Pharmacognostic studies of Pavonia senegalensis (cav.) Liestner (malvaceae) leaf

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Abstract

Pavonia senegalensis (Cav.) Liestner (Malvaceae) is a plant used in African Traditional medicine. The aim of this study was to carry out pharmacognostic studies and HPLC fingerprinting on the leaves of *P. senegalensis* for standardization purposes. Macroscopic, organoleptic, microscopic and physico-chemical evaluation were carried out on the leaf of the plant. The diagnostic microscopic features of the leaf include anomocytic stomata, unicellular covering stellate trichomes, polygonal epidermal cells with beaded anticlinal walls. The powdered leaves under light microscope showed the presence of numerous unicellular covering trichomes and clusters of rosette calcium oxalate crystals with groups of epidermal and parenchyma cells. The physico-chemical characteristics determined are total ash 10.53 %, acid-insoluble ash 4.83 %, moisture content 7.26 %, water-soluble and alcohol soluble-extractive 15.15 and 12.25 % respectively. The presence of heavy metals determined in the leaves of *P. senegalensis* were zinc and lead which were 0.24 and 4.00 $mgkg^{-1}$ respectively while cadmium, arsenic and mercury were not detected. The phytochemical screening of the aqueous ethanol leaf extract of *P. senegalensis* showed the presence of phenolic compounds (flavonoids and tannins) and steroids/triterpenes (saponins). The HPLC fingerprints of the aqueous ethanol leaf extract determined under optimized conditions showed 5 peaks with retention times 1.26, 1.56, 2.18, 3.10 and 4.34 mins. The result of this study can be used to evaluate any sample or products labelled as *Pavonia senegalensis.*

Key Words: Microscopic, trichomes, stomata, ash, HPLC.

Introduction

In developing countries, a large number of people depend on the traditional system of medicine. According to World Health Organization (WHO), 80% of the people living in rural areas depend on medicinal herbs as primary healthcare system (Tatiya, 2012). The Food and Agriculture Organization (FAO) estimated in 2002 that over 50,000 medicinal plants are used across the world (Smith-Hall *et al.,* 2012). The Royal Botanic Gardens, Kew estimated conservatively in 2016 that 17,810 plant species have a medicinal use, out of some 30,000 plants for which a use of any kind is documented (RBG Kew, 2016).

With increasing commercialization and usage of herbal medicine, assurance of safety, quality and efficacy of medicinal plants and herbal products has become an important issue. The herbal raw material is prone to a lot of variation due to several factors, the important ones being the identity of the plants. Seasonal variation (which has a bearing on the time of collection), the ecotypic, genotypic and chemotypic variations, drying and storage conditions and

the presence of xenobiotic are factors that affect the identity of plant samples (Dixit and Yadav, 2008). These factors are also considered in the standardization of plant products. Standardization as defined by American herbal product association refers to the body of information and control necessary to produce material of reasonable consistency. This is achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes (Waldesch *et al.,* 2003).

Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic properties, pharmacognostic characters, volatile matter content, quantitative parameters namely ash values, extractive values, phytochemical constituents, presence of xenobiotics, microbial load, toxicity, and biological activity.

Pavonia senegalensis (Cav.) Liestner synonyms *P. hirsuta* Gull. & Perr., *P. arabica* Hoschst ex Steud and *P. argentina* Gurke. It is called *Tsu* in Hausa. It is found in drier parts of Tropical Africa. *Pavonia senegalensis* is usually an annual plant, but occasionally lives longer. A spreading, short-lived perennial with semi-prostrate to ascending branches, up to 1.25 m. Stems are somewhat angular with harsh stellate hairs. Leaves are suborbicular in outline, angular to shallowly lobed; lower surface densely stellate-hairy. Flowers are solitary in the leaf axils, up to 8 cm in diameter sulphur-yellow with a maroon centre (Heywood, 1979). The roots are macerated in cold water and the infusion is taken as a remedy for diarrhoea in South and East Africa (Neuwinger, 2000). The powdered seed is taken with milk and used as a contraceptive in Sokoto North-west Nigeria (Adebisi and Alebiosu, 2014). The infusion of the roots is used in antenatal care for general wellbeing in Katsina North-west Nigeria and the maceration of the leaves is used in Zaria North-West Nigeria to treat wounds and bone infections (Kankara *et al.,* 2015). A cold-water infusion of the dry roots is taken to induce labour in Botswana, particularly if the onset is being delayed (Burkill, 1997).

Previous studies carried out on the leaves of the *P. senegalensis* to evaluate the ethnobotanical use of the plant in our laboratory showed that the aqueous ethanol extract of the leaves is non-toxic when given orally over a short period (acute toxicity) but the sub-chronic (28 days) toxicity study showed that the extract is nephrotoxic and non-significantly hepatotoxic in rats (Shehu *et al.,* 2019a). The hydro-alcoholic leaf extract and fractions (n-hexane, ethyl acetate and n-butanol fractions) of the plant were shown to be effective against both acute and chronic inflammation in a dose related manner in rats (Shehu *et al.,* 2019b). The alcoholic extract of the leaves of the plant was shown to be bacteriostatic against *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typi*, *S. pyrogens* and Vancomycin resistant enterococci (VRE) (Shehu *et al.,* 2021).

The aim of this study is to establish pharmacognostic parameters that can be used in the identification, authentication, standardization and quality control of the leaves of *P. senegalensis* because of its importance as medicinal plant.

Materials and Methods

Plant Collection, Preparation and Identification

Plant samples consisting of leaves and flowers of *P. senegalensis* were collected from Rafin Yashi, Giwa Local Government Area of Kaduna State in November, 2020.

The plant was identified and authenticated by a Taxonomist: U.S Gallah at National Research Institute for Chemical Technology (NARICT), Zaria, Kaduna State, Nigeria and assigned a voucher number NARICT 24011.

Macroscopical evaluation of the leaves

An initial macroscopical study was carried out on the whole and powedered leaves using mainly organoleptic methods. This was based on the colour, surface characteristics, odour and taste (WHO, 2011)**.**

Microscopical examination of the leaves

Microscopical examination of the fresh leaves.

Surface preparations of the upper and lower epidermis; and the transverse sections through the mid-rib of leaf of the plant were prepared with the aid of a razor blade. The sections were cleared with a few drops of dilute sodium hypochlorite and rinsed with distilled water to remove traces of the clearing agent. The cleared sections were then mounted with dilute glycerol on a slide covered with glass cover slip and viewed under the light microscope with x4, x10 and x40 objective lens. The observed features were photomicrograped. Measurement of diagnostic features was carried out using digital eyepiece and the S-EYE 1.3.2.297-blang software (Dhanabal *et al*., 2005).

Quantitative leaf microscopic determination

Stomatal number, stomatal index, veinlet termination number and vein-islet number were determined accordingly using a modified light microscope fitted with a camera lucida according to methods described by Dhanabal *et al*., 2005.

Powdered microscopic and chemomicroscopic examination

Powdered leaves of *P. senegalensis* were cleared and observed under the microscope. The leaf powder was then treated with different reagents namely: N/50 iodine, concentrated HCl, phloroglucinol, 5% ferric chloride, Sudan IV etc, mounted and observed under the microscope (Dhanabal *et al*., 2005).

Physico-chemical evaluation of the powdered leaves

Total ash, acid-insoluble ash, water-soluble ash, moisture content, extractive (alcohol and water-soluble) values were determined on the powdered leaves using the methods described by WHO, 2011.

Heavy metals analysis of *P. senegalensis*

Digestion of the powdered leaves of the plant was carried out using the method described by Saeed *et al.,* (2011). One gram of the powdered plant was taken in a beaker; 10 ml of concentrated nitric acid (67%) was added and kept at room temperature for 24 hours in a fume cupboard. Perchloric acid (4 ml) was added to the sample and concentrated on a hot plate at 60^{o}C until a suspension of approximately 1 ml was left in the beaker. The liquid was cooled, diluted with deionised water up to 50ml and filtered through whatman filter paper No. 42. Sufficient deionised water was added to make the volume up to100ml. The concentrations of heavy metals in the sample were determined using atomic absorption spectrophotometer (280FSAA Agilent Technologies USA).

Phytochemical Screening and HPLC fingerprinting of P. senegalensis

Plant extraction

One (1) kilogram of the powdered leaves was weighed and macerated in 70% ethanol for 72 hours with occasional shaking. The macerate

was filtered through a No. 6 Whatsman filter paper and the filtrate was evaporated under reduced pressure using a rotary evaporator at 65°C. The dried extract was kept in a desiccator until use.

Phytochemical screening

The extract of the plant was subjected to phytochemical screening test for the presence secondary metabolites using methods described by Kokate *et al*., (2016).

Sample preparation for HPLC Analysis

A portion of each extract and fraction (100 mg) was reconstituted in 10 ml methanol and filtered through 0.45 µm membrane filter (Hach, USA) before injecting into the HPLC machine.

HPLC Analysis

Agilent technologies 1260 infinity HPLC Series system (Agilent, Germany) equipped with a degasser, binary gradient pump, column thermostat, autosampler, and UV detector was used for the HPLC profiling of the plant extract. For the separation, a reverse-phase analytical column, Techsphere C_{18} 4.6 x 250, 5µm was used. Analysis was carried out at 25°C while separation monitored at 350 nm. The mobile phase programme was isocratic. A mixture of methanol and water at the ratio of 60:40 was used for the separation. Before sample injection, mobile phase was degassed through an online degasser and the base line was allowed to equilibrate for minimum of 10 minutes. The chromatographic data was processed using ChemStation and Data Analysis software from Agilent, Germany.

RESULTS

Pharmacognostic Evaluation of the Leaves of *P. senegalensis*

The leaf lamina is 2-7 x 3-8 cm, suborbicular, 5-6 palmati-lobed, the apex is acute, the margin is serrate, both the upper and lower epidermis are stellate-hairy, the petiole is 2-10 cm long (Figure I). The powdered plant was light green in colour, odourless with a bland taste and causes slight itching when it touches the skin.



Figure I: Leaves of *P. senegalensis* collected from Rafin Yashi Giwa L.G.A of Kaduna State

Microscopical examination of the leaves

Microscopical examination of its fresh leaves

The microscopic features of the epidermal layer of the leaf include anomocytic stomata, unicellular covering trichomes, polygonal epidermal cells with beaded anticlinal walls (Table 1, Figure II). The sizes of the epidermal characters like epidermal cells, trichomes, calcium oxalate crystals and guard cells area are shown in Table 3. The transverse section of the mid-rib of the leaf shows dorsiventral arrangement of tissue and the outline of the transverse section is concave on the lower epidermis and convex on the upper epidermis (Figure III).

Table 1: Microscopic epidermal characters of *P. senegalensis* leaf

Description	Characters observed			
	Cells	Stomata	Trichomes	Calcium oxalate
Shape:	Polygonal	-	-	Clusters/Rosette
Anticlinal walls:	Beaded	-	-	-
Type:	-	Anomocytic	Unicellular covering and stellate	-
	-			
Frequency:		Numerous	Numerous	Numerous

(-) – Not applicable

Table 2: Micrometric Determination of the Epidermal Characters of *P. senegalensis* Leaf

Character	Length (µm)	Breath (µm)	Area (μm^2)
Epidermal cells	40 – 80	36 – 65	-
Guard cells area	-	-	185 – 220
Trichomes	55 – 120	2 – 8	-
Calcium oxalate crystals	13 – 17	7 – 10	-

(-) – Not applicable, n = 30. Magnification x100

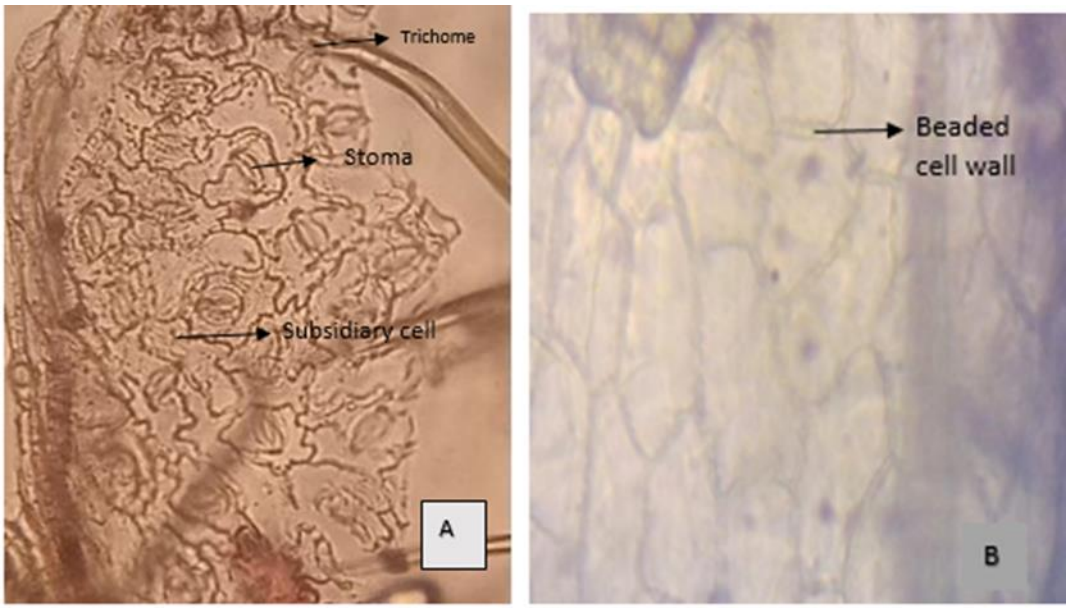


Figure II: Photomicrograph of the Surface Preparation of the Lower Epidermis of *P. senegalensis* Leaf. (A)-The surface preparation of the lower epidermis of the leaf of *P. senegalensis* x100. (B)- The beaded epidermal cell walls of *P. senegalensis* x400.

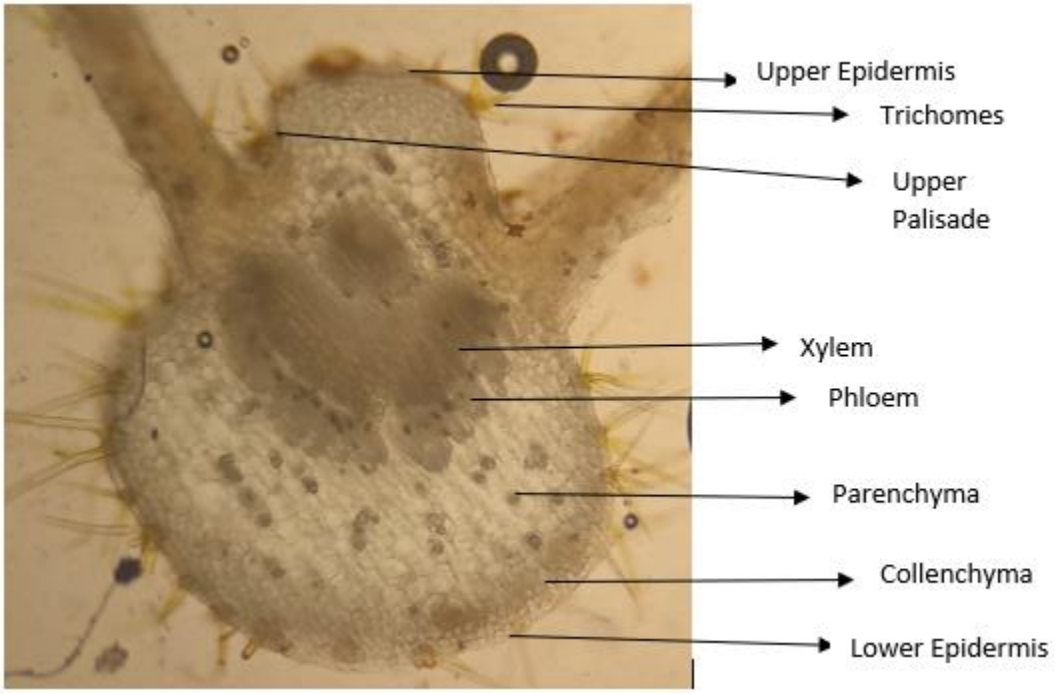


Figure III: The transverse section of the mid-rib of *P. senegalensis* Leaf x40

Quantitative leaf microscopic determination

The stomatal number, stomatal index, vein islet and veinlet termination number are shown in Table 3.

Table 3: Quantitative Microscopy of *P. senegalensis* Leaf

Parameters	Value		
Stomatal number (lower epidermis)	41.22	48.50	55.78
Stomatal number (Upper epidermis)	26.45	23.00	26.45
Stomatal index (Lower epidermis)	10.20	12.00	13.80
Stomatal index (Upper epidermis)	7.45	8.75	10.05
Vein islet number	6.09	6.00	5.10
Veinlet termination number	10.67	9.33	7.99

n = 4. Magnification x100.

Powdered leaves microscopic and chemomicrosscopic examination

The powdered plant under the light microscope showed the presence of numerous unicellular covering and stellate trichomes and clusters of calcium oxalate crystals (Figure IV) with groups of epidermal and parenchyma cells. The chemomicroscopy of the powdered leaves showed the presence of mucilaginous materials on the epidermal cells and lignification of the vascular tissues of the mid-rib. The cell inclusions included calcium carbonate, crystals of calcium oxalate, starch grain, tannins and fats and oils.

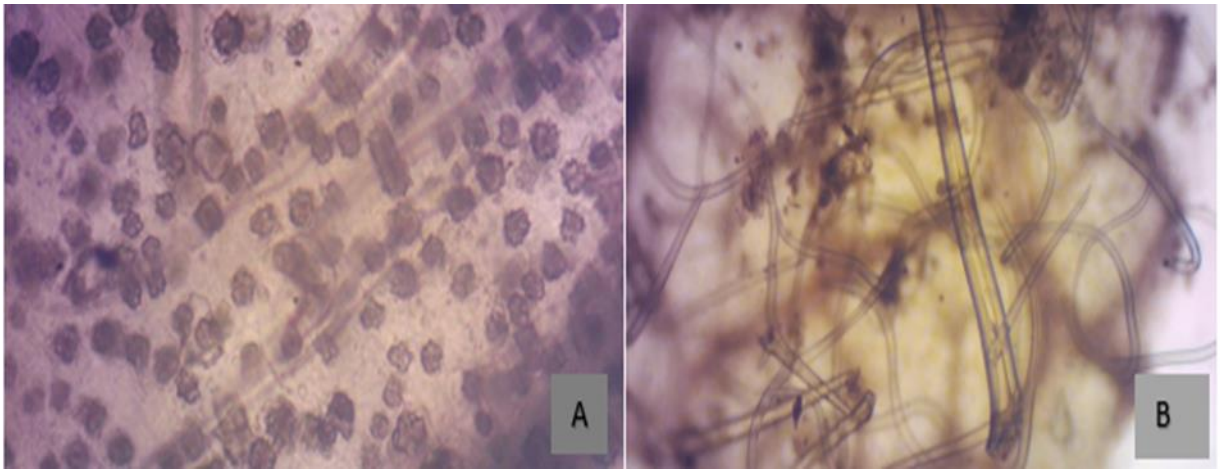


Figure IV: Features from the photomicrograph of Powdered leaves of *P. senegalensis.* (A)-Clusters (rosette) of calcium oxalate crystals from powdered leaves of *P. senegalensis* x100, (B)-Unicellular covering trichomes from powdered leaves of *P. senegalensis* x100

Physico-chemical evaluation of the Powdered Leaves of *P. senegalensis*

The various physico-chemical properties namely: moisture content, total ash, acid insoluble ash, water soluble ash and extractive values determined for the powdered leaves of *P. senegalensis* as shown in Table 4.

Table 4: The Physico-Chemical Characteristics of Powdered Leaves of *P. senegalensis*

Parameters	Value ($\%^{w}/_{w}$) Mean ± SEM
Total ash	10.67 ± 0.03
Water soluble ash	1.25 ± 0.67
Acid insoluble ash	4.83 ± 0.34
Moisture content	7.26 ± 0.12
Water extractive	15.15 ± 0.17
Alcohol extractive	12.51 ± 0.13

n = 3, SEM – Standard error of mean.

Heavy metals concentration of the leaves of *P. senegalensis*The concentrations of zinc (Zn), lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) determined in the leaves of *P. senegalensis* are shown in Table 5.

Table 5: The Concentration of Heavy Metals in the Leaves of *P. senegalensis*

Heavy Metals	Concentration ± SD $mgkg^{-1}$	FAO/WHO Permissible Limits $mgkg^{-1}$
Zinc (Zn)	0.24 ± 0.002	50
Cadmium (Cd)	ND	0.3
Lead (Pb)	4.00 ± 0.002	10
Arsenic (As)	ND	10
Mercury (Hg)	ND	0.1

SD - Standard deviation, ND - Not detectable, FAO - Food and Agriculture Organisation, WHO - World Health Organisation.

Phytochemical screening

The phytochemical screening using different reagents detected saponins, flavonoids, tannins and steroids/terpenes in the aqueous ethanol extract of the plant. The results are shown in Table 5.

Table 5: Phytochemical screening of the Aqueous Ethanol leaf extract and fractions of *P. senegalensis.*

S/no	Phytochemical class	Name of test	Inference
1.	Flavonoids	Ferric chloride test	Positive
		Shinoda test	Positive
		Alkaline reagent test	Positive
2.	Saponins	Frothing test	Positive
		Haemolysis test	Positive
3.	Anthraquinones	Borntrager's test	Negative
4.	Cardiac glycosides	Kella-Kiliani test	Negative
5.	Tannins	Lead subacetate test	Positive
6.	Alkaloids	Dragendorff`s test	Negative
		Mayer`s test	Negative
7.	Steroids and Triterpenes	Liebermann Burckhard test	Positive

High performance liquid chromatography (HPLC) Fingerprinting of the aqueous ethanol leaf extract and fractions of *P. senegalensis.*

The HPLC fingerprint of the plant extract determined under optimized conditions showed the following: the chromatogram of the extract showed 5 peaks with retention times in minutes and percentage areas 1.26 (61.64%), 1.56 (13.91%), 2.18 (8.04%), 3.10 (14.20%) and 4.34 (2.21%). The optimized conditions and chromatogram are shown in Table 6 and Figures V.

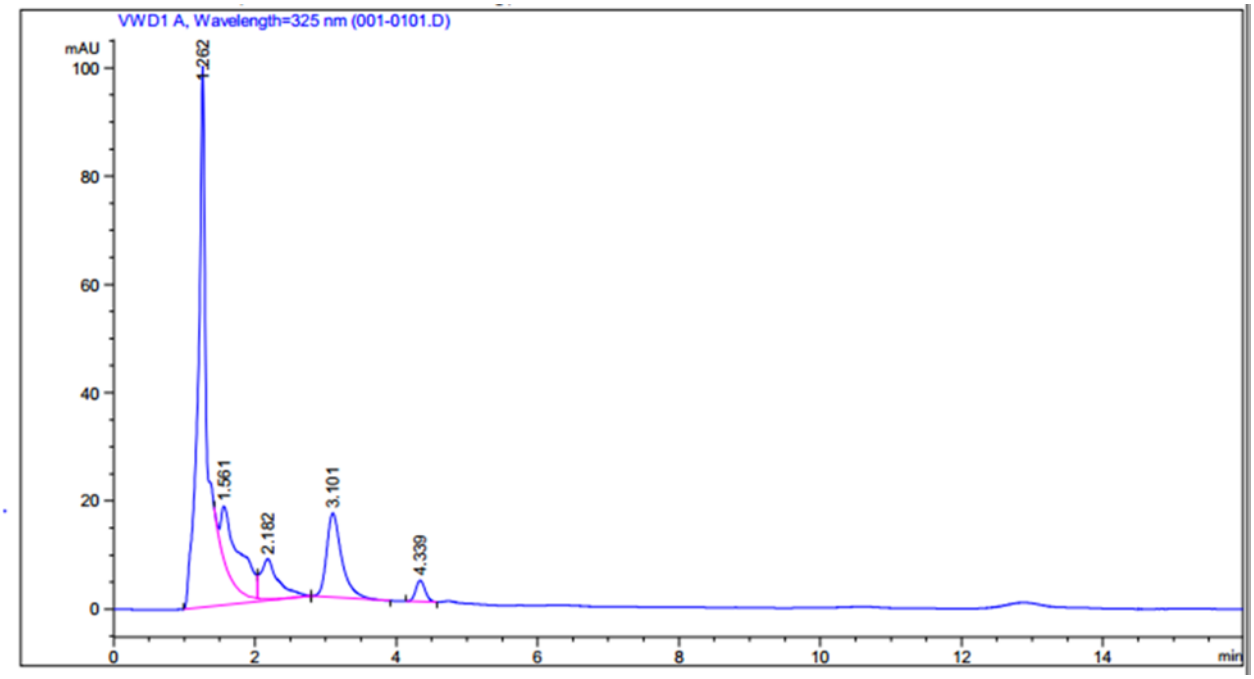


Figure V: HPLC chromatogram of aqueous ethanol leaf extract of *P. senegalensis.*
Mobile phase programme: MeoH: H_2O (60:40) isocratic programme modified with 0.1% orthophosphuric acid; flow rate 1.5 ml/min; Injection volume, 10 µl.

Discussion

In the microscopical studies of the whole leaf of the plant, the epidermal characters and transverse section of mid-rib of the plant were evaluated. In the epidermal characters unicellular covering stellate trichomes which were numerous were observed in both the upper and lower epidermis of the plant. Trichomes may be an effective defense mechanism in plants. They may be involved in both chemical and mechanical defense, negatively affecting the oviposition rate and feeding of herbivorous insects and nutrition of larvae (Lokesh and Singh, 2005). The morphological variation and distribution of these trichomes on the leaf surface are useful diagnostic characters for the sub-generic classification in Malvaceae family (Rédon-Carmona *et al.*, 2006).

The cell wall of the epidermal cells was observed to be beaded which could be diagnostic in the identification of the plant. The stomata were numerous in the lower epidermis and are of the anomocytic type. The stomatal structure is more important in assessing taxonomic relationships and

evolutionary trends than any other character of the leaf because it does not vary due to environmental conditions (Haruna and Ashir 2017). The quantitative microscopic determination of the epidermal characteristics as well as the micrometric determination of the epidermal cells, trichomes, calcium oxalate crystals, guard cell area of the stomata were carried out to provide data that can be used in distinguishing the plant from other closely related species. The report of Tahir and Rajpat (2009) showed that the size and shape of the stomata are taxonomically important characters and the stomata index could be valuable and very reliable in distinguishing some medicinal plant species (Olowokudejo, 1990). The findings of Haruna and Ashir (2017) revealed that the combination of micro-morphological characters such as stomata size, epidermal cell size, veinlet termination and other epidermal characters can be used for the delimitation of species.

The transverse section of the mid-rib of the leaf of the plant was examined, the arrangement of tissues was found to be dorsiventral, where the palisade is located only beneath the upper epidermis. This is used to confirm dicotyledonous plant. The shape and arrangement of the cells and tissues of the mid-rib are very valuable in the classification of plants (Brain and Turner, 1975). The outline of the transverse section of the mid-rib was found to be convex on the upper epidermis and concave on the lower epidermis. Bačić *et al.,* (1992) compared the mid-ribs of three species of *Arbutu*s (Ericaceae). Also, Woltz *et al*., (1987) described the mid-rib outline prominence or concavity for 112 of the 184 species of Podocarpineae. Both authors concluded that the midrib outline is a useful character for taxonomy. In Araceae, Engler (1905), Croat (1997), Keating (2002), and others have reported the usefulness of the mid-rib outline for the diagnosis of pant species.

The powdered microscopy showed the presence of numerous trichomes and calcium oxalate crystals while the chemo-microscopy of the leaves of the plant shows the presence of different cell wall materials such as mucilage and lignin on the epidermal surface of some cells. The presence of mucilage on the epidermal cells have been reported in Malvaceae family (Lersten and Curtis 1997; Hussin and Sani 1998 and Pimentel *et al.,* 2011). Calcium oxalate crystals in plants play both physiological functions and ecological roles as static or active defense structures, their synthesis depends on calcium levels but can also be influenced by external pressures such as herbivory (Franceschi and Nakata, 2005). Calcium oxalate crystals have been shown to be of diagnostic importance, their presence, absence and dimensions are useful the identification of the medicinal plants (Anitha and Sandhiya, 2014).

The moisture content of the powdered leaves was determined to be 7.62 %. The general requirement for moisture content in crude drug is not more than 14 % (African Pharmacopoeia, 1986). The lower the moisture content, the higher will be the stability of that drug and lesser chance of microbial growth and activation of endogenous enzymes (Ehiabhi, 2010). Total ash value of the leaves was determined to be 10.53 % which is within 14 % maximum limit for total ash in powdered medicinal plants (European Pharmacopoeia, 2007). The evaluation of total ash value can be used to detect foreign organic and inorganic matter and adulteration by sand or earth (Kunle *et al.,* 2002). The acid-insoluble ash was

determined to be 4.83 % which is above the 2 % maximum limit recommended by European Pharmacopoeia (European Pharmacopoeia, 2007). The acid-insoluble ash consists mainly of silica and indicate contamination with earthy material (Tatiya *et al.,* 2012) indicating that the plant sample may be contaminated with silica during collection or preparation. The extractive values (alcohol and water soluble) determined are useful in detecting exhausted and low-quality plant material (Ehiabhi, 2010).

The presence of heavy metals was quantitatively determined in the leaves of *P. senegalensis.* Cadmium, arsenic and mercury were not detected while zinc and lead were 0.24 and 4.00 $mgkg^{-1}$ respectively. The four major heavy metals of health concern are arsenic, cadmium, mercury and lead (Jarup, 2003). They are biologically non-essential and toxic (Shukla *et al.,* 2007). According to the World Health Organisation (WHO) and Food and Agricultural Organisation (FAO) the permissible limits for arsenic, cadmium, lead and mercury in herbal medicines and products are 10.00, 0.30, 10.00 and 0.10 $mgkg^{-1}$ respectively (WHO 2007) indicating that the leaves of *P. senegalensis* collected was not contaminated with these heavy metals.

The phytochemical screening of the aqueous ethanol leaf extract of *P. senegalensis* showed the presence of phenolic compounds and steroids/triterpenoids (saponins) in the extract. Therapeutic properties of medicinal plants are due to their secondary metabolites and these metabolites can be used as a drug, drug precursors, drug prototypes, and pharmacological probes. Some others may have accessory pharmaceutical and culinary importance (Butler, 2004).

The chromatographic fingerprinting of the extracts of the plant was carried out so as to determine the pattern of the phytochemicals in the plant using the high-performance liquid chromatography (HPLC). The HPLC fingerprint of the aqueous ethanol leaf extract of the plant was carried out under optimized conditions showing different retention times and their percentage area on the chromatogram which can be used in the identification and authentication of the plant based on the pattern of the phytochemical constituents. The HPLC is undoubtedly one of the most popular and widely used chromatography fingerprints for the analysis of herbal medicines (Fu *et al.,* 2009; Weon *et al.,* 2012). Since 1991, the WHO has accepted this technology as a strategy for identification and quantification of herbal medicine (Liu *et al.,* 2007; Goodrazi *et al.,* 2013). High reproducibility, sensitivity, selectivity, and the ability to analyse a number of constituents in herbal medicines are among the great advantages of using HPLC techniques (Snyder, *et al.,* 2011).

Conclusion

The results determined from this study can serve as parameters that can be used in identification, authentication, standardization and quality control of the leaves of *P. senegalensis* in its development as an herbal medicine.

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