African Journal of Agriculture and Food Science
Volume 2, Issue 1, 2019 (pp. 15-27)

www.abjournals.org

PHYTOCHEMICAL SCREENING, MINERAL DETERMINATION AND ANTIMICROBIAL SCREENING OF THE LEAVES EXTRACTS OF *PILIOSTIGMA THONNONGII* (MATURED AND YOUNG) LEAVES

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ABSTRACT: *This paper dwelled on a plant Piliostigma thonningii with reference voucher FNS/0013/ibbu/ 018. The young and matured leaves of Piliostigma thonningii were used in ethno-medicine for the treatment of wounds, ulcers, and gingivitis by some communities in Agaie, Lapai and Bida LGA of Niger State, Nigeria separately. There is no reported scientific register that implicate the equivocal chemical constituent of the leaves. Against this backdrop the young and matured leaves were investigated for chemical constituents, antimicrobial activity and mineral composition. The leaves of the plant were subjected to solvent extraction for ethanolic and aqueous extracts. Both extracts were then subjected to preliminary Phytochemical screening and it was found that the young and matured leaves of the plant both contained alkaloids, flavonoids, Saponins, tannins, Terpenoids, Steroidal nucleus and cardiac glycoside and the absence of Anthraquinone. The matured leave was richer in chemical content than the young leaves. The antimicrobial activity of ethanol and aqueous extracts were studied using isolates of three pathogenic microorganisms and the extracts exhibited activities against two of the three microorganisms with zone of inhibition ranging from 11- 40mm. Estimation of Na, Ca, P, and K were carried out by flame photometer and the mineral assessment result revealed that Na, Ca, P, and K were present in the concentration 240, 82, 39, 11 and 30, 78, 10, 18ppm for matured and young leaves respectively. This clearly indicated that the matured leaves are richer in mineral content than the young leaves except for the potassium (K) content as against the assertion by the local traditional healers.*

KEYWORDS: Phytochemicals, Microorganism, Mineral, Piliostigma Thonningiim Screening

INTRODUCTION

The use of plant materials in the treatment of diseases has been demonstrated to be as a result of the presence of chemical compounds in plants, which include flavanoids, alkaloids, steroids, tannins, and Saponins. Phytochemicals are not specific in their use therefore could exhibit several functions; bacterial, antifungal and antiviral (Deshi *et al.*, 2014).

Plant products play an important role in the health care delivery as therapeutic remedies in the world, especially in developing countries. Akinpelu et al., (2014) reported that plants product has made phytomedicine to become an integral part of the health care system of

many nations. Plants derived products are also use to control pest in developing countries before the discovery of pesticides (Deshi *et al.,* 2014).

Medicinal Plants

Medicinal plants are plants which contain substances that could be used for therapeutic purposes which may include synthesis of useful drugs (Abolaji *et al.,* 2000). Medicinal plants are rich sources of bioactive Phytochemicals and bionutrients. Studies carried out in the recent past decades have shown that these Phytochemicals play an important role in preventing chronic diseases like cancer, diabetes and coronary heart diseases. The major classes of Phytochemicals with disease preventing functions are all parts of the plant (Sexana *et al*., 2013).

Medicinal plants have been the mainstay of traditional herbal medicine among rural dwellers worldwide since antiquity till date. Doughari (2012) reported that according World Health Organization (WHO, 2000), a medicinal plant is any plant in which one or more of its parts contains substances that can be used for therapeutic purposes. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds employed in the control or treatment of a disease condition and therefore contain chemical components that are of medicinal value.

Medicinal plants are increasingly gaining acceptance even among the literates in urban settlement, probably due to increasing resistance by microbes on many modern drugs used for the control of infections such as typhoid fever, tuberculosis, respiratory ailment, snake bites skin infections. World Health Organization (WHO) have demonstrated that 80% of the world's population depends on medicinal plants for their primary health care. This has prompted increased efforts to the adoption and integration of herbal practices in health systems (Njeru *et al.,* 2015).

Medicinal plants are rich in bio-resources of drugs, and these chemical compounds play a definite physiological action in the human body system. Investigation of medicinal plants by ethno-botanist reveals that it can serve as an alternative to the existing synthetic medicines, this is because of the fact that most synthetic products are progressively losing their potency and pathogens developing resistance. In addition to the multiple resistance development by pathogens against the synthetic antibiotics, it has been reported that most of these drugs have a major setback due to the side effects on the patients. Medicinal plants are likely to be more effective by humans than the synthetic antimicrobial agents because plants are product of nature (Akinpelu *et al.,* 2014).

Piliostigma Thonningii

Piliostigma thonningii is also known as camel's foot, monkey bread, Rhodesian bauhinia. Locally the plant is called" Baffin, Abafe, Kaloo and Okpoatu in Nupe, Yoruba, Hausa and Igbo respectively in Nigeria (Deshi *et al.,* 2014). *Piliostigma. thonningii* is a dioecious tree with male and female flowers, common throughout the Sudan Savanna and it extend to the boarder of the Guinean rainforest. It is a leguminous plant that belongs to the *Caesalpiniodeae* family which consist of about 133 species, the plant is perennial in nature and its flowers which are produced between November to December with white pink colour, it bears hairy flat-pod fruits that turn nasty-brown and woody on maturity and usually persist on the plant till between June and September (Kwaji *et al.,* 2010).

Different parts of *P. thonningii* scum (caesalpiniodeae) have been reported to be used medicinally, the roots and twigs have been used locally in the treatment of dysentery, fever, respiratory ailments, snake bites, hookworm and skin infections in Eastern Nigeria. The leaf extract has been used for various purposes including the treatment of malaria all over Eastern Nigeria (Kwaji *et al.,* 2010).

P. thonningii is a multipurpose tree of high priority in Nigeria, almost all its parts are used in traditional medicine and its seed are good source of antioxidants, micronutrients, rich in crude protein and carbohydrates. This evergreen species is a good shade tree that fixes nitrogen and plays vital ecological roles in nutrient recycling from deep soil. The wood is use as fuel wood while salt can be extracted from its ash, the ashes and fresh pods are used in soap making (Ayisire *et al.,* 2008).

P. thonningii is used traditionally in the management of fever, cough, wounds and various ulceration. Malaria is an endemic infectious disease that is wide spread in tropical and subtropical regions of the Africa and one of the six most important parasitic disease of man. *Piliostigma thonningii* is found growing abundantly as a wild uncultivated tree in many part of Nigeria such as; Minna, Lapai, Agaie, Zaria, Bauchi, Illorin, Plateau, Lagos and Abeokuta. The powdered bark or the young inner bark and the scurf scrapped from the surface of the pods are applied to dressing for wounds. The bark is also chewed for relief of cough, the Leaves and bark are believed to have expectorant property and are used in infusions or chewed for chest pains, intestinal upset, diarrhea and dysentery (Jimoh, 2005; Madara *et al.,* 2010).

The use of chemotherapeutic agents to manage severe and life threatening bacterial infections has grown increasingly over the years because of its effectiveness, mode of administration, packaging and ease to access have made it a more user friendly. Due to curative nature, more people subscribe to the usage of this therapy. One of the antibacterial drug which is popularly used in the treatment of typhoid fever both in the developed and under developed countries of the world like the tropical Africa is known as Pefloxacin also called the fluoroquinoline derivative. Pefloxacin is to be considered to be very effective therefore could be of last resort when all antibiotics have failed. This drug is well absorbed by the oral route, the availability is 100%, protein binding is 20-30%, it undergoes hepatic metabolism with a half-life of 8.6hours and is excreted mostly through renal and biliary clearance. Schreiberbike (2007) reported that with these broad spectrum of advantage of pefloxacin, it has a very harmful adverse effect that is generally referred to as fluoroquinoline derivative toxicity. Researches have been reported that some plants like *P. thonningii* which have antioxidant properties that could help reduce the side effect (*Saxena et al.,* 2013).

Plate 1: Piliostigma Thonningii Tree

P. thonningii is a species of flowering plant in the legume family, *fabaceae*. It belongs to the sub family *Caesalpiniodeae.* Common names of this tree include Camel's foot tree, Monkey bread, Rhodesian bauhinia and Wild bauhinia (Bike, 2007).

Plate 2: *P. thonningii* (matured) leaves

Plate 3: *P. thonningii* (matured) leaves

MATERIALS AND METHODOLOGY

Collection of Plant Materials

The plant of was collected from uncultivated farm lands in Lapai, Niger state Nigeria. The plants were identified and authenticated by Mal. Isah Legbo Muhammad in the Department of Biological Sciences Ibrahim Badamasi Babangida University, Lapai. The plants (both the young and matured leaves) were rinsed with distilled water before cutting them into tiny pieces and were air dried at room temperature for a week.

Extraction Procedure

The air dried leaves of *piliostigma thonningii* leaves, both young and matured leaves separatly were pounded into a coarse powder form. 30g and 20g of the powdered sample i.e. matured and young respectively were extracted with ethanol by Soxhelation. An aqueous solution was obtained which was then subjected to a steam bath which helps in getting the crude extract by evaporating the ethanol in a steam bath (Harbone, 1973).

Phytochemical Screening

The fractions of various plant powder extracts of *piliostigma thonningii* were subjected to qualitative phytochemical analysis to identify the phytochemicals present. The extract was used for phytochemical screening as described by Soforowa (1993); Trease & Evans (1996) and Harbone (1998).

Cardaic Glycosides Test (Killer-Killiani Test)

Plant extract was added to $2cm^3$ of glacial acetic acids containing a drop of ferric chloride solution. The solution was underplayed with $1cm^3$ concentrated sulphuric acid. A brown colour ring indicates the presence of positive test (Trease & Evans, 1996).

Terpenoid Test (Salkowski Test)

$2cm^3$ of chloroform was added to about $1cm^3$ of plant extract. To this mixture, about 3cm3 of concentrated sulphuric acid (H_2SO_4) was added carefully without jerking. Development of reddish brown coloration at the interface indicates the presence of terpenoid (Sofowora, 1993).

Flavonoid Test

$5cm^3$ of dilute ammonia solution was added to a portion of plant extract followed by addition of concentrated sulphuric acid (H_2SO_4). A yellow coloration observed indicates the presence of flavonoid and the yellow coloration disappeared on standing (Sofowora, 1993).

Test for Tannins (Ferric Chloride Test)

A fraction of plant extract was dissolves in $10cm^3$ of distilled water then filtered. Few drops of ferric chloride solution were added to the filtrate. Formation of blue-black precipitation observed indicates the presence of hydrolysable tannins and green precipitate indicates the presence of condensed tannins (Trease & Evans, 1996).

Test for Alkaloid (Mayer's test)

Few drops of Mayer's reagent were added to the extract and the solution was shaking vigorously in a test tube. A cream colored precipitate indicates the presence of Alkaloids (Trease & Evans, 1996).

Test for Saponins

About 2g of the powdered sample was boiled in $20cm^3$ distilled water in a water bath and filtered. $10cm^3$ of the filtrate was mixed with 5cm3 of distilled water and shaken vigorously for a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and shaken vigorously, and the formation of emulsion was observed. (Trease & Evans, 1996).

Test for Steroids Nucleus

The extract was dissolved in $2cm^3$ of chloroform, $2cm^3$ of sulphuric acid was added carefully to form a layer. A reddish brown color at the interface indicates the presence of steroidal ring (Harbone, 1998).

Test for Anthraquinones

The plant extract was shaken with 10cm^3 benzene, and 5cm3 of 10% ammonia solution was added. The mixture was shaken vigorously. The presence of pink, red, or violet color in the lower phase indicates the presence of anthraquinones (Harbone, 1998).

Determination of Minerals

Mineral analysis was carried out after wet digestion with a digestion mixture containing concentrated nitric acid and concentrated tetraoxosulphate (VI) acid in a ratio of 3:1. 0.2g of the powdered samples was weighed into conical flask and 5cm^3 of digestion mixture was added, which was then placed in a fume cupboard for digestion. The mixture was digested for 2 hrs at a temperature of 150-200^{0}C, the digests were allowed to cool and 30cm^3 of distilled water was added. It was shaken vigorously and filtered and made up to mark into a 100cm^3 volumetric flask. The digests were then used for the determination of Sodium, Calcuim, potassium and phosphorous using Flame photometer while calcium was determined using EDTA complexometric titration (ASTM, 2004).

Anti-Microbial Screening

Pure culture and sub-culture of the bacterium, Extracts, Sterile Petri dishes, Anaerobic jar, Wire loop (inoculating loop), Bunsen burner, Autoclave, Hot air oven at 160^{0}C, Conical flask, test tubes, cotton wool, disposable syringe Test tubes rack, Aluminuim foil paper, Masking Tape, Incubator at 37%C, Disinfectant (Detol) and Cork Brorer.

Organisms and Microbial Cultures Used

Three bacterial species were employed in this work. These organisms were selected because they are pathogenic and cause a wider range of disease to the biotic organism. The bacterial cultures used are the pure isolates of the following: *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia.*

Anti-Biogram

This was employed with the use of plant to test against micro-organisms in the laboratory. The pure isolate of the micro-organisms was obtained by culturing and sub culturing (Flavia *et al.,* 2008).

Nutrient Broth

1.4g of the powdered commercially prepared broth was weighed and dissolved in 50cm^3 of distilled water, 9cm3 of the nutrient broth was then distributed into each test tubes and autoclaved for 15min at 121^{0}C after which was allowed to cool, the organisms which was sub-cultured was placed into the test tubes using sterile wire loop and then incubated for 24hrs (Flavia *et al.,* 2008).

Nutrient Agar

1.4g of the powdered commercially prepared nutrient agar was weighed and dissolved in 50cm^3 of distilled water and was autoclaved at standard condition. The nutrient agar was

cooled to 50^0C and was poured into the plates which were allowed to solidify (Flavia *et al.*, 2008).

Sample and Serial Dilution Preparation

1g of each of the plant extracts were diluted with 100cm^3 of distilled water to yield a sample concentration of 10mg/cm^3 and serial dilutions of 10mg/cm^3, 5mg/cm^3, 2.5mg/cm^3 and 0.625mg/cm^3 were used for each plant extract (Flavia *et al.*, 2008).

Plate Inoculation Method

The method of inoculation used in this work was the cork plate method. It involves the use of sterile loop to smear a loop full of the test organism on the surface of the medium. The loop was sterilized in the Bunsen flame and when cooled, streaked all over the plate to cover the plate surface. Holes were bored on the solidified agar using a sterile cork borer of 7.0mm in diameter. 0.3cm^3 of each of the extract in the test tubes prepared by serial dilution was dispersed into the holes made on the nutrient agar by using disposable syringe. After dispensing, the extract was allowed to diffuse into the agar for 2hrs and finally incubated in an incubator for 16-18hrs. After incubation, the diameter of zone of inhibition was measured to the nearest millimeter (Flavia *et al.*, 2008).

RESULTS AND DISCUSSION

Ethanolic extraction of the matured leaves of *Piliostigma thonningii* yield 5.6g from 30g of the powdered *piliostigma thonningii* leaves.

$$\text{Percentage yield} = \frac{5.6}{30} x\ 100$$

$$= 18.7\%$$

Ethanolic extraction of the young leaves of *Piliostigma thonningii* yield 3.1g from 20g of the powdered *piliostigma thonningii* leaves.

$$\text{Percentage yield} = \frac{3.1}{20} x\ 100$$

$$= 15.5\%$$

Table 1: Indicate the result of the phytochemical analysis of the ethanolic extract of both the matured and young leaves of *piliostigma thonningii.* The result of the ethanolic plant extract of the matured and young leaves indicated the presence of Flavonoid, Saponins, Tannins, Alkaloids, Cardiac Glycosides, Terpenoids, and Steroid nucleus. But the absence of Anthraquinone was found in both plants extract

Table 1: Phytochemical Screening of Ethanolic Extract of Both Matured and Young Leaves of *Piliostigma Thonningii*

TEST	INFERENCE MATURED	INFERENCE YOUNG
Flavonoid	+	+
Saponins	+	+
Tannins	+	+
Alkaloid	+	+
Cardiac glycoside	+	+
Steroidal nucleus	+	+
Anthraquinone	-	-
Terpenoids	+	+

Key: + = Present, - = Absent

Aqueous extraction of the matured leaves of Piliostigma thonningii yield 2.5g from 30g of the powdered piliostigma thonningii leaves.

$$\text{Percentage yield} = \frac{2.5}{30} \times \frac{100}{1}$$

$$= 8.3\%$$

Aqueous extraction of the matured leaves of Piliostigma thonningii yield 4.7g from 15g of the powdered piliostigma thonningii leaves.

$$\text{Percentage yield} = \frac{4.7}{15} \times \frac{100}{1}$$

$$= 31.3\%$$

Table 2: Shows the result of the phytochemical analysis of the aqueous extract of both the matured and young leaves of *piliostigma thonningii.* The result of the aqueous plant extract of the matured and young leaves indicated the presence of Flavonoid, Saponins, Tannins, Alkaloids, Cardiac Glycosides, steroidal nucleus and Terpenoids. However, the absence of Anthraquinone was found in both plants extract.

Table 2: Phytochemical Screening of Aqueous Extract of Both Matured and Young Leaves of *Piliostigma Thonningii*

TEST(S)	INFERENCE MATURED	INFERENCE YOUNG
Flavonoid	+	+
Saponins	+	+
Tannins	+	+
Alkaloid	+	+
Cardiac glycoside	+	+
Steroidal nucleus	+	+
Anthraquinone	-	-
Terpenoids	+	+

Key: + = Present, - = Absent

Table 3, 4, 5 and 6 shows the susceptibility test of the ethanolic and aqueous extract of both the matured and young leaves of *Piliostigma thonningii* on the test organism, the plant were found to inhibit the growth *E. coli* and *S. enterica* Typhi at 10, 5, 2.5, 1.25 and 0.625mg/cm^3 concentrations and not *P. aeruginosa*.

Table 3: Antimicrobial Activities of Ethanolic Extract of the Matured Leaves of *Piliostigma Thonningii*

Test Organism	Diameters of Zone of Inhibition (mm) Various Concentration of the Extract					Sparfloxacin
	10	5	2.5	1.25	0.625	
Escherichia coli	18	-	-	-	-	40
S. enterica Typhi	40	16	-	-	-	42
Pseudomonas aeruginosa	-	-	-	-	-	40

Table 4: Antimicrobial Activities of Ethanolic Extract of the Young Leaves of *Piliostigma Thonningii*

Test organism	Diameters of zone of inhibition (mm) Various concentration of the extract					Sparfloxacin
	10	5	2.5	1.25	0.625	
Escherichia coli	-	-	-	-	-	40
S. enterica Typhi	-	-	-	-	-	42
Pseudomonas aeruginosa	-	-	-	-	-	40

Table 5: Antimicrobial Activities of Aqueous Extract of the Matured Leaves of *Piliostigma Thonningii*

Test organism	Diameters of zone of inhibition (mm) Various concentration of the extract					Sparfloxacin
	10	5	2.5	1.25	0.625	
Escherichia coli	-	-	-	-	-	40
S. enterica Typhi	-	-	-	-	-	42
Pseudomonas aeruginosa	-	-	-	-	-	40

Table 6: Antimicrobial Activities of Aqueous Extract of the Young Leaves of *Piliostigma Thonningii* at 10mg/cm^3

Test organism	Diameters of zone of inhibition (mm) Various concentration of the extract					Sparfloxacin
	10	5	2.5	1.25	0.625	
Escherichia coli	18	-	-	-	-	40
S. enterica Typhi	14	11	-	-	-	42
Pseudomonas aeruginosa	-	-	-	-	-	40

Table 7: Minimum Inhibition Concentration (MIC) of both the Ethanolic and Aqueous Extract of the Matured and Young Leaves of *Piliostigma Thonningii* from the tables above

Test organism	Ethanol Matured	Ethanol Young	Aqueous Matured	Aqueous young
Escherichia coli	10	-	-	10
S. enterica Typhi	5	-	-	5
P. aeruginosa	-	-	-	-

Table 8: shows the result of the mineral compositions of the matured and young leaves of *Piliostigma thonningii.* The result shows that the concentration of sodium of the matured leaves is compared to the concentrations of others in the plants.

Table 8: Represent the Mineral Compositions of the Matured and Young Leaves of *Piliostigma Thonningii*

Element	*P. thonningii* Matured (ppm)	*P. thonningii* Young (ppm)
Sodium	240	30
Calcium	82	78
Phosphorous	39	10
Potassium	11	18

DISCUSSION

Preliminary Phytochemical Studies, the result of phytochemical screening carried out on the crude ethanol and aqueous extracts revealed the presence of Flavonoids, Tannins, Alkaloids, Saponins, Terpenoids and cardiac glycosides, Steroidal nucleus, However Anthraquinone was found absent in both extracts. The presence of phytochemical constituent listed supports its use to treat hepatobiliary disorders and anti-inflammatory property. This result is corroborated with report of studies (Ibewuike *et al.,* 1996, Trease & Evans, 2002 & Adihechu, 2014).

The presence of tannins supports the use of this plant to treat bacterial infection of the bladder (Avorn *et al.,* 1994). Similarly, the presence of flavonoids supports anti-tumor, antibacterial or antifungal properties which are used in domestic veterinary medicine, popularly in the form of ointment for treating dermal diseases (Trease & Evans, 1996).

The presence of these bioactive compounds has been linked to the antibacterial activity such as inhibition of growth of some microbes and offering some protection against microbial attack (Farnsworth, 1982; De & Ifeoma, 2002).

Antimicrobial Studies, the crude ethanolic and aqueous extract of both the matured and young leaves of *P. thonningii r*espectively revealed its strong activity against *Salmonella* enterica Typhi and *Escherichia coli.* The two extracts have no activity against *Pseudomonas aeruginosa*. *Salmonella enterica* Typhi is known to cause typhoid fever in humans and also cause self-limiting gastointestestinal diseases. So strong activity of both the ethanolic and aqueous extracts indicates that the plant can be effective against typhoid fever, and gastrointestinal diseases.

Escherichia coli are known to cause gastroenteritis, urinary tract infections and neonatal meningitis, it can also be characterized by severe abdominal cramps, diarrhea that typically turns bloody within 24hrs and sometimes fever (Jann & Jann, 1992).

In terms of concentration, the ethanolic and aqueous extracts of the matured and young leaves of *Piliostigma thonningii* inhibits growth of *E. coli* at only higher concentration of 10mg/cm^3 and inhibits growth of salmonella at concentrations of 10 and 5mg/cm^3, however the ethanolic extract of the matured leaves is more susceptible to salmonella by inhibiting growth of 40mm at 10mg/cm^3.

Mineral Composition, the sodium content is lower in the young leaves of *Piliostigma thonningii* than in the matured leaves of *Piliostigma thonningii* which is a characteristic of plant product (Olaofe *et al.*, 1994). The higher concentration of sodium in the matured leaves is an indication that the plant is not good for hypertensive patient which could result in high blood pressure, however, the presence of these minerals (Na, Ca, P and K) makes them a good traditional medicine for the treatment of diseases.

CONCLUSION

Based on the findings in this work, it can be concluded that that the use of *piliostigma thonningii* in the treatment of diseases has scientific basis. The phytochemical screening carried out on the matured and young leaves of *P. thonningii* revealed that the plants possesses Terpenoids, Alkaloids, tannins, Saponins, Cardiac glycoside, Steroidal nucleus, Flavonoids respectively. The investigation on antimicrobial activity revealed that at concentration >10mg/cm^3, the plant will also be effective in the treatment of diseases caused *E. coli* and *Salmonella enterica* Typhi. This result on antimicrobial activities is in agreement with work that has been carried out before by (Ibewuike *et al.*, 1996 and Adihechu, 2014).

RECOMMENDATION

It is therefore recommended that further work should be aimed at isolation and characterization of *piliostigma thonningii* to explore other medicinal uses of the plant, The phytochemical analysis reveals that these plants extracts contains secondary metabolites like Tannins, Alkaloids and Cardiac glycosides which are bioactive compounds which are of health benefit to man.

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