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Gas Chromatography-Mass Spectrometry (GC-MS) Determination of Bioactive Components from *Corallocarpus epigaeus* L Rhizome.

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Abstract

Corallocarpus epigaeous L. plant belongs to the family *Cucurbitaceae* is widely distributed in tropical Africa, Persian Gulf region and India (Andhra Pradesh, Assam, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, and West Bengal). Ethno medical information suggests that the roots are used as Laxative and antihelminthic. Syphilitic rheumatism, Venereal complaints, and later stages of dysentery. Yet no further characteristic study has been conducted from alcoholic extract of this species, therefore in this present study we seek to identify and evaluate the bioactive compounds from the ethanolic extracts of *Corallocarpus epigaeus* L (root) by using the GCMS. Result of this research work reports twenty-six compounds. The identified bioactive components and correlated with the NIST Mass Spectrum Library. In conclusion, we seek to provide additional information and pharmacological information associated with this rhizome.

Keywords

Corallocarpus epigaeus L, GC-MS, Traditional uses, Indolizine, Nonadecanoic acid

Introduction

Corallocarpus Epigaeus L. belongs to the family *Cucurbitaceae* is commonly known as Akasgaddah in Hindi and Akashagaruda in Tamil, and is distributed in tropical and temperate regions of India, Ceylon, Deccan, and South Maratha country.[1] In folk medicine this rhizome is especially used for the treatment of various ailments, including, lateral stage of dysentery, enteritis, laxative, rheumatism, syphilis and venereal complaints. It is used as a remedy for snake bites. [2,1] In this current research work, we attempt to compile and document information from the alcoholic extract of the whole plant, using GC / MS to evaluate and identify bioactive compounds to demonstrate the biological properties required for the research purpose.

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Materials and Methods

Raw materials

Dried rhizomes of *Corallocarpus epigaeus* L. were collected from Kancheepuram district, Tamil Nadu, India. The plant materials were authenticated based on organoleptic, macroscopic examination and certified (Authenticated No. PARC/2021/4532) by Professor P.Jayaraman, Director, IHB, Plant Anatomy Research Centre, Tambaram, Chennai-45, India.

Formation of Ethanolic extract:

Extracts were prepared as described by the standard method.[3] Initially, the collected rhizomes were chopped into tiny bits and allowed to dry for a few days in a sunshade so that the muddy portions were removed. Then the dried materials were roughly grinded by using a mixture. 50g of powdered material was soaked with 300ml of ethanol for 72 hours with intermediate shaking in a beaker. The filter was filtered through paper and extracted with the Soxhlet apparatus. The last traces of solvent were removed by transferring them into a china dish and then allowed to heat through a sand bath at normal temperature carefully in order to prevent charring or denature of the compound due to overheating. [4,5] The yield of ethanolic extracts (1.5mg) was noted for future reference. The dried crude extract was stored in sterile amber-colored bottles or vials and stored in the refrigerator until used for this work.

GC-MS:

Gas-chromatography and Spectrometry are to recognize diverse materials present in the unknown samples. It includes diagnosis of drugs, unidentified specimens, fire investigation and environmental analysis.[6] GC-MS-a combination of Gas Chromatography and Mass Spectrometry, is used to analyze complex organic and biochemical mixtures.[7] The sample is then injected into the column and the carrier gas helium is used to flow at a rate of 1ml/minute. The injector is operated to inject the sample into the column at an injection mode of 10°C/minute. The oven temperature is programmed at 50-250°C. The ionization voltage of 70eV for ions and the mass range of 50-600 units of mass are used for chromatographic conditions. The National Institutional Standards and Technology (NIST) database of over 62,000 formats was used to describe the compounds isolated by GC-MS. Isolated components were then compared with the known mass spectrum of the NIST library.

Results

Table 1: Isolation and identification of active principle compounds from alcoholic extract from the root of *Corallocarpus epigaeus* L. based on Retention time, Molecular formula, and Molecular weight by using (GC/MS).

SNo	Isolated Compound	Empirical formula	Retention time	MW g/mol
1	3-Dimethylsilyloxypentadecane	$C_{17}H_{38}OSi$	5.986	286
2	O-Toluidine	C_7H_9N	7.463	107
3	Glycerin,	$C_3H_8O_3$	8.560	92
4	Acetic acid, 3-acetoxy-1-ethyl-2-nitrobutyl ester,	$C_{10}H_{17}NO_6$	8.945	247
5	2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester	$C_7H_{12}O_3$	9.429	144
6	2H-Pyran-2-one, 5,6-dihydro-6-pentyl-	$C_{10}H_{16}O_2$	10.345	168
7	Indolizine	C_8H_7N	11.025	117
8	Butanoic acid, 2-(hydroxymethyl)-3-methyl-, ethyl ester, (R)-	$C_8H_{16}O_3$	12.164	160.
9	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	13.411	206
10	2H-Pyran-2-one, tetrahydro-4-hydroxy-6-pentyl-	$C_{10}H_{18}O_3$	13.933	186
11	3-Hexadecene, (Z)-	$C_{16}H_{32}$	14.449	224
12	Ethyl α-d-glucopyranoside	$C_8H_{16}O_6$	15.905	208
13	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	17.184	310
14	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	17.298	296
15	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	17.458	270

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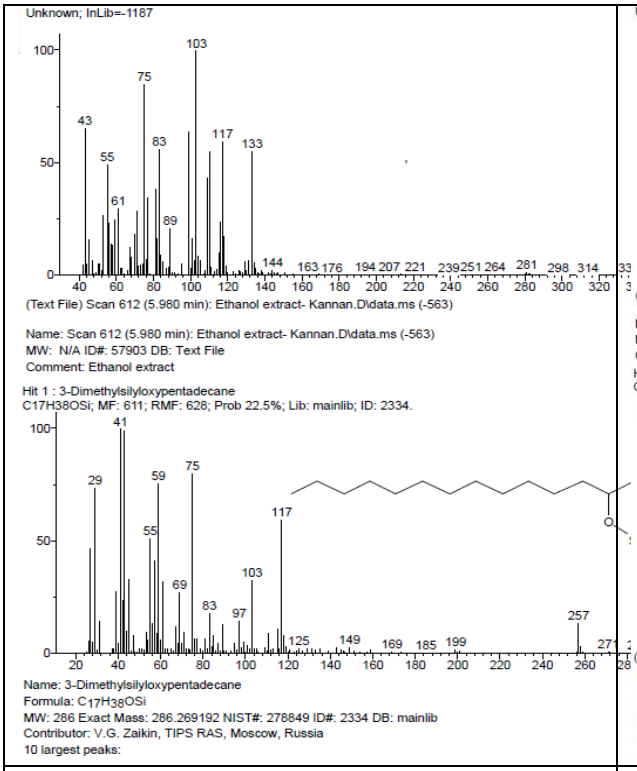
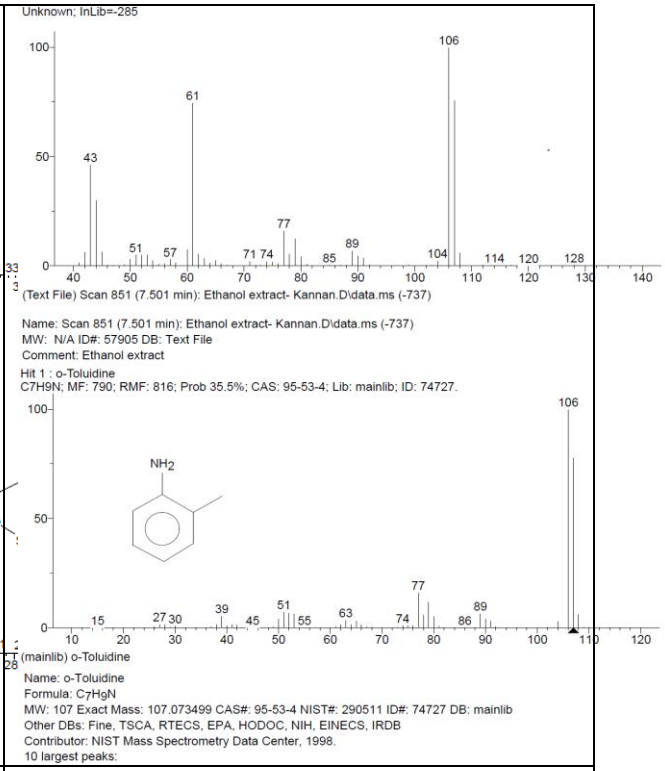
16	cis-10-Heptadecenoic acid	$C_{17}H_{32}O_2$	19.207	268
17	Heptadecanoic acid	$C_{17}H_{34}O_2$	19.360	270
18	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	21.010	340
19	Nonadecanoic acid, ethyl ester	$C_{21}H_{42}O_2$	21.359	326
20	Vitamin E	$C_{29}H_{50}O_2$	21.631	430
21	Methyl 19-methyl-eicosanoate	$C_{22}H_{44}O_2$	22.554	340
22	2-Hydroxy-4-methoxy-7-methyl-7,8,9,10,11,12,13,14-octahydro-6-oxabenzocyclododecen-5-one	$C_{17}H_{24}O_4$	24.507	292
23	2-Hydroxy-4-methoxy-7-methyl-7,8,9,10,11,12,13,14-octahydro-6-oxabenzocyclododecen-5-one	$C_{17}H_{24}O_4$	24.621	292
24	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	25.862	368
25	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	28.209	436
26	9,12-Octadecadienoic acid (Z, Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	28.782	354



Compound No 1. Name: 3-Dimethylsilyloxypentadecane



Compound No 2: Name: O-Toluidine

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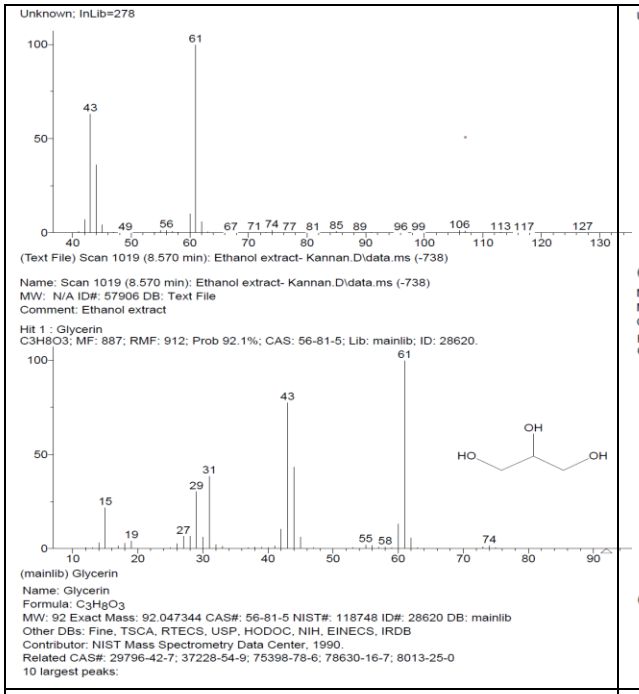
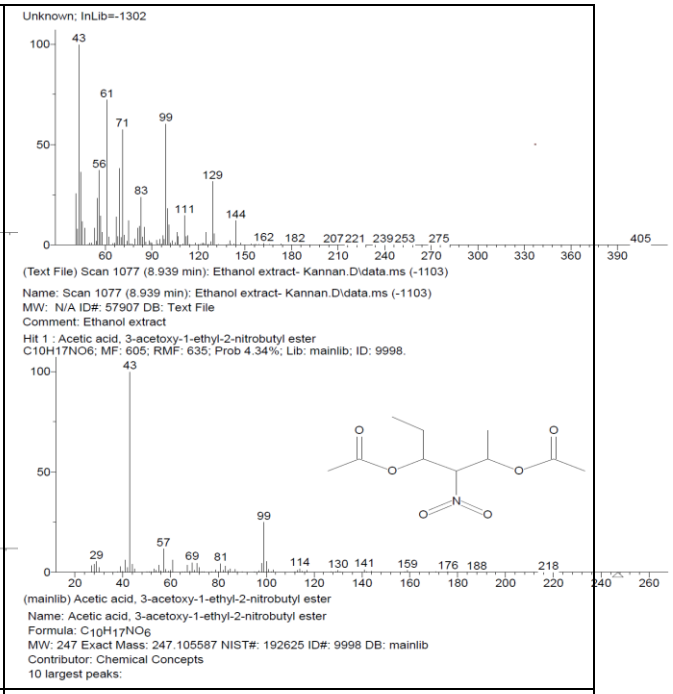
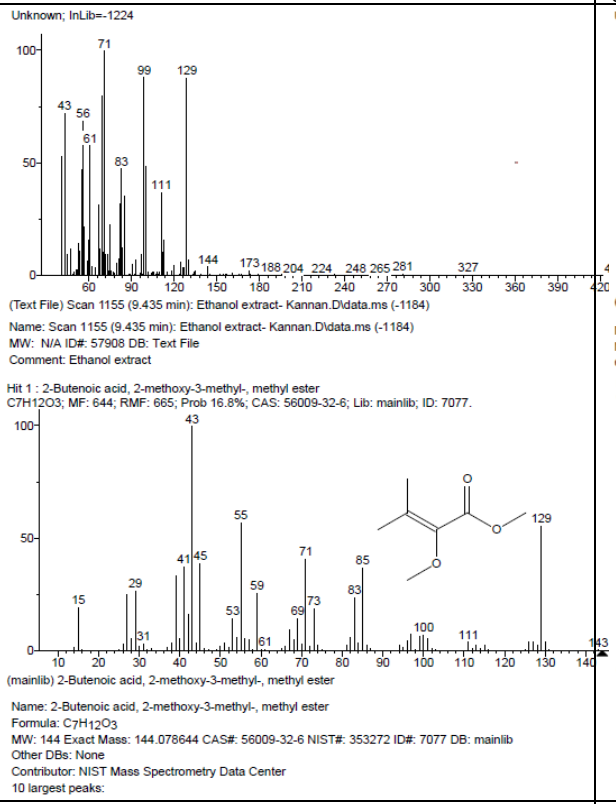
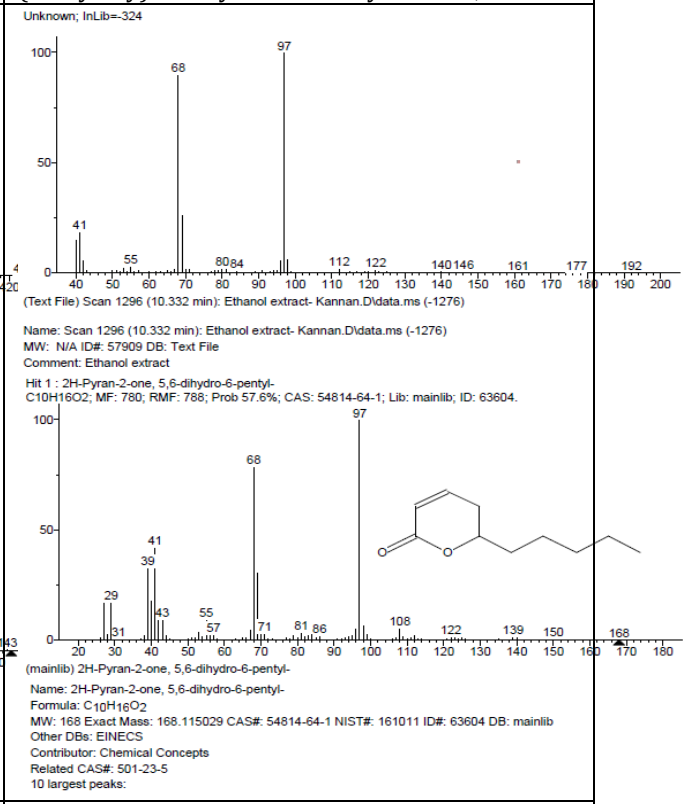




Compound No 3: Name: Glycerin,

Compound No 4: Name: Acetic acid, 3-acetoxy-1-ethyl-2-nitrobutyl ester, Synonyms: 1.3-(Acetyloxy)-1-ethyl-2-nitrobutyl acetate,





Compound No 5: Name: 2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester

Compound No 6: Name: 2H-Pyran-2-one, 5,6-dihydro-6-pentyl-

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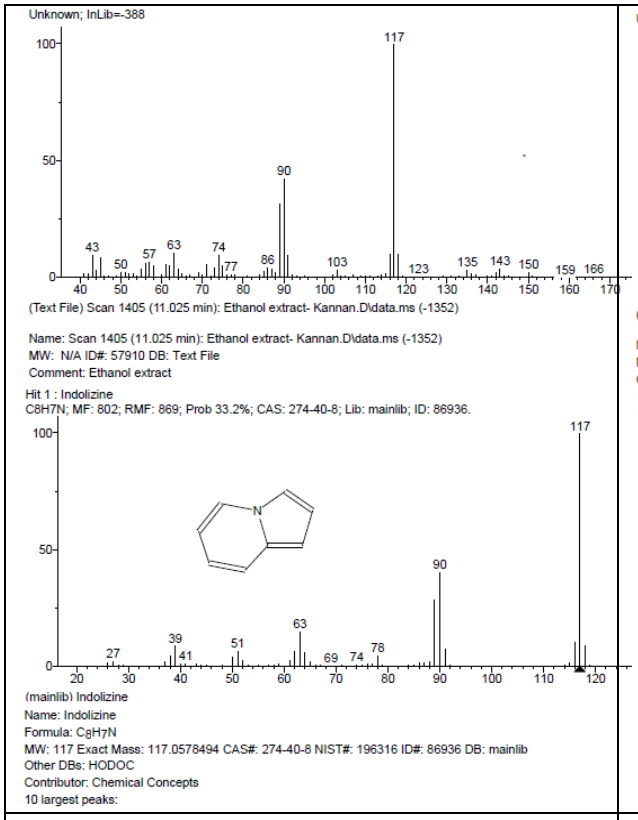
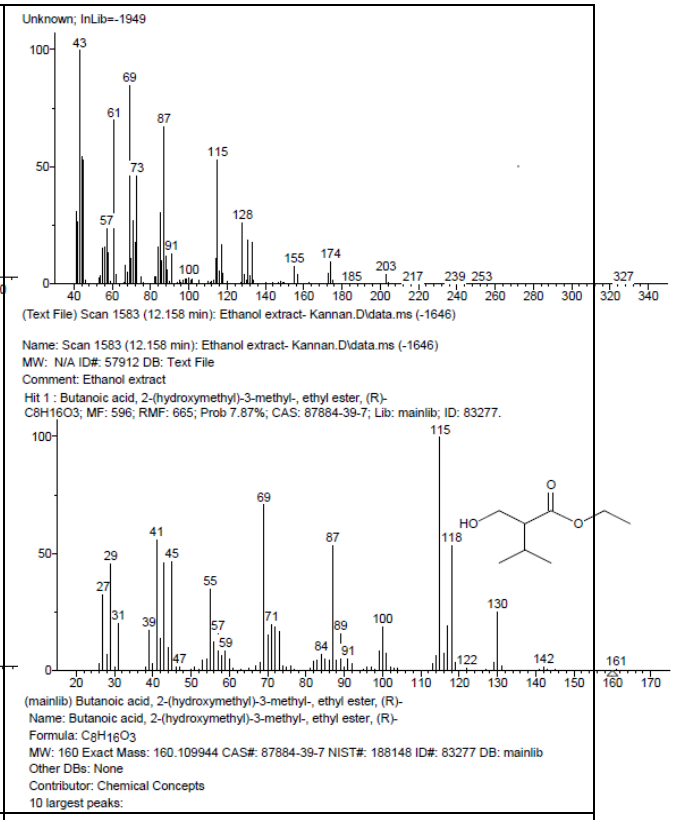
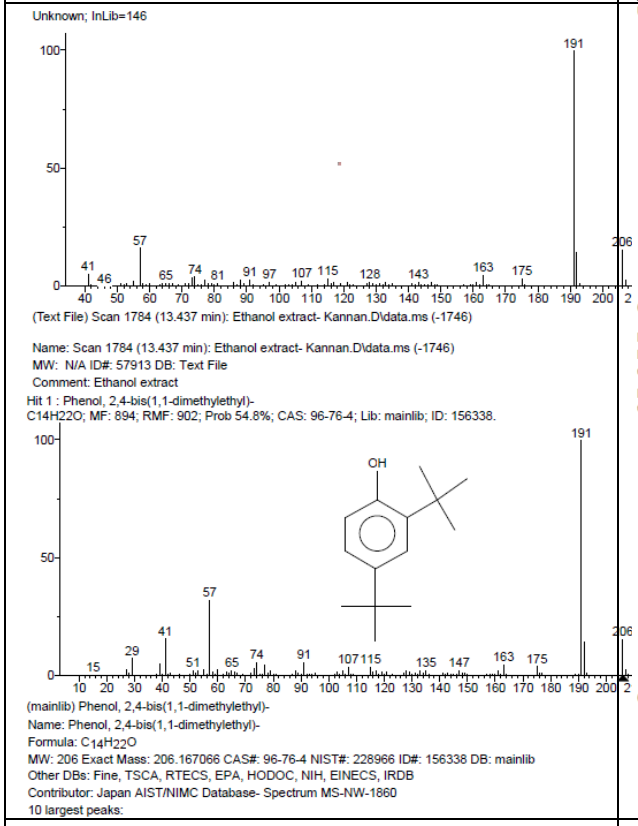


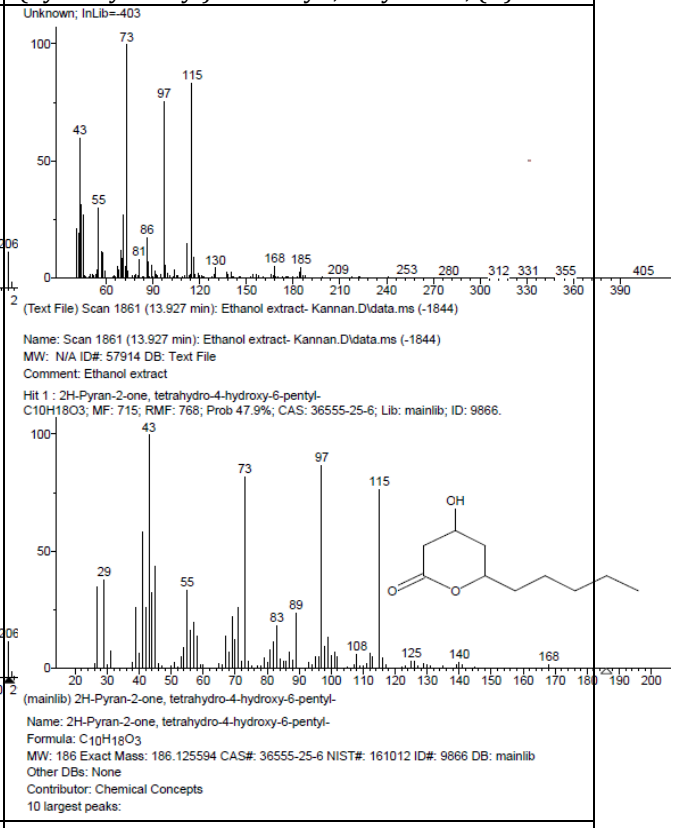
Compound No 7: Name: Indolizine



Compound No 8: Name: Butanoic acid, 2-(hydroxymethyl)-3-methyl-, ethyl ester, (R)-



Compound No 9: Name: Phenol, 2,4-bis(1,1-dimethylethyl)-



Compound No 10: Name: 2H-Pyran-2-one, tetrahydro-4-hydroxy-6-pentyl-

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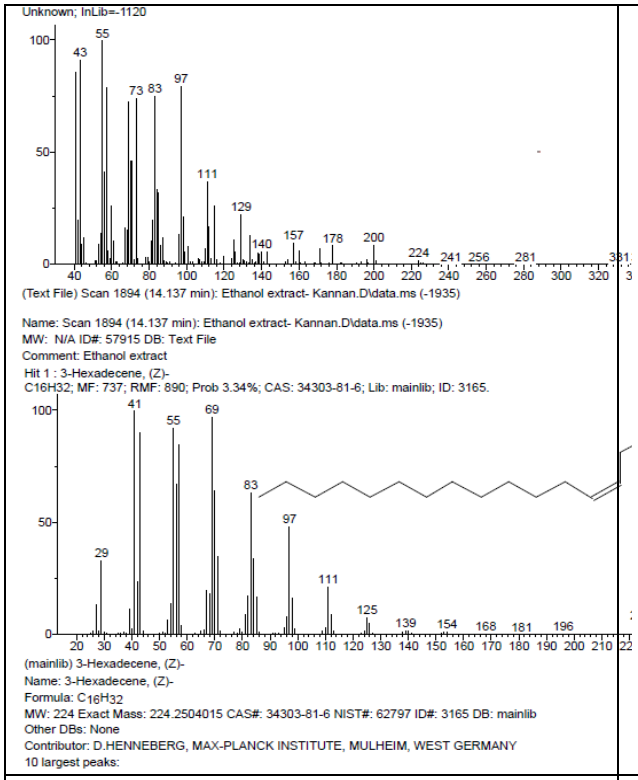
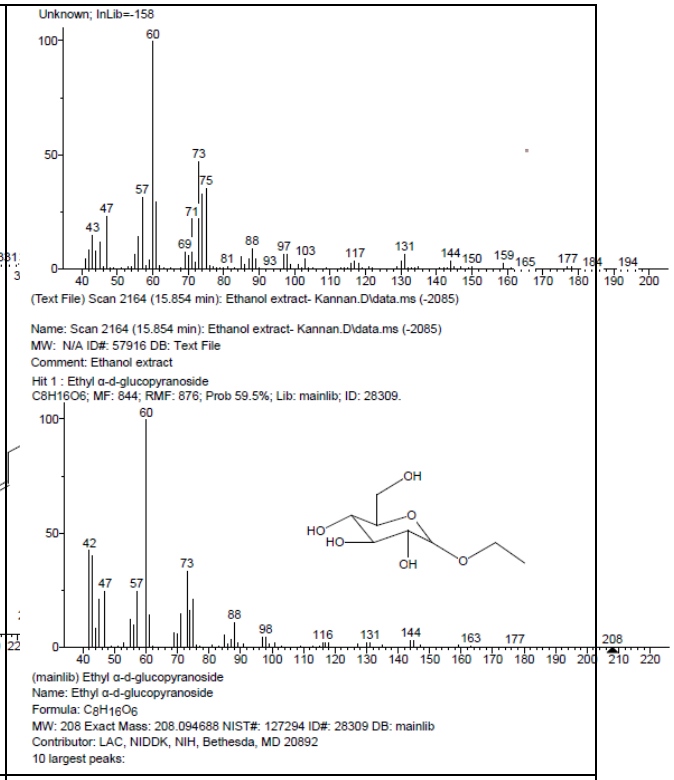
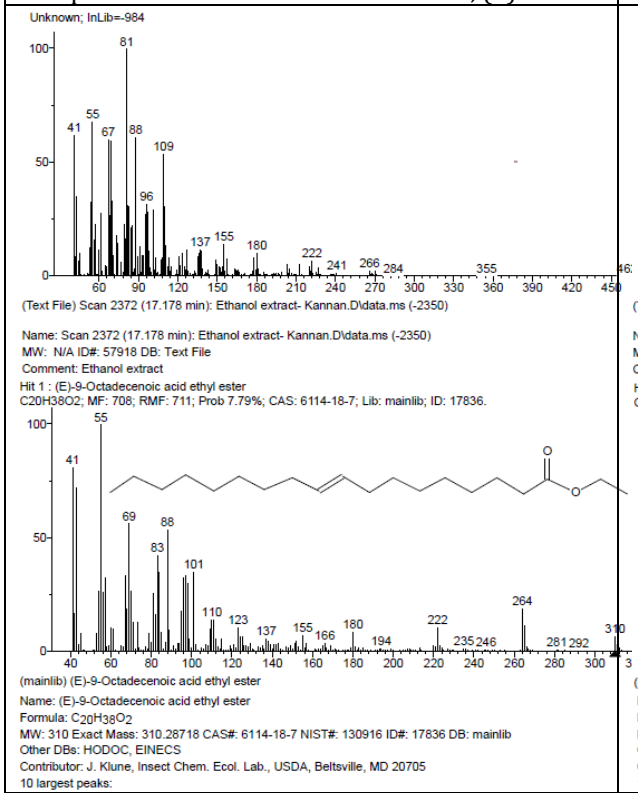


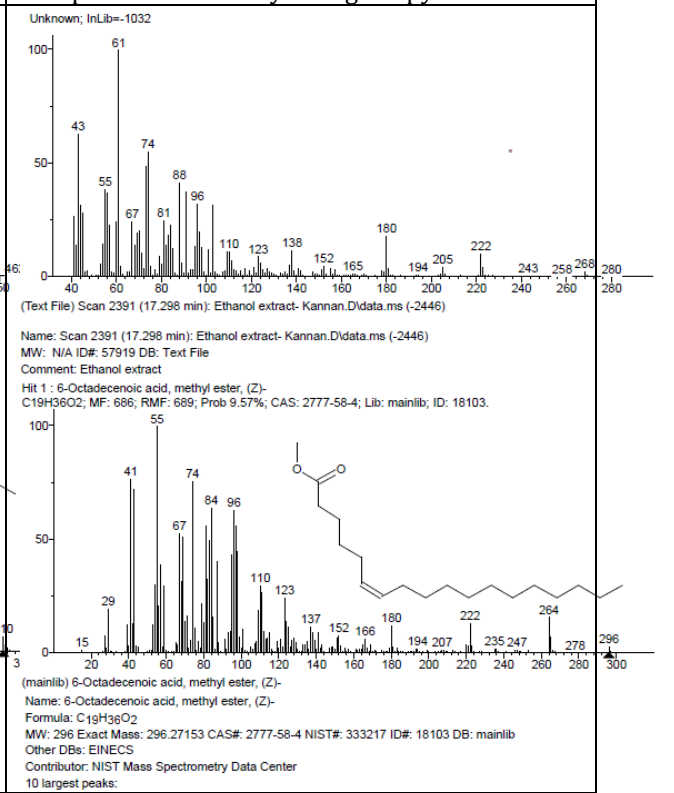
Compound No 11: Name: 3-Hexadecene, (Z)-



Compound No 12: Ethyl α-d-glucopyranoside



Compound No 13: Name: E)-9-Octadecenoic acid ethyl ester



Compound No 14: Name: 6-Octadecenoic acid, methyl ester, (Z)-

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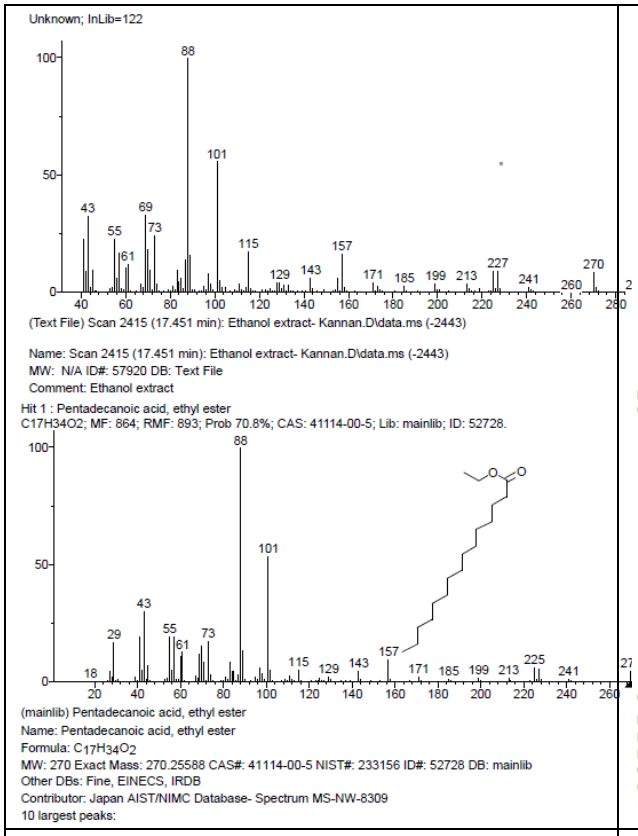
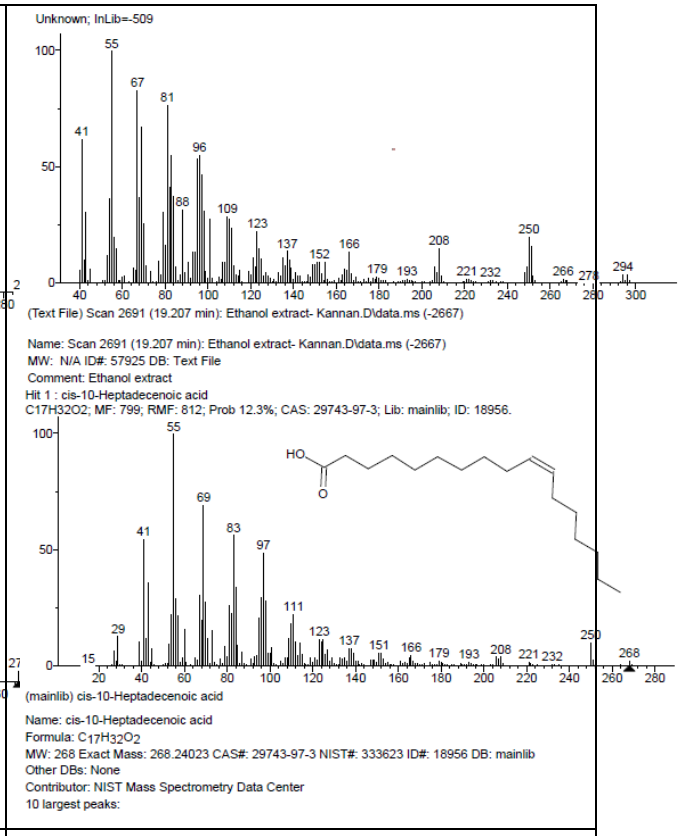
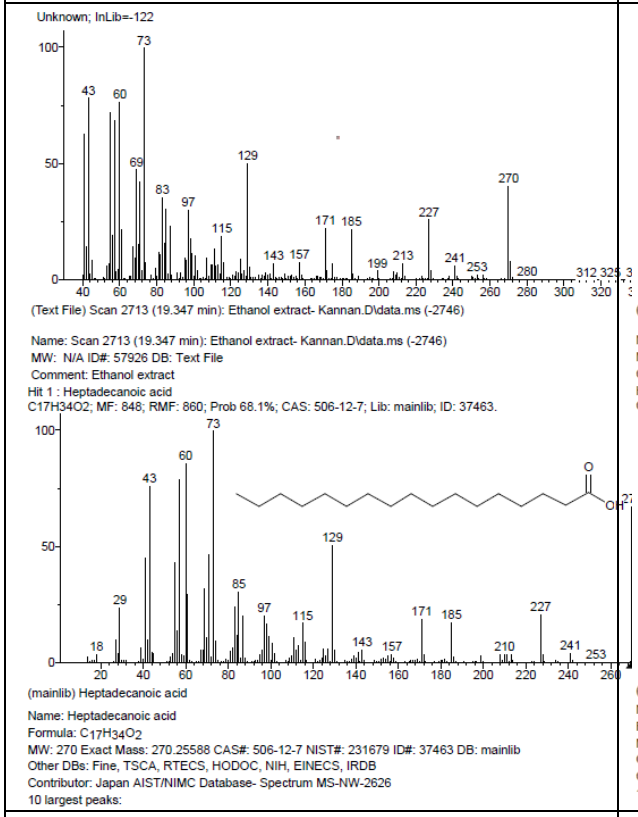


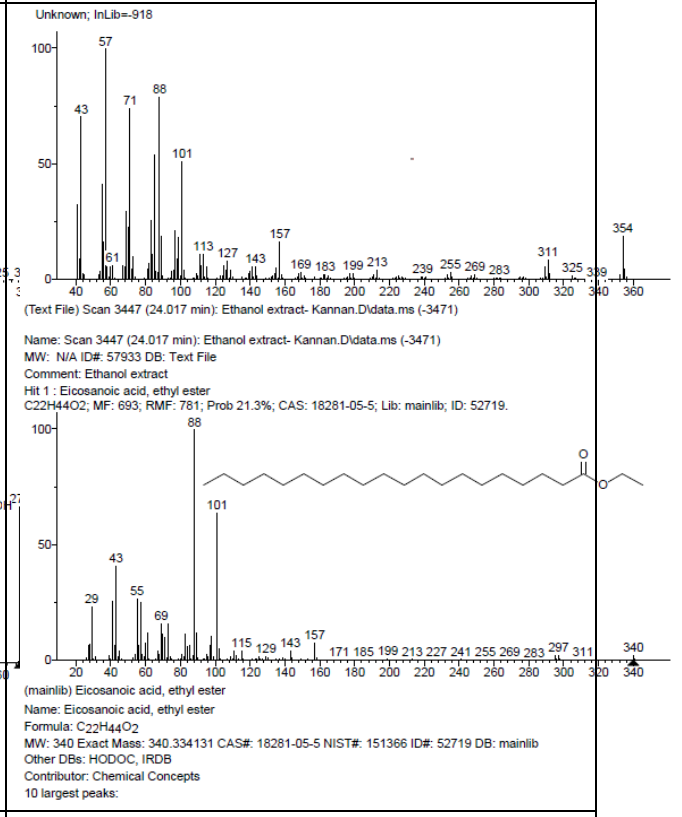
Compound No 15: Pentadecanoic acid, ethyl ester



Compound No 16: Name: cis-10-Heptadecenoic acid



Compound No 17: Name: Heptadecanoic acid



Compound No 18: Name: Eicosanoic acid, ethyl ester

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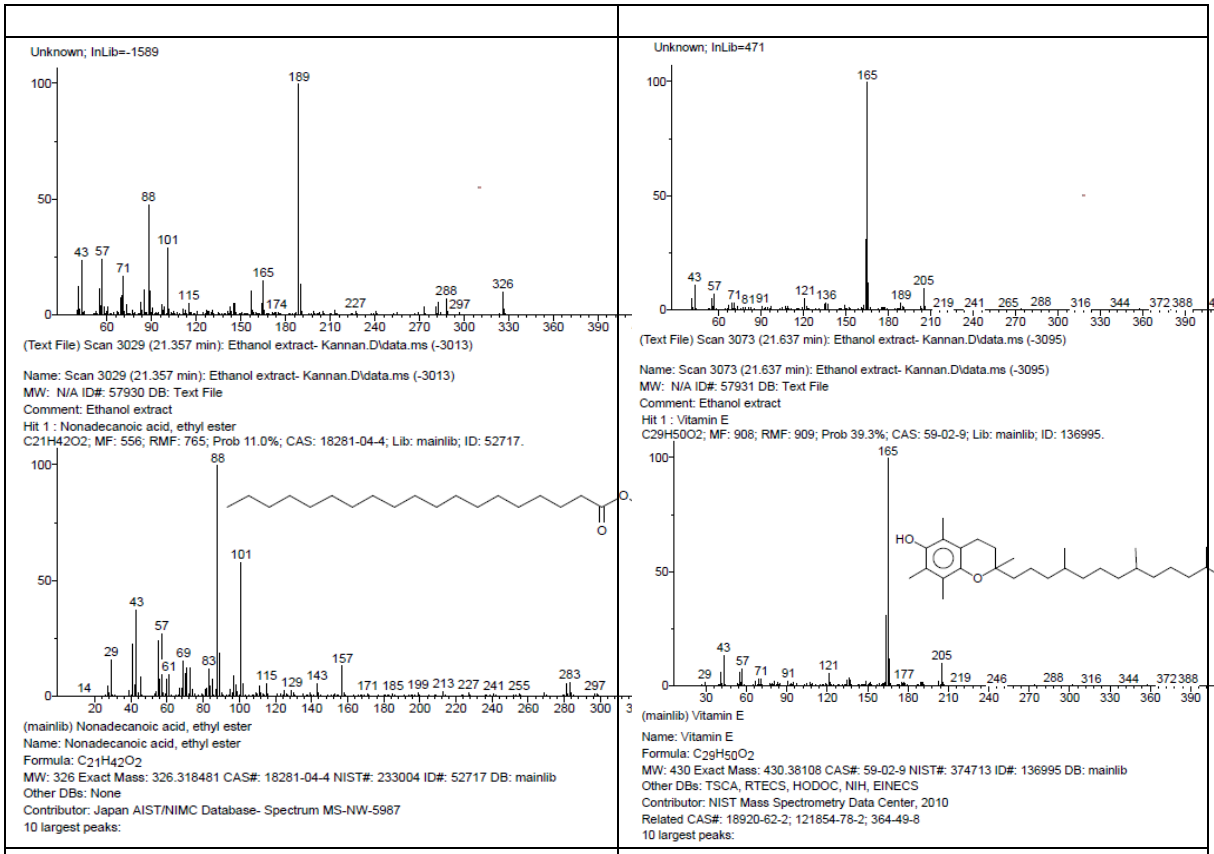
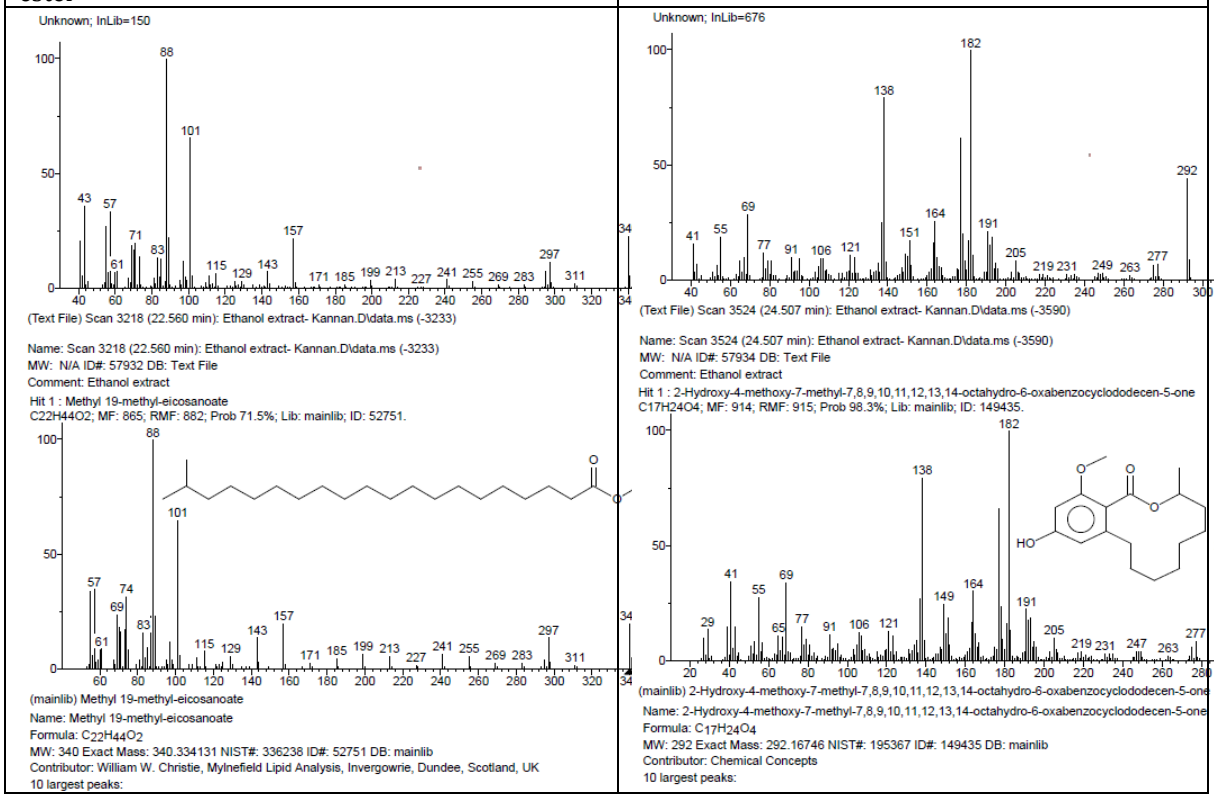


Compound No 19: Name: Nonadecanoic acid, ethyl ester

Compound No 20: Name: Vitamin E



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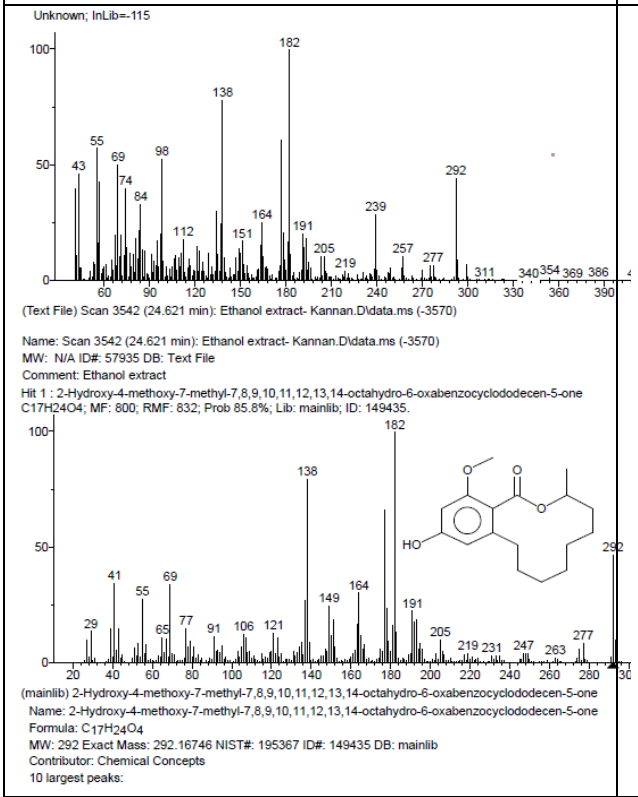
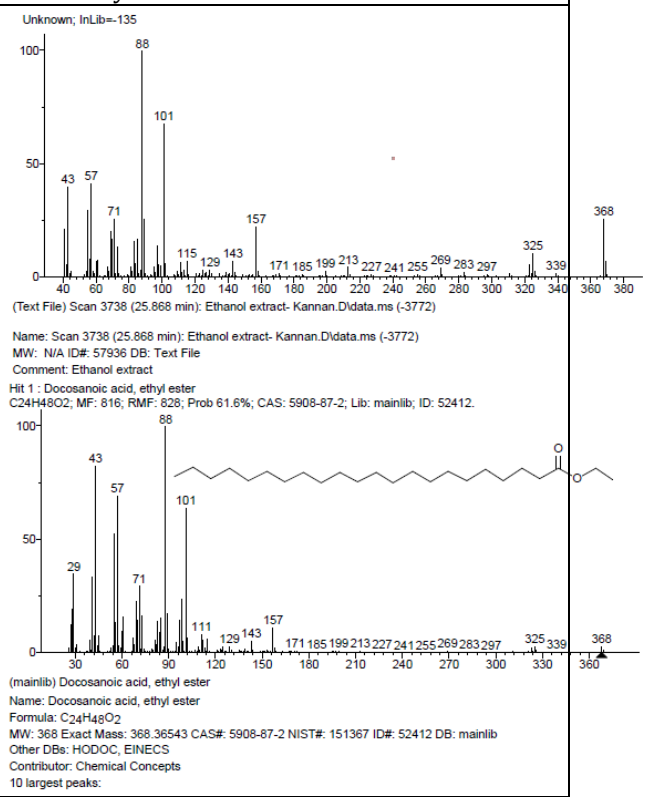
Compound No 21: Name: Methyl 19-methyl-eicosanoate	Compound No 22: Name: 2-Hydroxy-4-methoxy-7-methyl-7,8,9,10,11,12,13,14-octahydro-6-oxabenzocyclododecen-5-one
	
Compound No 23: Name: 2-Hydroxy-4-methoxy-7-methyl-7,8,9,10,11,12,13,14-octahydro-6-oxabenzocyclododecen-5-one	Compound No 24: Name: Docosanoic acid, ethyl ester

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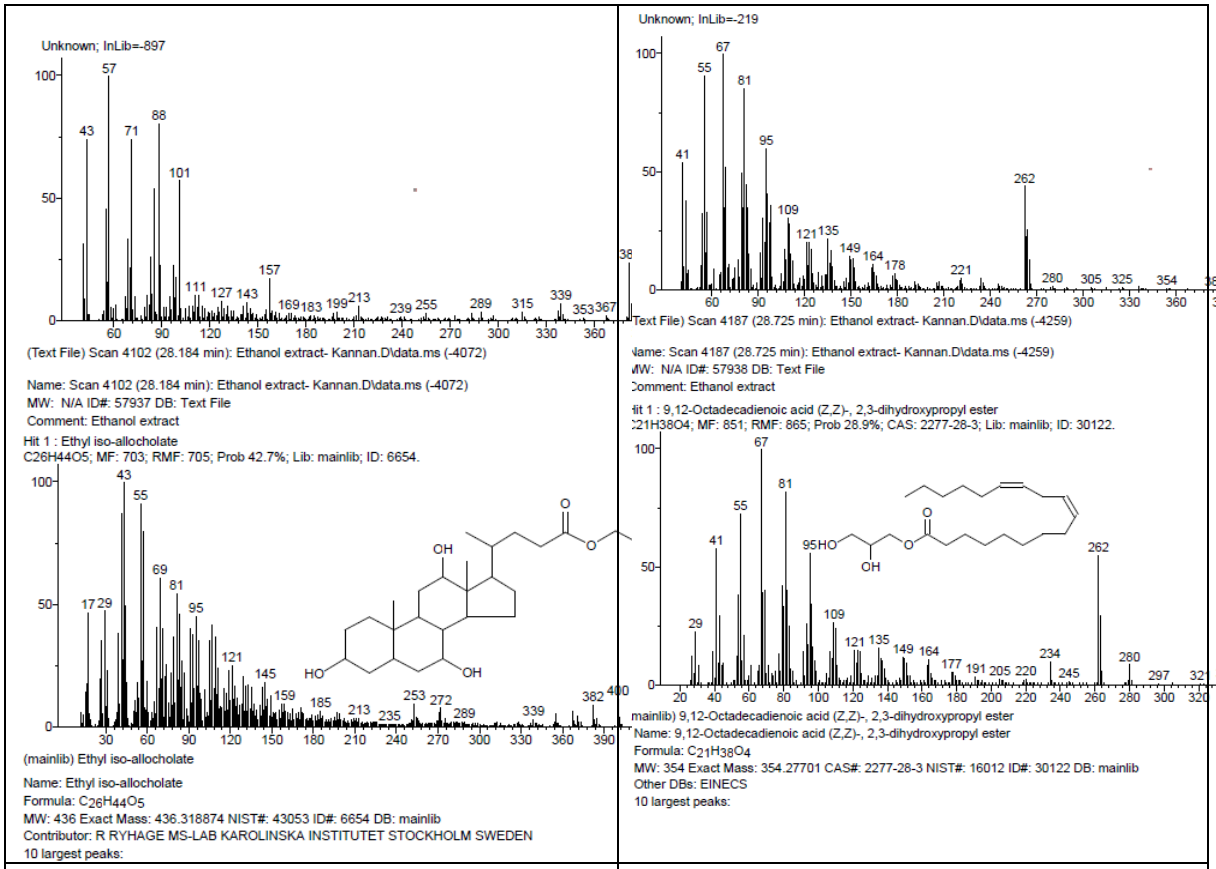

Compound No 25: Name: Ethyl iso-allocholate	Compound No 26: Name: 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester

Figure 1: Ion chromatogram structure of individual isolated compounds of alcoholic extract from the root of *Corallocarpus epigaeus* L.

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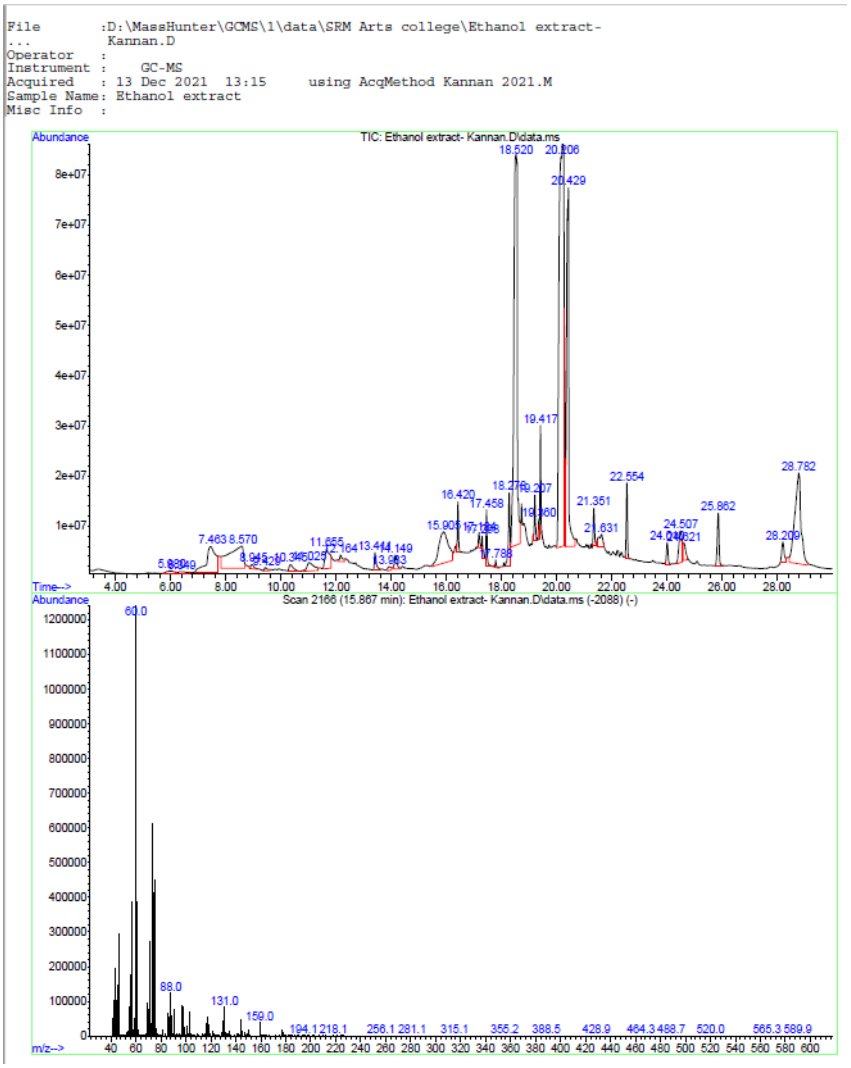

Figure 2: Total ionic chromatogram (GC/MS) of alcoholic extract from the root of *Corallocarpus epigaeus* L. obtained with 70eV using an Elite-1 fused silica capillary column with He gas as the carrier.

Discussion

In this present study, we observed and reported about plenty of bioactive compounds by GC/MS analysis of ethanolic extracts from *Corallocarpus epigaeus* L. The identified bioactive compounds were compared & confirmed, with a mass spectral library of NIST. The names of isolated compounds along with the IUPAC name, composition, molecular formula, and molecular weight were shown in Tables 1 and Figure 1. Each compound has a unique property and its uses and nature were collected from PUBCHEM and PUBMED.[8] The most important applications of isolated compounds were emphasized here. Pentadecanoic acid, ethyl ester, heptadecanoic acid acts as antioxidant.[9] Phenol, 2,4-bis(1,1-dimethylethyl)- acts as antifungal and antioxidant activity.[10] Vitamin E also called as tocopherol act as antineoplastic,[11] anti-carcinogenesis,[12] anti-inflammatory,[13] and antioxidant.[14,15] It also helps to maintain healthy skin and eyes, and strengthen the body's natural defense against illness and infection (the immune system).[16] (E)-9-Octadecenoic acid ethyl ester and 6-Octadecenoic acid, methyl ester, (Z)- is a fatty acid ester has antioxidant and antimicrobial activity.[17] Ethyl iso-allocholate is a steroid molecule that has antimicrobial, diuretic, anti-inflammatory properties, and it is used as an anti-asthma (drug that relieves the symptoms of asthma).[18] Eicosanoic acid, ethyl ester is an arachidic acid which has and activity of α-glucosidase inhibitors activity (Adverse effects of undigested carbohydrates in the lower gastrointestinal tract include flatulence, diarrhoea, and

abdominal pain).[19] Glycerin is used as Flavouring agent.[20] Nonadecanoic acid is a 19-carbon saturated fatty acid that is found in fats and vegetable oils. It has been shown to inhibit HL-60 cancer cell proliferation with an IC50 value of 68 µM.1. [21] *Cis*-10-Heptadecenoic acid is a C17:1 monounsaturated fatty acid that is a minor constituent of ruminant fats. [22] It has been examined for potential antitumor activity and was reported to inhibit HL-60 cell proliferation with an IC_{50} value of 302 µM and to prevent LPS-induced tumour necrosis factor production from mouse macrophages.[21] Ethyl α-d-glucopyranoside (sugar moiety) is a preservative.[23] Indolizine has various pharmacological properties which led to the development of new drugs such as CNS depressant, analgesic and anti-inflammatory, anticancer, antibacterial, antioxidant, larvicidal and anti-HIV.[24] 3-Hexadecene, (Z)- has insecticide, antibiotic, antioxidant, hypocholesterolemic activity. [25] The result of this present studies reveals that rhizome comprise antitubercular, anticancer, antifungal, antibacterial, antioxidant, and anti-inflammatory. Hence, *Corallocarpus epigaeus* L. may be an excellent remedy for the treatment of various diseases.

Conclusion

In conclusion, after systematic analysis of this research work, plenty of bio active compounds that have been identified and documented. It will provide additional information about the efficacy of diverse biological properties such as antioxidant, anticancer, antifungal, antibacterial and anti-inflammatory activities related to this plant. This documentation and valuable information will support the efficacy of *Corallocarpus epigaeus* L.

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