International Journal of Biological and Pharmaceutical Sciences Archive

ISSN: 0799-6616 (Online)

Journal homepage: https://ijbpsa.com/

(RESEARCH ARTICLE)

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Pharmacognostic and Physicochemical evaluation of *Harungana madagascariensis* Lam. Ex Poir fruit (Hypericaceae)

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International Journal of Biological and Pharmaceutical Sciences Archive, 2023, 05(02), 024–031

Publication history: Received on 18 February 2023; revised on 19 April 2023; accepted on 21 April 2023

Article DOI: https://doi.org/10.53771/ijbpsa.2023.5.2.0026

Abstract

Medicinal plant materials are being adulterated and substituted in commerce due to many reasons such as similar morphological features, same name as written in classical text, presence of similar active principles in the substituted plant which may badly affect the therapeutic activity of the finished products. As a result, authentication of medicinal plants is of paramount importance in ensuring quality and safety of crude drugs and herbal products. This research was aimed to evaluate and document the pharmacognostic standards for the fruits of *Harungana madagascariensis*. The plant sample was identified, collected and authenticated by a Taxonomist. Pharmacognostic and physicochemical evaluation were carried out on the powdered fruits of *Harungana madagascariensis* using standard methods. The result showed the presence of starch grains, lignified tissues, cellulose, and proteins while calcium oxalate and mucilage were absent. The moisture content (8.8±0.02)%, total ash value (5.2±0.005)%, acid insoluble ash (2.5±0.0)%, water soluble ash (2.2±0.01) % and extractive value were obtained. The n-hexane, ethyl acetate, n-butanol, ethanol and water soluble extractive values(3±0.00, 3.75±0.25, 4.50, 8.5±0.1, 6.25±0.25 %w/w respectively which were significantly different (at p set at 0.05). The revealed pharmacognostic features, physico-chemical and microscopy/chemomicroscopy properties of *H. madagascariensis* could be useful in the preparation of the herbal Pharmacopoeia.

Keywords: *Harungana madagascariensis*; Fruits; Pharmacognostic parameters; Standardization

1. Introduction

A key obstacle, which has hindered the acceptance of alternative medicines in the developed countries, is the lack of documentation and quality control guidelines. Correct identification of the starting materials among others, is an essential pre-requisite to ensure reproducible quality of herbal medicines[1] which will contribute to its safety and efficacy. Medicinal plant materials and products derived from them are being adulterated in commerce due to many reasons including similar morphological features, and presence of similar active principles in the substituted plant which may badly affect the therapeutic utility of the finished products [2-3]. Substitution or adulteration of a particular genuine drug with other species due to demand exceeding the supply of original species, is rampant in the present trade scenario. With this backdrop, it has becomes extremely important to make an effort towards standardization of the plant material to be used as medicine.

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Pharmacognostic study ensures plant identity and lays down standardization parameters which help in preventing adulterations [4]. These include physico-chemical, microscopic, macroscopic, and organoleptic properties among others. Physico-chemical parameters which are studied include moisture content, loss on drying, ash values and extractive values. These studies help in authentication of the plants and ensure reproducible quality of herbal products which will lead to safety and efficacy of natural products [4]. These standardized qualitative and quantitative information on the plant material can act as reference information for correct identification of the particular plant and also will be useful in making a monograph for the plant. Further, it will act as a tool to detect adulterants and substituent thus helping in maintaining the quality, reproducibility and efficacy of natural drugs [4].

Harungana madagascariensis Lam. ex Poir (Hypericaceae) locally known as "Nketto" by the Bamileke tribe in Cameroon, or "Aranje" and "Orturu" respectively by the Yoruba and Ibo tribes in Nigeria. is a flowering plant which is commonly known as the dragon's blood tree, orange-milk tree or haronga. The "harungana" plants originally came from Africa and Madagascar and are widely distributed from South Africa to Sudan. In ethnomedicine practice**,** *H. madagascariensis* have been used over many years in African herbal medicine for the treatment of a wide range of human illnesses such as: decoctions of the leaves are used to cure dysentery, diarrhea, anemia, typhoid and some heart ailments such as tachycardia. The bark and roots, either alone or in association with other plants, are prescribed for ailments such as gonorrhea, leprosy, hemorrhoids and to facilitate childbirth [5]. Additionally, the leaves are used in Ghana for the treatment of chest problems, dysentery while the latex of the plant is employed for treating skin diseases and as a dressing material for wounds. In Sierra Leone, post-partum bleeding is arrested by the juice of the leaves and stem bark, while the same juice is used to treat indigestion and poor pancreatic function in European herbal medicine [5]. Other documented uses of *H. madagascariensis* include the use of its stem bark as a local dye for clothing and traditional costumes by natives in Cameroon as well as the use of its dried and crushed seeds in cooking as a spice for the preparation of soups. A bark decoction of the root, is widely deemed helpful as a remedy for a range of ailments in which blood is manifest including haematuria; dysentery and piles; as an emmenagogue and oxytocic for a range of gynaecological conditions including expelling the placenta, miscarriage, dysmenorrhoea, irregular or painful menstruation; cough with bloody sputum [6].

2. Material and methods

Electrical weighing balance (M-Metlar), Conical Flasks (500ml and 1000ml), Measuring Cylinders (100 ml and 500 ml), test tubes, glass slides, glass petri dishes, slide. The reagents employed in this research work include: 70% chloral hydrate solution**,** dilute glycerol, N/50 iodine, 66% sulphuric acid, phloroglucinol, HCl, ruthenium red, 5% ferric chloride solution, Sudan (IV) reagent, Distilled Water, immersion oil, Lugol's Iodine, acetone, ethanol, safranin stain, crystal violet stain, tetramethyl-p-phenylenediamine powder (Merck,Germany).

2.1. Sample collection and identification

H. madagascariensis fruit was collected during its fruiting stage from the medicinal plant garden of the Department of Pharmacognosy and Phytotherapy, University of Port Harcourt, Nigeria in the month of August and identified by a Taxonomist. Voucher specimen number UPHH0585 was deposited in the herbarium of the same department. It was air dried for three weeks, pulverized and sieved to reduce the particle size, and stored in an air-tight ziplock bag until required for analysis.

2.2. The chemo-microscopic examination

The powdered samples of *H. madagascariensis* was studied to detect the presence of cell wall materials and cell inclusions. This was carried out to determine the presence or absence of starch grain, protein, lignin, fats/oil, calcium carbonate, and calcium oxalate crystals using standard techniques in accordance to the prescribed standard methods [7-9]. Briefly, finely ground samples of the plant were cleared in a test tube containing 70% chloral hydrate solution. They were boiled on a water bath for about thirty minutes to remove obscuring materials. The cleared samples were then mounted with dilute glycerol onto a microscope slide and the tests below carried out.

2.2.1. Test for Cellulose

Small amount of the cleared powdered fruit were separately placed on a slide and a drop of N/50 iodine added and left for a minute, followed by a drop of 66% sulphuric acid. The appearance of bluish colour was considered positive for cellulose on cell walls of the cells.

2.2.2. Test for Lignin

Small amount of the cleared powered fruit was placed on a slide and a drop of phloroglucinol added followed by a drop of concentrated HCl. Appearance of red stain or coloration was considered as positive for lignins.

2.2.3. Test for Gums and Mucilage

To a small portion of the cleared powdered fruit, a drop of ruthenium red was added. Appearance of pink colouration was considered positive for gums and mucilage

2.2.4. Test for Starch

To a small portion of the cleared powdered fruit, N/50 iodine was added. Appearance of blue-black or reddish-blue colouration on some grains was considered positive for starch.

2.2.5. Test for Fats and Oils

To a small portion of the cleared powdered fruit, a drop of Sudan (III) reagent was added and allowed to stand for a minute. Appearance of orange red was considered positive for fatty substances.

2.2.6. Test for Calcium Oxalates and Calcium Carbonates

To a small portion of the cleared powdered fruit, iodine and concentrated Sulphuric acid was added, dissolution of crystals in the powdered drug without effervescence was considered positive for calcium oxalate. While slow dissolution with effervescence was considered as positive for calcium carbonate.

2.2.7. Test for proteins

To a small portion of cleared powdered fruit, Biuret and ninhydrin reagents was separately added. Appearance of violet/mauve(Biuret) and purple/red(ninhydrin) color was considered positive for protein.

2.3. Physico-chemical evaluation

The physicochemical parameters determined for the sample include moisture content**,** ash values (total ash value**,** acid insoluble ash, and water soluble ash value) and extractive values using n-hexane, ethyl acetate, n-butanol, absolute ethanol and water as solvent. Three different determinations were carried out for each parameter and the average taken using the methods outlined by [9] on quality control methods for medicinal plant materials.

2.4. Extractive values

This is the amount of extractive in percentage of a plant sample using different solvents. 4g of the plant material each was weighed into 5 separate conical flasks which were labeled in line with the respective solvents. 100ml of the solvent (n-hexane, ethyl acetate, n-butanol, absolute ethanol and water) were respectively added to the sample in the separate conical flasks. Each was macerated for 24 hours, during which the mixture was frequently shaken within the first 6hours using a mechanical shaker. It was filtered and 25ml of the filtrate transferred into an evaporating dish of known weight and evaporated to dryness on a water bath. It was dried to a constant weight, the percentage of each solvent-soluble extractive value was then determined for the plant as

$$\text{Extractive Value (\%)} = \frac{\text{Weight of Residue in 25ml extract x 400}}{\text{Initial weight of sample}}$$

2.5. Data analysis

Where applicable, values for evaluated parameters are expressed as mean ± SEM and the data subjected to statistical test for significance (p=0.05) using one way Analysis of Variance and student t-test.

3. Results and discussion

3.1. Microscopic/Chemomicroscopic Studies

Microscopic/chemomicroscopic evaluation of the powdered fruit showed the presence of lignin, cellulose (major component of the cell wall), proteins, oil globules and starch grains (which appeared in oval shape) as shown in Table 1. Although the starch is the main form in which plants store carbon, they are sometimes converted into sugar by

amyloplasts when the plant needs energy. There were polygonally-shaped epidermal cells of the epicarp with thick and lignified cell walls (Figure 1), isolated fibre elements with thin cell wall (Figure 2), mass of collenchymatous cells of the mesocarp with thick cell walls and heavy lignifications (Figure 3), mass of parenchymatous cells of the endocarp with little to no lignification (Figure 4), isolated stone cells (Figure 5), and a vessel element (black arrows) with an oil gland (red arrow) (Figure 6).

3.2. Physico-chemical studies

The moisture content for *H. madagascariensis* was calculated to be 8.8 ± 0.02%. Therefore the moisture content of the plant is not too high falling within the reported upper limit of 13-15% [10-13], indicating less probability of microbial degradation and that the powder of this herb can be stored for a longer period of time without spoilage [14]. Total ash value is 5.2 ± 0.005%, which can be used to detect foreign and toxic heavy metals contaminants and adulteration of sand or earth. Acid insoluble ash value is 2.5 ± 0.01% which is not more than 4% hence the herb was in good physiological condition and contains less extraneous matter in the form of contamination with silicious materials (earth and sand). Water soluble ash value is 2.2 ± 0.01%. The water soluble ash is used to estimate the amount of inorganic compounds usually water soluble metal/metal oxides present in drugs [14]. This parameter is used to detect the presence of materials exhausted by water [15].

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phyto-constituents [15]. Percentage of extractives in different solvent helped us to determine the quantity and nature of active phyto-constituents in the extract. The water soluble extractive value indicates the presence of sugar, acids and inorganic compounds while alcohol soluble extractive values indicates the presence of polar constituents like phenols, glycosides and flavonoids [16] with n-hexane for non-polar constituents like lipids, and steroids. The trend in extractive value (%w/w): n-hexane (3.00±0.00) < ethyl acetate (3.75±0.025) < n-butanol (4.50±0.00)<water(6.25±0.25)< ethanol(8.5±0.1) and were significantly different $p < 0.05$. This suggests that the use of ethanol as an extractive solvent is a better choice for the polar metabolites present in the plant.

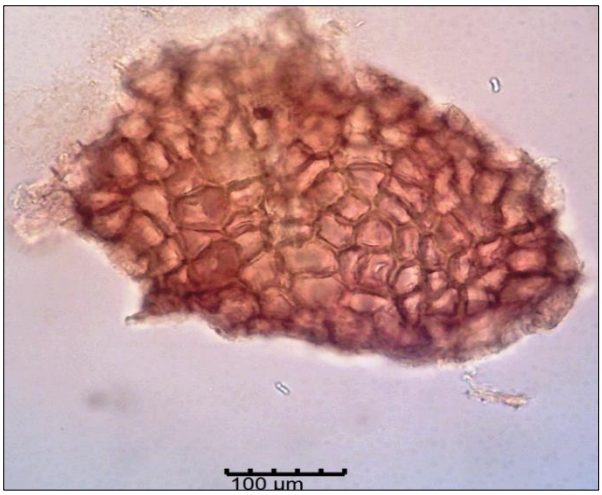
Table 1 Cell wall constituents and Cell inclusions present in *H. madagascariensis* fruit

Parameter	Reagent(s)	Color observed	Result
Starch grains	Iodine solution	Bluish-black	Present and oval in shape
Lignified tissues	Conc. HCl + Phloroglucinol	Red	Present in some tissues
Calcium oxalates	Iodine solution Conc. Sulphuric acid	No color or crystals or effervescence	Absent
Cellulose	Zinc chloride; Conc. Sulphuric acid	Blue	Present; major component of the cell walls
Gum/Mucilage	Ruthenium red	Pink color absent	Absent
Oil globules/cells	Sudan III reagent	Orange-red	Present; oil glands visibly seen
Protein	Biuret reagent; Nihydrin	Red	Present

Table 2 Physico-chemical parameters of *H. madagascariensis* powdered fruit

Moisture content (%)	Ash value (%)	Acid insoluble ash (%)	Water soluble ash (%)
8.8±0.020	5.200±0.005	2.500±0.010	2.200±0.010

Note: all values are expressed as mean ± SEM



X400

Figure 1 Chemomicroscopy of the fragment showing polygonally-shaped epidermal cells of the epicarp with thick and lignified cell walls

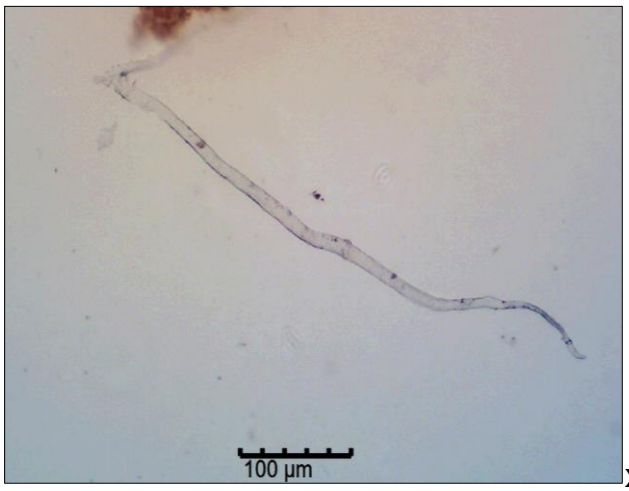


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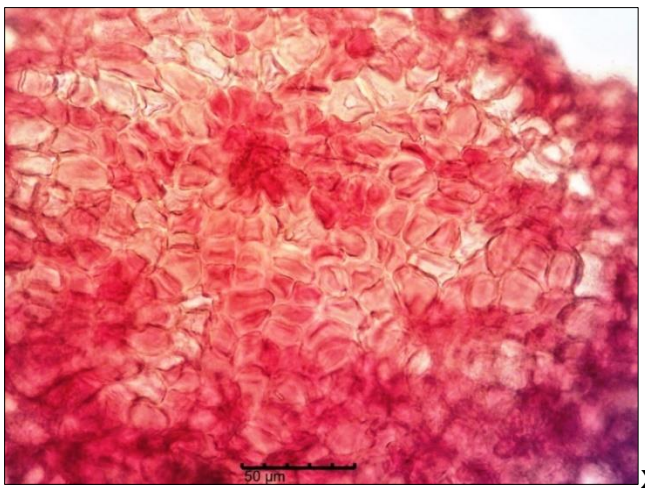
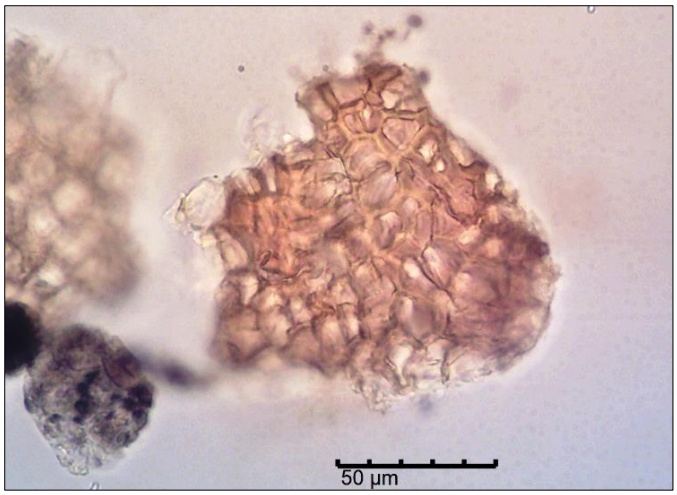
Figure 2 Chemomicroscopy of the powdered drug showing isolated fibre element with thin cell wall



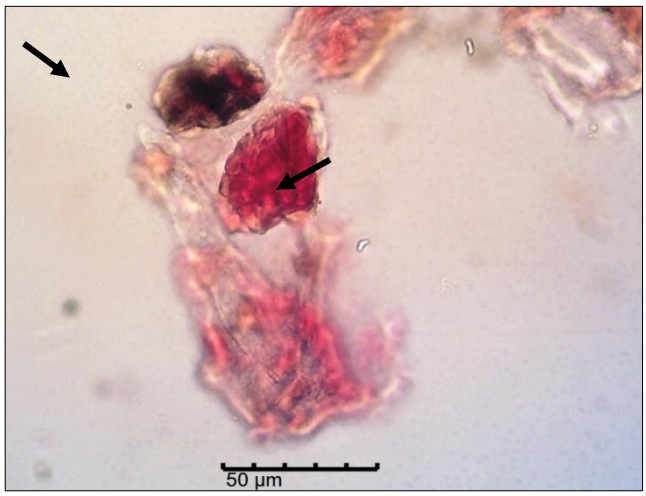
X400

Figure 3 Chemomicroscopy of the drug sample showing a mass of collenchymatous cells of the mesocarp with thick cell walls and heavy lignification



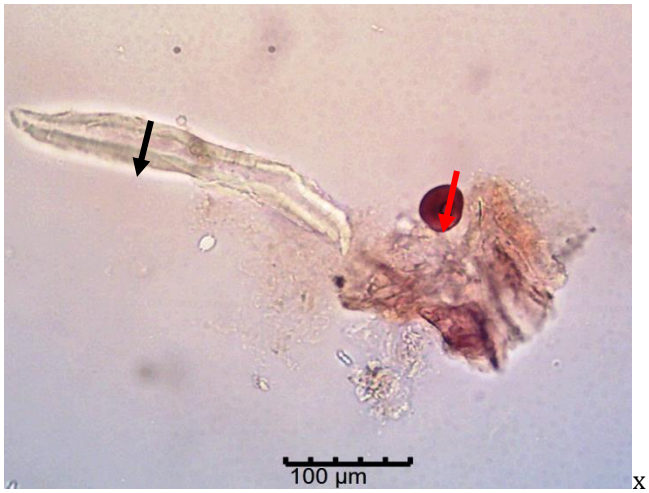
X400

Figure 4 Chemomicroscopy of the drug sample showing a mass of parenchymatous cells of the endocarp with little to no lignification



x 400

Figure 5 Chemomicroscopy of the drug sample showing isolated stone cells (black arrows)



x 400

Figure 6 Chemomicroscopy of the drug sample showing a vessel element (black arrows) and an oil gland (red arrow)

Table 3 Extractive values of *H. madagascariensis* powdered fruit

n-Hexane extractive. value (%) ± SEM	Ethyl acetate extractive value (%) ± SEM	n-Butanol extractive value (%) ± SEM	Ethanol extractive value (%) ± SEM	Water extractive value (%) ± SEM
3.00±0.00	3.75±0.25	4.50±0.00	8.50±0.10	6.25±0.25

Note: all values are expressed as mean ± SEM and were significantly different $p < 0.05$

4. Conclusion

The assurance of the safety and efficacy of a herbal drug requires monitoring of the quality of the product from collection through processing to the finished packaged product[17]. The current investigation reveals the pharmacognostic features, physico-chemical and microscopy/chemomicroscopy properties of *H. madagascariensis*. These parameters could be useful in the preparation of the herbal section of proposed Nigerian Pharmacopoeia. Any crude drug which is claimed to be *H. madagascariensis* but whose characters significantly deviate from the experimentally validated standards above would then be rejected as contaminated, adulterated or fake.

Compliance with ethical standards

Acknowledgments

The authors are grateful to the Department of Pharmacognosy and Environmental Medicine where the microscopic evaluation was done.

Disclosure of conflict of interest

As regards this work, the authors declare a no conflict of interest.

References

[1] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in pharmacology, 2014; 4:177.

[2] Santhosh Kumar, JU., Krishna, V., Seethapathy, GS., Ganesan, R., Ravikanth, G.,Shaanker, RU. Assessment of adulteration in raw herbal trade of important medicinal plants of India using DNA barcoding. Biotech, 2018; 8(3), 135

[3] Wijayasiriwardena, C., Premakumara, S. Comparative powder microscopy of Alpinia calcarata Roscoe and Alpinia galanga (Linn.) Willd. Ayu, 2012; 33(3), 441–443.

[4] Chanda S. Importance of pharmacognostic study of medicinal plants: An overview. Journal of Pharmacognosy and Phytochemistry. 2014; 2(5): 69-73

[5] Happi, GM., Tiani, GLM., Gbetnkom, BYM., Hussain, H., Green, IR., Ngadjui, BT., Kouam, SF. Phytochemistry and pharmacology of *Harungana madagascariensis*: mini review. Phytochemistry Letters, 2020; 103–112.

[6] Burkil,H.M. (1985). The useful plants of West Africa. Royal Botanic Garden Kew.

[7] Chumbhale, DS., Upasani, CD. Pharmacognostic standardization of stems of Thespesia lampas (Cav.) Dalz & Gibs. Asian Pacific Journal of Tropical Biomedicine, 2012; 2(5), 357–363.

[8] Kokate CK. Practical pharmacognosy. 4th ed. Vallabh Prakashan, New Delhi. 2010. pp. 17–26

[9] Quality control methods for medicinal plant materials. The World Health Organization Geneva. 1998.

[10] Cheng, WC., Ng, CS., Poon, NL. Herbal Medicines and Phytopharmaceuticals – Contaminations. In J. Siegel, P.Saukko, M. Houck (Eds.), Encyclopedia of Forensic Sciences. 2nd edition. Elsevier, Amsterdam, Netherlands:. 2013. pp. 280–288

[11] Kaur, L., Jayasekera, S., Moughan, PJ. Antioxidant Quality of Tea (Camellia sinensis) as Affected by Environmental Factors. In: V. Preedy (Ed.), Processingand Impact on Antioxidants in Beverages. Academic Press, Massachusetts, United States. 2014. pp. 121-129.

[12] Mandal, M.,Misra, D., Ghosh, NN., Mandal, V. Physicochemical and elemental studies of Hydrocotyle javanica Thunb. For standardization as herbal drug. Asian Pacific Journal of Tropical Biomedicine, 2017; 7(11), 979–986.

[13] Teles, S.,Pereira, JA., Santos, CHB., Menezes, RV., Malheiro, R., Lucchese, AM., Silva, F. Geographical origin and drying methodology may affectthe essential oil of Lippia alba (Mill) N.E. Brown. Industrial Crops and Products, 2012; 37(1),247–252.

[14] Bruce, SO., Onyegbule, FA., Ezugwu, CO.Pharmacognostic , physicochemical and phytochemical evaluation of the leaves of Fadogia cienkowski Schweinf (Rubiaceae). Journal of Pharmacgnosy and Phytotherapy, 2019; 11(3): 52–60.

[15] Abdullahi, MN., Ilyas, N., Hajara, I., Kabir, Y M. Pharmacognostic evaluation of the leaf of Microtrichia perotitii DC. (Asteraceae). Journal of Pharmacognosy and Phytotherapy.2018; 10(4), 76–84.

[16] Sonowal, N., Syndor, A, Lapasam P, Boruah A, Sarkar, BR., Bhattacharjee A, Dey, BK. Evaluation of Pharmacognostical parameters and Preliminary Phytochemical Screening of leave of the plant Myrica esculenta. International Journal of Research in Pharmacy and Pharmaceutical Sciences, 2018; 3(4): 07-12

[17] Kunle.Standardization of herbal medicines - A review. International Journal of Biodiversity and Conservation, 2012; 4(3), 101–112