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Research Paper

GC-MS Profiling of Aqueous and Methanolic Extracts of Meyna Laxiflora Robyns Leaf

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ABSTRACT

Plants are the good source of various phytochemicals and secondary metabolites which are used in medicine and environmental sectors as well as being widely used in commercial and pharmaceutical products. In our earlier study we have reported the preliminary phytochemical screening and antioxidant activity of aqueous and methanolic extracts of Meyna laxiflora Robyn leaf. The current study was carried out to analyze the active phytoconstituents present in the aqueous and methanolic extracts of leaf of Meyna laxiflora Robyns using GC-MS and the spectrum of unknown compounds was compared with the compounds stored in the National Institute of Standards and Technology Mass Spectral database (NIST). The gas chromatography with mass spectroscopic analysis showed thirtyeight compounds in aqueous extract and twentysix compounds in methanolic extract of Meyna laxiflora Robyns leaf.

INTRODUCTION

The search for Ethnomedicinal plants sets the basis for discovering new compounds of medicinal importance [1]. With the passage of time, a large amount of evidence has been gathered that demonstrates the use of plants in the field of pharmacology [2]. Since the appearance of life on Earth, plants are a major source of food, clothing, shelter, and medicine [3,4]. Due to their nutritive and medicinal potential, plants have been playing a vital role in human life [5]. From the start of life, human civilization has been using plants, mainly as medicines, and most civilizations still use them [6]. Currently, 25% of pharmaceutical drugs are derived from plants [7]. In developing countries, about 70%–95% population uses medicinal plants to treat health issues [8]. Phytobioactive compounds are plant secondary metabolites and have remarkable therapeutic potential. Polyphenols, alkaloids, terpenes, and polysaccharides isolated from medicinal plants showed remarkable antioxidant, anticancer,

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cytotoxic, anti-inflammatory, cardioprotective, hepatoprotective, immunomodulatory, neuroprotective, and antidiabetic activities. Phytobioactive compounds may be used as a potential alternative to synthetic compounds and as therapeutic agents for the treatment of various diseases. *Meyna laxiflora* is a tree of small or medium size distributed in tropical and subtropical regions. They are found mainly in North-east, West Bengal, Western UP and Deccan Peninsula etc. [9]. Since time immemorial different parts of the plant were used in the treatment of boils, dysentery, diphtheria etc. Fresh leaves with dry fish, little common salt and chillis are eaten as blood purifier. Dry fruits are consumed as such in case of boils and dysentery [10]. Fresh leaves smeared with coconut oil and then slightly heated are wrapped on goiter or swellings [11]. Five pinches of seed powder is mixed in water thoroughly and is given twice a day for 10-15 days to treat kidney stone [12]. From ancient time in Manipur, fresh leaves of *M. laxiflora* are boiled with rice water and used for washing hair to give soft and lustrous hair, oil extract from fruit pulp are applied on skin to prevent skin from dryness and local people consume its leave and fruit frequently in various ways. In continuation of our previous findings, GC-MS profiling of aqueous and methanol extracts of *M. laxiflora* leaf was analyzed in the present study. Until now, huge development has been made in the identification and functional characterization of bioactive compounds of medical importance. The phytochemical screening approach includes qualitative and quantitative chemical class profiling of ethnomedicinal plant species. Methanolic and aqueous extraction coupled with gas chromatography–mass spectrometry (GC-MS) has been widely used to identify phytochemicals of clinical importance [13]. Gas chromatography – Mass spectrometry is an important technique that has been adapted to evaluate different phytoconstituents present in various plant extracts with their structures. This technique has superior separation potency that leads to produce a high accuracy and precision of chemical fingerprint. Moreover, quantitative data along with the coupled mass spectral database can be given by GC-MS that is of tremendous value for achieving the correlation between bioactive compounds and their applications in pharmacology [14].

MATERIALS AND METHODS

M. laxiflora leaf was collected from Imphal West district of Manipur, Northeast India. The plant species was identified by L. Somarjit Singh, retired Associate Professor, Department of Botany, Imphal College, Imphal. Leaves were washed with tap water and then rinsed with distilled water and were dried in shade. Completely dried leaves were ground into powder.

Sample Preparation for GC-MS

Gas Chromatography mass spectrum analysis was done using GCMS-QP2010 Ultra at Aakaar Biotechnologies Private Limited, Lucknow.

Aqueous extract: 1 gm of sample was taken and mixed with 100 ml of demineralized water. Sample mixture was boiled until the volume is reduced to 25ml in conical flask. Then extract was centrifuged at 6000rpm for 5 min to get the clear supernatant. The extract was completely dried in the oven at 40-60°C. Extract were collected in micro centrifuge tube and stored at 4°C. Methanol extract: 1 gm of samples was taken and mixed with 10 ml of solvent (absolute methanol). Sample mixture was incubated on a rocker shaker for 24 hours. Then extract was filtered through Whatman filter paper 1 and the extract was completely dried in the oven at 40°C. Extract were collected in micro centrifuge tube and stored at 4°C.

Separately 10 µl of each sample (50 mg/ml) was taken in a separating funnel and shaken by adding 10 ml of water and ethyl acetate in the ratio of 1:4 (add 2.5 µl water to 7.5 µl ethyl acetate). Upper layer was collected and concentrated to 1 ml in the rotary evaporator. 50 µl N, O-Bis (trimethylsilyl) trifluoroacetamide and trimethylchlorosilane (BSTFA+TMCS) were added and then finally 10µl of Pyridine was also added. 100µl BSTFA+TMCS solution was prepared by mixing 99µl of BSTFA and 1µl of TMCS. Samples were transferred in GC vial and dried using nitrogen gas. Finally, samples were dissolved in methanol before GC-MS analysis. Acquired samples were programmed as described below. Analytical Conditions are ion source temperature: 220°C, interface temperature: 270°C, column oven temperature: 120°C, injection temperature: 260°C, split injection volume: 2 µl, flow control mode: linear velocity, pressure: 99.3 kPa, total flow: 16.3 mL/min, column flow: 1.21 mL/min linear velocity: 41.3 cm/sec, purge flow: 3.0 mL/min, split ratio: 10.0, high pressure injection OFF, carrier gas saver OFF, Carrier Gas: Helium, splitter hold OFF, oven temperature program: rate: 10.00, temperature : 300°C, hold time: 20 min, solvent cut time: 3.50 min, detector gain mode: relative, detector gain: +0.00 kV, threshold: 1000. MS start time: 4.30 min, end time: 30 min, ACQ mode: Scan, event time: 0.20sec, scan speed: 3333, start m/z: 40.00 and end m/z: 650.00.

RESULTS AND DISCUSSION

Phytochemical screening of aqueous and methanol extracts of *M. laxiflora* leaf showed the presence of many phytochemicals as reported earlier. The total phenolic content in aqueous and methanolic leaf extracts in terms of gallic acid equivalent was 100.50 and 111.86 mg/g of extract respectively and that of total flavonoid content was 21.26 µg/100g and 80.45 µg/100g of dried extract in terms of quercetin equivalent. The total antioxidant activity assay also indicates a dose dependent manner with methanol extract having higher activity than aqueous extract [15].

GC-MS chromatograms of aqueous and methanol extracts of *M. laxiflora* leaf are shown in Fig 1 and 2 respectively. The identification of the phytochemical compounds was based on the peak area, retention time and molecular formula. Table 1 and 2 indicates peak retention time, peak area (%) and structure of bioactive compounds with their medicinal properties compare to that of the known compounds described by the National Institute of Standards and Technology (NIST) library.

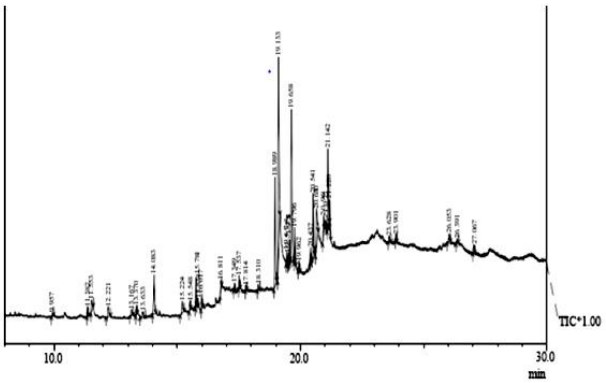


Fig. 1: GC-MS chromatogram of *M. laxiflora* aqueous leaf extract

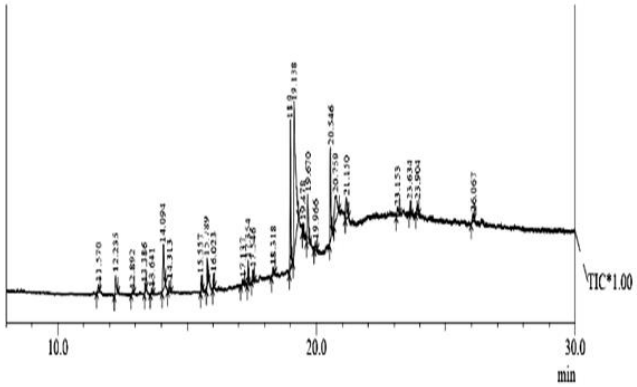
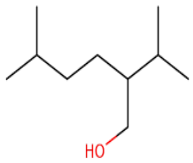
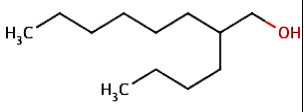


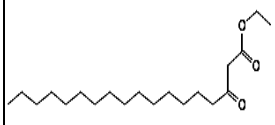
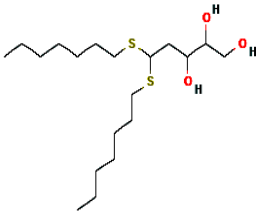
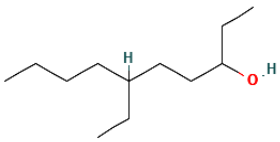
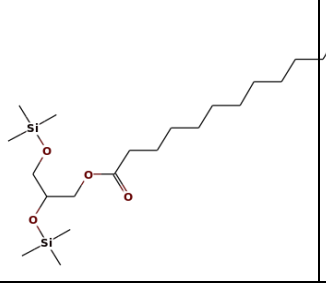
Fig. 2: GC- MS chromatogram of M. laxiflora methanol leaf extract

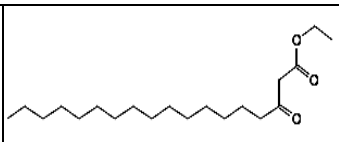
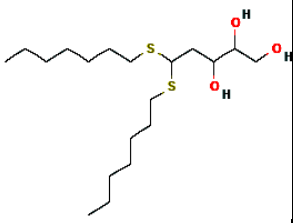
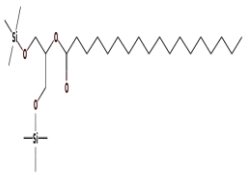
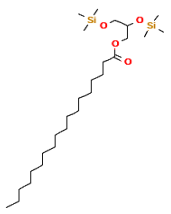
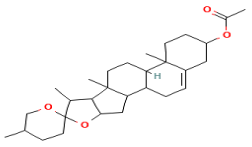
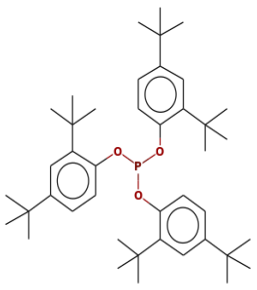
GCMS chromatogram of M. laxiflora aqueous leaf extracts shows 38 peaks (Fig.1) while that of methanolic leaf extract of M. laxiflora recorded a total of 26 peaks (Fig.2). For aqueous extract among the 38 compounds, 19 were found to be major compounds, viz.: 1-Hexanol,5-Methyl-2-(1-Methylethyl); 1-Octanol, 2-Butyl; Hexadecanoic Acid, Methyl Ester; Palmitic Acid, TMS Derivative; (Z)-Methyl Heptadec-9-Enoate; Octadecanoic Acid, Methyl Ester; Glycidyl Palmitate; OctadecanoicAcid,3-Oxo-,EthylEster; D-Ribose,2-Deoxy-Bis(Thioheptyl)-Dithioacetal; 6-Ethyl-3-Decanol,TMS Derivative; HexadecanoicAcid,4[(Trimethylsilyl)Oxy]ButylEster; 1-Monopalmitin,2TMSDerivative; OctadecanoicAcid,3-Oxo-,EthylEster; D-Ribose, 2-Deoxy-Bis(Thioheptyl)-; 2-Monostearin,2TMS Derivative; Heptadecanoic Acid, Trimethylsilyl Ester;2,3Bis[(Trimethylsilyl)Oxy]PropylStearate; Spirost-5-En-3-YlAcetate; Phenol,2,4-Bis(1,1-Dimethylethyl)-,Phosphite(3:1).

Table1: Major compounds identified in the aqueous leaf extract of *Meyna laxiflora*

	Peak Report of Aqueous Extract of *Meyna Laxiflora* leaf						
Sl. No.	Name of the compound	RT	Area %	MW (g/mol)	MF	Structure	Medicinal uses
1.	1-Hexanol,5-Methyl-2-(1-Methylethyl)	11.553	1.29	158.28	$C_{10}H_{22}O$		Unknown
2.	1-Octanol, 2-Butyl	12.221	1.43	186.33	$C_{12}H_{26}O$		Unknown

3.	Hexadecenoic Acid, Methyl Ester	14.083	4.44	270.45	$C_{17}H_{34}O_2$		Antioxidant, mematicide, insecticide, lubricant, antiandrogenic, hemolytic, hypo – cholesterolemic [16], antibacterial and antifungal activities [17] reducing blood cholesterol, and anti-inflammatory agent [18]. Nematicide, pesticide, antiarthritic, antitumor, anticoronary, hepatoprotective [19].
4.	PalmiticAcid,TMS Derivative	15.224	1.45	328.60	$C_{19}H_{40}O_2Si$		Unknown
5.	(Z)-Methyl Heptadec-9-Enoate	15.781	2.22	282.46	$C_{18}H_{34}O_2$		Antibiotic and antimicrobial properties [20]
6.	OctadecanoicAcid,Methyl Ester	16.017	1.18	296.48	$C_{19}H_{36}O_2$		antioxidant, anti-inflammatory and antimicrobial activity [16]

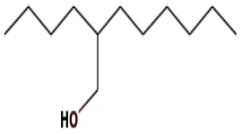
7.	GlycidylPalmitate	17.537	1.03	312.5	$C_{19}H_{36}O_3$		Antioxidant, antibacterial, and antifungal activities, used in preparation of isophosphatidic acid which inhibits apoptosis [21] [22]
8.	OctadecanoicAcid,3-Oxo-,EthylEster	18.989	8.59	326.5	• $C_{20}H_{38}O_3$		Antioxidant and anti-inflammatory activities [16]
9..	D-Ribose,2-Deoxy-Bis(Thioheptyl)-Dithioacetal	19.133	21.08	380.7	• • • $C_{19}H_{40}O_3S_2$		Unknown
10.	6-Ethyl-3-Decanol,TMS Derivative	19.580	1.45	186.33	$C_{12}H_{26}O$		Unknown
11.	HexadecanoicAcid,4-[(Trimethylsilyl)Oxy]Butyl Ester	19.658	18.02	400.7	$C_{23}H_{48}O_3Si$		Unknown
12.	1-Monopalmitin,2TMSDerivative	19.796	2.29	474.86	$C_{25}H_{54}O_4Si_2$		Antioxidant [16]

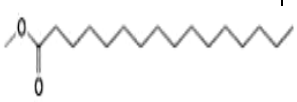
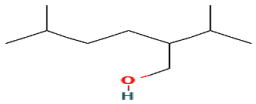
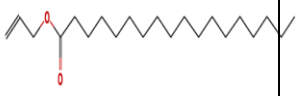
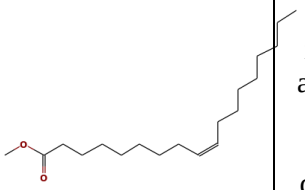
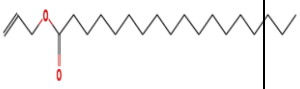
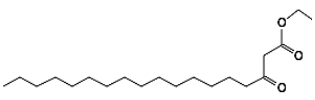
13.	OctadecanoicAcid,3-Oxo-,EthylEster	20.541	5.30	326.5	• $C_{20}H_{38}O_3$		Antioxidant and anti-inflammatory activities [16]
14.	D-Ribose, 2-Deoxy-Bis(Thioheptyl)-	20.680	5.03	380.7	• • • $C_{19}H_{40}O_3S_2$		Unknown
15.	2-Monostearin,2TMS Derivative	20.968	1.16	502.91	$C_{27}H_{58}O_4Si_2$		Unknown
16.	Heptadecanoic Acid, Trimethylsilyl Ester	21.142	7.97	342.63	$C_{20}H_{42}O_2Si$		Unknown
17.	2,3-Bis[(Trimethylsilyl)Oxy]PropylStearate	21.220	1.65	502.9	$C_{27}H_{58}O_4Si_2$		Unknown
18.	Spirost-5-En-3-YlAcetate	26.053	1.08	456.7	$C_{29}H_{44}O_4$		Unknown
19.	Phenol,2,4-Bis(1,1-Dimethylethyl)-,Phosphite (3:1)	27.067	1.15	646.92	$C_{42}H_{63}O_3P$		Antibacterial and antioxidant activity [23]

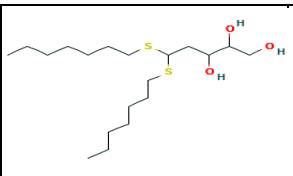
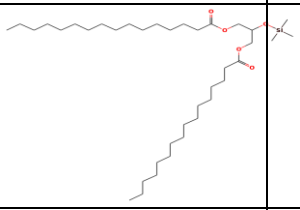
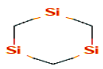
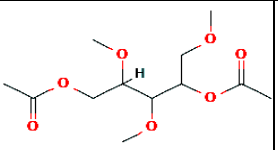
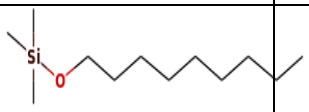
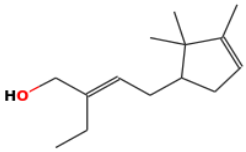
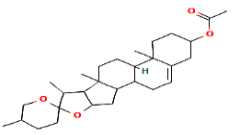
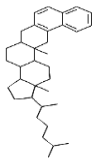
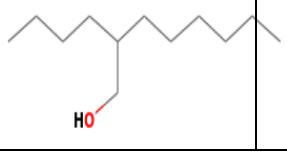
MF: Molecular Formula, MW: Molecular Weight, RT: Retention Time GC-MS result revealed that among the 19 major compounds Phenol,2,4-Bis(1,1-Dimethylethyl)-,Phosphite (3:1) had the

highest retention time and the highest molecular weight while D-Ribose,2-Deoxy-Bis (Thioheptyl)- Dithioacetal had the highest peak area. The GC–MS chromatogram of methanolic leaf extract of *Meyna laxiflora* recorded a total of 26 peaks among which18 were found to be major compounds viz: 1-Octanol,2-Butyl-; Hexadecanoic Acid, Methyl Ester; 1-Hexanol,5-Methyl-2-(1-Methylethyl)-; Octadecanoic Acid, 2-Propenyl Ester; 9-OctadecenoicAcid(Z)-,Methyl Ester; Octadecanoic Acid, Methyl Ester; Octadecanoic Acid, 2-Propenyl Ester; Hexadecanoic Acid,1-(Hydroxymethyl)-1,2-Ethanediyl Ester; OctadecanoicAcid,3-Oxo-,Ethyl Ester; D-Ribose,2-Deoxy-Bis (Thioheptyl)-Dithioacetal; 1,3-Dipalmitin,TMSDerivative; 1,3,5-Trisilacyclohexane; 1,4-Di-O-Acetyl-2,3,5-Tri-O-Methylribitol; O1-Nonanoyl-O3-Octanoyl-Glycerol,TMSDerivative; 2-Buten-1-Ol,2-Ethyl-4-(2,2,3-Trimethyl-3-Cyclopenten-1-Yl; Spirost-5-En-3-Ol,Acetate,(3.Beta.,25R)-; Cholest-1-Eno[2,1-A]Naphthalene,3',4'-Dihydro- and 1-Octanol,2-Butyl-.

Table2: Major compounds identified in the methanolic leaf extract of *Meyna laxiflora*

	Peak Report of Methanolic Extract of *Meyna Laxiflora* leaf						
Sl. No.	Name of the compound	RT	Area %	MW (g/mol)	MF	Structure	Medicinal uses
1.	1-Octanol,2-Butyl-	12.235	2.97	186.33	$C_{12}H_{26}O$		Metabolite observed in cancer metabolism. It has a role as a human metabolite. Used in the production of surfactants, emulsifiers, and cosmetics formulations due to its emulsifying properties. It is used to improve the wetting properties and effectiveness in cleaning products and also helps improve the efficacy of herbicides and insecticides by enhancing their ability to spread and penetrate [24]

2.	HexadecanoicAcid,MethylEster	14.094	5.95	270.45	$C_{17}H_{34}O_2$		Antioxidant, Nematicide, Insecticide, Lubricant, Antiandrogenic, Hemolytic, Hypo – cholesterolemic [16]
3.	1-Hexanol,5-Methyl-2-(1-Methylethyl)-	14.313	1.07	158.28	$C_{10}H_{22}O$		Unknown
4.	OctadecanoicAcid,2-PropenylEster	15.557	1.29	324.54	$C_{21}H_{40}O_2$		Antibacterial activity [16]
5.	9-OctadecenoicAcid(Z)-,MethylEster	15.789	2.11	296.48	$C_{19}H_{36}O_2$		Anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, hypocholesterolemic, 5-Alphareductase inhibitor anemiagenic, insectifuge properties and as flavouring agent [25]Antioxidant, anti-inflamatory activities [26]
6.	OctadecanoicAcid,MethylEster	16.023	1.18	294.47	$C_{19}H_{34}O_2$		Anticancer, antiviral
7.	OctadecanoicAcid,2-PropenylEster	17.354	1.67	324.54	$C_{21}H_{40}O_2$		Antibacterial activity [16]
8.	HexadecanoicAcid,1-(Hydroxymethyl)-1,2-Ethanediyl Ester	17.546	1.24	568.9	$C_{37}H_{72}O_4$		Anti-inflammatory, antioxidant, antihelmintic [16]
9.	OctadecanoicAcid,3-Oxo-,EthylEster	18.991	12.37	326.5	$C_{20}H_{38}O_3$		Antibacterial, antiviral, antioxidant activities [16]

10.	D-Ribose,2-Deoxy-Bis(Thioheptyl)-Dithioacetal	19.138	35.48	380.7	$C_{19}H_{40}O_3S_2$		Unknown
11.	1,3-Dipalmitin,TMSDerivative	19.670	6.72	641.09	$C_{38}H_{76}O_5Si$		Unknown
12.	1,3,5-Trisilacyclohexane	20.546	8.15	126.33	$C_3H_6Si_3$		Antibacterial and antibiofilm activities [27]
13.	1,4-Di-O-Acetyl-2,3,5-Tri-O-Methylribitol	20.759	7.47	278.30	$C_{12}H_{22}O_7$		Unknown
14.	O1-Nonanoyl-O3-Octanoyl-Glycerol,TMSDerivative	21.150	3.70	216.43	$C_{12}H_{28}OSi$		Unknown
15.	2-Buten-1-Ol,2-Ethyl-4-(2,2,3-Trimethyl-3-Cyclopenten-1-Yl	23.153	1.14	208.33	$C_{14}H_{24}O$		Unknown
16.	Spirost-5-En-3-Ol,Acetate,(3.Beta.,25R)-	23.634	1.08	456.7	$C_{29}H_{44}O_4$		Unknown
17.	Cholest-1-Eno[2,1-A]Naphthalene,3',4'-Dihydro-	23.904	1.21	470.8	$C_{35}H_{50}$		Unknown
18.	1-Octanol,2-Butyl-	12.235	2.97	186.33	$C_{12}H_{26}O$		Metabolite observed in cancer metabolism

MF: Molecular Formula, MW: Molecular Weight, RT: Retention Time GC-MS result showed that among the 18 major compounds Cholest-1-Eno[2,1-A]Naphthalene,3',4'-Dihydro- had the highest retention time and 1,3-Dipalmitin,TMSDerivative had the highest molecular weight while D-Ribose,2-Deoxy-Bis(Thioheptyl)-Dithioacetal had the highest peak

area. Both the extracts showed the presence of hexadecanoic acid and Octadecanoic Acid, 3-Oxo-EthylEster which may be responsible for the antioxidant activity of *Meyna laxiflora* leaf which was earlier reported. The compounds present include esters, alcohols, acetals and carboxylic acid groups. The GC-MS analysis of aqueous and methanolic extracts of *Meyna laxiflora* reveals the presence of 19 major compounds in aqueous and 18 in methanolic extract, which may be responsible for the medicinal properties of this plant as practiced by our traditional healers. There is no report on GC-MS-based plant metabolic characterization of *Meyna laxiflora* leaf. Therefore, the study has generated baseline data for further characterization of fruit pulp and seed extracts of this plant.

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