

Development of Quality Standards of *Prosopis africana* (Fabaceae) Stem-Bark

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Abstract

Prosopis africana (Guill. & Perr.) Taub. (Fabaceae) is the only known species of its genus found in Africa. Almost all parts of the tree are used in medicine namely as remedies for skin diseases, caries, fevers, gonorrhoea, tooth and stomach-ache, dysentery and bronchitis. It is therefore considered worthwhile to establish quality standard for the stem bark. Pharmacognostic standardization was carried out on the pulverized stem-bark and its anatomical section, by using standard experimental procedures to determine the macro and micro morphological characters, as well as other quantitative and qualitative parameters. The results of this study produced vital data that could be useful in setting some diagnostic indices that could be included in the preparation of a monograph of the plant stem bark.

Keywords: Quality standards, development, stem-bark, Mimosoidae

1.0 Introduction

Many herbs are moving from fringe to main stream use with a great number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plants-based products for the prevention and cure of different human diseases. With the present surge of interest in the 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 World Health Organization (WHO) 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[1].

Prosopis are pod bearing trees or shrubs consisting of 44 reported species which are found in arid and semiarid regions of the world[2]. *P. africana* is the only tropical African Prosopis species, occurring from Senegal to Ethiopia in the zone between the Sahel and savannah forests. *Prosopis africana* belongs to the family Fabaceae, sub-family Mimosoideae. The common names include, Iron wood, locust beans (English), Ubwa (Ibo), Kiriya (Hausa), Okpehe (Idoma)[3, 4].

Aqueous and ethanol extract of the stem bark indicates the presence of Saponins, tannins and alkaloids in high concentration. Phenols and steroids were also present in the investigated plant parts[5]. In many areas, the fermented seeds are used as a food condiment. In Northern and the Middle Belt of Nigeria, the seeds are boiled and made into 'daddawa' (Hausa) 'okpehe' (Idoma), a product used for flavouring local dishes[6]. Almost all parts of the tree are used in medicine, the leaves and bark are combined to treat rheumatism. 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. In Mali the leaves, bark, twigs and roots are used to treat and relieve bronchitis, dermatitis, tooth decay, dysentery, malaria and stomach cramps. This paper deals with determining the requisite anatomical feature of *P. africana* stem bark so as to provide pharmacognostic and physicochemical information for its correct identification.

2.0 Materials and Methods

2.1 Collection and Preparation of *P. africana* stem-bark

Stem-bark, fresh mature leaves and pods were collected from fully-grown plant in the field at the outskirts of Zuru town in Kebbi State, North Western Nigeria. The identity and authenticity of the plant was established by comparing its morphological characters with available literature [7] and by a taxonomist of the Herbarium Unit, Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen numbered PCG/UDUS/Mim/004 is kept in the same herbarium unit.

The plant stem bark was shade dried for 7 days and pulverized using mortar and pestle to obtain a coarse powder. The powder was stored in appropriate container until required for use.

2.2 Macroscopic Evaluation of *P. africana* stem-bark

The macroscopic and organoleptic properties were evaluated for the stem bark using standard methods[1].

Colour: The untreated powder was examined under bright daylight.

Texture and Fracture Characteristics: The untreated stem-bark was examined by touching to determine if it is soft or hard. The bark was also bent and ruptured to obtain information on brittleness and the appearance of the fracture plane.

Odour: A small portion of the powdered bark was placed in the palm of the hand and the air over the material was repeatedly inhaled. The strength and sensation of the odour were hence determined.

Taste: Small quantity of the powdered bark was placed on the tongue to determine the taste.

2.3 Microscopic Inspection of *P. africana* stem-bark

The following equipment were used for the microscopic inspection which includes Kyowa Microscope Model number XSZ-21, Electronic eyepiece YJ EYE model 10-130, Bunsen burner, slides and cover-glasses.

Determination of microscopic features: A needle tip load of the powder was transferred into 2 drops of glycerol on a slide and stirred slowly. The specimen was freed of air bubbles by boiling over a small flame of micro-burner and observed under the microscope for diagnostic characters (Phloem, Starch grains, Aleurone grains, Calcium Oxalate crystals, Cork Cells and Sclerids) and the above procedure was repeated with different slides (WHO, 1998)

Chemomicroscopic Evaluation: Drops of the Reagents were applied to small amount of powdered bark mounted on a slide and then irrigated using strip of Watman's Filter Paper (World Health Organization, 1998).

Test for 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 added.

Test for Lignin: 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.

Test for Calcium Carbonate: A few drops of hydrochloric acid were added to a mounted and cleared powdered bark and observed for slow dissolution of crystals with effervescence which will indicate the presence of Calcium carbonate.

Test for Subererin: 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.

*Microscopic Evaluation of *P. africana* Cell Content (Chemomicroscopy)*

Small amount of the *P. africana* powder was mounted on a slide as above and the following tests were conducted to detect cell contents.

Test for proteins: A drop of 1% Picric Acid in water was added to a mounted and cleared powdered bark and observed for yellow colouration.

Test for Tannin: A drop of Ferric Chloride was added to a mounted and cleared powdered bark and observed for bluish black or green black colouration.

Test for Starch: The powdered stem bark 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.

2.4 Physicochemical Analysis on *P. africana* Stem-bark

The methods adopted for the physicochemical analysis on the plant bark powder, are as described in Quality Control Methods for Medicinal Plant Materials (World Health Organization, 1998).

Determination of Moisture Content: The moisture content was determined by Loss on Drying method (gravimetric determination). 3 g of air-dried powdered plant bark 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 °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 as above until two consecutive constant weights were achieved. The moisture content (loss of weight) was calculated using the following formula:

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

Determination of Total Ash Value: A Nickel crucible was heated red hot, cooled in a desiccator and quickly weighed. Exactly 3 g of the air-dried powdered bark was weighed into the previously heated crucible. The powder was spread in an even layer and ignited by gradually increasing the heat to 600 °C 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:

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

Determination of Acid-insoluble Ash: To the crucible containing the total ash, was added 25 mL 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 5 mL of hot water and added to the crucible. The mixture was filtered through a 125 mm 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 and quickly weighed. The acid insoluble value was calculated as follows:

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

Determination of Water Soluble Ash: To the nickel crucible containing the total ash, was added 25 mL of water and boiled on a water bath for 5 minutes. The mixture was filtered through a 125 mm 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:

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

Determination of Extractive Values

Water Extractive Value: Exactly 4 g of air-dried powdered bark was weighed, into a glass stoppered conical flask. 100 mL of water was added and weighed to obtain the total weight including the flask and powder. 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. The weight was readjusted to the original total weight with water, stirred and filtered rapidly through a dry Whatman® Filter paper. A 25 mL volume of the filtrate was evaporated to dryness on hot water bath; further dried in the oven at 105 °C for 6 h, cooled in a desiccator for 30 minutes and weighed without delay. The percentage water extractive value was calculated using the following formula:

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

The process was as described for water above but with 96% ethanol as the substitute solvent.

2.5 Chromatographic Evaluation of *Prosopis africana*

2.5.1 Preparation of *P. africana* Extract

About 150 g of the pulverized stem bark of *P. africana* was extracted with ethyl acetate using soxhlet extractor for 4 h at 80 °C to obtain the ethyl acetate crude extract of the plant.

2.5.2 Thin Layer Chromatographic (TLC) Finger Print Profile

Ethyl acetate solution of the *P. africana* crude extract was spotted on a pre-coated Thin Layer Chromatographic (TLC) plate (Silica gel 60); and developed using a solvent system composed of Petroleum Ether, Chloroform, Methanol 4:6:1 (V/V/V). The developed TLC plate was air dried and visualized by spraying with 5% sulphuric acid and heating in an oven at 105 °C for about 10 minutes. The chromatograph was photographed. The crude extract was fractionated into petroleum ether and chloroform fractions. The TLC profiles of both fractions were determined adopting the procedure above. The R_f value for each spot was calculated using the following formula.

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

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;

3.0 Results

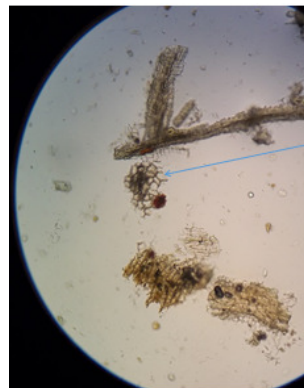
3.1 Macroscopic Evaluation of *Prosopis africana* stem-bark

Table 1: Macroscopic Analysis of *Prosopis africana* stem-bark

Parameter	Observation
Colour	Dark with White/Ash coloured patches. Reddish brown underneath the scale
Internal colour	Reddish brown
Texture	Hard
Odour	Weak, Musty and characteristic
Taste	Characteristic
Fracture	Rough
Internal surface	Longitudinally Straited
Powder colour	Reddish brown



Plate I: *Prosopis africana* Stem-Bark



X100 magnification

Cork cells

Uniformly thickened and radially arranged with thickened sclerified walls



X400 magnification

Starch grains



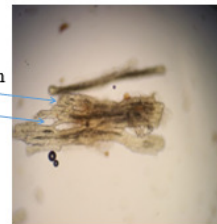
X100 magnification

Non collapsed secondary phloem

Sieve plate with sieve area

Fibre

Sclerid

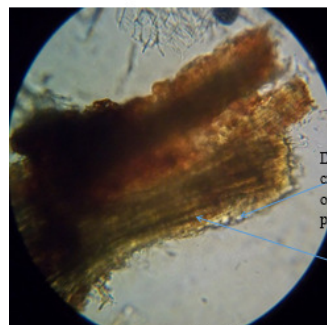


X400 magnification

Druse prismatic Calcium Oxalate crystals

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

Phloem



X400 magnification



X400 magnification

Plate I: Photomicrograph of the plant powder

3.2 Chemomicroscopic Analysis of the Stem-Bark Powder
3.2.1 Microscopic Evaluation of *P. africana* Cell Wall Materials

Table 2: Chemomicroscopic data of cell wall materials of *P. africana*

Test	Observation	Inference
Powder + iodinated ZnCl + 66% H ₂ SO ₄	Blue-violet	Cellulose is present
Powder + Phloroglucinol + Conc. HCl	Deep red	Lignin is present
Powder + Sudan red	Red stain	The cell wall is Suberized

3.2.2 Microscopic Evaluation of *P. africana* Cell Contents

Table 3: Chemomicroscopic data of cell contents of *P. africana* bark

Test	Observation	Inference
Powder + 80% H ₂ SO ₄	Un-dissolved crystals	Calcium oxalate crystals is present
Powder + HCl	No effervescence	Calcium carbonate is not observed
Powder + 1% Picric acid	Yellow colouration	Aleurone grains are present
Powder + FeCl ₃	Greenish black	Tannin is present
Powder + KOH (55g/L)	Red stain	Hydroxyanthraquinones is present

3.3 Physicochemical analysis of *Prosopis africana* stem-bark

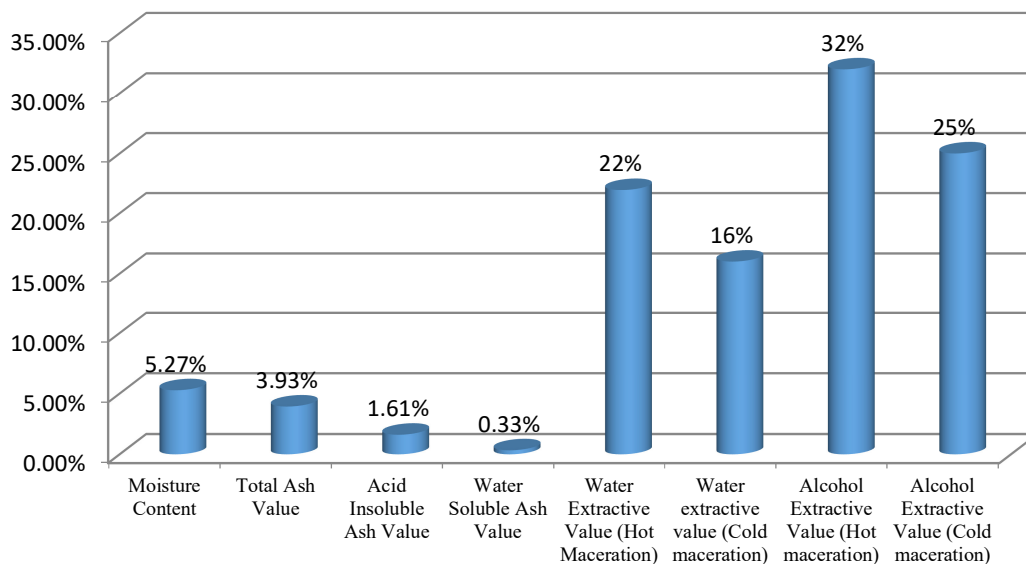


Fig. 1: Physicochemical parameters of *Prosopis Africana*

3.4 Thin Layer Chromatographic Finger print of *P. africana* bark extract

Table 5: TLC profile of the stem bark extract and fractions of *P. africana*




	s/n	Spot Colour	R _f value	Fraction Analysed		
				Crude	Chloroform	Petroleum
				Ethylacetate Extract	Fraction	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	+	+	-

Plate III: A, B and C above shows the chromatograms of Crude extract, Chloroform fraction and Petroleum Ether fractions respectively.

4.0 Discussions

The following investigated anatomical and phytochemical features of *P. africana* are the key features that can be used to diagnose it. Macroscopic inspection of the plant bark (Table 1) revealed Dark 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. Observed microscopic features in the stem bark powder 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. 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[8];[9]. Thus the constancy of crystal type and distribution may be considered a taxonomic character for classification of species[9]. The crystal pattern is therefore an important identification tool. The crystal pattern is also often stable within a genus[10]. 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 put together provides a collective identification and standardization parameter for *P. africana* stem bark.

Chemomicroscopic analysis of the plant (tables 2 and 3) reveals the presence of lignin, tannins, saponin, suberized cell wall, protein grains calcium oxalate crystals and hydroxyanthraquinones. [5]previously reported the presence of significant quantity of saponin and tannins in *P. africana* stem bark.

Fig. 1, shows the Physicochemical parameters obtained from this study (Fig.1), and may serve as composite 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[11]. Total Ash value is particularly important in the evaluation of purity and quality of drugs[12]. 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[13]. The total ash usually consists of both physiologic ash and non-physiologic ash which include carbonates phosphates, silicates and silica. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing[14]. 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 the polar or nonpolar extractable compounds in a plant material. So, it gives an idea of the chemical composition of the crude drug. Moisture content value obtained in this study (5.27%) was indicative that the material could be preserved over a long period of time without deterioration of the drug[15]. The general requirement for moisture content in crude drugs is not more than 14%[16].

TLC finger print profile in plate III (A, B, C) table 5 provides reference data for quick quality and identity assessment of *P. africana* crude drug. The combination of the results obtained from macroscopic, histochemical studies and physico-chemical parameters can be used to standardize stem bark of *Prosopis africana*.

5.0 Conclusion

The findings in this study could be used as a requisite diagnostic tool for the identification and standardization of this medicinal plant and will be helpful in identifying the possible adulterants of the crude drug.

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