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Phytochemical Screening and Acute Toxicity Study of Methanolic Extract of *Pericopsis Laxiflora* Leaf in Wistar Rat

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Abstract

Background: Plants are widely used to treat and prevent various illnesses. The phytoconstituents in plants are often viewed as natural and low in toxicity; they can be beneficial at specific doses, while overdoses may lead to toxicity in vital organs. This study aimed to analyse the methanolic extract for phytochemicals, determine total phenol and flavonoid contents, characterise the chemical compounds using GC-MS, and conduct an acute toxicity study in male Wistar rats.

Materials and Methods: 500 g of the powdered leaves was extracted with 2.5 litres of methanol using the cold maceration method for 24 hours. 40.50 g of the crude extract was serially partitioned with acetyl acetate, n-hexane, and distilled water to obtain fractions. The total phenol and flavonoid contents were quantified by HPLC, and GC-MS was used to characterise the chemical compounds. Lörke's method was used to determine the LD50 in male Wistar rats.

Results: The phytochemical screening revealed alkaloids, saponins, flavonoids, tannins, phenols, flavonoids, and cardiac glycosides. Total phenol content was 339.52±0.8 mg/g Gallic Acid Equivalents, and flavonoid content was 276.67±3.9 mg/g quercetin equivalent. The GC-MS analysis identified and characterised 72 compounds: 21 from the methanolic extract, 24 from the ethyl acetate fraction, and 27 from the n-2-hexane fraction. The acute toxicity showed an LD50 $\geq$ 5000 mg/kg and significantly increased body weight in group 1 of the phase 1 study, two weeks post-treatment.

Conclusion: The acute toxicity study of the plant revealed a substantial margin of safety, suggesting its potential efficacy. The presence of bioactive compounds supports the folklore use of plants in the treatment of many diseases.

KEYWORDS: *P. laxiflora, acute toxicity, clinical signs, phytoconstituents, body weights, Wistar rat*

Introduction

Before the development of most existing pharmaceuticals, traditional medicines were widely used globally to manage various diseases. These medications are considered to have fewer adverse effects, are more affordable, and remain culturally acceptable (1). As a result, more than 80% of people in developing countries still rely on traditional healers and medicinal plants for their primary healthcare needs (2). This study aimed to conduct a phytochemical analysis, quantify the total flavonoid and phenolic contents, GC-MS analysis, and evaluate the acute toxicity of *Pericopsis laxiflora* (*P. laxiflora*) leaf extract in male Wistar rats.

Pericopsis is a genus in the Fabaceae family and includes species such as *Pericopsis angolensis* (Baker) Meeuwen, *Pericopsis elata* (Harms) Meeuwen (African teak), and *Pericopsis laxiflora* (Baker) Meeuwen. *P. laxiflora* is a savannah, perennial, woody deciduous shrub or tree belonging to the Fabaceae family. Although native to tropical America, the plant is now widely distributed across tropical rainforests and is commonly cultivated in communities due to its ability to provide shade (3). It is known by several local names: Satinwood (English), Makarfo (Hausa), Abua-ocha (Igbo), and Ayan/Sedun (Yoruba) (4). In Ghana, *P. laxiflora* is traditionally used to treat jaundice and body weakness (4, 5). In Côte d'Ivoire, it is used for headaches, stomach ulcers, abdominal pain, gastritis, and enteritis (5). In Guinea, it is applied in the management of shigellosis and colibacillosis, while in the Benue region of Nigeria, it is commonly used as an anti-ulcer remedy (3, 5).

Previous studies have reported several biological activities of extracts from different parts of this plant, including antimicrobial and antioxidant properties (4, 6). Some plants have been found to contain toxic secondary metabolites regardless of their traditional use; therefore, toxicity studies are essential for evaluating the safety and potential health risks of any medicinal plant (6). Despite these findings, there is limited information on the phytochemical constituents, total phenol and flavonoid contents, GC-MS profile, and acute toxicity of the leaf extract of *P. laxiflora*. This study, therefore, seeks to fill this knowledge gap by providing detailed phytochemical characterisation and evaluating the safety profile of its methanolic leaf extract.

Materials and Methods

Wistar rat, oral cannula, conical flask (MBL, England), distilled water (Pharmacology, Lab UDUS) Centrifuge machine, formalin, n-hexane, ethyl acetate, methanol (Sigma Aldrich, USA), oven drier (NUVE Sanayi, Turkey), Electronic weighing scale (Mettler Toledo), Whatman filter paper (Whiteman International Ltd,

England), Kerro electronic compact (weighing scale) Mxrady Lab solutions Pvt Ltd. China.

Ethical approval

The guidelines of the Usmanu Danfodiyo University Sokoto Health Research Ethics Committee for the care and use of laboratory animals were followed, with approval and registration number UDUTH/HREC/2023/1312/V1.

Drugs/chemicals/ Equipment

All the Drugs/Chemicals/equipment used for this study are of high analytical grade**.**

Methods

Sample collection and identification of Plant material

Fresh leaves of *Pericopsis laxiflora* without stalks were collected from uncultivated farmland in June 2021 at Anchawu, a community in Danko Wasagu Local Government Area of Kebbi state, identified and authenticated at the Department of Pharmacognosy, Faculty of Pharmaceutical Science, Usmanu Danfodiyo University, Sokoto. Moreover, a voucher number PCG/UDUS/Fabacea/0015 was issued. A sample was deposited for future reference.

Preparation of plant extract

Fresh leaves of *Pericopsis laxiflora* harvested from the farm were thoroughly washed with tap water to remove dirt, then shade-dried for six months at room temperature, and pounded into a fine powder with a mortar and pestle. The measured weight of the powdered leaf material was 2500 g; 500 g of the powder was processed and used for this study, while the remaining powder was preserved for further use. Then, 500 g of the powdered materials were homogenised in 2.5L of methanol using the cold maceration method for 24 hours. The extract was filtered using what man No. 1 filter paper. The filtrate obtained was concentrated using a rotary evaporator (RE-6000) at 45 °C to obtain 40.50 g, corresponding to an 8.10% yield of the crude extract (6). The extract was packed in a properly labelled glass bottle and kept at 4 °C, away from light, by wrapping it in aluminium foil until required for use (7).

The percentage yield of the extract was calculated as:

Weight of extract/weight of sample used x 100

=40.50g/500g X 100 = 8.10

Fractionation of crude extract

A total of 30 grams of crude extract was dissolved in 300 millilitres of distilled water and filtered using Whatman No. 1 filter paper. The resulting filtrate was transferred to a separating funnel and subjected to serial partitioning with ethyl acetate (semi-polar), N-hexane (non-polar), and distilled water (polar) to obtain different fractions. The fractionation process involved mixing the extract with 500 millilitres of n-hexane (CAS 110-54-3, EMSURE®) and homogenising it in the separating funnel by horizontal rotation for 5 minutes. The funnel tap was then opened intermittently to release trapped air and reduce pressure. After allowing the mixture to settle, distinct layers of n-hexane and water were formed. These layers were separated into different containers. This fractionation process was repeated up to three times, and similar steps were followed for ethyl acetate (CAS 141-78-6, Merck) and the distilled water. Additionally, the n-hexane, ethyl acetate, and aqueous fractions were concentrated using a rotary evaporator (1110S-WD EYELA) at 45 °C. The yields of the fractions were as follows: n-hexane was 4.21 g, equivalent to 0.84%, ethyl acetate was 4.99 g, equivalent to 0.998%, and the aqueous portion was 28.8 g, equivalent to 5.76% (8, 9).

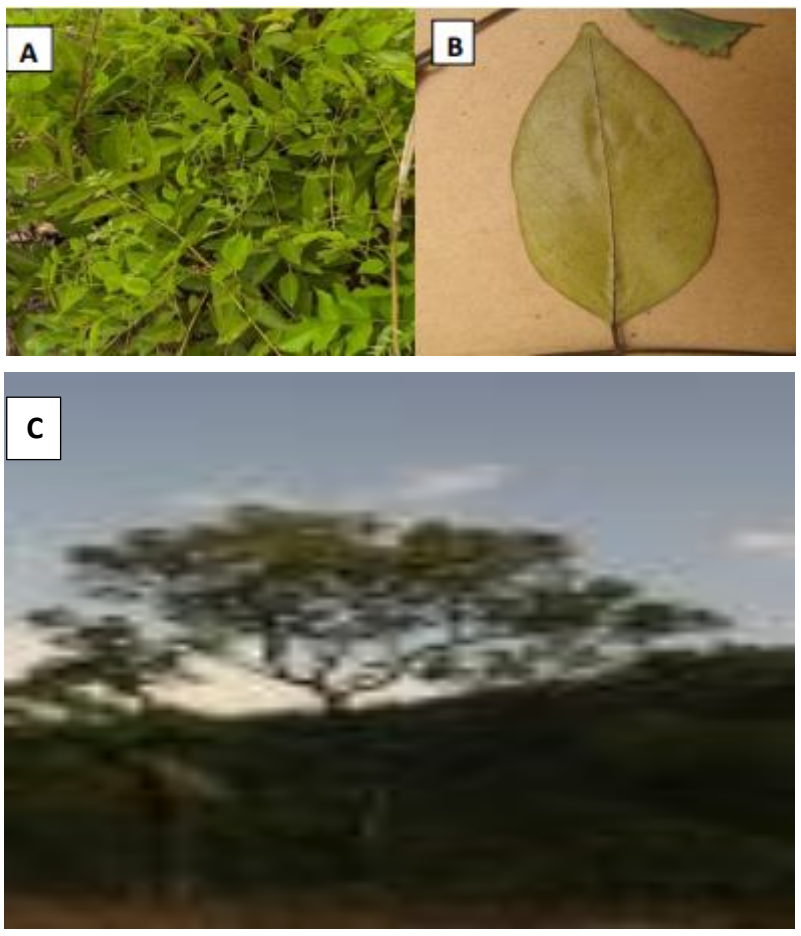


Figure 1: showing P. *laxiflora* (a) Fresh Leaves, (b) Dried leaf, and (c) Tree

Qualitative phytochemical screening of leaf extracts

Test for alkaloids (Mayer's test)

To test for the presence of alkaloid in the crude extract, 500 mg was dissolved in 5 mL of methanol and mixed with 5 mL of 1% aqueous hydrochloric acid over a water bath. Then, a few drops of Mayer's reagents were added to 1 mL of the solution. The presence of a fluorescent, brownish precipitate indicates the presence of alkaloids (11).

Test for saponin (Foam test)

The presence of saponin was determined according to the method of Edeoga et al., (11) for the determination of saponin. 500 mg of the extract was mixed with distilled water to a final volume of 25 mL, and the mixture was stirred for 10 minutes. After that, a layer of foam formed, indicating the presence of saponin or persistent frothing upon warming. Further, the supernatant was boiled with 50 mL phosphate buffer and was passed through an asbestos disc already soaked with cholesterol in ether. The disc was washed, dried, boiled in 20 mL of xylene, washed in ether, and then placed on a 7% blood nutrient agar plate. Complete haemolysis of red blood cells around the disc after 6 hours confirms the presence of saponin.

Test for tannin (Ferric Chloride Test)

The presence or absence of tannin was determined according to the method of Trease and Evans (8). 5 g of extract was mixed with 10 mL of distilled water and filtered with ferric chloride reagent. A blue-black, green, or blue-green precipitate indicates the presence of tannins.

Test for anthraquinone:

Test for anthraquinone: 500 mg of the methanol extract of *P. laxiflora* was mixed with 10 mL of benzene and filtered. To the filtrate, a 10% ammonia solution was mixed, and the development of pink colour confirms the presence of anthraquinone (11)

Test for flavonoids:

The test for flavonoids was done by mixing 3 mL of the methanolic extract of *P. laxiflora*, a magnesium ribbon, and 1 mL of concentrated HCl. The appearance of red colour verifies the presence of flavonoids (12).

Test for cardiac glycosides (Kellar–Kiliani test)

The test for cardiac glycosides, also known as the Kellar–Kiliani test, was performed according to the method of Parekh and Chanda (13). To 2 mL of the methanolic extract of *P. laxiflora* filtrate, 1 mL each of glacial acetic acid, ferric chloride, and concentrated sulphuric acid was added. A change in the mixture of colour to green-blue indicates the presence of cardiac glycosides.

Test for steroids/tri-terpenoids (Salkowski test)

In 1 ml of each plant extract (leaf, stem, root), add a few drops of conc. H2SO4, which forms a lower red colour, indicates the presence of steroids, whereas a yellow colour indicates the presence of tri- terpenoids. (14)

Test for phenols

To 2 ml of methanolic extract, a few drops of FeCl3 were added, and the solution turned blue, indicating the presence of phenols (14).

High Performance Liquid Chromatography (HPLC) of Total Flavonoid and Phenol Contents of the leaf extract.

Determination of total Flavonoid Content of the Extract.

The total flavonoid content was determined by aluminium chloride colourimetric assay. 1 mL of the extract and 4 mL of distilled water are mixed in a 10 mL volumetric flask. Then, 0.3 mL of 5% sodium nitrite solution was added and allowed to stand for 5 minutes, followed by the addition of 0.3 mL of 10% aluminum chloride solution. This was allowed to stand for 5 minutes. Then, 2 mL of 1M NaOH solution was added to it to make the mark with distilled water. A standard curve for quercetin was developed using the same method (Figure 1). The absorbance of the test and standard solutions was determined against the reagent blank at 510nm. The total flavonoid was expressed as milligrams of quercetin equivalent to QE/g of the extract (15, 16). TFC, y = 0.002x + 0.0399, where R2 = 0.9971. The result is presented in Table 2.

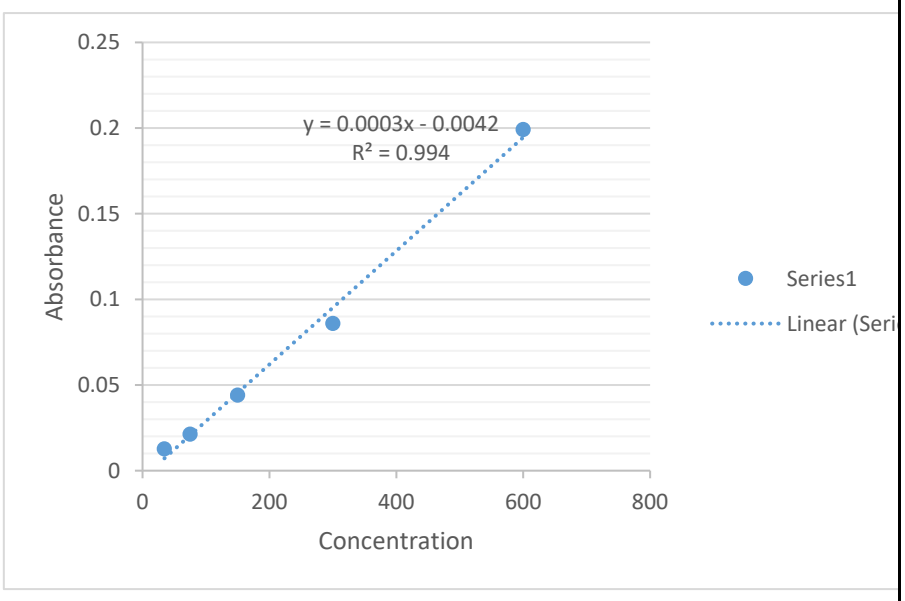


Figure 1: Quercetin Calibration Curve

Determination of Total Phenol Content of Crude Extracts

The total phenol content was determined according to the procedure of (15, 16). 1 mL of the extract and 9 mL of distilled water were placed in a 25 mL volumetric flask. 1 mL of Folin-Ciocalteu phenol reagent was added, and the mixture was shaken well with 10 mL of 7% sodium bicarbonate to mark. A standard curve for gallic acid was developed using the same method. The mixture was incubated for 90 min, and the absorbance of the reaction mixture was measured at 765 nm. The phenol content is expressed as milligrams of gallic acid equivalent to GAE/g of the extract. The total phenol content (TPC) and total flavonoid content (TFC) of the methanol extract were determined using the Folin-Ciocalteu and aluminium chloride colourimetric assay, respectively. Then, different reagents for TPC and TFC determinations were added, resulting in the formation of blue and yellow colours. The equation of the standard curve for the determination of TPC was y = 0.0023x − 0.0693, where R2 = 0.9981 (Figure 2). The result is presented in Table 2.

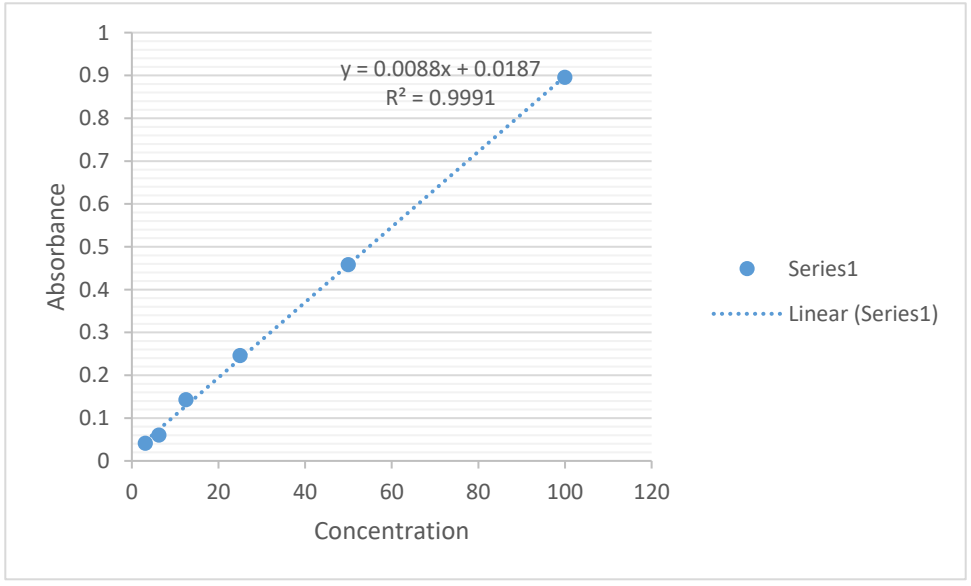


Figure 2: Gallic acid calibration curve

Gas Chromatography-Mass Spectrometry of Extract Fractions (GC-MS)

The GC-MS analysis was conducted at the Multi-user Science Research Laboratory, Ahmadu Bello University, Zaria, Kaduna, Nigeria, according to methods previously described in the literature (15, 16). Helium gas with 99.999% purity was used as the carrier gas at a constant flow rate of ±1 mL/min. The mass transfer line and injector temperature were set at 220 and 290 °C, respectively. An electron ionisation system with an ionisation energy of 70 eV was utilised to detect the chemical compounds from the methanol, ethyl acetate, and n-hexane fractions. The oven temperature program was set to 50-150 °C at 3 °C/min, then the isothermal temperature was held for 10 minutes, and finally the temperature was raised to 250 °C at 10 °C/min. The extracts were diluted 100-fold with methanol, ethyl acetate, and n-hexane, and 1 µl of the diluted samples was injected in split mode with a split ratio of 120:1. The delay time was 2 minutes. The total running time was 75 minutes (the retention time was between 9 and 29 minutes). The relative proportions of the chemical compounds in the extracts were expressed as area percentages. The chromatogram was interpreted using the National Institute of Standards and Technology (NIST) library (15).

Determination of Acute Toxicity of the Extract

Animal

Twelve healthy male Wistar rats of about 6- 8 weeks, weighing between 190 and 210 g, were purchased from the animal house of the faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria. The animals were kept under standard laboratory conditions (24 ± 2 °C, 12-h light/dark cycle) with free access to food (Vital Feeds PLC, Nigeria) and clean water *ad libitum* from the time of acclimatisation until the end of the experiment.

Experimental design

Acute toxicity was assessed using Lörke's method (17). A total of 12 healthy male Wistar rats were used for this experiment and the choice for male Wistar rats in acute toxicity study has previously been reported (18, 19) The experiment consist of two phases; phase 1 consist of three groups of three rat each, where group one received 10mg/kg, group II received 100mg/kg, and group III received 1000mg/kg. Phase 2 consists of three groups of three rats each, where group 1 received 1600 mg/kg, group II received 2900mg/kg, and group III received 5000 mg/kg. The rats were assigned to groups using simple random sampling. To ensure stomach emptying and prevent food interactions, the animals were fasted overnight. Before the experiment began, the animals in phase one were weighed, administered oral doses of the extract, and observed for 24 hours for signs of toxicity or mortality. When major signs or mortality were not observed, a similar procedure was repeated in phase two of the study. In the absence of major toxicity symptoms and mortality in both phases of the experiments, the LD50 value was calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (with deaths).

The Formula; $LD50 = \sqrt{D0} \times \sqrt{D100}$.

Where LD50 = Lethal dose, D0 = Highest dose that the animal survives, and D100 = Lowest dose that produces mortality.

Animals were continuously observed for 24 hours for signs of toxicity or mortality, and all animals were kept under observation for 2 weeks for any body weight changes, with access to food and clean water.

Statistical analysis

Data are represented as mean ± SD and mean ± SEM. The significance of differences between means was assessed using the Student's t-test in GraphPad Prism version 8. $P<0.05$ was considered statistically significant.

RESULTS

Phytochemical screening

The result of the phytochemical screening of the methanolic extract of *P. laxiflora* leaf is presented in Table 1.

Table I. Phytochemical analysis and fractionation of methanolic leaf extract of *P. laxiflora*

Phytochemical Constituents	Crude methanolic fraction	N-hexane fraction	Ethyl acetate fraction	Aqueous fraction
Carbohydrate	++	-	-	++
Alkaloids	+	-	-	+
Flavonoids	++	-	-	++
Tannins	++	-	-	++
Phenolic compound	++	-	-	++
Saponin	++	-	-	++
Anthraquinones	-	-	-	-
Cardiac glycosides	+++	-	-	+
Steroids/triterpenoids	+++	++	+++	+

Keys

[-] Absence
[+] Trace presence
[++] Moderate presence
[+++] Abundant presence

The result of high-performance liquid chromatography of total flavonoids and phenol contents of the methanolic extract of *P. laxiflora* leaf is presented in Table 2

Table 2: Total Flavonoid and Phenol contents of methanolic leaf extract compared to standard drugs.

Samples	Flavonoid content (mg/g QE) (Mean ± SD)	Phenol content (mg/g GAE) (Mean ± SD)
Quercetin (standard)	5.02 ± 0.1	-
Gallic acid	-	4.38 ± 0.1
Methanol extract	276.67±3.9	339.52±0.8

Standard drugs (Gallic acid and Quercetin) QE: quercetin equivalent; GAE: Gallic acid equivalent.

GC-MS analysis

The chemical compounds of the methanolic extract, ethyl acetate, and n-hexane fractions were listed by retention time, peak height, molecular formulae, compound names, and percentage composition, as shown in Tables 3-5.

Table 3: Chemical compounds of the methanolic leaf extract of *P. laxiflora*

S/N	RT	Peak Height (Area%)	Molecular formula	Compound name	% Quality
1	15.721	0.07	$C_{14}H_{26}O_2$	E-9-Tetradecenoic acid	80
2	16.109	0.76	C18H34O2	trans-13-Octadecenoic acid	99
3	16.987	0.26	$C_{29}H_{50}O$	beta-Sitosterol	95
4	18.009	0.04	$C_{16}H_{28}$	4-Hexadecen-6-yne, (E)-	49
5	18.305	0.06	$C_{22}H_{42}O_4$	Diisooctyl adipate	83
6	18.478	0.06	$C_{16}H_{28}O$	cis, cis-7,10-Hexadecadienal	80
7	18.887	0.07	$C_{19}H_{34}O_2$	9,12-Octadecadienal	99
8	15.731	21.53	$C_{18}H_{52}O_7Si_7$	3-Isopropoxy-1,1,1,7,7,7-hexamethy-3,5,5-tris(trimethylsiloxy)tetra siloxane	40
9	20.507	0.07	$C_{19}H_{38}O$	Disparlure	90
10	21.403	0.05	$C_{13}H_{39}O_5Si_6$	1,1,1,5,7,7,7-Heptamethyl-3,3 bis(trimethylsiloxy) tetrasiloxane	98
11	21.716	0.02	C_8H_{16}	1-Hexene, 3,3-dimethyl-	46
12	22.001	0.09	$C_{19}H_{34}$	Cyclohexene,4-(4 ethylcyclohexyl) -1-pentyl	27
13	22.327	0.03	$C_{18}H_{34}O$	13-Octadecenal, (Z)-	55
14	22.456	0.03	$C_{18}H_{54}O_9Si_{91}$ 3	Cyclononasiloxane, octadecamethyl	72
15	24.226	0.31	$C_{18}H_{34}O_2$	Oleic Acid	90
16	24.574	0.37	$C_{21}H_{40}O_4$	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	91
17	26.383	37.11	$C_{16}H_{28}O$	7,11Hexadecadienal	
18	27.448	0.53	$C_{21}H_{40}O_4$	9-Octadecenoic acid (Z)-, 2-hydroxy-1(hydroxymethyl)ethyl ester	86
19	27.786	1.43	$C_{18}H_{34}O_2$	9-Octadecenoic acid, (E)-	41
20	28.809	5.39	$C_{21}H_{40}O_4$	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	95
21	29.809	52.99	$C_1H_{50}O7Si8$	1,4-Bis(trimethylsilyl)benzene	74

RT = Retention time, which represents the time these compounds were eluted.

The result of GC-MS analysis of the ethyl acetate fraction of *P. laxiflora* is presented in Table 4.

Table 4: Chemical compounds of the ethyl acetate fraction.

S/N	RT	Peak Height (Area%)	Molecular formula	Compound names	% Quality
1	13.078	0.46	$C_{10}H_{18}$	Bicyclo [3.1.1] heptane, 2,6,6-trimethyl	64
2	14.063	0.37	$C_8H_{14}O$	9-Oxabicyclo [6.1.0] nonane, cis	60
3	14.063	7.90	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	97
4	14.443	1.21	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	64
5	15.524	1.33	$C_{18}H_{34}O_2$	Oleic Acid	83
6	15.544	0.47	$C_{18}H_{34}O_2$	trans-13-Octadecenoic acid	64
7	15.639	7.45	$C_{19}H_{34}O_2$	10,13-Octadecadienoic acid, methyl ester	99
8	15.731	21.53	$C_{18}H_{34}O_2$	13-Octadecenoic acid, methyl ester	99
9	15.783	0.76	$C_{18}H_{34}O_2$	cis-10-Heptadecenoic acid, methyl ester	90
10	6.019	7.41	$C_{18}H_{34}O_2$	Heptadecanoic acid, 16-methyl-, methyl ester	98
11	16.104	35.80		cis-Vaccenic acid	99
12	17.816	0.77	$C_{23}H_{44}O_2$	13-Docosenoic acid, methyl ester	80 64
13	18.303	1.08	$C_{22}H_{42}O_4$	Hexane dioic acid, bis (2ethylhexyl) ester	91
14	18.480	0.86	$C_{16}H_{28}O$	cis, cis-7,10, -Hexadecadienal	89
15	27.063	0.06	$C_{16}H_{48}O_6Si_7$	Heptasiloxane1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	50
16	27.594	-0.25	$C_{12}H_{22}Si_2$	1,2-Bis(trimethylsilyl)benzene	38
17	27.864	0.11		Methyltris (trimethylsiloxy)silane	58
18	28.189	0.10	$C_{11}H_{17}NOSi$	Acetamide, N-[4-(trimethylsilyl)phenyl]-	47
20	28.292	0.17	$C_{10}H_{30}O_3Si_4$	3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	35
21	28.703	7.98		Tetrasiloxane, Decamethyl-	50
23	28.821	1.15	$C_{12}H_{22}Si_2$	1,4-Bis(trimethylsilyl)benzene	64
24	28.857	0.65	$C_{18}H_{54}O_7Si_8$	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	91

Peak numbers = Peak height (area %) represents the relative concentrations of compounds. RT = Retention time, which represents the time these compounds were eluted.

The GC-MS analysis of the n-hexane fraction of *P. laxiflora* is presented in Table 5.

Table 5: Chemical compounds of n- Hexane fraction

SN	RT	Peak Height (Area%)	Molecular formula	Compound names	% Quality
1	9.563	0.35	$C_{13}H_{26}O_2$	Dodecanoic acid, methyl ester	83
2	14.062	0.45	$C_{13}H_{26}O_2$	Hexadecenoic acid, methyl ester	93
3	15.727	1.10	$C_{13}H_{26}O_2$	12-Octadecenoic acid, methyl ester	98
4	16.016	0.33	$C_{19}H_{38}O_2$	Methyl stearate	70
5	16.092	1.09	$C_{18}H_{34}O_2$	6-Octadecenoic acid, (Z Oleic Acid	97
6	17.335	0.34	$C_{13}H_{24}Cl_2O_2$	Chloromethyl 10-chlorododecanoate	58
7	18.311	0.58	$C_{22}H_{42}O_4$	Diisooctyl adipate	86
9	18.521	0.51	$C_{21}H_{40}O_4$	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	80
10	18.891	0.49	$C_{15}H_{28}$	1-Cyclohexylnonene	43
11	19.408	0.23	$C_{11}H_{12}O_4$	1,4-benzenedicarboxylic acid, mono (1-methylethyl) ester	60
12	21.726	0.70	$C_{14}H_{24}O$	6,11-Dimethyl-2,6,10-dodecatrien-1 -ol	50
13	22.387	0.36	$C_{19}H_{36}O_2$	15-Octadecenoic acid, methyl ester	45
14	22.954	0.21	$C_8H_{10}O_2$	2-Hydroxyphenethyl alcohol,	44
15	27.063	0.06	$C_{11}H_{14}N_2OS$	Thieno [2,3-c] furan-3-carbonitrile,2-amino-4,6-dihydro-4,4,6,6-tetra methyl-	46
16	23.091	0.38	$C_9H_{10}FN_5$	4H-1,2,4-triazole-3,5-diamine, N3-(4-fluorophenyl)-N5-methyl-	46
17	23.216	1.05	$C_6H_{18}O_3Si_3$	Cyclotrisiloxane, hexamethyl-	43
18	23.272	0.87	$C_{13}H_{22}OSi_2$	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl	53
19	23.313	1.00	$C_{10}H_{30}O_3Si_4$	Methyltris (trimethylsiloxy)silane	47
20	23.346	0.54	$C_{13}H_{19}BrO_2$	Methyl 3-bromo-1-adamantaneacetat	35
21	23.489	2.24	C_2H_6OS	Mercaptoethanol, 2TMS	55
22	23.600	1.64	$C_{12}H_{14}O_4$	1,3-Benzene dicarboxylic acid, 5-(1,1-dimethylethyl)-	46
23	23.628	0.96	$C_{12}H_{16}O_2$	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative	35
24	23.662	1.48	$C_{10}H_{28}O_4Si_3$	Silicic acid, diethyl bis (trimethyl silyl) ester	50
25	23.759	3.93	$C_{12}H_{36}O_4Si_5$	Penta siloxane, 1,1,3,3,5,5,7,7,9,9 -Deca methyl-	46
26	24.047	2.28	$C_9H_9N_3O_3$	Formic acid, 1-(4,7-dihydro-2-methyl-7-oxopyrazolo[1,5-a]pyrimidin-5-yl)-, methyl ester	38
27	31.321	0.08	$C_{17}H_{15}NO_3$	9H-Fluorene-2-carboxylic acid, 9-oxo-, (2-hydroxyethyl) (methyl)amide	47

Peak Height = Peak height (area %), which represents the relative concentrations of compounds. RT = Retention time, which represents the time these compounds were eluted.

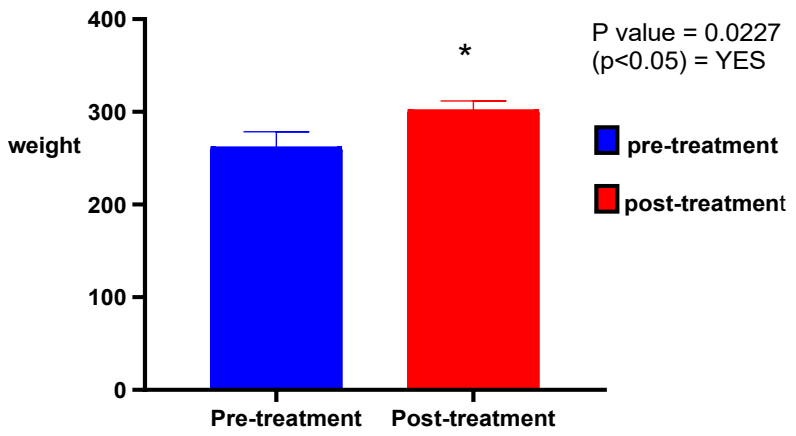
Acute Toxicity Study

The result of the acute toxicity study of the methanolic leaf extract of *P. Laxiflora* in Wistar rats is presented in Table 6.

Table 6: Result of acute toxicity test in male Wistar rats

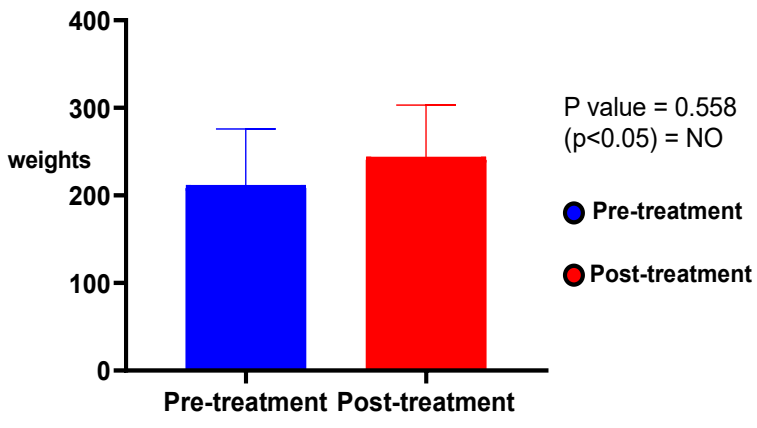
Experimental phase	Groups	No. of animals	Doses (mg/kg)	Mortality after 24 hrs
Phase one	1	3	10	0/3
	2	3	100	0/3
	3	3	1000	0/3
Phase Two	1	1	1600	0/1
	2	1	2900	0/1
	3	1	5000	0/1

Phase one (n = 3), Phase two (n = 1), 0/1 and 0/3: no mortality per group.



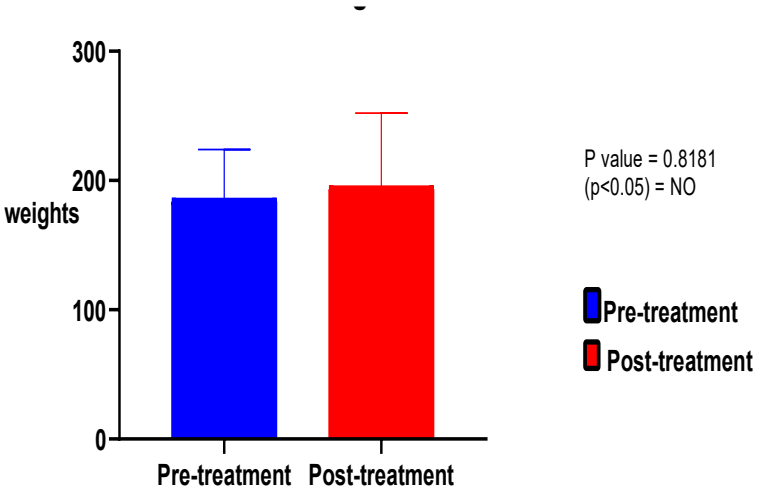
Dose (10 mg/kg)

Figure 3: Effect of methanolic extract on animal body weights in phase one experiment. Values are presented as mean ± SEM. Rats per group (n = 3)



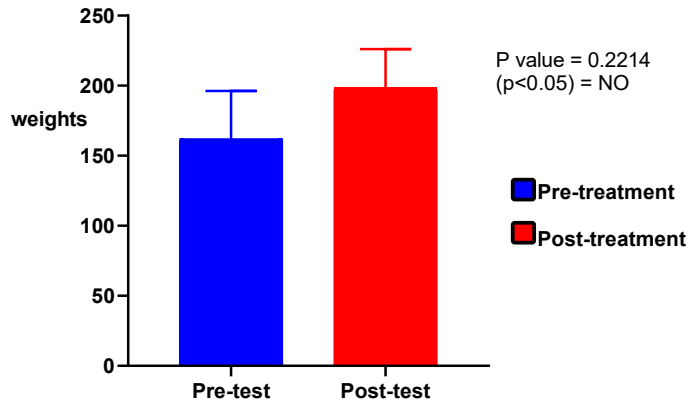
Dose (100mg/kg).

Figure 4: Effect of methanolic extract on animal body weights in phase one experiment. Values are presented as mean ± SEM. Rats per group (n = 3).



Dose (1000mg/kg).

Figure 5: Effect of methanolic extract on animal body weights in phase one experiment. Values are presented as mean ± SEM. Rats per group (n = 3) in phase one.



Doses (1600/2900/5000mg/kg)

Figure 6: Effect of methanolic extract on animal body weights in phase two. Values are presented as mean ± SEM. Rats per group (n = 1) in phase two.

Discussion

Medicinal plants continue to attract considerable interest due to their potential as alternative therapies to conventional drugs (20). However, they are not substitutes for modern medical treatments for serious health conditions (21). The therapeutic potential of medicinal plants is largely attributed to their bioactive secondary metabolites (22). In this study, preliminary phytochemical screening of the methanolic leaf extract of *P. laxiflora* and its ethyl acetate and n-hexane fractions revealed the presence of carbohydrates, alkaloids, flavonoids, phenolic compounds, tannins, saponins, cardiac glycosides, and steroids/triterpenoids (Table 4). The methanolic extract and aqueous fraction shared similar metabolite profiles, while ethyl acetate and n-hexane fractions also shared similar profiles, which likely reflect the polarity differences of the solvents (22). This finding aligned with a previous study of phytochemical screening of the stem bark extract of *P. laxiflora* (21, 22).

The current study found that the methanolic leaf extract contained higher concentrations of saponins, steroids, and tannins than the stem bark extract of *P. laxiflora*. This difference in metabolite composition may arise from variations in extraction methods, solvent used, harvest periods, climatic conditions, and genetic factors (23). Methanol, being highly polar, efficiently extracts polar compounds, whereas n-hexane and ethyl acetate extract non-polar and semi-polar compounds, respectively. These results suggest that the leaf contains predominantly polar bioactive constituents.

HPLC analysis revealed substantial levels of total flavonoids and total phenolic compounds present in the methanolic leaf extract of P. *laxiflora*. This result is consistent with previous studies on the total flavonoid and phenol contents of the extract, when compared with the standard quercetin and gallic acid equivalences (15, 24). Polyphenolic compounds, including flavonoids and phenolic acids, are well-known for their antioxidant, anti-lipoxygenase, and anticancer properties (24). Their redox properties, facilitated by hydroxyl groups, enable scavenging of free radicals, mitigating oxidative stress-related damage (25, 26).

GC-MS analysis identified 72 chemical compounds across the methanol extract with 21 compounds, ethyl acetate with 24 compounds, and n-hexane fraction with 27 compounds. Major compounds identified in the methanolic extract included: the 7, 11 hexadecadienal (37.11%), 1,4-Bis (trimethylsilyl) benzene (29.80 %), and 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (28.89 %). The ethyl acetate fraction included Cis-Vaccenic acid (35.80%) and 13-Octadecenoic acid, methyl ester (21.53%). Furthermore, in the n-hexane fraction, the compounds are Penta siloxane, 1,1,3,3,5,5,7,7,9,9 -Deca methyl (3.93 %) and 1-(4,7-dihydro-2-methyl-7-oxopyrazolo[1,5-a]pyrimidin-5-yl)-methyl ester (2.28 %). This result is consistent with previous GC-MS analyses of volatile oil components extracted from the leaves and stems of *P. laxiflora* (27, 28). However, the biological activity of some of these bioactive compounds in this plant has been reported. 7, 11-Hexadecadienal is a polyunsaturated aldehyde whose therapeutic component used in humans has not been reported. However, it is used in agricultural biotechnology as a safer alternative to insecticides **(**29). Cis-vaccenic acid is a natural monounsaturated fatty acid found in tissues and dairy products, with anti-inflammatory, cardioprotective, neuroprotective, anticancer, antimicrobial roles in bacteria, and anti-diabetic / metabolic regulation properties (30). Penta-siloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl- (Decamethylpentasiloxane / D5) is a cyclic or linear siloxane**.** It is not a pharmacologically active compound in the therapeutic sense, but it has anti-irritant**,** skin-protective, and weak estrogenic activities (31). Previous studies have demonstrated that combining quantitative data with mass spectral databases via GC-MS provides valuable insights into correlating bioactive compounds with their pharmacological potential (27).

Acute toxicity studies revealed no mortality or significant behavioural or physiological changes in rats at doses up to 5000 mg/kg, indicating a high safety margin. Observed parameters included fur, skin, ocular mucous membranes, behaviour, food and water intake, and absence of tremors, salivation, or diarrhoea. A significant increase in body weight ($p < 0.05$) was observed only in the 10 mg/kg group, suggesting that the extract does not adversely affect appetite or metabolism. These findings are consistent with previous toxicity studies of medicinal plant extracts in male rats (32, 33). This is because changes in body weight are commonly used to assess potential adverse effects of drugs or chemicals, with weight loss exceeding 10% of initial weight considered significant (33).

Overall, the study demonstrates that the methanolic leaf extract of *P. laxiflora* is rich in bioactive phytoconstituents and exhibits a wide safety margin in male Wistar rats, supporting its traditional use in managing various ailments. The data also provide a foundation for further pharmacological and clinical investigations.

Summary and conclusion

The methanolic leaf extract of *Pericopsis laxiflora* contains a diverse range of bioactive secondary metabolites, including alkaloids, saponins, flavonoids, tannins, phenols, and cardiac glycosides. Quantitative analysis revealed high levels of total phenolics and flavonoids. GC-MS profiling identified 72 chemical compounds across the methanol, ethyl acetate, and n-hexane fractions, reflecting the plant's rich chemical diversity. Acute toxicity studies in male Wistar rats indicated an LD_{50} greater than 5000 mg/kg, with no significant adverse effects on behaviour, physiology, or body weight. These findings suggest that the extract is safe and possesses a wide margin of safety. In conclusion, the methanolic leaf extract of *P. laxiflora* is relatively safe and contains diverse phyochemical compounds with wide-ranging potential pharmacological properties. Further studies are warranted to explore its therapeutic applications and clinical relevance.

Limitations of the study

As part of this study's limitations, the exclusive use of male Wistar rats and the absence of a control group in the acute toxicity study were noted.

Conflict of interest

There is no conflict of interest by the authors.

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