

Structural elucidation of chemical constituents from *Benincasa hispida* seeds and *Carissa congesta* roots by gas chromatography: Mass spectroscopy

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ABSTRACT

Background: *Benincasa hispida* (BH) and *Carissa congesta* (CC) are regarded as ethnopharmacological imperative plants in Asian countries. **Objective:** Phytochemical screening of the extracts has shown the presence of steroids, flavonoids, saponins, glycosides, tannins, phenolic compounds, fixed oils, and fats in the BH and CC extracts. The presence of lupeol has been reported previously by us using high-performance thin-layer chromatography and high-performance liquid chromatography. **Materials and Methods:** Present research studies encompasses identification of chemical constituents in BH seeds and CC roots petroleum ether extracts by hyphenated technique such as gas chromatography-mass spectroscopy (MS) which when coupled gives a clear insight of constituents. **Results:** The components were identified by matching mass spectra with MS libraries. There were 13 and 10 different compounds analyzed from CC and BH, respectively. The components present were Pentanoic acid, 5-hydroxy, 2,4-butylphenyl; *n*-Hexadecanoic acid (Palmitic acid); Sulfurous acid, 2-ethylhexylheptadecyl ester; *n*-Tridecane; 6-methyltridecane; (9E, 12E)-9,12-Octadecadienyl chloride, Hexadecanoic acid, 3-(trimethylsilyl)-oxy] propyl ester; 9,12-Octadecadenoic acid, 2 hydroxy-1-(hydroxymethylethyl) ester; 9,12-Octadecadienoic acid, 2,3 dihydroxypropyl ester; *n*-Propyl-9,12-Octadecadienoate, Lupeol; Taraxasterol; 6a, 14a-Methanopipene, perhydro-12,4a, 61a, 9,9,12a-heptamethyl-10-hydroxy and 9-Octadecene; 2-Isopropenyl-5-methyl-6-hepten-1-ol; *n*-Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ether; Butyl-9,12-Octadecadienoate; Friedoolean-8-en-3-one; friedours-7-en-3-one; 13,27-Cyclosuran-3-one; Stigmaste-7,25-dien-3-ol (3 β , 5 α); Stigmasta-7,16-dien-3-ol; chondrillasterol in BH seeds and CC roots extracts respectively. **Conclusion:** Eluted components from the extracts could provide further researchers to work with various pharmacological activities related models and studies.

Key words: Apocynaceae, *Benincasa hispida*, *Carissa congesta*, chondrillasterol, *Curcubitaceae*, gas chromatography, mass spectroscopy, lupeol, Stigmaste-7, 25-dien-3-ol

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INTRODUCTION

Discovery of natural plant-derived drugs from natural botanical herbarium has been the major breakthrough in paving the way for natural product chemistry.^[1] Plants

are common and important sources in contrast to marine organisms, microorganisms and fungi which are studied to the highest level in order their phytoconstituents.^[2,3] But very few research has been done in the field of natural product chemistry, hence it is an indeed a need of an hour to explore these resources in search of new products which have significant pharmacotherapeutic potential as anticancer agents.^[1,4-7] In recent times, the research on plants as medicines has been focusing on isolation and characterization of bioactive constituents and secondary metabolites.^[8,9]

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Plant kingdom exhibiting triterpenes in ample quantity shows structural multiplicity. Terpenoids have common biosynthetic origin based on isoprene units [$\text{CH}_2 = \text{C}(\text{CH}_3) - \text{CH} = \text{CH}_2$] (1). Carbon skeletons in them are built from the union of two or more of C_5 units. The classification is based on number of units as follows: C_{10} -Monoterpenoids (2 carbon atoms), C_{15} -Sesquiterpenoids (3 carbon atoms), C_{20} -Diterpenoids (4 carbon atoms), C_{30} -Triterpenoids (6 carbon atoms) from squalene parent molecule and C_{40} -Tetraterpenoids (8 carbon atoms).

Pentacyclic triterpenes such as α - and β -amyrins and derived acids such as oleanolic acid and ursolic acid are found in waxy coatings of leaves and fruits like apple and pear as well as in resins, latex and bark of trees. Lupeol, betulin and betulinic acid of lupane class, oleanane or ursane groups have shown enormous pharmacological potential comprising of antiviral,^[10-12] anti-inflammatory^[13] and antitumor activity. Stigmasterol and its analogues have shown potentiality of acting as chemopreventive agent in cancer by inhibiting the cells in colon and breast cancer.^[14-22]

During last few decades, infrared spectroscopy has been introduced as a very efficient and nondestructive analytical

tool for the reliable identification of triterpenes.^[23] Our previous studies^[24,25] confirmed the presence of lupeol by high performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) in *Benincasa hispida* (BH) and *Carissa congesta* (CC) petroleum ether plant extracts. Gas-liquid chromatography is an important hyphenated technique which provides an insight to qualitative, as well as quantitative estimation for volatile components such as terpenes.^[1] In the present work, we identified and confirmed the structures of the different elucidated constituents by gas chromatography-mass spectroscopy (GC-MS) from the petroleum ether extracts of BH seeds and CC roots for the first time.

MATERIALS AND METHODS

Part A: Collection, authentication, extraction and preliminary phytochemical identification

Fresh selected parts of CC and BH were collected, authenticated, extracted, preliminary phytochemical constituents were recognized followed by HPTLC and HPLC of lupeol as reported by us.^[24,25]

Table 1: Various components and their fragments in BH extract

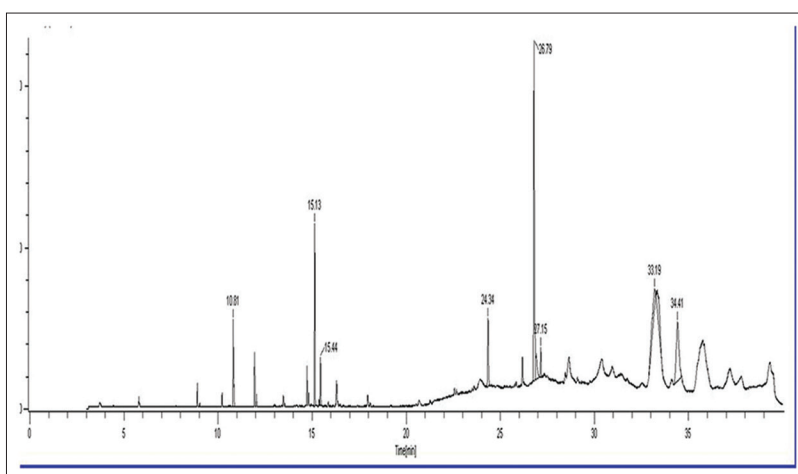
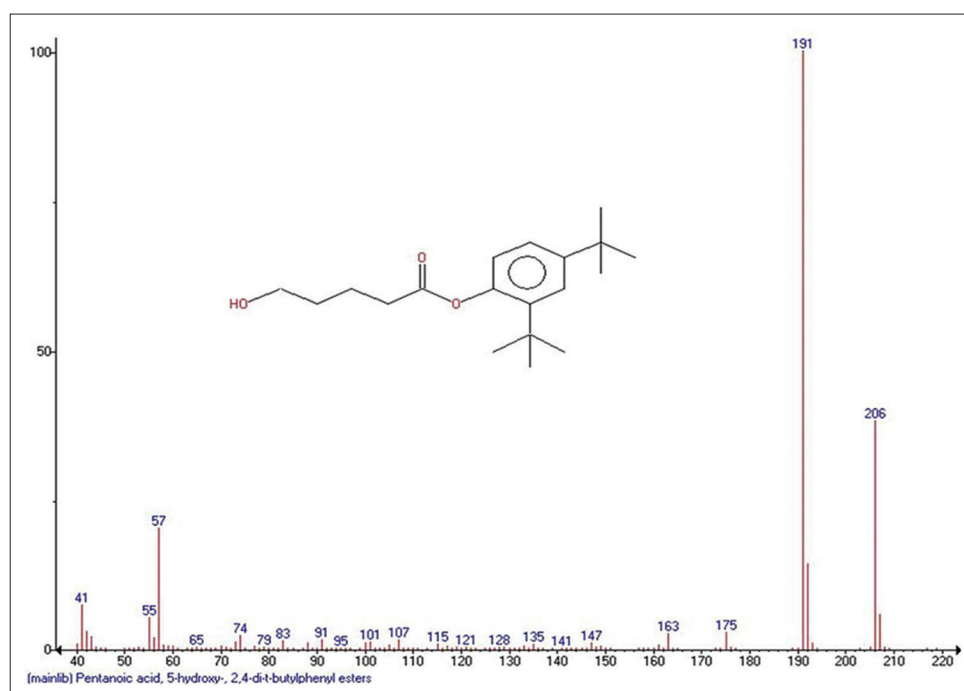
Retention time	Identity	Molecular formula	m/z	Synonyms	Figure number
10.79	(2)	$\text{C}_{19}\text{H}_{30}\text{O}_3$	306	(2,4-ditert-butylphenyl)-5-hydroxypentanoate	2
17.78	(3)	$\text{C}_{16}\text{H}_{32}\text{O}_2$	256	Palmitic acid, Cetylic acid, <i>n</i> -Hexadecanoic acid, Hexadecylic acid, Hydrofol, <i>n</i> -Hexadecanoic acid, 1-Pentadecanecarboxylic acid, hexaectylic acid, Pentadecanecarboxylic acid, Industrene 4516, Emersol 140, Emersol 143, Hystrene 8016, Hystrene 9016, Prifac 2960, Glycon P-45, Univol U 332	3
24.43	(4)	$\text{C}_{25}\text{H}_{52}\text{O}_3\text{S}$	432.74	2-ethylhexylheptadecyl sulfite	4
26.83	(5)	$\text{C}_{14}\text{H}_{30}$	198	6-methyltridecane	5
26.91	(6)	$\text{C}_{13}\text{H}_{28}$	184	<i>n</i> -Tridecane	6
27.15	(7)	$\text{C}_{18}\text{H}_{31}\text{ClO}$	298	(9E, 12E)-9,12-Octadecadienoyl chloride, Linoleic acid chloride, Linoleoyl chloride	7
28.37	(8)	$\text{C}_{22}\text{H}_{46}\text{O}_3\text{Si}$	386	2,3-[(trimethylsilyl) oxy] propyl palmitate, 1,1-Palmitoyl 1,3propanediol-trimethylsilyl	8
29.15	(9)	$\text{C}_{21}\text{H}_{38}\text{O}_4$	354	Glycerol Monooleate; Olein, 1-mono-; α -Monoolein; Aldo HMO; Aldo MO; Glycerin	9
29.88	(10)	$\text{C}_{21}\text{H}_{40}\text{O}_4$	356	1-monooleate; Glycerol α -cis-9-octadecenate; Glycerol α -monooleate; Glycerol 1-monooleate; Monoolein; 1-Glycerol oleate; 1-Monoolein; 1-Monooleoylglycerol; 1-Oleoylglycerol; 1-Oleylglycerol; dl- α -Monoolein; 1-Monooleoyl-rac-glycerol; Ablunol GMO; Glycerol-1-oleate; Glycerol oleate; Glycerol oleate; Witconol 2421; 9-Octadecenoic acid (9Z)-, 2,3-dihydroxypropyl ester; 1-Mono (cis-9-octacenoil) glycerol; 2,3-Dihydroxypropyl oleate; Danisco MO 90; rac-1-Monoolein; rac-1-Monooleoylglycerol; 2,3-Dihydroxypropyl (9Z)-9-octadecenoate	10
29.72	(11)	$\text{C}_{21}\text{H}_{38}\text{O}_2$	322	Linoleate	11
32.09	(12)	$\text{C}_{30}\text{H}_{50}\text{O}$	426.7	Lup-20 (29)-en-3-ol Lup-20 (29)-en-3 β -ol Lup-20 (29)-en-3 α -ol Lupeol, (3 α)-isomer Lupeol, (3 β , 18 β , 19 β)-isomer	12
32.09	(13)	$\text{C}_{30}\text{H}_{50}\text{O}$	426.7	Taraxasterol	13
32.09	(14)	$\text{C}_{30}\text{H}_{50}\text{O}$	426.7	6a, 14a-Methanopicene, 13,27-cyclooleanane derivatives; 6a, 14a-Methanopicene, eicosahydro-2,2,4a, 6b, 9,9,12a-heptamethyl-, [4aR-(4aa, 6ab, 6ba, 8ab, 12aa, 12bb, 14ab, 14ba)]-; [4aR-(4aa, 6ab, 6ba, 8ab, 12aa, 12bb, 14ab, 14ba)]-Eicosahydro-2,2,4a, 6b, 9,9,12a-heptamethyl-6a, 14a-methanopicene	14

BH=*Benincasa hispida*

Table 2: Various components and their fragments in CC extract

Retention time	Identity	Molecular formula	Molecular weight	Synonyms	Figure number
10.81	(15)	C ₁₈ H ₃₆	252	(9E)-9-Octadecene	16
15.13	(16)	C ₁₁ H ₂₀ O	168	2-Isopropenyl-5-methyl-6-hepten-1-ol	17
15.44	(17)	C ₁₉ H ₃₈ O ₄	330	Palmitin, 2-mono Palmitic acid β-Monoglyceride, 2-Hexadecanoyl glycerol, 2-Monoplamitin, 2-Monopalmitoyl-sn-glycerol, 1,2,3-Propanetriol, 2-hexadecanoyl ester, Glycerol β-palmitate, 2-hydroxy-1-(hydroxymethyl) ethyl palmitate	18
24.34	(18)	C ₂₂ H ₄₀ O ₂	336	Butyl-9,12-octadecadienone	19
26.79	(19)	C ₃₀ H ₄₈ O	(205),424	Isomultiflorenone; D:C-Friedoolean-8-en-3-one	20
26.92	(20)	C ₃₀ H ₄₈ O	(245), 424	Bauer-7-en-3-one; Bauerenone; D:C-Friedours-7-en-3-one	21
27.15	(21)	C ₃₀ H ₅₀ O	(205), 424	13,27-Cyclosuran-3-one	22
33.19	(22)	C ₂₉ H ₄₈ O	(271), 412	5α-Stigmasta-7,25-dien-3 β-ol; δ7,25-Stigmastadienol; 24-Ethyl-5-α-cholest-7,25-dien-3	23
33.34	(23)	C ₂₉ H ₄₈ O	(271), 412	-β-ol; 24 β-Ethyl-5α-cholesta-7,25-dien-3 β-ol; Stigmasta-7, 25 (27)-dien-3-ol, (3 β,5α)-	24
34.41	(24)	C ₂₉ H ₄₈ O	(271), 412	Stigmasta-7,22-dien-3-ol, (3 β,5α,22E,24R)-; 5α-Stigmasta-7,22-dien-3 β-ol, (24R)-	25

The bracket values indicate ions with highest relative intensity. CC=*Carissa congesta*

**Figure 1: Mass spectrum of *Benincasa hispida* extract****Figure 2: Mass spectrum showing presence of 2,4-di-tert-butylphenyl 5-hydroxypentanoate (2) in *Benincasa hispida* extract**

Part B: Gas chromatography-mass spectroscopy

Mobile phase: Petroleum ether (BH and CC) electron impact (EI)-MS Spectrum was scanned at 70 eV with instrument details as follows:

Model of MS: Joel

Model: Accu time of flight analyzer gross calorific value analyzer

Specification: Mass range-10–2000 amu and resolution-6000.

Make of GC: Agilent 7890

Detector: Flame ionization detector. Run time: 40 minutes

Gas chromatography-MS analysis was performed by splitless injection of 1.0 μL of the sample in petroleum ether on a Hewlett Packard 6890 (USA) gas chromatograph fitted with

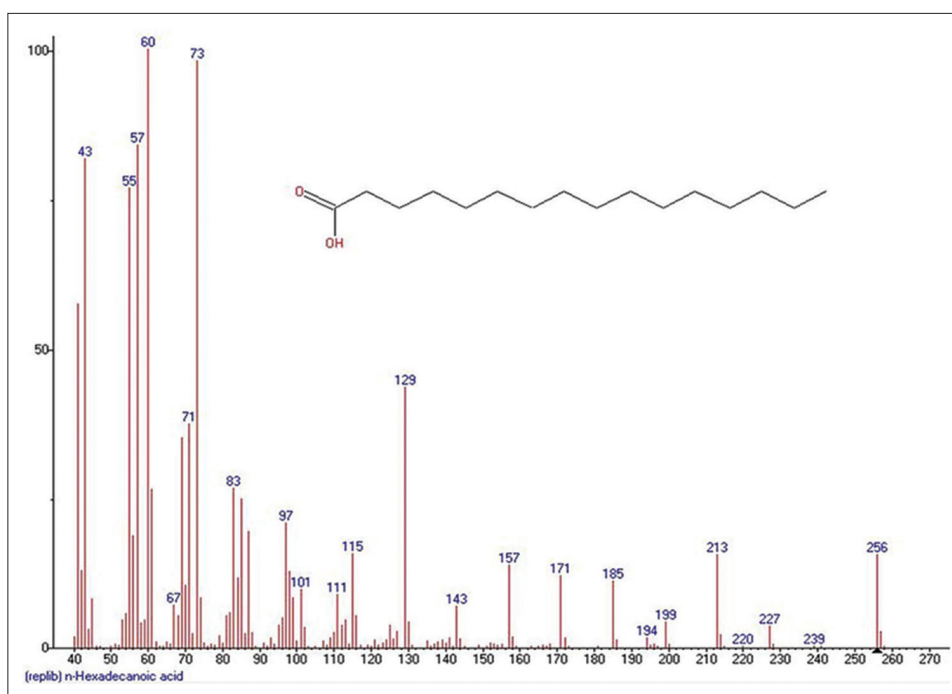


Figure 3: Mass spectrum showing presence of Palmitic acid (3) in *Benincasa hispida* extract

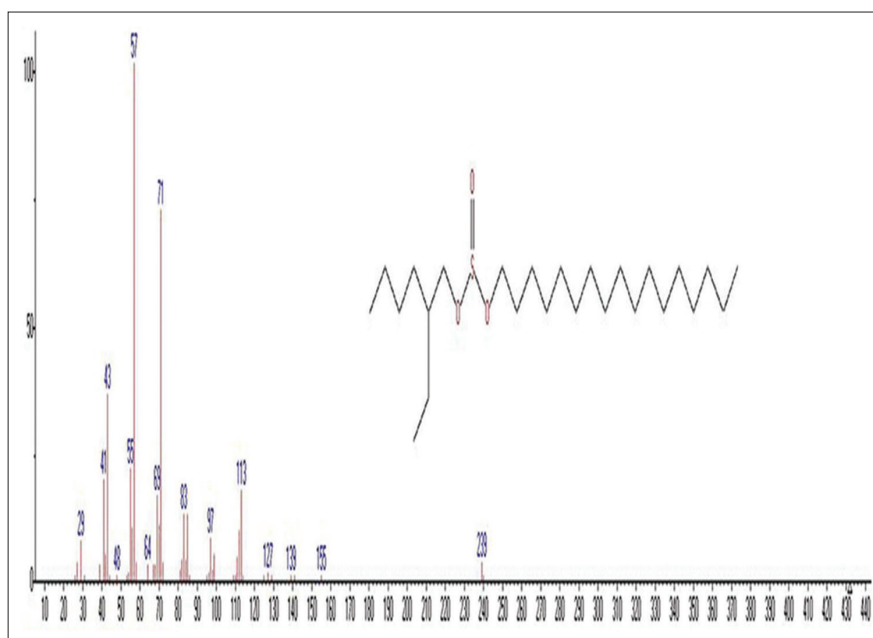


Figure 4: Mass spectrum showing presence of 2-Ethylhexyl heptadecyl sulfite (4) in *Benincasa hispida* extract

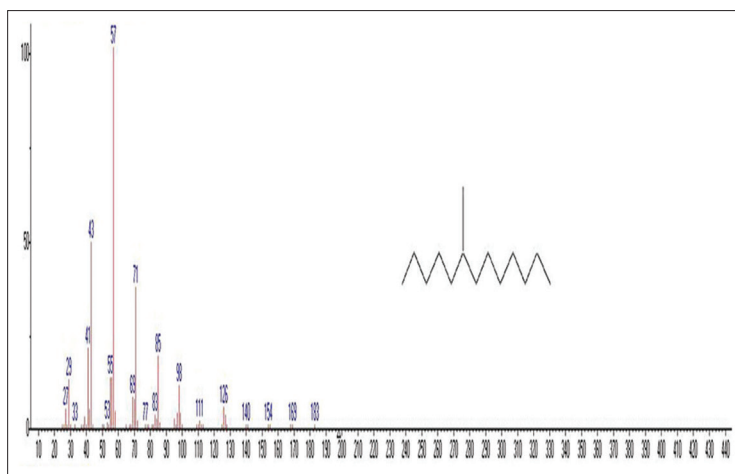


Figure 5: Mass spectrum showing presence of 6-Methyltridecane (5) in *Benincasa hispida* extract

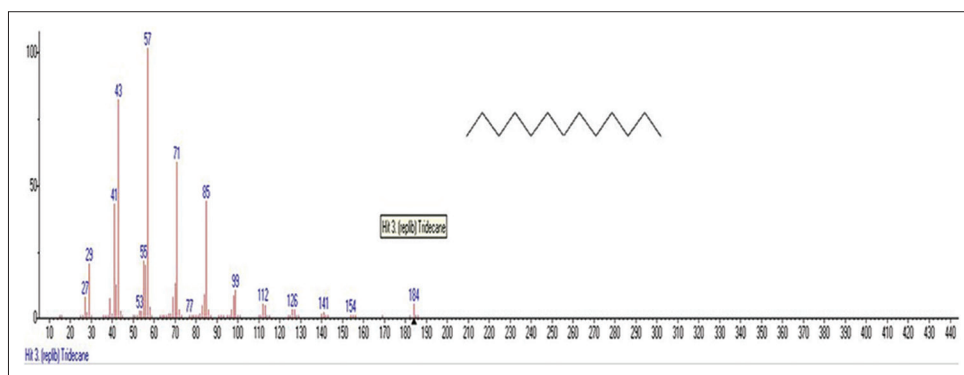


Figure 6: Mass spectrum showing presence of Tridecane (6) in *Benincasa hispida* extract

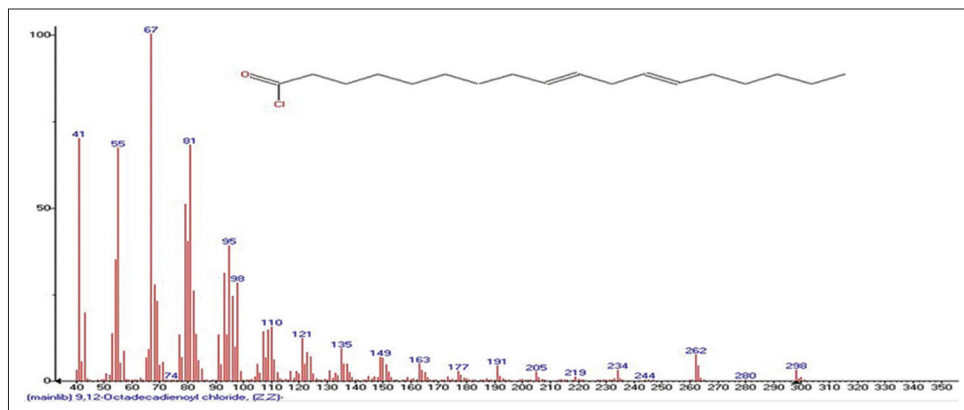


Figure 7: Mass spectrum showing presence of 9,12-Octadeca-9,12-dienoyl chloride (7) in *Benincasa hispida* extract

a cross-linked 5% phenyl methyl siloxane HP-5 MS capillary column (30 m × 0.32 mm × 0.25 μm coating thickness), coupled with a mass detector. GC-MS operating conditions were as follows: Injector temperature 200°C, transfer line 230°C, oven temperature program 60°C–280°C with ramping 5°C min⁻¹, carrier gas: Helium at 1.5 mL min⁻¹, mass spectra: EI⁺, ion source temperature: 280°C. Individual components were identified by NIST MS 2.0 f Structural Library.

RESULTS AND DISCUSSION

We have already reported BH and CC extracts yield in our previous studies.^[24,25] In the present study, constituents such as lupeol and stigmasterol were eluted in GC-MS studies. The GC-MS analysis in the recent research studies reveals that the identified compounds belong to the class of triterpenoids has a potential pharmacological role.

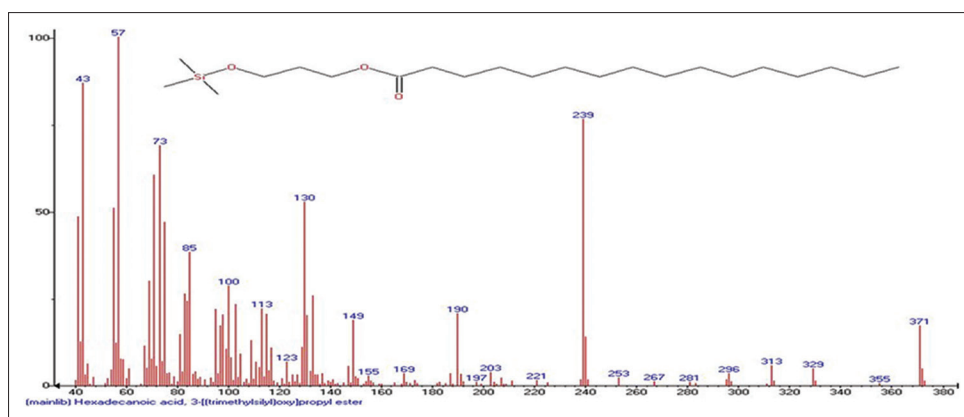


Figure 8: Mass spectrum showing presence of Hexadecanoic acid, 3-((trimethylsilyl)oxy)propyl ester (8) in *Benincasa hispida* extract

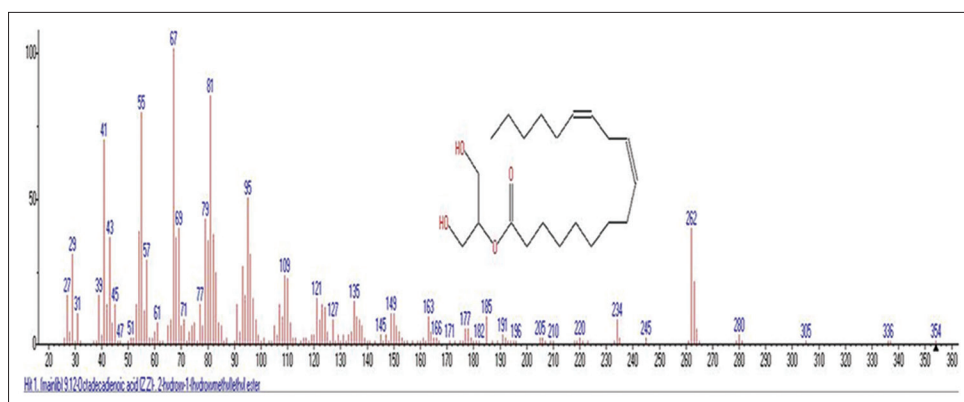


Figure 9: Mass spectrum showing presence of 9,12-Octadecadenoic acid (Z,Z), 2-Hydroxy-1-(hydroxymethylethyl) ester (9) in *Benincasa hispida* extract

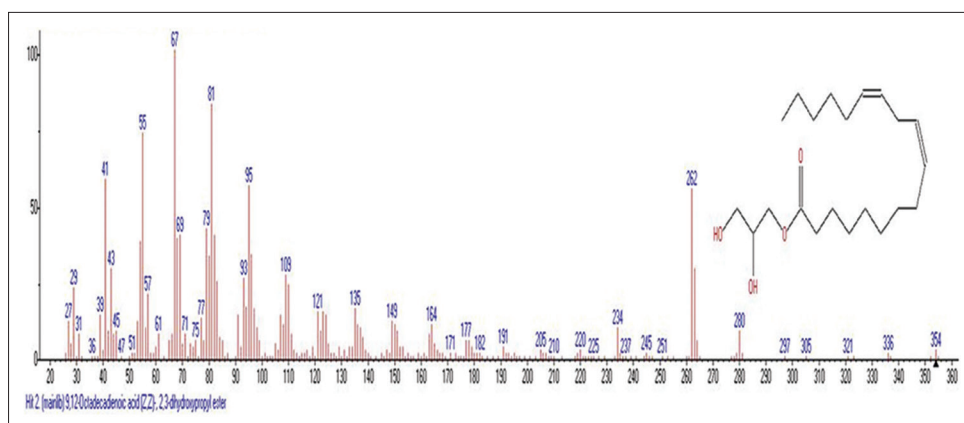


Figure 10: Mass spectrum showing presence of 9,12-Octadecadenoic acid (Z,Z), 2,3-dihydroxy propyl ester (10) in *Benincasa hispida* extract

The identified compounds are probably lipid soluble and found in the plant cell's cytoplasm. The general procedure for their extraction is using light petroleum ether or chloroform since these solvents help to separate the compounds by suitable chromatography techniques. GC is considered as a vital technique to interpret essential oils and volatile compounds.^[1] In the current research study, various compounds [Tables 1 and 2] were elucidated by

GC-MS technique from BH and CC petroleum ether extracts.^[10-22] Mass spectrum peaks of BH and CC extracts [Figures 1 and 15].

Pentanoic acid, 5-hydroxy-2,4-dibutylphenyl esters [Figure 2] [33.5%] with m/z 206 and fragment ions of 41, 57, 74, 91, 107, 115, 163, 175, 191 is seen most commonly from seeds of BH (*Cururbitaceae*). Main

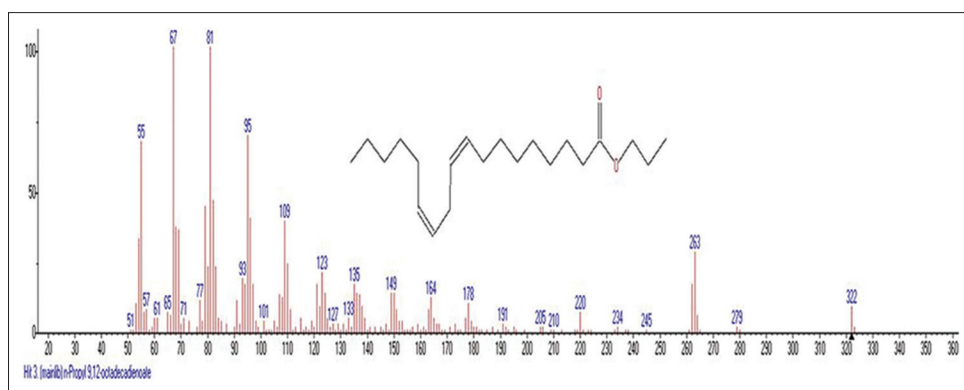


Figure 11: Mass spectrum showing presence of n-propyl-9,12-octadecadienoate (11) in *Benincasa hispida* extract

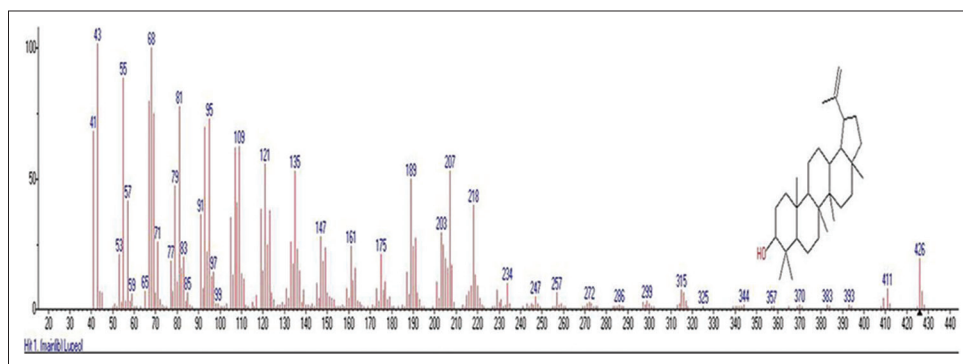


Figure 12: Mass spectrum showing presence of lupeol (12) in *Benincasa hispida* extract

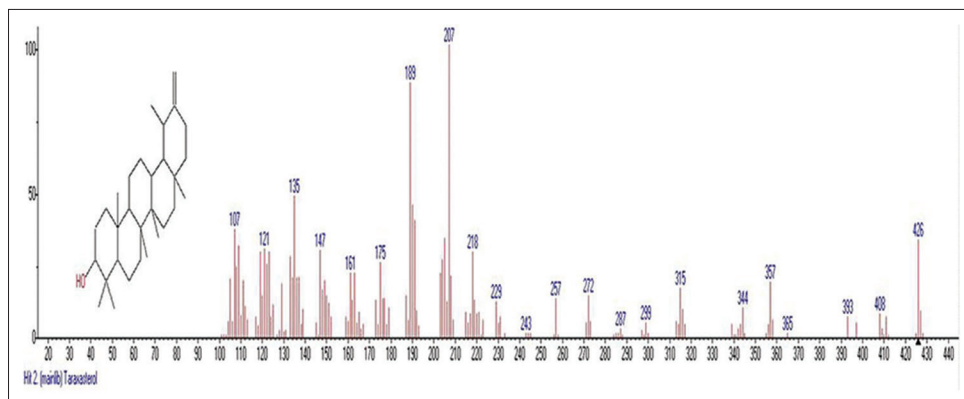


Figure 13: Mass spectrum showing presence of Taraxasterol (13) in *Benincasa hispida* extract

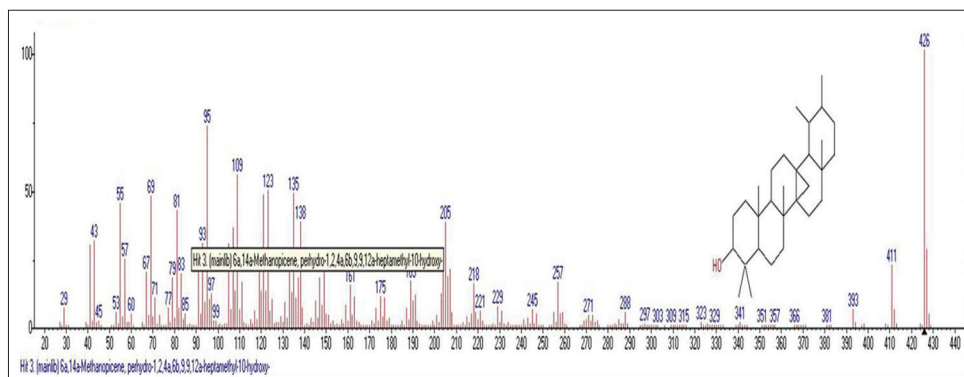


Figure 14: Mass spectrum showing presence of 6a,14a-Methanopicene (14) in *Benincasa hispida* extract

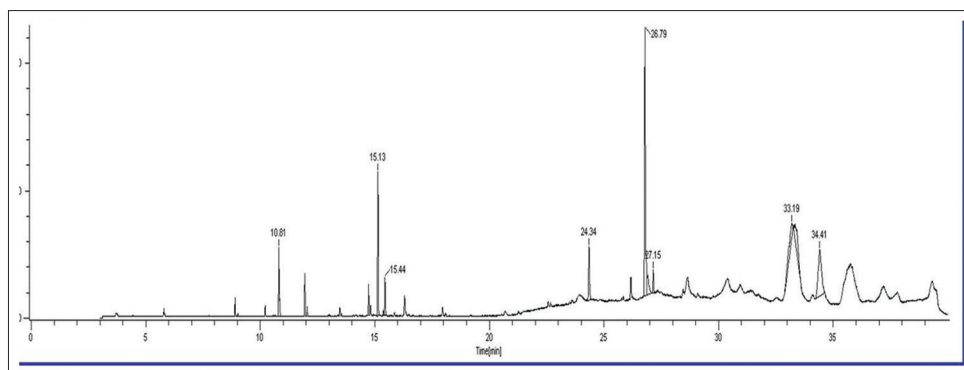


Figure 15: Mass spectrum of *Carissa congesta* extract

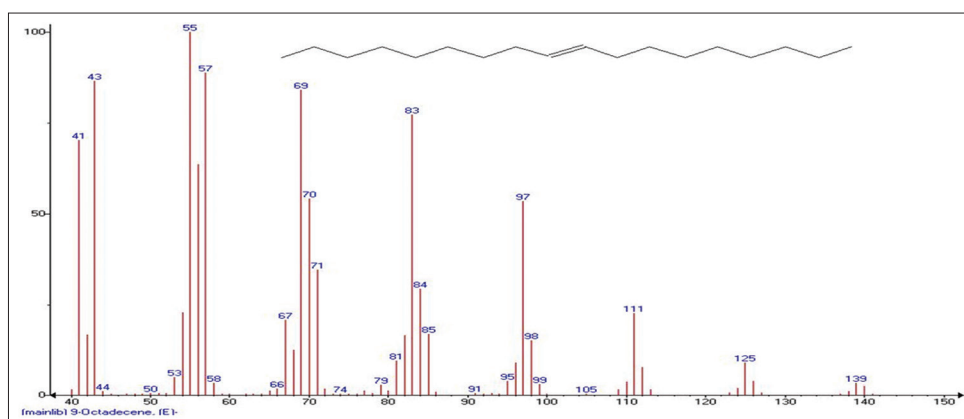


Figure 16: Mass spectrum showing presence of 9-Octadecene (15) in *Carissa congesta* extract

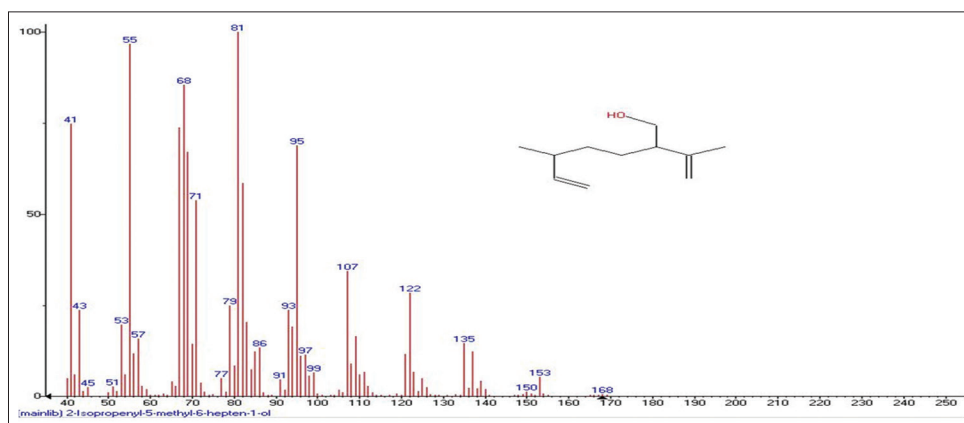


Figure 17: Mass spectrum showing presence of 2-Isopropenyl-5-methyl-6-hepten-1-ol (16) in *Carissa congesta* extract

component elucidated was Palmitic acid or n-Hexadecanoic acid Figure 3 [86.6%] with m/z 256 is found commonly in plants and animals with fragment ions of 41, 43, 45, 57, 60, 67, 71, 73, 83, 87, 97, 101, 111, 115, 125, 129, 143, 157, 171, 185, 194, 199, 213, 227. Sulfurous acid, 2-Ethylhexylheptadecyl ester Figure 4 with m/z 239 and fragment ions of 29, 41, 43, 48, 55, 64, 69, 71, 83, 97, 113, 127, 139, 155 along with 6-Methyltridecane Figure 5 as m/z 183 and Tridecane Figure 6 as m/z 184 with fragment ions of 27, 29, 33, 41, 43, 53, 55, 57, 69, 71, 77, 83, 85, 98, 111,

126, 140, 154, 169 and 27, 29, 41, 43, 53, 55, 57, 71, 77, 85, 99, 112, 126, 141, 154, respectively, are also seen prominently. 9,12-Octadeca-dienylchloride Figure 7 [9.97%] with m/z 263 and fragment ions of 41, 43, 55, 57, 65, 67, 71, 77, 79, 81, 87, 95, 109, 121, 135, 149, 155, 163, 177, 185 is also characterized. Hexadecanoic acid, 3-(trimethylsilyl) oxy] propyl ester Figure 8 having m/z 371 with fragment ions of 41, 43, 55, 59, 67, 69, 73, 81, 85, 95, 109, 117, 130, 149, 190, 203, 239, 267, 315 and 9,12-Octadecadenoic acid, 2-Hydroxy-1-(hydroxymethylethyl) ester Figure 9

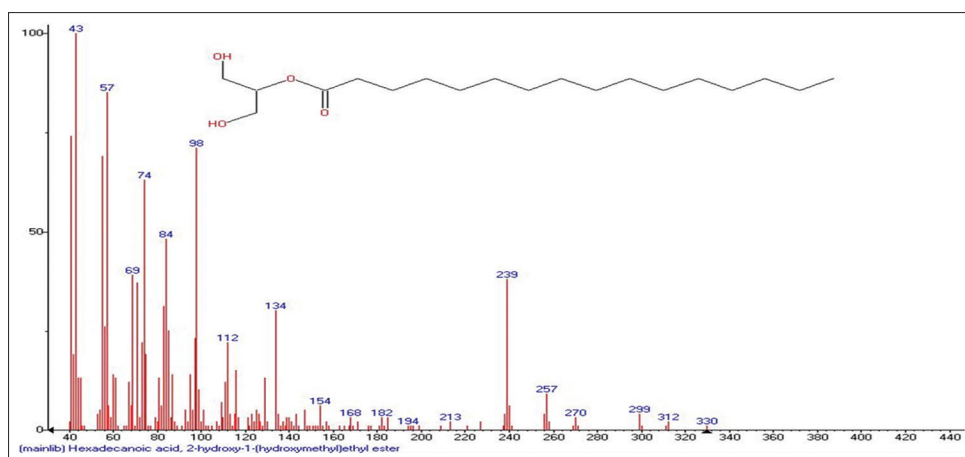


Figure 18: Mass spectrum showing presence of 2-Hydroxy-1-(hydroxymethyl)ethyl ether (17) in *Carissa congesta* extract

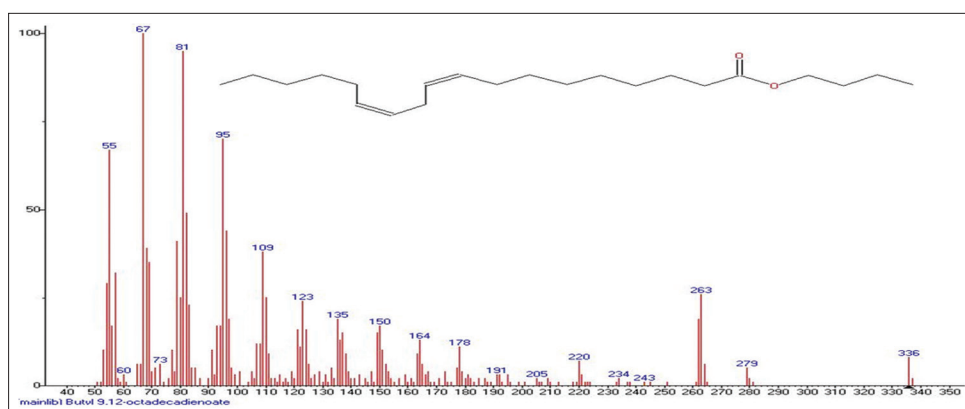


Figure 19: Mass spectrum showing presence of Butyl 9,12-octadecadienoate (18) in *Carissa congesta* extract

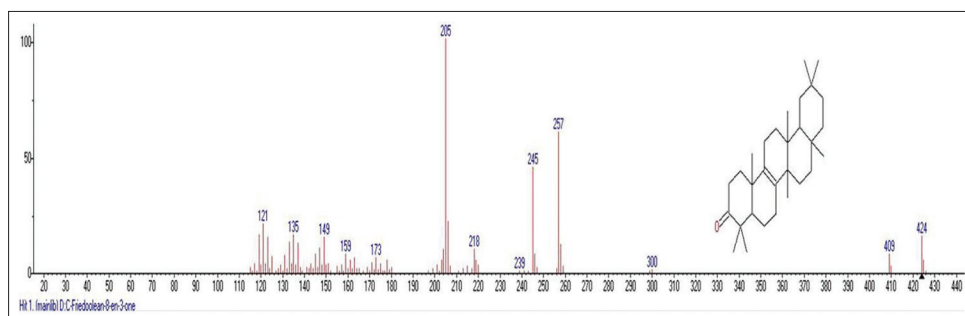


Figure 20: Mass spectrum showing presence of Friedoolean-8-en-3-one (19) in *Carissa congesta* extract

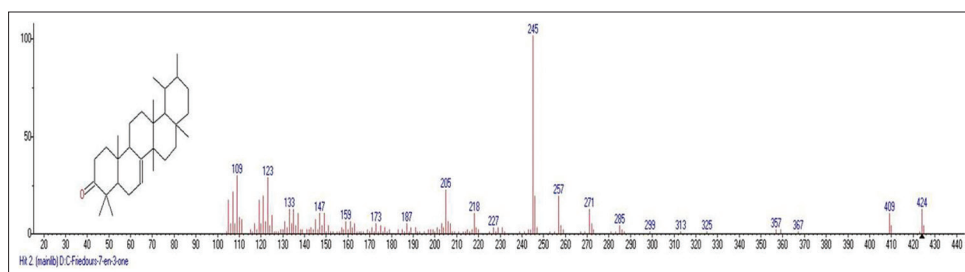


Figure 21: Mass spectrum showing presence of Friedours-7-en-3-one (20) in *Carissa congesta* extract

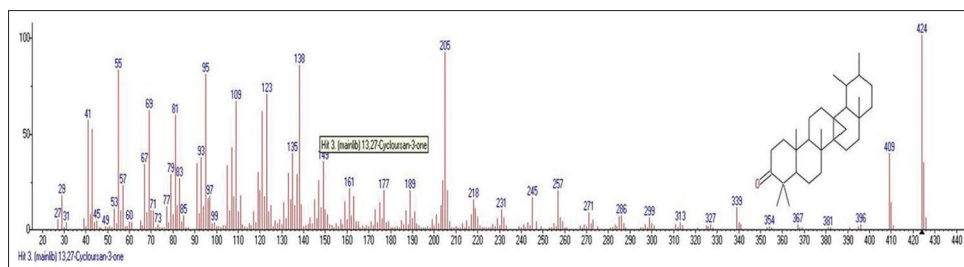


Figure 22: Mass spectrum showing presence of 13,27-Cyclosuran-3-one (21) in *Carissa congesta* extract

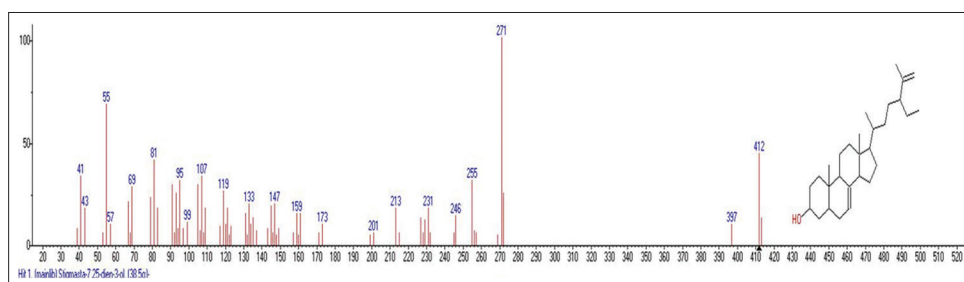


Figure 23: Mass spectrum showing presence of (3β,5α)-Stigma-7,25-dien-3-ol (22) in *Carissa congesta* extract

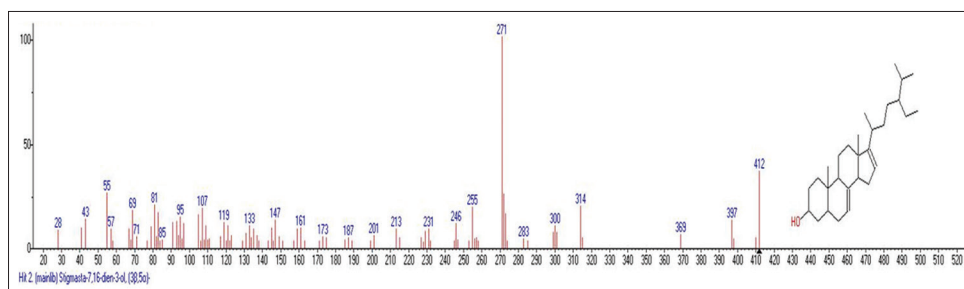


Figure 24: Mass spectrum showing presence of (3β,5α)-Stigma-7,16-dien-3-ol (23) in *Carissa congesta* extract

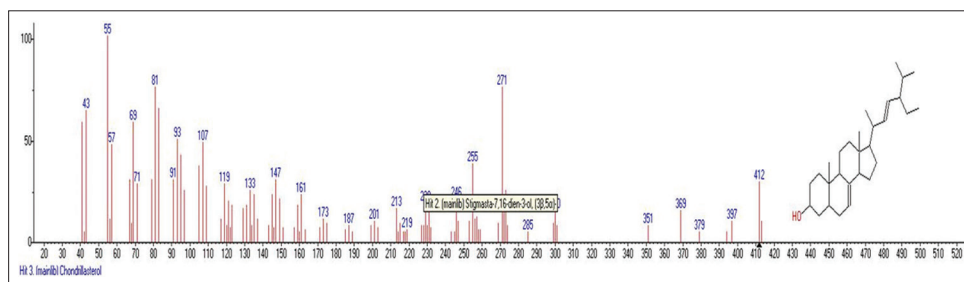


Figure 25: Mass spectrum showing presence of chondrillasterol (24) in *Carissa congesta* extract

having m/z 354 with fragment ions of 27, 29, 31, 39, 41, 43, 45, 47, 51, 55, 57, 61, 67, 69, 71, 77, 79, 81, 95, 109, 121, 127, 135, 145, 149, 163, 166, 171, 177, 182, 185, 191, 196, 205, 210, 220, 234, 245, 262, 280, 305, 336 are also characterized. 9,12-Octadecadenoic acid, 2,3-Dihydroxypropyl ester Figure 10 having m/z 354 with fragment ions of 27, 29, 31, 39, 41, 43, 45, 47, 51, 55, 57, 61, 67, 69, 71, 75, 79, 81, 93, 95, 109, 121, 135, 149, 164, 171, 177, 182, 191, 205, 210, 225, 234, 237, 245, 251, 262, 280, 297, 305, 321, 336 is seen. Another compound

called n-propyl-9,12-octadecadienoate Figure 11 having m/z 322 with fragment ions of 51, 55, 57, 61, 65, 67, 71, 77, 81, 93, 95, 101, 109, 123, 127, 133, 135, 149, 164, 178, 191, 205, 210, 234, 245, 263, 279 was obtained. Lupeol Figure 12 having m/z 426 with fragment ions of 41, 43, 53, 55, 57, 59, 65, 68, 71, 77, 79, 81, 83, 85, 91, 95, 97, 99, 109, 121, 135, 147, 161, 175, 189, 203, 207, 218, 234, 247, 257, 272, 286, 299, 315, 325, 344, 357, 370, 383, 393, 411 and Taraxasterol Figure 13 having m/z 426 with fragmentations of 107, 121, 135, 147, 161, 175, 189,

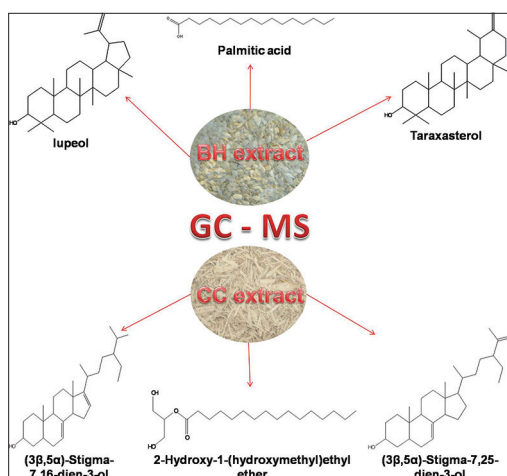


Figure 26: Graphical Abstract

207, 218, 229, 243, 257, 272, 287, 299, 344, 357, 365, 393, 408. 6a, 14a-Methanoplicene Figure 14 having m/z 426 with fragment ions of 29, 43, 45, 53, 55, 57, 60, 67, 69, 71, 77, 81, 83, 85, 93, 95, 97, 99, 109, 123, 135, 138, 161, 175, 190, 205, 208, 221, 229, 245, 257, 271, 288, 297, 303, 309, 315, 323, 329, 341, 351, 357, 366, 381, 399, 411 was also present. All the components and their fragments are depicted in Table 1.

Carissa congesta (*Apocynaceae*) showed the presence of 9-Octadecene Figure 16 [4.62%] having m/z 139 with fragment ions of 41, 43, 44, 50, 53, 55, 57, 58, 66, 67, 69, 70, 71, 74, 79, 81, 83, 84, 85, 91, 95, 97, 98, 99, 105, 111, 125 and 2-Isopropenyl-5-methyl-6-hepten-1-ol Figure 17 [8.64%] having m/z 168 with fragment ions of 41, 43, 45, 51, 53, 55, 57, 68, 71, 77, 79, 81, 86, 91, 93, 95, 97, 99, 107, 122, 135, 150, 153 were characterized. Hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl) ethyl ether Figure 18 [13.9%] having m/z 330 with fragment ions of 43, 57, 69, 74, 84, 98, 112, 134, 154, 168, 182, 194, 213, 239, 257, 270, 299, 312 along with Butyl-9,12-octadecadecionate Figure 19 (8.45%) having m/z 336 with fragment ions of 55, 60, 67, 73, 81, 95, 109, 123, 135, 150, 164, 178, 191, 205, 220, 234, 243, 263, 279 were also characterized. Friedoolean-8-en-3-one Figure 20 having m/z 424 with fragment ions of 121, 135, 149, 159, 173, 206, 218, 239, 245, 257, 300, 409 was also seen. Friedours-7-en-3-one Figure 21 having m/z 424 with fragment ions of 109, 123, 133, 147, 159, 173, 187, 205, 218, 227, 245, 257, 271, 285, 298, 313, 325, 357, 367, 408 and 13,27-Cyclosuran-3-one Figure 22 having m/z 424 with fragment ions of 27, 29, 31, 41, 45, 49, 53, 55, 57, 60, 67, 69, 71, 73, 77, 79, 81, 83, 85, 93, 95, 97, 99, 109, 123, 135, 138, 148, 161, 177, 189, 206, 218, 231, 245, 257, 271, 286, 299, 313, 327, 339, 364, 367, 381, 396, 408 were characterized. 3 β ,5 α -Stigma-7,25-dien-3-ol Figure 23 having m/z 412 with fragment ions of 41, 43, 55, 57, 69, 81, 95, 99, 107,

119, 133, 147, 159, 173, 201, 213, 231, 246, 255, 271 and 3 β ,5 α -Stigma-7,16-dien-3-ol Figure 24 having m/z 412 with fragment ions of 28, 43, 55, 57, 69, 71, 81, 85, 95, 107, 119, 133, 147, 161, 173, 187, 201, 213, 231, 246, 255, 271, 283, 300, 314, 369, 397 were characterized along with chondrillasterol Figure 25 with fragment ions of 43, 55, 57, 69, 71, 81, 91, 93, 107, 119, 133, 147, 161, 173, 187, 201, 213, 219, 238, 246, 255, 271, 285, 300, 351, 369, 379, 397 were also obtained as some of the compounds. The elucidated important compounds are depicted in graphical abstract with Figure 26.

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