IJBPAS, January, 2017, 6(1): 43-54

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHARMACOGNOSTIC EVALUATION OF THE LEAF OF *TREMA ORIENTALIS* (FAMILY: ULMACEAE)

***UGWOKE, CHRISTOPHER EMEKA CHUKWUNONYE [1], ANZE, SUNDAY PATRICK GAZA[1], NWEZE, EJIOFOR ANTHONY [2], CHUKWUBE, VINCENT OBISIKE [1]**

***Corresponding Author: E Mail: Christopher.ugwoke@unn.edu.ng**

1: department of pharmacognosy and environmental medicines, university of nigeria nsukka, Nigeria

2: department of plant science and biotechnology, university of nigeria nsukka, Nigeria

Received 24th Aug. 2016; Revised 27th Sept. 2016; Accepted 4th Nov. 2016; Available online 1st Jan. 2017

ABSTRACT

Background: *Trema orientalis* is native to India and widely used in Nigeria especially as it is applied topically on children to remedy skin blemishes due to infections. Various other uses have been recorded both locally and elsewhere in Africa. Pharmacognostic studies on the plant would enhance its therapeutic applications by setting quality control measures to ensure correct identity and to checkmate adulteration. **Materials and Methods:** Macro and micro-morphological studies, including anatomical sections, photomicrographs, numerical leaf microscopy and evaluation of physical constants as well as chemo-microscopy and fluorescent studies were carried out. **Results:** The leaves were simple, green, serrulate, acuminate, petiolate and lanceolate. It was dorsoventral with anomocytic stomata with somewhat polygonal and irregular epidermal cells, several trichomes and starch grains, rosettes of calcium oxalate crystals and established spongy parenchymatous cells and vascular tissues were observed. Lignins, tannins, proteins, starch, fats and fatty oils were detected. Average moisture content was 8.81 %, Total Ash value (8.33), Acid-insoluble Ash (1.16), Water-soluble Ash (2.86), Water and Alcohol soluble extractive values (4.87 and 2.53 % respectively). Palisade ratio was 44.7 %, vein islet number 34, veinlet termination 7, stomata number for upper and lower epidermis 0.5 and 4.5 respectively, while stomatal index was 2.44 and 18.37 for upper epidermis and lower epidermis respectively. **Conclusion:** The diagnostic macro and microscopic anatomical features,

chemomicroscopic, characteristic fluorescent colours and the numerical standards could be useful in quality control and to supplement a monograph for *Trema orientalis*.

Key words: *Trema orientalis*, pharmacognostic, numerical leaf microscopy, serrulate, vein islet

INTRODUCTION

Standardization of herbal medicines is a set of specific derived from experimentation and observations, with a view to specifying a set of characteristics of a particular herbal medicine [1]. This quality control measure is achieved by means of pharmacognostic evaluation of crude plant drugs [2]. Pharmacognostic evaluation involves macro and micro morphological, anatomical sectioning and other qualitative and quantitative microscopic characterization of the plant, fluorescent characteristics and phytochemical screening [3] among other parameters. The Geographical location of a plant is affects its diagnostic pharmacognostic features [3, 1] and so the same plant species growing in different geographical locations could possess different pharmacognostic features. *Trema orientalis* (Fig. 1) is an evergreen medium sized [4] tree of the family Ulmaceae [5]. It is a fast growing [6] plant that is cosmopolitan [5] and commonly found in tropical countries [1]. It has an extensive root system that enables it survive long periods of drought, and can grow on a wide range of soils from heavy clay to light sand [4]. It is commonly called "*Nri-nnunu*", in Igbo Language meaning bird's food. It is valued for pulp wood production [6] and widely used as medicine in various parts of Africa [7, 8, 9]. It contains tannins, saponins, flavonoids, triterpenoids and phytosterols [7, 10] and several isolated compounds [11, 12]. Reported medicinal uses of *T. orientalis* include anti-inflammatory, anthelminthic [13, 5], vermifuge, febrifuge, anti-dysentery, antibacterial, anti-amoebic, anti-convulsion, anti-diabetic, anti-diarrhoeal, analgesic, anti-sickling [14, 15, 16, 17, 5] and also to treat jaundice, bronchitis, pneumonia, pleurisy, toothache and venereal diseases [18, 4, 19, 5].

Incidentally, no pharmacognostic evaluation has been carried out on this plant sourced in Africa, except elsewhere in Idar, Gujar, India in 2008 by Panchal *et al*., [10]. In view of the distance and difference in climate between India (Asia) and Nigeria (Africa), this study aimed at investigating the peculiar pharmacognostic features of *T. orientalis* growing in Nsukka Enugu State, Nigeria.

Fig. 1: A Twig of *Trema orientalis*

MATERIALS AND METHODS

Collection, identification and preparation of the plant material

Fresh leaves and twigs of *T. orientalis* were collected from opposite Ikenga hotel in Nsukka local Government Area in Enugu State, Nigeria. A sharp knife was used to cut the leaves and allowed to fall into large black polythene bags to avoid contact with the skin. This was to avoid irritation caused by the plant latex. The collection was done at about 11:00 hrs in August, 2015. Some leaves were stored in a glass jar under refrigeration and used within 24 hrs for the preparation of anatomical sections [20]. Identification and authentication was done by Mr. AO Ozioko of the International Centre for Ethnomedicine and Drug Development InterCEDD, Nsukka, where voucher specimens were deposited (INTERCEDD/Ulma./082015). The leaves were dried under shade in an open air and then pulverized using mortar and pestle [21]. The powder was stored in airtight containers to avoid absorption of moisture.

Macroscopy

This was based on macroscopical features, including organoleptic properties of the leaf as described by Kokate [22] and Evans [21] viz. leaf viz. size and shape, colour, surfaces,

venation, presence or absence of petiole, nature of apex, margin, base, lamina, texture, odour and taste.

Quantitative leaf microscopy

Fragments of the outer epidermal membranous layer were cleared in chloral hydrate by boiling until transparent, mounted with glycerin and observed under a compound microscope. The presence or absence of the following was observed and captured: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution) [23, 21].

Permanent slide preparation: This was prepared according to the method reported by Sani *et al*., [24]. The prepared permanent slides were viewed for the microscopic characters [25]. Photomicrographs were supplemented using an optical microscopic camera.

Temporary slide preparation: A transverse section (TS) of the leaf through the lamina and the midrib was taken using freehand. It was cleared with chloral hydrate, treated (stained) with phloroglucinol and a drop of concentrated hydrochloric acid to stain the lignified elements [21, 26]. The prepared slide was observed under a compound light microscope [23, 20] and it was supplemented with photomicrographs.

Similarly, 2 mm square pieces of the leaf were placed in a solution of chloral hydrate in a test tube. It was boiled in a water bath until sufficiently transparent. It was then mounted in a mixture of equal parts of glycerin and chloral hydrate solution and examined under a compound light microscope [23].

Crude drug powder microscopy

About 5 g of the dried leaf powder was cleared with 10 ml chloral hydrate [23, 20]. Various microscopic features of diagnostic significance were then observed under the compound light microscope, with photomicrographs taken.

Chemomicroscopic examination of the powdered leaf

Standard procedures [25, 23, 21] were used to detect the presence of various chemical substances such as cellulose, lignin, tannins, starch grains, fats and fatty oils, proteins, calcium oxalate and calcium carbonate.

Analytical evaluation of the crude drug

The crude plant drug was evaluated for moisture content (Loss on Drying, LOD method), ash values (total ash, acid-insoluble ash and water soluble ash) and extractive values (water and alcohol) based on standard methods [3, 23, 21, 27, 28, 24].

RESULTS

The Transverse section (Fig. 2) through the lamina revealed a dorsiventral leaf type with several unicellular multiseriate trichomes on both upper epidermis (EP1) and lower epidermis (EP2). Vascular bundles were arranged in a concentric manner. Spongy parenchymatous cells and collenchymatous cells were well established. Calcium oxalate crystal was seen in the parenchyma. Epidermal cells are somewhat polygonal and irregular, stomata was anomocytic and found on both epidermal surfaces (Fig. 3). Rosettes of calcium oxalate crystals (Fig. 4) and starch grains (Fig. 5) were observed. Quantitative leaf microscopic analysis revealed the presence of stomata (though fewer in relation to the lower leaf surface) on the upper surface, Macroscopic evaluation revealed characteristic features as represented in below (Table 1). Quantitative microscopic leaf characteristics are shown in Table 2.

Chemo microscopic substances detected in the leaf powder are shown in Table 3. Under short and long wavelengths of fluorescence, the plant powder dissolved in various solvents exhibited characteristic colours (Table 4). The average physicochemical parameters of the coarse leaf powder of *T. orientalis* are shown in Table 5.

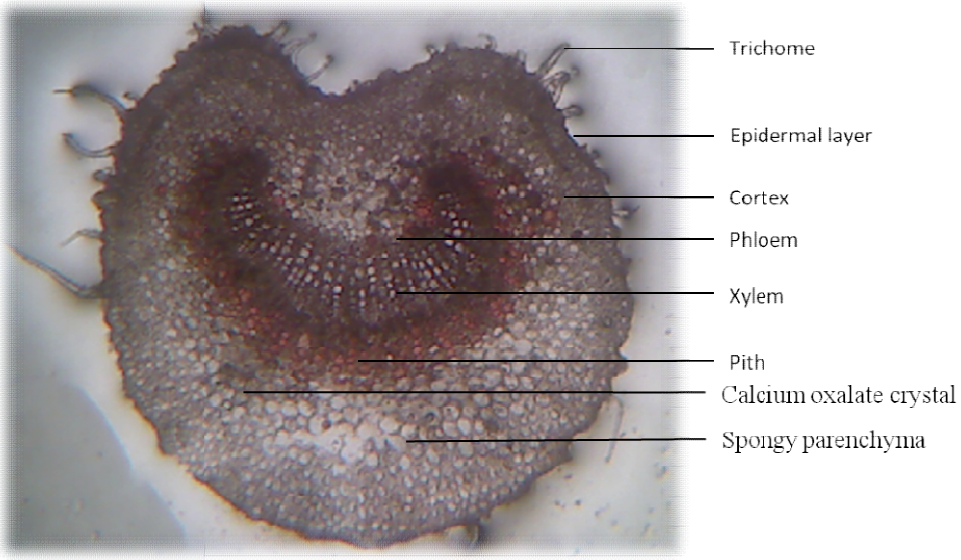

Fig. 2: Petiole of *Trema orientalis* in TS

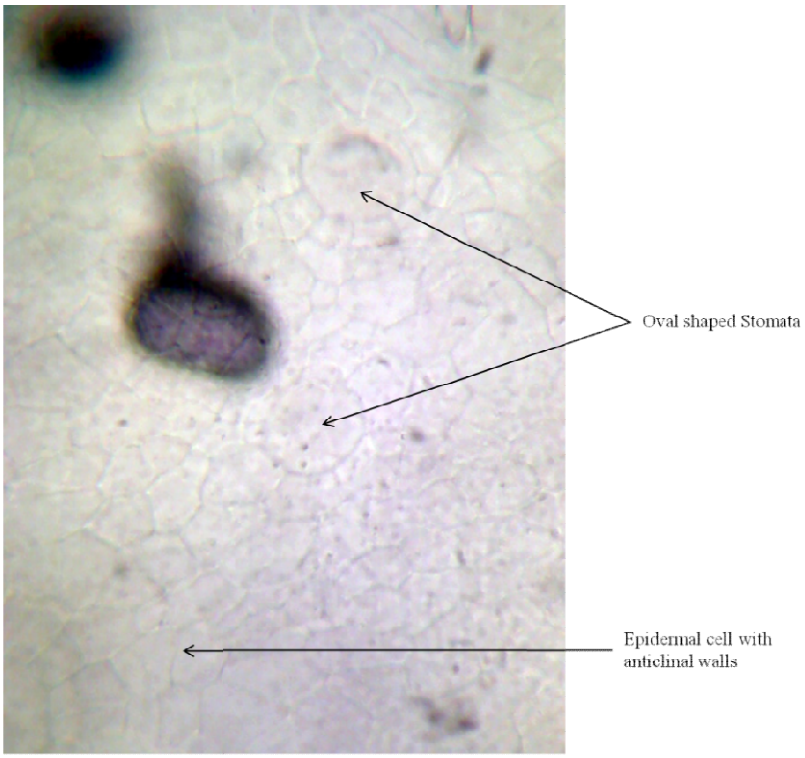

Fig 3: Stomata and epidermal cells of *Trema orientalis* leaf

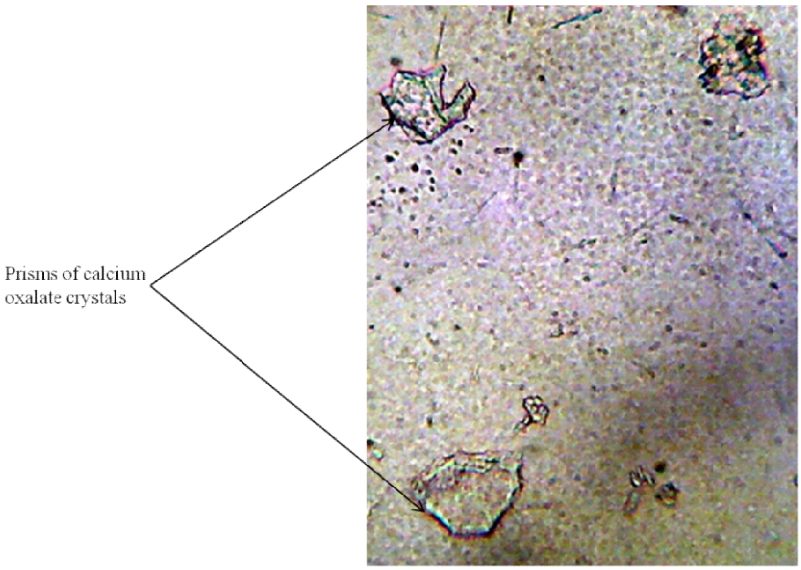

Fig. 4: Calcium oxalates of *T. orientalis* powdered leaf

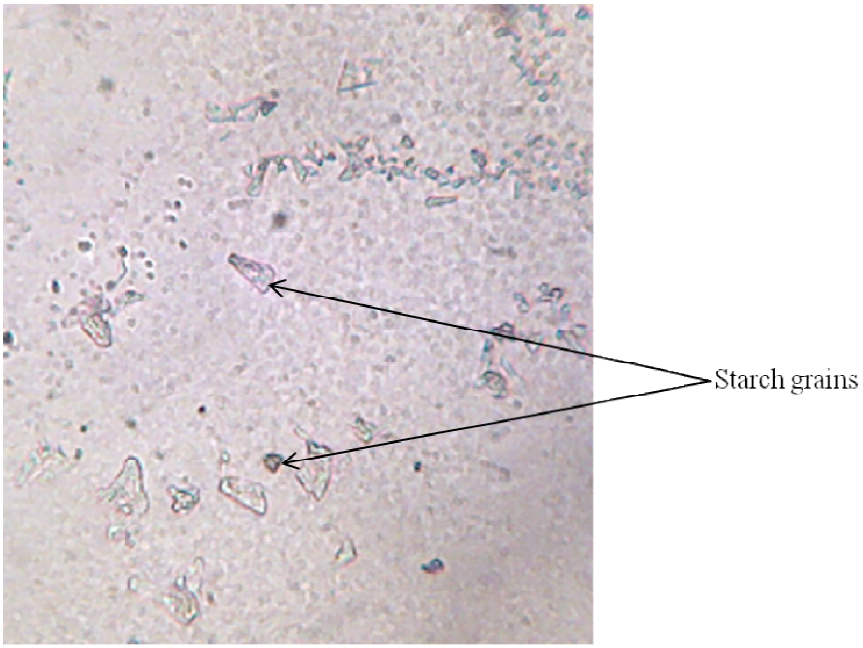

Fig. 5: Starch grains from *T. orientalis* powdered leaf

Table 1: Result of Macroscopical Examinations of *C. aconitifolius*

S/N	Character	Observation
1	Colour	Light green
2	Odour	Characteristic
3	Taste	Bland
4	Length (average)	12.40 cm
	Width (average)	6.23 cm
5	Shape	Lanceolate
6	Texture	Rough
7	Surface	Pubescent
8	Apex	Acuminate
9	Base	Cordate
10	Margin	Serrulate
11	Leaf arrangement	Alternate
12	Venation	Reticulate
13	Presence/absence of petiole	Petiolate
14	Lamina	Intact
15	Presence/absence of stipule	Stipulate
16	Leaf type	Simple

Table 2: Quantitative Microscopy of Leaf of *T. orientalis*

Parameter	Mean (N=3)
Palisade ratio	44.7
Stomata number Upper surface	0.5
Stomata number Lower surface	4.5
Stomatal index Upper surface	2.44
Stomatal index Lower surface	18.37
Vein islet number	34
Veinlet termination number	7

Table 3: Result of Chemo microscopy of *T. orientalis* leaf powder

Chemicals	Reagents	Observations	Inference
Cellulose	N/50 Iodine + 80 % Sulphuric acid	Blue-black colour	Present
Tannins	70 % Methanol + dil. Ferric chloride	Blue-black colour	Present
Calcium carbonate	Acetic acid + 50 % Sulphuric acid	Effervescence +needle-shaped crystals separated	Present
Fats and fatty oils	Sudan IV + heat	Orange-red or brick red substances	Present
Proteins	Few drops of Ninhydrin + gentle warming for 5 min	Yellow colour	Present
Lignin	Few drops of phloroglucinol + stand for 2 – 3 min + drop of con. Hydrochloric acid	Pink or cherry red colour	Present
Starch	Few drops of N/50 Iodine	Deep blue to pinkish colour	Present

Table 4: Fluorescence analysis of *T. orientalis* leaf powder

Detection reagents	UV wavelength / Colour exhibited	
	254 nm	365 nm
Petroleum ether	Dark red	Dark brown
Methanol	Brick red	Dark red
50 % Hydrochloric acid	Dark green	Deep brown
50 % Sulphuric acid	Very green	Dark green
Ammonia	Dark green	Black
Ethyl acetate	Brick red or Pink	Dark brown

Table 5: Physicochemical properties of *T. orientalis*

S/N	Parameters	Leaf powder
1	Moisture content (LOD) (% w/w)	08.81 ±1.02
	Ash Values	
2	Total ash (% w/w)	08.33 ±0.29
3	Acid-insoluble ash (% w/w)	01.16 ±0.29
4	Water soluble ash (% w/w)	02.86 ±0.03
	Extractive values	
5	Water soluble extractive value (% w/w)	04.87 ±0.26
6	Alcohol soluble extractive value (% w/w)	02.53 ±0.33

N = 3

DISCUSSION

Macroscopic description of a plant gives a firsthand guide to identification of the plant. Thus the macroscopic features as presented (Table 1) are essential. Vein islet and vein termination numbers are relatively constant for different plants and can be useful to differentiate closely related species. Similarly, stomatal index is relatively constant for a particular plant and therefore is essential in quality control to ensure correct identity and check adulteration [3]. Another advantage is that palisade ratio, stomata number and stomatal index can be determined on much smaller fine powders of crude drug [29], and because crude drugs are mostly obtained in powdered form, these parameters are indispensable. The presence of stomata on the upper epidermal layer is in contrast to the findings of Panchal *et al*., [10] in India. The observed difference may be due to climatic difference. Ash value gives the amount of organic matter in the crude drug and is useful in the detection of adulteration with foreign matter or improper processing of herbal drugs [3]. The knowledge of physiological and non-physiological ash assists in this regard. The soluble extractive yields (water and alcohol) are also useful to detect inclusion of inferior materials in herbal drugs. This is because it gives the average amount of possible extractable yield. The moisture content guide on the extent of care needed to avoid breakdown of important plant constituents responsible for its therapeutic effect. If the moisture content is above the maximum standard value in crude drugs, more frequent drying must be done in long storage to discourage yeast and fungal infestation. The African pharmacopoeia [25] stated that the moisture content should be reduced to about 14 % to preserve the medicinal constituents of herbal drugs. Fluorescence studies are also useful for quality control by checking for correct identity via the characteristic colour exhibited by a particular plant powder [3].

CONCLUSION

The macro and micro-morphological, as well as numerical standards are useful diagnostic tools to control plant drug quality. These could therefore be useful for the compilation of a suitable monograph for *Trema orientalis*.

ACKNOWLEDGEMENT

We acknowledge the contributions of the following the entire staff of the Department of Pharmacognosy and Environmental Medicines, and of Plant Anatomy Laboratory, Department of Plant Science and Biotechnology University of Nigeria Nsukka, Nigeria.

REFERENCES

[1] Kunle OF, Egharevba HO, Ahmadu PO, Standardization of herbal medicines – A review. International Journal of Biodiversity and Conservation, 4(3), 2012, 101 – 112.

[2] Nivedithadevi D, Somasundaram R, Pharmacognostical and qualitative phytochemical studies on the aerial parts of *Tephrosla purpurea* (L), International Journal of Research in Biological Sciences, 2(2), 2012, 48 – 53.

[3] Mukherjee PK, Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals, 1st Ed., Business Horizon Publications, New Delhi, India, 2002, 185 – 186, 193, 541 – 542.

[4] Smith CA, Common names of South African plants. Memoirs of the Botanical Survey of South Africa 35, Department of Agricultural Technical Services, Pretoria 1966.

[5] Michael BA, Isaac KG, Nicholas OA, *Trema orientalis* (Linn.) Blume: A potential for prospecting for drugs for various uses, Pharmacognosy Review, 7(13), 2013, 67 – 72.

[6] Jahan MS, Mun SP, Effect of tree age on the soda-anthraquinone pulping of Nalita wood (*Trema orientalis*), Journal of Industrial and Engineering Chemistry, 10(5), 2004, 766 – 771.

[7] Ghana Herbal Pharmacopoeia, The Adventist Press Accra Ghana, 1992, 141 – 143.

[8] Iwu MM, Handbook of African Medicinal Plants, CRC Press Inc., Boca Raton, Florida, 1993, 251 – 252.

[9] Yanes CV, Germination of a pioneer tree from Equatorial Africa, Turrialba, 27, 2007, 301 – 302.

[10] Panchal HS, Master SM, Saluja AK, Patel MR, Pharmacognostic and phytochemical evaluation of *Trema orientalis* leaf, International Journal of Pharmacy and Pharmaceutical Sciences, 7(5), 2015.

[11] Noungoue TD, Cartier G, Dijoux Franca MG, Tsamo AM, Xanthones and other constituents of *Trema oreintalis*, Pharmaceutical Biology, 39, 2001, 202 – 205.

[12] Dijoux-Franca MG, Noungoué Tchamo D, Cherel B, Cussac M, Tsamo E, Mariotte AM, New dihydrophenanthrene and phenyldihydroisocoumarin constituents of *Trema orientalis*, Journal of Natural Products, 64, 2001, 832 – 835.

[13] Diehl MS, Atindehou KK, Téré H, Betschart B, Prospect for anthelminthic

plants in the Ivory Coast using ethnobotanical criteria, Journal of Ethnopharmacology, 95, 2004, 277 – 284.

[14] McGaw LJ, Jäger AK, van Staden J, Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants, Journal of Ethnopharmacology, 72, 2000, 247 – 263.

[15] Chowdhury A, Islam MS. Antibacterial activity of *Trema orientalis*, Dhaka University Journal of Pharmaceutical Sciences, 3, 2004, 115 – 117.

[16] Panchal HS, Master SM, Shah UD, Saluja AK, Dholwani KK, Anti-convulsion activity of leaf of *Trema orientalis*, International Journal of Pharmaceutical Research, 2, 2010, 53 – 55.

[17] Abiodun O, Gbotosho G, Ajaiyeoba E, Happi T, Falade M, Wittlin S, et al., *In vitro* antiplasmodial activity and toxicity assessment of some plants from Nigerian ethnomedicine, Pharmaceutical Biology, 49, 2011, 9 – 14.

[18] Githens TS, Drug plants of Africa In: African Handbooks Vol. 8. University of Pennsylvania Press, Lancaster Press, Lancaster, United States, 1948. 125.

[19] Katende AB, Useful trees and shrubs for Uganda: Identification, Propagation and Management for Agricultural and Pastoral Communities, Regional Soil Conservation Unit (RSCU), Swedish International Development Authority (SIDA), 1995.

[20] Birdi TJ, Brijesh S, Daswani PG, Approaches towards the Preclinical Testing and Standardization of Medicinal Plants 2008.

[21] Evans WC, Trease and Evans pharmacognosy, 15th Ed., Rajkamal Electric press, Delhi, India, 2005, 516 – 536.

[22] Kokate CK, Practical Pharmacognosy, 4th Ed., Vallabh Prakashan, Delhi, 1997, 107 – 111.

[23] Khandelwal KR, Practical pharmacognosy, Nirali Prakashan, 12th Ed., 2004.

[24] Sani A, Agunu A, Danmalam HU, Ibrahim H, Pharmacognostic studies of the stem bark of *Detarium microcarpum*-Guill. and Perr. (Fabaceae), Natural Products Chemistry and Research, 2014, S1:004.

[25] African Pharmacopoeia, Determination of Extractives, Scientific Publication, No 3, Lagos, Nigeria, 1986, 78.

[26] Rachh PR, Dharamsi A, Pharmacognostic studies of *Moringa oleifera* leaflet, Pharma Science Monitor: an International Journal of Pharmaceutical Sciences, 3(3), 2012, 125 – 126.

[27] Vinotha S, Ira T, Sri Ranjani S, Phyto, physicochemical standardization of medicinal plant *Enicostemma littorale* Blume, IOSR Journal of Pharmacy, 3(2), 2013, 52 – 58.

[28] Kadam VB, Momin RK, Wadikar MS, Tambe SS, Determination of water soluble ash values of some medicinal plants of genus *Sesbania*, Journal of Pharmaceutical and Biological Research, 1(1), 2013, 1 – 4.

[29] DanMalam HU, Abubakar Z, Katsayal UA, Pharmacognostic studies on the leaves of *Moringa oleifera*, Nigerian Journal of Natural Products and Medicines, 05, 2001, 48.