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Phytochemical Screening and Antimicrobial Activity of Methanol Extract and Fractions of the Leaf of *Piliostigma thonningii* Schum (Caesalpiniaceae)

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Abstract: Background: Plants contain chemical substances that protect the plant against microbial infections among other action and confer a therapeutic benefit to humans. These medically active substances are non-nutritive and are referred to as phytochemicals. *Objective*: To detect the phytochemical composition and evaluate the antimicrobial and potentials of the leaf of Piliostigma thonningii**.** Materials and Methods: Plant materials were powdered and macerated with methanol. The crude extract was subjected to column chromatography using n-hexane, chloroform, ethyl acetate and methanol successively on silica gel. Phytochemical screening was by standard qualitative test procedures for basic plant secondary metabolites. The crude extract and fractions were respectively reconstituted in dimethylsulphoxide (DMSO) to 100 mg/ml and screened for antimicrobial sensitivity on clinical strains of enteric microorganisms using the Agar well Diffusion method. Reference antibiotics used were Gentamicin and Ketonazole. A further two-fold dilution from 100 – 3.125 mg/ml and sensitivity test was done for effective plant drugs to determine the Minimum Inhibitory Concentration (MIC). Statistical significance was at $p < 0.005$. *Results*: Phytochemical screening revealed the presence/absence of alkaloids, saponins, tannins, flavanoids, steroids, terpenoids, proteins, carbohydrates and reducing sugars. The results of the antimicrobial assay revealed potency against Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pneumonia, Candida albicans and Aspergillus niger. The antimicrobial activities (at the applied concentrations) were comparable to that of Gentamicin and Ketoconazole. *Conclusion*: This finding justifies the folkloric uses of Piliostigma thonningii in the treatment of infectious diseases.

Key words: Agar well · Dimethylsulphoxide · Enteric · Gentamicin · *Piliostigma thonningii*

INTRODUCTION

In time immemorial, man had used plants of different classes, habits and life forms in combating and managing infectious and non infectious diseases. It is established that most diseases of man are caused by microorganisms and the potency of drugs from plant origin in checkmating the activities of disease causing microorganisms is an attribute to its phytochemical composition [1]. Plant based antimicrobials are yet to be fully utilized and the few that have been utilized gave a promising future for herbal medicines as a complete replacement for synthetic antibiotic drugs. This is due to their enormous therapeutic effects and relative safety in contrast to side effects, which are mostly associated with synthetic drugs [1].

Piliostigma thoningii is a deciduous, single-stem leguminous tree belonging to the family Caesalpiniaceae. It is a perennial in habit with large, simple, two-lobed, leathery leaves which resemble a camel's foot and account for the common name – 'Camel foot'. The name '*Pilio-stigma*' means cap-shaped stigma, while specific name, *thonningii* was given after the Danish Botanist, Peter Thonning. It was formerly called *Bauhinia thonningii,* but later differentiated from *Bauhinia* by its

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unisexual flowers and indehiscent pods. The flowers have five white to pinkish pendulous petals with male and female organs on separate trees produced during November and April [2]. 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 [3]. *Piliostigma thonningii* is also known across Africa and other sub-Saharan countries as Mukolokote (Venda); Mokgoropo (North Sotho) [4]. In Nigeria, the plant bears local names such as abefe (Yoruba), kalgo (Hausa) Okpoatu (Igbo), ejei-jei (Igala), omepa (Igede) and nyihar (Tiv) [4]. It grows abundantly in the wild in some parts of Nigeria such as Zaria, Bauchi, Ilorin, Plateau, Lagos and Abeokuta [5, 6].

In Africa, parts of *Piliostigma thonningii* are reckoned for therapeutic significance as antimicrobial: the root and twig have been used for the treatment of dysentery, fever, respiratory ailments, snake bites, hookworm and skin diseases [2]. It is also used in the treatment of malaria fever, wounds, ulcers, gastric and heart pain, arthritis, headache, hemorrhoids backache and gingivitis [7]. The stem bark, in addition to dysentery, toothache and snakebite, is also used as an anthelmintic [8]. An isolated compound from the stem bark of *Piliostigma thonningii*, *D-3-0 methylchiroinositol*, an anthelmintic, induced approximately 60% larval paralysis within 24 hrs of contact with *Haemonchus contostus* larva at 4.4 mg/ml [9]. It is thus used to treat helminthiasis in African traditional medicine [9]. Traditional healers in "Doila" refer to this plant as "child remedy" as it is mainly used as a remedy for children [7]. The bark of *Piliostigma thonningii* is used as a remedy for cough, usually as an infusion or by chewing. A common use in Uganda is to stop diarrhoea, dysentery and intestinal upsets [10, 11]. Also, the infusion is also used in the treatment of malaria and leprosy. Other uses of the bark include analgesic, remedy for sore throat, toothache, stomachache and earache [12]. These claims necessitate a scientific proof aimed at facilitating standardization of the plant medicine for optimum health benefits.

MATERIALS AND METHODS

Collection and Authentication of Plant Material: Fresh leaves of *Piliostigma thonningii* Schum were collected from Orba 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 (InterCEDD), Nsukka. A voucher specimen was deposited with number INTERCEDD042014.

Fig. 1: Sectional view of *Piliostigma thonningii* tree

Preparation of Plant Extract: An 800 g of pulverized leaf was macerated in 2400 ml of methanol for 48 hours and then filtered using whattman (No.1) filter paper. This was done in batches, with addition of fresh solvent at each batch until all the extractable constituents were completely washed out as judged by loss of colour of the filtrate. The combined filtrates were concentrated in a vacuum evaporator to obtain a dried extract. The weight of the dried extract was obtained using a sensitive chemical balance and the percentage yield was calculated.

Preparation of Plant Fractions: The dried methanol extract was successively washed with n-hexane, chloroform, ethyl acetate and methanol using a column chromatography with silica gel of 60 – 120 mesh size as the stationary phase.

Phytochemical Studies: The phytochemical screening was carried out using standard procedures [13, 14] to detect the presence of alkaloids, saponins, tannins, flavanoids, steroids, terpenoids, proteins, carbohydrates and reducing sugars.

Preparation of Test Microorganisms and Plant Drug Materials: Clinical isolates of *Bacillus subtilis, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa*, *Candida albicans and Aspergillus niger* were obtained from the Madonna University Teaching Hospital, Elele, Rivers State. These were purified in Nutrient Agar (NA) and Saboraud's Dextrose Agar (SDA). The pathogens were diluted to 0.5 McFarland turbidity standards. Standard solutions of the extract and fractions were prepared in Dimethylsulphoxide (DMSO). 500 mg of each of the extract and fractions were carefully weighed and transferred into different test tubes

previously labelled. A 5 ml DMSO was transferred into each test tube containing the weighed extract and fractions using sterile pipettes to dissolve the extracts. To ensure homogenous mixture a glass stirring rod was used to stir the mixtures.

Antimicrobial Screening: The agar well diffusion method [15] was used to evaluate the sensitivity of the test microorganisms to the plant extract and fractions. A 10 ml of molten Nutrient Agar and Saboraud's Dextrose Agar (at 45°C) were inoculated with 0.1 ml of each bacterium and fungus or yeast cultures respectively and poured into sterile Petri-dishes. The contents were gently swirled and allowed to solidify. A 0.5 ml of each component of the reconstituted plant drug materials were transferred to each agar well. The plates were allowed to stand at room temperature for about 15 minutes and then incubated at 37°C for 24 hours (for bacteria) and 48 hours (for fungi). The Inhibition Zone Diameter (IZD) was measured. Gentamicin and Ketoconazole were used as standard antibacterial and antifungal respectively.

The Minimum Inhibitory Concentration (MIC) of the extract and fractions that were sensitive to the test organisms was determined using the serial dilution method [16]: A two-fold serial dilution of 100 mg/ml of each of the extract and fractions were carried out to obtain 50, 25, 12.5, 6.25 and 3.125 mg/ml in DMSO. Five wells were bored onto the seeded agar media using a sterile cork borer of 9 mm diameter and labelled accordingly. The serial diluted extract and fractions were applied into the wells and after pre-diffusion for 15 minutes, were incubated as above and examined for clear zones of inhibition and the MIC was noted.

Statistical Analysis: Data obtained were analyzed statistically using a one-way analysis of variance (ANOVA). Differences between Means were accepted significant at $p < 0.005$. Results were expressed as Mean ± SEM (Standard Error of Mean).

RESULTS

Various basic phytochemicals were detected in the plant as shown (Table 1).

All the test microorganisms were susceptible to the methanolic crude extract. The ethyl acetate fraction likewise inhibited the microorganisms except *C. albicans*. N-hexane and chloroform fractions were ineffective on all test bacterial strains. *A. niger* was resistant to only the chloroform fraction. On the other hand, Gentamicin inhibited only bacterial strains while Ketoconazole inhibited only the fungi as expected (Table 2).

The MIC result (Table 3) indicated that with a relatively lowest MIC of 12.98 mg/m, the methanol crude extract had its most inhibition against *A. niger*. The methanol fraction, with a lower MIC of 11.63 mg/ml was better effective than the crude extract. Accordingly, with an MIC of 8.11 mg/ml, the ethyl acetate fraction was most effective against *S. typhi*.

DISCUSSION/CONCLUSION

The results of the phytochemical screening revealed that the methanol extract and some fractions (methanol and ethyl acetate fractions) contain alkaloids and the glycosides – saponins, flavanoids and tannins in addition to other metabolites (Table 1). Plants containing phenolic

Table 1: Results of Phytochemical Screening

	Observation				
Phytochemicals	MCE	HF	CF	EF	MF
Alkaloids	+	-	+	+	++
Saponins	+++	-	-	+	++
Tannins	++	-	-	++	+++
Flavonoids	+++	-	+	+++	+
Reducing sugars	+	-	-	+	++
Proteins	++	+	+	++	++
Steroids	+	++	+	+	+
Terpenoids	+	+	+	-	-
Carbohydrates	++	-	-	+	++

N=3; values were expressed as Mean ± SEM

Key: + = Sparingly present, ++ = Moderately present, +++ = Abundantly present

Table 2: Result of Antimicrobial Sensitivity Test using Agar well (9 mm) Diffusion

	Plant Extract/Fractions (100mg/ml)					Standard Antibiotics (100µg/ml)	
Microorganisms	MCE	HF	CF	EF	MF	Gentamicin	Ketoconazole
Salmonella typhi	+	-	-	+	-	+	-
Pseudomonas aeruginosa	+	-	-	+	+	+	-
Escherichia coli	+	-	-	+	+	+	-
Staphylococcus aureus	+	-	-	+	-	+	-
Streptococcus pneumonia	+	-	-	+	-	+	-
Candida albicans	+	+	+	-	-	-	+
Aspergillus niger	+	+	-	+	+	-	+

N=3; values were expressed as Mean ± SEM
Key: + = Sensitive; - = Not Sensitive.

Table 3: Result of Minimum Inhibitory Concentration (MIC)

Microorganisms	MCE	HF	CF	EF	MF	Gentamicin	Ketoconazole
Salmonella typhi	23.50±0.49	–	–	8.11±0.13	–	5.23±0.25	–
Pseudomonas aeruginosa	15.72±0.46	–	–	17.39±1.21	14.69±0.29	18.76±0.44	–
Escherichia coli	13.17±0.45	–	–	14.02±0.6	17.81±0.15	18.07±1.92	–
Staphylococcus aureus	22.80±0.58	–	–	22.80±0.55	–	21.00±0.64	–
Streptococcus pneumonia	13.15±0.085	–	–	21.54±1.02	–	11.47±0.21	–
Candida albicans	22.88±0.74	25.01±0.50	23.48±0.34	–	–	–	12.09±0.91
Aspergillus niger	12.98±0.22	24.27±0.55	–	22.23±0.09	11.63±0.17	–	10.83±0.35

N=3; values were expressed as Mean ± SEM Key: – = No reaction

compounds such as tannins and flavonoids have been known and reported to possess antimicrobial activities [17, 18]. Saponins are surface active agents which alter the permeability of the cell wall of organisms most especially in fungi and bacteria by producing microporation that facilitate the entry of toxic materials or leakage of vital constituents from the cell thus induces cell lyses [19, 20]. This property confirms saponins as potent antimicrobial agent. The presence of these may have acted in synergy to inhibit the pathogens. The results of the antimicrobial screenings showed that activities vary with fractions (Table 2). The observed difference can be attributed to the variation in the distribution of active principles according to their affinity for the solvent used in fractionation. Also observed (Tables 2 and 3) was that the steroidal components in n-hexane and chloroform fractions were effective against the fungi only. The antimicrobial activities of plant extract is considered significant if the Minimum Inhibition Concentration (MIC) of the extract is less than or equal to 200 mg/ml [21]. Therefore based on the MIC result (Table 3), the test plant may be considered to possess significant antimicrobial activity. On the basis of the overall finding from this investigation, the traditional uses of *Piliostigma thonningii* in the treatment of infections is justified.

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REFERENCES

1. Iwu, M.M., A.R. Duncan and C.O. Okunji, 1999. New antimicrobials of plant origin. In: Janick J., ed. Perspectives on New Crops and New Uses. Alexandria (VA): ASHS Press, pp: 457-462.
2. Jimoh, F.O. and A.T. Oladeji, 2005. Preliminary studies of *Piliostigma thonningii* seeds: Proximate analysis, mineral composition and photochemical screening. Afr. J. Biotechnol., 4(12): 1439-1442.
3. Lock, J.T. and M.J. Simpson, 1999. Legumes of West Tropical Asia. 3rd ed. London: Academic Press Inc.; pp: 216-220.
4. Dasofunjo, K., F.O.C. Nwodo, S.S. Ipav and Z.L. Barminas. 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.

5. Schultes, R.E. and A. Hofmann, 1973. The botany and chemistry of hallucinogens, Charles C. Thomas: Spring Fields III; 1973. p. 267.
6. Djuma. Djuma Game reserve. Copyright(C) 1998-2003; 2003.
7. Egharevba, H.O. and F.O. Kunle, 2010. Preliminary phytochemical and proximate analysis of the leaves of *Piliostigma thonningii* (Schumach.) Milne-Redhead. Ethnobotanical Leaflets, 14: 570-77.
8. Lewis, W.H. and M.P.F. Elvin-lewis, 1979. Medical botany. New York: John Wiley and Sons, pp: 236- 261.
9. Irvine, F., 1961. Woody plants of Ghana. London: Oxford Univ. Press; pp: 687-688.
10. Ajali, U., 2002. Chemistry of bio-compounds. 1st ed. Enugu (Nigeria): Rhyce Kerex Publishers, pp: 60-178.
11. Burkill, H.M., 1995. The useful plants of West Tropical Africa: Caesalpinioideae. kew (London): Royal Botanic Garden; 3: 50.
12. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Review, 12(4): 564-582.
13. Evans, W.C., 2005. Trease and Evans Pharmacognosy. 15th ed. Delhi (India): Rajkamal Electric Press; pp: 14.
14. Haborne, J.B.C., 1973. Phytochemical methods. London: Chapman and Hall; pp: 279.
15. White, L.O. and D.S. Reeve, 1987. Assaying antibiotics in pharmaceutical microbiology. New York: McGraw-Hill Company; pp: 140-145.
16. Deeni, Y. and H. Hussain, 1991. Screening for antimicrobial activity of alkaloids of *Nuclea latifolia*. J. Ethnopharmacol., 35: 91-96.
17. Musa, K.Y., A. Ahmed, H. Ibrahim, G. Arowosaiye and O.S. Olonitola, 2000. Phytochemical and antimicrobial studies of leaves of *Acalypha racemosa.* Nig. Journal of Natural Products and Medicine, 4: 67-69.
18. Abere, T.A. and F.O. Agoreyo, 2006. Antimicrobial and toxicological evaluation of the leaves of *Baissea axillaries* used in the management of HIV/AIDS patients. *BMC* Complementary and Alternative Medicine, 6: 22.
19. Daniyan, S.Y., M. Galadima, U.J.J. Ijah, L.E. Odama, A.Y. Yusuf and Y. Abbas, 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.
20. Sodipo, O.A., M.A. Awanji and F.B. Kolawole, 19991. Odunuga AA. Saponin is the active fungal principle in *Garcinia kola* seeds. Biological Research Communication, 3: 171.
21. Suffredin, B., M.L.B. Pacienca, D.C. Nepomuceno, R.N. Younes and A.D. Varella, 2006. Antibacterial and cytotoxic activity of Brazilian plant extracts. Clusiaceae. Mem. Inst. Oswaldo Cruz, 107: 287-290.