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GC-MS PROFILING OF ETHANOLIC EXTRACT OF *MORINGA OLEIFERA* LEAF

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ABSTRACT

To analyze and characterize the phytochemical compounds of ethanolic leaf extract of*oringa oleifera* using GC-MS. The phytochemical screening of ethanolic extract was carried out according to standard procedures stated by WHO guidelines. The various bioactive compounds of the extract weredentified by GC-MS technique.The results of the GCMS analysis revealed 35 compounds in the ethanolic extract of *oringa oleifera* leaves. Among the 35 compounds the following 15 major compounds revealed iththe maximum peak percentage area in parenthesis shown in table 1 : 1,2,3-Cyclopentanetriol 1.63), L-Galactose, 6- eoxy (4.35), n-Hexadecanoic acid (28.84), Tetradecanoic acid (2.12), cis- accenic acid (26.45), Octadecanoic acid (4.91), Palmitoyl chloride (1.56), 3-Chloro-N- sochroman-1-ylmethyl-propionamide (5.61), 2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester 1.77) , 3,4- ichlorobenzonitrile (2.21), Mannitol,1,4-di-O-methyl-, tetraacetate (1.09), beta.-l- amnofuranoside, 5-O-acetyl-thio-octyl (1.01), Vitamin E(2.07), gamma.-Sitosterol (2.23), Pregn- ,7-diene-3-ol-20-one (1.47). Their respective retention times being as. follows:13.03,15.28,18.62,18.67,20.35,20.52,21.62,23.36,23.46,23.67,23.90,23.94,27.85,29.16 29.26. The minor compounds are 4,5-Diamino-2- ydroxypyrimidine (0.45), 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl (0.93), enzofuran,2,3-dihydro (0.64), Benzeneacetonitrile,4-hydroxy (0.64), 2- Cyclohexenone,4-cetamido(0.68),4-((1E)-3-Hydroxy-1-propenyl)- -ethoxyphenol(0.39), Cyclopropaneoctanal,2- ctyl (0.23), 5-Eicosene, (E) (0.54), Octadec-9-enoic acid (0.74), 5-Eicosene, (E) (0.54), Oxirane, tetradecyl (0.64), trans-13-Octadecenoic acid (0.41), 6- ctadecenoic acid, (Z) (0.28), cis-9- exadecenal (0.21), Cyclopentadecanone,2-hydroxy (0.25), Cyclopropaneoctanal,2-octyl (0.26), leic Acid (0.35), Oleylalcohol, heptafluorobutyrate (0.90),7- entadecyne (0.70),trans-13- ctadecenoic acid (0.60).The ethanolic leaf extract of *Moringa oleifera* proved to be a reservoir of bioactive compounds identified by GC-MS which could prove effective in the treatment of various diseases.

KEYWORDS: ***Moringa oleifera* leaf, GC-MS analysis**

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INTRODUCTION

Better cultural acceptability, better compatibility with the human body and lower incidence of side effects are the well known attributes of herbal medicine, accounting for 70 to 80% of the world population mainly in the developing countries [1]. India plays a major role in the herbal drug market and making a good turnover from the export of herbal products. However, based on the rich traditional knowledge on herbal medicine in India, this profit is meagre. To encourage the growing industry, it is important for us to export more herbal drugs and isolate the bioactive phytochemical compounds of the herbal extract using various advanced chromatographic techniques. Mass spectrometry, coupled with Gas chromatography (GC/MS) is normally used for analysis of compounds present in traditional herbal medicines.In this study we are using an ethnopharmacologically important plant, *Moringa oleifera*. *Moringa oleifera* is commonly known by various local names like Miracle tree, Horseradish tree, and Ben oil tree[2]. Needless to say these names suggests the value and the multitude of uses of this plant. It is a soft wooded tree, whose leaves are used for traditional and industrial uses like domestic cleaning agent, fertilizer, ornamental planting, pulp, rope, water purification, perfume)[4][5]. It is a medium sized tree, 10 m in height, widely grown, easily cultivable and found mainly in the tropical and subtropical regions worldwide [6]. The leaves are highly nutritious and also a source of beta carotene amino acids like methionine, cysteine, tryptophan, lysine, vitamin C, vitamin B_1, vitamin B_2, vitamin B_3, iron, potassium, calcium, zinc, sodium and also a potential source of natural antioxidants[7],[8],[9]. The plant also contains flavonoids, anthocyanin, cinnamates and proanthocyanidins, 4-hydroxymellein,ß-sitosterol, vanillin[6]. The leaves have a number of activities like anticonvulsant, antidepressant, antipyretic, anti-asthmatic, anti-inflammatory, antiarthritic, analgesic, and neuroprotective in Alzheimer's disease[10],[11],[12],[3]. Previous phytochemical studies on the ethanolic leaf extract of *Moringa oleifera* revealed the presence of tannins, alkaloids, phenol, glycoside, flavonoids, and glycosides[13],[14]. Karthika *et al* reported a total of 28 compounds present in ethyl acetate extract of *Moringa oleifera* leaves by GC-MS study[15]. Previous studies on GC-MS of ethanolic leaf extract of *Moringa oleifera* has been done and a few bioactive principles were isolated and identified [16]. The aim of the present study is to isolate, investigate and characterize all possible bioactive phytocompounds in ethanolic extract of *Moringa oleifera* leaf by GC-MS analysis.

MATERIALS AND METHODS

Collection of Plant material

The fully mature leaves were collected from the local areas of Syampur, Bhubaneswar, Odisha, India. The plant was identified and authenticated by Dr. P. C. Panda, Senior Scientist, Taxonomy and Conservation Division, Regional Plant Resource Centre, Bhubaneswar, Odisha. The collected leaves were free from diseases and other undesired plant parts. A voucher specimen was deposited in the herbarium of Department of Pharmacognosy, Siksha O Anusandhan University, Bhubaneswar, Odisha.

Preparation of plant extract

Fresh leaveswere collected dried in shade and powdered. The powder (100 g) was extracted with 90% ethanol using a continuous hot percolation method in a Soxhlet apparatus for 18 hrs. The extract was filtered using whatman filter paper no 1 and concentrated in a rotary evaporator to yield a semi solid mass of 9.45 g (yield 9.45 % w/w). Extract stored in refrigerator at 4^0 C and used for oral administration.

Phytochemical screening

The ethanolic extract was tested for various phytoconstituents such as steroids, triterpenoids, flavonoids, tannins using standard methods.[14].

GC-MS analysis

The GC – MS analysis was carried out using a Clarus 500 Perkin –Gas Chromatograph coupled to a mass detector, Turbo mass gold – Perkin Elmer Turbomass 5.1 spectrometer with an Elite- (100% Dimethyl poly siloxane),

30m x 0.25 mm ID x 0.25µm of capillary column.Injection temperature was maintained at 250 °C, Helium flow rate as 1.5 ml/min and ion source temperature at 230 °C. Injection was performed in the split less mode and the volume was 1 µL. The instrument was set to an initial temperature of 70°C, and maintained at this temperature for 3 min. At the end of this period the oven temperature was arisen up to 300°C, at the rate of an increase of 10°C/min, and maintained for 9 min. The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV, and the detector operated in scan mode from 40 – 700 m/z. The MS start time was 3 min, end time was 35 min with solvent cut time was about 3 min. Identifications were based on mass spectral matching with standard compounds in NIST library, while identification of Deoxyelephantopin isomers and analogs were based on the molecular structure, molecular mass and calculated fragmentations. The essential chemical constituents were identified by matching mass spectra with spectra of reference compounds in mass spectral library of the National Institute of Standards and Technology (NIST 11). The relative amounts of individual components were expressed as percent peak areas relative to the total peak area.

Identification of components

The chemical constituents were identified by matching mass spectra with spectra of reference compounds in mass spectral library of the National Institute of Standards and Technology (NIST 11). The name, molecular weight and structure of the components of the test materials were to be identified.

Figure 1

GC-MS chromatogram of ethanolic extract of Moringa oleifera leaf.

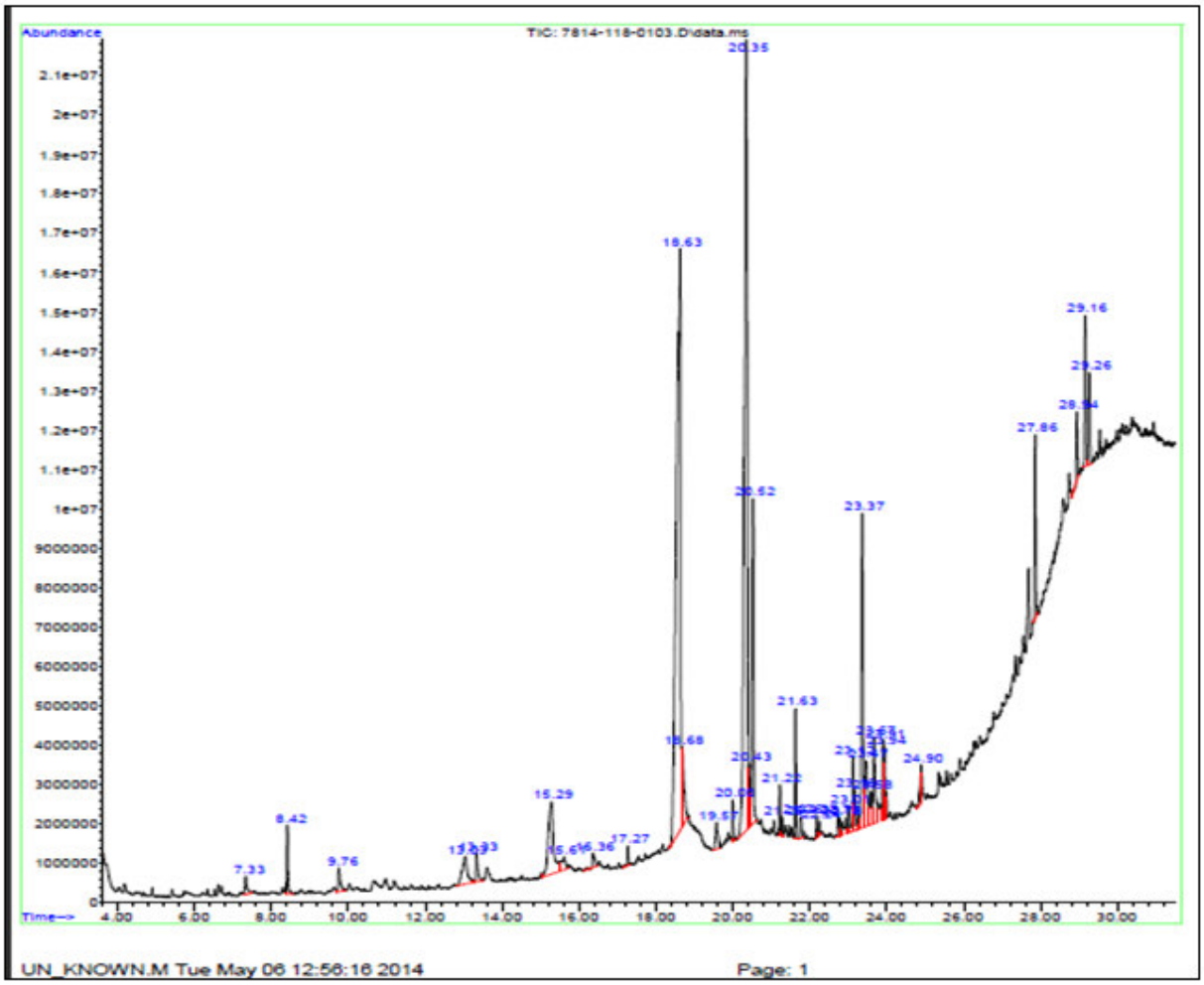


Figure 2A
Mass spectrum of 1,2,3-Cyclopentanetriol

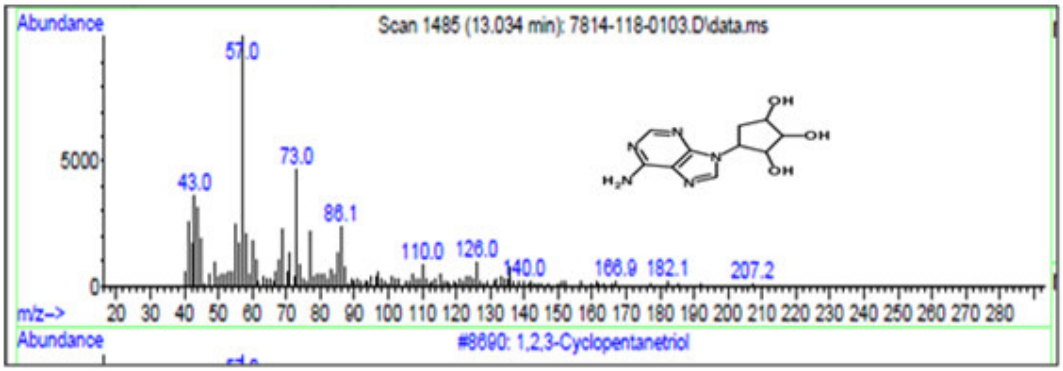


Figure 2B
Mass spectrum of L-Galactose, 6-deoxy

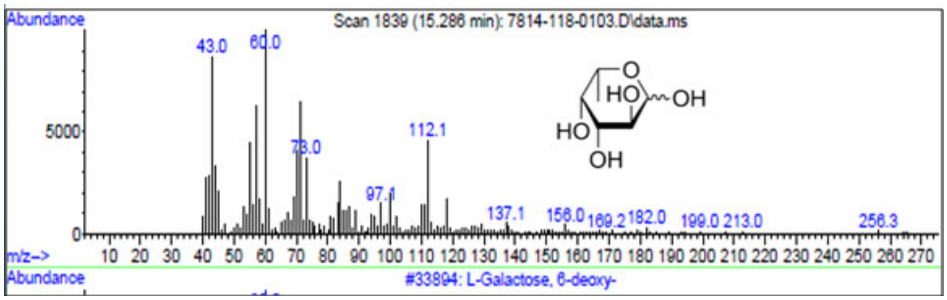


Figure 2C
Mass spectrum of n-Hexadecanoic acid

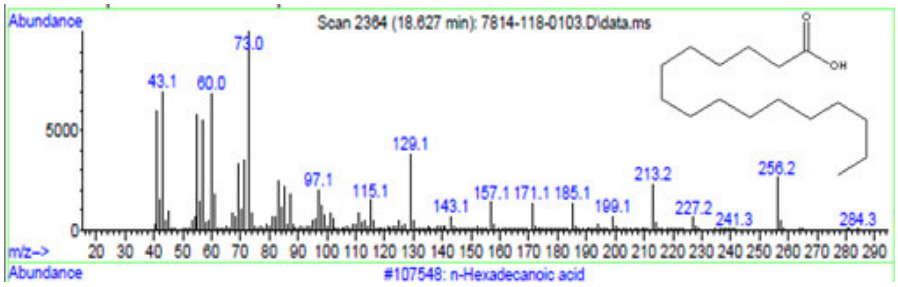


Figure 2D
Mass spectrum of Tetradecanoic acid

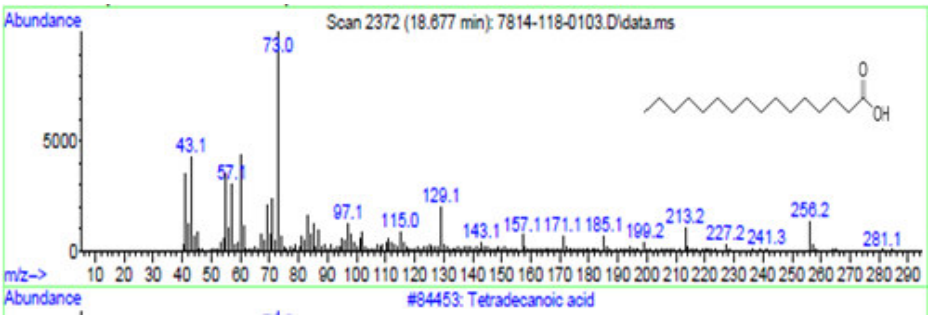


Figure 2E
Mass spectrum of cis-Vaccenic acid

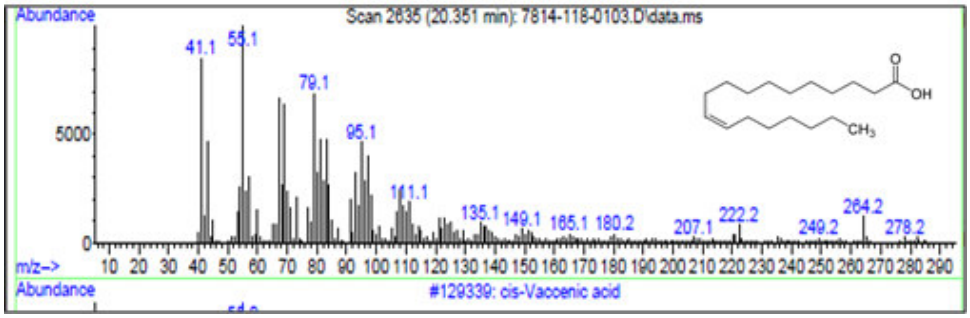


Figure 2F
Mass spectrum of Octadecanoic acid

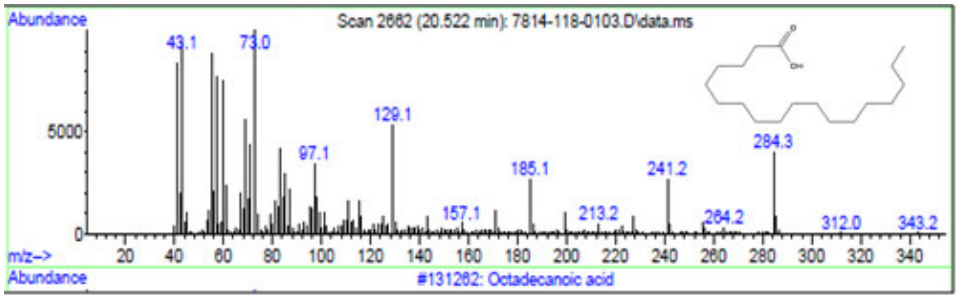


Figure 2G
Mass spectrum of Palmitoyl chloride

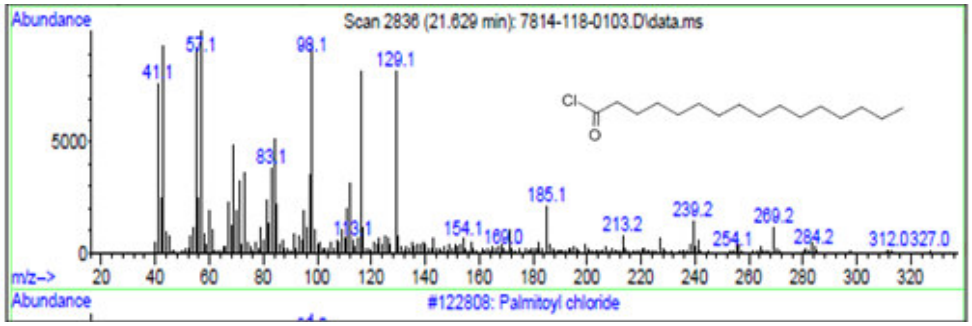


Figure 2H
Mass spectrum of 3-Chloro-N-isochroman-1-ylmethyl-propionamide

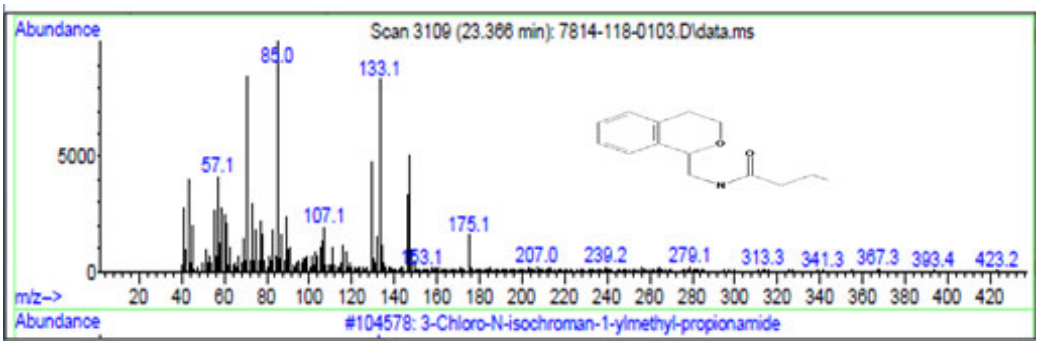


Figure 2I
Mass spectrum of 2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester

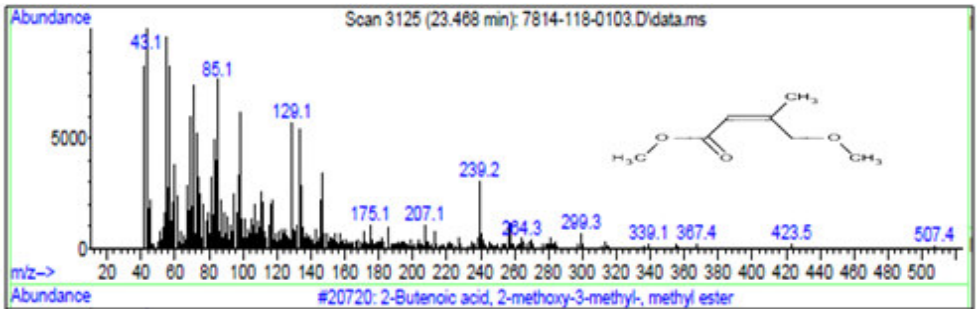


Figure 2J
Mass spectrum of 3,4-Dichlorobenzonitrile

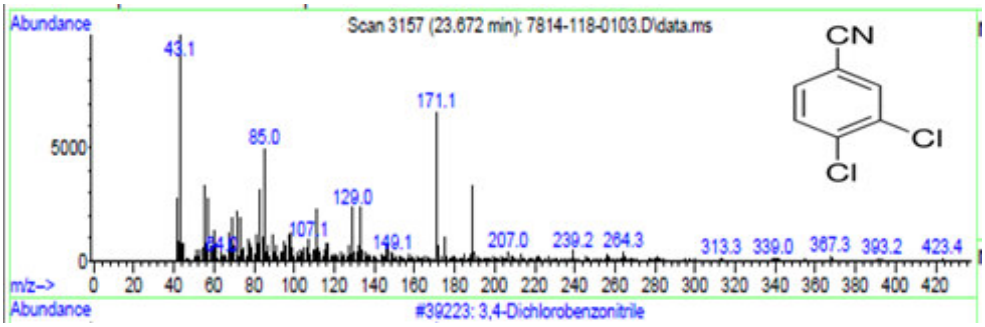


Figure 2K
Mass spectrum of Mannitol,1,4-di-O-methyl-, tetraacetate

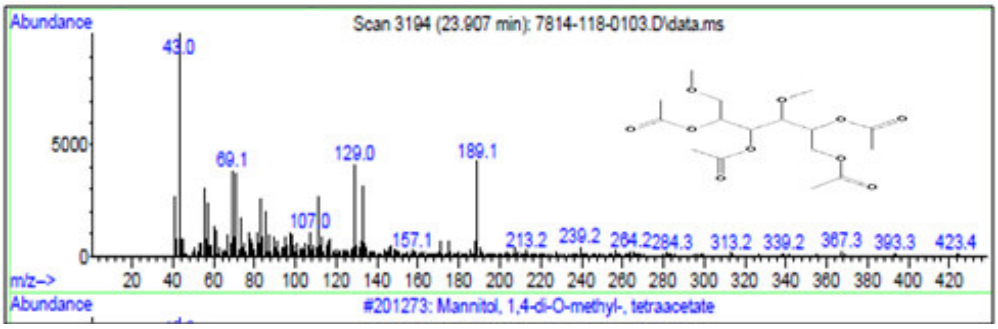


Figure 2L
Mass spectrum of beta.-l-Rhamnofuranoside, 5-O-acetyl-thio-octyl

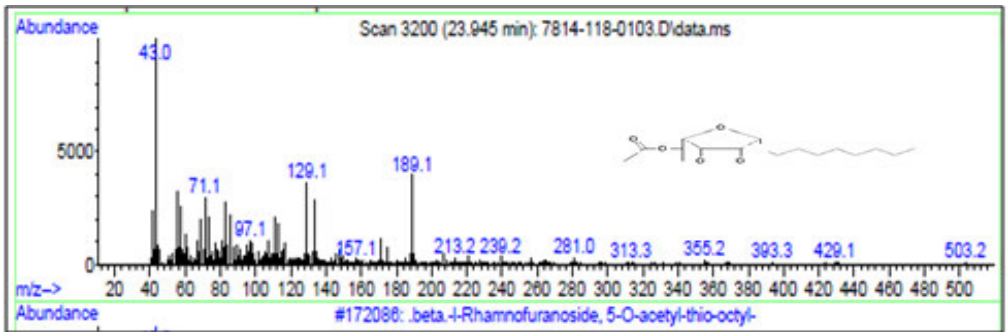


Figure 2M
Mass spectrum of Vitamin E

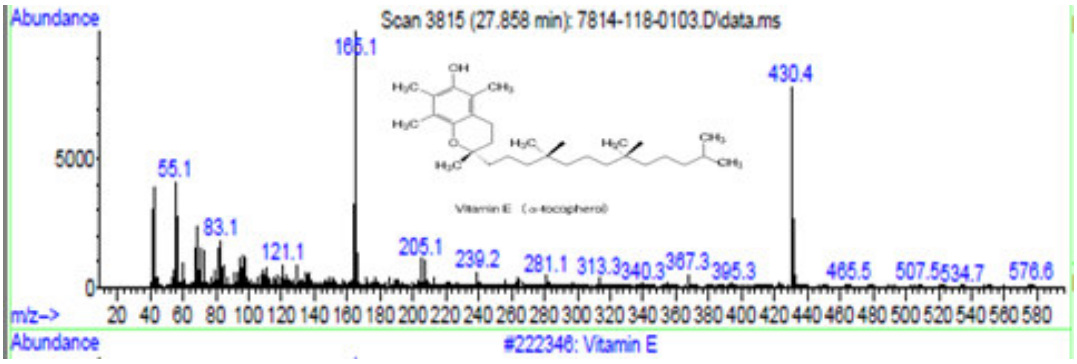


Figure 2N
Mass spectrum of gamma.-Sitosterol

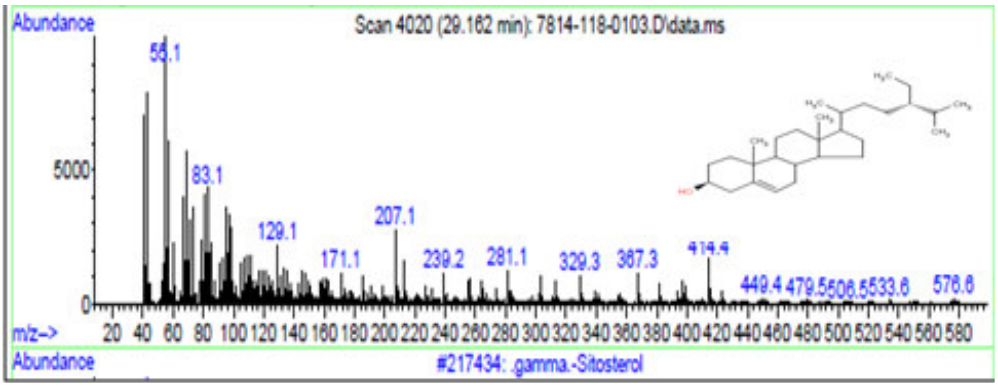


Figure 2O
Mass spectrum of Pregn-5,7-diene-3-ol-20-one

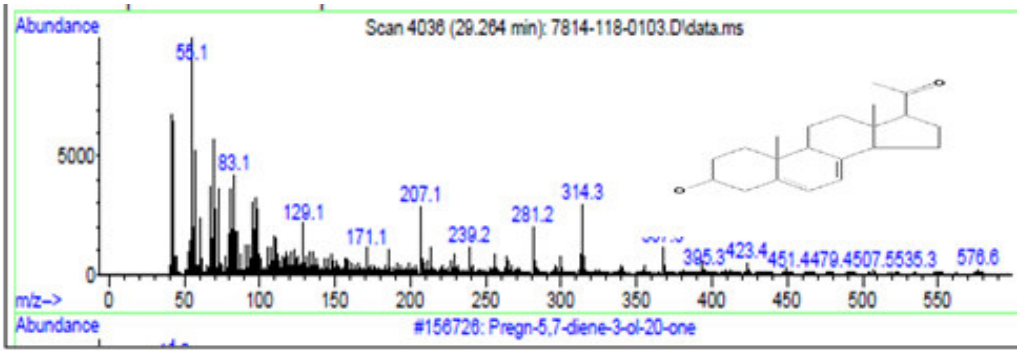


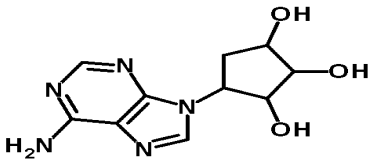
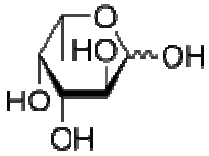
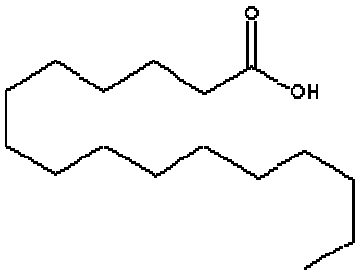
Table 1
Major phytochemical compounds identified in ethanolic extract of Moringa oleifera leaf

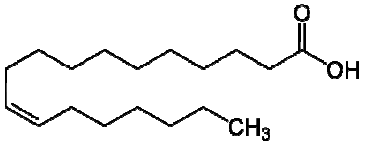
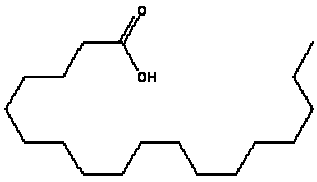
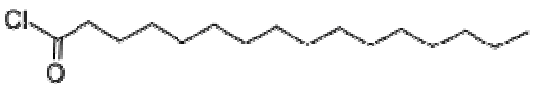
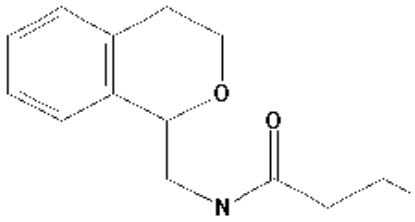
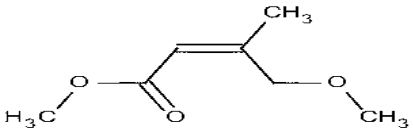
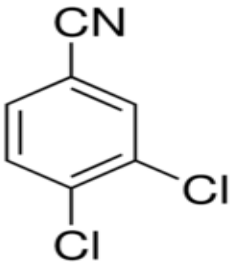
No.	RT(min)	Name of the compound	Molecular Formula	Molecular weight	Peak area (%)	Nature of the compound
1	13.03	1,2,3-Cyclopentanetriol	$C_5H_{10}O_3$	118.13	1.63	Fatty acid ester
2	15.28	L-Galactose, 6-deoxy-	$C_6H_{12}O_5$	164.15	4.35	Sugar
3	18.62	n-Hexadecanoic acid	$C_{16}H_{31}O_2$	255.41	28.84	Saturated fatty acid
4	18.67	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	2.12	Saturated fatty acid

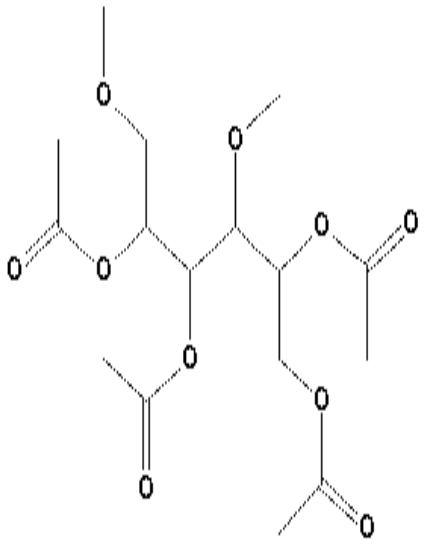
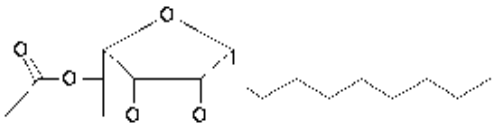
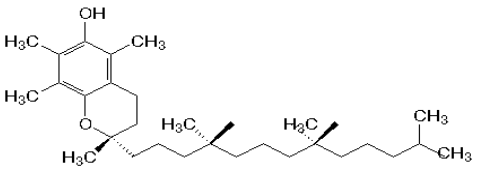
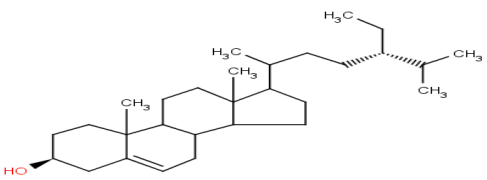
5	20.35	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.46	26.45	fatty acid
6	20.52	Octadecanoic acid	$C_{18}H_{36}O_2$	284.47	4.91	Fatty acid
7	21.62	Palmitoyl chloride	$C_{16}H_{31}ClO$	274.86	1.56	Fatty acid chloride
8	23.36	3-Chloro-N-isochroman-1-ylmethyl-propionamide	$C_{13}H_{16}ClNO_2$	253.72	5.61	Amide
9	23.46	2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester	$C_7H_{12}O_3$	144.16	1.77	Ester
10	23.67	3,4-Dichlorobenzonitrile	$C_7H_3Cl_2N$	172.01	2.21	Aromatic compopund
11	23.90	Mannitol,1,4-di-O-methyl-, tetraacetate	$C_{16}H_{26}O_{10}$	378.37	1.09	Alcohol ester
12	23.94	beta.-l-Rhamnofuranoside, 5-O-acetyl-thio-octyl-	$C_{16}H_{30}O_5S$	334.47	1.01	Glycoside
13	27.85	Vitamin E	$C_{29}H_{50}O_2$	430.70	2.07	Alcoholic compound
14	29.16	gamma.-Sitosterol	$C_{29}H_{50}O$	414.70	2.23	Steroid
15	29.26	Pregn-5,7-diene-3-ol-20-one	$C_{21}H_{30}O_2$	314.4617	1.47	Steroid

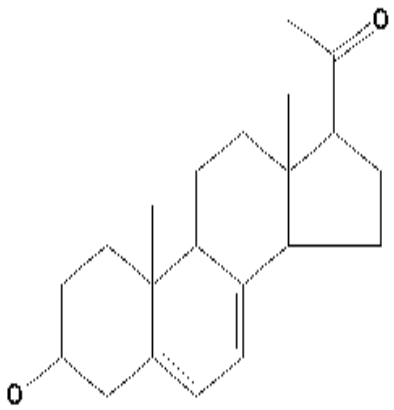
Table 2

Structure and Pharmacological actions of the phytochemical cmpounds identified in ethanolic extract of Moringa oleifera leaf

RT(min)	NAME OF COMPOUNDS	CHEMICAL STRUCTURE	PHARMACOLOGICAL ACTIONS
13.03	1,2,3-Cyclopentanetriol		Antiviral property.
15.28	L-Galactose, 6-deoxy		Anti-aging, Anti-cancer, Anti-oxidant properties
18.62	n-Hexadecanoic acid		Antioxidant, hypocholesterolemic, nematicide, hemolytic, 5-alpha,antiandrogenic, reductase inhibitor,pesticide,lubricant antiinflammatory
18.67	Tetradecanoic acid		Antioxidant, cancer preventive, nematicide, hypercholesterolemic, Lubricant

20.35	cis-Vaccenic acid		Hypolipidaemic, antihypertensive
20.52	Octadecanoic acid		Antibacterial action, soap lubricant, cosmetics
21.62	Palmitoyl chloride		Anticancer
23.36	3-Chloro-N-isochroman-1-ylmethyl-propionamide		No reported activity
23.46	2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester		No reported activity.
23.67	3,4-Dichlorobenzonitrile		No reported activity.

23.90	Mannitol,1,4-di-O-methyl-, tetraacetate		No reported activity.
23.94	beta.-l-Rhamnofuranoside, 5-O-acetyl-thio-octyl-		Analgesic, Anti-inflammatory, Antipyretic, Antioxidant, Muscle relaxant
27.85	Vitamin E		Antioxidant
29.16	gamma.-Sitosterol		Anticancer , Antioxidant

29.26	Pregn-5,7-diene-3-ol-20-one		Anti-infertility agent

RESULTS AND DISCUSSION

The results of the GCMS analysis revealed the presence of 35 compounds in the ethanolic extract of *Moringa oleifera* leaves. Among the 35 compounds the following 15 major compounds revealed with the maximum peak percentage area in parenthesis shown in table 1 : 1,2,3-Cyclopentanetriol (1.63), L-Galactose, 6-deoxy (4.35), n-Hexadecanoic acid (28.84), Tetradecanoic acid (2.12), cis- accenic acid (26.45), Octadecanoic acid (4.91), Palmitoyl chloride (1.56), 3-Chloro-N-isochroman- -ylmethyl-propionamide (5.61), 2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester (1.77) , 3,4- ichlorobenzonitrile (2.21), Mannitol,1,4-di-O-methyl-, tetraacetate (1.09), beta.-l- hamnofuranoside, -O-acetyl-thio-octyl (1.01), Vitamin E(2.07), gamma.-Sitosterol (2.23), Pregn-5,7-diene-3-ol-20-one 1.47). Their respective retention times being as.

follows

3.03,15.28,18.62,18.67,20.35,20.52,21.62,23.36,23.46,23.67,23.90,23.94,27.85,29.16,2.26. The minor compounds are 4,5-Diamino-2-hydroxypyrimidine (0.45), 4H-Pyran-4-one,2,3- dihydro-3,5dihydroxy-6-methyl (0.93),Benzofuran,2,3dihydro(0.64),Benzene acetonitrile,4hydroxy(0.64),2Cyclohexenone, 4-acetamido(0.68),4-((1E)-3-Hydroxy-1-propenyl)ethoxyphenol(0.39),Cyclopropaneoctanal,2-octyl (0.23), 5-Eicosene, (E) (0.54), Octadec-9-enoic acid (0.74), 5-Eicosene, (E) (0.54), Oxirane, tetradecyl (0.64), trans-13-Octadecenoic acid (0.41), 6-Octadecenoic acid, (Z) (0.28), cis-9-Hexadecenal (0.21), Cyclopentadecanone,2-hydroxy (0.25), Cyclopropaneoctanal,2-octyl (0.26), Oleic Acid (0.35), Oleylalcohol, heptafluorobutyrate (0.90),7-Pentadecyne (0.70),trans-13-Octadecenoic acid (0.60). Among the identified compounds,1,2,3Cyclopentanetriol and L-Galactose, 6- deoxy have antiviral, anti-aging, anti-cancer and antioxidant properties [17],[18],[19],[20]. n-Hexadecanoic acid, Octadecanoic acid, Tetradecanoic acid and cis-Vaccenic acid have been reported to have antioxidant, hypocholesterolemic, hemolytic, antiinflammatory, anticancer, antibacterial, nematicide and antihypertensive activities [21],[22],[23],[24]. Palmitoyl chloride and gamma.-Sitosterol have the property of anticancer activity[25]. Vitamin E and beta.-l-Rhamnofuranoside, 5-O-acetyl- hio-octyl- have been found to act as antioxidant, analgesic, anti-inflammatory, antipyretic agents. Pregn-5,7-diene-3-ol-20-one is used as a precursor in the manufacture of semi synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue

rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of Vitamin D3 [26]. Here, it is important to mention that various authors have reported analgesic properties from other common Indian plant, Azadirachta Indica seed oil [27].

CONCLUSION

The leaf extract of *Moringa oleifera* proved to be a reservoir of bioactive constituents, which could be used in various diseases in future. However, isolation of individual compounds and their biological activities needs to be uncovered further to enhance its pharmacological importance and open new avenues in research. It could be concluded that, *Moringa oleifera* contains various bioactive compounds and may be recommended as a plant of phytopharmaceutical importance.

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