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PHARMACOGNOSTIC STUDIES AND PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAF OF *MAYTENUS SENEGALENSIS* (LAM.) EXELL CELASTRACCEAE

Ahmed MA[1]*, Garba I[2], Abubakar A[3], Abdullahi AM [1], Yusha'u HM[1]

[1]*Department of Pharmacognosy and Drug Development, Kaduna State University, Kaduna*
[2]*Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Kaduna.*
[3]*Department of Science laboratory technology, Nuhu Bamalli Polytechnic, Zaria, Kaduna, Nigeria*.

For correspondence: murjanatu.ahmed@kasu.edu.ng, +234-8037651813

ABSTRACT

Aim: The aim of the study was to evaluate the pharamacognostic parameters for the plant *M. senegalensis*, to ensure its purity, quality and safety.

Place and duration of study: this study was conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. From August to December 2021.

Methodology: Whole powdered leaves and methanolic extract were used. Pharmacognostic standards were determined according to the guidelines given by the World Health Organization (WHO). Parameters determined are macroscopic and microscopic characters (quantitative and qualitative), physicochemical parameters as well as preliminary phytochemical tests.

Results: Macroscopically the leaves of *M. senegalensis* are alternately arranged with glabrous surface, serrated margin, and a characteristic taste and odor. Microspically, the leaves have stomata that is anomocytic type on both upper (SN= 80.50-70.00-59.50, SI= 10.81-12.72-14.63) and lower epidermis (SN= 101.78-88.50-75.23, SI= 10.65-12.53-14.41), the epidermal cells were polygonal in shape and trichomes were absent. The vein islet number was determined to be 23.46-20.40-17.34 and the vein let termination number was 17.94-15.60-13.26. Transverse sections of the leaves revealed a dorsiventral type having a singled layered epidermis, palisade cell beneath the upper epidermis, with some vascular bundles at the center covered by a bundle carp, and some spongy mesophyll. Chemo-microscopical study revealed the presence of cellulose cell wall, lignified fibers, starch grains, calcium oxalates, fixed oil and fats. Other determinations include: moisture content (9.33 % w/w ± 0.01), total ash (7.83.00 % w/w ± 0.004) acid-insoluble ash (01.67% w/w ± 0.01), alcohol-soluble extractive values (12.00% w/w ± 0.10), and water-soluble extractive values (13.25%w/w ± 0.04). Preliminary phytochemical screening of the methanolic leave extract reveals the presence alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, carbohydrates and flavonoids.

Conclusion: The drug can be stored in powdered form for a long time without worrying about its purity based on the moisture content value obtained. The high digestibility of the plant when eaten is indicated by the low total ash and acid-insoluble ash values obtained (i.e can be safely eaten). The drug may be very significant in the development of phytomedicines, according to the results of the phytochemical screening.

Key words: macroscopic, microscopic, physicochemical, phyto-chemical

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INTRODUCTION

All medicines, be it synthetic or of plant origin, should fulfill the basic requirement of being safe, and effective [22]. Due to cultural and historical factors, dependency on medicinal plants has become a topic of global importance in both the developing and developed countries [7]. In most countries, herbal products are launched into the market without proper scientific evaluation; consumers can buy herbal products without a prescription and might not recognize the danger in an inferior product [13]. The major source of herbs for local people and herbal industries is wild source, adulteration is mostly found in the raw materials when purchased from the market [9]. There is also report that the herbal industries and local residents face the problems of adulteration and substitution at a raw material stage [2]. The first step towards the identification of crude medicinal plant is authentication, and despite modern techniques, pharmacognostic identification of plant drugs is more reliable. Pharmacognostic studies ensure plant identity hence its authentication, there by leading to the production of quality herbal products [14].

The Celastraceae family comprises approximately 106 genera and 1300 species that are widely distributed in tropical and subtropical regions of the world [18]. Most members of the family are shrub to small trees, although members of some genera such as *Bhesa, Koona,* and *Lapophetalum,* reach up to 50 m high and have buttressed trunk. Most Celastraceae are erect but members of some genera such as *Celastrus, Euonymus, Maytenus,* etc are scandent [5].

The genus has been reported to contain various phytoconstituents such as flavonoids, phenolic glucosides, triterpenes. Pentacyclic triterpenes- 3-oxofriedelane and 3ß-hydroxyfriedelane are said to be the chemotaxonomic marker of the genus. Compounds isolated from the *Maytenus* genus include the ansa macrolide, maytansine, and related macrolides such as normaytansine, maytanprine and maytanbutine [11]. Other isolated compounds include spermidine alkaloids (celacinnine and celallocinine) and nicotin sesquiterpene alkaloids (maytoline and maytolidine) as well as catechin, procyanidins and phenoldienone triterpenoids [10].

In African traditional medicine, root of *Maytenus senegalensis* is used in the treatment of cancer; in Asia it is used as an insect repellant while the South American people use it for treating gastro intestinal disorders. The plant is one of the most frequently used species [17]. It has a long history of usage by the 'Hausa' community in traditional medicine and known to exhibit a wide range of biological activities such as anti-inflammatory, analgesic, antibacterial activities. Literature review shows that a systemic standardization on the plant is still inadequate; therefore, this investigation was carried out to establish pharmacognostic and phytochemical standards that can be helpful in identification as well as checking adulteration which will help in quality assurance of finished products.

MATERIALS AND METHODS

Collection and Identification of Samples

Leaves were collected from Ahmadu Bello University dam, Samaru, Zaria, Nigeria. It was identified by taxonomist, Malam Musa Muhammad of the herbarium section, Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State. Voucher Number in the Herbarium.

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Macroscopical Studies of the Leaves
Fresh samples were used for this analysis. All distinguishable macroscopic features of the leaves such as shape, color, odor, e.t.c., were observed, noted and described with appropriate terms as described in quality control methods for medicinal plants [21].

Microscopical Studies
Cleared fresh samples of the leaves were prepared and observed under a compound microscope under magnification (x100). The features observed such as stomata, epidermal cells, e.t.c; were analyzed, drawn and labeled [6] [21]. Four physical constants were determined for the leaf. These are: stomatal number, stomatal index, vein islet number, vein-let termination number. The determinations were carried out three times using surface preparations and cut sections [22].

Anatomical Studies
Transverse and longitudinal sections of the leaves were studied. Thin sections were placed in a test-tube containing 70% chloral hydrate and boiled for a few minutes until they become transparent. Cleared sections were mounted and observed under microscope, magnification (x100). Features observed were photographed and labeled.

Chemo-microscopical Studies
Powdered material of the leaves was used for this study in order to detect the presence of cell wall material and ergatsic substances.

Test for starch:
A small amount of the cleared leaves powder was stained with N/50 iodine on a glass slide. A drop of glycerol was added and the sample was observed under a compound microscope (x400). Appearance or absence of blue-black coloration indicated the presence or absence of starch grains.

Test for Tannins:
A small quantity of the cleared leaves powder was mounted in 5% ferric chloride; one drop of glycerol was then added, the mounted sample was analyzed under microscope. Appearance of bluish or greenish-black coloration indicated the presence of tannins.

Test for Lignin
Cleared small quantity of leaves powder was placed on slides and a drop of phloroglucinol was added followed by a drop of conc. Hydrochloric acid. Appearance of red coloration indicated the presence of lignin.

Test for Gums and Mucilage
Small quantity of cleared leaves powder was placed on slides and a drop of ruthenium red was added. Appearance of pink coloration indicated the presence of gums and mucilage.

Test for Oils
Small quantity of the cleared leaves powder was placed on a slide and a drop of Sudan (IV) reagent was added. Appearance of pinkish color indicated the presence of oils.

Test for Cellulose
Small quantity of the cleared leaves powder was placed on a slide and a drop of N/50 iodine was added, followed by a drop of 66% sulphuric acid. The appearance of bluish or violet coloration indicated the presence of cellulose.

Test for Calcium Oxalates and Calcium Carbonates
Small quantity of the cleared leaves powder was placed on a slide this was viewed under microscope and some crystals were observed which dissolved in sulphuric acid without

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effervescence and later formed some needle-shaped crystals. Thus, indicating the presence of calcium oxalates. Calcium carbonate only dissolves in acetic acid or conc. Hydrochloric acid.

Determinations of Microscopical Physical Constants

Stomatal Number

A cleared surface section of the upper and lower epidermis of the leaf was mounted using glycerol, this was analyzed under camera Lucida, the size, type and the dimension of the stomata and epidermal cells per square mm was counted (5×). The stomatal numbers of the section were calculated using the formula: 15%mean ± SEM.

Stomatal Index

The stomata and epidermal cells per squared mm were counted with the aid of camera lucida and the stomatal index was calculated as follows:

$$[\text{Stomatal index} = \frac{S}{E+S} \times 100]$$

Where S= total number of stomata per mm^2, E= total number of epidermal cells per mm^2.

Vein-islet Number

It is the number of vein-islet per square mm of the leaf surface midway between midrib and margin.

Thin section of the leaf was cleared and mounted in dilute glycerol. Vein islet per four contagious mm^2 in the central part of the lamina of the leaf midway between the mid rib and margin was analyzed under camera Lucida and calculated.

Vein let Termination Number

This was also achieved with the aid of camera Lucida. The portion where veins terminate are noted, traced and counted.

Determinations of Physicochemical Constants of the Leaf of *Maytenus senegalensis*

Five physical constants were determined for the plant material. These include: moisture content; ash value; acid insoluble ash value; alcohol extractive value and water extractive value [21]. Three different determinations were carried out for each of the parameter and average was taken as shown in the appendix one. Physico-chemical values obtained were calculated in terms of air-dried weight samples of the leaves.

Moisture Content

It is the quantity of moisture contained in a plant material. Moisture content of *Maytenus senegalensis* was determined by loss on drying method. 3.0g of the leaves powder was accurately weighed and placed in clean, dried evaporating dish of known weight. Dish was placed in an oven and heated at a temperature of 105^0C. After 30 mins, the weight of the drug and the dish was determined and returned to oven. The weight was taken subsequently after every 30 mins until the weights become constant. The weight of water loss on drying was computed following the formula below:

% Moisture content =

$$\frac{wt.of\ moisture}{original\ wt.\ of\ sample} x\ 100$$

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Total Ash Value

Total ash is the ash remaining after ignition of medicinal plant materials. Powdered plant material (2.0g) was accurately weighed and placed in a crucible of known weight. The material was in an even layer and ignited and the heat was gradually increased until it is carbon white. It was allowed to cool in a desiccator and weighed. The ash was again heated and weighed. The heating and weighing continue until a constant weighed is obtained. The ash value was then calculated using the formula below:

$$\% \text{ Ash value } = \frac{wt.of\ residual\ ash}{wt.of\ original\ sample} \times 100$$

Acid-insoluble Ash Value

This is the residue that remains after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. Conc. Hydrochloric acid (25ml) was added to the crucible containing the ash; it was then covered with a watch-glass and gently boiled for 5 mins. The watch glass was rinsed with a 5ml of hot water, which was added to the crucible. It was filtered with ash less filter-paper; the filter paper and the filtrate are placed back into the crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in desiccator for 30 mins, and then weighed without delay. The acid-insoluble ash value was then calculated using the formula below:

$$\% \text{ Acid} - \text{insoluble ash value}$$

$$= \frac{\text{wt. of residual ash}}{\text{wt. of original sample}} \times 100$$

Alcohol Extractive Value

The powdered plant material (4.0g) was accurately weighed in a conical flask. It was macerated with 100ml of 95% ethanol for 6 hours, shaken frequently with mechanical shaker and later allowed to stand for 18 hours. It was filtered and 25ml of the filtrate was transferred into an evaporating dish of known weight and evaporated to dryness on a water bath. Dried at 105^0C for 6 hrs and weighed after it was cool. The alcohol extractive value was calculated using the following formula:

$$\% \text{ Alcohol soluble ext. value } = \frac{wt\ of\ residue\ in\ 25ml\ extract}{initial\ wt\ of\ sample} \times 100$$

Water Soluble Extractive Value

The procedure for water soluble extractive value is the same with that of alcohol soluble extractive value. The solvent for extraction here is water.

Extraction of the Powdered Leaf of *M. senegalensis*

The leaves powder (300g) was extracted with aqueous methanol (70:30 v/v) using maceration method for 96 hours. The extract was concentrated using water bath at the temperature of 45^0C.

Phytochemical Analysis on Methanolic Extract of Leaf of *M. Senegalensis*

Phytochemical Analysis was carried out using leaf's extract to detect the presence of various phytoconstituents such as alkaloid, saponins, tannins, anthraquinones, glycosides, carbohydrate, and flavonoids according [6].

Test for Alkaloids

About 0.5g of each extract was stirred with 5ml of 1% aqueous hydrochloric acid on a water bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated with Dragendorff's reagent. Turbidity or

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precipitate with either of these reagents may be due to the presence of alkaloids in the extracts.

Test for Saponins

The extract (0.5g) was shaken with water in a test tube followed by warming on a water bath. Frothing which persists on warming was taken as preliminary evidence for the presence of Saponins.

Test for Tannins

Ferric Chloride Test

The extract (0.5g) was stirred with 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

Bromine Water Test

Few drops of bromine water was added to the extract in test tube, formation of a buff colored precipitate indicates the presence of condensed tannins while none color reaction indicates the presence of hydrolysable tannins.

Test for Anthraquinones

To the plant extract (5g) in the test tube, 10ml of benzene was added and was shaken for 5 minutes. The extract was then filtered and shaken with 5ml of 10% ammonia solution. Formation of a bright color in the upper part of the aqueous layer indicates the presence of free anthraquinones.

Test for Cardiac Glycosides

Keller-Keliani Test

Small quantities of the extract (0.5g) was dissolved in 2ml of glacial acetic acid containing a drop of ferric chloride solution; 1ml of conc. Sulphuric acid was carefully added to form a lower layer. A brown ring formed at the interface indicates the presence of desoxy sugar. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer, indicates the presence of cardenolides.

Salkowski's Test

Five milliliter of extracts each were mixed in 2 ml of chloroform and filtered. Concentrated sulphuric acid (3ml) was carefully added to the filtrate to form a layer. A reddish-brown coloration at the interface indicates presence of steroids/Terpenes.

Test for Carbohydrate

Molisch Test

The plant extract (0.5g) was dissolved in distilled water in a test tube and filtered. Four drops of Molisch reagent and 4 drops of conc. Sulphuric acid were added to the filtrate without mixing. Appearance of purple ring at the interface as a result of interaction between Molisch reagent and 5-hydroxymethylfurfural produced by dehydration of saccharides indicates the presence of carbohydrates.

Fehling's Test

The plant extract (0.5g) was dissolved in distilled water in a test tube. The test tube was then placed on a water bath to heat. Equal volumes of Fehling's solution A and B were added drop by drop into the test tube. Appearance of brick-red precipitation of cuprous oxide indicates the presence of carbohydrate.

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Test for Flavonoids

Shinoda Test

The plant extract (0.5g) was dissolved in 2ml of methanol and 4 drops of concentrated hydrochloric acid was added followed by some chips of magnesium metal. Appearance of orange color indicates flavones, red-crimson indicates flavonols and pink-magneta indicates flavonones.

Sodium Hydroxide Test

The plant extract reconstituted was in water and filtered, (5ml) each was shaken with 10% sodium hydroxide. A yellow solution which becomes colorless on addition of dilute hydrochloric acid indicates the presence of flavonoids.

RESULTS

Macroscopical Evaluations on the Leaf of *M. senegalensis*

Organoleptic properties of *Maytenus senegalensis* were assessed. Leaves: The ovate-shaped leaf is organized alternately. The lower surface is pale green and the upper surface is dark green, with a length that varies from 12.5 to 22 cm. The leaf's surface is glabrous, with serrated margin, acute apex and a symmetrical base. Petiolate attachment and with parallel venation. Leaf has a characteristic odor and a characteristic taste (Table 1).

Table 1. Macroscopical Evaluations on the Leaf of *M. senegalensis*

Evaluative Parameters	Results
Arrangement	Alternate
Shape	Ovate
Size	12.5-22cm in length
Color	Pale green (LS), green (US)
Surface	Glabrous
Margin	Serrate
Apex	Acute
Base	Symmetrical
Attachment	Petiolate
Venation	Parallel
Odor	Characteristic odor
Taste	Characteristic Taste

Microscopical Features of the Leaf of *M. Senegalensis*

The surface peel of leaf showed the presence of stomata and epidermal cells, trichomes are absent on the epidermal surface. The stomata are anomocytic type and are present on both upper and lower epidermis with diameter of 48μ, but it is more frequent on the lower epidermis. The epidermal cells are straight walled, polygonal (5 – 6 sided) cells. The mean values of the stomatal number, stomatal index, vein islet and vein let termination numbers are given in table 2 and the photomicrograph stomata are shown in plate I and II. The transverse sections of the leaf is dorsiventral showing single layered upper and lower epidermis with a layer of compact elongated palisade cell below the upper epidermis, an anomocytic type of stoma and arc shaped vascular bundles that were

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surrounded by a bundle carp with some spongy mesophyll in the lamina (plate III). The longitudinal sections of the leaf showed the presence of single layered epidermis and some collenchymas (plate. IV).

Table 2: Microscopical Physical Constants of *M. Senegalensis*

Leaf's constants	Values	
	Upper epidermis	**Lower epidermis**
Stomatal Number	80.50-70.00-59.50	101.78-88.50-75.23
Stomatal Index	10.81-12.72-14.63	10.65-12.53-14.41
Vein-Islet Number	23.46-20.40-17.34	
Vein let Termination Number	17.94-15.60-13.26	

*n=5

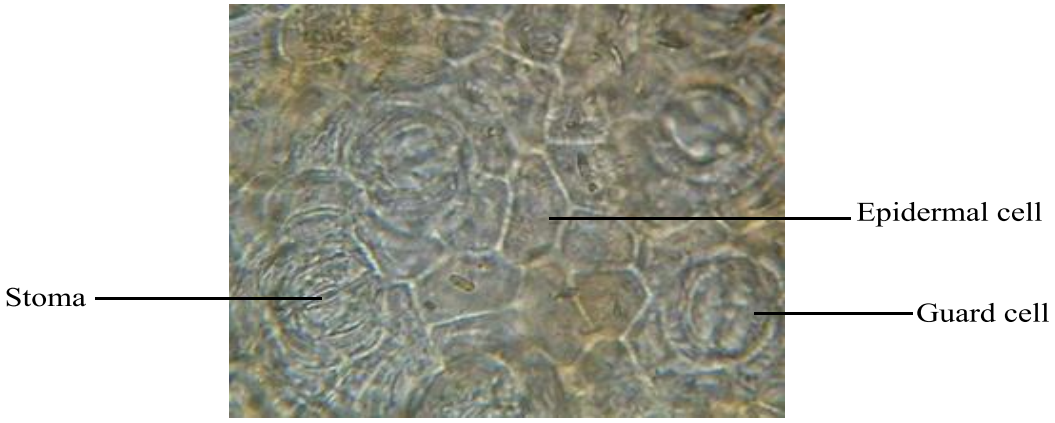

Plate I: Photomicrograph of the Lower Epidermis of the Leaf of *M. senegalensis* (×100)

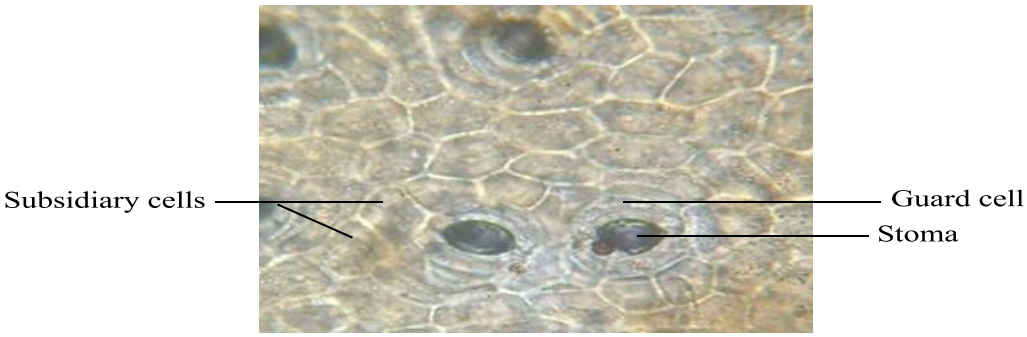

Plate II: Photomicrograph of the Upper Epidermis of the Leaf of *M. senegalensis* (×100)

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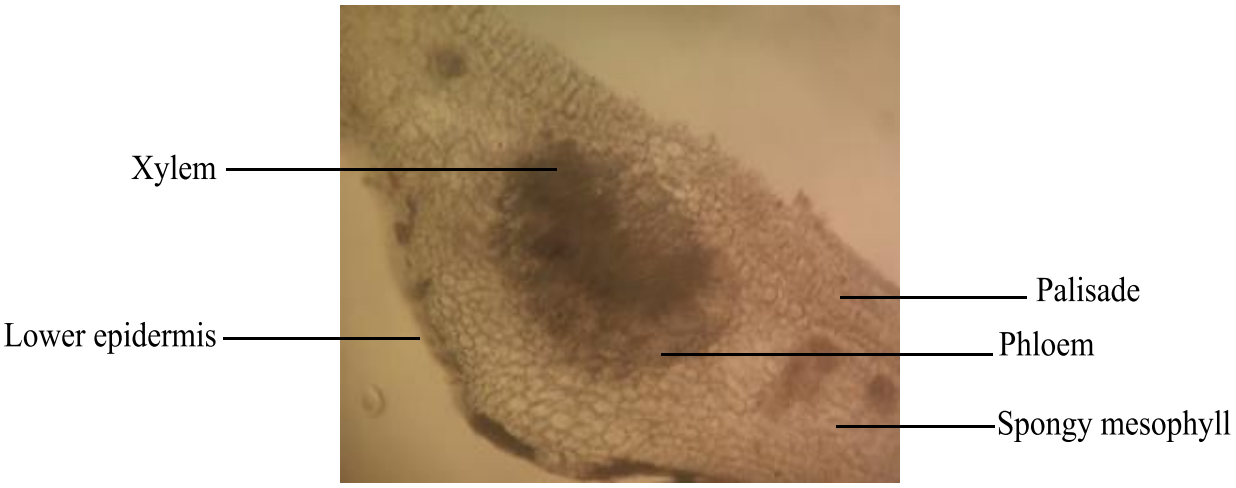

Plate III: Photomicrograph of the Transverse Section of the Leaf of *M. senegalensis* (×100)

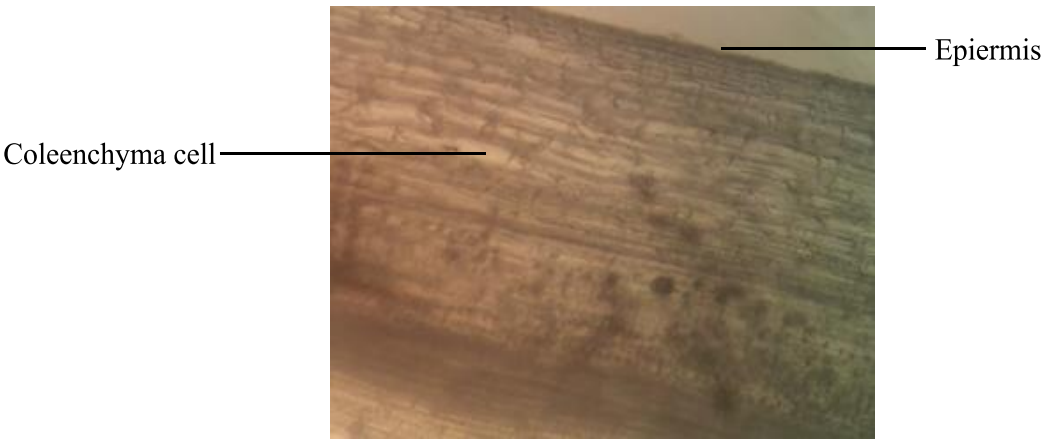

Plate IV: Photomicrograph Showing Longitudinal Section of the Leaf of *M. senegalensis* (×100)

Chemo-Microscopical Evaluations of Leaf of *M. senegalensis*

Crystals of calcium oxalate as well as cellulose, lignin, starch, and protein were found after chemomicroscopic examination (Table 3).

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Table 3: Chemo-Microscopical Characters of Leaf of *M. senegalensis*

Constituents/reagents	Observations	Inference
Cellulose N/50 iodine + HCl + 66% H2SO4	Violet coloration observed on outer wall of some cells and fibers	Cellulose is confirmed to be present
Lignin	Some fibers stained pink	Lignified fibers are present
Starch N/50 iodine	Some grains in parenchyma cells stained blue	Starch grains are present
Protein Millions reagent	No reaction	Protein is absent
Calcium oxalate crystals Conc. H_2SO_4	clustered crystals dissolved without effervescence	Calcium oxalate crystals are present
Hydroquinone A drop of KOH	No reaction was observed	Hydroquinones are absent
Tannins 1 drop of $FeCl_3$	Green coloration was observed in some parts of the parenchyma cells	Tannins are present
Fixed oil and fat A drop of sudan IV	Red stains were observed in some parts of the cytoplasm of the parenchyma cells	Fixed oil and fats are present
Gums and mucilage Red stains	Red stains were observed in some parts of the cell	Gums and mucilages are present

Determinations of Physico-chemical Constants of the Powdered Leaf of *M. Senegalensis*

Moisture content was found to be 9.33% ± 0.01, ash values 7.83 ± 0.00, insoluble ash values 1.67% ± 0.01, alcohol extractive value 12.00% ± 0.01, and water-soluble extractive value 13.25 ± 0.04.

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Table 4: Physico-Chemical Parameters of the Powdered Leaf of *M. Senegalensis*

Evaluative Parameters	Values %w/w ± SEM Leaves
Moisture Content	09.33 ± 0.01
Total Ash Value	07.83 ± 0.00
Acid Insoluble Ash	01.67 ± 0.01
Alcohol Extractive Value	12.00 ± 0.10
Water-soluble Extractive Value	13.25 ± 0.04

*Each value is a mean of three determinations ± SEM

Phytochemical Analysis on Leaf Methanolic Extracts of *M. Senegalensis*

Phytochemical screening of methanolic extracts of leaf of the plant showed the presence of alkaloid, saponins, tannins, cardiac glycosides, carbohydrates, and flavonoids while anthraquinones were found to be absent. The result is shown in table 5.

Table 5: Result of Preliminary Phytochemical Screening on Leaf Methanolic Extracts of *M. Senegalensis*

Phyto-constituents	Tests	Observations	Inference
Alkaloids	Dragendorff's reagent	Orange-red Precipitate	Present
	Mayer's reagent	Cream coloured Precipitate	Present
Saponins	Frothing test	Frothing persisted on warming	Present
Tannins	Ferric chloride test	Green Precipitate	Present
	Bromine water test	Buff coloured precipitate	Present
Anthraquinones	Borntrager's test	No reaction	Absent
Cardiac glycosides	Keller-Kiliani test	Brown ring at interface	Present
	Salkowski's test	Reddish brown color at interface	Present
	Lieberman-buchard's test	Color changes from violet to blue to green	Present
	Molisch's test	Purple to violet coloration	Present
Carbohydrates	Fehling's solution	Brick red Precipitate	Present
	Shinoda test	Pink color	Present
Flavonoids	Sodium hydroxide (Na_2OH) test	Yellow color formed	Present

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DISCUSSION

The pharmacognostic studies carried out showed that leaves are with spikes, a feature that protects the plant from predators. The plant is commonly referred to as Red spike thorn which is attributed to the presence of these spikes and can be a distinguishing feature of the plant. Some organoleptic features of the leaves were reported to be petiolate, alternate or fascicled with pale green lamina features that were similarly observed in this research [4]. The presence of anomocytic-type stomata, which are more frequent on the lower surface of the plant as observed in this research, can be used as a character of identification. Dorsiventral leaves are a characteristic of dicotyledonous plants therefore, can be used as an identification tool. In the present study, prisms of calcium oxalate crystals were found either scattered or clustered in parenchymatous and epidermal cells in the leaves. Calcium oxalate crystals in higher plants are most common in form of minerals and are usually formed from environmentally derived calcium and biologically synthesized oxalates and are deposited inside intra-vacuolar membrane chambers of specialized cells in any organ or tissue [16] [20]. It was reported that differentiation of cell into a crystal idioblast is surely under genetic control [8]. Therefore, the shape of the crystals may also be governed genetically. Functions of calcium oxalates can be related to ionic balance and osmo regulation, storing form of calcium oxalates, mechanical support and protection against foraging animals [12]. Chemomicroscopical features are unique to a particular plant and are employed in standardization. Allied substances when mixed with original drugs as adulterants can only be detected by chemomicroscopical analysis. Leaves physical parameters determined in quantitative microscopy are relatively constants for plants and can be used in distinguishing members of closely related species. The result of the moisture content of the leaves was determined to be 9.33%. Moisture of the leaves is not high, meaning less chance of microbial growth. The general requirement of moisture content in crude drugs should not exceed 14% [1], at this value of moisture content, drugs can be stored for a long period of time without fear of microbial degradation. Total ash determined for the leaves was 7.83%, this result implies that both the leaves have low content of organic and inorganic matter. The accepted range of total ash is 22% [3], this signifies that total ash for leaves is within the accepted limit. This parameter is particularly important in determination of purity of crude drugs. The result of acid insoluble ash values for the leaves was 1.67%, this result is also within the range of the accepted limit of the acid insoluble ash value. Total ash determines presence or absence of foreign organic matter [15]. The alcohol and water soluble extractive values determined for leaves were 12% and 13.25% respectively, alcohol and water soluble extractive values suggest total solvent soluble phytochemical constituents [19]. Therefore, from the result, the leaves have high water soluble extractive value, meaning water may be a better extractive solvent for the leaves of *Maytenus senegalensis*. Quantitative evaluation as an important parameter in setting standard for crude drugs [6].

CONCLUSION

Standardization is essential measure for quality, purity and identification. Macromorphology and microscopy along with the quantitative analytical microscopy is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. Physiochemical and

chemical analysis of the leaves confirm the quality and purity of plant and its identification. This work can be used as a guide to identifying and differentiating *M. senegalensis* from the members of its family.

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