air dried powder was therefore calculated using the following formula:

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

*3.2.3.5 Determination of Extractive Values*

*Water Extractive Value: Hot Extraction method*

Exactly 4g of air-dried stem bark powder was weighed into a glass stoppered conical flask. 100ml of water was added and the total weight including the flask and powder was determined. The flask was well shaken and allowed to stand for one hour. The flask's content was gently boiled on a hot water bath for one hour, cooled and weighed. This weight was readjusted to the original total weight with water. This was well shaken and filtered rapidly through a dry Whatman® filter paper. A 25ml volume of the 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 $105^0$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:

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

**Water Extractive Value: Cold Maceration Method**

Exactly 4g of air-dried stem bark powder was weighed into a 250ml glass stoppered conical flask and 100ml of water was added to macerate the powder for 6 hours with frequent shaking. The flask was allowed to stand for 18 hours. This was well shaken and filtered rapidly through a dry Whatman® filter paper. A 25ml volume of the 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 $105^0$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:

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

**Alcohol Extractive Value: Hot Extraction method**

The process is as described for water above but with 96% ethanol as the extracting solvent.

**Alcohol Extractive Value: Cold Maceration Method**

The procedure is as adopted as for water above, but with 96% ethanol as the extracting solvent.

## 3.3 Specific Aim 2: Acute toxicity and antiulcer evaluation of *P. africana*

### 3.3.1 Acute toxicity evaluation of the crude extract

#### *3.3.1.1Animal Stock*

White Wistar rats of both sexes weighing 160 – 220g were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dose administration to allow for acclimatization to laboratory conditions. All the animals were housed in a cross ventilated room with temperature of about 37 ± $2^0$C, 12 hours light/12 hours dark cycle. The rats were fed with standard (Grower) mash (vital feed, Jos, Nigeria) and water ad-libitum (Nwafor, 2000).

#### *3.3.1.2Preparation of plant extract*

Using the same method as in 3.3.1, 500g of the stem bark powder of *Prosopis africana* was extracted with ethylacetate. The filtrate was concentrated in vacuo to obtain the *Prosopis africana* stem bark ethylacetate extract.

#### *3.3.1.3Acute Toxicity Testing:*

The acute toxicity testing was performed as described in OECD 425, (2008). A 300 mg/ml concentration of the aqueous extract of *Prosopis africana* was prepared and administered (3000 mg/kg dose) to each of the test animals. A single bolus dose of 3000 mg/kg body weight was administered to an overnight fasted rat orally using an oral feeding tube and observed for signs of toxicity or death during the first 30 minutes after administration and periodically during the first 24 hours with special attention during the first 4 hours. Food and water were withheld for the first 4 hours after administration. After 48 hours of no death occurring, the next rat was similarly administered 3000 mg/kg of the extract and similarly observed. Sequentially, 3 other rats received similarly doses and observed. All the 5 rats were individually observed once daily for signs of toxicity or death for a period of 14 days. Food and water were provided throughout the 14 day observation period. The rats were observed for signs of toxicity, such as, excitation, tremors, twitches, motor coordination, righting reflex and respiratory changes.

The toxicological effect was assessed on the basis of mortality and expressed as $LD_{50}$ and calculated using Up and Down procedure (OECD 425, 2008). The results were evaluated as follows (O=survival, X=death). The $LD_{50}$ is less than the test dose (3000 mg/kg) when three or more animals die. The $LD_{50}$ is greater than the test dose (3000 mg/kg) when three or more animals survive.

### 3.3.2 Antiulcer evaluation of the crude ethylacetate extract

*3.3.2.1Animals*

Healthy adult male and female Wistar rats between 2 and 3 months of age and weighing about 150 – 200g were used for the study. The rats were maintained under standard

conditions (12hr light: 12hr dark cycle; 25±3$^0$C; 35 – 60% humidity), and fed with standard rat pellet diet.

### *3.3.2.2Gastroprotective effect of Prosopis africana using the absolute ethanol induced ulcer model*

The modified method of Nwafor, (2000) was adopted. The rats were randomly divided into 5 groups of five rats each. The rats were fasted for 24 hours before the experiment, but were allowed free access to drinking water until 2 hours before the experiment. Group 1 rats were pretreated with 1ml of water orally using an orogastric cannula. In the same way, groups 2, 3, and 4 were pretreated with 10%, 20% and 30% respectively of the limit dose (3000 mg/kg) of the ethylacetate extract of *Prosopis africana*. Group 5 rats were pretreated with Omeprazole (Ompraxin® by Jiangsu Ruinian Qjanjin Pharm. Co. Ltd. China) 20 mg/kg body weight. After 30 minutes, gastric lesions were induced in all the five groups with absolute ethanol at a dose of 8 ml/kg (Mizui, 1987) administered by orogastric intubation. After an interval of 2 hours the rats were sacrificed by cervical dislocation, their stomachs removed and opened along the greater curvature. Each stomach was gently rinsed with water to remove the gastric contents and blood clots for subsequent ulcer scoring. The ulcer score was determined by measuring the ulcer index (Darbar, 2010).

**Measurement of Ulcer Index:** The lesion was examined under a 10 x dissecting microscope. Long lesions were counted and measured along their greater length. Petechial lesions were counted. Each five petechial lesions were taken as 1mm of ulcer (Darbar, 2010). The sum of the total length of long ulcers and petechial lesions in each

group of rats was divided by its number to calculate the ulcer index (mm). The macroscopic curative ratio was determined using the formula:

$$\text{Curative ratio (\% Gastroprotection)} = \frac{(\text{Control ulcer index}) - (\text{Test ulcer index})}{(\text{Control ulcer index})} \; X\; 100$$

(Navarrete, 1998)

**Statistical analysis:** The values were expressed as mean ± SDev for five rats in each group. All the data were analyzed with Microsoft Excel 2010 software. One way analysis of variance (ANOVA) was applied as the Hypothesis testing method. A p-value of less than 0.05 was considered to indicate statistical significance.

### *3.3.2.3Gastroprotective effect of Prosopis africana pre-treatment using the indomethacin induced ulcer model*

The method of Nwafor (2000), was slightly modified and adopted for this work. The rats were divided into 5 groups of five rats each. The rats were fasted for 24 hours, but were allowed free access to drinking water until 2 hours before the experiment. Group 1 rats were pretreated with 1ml of water orally. Groups 2, 3, and 4 were pretreated with 10%, 20% and 30% respectively of the limit dose (3000 mg/kg) of the ethylacetate extract of *Prosopis africana*. Group 5 rats were pretreated with Omeprazole 20 mg/kg body weight. After 1 hour, gastric lesions were induced in all the five groups with 100 mg/kg of indomethacin administered by orogastric intubation. After 4 hours, the animals were sacrificed by cervical dislocation and the gastric ulcer scores determined as described previously.

### 3.3.3 Antiulcer Evaluation of Chloroform and Methanol fractions of the crude extract

Using the methods described in 3.4.2.1 and with slight modifications of 3.4.2.3 above, the gastroprotective property of the chloroform and methanol fractions were investigated. For the chloroform fractions, group 1 rats were pretreated with carboxy methylcellulose (CMC) 0.5% (0.3 ml/100g rat). Groups 2 and 3 rats were pretreated with 600 mg/kg chloroform fraction in 0.5% CMC and 900 mg/kg chloroform fraction in 0.5% CMC respectively. For the methanol fraction, group 1 rats were pretreated with 1 ml water while groups 2 and 3 rats were pretreated with 600 mg/kg and 900 mg/kg of the methanol fraction respectively.

## 3.4 Specific Aim 3: Chemical and chromatographic evaluation of *P. africana*

### 3.4.1 Preparation of *P. africana* extract

The method of Jean de Dieu, *et al*., 2012) was adopted for the extraction process. About 300g of the stem bark powder of *Prosopis africana* was extracted with ethylacetate using a Soxhlet extractor to obtain the crude ethylacetate extract of the plant.

### 3.4.2 Thin layer chromatographic (TLC) analysis of the crude ethylacetate extract

An 11ml volume of 4:6:1 (V/V/V) Petroleum Ether, Chloroform, Methanol solvent system was placed in a 250ml capacity chromotank. The sides of the tank were lined with filter paper and covered for saturation. The crude ethylacetate extract of *Prosopis africana* was spotted at a 1cm mark from the bottom on a pre-coated Thin Layer Chromatographic (TLC) plate; and allowed to migrate to about 1cm from the top of the

plate. The above procedure was repeated while spotting with the petroleum ether and Chloroform fractions respectively.

*3.4.2.1Detection of spots on the Chromatogram*

The plates were sprayed with 5% sulphuric acid and heated in an oven at $105^0$C for about 10 minutes. The developed spots were observed and photographed. The $R_f$ value for each spot was calculated using the following formula.

$$Rf = \frac{a}{b}$$

Where *a* = the distance between the point of application and the centre of the spot being examined;

*b* = the distance between the point of application and the solvent front;

The above procedures were repeated 4 times to check for consistency of observation.

### 3.4.3 Fractionation of the crude ethylacetate extract of *P. africana*

Using Kern EW Electronic Scale, 4.0g of the crude ethylacetate extract was weighed and suspended in water and partitioned successively with petroleum ether, chloroform and methanol to obtain their respective fractions. The fractions were concentrated and weighed.

### 3.4.4 Column chromatographic separation of the fractions

A 100ml capacity chromatographic column was packed with 60gm of 60-120 mesh silica gel in n-hexane solvent adopting the wet packing procedure and allowed to stand for 4 hours. The 1.24gm chloroform fraction was packed in the column. The column was eluted by gradient elution method starting with 100% n-hexane then finally with 0-

100% ethylacetate. Successive fractions of 10 ml each were collected in numerically labelled collection bottles. The fractions were monitored for purity using TLC with 4:6:1 (V/V/V) Petroleum Ether, Chloroform, Methanol solvent system. Fractions which showed single and identical spots on the chromatogram were pooled together to obtain a 12 mg sample for further analysis. This sample was designated J29.

### 3.4.5 Melting point determination of the isolate

A small amount of compound J29 was pushed into the open end of a capillary tube and tapped at the sealed end several times to fill and pack the sample well to the bottom. This step was repeated until the sample accumulated to about 2 mm (≈ 0.5 cm) high in the bottom of the tube. The tube was placed in Digimelt® melting point apparatus (model MPA 161) and the temperature raised at the rate of about 5°C/minute. The temperatures at which the first crystals melted and at which the sample completely melted were noted as the melting point of compound J29.

### 3.4.6 Spectroscopic analysis for characterization of the isolate

The following spectroscopic methods were adopted to ascertain the identity of compound J29.

#### *3.4.6.1Infrared (IR) Spectroscopy of the isolate*

Fourier Transform Infrared Spectroscopy (FTIR) experiments were performed using Shimadzu (Model 4800S) instrument controlled by IR solution software set at spectra

resolution of 4$cm^{-1}$. A KBr disc was first prepared to serve as a blank then, the sample AJ29 was dissolved in Chloroform and grounded with KBr and pressed to make the sample disc (window). The prepared sample window was scanned between 400 – 4000$cm^{-1}$ twenty times and the mean was printed.

### *3.4.6.2Nuclear Magnetic Resonance (NMR)of the isolate*

Both $^{1}$H-NMR and $^{13}$C-NMR analyses were performed on the 12mg of sample AJ29 using Topspin 300 MHz, Bruker Germany at University of Pretoria, South Africa. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded using $CDCl_3$ as solvent that dissolved the sample on Bruker and TMS as internal standard reference which was added to the sample before recording.

# CHAPTER FOUR

# RESULTS

## 4.1 Macroscopic Examination of *Prosopis africana* Stem Bark

Table 4.1: Macroscopic analysis of *Prosopis africana* stem bark

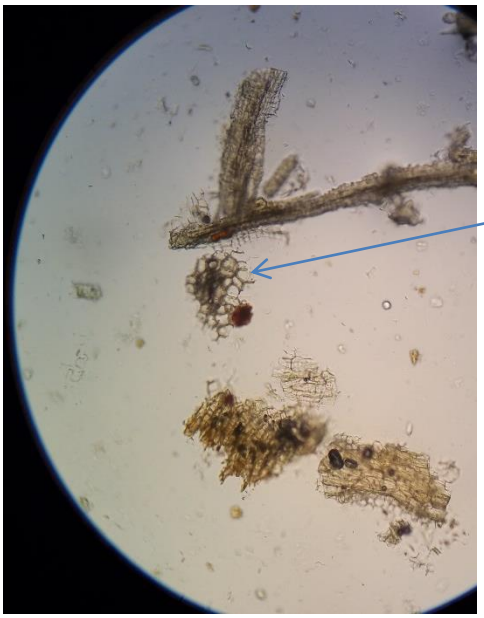
| **Test Parameter** | **Observation** |
|---|---|
| Colour | Dark grey with White/Ash coloured patches. Reddish brown underneath the scale |
| Internal colour | Reddish brown |
| Texture | Hard |
| Shape | Curved |
| Odour | Weak, musty and characteristic |
| Taste | Characteristic |
| Fracture | Outer bark is short and rough in the inner bark |
| Thickness | 4mm – 7mm |
| Internal surface | Longitudinally striated |
| Powder colour | Reddish brown |



Plate III: *P. africana* stem bark

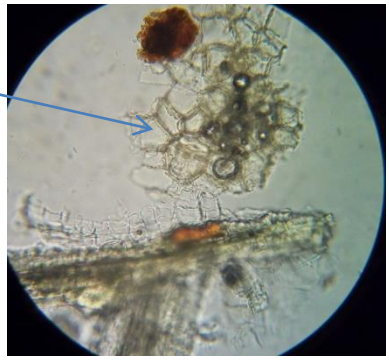
### 4.2 Results of Microscopic Examination of The Stem Bark Powder

Observed microscopic features in the stem bark power were recorded thus: radial bands of rectangular, tangentially elongated, thin-walled cork cells in rows. Secondary phloem composed of phloem fibres in small patches with thin walled parenchyma in between. Few druses type of calcium oxalate crystals were observed as shiny prisms with unequal axes. Fibres observed are elongated cells with blunt end and thick walls. Sclerids are isodiametric in shape. The walls are thick, lignified, showing well marked stratification. Starch grains are present. Calcium carbonate was not observed.



X100 magnification

Cork cells
Uniformly thickened and radially arranged with thickened sclerified walls



X400 magnification

Starch grains



X100 magnification

Non collapsed secondary phloem

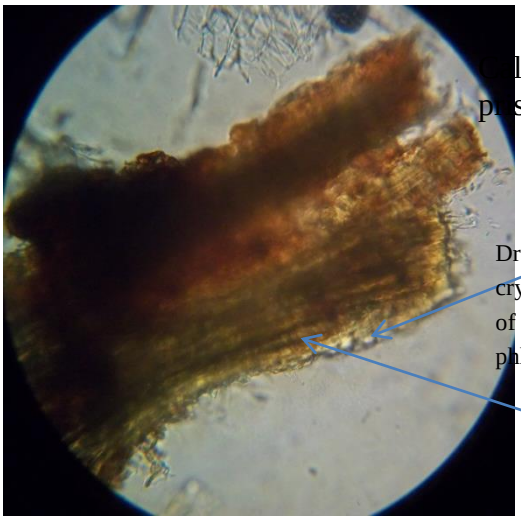
Sieve plate with sieve area

Fibre

Sclerid



X400 magnification



X400 magnification

Calcium Oxalate Druse prismatic crystals

Druse prismatic crystals at the boarders of the non- collapsed phloem fibre

Phloem



X400 magnification

**Plate IV: Photomicrograph of the stem bark powder**

## 4.3 Chemomicroscopical Analysis Of The Stem Bark Powder

### 4.3.1 Microscopic evaluation of *P. africana* cell wall materials

Table 4.2: Chemomicroscopic data of cell wall materials of *P. africana*

| **Test** | **Colour in ordinary light** | **Inference** |
|---|---|---|
| Powder + iodinated ZnCl + 66% $H_2SO_4$ | Blue-violet | Cellulose present |
| Powder + Phloroglucinol + Conc. HCl | Deep red | Lignin present |
| Powder + Sudan red | Red stain | Cell wall Suberized |

### 4.3.2 *Microscopic Evaluation of P. africana Cell Contents*

Table 4.3: Chemomicroscopic data of cell contents of *P. africana* bark

| **Powder and Reagent** | **Observation** | **Inference** |
|---|---|---|
| Powder + 80% $H_2SO_4$ | Un-dissolved crystals | Calcium oxalate crystals present |
| Powder + HCl | No effervescence | Calcium carbonate absent |
| Powder + 1% Picric acid | Yellow colouration | Aleurone grains present |
| Powder + $FeCl_3$ | Greenish black | Tannins present |
| Powder + N/50 iodine | Blue | Starch present |
| Powder + KOH (55g/L) | Red stain | Hydroxyanthraquinnones present |

## 4.4 Results Of The Physicochemical Analysis

The values of this analysis are presented in Table 4.4.

Table 4.4: Physicochemical parameters of *P. africana*

| **Physicochemical parameters** | **Constant value** |
|---|---|
| Moisture Content | 5.27% |
| Total Ash Value | 3.93% |
| Acid Insoluble Ash Value | 1.61% |
| Water Soluble Ash Value | 0.33% |
| Water Extractive Value (Hot Maceration) | 22% |
| Water extractive value (Cold maceration) | 16% |
| Alcohol Extractive Value (Hot maceration) | 32% |
| Alcohol Extractive Value (Cold maceration) | 25% |

## 4.5 Acute Toxicity Profile of the Crude Ethylacetate Extract

Table 4.5: Response of wistar rats treated with 3,000 mg/kg, ethylacetate extract of *Prosopis africana* stem bark in acute toxicity test

| **Test sequence** | **Test animal ID** | **Weight After Fasting (g)** | **Dose of *P. africana* required** | **Volume of Preparation Administered** | **Short Term outcome** | | **Long term outcome** | |
|---|---|---|---|---|---|---|---|---|
| | | | | | After 30 Minutes | After 24 hours | After 48 hours | After 14 days |
| **1.** | J1 | 199.03 | 597 | 2ml | O | O | O | O |
| **2.** | J2 | 155.41 | 466 | 1.6ml | O | O | O | O |
| **3.** | J3 | 172.69 | 518 | 1.7ml | O | O | O | O |
| **4.** | J4 | 159.02 | 477 | 1.6ml | O | O | O | O |
| **5.** | J5 | 176.35 | 529 | 1.8ml | O | O | O | O |

The concentration of *P. africana* extract in water used = 300mg/ml

## 4.6 Antiulcer Profile of *Prosopis africana*

Table 4.6: Antiulcer activity of *Prosopis africana* Stem bark ethylacetate extract

| | **Absolute Ethanol (8 ml/kg)** | | | **Indomethacin (100 mg/kg)** | | |
|---|---|---|---|---|---|---|
| | **n** | **Ulcer index** | **% protection** | **N** | **Ulcer index** | **% protection** |
| **Control** | 12 | 7.92±0.58 | 0 | 13 | 2.50±0.22 | 0 |
| PAE 300 mg/kg | 6 | 6.41±1.87 | 19 | 12 | 1.94±0.36 | 22 |
| PAE 600 mg/kg | 4 | 2.32±0.88 | 71 | 8 | 1.42±0.18 | 43 |
| PAE 900 mg/kg | 4 | 1.50±0.35 | 81 | 5 | 1.10±0.14 | 56 |
| Omeprazole 20 mg/kg | 12 | 6.70±0.30 | 15 | 11 | 1.79±0.47 | 28 |
| One way ANOVA | F | 43.1 | | F | 15.7 | |
| | P | 0.000 | | P | 0.000 | |

Ulcer index Values are mean±SDev; significantly different from control at *P value <0.05, (Dunnett's test for ulcer index). n is the number of ulcer points.

## 4.7 Antiulcer Evaluation of Chloroform and Methanol fractions of the crude extract

Table 4.7: Antiulcer activity of the chloroform fraction of *Prosopis africana* extract

| | | Indomethacin (100 mg/kg) | |
|---|---|---|---|
| | **N** | **Ulcer index** | **% protection** |
| **Control CMC 0.5%** | 11 | 2.31±0.23 | 0 |
| CFPA 600 mg/kg | 7 | 1.46±0.15 | 37 |
| CFPA 900 mg/kg | 5 | 1.22±0.08 | 47 |
| Omeprazole 20 mg/Kg | 9 | 1.59±0.31 | 31 |
| One way ANOVA | F | 24.9 | |
| | P | 0.000 | |

CMC- Carboxy methyl cellulose (control), CFPA- Chloroform fraction of *Prosopis africana* stem bark

Table 4.8: Antiulcer activity of the methanol fraction of *Prosopis africana* extract

| | | Indomethacin (100 mg/kg) | |
|---|---|---|---|
| | **N** | **Ulcer index** | **% protection** |
| **Control** | 13 | 2.50±0.22 | 0 |
| MFPA 600 mg/kg | 9 | 1.50±0.16 | 40 |
| MFPA 900 mg/kg | 6 | 1.20±0.10 | 52 |
| Omeprazole 20 mg/Kg | 11 | 1.79±0.47 | 28 |
| One way ANOVA | F | 20.5 | |
| | P | 0.000 | |

MEPA- Methanol fraction of *Prosopis africana* stem bark

## 4.8 Chromatographic evaluation of *P. africana*

### 4.8.1 *Thin Layer Chromatographic Profile of P. africana bark extract*

The data of qualitative profile of the stem bark extract, of *P. africana* by thin layer chromatography is presented in the Table 4.9.

Table 4.9: TLC profile of the stem bark extract of *P. africana*

| s/n | Spot Colour | $R_f$ value | Fraction Analysed | | |
|---|---|---|---|---|---|
| | | | Crude Ethylacetate Extract | Chloroform Fraction | Petroleum Ether Fraction |
| **1.** | Ash | 0.92 | + | + | + |
| **2.** | Reddish brown | 0.88 | + | + | + |
| **3.** | Green | 0.77 | + | + | + |
| **4.** | Light yellow | 0.62 | + | + | + |
| **5.** | Light Ash | 0.32 | + | - | - |
| **6.** | Purple | 0.29 | + | + | + |
| **7.** | Light ash | 0.25 | + | - | - |
| **8.** | Purple | 0.22 | + | + | - |
| **9.** | Ash | 0.15 | + | + | - |

Key: + implies the presence of the spot with the corresponding colour and $R_f$

- Implies absence of the spot with the corresponding colour and $R_f$.

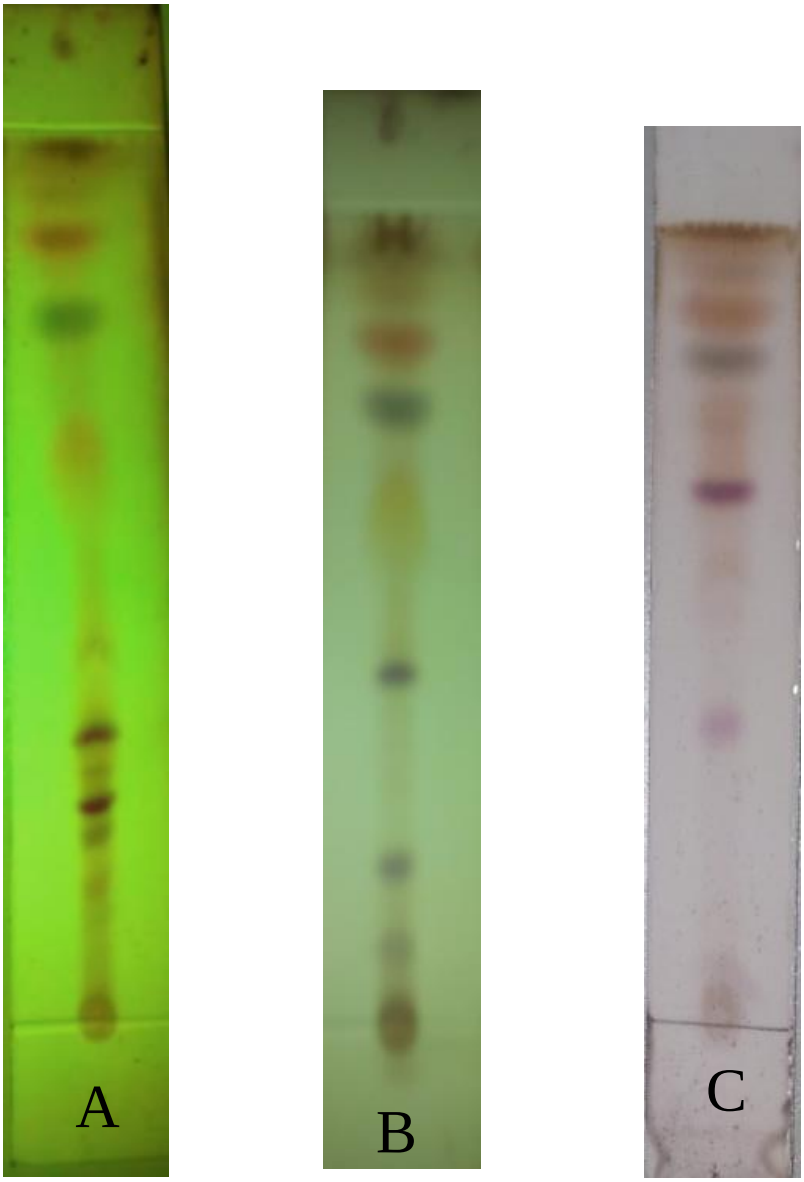



Plate V: A, B and C above show the chromatograms of crude ethylacetate extract, chloroform and petroleum ether fractions respectively of *P. africana* stem bark.

### 4.9 Column Chromatographic Separation of the Ethylacetate Extract of *P. africana* Stem Bark

Table 5.0: Column chromatographic isolate from the Stem Bark of *P. africana*

| Fraction type loaded | Quantity loaded | Quantity of isolate | Yield |
|---|---|---|---|
| Chloroform | 1.24 g | 0.012 g | 0.97% |

### 4.10 Melting point analysis

Melting point analysis of the isolate (J29) was found to be 161 -163$^0$C

## 4.11 Spectroscopic Analysis of J29

The various spectroscopic evaluation of sample J29 showed the following results.

### 4.11.1 Infra-Red Spectroscopic analysis of J29

Table 5.1: The IR spectrum of J29

| Band type | Wavenumber $Cm^{-1}$ | Possible Bond |
|---|---|---|
| Sharp | 3254.05 | $CH_3$ Stretching |
| Sharp | 2899.11 | C –H Asymmetric and Symmetric Stretching |
| Sharp | 1751.05 | C=O Stretching for Ketone |
| Sharp | 1498.74 | C-H Bending for $CH_3$ |

4.11.2 NMR Spectroscopic Analysis of J29

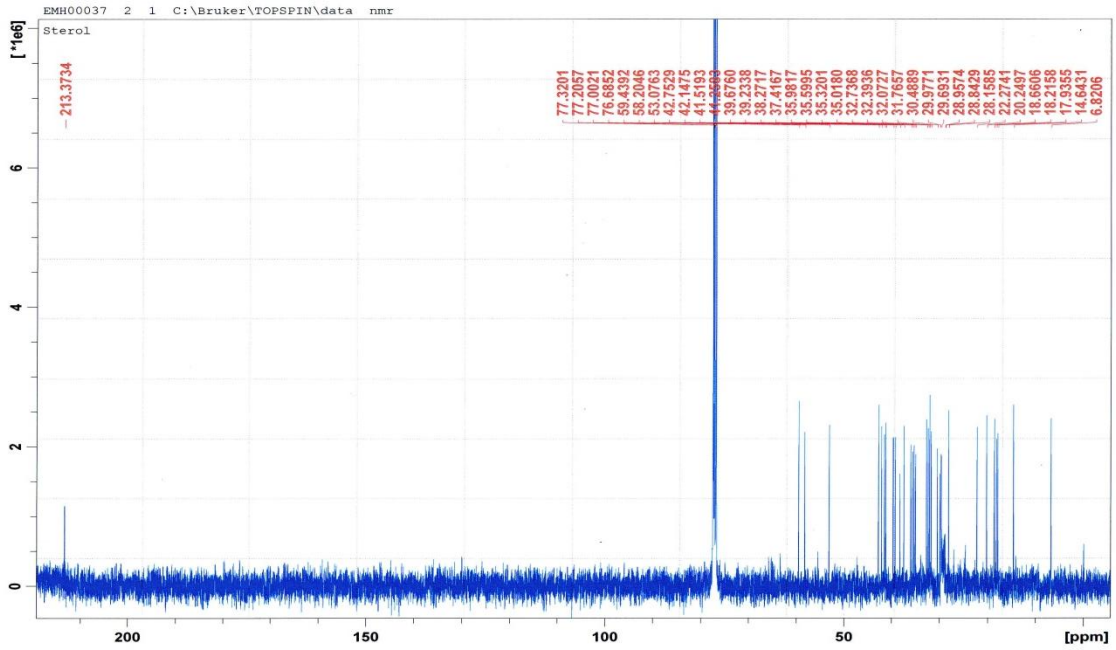



Figure 3: $^{13}C$ Spectrum of J29 isolate from *P. africana* stem bark

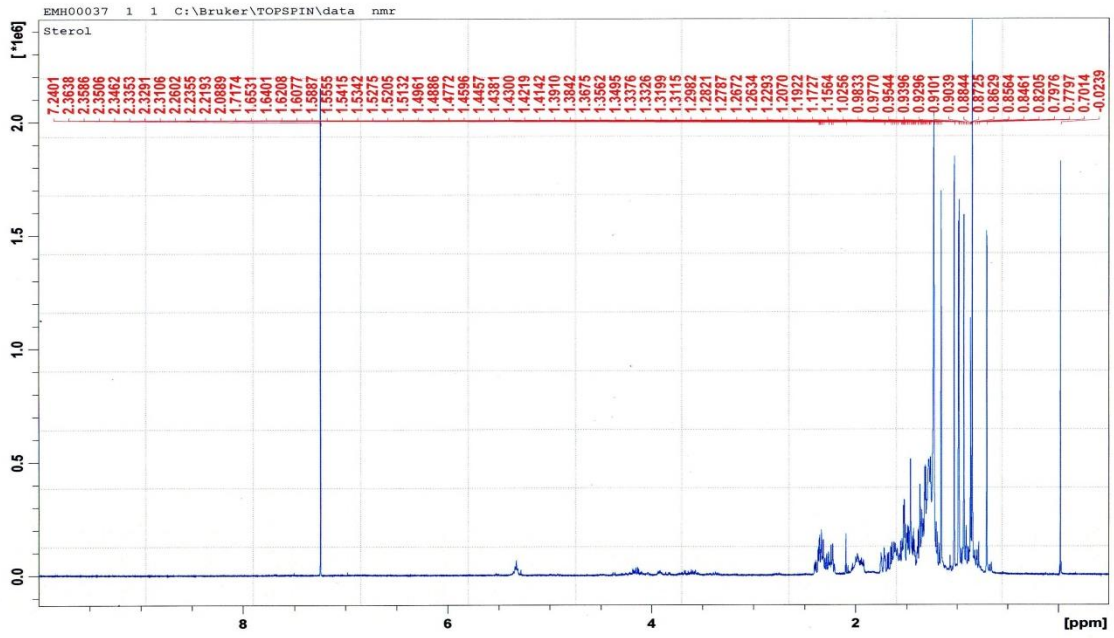



Figure 4: [1]H NMR Spectrum of J29 isolate from *P. africana* stem bark

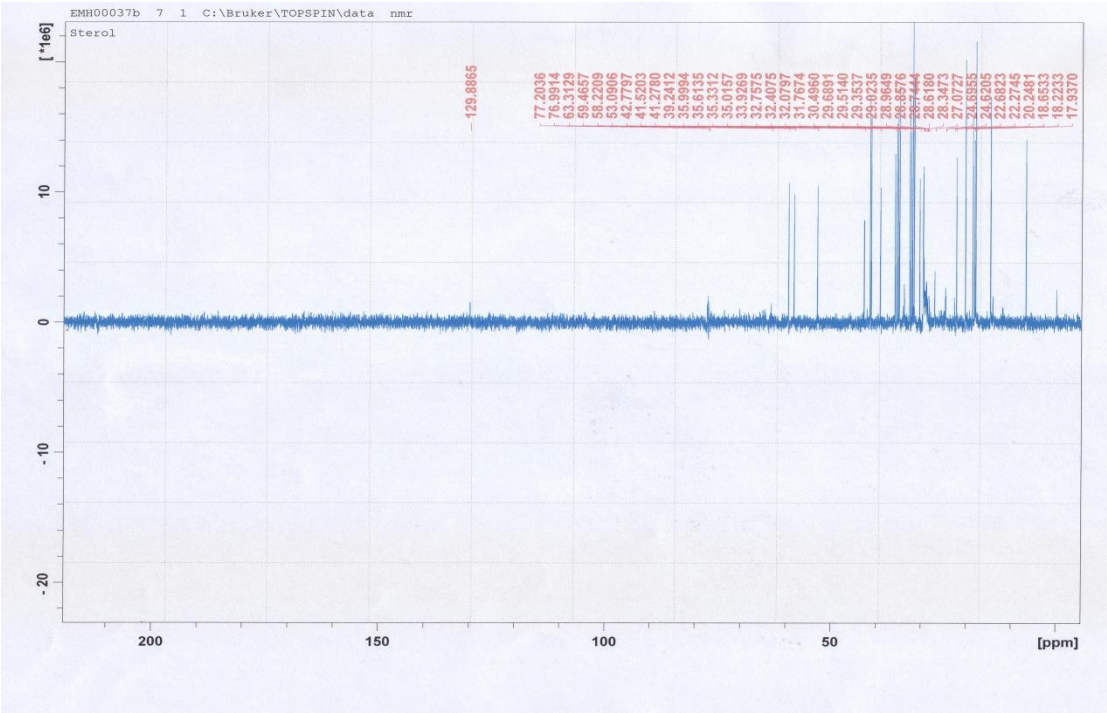



Figure 5: DEPT Spectrum of J29 isolate from *P. africana* stem bark

Table 5.2: $^{1}$H-NMR and $^{13}$C-NMR Chemical Shift of J29

| H/C Position | δc (ppm)Ref. | δc (ppm)J29 | δH (ppm)Ref. | δH (ppm) J29 | Multiplicity |
|---|---|---|---|---|---|
| 1 | **22.3** | 22.27 | **1.90,1.6** | 2.09,1.61 | 2H,m |
| 2 | **41.5** | 41.52 | **2.22,2.32** | 2.22,2.33 | 2H,m |
| 3 | **213.2** | 213.37 | _ | _ | _ |
| 4 | **58.2** | 58.20 | **2.23** | 2.24 | 1H,m |
| 5 | **42.1** | 42.15 | _ | _ | _ |
| 6 | **41.3** | 41.26 | **1.66,1.21** | 1.65,1.21 | 2H,m |
| 7 | **18.2** | 18.20 | **1.36,1.46** | 1.36,1.46 | 2H,m |
| 8 | **53.1** | 53.07 | **1.36** | 1.37 | 1H,m |
| 9 | **37.4** | 37.42 | _ | _ | |
| 10 | **59.4** | 59.44 | **1.50** | 1.50 | 1H,m |
| 11 | **35.6** | 35.60 | **1.23,1.25** | 1.26,1.28 | 2H,m |
| 12 | **30.5** | 30.49 | **1.23,1.25** | 1.23,1.26 | 2H,m |
| 13 | **39.7** | 39.68 | _ | _ | _ |
| 14 | **38.3** | 38.27 | _ | _ | _ |
| 15 | **32.4** | 32.39 | **1.36,1.46** | 1.38,1.48 | 2H,m |
| 16 | **36** | 35.98 | **1.36,1.46** | 1.35,1.45 | 2H,m |
| 17 | **30** | 29.98 | _ | _ | |
| 18 | **42.8** | 42.75 | **1.40** | 1.41 | 1H,m |
| 19 | **35.3** | 35.32 | **1.60,1.20** | 1.59,1.19 | 2H,m |
| 20 | **28.1** | 28.16 | _ | _ | |
| 21 | **32.7** | 32.74 | **1.41,0.90** | 1.42,0.90 | 2H,m |
| 22 | **39.2** | 39.23 | **1.41,0.90** | 1.39,0.91 | 2H,m |
| 23 | **6.8** | 6.82 | **0.81** | 0.82 | 3H,s |
| 24 | **14.6** | 14.64 | **0.65** | 0.70 | 3H,s |
| 25 | **17.9** | 17.94 | **0.80** | 0.79 | 3H,s |
| 26 | **20.2** | 20.25 | **0.98** | 0.98 | 3H,s |
| 27 | **18.6** | 18.66 | **0.94** | 0.94 | 3H,s |
| 28 | **32.1** | 32.07 | **1.11** | 1.15 | 3H,s |
| 29 | **35** | 35.60 | **0.93** | 0.93 | 3H,s |
| 30 | **31.8** | 31.77 | **0.88** | 0.88 | 3H,s |

The reference used is (Rajesh, 2012).

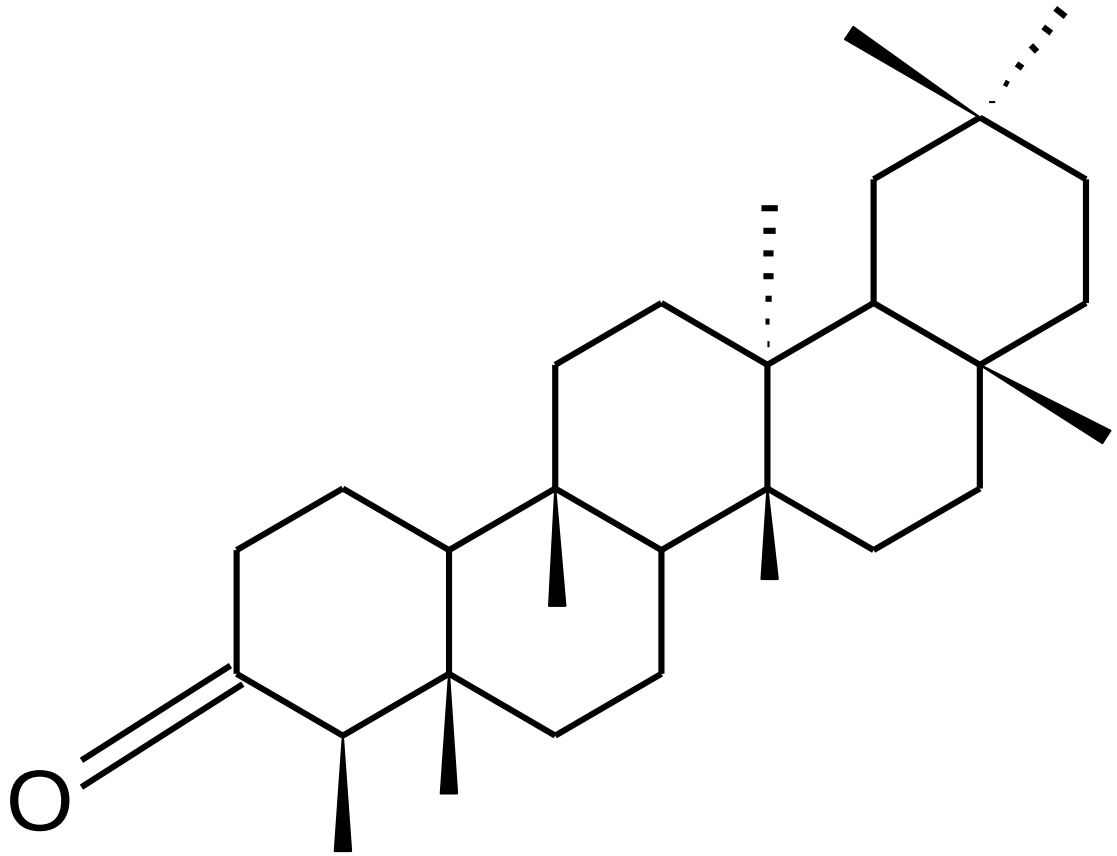



Figure 6: Friedelin

The triterpenoid compound friedelin was isolated and identified from *Prosopis africana*. Friedelin is found to be present in many plants (Chang *et al*., 2003).

# CHAPTER FIVE

## DISCUSSIONS

*Pharmacognostic evaluations:* Accurate morphological and anatomical standardization of drug plants is very essential. In this study, the pharmacognostic standardization and physico-chemical evaluations of the stem bark of *Prosopis africana* were undertaken. The results of this study can be used as diagnostic tool for the standardization of *Prosopis africana* and will be helpful in the characterisation of the crude drug. According to World Health Organization (WHO, 1988) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken (WHO, 1998).

The following investigated anatomical and phytochemical features of *Prosopis africana* are the key features that can be used to diagnose it. Macroscopic examination of the stem bark (table 4.1 and Plate III) revealed dark grey coloured bark with white/ash coloured patches having reddish brown colouration underneath the scale. The odour is weak, musty and characteristic. The taste is characteristic and the bark has a rough fracture with longitudinally striated internal surface. The stem bark powder is reddish brown in colour. Observed microscopic features (Plate IV) in the stem bark power were recorded thus: radial bands of rectangular, tangentially elongated, thin-walled cork cells in rows. secondary phloem composed of phloem fibres in small patches with thin walled parenchyma in between. Few druse type of calcium oxalate crystals were observed as shiny prisms with unequal axes. This feature is significant in that the morphology and distribution of crystals is constant within a species. Their presence, morphology and

distribution in a species are under genetic control (Ilarslan, 2001); (Franceschi, 2005). Thus the constancy of crystal type and distribution may be considered a taxonomic character for classification of species (Franceschi, 2005). The crystal pattern is therefore an important identification tool. The crystal pattern is also often stable within a genus (Lersten, 2000). Fibres observed are elongated cells with blunt end and thick walls. Sclerids are isodiametric in shape. The walls are thick, lignified, showing well marked stratification. Starch grains are present. Calcium carbonate was not observed. All these features are composite units of the data that are required to provide collective identification and standardization parameter for *P. africana* stem bark.

Histochemical (chemomicroscopic) examination of the stem bark (table 4.3) revealed the presence of lignin, tannins, saponins, suberized cell wall, aleurone grains, calcium oxalate crystals and hydroxyanthraquinones. Kolapo (2009), reported the presence of significant quantity of saponin and tannins in *P. afriana* stem bark. From table 4.4, the physicochemical parameters for the stem bark powder were established as 5.27% moisture content, 3.93% total ash value, 1.61% acid insoluble ash value, 0.33% water soluble ash value, 22% water extractive value (hot maceration), 16% water extractive value (cold maceration), 32% alcohol extractive value (hot maceration). The above parameters serve as referential data for the identification and quality control of *P. africana* crude drug. The physicochemical analysis of plant drugs is an important tool for detecting adulteration or improper handling of drugs (Javed, 2012). The total ash is particularly important in the evaluation of purity and quality of drugs. In this study, the ash value was determined by 3 different methods, which measured total ash, acid insoluble ash, and water soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition (Singh, 2010). The total ash which

include both physiologic ash and non-physiologic ash usually consists of carbonates, phosphates, silicates and silica,. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing (Mukherjee, 2002). Acid insoluble ash indicates contamination with silica, for example, earth and sand. Comparison of this with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug. Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water soluble salts in the drug. Extractive values are representative of the presence of polar or nonpolar extractable compounds in a plant material. Moisture is an inevitable component of crude drugs, which must be eliminated as much as possible. Moisture content value obtained in this study (5.27%) shows that the material could be preserved over a long period of time without deterioration of the drug (Prohp, 2011). Insufficient drying leads to spoilage by moulds and bacteria and makes possible the enzymatic destruction of active principles (Mukherjee, 2002). TLC finger print profile in Plate V, (A, B, C) and table 4.4, provides reference data for quick quality and identity assessment of *Prosopis africana* crude drug. The combination of the data obtained from macroscopic, histochemical and physico-chemical analyses obtained in this study can be used as standardization parameters of *Prosopis africana* stem bark.

*Acute toxicity profile:* Data from table 4.5 indicate that the limit dose of 3000 mg/kg of *P. africana* ethanolic extract did not cause any mortality or any sign of acute toxicity in any of the five test animals, from the time of administration of the extract and the 14 day period of observation. All rats treated with 3000 mg/kg limit test dose were initially hypoactive for about 10 minutes post administration of the extract but subsequently were generally active and exhibited normal rat behaviour throughout the 48 hours and

up to the 14 day of observation period. This result therefore suggests that *P. africana* extract has an $LD_{50}$ greater than 3000 mg/Kg (OECD 425, 2008). A limit test can be used efficiently to identify chemicals that are likely to have low toxicity. An $LD_{50}$ of 3000 mg/kg is therefore an indication that *P. africana* stem bark ethanolic extract has a low toxicity profile. The gram equivalent of the $LD_{50}$ of the extract in an adult male would be over 210 g, that is, a plate full of the extract, making it relatively safe. Likewise, the extract was administered orally to the test animals. This way the same route used by the local consumers of the plant products was used in the test animals. This finding could be extrapolated in human subjects. In any case the extract was not pure enough for parenteral route administration.

*Antiulcer profile:* Results of this study (table 4.6), demonstrate a gastro protective action of *Prosopis africana* extract (PAE) on the gastric mucosa of rats against both ulcerogen models vis ethanol (8 ml/kg) and endomethacin (100 mg/kg). The curative ratio of the PAE 300, 600 and 900 mg/kg doses were 19%, 71% and 81% respectively. The curative ratio profile increased with increase in doses of PAE. It is remarkable that the 600 mg/kg and 900 mg/kg doses of PAE produced greater gastro protection than omeprazole 20 mg/kg in both ulcerogen models. PAE at a 300 mg/kg dose produced slightly greater gastro protection (19%) against ethanol induced ulcer model than Omeprazole 20 mg/kg (15%) and this is reflected in the number of ulcer points (n) of 6 and 12 respectively. One way analysis of variance (ANOVA) of the mean ulcer indexes of the verified samples in both models produced a $p<0.05$ which is an indication of statistical significance. Ethanol induced both long ulcers and petechial lesion within a short time, which makes the technique suitable for investigating antiulcer drugs.

The aetiology of gastric ulceration is multifactorial and not 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 protective effects of mucous barrier depend not only on the gel structure but also on the amount or thickness of the layer covering the mucosal surface. The ability of the gastric mucosa to resist injury caused by endogenous secretions (acid, pepsin and bile) and by ingested irritants such as alcohol, aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) can be attributed to a number of factors that have been generally referred to as mucosal defence (Wallace, 2001).

Ulcers caused by ethanol are due to superficial damage to the mucosal cells. Exposure to ethanol increases the extension of cellular damage in a dose dependent manner. Ethanol induced gastric damage may be due to stasis in gastric blood flow, which contributes to the development of haemorrhage and necrotic aspects of tissue injury. This action is direct on the gastric epithelium also causing perturbation of mast cells and release of a vasoactive mediator such as histamine (Iyyam, 2010). Some reports show that changes in gastric circulation after ethanol administration remains unknown, but it has been reported that microcirculation damage can be prevented by prostaglandin administration (Guth, 1984). On the other hand, ethanol induced gastric lesions are

thought to arise as a result of direct damage of gastric mucosal cells, resulting in the production of free radicals and hyper oxidation of lipids (Iyyam, 2010). It is suggested that antioxidant compounds could be active in producing antiulcerogenic effects referred to as cytoprotection (Salim, 1990).

The results of the present study indicate that *Prosopis africana* extract displays an antiulcerogenic effect related to its cytoprotective activity, since it significantly reduced ethanol induced ulcers.

Therefore the observed antiulcer activity of *Prosopis africana* extract against the alcohol induced ulcer could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection.

The result of the present study (table 4.7) also shows that the chloroform fraction exhibits potent and dose related anti-ulcerogenic activities at doses of 600 mg/kg and 900 mg/kg. The methanolic fraction (table 4.8) of the extract also shows a potent and dose related anti-ulcerogenic activity. The methanolic fraction however, produced a more effective cytoprotection to the mucosa when compared to the chloroform fraction at equivalent doses.

Reduction of ulcers in indomethacin ligation model usually signifies a protective action and possibly an anti-secretory effect. (Atay, 2000).

Indomethacin is known to induce gastric ulcer by inhibition of prostaglandins which are cytoprotective to gastric mucosa (Wallace, 2001), particularly due to the inhibition of cyclooxygenase pathway of arachidonic acid metabolism resulting in excessive production of leukotrienes and other products of 5-lipoxygenase pathway (Rainsford,

1987). In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow, and regulating mucosal cell turnover and repair (Hayllar, 1995). Thus, the suppression of prostaglandin synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastroduodenal ulceration. Several studies have indicated that gastroduodenal protection by prostaglandins is due to increase in mucosal resistance as well as the decrease in aggressive factors, mainly acid and pepsin (Aly, 1987). The observed antiulcerogenic property of *Prosopis africana* may be due to increased synthesis of mucous and/or prostaglandins or could possibly be due to its 5-lipoxygenase inhibitory effect. *Prosopis africana* stem bark contains phenols, saponins and tannins (Kolapo, 2009). 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). Saponins have antioxidant properties and antioxidants generally have cytoprotective effect which may also account for the antiulcer activity observed with *Prosopis africana* stem bark extract.

*Isolation of Natural compound:* from the chloroform fraction which also showed a significant antiulcer activity, Compound J29 was isolated as white needles. The melting point of J29 is 261 – $263^0$C and it is soluble in chloroform. Compound J29 was identified by comprehensive analysis of its NMR spectra in comparison with published data, (Rajesh, 2012). From table 5.2, $^1$HNMR showed seven singlet Methyl ($CH_3$) signals at $\delta_H$ (ppm) 1.15 (3H, 28), 0.98 (3H, 26), 094 (3H, 27), 0.93 (3H, 29), 0.88 (3H, 30), 0.79 (3H, 25), 0.70 (3H, 24), and a doublet methyl at 0.82 (3H, d, 23). It reveals methine proton at 2.24 (1H, m, 4), 1.37 (1H, m, 8), 1.50 (1H, m, 10), 1.41(1H, m, 18).

The $^{13}$CNMR (Figure 3) and DEPT (Figure 5) spectra of J29 confirmed the presence of 30 carbons one of which is $\delta_C$ (ppm) 213.37; diagnostic for carbonyl carbons. The IR spectrum (table 5.1) of the compound J29 showed characteristic absorption peak at $V_{max}$ 1715.05cm$^{-1}$, which is a ketone stretching signal. This suggests that the carbonyl group is a ketone. The NMR data of J29 were identical with those reported in literature for friedelin (Rajesh, 2012) (Table 5.2). Therefore, compound J29 was identified as Friedelin (fig.6).

# CHAPTER SIX

# SUMMARY, CONCLUSION AND RECOMMENDATION

## 6.1 Summary

The pharmacognostic standard for the stem bark of *Prosopis africana* is established the first time in this study and these data could be used as a diagnostic tool for the standardization of this medicinal plant and will be helpful in characterization of the crude drug. These parameters could be useful in the preparation of its monograph in the African Pharmacopoeia. Any crude drug which is claimed to be *Prosopis africana* but whose characters significantly differ from the accepted standard would then be rejected as contaminated, adulterated or downright fake.

From this study, *P. africana* stem bark ethylacetate extract has an $LD_{50}$ greater than 3000 mg/kg. An $LD_{50}$ of 3000 mg/Kg is an indication that *P. africana* stem bark ethylacetate extract has a low toxicity profile. The gram equivalent of the $LD_{50}$ of the extract in an adult man would be over 210 g, that is, a plate full of the extract, making it relatively safe. It can be concluded that the ethylacetate extract of *P. africana* is relatively safe within the normal doses.

The extract of *Prosopis africana* stem bark demonstrated protective activity against indomethacin and ethanol induced gastric ulcer lesions in rats. This is evidenced in the significant and increased preventive index with doses (100, 300 and 900 mg/kg) of the extract. The chloroform and methanolic fractions of the extract also demonstrated significant antiulcer activity which increased with dose. Non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin are known to induce gastric ulceration. The reason is attributed principally to inhibition of biosynthesis of

'cytoprotective prostaglandins' (by inhibition of cyclo-oxygenase pathway of arachidonic acid metabolism), resulting in over production of leukotrienes and other products of 5-lipoxygenase pathway (Rainsford, 1987). Hence, the protective action of *Prosopis africana* extract and its methanolic and ethylacetate fractions, against indomethacin-induced gastric lesions could possibly be due to its 5-lipoxygenase inhibitory effect.

From this study, friedelin was isolated and characterized from the chloroform fraction of the *Prosopis africana* stem bark ethylacetate extract. Friedelin is a known pentacyclic triterpenoid.

## 6.2 Conclusion

The result of this research provides diagnostic parameters for the proper identification of *Prosopis africana* stem bark. *Prosopis africana* stem bark crude ethylacetate extract shows significant antiulcer activity. The methanol and chloroform fractions of the extract also exhibited significant antiulcer activity. The pentacyclic triterpenoid friedelin, was isolated from the stem bark of the plant.

## 6.3 Recommendation

However, it is recommended that:

a. Further toxicity studies using different animal species is necessary.
b. Sub-acute and chronic toxicity tests is planned in order to determine the long-term effects of the extract.

c. Further studies to ascertain the actual antiulcer mechanism of action of the plant extract and fractions.

d. Bio assay guided isolation of the actual antiulcer compound(s) from the plant.

e. Isolation of other pharmacologically active compounds is carried out from the plant.

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## APPENDIX

## TWO DIMENSIONAL SPECTRA OF J29

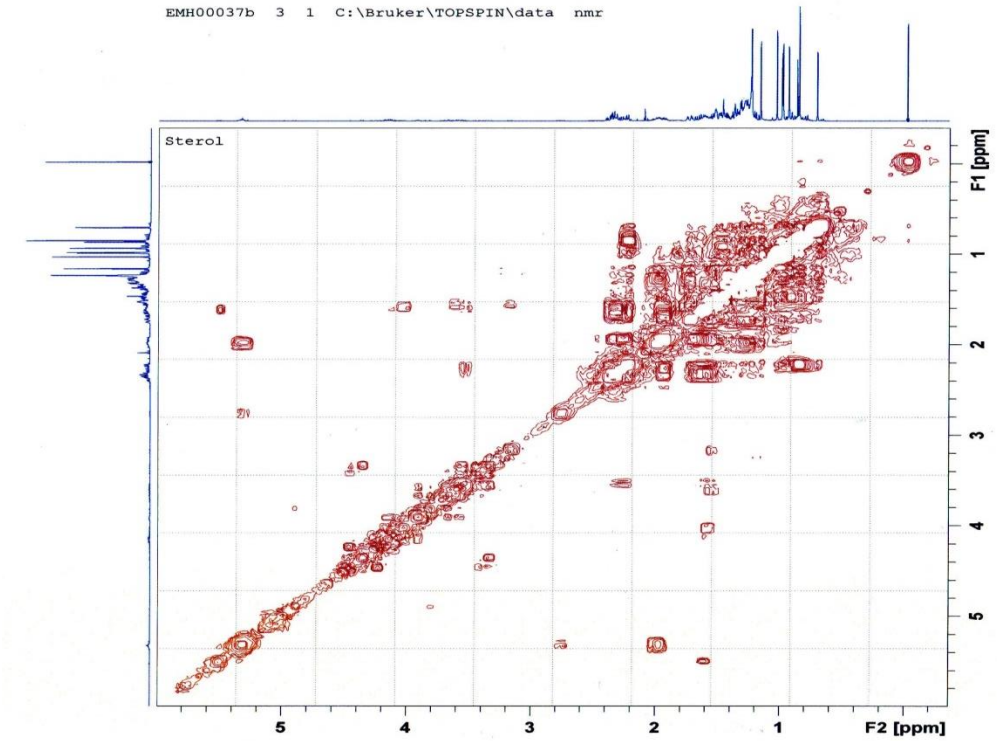



FIG. 5.0: COESY SPECTRUM OF J29

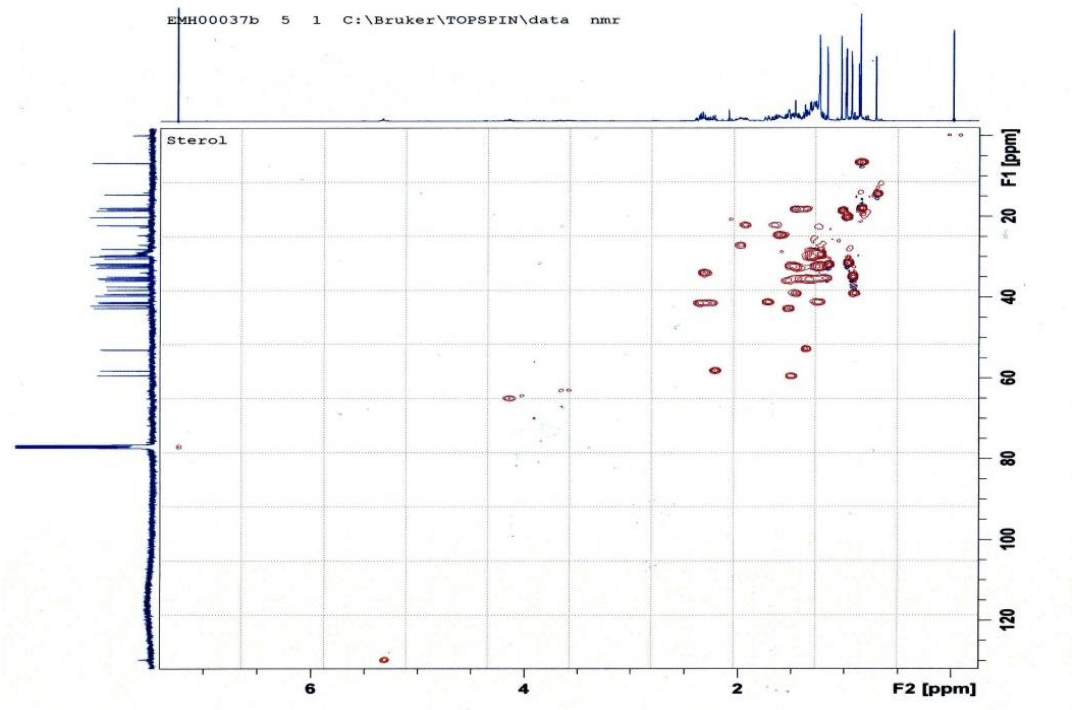



FIG. 5.1: HSQC SPECTRUM OF J29

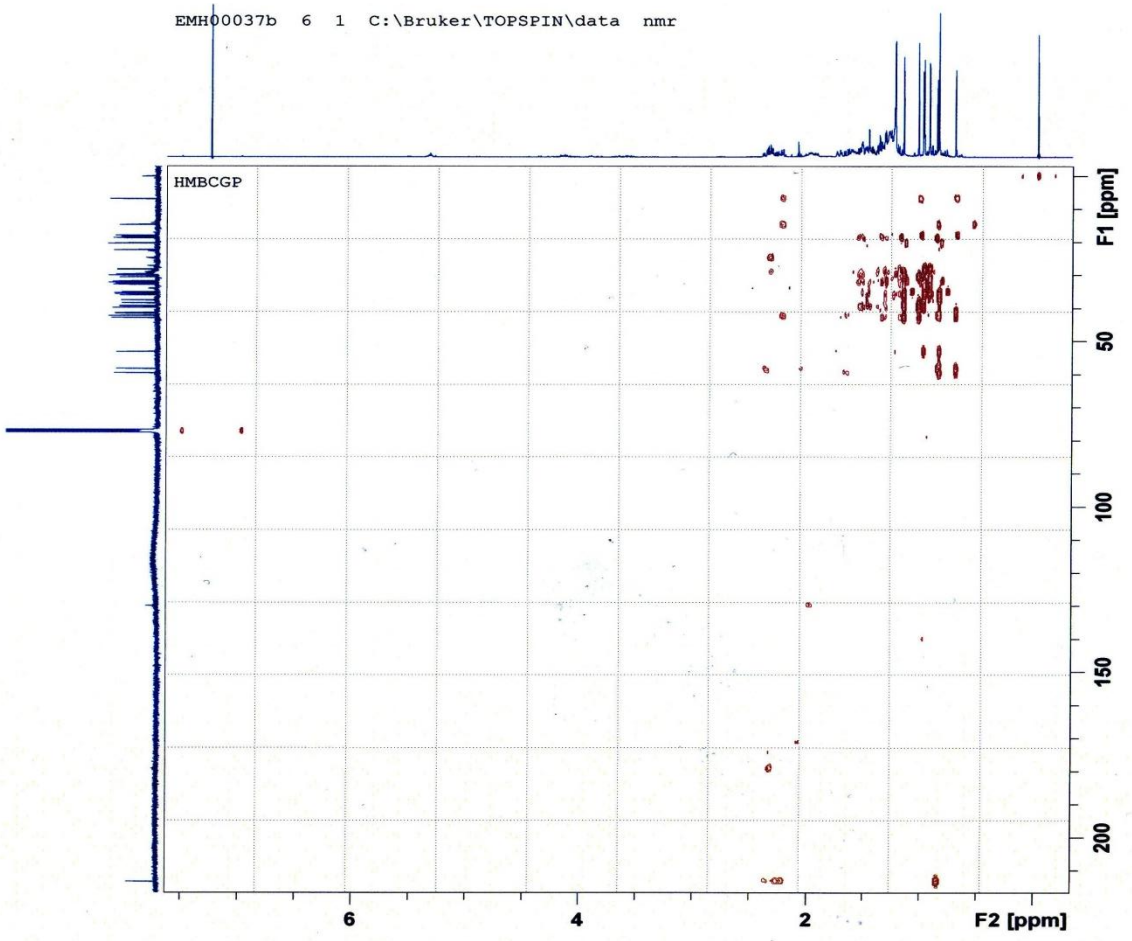


FIG. 5.2: HMBC SPECTRUM OF J29