

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

ABSTRACT

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 has led to misidentification and replacement of one species/variety of plant for another. 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.

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 Caesalpinaceae. 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, *thonningii* was given after the Danish Botanist, Peter Thonning (Ugwoke *et al.*, 2017). It was formerly called *Bauhinia thonningii*, 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 thonningii* 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 thonningii* Schum have been used to treat dysentery, fever, respiratory ailments, snake bites, hookworm and skin diseases (Ogbiku *et al.*, 2021; 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 thonningii* 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 thonningii*, *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 thonningii* leaf that will be helpful for the correct botanical identification of the plant.

MATERIALS AND METHODS

Fresh leaves of *Piliostigma thonningii* 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

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.

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.

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.

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.

RESULTS

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

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

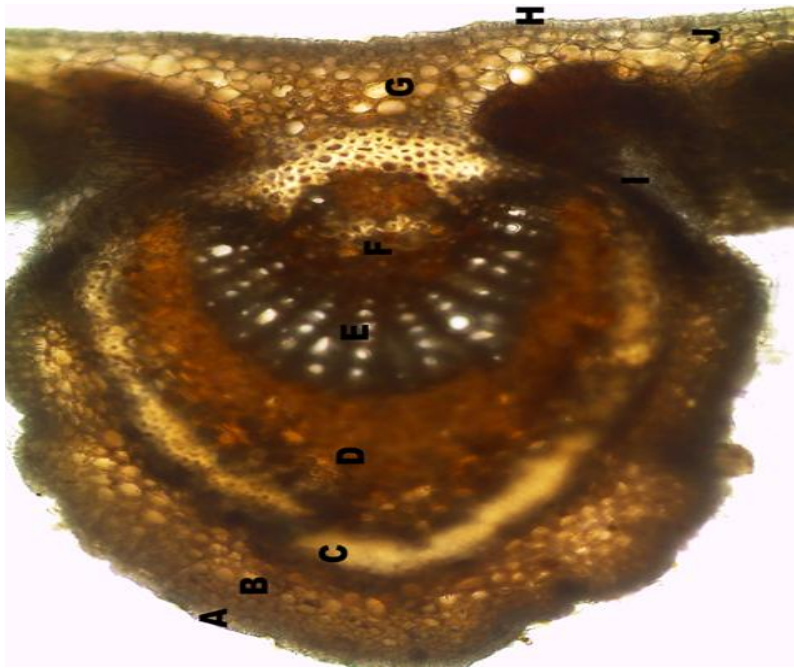
- f. Venation – Reticulate
- g. Leaf base – Cordate
- h. Odour – Characteristic odour
- i. Colour – Dark green on the upper epidermis pale green on the lower epidermis
- j. Texture – Leathery



Figure 1: Photograph of *Piliostigma thonningii* flower buds



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



- 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

Figure 3: Photomicrograph of the Transverse section of *Piliostigmathonningii* leaf

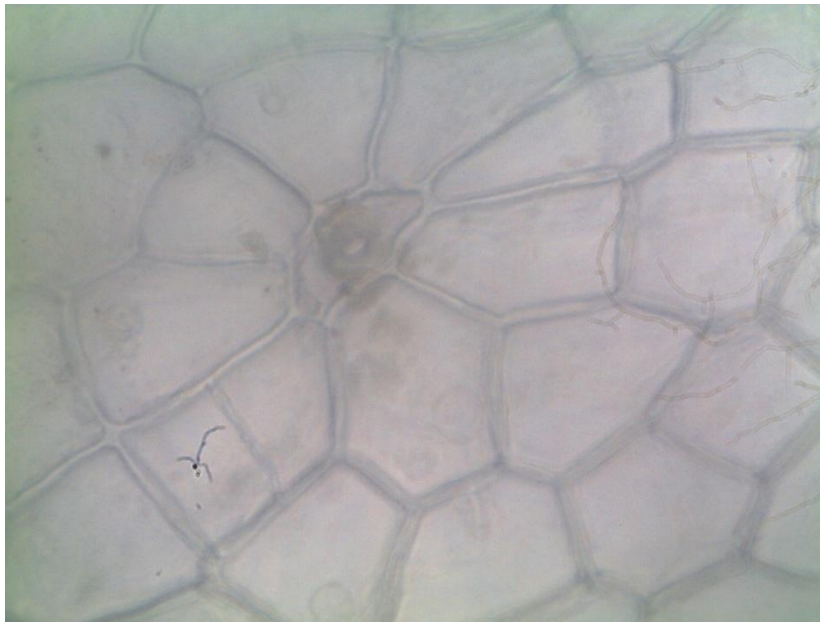


Figure 4: Photomicrograph of fresh *Piliostigma thonningii* leaf showing a closer view of actinocytic stoma

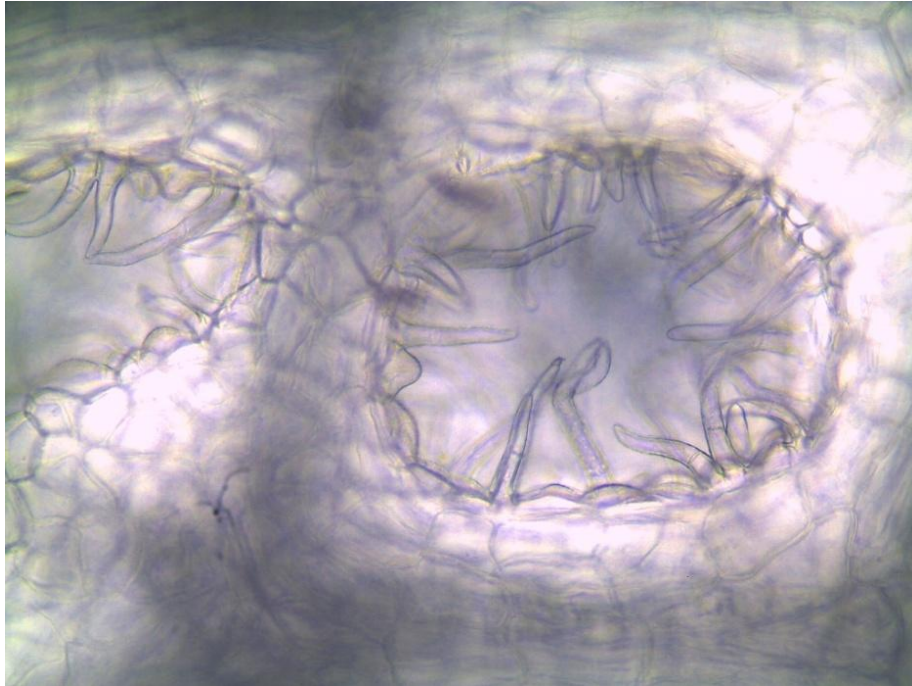


Figure 5: Photomicrograph of schizogenous cavities with uniseriate trichomes on the lower epidermis of fresh *Piliostigma thonningii* leaf

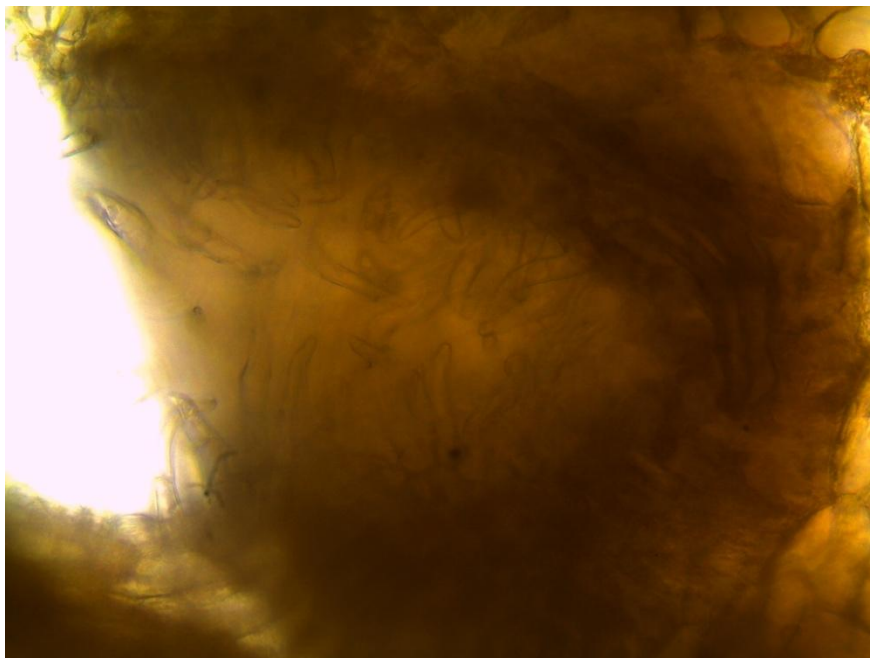


Figure 6: Photomicrograph of the sectional view of the transverse section of *Piliostigma thonningii* leaf showing the Schizogenous cavity on the lower epidermis

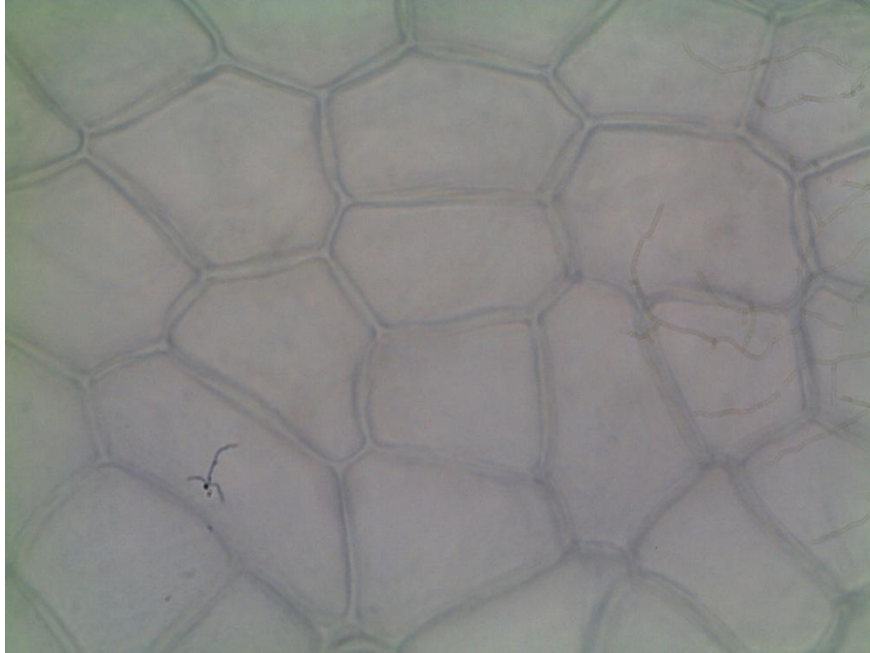


Figure 7: Photomicrograph of the upper epidermis of *Piliostigma thonningii* leaf showing straight walled epidermal cells



KEY
A = Scalariform xylem vessel

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



KEY
A = Rosette calcium oxalate

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



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



Figure 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

Number of replicates (N) = 3, Mean±SEM

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

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

TEST COMPOUNDS	RESULT
Saponins	+
Tannins	+
Flavonoids	+
Steroids	+
Terpenoids	+
Alkaloids	+
Glycosides	+

Key: + = present

DISCUSSION

The results of the macroscopic and organoleptic evaluation of *Piliostigma thonningii* leaf which involves the visual and sensory characters as shown in Figure 1 and 2 above 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 as shown in figure 3 to 11 revealed some structurally distinct features and cell inclusions such as actinocytic stomata, uniseriate trichomes, schizo-lyigenous 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.

The result of the phytochemical screening of *Piliostigma thonningii* leaf as shown in table 3 revealed the presence of 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.

Ethical Approval:

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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