

## Exploration Of *Piliostigma Thonningii* (Schum.) A Tropical Tree For Possible Utilization As A Plant-Derived Pesticide

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### Abstract

The use of plant-derived pesticides against crop pests both on the field and during post-harvest is now emerging as one of the most important means of crop protection under an Integrated Pest Management framework following the multiple global challenges created by synthetic chemical pesticides. Studies on the phytochemicals, chemomicroscopic screening and mineral composition of *Piliostigma thonningii* (Schum.) were investigated for its potential useful bioactive compounds resource for possible utilization as plant-derived pesticides. The aqueous screening using reported methodologies for phytochemical screening revealed the presence of alkaloids, anthraquinones, carbohydrates, glycosides, flavonoids, saponins, steroids and tannins. Mineral analysis of both bark and leaves revealed that it contain substantial amount of nutrients evaluated. The results of this study suggested that *P. thonningii* (Schum.) have great potential as plant-derived pesticidal agent. Knowing that *P. thonningii* (Schum.) have bioactive properties is not enough, more research work is recommended on the various plant parts for isolation and characterization of bioactive compounds that may be utilize for pest control of agricultural plants.

**Keywords:** exploration, secondary metabolites, mineral analyses, *Piliostima thonningii*, plant-derived pesticide.

### 1. Introduction

*Piliostigma thonningii* (Shumach) Milne-Redh is also known as Camel's foot, Monkey bread, Rhodesian bauhinia. Locally the plant is called "abefe"; "Kaloo" and "Okpoatu" in Yoruba, Hausa and Igbo respectively in Nigeria (Jimoh and Oladeji, 2005). It is a species of flowering plants in the legume family, Fabaceae. It belongs to the order Caesalpinioideae which consists of about 133 genera. A dioecious tree with male and female flowers on different trees (Orwa, Mutua, Kindt, et al. 2009). Found in woodland, common throughout the Sudanian Savannas, it extends to the border of the Guinean rainforest and forest galleries, tends to colonize clearings and fallows. Found throughout tropical Africa (Orwa et al, 2009); yielding heavy crops of pods, which are eaten by livestock, game monkeys, and also by humans.

The use of plants and plants-derived products to control pests in developing world is well known and, before the discovery of synthetic pesticides, plants or plants-derived products were the only pest managing agents available (Owen, 2004). For self defense purposes, many plants generate chemicals that are toxic to pests and since these naturally occurring pesticides are derived from plants, they are called Botanicals (Okrikata and Oruonye, 2012). The potential of using plant material as deterrent against pest in crops, on the field and during post-harvest period is presently gaining acceptance as a result of the indiscriminate use of chemical pesticides which have given rise to many well-known problems, including genetic resistance of pest species, toxic residues in stored products, increasing cost of application, hazards from handling and environmental pollution.

The usefulness of plant materials in the treatment of diseases has been demonstrated to be as a result of the presence of certain chemical compounds in plants, which include flavonoids, alkaloids, steroids, tannins and saponins. Phytochemical compounds are non specific in their action and can exhibit several functions viz anti bacterial, antifungal, antiviral and anti-spasmodic (Ukwuani, Ihebunna, Samuel et al 2012).

In the present study four different parts (bark; flower, fruit and leaves) of *P. thonningii* (Schum.) were screened:

phytochemical test, chemo-microscopy and nutrient analysis were investigated. Camel's foot has been claimed on empirical grounds to have some pesticidal properties which have been used in Nigeria as an integral part of farming culture, but most of these claims have not been scientifically validated.

## 2. Materials And Methods

### 2.1 Plant Materials

The plant parts (Bark, flower, fruit and leaf) were obtained from the premises of the Federal College of Education Pankshin, Plateau State in the month of March 2013 and were identified and authenticated by the Department of Pharmacognosy, Faculty of Pharmacy, University of Jos where a herbarium specimen exists. Identification was further confirmed at the Department of Plant Science and Technology, University of Jos alongside internet photographs of the plants from a recognized website for plant identification. Phytochemical and chemomicroscopy analysis were carried out at the Pharmacological Laboratory, University of Jos while quantitative screening was done at the National Metallurgical Development Centre, Jos, Plateau State Nigeria.

### 2.2 Chemicals and Reagents

All the chemicals and solvents used in this study were standard and of analytical grade.

### 2.3 Preparation of Extracts

Fresh plant parts were collected, weighed and shade-dried. They were ground to fine powder using sterilized pestle and mortar. The powder was stored in air tight bottles.

### 2.4 Phytochemical Screening:

The fractions of various plant powder extracts were subjected to preliminary phytochemical screening to identify the secondary metabolites present. The methods of analysis employed were those described by Trease and Evans (1996); Harbone (1998) and Soforowa (1993). Using Conventional protocols for detecting the presence of alkaloids, tannins, carbohydrates, saponins, flavonoids, steroids, anthraquinones and cardiac glycosides.

### 2.5 Alkaloids:

A quantity (3ml) of 1% aqueous hydrochloric acid on a steam bath; 1ml each of the filtrate was treated with a few drops of Mayer's reagent, Dragendorff's reagent and picric acid solution. Precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract.

### 2.6 Anthraquinones:

Borntrager's test was used for the detection of anthraquinones. 1g of each powdered sample was taken into a dry test tube and 5ml of chloroform was added and shaken for 5 minutes. The extract was filtered, and filtrate shaken with an equal volume of 100% ammonia solution. A pink violet or red colour in the ammoniacal layer indicates the presence of free anthraquinones.

### 2.7 Carbohydrates:

100mg of each extract was dissolved in 3ml of distilled water and mixed with a few drops of Molisch reagent (10% solution of  $\alpha$ -naphthol in alcohol). Then 1ml of concentrated sulphuric acid was carefully added down the side of the inclined tube so that the acid forms a layer beneath the aqueous solution without mixing it. A reddish or violet ring at the junction of the liquids was observed indicating the presence of carbohydrate.

## 2.8 Cardial Glycosides:

Keller-Killani Test: Plant extract treated with 2ml glacial acetic acid containing a drop of  $\text{FeCl}_3$ . A brown colour ring indicates the presence of positive test.

## 2.9 Flavonoids:

1g of each powdered samples was completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone on a water bath. The mixture was filtered while hot. The filtrate was cooled and used for Shinoda test and Alkaline reagent test ( Sodium hydroxide test). In the first test, the extracts were treated with few drops of concentrated HCL and Magnesium ribbon. The appearance of pink or tomato red colour within few minutes indicated the presence of flavonoids, while in the later test, the extracts were treated with few drops of diluted sodium hydroxide (Na OH) separately. Formation of intense yellow colour which turned colourless on addition of few drops of diluted HCL indicated presence of flavonoids.

## 2.10 Saponins:

1g of each plant powder was shaken with water in a test tube and filtered. The filtrate was shaken vigorously and frothing which persist on warming was taken as preliminary evidence for the presence of Saponins.

## 2.11 Steroids:

1ml extract was dissolved in 10ml of Chloroform and equal volume of concentrated  $\text{H}_2\text{SO}_4$  acid was added from the side of test tube. The upper layer turns red and  $\text{H}_2\text{SO}_4$  layer showed yellow with green fluorescence. This indicated the presence of steroid.

## 2.12 Tannins:

1g of each plant powder was stirred with 1ml of distilled water and filtered, and ferric chloride reagent added to the filtrate. A blue-black, green or blue green precipitate was taken as evidence for the presence of tannins.

## 2.13 Chemo-microscopical Examination:

### 2.13.1 Test for Starch:

A small quantity of the powdered sample was mounted in a little N/50 iodine solution and observed under the microscope. A blue-black colouration indicated the presence of starch.

### 2.13.2 Test for Protein:

A small quantity of the powdered sample was placed on a glass slide and mounted using Millon's reagent. A dark-pink or red colour indicated the presence of protein.

### 2.13.3 Test for Oils:

A small quantity of the powdered sample was placed on a filter paper. The paper was then firmly used to wrap the content. The presence of translucent stain is indicative of fixed oils. The powder was also cleared and mounted in Sudan III solution. Red or pink colour indicated the presence of oil globules.

### 2.13.4 Test for Lignin:

A small quantity of the powdered sample was mounted in Phloroglucinol and allowed to dry. Then a few drops of concentrated HCL was added unto the slides and then viewed under the microscope. The presence of red or pink stained

structures indicated the presence of lignin.

#### 2.13.5 Test for Calcium Oxalates:

A small quantity of the powdered sample was mounted in Phloroglucinol and observed for bright structures of calcium oxalate crystals. Then a few drops of concentrated HCL was then added and presence or absence of effervescence observed under the microscope. The disappearance of the calcium oxalate crystals without effervescence indicated their presence.

#### 2.14 Quantitative Analysis:

The quantitative analysis of ground samples of leaves and flowers were carried out using an X-Ray fluorescence machine, Model: PWi 660, XRA, with the made: PHILIPS, ENGLAND. Powdered samples of 200 mesh or finer paste were used, and pressed into pellet for analysis. The X-ray fluorescence involved the emission of characteristic "secondary"x-. The machine automatically within a period of time gave out the analysis of each of the samples.



Plate 1: *P. thonningii* tree



Plate 2: *P. thonningii* fruit



Plate 3: *P. thonningii* bark



Plate 4: *P. thonningii* pink flower



Plate 5: *P. thonningii* white flower



Plate 6: *P. thonningii* leaves

### 3. Results

Preliminary phytochemical screening of eight different secondary metabolites (alkaloids, anthraquinones, carbohydrates, glycosides, flavonoids, saponins, steroids and tannins) were tested from four different parts of *P. thonningii* extracts. Thus out of (1x4x8=32) tests for the presence or absence of above compounds, 29 tests conferred positive results and the remaining 3 gave negative results. The results of phytochemical screening of different secondary metabolites of *P. thonningii* were illustrated in Table 1. The bark had high presence of carbohydrates and tannins; anthraquinones and flavonoids moderately present and the remaining slightly present. The flower had 3 of the metabolites highly present; 2 each moderately and slightly present respectively.

**Table 1:** Phytochemical screening of the various parts of *Piliostigma thonningii* (Schum.)

Secondary Metabolites	Plant parts			
	Bark	Flower	Fruit	Leaf
Alkaloides	+	—	+++	++
Anthraquinones	++	+	—	+
Carbohydrates	+++	+	++	++
Cardial Glycosides	+	+++	++	++
Flavonoids	++	+++	+++	+++
Saponins	+	++	—	+
Steroids	+	+++	+	+
Tannins	+++	++	+	++

Key: += Slightly present, ++= Moderately Present, +++= Highly Present. -= Not detected.

The result of chemo-microscopy of the bark, flower, fruit and leaf of *P. thonningii* (Table 2), revealed that protein was highly present in flower and fruit; Lignin highly present in fruit and moderately present in the other 3 parts. On the whole, four of the primary metabolites were present except fats/oils that were only detected moderately on fruit and absent on all the others.

**Table 2:** Chemo-microscopy of various parts of *P. thonningii* (Schum.).

Primary metabolites/ Constitutes	Plant parts			
	Bark	Flower	Fruit	Leaf
Starch	+	+	++	+
Protein	+	+++	+++	+
Fat/Oils	—	—	++	—
Lignin	++	++	+++	++
Calcium Oxalates	+++	+	++	+

Key: += Slightly present, ++= Moderately Present, +++= Highly Present. -= Not detected.

Investigation into mineral analyte concentration of the bark and leaf of *P. thonningii* revealed that 22 analytes were detected (Table 3), out of which 4 (P, Cr, Eu and Os) were not detected in the bark. The leaf was not found to contain 5 (Ru, Pd, Ce, Y and Zr). It revealed the high concentration of CaO 81.7% and 57.8% in bark and leaves respectively. This is followed by K<sub>2</sub>O 5.70% and 27.7% respectively.

**Table 3:** Mineral Analyte Concentration of *P. thonningii* bark and leaves.

Mineral Elements	Concentration/Plant Parts (%)	
	Bark	Leave
P (P <sub>2</sub> O <sub>5</sub> )	-	3.3
S (SO <sub>3</sub> )	0.49	3.7
K (K <sub>2</sub> O)	5.70	27.7
Ca (CaO)	82.7	57.8
Ti(TiO <sub>2</sub> )	0.47	0.3
Cr (Cr <sub>2</sub> O <sub>3</sub> )	-	0.17
Mn(MnO)	0.43	0.90
Fe(Fe <sub>2</sub> O <sub>3</sub> )	3.00	2.39
Ni(NiO)	0.11	0.18
Cu(CuO)	0.58	1.1
Zn(ZnO)	0.12	0.16
Sr(SrO)	0.49	0.29
Ba(BaO)	0.8	0.6
Eu(Eu <sub>2</sub> O <sub>3</sub> )	-	0.2
Re(Re <sub>2</sub> O <sub>7</sub> )	0.3	0.5
Os(OsO <sub>4</sub> )	-	0.2
Au (Au)	0.26	0.49
Ru(RuO <sub>2</sub> )	4.48	-
Pd(PdO)	0.87	-
Ce( )	0.05	-
Y(Y <sub>2</sub> O <sub>3</sub> )	0.15	-
Zr (ZrO <sub>2</sub> )	0.01	-

#### 4. Discussion

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "secondary metabolites" of which there are several classes including alkaloids, flavonoids, glycosides, tannins (Madara, Ajayi, Salawu et al, 2010; WHO, 2002; Ozolua&Alonge, 2008). They are present in a variety of plants and are utilized as important components. Botanists, microbiologists, and natural product chemists are searching the earth for phytochemicals which could be developed for treatment of pathogenic organisms especially in the light of the emergence of drug-resistant micro organisms and the need to produce more effective anti microbial agents (Jimoh and Oladeji, 2005; Asuzu&Nwaehujor, 2013 and Orji, et al. 2003). The results of the phytochemical analysis of extracts of *P. thonningii* revealed the presence of various primary and secondary metabolites at varying degrees in the parts studied which is suggestive of varying degrees of effectiveness of the parts under investigation in this regard.

Plants have evolved the ability to synthesize chemical compounds that help in fighting against a wide range of predators such as insects, fungi, and herbivorous mammals. These compounds are secondary metabolites (Daniyan&Abalaka, 2002). Unlike pharmaceutical drugs which are mainly synthetic, herbs are easily assimilated in the body, they are eliminated and do not usually accumulate, hence can be said to be relatively more suitable in the control of such pests especially as it relates to associated health hazards.

The discovery of biocontrol agents have been reported to begin with the discovery of a useful naturally occurring product (Chaube&Pundhir, 2005). In plants, high concentration of secondary metabolites tend to accumulate in specific cell types at specific developmental stages. Plants may contain compounds that are potential drug candidates or drug leads. *D-3-0-methylchiroinositol* has been isolated from the stem bark of *P. thonningii* (Asuzu&Nwaehujor, 2003). They reported that *D-3-0-methylchiroinositol*, being a natural product does not have the tendency to cause toxicity when used over a long period. The use of plants in traditional system is excellent proof of clinical efficacy and safety of medical plants.

In Nigeria, particularly the northern region, there is a long tradition of using medicinal plants for the treatment of pest/diseases as an integral part of farming culture. Some of these plants have been claimed, on empirical grounds to have somepesticidal properties. Efforts in recent times to identify plants with medicinal values tend to show that most plants in the tropical forest has one bioactive value or the other. The plants usually have active ingredients which may be found in a particular part of the plant or all over its body (Orji et al. 2003).

George and Pamplona-Roger (2006) documented that not every plant of the same species always produces

the same amount and concentration of active substances. Active substances are unequally distributed among the diverse parts or organs of a plant, because of the specialization of its cells. This may explain the varying concentrations of the active substances in the different plants parts in the present investigation. This serves as a guide as to which part of the plant should be used for a more effective result. Accordingly the present investigation presents Bark as having the highest potential followed by leaf, flower and fruit in that order for more promising result. Knowing that *P. thonningii* has bioactive properties is not enough, we must know which part of the plant should be used. These results also supplemented the previous observations (findings) and provided similarity and variations. Thus the present studies on *P. thonningii* (Schum.) produced novel plant resources as potential target useful naturally occurring products.

## 5. Conclusion

The results of the present study confirmed the presence of primary and secondary metabolites with varied degree of concentration. In addition to this, mineral analytes were also confirmed. Natural plant chemicals will undoubtedly play a significant role in the future of pest control and can significantly reduce amount of pesticides and thereby contribute to sustainable development of agriculture. More research work is recommended on the various plant parts for isolation and characterization of bioactive compounds that may be utilized for pest control of agricultural plants.

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