**IJPSR (2025), Volume 16, Issue 6** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 January 2025; received in revised form, 04 February 2025; accepted, 14 February 2025; published 01 June 2025

# GC-MS PROFILING OF AQUEOUS AND METHANOLIC EXTRACTS OF *MEYNA LAXIFLORA* ROBYNS FRUIT PULP

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**Keywords:**

*Meyna laxiflora*, GC-MS, Fruit pulp

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**ABSTRACT:** Medicinal plants are of great importance to researchers in the field of pharmacology as most pharmaceutical industries depend on medicinal plant for their raw materials. *Meyna laxiflora* belongs to the family Rubaceae and is well known for its medicinal properties. The present study was carried out in continuation of our previous findings to determine the possible bioactive components present in the aqueous and methanolic extracts of *Meyna laxiflora* fruit pulp. The gas chromatography - Mass spectrometry was used for identification of the bioactive compounds in the extracts and the spectrums of unknown compounds were compared with the compounds stored in the National Institute of Standards and Technology Mass Spectral database (NIST). The GC-MS analysis revealed the presence of 37 peaks in aqueous extract among these 19 were found to be major compounds while that of methanolic extract showed 24 peaks of which 15 were found to be major compounds. Hence, the present study has generated baseline data for further characterization of fruit pulp extract of this plant.

**INTRODUCTION:** Medicinal plants are the major source of bioactive secondary metabolites. They possess phytoconstituents having biological activities such as antioxidant, anti-inflammatory, anticancer, antiviral, antifungal, and antibacterial [1]. The plants are used to cure several illness and manage various pathogenic agents. Phyto-chemicals that are considered as bioactive compounds in plants have been confirmed to be safe, effective, relatively cheap, and recently predicted as a suitable substitute to antibiotics [2]. Geographically, *Meyna laxiflora* is distributed in tropical and subtropical regions. They are small or medium size trees. In India, it is found mainly in North-east, West Bengal, Western UP and Deccan Peninsula *etc* [3]. In ancient times different parts of the plant were used in the treatment of boils, dysentery, diphtheria *etc* [3, 4]. Dry fruits are also consumed in case of constipation, to control diabetes and as narcotics [3, 5]. It is also used as abortifacient [3, 6]. Fruits are fleshy dupes, smooth globose with 4-5 one-seeded pyrenes, oblong-reniform shape. They are green in colour at maturity and yellow brownish at the ripe stage. Ripe fruits are edible.

Fruit size is 3-7 cm in diameter. Fruiting pedicel is 1.6-2 cm long. Seeds are albuminous with a membranous testa. Fruit set occurs during March-April and attains ripens maturity and ripen during May-July [7]. From ancient times in Manipur, oil extract from fruit pulp is applied on skin to prevent skin from dryness. The preparation of jam from this

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.16(6).1608-15

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.16(6).1608-15

fruit has been successfully standardized by Dhodade *et al.,* 2019 [8]. Among the tribes of Khasi and Jaintia, fresh ripened fruits are eaten as a dessert. The ripened fruits are also used for wine preparation. The wine prepared from this fruit showed unique colour and aroma [7].

Preliminary phytochemical screening and antioxidant activity of the aqueous and methanolic extracts of *M. laxiflora* fruit pulp was done in the earlier part of our study and was reported [9]. Methanolic and aqueous extraction coupled with gas chromatography mass spectrometry (GC-MS) has been widely used to identify phytochemicals of pharmacological significance. Gas chromatography – Mass spectrometry is an important technique that has been used to assess different phytoconstituents 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 [10]. In continuation of our previous findings, GC-MS profiling of aqueous and methanolic extracts of *M. laxiflora* fruit pulp was analyzed in the present study.

**MATERIALS AND METHODS:** Fruits of *Meyna laxiflora* were collected from Imphal West district of Manipur, Northeast India. Identification of plant specimen was done by L. Somarjit Singh, retired Associate Professor, Department of Botany, Imphal College, Imphal. The plant samples were washed with tap water and then rinsed with distilled water and shade dried. Fruit pulp was separated out from seed then dried separately and ground into fine powder.

**Sample Preparation for GC-MS Analysis:** Gas Chromatography mass spectrum analysis was done using GCMS-QP2010 Ultra at Aakaar Biotechnologies Private Limited, Lucknow.

**Aqueous Extract:** 1gm of sample was taken and mixed with 100 ml of demineralized water. The 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). The sample mixture was incubated on a rocker shaker for 24 hours. Then the 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.

10 µl of sample (40mg/ml) was taken in a separating funnel and shaken by adding 10 ml of water and ethyl acetate in the ratio of 1:3 (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. For BSTFA+TMCS, make 100µl solution of 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:** Preliminary phytochemical analysis of aqueous and methanol extracts of fruit pulp of *M. laxiflora* indicates the presence of many phytochemicals as reported earlier. The total phenolic content in aqueous and

methanolic fruit pulp extracts in terms of gallic acid equivalent was 94.18 and 129.54 mg/g of extract respectively and that of total flavonoid content was 19.51 µg/100g and 51.70 µg/100g of dried extract in terms of quercetin equivalent. The total antioxidant activity assay indicates a dose dependent manner with methanol extract having higher activity than aqueous extract [9].

GC-MS analysis of aqueous and methanolic fruit pulp extracts of *M. laxiflora* revealed the presence of various bioactive compounds having medicinal properties and is shown in **Table 1** and **2** respectively. GC-MS chromatogram of aqueous and methanolic fruit pulp extracts of *M. laxiflora* are illustrated in **Fig. 1** and **2.**

The GC–MS chromatogram of aqueous and methanolic fruit pulp extracts of *Meyna laxiflora* recorded a total of 36 peaks and 24 peaks respectively corresponding to the bioactive compounds that were recognized by relating their peak retention time, peak area (%), height (%) and mass spectral fragmentation patterns to that of the known compounds described by the National Institute of Standards and Technology (NIST) library.

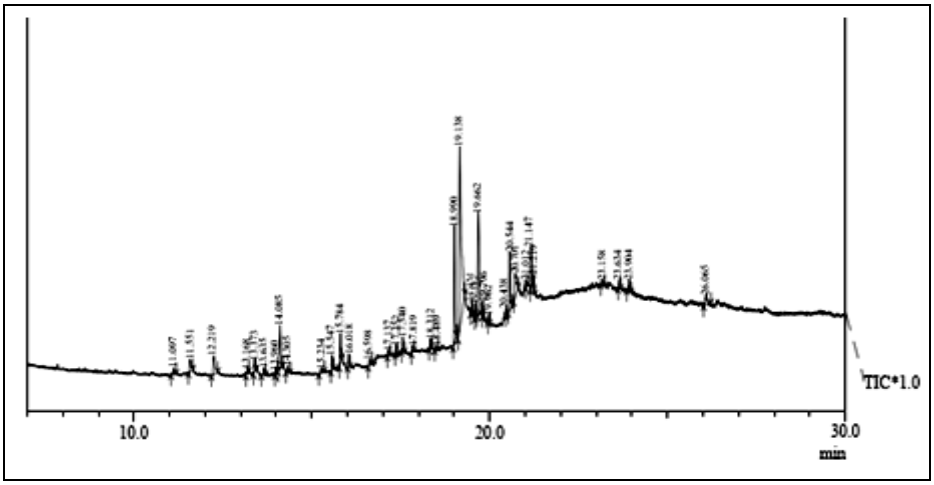



**FIG. 1: GC-MS CHROMATOGRAM OF *M. LAXIFLORA* AQUEOUS FRUIT PULP EXTRACT**

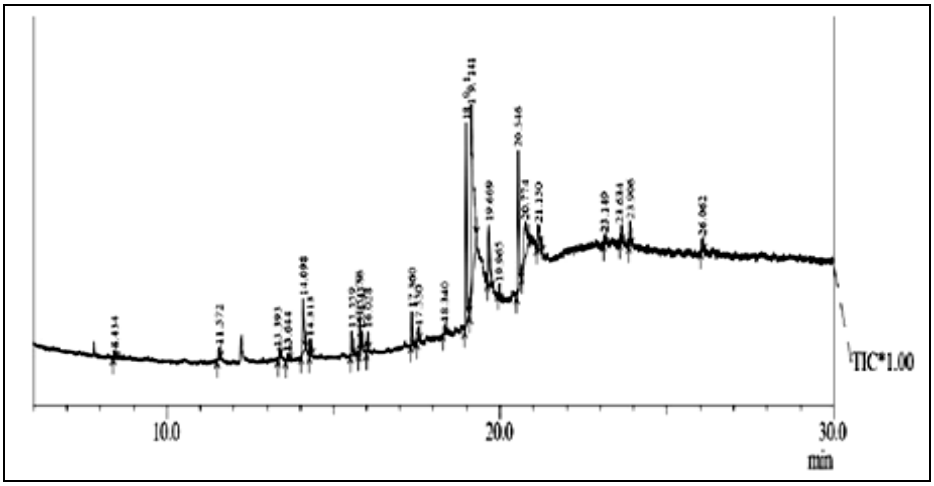
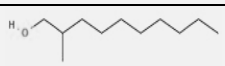
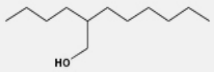
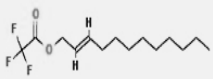
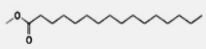


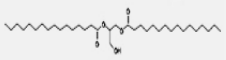
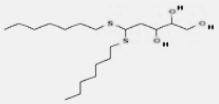
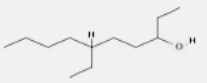
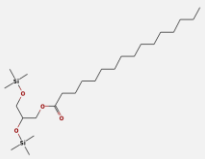
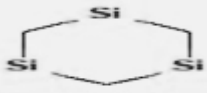
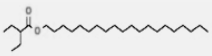
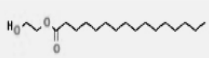
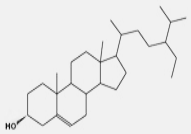

**FIG. 2: GC-MS CHROMATOGRAM OF *M. LAXIFLORA* METHANOLIC FRUIT PULP EXTRACT**

GCMS results showed the plant extracts had many compounds, and a literature review showed that some of the compounds had pharmacological properties. For example, some of the aqueous extract compounds reported in the literature had anti-inflammatory, anticancer, antioxidant, antidepressant anxiolytic, antipyretic, antiulcer and antiarthritic activities. On the other hand, some compounds from methanol extract reported in the literature had anti-inflammatory, antioxidant and antimicrobial activities. Review of the literature about the *M. laxiflora* fruit pulp showed that there was no report on GC-MS-based plant metabolic characterization of its bioactive compounds.

Therefore, the study has generated baseline data for further studies of the *M. laxiflora* fruit pulp. Major compound indentified in the aqueous fruit pulp extract of *M. laxiflora* are namely 1-Decanol,2-Methyl; 1-Octanol,2-Butyl; Trans-2-Dodecen-1-Ol,Trifluoroacetate; Hexadecanoic Acid, Methyl Ester; Octadecanoic Acid, 2-Propenyl Ester; 9,12 Octadecadienoyl Chloride,(Z,Z); Eicosanoic Acid, Methyl Ester; Glycidyl Palmitate; Hexadecanoic Acid, 1-(Hydroxymethyl)-1, 2-Ethanediyl Ester; Octadecanoic Acid,3-Oxo-, Ethyl Ester; D-Ribose, 2-Deoxy-Bis (Thioheptyl)-Dithioacetal; Eicosanal-; 6-Ethyl-3-Decanol, TMS Derivative; Hexadecanoic Acid, 2,3-Bis[(Trimethylsilyl); 1-Monopalmitin, 2TMS Derivative; 1,3,5-Trisilacyclohexane; 2-Ethylbutyric Acid, Eicosyl Ester; 2 Hydroxyethyl Palmitate, TMS Derivative and Stigmast-5-En-3-Ol,(3.Beta.). GC-MS result for aqueous fruit pulp extract of *M. laxiflora* revealed that among the 19 major compounds Stigmast-5-En-3-Ol, (3.Beta.) had the highest retention time and the highest molecular weight while D-Ribose, 2-Deoxy-Bis (Thioheptyl)-Dithioacetal had the highest peak area.

**TABLE 1: MAJOR COMPOUNDS IDENTIFIED IN THE AQUEOUS FRUIT PULP EXTRACT OF *MEYNA LAXIFLORA***

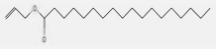
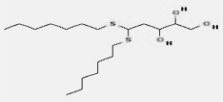
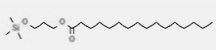
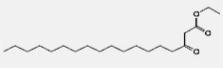
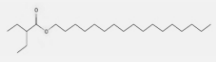
| Peak Report of Aqueous Extract of *Meyna Laxiflora* fruit pulp | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Sl. no.** | **Name of the compound** | **RT** | **Area %** | **MW (g/mol)** | **MF** | **Structure** | **Medicinal uses** |
| 1. | 1-Decanol,2-Methyl | 11.551 | 1.83 | 172.31 | $C_{11}H_{24}O$ |  | A precursor for the synthesis of biologically active compound Antioxidant [11] |
| 2. | 1-Octanol,2-Butyl- | 12.219 | 2.77 | 116.20 | $C_{12}H_{26}O$ |  | 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 [12,13] |
| 3. | Trans-2-Dodecen-1-Ol, Trifluoroacetate | 13.373 | 1.07 | 280.33 | $C_{14}H_{23}F_3O_2$ |  | Unknown |
| 4. | Hexadecanoic Acid, Methyl Ester | 14.085 | 5.23 | 280.33 | $C_{17}H_{34}O_2$ |  | Antioxidant, mematicide, insecticide, lubricant, antiandrogenic, hemolytic, hypo –cholesterolemic [11], antibacterial and antifungal activities [14] reducing blood cholesterol, and anti-inflammatory agent [15] Nematicide, pesticide, antiarthritic, antitumor, anticoronary, hepatoprotective [16] |
| 5. | OctadecanoicAcid,2-PropenylEster | 15.547 | 1.21 | 324.54 | $C_{21}H_{40}O_2$ |  | Antibacterial activity [11] |
| 6. | 9,12-Octadecadienoyl Chloride,(Z,Z) | 15.784 | 2.18 | 298.9 | $C_{18}H_{31}ClO$ |  | Antioxidant properties [17] |
| 7. | Eicosanoic Acid, Methyl Ester | 16.018 | 1.07 | 326.55 | $C_{21}H_{42}O_2$ |  | Alpha-glucosidase inhibitors activity [18] |

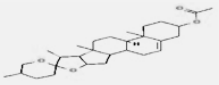
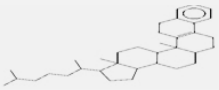
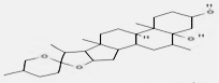
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 8. | Glycidyl Palmitate | 17.540 | 1.25 | 312.5 | $C_{19}H_{36}O_3$ |  | Antioxidant, antibacterial, and antifungal activities, used in preparation of isophosphatidic acid which inhibits apoptosis [19, 20] |
| 9. | HexadecanoicAcid,1-(Hydroxymethyl)-1,2-EthanediylEster | 18.312 | 1.05 | 581.0 | $C_{35}H_{68}O_5$ |  | Antioxidant [11] |
| 10. | OctadecanoicAcid,3-Oxo-,EthylEster | 18.990 | 9.07 | 326.5 | $C_{20}H_{38}O_3$ |  | Antioxidant and anti-inflammatory activities [11] |
| 11. | D-Ribose,2-Deoxy-Bis(Thioheptyl)-Dithioacetal | 19.138 | 30.47 | 380.7 | $C_{19}H_{40}O_3S_2$ |  | Unknown |
| 12. | Eicosanal- | 19.475 | 1.10 | 296.53 | $C_{20}H_{40}O$ |  | Antioxidant, antidepressant and anxiolytic [11] |
| 13. | 6-Ethyl-3-Decanol, TMS Derivative | 19.582 | 1.20 | 186.33 | $C_{12}H_{26}O$ |  | Antimicrobial [21] |
| 14. | HexadecanoicAcid,2,3-Bis[(Trimethylsilyl) | 19.662 | 12.94 | 474.9 | $C_{25}H_{54}O_4Si_2$ |  | Unknown |
| 15. | 1-Monopalmitin,2TMSDerivative | 19.796 | 1.31 | 474.86 | $C_{25}H_{54}O_4Si_2$ |  | Antioxidant [11] |
| 16. | 1,3,5-Trisilacyclohexane | 20.544 | 4.88 | 126.3 | $C_3H_6Si3$ |  | Unknown |
| 17. | 2-Ethylbutyric Acid,Eicosyl Ester | 20.701 | 4.30 | 396.7 | $C_{26}H_{52}O_2$ |  | Unknown |
| 18. | 2-Hydroxyethyl Palmitate,TMS Derivative | 21.147 | 4.27 | 300.5 | $C_{18}H_{36}O_3$ |  | Unknown |
| 19. | Stigmast-5-En-3-Ol, (3.Beta.) | 23.904 | 1.43 | 681.2 | $C_{29}H_{50}O$ |  | Anti-inflammatory, Antipyretic, Anti-ulcer, Antiarthritic [11] |

MF: Molecular Formula, MW: Molecular Weight, RT: Retention Time

Major compound indentified in the methanolic fruit pulp extract of *M. laxiflora* viz. Methyl 11-(2,3-Dideuterocyclopentan-1-Yl) Undecanoate; 1,1-Dideutero Hexadecanol; Octadecanoic Acid,2-Propenyl Ester; 9-Octadecenoic Acid(Z)-, Methyl Ester; Heneicosanoic Acid, Methyl Ester; Octadecanoic Acid, 2-Propenyl Ester; Octadecanoic Acid, 3-Oxo-, Ethyl Ester; D-Ribose,2-Deoxy-Bis(Thioheptyl)-Dithioacetal; 3-Hydroxypropyl Palmitate, TMS Derivative; Octadecanoic Acid,-Oxo-, Ethyl Ester; 2-Ethylbutyric Acid, Heptadecyl Ester; 1-S-Hexyl-1-Thio-D-Glucitol; Spirost-5-En-3-Ol, Acetate, (3Beta, 25R)-; Cholest-1-Eno[2,1-A] Naphthalene, 3', 4'-Dihydro- and 5Alpha – Hydroxy - 6Beta - Methyltigogenin.

**TABLE 2: MAJOR COMPOUNDS IDENTIFIED IN THE METHANOLIC FRUIT PULP EXTRACT OF *MEYNA LAXIFLORA***

| Peak Report of Methanolic Extract of *Meyna laxiflora* fruit pulp | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Sl. no.** | **Name of the compound** | **RT** | **Area %** | **MW (g/mol)** | **MF** | **Structure** | **Medicinal uses** |
| 1. | Methyl 11-(2,3-Dideuterocyclopentan-1-Yl) Undecanoate | 14.098 | 6.45 | 280.4 | $C_{17}H_{28}O_3$ |  | A precursor for the synthesis of biologically active compound Antioxidant [11] |
| 2. | 1,1-DideuteroHexadecanol | 14.313 | 1.19 | 242.44 | $C_{16}H_{34}O$ |  | 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 [12,13] |
| 3. | OctadecanoicAcid,2-PropenylEster | 15.559 | 1.68 | 324.54 | $C_{21}H_{40}O_2$ |  | Unknown |
| 4. | 9-OctadecanoicAcid(Z)-,MethylEster | 15.793 | 1.98 | 296.48 | $C_{19}H_{36}O_2$ |  | Antioxidant, mematicide, insecticide, lubricant, antiandrogenic, hemolytic, hypo –cholesterolemic [11], antibacterial and antifungal activities [14] reducing blood cholesterol, and anti-inflammatory agent [15] Nematicide, pesticide, antiarthritic, antitumor, anticoronary, hepatoprotective [16] |
| 5. | HeneicosanoicAcid,MethylEster | 16.028 | 1.19 | 340.58 | $C_{22}H_{44}O_2$ |  | Antibacterial activity [11] |
| 6. | OctadecanoicAcid,2-PropenylEster | 17.360 | 1.96 | 324.54 | $C_{21}H_{40}O_2$ |  | Antioxidant properties [17] |
| 7. | OctadecanoicAcid,3-Oxo-, EthylEster | 18.991 | 14.40 | 326.5 | $C_{20}H_{38}O_3$ |  | Alpha-glucosidase inhibitors activity [18] |
| 8. | D-Ribose,2-Deoxy-Bis(Thioheptyl)-Dithioacetal | 19.141 | 32.15 | 380.7 | $C_{19}H_{40}O_3S_2$ |  | Antioxidant, antibacterial, and antifungal activities, used in preparation of isophosphatidic acid which inhibits apoptosis [19, 20] |
| 9. | 3-HydroxypropylPalmitate, TMS Derivative | 19.669 | 7.04 | 386.7 | $C_{22}H_{46}O_3Si$ |  | Antioxidant [11] |
| 10. | OctadecanoicAcid,3-Oxo-,Ethyl Ester | 20.546 | 10.32 | 326.5 | $C_{20}H_{38}O_3$ |  | Antioxidant and anti-inflammatory activities [11] |
| 11. | 2-Ethylbutyric Acid, Heptadecyl | 20.774 | 8.40 | 354.61 | $C_{23}H_{46}O_2$ |  | Unknown |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Ester | | | | | | |
| 12. | Spirost-5-En-3-Ol, Acetate,(3.Beta.,25R)- | 23.634 | 1.21 | 352.5 | $C_{29}H_{44}O_4$ |  | Antioxidant, antidepressant and anxiolytic [11] |
| 13. | Cholest-1-Eno[2,1-A]Naphthalene,3',4'-Dihydro- | 23.906 | 1.69 | 472.8 | $C_{35}H_{52}$ |  | Antimicrobial [21] |
| 14. | 5Alpha-Hydroxy-6Beta-Methyltigogenin | 26.062 | 1.16 | 446.7 | $C_{28}H_{46}O_4$ |  | Unknown |
| 15. | Methyl 11-(2,3-Dideuterocyclopentan-1-Yl) Undecanoate | 14.098 | 6.45 | 280.4 | $C_{17}H_{28}O_3$ |  | Antioxidant [11] |

MF: Molecular Formula, MW: Molecular Weight, RT: Retention Time

Whereas GC-MS result for methanolic fruit pulp extract of M. *laxiflora* showed that among the 24 compounds 5Alpha - Hydroxy - 6Beta – Methyl - tigogenin had the highest retention time, Cholest-1-Eno[2,1-A] Naphthalene,3',4'-Dihydro- had the highest molecular weight and D-Ribose,2-Deoxy-Bis(Thioheptyl)-Dithioacetal had the highest peak area.

**CONCLUSION:** The GC-MS profiling of aqueous and methanolic extracts of *Meyna laxiflora* fruit pulp revealed the presence of bioactive compounds with important medicinal properties. The present investigation concluded that the aqueous and methanolic extracts have 19 and 15 major compounds respectively. Hence, the presence of these phytochemicals could be responsible for the therapeutic effects of the plant as practiced by our local traditional healers. There is no report on GC-MS-based plant metabolic characterization of *Meyna laxiflora* fruit pulp till date. Therefore, the study has generated baseline data for further characterization of fruit pulp extract of this plant.

**ACKNOWLEDGEMENT:** We are thankful to the Department of Biotechnology, Govt. of India for financial assistance and also to L. Somarjit Singh, retired Associate Professor, Department of Botany, Imphal College for identification of the plant specimen.

**CONFLICTS OF INTEREST:** Nil

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**How to cite this article:**
Bhaigyabati T, Sanasam S and Singh LR: GC-MS profiling of aqueous and methanolic extract of *Meyna laxiflora* robyns fruit pulp. Int J Pharm Sci & Res 2025; 16(6): 1608-15. doi: 10.13040/IJPSR.0975-8232.16(6).1608-15.