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The Proximate and Phytochemical Analyses of Aqueous Root Extract of *Piliostigma thonningii* and its Effects on Indices of Well-being in Experimental Animals

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ABSTRACT: Plants have constituted a significant quota to the sustainability of humans as food, medicines and raw materials for other uses. Increased awareness of the significance of medicinal plants led to more studies focused on the phytochemistry and proximate analysis of plants. The current study explored the proximate and phytochemical analyses of the aqueous root extract of *Piliostigma thonningii* and its effects on the haematology, hepatic function indices and histopathology of liver and kidney of male rabbits. The rabbits were administered 100 mg of the aqueous root extract of *P. thonningii*/kg body weight of rabbit once daily for twenty-two days. The sample had high carbohydrate and crude fibre contents of 55.63 % and 28.80 % respectively. The aqueous root extract of *P. thonningii* is rich in saponins, tannins and flavonoids. The extract caused no significant change in the concentration of leukocytes, erythrocytes, haemoglobin and platelets of the animals. *P. thonningii* significantly ($p < 0.05$) increased the level of total protein in the administered animals. The photomicrographs of the liver and kidney of some animals administered the aqueous root extract of *P. thonningii* revealed minor injuries in the organs examined, which were absent in the control group.

Keywords: Phytochemical analysis, proximate analysis, haematology, hepatic, histopathology

Introduction

Increased awareness of the significance of plants in the health and nutrition of humans necessitated the need for knowledge of the phytochemicals and nutrients present in their various organs. The phytochemicals contained in plants are mainly responsible for the definite physiological activities they exert on the human body (Ighodaro *et a*l., 2009). Many plants are known for their medicinal and nutritional usefulness, and one such is *Piliostigma thonningii*.

Piliostigma thonningii Schum is a leguminous plant that belong to the leguminosae-ceasalpiniodeae family (Ighodaro and Omole, 2012). It is native to tropical Africa and widespread in the Sudano-Guinean region from Senegal eastward to Eritrea, southward to Namibia, Botswana, Mozambique and South Africa. The name "Piliostigma" means "cap-like stigma", and the species "thonningii' is named after the Danish plant collector, Peter Thonningii, who collected the plant in present-day Ghana (Orwa *et al.*, 2009). *P. thonningii* is commonly called camel's foot in English and “kalgo”, “abafe”, “okpoatu”, “okhorior” and “nyihar” in Hausa, Yoruba, Igbo, Edo and Tiv tribes of Nigeria, respectively (Jimoh and Oladiji, 2005)

In the folklore, the roots and twigs of *P. thonningii* have been used as laxative, anthelminthic and anti-inflammatory agents and in the treatment of dysentery, fever, respiratory ailments, snake bites, hookworm and skin infections (Fakae *et al.*, 2000; Igoli *et al.*, 2005; Kwaji *et al*., 2010). The bark infusion is used to treat malaria, leprosy, diarrhoea, ear ache, intestinal problems, cough and other respiratory problems (Hutchinson *et* al., 1958; Burkill, 1995). The pods and foliage of *P. thonningii* are rich sources of antioxidants, micronutrients,

crude protein and carbohydrates and are relished by cattle, antelopes and elephants (Burkill, 1995; Pomeraz *et al.*, 2004). The inner bark is used to make rope for tying in huts, fences and bridge building, while the roasted seeds and root can also be used in dye production (Burkill, 1995). The leaves are used for wrapping food, and twigs and roots serve as toothbrushes. The dried fruit is used to smooth pottery, and the roots are used for glazing gourds. The bark provides a tanning material and yields a red-brown dye for cloth and wooden tools. The root yields a red-brown or black dye, and the fruit and seeds a black or blue one used as cosmetics (Burkill. 1995). The fruit is eaten as a snack or emergency food, especially by children and herders. The fresh leaves and flowers of *P. thonningii* can be chewed to relieve thirst. The unripe pods can be used as a soap substitute (Odukoya, 2002).

The leaf extract of *P. thonningii* have been used in the treatment of malaria, wounds, itching, snake bite, HIV, and Hepatitis B and C (Cowan, 1999; Kwaji *et al*., 2010). The antibacterial activity of the stem bark extract of *P. thonningii* against *Bacillus subtilis*, *Staphylococcus aureus*, *Shigella dysenteriea*, *Escherichia coli* and *Proteus* v*ulgaris* was reported by Akinpelu and colleagues (2000). D-3-O-Methylchiroinositol isolated from *P. thonningii* paralyzed about 60% of *Haemonchus contortus* larvae at 4.4 mg/mL within 24-hr contact (Asuzu *et al*., 1999). In addition, the root, bark and leaf extracts of *P. thonningii* were reported to exhibit antiviral activity against *Herpes Simplex* Virus Type 1 and 2, HIV and different strains of influenza and syncytial viruses (Silva *et al.*, 1997).

The present study focused on the phytochemical and the proximate analyses of the aqueous root extract of *P. thonningii* and the effect of this extract on haematology, liver function indices and histopathology of the liver and kidney tissues of normal male rabbits.

Materials and methods

Collection of plant: The roots of *Piliostigma thonningii* were collected from Auchi, Edo State, Nigeria. The roots were identified and authenticated by Dr. H. A. Akinnibosun, a plant taxonomist of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Preparation of aqueous extract

The roots were cut into smaller pieces, air-dried for two weeks and thereafter, pulverized using a milling machine. The pulverized sample (758 g) was soaked in 7.5 L of distilled water for forty-eight hours (48 h) with occasional stirring and filtered using Whatmann No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 60 °C. Thereafter, it was evaporated to dryness over a water bath to yield a dark brown extract and stored under refrigeration.

Proximate analysis

Moisture content: The moisture content of *P. thonningii* was determined according to the AOAC method (1990). Ten gram (10 g) of the extract was weighed in a pre-weighed ceramic crucible on an analytical balance and dried to a constant weight at 105 °C for 24 h in an oven. The sample was allowed to cool to room temperature in a desiccator and was accurately weighed to determine the dry weight. The loss in weight was attributed to moisture content. Moisture content was calculated as follows:

$$\% \text{ moisture} = \frac{W2-W3}{W2-W1} * 100\%$$

W1= Weight of empty crucible (g)
W2= weight of sample+ crucible before drying (g)
W3= Weight of sample+ crucible after drying (g)

Ash content: A crucible dish was cleaned, dried, ignited, cooled and weighed as W 1. The sample was weighed accurately and directly in the crucible, i.e. W2. The sample was dried in a boiling water bath and then charred over a hot plate in a fume cupboard until no more soot was given out. Then it was then ashed with a muffle furnace at 500^0C to obtain W3 (AOAC, 1990). Percentage ash is calculated as shown below:

$$\% \text{ Ash} = \frac{W3-W1}{W2-W1} * 100\%$$

W3= Weight of crucible + ash (g)
W1= weight of the crucible (g)
W2= Weight of crucible + sample before ashing (g)

Crude protein: Crude protein was determined using the Kjeldahl method (AOAC, 1990). One gram of the sample was weighed into the digestion flask and Kjedahl catalyst (5 selenium tablets) was added to the sample. About 20 mL of concentrated tetraoxosulphate VI acid was added to the sample and then fixed for 8 hours in the digestion unit (450^{o}C) of the Kjedahl apparatus in a fume cupboard. After cooling, the digest, pure yellow, changed into a colourless liquid that was transferred into a 100 mL volumetric flask and made up to mark with

distilled water. About 20 mL of 4% boric acid solution was pipetted into a conical flask. A drop of methyl red was added to the flask as an indicator. The sample was diluted with 75 mL of distilled water. About 10 mL of the digest was made alkaline with 20 mL of NaOH (20%) and distilled. The steam exit of the distillatory was closed, and the colour change of boric acid solution to green was timed. The mixture was distilled for 15 minutes (AOAC, 1990). The filtrate was then titrated against 0.1 N HCl. The protein content was calculated using:

$$\%\text{Nitrogen} = \frac{Titre\ value * normality\ of\ acid * 0.014}{weight\ of\ sample} * 100$$

Protein conversion factor = 6.25
Normality of acid (HCl) = 0.1 N
Sample weight = 1.0 g
Protein (%) = % Nitrogen x 6.25

Crude fibre: Ether extracted sample was refluxed first with 1.25 per cent sulphuric acid and subsequently with 1.25 per cent sodium hydroxide for 30 mins each to dissolve acid and soluble alkali components. The residue containing crude fibre and ash was dried to a constant weight. The weight loss on ignition in a furnace was calculated to express its crude fibre.

$$\text{Crude fibre (\%)} = \frac{loss\ in\ weight}{weight\ of\ sample} * 100$$

Fat content: Crude lipids content of the root of *P. thonningii* was determined according to a modified method originally recommended by AOAC (1990). A total of 5 g of the sample was weighed and put in a thimble using dry paper and plugged with cotton wool. The thimble was dried and inserted into a Soxhlet system. Two round-bottom flasks were dried and weighed, and then 200 mL of (petroleum ether) was added to each of them. The round-bottom flasks were inserted into the Soxhlet. The samples were defatted by refluxing with 200 mL petroleum ether for 3 hours in a boiling position. The defatted thimble and round-bottom flasks were then dried at 105°C for 24 h, cooled and reweighed.

$$\%\ \text{fat} = \frac{Weight\ of\ extracted\ crude\ fat}{weight\ of\ sample} * 100$$

Determination of carbohydrate content: The carbohydrate content was calculated as follows;

Carbohydrate content = 100- (% ash + % crude fibre + % crude protein + % fat + % moisture).

Qualitative phytochemical analyses: *P. thonningii* root extract (2 g) was boiled with 20 mL of distilled water in a water bath and filtered and it was screened for the presence of phytochemicals.

Test for alkaloids: Four protocols were employed in checking for the presence of alkaloids in *P. thonningii* root extract:

i) *P. thonningii* root extract (0.5 g) was dissolved in 5 mL dilute HCl in a steam bath and filtered (Harborne, 1973).
ii) One millilitre of the filtrate was treated with a few drops of Mayer's reagent, to give a cream or pale-yellow precipitate (Edeoga *et al.*, 2005).
iii) One millilitre of filtrate was treated with a few drops of Dragendoff's reagent, giving an orange precipitate (Edeoga *et al.*, 2005).
iv) Then, 1 mL of filtrate was treated with Wagner's reagent which gave a brown or reddish-brown precipitate (Edeoga *et al.*, 2005).

Test for tannins: To 1mL of the extract wad added few drops of 0.1% ferric chloride and observed for brownish green or a blue-black colouration (Edeoga *et al*., 2005).

Test for saponins: To 1mL of the filtrate was added 5 mL of distilled water in a test tube and shaken vigorously to obtain a stable, persistent froth. The frothing is then mixed with three drops of olive oil and observed for formation of an emulsion, which indicates the presence of saponins (Edeoga *et al.*, 2005).

Test for flavonoids: A few drops of 1% NH_3 solution are added to the aqueous extract of *the P. thonningii* sample in a test tube. A yellow colouration observed indicated the presence of flavonoid compounds (Edeoga *et al.*, 2005).

Test for cardiac glycosides: *P. thonningii* aqueous extract (5 ml) was mixed with 2 mL of glacial CH_3CO_2H containing one drop of $FeCl_3$. The above mixture is carefully added to the 1 mL of concentrated H_2SO_4 so that the concentrated H_2SO_4 is underneath the mixture. The formation of a brown ring indicates the presence of cardiac glycosides (Edeoga *et al*., 2005).

Quantitative phytochemical determinations

Determination of saponin content: Saponin content determination was done using the method of AOAC (1990). Two gram of the sample was folded into a thimble and inserted in a soxhlet extractor, and a reflux condenser was fitted on top. Extraction was done with acetone in a 250 mL capacity round-bottom flask for 3 hours, after which the apparatus was dismantled, and another 150 mL round-bottom flask containing 100 mL of methanol was fitted to the extractor and extraction was carried on for another 3 hours. The weight of the flask was taken

before and after the second extraction to note the weight change. At the end of the second extraction, the methanol was recovered by distillation, and the flask was oven-dried to remove remaining solvent from the flask. The flask was then allowed to cool, and the weight of the flask was taken. The saponin content of the sample was calculated thus:

$$\% \text{ Saponin} = \frac{Weight\ of\ saponin}{weight\ of\ sample} * 100$$

Determination of alkaloid content: The gravimetric method of Harborne (1973) was used to determine the total alkaloid content of the aqueous root extract of *Piliostigma thonningii*. Five gram of the sample was dispersed into 50 mL of 10% acetic acid solution in ethanol. The mixture was well shaken and allowed to stand for 4 hours before filtering, after which it was evaporated to one-quarter of its original volume. Ammonium hydroxide was added dropwise to precipitate the alkaloids. The precipitate was filtered off with a pre-weighed filter paper and washed with 1% ammonium hydroxide solution. The precipitate was oven dried for 30 minutes at 60 ^{o}C and reweighed. The alkaloid contents of the samples were determined by difference using the equation:

$$\% \text{ Alkaloid} = \frac{W2-W1}{W} * 100$$

where W = weight of sample; W1 = weight of blank filter paper; W2 = Weight of paper + precipitate.

Flavonoid content determination: One gram of the sample was weighed, 20 mL of ethyl acetate was added, then shaken for 5 minutes and filtered. Five millilitres of the filtrate was added to 5 mL of dilute ammonia. It was shaken for 5 minutes and the upper layer was collected. The absorbance at 490 nm was read and the percentage concentration of flavonoid was then extrapolated from the standard curve.

Reducing sugar content determination: One gram of the sample was weighed, macerated with 20 mL of distilled water and filtered. One millilitre of the filtrate was pipetted and 1 mL of alkaline copper reagent was added. It was boiled for 5 minutes and cooled. Phosphomolybdic acid reagent (1 mL) was added as well as 7 mL of distilled water. The absorbance was read at 420 nm and the percentage concentration then extrapolated from the standard glucose curve.

Glycosides content determination: Glycoside content was determined using the method outlined by (El-Olemy *et al*. 1994). One gram of the sample was weighed and macerated with 2.5 mL of 15 % lead acetate and filtered. Thereafter, 2.5 mL of chloroform was added and shaken vigorously. The lower layer was collected and evaporated to dryness. The residue was dissolved with 3 mL of glacial acetic acid and 0.25 mL of concentrated H_2SO_4 was then added. It was shaken vigorously and kept in the dark for 2 hours. The absorbance was read at 530 nm and yhe percentage concentration extrapolated from the standard glycoside curve.

Tannin content determination: Tannin content was determined using the method outlined by Van-Burden and Robinson (1981). To 1 g of the sample, 50 mL of methanol was added and then shaken thoroughly for 1 hour, after which the content was filtered. Five millilitres (5 mL) of the filtrate was pipetted, and 0.3 mL of 0.1 N ferric chloride ferric cyanide was added. The absorbance at 720 nm was taken. The percentage concentration was extrapolated from the standard tannin curve.

Animal study

Experimental design: Eighteen (18) Male New Zealand breeds of rabbits were obtained from the animal farm of the Faculty of Agriculture, University of Benin, Benin City Edo State, for the experiment. The animals were acclimatized to laboratory conditions for 14 days in the Department of Biochemistry's Animal House. Each rabbit was housed in a wooden cage. The animal room was well-ventilated and maintained at room temperature with 12 h of the natural light-dark cycle and allowed free access to food and water *ad libitum*. Good hygiene was maintained by constantly cleaning and removing faeces and spilt feeds from cages daily. The animals were handled following the guidelines of the European Convention for the Protection of vertebrate animals and other scientific purposes (ETS-123, 2005).

The rabbits were grouped into four according to their weights. The animals in groups A-C were administered 5 mL of the extract corresponding to 100 mg/kg body weight, while the control animals were administered 5 mL of distilled water orally.

- Control was given distilled water orally, once daily, for 22 Days.
- Group A was given 155.25 mg of the root extract of *Piliostigma thonningii* orally, once daily for 22 days, based on the average body weight of 1552.5 g of the animals.
- Group B was given 131.14 mg of the root extract of *Piliostigma thonningii* orally, once daily for 22 days, based on the average body weight of 1311.4 g of the animals.
- Group C was given 111.81 mg of the root extract of *Piliostigma thonningii* orally, once daily for 22 days, based on the average body weight of 1118.1 g of the animals.

Blood sample collection: The animals were sacrificed 24 h after the last administration of the aqueous root extract of *Piliostigma thonningii*. Blood was withdrawn by cardiac puncture from the rabbits under chloroform anaesthesia. Blood samples were dispensed in plain, sterile bottles and centrifuged at 3000 rpm for 10 min using UniScope Laboratory Centrifuge. The serum was aspirated to determine total protein and albumin levels. The

blood samples used for haematological analysis were dispensed in ethylene-diamine-tetraacetic acid (EDTA) bottles.

Haematology: Sysmex haematology analyzer was used to determine haematological parameters in the whole blood samples. The haematological analysis carried out by the haematology analyzer include direct measurement of white blood cell count (WBC), red blood cell count (RBC), Hematology (HGB), Hematocrit (HCT), Platelets (PLT), Neutrophil count (NEUT), absolute Lymphocytes count (LYM). The other parameters were derived which include mean cell volume (MCV), mean platelet volume (MPV), percentage lymphocyte (LMP %), RBC distributor width – standard deviation (RDW-SD), red blood cell (RBC) distribution width-coefficient of variation (RDW-CV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR) (Campbell and Neil, 2008).

Collection of organs: The (organs) liver and kidney of the rabbits were excised, blot-dried and fixed in 10 % buffered formalin for routine histological techniques.

Histological study: The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 7 microns thick were obtained using a rotatory microtome. The paraffined sections were stained routinely with haematoxylin and eosin (Drury *et al*., 1976). Photomicrographs of the stained organ sections were obtained using a photographic research microscope in the Department of Chemical Pathology, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria.

Biochemical Assays: The methods of described by Tietz, (1976) were used to determine the concentration of bilirubin and the activities of ALT and AST. The bromocresol purple (Lasky *et al*., 1985) and biuret methods (Okutucu *et al*., 2007) were used to determine the concentration of albumin and total protein in the serum.

Data analysis: The data obtained in this study were expressed as Mean ± SEM. Differences between groups were tested by one-way analysis of variance (ANOVA), and the means were compared using Duncan's multiple range test with $p < 0.05$ significance level.

Results

The proximate analysis of *P. thonningii* (Table 1) shows that the sample is high in carbohydrates and crude fibre, and low in moisture and crude fat contents.

The phytochemical analysis of the aqueous root extract of *P. thonningii* revealed that it is rich in saponins, tannins and flavonoids (Table 2).

Table 1: Proximate analysis of aqueous root extract of *P. thonningii*

Parameters	**Percentage Yield (%)**
Total ash content	7.20 ± 0.03
Carbohydrate	55.63 ± 0.37
Crude fibre	28.80 ± 0.05
Crude protein	5.07 ± 0.00
Lipid content	1.80 ± 0.05
Moisture content	1.50 ± 0.03

Values are expressed as Mean ± SEM, n=3

Table 2: Phytochemical analyses of the aqueous root extract of *P. thonningii*

Phytochemical	**Qualitative**	**Quantitative (%)**
Saponins	+++	33.83 ± 0.36
Tannins	++	18.47 ± 0.64
Alkaloids	+	0.49 ± 0.01
Flavonoids	+	13.90 ± 0.22
Cardiac glycosides	+	6.33 ± 0.72
Reducing sugar	++	21.33 ± 0.54

Values are expressed as Mean ± SEM (n=3).

Table 3 depicts the effect of the aqueous root extract of *P. thonningii* on the haematological parameters of the experimental animals. There was no significant difference ($p > 0.05$) in the levels of the parameters in the animals administered with the extract of *P. thonningii* and the normal control.

Table 3: Effect of aqueous root extract of *P. thonningii* on haematological indices of normal male rabbits

Parameter	Control	Group A	Group B	Group C
WBC (1000 ul)	4.87 ± 1.69[a]	7.26 ± 1.67[a]	8.73 ± 1.68[a]	9.13 ± 1.83[a]
Lymphocytes (1000 ul)	2.10 ± 0.15[a]	2.10 ± 0.48[a]	1.93 ± 0.55[a]	3.30 ± 1.36[a]
Monocytes (1000 ul)	0.63 ± 0.20[a]	0.90 ± 0.10[a]	0.90 ± 0.15[a]	0.90 ± 0.15[a]
Granulocytes (1000 ul)	2.17 ± 1.44[a]	4.30 ± 1.13[a]	5.90 ± 1.92[a]	4.90 ± 2.47[a]
RBC (ul)	4.55 ± 0.25[a]	5.77 ± 1.07[a]	6.05 ± 0.70[a]	5.74 ± 0.26[a]
HGB (g/dl)	9.67 ± 0.92[a]	11.37 ± 1.93[a]	12.63 ± 1.62[a]	11.40 ± 0.93[a]
HCT (%)	33.20 ± 3.76[a]	38.00 ±7.12[a]	40.07 ± 5.13[a]	40.07 ± 3.85[a]
MCV (fl)	72.60 ± 5.40[a]	64.88 ± 1.23[a]	65.93 ± 1.33[a]	69.70 ± 5.50[a]
MCH (pg)	21.20 ± 1.66[a]	19.58 ± 0.40[a]	20.80 ± 1.10[a]	19.80 ± 1.05[a]
MCHC (g/dl)	29.30 ± 1.51[a]	32.18 ± 1.92[a]	31.50 ± 1.08[a]	28.50 ± 0.84[a]
RDW (%)	16.67 ± 0.75[a]	15.94 ± 0.46[a]	16.33 ± 0.60[a]	19.43 ± 1.09[b]
Platelet (ul)	246.33 ± 90.82[a]	364.80 ± 67.90[a]	275.67 ± 56.52[a]	453.00 ± 49.37[a]

In Table 4, there was a significant ($p < 0.05$) increase in the level of total protein of the groups administered the *P. thonningii* extract when compared with the control group. Though, there was no significant difference ($p > 0.05$) in the level of albumin in the groups administered with *P. thonningii* when compared with control group, a significant ($p < 0.05$) increase was observed in the level of globulin in the groups administered extract when compared with control.

Table 4: Effect of aqueous root extract of *P. thonningii* on the level of total protein, albumin and globulin in male rabbits

Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Control	5.07 ± 0.61[a]	4.52 ± 0.22[a]	0.55 ± 0.39[a]
Group A	6.00 ± 0.32[ab]	4.15 ± 0.27[a]	1.84 ± 0.45[b]
Group B	6.96 ± 0.54[b]	4.47 ± 0.18[a]	2.49 ± 0.53[b]
Group C	5.97 ± 0.[27ab]	3.95 ± 0.41[a]	2.02 ± 0.47[b]

Values are expressed as Mean ± SEM (n=3). Values with different superscripts significantly differ from the control in a column at $p < 0.05$ significance level.

The activities of ALT and AST are shown in Table 5. There was no significant difference ($p > 0.05$) in the activity of ALT in the groups administered with the extract of *P. thonningii* and that of the normal control. There was, however, a significant increase ($p < 0.05$) in the activity of AST in the groups administered the root extract of *P. thonningii* when compared with the control.

Table 5: Effect of aqueous root extract of *P. thonningii* on the activity of alanine aminotransferase and aspartate aminotransferase

Groups	Alanine aminotransferase (U/L)	Aspartate aminotransferase (U/L)
Control	32.33 ± 1.67[a]	19.67 ± 9.94[a]
Group A	30.60 ± 4.09[a]	37.60 ± 8.53[b]
Group B	29.67 ± 4.67[a]	20.00 ± 4.04[a]
Group C	27.00 ± 7.57[a]	28.00 ± 6.80[ab]

Values are expressed as Mean ± SEM. Values with different superscripts in a column are significantly different ($p < 0.05$) from one another.

The levels of total bilirubin, conjugated bilirubin and unconjugated bilirubin are shown in Table 6. There was a significant increase ($p < 0.05$) in the levels of total, conjugated and unconjugated bilirubin in the groups treated with the aqueous extract of *P. thonningii* when compared with control.

Table 6: Effect of aqueous root extract of *P. thonningii* on the level of total bilirubin, conjugated bilirubin and non-conjugated bilirubin

Groups	Total bilirubin (mg/dl)	Conjugated bilirubin (mg/dl)	Non-conjugated bilirubin (mg/dl)
Control	0.50 ± 0.06[a]	0.27 ± 0.03[a]	0.23 ± 0.03[a]
Group A	1.08 ± 0.17[b]	0.82 ± 0.20[b]	0.26 ± 0.04[a]
Group B	0.83 ± 0.27[b]	0.40 ± 0.25[ab]	0.43 ± 0.24[b]
Group C	1.00 ± 0.44[b]	0.37 ± 0.12[ab]	0.63 ± 0.31[b]

Values are expressed as Mean ± SEM. Values with different superscripts in a column are significantly different from one another at $p < 0.05$ significance level.

The histopathological examination of the liver and kidney tissues of the animals administered with the aqueous extract of *P. thonningii* revealed minor alterations when compared with the control (plates 1-8).

Histopathology results

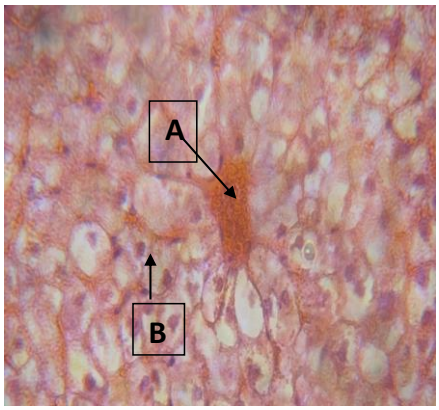

Plate 1: Section of rabbit Liver (control) shows normal central vein (A) and hepatocytes (B) H&E, X400

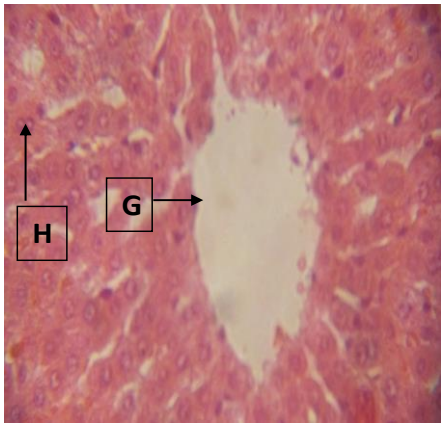

Plate 2: Section of rabbit liver (Group A) showing central vein (G) and hepatocytes (H) H&E, X400

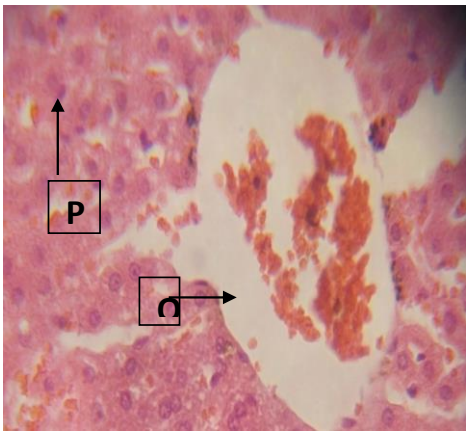

Plate 3: Section of rabbit Liver (Group B) showing central vein (O) and hepatocytes (P). H&E, x400

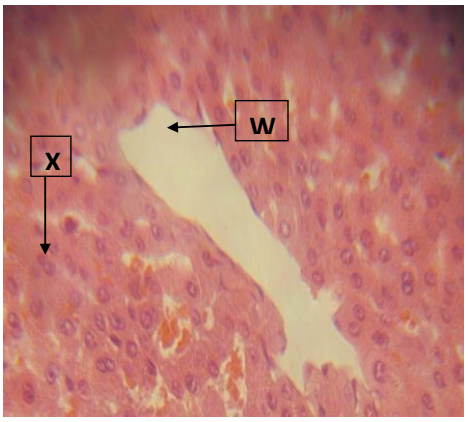

Plate 4: Section of rabbit Liver (Group C) showing central vein (w) and hepatocytes (x). H&E, x400

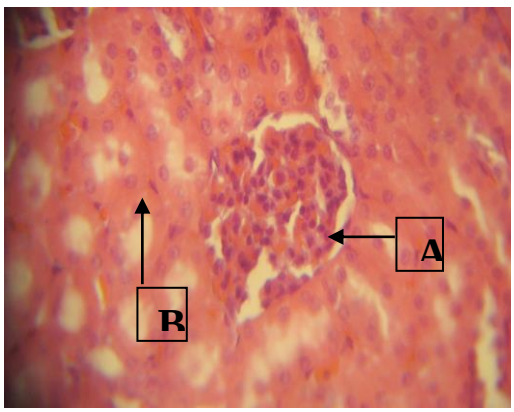

Plate 5: Section of rabbit Kidney (Control) shows normal kidney with glomerulus (A) and convoluted tubule (B) H&E, x 400

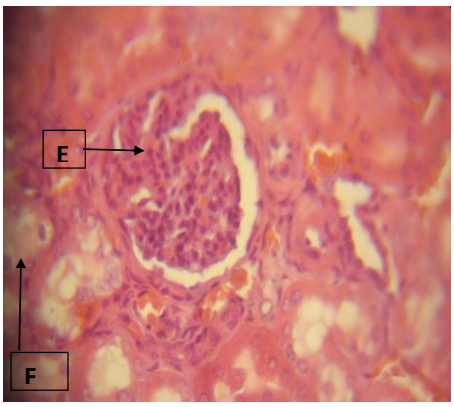

Plate 6: Section of (Group A) rabbit kidney showing glomerulus (E) and convoluted tubule (F) H&E, x400

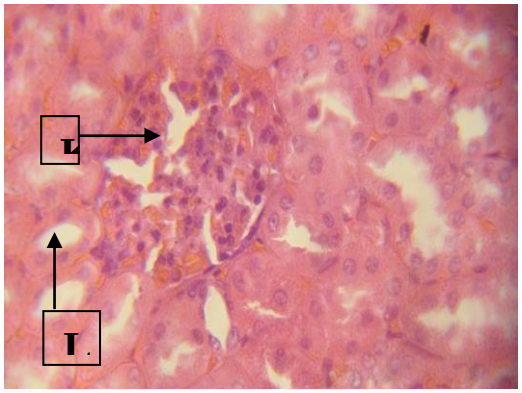

Plate 7: Section of (Group B) rabbit Kidney 0.13mg root extract given. Glomerulus (K) convoluted tubule (L) H&E, X 400

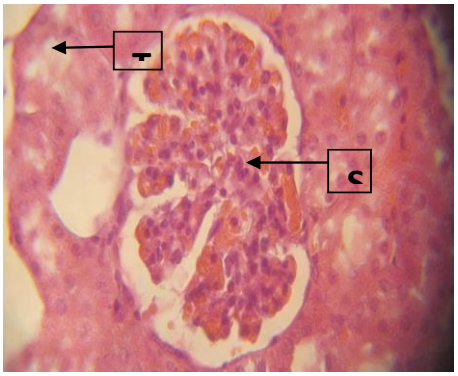

Plate. 8: Section of (Group C) rabbit Kidney showing glomerulus (S) convoluted tubule (T) H&E, X400

Discussion

The proximate analysis revealed that the root of *P. thonningii* is rich in carbohydrates and crude fibre, moderately low in ash and protein, whereas lipids and moisture are present in minute quantities. This root sample could serve as a source of energy and nutrients for herbivores. This plant may have longer shelf life than most plants because of its low moisture content. The *P. thonningii* seed was reported to have lower moisture content than most legume seeds (Giani, 1993; Temple and Bassa, 1991). The root of *P. thonningii* has low fat and high fibre content. Crude fibre enhances digestion, though, on the other hand, a high level of fibre in the diet can lead to lower digestibility, intestinal irritation and decreased absorption of nutrients (Oyenuga and Fetuga, 1975).

The phytochemical analysis of the aqueous root extract of *P. thonningii* revealed the presence of flavonoids, saponins, tannins, alkaloids and cardiac glycosides. Phytochemicals have been implicated in various medicinal and pharmacological properties of plants. Saponins have anticarcinogenic, immuno-modulatory, antifungal and cholesterol-lowering activities and regulate cell proliferation (Seigler, 1998; Sodipo *et al.*, 1991). Some saponins are cardiotonics and others are contraceptives and precursors for sex hormones (Evans, 2002). Cardiac glycosides are inhibitors of Na^+/K^+ ATPase in the heart (Koh *et al.*, 2017). Inhibition of the Na^+/K^+ pump leads to an increase in the level of Na^+ in the myocytes and a rise in the Ca^{2+} level, thus, increasing the level of Ca^{2+} available for the contraction of the heart muscle. Thus, improving cardiac output and reducing distension of the heart; hence, cardiac glycosides are used in the treatment of heart failure and arrhythmia (Hauptman and Kelly, 1999). Tannins possess diuretic, antibacterial, antiviral and anti-tumour activities (Dulay and De Castro, 2016). Plant tannins are commercial sources of tannic acids and tanning agents (Evans, 2002). Flavonoids are immunomodulatory, anti-allergic, anti-inflammatory, antiviral and anti-cancer agents (Evans, 2002).

The effect of plant extracts on the haematological parameters of experimental animals is used to evaluate their toxicity or haematopoietic property (Yakubu *et al.*, 2008). The extent of harm caused by the extracts on the blood cells of the animals is an index of their toxic effect (Yakubu *et al.*, 2007). The Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Volume (MCV) give information about individual red blood cells, while haemoglobin (Hgb), red blood cell (RBC) and packed cell volume (PCV) or haematocrit (HCT) provide information about the total population of red blood cells in the blood (Campbell and Neil, 2008). The aqueous root extract of *P. thonningii* caused no significant increase ($p > 0.05$) in the concentration of leukocytes, erythrocytes and haemoglobin.

The total protein is a valuable indicator of the synthetic function of the liver (Katharine and Charles, 2001). The total protein level of the groups administered the extract was significantly different from the control group ($p < 0.05$). There was no significant difference between the levels of serum albumin of the treatment groups and that of the control group ($p > 0.05$). There was a significant ($p < 0.05$) increase in the globulin level of the administered groups when compared with that of the control.

The activities of ALT and AST, along with other enzymes and parameters, are used in diagnosing liver diseases (Hall and Cash, 2012). In this study, the activity of alanine aminotransferase (ALT) in the serum of the rabbits administered *P. thonningii* extract was not significantly different from that of the control group ($p > 0.05$). Significant increases ($p < 0.05$) were observed in the activity of AST in groups A and C when compared with that of the control group.

The serum bilirubin levels of the rabbits administered the plant extract was significantly different from that of the control group ($p < 0.05$). The highest level of total bilirubin was observed in group A, which had the highest average weight of the four groups. This slight increase was probably due to the weight of the animals in this group (Salvaggio *et al*., 1991). Bilirubin is known to be an important antioxidant in the serum (Stocker *et al*., 1987). In epidemiological studies, mild increases in the level of bilirubin gave rise to low risk of some cardiovascular diseases and better receipt of organ transplant (Adin, 2021)

The photomicrographs of the liver and kidney of animals administered the aqueous root extract of *P. thonningii* revealed minor injuries. The control group had typical features in the liver and kidney. The kidney micrograph of group A showed essentially normal glomerulus and convoluted tubules and the liver also showed normal hepatocytes and central vein. However, slight histological changes were seen in groups B and C. Although no alteration was seen in the liver of group C, kidney damage is suspected as an increase in the size of the glomerulus was observed, and there was mild congestion of the intraglomerular space.

Conclusion

The proximate and phytochemical analyses of the aqueous root extract of *P. thonningii* have revealed the nutritional and physiological bases of *P. thonningii.* The administration of the aqueous root extract of *P. thonningii* resulted in an insignificant change in the haematological parameters and significant increases in the level of total protein and total bilirubin level in the rabbits. Minor injuries were observed in the photomicrographs of the liver and kidney of the rabbits administered the aqueous root extract of *P. thonningii;* hence the use of high doses of the plant is discouraged.

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