

Original Research Article

PHARMACOGNOSTIC STUDY OF THE LEAVES OF *Piliostigma thonningii* Schum (CEASALPINIACEAE)

ABSTRACT

Due to the existence of huge species and varieties of plants that bear resemblance in their macro-morphological features but differs in micro-morphology and phytochemical constituents, misidentification and replacement of one species/variety of plant for another is so rampant. Hence, the need for standard documentation of the macro and micro morphological features, unshared and unique characters and phytochemical compositions of individual plant drug cannot be over emphasized. This research studied the pharmacognostic profile of *Piliostigma thonningii* Schum leaf using pharmacognostic tools for crude drug standardization such as macromorphological / organoleptic evaluation, qualitative and quantitative microscopy, analytical evaluation (physicochemical constants) and phytochemical screening. The results of the microscopical evaluation of *Piliostigma thonningii* leaf showed that it has actinocytic stomata on the upper epidermis, uniseriate trichomes and schizo-lysigenous cavities on the lower epidermis, reticulate and spiral xylem vessels and rosette shape calcium oxalate crystals. While the analytical standard of the powdered drug revealed 9 moisture content, 4.1 total inorganic ash, 2.2 water soluble ash, 0.85 acid insoluble ash, 4.8 sulphated ash, 13 alcohol extractive, 11.5 water extractive and 4.61% n-hexane extractive. The phytochemical screening revealed the presence of saponins, alkaloids, tannins, flavonoids, steroids, glycosides and terpenoids. The overall results of this research can serve as reference standard for proper identification of *Piliostigma thonningii* plant.

Comment [U1]: Rephrase

Comment [U2]: Remove extra space

KEYWORDS: *Piliostigma thonningii*, acid insoluble ash, water soluble ash, calcium oxalate, stomata, trichome

INTRODUCTION

Standardization of herbal medicine helps to reveal and assemble characteristics that are inherently peculiar through series of laboratory experiments and pharmacognostic standardization of crude drugs involve the evaluation of a set of inherent characteristics that are either constant parameters, definitive, qualitative and quantitative values or unique and unshared features on the basis of which similar herbal medicines, claimed to be the same, can be compared for the purpose of authenticity, purity, genuineness and overall quality assurance (Elujoba, 1996; Inya-Agha, 2006).

Piliostigma thonningii Schum is a deciduous, single stem; leguminous tree belonging to the family Caesalpiniaceae. It is a perennial plant with large, simple, two-lobed, leathery leaves which resemble a camel's foot which account for the common name. The name '*Pilio-stigma*' means

cap-shaped stigma, while specific name, *thoningii* was given after the Danish Botanist, Peter Thoning (Ugwoke *et al.*, 2017). It was formerly called *Bauhinia thoningii*, but later differentiated from *Bauhinia* by its unisexual flowers and indehiscent pods (Ugwoke *et al.*, 2017). The tree bears flowers with five white to pinkish pendulous petals and are unisexual with male and female on a separate tree produced during November and April (Jimoh and Oladeji, 2005). The fruit is a hairy, hard, flattish pod which turns rusty brown at ripening and split; it is usually persistent on the tree and produced between June and September (Lock and Simpson, 1999). *Piliostigma thoningii* Schum is a common plant across most sub-Saharan African Countries. In Nigeria, It grows abundantly in the wild in some places such as Enugu, Nsukka, Zaria, Bauchi, Ilorin, Plateau, Lagos and Abeokuta (Schultes and Hofmann, 1973; Djuma, 2003; Ugwoke 2017).The plant is commonly called Okpoatu, abefe and kalgo in Igbo, Yoruba and Hausa languages respectively. (Dasofunjo *et al.*, 2012).

The root and twig of *Piliostigma thoningii* Schum have been used to treat dysentery, fever, respiratory ailments, snake bites, hookworm and skin diseases (Jimoh and Oladeji, 2005). It is also used in the treatment of malaria fever, wounds, ulcers, gastric and heart pain, arthritis, headache, hemorrhoids backache and gingivitis (Egharevba and Kunle, 2010). Traditional healers in “Doila” refer to this plant as “child remedy” as it is mainly used as a remedy for children (Egharevba and Kunle, 2010). The bark of *Piliostigma thoningii* is used as a remedy for cough, usually as an infusion or by chewing (Ugwoke, 2017). An isolated compound from the stem bark of *Piliostigma thoningii*, *D-3-O methylchiroinositol*, has been reported to possess anthelmintic analgesic, antipyretic, antidiabetic, antioxidant, and antilipidemic properties (Asuzu and Nwaehujor, 2013; Nwaehujor *et al.*, 2015; Ugwoke *et al.*,2017). The basic rationale of this study is to study the inherent characteristics of *Piliostigma thoningii* leaf that will be helpful for the correct botanical identification of the plant.

MATERIALS AND METHODS

Fresh leaves of *Piliostigma thoningii* Schum were collected from Oba town in Nsukka Local Government Area, Enugu State and were authenticated by Mr. A. O. Ozioko of the International Centre for Ethnomedicine and Drug Development (Inter CEDD), Nsukka, Enugu State. A voucher specimen number ESUT/COG/208 was preserved in Department of Pharmacognosy herbarium, Enugu State University of Science and Technology, Enugu, Nigeria.

Preparation of Plant Material For Phytochemical Analysis

Fresh leaf sample was air dried at room temperature for 10 days to remove sufficient moisture. The dried leaf sample was cut into smaller pieces and further pulverized with the aid of electrical blender. The powdered plant sample was stored in an air tight container for phytochemical screening.

Phytochemical Screen

Comment [U3]: F should be small

The phytochemical screening was carried out using standard procedures outlined by Evans (2009) and Haborne (1998) to detect the presence of glycosides, tannins, alkaloids, flavonoids, steroids and saponins.

Preparation of Plant Material For Macroscopic Examination

The fresh plant material was visually examined and the morphological features (such as leaf size, shape, type of venation, margin, leaf base, leaf apex etc.) and organoleptic features (such as colour, odour, and taste of the leaf) were evaluated and noted.

Comment [U4]: F should be small

Qualitative microscopic examination of fresh plant material

The fresh leaf sample was washed, cut into smaller pieces and placed in 70% chloral hydrate solution in a test tube and heat in a water bath to clear the cells. The cleared leaf sample was then placed on a slide and viewed under the microscope.

Comment [U5]: Inconsistent heading format

Microscopic examination of powdered leaves

A small quantity of the powdered crude drug was placed on a slide and few drops of chloral hydrate solution were added to it. The mixture was passed across the flame of a Bunsen burner repeatedly until bubbles occurred and allowed to cool for proper clearing of the sample. Two drops of glycerine were added to the slide as mountant and the slide was covered with cover slip and viewed under the microscope. The microscopic characters (such as cork cells, sclereids, fibres, calcium oxalate crystal etc.) were observed and noted.

Comment [U6]: Heading style should be same in all headings

Transverse section of the leaf

A sledge microtome machine was used to get a thin transverse section of a fresh leaf that was collected in a petri dish containing 70% ethanol. The sectioned materials were transferred into a staining jar containing safranin solution and allowed to stand for 5 minutes after which the safranin was drained off and the section washed 3 times in distilled water. The section was washed twice in 97% alcohol and rewashed with absolute ethanol for additional two times to achieve dehydration. The section was counter stained in 1% fast green for 5 minutes and washed in absolute alcohol and clove oil at ratio of 3:1 for 3-4 times at 2 minutes interval. The section was transferred into another staining jar containing 50/50 alcohol/xylene solution for preliminary clearing. Pure xylene was used to clear the section finally and Canada balsam was used as mountant for permanent slide preparation of the sectioned leaf material.

Result of Macroscopic Examination / Organoleptic Evaluation of *Piliostigma thonningii* Leaf

Comment [U7]: Clearly mention heading of RESULTS

The macroscopic examination / organoleptic evaluation of *Piliostigma thonningii* leaf showed the following:

- a. Leaf type – Simple leaf
- b. Leaf shape – Two lobed (Oblong)

- c. Leaf size – 7.5 to 15cm long
- d. Leaf margin – Entire
- e. Leaf apex – Emarginate
- f. Venation – Reticulate
- g. Leaf base – Cordate
- h. Odour – Characteristic odour
- i. Colour – Dark green on the upper epidermis pale green on the lower epidermis
- j. Texture – Leathery



Plate 1: Photograph of *Piliostigma thonningii* flower buds

Comment [U8]: Figure 1, caption missing. Plate 1 is not caption for an article.

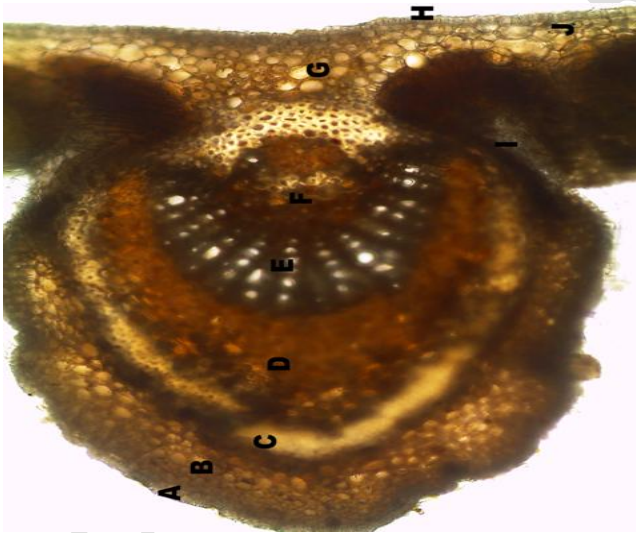
Comment [U9]: This is not plate.



Plate 2: Photograph of *Piliostigma thonningii* fruit (pod)

Comment [U10]: Caption missing

Comment [U11]: This is not plate



- KEY**
- A = Lower epidermis
 - B = Collenchyma
 - C = Starch granules
 - D = Parenchyma
 - E = Xylem
 - F = Phloem
 - G = Collenchyma
 - H = Upper epidermis
 - I = Schizogenous cavity
 - J = Palisade Mesophyl

Plate 3: Photomicrograph of the Transverse section of *Piliostigma thonningii* leaf

Comment [U12]: All figures are missing caption.

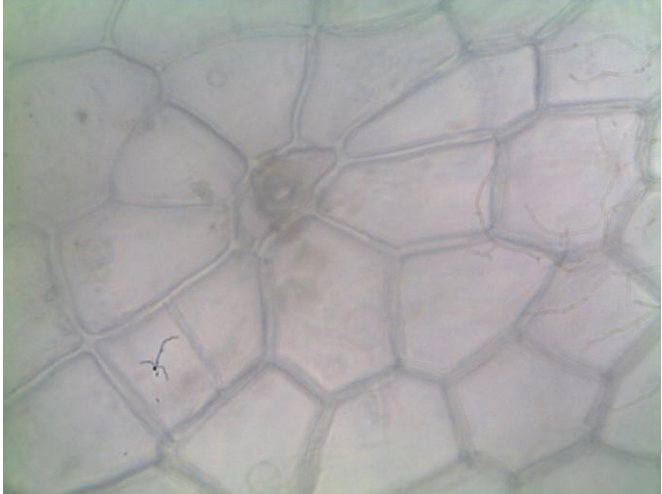


Plate 4: Photomicrograph of fresh *Ptilostigma thonningii* leaf showing a closer view of actinocytic stoma

Comment [U13]: caption

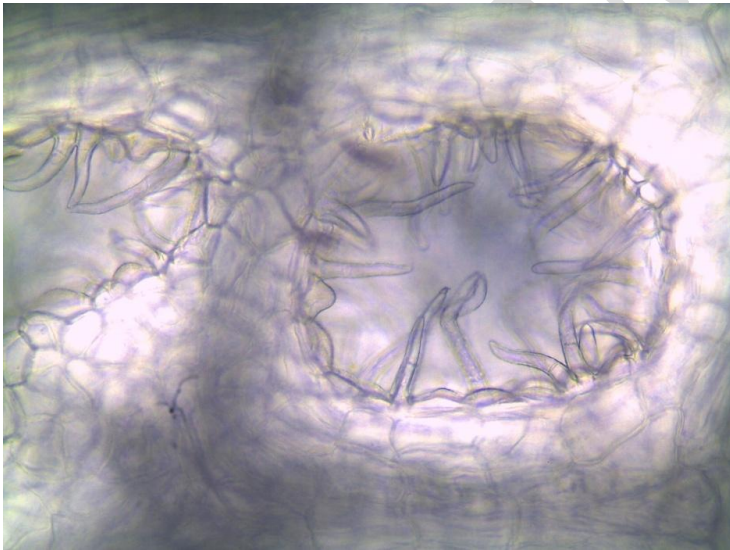


Plate 5: Photomicrograph of schizogenous cavities with uniseriate trichomes on the lower epidermis of fresh *Ptilostigma thonningii* leaf

Comment [U14]: check all document for figure captions

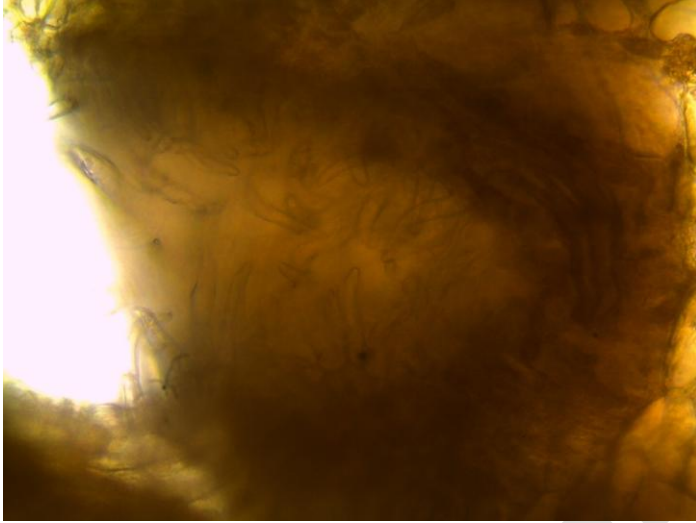


Plate 6: Photomicrograph of the sectional view of the transverse section of *Piliostigma thonningii* leaf showing the Schizogenous cavity on the lower epidermis

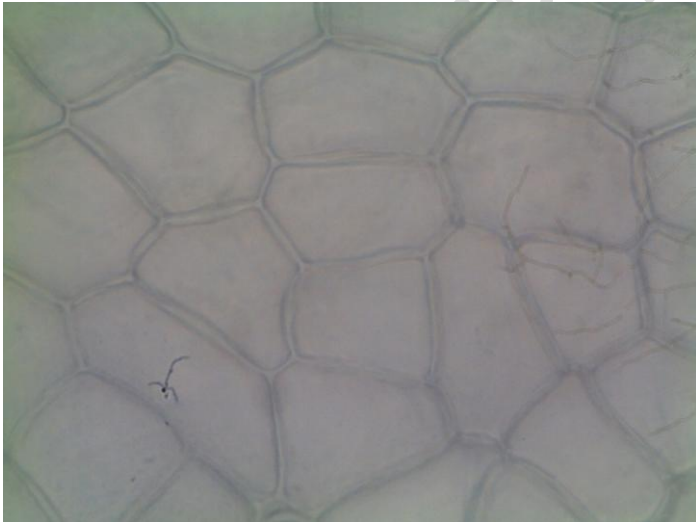
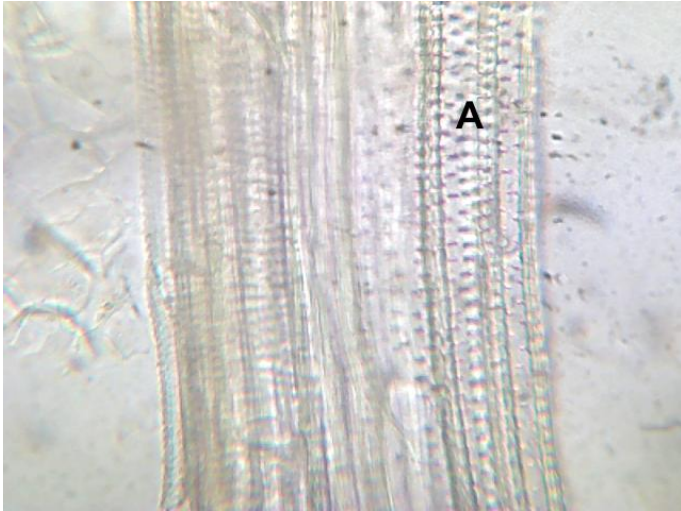
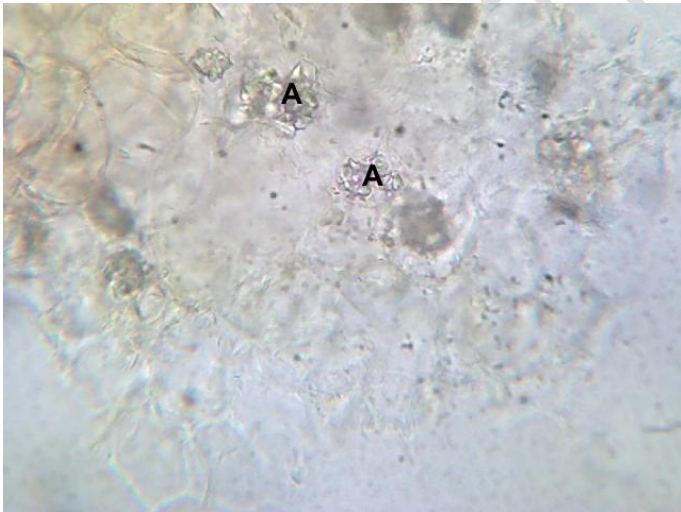


Plate 7: Photomicrograph of the upper epidermis of *Piliostigma thonningii* leaf showing straight walled epidermal cells



KEY
A = Scalariform xylem vessel

Plate 8: Photomicrograph *Piliostigma thonningii* leaf powder showing scalariform xylem vessel



KEY
A = Rosette calcium oxalate

Plate 9: Photomicrograph *Piliostigma thonningii* leaf powder showing rosette calcium oxalate crystals



Plate 10: Photomicrograph *Piliostigma thonningii* leaf powder showing spiral xylem vessel



Plate 11: Photomicrograph of *Piliostigma thonningii* leaf powder showing non glandular uniseriate trichome

Table 1: Results of Quantitative microscopy of *Piliostigma thonningii* leaf

Quantitative Standards	Composition (mm ²)
Vein-islet number	9.25 ± 0.61
Veinlet termination number	6.00 ± 0.82
Palisade ratio	13.67 ± 1.25
Stomatal number	4.33 ± 0.47
Stomatal index	0.0041 ± 0.0017

N = 3, Mean ± SEM

Comment [U15]: You haven't mentioned/referred table in text.

Comment [U16]: What is N?

Table 2: Results of Physico-chemical parameters of *Piliostigma thonningii* leaf

PARAMETER	VALUE (%)
Percentage yield	19.80
Alcohol extractive value	13.00
Water extractive value	11.50
N-hexane extractive value	4.61
Moisture content	9.00
Total ash	4.10
Water soluble ash	2.20
Acid insoluble ash	0.85
Sulphated ash	4.8

Comment [U17]: You haven't mentioned/referred table in text.

TABLE 3: Results of phytochemical screening of *Piliostigma thonningii* leaf

S/N	TEST COMPOUND	RESULT
1.	Saponins	present
2.	Tannins	present
3.	Flavonoids	present
4.	Steroids	present
5.	Terpenoids	present
6.	Alkaloids	present
7.	Glycosides	present

Comment [U18]: All heading should be in same format.

Comment [U19]: All tables should be mentioned in respective paragraphs.

Comment [U20]: Try to have all tables in same format

Comment [U21]: You may consider using + sign to represent present, – for absence. And mention that in key

RESULTS AND DISCUSSION

The results of the macroscopic and organoleptic evaluation of *Piliostigma thonningii* leaf which involves the visual and sensory characters revealed that the leaf is simple with oblong shape, entire margin, emarginated apex, leathery texture and leaf size of 7.5 cm to 15 cm. These parameters provide the simplest and quickest indication of the identity and quality of *Piliostigma thonningii* leaf drug when compared with official monographs.

The anatomy of a leaf has basic structural pattern yielding characters that help to detect a leaf in a powder. Characters that are not too common in other plant leaves give distinctions between plants of different class and the more detailed anatomical characters when put together help in the identification of a leaf up to genus and species level. Conversant knowledge of all the diagnostic characters of any leaf helps in the detection of contaminants and substitutes (Elujoba, 2006; Evans, 2009). The results of the anatomical sectioning and qualitative microscopy of *Piliostigma thonningii* leaf revealed some structurally distinct features and cell inclusions such as actinocytic stomata, uniseriate trichomes, schizo-lysigenous cavities on the lower epidermis, mesophyll cells and straight wall epidermal cells. Microscopy of the powdered leaf sample of *Piliostigma thonningii* further revealed the presence of spiral and scalariform xylem vessels and rosette shape calcium oxalate crystals. These features help in distinguishing *Piliostigma thonningii* leaf from closely related species.

Quantitative microscopy of a leaf helps to give accurate cellular micrometry of all the tissues and also to determine the proportions of the substances present (starch grains) by means of microscope (Wallis, 1920). The results of the quantitative microscopy of *Piliostigma thonningii* leaf sample as listed in table 1 of this study can be set as a reference standard to assist future researchers to distinguish the leaf from other closely related plant species.

Physiochemical constant parameters such as extractive values, moisture content, total ash, water soluble ash, acid insoluble ash, sulphated ash as listed in table 2 can serve as a guide to assess the quality and purity of *Piliostigma thonningii* leaf drug. The total ash of crude drug is mostly carbonates, phosphates and silicates that are either plant derived or residues such as soil contaminants that may adhere to the plant surface (Mukherjee, 2002). The total ash value of *Piliostigma thonningii* leaf (4.1%) revealed that it contains significant amount of either carbonates, phosphates, silicates or mixture of them. The value of acid insoluble ash (0.85%) showed that the leaf has negligible amount of silicious contaminants because acid insoluble ash value gives an idea of the amount of silicious material contamination (Elujoba, 2006). Water soluble ash (2.20%) is component of the total ash that are soluble in water. It helps to detect incorrect preparation and presence of adulterants in crude drug (Mukherjee, 2002). The sulphated ash (4.80%) produces more consistent ash than the total ash because all oxides and carbonates are converted to sulphates at high temperature.

Comment [U22]: Ypu have already written results above.

Comment [U23]: Missing t in that...

The result of the phytochemical screening of *Piliostigma thonningii* leaf showed that it contains secondary metabolites such as flavonoids, tannins, alkaloids, terpenoids, steroids, glycosides and saponins. Edible plant materials containing tannins are known to be astringent, and are used for treating intestinal disorders such as diarrhea and dysentery (Smirnoff, 2001; Li *et al.*, 2003; Cho *et al.*, 2004). Polyphenolics such as flavonoids possess strong antioxidants property (Rong, 2010) while some triterpenoids are known natural antioxidants and most pentacyclic triterpenes ameliorating activities on lipoprotein lipase expression, dyslipidemia and insulin sensitivity have been reported (Eu *et al.*, 2010; Gao *et al.*, 2009; Sheng and Sun, 2011; Takagi *et al.*, 2010). Saponins are surface active agents which alter the permeability of organism's cell wall by causing microporations that facilitate the entry of toxic materials or leakage of vital constituents from the cell thus inducing cell lyses (Daniyan *et al.*, 2010; Sodipo *et al.*, 1991).

CONCLUSION

Based on the results of extractive values and phytochemical screening of *Piliostigma thonningii* leaf sample, alcohol extractive gave the highest yield while most of the secondary metabolites that tested positive in the phytochemical screening are soluble in polar solvents. These observations suggested the reason why most trado-medicinal preparations from this plant are done using either alcohol (ethanol) or water. The overall results from this study will help to evaluate the purity, quality and precise identification of *Piliostigma thonningii* leaf drug.

REFERENCES

- Inya-Agha, S. I. (2006). *Phytochemistry and Therapeutics in the Tropics*. MASON Printer and Publishers, Enugu, 20-34p.
- Jimoh, F. O. and Oladeji, A. T. (2005). Preliminary studies of *Piliostigma thonningii* seeds. Proximate analysis, mineral composition and phytochemical screening. *Afr. J. Biotechnol.*, 4(12): 1439-1442.
- Habourne, J. B. C. (1973). *Phytochemical Methods*. Chapman and Hall, London, 279p.
- Evans W. C. (2009). *Text Book of Pharmacognosy*. 16th edn., Elsevier Limited, China, 14-125p.
- Smirnoff, N. (2001). L-ascorbic acid biosynthesis. *Vitamins and Hormones*, 61: 241-266.
- Li, H., Wang, Z., Liu, Y. (2003). Review in the studies on tannins activity of cancer prevention and anticancer. *Zhong-Yao-Cai*, 26(6): 444-448.
- Cho, E., Seddom, J., Ronser, B., Willer, W. and Hankinson, S. (2004). Prospective study of intake of fruits, vegetables, vitamins and carotenoids and related muscupathy. *Archeological Ophthalmology*, 122: 882- 892.
- Rong Tsao, 2010. Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients*. 2(12): 1231–1246.

- Eu C.H., Lim W.Y., Ton S.H., bin Abdul Kadir K., 2010. Glycyrrhizic acid improved lipoprotein lipase expression, insulin sensitivity, serum lipid and lipid deposition in high-fat diet-induced obese rats. *Lipids Health Dis.* 9:81–90.
- Gao D., Li Q., Li Y., Liu Z., Fan Y., Liu Z., Zhao H., Li J., Han Z., 2009. Antidiabetic and antioxidant effects of oleanolic acid from *Ligustrum lucidum* Ait in alloxan-induced diabetic rats. *Phytother Res.* 23:1257–1262.
- Sheng H, Sun H., 2011. Synthesis, biology and clinical significance of pentacyclic triterpenes: a multi-target approach to prevention and treatment of metabolic and vascular diseases. *Nat Prod Rep.* 28:543–593.
- Wallis, T. E. (1920). Lycopodium method of quantitative microscopy. *Journal Roy. Micro. Sc.*, 69(3): 15-21.
- Takagi S., Miura T., Ishihara E., Ishida T., Chinzei Y., 2010. Effect of corosolic acid on dietary hypercholesterolemia and hepatic steatosis in KK-Ay diabetic mice. *Biomedical Research.* 31:213–218.
- Ugwoke, C.E.C., Obi, P.E., Tchimine, M.K., Anze, S. P.G. (2017). Phytochemical Screening and Antimicrobial Activity of Methanol Extract and Fractions of the Leaf of *Piliostigma thonningii* Schum (Caesalpiniaceae). *World Applied Sciences Journal* 35 (4): 621-625, 2017
- Daniyan, S.Y., Galadima, M., Ijah, U.J.J., Odama, L.E., Yusuf, A.Y., Abbas, Y. 2010. *In vitro* antibacterial screening of *Piliostigma thonningii* leaf extract against clinical isolates of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* International Journal of pure and Applied Sciences, 4(1): 88-94.
- Sodipo, O.A., M.A. Awanji M.A., Kolawole, F.B. 1999. Odunuga AA. Saponin is the active fungal principle in *Garcinia kola* seeds. *Biological Research Communication*, 3: 171.
- Elujoba, A.A. 1996. *Pharmacognosy and Therapeutics in the Tropics*. Mason Printers and Publishers, Enugu, Nigeria. 5-12p.
- Mukherjee, P. K. (2002). *Quality Control of Herbal Drugs*. Business Horizons, New Delhi, 22-30p.
- Schultes, R. E., Hofmann, A. (1973). *The Botany and Chemistry of Hallucinogens*, Charles C. Thomas, Springfield III, 267p.
- Djuma (2003). *Djuma Game Reserve Copyright(C) 1998-2003*.
- Dasofunjo, k., Nwodo, F. O. C., Ipav S. S., Barminas Z. L. (2012). Effect of the ethanolic extract of *Piliostigma thonningii* leaves on kidney function indices and haematological parameters of male albino wistar rats. *J. Nat. Prod. Plant Resour.*, 2 (6):670-674.

Egharevba, H.O., Kunle F.O., 2010. Preliminary phytochemical and proximate analysis of the leaves of *Piliostigma thonningii* (Schumach.) Milne- Redhead. *Ethnobotanical Leaflets*, 14: 570-77.

Asuzu I.U., Nwaeujor C.O., The Anti-diabetic, hypolipidemic and anti-oxidant activities of D-3-O-methylchiroinositol in alloxan-induced diabetic rats, *Hygeia Journal of Drugs and Medicines*, 5, 2013, 27–33.

Nwaeujor C.O., Udegbumam R., Asuzu I.U., Analgesic, anti-inflammatory and anti-pyretic activities of D-3-O-methylchiroinositol isolated from stem bark of *Piliostigma thonningii*, *Medicinal Chemistry Research*, 24, 2015, 4139–4145.

UNDER PEER REVIEW