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Phytochemical determination and LCMS analysis of the aqueous ethanol leaf extract of *Pavonia senegalensis* (cav.) Liestner (Malvaceae)

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ABSTRACT

Background & Aim: The leaves of *Pavonia senegalensis* are used in African traditional medicine in the treatment of wounds and bone infection. The aim of this study is to quantify the phytochemical constituents and establish the LCMS profile of the hydroalcoholic extract of the leaves of *P. senegalensis* which can be used in the authentication, standardization and quality control of herbal products labelled as the leaves of *P. senegalensis.*

Experimental: Qualitative and quantitative phytochemical screening of the aqueous ethanol leaf extract were carried out using standard methods. LCMS analysis was carried out for identification of phytochemical compounds in the aqueous ethanol leaf extract of the plant.

Results: 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 quantitative phtytochemical determination per gram of the plant extract revealed total phenolics 152.5 mg/g, flavonoids 133.4 mg/g, tannins 24.50 mg/g and saponins 81.9 mg/g. Eight compounds were tentatively identified from the LCMS analysis of the plant extract which included the flavonoid glycosides: kaempferol-3-O-glucoside, quercetin-3-O-glucose-6"-acetate, luteolin-8-C-glucoside, daidzein O-di-hexosidemalonylated, kaempferol hexose glucuronide, malvidin 3-O-glucoside and Dihexosyl luteolin sulfate; and the saponin Oleanolic acid 3-O-glucose acid.

Recommended applications/industries: The results of this study can be used in the identification process and help in quality control for authentication and detection of adulteration of herbal medicines labelled as *P. senegalensis*.

1. Introduction

Phytochemicals are natural compounds found in plants that have potential therapeutic properties. Determining the presence and quantity of phytochemicals in medicinal plants is important for several reasons. Firstly, phytochemicals can help to identify the medicinal properties of a plant. For example, alkaloids found in the plant Vinca minor have been shown to have anti-cancer properties (Kumar and Singh, 2015), while the flavonoids in Ginkgo biloba have been shown to improve cognitive function (Smith and Luo, 2003). Secondly, the determination of phytochemicals can help to standardize the quality of herbal products. As natural products, the composition of medicinal plants can vary based on factors such as growing conditions, harvesting methods, and storage conditions. Thirdly, the determination of phytochemicals is important for safety reasons. Some phytochemicals can be toxic or have adverse effects when consumed in large

quantities. For example, pyrrolizidine alkaloids found in certain plants such as comfrey and coltsfoot can cause liver damage and other health problems (Edgar and Molyneux, 2016).

Liquid chromatography-mass spectrometry (LCMS) is a powerful analytical technique that is widely used for the determination of phytochemicals in medicinal plants. LCMS combines the separation capabilities of liquid chromatography with the detection and identification capabilities of mass spectrometry, allowing for the identification and quantification of a wide range of phytochemicals in complex plant extracts. This information can be used to develop new drugs, supplements, and other natural products based on these compounds (Ma *et al.*, 2019). LCMS is also important for the standardization of herbal products. By identifying and quantifying specific phytochemicals in medicinal plants, LCMS can help ensure consistency in the quality and potency of herbal products. This can be particularly important in the case of plant-based medicines, where the active compounds are often present in low concentrations and may vary based on factors such as harvesting and processing methods (Shao *et al.*, 2020).

The study plant *Pavonia senegalensis* (Cav.) Liestner family Malvaceae commonly called dogstooth in English is found in drier parts of Tropical Africa. *P. senegalensis* is usually an annual plant, but may live longer. It is a spreading, shortlived perennial with semi-prostrate to ascending branches with harsh stellate hairs, up to 1.25 m. Leaves are suborbicular in outline, angular to shallowly lobed with lower surface densely stellate-hairy. Flowers are solitary in the leaf axils, up to 8 cm in diameter sulphur-yellow with a maroon center (Heywood, 1979). In traditional medicine, 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), 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).

Studies carried out on the leaves of the *P. senegalensis* to evaluate the ethnobotanical claim 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 quantify the phytochemical constituents and establish the LCMS profile of the hydroalcoholic extract of the leaves of *P. senegalensis* which can be used in the authentication, standardization and quality control of herbal products labelled as the leaves of *P. senegalensis.*

2. Materials and Methods

2.1. 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. 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.

The collected leaves were air dried at room temperature for seven days and size reduced to powdered form using pestle and mortar.

2.2. Extraction and solvent-solvent partioning of plant material

One kilogram of the powdered leaves was weighed and macerated in 70% ethanol for 72 h 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.

2.3. 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).

2.4. Quantitative phytochemical analysis using UV spectrophotometry

2.4.1. Determination of total phenol content

Estimation of total phenol content in *P. senegalensis* aqueous ethanol leaf extract was measured by Folin-Ciocalteu colorimetric method, using gallic acid as the standard and expressed results as gallic acid equivalent per gram of Sample. Different concentrations (10-100 µg/mL) of gallic acid were prepared in methanol. Aliquots of 0.5 mL of the test sample and each sample of the standard solution was taken, mixed with 2 mL of Folin-Ciocalteu reagent (1:10 in deionized water) and 4 mL of Saturated solution of sodium carbonate (7.5 % w/v). The tubes were covered with silver foils and incubated at room temperature for 30 min with intermittent shaking. The absorbance was taken at 765 nm using methanol as blank. All the samples were analyzed in three replicates. The total phenol was determined with the help of standard curve prepared from pure phenolic standard (Gallic acid) (Savitree *et al.*, 2004).

2.4.2. Determination of total flavonoid content

The total flavonoid content of *P. senegalensis* aqueous ethanol leaf extract was determined by aluminum chloride colorimetric assay (Zhishen *et al.*, 1999). 0.5 mL aliquots of the samples and standard solution (10-100 µg/mL) of quercetin was added with 2 mL of distilled water and subsequently with 0.5ml of 5% sodium nitrite (5% $NaNO_2$ w/v) solution was added. After 6 minutes, 0.15 mL of 10% $AlCl_3$ w/v solution was added. The solutions were allowed to stand for 6 minutes and after that 2 mL of sodium hydroxide (4% NaOH w/v) solution was added to the mixture. The final volume was adjusted to 5 mL with immediate addition of distilled water, mixed thoroughly and allowed to stand for another 15 minutes. The absorbance of each mixture was determined at 510 nm against the same mixture. The total flavonoid content was determined as milligram of quercetin equivalent per gram of sample with the help of calibration curve of quercetin. All determinations were performed in triplicate.

2.4.3. Determination of tannin content

The tannin content was determined by Folin - CioCalteu method. About 0.1 mL of the sample extract was added to a volumetric flask (10 mL) containing 7.5 mL of distilled water and 0.5 mL of Folin –Ciocalteu reagent and 1ml of 35% Na_2CO_3 solution. The mixture was made up to 10 mL with distilled water. The mixture was shaken and kept at room temperature for 30 minutes. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg /mL) was prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with Uv/Visible spectrophotometer. The tannin content was expressed in terms of milligram equivalent of gallic acid per gram of extract (Rajeev *et al.*, 2012).

2.4.4. Determination of saponins content

Total saponins content was determined according to method described by Makkar *et al.* (2007). 1g of extract was dissolved in aqueous 50% methanol and an aliquot (5 mg/ml) was taken. Vanillin reagent (0.25 mL; 8%) was added followed by sulphuric acid (2.5 mL; 72% v/v). The reaction mixtures were mixed well and incubated at 60°C in a water bath for 10 min. After incubation, the reaction mixtures were cooled on ice and absorbance at 544 nm (UV visible spectrophotometer) was read against a blank that does not contain extract. The standard calibration curve was obtained from suitable aliquots of diosgenin (0.5 mg/mL in 50% aqueous methanol). The total saponins content was expressed as milligram of diosgenin equivalents per gram of extract.

2.5. Liquid chromatography mass spectrometry (LCMS) profiling of the of the aqueous ethanol leaf extract of P. senegalensis

Separation of compounds from ethanol extract was conducted on an Agilent technology 6460 Triple Quad LC/MS/HPLC-1290 infinity equipped with an Agilent SB-C18 column (2.1 x 50 mm, 1.8 µm). Acidified water (0.1 % formic acid, v/v) and methanol were used as mobile phase A and B respectively. Sample preparation was done as in 3.7.3 above.

The gradient elution was programmed as follows: 0 min, 70% A and 30% B; 30 min, 100% B; 33 min, 100% B; 35 min, 70% A and 30% B; 40 min 70% A and 30% B; The flow rate was set at 1 mL/min throughout the elution. The flow from the HPLC system into the ESI-MS detector was 0.2 mL/min. The injection volume was 10 µL and the column temperature was maintained at 25˚C. The HPLC system was coupled to a mass spectrometer and was

operated with electrospray ionization (ESI) in positive mode at the mass resolution of 100-1000, gas temperature of 250 ^{0} C and gas flow of 16 L/min were used, nebulizer pressure was set at 40 psi, the capillary voltage of 3000V was used, sheath gas temperature was 400ºC with a sheath gas flow of 12 L/min and the fragmentor voltage was set at 30 to 150V.

The chromatographic data was processed using ChemStation and Data Analysis software from Agilent, Germany. Identification of compounds from the mass spectrum were carried out by comparing with spectra of known compounds using the Global Natural Products Social Molecular Networking (GNPS) spectral library (online).

3. Results and discussion

3.1. Phytochemical screeningand quantitative phytochemical analysisof P. senegalensis leaves

The phytochemical screening of the aqueous ethanol leaf extract of *P. senegalensis* and its fractions showed the presence of phenolic compounds and steroids/triterpenoids (saponins) in the extract (Table 1).

Table 1. Phytochemical screening of the aqueous ethanol leaf extract and fractions of *P. senegalensis.*

S/no	Phytochemical class	Test	Inference
1	Flavonoids	Ferric chloride test Shinoda test Alkaline reagent test	Positive Positive Positive
2	Saponins	Frothing test Haemolysis test	Positive 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 Mayer`s test	Negative Negative
7	Steroids and Triterpenes	Liebermann Burckhard test	Positive

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 quantitative determination of total phenolics, flavonoids, tannins and saponins carried out on the extract showed the variations of these phytochemicals (Table 2). The quantification of these phytochemicals is useful in determining the quality of the plant as a source of drug and as such can be used as a quality control mechanism in standardization based on minimum concentrations that will elicit activity.

Table 2. The quantitative determination of phytochemicals in the aqueous ethanol leaf extract of *P. senegalensis*

Phytochemical	Quantity (mg/g) ± SD
Total phenolics	152.50 ± 1.71
Flavonoids	133.40 ± 0.14
Tannins	24.50 ± 3.54
Saponins	81.90 ± 0.45

Total phenolics and Tannins as mg equivalent of gallic acid, Flavonoids as mg equivalent of quercetin and Saponins as mg equivalent of diosgenin. SD: standard deviation, n = 3.

3.2. Liquid chromatography mass spectrometry (LCMS) analysis of the aqueous ethanol leaf extract of P. senegalensis

The chromatogram from the liquid chromatography mass spectrometry (LCMS) of the plant extract showed ten different retention times (Figure 1) and the mass spectrum from this retention times were compared with documented mass spectra of known compounds of plants origin from the Global Natural Products Social Molecular Networking (GNPS) spectral library (online). Nine compounds were proposed based on spectra match, eight of the compounds were tentatively identified to be flavonoids glycosides and the other was a triterpenoid glycoside. The flavonoid glycosides were kaempferol-3-O-glucoside, quercetin-3-O-glucose-6"-acetate, luteolin-8-C-glucoside, daidzein O-di-hexosidemalonylated, kaempferol hexose glucuronide, malvidin 3-O-glucoside and Dihexosyl luteolin sulfate while the saponin was Oleanolic acid 3-O-glucose acid a triterpenoidal saponin (Table 3). Flavonoids, such as quercetin, luteolin, and kaempferol were commonly found among the members of the Malvaceae family. Flavonol and flavones are characteristic of this family, demonstrating chemotaxonomic significance (Vadivel *et al.,* 2016). The presences of saponins had been reported in the Malvaceae family (Silva *et al.,* 2009). The classes of the proposed compounds are in agreement with the result of the phytochemical screening carried out. The LCMS 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). High reproducibility, sensitivity, selectivity, and the ability to analyse a number of constituents in herbal medicines are among the great advantages of using the techniques (Snyder *et al.,* 2011).

Table 3. Proposed compounds from the LCMS analysis of the aqueous ethanol leaf extract of *P. senegalensis.*

RT (min)	Fragmentation pattern (m/z)	Proposed compound	Chemical formula	Compound Class	Reference
1.179	444.5, 352.6, 274.7, 174.9, 118.1	kaempferol-3-O-glucoside	$C_{21}H_{20}O_{11}$	Flavonoid (flavonol) glycoside	Mastuda *et al.*, 2009
1.783	508.8, 427.0, 352.6, 274.7, 153.8, 117.8	Quercetin-3-O-glucose-6"-acetate	$C_{23}H_{22}O_{13}$	Flavonoid (flavonol) glycoside	Sawada *et al.*, 2009
2.536	442.9, 352.8, 274.4, 174.8	Luteolin-8-C-glucoside	$C_{21}H_{20}O_{11}$	Flavonoid (flavone) glycoside	Sawada *et al.*, 2009
2.708	745.1, 669.7, 573.9, 428.2, 352.8, 267.8, 197.2	Kaempferol-3-Glucoside-2-p-coumaroyl-7-Glucoside	$C_{36}H_{36}O_{18}$	Flavonoid (flavonol) glycoside	Mastuda *et al.*, 2009
3.215	699.1, 589.0, 471.0, 372.6, 308.8, 274.7, 175.0, 139.6	Unknown	Unknown		
3.469	663.9, 547.7, 426.5, 372.8, 274.8, 196.8	Daidzein O-di-hexosidemalonylated		Flavonoid (flavone) glycoside	Cavaliere *et al.*, 2007
3.723	743.0, 624.6, 455.0, 410.4, 352.9, 258.0, 175.0	Kaempferol hexose glucuronide	$C_{27}H_{28}O_{17}$	Flavonoid (flavonol) glycoside	Hanhineva *et al.*, 2008
4.029	793.6, 680.8, 520.2, 410.7, 374.1, 274.4, 211.0, 174.5	Oleanolic acid 3-O-glucose acid	$C_{36}H_{56}O_9$	Triterpenoid glycoside (saponin)	Madl *et al.*, 2006
4.424	744.9, 653.5, 586.1, 508.6, 428.7, 372.9, 274.5, 176.4	Malvidin 3-O- glucoside	$C_{40}H_{35}O_{15}$	Flavonoid (anthocyanin) glycoside	Tourino *et al.*, 2008
4.707	686.6, 520.7, 430.6, 352.7, 274.7, 175.0	Dihexosyl luteolin sulfate	$C_{27}H_{30}O_{19}S_1$	Flavonoid (flavone) glycoside	Hong *et al.*, 2006

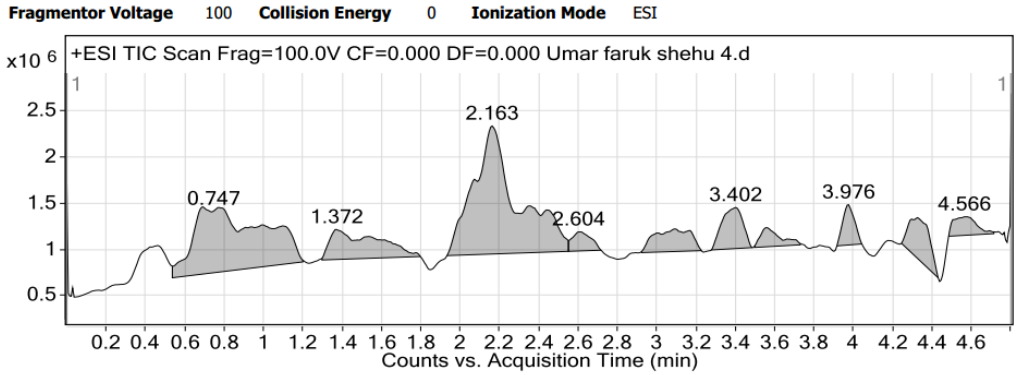

Figure 1. The Chromatogram of the LCMS analysis of aqueous ethanol leaf extract of *P. senegalensis.*

4. Conclusion

The phytochemical determination of the aqueous ethanol leaf extract of *P. senegalensis* revealed the presence of phenolic compounds (tannins and flavonoids) and steroids/triterpenes (saponins) with their variations while the LCMS analysis was used to tentatively identifyeight flavonoid glycosides and a triterpenoidal saponin from the extract.The result determined from this studycan be used in the identification process and help in quality control for authentication and detection of adulteration of herbal medicines labelled as *P. senegalensis.*

5. Acknowledgments

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