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GC-MS ANALYSIS OF METHANOLIC EXTRACT OF *UROCHLOA DISTACHYA* (L.) T. Q. NGUYEN, LEAVE

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Urochloa distachya, methanolic, GC-MS analysis

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ABSTRACT: *Urochloa distachya* (Poaceae), commonly known as Signal grass 20-60 cm long found in tropical Asian origin, Africa, and other countries. Generally, it has been used in fodder, animal feed etc., and least and no therapeutic activities were reported. The present study was established to identify the phytochemicals by using GC-MS of the *U. distachya* leaves part. The leaves part of the plant was extracted in methanol by using the Soxhlet apparatus. The GC-MS Methanolic extract of *U. distachya* identified 26 phytochemicals; Z-7-Pentadecenol; t-Butyl hydrogen phthalate, Ricinoleic acid; Methyl -12-hydroxy-9-octadecenoate; Octadecanoic acid; Glycerin; 4, 6-Diphenyl-2-(2-hydroxyphenyl) pyrimidine, Eicosanoid acid, methyl ester, 2-Naphthalenol 2, 3, 4, 4a, 5, 6, 7-octahydro-1, 4-dimethyl-7-(2-hydroxy-1-methylethyl); Cholest-5-en-3-ol (3 α), tetradecanoate; 6-Octadecenoic Acid; Oleic acid, 3-hydroxypropyl ester; Octadecanoic acid, 2, 3-dihydroxypropyl ester; Eicosane; Tert-Hexadecanethiol; 2-[4-methyl-6-(2, 6, 6-trimethylcyclohex-1-enyl) hexa-1, 3, 5-trienyl] cyclohex-1-en-1-carboxaldehyde; Spiro [4.5] decan-7-one, 1, 8-dimethyl-8, 9-epoxy-4-isopropyl; 9, 12, 15-Octadecatrienoic acid, 2, 3-bis [(trimethylsilyl) oxy] propyl ester, (Z, Z, Z). The phytoconstituents of methanolic extract of *U. distachya* has shown remarkable activities like antimicrobial, antioxidant, anti-inflammatory and anticancer etc.

INTRODUCTION: Medicinal plants are of great importance in the field of treatment and cure of diseases. The medicinal value of plants depends on the presence of certain chemical substances, so scientific research defines the chemical effects and composition of active ingredients, which determine the medicinal properties of plants.

HPLC, HPTLC and GC-MS are a modern analytical technique that provides superior separation of plant extract sample. High-performance liquid chromatography (HPLC) is a separation technique used to identify, purify, and quantify the individual components of the mixture sample ¹.

High-performance thin-layer chromatography (HPTLC) is very useful for qualitative as well as quantitative analytical works. This technique plays a vital role in phytochemical analysis, biochemical analysis, fingerprint analysis, herbal drugs quantification, etc. ².

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Gas chromatography-mass spectroscopy is a combined analytical technique used to detect and identify the chemical component present in the plant extract. The GC-MS studies are essential for the determination of biologically active components from the plant sample. *Urochloa distachya* (L.) T. Q. Nguyen (Poaceae) is generally known as signal grass. It is an annual grass, with 20-60 cm high, lance-shaped leaves, 2-3 racemes, bearing spikelet, found in tropical Asian origin, Africa, and other countries. This plant is adapted to the warm tropics, particularly in monsoon environments. This species flowers throughout the year. It reproduces vegetative, developing roots from nodes in culms in contact with soil. Stolons develop into new culms and form clones. At 26°C of soil temperature gets geminate. The plant has been used in fodder, animal feed, etc. there is less or no biological activities are reported³. Literature review revealed that there was no HPLC, HPTLC, and GC-MS was not carried out for the standardization of *Urochloa distachya*. Therefore, the present investigation was focused on analyzing the qualitative estimation of phytoconstituents present in the plant by GCMS analysis.

MATERIAL AND METHOD:

Collection and Preparation of Plant Materials:

The fresh plant was collected from Hatgaon, Rusuda, Bargarh, Odisha, India and authenticated by a Botanical survey of India, Kolkata, India, bearing reference number: CNH/Tech.II/2019/77. The leaves parts of the plant were dried under shade. The dried leaves were ground into powder by using mechanical grinder. The powder material was stored in an airtight container for further use.

Preparation of Extract: The dried leave powder weighing about 200 gm was mounted in the Soxhlet apparatus and extracted with methanol (60-65°C). The extract was concentrated with the help of a rotary evaporator and stored in the desiccator at a temperature 4°C for further use.

GC-MS: The methanolic extract of *U. distachya* was subjected to Thermo Scientific TSQ 8000 Gas Chromatograph - Mass Spectrometer. MS part consists of Triple Quadrupole, and the GC part consists of Split/Split less Injectors and multi-mode (including on-column) Programmed Temperature Vaporizing (PTV), the column temperature is 400

°C. The mass spectrometer joined with the TRACE 1300 GC along with Auto-sampler for automated sample handling. About 1 µl of the methanol extract was injected into the GC-MS using a microsyringe and the scanning was done for 31.08 minutes. The carrier gas was used as Helium gas at a constant flow rate of 1 ml/minute.

The ion source was programmed to 350°C. The name, molecular formula, and molecular weight of the components were referred from the National Institute of Standard and Technology (NIST).

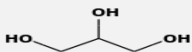
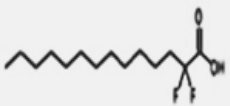

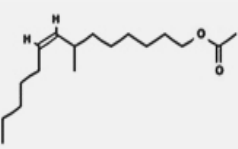
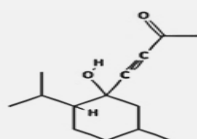
RESULTS AND DISCUSSION: Gas chromatography and mass spectroscopy are very efficient and effective methods for separating and detecting a mixture of phytochemicals. The GCMS analysis of a methanolic extract of *U. distachya* was depicted in **Fig. 1**. The retention time (RT), structure, molecular formula, and percentage peak areas of the identified compounds are given in **Table 1**. The methanolic extract of *U. distachya* showed the presence of 26 compounds.

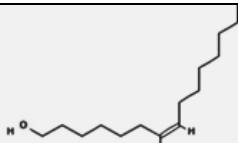
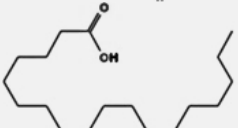
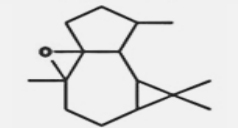
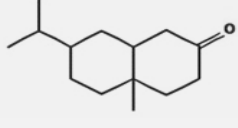
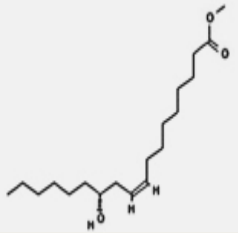
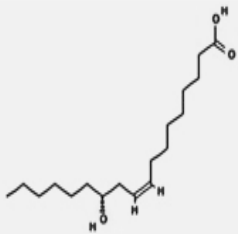
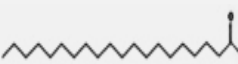
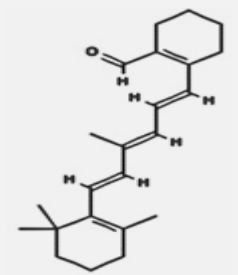
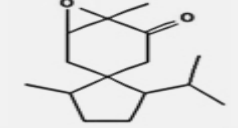
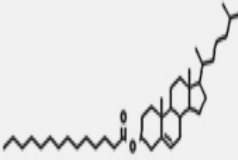
Out of these compound four compounds were determined as major compound Z-7-Pentadecenol (31.37%) **Fig. 7**, t-Butyl hydrogen phthalate (13.90%) **Fig. 20**, Ricinoleic acid (11.88%) **Fig. 12**, Methyl -12-hydroxy-9-octadecenoate (9.72%) **Fig. 11** and rest compound were minor compound Octadecanoic acid (3.16%) **Fig. 8**, Glycerin (2.69%) **Fig. 1**, 4,6-Diphenyl-2-(2-hydroxyphenyl) pyrimidine (2.82%) **Fig. 23**, Eicosanoid acid, methyl ester (2.62%) **Fig. 13**, 2-Naphthalenol, 2, 3, 4, 4a, 5, 6, 7 -octahydro1, 4adimethyl-7-(2-hydroxy-1-methylethyl) (2.34%) **Fig. 10**, Cholest-5-en-3-ol (3á), tetradecanoate (2.26%) **Fig. 16**, 6-Octadecenoic Acid (2.10%) **Fig. 25**, Oleic acid, 3-hydroxypropyl ester (1.92%) **Fig. 24**, Octadecanoic acid, 2, 3-dihydroxypropyl ester (1.92%) **Fig. 26**, Eicosane (1.59%) **Fig. 21**, Tert-Hexadecanethiol (1.34%) **Fig. 18**, 2 - [4 - methyl - 6 - (2, 6, 6-trimethylcyclohex - 1 - enyl) hexa-1, 3, 5-trienyl] cyclohex1en-1-carboxaldehyde (1.21%) **Fig. 14**, Spiro [4.5] decan-7-one, 1, 8-dimethyl-8,9-epoxy-4-isopropyl (0.98%) **Fig. 15**, 9, 12, 15-Octadecatrienoic acid, 2, 3-bis [(trimethylsilyl) oxy] propyl ester, (Z, Z, Z) (0.98%) **Fig. 22**, 2, 3-Dihydroxypropyl elaidate (0.79%) **Fig. 4**, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (0.79%) **Fig. 19**, Oleic acid, 3-

(octadecyloxy) propyl ester (0.72%) Figure 17], Diisooctyl phthalate (0.69%) Fig. 27, 2,2-Difluoroheptacosanoic acid (0.65%) Fig. 3, Ledene oxide (II) (0.58%) Fig. 9, Menthol, 1'-(butyn-3-one-1-yl), (1R,2S,5R) (0.52%) Fig. 6, 7-methyl-z-tetradecen-1-ol acetate (0.49%) Fig. 5. The GC-MS investigation compounds of the methanolic extract of *U. distachya* (L.) showed various biological activities, which were presented in Table 2. 2, 3-Dihydroxypropyl elaidate, 9, 12, 15 – Octadecatrienoic acid, 2, 3 – bis [(trimethylsilyl) oxy] propyl ester, (Z, Z, Z), and Oleic acid, 3 – hydroxypropyl ester are fatty acid in nature, and they showed antioxidant activity^{6, 22, 23}. 2, 2-Difluoroheptacosanoic acid is an acid compound stated to possess antimicrobial activity⁵. 2, 3-Dihydroxypropyl elaidate, 7-Methyl-Z-tetradecen-1-ol acetate, Menthol, 1'-(butyn-3-one-1-yl), (1S, 2S, 5R) and 6-Octadecenoic acid are fatty acid, acetate compound, monoterpene and fatty acid in nature respectively which showed anti-cancer activity^{6, 7, 10, 24}. Apart from anti-cancer activity of 7-Methyl-Z-tetradecen-1-ol acetate also indicate anti-inflammatory and hepatoprotective activities⁷. 9, 12, 15-Octadecatrienoic acid, 2, 3-dihydroxypropyl ester, (Z, Z, Z) is Linolenic acid

ester compound that exhibits antioxidant, antimicrobial activity, anti-inflammatory, nematocide, antihistaminic antieczemic, insectifuge⁸, hypo-cholesterolemia nematocide antiarthritic activities⁹. Antimicrobial, dietary supplements, softening agents, and surfactant activities showed by Octadecanoic acid, which is a fatty acid in nature¹¹. Ledene oxide (II) is sesquiterpenoid in nature, and the activities are indicated antibacterial, antioxidant¹². 2- [4-methyl-6-(2, 6, 6-trimethylcyclohex-1-enyl) hexa 1, 3, 5-trienyl] cyclohex-1-en-1-carboxaldehyde is an aldehyde compound that showed antimicrobial and anti-inflammatory activity¹⁶. Oleic acid, 3-(octadecyloxy) propyl ester revealed antifungal activity, a fatty acid in nature¹⁸. Eicosane produced antifungal, antibacterial, antitumor, and cytotoxic activity²¹. Diisooctyl phthalate revealed antimicrobial and antifouling activity²¹. The CG-MS of *U. distachya* proposed that the biological properties of several components possess antioxidant, anti-inflammatory, antimicrobial, and anticancer activities, so further investigation and isolation of the plant can reveal newer molecules that will be helpful for the study of therapeutic and pharmacological activities.

TABLE 1: GC-MS ANALYSIS OF METHANOLIC EXTRACT OF *U. DISTACHYA*

Sl. no.	RT	Name of the compound	Chemical structure	Molecular formula	Molecular weight	Peak area %
1	10.52	Glycerin		C ₃ H ₈ O ₃	92.0938	2.69
2	17.28	2,2-Difluoroheptacosanoic acid		C ₁₄ H ₂₆ F ₂ O ₂	264.35	0.65
3	17.90	2,3-Dihydroxypropyl elaidate		C ₂₁ H ₄₀ O ₄	356.5	0.79
4	18.09	7-methyl-z-tetradecen-1-ol acetate		C ₁₇ H ₃₂ O ₂	268.4	0.49
5	19.48	Menthol, 1'-(butyn-3-one-1-yl), (1R,2S,5R)		C ₁₄ H ₂₂ O ₂	222.32	0.52

6	19.94	Z-7-Pentadecenol		$C_{15}H_{30}O$	226.4	31.37
7	20.34	Octadecanoic acid		$C_{18}H_{36}O_2$	284.4772	3.14
8	20.78	Ledene oxide(II)		$C_{15}H_{24}O$	220.3505	0.58
9	21.56	2-Naphthalenol, 2,3,4,4a,5,6,7-octahydro1,4adimethyl-7-(2-hydroxy-1-methylethyl)		$C_{15}H_{26}O_2$	238.37	2.34
10	22.26	Methyl -12-hydroxy-9-octadecenoate		$C_{19}H_{36}O_3$	312.5	9.72
11	22.38	Ricinoleic acid		$C_{18}H_{34}O_3$	298.5	11.88
12	22.97	Eicosanoic acid, methyl ester		$C_{21}H_{42}O_2$	326.5570	2.62
13	23.41	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hex-1-enyl]cyclohexen-1-carboxaldehyde		$C_{23}H_{32}O$	324.5	1.21
14	24.56	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl		$C_{15}H_{24}O_2$	236.35	0.98
15	24.94	Cholest-5-en-3-ol (3á), tetradecanoate		$C_{41}H_{72}O_2$	597	2.26



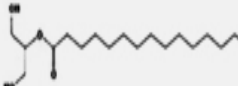
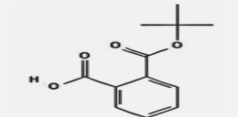

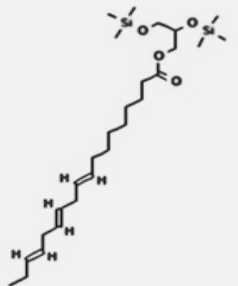
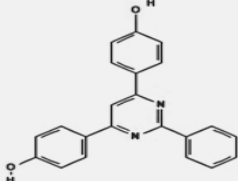
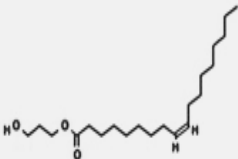


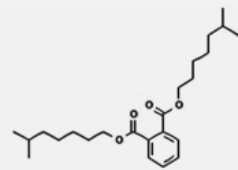
16	25.79	Oleic acid, 3-(octadecyloxy) propyl ester		$C_{39}H_{76}O_3$	593	0.72
17	26.08	Tert-Hexadecanethiol		$C_{16}H_{34}S$	258.511	1.34
18	26.42	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester		$C_{19}H_{38}O_4$	330.5026	0.79
19	26.68	t-Butyl hydrogen phthalate		$C_{12}H_{14}O_4$	222.24	13.90
20	28.34	Eicosane		$C_{20}H_{42}$	282.5	1.59
21	28.88	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)		$C_{27}H_{52}O_4$ Si_2	496.9	0.98
22	29.51	4,6-Diphenyl-2-(2-hydroxyphenyl) pyrimidine		$C_{22}H_{16}N_2$ O	340.4	2.82
23	30.96	Oleic acid, 3-hydroxypropyl ester		$C_{21}H_{40}O_3$	340.5	1.92
24	31.15	6-Octadecenoic acid		$C_{18}H_{34}O_2$	282.5	2.10
25	31.78	Octadecanoic acid, 2,3-dihydroxypropyl ester		$C_{21}H_{42}O_4$	358.5558	1.92
26	32.41	Diisooctyl phthalate		$C_{24}H_{38}O_4$	390.6	0.69

TABLE 2: BIOLOGICAL PROPERTIES OF THE PHYTOCOMPOUNDS

Sl. no.	Name of the compound	Nature of the compound	Biological activity
1	Glycerin	Simple polyol	Potent osmotic dehydrating agent with additional effects on brain metabolism, decreases intracranial pressure (Reye's syndrome, stroke, encephalitis, meningitis, pseudotumor cerebri, central nervous system tumor, and space-occupying lesions), glaucoma ⁴
2	2,2-Difluoroheptacosanoic acid	Acid	Antimicrobial activity ⁵
3	2,3-Dihydroxypropyl elaidate	Fatty acid	Anticancer and antioxidant activity ⁶
4	7-Methyl-Z-tetradecen-1-ol acetate	Acetate compound	Anti-cancer, anti-inflammatory, hepatoprotective ⁷
5	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z)	Linolenic acid ester compound	Antioxidant, antimicrobial activity, anti-inflammatory, nematocide, antihistaminic antieczemic, insectifuge ⁸ , hypocholesterolemia nematocide Antiarthritic ⁹
6	Menthol, 1'-(butyn-3-one-1-yl), (1S,2S,5R)	monoterpene	Anticancer activity ¹⁰
7	Octadecanoic acid	Fatty acid	Antimicrobial activity, dietary supplements, softening agent, surfactant ¹¹
8	Ledeneoxide (II)	Sesquiterpenoid	Antibacterial, antioxidant ¹²
9	2-Naphthalenol,2,3,4,4a,5,6,7 octahydro1,4adimethyl-7-(2-hydroxy- 1-methylethyl)	-	Anti-inflammatory ¹³
10	Ricinoleic acid	Fatty acid	Gaucher disease treatment pulmonary hypertension treatment skin diseases treatment Ophthalmic drug anti-seborrheic ¹⁴
11	Eicosanoic acid, methyl ester	Arachidic acid	Alpha-glucosidase inhibitors activity ¹⁵
12	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-carboxaldehyde	Aldehyde compound	Antimicrobial, anti-inflammatory ¹⁶
13	Spiro[4.5]decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl	-	Anti-inflammatory activity ¹⁷
14	Oleic acid, 3-(octadecyloxy)propyl ester	Fatty acid	Antifungal ¹⁸ .
15	Tert-Hexadecanethiol	-	Enzyme activators ¹⁹ .
16	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Amino compound	Hemolytic, pesticide, flavor, antioxidant ²⁰
17	Eicosane	Alkane	Antifungal, antibacterial, antitumor, cytotoxic ²¹
18	9,12,15-Octadecatrienoic acid,2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)	Fatty acid	Antioxidant, antidiabetic, anti-inflammatory ²²
19	Oleic acid, 3-hydroxypropyl ester	Fatty acid	Antimicrobial and antioxidant ²³
20	6-Octadecenoic acid	Fatty acid	Anti-cancerous ²⁴
21	Octadecanoic acid, 2,3-dihydroxy propyl ester	Fatty acid	Food additive in dairy, surfactants, antiviral ²⁵
22	Diisooctyl phthalate	-	Antimicrobial and antifouling ²⁶

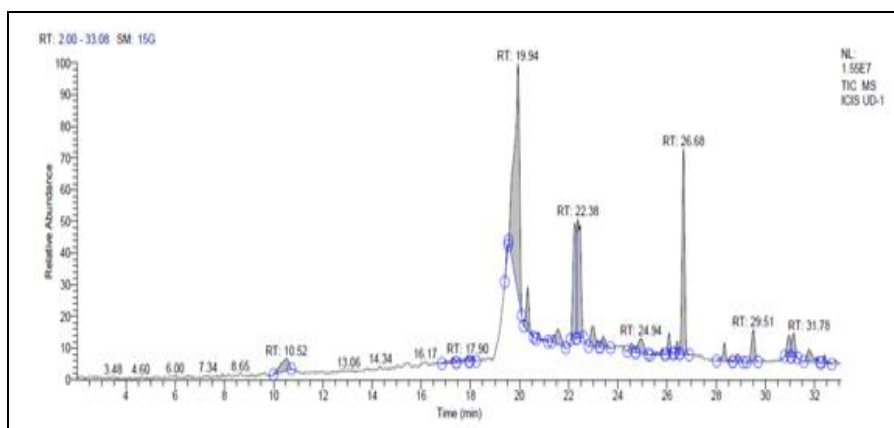


FIG. 1: GC-MS CHROMATOGRAM OF THE METHANOLIC LEAVES EXTRACT OF *U. DISTACHYA*

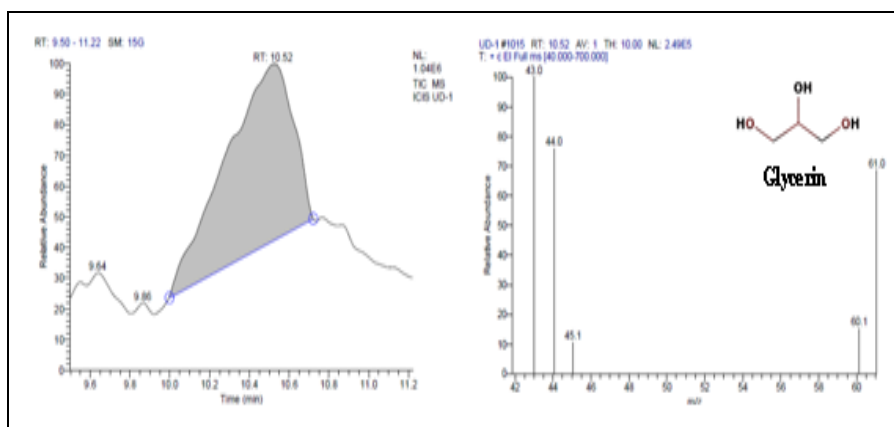


FIG. 2: GC-MS CHROMATOGRAM OF GLYCERIN (10.52)

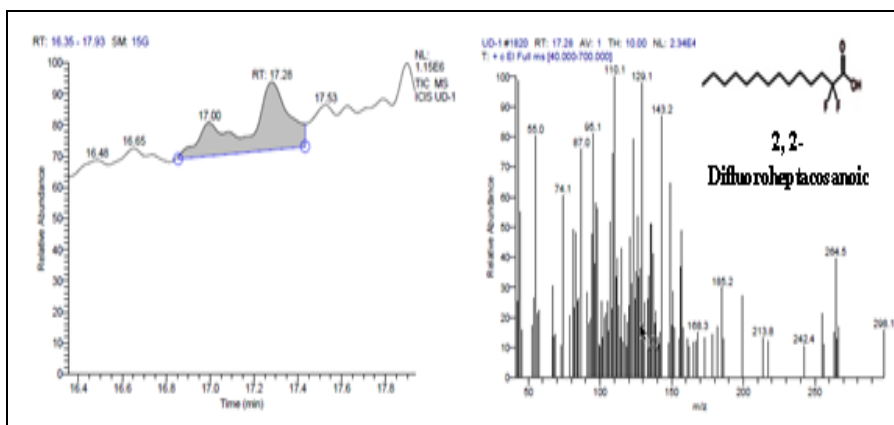


FIG. 3: GC-MS CHROMATOGRAM OF 2, 2-DIFLUOROHEPTACOSANOIC ACID (17.28)

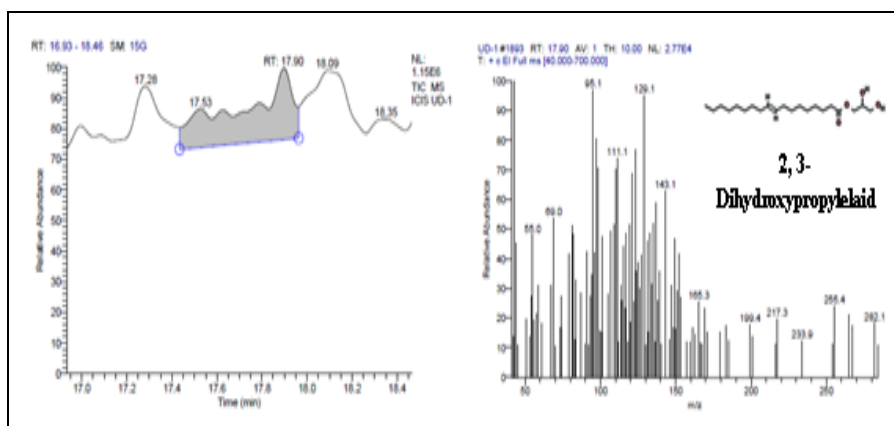


FIG. 4: GC-MS CHROMATOGRAM OF 2, 3-DIHYDROXYPROPYL ELAIDATE (17.90)

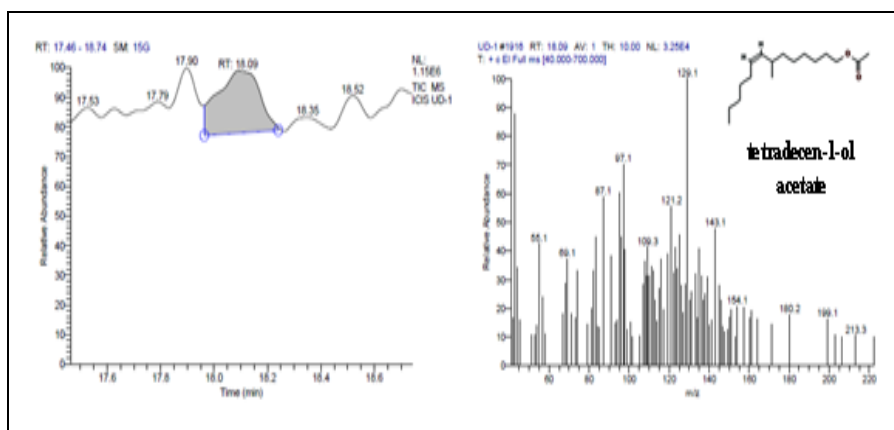


FIG. 5: GC-MS CHROMATOGRAM OF 7-METHYL-Z-TETRADECEN-1-OL ACETATE (18.09)

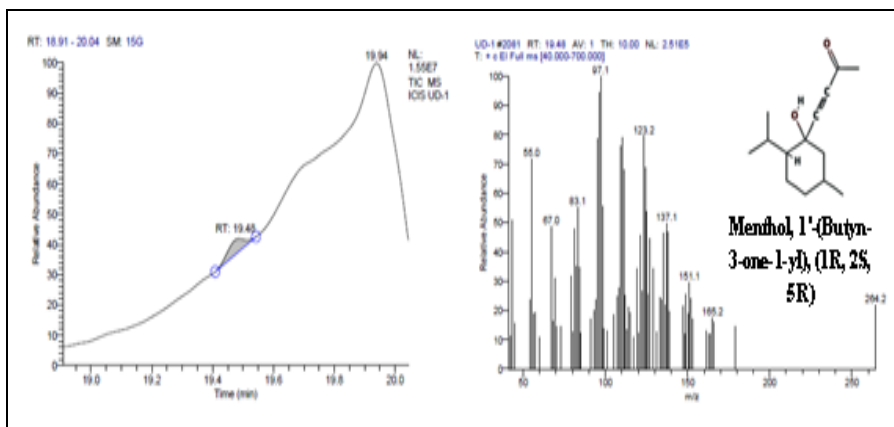


FIG. 6: GC-MS CHROMATOGRAM OF MENTHOL, 1'-(BUTYN-3-ONE-1-YL), (1R, 2S, 5R) (19.48)

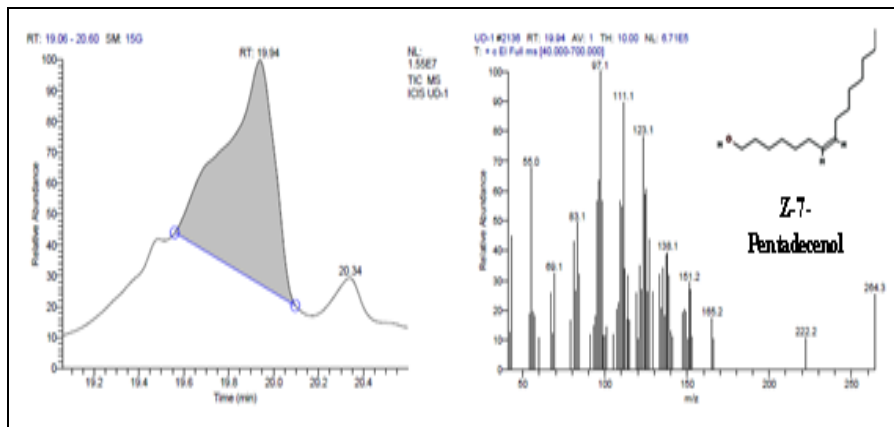


FIG. 7: GC-MS CHROMATOGRAM OF Z-7-PENTADECENOL (19.94)

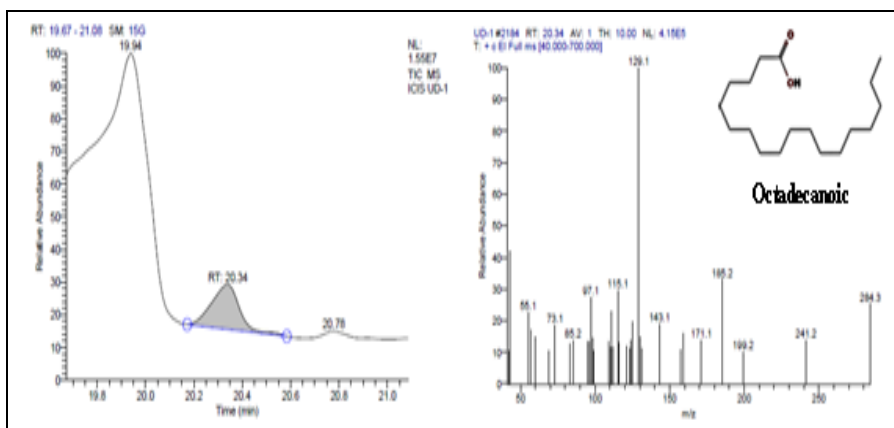


FIG. 8: GC-MS CHROMATOGRAM OF OCTADECANOIC ACID (20.34)

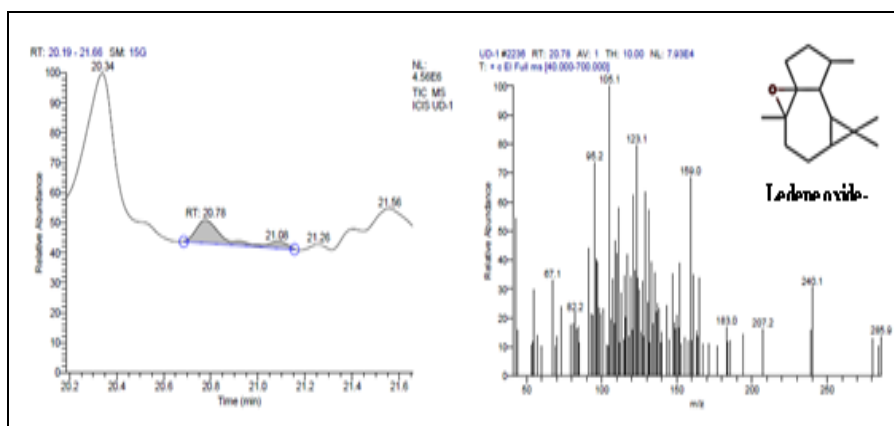


FIG. 9: GC-MS CHROMATOGRAM OF LEDENE OXIDE-(II) (20.78)

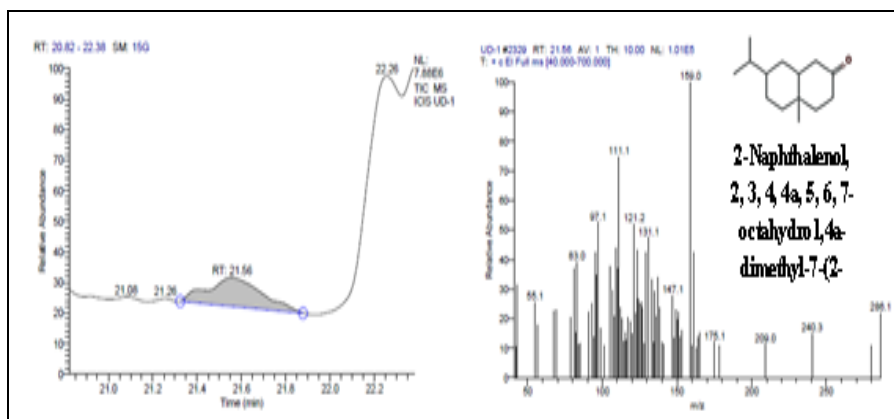


FIG. 10: GC-MS CHROMATOGRAM OF 2-NAPHTHALENOL, 2, 3, 4, 4A, 5, 6, 7-OCTAHYDRO1, 4A-DIMETHYL-7-(2-HYDROXY- 1-METHYL ETHYL) (21.56)

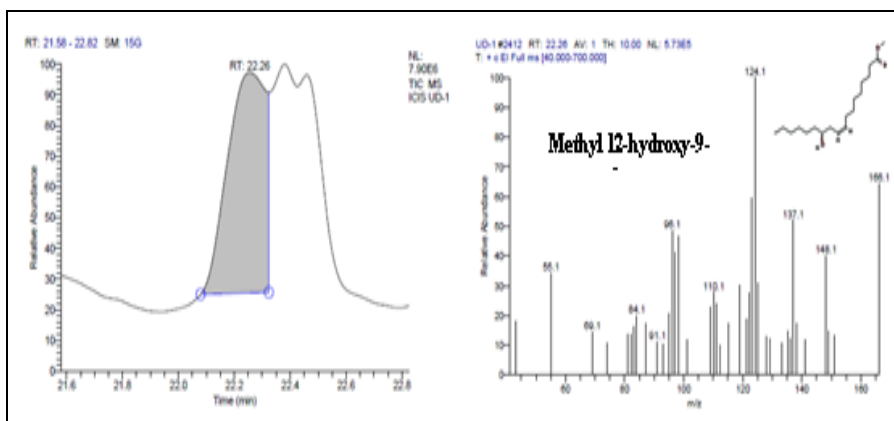


FIG. 11: GC-MS CHROMATOGRAM OF METHYL 12-HYDROXY-9-OCTADECENOATE (22.26)

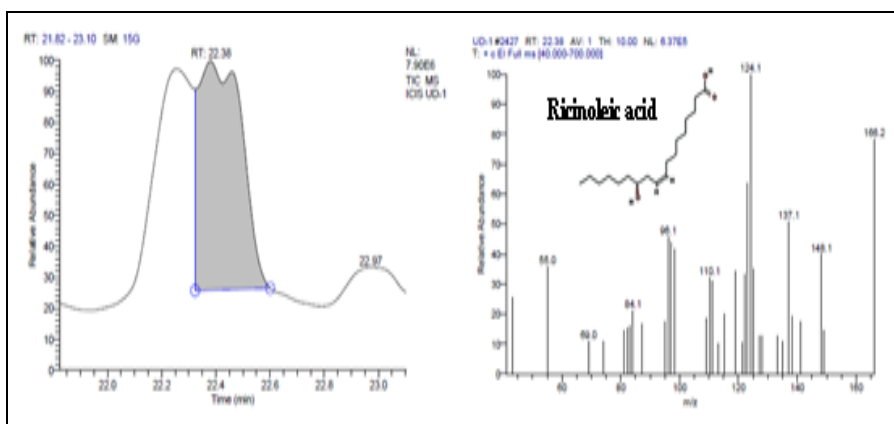


FIG. 12: GC-MS CHROMATOGRAM OF RICINOLEICACID (22.38)

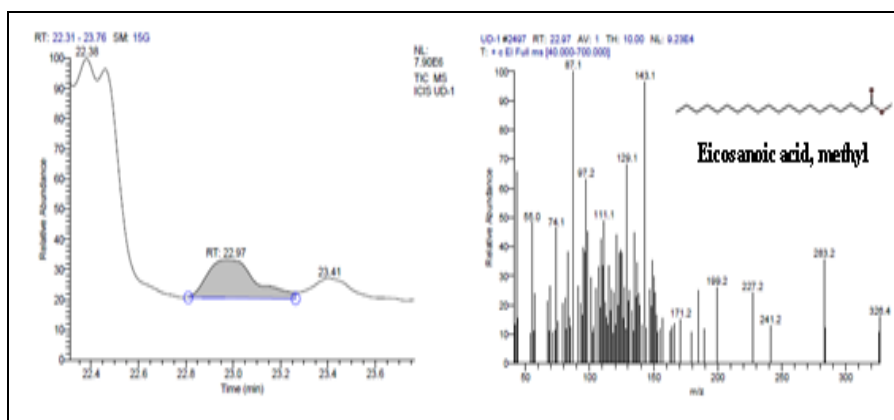


FIG. 13: GC-MS CHROMATOGRAM OF EICOSANOICACID, METHYL ESTER (22.97)

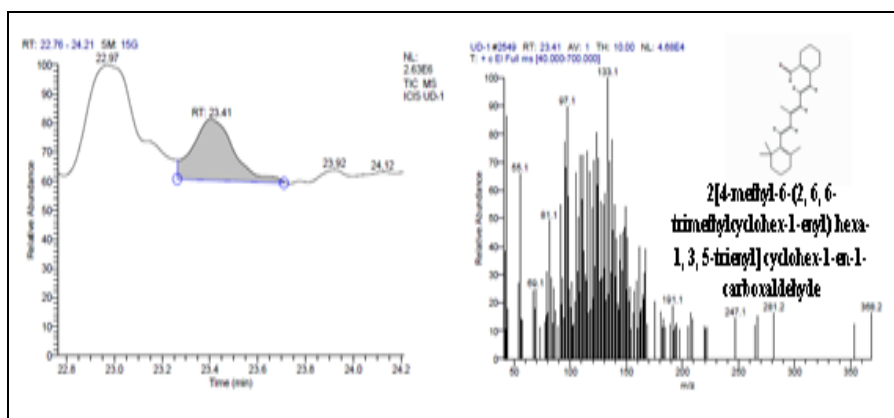


FIG. 14: GC-MS CHROMATOGRAM OF 2[4-METHYL-6-(2,6,6-TRIMETHYLCYCLOHEX-1-ENYL)HEXA-1,3,5-TRIENYL]CYCLOHEX-1-EN-1-CARBOXALDEHYDE (23.41)

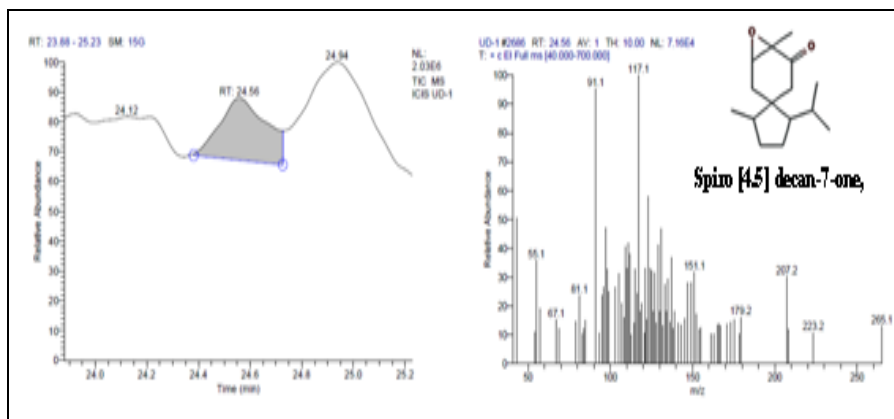


FIG. 15: GC-MS CHROMATOGRAM OF SPIRO [4.5] DECAN-7-ONE, 1,8-DIMETHYL-8,9-EPOXY-4-ISOPROPYL (24.56)

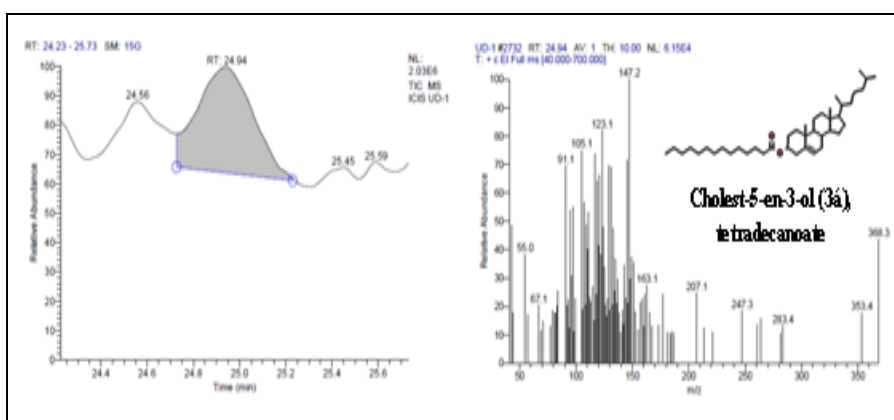


FIG. 16: GC-MS CHROMATOGRAM OF CHOLEST-5-EN-3-OL (3A), TETRADECANOATE (24.94)

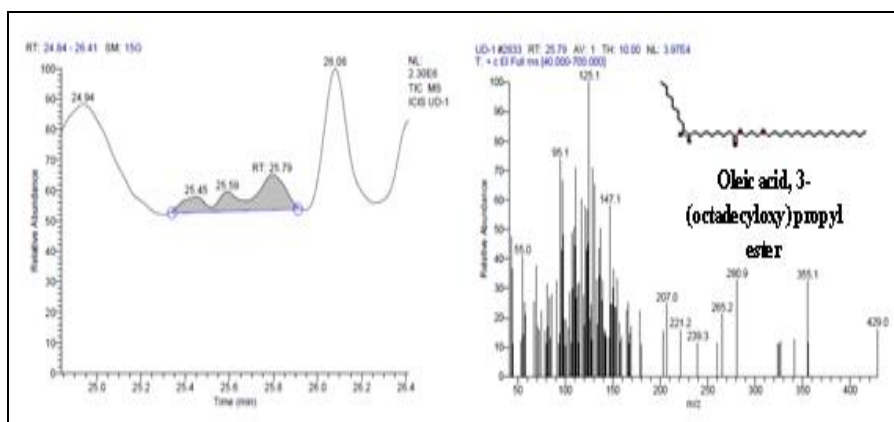


FIG. 17: GC-MS CHROMATOGRAM OF OLEIC ACID, 3-(OCTADECYLOXY) PROPYL ESTER (25.79)

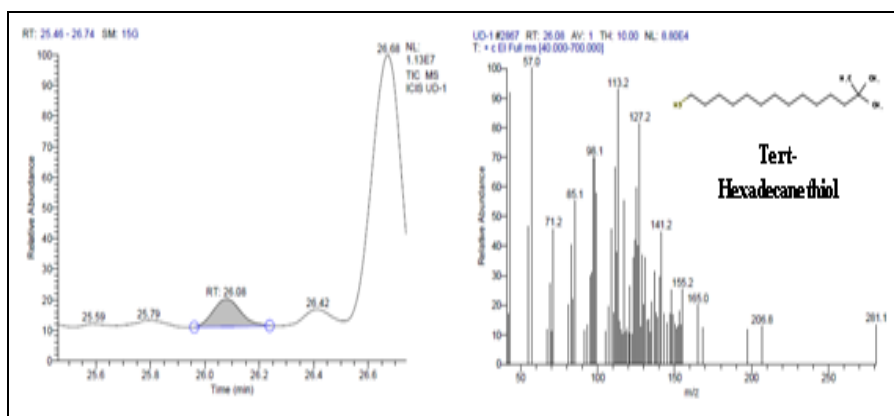


FIG. 18: GC-MS CHROMATOGRAM OF TERT-HEXADECANETHIOL (26.08)

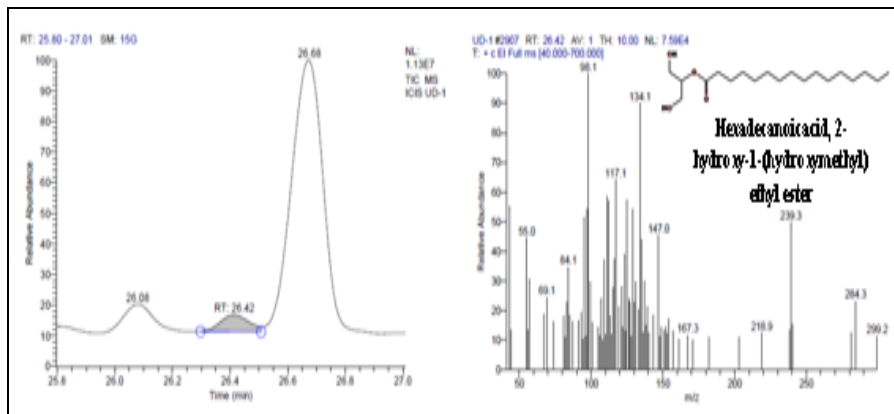


FIG. 19: GC-MS CHROMATOGRAM OF HEXADECANOICACID, 2-HYDROXY-1-(HYDROXYMETHYL) ETHYL ESTER (26.42)

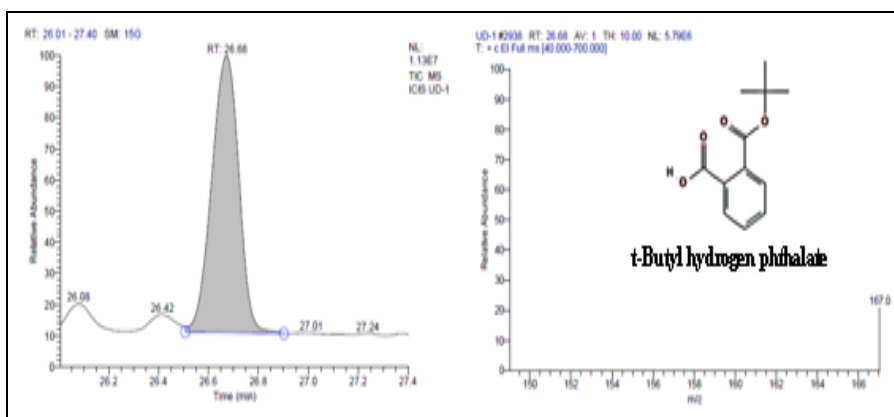


FIG. 20: GC-MS CHROMATOGRAM OF T-BUTYL HYDROGEN PHTHALATE (26.68)

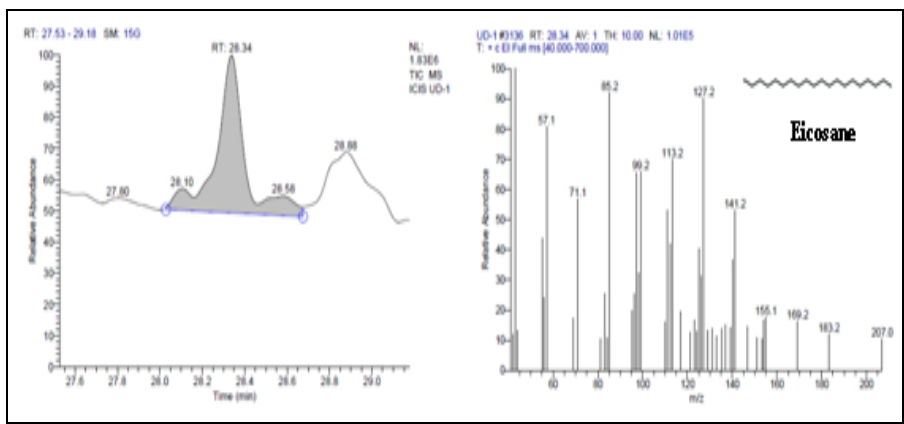


FIG. 21: GC-MS CHROMATOGRAM OF EICOSANE (28.34)

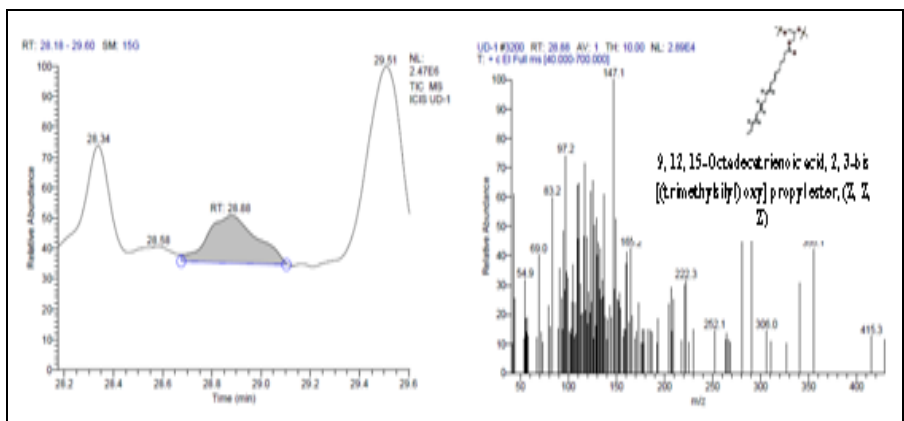


FIG. 22: GC-MS CHROMATOGRAM OF 9, 12, 15-OCTADECATRIENOIC ACID, 2, 3-BIS [(TRIMETHYLSILYL) OXY] PROPYL ESTER, (Z, Z, Z) (28.88)

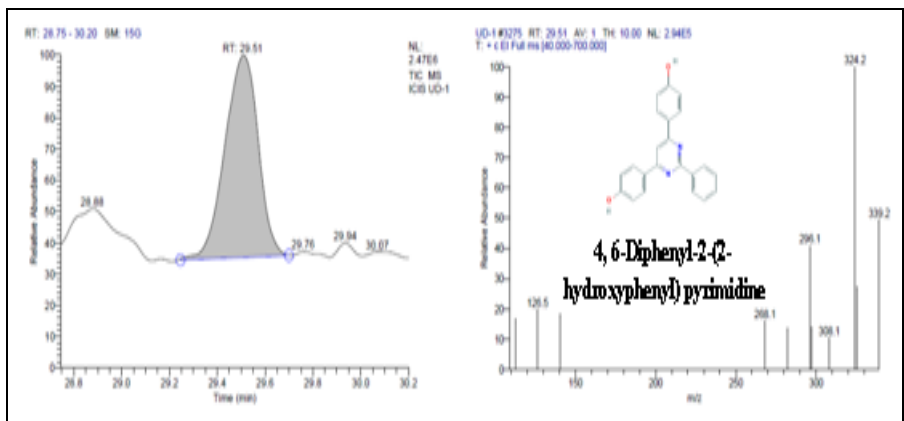


FIG. 23: GC-MS CHROMATOGRAM OF 4, 6-DIPHENYL-2-(2-HYDROXYPHENYL) PYRIMIDINE (28.88)

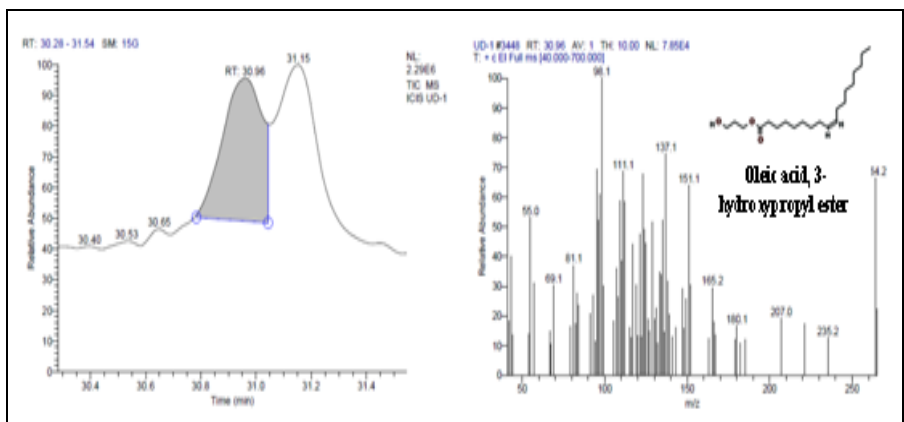


FIG. 24: GC-MS CHROMATOGRAM OF OLEIC ACID, 3-HYDROXYPROPYL ESTER (30.96)

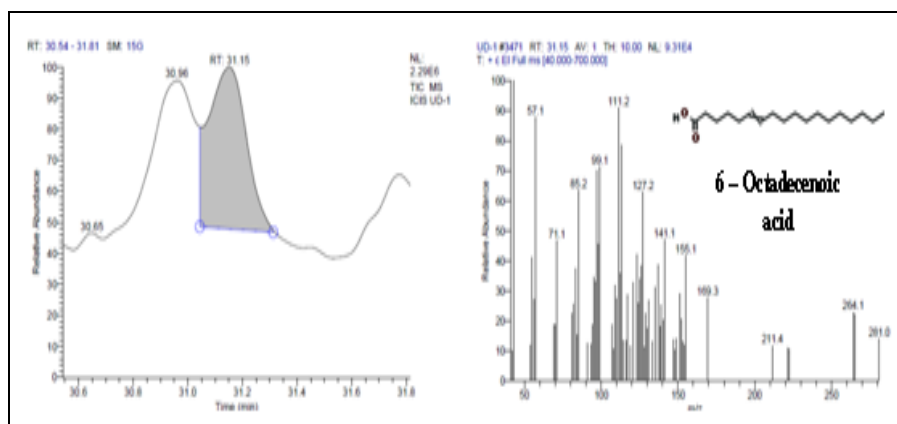


FIG. 25: GC-MS CHROMATOGRAM OF 6 – OCTADECENOIC ACID (31.15)

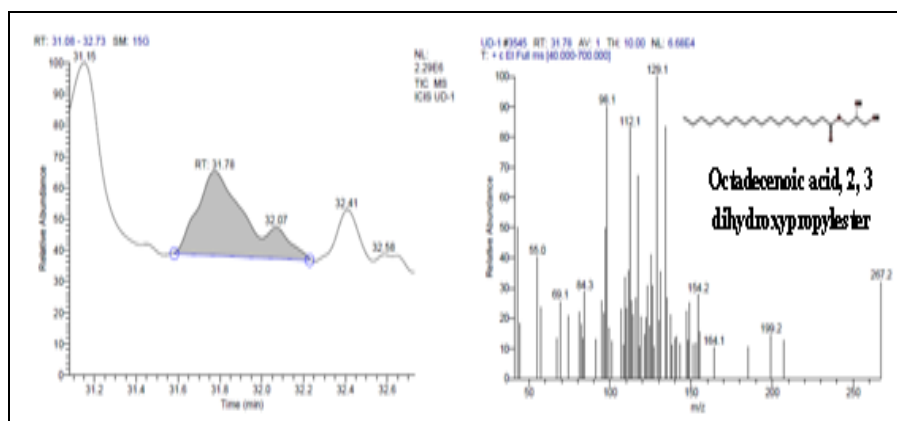


FIG. 26: GC-MS CHROMATOGRAM OF OCTADECENOIC ACID, 2, 3 DIHYDROXYPROPYLESTER (31.78)

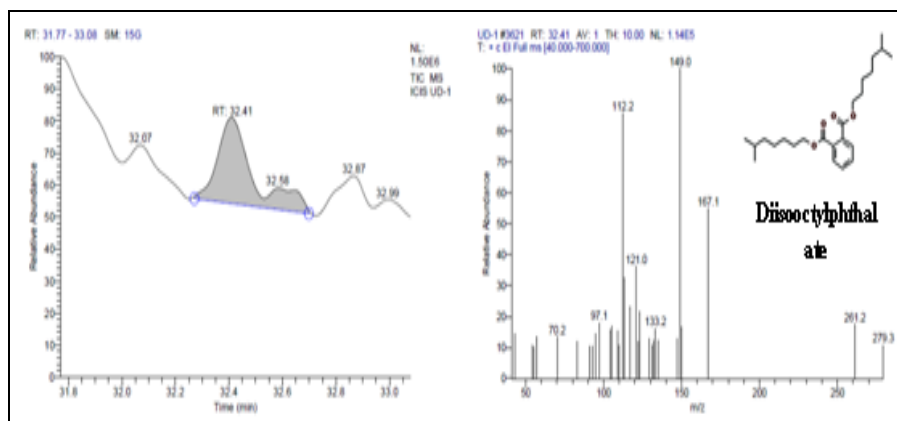


FIG. 27: GC-MS CHROMATOGRAM OF DIISOOCTYLPHTHALATE (32.41)

CONCLUSION: The present investigation concluded that the methanolic extract of leaves of *U. distachya* has several bio-active phyto-components which are responsible for various biological activities. The local tribals and Vaidyas are used in some diseases and disorders. So, further investigation and isolation of newer compounds will be helpful for the study of pharmacological activities.

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