Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 6 (7): 537-543, 2012
ISSN 1819-3455 / DOI: 10.3923/rjmp.2012.537.543

Evaluation of the Chemical, Nutritional, Antimicrobial and Antioxidant-vitamin Profiles of *Piliostigma thonningii* Leaves (Nigerian Species)

O.M. Ighodaro, S.O. Agunbiade, J.O Omole and O.A. Kuti
Department of Biochemistry and Microbiology, Lead City University, Ibadan, Nigeria

Corresponding Author: O.M. Ighodaro, Department of Biochemistry and Microbiology, Lead City University, Ibadan, Nigeria

ABSTRACT

In this study, the antioxidant-vitamin, phytochemical, mineral and proximate compositions as well as the antimicrobial sensitivity of *Piliostigma thonningii* leaves were evaluated. The dry leaf powder was found to contain alkaloids, saponins, flavonoids and tannins as phytochenicals in amounts between 0.2 to 2.1 g/100 g. The proximate analysis showed that they are rich in carbohydrate (72.17%). Other food nutrients include crude protein (10.09%), dietary fibre (23.05%), ash (9.13%), moisture (5.57%) and low amount of crude fat (2.81%). Ca, Mg, K, Na, Fe, Zn, Pb, Mn and Cu were the detectable mineral elements in the *P. thoningii* leaves. Ca and Pb had the highest (1740 mg/100 g) and lowest (0.4 mg/100 g) values, respectively. The plant leaves were also found to contain vitamin C (17.80 mg/100 g), vitamin E (3.29 mg/100 g) and beta carotene (12.5 mg/100 g). The results of the antimicrobial screening of the aqueous and ethanolic extracts of the leaves against eight human pathogenic microbes, five bacteria and three fungi showed that at 25 mg mL^{-1} concentration, both extracts exhibited potency range of 30.43 to 45.28% relative to a reference antibiotic (tetracycline). The ethanolic extract showed higher antimicrobial activities at all concentrations as compared to the aqueous extract. Results from the study have shown that *Piliostigma thonningii* leaves are a good source of some minerals, antioxidant-vitamins and efficient source of energy (energy value = 404.05 kcal/100 g).

Key words: *Piliostigma thonningii,* antioxidant-vitamin, phytochemical, minerals, proximate, analysis antimicrobial-sensitivity

INTRODUCTION

Increased awareness of the significance of medicinal plants and nutrition to the health of individuals and communities has necessitated the need for knowledge of the food nutrients and phytochemicals present in the various parts of different plants. The phytochemicals contained in plants are largely responsible for the definite physiological action they exert on the human body (Ighodaroro *et al.*, 2009) and their nutritional value is determined by the food nutrient they contain. Considerable information exists on the nutrient composition and medicinal value of most well known and easily cultivated plants. *Piliostigma thonningii* is an underexplored leguminous plant that belongs to the family of caesalpi niacea. The tree is perennial in nature and the color of its petals varies from white to pink (Simpson, 1999). It is found growing abundantly as a wild uncultivated tree in many parts of Nigeria and is widely distributed in Africa and Asia in open

woodlands and savannah regions that are moist as well as wooded grasslands in low to medium altitudes (Jimoh and Oladiji, 2005). Locally, the seed is called "abefe", "kalgo" and "okpoatu" in Yoruba, Hausa and Ibo lands, (Nigeria), respectively. The common names include monkey bread and camel's foot.

In the developing countries of the world, traditional herbal medicine is often used side by side western medicine with herbal medicine taking the upper hand when the cost of western medicine is beyond reach (Busia, 2005). *Piliostigma thonningii* Schum. is one of the plants with diverse ethnomedical applications (Togola *et al*., 2005). Different parts of the plant have been described as useful medicinally. Its root and twig have been used for the treatment of dysentery, fever, infections, snake bites, hookworm and skin disease as well as laxative, antihelmintic and anti-inflammatory agents (Igoli *et al*., 2005; Fakae *et al*., 2000). The seeds which are a good source of antioxidant micronutrients and rich in crude protein and carbohydrate, are eaten by African antelope and elephants (Jimoh and Oladiji, 2005).

However, there is paucity of documented information on the nutritional and medicinal value of the leaves. The purpose of this work was therefore to identify and quantify the phytochemicals, antioxidant-vitamins and food nutrients (carbohydrate, protein, minerals, fat etc.) present in *Piliosigma thonningii* leaves, as well as to evaluate the antimicrobial activities of the leaf extracts.

MATERIALS AND METHODS

The plant material: Fresh *Piliostigma thonningii* leaves were harvested as one batch at Ijaiye, Ibadan, Nigeria and the botanical identification and authentication was done at the department of Botany, University of Ibadan, Nigeria.

Preparation of plant extract: The leaves were freed of extraneous materials, air dried at room temperature and ground into a uniform powdery form using a milling machine. The aqueous and ethanolic extracts were prepared by soaking 300 g of the dried powdered leaves separately in 500 mL of distilled water and ethanol (80% v/v, BDH) for 24 h. They were thereafter filtered with Whatman filter paper No. 42 (125 mm). The filtrates were then concentrated at 45°C in a rotary evaporator and stored in air tight bottles until used.

Phytochemical screening: Chemical tests were carried out on the aqueous extract and dried powdered specimens using standard procedures to identify the constituents as described by Harborne (1973), Sofowora (1993) and Trease and Evans (1989).

Qualitative photochemical analysis: Qualitative analysis was carried out to identify the phytochemicals present in the leaves prior to quantitative estimation. The methods used and their corresponding inferences are shown in Table 1.

Quantitative analysis phytochemicals: This was done to determine the quantity of the phytochemicals contained in *Piliostigma thonningii*. Fat free samples were prepared by defatting some of the dried leaf powder with diethyl ether in the ratio of 1 g of sample to 50 mL of solvent (1:50, g/v) using soxhlet apparatus for 24 h.

Table 1: Qualitative analysis of phytochemicals

Phytochemical	Test	Observation	Inference
Cardiac glycosides	Keller-Kullam	Brown ring at the interface	Cardenolide is present
Flavonoids	Sodium hydroxide acid test	Yellow color which disappeared on addition of acid	Flavonoid is present
Alkaloids	Wagner	Brown precipitate which turns intense yellow with picric acid	Alkaloid is present
Tannins	Ferric chloride test	Greenish-black ppt.	Tannin is present
Terpenoids	Salkowski test	Reddish brown color	Terpenoid is present
Steroids	Liberman Burchard test	Brownish color	Steroid is present
Phlobatannins	Hydrochloric acid test	Red precipitate	Phlobatannins is present
Anthraquinones	Benzene test	Violet color in the layer	Anthraquinones is present
Saponins	Frothing test	Stable froth	Saponin is present

Harborne (1973), Trease and Evans (1989) and Sofowora (1993)

Estimation of flavonoids: This was determined according to the method of Harborne (1973).

Determination of alkaloids: Alkaline precipitation gravimetric method described by Harborne (1973) was used.

Determination of saponin: The method of Obadoni and Ochuko (2001) was used.

Proximate analysis: Proximate analysis of the leaf powder of *Piliostigma thonningii* was carried out by the methods of Association of Official Analytical Chemists (AOAC, 1992a). Crude Protein (CP) was determined by multiplying crude nitrogen by 6.25 while total carbohydrate was obtained by simple difference.

The energy value was calculated using Atwater factor method by multiplying the fat, protein and carbohydrate by their respective physiological fuel values of 9.0, 4.0 and 4.0 kcal g^{-1}, respectively and taking the sum of the products (Osborn and Voogt, 1978; Eneche, 1999).

The moisture content (hot air oven method), crude fat (soxhlet extraction method) were determined by the method of Pearson (1978).

Mineral elements were estimated after wet oxidation of samples (2 g) using concentrated Nitric acid and Perchloric acid as described by Osborn and Voogt (1978). The concentrations of the minerals: Ca, Mg, Cu, Pb, Zn, Mn and Fe in the digested sample were estimated with the Pye-Unicam Atomic Absorption Spectrum. K and Na were determined using flame photometer.

Determinaion of antioxidant-vitamins: The beta carotene content of fresh *Piliostigma thonningii* leaves was estimated by high performance liquid chromatography method. Vitamine C was determined by the titration methods using the 2,6 dichlorophenol indophenols method AOAC (1992b). Estimation of vitamin E (tocopherols) was done by the method of Emmerie and Engel (1939) with slight modification. This depends upon the oxidation of the tocopherol in alcoholic solution by ferric chloride and the subsequent spectrophotometric measurement of the red colour produced when the resultant ferrous iron reacts with $\alpha\alpha$-dipyridyl.

Antimicrobial assay: The 25 mg mL^{-1} of *P. thonningii* extract solution was used as standard extract concentration. This was obtained by dissolving 0.25 g of the extract in 10 mL each of sterile distilled water and ethanol (80% BDH). The 12.5, 6,25 and 3.01 mg mL^{-1} test solutions were subsequently prepared by serial dilution, using water and ethanol as the diluents in each case. The culture media used were carefully handled and prepared according to the manufacturer's instruction. They were all commercial products of Oxoid Ltd. Company, England. Five bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Proteus mirabilis*) and three fungi (*Rhizopus nigricans*, *Fusarium oxysporum* and *Aspergillus niger*) were used for this assay. The antibacterial and antifungal activities of the test sample were done using the agar well diffusion method (Stoke and Ridgeway, 1980). The inhibitory zones were measured in millimeters. Negative results were regarded as zones where no inhibition was observed.

RESULTS AND DISCUSSION

The phytrochemical screening and quantitative estimation of the percentage crude yield of the chemical constituents of *Piliostigma thonningii* showed that the leaves contain alkaloids (1.2 g/100 g), saponins (2.1 g/100 g), flavonoids (1.3 g/100 g) and tanins (0.8 g/100 g), as shown in Table 2. Saponin content was the highest. These phytochemicals are bioactive and responsible for the definite physiological effects exerted on the human body by various parts of plants (Ighodaroro *et al*., 2009). Moreover, some of these biochemical components have been reported to exert multiple biological and pharmacological effects (antibacterial, anti-hypertensive and anti-inflammatory activities etc. (Middleton and Kandaswanmi, 1992). The presence of these chemicals in *P. thonningii* leaves is therefore a strong indication that the leaves possess valuable medicinal properties which are yet to be explored.

In the proximate analysis of *Piliostigma thonningii* leaves, the crude fat component recorded the lowest value (2.81%) while the carbohydrate content was found to be the highest (72.17%) among other nutrient compositions. This value along with an energy value of 404.05 kcal/100 g (Table 3) presents *P. thonningii* leaves as good and efficient source of energy. The protein content

Table 2: Quantitative values of the phytochemicals present in *Piliostigma thonningi* leaves

Phytochemical	Amount (mg/100 g)
Saponins	2.1
Alkaloids	1.2
Flavonoids	1.3
Tanins	0.8

Values are means of triplicate determinants

Table 3: Proximate composition of *Piliostigma thonningi* leaves

Parameter	Composition (%)
Carbohydrate	72.17
Protein	10.09
Ash	6.10
Crude fibre	5.23
Moisture	3.11
Fat	2.81
Energy value	404.05 kcal/100 g

Values are means of triplicate determinants

Table 4: Mineral composition (mg/100 g) of *Piliostigma thonningii* leaves

Mineral	Ca	K	Mg	Na	Fe	Mn	Zn	Cu	Pb
Amount	1740.30	787.70	293.47	47.35	26.25	4.45	2.51	1.19	0.40

Values are means of triplicate determinants

Table 5: Antioxidant vitamin content of *Piliostigma thonningii* leaves

Parameter	Amount (mg/100 g)
Vitamin C	17.80
Vitamin E	3.29
Beta carotene	12.25

Values are means of triplicate determinants

of the leaves was 10.09%. *Piliostima thonningi* leaf due to its high protein may be utilized as a source of plant protein. The moisture, crude fibre and ash contents were found to be 3.11, 5.23 and 6.10%, respectively (Table 3).

The mineral compositions of *Piliostigma thonningii* leaves showed high levels of calcium, potassium and magnesium (1470.30, 787.70 and 293.47 mg/100 g) as shown in Table 4. The calcium content is incomparably high. This result suggests that the leaves may be of great physiological significance especially in part of the world were muscle weakness, increased nervous system irritability and spontaneous action potential generation in neurons are relatively rampant. *Piliostigma thonningii* leaves are also sources of sodium (47.37 mg/100 g), iron (26.25 mg/100 g). The levels of manganese (4.45 mg/100 g), copper (1.19 mg/100 g), Zinc (2.51 mg/100 g) and lead (0.4 mg/100 g) were quite low. The low content of heavy metals in the leaves is a beneficial in the light of the toxicity associated with heavy metal accumulation in the body.

The leaf of *Piliostigma thonningii* plant has also been found to be rich in vitamin E (3.29 mg/100 g), vitamin C (17.80 mg/100 g) and beta carotene (12.25 mg/100 g) as shown in Table 5. These are antioxidant nutrients which when present at low concentration compared to oxidizable substrates significantly delay or prevent the oxidization of these substrates (Halliwell and Gutteridge, 1999). There is an increasing body of evidence that natural antioxidants such as vitamin C, E and beta carotene protect the body against a number of degenerative diseases such as atherosclerosis, aging and certain types of cancer (Pratt, 1990). The substantial level of these molecules in *P. thonningii* leaves is indicative of the plant nutritional and medicinal significance.

The results of the antimicrobial screening of the aqueous and ethanolic extracts of the leaves against eight human pathogenic microbes, five bacteria and three fungi showed that both extracts were less effective as compared to a reference antibiotic, tetracycline. *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis* among other microbes were comparatively more inhibited by both extracts (Table 6a, b). These microbes are all bacteria, indicating that the leaf extract of *P. thonningii* has more antibacterial than antifungal properties .The ethanolic extract exhibited higher antimicrobial activities at all concentrations compared to the aqueous extract (Table 6a, b). The microbes against which the *P. thonningii* leaf extracts were effective are pathogens already implicated in the etiology and severity of human diseases. Thus, the plant extract may be useful in pharmaceutical and medical formulations. However, the possibility of further purification and formulation into antibiotics may be considered later.

Table 6a: Diameters (mm) of inhibition zones of microbial growth by *P. thonningii* ethanolic extract

			Concentration of *P. thonningii* extract (mg mL^{-1})			
Organism	-ve control	+ve control	25	12.5	6.25	3.01
Staphylococcus aureus	0	17.50	6.40	4.80	2.50	1.60
Escherichia coli	0	22.40	7.28	5.27	2.80	1.40
Bacillus cereus	0	14.30	4.907	2.80	1.94	1.14
Pseudomonas aeruginosa	0	14.60	5.20	3.90	2.10	1.10
Proteus mirabilis	0	12.60	5.70	3.90	2.80	1.30
Fusarium oxysporium	0	13.80	4.20	2.30	1.40	0.30
Aspergillus niger	0	11.70	3.60	2.10	10.6	0.60
Rhizopus nigricans	0	12.60	4.75	2.83	1.75	1.13

Values are means of triplicate determinants

Table 6b: Diameters (mm) of inhibition zones of microbial growth by *P. thonningii* aqueous extract

			Concentration of *P. thonningii* extract (mg mL^{-1})			
Organism	-ve control	+ve control	25	12.5	6.25	3.01
Staphylococcus aureus	0	17.50	3.70	2.00	1.00	0.40
Escherichia coli	0	22.40	3.40	2.20	1.20	0.40
Bacillus cereus	0	14.30	2.80	1.4	0.80	0.30
Pseudomonas aeruginosa	0	14.60	2.90	2.10	1.40	0.48
Proteus mirabilis	0	12.60	3.20	1.80	1.10	0.35
Fusarium oxysporium	0	13.80	1.40	0.60	0.40	0.20
Aspergillus niger	0	11.70	1.80	1.20	0.50	0.20
Rhizopus nigricans	0	12.60	1.60	1.08	0.48	0.35

Values are means of triplicate determinants

REFERENCES

AOAC, 1992a. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC.

AOAC, 1992b. Official Methods of Analysis. 13th Edn., Association of Official Analytical Chemists, Arlington, TX. USA.

Busia, K., 2005. Medical provision in Africa-Past and present. Phytotherapy Res., 19: 919-923.

Emmerie, A. and C. Engel, 1939. Colorimetric determination of alpha tocopherol (vitamin E). II Adsorption experiments. Rec. Trav. chim. PayB-Bas, 58: 283-289.

Eneche, E.H., 1999. Biscuit-making potential of millet/pigeon pea flour blends. Plant Foods Hum. Nutr., 54: 21-27.

Fakae, B.B., A.M. Cambell, J. Barrett, I.M. Scott, P.H. Teesdale-Spittle, E. Liebau and P.M. Brophy, 2000. Inhibition of gluthathione S-transferase (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. Phytother. Res., 14: 630-634.

Halliwell, B. and M.C. Gutteridge, 1999. Free Radical and Other Reactive Species and Disease. 3rd Edn., Oxford University Press, Oxford, pp: 639-646.

Harborne, J.B., 1973. Phytochemical Methods. Chapman and Hall, Ltd., London, pp: 49-188.

Ighodaroro, O.M., J.P. Mairiga and A.O. Adeyi, 2009. Reducing and Anti-proxidant profiles of flavonoids in *Ocimum gratissimum*. Int. J. Chem. Sci., 2: 85-89.

Igoli, J.O., O.G. Ogali, T.A. Tor-Anjiin and N.P. Logli, 2005. Traditional medicine practice amongst the Igede people of Nigeria Part II. Afr. J. Trad. CAM, 2: 134-152.

Jimoh, F.O. and A.T. Oladiji, 2005. Preliminary studies on *Piliostigma thonningii* seeds: Proximate analysis, mineral composition and phytochemical screening. Afr. J. Biotechnol., 4: 1439-1442.

Middleton, E. Jr and C. Kandaswanmi, 1992. Effects of flavonods on immune and inflammatory cell function. Biochem. Pharmacol., 43: 1167-1179.

Obadoni, B.O. and P.O. Ochuko, 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global J. Pure Applied Sci., 8: 203-208.

Osborn, D.R. and P. Voogt, 1978. The Analysis of Nutrients in Foods. Academic Press, London, pp: 166-182.

Pearson, D., 1978. Chemical Analysis of Foods. 7th Edn., Churchill Livingstone, Edinburgh, New York, pp: 194.

Pratt, D.E., 1990. Natural Antioxidants Not Exploited Commercially. In: Food Antioxidants, Hubson, B.J.F. (Ed.). Ist Edn., Elsevier Applied Science, Amsterdam, USA., ISBN: 9781851664405, pp: 171-192..

Simpson, D.P., 1999. Cassell's Latin Dictionary.` Cassell Ltd., London.

Sofowora, A., 1993. Medicinal Plants and Traditional Medicine in Africa. 2nd Edn., Spectrum Books Ltd., Ibadan, Nigeria, ISBN-13: 9789782462190, Pages: 289.

Stoke, J.E. and G.L. Ridgeway, 1980. Clinical Bacteriology. Edward Arnold Ltd., London.

Togola, A., D. Diallo, S. Dembele, H. Barsett and B.S. Paulsen, 2005. Ethnopharmacological survey of different uses of seven medicinal plants from Mali (West Africa) in the regions Diola, Kolokani and Siby. J. Ethnobiol. Ethnomed., 1: 7-7.

Trease, G.E. and W.C. Evans, 1989. Pharmacognosy. 11th Edn., Macmillan Publishers, London.