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# Proximate Analysis and Chemical Constituents of *Psychotria latistipula Benth*. (Rubiaceae) Leaves

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**Abstract**

*Psychotria latistipula* Benth. (Rubiaceae), a traditional Nigerian medicinal plant recognised for its effectiveness in treating cancer, tumours, bronchial and gastrointestinal disorders was investigated for its chemical constituents by Gas Chromatography-Mass Spectrometry (GC-MS) technique; also, proximate analysis was done to determine the composition of moisture, crude protein, crude fiber, fat, carbohydrate, and ash contents, which had not been previously reported. *P. latistipula* leaves were extracted by maceration using acetone and hexane (1:1). The proximate analysis indicated that the leaves of *P. latistipula* contain moisture, crude protein, crude fiber, fat, carbohydrate, and ash contents of 8.91±0.14, 12.43±0.05, 20.78±0.66, 2.28±0.06, 50.34±0.79, and 5.27±0.16%, respectively, demonstrating a notably high carbohydrate content. The GC-MS identified twenty-eight compounds, making up 93.08% of the total. The dominant classes of these compounds included fatty acid esters, saturated fatty acids, fatty acid methyl esters, and unsaturated fatty acid aldehydes accounting for 19.27%, 18.71%, 13.77% and 13.36% of the total, respectively. Octadecanoic acid (14.03%) was the major compound in *P. latistipula* leaves acetone-hexane extract. Notably, Octadecanoic acid is known for its potential anti-inflammatory, anticancer and antioxidant properties, which indicates *P. latistipula* could serve as an anti-inflammatory, antioxidant and anticancer agents further justifying its ethnomedicinal use.

**Keywords:** Fatty acid esters; GC-MS; Octadecanoic acid; *P. latistipula; Rubiaceae*; Secondary metabolites.

**Abbreviations**: *P. latistipula*: *Psychotria latistipula*; GC-MS: Gas Chromatography-Mass Spectrometry; RT: Retention time; mins: Minutes; MF: Molecular formula; MM: Molecular mass; g/mol: grams per mole.

## INTRODUCTION

Plant extracts and their constituents have demonstrated potential for being developed into new treatments with anticancer, antioxidant, antidiabetic, antimalarial, antimicrobial, and anti-inflammatory properties, among others (Riaz et al., 2023). They offer advantages over synthetic medicine regarding safety, local availability, and cost-effectiveness (Ansari et al., 2025). The screening of plant extracts represents an innovative approach to discovering medicinally important compounds that can facilitate the development of new pharmaceuticals (Chaachouay & Zidane, 2024). In the study of natural products, such as plant extracts, Gas Chromatography-Mass Spectroscopy is invaluable for characterising the bioactive compounds present; It can be used for the analysis of a wide range of biological compounds including Fatty acids, essential oils, eicoscanoids, wax, esters by the selection of suitable columns, thereby informing pharmacological studies and potential therapeutic applications (Santhiya & Ramasamy, 2019; Olaoluwa et al., 2019; Ranjan et al., 2023; Takuma et al., 2025).

The family Rubiaceae comprises 620 genera totaling about 13526 species, distributed worldwide. Psychotria is the largest of the Rubiaceae, possessing more than 2000 species, mainly found in tropical and subtropical regions (Mohamad et al., 2025). Several Psychotria species are widely used in folk medicine around the world to treat various illnesses (Sangeetha et al., 2020). *Psychotria latistipula* Benth, the type species of the genus Psychotria and from the family Rubiaceae, is a shrub of 5–6 feet or a small tree with branches subterete, glabrate, at the extremities somewhat herbaceous and compressed, puberulous and its leaves are elliptical or oval, acuminate, wedge-shaped at the base, nearly glabrous or puberulous on the veins beneath. It is native to Nigeria, Cameroon, Central African Republic, Congo, Gabon, Gulf of Guinea Is., and Togo (POWO, 2025). The leaves treat cancer and tumours (Burkill, 1985). Also, the genus Psychotria, to which *P. latistipula* belongs, is used in traditional medicines for treating bronchial and

gastrointestinal disorders, such as cough, bronchitis, ulcer, stomach ache, and infections of the reproductive system (Ngnokam-Jouogo et al., 2022). Psychotria (Rubiaceae) possesses various biological properties, ranging from phytochemical and pharmacological properties of their chemical constituents to traditional medical applications (Aureada et al., 2023).

Despite their high diversity, most Psychotria species, including *P. latistipula*, remain largely unstudied. Hence, to gain insights into its chemical composition, this study conducted a GC-MS analysis to identify the presence of various chemical constituents in the acetone-hexane extract of *P. latistipula* leaves and also a proximate analysis to determine the moisture, crude protein, crude fiber, fat, carbohydrate and ash contents.

## MATERIALS AND METHODS

### Plant material and Extraction

Leaves of *P. latistipula* (Figure 1) were obtained from the Arboretum of the Forestry Research Institute of Nigeria (FRIN), Jericho Ibadan, Oyo State. The plant was identified and authenticated at the Forestry Herbarium in Ibadan (FHI), voucher number FHI 1142147 was issued for reference use. The leaves were then air-dried for about 2 weeks to remove all moisture and subsequently pulverized.

Air-dried ground leaves of *P. latistipula* (5 g) was measured and placed into a 250 mL conical flask with a lid. Next, 40 mL of a 1:1 mixture of acetone and hexane was added to the sample in the flask, which was then ultrasonicated at 27ºC for 15 minutes. The resulting suspension was filtered, and the filtrate was concentrated in vacuo using a rotary evaporator. For the GC-MS analysis of various compounds, 1 µL of the sample was utilized.

**Figure 1.** *Psychotria latistipula* leaves

### Proximate Analysis of P. latistipula leaves

#### *Determination of Moisture content*

The moisture content was determined by the gravimetric method. A measured weight of each sample (2g) was weighed into a weighed moisture can. The can and its sample content were dried in the oven at 105ºC for 3 hours. It was then cooled in a dessicator and reweighed. The weight was recorded while the sample was returned to the oven for further drying. The drying, cooling and weighing were done repeatedly until a constant was obtained. The moisture content was calculated.

#### *Determination of crude protein content*

Crude protein was determined from organic nitrogen using the macro-Kjeldhal method (Adegbaju et al., 2019).

#### *Determination of crude Fibre content*

For crude fiber, 3 g of each sample was defatted (during fat analysis). The defatted sample was boiled in 200 mL of 1.25% $H_2S0_4$ solution under reflux for 30 minutes. After that, the sample was washed with several portions of hot boiling water using a two-fold muslin cloth to trap the particle. The washed samples were carefully transferred quantitatively back to the flask and 20 mLof 1.25% NaOH solution added to it. Again the sample was boiled for 30 minutes and washed as before with hot water. Then was carefully transferred to a weighed proclaim crucible and dried in the oven at 105ºC for 2 hours. Fiber content was calculated by dividing loss in weight of the sample by the weight of the sample (AOAC International, 2016).

#### *Determination of Fat content*

The fat content of the sample was determined by the continuous solvent extraction method using a soxhlet apparatus by extracting with petroleum ether. The extract was reduced to half by evaporation and dried at 60º C for 3 minutes.The percent fat content was calculated by dividing the weight obtained after drying with the weight of the sample to estimate the fat content (James et al., 2015).

#### *Determination of carbohydrate content*

The carbohydrate content was calculated by difference as the nitrogen free extractive (NFE). The carbohydrate content was calculated from the differences protein, fat, moisture, and ash content subtracted from 100 (Okunola et al., 2019).

#### *Determination of ash content*

The ash content was determined using the furnace incineration gravimetric method. The sample (1g) sample was put in a precious weighed porcelain crucible. The sample in crucible was put in a muffle furnace at 550º C and allowed to burn for 2 – 3 hours (until the sample became a gray ash). The sample in crucible was very

carefully removed from the furnace (taking care not to blow air into the ash) and cooled in a dessicator. It was reweighed by difference and the weight of the ash in percentage was obtained by dividing loss in weight of the sample by the weight of the sample (AOAC International, 2016)

**Data analysis**

The proximate analysis was done in triplicates and reported as mean ± standard deviation.

***GC-MS Analysis***

The GC-MS analysis of the acetone-hexane extract of *P. latistipula* leaves was carried out using an Agilent 7820A gas chromatograph fixed to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies Capillary column (HP-5) coated with 5% Phenyl Methyl Siloxane (30 m length x 0.32 mm diameter x 0.25 µm film thickness) was the stationary phase of separation of the compounds. Helium was used as the carrier gas at constant flow of 1.4871 mL/min at an initial nominal pressure of 1.4902 psi and average velocity of 44.22 cm/sec. At an injection temperature of 300 °C, an injection volume of 1µL of the sample was introduced in splitless mode. While the gas saver mode was turned off, the purge flow to spilt vent was 15 mL/min at 0.75 min with a total flow of 16.654 mL/min. Oven was initially auto regulated at 40 °C for (1 min) then ramped at 12 °C/min to 300 °C (10 min). The run time was 32.667 min with a 5 min solvent delay. The mass spectrometer was utilized in electron-impact ionization mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 280 °C. Acquisition of ion was through Scan mode (scanning from m/z 45 to 550 amu at 2.0s/scan rate). The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. National Institute Standard and Technology (NIST) 14.L library (2018) was then searched to compare the structures of the compounds with that of the NIST database. Compounds were then identified based on the retention times and mass spectra with already known compounds in the NIST library (C: \ Database \ NIST14.L).

## RESULTS AND DISCUSSION

**The Proximate analysis of *P. latistipula* leaves**

The proximate analysis revealed *P. latistipula* leaves to contain moisture, crude protein, crude fiber, fat, carbohydrate, and ash contents of 8.91±0.14, 12.43±0.05, 20.78±0.66, 2.28±0.06, 50.34±0.79, and 5.27±0.16%, respectively, demonstrating a notably high carbohydrate content and an appreciable amount of crude fibre (Table 1).

**Table 1.** Proximate composition of *P. corymbose*.

| S/N | Constituents | Percentage composition |
|---|---|---|
| 1 | Moisture | 8.91 ± 0.14% |
| 2 | Ash | 5.27 ± 0.16% |
| 3 | Fat | 2.28 ± 0.06% |
| 4 | Crude fibre | 20.78 ± 0.66% |
| 5 | Crude protein | 12.43 ± 0.05% |
| 6 | Carbohydrate | 50.34 ± 0.79% |

Values are the mean and standard deviation of three determinations.

**GC-MS profile of *P. latistipula* leaves acetone-hexane extract**

The chemical constituents of plants play an essential role in determining their biological attributes, influencing not only their physiological functions but also their interactions with the environment. Additionally, the presence and concentration of specific chemical constituents can affect the plant's medicinal properties, making them valuable in traditional and modern medicine.

Twenty-eight chemical components were identified from the acetone-hexane (1:1) extract of *P. latistipula* leaves representing a total of 93.08% (Table 2a; Figure 1). The acetone-hexane components of *P. latistipula* leaves were dominated by fatty acid esters, saturated fatty acids, fatty acid methyl esters, and unsaturated fatty acid aldehydes accounting for 19.27%, 18.71%, 13.77% and 13.36% of the total, respectively (Table 2b). The major compounds found were Octadecanoic acid (14.03%), 9- Octadecenoic acid methyl ester (E)- (12.36%), 9-Octadecenal, (Z)- (9.47%), Heptadecanoic acid, 14-methyl-methyl ester (6.97%), Pentadecanoic acid, 14-methyl-, methyl ester (9.80%), Oleic acid (6.19%), n-Hexadecanoic acid (4.68%), Neophytadiene (4.32%), Heptyl octadecyl ether (3.86) and (Z)-Methyl heptadec-9-enoate (3.02%) (Figure 2).

The most abundant compound in *P. latistipula* leaves acetone-hexane extract is Octadecanoic acid, also known as Stearic acid, a long-chain saturated fatty acid, has been identified as a key phytochemical compound in various plant extracts exhibiting various biological activities. Research indicates that Octadecanoic acid and its methyl esters have been shown to possess antioxidant properties, helping to scavenge free radicals and protect against oxidative stress; exhibits antitumor activity in mouse models and selective cytotoxicity against certain cancer cells; exhibits anti-inflammatory effect; and serves as androgenic flavor, hemalytic, 5α reductase inhibitor (Duke, 2016; Balasundari & Boominathan, 2018). 9-Octadecenoic acid methyl ester (E)- (12.36%) possesses antioxidant, anemiagenic, anti-inflammatory, anti-hypertensive, anticarcinogenic activities, exist in human red blood cells and serve as endogenous peroxisome proliferator-activated receptor ligand, dermatigenic flavour, increase high-density lipoprotein (HDL) cholesterol and decreases low-density lipoprotein (LDL) cholesterol (Singh et al., 2008; Akpuaka et al., 2013). 9-

Octadecenal, a monounsaturated fatty aldehyde, exhibits various biological activities, including antimicrobial, anti-inflammatory, and antioxidant properties.

9-octadecenal found in plant extracts demonstrates antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella sp*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*; also can neutralize free radicals and reduce inflammation serving as an antioxidant and anti-inflammatory agents; additionally, can act as a 5α reductase inhibitor and a percutanea stimulant; also possesses mild anti-infective and anti-fungi activity (Selvi & Basker, 2012; Mangrove-Abayomi et al., 2014; Atni et al., 2024). Hexadecanoic acid, also known as palmitic acid showed anticancer, anthelmintic, antioxidant, hypocholesterolemic, nematicide and pesticide (Sheela & Uthayakumari, 2013; Elufioye et al., 2019). Duke (2016) also reported Hexadecanoic acid having antidiarrheal activity. Neophytadiene, a diterpene, exhibits diverse biological activities, including anti-inflammatory, antimicrobial, anti-cancer, anxiolytic-like, and anticonvulsant properties (Selmy et al., 2023; Gonzalez-Rivera et al., 2023; Endris et al., 2024).

Oleic acid, a naturally occurring omega-9 fatty acid, exhibits a range of biological activities, including antimicrobial, antioxidant, and potential roles in reducing cholesterol and inflammation, as well as being a key component of cell membranes and potentially influencing brain development (Alabi et al., 2018; Ramadan et al., 2024). (Z)-Methyl heptadec-9-enoate was reported to possess antibiotic properties (Elufioye et al., 2019). Among the minor compounds identified from the acetone-hexane extract of *P. latistipula* leaves were: Z-10-Methyl-11-tetradecen-1-ol propionate (1.88%), Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]- (1.77%), cis-Vaccenic acid (1.61%), ar-Turmerone (1.49%), 1-Methoxy-3-(2-hydroxyethyl) nonane (1.46%), 2,6,10-Cycloundecatrien-1-one,2,6,9,9-tetramethyl-, (E,E,E)- (1.30%), Eicosane (0.97%). However, minor components, even in small concentrations, can also play a crucial role in the overall efficacy and functionality of the extract; reported bioactivity of these minor compounds based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA are listed in Table 3.

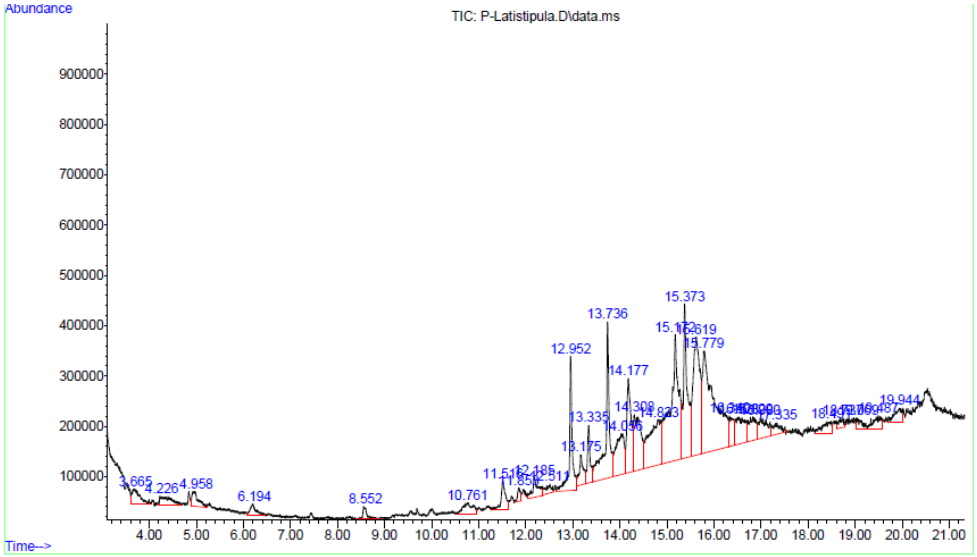



**Figure 1.** GC–MS chromatogram of acetone-hexane extract of *Psychotria latistipula* leaves

**Table 2a.** Identified compounds from the acetone-hexane Extract of *Psychotria latistipula* Leaves Using GC-MS.

| S/N | RT (mins) | Compound | Class of compound | MF | MM (g/mol) | Peak Area (%) |
|---|---|---|---|---|---|---|
| 1 | 3.665 | Benzene, 1-ethyl-4-methyl- | Aromatic hydrocarbon | $C_9H_{12}$ | 120.1 | 1.24 |
| 2 | 4.226 | 2(1H)-Benzocyclooctenone, decahydro-4a-methyl-, trans-(-)- | Bicyclic hydrocarbon | $C_{13}H_{22}O$ | 194.3 | 1.06 |
| 3 | 4.958 | Undecane | Acyclic saturated hydrocarbon | $C_9H_{12}$ | 156.3 | 1.06 |
| 4 | 6.194 | Heptanoic acid, ethyl ester | Fatty acid ester | $C_9H_{18}O_2$ | 158.2 | 0.82 |
| 5 | 8.552 | Decanoic acid, ethyl ester | Fatty acid ester | $C_{12}H_{24}O_2$ | 200.3 | 0.62 |
| 6 | 10.761 | 2-Acetylbenzoic acid | Carboxylic acid | $C_9H_8O_3$ | 164.1 | 1.21 |

| S/N | RT (mins) | Compound | Class of compound | MF | MM (g/mol) | Peak Area (%) |
|---|---|---|---|---|---|---|
| 7 | 11.516 | ar-Turmerone | Sesquiterpenoid | $C_{15}H_{20}O$ | 216.3 | 1.49 |
| 8 | 11.853 | Cyclooctaneacetic acid, 2-oxo- | Carboxylic acid | $C_{10}H_{16}O_3$ | 184.2 | 0.43 |
| 9 | 12.185 | 2,6,10-Cycloundecatrien-1-one,2,6,9,9-tetramethyl-, (E,E,E)- | Sesquiterpenoid | $C_{15}H_{22}O$ | 218.3 | 1.30 |
| 10 | 12.511 | Undec-10-ynoic acid, undecyl ester | Fatty acid ester | $C_{22}H_{40}O_2$ | 336.5 | 0.57 |
| 11 | 12.952 | Neophytadiene | Diterpene | $C_{20}H_{38}$ | 278.5 | 4.32 |
| 12 | 13.175 | 1-Methoxy-3-(2-hydroxyethyl)nonane | Alkoxy alcohol | $C_{12}H_{26}O_2$ | 202.3 | 1.46 |
| 13 | 13.335 | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]- | Diterpenoid | $C_{22}H_{42}O_2$ | 338.6 | 1.77 |
| 14 | 13.736 | Pentadecanoic acid, 14-methyl-, methyl ester | Fatty acid methyl ester | $C_{17}H_{34}O_2$ | 270.4 | 6.80 |
| 15 | 14.056 | Heptyl octadecyl ether | Ether | $C_{25}H_{52}O$ | 368.6 | 3.86 |
| 16 | 14.177 | n-Hexadecanoic acid | Saturated Fatty acid | $C_{16}H_{32}O_2$ | 256.4 | 4.68 |
| 17 | 14.833 | Oleic Acid | Monounsatutared fatty acid | $C_{18}H_{34}O_2$ | 282.5 | 6.19 |
| 18 | 15.172 | 9-Octadecenoic acid, methyl ester, (E)- | Fatty acid ester | $C_{19}H_{36}O_2$ | 296.4 | 12.36 |
| 19 | 15.373 | Heptadecanoic acid, 14-methyl-, methyl ester | Fatty acid methyl ester | $C_{18}H_{36}O_2$ | 284.4 | 6.97 |
| 20 | 15.619 | 9-Octadecenal, (Z)- | Unsaturated fatty aldehyde | $C_{18}H_{34}O$ | 266.5 | 9.47 |
| 21 | 15.779 | Octadecanoic acid | Saturated Fatty acid | $C_{18}H_{36}O_2$ | 284.5 | 14.03 |
| 22 | 16.340 | 8-Hexadecenal, 14-methyl-, (Z)- | Unsaturated fatty aldehyde | $C_{17}H_{32}O$ | 252.4 | 1.43 |
| 23 | 16.328 | (Z)-Methyl heptadec-9-enoate | Fatty acid ester | $C_{18}H_{34}O_2$ | 282.4 | 3.02 |
| 24 | 16.980 | Z-10-Methyl-11-tetradecen-1-ol propionate | Fatty acid ester | $C_{18}H_{34}O_2$ | 282.5 | 1.88 |
| 25 | 17.335 | Eicosane | Acyclic saturated hydrocarbon | $C_{20}H_{42}$ | 324.8 | 0.97 |
| 26 | 18.491 | 13-Octadecenal, (Z)- | Unsaturated fatty aldehyde | $C_{18}H_{34}O$ | 266.5 | 1.26 |
| 27 | 19.069 | cis-Vaccenic acid | Monounsaturated fatty acid | $C_{18}H_{34}O_2$ | 282.5 | 1.61 |
| 28 | 19.944 | E-15-Heptadecenal | Unsaturated fatty aldehyde | $C_{17}H_{32}O$ | 252.4 | 1.20 |
| | | **Total** | | | | **93.08%** |

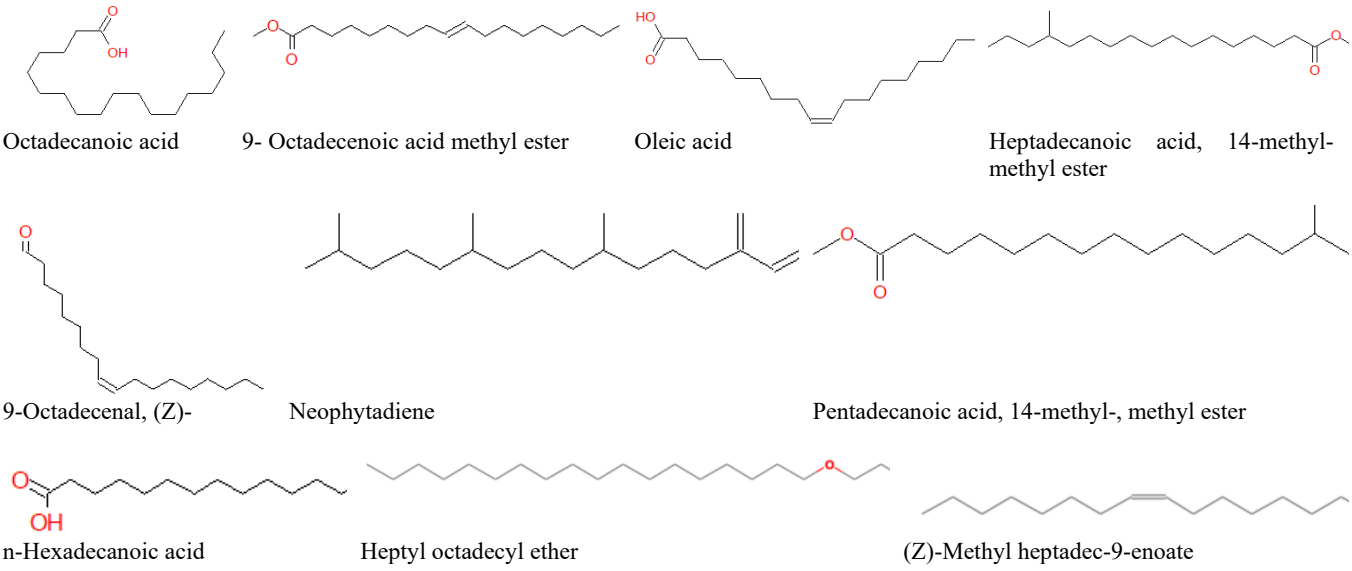
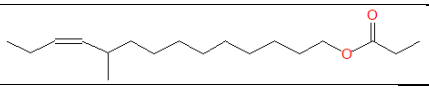
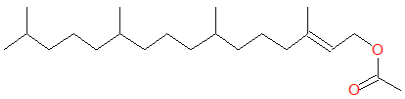
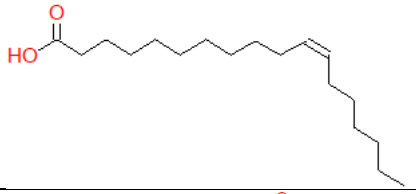
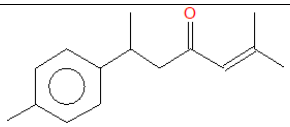
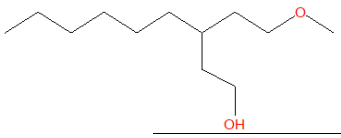
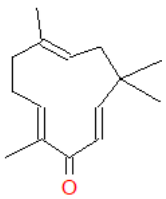



**Figure 2:** Some major constituents found in acetone-hexane extract of *Psychotria latistipula* leaves.

**Table 2b.** Classes of Compounds identified from and their compositions.

| S/N | Class of compounds | Peak Area % composition |
|---|---|---|
| 1. | Sesquiterpenoids | 2.79 |
| 2. | Diterpenes | 4.32 |
| 3 | Diterpenoids | 1.77 |
| 5 | Saturated fatty acids | 18.71 |
| 6 | Monounsaturated fatty acids | 7.80 |
| 7 | Fatty acid esters | 19.27 |
| 8 | Fatty acid methyl ester | 13.77 |
| 9 | Aromatic hydrocarbons | 1.24 |
| 10 | Bicyclic hydrocarbons | 1.06 |
| 11 | Acyclic saturated hydrocarbons | 2.03 |
| 12 | Unsaturated fatty aldehyde | 13.36 |
| 13 | Ethers | 3.86 |
| 14 | Alkoxy alcohols | 1.46 |
| 14 | Carboxylic acid derivatives | 1.64 |

**Table 3.** Some identified minor compounds in the acetone-hexane extract of *Psychotria latistipula* leaves and their reported biological activity.

| S/N | Compounds | Structure | Reported Biological activity* |
|---|---|---|---|
| 1. | Z-10-Methyl-11-tetradecen-1-ol propionate |  | 5α reductase inhibitor, antipsychotic. |
| 2. | Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]- |  | Antioxidant, anti-inflammatory, and neuroprotective effects. |
| 3 | cis-Vaccenic acid |  | Antibacterial, anti-inflammatory, and hypolipidemic effects |
| 4 | ar-Turmerone |  | Anti-inflammatory, antioxidant, anti-angiogenic, neuroprotective, anti-cancer, anti-plasmodial, antiaging, antidepressant, antiepileptic, anti-dermatophyte, and antiplatelet activities. |
| 5 | 1-Methoxy-3-(2-hydroxyethyl) nonane |  | Antimicrobial and anti-inflammatory activities. |
| 6 | 2,6,10-Cycloundecatrien-1-one,2,6,9,9-tetramethyl-, (E,E,E)- |  | Anticancer, anti-inflammatory activities |
| 7 | Eicosane |  | Antifungal and potentially neuroprotective and anti-inflammatory properties |

* Source of reference: Dr. Duke's Phytochemical and Ethnobotanical Databases, 1992-2016

## CONCLUSIONS

The leaves of *P. latistipula* contain a high level of carbohydrate and crude fibre. Gas chromatography-mass spectrometry analysis of the acetone-hexane extract from the leaves of *P. latistipula* has identified a range of bioactive compounds, primarily consisting of fatty acid esters, saturated fatty acids, fatty acid methyl esters, and unsaturated fatty acid aldehydes, which possess notable medicinal properties. These bioactive constituents have been reported to effectively combat bacteria, fungi, inflammation, oxidative stress, and cancer, reinforcing

the ethno medicinal uses of *P. latistipula*. Isolating individual bioactive compounds holds great promise for advancing drug development, potentially leading to innovative therapies that could enhance treatment options for various diseases.

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***Authors' Contributions:*** Conceptualization and literature search were done by Samuel Akinniyi Odewo & Olayombo Margaret Banwo. Methodology and analysis was carried by Samuel Akinniyi Odewo. Interpretation of data were done by Samuel Akinniyi Odewo & Olayombo Margaret Banwo. Both authors contributed to the writing of the manuscript. Both authors read and approved the final version of the manuscript.

***Competing Interests:*** The authors declare that there are no competing interests.

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