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PHARMACOGNOSTIC STANDARDIZATION ON THE LEAF OF *BREONADIA SALICINA* HEPPER AND J. R. I. WOOD (RUBIACEAE)

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ABSTRACT

Aim: This study was designed to evaluate the macroscopical, microscopical, physicochemical studies and preliminary phytochemical screening on the leaves of *Breonadia salicina*.

Study design: The study include collection and identification of the plant, macroscopic and microscopic leaf examination as well as preliminary phytochemical and physico-chemical evaluations.

Place and duration of the study: The study was carried out in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna. The study was conducted from September to December, 2022.

Methodology: The plant was collected around Kudingi Village, Giwa Local Government Area, Kaduna state, Nigeria. Sample was then identified and authenticated in the Department of Botany, Ahmadu Bello University, Zaria. Macroscopic and microscopic leaf examination as well as preliminary phytochemical and physico-chemical evaluations were carried out using standard methods.

Results: Macroscopically, the leaves of the plant was observed to be of pinnate arrangement with dimension up to 38.5 cm; lamina is lanceolate, margin of the lamina is entire, apex is acute and the base is symmetrical while petiole is long up to 4.8 and light green in colour. The leaf has characteristic odour and leathery texture while the surfaces are glaborous. Microscopically, polygonal epidermal cells, slightly wavy anticlinal walls, anomocytic types of stomata observed. Phytochemicals present include flavonoids, saponins and tannins, while alkaloids were absent. Physicochemical parameters showed moisture contents of 13.50 ± 0.34; percentage yield of total ash was 9.0 ± 0.20. For the acid insoluble ash, the value was 2.2 ± 0.12, while for water-soluble ash, the values determined was 1.2 ± 0.12. The percentage ethanol and aqueous extractive values of the sample was 12.3 ± 0.10 and 15.7 ± 0.10.

Conclusion: The research conducted could be utilized for the identification, evaluation and standardization of this plant species.

Keywords: *Breonadia salicina*, Macroscopy, Microcopy, Physico-chemical, Pre-liminary phytochemical

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INTRODUCTION

Standardization of herbal drugs is desirable or even necessary to ascertain the physical, physicochemical, biochemical as well as the biological properties of such drugs [1]. This is because herbal drugs are susceptible to a number of physical and chemical changes especially if they are not to be immediately utilize or may even needed to be stored a longer period of time [2]. Standardization and authentication of the herbals are cogent in trading of such products providing information to consumers [3]. In spite of the modern techniques, standardization and authentication of plant drugs by pharmacognostic procedures is still relevant. The morphological and microscopical description of a medicinal plant is needed first before commencement of processes for the detection of adulteration and impurities [4]. The macroscopic identity include shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface. Yet, microscopic examination is indispensable to substantiate the findings and sometimes comparison with a reference material is necessary [5] *Breonardia salicina* is an evergreen plant that grows up to 20 m in height [8]. Commonly called Matumi and locally *Kadanyar rafi* by Hausa communities of Northern Nigeria [8]. It is widely distributed in tropical and subtropical parts of the world including but not limited to Saudi Arabia, Yemen, Madagascar, Ethiopia and Nigeria. The plant is used in ethno-medicine to treat cancer, gastrointestinal diseases, fevers, headaches, arthritis, diabetes, inflamed wounds and ulcers [9].

MATERIALS AND METHODS

Identification, Collection and Preparation of the Leaves of *Breonadia salicina*

Breonadia salicina was first identified on the field using its morphological features around Kudingi Village, Giwa Local Government Area, Kaduna state, Nigeria. Sample of the plant was then collected and transported to Herbarium Unit of the Department of Botany, Ahmadu Bello University, Zaria for proper identification and authentication. After authenticating the identity of the *Breonadia salicina*, sufficient quantities of the leaves were then obtained for further studies.

Macroscopic Examination

Macroscopic observations were conducted on the whole plant leaf which include; leaf colour, odour, leaf taste, shape, venation, margin, leaf surface, leaf apex, texture, and leaf arrangement.

Colour: The colour of the crude leaves was determined under diffused daylight

Odour: A small sample of leaf crushed between thumb and index finger and between palms of the hands, using gentle pressure and the strength of the odour was determined as weak or as strong.

Taste: A small sample of the leaf was tasted using organ of taste (tongue).

Surface texture: The surface characteristics of the leaves was determined as softness or hardness; bend or rupture by touching of surface of the leaves

Size: A graduated ruler in centimetres/millimetres used for the

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measurement of length and width of the leaf with the aid of a thread.

Microscopical Examination of Leaf of *Breonardia salicina*

Anatomical transverse section (T.S) and Longitudinal section (L.S) of the leaf sample were prepared and examined under microscope. This procedure was carried out according to Evans (2002) as outlined below.

Leaf sample Sectioning: A fresh leaf of *B. salicina* was firmly held against the side of the first finger with the assistance of the thumb and a drop of fresh water was smeared on a razor blade to avoid friction while cutting the leaf spacemen. The razor blade was then quickly moved across the top of the leaf spacemen in such a manner that one single stroke cut was obtained. Many more uniform strokes were made to obtain several sections at a time. The sections was thereafter transferred to water in a transparent beaker using a brush.

Clearing: The thinnest and most transparent sections was transferred onto a clean glass slide and three drops of chloral hydrate solution was added into each of the slide containing the thin and transparent sections, and then the slides was gently passed back and forth over a low flame.

Staining: A drop of safranin staining reagent added, followed by a drop of glycerine then covered slowly with a coverslip held at an angle on one edge of the slide to avoid air bubbles. The prepared slide allowed standing for few minutes to allow for effective staining of the microscopic features.

Mounting: The slides mounted on a binocular compound microscope for observations. The specimens on the slides were focused using scanning objective lens (x4 objective), followed by high power and very high power objectives (x10 and x40). Several pictures of the microscopic features captured.

Determination of Physico-chemical Parameters of the Powdered Leaves of *Breonadia salicina*

The quantitative physical standard (moisture content, solvent extractive values and ash values) of the leaves of the *B. salicina* were determined.

Moisture Content Determination

Crucibles were heated to a constant weight and allowed to cool in a dessicator. The powdered samples of the leaves of the plant (3g) was weighed and transferred in to the crucibles. The crucibles with the plant samples were then heated for another 1hour in an oven maintained at 105^0C. The admixture was allowed to cool in a dessicator and re-weighed. The procedures were repeated until no further loss in weight was obtained for all the samples. The moisture content was then determined in percentage using the formula below:

$$\text{Moisture Content (\%)} = \frac{Weight\ of\ WaterLost}{Original\ Weight\ of\ Sample} \times 100$$ Ash Values

(i) Total Ash

Nickel crucibles was heated at 105^0 C to a constant weight and cooled in a dessicator. The powdered samples of the leaves (3g) was then weighed and transferred in to the pre-weighed crucibles. The crucibles with the powdered samples were then heated into

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ashes until it became free of carbons and allowed to cooled in the dessicator and weighed. The total ash value was calculated in percentage as:

Total Ash Value (%) =

$$\frac{Weight\ of\ Residual\ Ash}{Weight\ of\ Sample} \times 100$$

(ii) Acid Insoluble Ash

Dilute HCl (25 ml) was added to the ash of the leaf obtained in (i) above, boiled for 5 minutes and filtered using ash less filter paper. The insoluble matter collected on ash less filter paper was transferred into a beaker. The insoluble matter was then washed in hot water and the washings passed through the filter paper. The washing continued until the residue was free of acid. The residue and filter paper were dried gently in an oven at 105^0C and ignited in the crucible and burnt completely, allowed to cool and weighed. The acid insoluble ash was calculated in percentage as:

Acid Insoluble Ash (%) = $\frac{Weight\ of\ ResidualAsh}{Initial\ Weight\ of\ Sample} \times 100$

(iii) Water Soluble Ash

The water-soluble ash value of the powdered leaves of the plant was determined. The ash obtained in (i) above was used. The procedure used to obtain the water insoluble ash was the same as in (ii) above except that water was used instead of dilute HCl. The water insoluble ash was determined in percentage as:

Water ash (%) =

$$\frac{Weight\ of\ Initialash - Weight\ of\ ResidualAsh}{Initial\ Weight\ of\ Sample} \times 100$$

Solvent Extractive Values

(i) Alcohol Soluble Extractive Value

The powdered samples of the leaves of the plant (5g) was weighed and macerated with 100 ml of alcohol in a stoppered flask. Agitated with the aid of a mechanical shaker for the first 6 hours and allowed to stand for 18 hours then filtered after 24 hours. The filtrate (20 ml) for each of the sample was evaporated to dryness in an evaporating dish. Alcohol soluble extractive values was calculated (in percentage) with reference to the initial weights of the extract, five determinations were done for all the samples and the average taken.

Alcohol Extractive Value (%) = $\frac{Weight\ of\ residure \times 5}{Weight\ of\ Sample} \times 100$

(ii) Water Soluble Extractive Value

The same procedure in (i) above was repeated in determining the water-soluble extractive value, using water as the solvent instead of alcohol. The water-soluble extractive value was determined using the formula below:

Water Soluble Extractive Value (%) = $\frac{Weight\ of\ Residue \times 5}{Weight\ of\ Sample} \times 100$

Phytochemical Screening of the Leaves of *Breonadia salicina*

Standard methods of preliminary phytochemical screening as described by Evans (2002) was followed in screening the powdered leaf sample, for the presence or absence of carbohydrates, cardiac glycosides, saponins, triterpenes and steroids, tannins, flavonoids, alkaloid and anthraquinones

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RESULTS

Macroscopical Features of the Leaves of *Breonadia salicina*

The leaves of the plant was observed organoleptically to be of pinnate arrangement with dimension up to 38.5 cm; lamina is lanceolate while margin of the lamina is entire. Apex was observed to be acute and the base was symmetrical while petiole was long up to 4.8 and light green in colour. The leaf had characteristic odour and leathery texture while the surfaces are glaborous. The organoleptic features observed in the leave is summarized as shown in Table I.

Table I: Macroscopic features of the Leaves of *Breonadia salicina*

Morphological character	Observation
Whole leaves Assessment	
Type	: Simple
Botanical Source	: *B. salicina*
Family	: Moraceae
Geographical source	: Kudingi-Samaru, Zaria
Condition	: Fresh
Lamina Assessment	
Shape	: Lanceolate
Size (Length)	: 19.25cm-(10.1cm)-4.9cm
Width	: 6cm-(4.7cm)-2.3
Colour	: Dark-green
Venation	: Reticulate
Margin	: Entire
Apex	: Acute
Base	: Symmetrical
Surface	: Glaborous
Petiole	: Long
(Length)	: 4.8cm-(3.0cm)-1.5cm
Attachment of Leaf	: Petiolate

Microscopical Features of *Breonadia salicina* Leaf

Microscopical examination of the leaf of *Breonadia salicina* revealed the presence of some important diagnostic characters on both upper and lower epidermal layers. These includes polygonal epidermal cells, slightly wavy anticlinal walls, and anomocytic types of stomata. However, trichomes and calcium oxalate crystals were not observed. Transverse section (T.S) of the leaf revealed a well formed and arranged vascular bundles. Xylem was observed to have spiral type of xylem vessels that are lignified while phloem was observed to have phloem parenchyma that is cylindrical and elongated (Table II).

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Table II: Microscopical Characteristics of Transverse Section (T.S) and Longitudinal Section (L.S) of *Breonadia salicina* Leaf

Microscopic character	Observation
Epidermal cells	Polygonal
Anticlinal walls	Slightly wavy
Xylem vessels	Spiral
Phloem parenchyma	Cylindrical
Stomata	Anomocytic
Trichomes	Not observed
Calcium oxalate crystals	Not observed

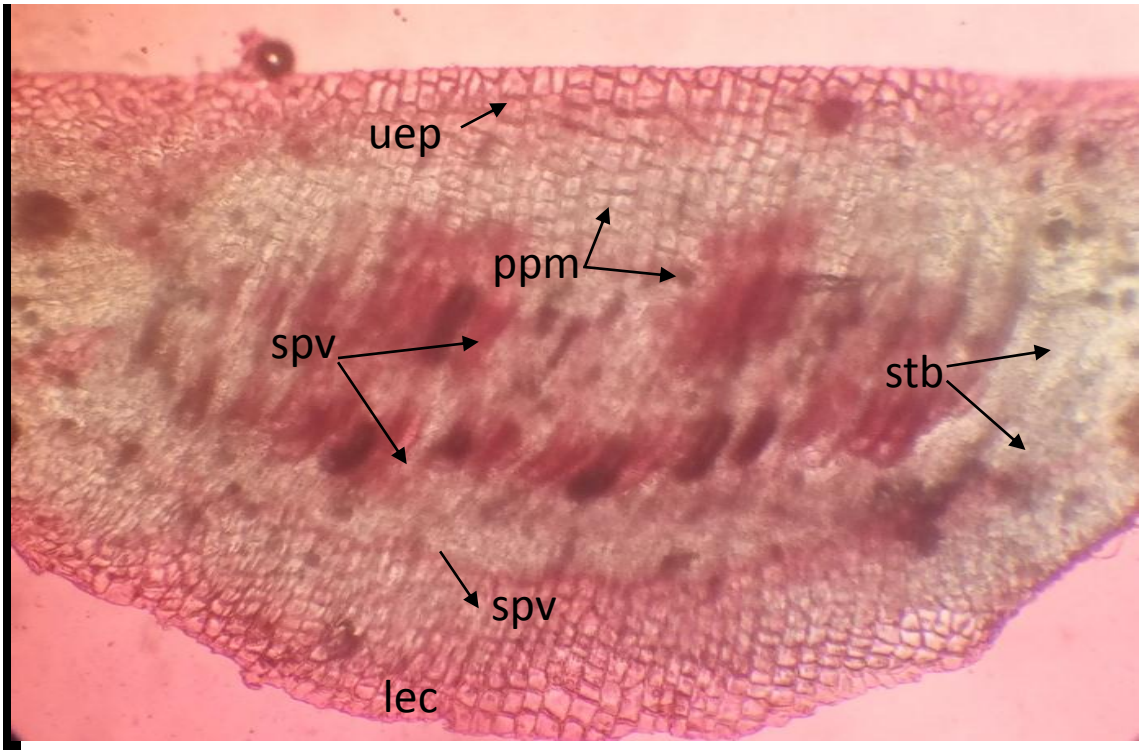

Plate I: Photomicrograph of the Transverse Section of the Leaf of *Breonadia salicina* at Magnification 100X.

Keys: ppm = phloem parenchyma, lep = lower epidermal cells, uep = upper epidermal cells, stb = sieve tubes

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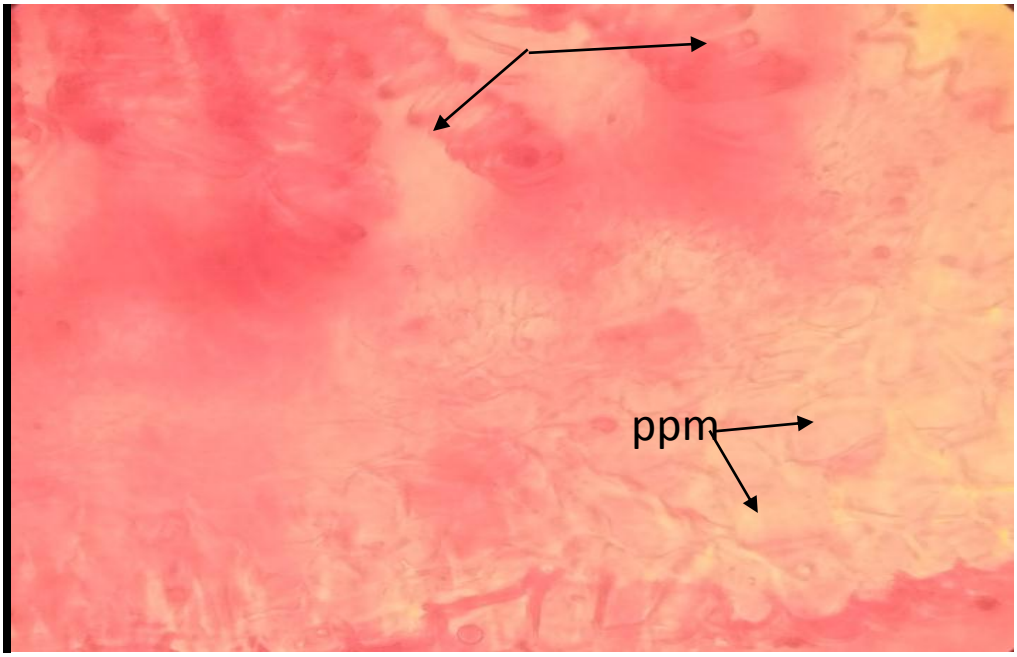

Plate II: Photomicrograph of the Transverse Section of the Leaf of *Breonadia salicina* at Magnification 400X.
Keys: spv –spiral vessels, ppm = phloem parenchyma

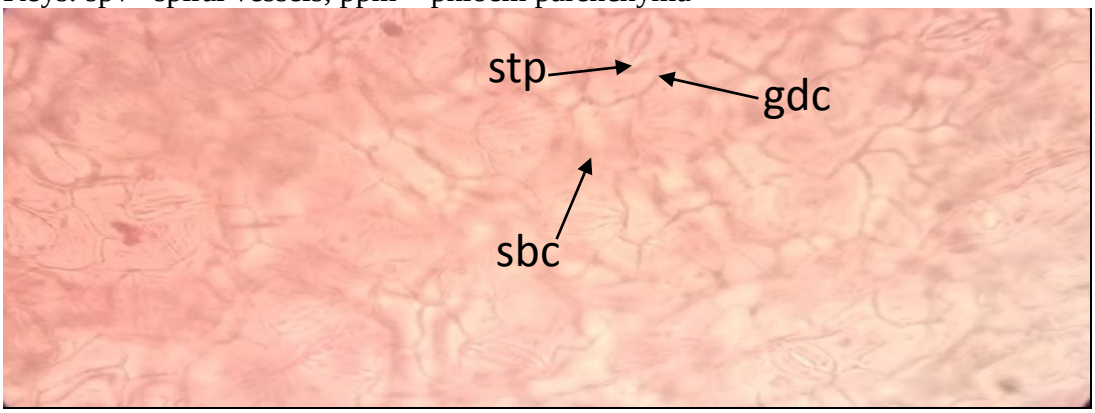

Plate III: Photomicrograph of the Anomocytic stomata on the Lower Surface for the Leaf of *Breonadia salicina* at Magnification 400X.
Keys: stp = stomatal pore, sbc = subsidiary cells, gdc = guard cells

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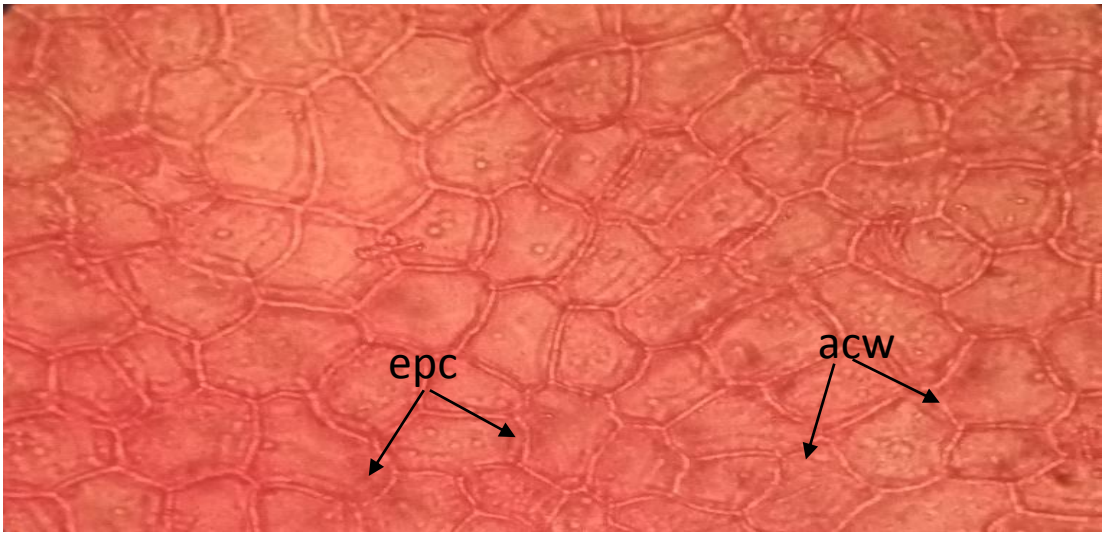

Plate IV: Photomicrograph of the upper Section of the Leaf of *Breonadia salicina* at Magnification 400X.
Keys: acw = anticlinal walls, epc = epidermal cells

Physicochemical Constants of the Powdered Leaf of *Breonadia salicina*

Moisture content value was 13.50 % ± 0.34. The percentage yield of total ash was 9.0 ± 0.20%. For the acid insoluble ash, the values obtained was 2.2 ± 0.12% while for water-soluble ash it was 1.2 ± 0.12%. The ethanol and aqueous extractive values of the powdered leaf of *B. salicina* obtained as percentage 12.3 ± 0.10 and 15.7 ± 0.10. Below (Table III) are the physico-chemical parameters evaluated:

Table III: Physico-chemical constants of the powdered Leaf of *Breonadia salicina*

S/N	Parameter	Leaf ± SEM	Stem bark ± SEM	Root ±SEM (%)
1	Moisture	13.50 ± 0.34	14.86 ± 0.46	15.30 ± 0.97
2	Total Ash	9.0 ± 0.20	10.2 ± 1.2	12.2 ± 0.76
3	Acid insoluble Ash	2.2 ± 0.12	3.6 ± 0.65	4.80 ± 0.67
4	Water soluble Ash	1.2 ± 0.12	1.4 ± 0.42	1.20 ± 0.57
5	Alcohol soluble extractive	12.3 ± 0.10	10.52 ± 0.66	10.08 ± 0.52
6	Water soluble extractive	13.7 ± 0.10	13.28 ± 0.18	12.56 ± 0.09

Key: Values are means ± SEM of 5 replicates

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Preliminary Phytochemical Screening of the Leaf of *Breonardia salicina*

The preliminary phytochemical screening of the powdered leaf, stem bark and the root of the plant indicated the presence of anthraquinones, cardiac glycosides, saponins, flavonoids, triterpenes, steroids, tannins and alkaloid (Table IV).

Table 1V: Phytochemical Screening of the Leaf of *Breonardia salicina*

Phytochemicals	Test	Inference
Anthraquinones		
Bontrager's test	A bright pink colour in the upper aqueous layer observed	+
Modified Bontrager's test	A rose pink at the lower layer observed	+
Cardiac glycosides		
Keller-Killiani test	A purple-brown ring at the interface and a pale green colour in the upper acetic acid layer observed	+
Legal test	A deep red colour observed	+
Kedde test	A purple-blue colour observed	+
Saponin glycosides		
Frothing test	A honey comb froth that persisted for 15 minutes observed	+
Flavonoids		
Ferric Chloride test	A greenish black colour observed	+
Sodium hydroxide test	A yellow solution observed	+
Shinoda's test	A pink colour solution observed	+
Steroids/Triterpenoids		
Libermann-Burchard test	A purple colour observed	+
Salkowski test	A cherry red colour at the interface observed	+
Tannins		
Ferric Chloride test	A green precipitate observed	+
Lead sub-acetate	A colour precipitate observed	+
Bromine water test	A buff colour precipitate observed	+
Alkaloids		
Meyer's test	A cream coloured precipitate observed	+
Dragendoff's test	An orange-red precipitate observed	+
Wagner's test	A reddish brown precipitate observed	+
1% Picric acid test	A yellow precipitate observed	+
10% Tannic acid test	A buff coloured precipitate observed	+

Keys: + = present, - = absent

DISCUSSION

Pharmacognostic evaluation of a plant or plant parts is considered an important step that provides valuable information in terms of its morphological and microscopical characteristics [7]. The macroscopic and microscopic evaluations of the fresh leaves of the plant revealed some features, which are of importance and applicable in establishing the identity of the plant. This is important in the study of crude drugs since the first step in the study of crude drug is establishing the correct identity of the plant [8]. Macroscopically, the leaves of the plant appeared to be dark green coloured, reticulate venation with lanceolate shape and entire margin. The leaf is 19.25cm-(10.1cm) - 4.9cm long and 6cm-(4.7cm)-2.3cm width while the apex is acute, symmetrical base

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with glaborous surface and long petiole of 4.8cm - (3.0cm) - 1.5cm as shown on (Table I). The macroscopic feature of the plant was in conformity with the characteristic macroscopic features of the plant as described by Gafar (2014). Microscopically, the laminar shows regular upper and lower epidermis with well-developed thin cuticle stomata. Parenchymatous mesophyll is also present. The midrib shows upper and lower epidermis layer continuous over the midrib followed by a patch of collenchyma cells below the upper above the lower epidermis. The epidermal cells show similar features as seen in the lamina region. Paranchyamatous tissue containing spiral vasc ular strands. The rest of the midrib is occupie d by the parenchyma cells. These features are consistent with other members of the family as described by Delprete (1996). The result of the moisture contents using loss on drying method determined to be 13.50 ± 0.34%. It is very essential to control moisture content since higher moisture content in plant material may lead to its deterioration and may therefore result in biodegradation of active constituents [10]. The ash values indicate inorganic residues obtained when vegetable drug incinerated. Total ash in a plant material includes both physiological as well as none physiological ash while acid insoluble ash is a part of total ash and is indicative of silica present especially in sand and siliceous earth whereas water soluble ash is the water soluble portion of the total ash [11]. The percentage yield of total ash was 9.0 ± 0.20. For the acid insoluble ash, the value obtained was 2.2 ± 0.12% while for water-soluble ash it was determined to be 1.2 ± 0.12%. The results showed the presence of lesser quantity of acid insoluble ash, it was observed that there was a reduction in extractive values with decrease in extractive solvent polarity. The ethanol and aqueous extractive values of the powdered leaf of the plant obtained were 12.3 ± 0.10 and 15.7 ± 0.10% respectively. Alcohol and water-soluble extractive values can serve as an indicator of adulteration [12],

Despite the high extractive value of water, also been a universal solvent and its use primarily as solvent by traditional medical practioners, alcohol is still given preference in terms of choice of solvent when it comes to medicinal plant researches. The choice of solvent in a research involving plants depend on so many factors among which include the diversity of different phyto compounds to be extracted. In spite, the higher yield of the extract from water solvent over alcohol; ethanol was chosen as the solvent of extraction in this study. This is due to its higher extracting capacity [13]. Moreover, water is a better medium for the occurrence of microorganisms as compared to ethanol [14]. Additionally, ethanol easily penetrates the cellular membrane to extract the inner cellular ingredients from the plant material [15]. Phytochemical screening is a technique that is use to carry out simple chemical tests on drug materials in order to establish the presence or absence of a particular bioactive chemical constituent [13]. The test is useful in the evaluation and identification of samples of drugs.

The phytochemical screening of the powdered leaf of the *Breonardia salicina* reveals the presence of anthraquinones, saponins, cardiac glycosides, triterpenoids, flavonoids, tannins and alkaloids as shown on (Table 1). This is consistent with the findings of [9].

CONCLUSION

The research conducted could be utilized for the identification, evaluation and standardization of this plant species.

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