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Research Article

Profile of Phytochemicals and GCMS Analysis of Bioactive Compounds in Natural Dried-Seed Removed Ripened Pods Methanolic Extracts of *Moringa oleifera*

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Abstract

Moringa oleifera has been reported to be the store-house of wide range of bioactive compounds. Most commonly used plant part has been the leaves which are reported to be rich in Vitamins, Carotenoids, Polyphenols, Phenolic Acids, Flavonoids, Alkaloids, Glucosinolates, Isocyanides, Tannins and Saponins. *Moringa* leaves are used as Keerai while, green pods are commonly used as vegetable in the traditional preparation of Sambar in South-India. MO is gaining popularity because of its nutrient-rich root, leaves, flowers and fruits, having immense traditional medicinal uses and proved pharmacological properties. Not much of work has been carried out on analysis of bioactive compounds present in the pods. In the present study an attempt has been made to screen and analyze the range of bioactive compounds present in *Moringa oleifera* seed removed ripened natural dried pods. Phytochemical screening and GCMS analysis revealed the presence of 12 compounds namely - 7-Octadecyne, 2-methyl- (C₁₉H₃₆); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (C₂₀H₄₀O); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (C₂₀H₄₀O); 6,9,12,15-Docosatetraenoic acid, me, me (C₂₃H₃₈O₂); Cyclohexanol, 5-methyl-2-(1-methylethyl)- (C₁₀H₂₀O); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (C₂₀H₄₀O); Palmitic acid vinyl ester (C₁₈H₃₄O₂); gamma-Tocopherol (C₂₈H₄₈O₂); Vitamin E (C₂₉H₅₀O₂); Cholesta-7,9(11)-dien-3-ol, 4,4-dim (C₂₉H₄₈O); gamma-Sitosterol (C₂₉H₅₀O); Stigmasta-5,24(28)-dien-3-ol, (3.β,24Z)- (C₂₉H₄₈O). Further, *in-silico* ADMET analysis is expected to provide in-depth physiochemical and biomolecular details of these molecules in order to exploit them for production of novel drugs for the pharma market with wide array of bio medical applications.

Keywords: Bioactive Compounds; GCMS; Phytochemical Screening; MOPME; Plant Based Natural Products;

INTRODUCTION

Moringa oleifera Lam. (Fam: Moringaceae) is a potential medicinal plant native to India, however, distributed in tropical and sub-tropical regions of the world. Furthermore, this plant has now been cultivated in other regions of the world¹. *M. oleifera* is considered to be ware-house of plant based natural products (PBNPs). The tree is endowed with far-fetched richness of bioactive compounds that serve as nutraceuticals and bio-pharmaceuticals. This tree is used as a natural source of nutrient supplement for women, infants and

children². MO has a wide range of culinary applications besides bioremediation and medicinal properties³. Edible part(s) of MO (leaves and green pods) contain - proteins, essential and non-essential amino acids, vitamins, minerals, antioxidants and phenolic compounds, Palmitic acid, Oleic acid, Linoleic acid, Gallic acid, p-Coumaric acid, Ferulic acid, Catechin, Quercetin, Kaempferol, Niazimicin, Vitamins (B, A, C, D and K). Quercetin, myricetin glycosides, caffeoylquinic acid, coumaroylquinic acid, hydroxybenzoic acid, kaempferol, glucotropaeolin, glucosinalbin, glucoraphanin, glucomoringin, glucoiberin, glucosinolates, apigenin, luteolin, lutein,

luteoxanthin, zeaxanthin, b-carotene and isothiocyanates were identified as the main compounds in the extracts from moringa. Phenolic compounds from *M. oleifera* seed, such as gallic acid, ellagic acid and kaempferol are endowed with significant antioxidant activity.

There are 13 species with in the genus *Moringa*, of all, *M. oleifera* is best known, widely distributed, and popular species due to its manifold uses⁵. Leaves, bark, flowers and green pods of MO has reported antioxidant, antidiabetic, antibacterial, antifungal, anti-tumor, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, hepatoprotective, antipyretic, antiepileptic, cardioprotective and cholesterol-lowering activities⁴.

Of all parts, the leaves are inexpensive and abundantly available but largely underutilized, ignored and often discarded. Different pharmaceutical products from this plant have been manufactured and marketed in both the Indian and worldwide markets due to these medicinal advantages. *M. oleifera* has been traditionally utilized in folk remedies to cure conjunctivitis, and given to lactating mothers for enhancing milk production. The juice obtained from leaves is used to normalize blood pressure and blood glucose levels⁵. Though, significant variation in composition of different species exists¹⁹ versatile nature of phytochemicals remains the key aspect of nutrition for people suffering from malnutrition³. Due to overwhelming nutritive and medicinal value of the pods, it is indicated that *Moringa* can be widely exploited for its nutritionally important phytoconstituents in the development of functional foods, nutraceutical products and therapeutic agent on a commercial by fortification to eradicate malnutrition^{20,21}. Several bioactive compounds have been isolated and identified from different parts of Moringa (leaves, seeds, bark, flowers, pods, and root). Prospecting BANPs in pods of MO using in-silico ADMET predictions is expected to chart-out a new road map for drug discovery is the basic aspect sustainable exploitation of bioactive natural products²²⁻²⁷. The aim of this study is to identify phytochemicals in the natural dried-seed removed ripened pods by phytochemical screening followed by GC-MS so as to exploit them for the development of novel leads considering it's nutritional and bio-pharmaceutical applications.

MATERIAL AND METHODS

COLLECTION OF THE PLANT MATERIAL

The plant specimen (dried pods) was collected from the fields (Organic Farms) near the Foothills of Alagarkovil Reserve Forest, Madurai, Tamil Nadu, India. The plant type specimen was identified and authenticated by Prof. Dr. S. Sutha at The Department of Medicinal Botany, Govt. Siddha Medical College, Palayamkottai, Tirunelveli District, Tamil Nadu, India.

PHYTOCHEMICAL SCREENING OF THE POD

The methanolic extracts were subjected to chemical tests for the detection of phytoconstituents using standard procedures¹⁵⁻²⁵.

TEST FOR ALKALOIDS

Mayer's test: Few drops of Mayer's reagent was added to 1 mL of plant extract, appearance of a deep yellow or white precipitate indicated the presence of alkaloids in the solution. (Mayer's reagent was freshly prepared by dissolving mercuric chloride (1.36 g) and potassium iodide (5.00 g) in 100 ml water).

Dragendorff's test: To 2 mL of the extract added 1 mL of Dragendorff's reagent along the side of the test tube. Formation of orange or orange reddish brown precipitate

indicated the presence of alkaloids. Dragendorff's reagent was prepared by Sol A: 0.85g bismuth subnitrate, 40mL water, and 10mL glacial acetic acid and Sol B: 8g potassium iodide and 20mL water. 5mL each of Sol A & B with 20mL of glacial acetic acid and 70-100 mL of water is mixed to prepare Dragendorff's reagent.

Hager's test: Hager's test was done by adding a few drops of Hager's reagent to plant extracts and appearance of a yellow-color precipitate indicated the presence of alkaloids in the solution. Hager's reagent is saturated solution of picric acid.

Wagner's test: Approximately, 1 ml of crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colour precipitate indicates the presence of alkaloids. Wagner's Reagent was prepared by mixing 2.5 gm iodine in 12.5 gm of potassium iodide (KI 2); add 250 ml of water to produce solution.

TEST FOR GLYCOSIDES

Test For Anthraquinones Glycosides

Bornträgers test: 0.5 g of extract was boiled with 10% hydrochloric acid for few minutes in water bath. It was filtered and allowed to cool. Equal volume of CHCl₃ was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. Formation of rose - pink color indicates of n-hexane, chloroform, ethyl acetate and methanol of the presence of the anthroquinones.

Baljet test: Part of plant containing cardiac glycoside is dipped in sodium picrate solution; formation of a yellow to orange colour indicates the presence of aglycones or glycosides in the plant tissues.

Legal's Test: To the concentrated ethanolic extract few drops of 10% NaOH were added, to make it alkaline. Then freshly prepared sodium nitroprusside was added to the solution. Presence of blue coloration indicated the presence of glycosides in the extract.

TEST FOR CARDIAC GLYCOSIDES

Keller-Kiliani test: 5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated sulphuric acid. A browning of the interface indicates a deoxy-sugar characteristic of carotenoids. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

TEST FOR CARBOHYDRATES

Molisch's test: Small portion of the plant extract was put in a test tube; 10 ml of distilled water was added and shaken vigorously and gently. The mixture was then filters and divided into two portions. To the first portion, two drops of Molish's reagent was added followed by few drops of concentrated sulphuric acid by the wall of the test tube. Formation of brown or purple ring at the interphase indicated the presence of carbohydrates.

Fehling's test Equal volume of Fehling A and Fehling B reagents were mixed together and then add 2ml of crude extract in it and gently boiled. A brick red precipitate appeared at the bottom of the test-tube indicates the presence of reducing sugars.

Benedict's test 1 ml of crude extract was mixed with 2ml of Benedict's reagent and boiled. A reddish brown precipitate was formed which indicates the presence of the carbohydrates.

TEST FOR PHYSTOSTEROLS

Libermann Burchard's Test: Dissolve one or two crystals of cholesterol in dry chloroform in a dry test tube. Add few drops of acetic anhydride and then 2 drops of concentrated H₂SO₄ and mix well. The formation of a green or green-blue colour after a few minutes indicates the presence of phytosterols. After the reaction, concentration of cholesterol can be measured spectrophotometry.

Salkowski's Test: On adding a few drops of conc. Sulphuric acid to the plant extract and allow the solution to stand for some time, formation of brown ring indicated the presence of phytosterols in the plant extract.

TEST FOR FLAVONOIDS

FeCl₃ Test: To 1 ml of the extract, 3 ml of distilled water followed by few drops of 10% aqueous Ferric chloride solution was added. Formation of blue or green colour indicates the presence of flavonoids. Shinoda Test: To 2 ml of the extract, 1 ml of 1% ammonia solution was added. Appearance of yellow colour indicates the presence of flavonoids.

Shinod's Test: In this test, four pieces of magnesium fillings (ribbon) are added to the ethanolic extract followed by a few drops of concentrated hydrochloric acid. A reddish colour indicates the presence of flavonoid.

TEST FOR FIXED OILS AND FATS

Spot test: Take the sample to be tested, press a little in the folds of the filter paper. On folding, if there is the appearance of greasy spot indicates the presence of oils or fats. The spot grows larger on heating and drying the filter paper.

Saponification: Take approximately 100 mg of oil or fat in a test tube. Add 3 mL of alcoholic-KOH and mix well. Place the tube in a boiling water bath for 15-20 min. Saponification value represents mg of potassium hydroxide required to saponify one gram of fat under the conditions specified. It is a measure of the average molecular weight of all the fatty acids present in the sample as triglycerides.

TEST FOR FREE AMINO ACIDS

Millon's reagent test: Millon's test is specific to phenol containing structures (tyrosine is the only common phenolic amino acid). Millon's reagent is concentrated HNO₃, in which mercury is dissolved. As a result of the reaction a red precipitate or a red solution is considered as positive test.

Ninhydrin reagent test: A 2% solution of ninhydrin is prepared by dissolving 0.2 grams of ninhydrin in 10ml of either ethanol or acetone. 1% solution of the amino acid (analyte) in distilled water is prepared, few drops of 2% ninhydrin solution is added to this solution. Test tube is kept in a warm water bath for 5 min; development of a deep blue/violet colour indicates presence of amino acids.

TESTS FOR FIXED OILS AND FATS

Spot test Take the sample and place it between the folds of filter paper and rub it lightly. Presence of translucent spots on the filter paper confirms the presence of fats in the plant material.

Saponification Take a sample a test tube, add strong alkali NaOH, boil the solution in a water bath for 5 min, add ethanol. Observe for the appearance of froth, formation of forth in the test tube indicates the presence of fat in the sample.

TEST FOR FREE AMINO ACID

Millon's test 1 ml of crude extract was mixed with 2ml of Millon's reagent; white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin test 1 ml of crude extract was mixed with 2ml of 0.2% solution of Ninhydrin and boiled. A violet colour precipitate was appeared suggesting the presence of amino acids and proteins.

TEST FOR TANNINS

5% Ferric chloride test: 5 mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black color indicates the presence of tannins.

10% Lead acetate test: 10 mg of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

TEST FOR SAPONINS

Foam Test: 2 ml of crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

GUMS & MUCILAGE

Ruthenium red test: 50 mg of dried mucilage powder was dissolved in 2 mL of distilled water, mixed with a few drops of Ruthenium red solution. Observed for pink color indicates the presence of gums and mucilage.

GC-MS-MS Analysis:

Pod samples of were collected from the fields (Organic Farms) near the Foothills of Alagarkovil Reserve Forest, Madurai, Tamilnadu, India. The samples were processed and the methanolic extractions were carried out as described previously for GCMS analysis. GC-MS-MS analysis was carried out using Varian 4000 Ion trap GC/MS/MS with Fused silica 15m x 0.2 mm ID x 1 μ m of capillary column. The instrument was set to an initial temperature of 110 °C, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280 °C, at the rate of an increase of 5 °C/min, and maintained for 9 min. Injection port temperature was ensured as 250 °C and Helium flow rate as 1 ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS-MS compounds present in the plants sample were identified.

Identification of Phytocompounds

Interpretation on mass-spectrum GC-MS-MS was conducted using the database of National institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

Phytochemical analysis of MOMPE revealed the presence of alkaloids, carbohydrates, coumarins, flavonoids, glycosides, phenol, proteins, quinones, saponins, steroids, tannins and terpenoids. However, gums were not detected in the samples analyzed (Table 1). GCMS analysis revealed the presence of the following 12 phyto-compounds 7-Octadecyne, 2-methyl-

(C₁₉H₃₆); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (C₂₀H₄₀O); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (C₂₀H₄₀O); 6,9,12,15-Docosatetraenoic acid, me (C₂₃H₃₈O₂); Cyclohexanol, 5-methyl-2-(1-methylethyl)- (C₁₀H₂₀O); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (C₂₀H₄₀O); Palmitic acid vinyl ester (C₁₈H₃₄O₂); gamma-Tocopherol (C₂₈H₄₈O₂); Vitamin E (C₂₉H₅₀O₂); Cholesta-7,9(11)-dien-3-ol, 4,4-dim (C₂₉H₄₈O); gamma-Sitosterol (C₂₉H₅₀O); Stigmasta-5,24(28)-dien-3-ol, (3.β,24Z)- (C₂₉H₄₈O) Table 1,2; Fig. 1,2.

DISCUSSION

Medicinal and biological activities of pod extract have been upheld by *in-vitro* assays^{26,33-42}. MOMP contains significantly high phenolic compounds responsible for antioxidant effects^{14,33,36}. Most of the compounds identified in MOMP are endowed with medicinal properties³³⁻⁴². For, instance, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol) is commonly used as a precursor for synthetic forms of vitamin E and vitamin K1. Furthermore, it has been shown to modulate transcription in cells via transcription factors PPAR-alpha and retinoid X receptor (RXR)⁴³. Gamma-tocopherol (γ-T), major form of vitamin E in seeds has been attracting increasing attention because of its health-promoting roles. γ-T has demonstrated antioxidant activities in food and *in-vitro* studies and showed higher activity in trapping lipophilic electrophiles and reactive nitrogen and oxygen species⁴⁴. Derivatives of tocopherols [α (15.38), γ (4.47), δ (15.51) mg/kg] have been reported from *Moringa oleifera* seed oil (MOSO) (variety Periyakulam 1) from India⁴⁵. However, Fejer et al. reported as high as 178.1 mg/kg in leaves and 220.6 mg/kg in seed samples.

CONCLUSION

Scientific studies illustrate that *M. oleifera* and its bioactive constituents could play a vital role in the prevention of several chronic and degenerative diseases associated with oxidation stress. Therapeutic potential's of PBNPs be better understood with proper screening and pre-clinical and clinical investigations. *M. oleifera* pod remains as an ideal sources of nutrients and BASM that can be used for the development of nutraceuticals, pharmaceuticals and functional foods. Compounds identified by GC-MS analysis of methanolic pod extracts of *M. oleifera* in the present study may be related to their applications in folklore medicine to prompt inspiration for further *in-silico* ADMET analysis as a potential source of natural drug leads of GRAS standard for next generation drug design, development and therapeutics.

REFERENCES

1. Airaodion AI, Ogbuagu U, Ogbuagu EO, Ekenjoku JA, Airaodion EO. Protective Effect of ethanolic leaf extract of *Moringa oleifera* on haematological indices of rats fed with crude oil-treated diet. International Journal of Bio-Science and Bio-Technology. 2019; 11(8):84-92.
2. Mahato DK, Kargwal R, Kamle M, Sharma B, Pandhi S, Mishra S, Gupta A, Mahmud MC, Gupta MK, Singha LB, Kumar P. Ethnopharmacological properties and Nutraceutical potential of *Moringa oleifera*. Phytomedicine Plus. 2022; 2(1):100168. <https://doi.org/10.1016/j.phyplu.2021.100168>
3. Gupta S, Jain R, Kachhwaha S, Kothari SL. Nutritional and medicinal applications of *Moringa oleifera* Lam.-Review of current status and future possibilities. Journal of Herbal Medicine. 2018; 11:1-1. <https://doi.org/10.1016/j.jhermed.2017.07.003>
4. Kou X, Li B, Olayanju JB, Drake JM, Chen N. Nutraceutical or pharmacological potential of *Moringa oleifera* Lam. Nutrients. 2018; 10(3):343. <https://doi.org/10.3390/nu10030343>
5. Padayachee B, Baijnath H. An overview of the medicinal importance of Moringaceae. Journal of Medicinal Plants Research. 2012; 6(48):5831-9.
6. Sahay S, Yadav U, Srinivasamurthy S. Potential of *Moringa oleifera* as a functional food ingredient: A review. Magnesium (g/kg). 2017; 8(9.06):4-90.
7. Tahir NA, Majeed HO, Azeez HA, Omer DA, Faraj JM, Palani WR. Allelopathic plants: 27. *Moringa* species. Allelopathy Journal. 2020; 50(1):35-48. <https://doi.org/10.26651/allelo.j/2020-50-1-1272>
8. Saini RK, Sivanesan I, Keum YS. Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. 3 Biotech. 2016; 6(2):1-4. <https://doi.org/10.1007/s13205-016-0526-3>
9. Rani EA, Arumugam T. *Moringa oleifera* (Lam)-a nutritional powerhouse. Journal of Crop and Weed. 2017; 13(2):238-46.
10. Rathnayake ARMHA, Navaratne SB, Uthpala TGG. *Moringa oleifera* plant and the nutritional and medicinal properties of *Moringa oleifera* leaves Trends Pros. Process. Hort. Crops (2019), pp. 251-268
11. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in *Moringa oleifera* leaves protect against chronic disease. Antioxidants. 2017; 6(4):91. <https://doi.org/10.3390/antiox6040091>
12. Gopalakrishnan L, Doriya K, Kumar DS. *Moringa oleifera*: A review on nutritive importance and its medicinal application. Food science and human wellness. 2016; 5(2):49-56. <https://doi.org/10.1016/j.fshw.2016.04.001>
13. Barichella M, Pezzoli G, Faierman SA, Raspini B, Rimoldi M, Cassani E, Bertoli S, Battezzati A, Leone A, Iorio L, Ferri V. Nutritional characterisation of Zambian *Moringa oleifera*: acceptability and safety of short-term daily supplementation in a group of malnourished girls. International journal of food sciences and nutrition. 2019; 70(1):107-15. <https://doi.org/10.1080/09637486.2018.1475550>
14. Luqman S, Srivastava S, Kumar R, Maurya AK, Chanda D. Experimental assessment of *Moringa oleifera* leaf and fruit for its antistress, antioxidant, and scavenging potential using *in vitro* and *in vivo* assays. Evidence-Based Complementary and Alternative Medicine. 2012; 2012. <https://doi.org/10.1155/2012/519084>
15. Mehta J, Shukla A, Bukhariya V, Charde R. The magic remedy of *Moringa oleifera*: an overview. International Journal of Biomedical and Advance Research. 2011; 2(6):215-27. <https://doi.org/10.7439/ijbar.v2i6.35>
16. Jung IL. Soluble extract from *Moringa oleifera* leaves with a new anticancer activity. PloS one. 2014; 9(4):e95492. <https://doi.org/10.1371/journal.pone.0095492>
17. Isitua CC, Ibeh IN. Toxicological assessment of aqueous extract of *Moringa oleifera* and *Caulis bambusae* leaves in rabbits. Journal of Clinical Toxicology S. 2013; 12:4. <https://doi.org/10.4172/2161-0495.S12-003>
18. Adedapo AA, Mogbojuri OM, Emikpe BO. Safety evaluations of the aqueous extract of the leaves of *Moringa oleifera* in rats. Journal of medicinal plants Research. 2009; 3(8):586-91.
19. Asiedu-Gyekye IJ, Frimpong-Manso SA, Awortwe C, Antwi DA, Nyarko AK. Micro-and macro-elemental composition and safety evaluation of the nutraceutical *Moringa oleifera* leaves. Journal of Toxicology. 2014; 2014. <https://doi.org/10.1155/2014/786979>
20. Manavalan N, Boopathi NM, Raveendran M. Medicinal and Therapeutic Properties of *Moringa*. In The *Moringa* Genome 2021 (pp. 31-39). Springer, Cham. https://doi.org/10.1007/978-3-030-80956-0_4
21. Boopathi NM, Raveendran M. *Moringa* and Its Importance. In The *Moringa* Genome 2021 (pp. 1-9). Springer, Cham. https://doi.org/10.1007/978-3-030-80956-0_1
22. Loganathan T, Barathinivas A, Soorya C, Balamurugan S, Nagajothi TG, Ramya S, Jayakumararaj R. Physicochemical, Druggable, ADMET Pharmacoinformatics and Therapeutic Potentials of Azadirachtin-a-Prenol Lipid (Triterpenoid) from Seed Oil Extracts of *Azadirachta indica* A. Juss. Journal of Drug Delivery and Therapeutics. 2021; 11(5):33-46. <https://doi.org/10.22270/jddt.v11i5.4981>
23. Kalaimathi RV, Jeevalatha A, Basha AN, Kandeepan C, Ramya S, Loganathan T, Jayakumararaj R. In-silico Absorption, Distribution, Metabolism, Elimination and Toxicity profile of Isopulegol from *Rosmarinus officinalis*. Journal of Drug Delivery and Therapeutics. 2022; 12(1):102-8. <https://doi.org/10.22270/jddt.v12i1.5188>

24. Jeevalatha A, Kalaimathi RV, Basha AN, Kandeepan C, Ramya S, Loganathan T, Jayakumararaj R. Profile of bioactive compounds in *Rosmarinus officinalis*. *Journal of Drug Delivery and Therapeutics*. 2022; 12(1):114-22. <https://doi.org/10.22270/jddt.v12i1.5189>

25. Ramya S, Murugan M, Krishnaveni K, Sabitha M, Kandeepan C, Jayakumararaj R. In-silico ADMET profile of Ellagic Acid from *Syzygium cumini*: A Natural Biaryl Polyphenol with Therapeutic Potential to Overcome Diabetic Associated Vascular Complications. *Journal of Drug Delivery and Therapeutics*. 2022; 12(1):91-101. <https://doi.org/10.22270/jddt.v12i1.5179>

26. Soorya C, Balamurugan S, Ramya S, Neethirajan K, Kandeepan C, Jayakumararaj R. Physicochemical, ADMET and Druggable properties of Myricetin: A Key Flavonol in *Syzygium cumini* that regulates metabolic inflammations. *Journal of Drug Delivery and Therapeutics*. 2021; 11(4):66-73 <https://doi.org/10.22270/jddt.v11i4.4890>

27. Sabitha M, Krishnaveni K, Murugan M, Basha AN, Pallan GA, Kandeepan C, Ramya S, Jayakumararaj R. In-silico ADMET predicated Pharmacoinformatics of Quercetin-3-Galactoside, polyphenolic compound from *Azadirachta indica*, a sacred tree from Hill Temple in Alagarkovil Reserve Forest, Eastern Ghats, INDIA. *Journal of Drug Delivery and Therapeutics*. 2021; 11(5-S):77-84. <https://doi.org/10.22270/jddt.v11i5-S.5026>

28. Boopathi NM, Abubakar BY. Botanical Descriptions of *Moringa* spp. In *The Moringa Genome 2021* (pp. 11-20). Springer, Cham. https://doi.org/10.1007/978-3-030-80956-0_2

29. Loganathan T, Barathinivas A, Soorya C, Balamurugan S, Nagajothi TG, Jayakumararaj R. GCMS Profile of Bioactive Secondary Metabolites with Therapeutic Potential in the Ethanolic Leaf Extracts of *Azadirachta indica*: A Sacred Traditional Medicinal Plant of INDIA. *Journal of Drug Delivery and Therapeutics*. 2021; 11(4-S):119-26. <https://doi.org/10.22270/jddt.v11i4-S.4967>

30. Krishnaveni K, Sabitha M, Murugan M, Kandeepan C, Ramya S, Loganathan T, Jayakumararaj R. vNNT model cross validation towards Accuracy, Sensitivity, Specificity and kappa performance measures of β -caryophyllene using a restricted-unrestricted applicability domain on Artificial Intelligence & Machine Learning approach based in-silico prediction. *Journal of Drug Delivery and Therapeutics*. 2022; 2(1-S):123-31. <https://doi.org/10.22270/jddt.v12i1-S.5222>

31. Ramya S, Jepachanderamohan PJ, Kalayanasundaram M, Jayakumararaj R. In vitro antibacterial prospective of crude leaf extracts of *Melia azedarach* Linn. against selected bacterial strains. *Ethnobotanical Leaflets*. 2009; 2009(1):32.

32. Kandeepan C, Kalaimathi RV, Jeevalatha A, Basha AN, Ramya S, Jayakumararaj R. In-silico ADMET Pharmacoinformatics of Geraniol (3, 7-dimethylocta-trans-2, 6-dien-1-ol)-acyclic monoterpene alcohol drug from Leaf Essential Oil of *Cymbopogon martinii* from Sirumalai Hills (Eastern Ghats), INDIA. *Journal of Drug Delivery and Therapeutics*. 2021; 11(4-S):109-18. <https://doi.org/10.22270/jddt.v11i4-S.4965>

33. Ramya S, Neethirajan K, Jayakumararaj R. Profile of bioactive compounds in *Syzygium cumini*-a review. *Journal of Pharmacy research*. 2012; 5(8):4548-53.

34. Ramya S, Krishnasamy G, Jayakumararaj R, Periathambi N, Devaraj A. Bioprospecting *Solanum nigrum* Linn. (Solanaceae) as a potential source of Anti-Microbial agents against selected Bacterial strains. *Asian Journal of Biomedical and Pharmaceutical Sciences*. 2012; 2(12):65.

35. Sundari A, Jayakumararaj R. Medicinal plants used to cure cuts and wounds in Athur region of Thoothukudi district in Tamil Nadu, India. *Journal of Drug Delivery and Therapeutics*. 2020; 10(6-s):26-30. <https://doi.org/10.22270/jddt.v10i6-s.4429>

36. Sundari A, Jayakumararaj R. Herbal remedies used to treat skin disorders in Arasankulam region of Thoothukudi District in Tamil Nadu, India. *Journal of Drug Delivery and Therapeutics*. 2020; 10(5):33-8. <https://doi.org/10.22270/jddt.v10i5.4277>

37. Rajasekaran C, Meignanam E, Vijayakumar V, Kalaivani T, Ramya S, Premkumar N, Siva R, Jayakumararaj R. Investigations on antibacterial activity of leaf extracts of *Azadirachta indica* A. Juss (Meliaceae): a traditional medicinal plant of India. *Ethnobotanical leaflets*. 2008; 2008(1):161.

38. Ramya S, Jayakumararaj R. In Vitro Evaluation of Antibacterial Activity Using Crude Extracts of *Catharanthus roseus* L.(G.) Don. *Ethnobotanical Leaflets*. 2008; 2008(1):140.

39. Shanmugam S, Sundari A, Muneeswaran S, Vasanth C, Jayakumararaj R, Rajendran K. Ethnobotanical Indices on medicinal plants used to treat poisonous bites in Thiruppuvanam region of Sivagangai district in Tamil Nadu, India. *Journal of Drug Delivery and Therapeutics*. 2020; 10(6-s):31-6. <https://doi.org/10.22270/jddt.v10i6-s.4432>

40. Ramya S, Alaguchamy N, Maruthappan VM, Sivaperumal R, Sivalingam M, Krishnan A, Govindaraj V, Kannan K, Jayakumararaj R. Wound healing ethnomedicinal plants popular among the Malayali tribes in Vattai Hills, Dharmapuri, TN, India. *Ethnobotanical Leaflets*. 2009; 2009(10):6.

41. Sivaperumal R, Ramya S, Ravi AV, Rajasekaran C, Jayakumararaj R. Herbal remedies practiced by Malayali to treat skin diseases. *Environ We Int J Sci Tech*. 2009; 4(1):35-44.

42. Kadhirvel K, Ramya S, Sudha TS, Ravi AV, Rajasekaran C, Selvi RV, Jayakumararaj R. Ethnomedicinal survey on plants used by tribals in Chitteri Hills. *Environ We Int J Sci Tech*. 2010; 5:35-46.

43. Kandeepan C, Sabitha M, Parvathi K, Senthilkumar N, Ramya S, Boopathi NM, Jayakumararaj R. Phytochemical Screening, GCMS Profile, and In-silico properties of Bioactive Compounds in Methanolic Leaf Extracts of *Moringa oleifera*. *Journal of Drug Delivery and Therapeutics*. 2022 Mar 15; 12(2):87-99. <https://doi.org/10.22270/jddt.v12i2.5250>

44. Kalaimathi RV, Krishnaveni K, Murugan M, Basha AN, Gilles AP, Kandeepan C, Senthilkumar N, Mathialagan B, Ramya S, Ramanathan L, Jayakumararaj R. ADMET informatics of Tetradecanoic acid (Myristic Acid) from ethyl acetate fraction of *Moringa oleifera* leaves. *Journal of Drug Delivery and Therapeutics*. 2022 Aug 20; 12(4-S):101-11. <https://doi.org/10.22270/jddt.v12i4-S.5533>

45. Meena R, Prajapati SK, Nagar R, Porwal O, Nagar T, Tilak VK, Jayakumararaj R, Arya RK, Dhakar RC. Application of *Moringa oleifera* in Dentistry. *Asian Journal of Dental and Health Sciences*. 2021 Dec 25; 1(1):10-3.

46. Parvathi K, Kandeepan C, Sabitha M, Senthilkumar N, Ramya S, Boopathi NM, Ramanathan L, Jayakumararaj R. In-silico Absorption, Distribution, Metabolism, Elimination and Toxicity profile of 9, 12, 15-Octadecatrienoic acid (ODA) from *Moringa oleifera*. *Journal of Drug Delivery and Therapeutics*. 2022 Apr 15; 12(2-S):142-50. <https://doi.org/10.22270/jddt.v12i2-S.5289>

47. Murugan M, Krishnaveni K, Sabitha M, Kandeepan C, Senthilkumar N, Loganathan T, Pushpalatha GL, Pandiarajan G, Ramya S, Jayakumararaj R. In silico Target Class Prediction and Probabilities for Plant Derived Omega 3 Fatty Acid from Ethyl Acetate Fraction of *Moringa oleifera* Leaf Extract. *Journal of Drug Delivery and Therapeutics*. 2022 May 20; 12(3):124-37. <https://doi.org/10.22270/jddt.v12i3.5352>

48. Krishnaveni K, Murugan M, Kalaimathi RV, Basha AN, Pallan GA, Kandeepan C, Senthilkumar N, Mathialagan B, Ramya S, Jayakumararaj R, Loganathan T. ADMET informatics of Plant Derived n-Hexadecanoic Acid (Palmitic Acid) from ethyl acetate fraction of *Moringa oleifera* leaf extract. *Journal of Drug Delivery and Therapeutics*. 2022 Sep 15; 12(5):132-45. <https://doi.org/10.22270/jddt.v12i5.5605>

49. Szanto A, Narkar V, Shen Q, Uray IP, Davies PJ, Nagy L. Retinoid X receptors: X-ploring their (patho) physiological functions. *Cell Death & Differentiation*. 2004 Dec; 11(2):S126-43. <https://doi.org/10.1038/sj.cdd.4401533>

50. Jiang Q, Im S, Wagner JG, Hernandez ML, Peden DB. Gamma-tocopherol, a major form of vitamin E in diets: Insights into antioxidant and anti-inflammatory effects, mechanisms, and roles in disease management. *Free Radical Biology and Medicine*. 2022 Jan 1; 178:347-59. <https://doi.org/10.1016/j.freeradbiomed.2021.12.012>

51. Lalas, S.; Tsaknis, J. Characterization of *Moringa oleifera* Seed Oil Variety "Periyakulam 1". *J. Food Compos. Anal.* 2002, 15, 65-77. <https://doi.org/10.1006/jfca.2001.1042>

52. Fejér J, Kron I, Pellizzeri V, Pluchtová M, Eliašová A, Campone L, Gervasi T, Bartolomeo G, Cicero N, Babejová A, Konečná M. First report on evaluation of basic nutritional and antioxidant properties of *Moringa oleifera* Lam. from Caribbean Island of Saint Lucia. *Plants*. 2019 Nov 23; 8(12):537. <https://doi.org/10.3390/plants8120537>

Table 1 Phytochemical profile of *M. oleifera* Methanolic Pod Extracts (MOMPE)

S. No	Plant constituents tested & Reagent used	<i>M. oleifera</i> Pod (Dried)	
		Observation/ Results of the Test	MOMPE
1	TEST FOR ALKALOIDS		
1.1	<i>Mayer's test</i>	Absence of Creamy White ppt	++
1.2	<i>Dragendorff's test</i>	Absence of Reddish Orange ppt	++
1.3	<i>Hager's test</i>	Absence of Yellow precipitate	++
1.4	<i>Wagner's test</i>	Absence of Reddish-Brown ppt	++
2.1	TEST FOR GLYCOSIDES - Anthroquinone		
2.1.1	<i>Borntrager's test</i>	Formation of Rose - Pink color	++
2.1.2	<i>Baljet test</i>	Formation of Yellow Orange color	+++
2.1.3	<i>Legal's test</i>	Formation of pink to red colour	++
2.2	TEST FOR GLYCOSIDES - Cardiac		
2.2.1	<i>Keller-Killani test</i>	Violet ring appears below brown	++
3	TEST FOR CARBOHYDRATES		
3.1	<i>Molish's test</i>	Formation of ring at junction	++
3.2	<i>Fehling's solution test</i>	Formation of red precipitate	+++
3.3	<i>Benedict's reagent test</i>	Formation of reddish-brown ppt	++
4	TEST FOR PHYTOSTEROLS		
4.1	<i>Libermann Burchard's</i>	Formation of a green-blue colour	+
4.2	<i>Salkowski's test</i>	Formation of clear Brown Ring	+
5	TEST FOR FLAVONOIDS		
5.1	<i>Ferric chloride test</i>	Appearance of Yellow Colour	++
5.2	<i>Shinod's test</i>	Formation of a Reddish Colour	++
6	TEST FOR FIXED OILS AND FATS		
6.1	<i>Spot test</i>	Appearance of Greasy Spot	-
6.2	<i>Saponification</i>	mg of KOH to saponify 1g of fat	-
7	TEST FOR FREE AMINO ACIDS		
7.1	<i>Millon's reagent test</i>	Red precipitate or a Red solution	+
7.2	<i>Ninhydrin reagent test</i>	Formation of a Blue/Violet color	+
8	TEST FOR TANNINS		
8.1	<i>5% Ferric chloride</i>	Development of bluish-black color	++
8.2	<i>10% Lead acetate</i>	Formation of a clear precipitate	++
9	TEST FOR SAPONINS		
9.1	<i>Foam test</i>	Formation of stable foam	++
10	GUMS & MUCILAGE	Formation of a Pink color	-

Note: (++) - Indicate active constituents in high amount; (+) - Indicate active constituents in low amount; (-) - Indicate the absence of active constituents.

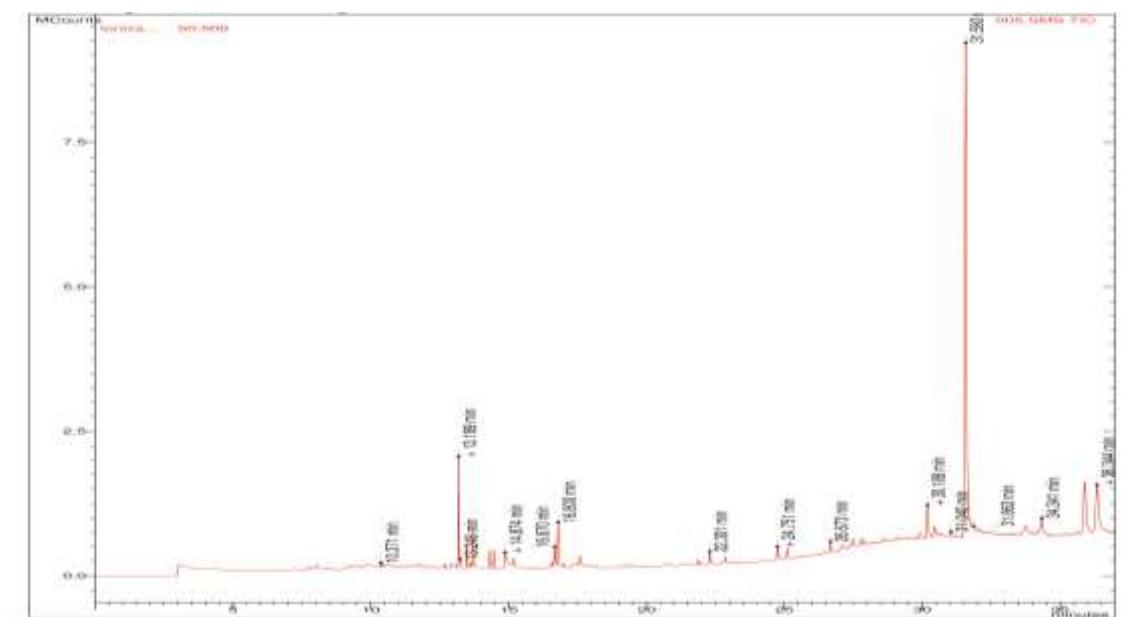
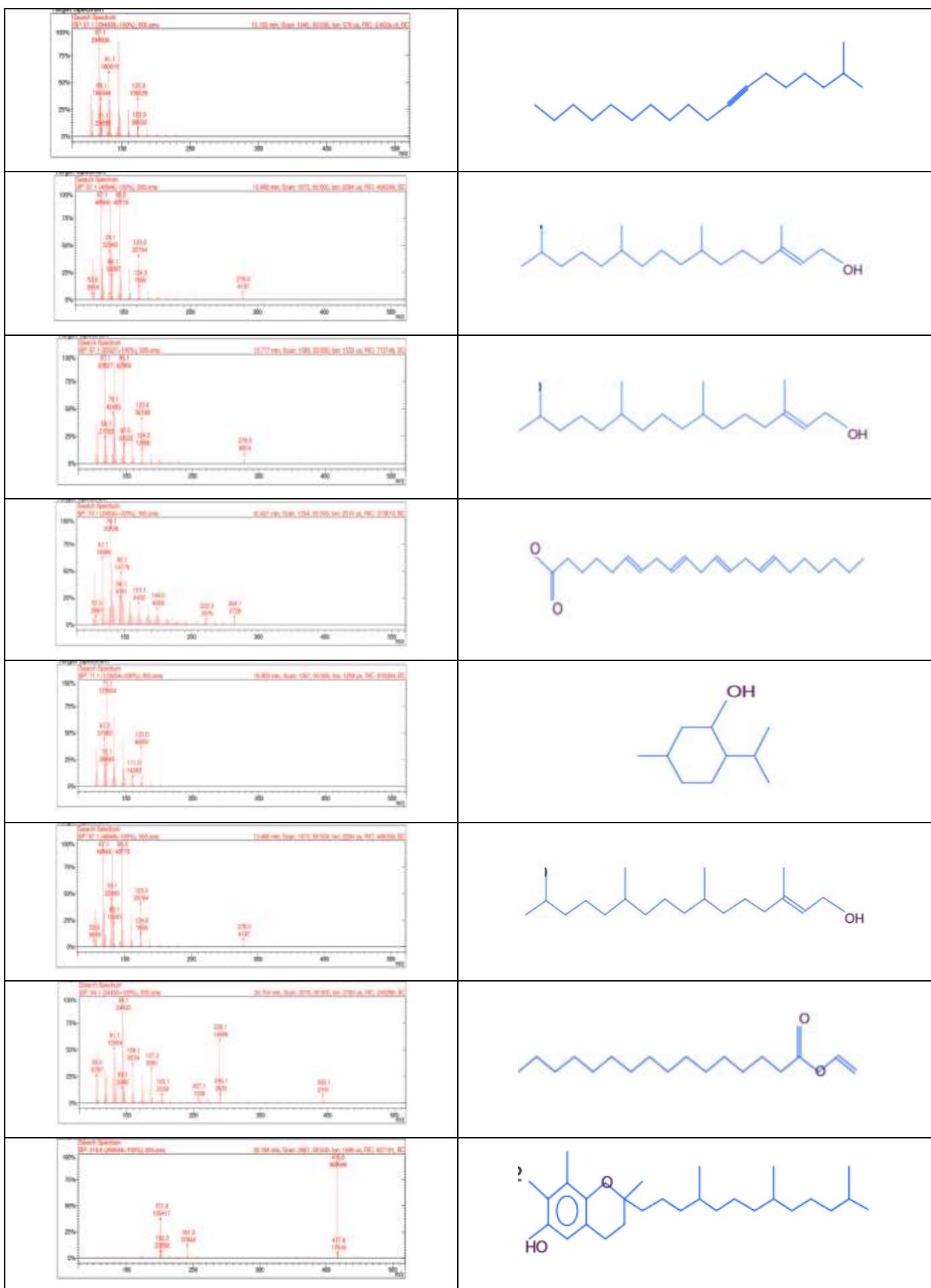
Figure 1: GCMS profile of *M. oleifera* Methanolic Pod Extracts (MOMPE)

Table 2: List of Bioactive Compounds in the GCMS profile of MOMPE

RT	COMPOUND NAME	MF	MW	PA%
13.189	7-Octadecyne, 2-methyl-	C ₁₉ H ₃₆	264.5	5.803
13.486	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.5	1.045
13.718	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.5	2.073
16.670	6,9,12,15-Docosatetraenoic acid, me	C ₂₃ H ₃₈ O ₂	332.5	1.420
16.800	Cyclohexanol, 5-methyl-2-(1-methylethyl)-	C ₁₀ H ₂₀ O	198.3	2.969
17.874	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.5	1.045
24.751	Palmitic acid vinyl ester	C ₁₈ H ₃₄ O ₂	282.5	1.051
30.188	gamma-Tocopherol	C ₂₈ H ₄₈ O ₂	416.7	3.184
31.580	Vitamin E	C ₂₉ H ₅₀ O ₂	430.7	55.380
34.341	Cholesta-7,9(11)-dien-3-ol, 4,4-dim	C ₂₉ H ₄₈ O	412.7	1.933
35.891	gamma-Sitosterol	C ₂₉ H ₅₀ O	432.7	8.456
36.344	Stigmasta-5,24(28)-dien-3-ol, (3beta.,24Z)-	C ₂₉ H ₄₈ O	412.7	8.810

Table 3 IUPAC Name, CID, and SMILES of Bioactive Compounds in MOMPE

IUPAC NAME	CID	SMILES
2-methyloctadec-7-yne	118810	CCCCCCCCCCCC#CCCC(C)C
(E)-3,7,11,15-tetramethylhexadec-2-en-1-ol	5366244	CC(C)CCCC(C)CCCC(C)CCC/C(=C/CO)/C
(E)-3,7,11,15-tetramethylhexadec-2-en-1-ol	5366244	CC(C)CCCC(C)CCCC(C)CCC/C(=C/CO)/C
docosa-6,9,12,15-tetraenoic acid	53867699	CCCCCCCC=CCC=CCC=CCC=CCCCC(=O)O
[(1R,2R,5R)-5-methyl-2-propan-2-ylcyclohexyl] acetate	88692	C[C@H]1CC[C@H]([C@H](C1)OC(=O)C)C(C)C
(E)-3,7,11,15-tetramethylhexadec-2-en-1-ol	5366244	CC(C)CCCC(C)CCCC(C)CCC/C(=C/CO)/C
ethenyl hexadecanoate	69658	CCCCCCCCCCCCCCCC(=O)OC=C
2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-3,4-	14986	CC1=C(C=C2CCCC(OC2=C1)C)CCCC(C)CCCC(C)C
(2R)-2,5,7,8-tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-ol	14985	CC1=C(C2=C(CC[C@H](O2)C)CCC[C@H](C)CCC[C@H](C)CC(C)C(=C1O)C)C
(3S,5R,10S,13R,14R,17R)-4,4,10,13-tetramethyl-17-[(2R)-6-methylheptan-2-yl]-1,2,3,5,6,12,14,15,16,17-	22296805	C[C@H](CCCC(C)C)[C@H]1CC[C@H]2[C@H]1(CC=C3C2=C C[C@H]4[C@H]3(CC[C@H](C4)O)C)C
(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5S)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol;hydrate	133082557	CC[C@H](CC[C@H](C)[C@H]1CC[C@H]2[C@H]1(CC[C@H]3[C@H]2CC=C4[C@H]3(CC[C@H](C4)O)C)C(C)C)O
(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(Z,2R)-5-propan-2-ylhept-5-en-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	5281326	C/C=C(/CC[C@H](C)[C@H]1CC[C@H]2[C@H]1(CC[C@H]3[C@H]2CC=C4[C@H]3(CC[C@H](C4)O)C)C)\C(C)C



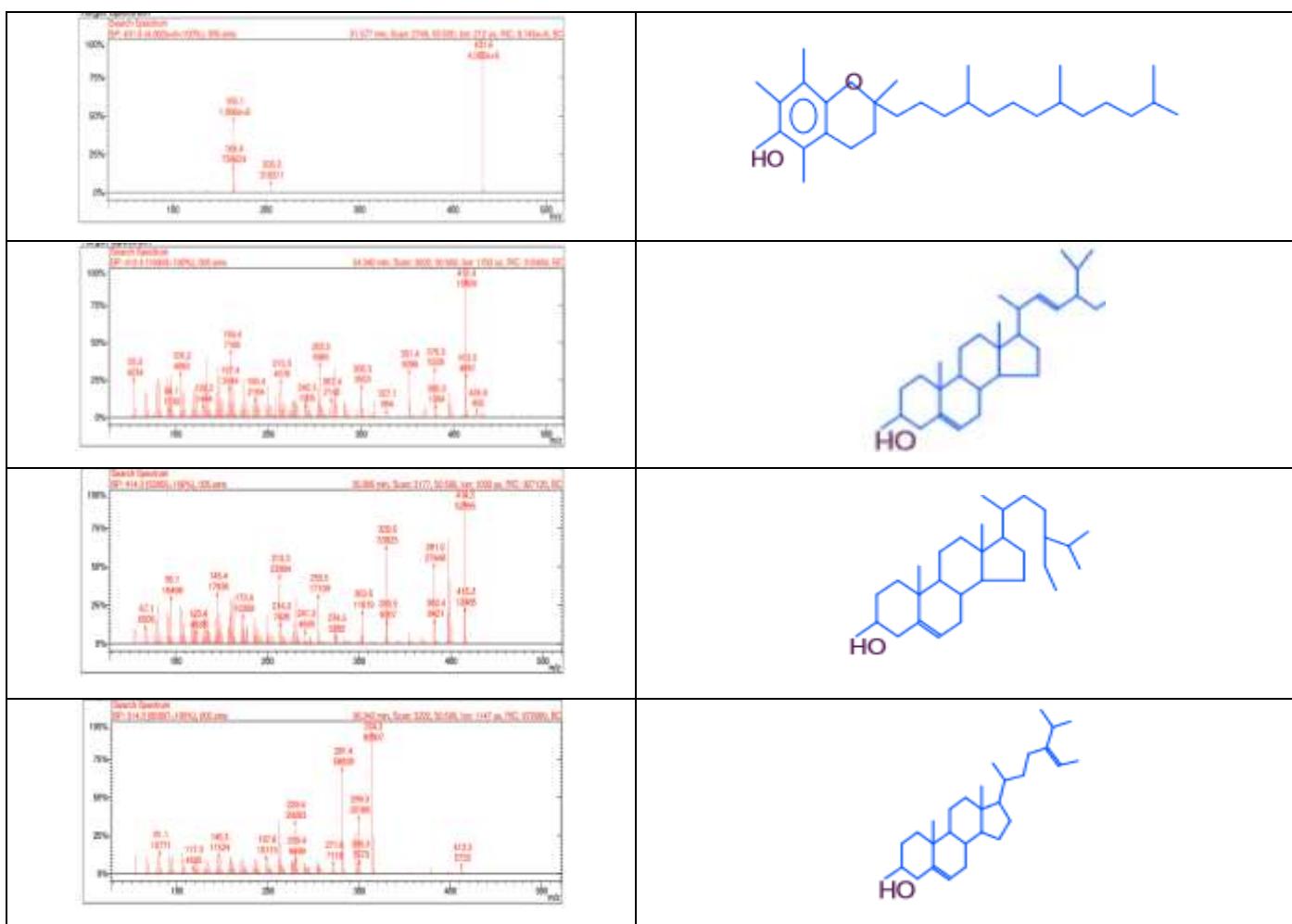


Figure 2: MS profile and 2D structure of Bioactive Compounds in MOMP