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Original Research Article

Piliostigma thonningii (Schum.) Milne-Redh*:* GC-MS profiling, *In vitro, In vivo* Antioxidant Potential and Toxicological Assessment in Female Wistar Rats

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ABSTRACT

Medicinal plants showed promising prospects in managing various diseases due to their rich bioactive content with *Piliostigma thonningii* being a notable example. This study evaluated the plant's *in vitro, in vivo* antioxidant potential, GC-MS profiling of its crude extract as well as the toxicological assessment to ascertain the plants toxicological profile in female wistar rat for a putative lead against uterine fibroids. GC-MS analysis identified 31 compounds, with 1,3-Dioxane-2-pentadecyl- (47.03%) as the most abundant. *In vitro* assays revealed that the crude extract (CE), chloroform (ChF), & ethyl-acetate fractions (EaF) demonstrated high Ferric reducing antioxidant power (FRAP) & 1,1- diphenyl-2-picrylhydrazyl (DPPH) activities, while n-hexane (HF) & aqueous fractions (AqF) presented greater Cupric ion Reducing Antioxidant Capacity (CUPRAC) activity. CE and EaF demonstrated low IC_{50} values for FRAP, while CE and ChF gave the lowest IC_{50} values for DPPH and finally, HF, showed better CUPRAC compared to the standard. Acute toxicity assessment of the plant's crude extract was evaluated at varying doses of 500, 1000 and 2000 mg/kg body weight and the results showed no significant changes in endogenous antioxidant enzymes, suggesting the plant does not induce oxidative stress in rats. The extract was not toxic to both kidney and liver as reported in previous studies. Histological analysis revealed normal ovarian architecture at 500 and 1000 mg/kg but tissue distortion at 2000 mg/kg. *P. thonningii* leaves exhibit high antioxidant activity, aligning with previous reports, and showed no oxidative stress to female wistar rats except at high dose (2000mg/kg b.w). Its high antioxidant potential maybe useful in managing oxidative stress associated with uterine fibroids.

***Keywords*:** *Pilostigma thonningii*, Fibroids, Antioxidants, Medicinal Plants, GC-MS.

Introduction

In the past, medicinal plants (Mps) have been employed in traditional medical practices as an all-natural cure for various ailments. Additionally, because these Mps contain active constituents that can be used as agents in the manufacture of drugs makes them useful raw materials by the pharmaceutical industry.[1] According to an account of the World Health Organization (WHO), 80 % of the world's population, including a large proportion of youth, are turning their gaze towards Mps for their main healthcare [2] thereby increasing the need for more research on Mps as a powerhouse of potential therapeutic agents. These plants are widely utilized and are often preferred due to their easy availability, low cost, perceived safety, effectiveness, and cultural significance, which can lead to a reliance on them for healthcare.[3] One important aspect for utilizing Mps is in treating ailments such as uterine fibroids brought about as a result of oxidative stress which could be managed by altering the oxidative stress/antioxidant ratio by either the use of dietary and/or pharmaceutical antioxidants as well as using medicinal plants with antioxidant activity. Oxidative stress a condition marked by the disparity between pro-oxidants and antioxidants [4] is commonly produced during normal metabolic processes due to high production of reactive nitrogen or oxygen species (RNS/ROS). [5]

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These radicals can be scavenged by the endogenous antioxidant system, maintaining the equilibrium between oxidation and anti-oxidation. [6] However, exposure to environmental stressors can trigger the production of increased levels of ROS and RNS, potentially bring about redox imbalance in addition to contributing to the etiopathogenesis of chronic and degenerative ailments.[6, 7] Additionally, exogenous antioxidants derived from medicinal plants could be harnessed to augment the work of the endogenous antioxidant thereby providing great potential to combat these free radicals generated within the body. Several Nigerian Mps containing bioactive compounds with significant antioxidant potential have been identified.[8, 9, 10, 11, 12] Beyond their antioxidant properties, these plants also exhibit other therapeutic potential which informed their usage in managing disease conditions.[13, 14, 17] *Piliostigma thonningii* is one of such Mps, commonly utilized in Nigerian ethno-medicine for treating various ailments such as boils, malaria, sterility, ulcers, and wounds.[14, 15, 16, 17] It is also employed as a remedy for dysentery, fibroids, syphilitic cancers, wound infections, fever, diarrhea, skin conditions, and cough.[18, 19] Crude extracts from *P. thonningii* have demonstrated anti-inflammatory, antibacterial,[20] antimicrobial,[13, 17] and antihelminthic[19] activities, also its activity against *Leishmania donovani* and *Trypanosoma brucei brucei* have also been recorded [21] as well as its antilipidemic activity.[14] Its toxicological profile has been reported in kidney[22, 23, 24] and liver[25, 26, 27] at different doses in both male and female rat models and in all, the studies revealed *P. thonningii* to be protective to both liver and kidney at varying doses. Phytochemical analysis of the plant has revealed various class of compounds, such as carbohydrates, tannins, flavonoids, terpenes, alkaloids, saponins, diterpenes, volatile oils, and kaurane, thought to bring about the therapeutic effects of the plant.[17, 65, 28, 29, 30] Its activity in addressing female gynecological conditions such as uterine fibroids has not yet been reported. Therefore, it is crucial to investigate its antioxidant potential as well as assess its toxicity in female rats, with the aim of evaluating its effectiveness in managing oxidative stress associated with uterine fibroids.

Materials and Methods

Plant material

Fresh leaf of *Piliostigma thonningii* (Schum) Milne-Redh was harvested in May, 2024 from a farmland along Zino block industry, Kuchikau, Karu local government area of Nasarawa state Nigeria. Plant authenticated was done at the herbarium unit of Nigerian Institute of Pharmaceutical Research and Development (NIPRD). A voucher specific number (NIPRD/H/7362) was given and deposited in the herbarium.

Crude extract preparation

The fresh leaves of *P. thonningii* were chopped, air-dried at room temperature and grinded into powder, and further air dried. The powdered samples were extracted following the method of Sumaiyah et al.[31] 2 kg of plant powder was macerated in 5 L of 96 % ethanol for 72 hours with intermittent stirring, then filtered using a cheese cloth and then finally filtered using Whatman No. 1 filter paper. The obtained filtrate was concentrated into a slurry using RE52A rotary evaporator (PEC Medical) to obtain a viscous ethanol crude extract.[31] Until it was required, the yield was preserved at 4 °C.

Plant fractionation

Plant crude extract (CE) was prepared using solvent/solvent separating method with 300 ml distilled water (aqueous solvent) and the organic solvents; n-hexane, chloroform, as well as ethyl acetate. Fractionation was done using Hostettmann[32] method with a few modifications. The crude extract of the plants was initially dissolved in 300 ml of distilled water and then poured into a separating funnel and partitioned using a total of 900 ml each of n-hexane, chloroform, ethyl acetate in a divided volume of 300 ml to give HF, ChF, EaF and finally AqF.

Chemical compounds identification by GC-MS profiling of Piliostigma thonningii ethanol crude extract (CE)

GC-MS analysis of the CE was done using the method of Anyanwu et al.[33] The analysis utilized an Agilent GC 7890B system with an MS detector (MSD 5977A, Agilent Technologies, USA), integrated with the MassHunter software (NIST 14.L library) to examine the CE of the plant samples. Detection was achieved via an electron ionization system set to 70 eV, with helium as the carrier gas flowing at the rate of 1 mL/min. The oven and inlet temperatures were maintained at 100 °C and 250 °C respectively for 1.5 minutes. The oven temperature was then gradually elevated to 270 °C at a rate of 5 °C per minute. Exactly, one microliter of dilute sample (1:100, v/v in suitable solvents) was injected for the analysis, and the scan range was set between 40 and 600.

In vitro antioxidant assay

FRAP determination

The method of Benzie and Strain[34] was used for determining FRAP with slight modification. The assay was performed on a 96 well micro titer plate by adding 150 µl of FRAP reagent to 5 µl of the samples. The reaction was incubated for 4 mins at 37 ºC and absorbance was read at 593 nm against reagent blank at varying time up to 30mins using Kayto RT 2100C Micro plate Reader. Afterwards, the percentage antioxidant activity was assessed in comparison with a positive control, which was a standard solution of ascorbic acid (5 mg/ml).

Determination of DPPH free radical scavenging ability

With a few modifications, the DPPH assay was done following the procedure outlined by Xu and Chang.[35] 180 µl of a 0.2 mM DPPH solution was combined with 50 µl of each plant extract and fraction. As the negative control, 50 µl of methanol was utilized, while the positive control was ascorbic acid at a concentration of 5 mg/ml. The Kayto RT 2100C Microplate Reader was used to measure absorbance at 492 nm after the solution had been left in the dark for 30 minutes.

CUPRAC determination

A modification of Apak et al.[36] was adopted for the determination of CUPRAC. Exactly 50 µl of Cu (II), Nucuproine (Nc), NH_4Ac buffer, & a total volume of 50 µl of antioxidant solution + Ethanol was added into a 96 wells micro titer plate. This was then measured at A450 against a reagent blank after 30 min of reagent addition using Kayto RT 2100C Micro plate Reader.

In vitro antioxidant inhibitory activity

The *In vitro* antioxidant inhibitory activities for FRAP, DPPH, and CUPRAC were conducted on the samples that exhibited 50% antioxidant activity. These assays were performed according to the standard methods for FRAP, DPPH, and CUPRAC at various concentrations, specifically 0.0391, 0.0782, 0.1564, 0.3128, 0.626, 1.25, 2.5, and 5 µg/ml.

In vivo antioxidant assay

Experimental Animals

For this investigation, twenty-four female Wistar rats that were not pregnant weighing between 150 and 200 grams each were chosen. The animals were housed in cages at the animal-care facility of Bingham University, Karu, Nasarawa State, after being purchased from the National Veterinary Research Institute (NVRI) in Vom, Plateau State, Nigeria. For two weeks before induction, they were allowed to adjust to a 12-hour light/dark cycle at ambient temperature, with unfettered supply of water & food.

All animal procedures were conducted in accordance with the *Principles of Laboratory Animal Care* by NIH with publication No. 85-23, which was revised in the year 1985. Approval was by the Bingham University Health Research Ethics Committee (Approval No. NHREC/21/05/2005/01406).

Experimental Design

A total of four groups of six rats each were set up from the rats. Group 1 (normal control) was not given any treatment and was allowed unlimited access to food and water. The crude extract was given at doses of 500, 1000, and 2000 mg/kg body weight to groups two, three, and four, respectively. Treatment was administered orally using a gavage tube. This was done for 28 days with body weight taken weekly before each administration.

Sample Collection

After a 12-hour fasting period following the final administration, the rats were anesthetized and humanely euthanized in accordance with ethical guidelines. Blood samples were collected via cardiac puncture into plain collection tubes. Following sample collection, the samples were centrifuged (time: 10 mins, rpm: 15, 000g), serum obtained, portioned and stored at -80 °C until needed. Ovaries were excised, weighed, measured, and comparisons were made between treatment and control groups. The tissues were then fixed in formalin for subsequent histological evaluation.

Measurement of serum antioxidant enzymes

The catalase assay was based on the reaction of ammonium molybdate, which generates a yellow complex observable at 405 nm, the breakdown rate of hydrogen peroxide ($H2O_2$) was observed in order to assess the catalase (CAT) activity spectrophotometrically[37]. Li's [38] method, which compares the dismutation of pyrogallol by SOD with its autoxidation by $O_2^{\bullet-}$, was used to measure the activity of superoxide dismutase (SOD). At 420 nm, the SOD-induced suppression of pyrogallol autoxidation was seen following the addition of a SOD chromogen solution. Ellman's method, which uses Ellman's reagent (5',5′-dithiobis-(2-nitrobenzoic acid)) to react with sulfhydryl (SH) groups to form a persistent yellow chromophore, 2-nitro-5-mercaptobenzoic acid, measured at 412 nm, was used to spectrophotometrically assess reduced glutathione (GSH) levels.[39] By measuring the amounts of malondialdehyde (MDA) using the thiobarbituric acid (TBA) reagent in an acid-heating reaction at 25 °C, lipid peroxidation (LPO) was evaluated. The MDA-TBA complex, a condensation product of the Knoevenagel type, is produced by this reaction and was detected at 532 nm.[40]

Histopathological analysis

Histopathological examination of the ovaries was conducted to evaluate changes in tissue morphology and identify any histological alterations resulting from the treatments. The ovaries were preserved in 10% buffered formalin for 24 hours. A Surgcare Microtome (Model 335A, USA) was used to cut 3 µm sections from the paraffin mass after

dehydration with three ethanol changes, clearing in xylene, and embedding in molten paraffin. Following deparaffinization, histological alterations were assessed after staining the tissue sections with hematoxylin and eosin. The observed changes were compared to those in the normal control group.[41]

Statistical Analysis
Mean ± S.E.M. was used to express all collected data. Percentage inhibition was performed with Graph-Pad Prism 8.0. One Way ANOVA was used to examine the statistical significance difference in parameters amongst the groups using SPSS version 26. $P<0.05$ was deemed significant.

Results and Discussion

Chemical compounds identified by GC-MS profiling of Piliostigma thonningii leaves ethanol crude extract (CE)
The GC-MS analysis of *P. thonningii* leaves CE is presented in Table 1. The result showed that thirty-one (31) compounds were identified from the CE. The most abundant compounds are: 1,3-Dioxane, 2-pentadecyl- (47.03 %), 9,17-Octadecadienal, (Z)- (7.03 %), and Linoelaidic acid (5.62 %). (R)-(-)-14-Methyl-8-hexadecyn-1-ol was the least abundant (0.48 %).

Table 1: Compounds detected in *P. thonningii* CE fraction using GC-MS

PK	RT	AREA%	COMPOUNDS
1	4.70	0.76	1,4-Benzenediol
2	6.87	0.63	Butanedioic acid, hydroxy-, diethyl ester
3	7.50	0.49	2-Methoxy-4-vinylphenol
4	7.89	0.77	Butanedioic acid, 2,3-dihydroxy-diethyl ester, [S-(R*,R*)]
5	9.68	1.23	.beta.-D-Glucopyranose, 1,6-anhydro-
6	10.88	1.31	1,3-Benzenediol, 4-propyl-
7	12.41	47.03	1,3-Dioxane, 2-pentadecyl-
8	12.70	2.33	9,12-Tetradecadien-1-ol, (Z,E)-
9	12.95	5.62	Linoelaidic acid
10	13.32	7.03	9,17-Octadecadienal, (Z)-
11	13.64	0.52	Cyclopropaneoctanal, 2-octyl-
12	13.82	2.68	n-Hexadecanoic acid
13	14.03	2.87	Undecanoic acid, ethyl ester
14	14.41	0.61	9,12-Octadecadienoic acid (Z,Z)-
15	14.61	2.67	2-Methyl-Z,Z-3,13-octadecadienol
16	14.88	1.26	9,12-Octadecadienoic acid (Z,Z)-
17	14.97	0.86	2-Methyl-Z,Z-3,13-octadecadienol
18	15.23	5.36	Oleic Acid
19	15.39	3.63	9-Eicosyne
20	15.59	1.71	Octadecanoic acid, ethyl ester
21	15.91	1.29	9,12-Octadecadienoic acid (Z,Z)-
22	16.32	0.96	9,17-Octadecadienal, (Z)-
23	16.43	0.80	9,12-Octadecadienoic acid (Z,Z)-
24	16.77	0.80	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
25	16.95	0.48	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
26	17.22	1.63	Undecanoic acid, ethyl ester
27	17.55	2.11	2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane
28	18.11	0.60	13-Tetradece-11-yn-1-ol
29	19.00	0.63	9,12-Octadecadienoic acid (Z,Z)-
30	19.28	0.64	9,12-Octadecadienoic acid (Z,Z)-
31	19.53	0.70	Phthalic acid, cyclohexyl 2-pentyl

In vitro Antioxidant activity
FRAP antioxidant potential of CE and fractions of P. thonningii
Figure 2 displays the FRAP antioxidant capacity of the ethanolic CE and fractions of *P. thonningii*. The CE exhibited the highest activity at 95.97%, while the HF recorded the least value (5.38%). All other fractions demonstrated less than 50% activity compared to the standard, which showed 100.00% FRAP antioxidant activity.

DPPH antioxidant potential of CE and fractions of P. thonningii
Figure 3 illustrates *P. thonningii*'s CE and its various fraction's DPPH antioxidant potential. CE, HF, ChF, and EaF gave high respective values of 55.12%, 51.14%, 72.64%, and 72.17% in contrast to AqF which showed the lowest DPPH activity at 44.66%.
CUPRAC antioxidant potential of CE and fractions of P. thonningii
Figure 4 presents the CUPRAC potential of CE and various fractions derived from *P. thonningii* leaf extract. The various activity values varied significantly, ranging from 8.90 % to 83.23 %. The samples displayed the following order of CUPRAC activity: HF > AqF > CE > ChF > EaF. Notably, HF demonstrated the most CUPRAC activity at 83.23 %, while EaF recorded the least (8.90 %). This activity was compared to the standard ascorbic acid, with an activity of 99.94 %.

In vitro antioxidant inhibitory activity
The *in vitro* antioxidant inhibitory activities of *Piliostigma thonningii* CE and its solvent fractions—n-hexane (HF), chloroform (ChF), ethyl-acetate (EaF), and aqueous (AqF)—were evaluated using three standard *in vitro* methods: FRAP, DPPH, and CUPRAC. The IC_{50} values obtained for each assay are summarized in Table 2. In the FRAP assay, which measures the ferric reducing antioxidant power, EaF exhibited the highest inhibition among the fractions (IC_{50} = 1.35 ± 0.09 µg/ml) followed by the crude extract (2.01 ± 0.08 µg/ml). In contrast, ChF showed markedly lower inhibitory capacity (11.89 ± 0.31 µg/ml). Ascorbic acid reference standard, displayed the most potent inhibition (1.153 ± 0.014 µg/ml). Following the DPPH radical scavenging activity, CE (0.24 ± 0.19 µg/ml), ChF (0.28 ± 0.05 µg/ml), and HF (0.37 ± 0.02 µg/ml) all exhibited moderate inhibitory activity, while the ethyl acetate fraction showed relatively lower activity (0.57 ± 0.09 µg/ml) compared the ascorbic acid which demonstrated superior radical scavenging capacity (0.16 ± 0.08 µg/ml). In the CUPRAC assay, only the n-hexane and aqueous fractions were tested. The HF exhibited a strong cupric ion reducing capacity (0.43 ± 0.15 µg/ml), even outperforming the standard (0.72 ± 0.18 µg/ml). In contrast, the aqueous fraction showed much lower inhibition (10.42 ± 0.71 µg/ml), indicating weaker antioxidant potential in this assay (Table 2).

Table 2: IC_{50} values of *P. thonningii* CE and fractions determined by FRAP, DPPH and CUPRAC method

Fraction	FRAP (µg/ml)	DPPH (µg/ml)	CUPRAC (µg/ml)
Ascorbic acid	1.15 ± 0.014	0.16 ± 0.08	0.72 ± 0.18
CE	2.01 ± 0.08	0.24 ± 0.19	-----
HF	-----	0.37 ± 0.02	0.43 ± 0.15
ChF	11.89 ± 0.31	0.28 ± 0.05	-----
EaF	1.35 ± 0.09	0.57 ± 0.09	-----
AqF	-----	-----	10.42 ± 0.71

The results were obtained at 5 mg/ml concentration. IC_{50}: concentration causing 50 % radical inhibition.

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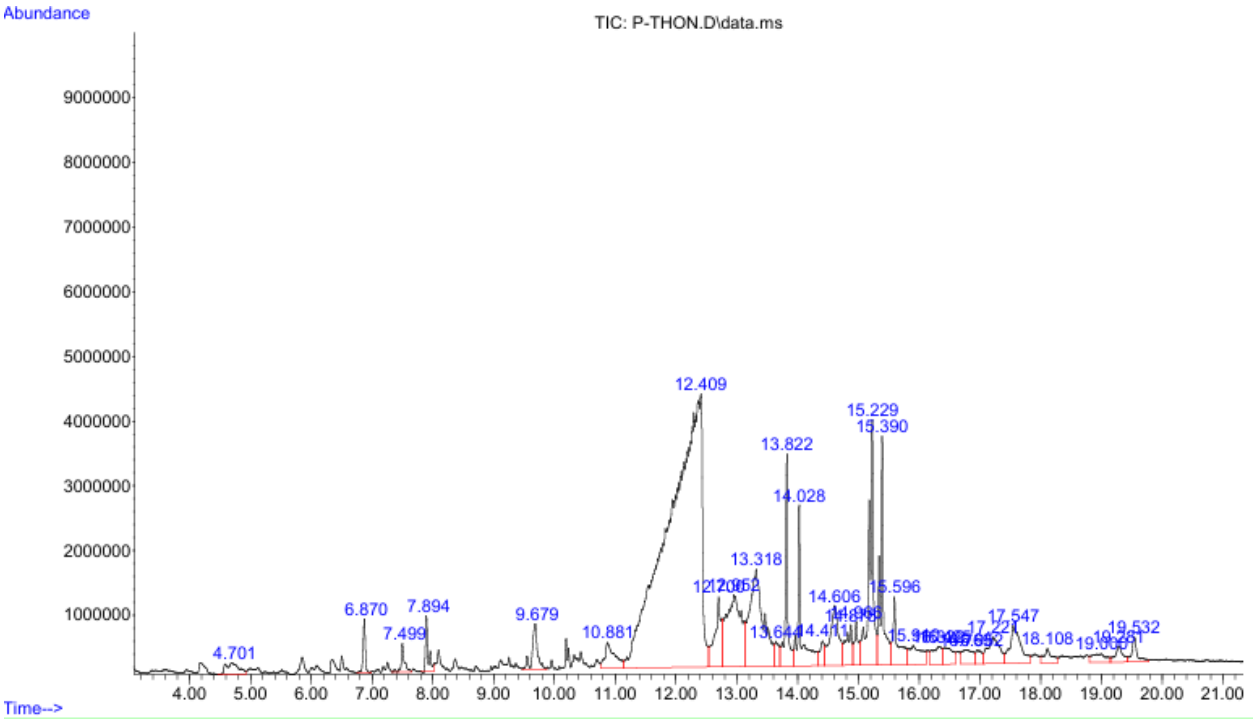

Figure 1: GC-MS base peak chromatograms of metabolites analyzed from *P. thonningii* ethanol crude extract

Body weight and organ weight measurements

Tables 4 and 5 show the results of *P. thonningii* CE on the body and ovary weights of experimental animals, respectively. As the administration progressed, it was found that there were no significant differences in the animals' body weights within groups. Similar trend was also observed in the ovary weight of the animals as no significant difference was also observed.

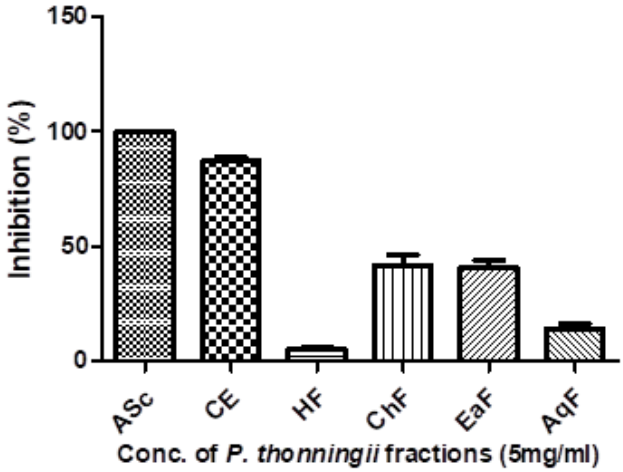

Figure 2: FRAP of P. *thonningii* CE and fractions at 5 mg/ml. Data are presented as mean ± SEM, for three determinations (n=3). Sample which did not give 50% activity are not active

Effects of P. thonningii crude extract on serum antioxidant enzymes

The result of the effect of *P. thonningii* on serum antioxidant enzymes of female wistar rat (table 3) revealed that, the administration of the CE did not significantly affect the activities of antioxidant enzyme (MDA, GSH, SOD, and CAT). For each antioxidant enzymes, no statistical difference was seen in the groups administered when compared with the normal control group for 28 days.

Histopathological result of the ovaries

The histopathological findings of the ovaries are presented in Figure 5. The photomicrograph of the NC group, stained with Haematoxylin and Eosin, revealed intact ovarian architecture, including a well-defined Graafian follicle (indicated by the red arrow) surrounded by normal stromal cells (black arrow). Similarly, the photomicrograph of group treated with 500 mg/kg b.w. of the CE displayed normal ovarian follicles (red arrow) and well-preserved stromal cells (black arrow), closely resembling the normal control group. A similar pattern was seen in animals treated with 1000 mg/kg b.w., where the photomicrograph showed healthy ovarian architecture with intact follicles (red arrow) and stromal cells (black arrow). However, the group treated with 2000 mg/kg b.w., there was significant disruption of ovarian structure. The photomicrograph revealed severe degeneration of ovarian cells (black arrow) and interstitial hemorrhage (red arrow), indicating considerable tissue damage.

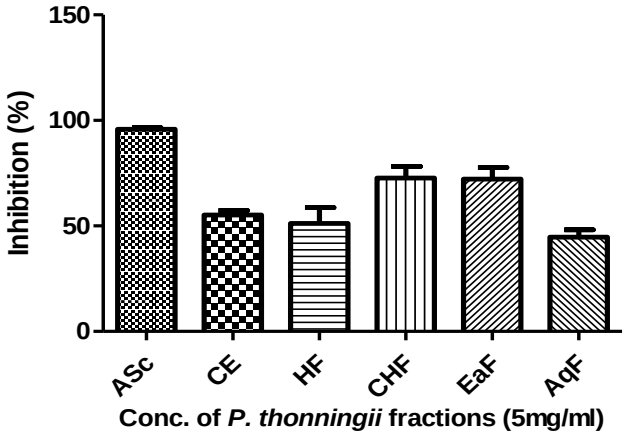

Figure 3: DPPH radical scavenging activity of *P. thonningii* CE and fractions at 5 mg/ml. Data are presented as mean ± SEM, for three determinations (n=3). Sample which did not give 50% activity are not active

Table 3: Effect of ethanolic crude extract of *P. thonningii* on body weight

Group	Week 1	Week 2	Week 3	Week 4
NC	131.00 ± 1.00[a]	134.67 ± 3.06a	135.00 ± 2.65[a]	136.00 ± 1.00[a]
500 mg/kg	173.67 ± 10.02[a]	174.33 ± 10.02[a]	178.33 ± 4.62[a]	185.00 ± 5.29[a]
1000 mg/kg	166.00 ± 8.19[a]	171.00 ± 13.53[a]	176.33 ± 14.22[a]	184.67 ± 16.29[a]
2000 mg/kg	132.33 ± 7.23[a]	146.33 ± 10.02[a]	148.67 ± 11.02[a]	146.00 ± 17.58[a]

Mean ± S.E.M. was used to express all collected data for six replicates. Values with the similar superscript are not statistically significant while those with different superscripts are statistically significant at $p<0.05$

Table 4: Effect of ethanolic crude extract of *P. thonningii* on weight of ovary

GROUPS	WEIGHT (g)
NC	0.14 ± 0.02[a]
500mg/kg	0.14 ± 0.03[a]
1000mg/kg	0.21 ± 0.01[a]
2000mg/kg	0.16 ± 0.03[a]

Mean ± S.E.M. was used to express all collected data for six replicates. Values with the similar superscript are not statistically significant while those with different superscripts are statistically significant at $p<0.05$

The GC-MS analysis of compounds in the CE of the study plant identified 31 compounds (Table 1), of which 9,12-Octadecadienoic acid, 1,4-Benzenediol, 1,3-Benezenediol-4-proyl, 13-Tetradece-11-yn-1-ol, n-Hexadecanoic and Oleic acid reported in this study, were among those identified in a study by Yinusa,[42] who identified 64 compounds from the ethanolic leaf extract of the plant. 9,12-Octadecadienoic acid is a polyenoic fatty acid known for its hepatoprotective,[23] antihistaminic, and hypo-cholesterolemic effects,[43] it was also reported to have anti-inflammatory, nematicide, anti-tumor activities.[44] n-Hexadecanoic acid a palmitic acid possesses anti-inflammatory, antioxidant,[45, 46] hypo-cholesterolemic and antidiabetic effects[47], in addition, it also possesses hemolytic 5-alpha reductase inhibitory activity, anti-androgenic, pesticide, nematicide activities and also antioxidant potential.[48] Dalla Lana et al.[49] reported that 1,4-benzenediol possess antimicrobial activity against *E. coli* (ATCC 25922) while 1,3-benzenediol, 4-propyl- reported by Deryabin & Tolmacheva [50] showed antibacterial potential. It has been demonstrated that the monounsaturated fatty acid (MUFA) oleic acid strengthens the immune system by regulating neutrophils, lymphocytes, and macrophages, among other cells.[51] MUFAs taken orally have been shown to decrease the LDL levels while increasing HDL levels.[52, 53] The primary omega-9 MUFA oleic acid has been found to have hypotensive properties and is utilized as an emulsifying agent.[54] Additionally, it is known to slow the growth of adrenoleukodystrophy, a serious condition that affects the adrenal glands and brain.[55] The most abundant compounds 1,3-Dioxane, 2-pentadecyl- (47.03 %) also has antibacterial property,[56] as well as 9,17-octadecadienal, (Z)- (7.03 %) an aldehyde also known as linolenic acid: a crucial omega-3 fatty acid with cholesterol lowering potential. 1,3-Dioxane, 2-pentadecyl also demonstrate neuroprotective qualities[57, 58, 59] in addition, 9,17-octadecadienal, (Z)- possess antimicrobial property as reported by Odiase-Omoighe et al.[60] Various studies have also reported the hepatoprotective properties of *P. thonningii* plant extract demonstrating that the plant is non-toxic to the liver and other organs at different doses.

Table 5: Effect of ethanolic crude extract of *P. thonningii* on serum antioxidants

Samples	SOD (U/ml)	CAT (U/mg protein)	MDA (μM)	GSH (mM)
NC	1.96 ± 0.51[a]	2.66 ± 1.48[a]	0.69 ± 0.01[a]	0.18 ± 0.03[a]
500mg/kg	1.43 ± 0.40[a]	2.41 ± 0.39[a]	0.67 ± 0.04[a]	0.14 ± 0.01[a]
1000mg/kg	0.67 ± 0.09[a]	2.51 ± 0.80[a]	0.79 ± 0.05[a]	0.14 ± 0.02[a]
2000mg/kg	2.13 ± 0.78[a]	2.95 ± 1.66[a]	0.73 ± 0.03[a]	0.17 ± 0.02[a]

Mean ± S.E.M. was used to express all collected data for six replicates. Values with the similar superscript are not statistically significant while those with different superscripts are statistically significant at $p<0.05$.

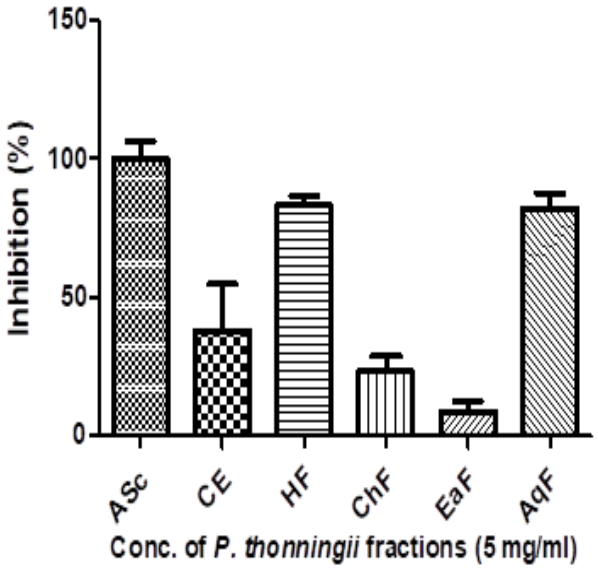

Figure 4 CUPRAC of *P. thonningii* CE and fractions at 5 mg/ml. Data are presented as mean ± SEM, for three determinations (n=3). Sample which did not give 50% activity are not active

The antioxidant activities of *P. thonningii* fractions were assessed using three assay models: CUPRAC, FRAP, and DPPH. Usin and Daramola [61] previously reported that *P. thonningii* leaves are rich in ascorbic acid, α-tocopherol, and β-carotene, which may account for the plant's ability to reduce FRAP and CUPRAC, as well as scavenge free radicals in the DPPH assay, as observed in the current study. The CE and the various fractions of the plant exhibited over 50 % inhibition, comparable to the ascorbic acid standard used. According to these results, *P. thonningii*'s CE and fractions showed significant antioxidant activity, which is in line with findings of Olela et al. Duenngai et al. Usin and Daramola, Seyi et al. [62, 63, 65, 64] Serum antioxidant enzyme activities presented in table 3 suggests that the effect of the study plant on the activities of the enzymes Superoxide Dismutase (SOD) [which helps convert superoxide radicals (a type of ROS) into H_2O_2 and oxygen (O_2)], and Catalase (CAT) [which breaks down H_2O_2 (a reactive molecule produced by SOD) into water and oxygen], was highest at the 2000 mg/kg dose. A Similar trend was observed for glutathione (GSH), a major intracellular antioxidant, whose elevation indicates that the plant extract is helping to replenish this vital molecule. Inhibition of lipid peroxidation was also achieved with both 500 and 2000 mg/kg group as seen in the MDA levels (a byproduct of lipid peroxidation, whose reduction in its levels indicates lower oxidative damage to cell membranes) although no statistical difference was also observed when compared with the NC. These findings suggest that at high dose, the plant may enhance antioxidant response. This implies that the extract may have a stimulatory impact on the body's antioxidant defense mechanism and may also have protective effect against oxidative stress, potentially helping to neutralize ROS and mitigate cellular damage. This findings are in line with those of Ibe et al. [25] and Ajiboye et al.[27] The histological study (figure 5a-d) did not show any significant changes with the 500 mg/kg and 1000 mg/kg groups, however, the 2000 mg/kg, showed significant change in the ovarian structure which could occur from chronic injury indicating that the plant extract may elicit tissue damage to the ovaries at high dose. Although this may seem contradictory because of the antioxidant activity at this dose, it is worthy to note that certain plant extracts contain compounds which acts as pro-oxidant and maybe cytotoxic at high doses and may cause effects to ovarian architecture. [65, 66,67,68,69]

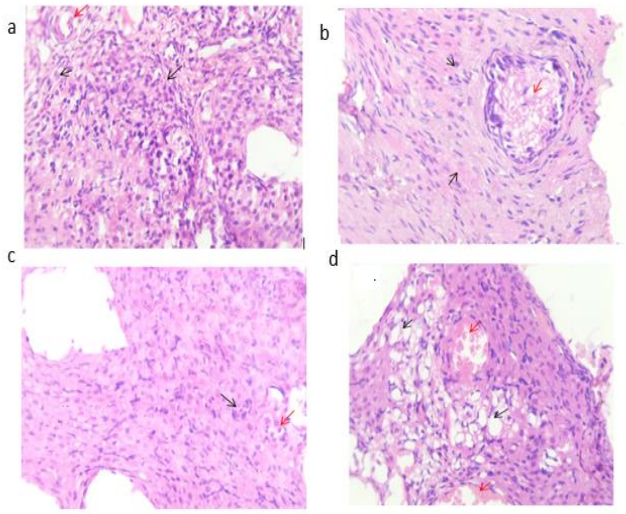

Figure 5a-d: Histopathological analysis of ovaries of rats administered *P. thonningii* crude extract after 28 days. (a) Normal control (b) 500 mg/kg b.w. (c) 1000 mg/kg b.w. and (d) 2000 mg/kg b.w. H&E Staining, Magnification X400.

Conclusion

Strong *in vitro* antioxidant activity was demonstrated by the *P. thonningii* leaf extract. This is probably because of the bioactive compounds it possesses. The plant extract also did not show any effect to the body and ovary weights of female Wistar rats and also did not induce any oxidative stress to the animals *in vivo*. While tissue damage may have been observed in the ovary of the rats administered 2000 mg/kg of the crude extract, at 500 and 1000 mg/kg, the ovarian structure was well preserved. Owing to its potential antioxidant activity the plant may be evaluated for its possible therapeutic potential against oxidative stress conditions associated with uterine fibroids.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors affirm to the originality of the content of this article and accept full responsibility for any claims arising from its content.

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