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RESEARCH ARTICLE

Metabolomic profiling, FT-IR and GC-MS characterization and bioactivity of *Psychotria adenophylla* leaf extracts

Debasish Dikshit*, Topi Karga & A Venkatesan

Department of Botany, Annamalai University, Annamalai Nagar, Cuddalore 608 002, India

*Correspondence email - debasishdikshit586@gmail.com

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Abstract

Psychotria adenophylla Wall. belongs to Rubiaceae family and known "Chelianthua" in Odisha. It's used as traditional medicine and an efficient analytical method for reliably assessing the quality of *P. adenophylla* leaves has yet to be clearly developed. The present study investigated the preliminary phytochemical screening, FT-IR and GC-MS characterization, anti-inflammatory**,** antidiabetic and anti-bacterial activity of different solvents leaves extract where it showed significant biological activities. Qualitative analysis was used to identify the secondary metabolites saponin, tannin, alkaloid, phenol, phlobatannin, steroid, terpenoid, glycosides, anthraquinone and flavonoids. Quantitative phytochemical screening of total phenolic content (TPC), total flavonoid content (TFC) and total steroid content (TSC) was done using different methods. FT-IR and GC-MS was done using crude extracts, viz., petroleum ether, chloroform and aqueous. *In-vitro* anti-inflammatory activity was done by albumin inhibition assay. *In-vitro* hypoglycemic effect was studied using α-amylase inhibition assay and α-glucosidase assay. Antibacterial potential was studied through agar well diffusion method, MIC and MBC were evaluated by serial dilution method. Result showed the presence of secondary metabolites viz. saponin, tannins, alkaloids, phenol, steroids, terpenoids, glycosides, anthraquinone and flavonoids. Total phenolic content was evaluated to be 118.76 mg GAE, total flavonoid content was 47.42 mg/g rutin equivalent and total steroid content was estimated to be 104.9 mg/g of diosgenin in petroleum ether (PA-Pe) extract which was higher than chloroform (PA-C) i.e 65.51 mg/g of diosgenin and 49.15 mg/g of diosgenin in aqueous extract (PA-A). GC-MS spectrum shows 25 compounds in PA-Pe crude extract, where PA-C showed 50 compounds. The petroleum ether extract demonstrated superior biological activity, exhibiting 73.79 % α-amylase and 71.55 % α-glucosidase inhibition (antidiabetic activity), along with significant antibacterial effects against gram-negative pathogens (18.32 ± 0.58 mm zone against *K. pneumoniae*) with MIC/MBC values of 37.5 µg/mL where in gram positive bacteria *B. subtilis* showed higher zone of inhibition of 17.39 ± 0.64. The lowest MIC and MBC were evaluated for both *K. pneumoniae* and *B. subtilis* i.e 37.5 µg/mL and > 37.5 µg/mL Every test revealed that petroleum ether extract was more active than chloroform and aqueous extracts. These findings highlight PA-Pe as the most effective extract, suggesting its potential as a source of natural anti-inflammatory, antimicrobial and antidiabetic agents.

Keywords: antidiabetic activity; FT-IR; GC-MS; MIC; MBC; *Psychotria adenophylla* wall

Introduction

In recent decades, diabetes mellitus (DM) has grown to be a significant worldwide health concern, mostly linked to modern eating habits (1). Approximately 90 % of individuals with diabetes worldwide have type 2 diabetes mellitus (T2DM), which affects an estimated 537 million people (2). Dysregulation of glucose and lipid homeostasis characterises diabetes mellitus (DM), a chronic metabolic disease that leads to persistent hyperglycemia, impaired insulin action and progressive islet cell failure. Long-term sequelae such as diabetic retinopathy, macrovascular and microvascular damage and an elevated risk of cardiovascular diseases (CVD) are all significantly influenced by chronic hyperglycemia (3).

The globe is still suffering greatly from infectious illnesses brought on by bacteria, viruses, fungus and other parasites. Both gram-positive and gram-negative bacteria can develop resistance mechanisms to environmental aggression (natural environment, competing bacteria, host defence, or antibiotics) by producing degradative enzymes or altering the sites of action of anti-infective molecules. Therefore, it is crucial to use natural plants in the development of new medications in order to combat antibiotic resistance and reduce unwanted side effects (4).

With an estimated 1645-2000 plant species worldwide, the genus *Psychotria*, which Linnaeus named in 1759, is one of the biggest angiosperm genera (5). Only a small number of species in this genus inhabit drier conditions; most of them are found in tropical areas and thrive in wet to seasonal forests (6). A cymose inflorescence, small white or yellow flowers, drupaceous fruits, two plano-convex pyrenes with a preformed germination slit, seeds with a ruminate endosperm and seed coat pigments that dissolve in ethanol are characteristics used to describe the majority of species in this genus, which are generally classified as shrubs, small trees, or climbing vines. A total of 27 *Psychotria* species and one variant are now recorded

in Vietnam, following the recent identification of *Psychotria ngotphamii* as a new species in 2023 (7).

Traditionally, various portions of these plants such as leaves, roots and rhizomes have been used to treat female gynaecological haemorrhage, ulcers, stomach-aches, bronchitis and fever (8). According to pharmacological research, *Psychotria* plants contain a range of biological properties, such as high cytotoxic effects against many cancer cell lines, analgesia, hypoglycemia, antibacterial and antiviral properties (9, 10). According to a thorough analysis of the genus *Psychotria*'s chemical data, the main constituents of this genus include terpenoids and alkaloids. *Psychotria* plant biomarkers include some alkaloids (11). The southern region of Vietnam is home to *Psychotria adenophylla* wall, however nothing is known about its phytochemical makeup. Eight sterols and triterpenes, including b-sitosterol, betulin, betulinic acid, α-amyrin, ursolic acid, friedelin, bauerenol and bauerenol acetate, were found in a study on the chemical contents of *P. adenophylla* growing in India (12). FT-IR and GC-MS characterisations, phytochemical screening and the antidiabetic and antibacterial properties of several solvent extracts such as chloroform, petroleum ether and aqueous of *Psychotria adenophylla* leaves, a plant used as traditional medicine in Odisha are the main focus of this study.

Materials and Methods

Plant sample

Psychotria adenophylla wall plant was collected from Kapilash Hill Range, Dhenkanal, Odisha (Lat 20.697442 ^{0}, Long 85.883254 ^{0}) by Debasish Dikshit on 07.03.2024 and was identified by Prof. (Dr.) D. Kumarasamy and verified by Prof (Dr.) L Mullainathan, Professor, Department of Botany, Annamalai University (Voucher: AUBOT#626) (Fig. 1). Approximately 1kg of fresh leaves was taken, shade dried, coarsely powdered using a mixer and stored in a zip-lock bag in a dark location. Petroleum ether, chloroform and aqueous solvents were used in a 1:10 (sample: solvent) ratio for the extraction process, which was carried out using a Soxhlet apparatus. Petroleum ether and chloroform extractions were performed at 40-60 ^{0}C temperature for 2 hr, whereas aqueous extraction was conducted at 80 ^{0}C for 4-6 hr.

Fig. 1. *Psychotria adenophylla* Wall (AUBOT#626).

Qualitative phytochemical analysis

Using standard procedures, the secondary metabolites alkaloids, flavonoids, glycosides, phenols, saponins, steroids, terpenoids, anthraquinone, phlobatannin and tannins were qualitatively identified from the crude plant extracts of *Psychotria adenophylla* in Petroleum ether, chloroform and aqueous (13).

Quantitative phytochemical analysis

Estimation of total phenol content (TPC)

This method was followed according to Slinkard and Singleton (14). Determination of total phenolics by spectrophotometric method: The Folin-Ciocalteu method was used to calculate the extracts total phenolic content. Test tubes were filled with varying amounts of working standard gallic acid solutions and crude plant extracts were utilised in place of gallic acid for the test samples. Each tube received 5 mL of Folin-Ciocalteu reagent, which was then left to stand in the dark for five min. After adding 4 mL of 7.5 % sodium carbonate, the tubes were left at 23 °C in the dark for 90 min. The tubes were mixed and allowed to stand for 1 min. At 765 nm, absorbance was measured. The total phenolic content was expressed as gallic acid equivalents (GAE) in micrograms per gram of tissue, as calculated from a standard gallic acid calibration curve. A range of concentrations of working standard gallic acid (20, 40, 60, 80 and 100 µg/mL) in the 20–100 µg/mL concentration range were generated in a series of test tubes for the standard curve. One millilitre of distilled water was used to create a blank individually. One millilitre of crude extract containing 100 µg/mL was utilised for the test. After adding four mL of sodium carbonate and five mL of Folin-Ciocalteu reagent, the mixture was incubated for ninety min at 23 °C. The tubes were mixed and allowed to stand for 1 min. The colour developed was measured by recording the absorbance at 765 nm.

$$\text{Total phenol content (TPC)} = \frac{CV}{M} \text{ Mg/gm} \qquad \text{(Eqn. 1)}$$

C = Concentration of gallic acid from calibration curve

V= Volume of extract in ml

M=Weight of plant extracts in gm.

Estimation of total flavonoid content (TFC)

Aluminum chloride colorimetric method was used for total flavonoids (15).

Estimation of total flavonoid content: In a 10 mL test tube, 1 mL of crude plant extract at a concentration of 100 µg/mL was used for the test. For the standard, different volumes (0.2, 0.4, 0.6, 0.8 and 1.0 mL) of 30 % methanolic quercetin were used, corresponding to a concentration range of 20–100 µg/mL. To each tube, 0.3 mL of $NaNO_2$ (0.5 M) and 0.3 mL of $AlCl_3$ (0.3 M) were added and mixed. After 5 in, 2 mL of NaOH (1 M) was added and the volume was made up to 10 mL with distilled water. The solution was mixed well and the absorbance was measured against a reagent blank at 506 nm. The standard curve for total flavonoids was prepared using quercetin standard solution (0.1 mg/mL) following the same

procedure as described above. The total flavonoid content was expressed as mg of rutin equivalents per gram of dried fraction.

$$\text{Total flavonoid content (TPC)} = \frac{\text{RxD.FxV}}{\text{W}} \text{ X } 100 \quad \text{(Eqn. 2)}$$

R = Results obtained from standard curve

D.F = Dilution factor

V = Volume of stock solution

100= 100 µg of dried extract

W= Weight of plant used in gm.

Quantification of total steroid

Quantification of total steroids in plant extracts was done by adopting method reported in an earlier study (16).

To measure total steroid content a spectrophotometric assay built on ferric-ferrous complex formation was used. Calibration graph was obtained using ethanolic solutions of Diosgenin as reference standard. The analytical technique for measuring total steroid content was achieved as follows:

A stock standard solution of 1000 µg/mL was prepared by dissolving 2 mg of the compound in 2 mL of ethanol. A series of working reference solutions was prepared by suitably diluting the stock solution with ethanol to obtain concentrations ranging from 20 to 100 µg/mL (20, 40, 60, 80 and 100 µg/mL). 1 mL of the test extract of the steroid solution was placed into 10 mL volumetric flasks. After adding and mixing sulphuric acid (4 N, 2 mL) and iron (III) chloride (0.5 % w/v, 2 mL), potassium hexacyanoferrate (III) solution (0.5 % w/v, 0.5 mL) was added. The mixture was maintained in a water bath at 70 °C for 30 min with intermittent shaking and then diluted to the mark with distilled water. The absorbance was measured at 780 nm. For the sample extract, a stock solution of 1000 µg/mL was prepared by dissolving 10 mg in 10 mL of water, followed by sonication for 30 min. The same procedure was then repeated using the above-mentioned steps.

Fourier Transform Infrared Spectroscopy (FT-IR) analysis and Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The functional groups in the plant extract were identified using a Fourier Transform Infrared (FTIR) spectrophotometer. The spectrum indicates the wavelength of absorbed light, which corresponds to specific chemical bonds. The FTIR spectrometer (Agilent) was employed to analyze different solvent extracts from the leaves of *Psychotria adenophylla* Wall. GC-MS analysis of the petroleum ether and chloroform leaf extracts was conducted at CIF, Central University of Punjab. The mass spectra of unknown compounds were compared with those of known compounds in the database to identify their molecular masses and were identified using the National Institute of Standards and Technology (NIST) library.

Anti-inflammatory activity

Two mL of 1 % bovine albumin fraction were mixed with 400 mL of plant extract at varying concentrations (50–150 millilitres). The pH of the reaction mixture was then brought down to 6-8 using 1N HCl. After incubating for 20 min at ambient temperature, the reaction was heated to 55 °C for 20 min in a water bath. After the mixture cooled to 27 ^{0}C, the absorbance value was measured at 600 nm. A similar amount of plant extract was substituted with DMSO as the control. Diclofenac sodium was used as a reference at several doses. The experiment was run three times to avoid standard error. Percentage of inhibition formula:

$$\% \text{ inhibition} = \frac{\text{Control O.D - Sample O.D}}{\text{Control O.D}} \text{ X } 100 \quad \text{(Eqn. 3)}$$

In- vitro hypoglycemic effects

α- Amylase inhibition assay

This test was conducted using the methodology described by a previous study (17). First, 500 µL of NaCl and 0.02 M sodium dihydrogen phosphate buffer were combined with 0.006 M. After adding 0.5 mg/mL of porcine pancreatic alpha-amylase to the mixture, it was incubated for 10 min at 25 °C. The mixture's pH was changed to 6.9. Following the first incubation, 50 µL of 0.006 M sodium phosphate buffer with 0.006 M NaCl and 1 % starch solution were added. The reaction mixture was incubated at 25 °C for an extra 10 min. After adding 1.0 mL of DNSA (3, 5-dinitrosalicylic acid), the mixture was boiled in water for 5 min. The combination was left to cool at room temperature. The reaction mixture was diluted with 10 mL of deionised water and the UV absorbance at 540 nm was measured. Acarbose an enzyme inhibitor served as the assay's control. IC50 value was determined, which indicates the sample concentration required to record 50 % inhibition of enzyme activity. The formula is used to determine the percentage of α-amylase activity inhibition.

% Inhibition of α - Amylase =

[(Absorbance of control- Absorbance of sample)/Absorbance of control] × 100 (Eqn. 4)

α-Glucosidase inhibition assay

According to the method outlined earlier research the tests were carried out (17). The tests were carried out 20 µL of plant extracts was added with 0.5 mg/mL of 0.1 M phosphate buffer at a pH of 6.9 in the first phase. At 25 °C, the produced solution was incubated for ten min. Following the addition of different crude extracts again the mixture was incubated at 25 °C for five min. Each reaction mixture's absorbance was determined at 405 nm using UV-V is spectrophotometry. In this assay, the enzyme serves as a control. IC50 value was calculated representing the concentration of sample which needed to inhibit 50 % of the enzyme activity was reported. The following formula was used to calculate the inhibition percentage of α-glycosidase activity.

% Inhibition of α- glycosidase =

[(Absorbance of control- Absorbance of sample)/ Absorbance of control] × 100 (Eqn. 5)

Antibacterial assay

The agar well diffusion experiment was used to determine the zone of inhibition of the extracts' sensitivity to various bacterial

strains (18). The extracts that would be tested in dimethyle sulfoxide (DMSO) were prepared at three distinct concentrations: 75, 150 and 300 µg/ml. A sterile cotton swab was used to remove an inoculum from the standardised culture. After that, it was added to a new batch of solidified Mueller-Hinton agar and let to stand for around fifteen min. A 6 mm sterile stainless-steel borer was used to make wells and different extract concentrations apply to each well created in the infected plates with positive control (PC) and negative control. After 15 min of pre-diffusion, the plates were incubated upright at 37 ^{0}C for 24 hr. The zone of inhibition that developed around the well was subsequently measured. Azithromycin for gram-positive bacteria, ciprofloxacin, a popular antibacterial drug, for gram-negative bacteria and DMSO as a negative control. Following three independent tests by the extracts, PA-Pe, PA-C and PA-A with antibiotics, the mean zones of inhibition for each extract and the reference antibiotic were calculated.

Determination of minimum inhibitory concentration (MIC)

Using the broth macro-dilution technique in MHB (Mueller Hinton Broth), the lowest inhibitory concentration of plant extracts for bacteria were evaluated (19). To make a 128 mg/ml (plant) stock solution, the extracts were dissolved in 5 % DMSO. 0.5 ml of stock and 0.5 ml of MHB for bacteria were mixed together to get concentrations of 150, 75, 37.5, 18.75, 9.38, 4.7, 2.34 and 0.58 µg/ml (for plant extracts). Each tube contained 5 0 µl of the test organism's standardised suspension. Only organisms and no plant extracts were present in the control tube. The bacteria in the culture test tubes were maintained at 37 ºC for 24 hr. The minimum inhibitory concentration (MIC) was defined as the lowest concentrations at which the tested organism did not grow when seen under a microscope.

Minimum bactericidal concentration (MBC)

The MBC of the extracts was determined using 100 µl samples from each MIC test tube with growth inhibition and freshly prepared Mueller Hinton Agar (for bacteria) (20). Bacteria were then cultivated on the plates at 37 ºC. The MBC was the smallest quantity of extract that prevented the growth of any detectable bacterial on the corresponding agar plate throughout the incubation time.

Statistical analysis

The findings from the experimental investigation were analysed statistically in triplicate. The mean and standard deviation were calculated using Duncan's Multiple Range Test (DMRT) and GraphPad Prism version 8. The antibacterial activity of the plant extracts was assessed by measuring the zone of inhibition of the test organisms and calculating the means, standard deviations at P- values significance to < 0.001.

Results

Qualitative phytochemical screening

Several secondary metabolites, including saponin, tannins, alkaloids, phenol, steroids, terpenoids, glycosides, anthraquinone and flavonoids, were identified by secondary phytochemical examination of *Psychotria adenophylla* leaves crude extract from various solvents, including chloroform, petroleum ether and aqueous (Table 1).

Quantitative analysis

Using standard gallic acid, the total phenolic content of several solvent leaf crude extracts of *P. adenophylla* showed that the greatest phenolic content was in PE-Pe>PE-C>PE-A, with PE-Pe having a phenolic content of 118.76 mg GAE/g (Fig. 2). The rutin -based standard curve (Fig. 3) revealed that PA-Pe had the greatest overall flavonoid equivalency content (PA-Pe > PA-C > PA-A), with PA-Pe exhibiting 70.33 mg/g rutin equivalent. In PA-C, the rutin equivalent was 65.51 mg/g, while in PA-A, it was 47.42 mg/g. The total steroid equivalency content, as determined by the standard curve using diosgenin, was found to be at PA-Pe crude extract, or 104.96 mg/g Diosgenin, which was higher than PA-C (63.054 mg/g Diosgenin) and PA-A (49.15 mg/g Diosgenin) (Fig. 4).

FT-IR analysis

FT-IR spectroscopy is frequently used to determine a material's inorganic functional groups. Ten frequencies 3377.0, 2922.0, 2855.1, 1692.2, 1461.1, 1379.1, 1215.1, 1020.7, 827.5 and 752.9 were identified with several types of compounds, including

Table 1. Phytochemical screening of different crude extract of *Psychotria adenophylla* leaves

Secondary metabolites	Tests Performed	Result		
		Petroleum ether	Chloroform	Aqueous
Saponin	Foam test	+	+	++
	Froth test	+	+	+
Tannin	$FeCl_3$ test	++	+	-
	1 % HCl test	++	+	+
Alkaloid	Mayer's test	++	+	+
	Wagner's test	++	+++	+
Phenol	Ellagic test	-	++	-
Phlobatannin	1 % HCl test	-	-	-
Steroids	Salkowski's test	++	+	+
	Liberman-Burchard's test	+	+	-
Terpenoids	Keller-Killiani test	++	+	+
	Salkowski's test	++	+	+
	Glacial acetic test	++	+	-
Glycosides	Conc. H_2SO_4 test	+	-	+
	Keller-Killiani test	+	-	-
Anthraquinone	Borntrager's test	+	+	-
Flavonoids	1 % ammonia test	+	+	+
	Shinoda test	++	-	+
	Lead-acetate test	+	-	-

+++Highly present; ++ Moderately present; + Lightly present; - Absent

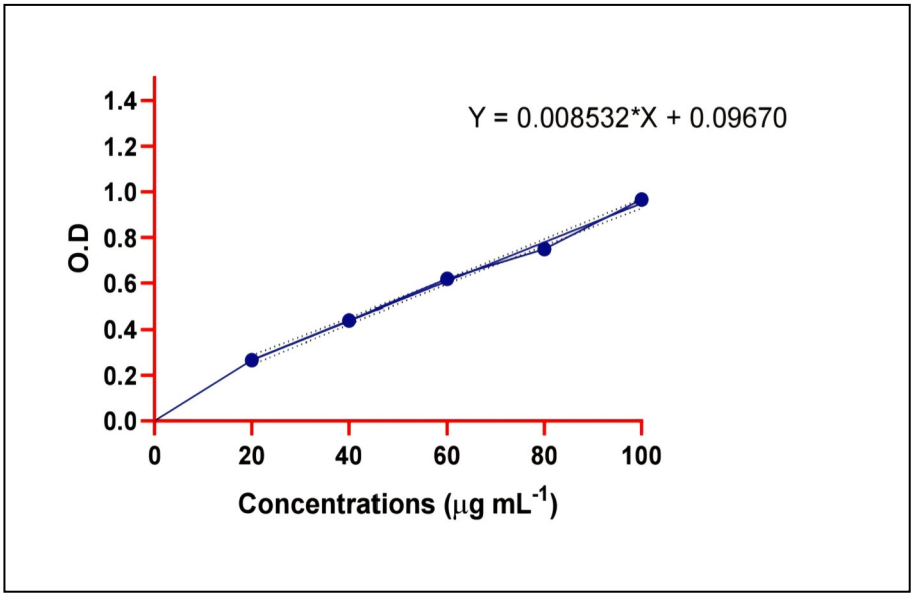


Fig. 2. Standard graph curve for total phenolic content of different plants with different solvents (Gallic Acid).

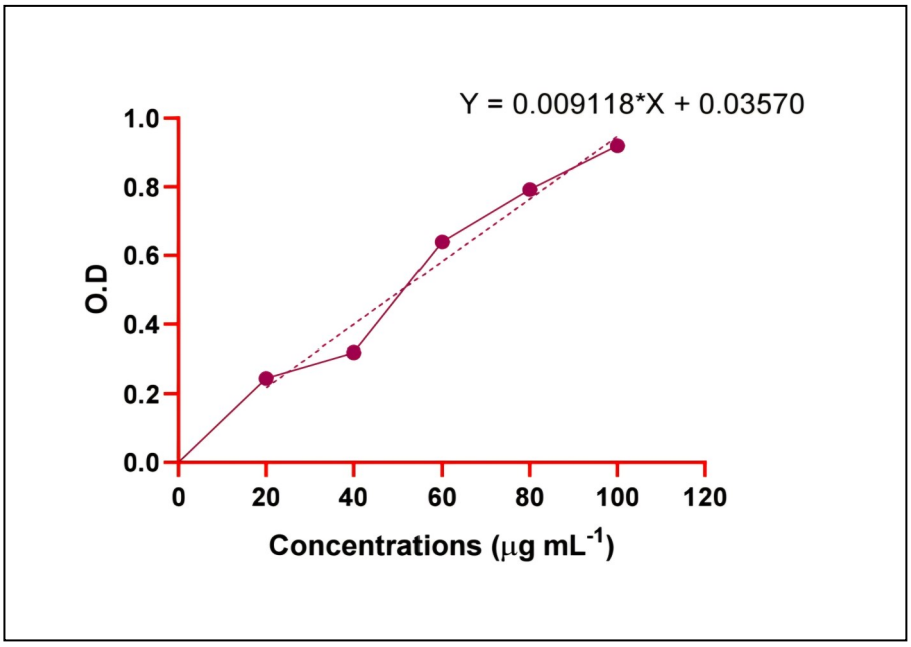


Fig. 3. Standard graph curve for total flavonoids contents of different plants with different solvents (Rutin).

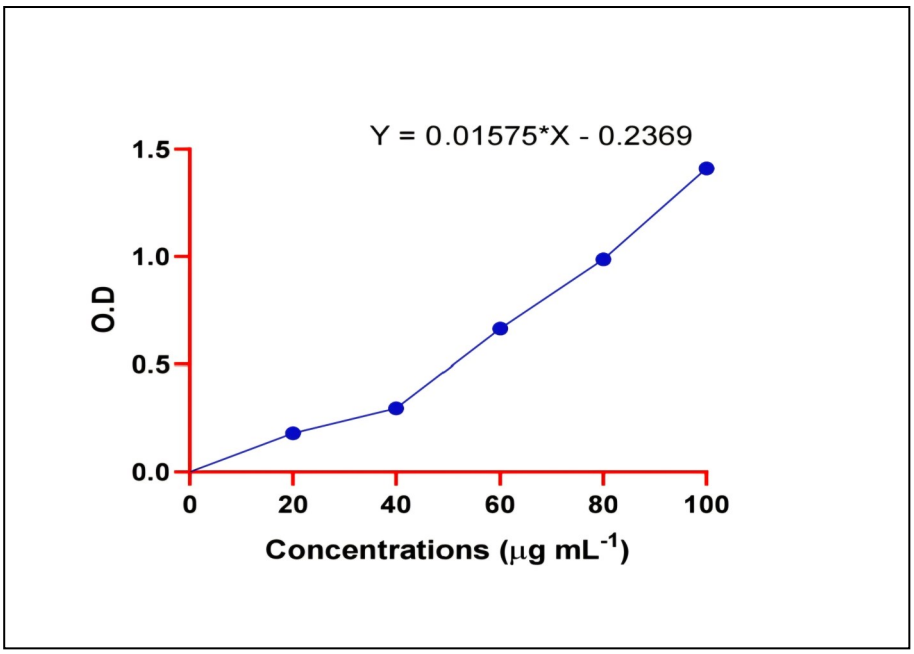


Fig. 4. Standard graph curve for total steroid contents of different plants with different solvents (Diosgenin).

Table 2. Functional group seen by FT-IR analysis of Chloroform extracts of *Psychotria adenophylla* leaves (PA-C)

Wavenumber (cm^{-1})	Vibration Type	Functional Group	References
3377.0	O–H Stretch (broad)	Alcohol	(21, 22)
2922.2	C–H Stretch	Alkane	(21–23)
2855.1	C–H Stretch (symmetric)	Aldehyde	(21–23)
1692.2	C=O Stretch	Imine	(21, 24)
1461.1	C–H Bending	Alkane	(21,22)
1379.1	O–H Bending	Phenol	(21–23)
1215.1	C–O Stretch	Amine	(21, 22)
1028.7	C–O Stretch	Alcohol	(21-23)
827.5	=C–H Bending	Alkene / Aromatic	(21, 22, 24)
752.9	C–H Rocking	Alkane (long-chain)	(21–23)

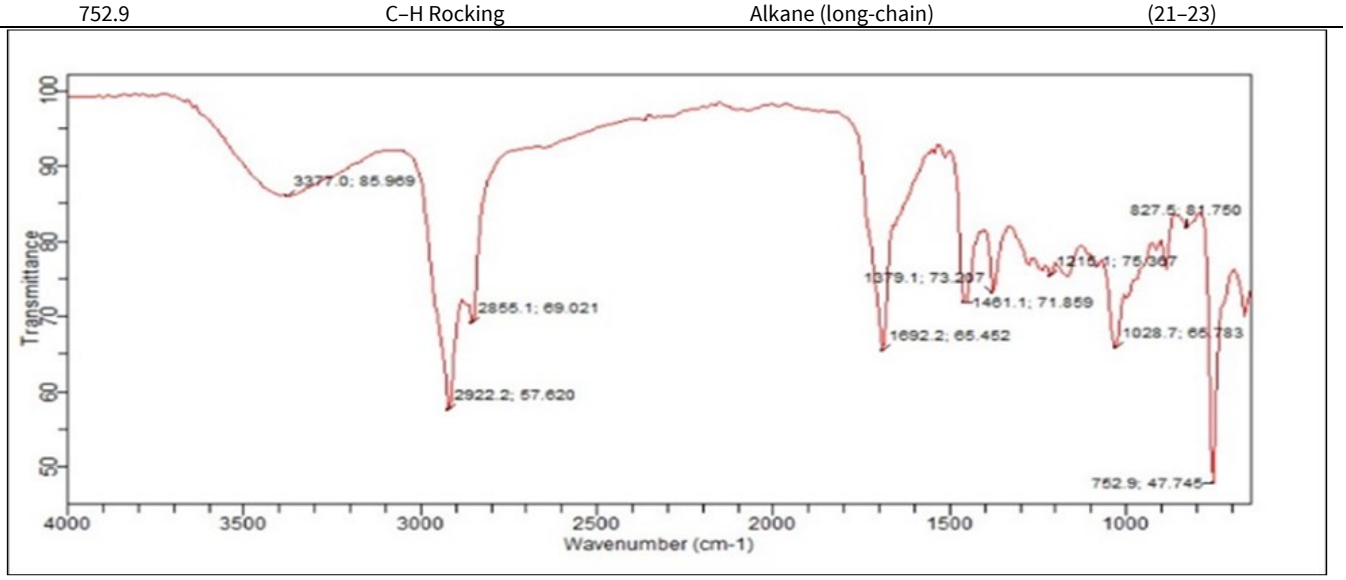


Fig. 5. FT-IR spectrum of leaves chloroform extracts (PA-C) of *Psychotria adenophylla* wall.

alcohol, alkane, imine/oxine, phenol, amine and alkene compound, according to the FTIR spectrum of chloroform leaves extract (Table 2 & Fig. 5). Twelve frequencies 3389.3, 2922.2, 2855.1, 1699.7, 1461.1, 1379.1, 1244.9, 1177.8, 1028.7, 887.1, 827.5 and 723.1 were identified to be present in the FTIR spectrum of leaves petroleum ether extract (PA-Pe) and are associated with a variety of chemicals, including amine salt, alkane, aldehyde, imine, phenol and alkene (Table 3 & Fig. 6). The FTIR spectrum of the aqueous leaf extract (PA-A) exhibited five prominent absorption frequencies at 3265.1, 1632.6, 1401.5, 1267.3 and 1073.5 cm^{-1}, which correspond to functional groups such as alcohols, imines, alkanes, amines and carbohydrate. These findings indicate the diverse range of bioactive functional groups present in the different solvent extracts of *P. adenophylla* leaves, contributing to its pharmacological potential (Table 4 & Fig. 7). The FT-IR analysis of PA-Pe extract exhibits both polar and non-polar compounds where PA-c extract also exhibit similar compounds with additional compounds like oxines and carboxylic acids. But in aqueous extract showed only polar compounds.

GC-MS analysis

Psychotria adenophylla extract's GC-MS spectra showed the peaks that denoted the presence of several chemicals. The chemicals with spectral fingerprints determined by molecular weight and data library are given in Table 5 for Petroleum ether (PA-Pe) and Table 6 chloroform (PA-C) extract, respectively.

Out of 25 compounds, 9 had high peak area (%) based on petroleum ether leaf extract, with Tris (2,4-di-tert-butylphenyl) phosphate and Lup-20(29)-en-3-one - 8.37 % having the highest peak area percentage i.e 42.3 % and 8.37 % respectively (Table 5 & Fig. 8). where 50 different types of chemicals were found to have a high peak area percentage in chloroform extract (PA-C) (Table 6 & Fig. 9). In this case, the peak area of hexadecanoic acid is 15.41 %, while that of tris (2,4 -di-tert-butylphenyl) phosphophaste is 19.58 %. The petroleum ether extract had tris(2,4-di-tert-butylphenyl) phosphate as the dominant compound at 42.31 %, whereas in the chloroform extract, its presence was lower (19.58 %). Fatty acids (e.g., hexadecanoic acid derivatives) were more abundant in the

Table 3. Functional group seen by FT-IR analysis of petroleum ether extracts of *Psychotria adenophylla* leaves (PA-Pe)

Wavenumber (cm^{-1})	Vibration Type	Functional Group	Reference
3389.3	N–H Stretch (broad)	Amine salt	(21, 22)
2922.2	C–H Stretch	Alkane	(21-23)
2855.1	C–H Stretch	Aldehyde/alkane	(22)
1699.7	C=O Stretch	Imine or Oxime	(21, 24)
1461.1	C–H Bending	Alkane	(21)
1379.1	O–H Bending	Phenol	(21-24)
1244.9	C–N Stretch	Amine	(21)
1177.8	C–N Stretch	Amine	(21, 23)
1028.7	C–N Stretch	Amine	(21, 23)
887.1	=C–H Bending	Alkene	(22)
827.5	=C–H Bending	Alkene	(22)
723.1	C–H Rocking	Alkane	(21-23)

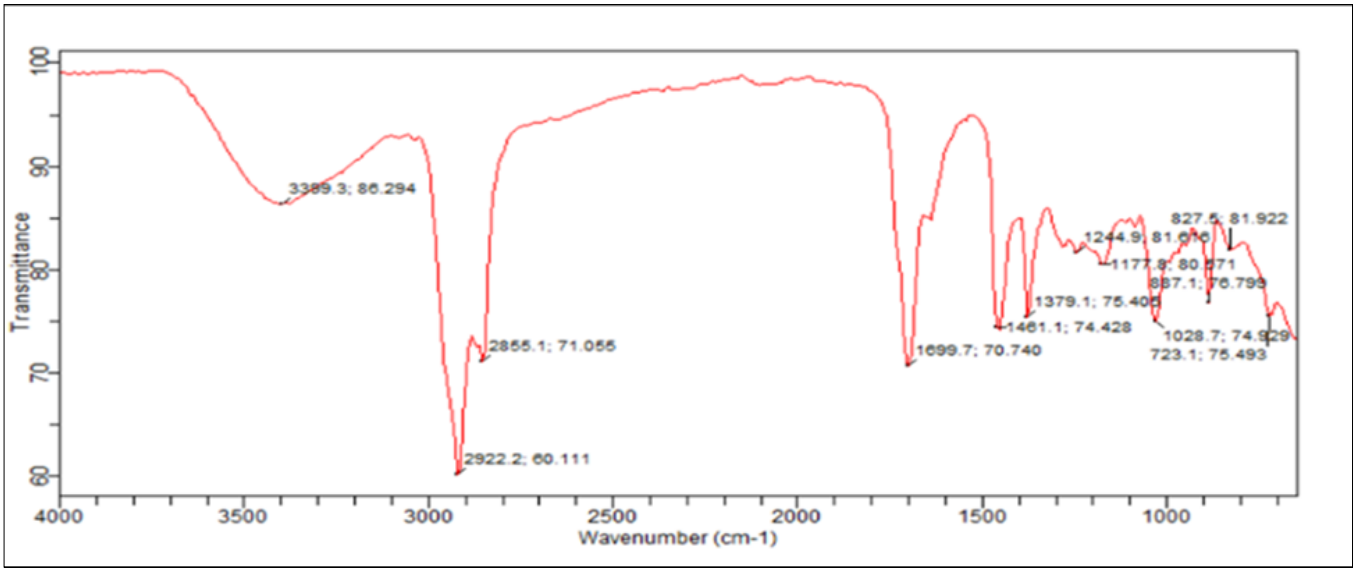


Fig. 6. FT-IR spectrum of leaves petroleum ether extracts (PA-Pe) of *Psychotria adenophylla* wall.

Table 4. Functional group seen by FT-IR analysis of aqueous extracts of *Psychotria adenophylla* leaves (PA-A)

Wavenumber (cm^{-1})	Vibration Mode	Functional Group	References
3265.1	O–H Stretch (broad)	Alcohol	(21,22)
1632.6	C=O Stretching	Imine or Oxime	(21,24)
1401.5	C–H Bending	Alkane	(21, 22)
1267.3	C–N Stretching	Amines	(21-23)
1073.5	C–O Stretching	c	(22)

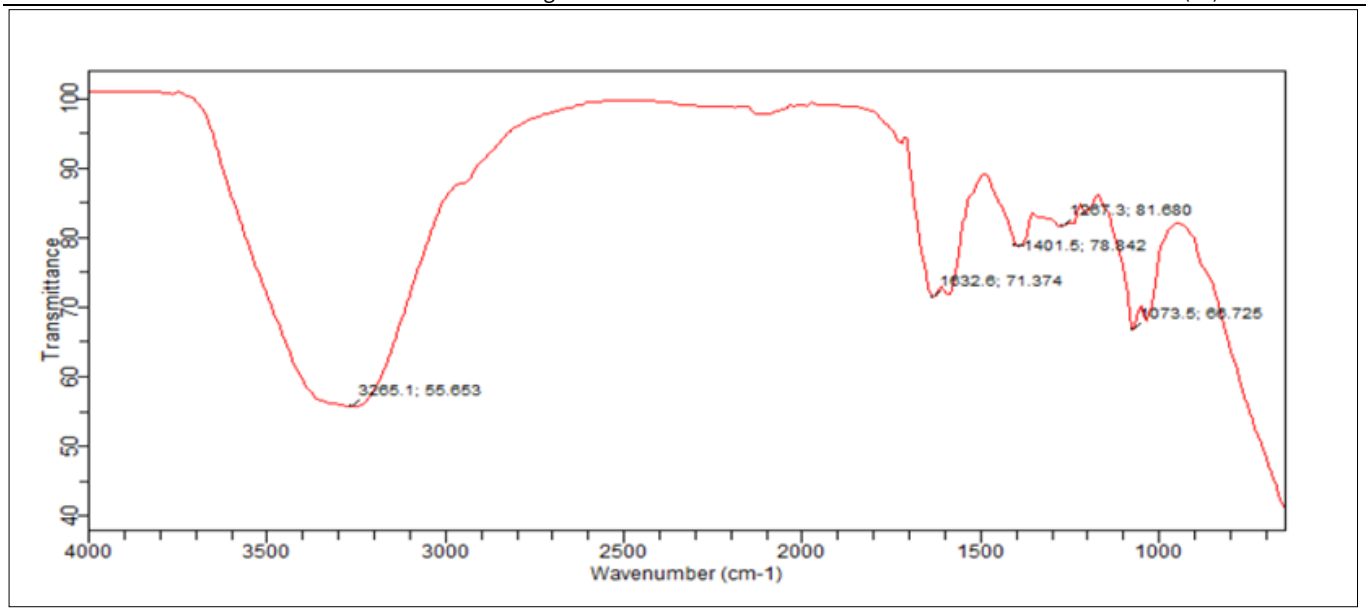


Fig.7. FT-IR spectrum of leaves aqueous extracts (PA-A) of *Psychotria adenophylla* wall.

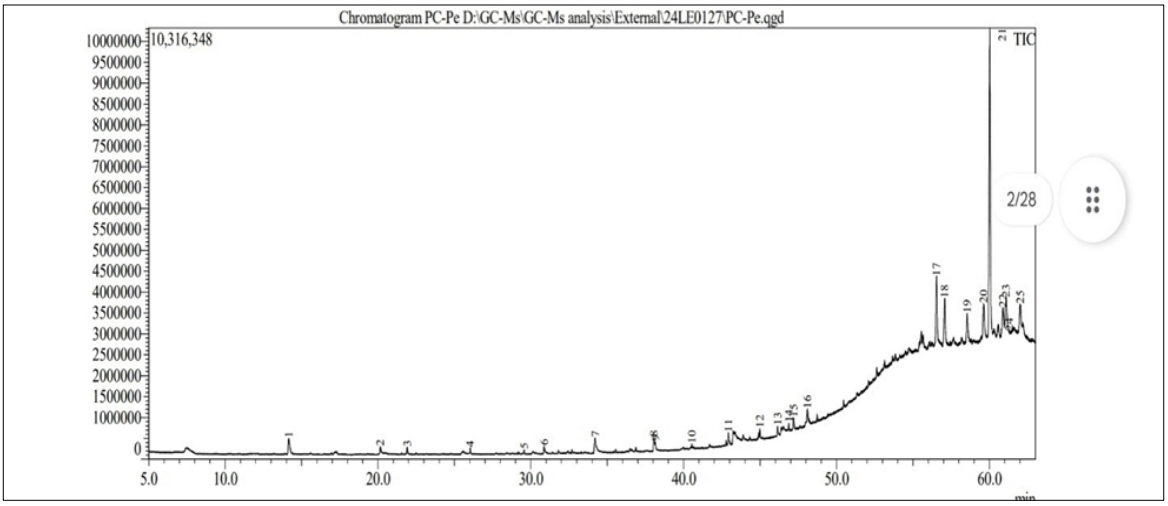


Fig. 8. Shows the GC-MS spectrum of petroleum ether leaves extracts of *Psychotria adenophylla* (PA-Pe).

Table 5. GC-MS analysis of petroleum ether leaves extracts of *Psychotria adenophylla*

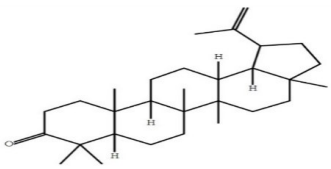
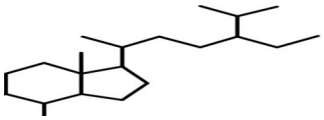
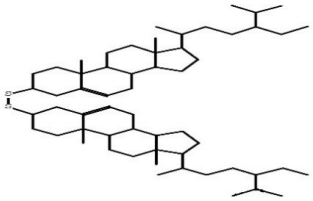
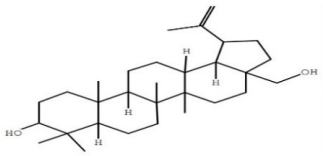
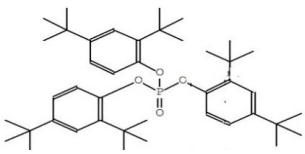
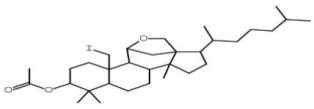
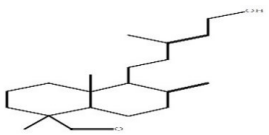
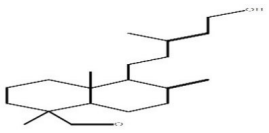
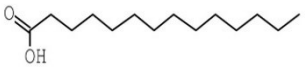
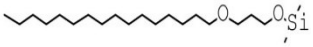
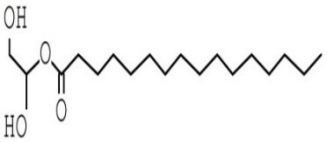
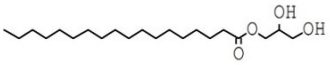
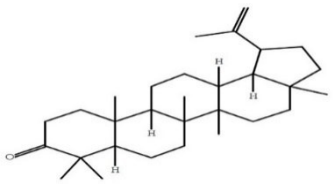
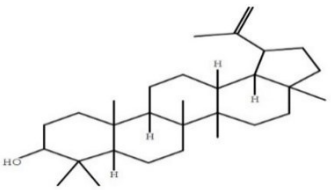
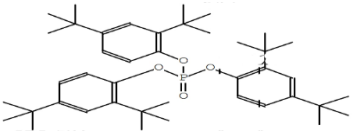
Sl No.	Compound Name	Molecular Formula	Molecular weight (g/mol)	Retention time (Min)	Peak area %	Structure
1	Dodecane	$C_{12}H_{26}$	170	14.164	2.40	
17	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424	56.539	8.37	
18	Lupeol	$C_{30}H_{50}O$	426	57.075	7.11	
19	Thiositosterol disulfide	$C_{58}H_{98}S_2$	858	58.547	4.52	
20	Betulin	$C_{30}H_{50}O_2$	442	59.619	6.46	
21	Tris(2,4-di-tert-butylphenyl) phosphate	$C_{42}H_{63}O_4P$	662	60.025	42.31	
22	Lanostan-3.beta.-ol, 11.beta.,18-epoxy-19	$C_{32}H_{53}IO_3$	612	60.882	4.74	
23	(1R,4aR,5S)-5-[(E)-5-Hydroxy-3-methylp	$C_{20}H_{32}O_2$	304	61.092	7.39	
25	(1R,4aR,5S)-5-[(E)-5-Hydroxy-3-methylpent-	$C_{20}H_{32}O_2$	304	62.011	5.08	

Table 6. GC-MS analysis of chloroform leaves extracts of *Psychotria adenophylla*

S. No.	Compound Name	Molecular Formula	Molecular weight (g/mol)	Retention time (Min)	Peak area %	Structure
7	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	29.325	2.75	
22	Tetracosane	$C_{24}H_{50}$	338	41.691	2.08	
23	3-(Hexadecyloxy)propan-1-ol, TMS	$C_{22}H_{48}O_2Si$	372	42.919	2.26	
24	Hexatriacontane	$C_{36}H_{74}$	506	43.364	4.13	
25	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	43.635	15.41	
26	Hexatriacontane	$C_{36}H_{74}$	506	44.970	3.44	
28	Hexatriacontane	$C_{36}H_{74}$	506	46.517	4.82	
29	Octadecanoic acid, 2,3-dihydroxypropyl	$C_{21}H_{42}O_4$	358	46.869	6.91	
31	Hexatriacontane	$C_{36}H_{74}$	506	48.007	3.55	
33	Tetrapentacontane	$C_{54}H_{110}$	758	49.443	2.51	
43	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424	56.571	3.65	
44	Lupeol	$C_{30}H_{50}O$	426	57.102	3.47	
47	Tris(2,4-di-tert-butylphenyl) phosphate	$C_{42}H_{63}O_4P$	662	60.136	19.58	

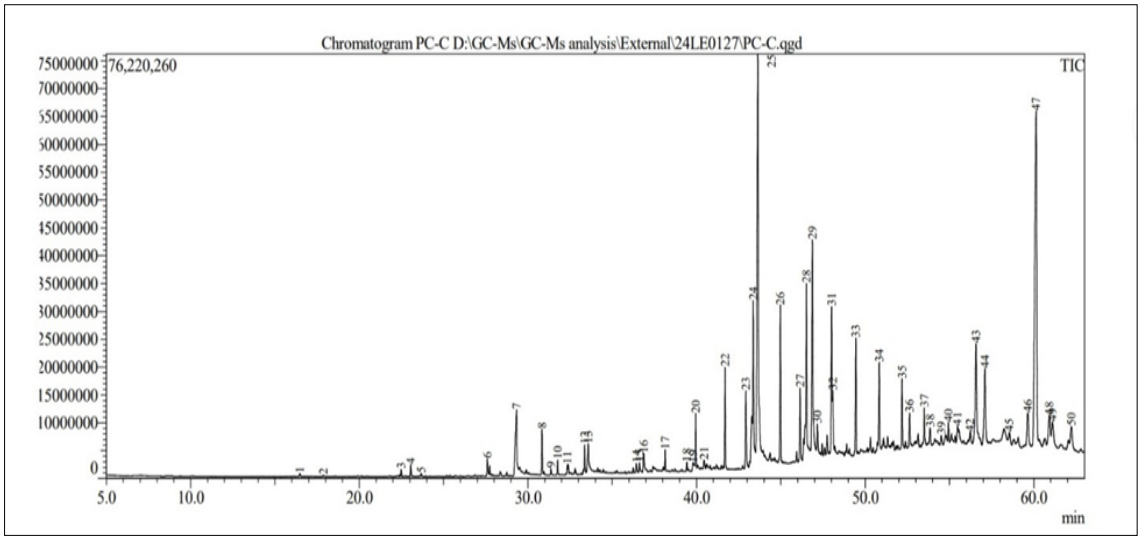


Fig. 9. Shows GC-MS spectrum of chloroform leaves extracts of *Psychotria adenophylla* (PA-C).

chloroform extract, with hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester being a major constituent (15.41 %). Sterols and triterpenoids (e.g., lupeol, betulin, lanostan-3β-ol) were more prominent in the petroleum ether extract, suggesting a preference for non-polar compounds in this solvent.

Anti-inflammatory activity

The anti-inflammatory properties of the PA-Pe, PA-C and PA-A extracts were assessed using the albumin denaturation test. With the greatest inhibition percentage of 55 %, the PA-Pe extract showed the most anti-inflammatory action at a dosage of 150 µg/mL. By contrast, the PA-C and PA-A extracts showed 48.5 % and 40.15 % inhibition rates, respectively. The percentage of inhibition of standard diclofenac showed highest at 150 µg/mL i.e. 88.33 % where at 50, 75, 100, 125 µg/mL it showed 35.3 %, 52.3 %, 66.03 % and 77.31 % respectively. These results point to the PA-Pe extract's potential for more research and use in therapeutic settings by indicating that it has a stronger anti-inflammatory impact than the other extracts (Fig. 10).

Antidiabetic activity

α-amylase assay

The α-amylase inhibition activity of *P. adenophylla* crude extract was evaluated using various solvents at concentrations of 100, 200, 300, 400 and 500 µg/mL. At a concentration of 500 µg/mL, the standard acarbose exhibited an inhibition of 90.76 %. Among the plant extracts, the petroleum ether extract demonstrated the highest inhibitory effect, with a percentage inhibition of 73.79 %, out-performing the chloroform extract (69.85 %) and the aqueous extract (66.21 %). The IC50 values, which represent the concentration required for 50 % inhibition, were calculated as 337.04 µg/mL for the petroleum ether extract, 358.81 µg/mL for the aqueous extract and 372.94 µg/mL for the chloroform extract. In comparison, the standard acarbose had an IC50 value of 226.21 µg/mL (Fig. 11 & 13).

α-glucosidase assay

The α-glucosidase inhibition activity of various solvent crude extracts of *P. adenophylla* was evaluated at a different concentration. The standard acarbose demonstrated an inhibition of 90.76 %. Among the plant extracts, the petroleum ether (PA-Pe) extract exhibited the highest inhibitory activity,

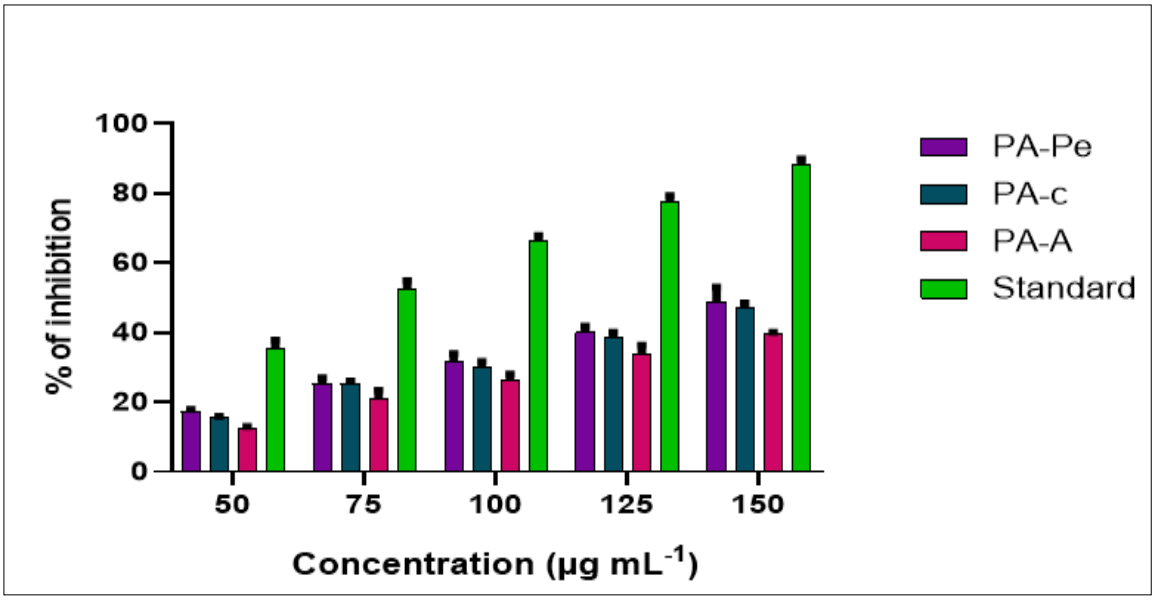


Fig. 10. Albumin inhibition assay of different solvent extracts of *P. adenophylla* standard (n=3), P=0.0034.

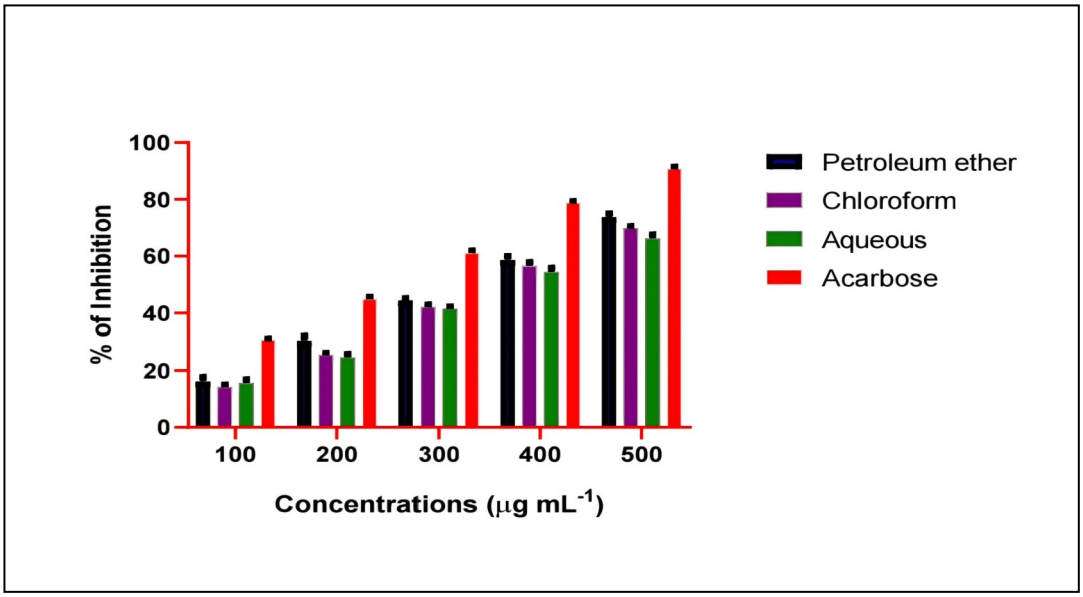


Fig. 11. α-amylase inhibition assay of different solvent extracts of *Psychotria adenophylla* with standard acarbose (n=3) P < 0.048.

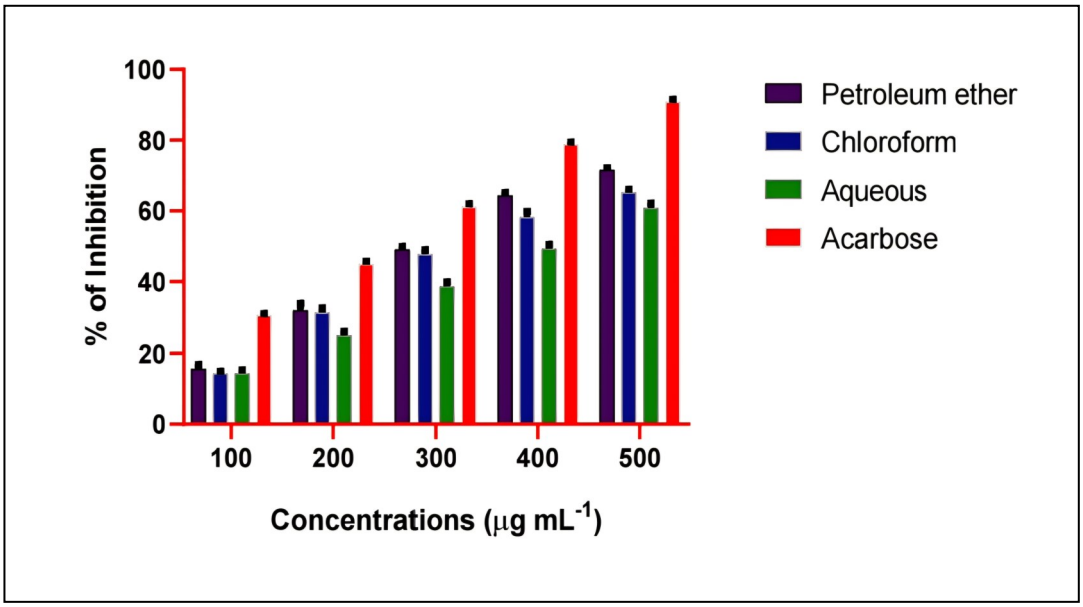


Fig. 12. α-glucosidase inhibition assay of different solvent extracts of *Psychotria adenophylla* with standard acarbose (n=3) P < 0.0384.

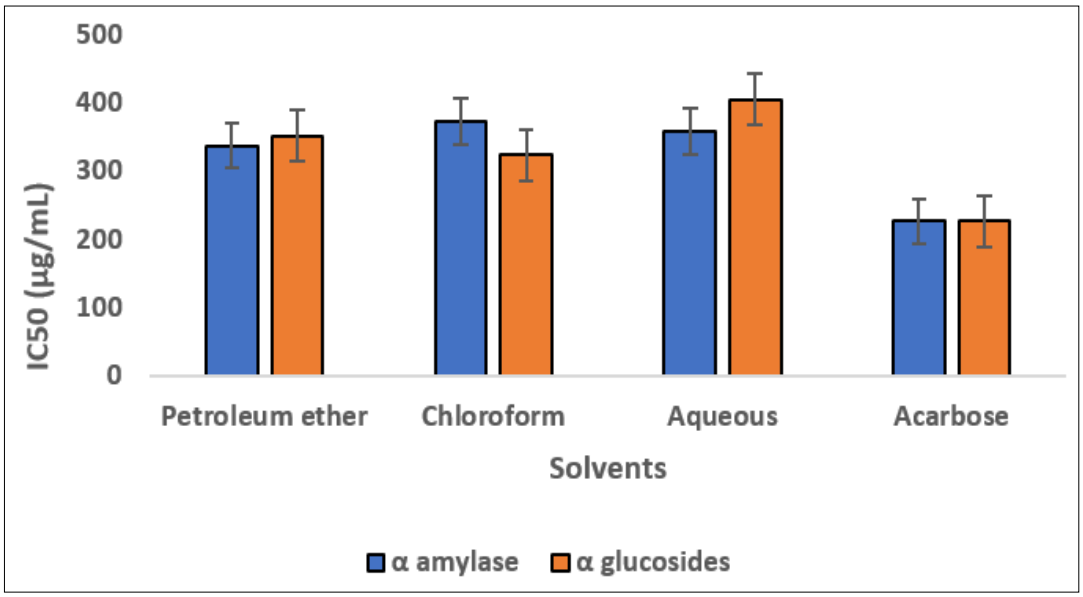


Fig. 13. IC50 values of α-amylase inhibition assay and α-glucosidase inhibition assay in different solvent extracts of *Psychotria adenophylla* with standard acarbose (n=3) P > 0.0001.

with 71.55 % inhibition, followed by the chloroform (PA-C) extract at 65.22 % and the aqueous (PA-A) extract at 60.75 %. The IC50 values, representing the concentration required for 50 % inhibition, were found to be 226.53 µg/mL for acarbose, 324.14 µg/mL for the PA-C extract and 405.95 µg/mL for the PA-A extract. These results indicate that the petroleum ether extract of *P. adenophylla* has a relatively strong α-glucosidase inhibitory effect, suggesting its potential for further exploration as a therapeutic agent for managing postprandial hyperglycemia (Fig. 12 & 13).

Antibacterial activity

Five bacterial pathogens, including gram-positive bacteria *B. subtilis* and *E. faecalis* and gram-negative bacteria like *K. pneumoniae, S. Typhi* and *Citrobacter*, were used to investigate the antibacterial activity of various crude leaf extracts.

The antibacterial activity of *P. adenophylla* extracts was assessed, with the petroleum ether extract (PA-Pe) demonstrating stronger activity than both the chloroform (PA-C) and aqueous (PA-A) extracts. Among the tested pathogens, *Klebsiella pneumoniae* exhibited the highest mean zone of inhibition (18.32 ± 0.58 mm) against the PA-Pe extract at a concentration of 300 µg/mL, while *Enterococcus faecalis* showed the lowest inhibition (12 ± 0.56 mm). In comparison, the chloroform extract (PA-C) and aqueous extract (PA-A) produced zones of inhibition of 16.83±0.57 mm and 15.13 ± 0.45 mm, respectively, against *K. pneumoniae* and 13.15 ± 0.46 mm and 10.66 ± 0.34 mm, respectively, against *E. faecalis* at the same concentration. Additionally, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for the five tested pathogens. The PA-Pe extract showed an MIC of 37.5 µg/mL and an MBC of > 37.5 µg/mL against *K. pneumoniae*, while *E. faecalis* had an MIC and MBC of 75 µg/mL for both (Table 7).

Discussion

The phytochemical screening of *Psychotria adenophylla* leaf extracts obtained from different solvents, including chloroform, petroleum ether and aqueous extracts, revealed the presence of various secondary metabolites. These metabolites include flavonoids, anthraquinones, steroids, terpenoids, glycosides, alkaloids, phenols, tannins and saponins. Species within the *Psychotria* genus are recognized as rich sources of flavonoids, quinoline and isoquinoline alkaloids, indole and monoterpene indole compounds (8). The presence of alkaloids, flavonoids, reducing sugar, protein, amino acid, saponins, tanins, terpenoids and glycosides has been reported in *Psychotria octasulcata* (25). Alkaloids, flavonoids, glycosides, phenols, steroids and terpenoids is present in *Psychotria dalzellii* (26). These findings underscore the pharmacological significance of *P. adenophylla* and highlight its potential for further investigation in drug discovery and therapeutic applications.

The GC-MS Chromatogram of petroleum ether and chloroform leaf extracts revealed the presence of 25 and 50 bioactive compounds, respectively. Results showed that the PA-Pe and PA-C extracts have some different compounds as well. FT-IR and GC-MS analysis showed that those are rich in triterpenoids, sterols and lipophilic antioxidants, such as tris (2,4-ditert-butylphenyl) phosphate, a known industrial antioxidant in Petroleum ether extracts, where chloroform extract contains fatty acids, hydrocarbons and alcohol derivatives, including compounds like hexadecanoic acid derivatives. The presence of long-chain hydrocarbons suggests potential applications in lipid-based formulations and emollient properties Petroleum ether and other non-polar solvents are frequently praised for their capacity to extract lipophilic bioactive substances, such terpenes and essential oils, which have potent antibacterial and cytotoxic effects

Table 7. Antibacterial activity of different solvent leaves extract of *P. adenophylla* with MIC and MBC

Bacterial strains	Concentration (µg/mL)	Petroleum ether	Chloroform	Aqueous	MIC (µg/mL)	MBC (µg/mL)
	PC	23.01±0.43	25.43±1.07	22.77±0.32		
K. pneumoniae	**75**	8.53±0.39	9.73±0.54	7.07±0.53	37.5	>37.5
	150	12.64±0.53	14.63±0.43	10.76±0.36		
	300	18.32±0.58	16.83±0.57	15.13±0.45		
	Concentration	**Petroleum ether**	**Chloroform**	**Aqueous**		
	PC	23.12±0.42	24.43±0.71	21.48±1.05		
Citrobacter	**75**	7.8±0.54	8.31±0.61	7.46±0.73	75	37.5
	150	11.6±0.84	12.5±0.54	9.21±0.59		
	300	15.8±0.95	17.24±0.67	13.24±0.68		
	Concentration	**Petroleum ether**	**Chloroform**	**Aqueous**		
	PC	20.53±0.93	21.66±0.66	18.39±0.53		
S.typhi	**75**	7.62±0.73	8.34±0.70	7.47±0.43	75	75
	150	12.13±0.67	13.76±0.82	10.52±0.79		
	300	15.92±0.42	16.58±0.91	13.56±0.96		
	Concentration	**Petroleum ether**	**Chloroform**	**Aqueous**		
	PC	21.71±0.49	22.19±0.51	20.39±0.79		
B. subtilis	**75**	7.58±0.87	8.31±0.64	7.45±0.55	37.5	>37.5
	150	13.58±0.78	14.55±0.75	11.49±0.90		
	300	17.39±0.64	17.02±0.62	15.56±0.66		
	Concentration	**Petroleum ether**	**Chloroform**	**Aqueous**		
	PC	16.42±0.72	17.44±0.62	14.31±0.74		
E. faecalis	**75**	7.66±0.74	7.32±0.53	7.17±0.49	75	75
	150	9.273333±0.61	10.36±0.83	7.45±0.80		
	300	12±0.56	13.15±0.46	10.66±0.84		

because of their capacity to disrupt membranes (27). Polar solvents, including methanol and water, are excellent at extracting hydrophilic substances like glycosides and polyphenols, which have anti-inflammatory and antioxidant properties (28). The findings are consistent with the chemotaxonomy of *Psychotria* plants are a rich source of flavonoids, quinoline and isoquinoline alkaloids, indole and monoterpene indole (4). Furthermore, approximately 52 % of the metabolites reported were characterized as alkaloids, followed by triterpenes (12 %) and flavonoids (6 %) along with constituents of other classes (29). Out of which Tris(2,4-di-tert-butylphenyl) phosphate, a sterically hindered aromatic phosphate, exhibits biological activity due to its phenyl rings, which can engage in π–π stacking interactions with membrane proteins or nucleic acids. The phosphate group may mimic endogenous phosphate-containing molecules, thereby interfering with enzymatic or signaling pathway. The lipophilic property of Tris(2,4-di-tert-butylphenyl) phosphate suggests its high potential for bioaccumulation and having antioxidant activity (30). Lupeol has high anti-inflammatory and anticancer activity (31). Lup-20(29)-en-3-one exhibits anti-HIV activity, Betulin has antioxidant activity and Hexadecanoic acid shows anticancer, antioxidant and antibacterial properties. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester contains various hydrophobic moieties that influence membrane integration and disruption (32-36). And may be used as a modulating inflammatory mediator and contributing to wound healing and immune response. The lupane skeleton of luteol, a pentacyclic triterpenoid, has a single hydroxyl group at position C-3. It can effectively interact with lipid membranes due to its high lipophilicity, which compromises microbial integrity and alters cellular signalling in inflammatory pathways (37). Betulin, another lupane-type triterpene, bears two hydroxyl groups at C-3 and C-28, conferring increased hydrogen bonding potential and its anticancer activity (38, 39).

Research indicates the potential anti-diabetic effects of *P. malayana* leaf extracts using various methanol-water ratios (0 %, 25 %, 50 %, 75 %,and 100 % methanol-water) (23). The highest level of inhibitory action was demonstrated by the 100 % methanol-water extract. The functional groups that can cause α-glucosidase inhibition activity were found by comparing FTIR spectra with α-glucosidase inhibition activity. These groups include hydroxyl (O-H), alkenyl (C=C), methylene (C-H), carbonyl (C=O) and secondary amine (N-H) groups. Potential biological characteristics were displayed by other plants of the same species. For instance, neurodegenerative activity was found in *P. longipes* and *P. solitudinum*. *P. umbellata* and *P. brachyceras* were shown to have antioxidant and antimutagenic activities, respectively. Similarly, *P. capitata* and *P. forsteriana* leaves exhibited cytotoxic action and *P. suterela* and *P. stachyoides* crude extract tests demonstrated a favourable anti-inflammatory effect. *P. rostrata* shown significant antibacterial action against *Staphylococcus aureus* and *Escherichia coli*, whereas *P. spectabilis* demonstrated antifungal activity against the filamentous fungus *Cladosporium cladosporioides* (40). To identify novel sources of antimicrobial lead compounds, the extracts' antibacterial activity was assessed. This is due to the fact that almost all standard antibiotics were showing more negative side effects than the traditional therapies and developing resistance to certain bacterial strains (41). Approximately 700000 fatalities worldwide are attributed to antimicrobial-resistant bacteria each year (42). The extracts of *Psychotria dalzellii* have been reported to possess antioxidant and antidiabetic properties (43). The study demonstrated that extracts and chemical constituents of *Psychotria succulenta* possess significant antibacterial activity, supporting its potential use in treating bacterial infections. Previous pharmacological investigations have shown that the extracts of several other *Psychotria* species in this genus, including *P. gardineri* (leaves and branches*), P. microlabastra* (stem, leaves and root bark) and *P. nigra* (branches and leaves), have antibacterial qualities (44). The antibacterial efficacy of *Psychotria dalzellii* against Gram-negative pathogenic bacterial strains such as *Pseudomonas syringae*, *Klebsiella pneumoniae, Xanthomonas compestris* and *Escherichia coli* showed significant zone of inhibition (26, 45). Many other *Psychotria* species also exhibit biological activities, including *P. cupularis* (anti-inflammatory and antiproliferative), *P. sycophylla* (antibacterial), *P. capensis* (potential anti-inflammatory adjuvant) and *P. nuda* (anti-inflammatory) (46-49).

n-Hexadecanoic acid, identified in *Macrosolen parasiticus*, is reported to exhibit nematocidal, pesticidal, hypocholesterolaemic, antioxidant and anti-inflammatory properties (50). The majority of the phytoconstituents found in both extracts as a result of this investigation were previously reported to possess antibacterial activities, which may be related to this plant's antibacterial qualities. Furthermore, it has been shown that a number of *Psychotria* species possess antibacterial qualities (51–54). The presence of a zone of inhibition confirmed the inhibitory activity *of P. dalzellii* plant extracts. The activity of the sample was shown by the clear zone surrounding it on the plates and the zone of inhibition of many bacteria was observe. As the concentration of plant extracts increases, the zone of inhibition expands in all bacterial plates (55).

Conclusion

The present study highlights the pharmacological potential of *P. adenophylla* (Chelianthua), a traditionally used medicinal plant from the Rubiaceae family, by evaluating its biological activities through various solvent extracts. The petroleum ether extract demonstrated the highest antidiabetic activity, as evidenced by strong α-amylase and α-glucosidase inhibition, suggesting the presence of lipophilic bioactive compounds with potential hypoglycemic effects. The plant also exhibits significant anti-inflammatory activity, as demonstrated by the albumin inhibition assay. Similarly, the antibacterial analysis revealed that *K. pneumoniae* exhibited the highest inhibition among Gram-negative bacteria, while *B. subtilis* showed superior inhibition in Gram-positive bacteria, with MIC and MBC values of 37.5 µg/mL and >37.5 µg/mL both. These findings underscore the influence of solvent polarity on compound extraction and emphasize the potential of *P. adenophylla* as a source of natural anti-inflammatory, antidiabetic and antibacterial agents. To improve therapeutic applications, future research should concentrate on identifying

active ingredients, clarifying molecular processes, doing *in vivo* and clinical investigations and investigating synergistic effects with already available medications. Strong bioactivity, especially in the petroleum ether extract, suggests that it may be used in alternative therapeutics, nutraceutical formulations and medication development to manage bacterial infections and diabetes in a sustainable way.

Credit authorship contribution statement

DD contributed to the development of the methodology, software implementation, acquisition of resources, data curation and preparation of the original draft. TK performed data analysis. AV was involved in conceptualization, provided supervision and oversaw the final editing and approval of the manuscript. All the authors read and approved the final manuscript.

Declaration of competing interest

The final version was approved by all of the authors listed above, who all made major contributions to the work. This study has not been published anywhere and there are no conflicts of interest. The work complies with contemporary ethical standards. The manuscript conforms to contemporary ethical standards.

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Authors' contributions

DD carried out the methodology, software, resources, data curation and original draft writing. Data analysis was done by TK. AV conceived the study and reviewing, editing, project management and supervision. All author read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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