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Phytochemical Profiling, GC–MS Characterization, and Antioxidant Potential of *Meyna laxiflora* Robyns Leaf Extracts

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ABSTRACT
The present study aimed to evaluate the phytochemical profile, biochemical composition, GC–MS analysis, and antioxidant potential of methanol, chloroform, and aqueous leaf extracts of *Meyna laxiflora* Robyns, a common medicinal plant traditionally used by the ethnic communities of Assam against various ailments. Standard protocols were employed for all analyses. Preliminary phytochemical screening confirmed the presence of pharmaceutically significant compounds, including alkaloids, tannins, flavonoids, and phenols in all extracts. Quantitative biochemical analysis revealed that the methanolic extract contained the highest levels of total alkaloids (1.37 ± 0.1 mg/g), phenols (14.64 ± 0.3 mg/g GAE), and tannins (2.93 ± 0.1 mg/g), while flavonoid content was greater in the chloroform extract (8.00 ± 0.6 mg/g QE). Antioxidant activity assessed by the DPPH radical scavenging assay showed that the methanolic extract exhibited 48.88 ± 0.60% inhibition at 1.00 µg/ml, comparable to the standard ascorbic acid (51.38 ± 0.22%). The IC_{50} values of the methanolic extract (31.60 µg/ml) and ascorbic acid (20.35 µg/ml) indicated strong antioxidant potential. Similarly, in the hydrogen peroxide (H_2O_2) scavenging assay, both extracts and the standard showed dose-dependent activity, with IC_{50} values of 48.51 µg/ml and 29.49 µg/ml for the methanolic extract and standard, respectively. GC–MS analysis identified several biologically active compounds, including neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, phytol, squalene, and 9-eicosyne, which hold promise for pharmaceutical applications.

INTRODUCTION:

Phytochemicals are naturally occurring bioactive compounds found in plants. These non-nutritive substances, present in fruits, vegetables, grains, and various other plants, have been reported to exert beneficial effects on human health **(Nivya et al., 2012; Santhi et al., 2011)**. Beyond their role in human well-being, phytochemicals also function as protective agents for plants, defending them against pathogens, insects, and herbivores **(Taiz and Zeiger, 2002)**. Several of these compounds exhibit diverse biological activities and have been shown to safeguard humans against a range of diseases **(Ghasemzadeh et al., 2015; Khan and Javaid, 2020)**.

Plants synthesize antioxidants as secondary metabolites that neutralize free radicals and reactive oxygen species (ROS), thereby mitigating oxidative stress and cellular damage **(Zehiroglu et al., 2019; Khan and Javaid, 2019)**. Many such bioactive molecules are extracted, commercialized, and used as raw materials in herbal and pharmaceutical industries **(Renisheya et al., 2011)**. The rising concern over the adverse effects of synthetic drugs has further shifted scientific attention towards medicinal plants as safer alternatives **(Bushra et al.,**

2012).

Assam is endowed with rich biodiversity, including a wide array of medicinal plants and herbs. Knowledge regarding the therapeutic uses of many of these plants is confined primarily to indigenous and ethnic communities residing across the region. However, the active ingredients and pharmacologically important phytochemicals in several of these plants remain underexplored **(Sharma and Das, 2018)**.

Meyna laxiflora Robyns (Family: Rubiaceae) is a small to medium-sized evergreen tree commonly found in the natural forests and along roadsides of Assam. It is an underutilized medicinal plant widely used by the Rabha and Garo tribes of southern Kamrup district to treat ailments such as inflammation, gastrointestinal disorders, boils, and skin diseases. While the phytochemical and antioxidant properties of *M. laxiflora* fruits have been reported **(Dhodade et al., 2019)**, no comprehensive study on the leaves of this species has been conducted to date. Therefore, the present investigation was undertaken to analyze the leaf extracts of *M. laxiflora* with the aim of identifying active phytoconstituents and evaluating their potential pharmacological properties.

MATERIALS AND METHODS:

Collection and identification of plant material:
An ethnobotanical survey was carried out in the southern part of Kamrup district, Assam. Fresh leaf specimens of *Meyna laxiflora* were collected for the study. The plant was initially identified using standard literature, including Flora of Assam by **Kanjilal U.N. (1997)**. The identification was further authenticated by the Department of Botany, Gauhati University, where specimens were deposited for future reference.

Preparation of plant extracts: Fresh leaves were thoroughly washed under running tap water to remove surface impurities and shade-dried for 45 days. The dried material was ground into fine powder using a laboratory grinder. For organic extractions, 100 g of powdered leaves were soaked separately in 1000 ml of methanol and chloroform for 24 h in an orbital shaker at 150 rpm and 30 °C. The macerates were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated to dryness using a rotary evaporator (Buchi R-124). The condensed extracts were stored in airtight containers at 4 °C until further analysis.

For aqueous extraction, 100 g of powdered leaves were dissolved in 1000 ml distilled water and heated at 40 °C in a water bath for 1 h. The mixture was filtered, and the filtrate was freeze-dried. The dried extract was preserved in tightly closed glass vials at 4 °C for subsequent use.

Phytochemical screening:
Qualitative phytochemical analysis of the methanol, chloroform, and aqueous extracts of *M. laxiflora* was carried out to detect the presence of major classes of secondary metabolites, including alkaloids, tannins, saponins, glycosides, sterols, flavonoids, phenolic compounds, terpenoids, starch, and proteins. Standard protocols were followed as described by **Ogunjobi et al. (2020), Raaman (2006),** and **Tripathi and Mishra (2015)**.

Quantitative estimation of phytochemical constituents:
Total alkaloid content was determined by taking 2.5 g of powdered leaf sample and was extracted with 200 ml of 10% acetic acid (CH_3COOH) prepared in ethanol (C_2H_5OH) and allowed to stand for 4 h. The mixture was filtered, and the filtrate was concentrated by heating in a water bath. Concentrated ammonium hydroxide (NH_4OH) was then added dropwise until complete precipitation occurred. The precipitate was washed with 20 ml of 0.1 M ammonium hydroxide and filtered through Whatman No. 1 filter paper. The residue was oven-dried and weighed using an electronic balance. The total alkaloid content was calculated following the method of **Ogunjobi et al. (2020)** and **Harborne (1973)** as:

$$\text{Alkaloid (\%)} = \frac{\text{Final weight of the residue}}{\text{Initial weight of sample}} \times 100$$

Total flavonoid content was estimated by taking 2.5 g of powdered leaf sample and was mixed with 50 ml of 80% methanol (CH_3OH) in a 250 ml beaker and kept at 20 °C for 24 h. The filtrate was extracted three times with equal volumes of ethanol (C_2H_5OH). The combined extracts were filtered through Whatman No. 1 filter paper, concentrated by evaporation over a water bath, dried, and weighed using an electronic balance. The total flavonoid content was determined as described by **Ogunjobi et al. (2020)** and **Harborne (1973)** using the formula:

$$\text{Flavonoid (\%)} = \frac{\text{Final weight of the residue}}{\text{Initial weight of sample}} \times 100$$

The total phenolic content of the methanolic leaf extract was estimated using the Folin–Ciocalteu (FC) method with slight modifications **(Chethana et al., 2018; Jamuna et al., 2014)**. A 0.01 g sample of extract was dissolved in distilled water to make up 1800 µl. To this, 150 µl of FC reagent and 1 ml of 10% sodium carbonate (Na_2CO_3) solution were added, and the volume was adjusted to 3 ml. The reaction mixture was incubated in the dark for 40 min, after which absorbance was recorded at 765 nm using a UV–Vis spectrophotometer (Shimadzu A

125358) against a reagent blank. A calibration curve was prepared using gallic acid (10–50 µg/ml) as standard. The results were expressed as milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g dry extract), based on the average of three independent experiments.

Total tannin content was determined by the Folin–Denis (FD) method with slight modifications **(Padma et al., 2013; Polshettiwar** and **Ganjiwale, 2007)**. A 0.01 g extract sample was dissolved in distilled water to make up 1800 µl. Then, 150 µl of FD reagent and 1 ml of sodium carbonate (Na_2CO_3) solution were added, and the final volume was adjusted to 3 ml. The mixture was incubated in the dark for 40 min. Absorbance was measured at 760 nm against a blank using a UV–Vis spectrophotometer (Shimadzu A 125358). A calibration curve was constructed using tannic acid (10–50 µg/ml) as standard. The results were expressed as milligrams of tannic acid equivalents per gram of dried extract (mg TAE/g dry extract), based on the mean of three independent experiments.

Antioxidant analysis:

DPPH free radical scavenging activity

The free radical scavenging activity of the extracts was determined by the DPPH method as described by **Brand-Williams et al. (1995)**. A DPPH solution (6×10^{-5} M) was freshly prepared in methanol prior to analysis. Stock solutions of crude extracts were prepared by dissolving a known amount of dried extract in methanol, and serial dilutions were made to obtain concentrations of 2, 4, 8, and 10 µg/ml. Equal volumes of extract solution and DPPH solution were mixed (1:1, v/v), vortexed, and incubated at room temperature in the dark. Absorbance was measured at 517 nm at specified time intervals using a UV–Vis spectrophotometer. A decrease in the intensity of the purple color indicated an increase in scavenging activity.

The percentage inhibition of DPPH radicals was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A0-A}{A0} \times 100$$

where A_0 is the absorbance of the DPPH control (without extract) and A is the absorbance in the presence of extract/standard. The IC_{50} value (concentration of extract required to scavenge 50% of DPPH radicals) was determined from a plot of inhibition (%) versus concentration. Ascorbic acid and α-tocopherol were used as reference standards.

Hydrogen peroxide (H_2O_2) scavenging activity

The H_2O_2 radical scavenging activity of the extracts was assessed following the method of **Ruch et al. (1989)**, with ascorbic acid as standard. Different concentrations of ascorbic acid (200, 400, 600, 800, and 1000 µg/ml) were prepared in distilled water. Test solutions were prepared by mixing 0.6 ml of H_2O_2 solution (40 nM in phosphate buffer saline, pH 7.4) with 1 ml of sample solution at different concentrations. Blanks were prepared without H_2O_2, while control contained 0.6 ml of 40 mM H_2O_2 with 1 ml of phosphate buffer saline. The reaction mixtures were incubated for 10 min at room temperature, and absorbance was recorded at 230 nm using a UV–Vis spectrophotometer (Model 119, Systronics Ltd., India).

The scavenging activity of H_2O_2 was calculated as:

$$H_2O_2 \text{ radical scavenging activity (\%)} = \frac{A0-A}{A0} \times 100$$

where A_0 is the absorbance of the control and A is the absorbance of the extract/standard.

GC–MS analysis:

Gas chromatography–mass spectrometry (GC–MS) was employed to identify the major bioactive compounds present in the crude methanolic and chloroform leaf extracts of *Meyna laxiflora*. The analysis was performed using a PE Auto-System GC equipped with a built-in autosampler. The capillary column used measured 60 m in length and 250 µm in diameter, with vacuum compensation. The total run time was 56 min, and data were acquired at a sampling rate of 1.5625 pts/s. An injection volume of 1 µl was introduced in split mode (split ratio 10:1). Helium was used as the carrier gas at a constant flow rate of 1 ml/min.

The oven temperature program was as follows: initial temperature 60 °C (hold for 3 min), ramped at 6 °C/min to 200 °C (hold for 3 min), followed by a ramp of 6 °C/min to 300 °C (hold for 10 min). The equilibration time was maintained at 2 min. Identification of compounds was carried out by comparing the obtained mass spectra with those available in the NIST library **(Roy et al., 2019)**.

Statistical analysis:

All experiments were conducted in a completely randomized design with three replications. Results were expressed as mean ± standard error (SE). Data were analyzed using one-way analysis of variance (ANOVA), and significant differences among extracts were determined using Duncan's multiple range test and Tukey's HSD test at a significance level of $p < 0.05$. Statistical analyses were performed using the R software environment, while Microsoft Excel was used for preliminary calculations.

RESULTS AND DISCUSSION:

Phytochemical screening:

The preliminary phytochemical analysis of *Meyna laxiflora* leaf extracts revealed the presence of various bioactive secondary metabolites, including alkaloids, tannins, flavonoids, carbohydrates, proteins, saponins, steroids, phenols, and terpenoids, whereas glycosides were absent in all extracts

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(Table 1). The intensity of occurrence varied with the solvent system, with methanol and chloroform extracts showing a broader range of phytochemicals compared to the aqueous extract.

The presence of these compounds is significant, as they are known to exert diverse physiological and pharmacological effects in humans. Alkaloids and tannins are well-documented for their antimicrobial and anti-inflammatory activities; flavonoids and phenols act as potent antioxidants; while steroids and terpenoids play important roles in anti-inflammatory and anticancer pathways **(Ghaneian et al., 2015; Kabir et al., 2020)**. The detection of these phytochemicals in *M. laxiflora* supports its traditional medicinal use among ethnic communities of Assam.

Comparable findings were reported by **Ganesh et al. (2010)**, who identified alkaloids, saponins, glycosides, steroids, and carbohydrates in methanolic seed extracts of *M. laxiflora*. The present study extends these observations by confirming the phytochemical richness of the leaves, suggesting their potential as a source of pharmacologically important compounds.

Table 1. Phytochemical constituents in leaf extracts of *M. laxiflora*

Phytochemical constituent	Methanol	Chloroform	Water
Alkaloid	++	++	+
Tannin	++	++	+
Flavonoid	++	++	+
Carbohydrate	++	++	+
Protein	+	–	+
Glycoside	–	–	–
Saponin	+	+	–
Steroid	++	+	+
Phenol	++	++	+
Terpenoid	+	+	+

Note: "+" = present; "++" = strongly present; "–" = absent.

Quantitative estimation of phytochemical compounds:

The quantitative analysis of secondary metabolites revealed appreciable amounts of alkaloids, flavonoids, phenols, and tannins in *M. laxiflora* leaf extracts, with marked variation among solvents (Table 2). These phytoconstituents are considered potential reservoirs of pharmacologically active compounds that may contribute to the plant's traditional therapeutic applications **(Tanti et al., 2010; Olasunkanmi et al., 2022)**.

The highest alkaloid content was recorded in the methanol extract (1.37 ± 0.1 mg/g), whereas the aqueous extract contained the lowest concentration (0.74 ± 0.1 mg/g). Flavonoid content was maximum in the chloroform extract (8.00 ± 0.6 mg/g QE), followed by the methanol extract (5.99 ± 0.4 mg/g QE), while the aqueous extract exhibited the lowest level (1.21 ± 0.03 mg/g QE). Similarly, the phenolic content was found to be highest in methanol (14.64 ± 0.3 mg/g GAE), with chloroform (13.59 ± 0.7 mg/g GAE) and aqueous extracts (13.18 ± 0.7 mg/g GAE) showing slightly lower but comparable values. The tannin content followed a similar trend, being maximum in methanol (2.93 ± 0.1 mg/g TAE) and minimum in the aqueous extract (0.36 ± 0.5 mg/g TAE).

These findings highlight methanol and chloroform as more effective solvents for extracting bioactive compounds from *M. laxiflora* leaves compared to water. The high phenolic and flavonoid content suggests strong antioxidant potential, whereas the appreciable presence of alkaloids and tannins may account for antimicrobial and anti-inflammatory properties. The variation in solvent extraction efficiency could be attributed to the polarity differences of the phytochemicals.

Table 2. Phenolic, flavonoid, alkaloid, and tannin content of *M. laxiflora* leaf extracts (mean ± SE, n = 3)

Extract (Leaf)	Total Alkaloid content (mg/g)	Total Flavonoid content (mg/g QE)	Total Phenol content (mg GAE/g)	Total Tannin content (mg TAE/g)
Methanol extract	1.37 ± 0.1	5.99 ± 0.4	14.64 ± 0.3	2.93 ± 0.1
Chloroform extract	1.32 ± 0.1	8.00 ± 0.6	13.59 ± 0.7	1.21 ± 0.6
Water extract	0.74 ± 0.1	1.21 ± 0.03	13.18 ± 0.7	0.36 ± 0.5

Note: Samples were analyzed in triplicates; values are expressed as mean ± SE.

Antioxidant Analysis:

The antioxidant activity of *M. laxiflora* leaf extracts was assessed using DPPH and H_2O_2 radical scavenging assays, with ascorbic acid as the reference standard. The DPPH radical scavenging activity demonstrated that the methanol extract exhibited the highest inhibition, which was slightly lower than that of ascorbic acid, suggesting appreciable antioxidant potential. At 1.00 µg/mL, the scavenging activity of methanol extract and ascorbic acid was 48.88 ± 0.60% and 51.38 ± 0.22%, respectively (Table 3; Fig. 1). The IC_{50} value of the methanol extract (31.60 µg/mL) was higher than that of ascorbic acid (20.35 µg/mL), indicating good, though comparatively lower, radical scavenging capacity (Table 4).

The chloroform and aqueous extracts also exhibited dose-dependent activity but were less effective compared to methanol and the standard. The hydrogen peroxide (H_2O_2) scavenging assay further confirmed the antioxidant potential of *M. laxiflora*. The methanol extract showed considerable inhibition with an IC_{50} value of 29.49 µg/mL, which

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was comparable to but slightly higher than that of the standard (47.16 µg/mL). At 1.00 mg/mL concentration, the scavenging percentages were 35.77 ± 0.36% for methanol extract and 28.45 ± 0.11% for the standard (Table 5; Fig. 2).

The results corroborate earlier findings. **Roy et al. (2019)** reported IC_{50} values of 84.2 ± 2.1 µg/mL and 91.0 ± 3.0 µg/mL for DPPH and H_2O_2 assays, respectively, highlighting the radical scavenging potential of the species. Similarly, **Bag et al. (2016)** observed IC_{50} values of 34.95 µg/mL and 60.95 µg/mL for water and methanol leaf extracts, respectively. These comparative studies strengthen the evidence that *M. laxiflora* is a promising source of natural antioxidants, likely due to its rich phenolic and flavonoid content.

Table 3. DPPH radical scavenging activity of *M. laxiflora* leaf extracts (mean ± SE, n = 3)

Working Concentration (µg/mL)	Scavenging (%) – Ascorbic acid	Methanol Extract	Chloroform Extract	Water Extract
0.25	46.64 ± 0.27	41.46 ± 0.33	38.18 ± 1.06	33.05 ± 0.20
0.50	47.58 ± 0.25	47.80 ± 0.53	42.54 ± 0.60	33.87 ± 0.35
0.75	48.45 ± 0.29	47.80 ± 0.20	43.70 ± 0.87	37.15 ± 0.68
1.00	51.38 ± 0.22	48.88 ± 0.60	47.20 ± 0.85	40.34 ± 0.68

Note: Data represent mean ± SE of triplicate analysis.

Table 4. H_2O_2 radical scavenging activity of *M. laxiflora* leaf extracts (mean ± SE, n = 3)

Working Concentration (µg/mL)	Scavenging (%) – Ascorbic acid	Methanol Extract	Chloroform Extract	Water Extract
0.25	35.77 ± 0.36	28.45 ± 0.11	19.30 ± 0.22	18.72 ± 0.15
0.50	43.84 ± 0.35	37.05 ± 0.11	31.39 ± 0.11	28.74 ± 0.11
0.75	53.25 ± 0.39	45.14 ± 0.11	35.55 ± 0.15	34.75 ± 0.15
1.00	69.50 ± 0.40	51.00 ± 0.20	40.24 ± 0.14	37.83 ± 0.15

Note: Samples were analyzed in triplicate; values are expressed as mean ± SE.

Table 5. IC_{50} values (µg/mL) of *M. laxiflora* leaf extracts in DPPH and H_2O_2 radical scavenging assays

Sample Extract	DPPH IC_{50} (µg/mL)	H_2O_2 IC_{50} (µg/mL)
Ascorbic acid	20.35	29.49
Methanol Extract	31.60	48.51
Chloroform Extract	45.55	48.92
Water Extract	38.13	49.10

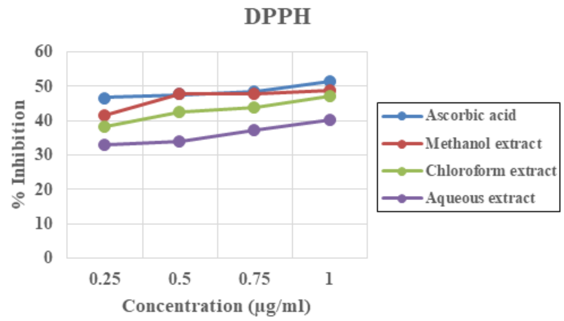


Fig 1. DPPH radical scavenging activity (%) versus different leaf extracts concentrations (µg/ml) versus Ascorbic acid (standard) of *M laxiflora*

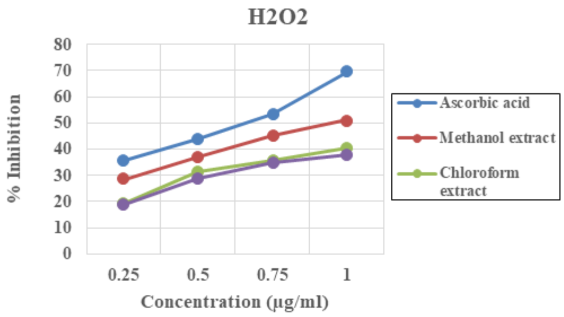
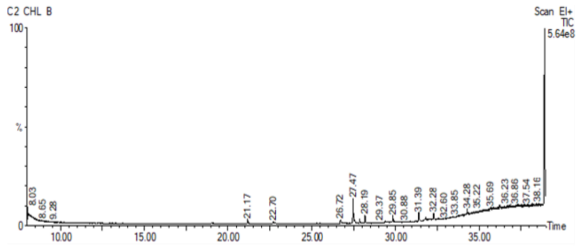


Fig 2. H_2O_2 scavenging activity of different leaf extracts concentrations (µg/ml) versus Ascorbic acid (standard) of *M laxiflora*

GC–MS Analysis:

Gas chromatography–mass spectrometry (GC–MS) is a widely used technique for identifying volatile compounds, long-chain and branched hydrocarbons, alcohols, acids, esters, and other bioactive molecules **(Rukshna et al., 2017)**. In the present study, GC–MS analysis of methanol and chloroform leaf extracts of *Meyna laxiflora* revealed multiple peaks, indicating the presence of several phytochemical constituents (Fig. 3). Major compounds detected included neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, phytol, squalene, and 9-eicosyne.

The compounds were identified using the NIST library, and their retention time (RT), molecular formula (MF), molecular weight (MW), peak area percentage, and reported bioactivities are summarized in Table 6. The results highlight the presence of phytochemicals with notable biological properties, including antimicrobial, antifungal, antioxidant, and anti-inflammatory activities.



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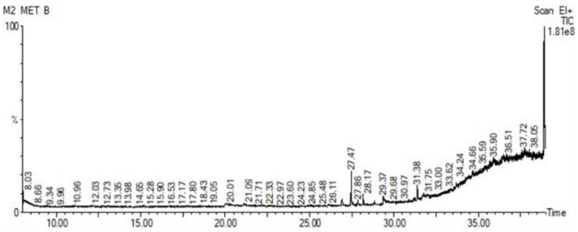

(a) Chloroform leaf extract
(b) Methanolic leaf extract

Fig. 3. GC–MS chromatograms of *M. laxiflora* leaf extracts: (a) Chloroform extract; (b) Methanolic extract

Table 6. Major phytocomponents identified in chloroform and methanol leaf extracts of M. laxiflora by GC–MS

Sl. No.	Compound Name	MF	MW	RT (min)	Peak Area (%)	Bioactivity
Chloroform extract						
1	Neophytadiene	C20H38	278	27.471	3.531	Anti-inflammatory and antimicrobial agent; alkene and diterpene **(Ratheesh et al., 2022)**
2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	27.471	3.531	Antifungal agent **(Nithya et al., 2018)**
3	Phytol	C20H40O	296	31.393	1.613	Antimicrobial agent **(Ganesh et al., 2010)**
4	Squalene	C20H50	410	38.906	1.330	Natural 30-carbon isoprenoid; intermediate in cholesterol synthesis **(Lozano-Grande et al., 2018)**
5	9-Eicosyne	C20H38	278	27.881	0.422	Antimicrobial and cytotoxic properties **(Paul et al., 2022)**
Methanol extract						
6	Neophytadiene	C20H38	278	27.466	2.465	Anti-inflammatory and antimicrobial agent **(Ratheesh et al., 2022)**
7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	28.172	0.700	Antifungal agent **(Nithya et al., 2018)**
8	Squalene	C30H50	410	38.896	1.330	Natural 30-carbon isoprenoid; intermediate in cholesterol synthesis **(Lozano-Grande et al., 2018)**

The GC–MS results demonstrate that *M. laxiflora* is a rich repository of structurally diverse bioactive molecules. Combined with the DPPH and H_2O_2 assays, which revealed significant antioxidant activity, these findings highlight the plant's potential utility in pharmaceutical and medicinal applications. Many pharmacologically active compounds in modern medicines are combinations of such bioactive constituents. Further studies are warranted to isolate, characterize, and evaluate the therapeutic potential of these compounds for drug development.

CONCLUSION:

This study successfully isolated and characterized key bioactive compounds from *Meyna laxiflora*, revealing appreciable amounts of alkaloids, flavonoids, phenols, and tannins. Among the solvents tested, methanol and chloroform were more effective in extracting bioactive constituents. Notably, the methanol extract demonstrated significant antioxidant potential. GC–MS analysis of methanol and chloroform leaf extracts indicated the presence of several phytochemical compounds, including neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, phytol, squalene, and 9-eicosyne. These phytochemicals are associated with diverse biological properties such as antimicrobial, antifungal, antioxidant, and anti-inflammatory activities. Overall, the findings highlight the pharmacological potential of *M. laxiflora* and suggest its promising applications in pharmaceutical and medicinal fields.

CONFLICT OF INTEREST:

The Authors do not have any conflict of interest.

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