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Research Article

**GC-MS analysis of ethanolic extract of *Amarantus
viridis* Linn.**

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ABSTRACT

Objective: To identify the chemical constituents of the entire plant powder of *Amarantus viridis* Linn.

Material; and Method; The dried powder of whole plant powder of *Amarantus viridis* were exhaustively by Soxhlet with ethanol extract. The prepared extract is also analysed by gas chromatography-mass spectrometry to identify and characterize the chemical compounds present in the extract.

Result: In this study, the bioactive compounds of the entire plant of ethanolic extract of *Amarantus viridis* was investigated using Gas Chromatography-Mass Spectroscopy analysis. Totally 30 chemical compounds were identified. Lupeyl acetate was found to be present as the major compound with peak area 66.88% and followed by Phytol (4.00%).

Keywords: GC -MS analysis, bioactive compounds, *Amarantus*, Ethanolic, Lupeyl acetate.

INTRODUCTION

Plants have been an important source of medicine for thousands of years. The rich resource is decreasing at an alarming rate as a result of over-exploitation. The medicinal value of plants is due to the presence of some chemical substances in the plant tissues which produce a definite physiological action on the human body. Very few of these chemicals are toxic also. Hence, preparation and administration of drugs should be done by experts only. Drugs may be obtained from various parts of the plant. So, an extensive study is required to detect the medical properties of the plant^{1,2} (Haraguchi *et al.*, 1999 and Sashikumar *et al.*, 2003). In general, secondary metabolites from plants were having interesting biological activities. These secondary metabolites are act as lead compound for new drugs because of its variety of structural arrangements and properties³ (de-Fatima *et al.*, 2006). Knowledge on the phytoconstituents of plants is desirable for the discovery of therapeutic agents, new sources of economic phytochemicals for the synthesis of complex chemical substances and for disclosing the actual significance of folkloric remedies⁴ (Milne 1993). The standardization of the natural drugs has emerged as a new branch of science as the phytochemicals have complementary and overlapping mechanism of action; hence a thorough validation of the herbal drugs was

emphasized and prioritized. Amaranthaceae family consists of about 180 genera and 2,500 species distributed mainly in cool temperate regions. This family represents the most species-rich lineage within the flowering plant order of Caryophyllales⁵ (Brown, 1810). *Amarantus viridis* is an annual herb and used as folk fore medicine as cooling, acrid, carminative, diuretic, urolithiasis and laxative. It promotes appetite, improves digestion. It is also used in calcium and vitamin A deficiency⁶ (Said *et al.*, 1986). The whole plant is used against burning sensation, dyspepsia, hemophilic conditions, urinary tract diseases and poisonous affections⁷ (Kurup *et al.*, 1979). The paste of the root is applied on scorpion sting. The plant use as potherb is in common practice in this area for the alleviation of heat from the body as well as in removing kidney and gall bladder stones. The plant is used as potherb and fodder to sheep and goats. Hence in the present study, the ethanolic extract of whole plant of *Amarantus viridis* was screened for GC-MS analysis.

MATERIALS AND METHODS

Collection and Preparation of Plant Extract:

The whole plant of *Amarantus viridis* Linn. was collected from Erode district, Tamil Nadu, India and

were authenticated and deposited at the PG and Research Department of Botany, Vellalar College for Women, Erode (Tamil Nadu), India. Fresh plants were collected and air-dried at room temperature and then homogenized to obtain coarse powder. The powder test plant was extracted⁸(Mukherjee, 2002) with the solvent ethanol by hot extraction using soxhlet apparatus, collected and stored in a vial for further analysis.

GC-MS Analysis:

Ethanol extract of whole plant of *Amarantus viridis* was analyzed for the presence of different volatile compounds by Gas chromatography-Mass spectroscopy (GC-MS) technique. GC-MS analysis of some of the plant extract was performed at The South India Textile Research Association (SITRA), Coimbatore (Tamil Nadu), India. Using a GC-MS (Model; Thermo Trace GC Ultra Ver.5.0) equipped with a DB-35MS fused silica capillary column (30m length X outside diameter 0.25 mm X internal diameter 0.25 µm) and gas chromatograph interfaced to a Mass Selective Detector (MS-DSQ-II) with XCALIBUR software. For GC-MS detection, an electron ionization system with ionization energy of -70eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1ml/min and the sample injected was 1µl; Injector temperature 250°C; Ion source temperature 200°C. The oven temperature was programmed from 70° to 200°C at the rate of 10°C/min, held isothermal for 1minutes and finally raised to 250°C at 10°C/min. Interface temperature was kept at 250°C. Total GC run time was 40.51 min. The relative percentage of each extract constituent was expressed as percentage with peak area normalization.

Identification of Components:

The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST⁹(Mc Lafferly, 1989), WILEY¹⁰(Stein, 1990) library sources were also used for matching the identified components from the plant material.

RESULT AND DISCUSSION

Since times immemorial medicinal plants have been nature's hidden and to a large extent unexplored pharmacy having been used virtually in all human cultures around the world as a source of safe and effective medicine. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases. The GC-MS analysis of the ethanolic extract of whole plant of *Amarantus viridis* revealed the presence of thirty bioactive compounds that could contribute to the medicinal value of the plant. GC and MS total running

time was 40.51minutes. The GC-MS chromatogram of the test plant is presented in Figure 1. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area are exhibited in Table 1. The structure and nature of the bioactive phytoconstituents uses are showed in Table 2. Some of the identified major components were Lupeyl acetate (66.88%), Lupeyl acetate (8.92%), Phytol (4.00%), 2, 6, 10, 14, 18, 22- tetracosahexaene, 2, 6, 10, 15, 19, 23- hexamethyl-, (all-E)- (3.55%), 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z) (3.20%), 1,5-Dichloro- 9, 10-bis (p- diphenyl)-9, 10- dihydroxyanthracene (2.83%), Hexadecanic acid (2.11%), Neophytadiene (1.18%), Lycoxanthin (1.10%) etc. The highest peak area percentage of 66.88% was obtained by lupeyl acetate (RT= 35.11min.) and lowest peak area percentage of 0.15% was obtained by hexadecane (RT= 8.48min.). Some of the pharmacologically important compounds like Hexadecanic acid, ethyl ester (0.39%), Hexadecanic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester (0.30%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol(0.34%), Benzene,(1-ethoxyethyl)-(0.34%), Chlorphen amine (0.20%) were also obtained. The identified phytochemical compounds have many biological properties.

For instance, Lupeyl acetate is one of the major compounds and noted for its potent antimicrobial, anti-inflammatory, anti-tumour, antiprotozoal, chemo preventive, skin cancer activity. The compound 9,12,15-Octadecatrienoic acid, (Z,Z,Z) is a linolenic acid is widely used as an anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocide, insectifuge, anti-histaminic, anti-eczemic, anti-acne, 5-alpha-reductase-inhibitor, anti-androgenic. Earlier reports that Velanganni and Kadamban (2011)¹¹ reported in the leaf of *Mallotus philippensis* and Anandhi and Pragasam (2013)¹² also reported in the methanolic extract of stem of *Tricalysia sphaerocarpa*.

Similarly, the presence of Phytol is a key acyclic diterpene compound which is reported to possess anti-microbial, anti-cancer, anti-inflammatory, diuretic agent¹³ (Praveen Kumar *et al.*, 2010) and precursor for vitamins E and K1¹⁴(Inoue *et al.*, 2005). Similarly, the presence of phytol was observed in the leaves of *Lantana camara*¹⁵ (Mariai *et al.*, 2011) and *Mimosa pudica*¹⁶(Sridharan *et al.*, 2011). Neophytadiene, a terpenoid compound has antipyretic, anti-inflammatory, anti-microbial and antioxidant activity¹⁷(Venkata Raman *et al.*, 2012). Correspondingly, ¹⁸Carretero *et al.* (2008) identified Neophytadiene in *Bursera simaruba* which were used as analgesic and vermifugic. Hexadecanoic acid, ethyl ester which is a palmitic acid compound found to be an antioxidant, hypocholesterolemic, nematocide, pesticide, lubricant activities and hemolytic

5-alpha is a reductase inhibitors. These results are strengthened by the findings of ¹⁹Sermakkani and Thangapandian (2012) who observed the presence of this compound in methanol extract of *Cassia italica* leaves. Pramitha and Sree Kumari (2016)²⁰ reported that GC-MS analysis revealed that ethyl acetate fraction of *Sargassum wightii* contains seventeen compounds and some of the major compounds detected were Bromoacetic acid, hexadecyl ester (94.98%), 1,4-Eicosadiene (87.16%), Eicosane (73.97%), 6-Octadecenoic acid, (Z)- (72.17%), n-Hexadecanoic acid (62.97%), Benzene, 1,2-dimethoxy-4-(1-propenyl)- (62.92%), Stigmasta-5,24(28)-dien-3-ol, (3.beta)- (61.06%), Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3(Phenyl methyl)- (56.62%) 2(1H)- Pyrimidinone, 4-amino-5-methyl- (41.97%) and 4-Methoxy-3Propoxy-Benzaldehyde (40.18%). *Cordia africana* Lam. (family- Boraginaceae) is a small to medium-sized evergreen tree, 4 to 15 (30) m high, heavily branched with a spreading, umbrella-shaped or rounded crown. Bole typically curved or crooked. Bark grayish-brown to dark brown, smooth in young trees, but soon becoming rough and longitudinally fissured with age; young branch lets with sparse long. Uses of *C. africana*: firewood, timber (furniture, beehives, boxes, mortars, church, drums), food (fruit), medicine (bark, roots), fodder (leaves), bee forage, mulch, soil conservation, ornamental, shade. Ethyl acetate fractions of *C. africana* leaves and stems were analyzed using Gas Chromatography Mass Spectroscopy. Emtinan *et al.*, (2016)²¹ observed that leaves ethyl acetate fraction showed the presence of forty eight compounds. The major compounds were 2-Hydroxy-4-methylbenzaldehyde (26.6%), Neophytadiene (17.2%), 9, 12, 15Octadecatrienoic acid, methyl ester (19.46 %), Pentadecanoic acid (14.01 %), 1, 2Benzenedicarboxylic acid, is (2-ethylhexyl) ester (7.86 %), and Octadecanoic acid (2.50 %). Stems ethyl acetate fraction showed thirteen compounds. Acetic acid, 2-methyl propyle ester (11.59 %), Butanoic acid, ethyl ester (0.84 %), Acetic acid, butyle ester(0.44 %), Butonic acid, 3-methyl-ethyl ester(0.88 %), 1-butanol,3-methylacetate 4.52, -butanol, 2-methyl acetate (4.52 %), Un known(0.35 %), Pentadecanoic acid 21.4, Octadec-9-penoic acid (7.31 %), Octadecanoic acid (5.74 %), 1,2-

Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (30.53 %) and unknown (5.36 %). Senthamil selvan and Velavan (2015)²² revealed the presence of various compounds like Tetradecanoic acid (19.658), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (20.921), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (21.144), Hexadecanoic acid, methyl ester (21.636), Oleic Acid (21.865), 1-(+)-Ascorbic acid 2,6-dihexadecanoate(22.057), 9-Octadecenoic acid (22.712), Andrographolide (22.947), Heptadecanoic acid (23.106), Octadecanoic Acid, methyl ester (23.817), 9,12-Octadecadienoic acid (24.552) and 22-Tricosenoic acid (26.836) in the methanolic extract of *Cissus vitiginea*. These findings support the traditional use of *Cissus vitiginea* in various disorders. Vinod *et al.*, (2016)²³ identified 62 phytochemicals were detected in the leaves of the six species analyzed: *Rumex dentatus* (17), *Achyranthes aspera* (26), *Alternanthera philoxeroides* (12), *Lantana camara* (20), *Erigeron bonariensis* (19) and *Sesbania bispinosa* (17). The major compounds detected were androstan-3-ol, 9-methyl-(3 beta, 5 alpha) (*R. dentatus*), 2-propenoic acid, 3phenyl-methyl ester, cinnamic acid methyl ester (*A. aspera*), benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester (*A. philoxeroides*), olean18-en-28-oic acid, 3-oxo-methyl ester methyl moronate (*L. camara*), 1-alpha-18O-1, 25-dihydroxycholecalciferol (*E. bonariensis*) and glaucic acid (*S. bispinosa*). The presence of various bioactive compounds justifies the use of entire plant various ailments by traditional practitioners.

CONCLUSION

From the present study, it was concluded that the plant *Amarantus viridis* are highly valuable in medicinal usage for the treatment of various human ailments along with clearly imply that the strength the chemical constituents present in it. Plants are important source of potentially useful compounds for the development of new chemotherapeutic agents.

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RT: 0.00 - 40.51 SM: 11G

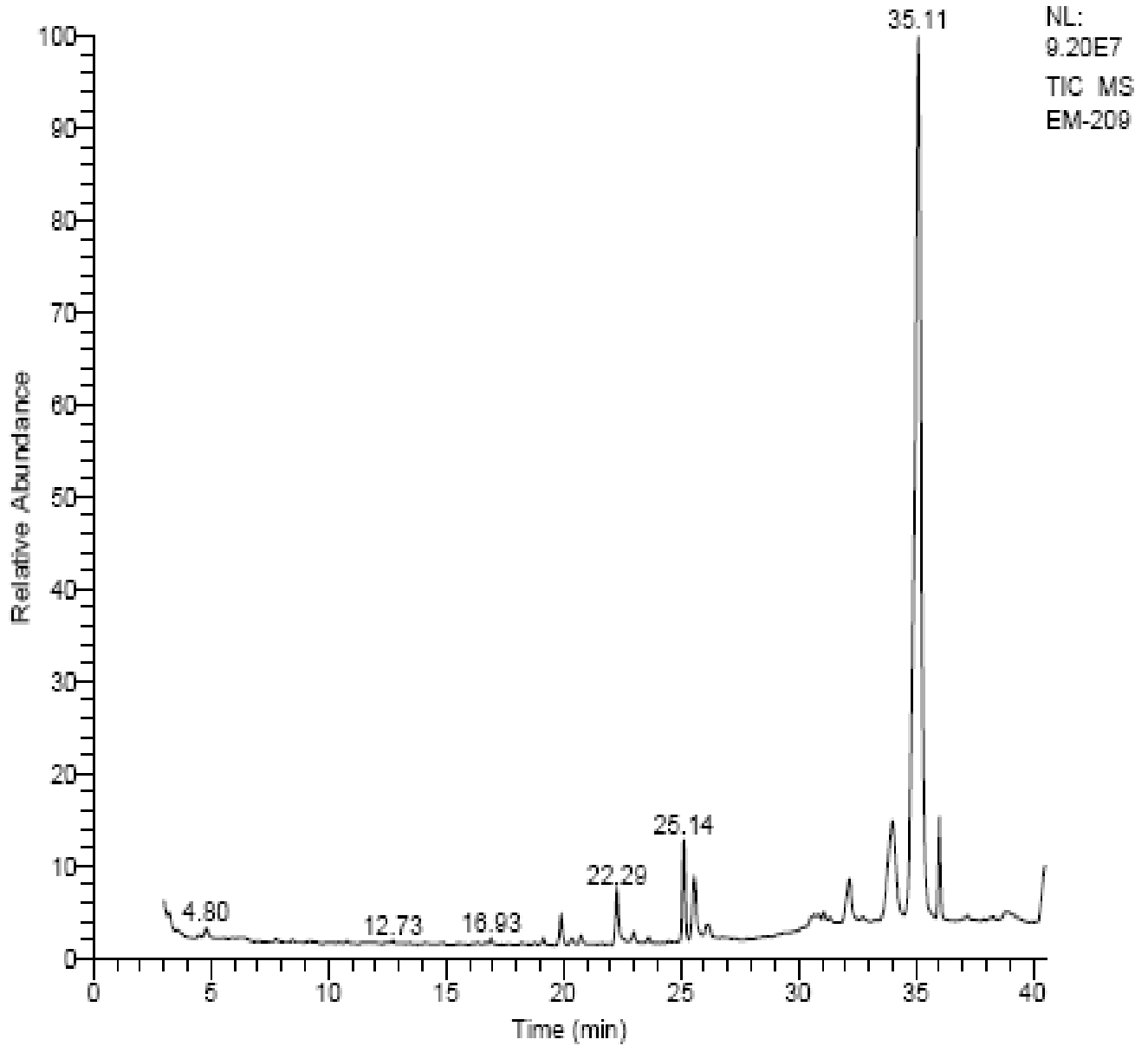
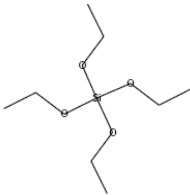
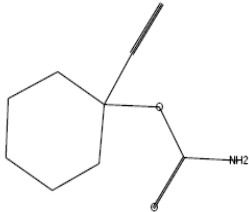
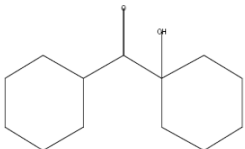








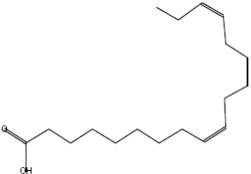

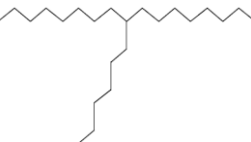


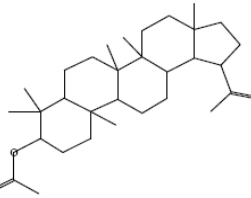
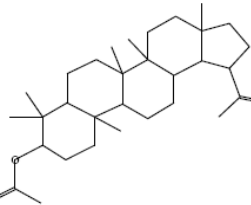
Figure 1
GC-MS Chromatogram of the ethanolic extract of entire plant of *Amarantus viridis* L.


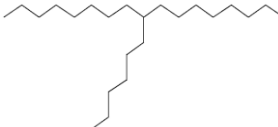
Table 1
Bioactive compounds identified in the ethanolic extract of entire plant parts of *Amarantus viridis* L. by GC-MS

S.No	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area%
1	3.24	Benzene, (1-ethoxyethyl)-	C ₁₀ H ₁₄ O	150	0.34
2	4.47	Silicic acid (H ₄ SiO ₄), tetraethyl ester	C ₈ H ₂₀ O ₄ Si	208	0.16
3	4.80	2-(2'-Nitro-2'-propenyl)-1-cyclohexanone	C ₉ H ₁₃ NO ₃	183	0.52
4	6.27	Cyclohexanol, 1- ethynyl-, carbamate	C ₉ H ₁₃ NO ₂	167	0.24
5	8.48	Hexadecane	C ₁₆ H ₃₄	226	0.15
6	9.26	1-(2',3':5',6'-di-o-ethyl-boranediy-l-a-d-manno furanosyl)-1,2,4-triazole	C ₁₂ H ₁₉ B ₂ N ₃ O ₅	307	0.24
7	12.73	Phenol, 4-(1,1-dimethylethyl)-2,6-dinitro-	C ₁₀ H ₁₂ N ₂ O ₅	240	0.15
8	16.93	Methanone, (1-hydroxycyclohexyl) phenyl –	C ₁₃ H ₁₆ O ₂	204	0.24
9	19.14	4,7-Methano-1H-indene,	C ₁₃ H ₁₈ O	190	0.23
10	19.90	Neophytadiene	C ₂₀ H ₃₈	278	1.18
11	20.39	3,7,11,15- Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	0.34
12	20.75	3,7,11,15- Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	0.34
13	22.29	Hexadecanic acid	C ₁₆ H ₃₂ O ₂	256	2.11
14	22.98	Hexadecanic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	0.39
15	23.64	Chlorphenamine	C ₁₆ H ₁₉ ClN ₂	274	0.20
16	25.14	Phytol	C ₂₀ H ₄₀ O	296	4.00
17	25.59	9,12,15- Octadecatrienoic acid, (Z,Z,Z)	C ₁₈ H ₃₃ O ₂	278	3.20
18	26.21	Ethyl linoleolate	C ₂₀ H ₃₆ O ₂	308	0.18
19	28.55	Heptadecane, 9-hexyl-	C ₂₃ H ₄₈	324	0.15
20	30.67	Lycoxanthin	C ₄₀ H ₅₆ O	552	1.10
21	31.08	Hexadecanic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	C ₁₉ H ₃₈ O ₄	330	0.30
22	32.16	1,5-Dichloro-9,10-bis(p-diphenyl)-9,10-dihydroxyanthracene	C ₃₈ H ₂₆ Cl ₂ O ₂	584	2.83
23	32.77	1,4-Bis(3,5-dibromo-2-thienyl)benzene	C ₁₄ H ₆ Br ₄ S ₂	554	0.17
24	34.01	Lupeyl acetate	C ₃₂ H ₅₂ O ₂	468	8.92
25	35.11	Lupeyl acetate	C ₃₂ H ₅₂ O ₂	468	66.88
26	35.99	2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(all-E)-	C ₃₀ H ₅₀	410	3.55
27	37.21	Heptadecane, 9-hexyl-	C ₂₃ H ₄₈	324	0.32
28	38.31	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-	C ₂₇ H ₅₄	378	0.23
29	38.82	Acetic acid, decyl ester	C ₁₂ H ₂₄ O ₂	200	0.43
30	40.45	Anodendroside-E2	C ₃₀ H ₃₈ O ₁₁	574	0.23

Table 2
The structure and nature of the bioactive phytoconstituents of ethanolic extract of entire plant parts of
***Amarantus viridis* L.**

S.No	RT	Name of the compound	Structure of Compound	Nature/Synonym	Bioactive/ Uses
1	4.47	Silicic acid (H ₄ SiO ₄), tetraethyl ester		Tetra ethyl ester, Silicic acid, Silane, Ethyl silicate	Carcinogenic
2	6.27	Cyclohexanol, 1-ethynyl-, carbamate		1-Ethynylcyclohexyl carbonate, Carbamic acid, 1-ethynylcyclohexyl ester	Anti-inflammation
3	16.93	Methanone, (1-hydroxycyclohexyl) phenyl –		1-Hydroxycyclohexyl phenyl ketone, Iegacure 184, 1-Hydroxycyclohexyl-1-phenyl methanone	Anti-cancer
4	19.90	Neophytadiene		2,6,10-trimethyl,14-ethylene-14-pentadecene	Antiproliferative
5	20.39	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		Terpene alcohol, Phytol, Diterpene	Cancer preventive, Anti-inflammatory, Fragrance compound, Anti-microbial
6	20.75	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		Terpene alcohol, Phytol, Diterpene	Cancer preventive, Anti-inflammatory, Fragrance compound, Antimicrobial
7	22.29	Hexadecanic acid		Palmitic acid, Fatty acid, White crystal	Antioxidant, Pesticide, Flavor, 5-Alpha-eductase-inhibitor, Antifibrinolytic, Hemolytic, Lubricant, Nematicide, Antiallopecic, Hypocholesterolemic, Anti-inflammatory, Anti-bacterial
8	22.98	Hexadecanic acid, ethyl ester		Palmitic acid, ester compound	Antioxidant, Hypocholesterolemic, Nematicide, Flavor, pesticide, Antiandrogenic, Hemolytic, 5-Alpha-Reductase- inhibitor, Lubricant

9	25.14	Phytol		Transphytol; 3,7,11,15-tetramethyl-2-hexadecene-1-ol; Diterpene	Anti-cancer, Antioxidant, Anti-inflammatory, Cytotoxicity, Diuretic, Anti-microbial, Cancer preventive
10	25.59	9,12,15-Octadecatrienoic acid, (Z,Z,Z)		Linolenic acid	Anti-inflammatory, Hypochloesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Nematicide, Antihistaminic, Antieczemic, Antiacne, 5-Alpha-Reductase- inhibitor, Antiandrogenic, Anticoronary
11	26.21	Ethyl linoleolate		Mandenol, rein, Ethyl linolate, ethyl ester fatty acid	Anti-microbial
12	28.55	Heptadecane, 9-hexyl-		9-n-Hexylheptadecane, Heptadecane	Carminative
13	30.67	Lycoxanthin		All-trans-Lycoxanthin; Rhodoxanthin; Aromatic or Aliphatic	Anti-cancer
14	31.08	Hexadecanic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester		Glycerol 1-Palmitate; Hexadecanic acid	Cytotoxicity; Anti-viral
15	34.01	Lupeyl acetate		Triterpenoid	Anti-microbial, Anti-inflammatory, Anti-tumour, Antiprotozoal, Chemopreventive, Skin cancer
16	35.11	Lupeyl acetate		Triterpenoid	Anti-microbial, Anti-inflammatory, Anti-tumour, Antiprotozoal, Chemopreventive, Skin cancer

17	35.99	2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(all-E)-		Squalene; Organic compound	Anti-bacterial, Antioxident, Anti-Tumor, Anti-inflammatory, Hypocholesterolemic, Immunostimulant
18	37.21	Heptadecane, 9-hexyl-		9-n-Hexylheptadecane, Heptadecane	Carminative

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