academicJournals

Vol. 8(2), pp. 25-48, February 2016
DOI: 10.5897/JPP2015.0367
Article Number: D4478E557364
ISSN 2141-2502

http://www.academicjournals.org/JPP

Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

Determination of metabolites products by *Cassia angustifolia* and evaluate antimicobial activity

Ali Hussein Al-Marzoqi [1], Mohammed Yahya Hadi[2] and Imad Hadi Hameed[1]*

[1]Department of Biology, Babylon University, Hilla City, Iraq.
[2]College of Biotechnology, Al-Qasim Green University, Iraq.

Received 26 August 2015; Accepted 20 October 2015

Phytochemicals are chemical compounds often referred to as secondary metabolites. Forty four bioactive phytochemical compounds were identified in the methanolic leaves extract of *Cassia angustifolia*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight and molecular formula. Gas chromatography-mass spectrometry (GC-MS) analysis of *C. angustifolia* revealed the existence of the 2,5-dimethyl-4-hydroxy-3(2H)-furanon, 2-propyl-tetrahydropyran-3-ol, estragole, benzene, 1-ethynyl-4-fluoro-, 5-hydroxymethylfurfural, anethole, 7-oxabicyclo[4.1.0]heptan-2-one,6-methyl-3-(1-methylethyl)-, 2-methoxy-4-vinylphenol, 1,2,2-trimethylcyclopentane-1,3-dicarboxylic acid, E-9-tetradecenoic acid, caryophyllene, cholestan-3-ol,2-methylene-, (3ß,5α)-, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-, ß-curcumene, 7-epi-cis-sesquisabinene hydrate, Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-m, octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a,-trimethyl, dodecanoic acid, 3-hydroxy, tetraacetyl-d-xylonic nitrile, 1-ethenyl 3, trans(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol, phen-1,4-diol,2,3-dimethyl-5-trifluoromethyl, 5-benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3,6-dime, 5-benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3,6-dime, phytol, acetate, desulphosiniqrin, oxiraneundecanoic acid, 3-pentyl-,methyl ester, cis,Phytol, 9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester, butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5adihydroxy-4-(h), 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E) and Diisooctyl phthalate. *C. angustifolia* was highly active against *Aspergillus terreus* (6.01±0.27).

Key words: Antifungal, gas chromatography-mass spectrometry, fourier-transform infrared spectroscopy, phytochemicals, *Cassia angustifolia*.

INTRODUCTION

Medicinal plants are those plants which contain substances that can be used for the therapeutic purposes in one or more of its organ or substances which are precursors for the synthesis of useful drugs (Sofowora, 1982; Bako et al., 2005; Altameme et al., 2015a). The use of medicinal herbs to relieve and treat diseases is

*Corresponding author. E-mail: imad_dna@yahoo.com. Tel: 009647716150716.

increasing because of their mild features and few side effects (Basgel and Erdemoglu, 2006). These plants are unlicensed and freely available, however, and there is no requirement to demonstrate efficacy, safety or quality (Ernst, 1998). The genus *Cassia* comprises 580 species of shrubs and trees which are widely distributed throughout the world, of which only twenty species are indigenous to India which belongs to the family Caesalpiniaceae, which generally consist of trees, shrubs and a few woody herbs. *Cassia angustifolia* Vahl (Family: Caesalpinaceae), popularly known as senna, is a valuable plant drug in Ayurvedic and modern system of medicine for the treatment of constipation. The pods and leaves of senna, as well as the pharmaceutical preparations containing sennosides A and B, are widely used in medicine because of their laxative properties. Senna is used in medicine as a cathartic; it is especially useful in habitual constipation. The laxative property of senna is based on two glycosides viz. sennoside A and sennoside B, whereas sennoside C and D have also been reported in the plant. Apart from sennoside, the pod and leaf also contain glycosides of anthraquinones rhein and chrysophenic acid, recently two naphthalene glycosides have also been isolated from leaves and pods (Gupta, 2010).

Antimicrobial activity has been reported in many plants by various workers (Sarin, 2005; Bansal et al., 2010; Chahal et al., 2010; Seth and Sarin, 2010; Malwal and Sarin, 2011; Hameed et al., 2015a). A new anthraquinone glycoside (emodin 8-0- sophorside) and seven known glycosides were isolated from the leaves of *C. angustifolia* and their structures were elucidated by spectral analysis (Kinjo et al., 1994). It has anti-inflammatory properties (Vanderperren et al., 2005), detoxification ability (Bournemouth, 1992) and also helps improve the function of the digestive system (Hoffmann, 1990). *Cassia senna* helps to reduce the nervous tension (Mills, 1993) and also helps in aiding the spleen and liver in production of blood and red blood cells (Spiller et al., 2003; Altameme et al., 2015b; Hamza et al., 2015). The present study was undertaken to investigate the antimicrobial activity and phytochemical analysis of *C. angustifolia*.

MATERIALS AND METHODS

Collection and preparation of plant material

C. angustifolia was purchased from local market in Hilla city, middle of Iraq. After thorough cleaning and removal of foreign materials, the seeds were stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use (Hameed et al., 2015b; Jasim et al., 2015).

Preparation of sample

About fifteen grams of methanolic leaves extract of *C. angustifolia* powdered was soaked in 30 ml methanol for ten hours in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture (Hussein et al., 2015; Hameed et al., 2015c).

Gas chromatography – mass spectrum analysis

The GC-MS analysis of the plant extract was made in a Agilent 7890 A instrument under computer control at 70 eV. About 1 µl of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected (Imad et al., 2014a; Kareem et al., 2015). The greater the concentration in the sample, the bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the retention time (RT). While the instrument was run, the computer generated a graph from the signal called chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the gas chromatography column into the detector (Mohammed and Imad, 2013; Imad et al., 2014b). The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing, them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass (Hameed et al., 2015d). The mass/charge (M/Z) ratio obtained was calibrated from the graph obtained, which was called the mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using gas chromatography and mass spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1 ml per minute. The electron gun of mass detector liberated electrons having energy of about 70eV. The column employed here for the separation of components was Elite 1 (100% dimethyl poly siloxane). The identity of the components in the extracts 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 (Imad et al., 2014c). Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries.

Determination of antifungal activity

Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 µl of the samples solutions (*C. angustifolia*) was delivered into the wells. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent (Hameed et al., 2015b). The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA), and differences among the means were determined for significance at P

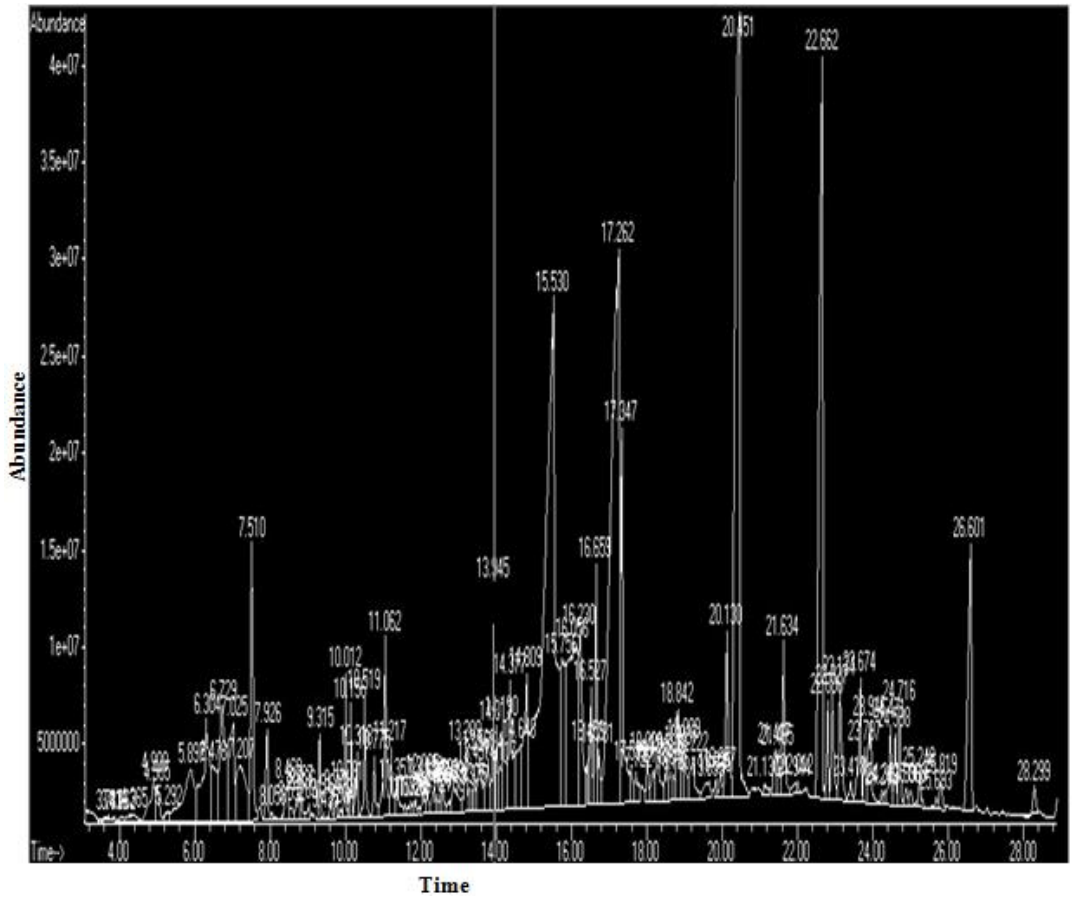


Figure 1. GC-MS chromatogram of methanolic extract of *Cassia angustifolia.*

< 0.05, using Duncan's multiple range test (by SPSS software) Version 9.1.

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *C. angustifolia*, as shown in Table 1. The GC-MS chromatogram of the forty four peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Althaea rosea* showed the presence of 44 major peaks and the components corresponding to the peaks were determined as follows. The first set up peaks were determined to be 2,5-dimethyl-4-hydroxy-3(2H)-furanon (Figure 2). The next peaks considered to be 2-Propyl-tetrahydropyran-3-ol, Estragole, Benzene, 1-ethynyl-4-fluoro-, 5-Hydroxymethylfurfural, Anethole, 7-Oxabicyclo[4.1.0]heptan-2-one,6-methyl-3-(1-methylethyl)-, 2-Methoxy-4-vinylphenol, 1,2,2-Trimethylcyclopentane-1,3-dicarboxylic acid, E-9-Tetradecenoic acid, Caryophyllene, Cholestan-3-ol,2-methylene-,(3ß,5α)-, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-, ß-curcumene, 7-epi-cis-sesquisabinene hydrate, Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-m, Octahydrobenzo[b] pyran,4a-acetoxy-5,5,8a,-trimethyl, Dodecanoic acid, 3-hydroxy, Tetraacetyl-d-xylonic nitrile, 1-Ethenyl 3,trans(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol, Phen-1,4-diol,2,3-dimethyl-5-trifluoromethyl, 5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3,6-dime, 5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3,6-dime, Phytol, acetate, Desulphosiniqrin, Oxiraneundecanoic acid, 3-pentyl-,methyl ester,cis, Phytol, 9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester, Butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5adihydroxy-4-(h), 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E) and Diisooctyl phthalate (Figures 3 to 45). Methanolic extraction of plant showed notable antifungal activities against *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus,* and *Aspergillus fumigatus* (Table 2). *C. angustifolia* was very highly active against *A. terreus* (6.01±0.27). *Aspergillus* was found to be sensitive to all test medicinal plants and mostly comparable to the standard reference antifungal drug amphotericin B and fluconazole to some extent.

Table 1. Major phytochemical compounds identified in *Cassia angustifolia.*

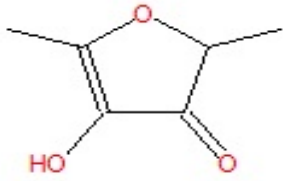
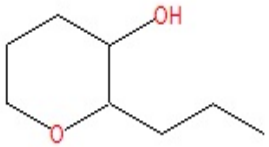
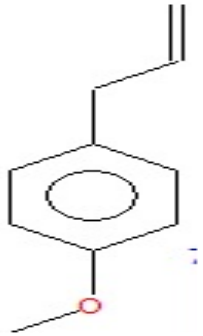
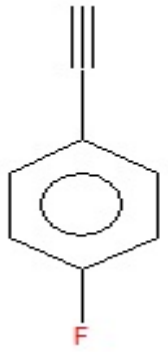
Serial No.	Phytochemical compound	RT (min)	Formula	Molecular weight	Exact mass	Chemical structure	MS Fragment- ions	Pharmacological actions
1	2,5-dimethyl-4-hydroxy-3(2H)-furanone	4.883	$C_6H_8O_3$	128	128.047344		57, 72, 85, 94, 109, 128	Antimicrobial effect
2	2-Propyl-tetrahydropyran-3-ol	5.908	$C_8H_{16}O_2$	144	144.115029		55, 73, 87, 101, 116, 144	Anti-infective agent in human microbial infections.
3	Estragole,	6.303	$C_{10}H_{12}O$	148	148.088815		51, 55, 63, 77, 91, 105, 121, 133, 148	Anti-inflammatory activity
4	Benzene , 1-ethynyl-4- fluoro	6.720	C_8H_5F	120	120.0375285		50, 63, 74, 81, 94, 100, 120	Antibacterial activity / Antifungal activity

Table 1. Cont'd

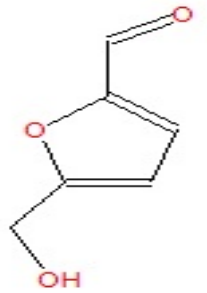
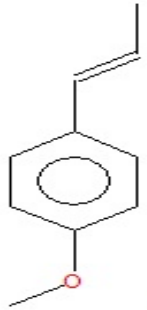
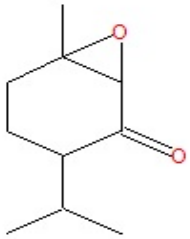
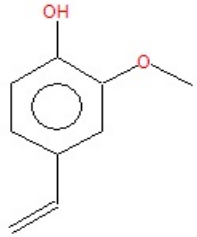
5	5-Hydroxymethylfurfural	7.247	$C_6H_6O_3$	126	126.0311694		53, 69, 81, 84, 97, 109, 126	Antioxidant and specific anti-cancer agents
6	Anethole	7.510	$C_{10}H_{12}O$	148	148.088815		51, 55, 63, 74, 77, 91, 105, 117, 133, 148	Antihyperglycemic effect
7	7-Oxabicyclo[4.1.0]heptan-2-one,6-methyl -3-(1-methylethyl)-	7.750	$C_{10}H_{16}O_2$	168	168.115029		55, 69, 83, 97, 111, 126, 139, 150, 168	New chemical compound
8	2-Methoxy-4-vinylphenol	7.933	$C_9H_{10}O_2$	150	150.06808		51, 77, 89, 107, 121, 135	Antioxidant, *anti* microbial and anti inflammatory

Table 1. Cont'd

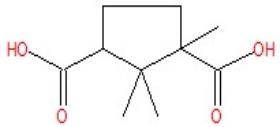
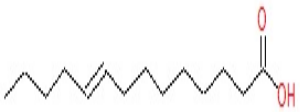
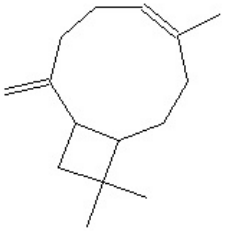
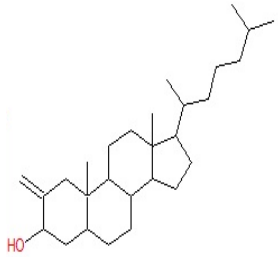
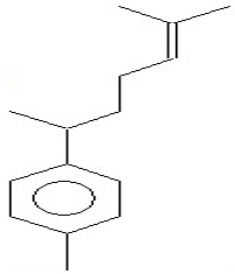
9	1,2,2-Trimethylcyclopentane-1,3-dicarboxylic acid	8.431	$C_{10}H_{16}O_4$	200	200.104859		55, 68, 82, 109, 136, 154, 182	Anti- fungal
10	E-9-Tetradecenoic acid	8.746	$C_{14}H_{26}O_2$	226	226.19328		55, 69, 83, 97, 110, 166, 208	Analgesic and *anti*-inflammatory effect
11	Caryophyllene	9.301	$C_{15}H_{24}$	204	204.1878		79, 93, 105, 120, 133, 147, 161, 175, 189, 204	Anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and local anaesthetic
12	Cholestan-3-ol,2-methylene-,(3ß,5α)-	9.616	$C_{28}H_{48}O$	400	400.370516		69, 81, 95, 175, 227, 260, 315, 400	Anti-oxidant
13	Benzene , 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	10.010	$C_{15}H_{22}$	202	202.172151		55, 65, 69, 77, 83, 91, 95, 105, 119, 132, 145, 159, 187, 202	Antimicrobial and anti-inflammatory

Table 1. Cont'd

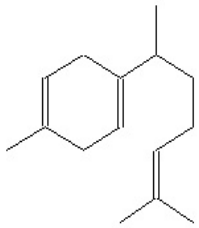
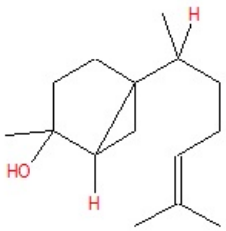
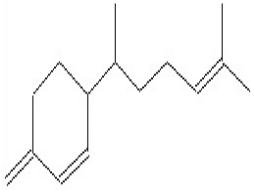
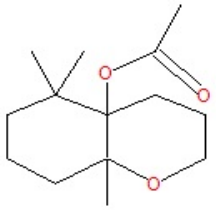
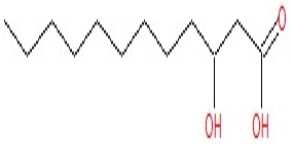
14	ß-curcumene	10.165	$C_{15}H_{24}$	204	204.1878		55, 69, 77, 93, 105, 119, 133, 147, 161, 176, 189, 204	Anti-tumor, anti-cancer, anti-repellent, antitussive and anti-platelet
15	7-epi-cis-sesquisabinene hydrate	10.274	$C_{15}H_{26}O$	222	222.198365		55, 69, 82, 93, 105, 119, 161, 175, 204, 222	nti-cancer
16	Cyclohexene ,3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-	10.508	$C_{15}H_{24}$	204	204.1878		55, 69, 77, 93, 109, 133, 147, 161, 175, 189, 204	Anti-bacterial and antifungal
17	Octahydrobenzo[b]pyran,4a-acetoxy-5,5,8a,-trimethyl	10.771	$C_{14}H_{24}O_3$	240	240.1725445		55, 69, 97, 111, 124, 137, 151, 165, 180, 197, 240	Anti-Candida and anti-inflammatory
18	Dodecanoic acid , 3-hydroxy	11.218	$C_{12}H_{24}O_3$	216	216.1725445		55, 69, 83, 96, 112, 126, 138, 151, 180, 200, 215	Antifungal activity

Table 1. Cont'd

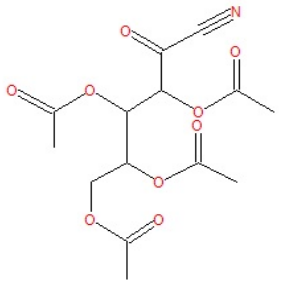
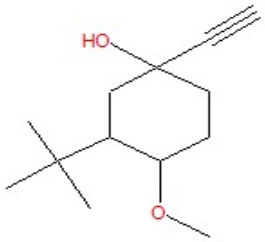
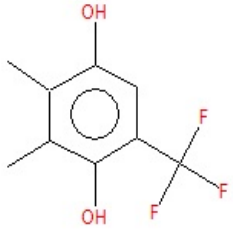
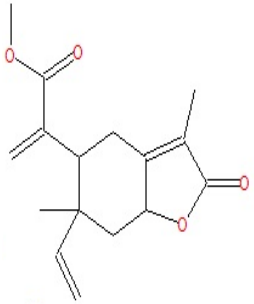
19	Tetraacetyl-d-xylonic nitrile	11.012	$C_{14}H_{17}NO_9$	343	343.090332		60, 73, 112, 133, 164, 197, 226, 270	Have anti-viral effects
20	1-Ethenyl 3,trans(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol	11.246	$C_{13}H_{22}O_2$	210	210.16198		57, 70, 79, 91, 104, 121, 137, 151, 163, 192, 210	Unknown
21	Phen-1,4-diol,2,3-dimethyl-5-trifluoromethyl	11.378	$C_9H_9F_3O_2$	206	206.055464		57, 69, 83, 91, 123, 149, 206	Antimicrobial effect
22	5-Benzofuranacetic acid,6-ethenyl -2,4,5,6,7,7a-hexahydro-3,6-dime	12.036	$C_{16}H_{20}O_4$	276	276.13616		53, 77, 91, 105, 121, 148, 176, 216, 244, 276	New chemical compound

Table 1. Cont'd

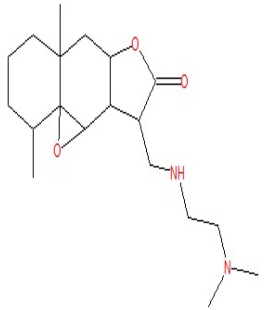
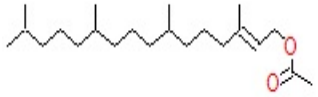
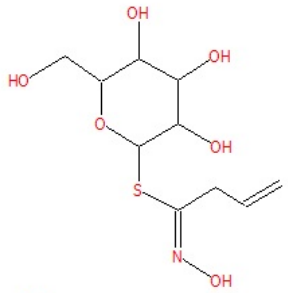
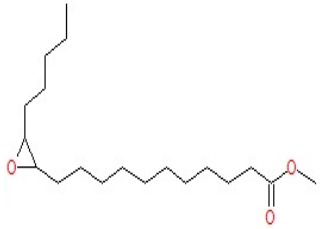
23	2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9-[[[2-(dimethylamin	12.877	$C_{19}H_{32}N_20_3$	336	336.241293		58, 71, 81, 91, 109, 123, 149, 166, 185, 204, 219, 233, 248	Antitumor, anti-inflammatory, antifungal, pesticidal and insecticidal
24	Phytol, acetate	13.953	$C_{22}H_{42}O_2$	338	338.318481		57, 68, 81, 95, 109, 123, 137, 151, 179, 208, 249, 278	Anti-inflammatory, antileishmanial and antitrypanosomal
25	Desulphosiniqrin	14.399	$C_{10}H_{17}NO_6S$	279	279.077658		60, 73, 85, 103, 127, 145, 163, 213, 262	New chemical compound
26	Oxiraneundecanoic acid ,3-pentyl-,methyl ester , cis	16.482	$C_{19}H_{36}O_3$	312	312.266445		55, 74, 87, 97, 111, 127, 155, 183, 199, 227, 264, 294	Anti-oxidant
27	Phytol	16.665	$C_{20}H_{40}O$	296	296.307917		57, 71, 81, 95, 111, 123, 137, 196, 221, 249, 278	Anti-cancer activities

Table 1. Cont'd

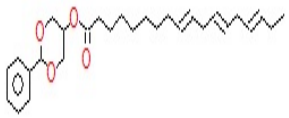
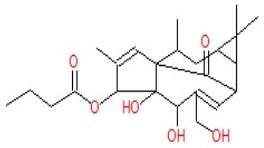
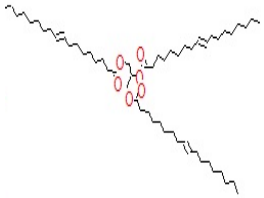
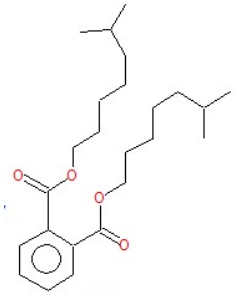
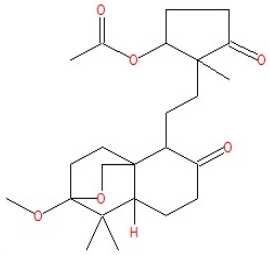
28	9,12,15-Octadecatrienoic acid , 2-phenyl-1,3-dioxan-5-yl ester	18.296	$C_{28}H_{40}O_4$	440	440.29266		55, 67, 79, 105, 129, 165, 185, 219, 265, 334, 440	Antiviral and anti-obesity properties
29	Butanoic acid ,1a,2,5,5a,6,9,10,10a-octahydro-5,5adihydroxy-4-(h)	18.874	$C_{24}H_{34}O_6$	440	440.29266		71, 91, 107, 122, 135, 151, 177, 213, 241, 299, 387, 418	Unknown
30	9-Octadecenoic acid , 1,2,3-propanetriyl ester , (E,E,E)-	19.846	$C_{57}H_{104}O_6$	884	884.78329		55, 69, 83, 98, 220, 264, 282, 339, 356, 393, 449, 489	Anti-spasmodic and immune modulators
31	Diisooctyl phthalate	20.373	$C_{24}H_{38}O_4$	390	390.27701		57, 71, 83, 113, 132, 149, 167, 279, 390	Antimicrobial activity and Anti oxidant.
32	8,14-Seco -3,19-epoxyandrostane-8,14-dione,17-acetoxy-3ß-methoxy	21.449	$C_{24}H_{36}O_6$	420	420.251188		55, 69, 83, 96, 111, 149, 177, 209, 265, 304, 360, 420	New chemical compound

Table 1. Cont'd

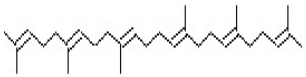
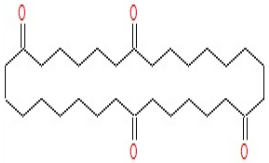
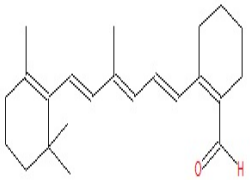
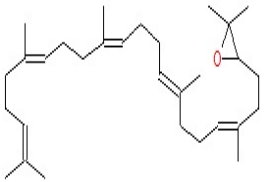
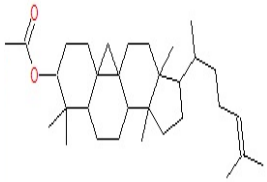
33	Squalene	22.604	$C_{20}H_{50}$	410	410.391253		69, 81, 95, 121, 149, 175, 203, 231, 257, 285, 341, 367, 395	Widely used in the cosmetics industry as an *anti*-wrinkle agent
34	Cyclopropanebutanoic acid ,2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclo	22.845	$C_{25}H_{42}O_2$	374	374.318481		74, 121, 227, 270, 298, 334	Used as a poultice as an *anti*-inflammatory
35	Cyclotriaconta-1,7,16,22,-tetraone	23.159	$C_{30}H_{52}O_4$	476	476.38656		55, 81, 125, 183, 239, 279, 321, 337, 379, 419, 458	New chemical compound
36	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclo	23.451	$C_{23}H_{32}O$	324	324.245316		555, 91, 135, 173, 187, 239, 324	Antimicrobials and anti-virals
37	Oxirane ,2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19,-hen	23.657	$C_{30}H_{50}O$	426	426.386166		69, 81, 95, 135, 203, 231, 271, 299, 357, 426	Anti-diarrhoeal activity
38	9,19-Cyclolanost-24-en-3-ol,acetate , (3ß)-	23.686	$C_{32}H_{52}O_2$	468	468.39673		55, 69, 81, 95, 109, 135, 203, 217, 286, 311, 365, 408, 424	Anti mosquito larvicidal activity

Table 1. Cont'd

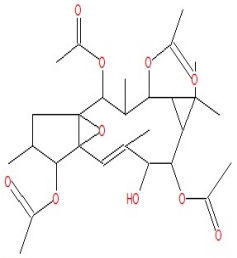
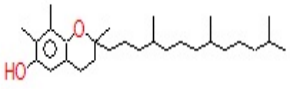
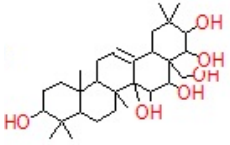
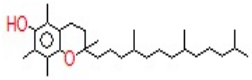
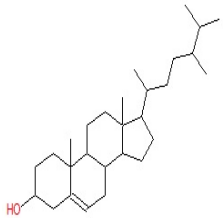
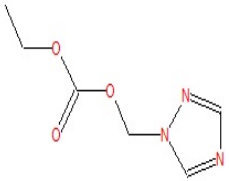
39	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	25.025	$C_{28}H_{40}O_{10}$	536	536.262146		55, 69, 122, 207, 236, 297357, 417, 477	Anti- inflammatory effects
40	y-Tocopherol	25.236	$C_{28}H_{48}O_2$	416	416.365543		57, 107, 151, 191, 205, 246, 274, 303, 344, 373, 416	anti-oxidant activity
41	Olean-12-ene-3,15,16,21,22,28-hexol,(3ß,15α,16α,21ß,22α)-	25.683	$C_{30}H_{50}O_6$	506	506.360739		135, 190, 207, 231, 249, 280, 298, 334, 352, 384, 439, 506	Anti-tumourogenic properties
42	Vitamin E	26.581	$C_{29}H_{50}O_2$	430	430.38108		57, 69, 91, 121, 165, 205, 246, 274, 302, 330, 358, 386	Anti-oxidant activity
43	Campesterol	28.315	$C_{28}H_{80}O$	400	400.370516		55, 71, 81, 145, 161, 213, 255, 289, 315, 382, 400	Campesterol is a phytosterol whose chemical structure is similar to that of cholesterol. have *anti*-inflammatory effects.
44	Carbonic acid , (ethyl)(1,2,4-triazol-1-ylmethyl)diester	3.224	$C_6H_9N_3O_3$	171	171.064391		55, 70, 82, 98, 112, 171	Anti-inflammatory

Table 2. Zone of inhibition (mm) of *Aspergillus Spp.* test to *Cassia angustifolia* bioactive compounds and standard antibiotics.

Plant/ antibiotics	*Aspergillus spp.*			
	Aspergillus niger	***Aspergillus terreus***	***Aspergillus flavus***	***Aspergillus fumigatus***
Cassia angustifolia	3.08±0.10	6.01±0.27	5.00±0.16	4.03±0.20
Amphotericin B	2.01±0.20	2.99±0.16	4.05±0.10	4.90±0.30
Fluconazol	4.08±0.61	2.96±0.14	3.00±0.81	4.90±0.40
Control	0.00	0.00	0.00	0.00

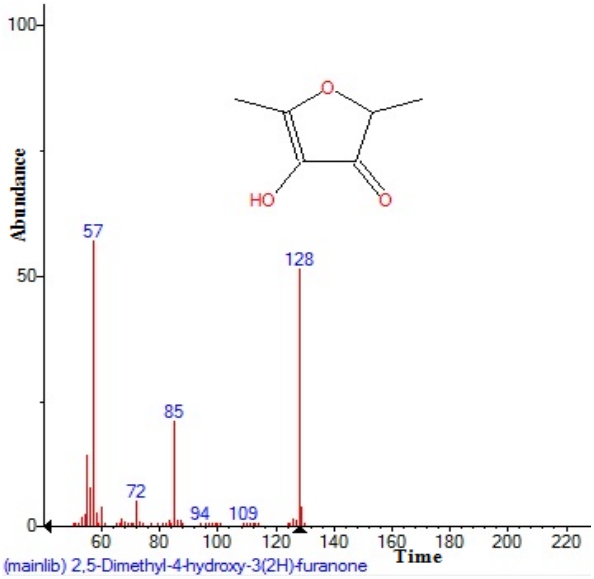


Figure 2. Structure of 2,5-dimethyl-4-hydroxy-3(2H)-furanone present in *Cassia angustifolia* with RT= 4.883 using GC-MS analysis.

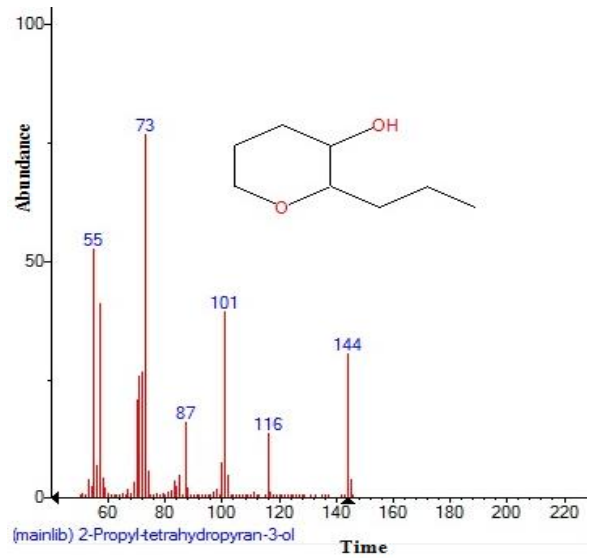


Figure 3. Structure of 2-Propyl-tetrahydropyran-3-ol present in *Cassia angustifolia* with RT= 5.908 using GC-MS analysis.

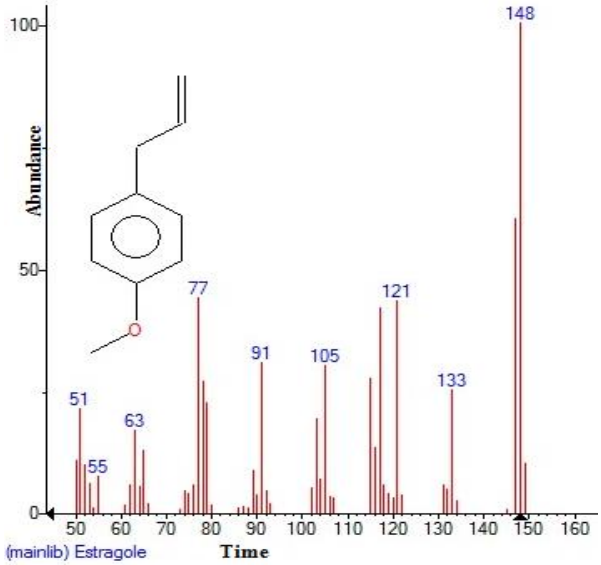


Figure 4. Structure of Estragole present in *Cassia angustifolia* with RT= 6.303 using GC-MS analysis.

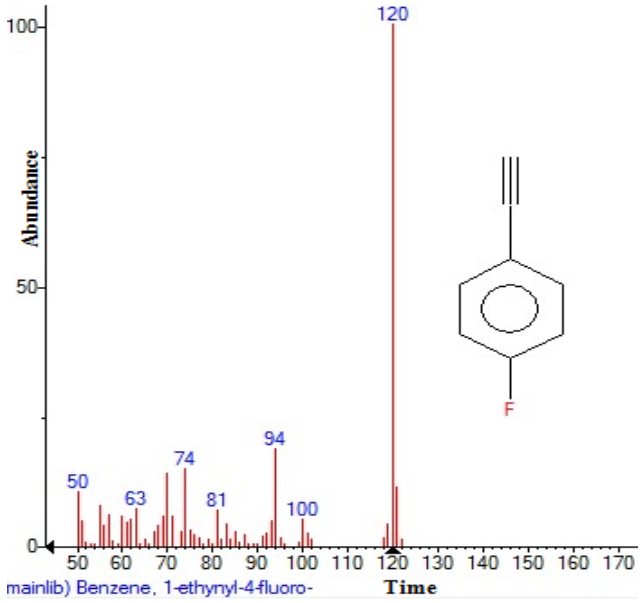


Figure 5. Structure of Benzene, 1-ethynyl-4- fluoro present in *Cassia angustifolia* with RT= 6.720 using GC-MS analysis.

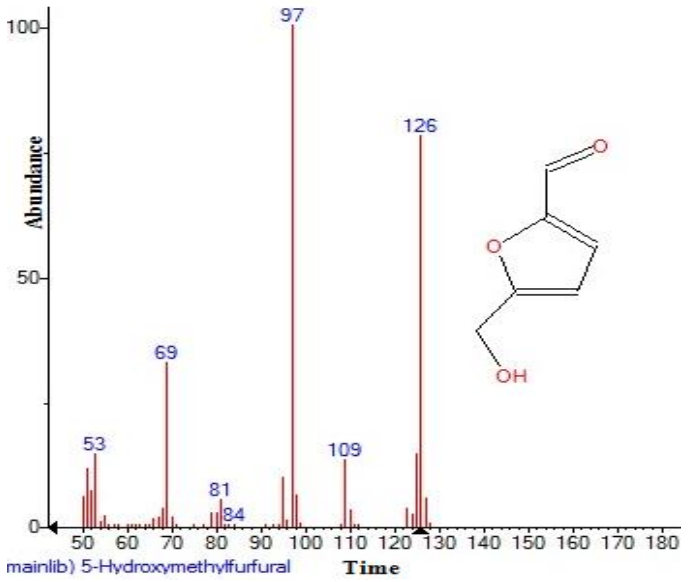


Figure 6. Structure of 5-Hydroxymethylfurfural present in *Cassia angustifolia* with RT= 7.247 using GC-MS analysis.

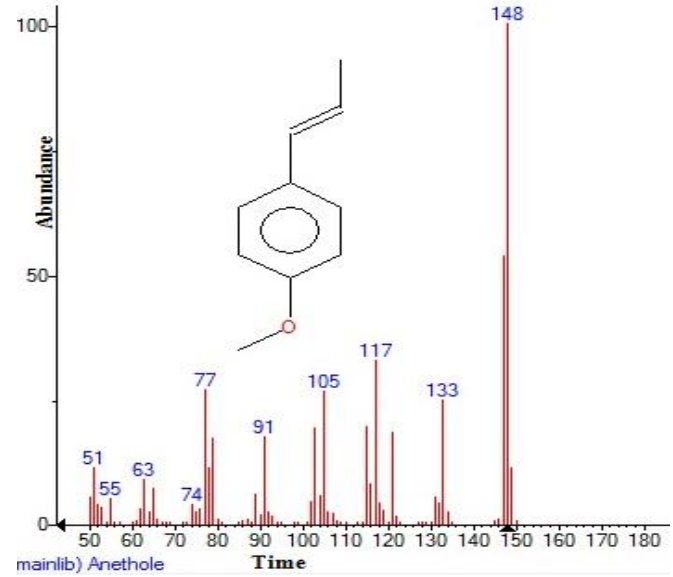


Figure 7. Structure of Anethole present in *Cassia angustifolia* with RT= 7.510 using GC-MS analysis.

Conclusion

From the results obtained in this study, it could be concluded that *C. angustifolia* possesses remarkable antimicrobial activity which is mainly due to 2-Propyl-tetrahydropyran-3-ol, 1,2,2-Trimethylcyclopentane-1,3-dicarboxylic acid and Diisooctyl phthalate. According to these findings, it could be said that the methanol extract act as antifungal agent.

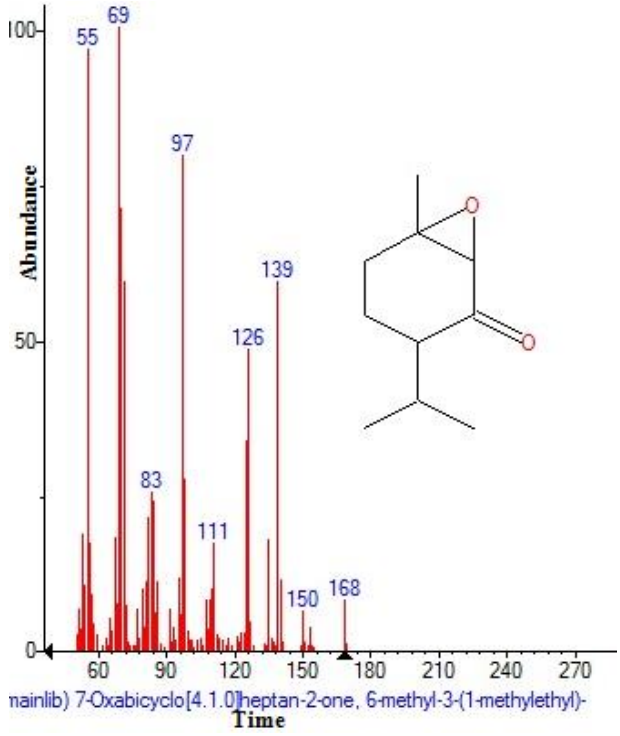


Figure 8. Structure of 7-Oxabicyclo[4.1.0]heptan-2-one,6-methyl -3-(1-methylethyl) present in *Cassia angustifolia* with RT = 7.750 using GC-MS analysis.

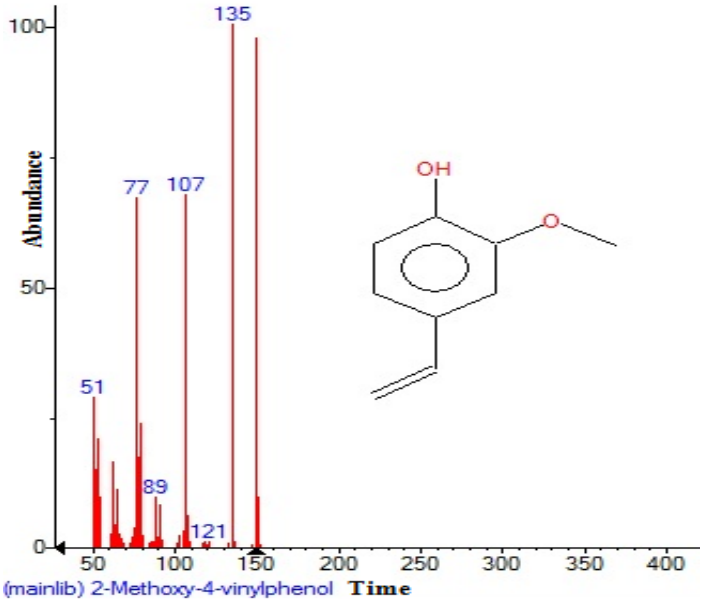


Figure 9. Structure of 2-Methoxy-4-vinylphenol present in *Cassia angustifolia* with RT= 7.933 using GC-MS analysis.

Conflict of Interests

The authors have not declared any conflict of interests.

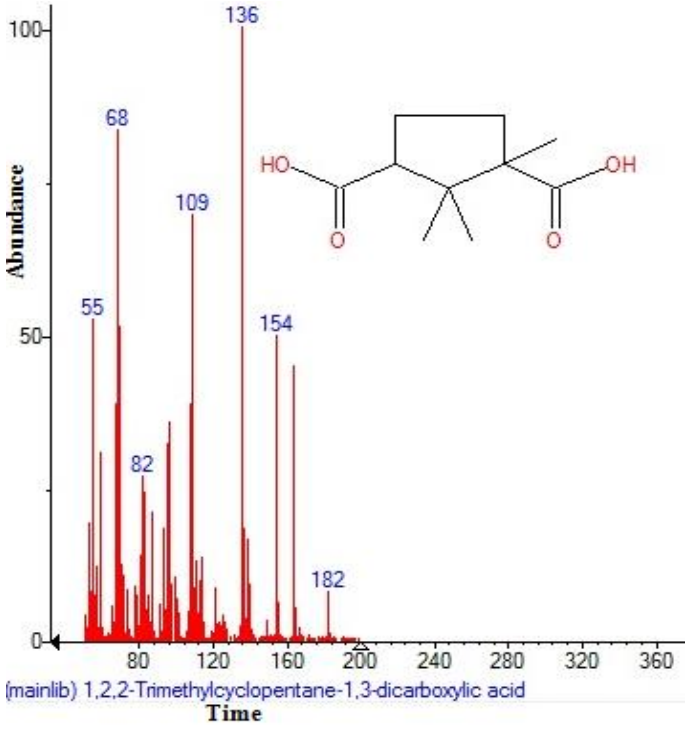


Figure 10. Structure of 1,2,2-Trimethylcyclopentane-1,3-dicarboxylic acid present in *Cassia angustifolia* with RT= 8.431 using GC-MS analysis.

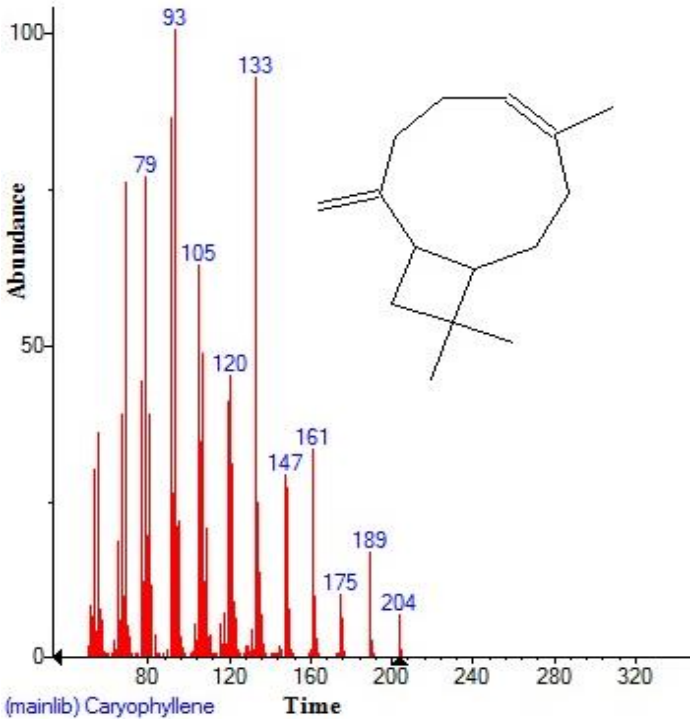


Figure 12. Structure of Caryophyllene present in *Cassia angustifolia* with RT= 9.301 using GC-MS analysis.

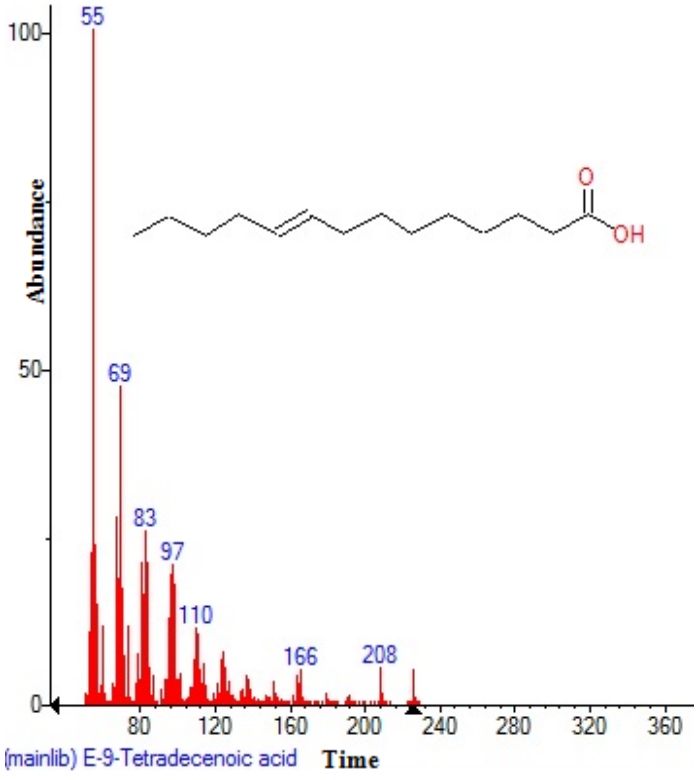


Figure 11. Structure of E-9-Tetradecenoic acid present in *Cassia angustifolia* with RT= 8.746 using GC-MS analysis.

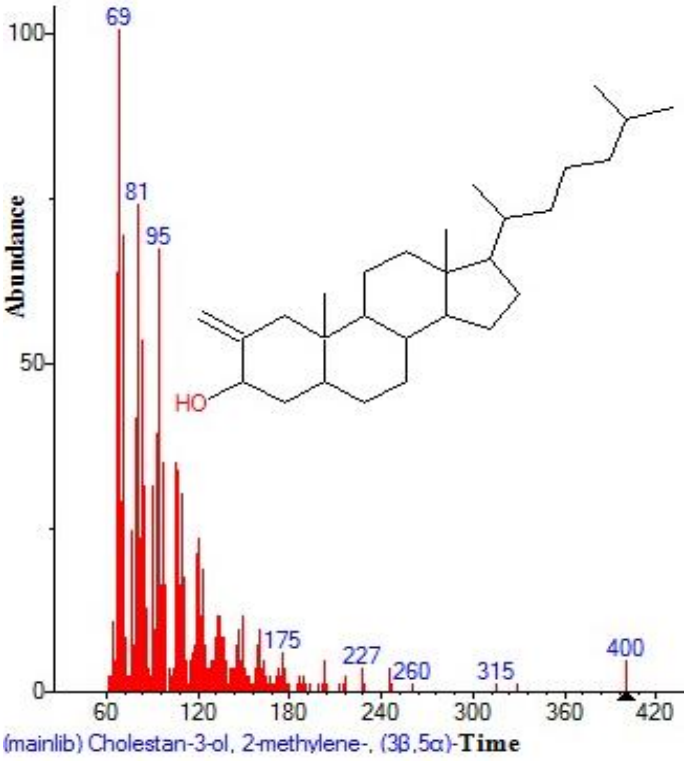


Figure 13. Structure of Cholestan-3-ol,2-methylene-,(3ß,5α) present in *Cassia angustifolia* with RT= 9.616 using GC-MS analysis.

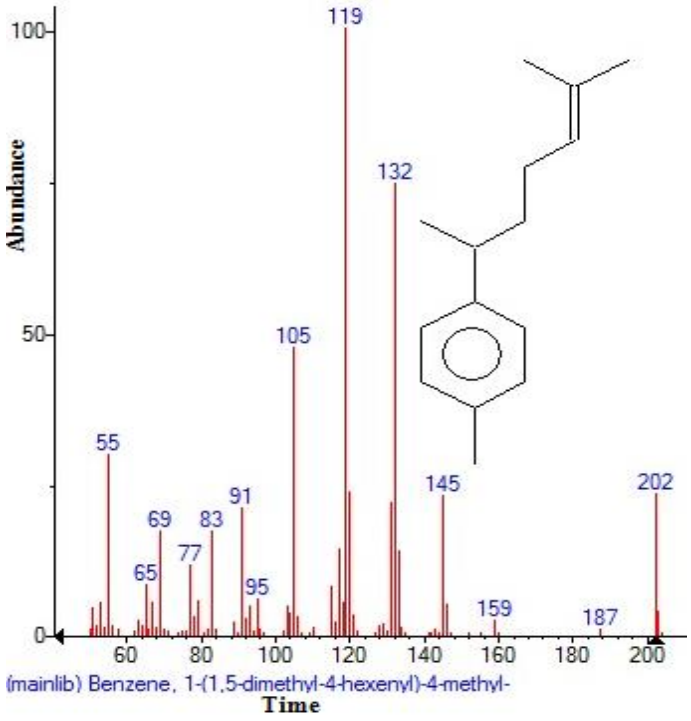


Figure 14. Structure of Benzene , 1-(1,5-dimethyl-4-hexenyl)-4-methyl present in *Cassia angustifolia* with RT= 10.010 using GC-MS analysis.

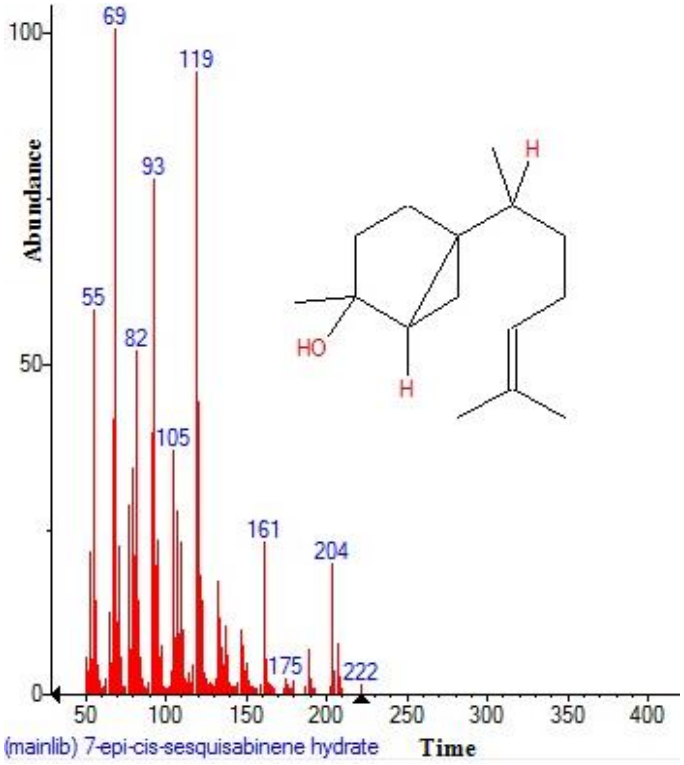


Figure 16. Structure of 7-epi-cis-sesquisabinene hydrate present in *Cassia angustifolia* with RT = 10.274 using GC-MS analysis.

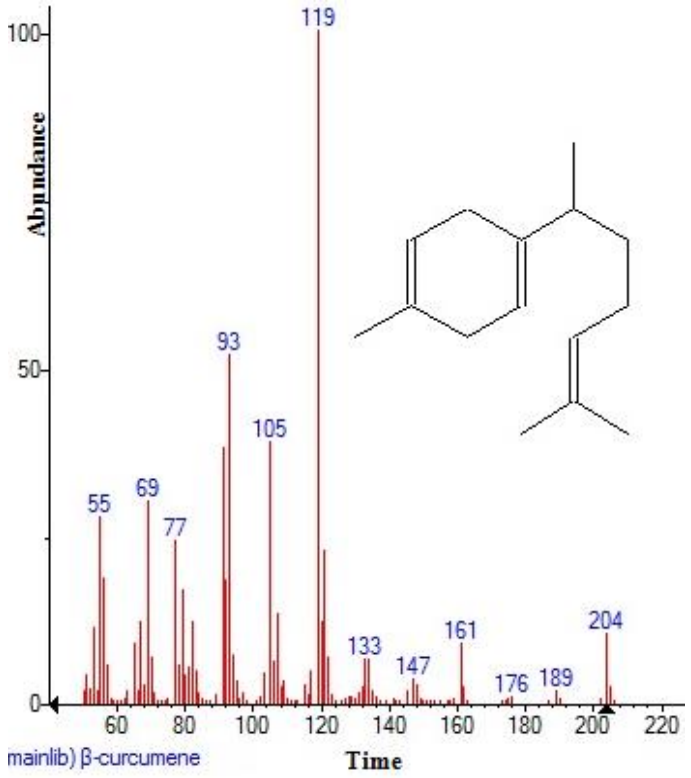


Figure 15. Structure of ß-curcumene present in *Cassia angustifolia* with RT= 10.165 using GC-MS analysis.

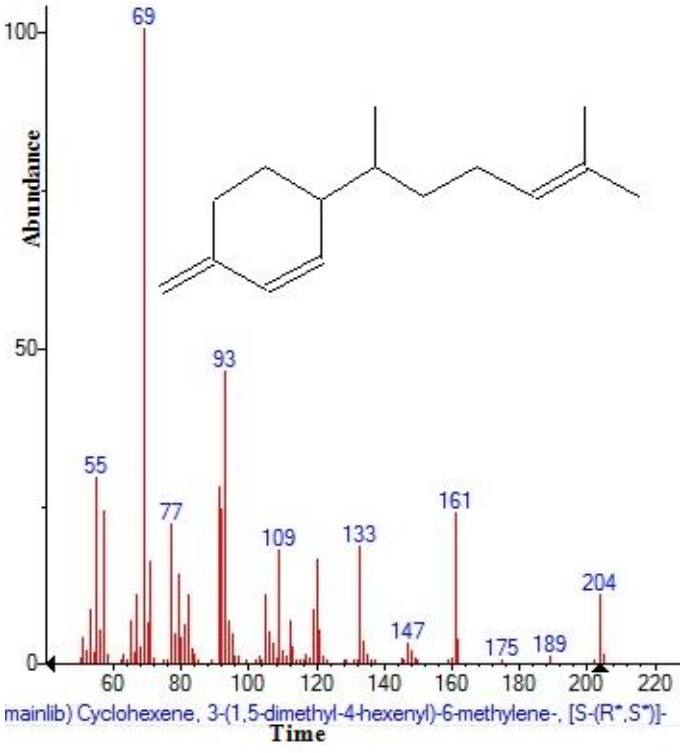


Figure 17. Structure of Cyclohexene ,3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)] present in *Cassia angustifolia* with RT= 10.508 using GC-MS analysis.

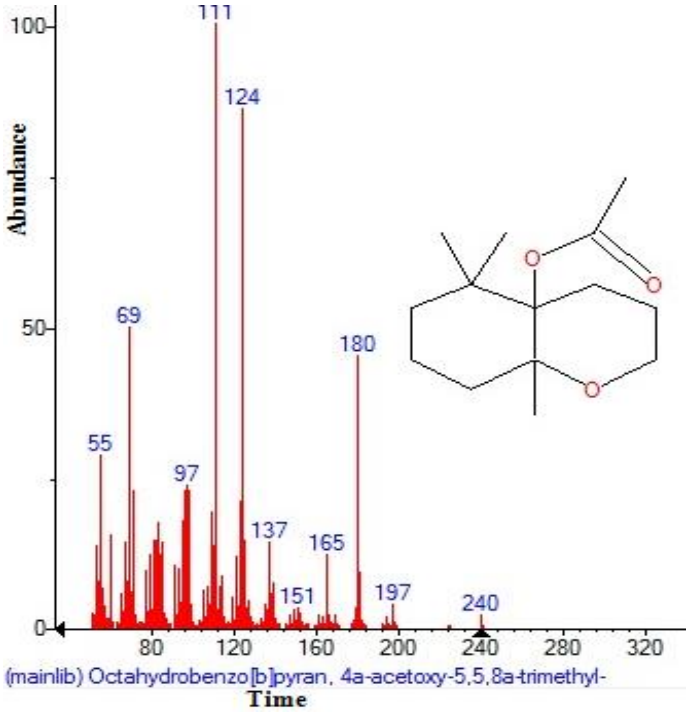


Figure 18. Structure of Octahydrobenzo[b]pyran,4a-acetoxy-5,5,8a,-trimethyl present in *Cassia angustifolia* with RT= 10.771 using GC-MS analysis.

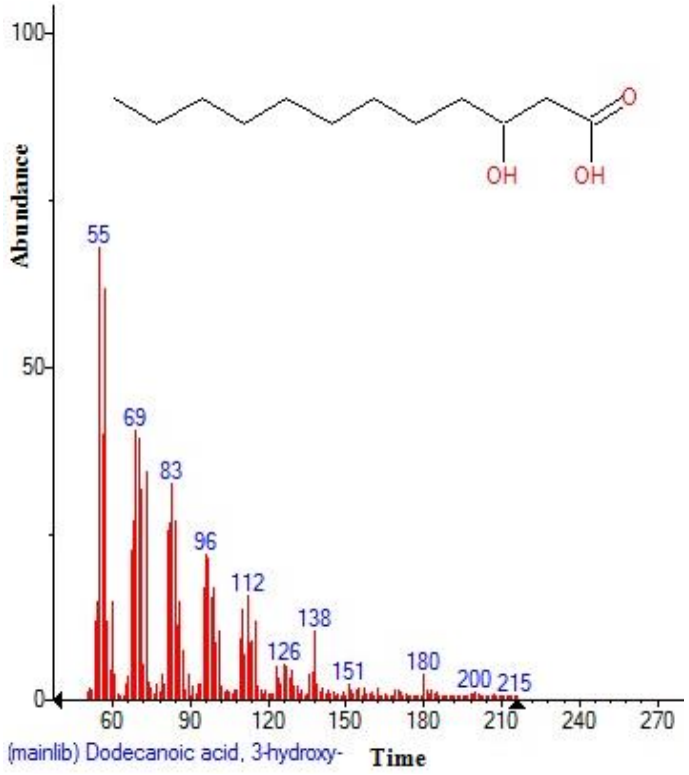


Figure 19. Structure of Dodecanoic acid , 3-hydroxy present in *Cassia angustifolia* with RT= 11.218 using GC-MS analysis.

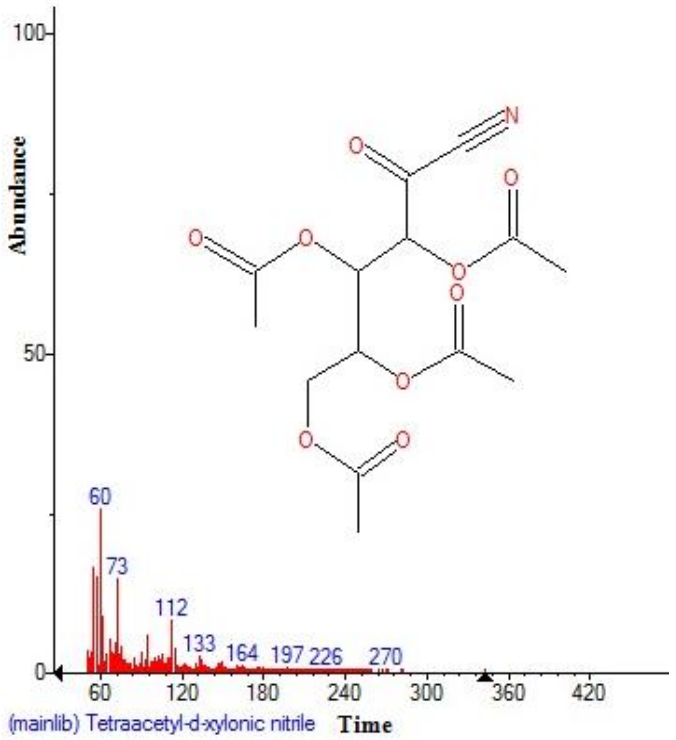


Figure 20. Structure of Tetraacetyl-d-xylonic nitrile present in *Cassia angustifolia* with RT= 11.012 using GC-MS analysis.

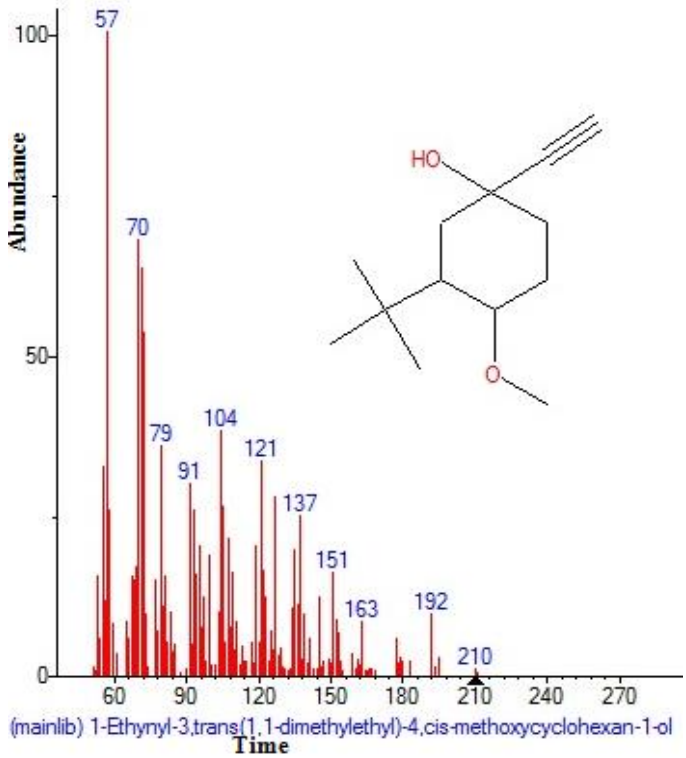


Figure 21. Structure of 1-Ethenyl 3,trans(1,1-dimethylethyl)-4,cis-methoxycyclohexan-1-ol present in *Cassia angustifolia* with RT= 11.246 using GC-MS analysis.

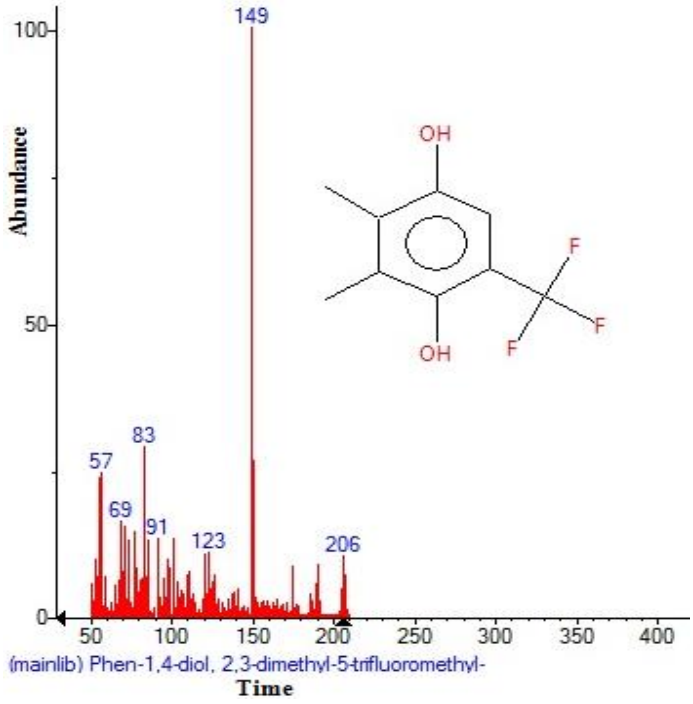


Figure 22. Structure of Phen-1,4-diol,2,3-dimethyl-5-trifluoromethyl present in *Cassia angustifolia* with RT= 11.378 using GC-MS analysis.

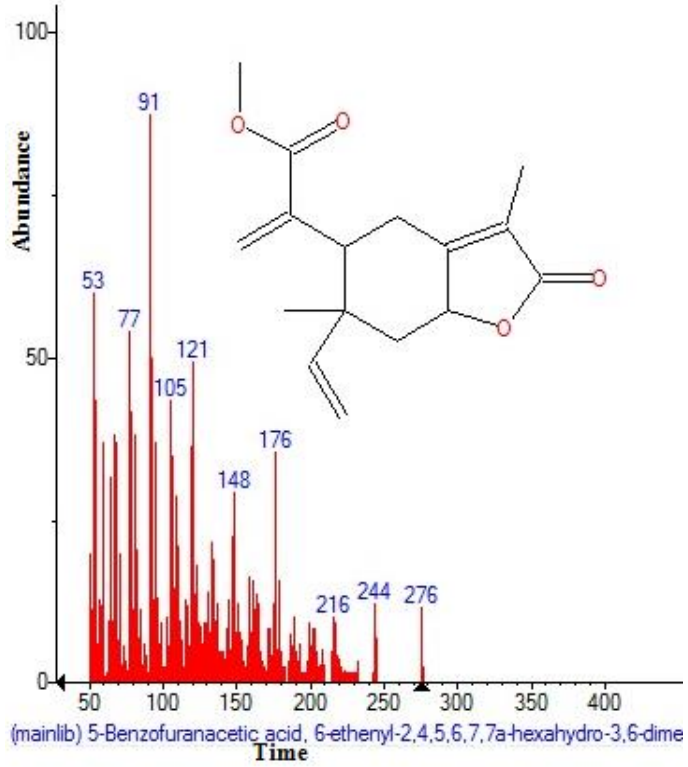


Figure 23. Structure of 5-Benzofuranacetic acid,6-ethenyl -2,4,5,6,7,7a-hexahydro-3,6-dime present in *Cassia angustifolia* with RT= 12.036 using GC-MS analysis.

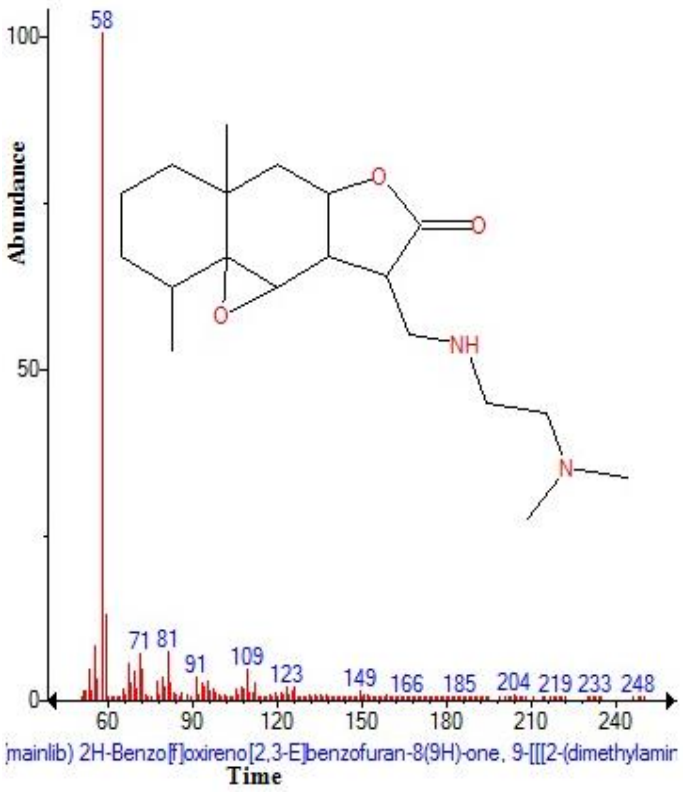


Figure 24. Structure of 2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9-[[[2-(dimethylamin present in *Cassia angustifolia* with RT= 12.877 using GC-MS analysis.

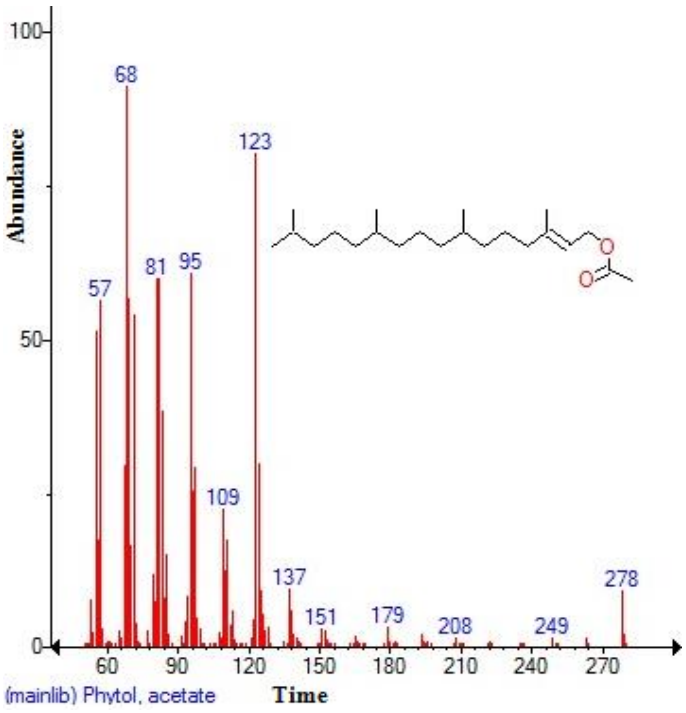


Figure 25. Structure of Phytol, acetate present in *Cassia angustifolia* with RT= 13.953 using GC-MS analysis.

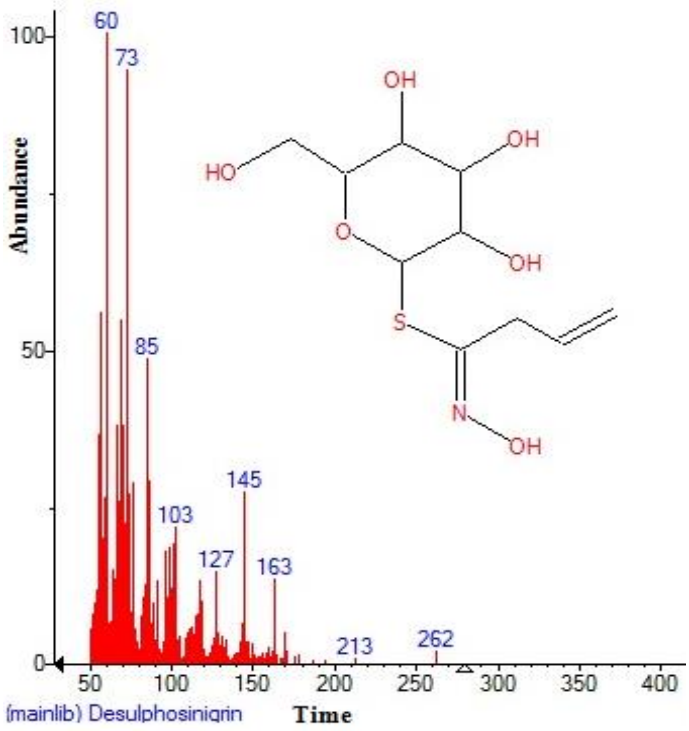


Figure 26. Structure of Desulphosiniqrin present in *Cassia angustifolia* with RT= 14.399 using GC-MS analysis.

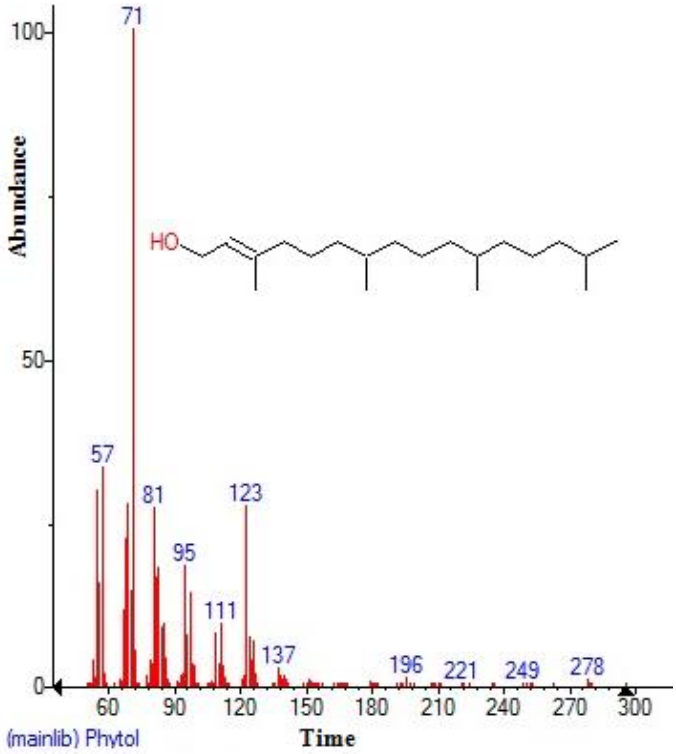


Figure 28. Structure of Phytol present in *Cassia angustifolia* with RT= 16.665 using GC-MS analysis.

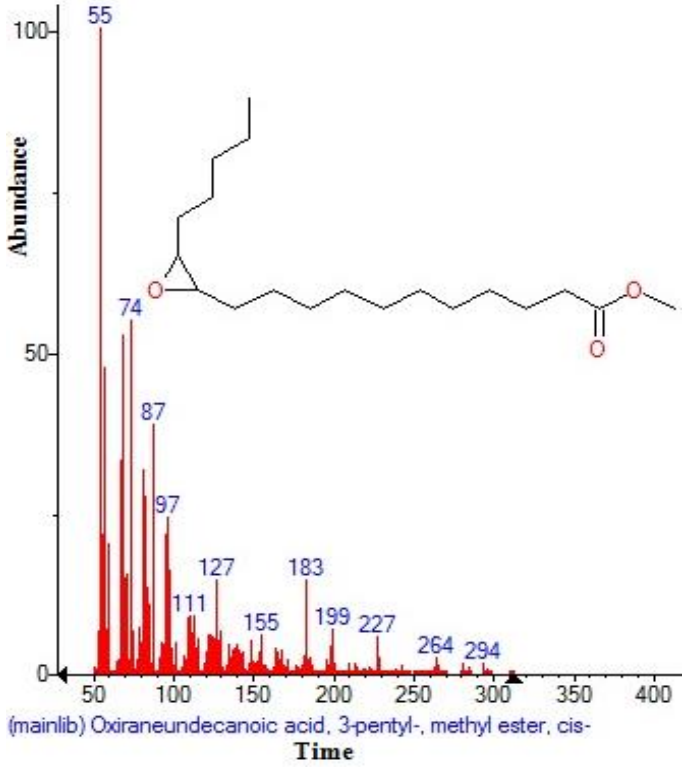


Figure 27. Structure of Oxiraneundecanoic acid ,3-pentyl-,methyl ester , cis present in *Cassia angustifolia* with RT= 16.482 using GC-MS analysis.

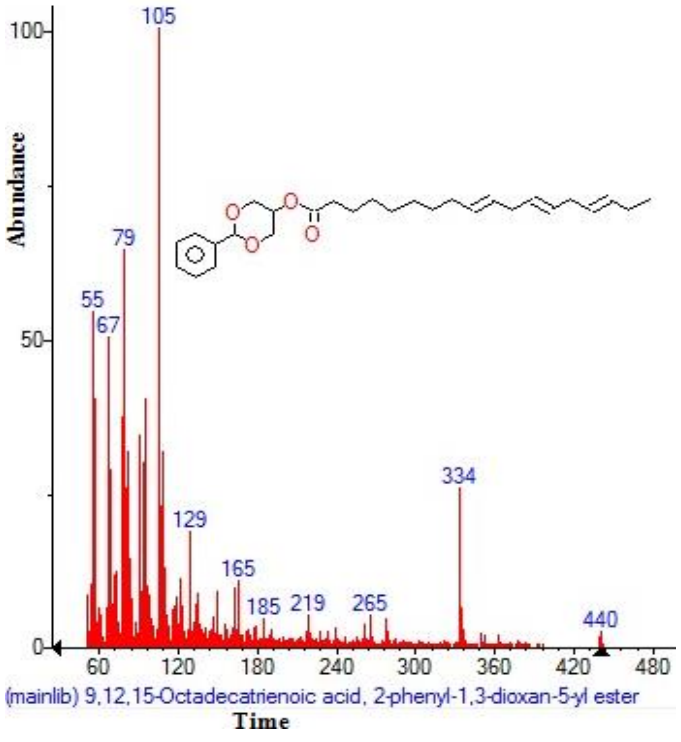


Figure 29. Structure of 9,12,15-Octadecatrienoic acid , 2-phenyl-1,3-dioxan-5-yl ester present in *Cassia angustifolia* with RT= 18.296 using GC-MS analysis.

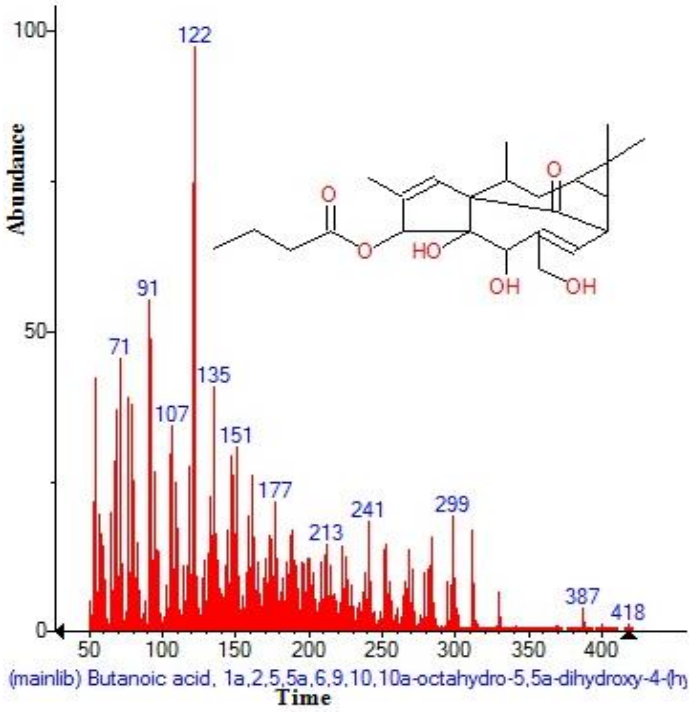


Figure 30. Structure of Butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5adihydroxy-4-(h) present in *Cassia angustifolia* with RT= 18.874 using GC-MS analysis.

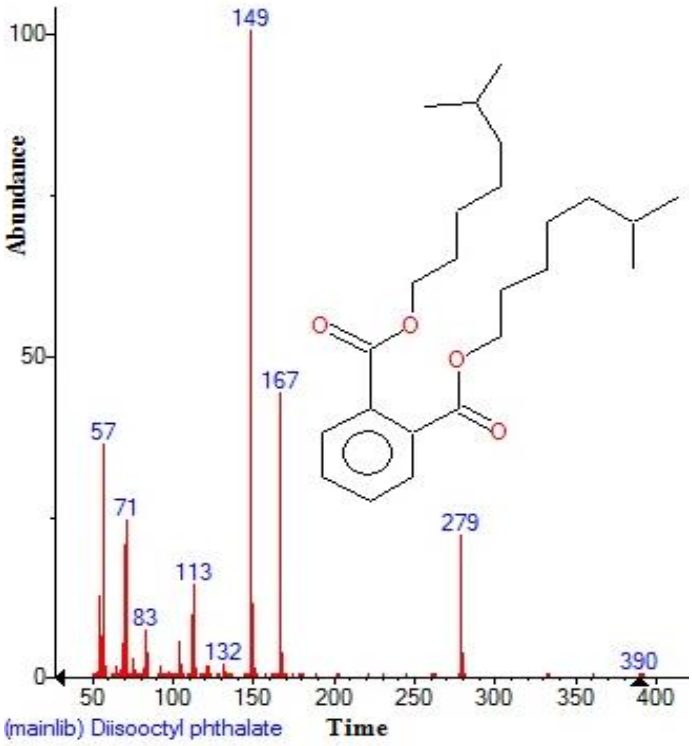


Figure 32. Structure of Diisooctyl phthalate present in *Cassia angustifolia* with RT= 20.373 using GC-MS analysis.

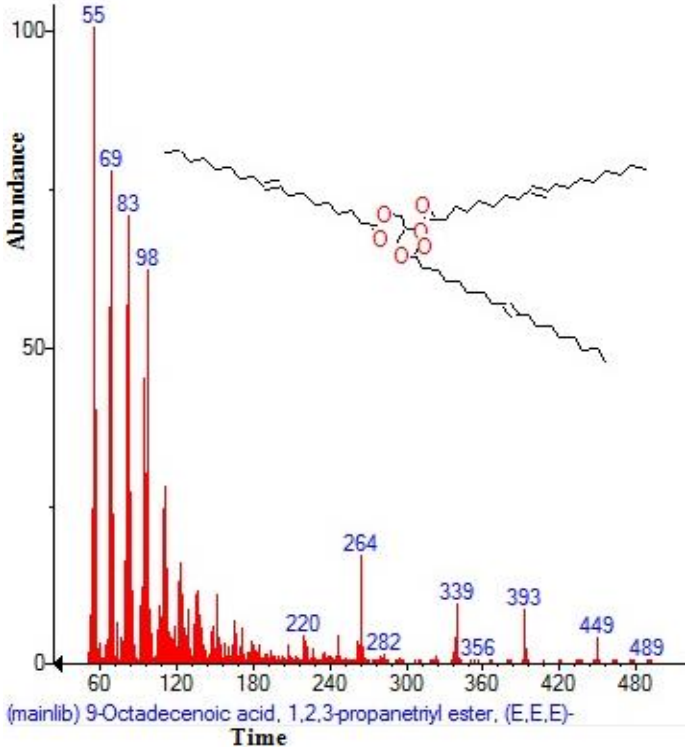


Figure 31. Structure of 9-Octadecenoic acid , 1,2,3-propanetriyl ester , (E,E,E) present in *Cassia angustifolia* with RT= 19.846 using GC-MS analysis.

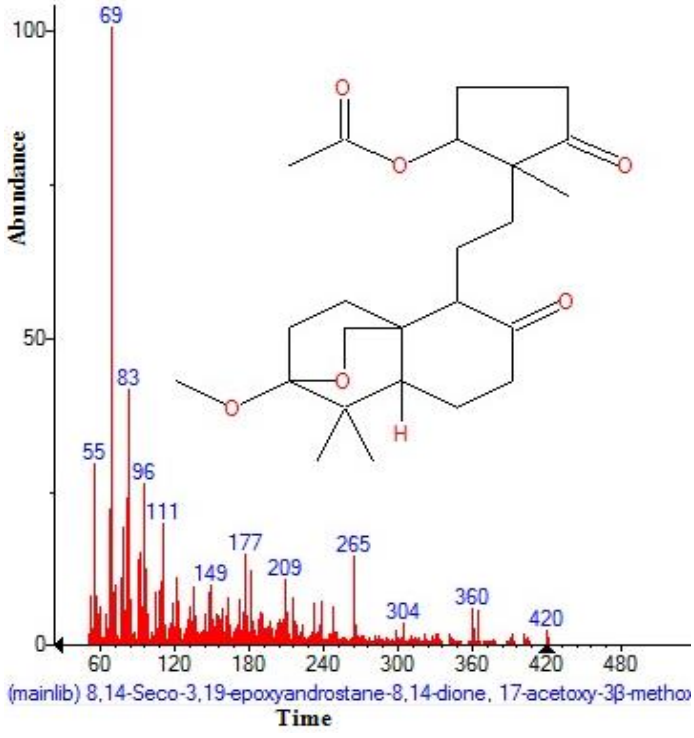


Figure 33. Structure of 8,14-Seco -3,19-epoxyandrostane-8,14-dione,17-acetoxy-3ß-methoxy present in *Cassia angustifolia* with RT= 21.449 using GC-MS analysis.

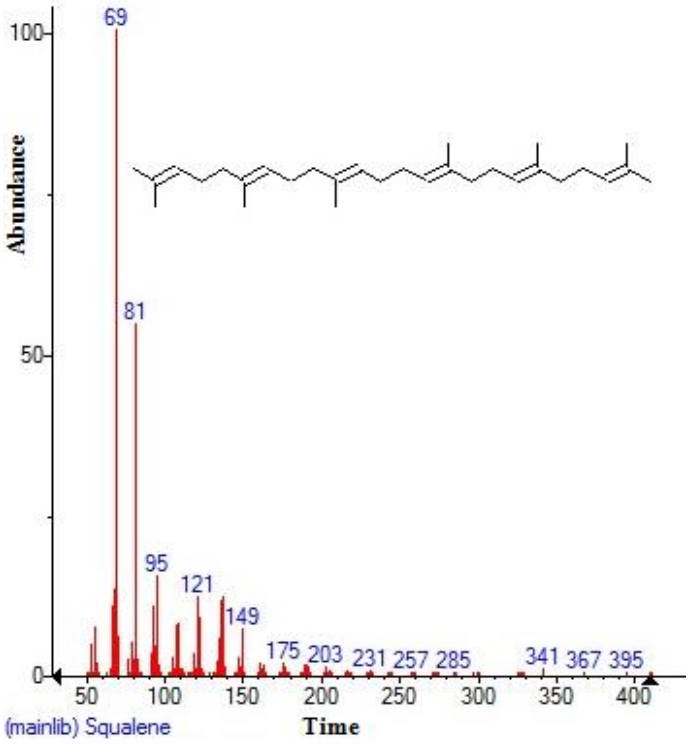


Figure 34. Structure of Squalene present in *Cassia angustifolia* with RT= 22.604 using GC-MS analysis.

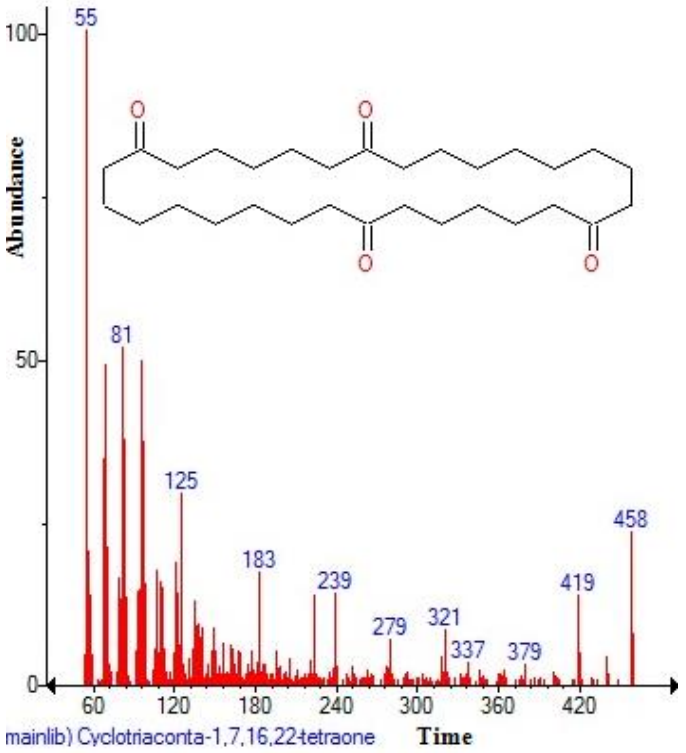


Figure 36. Structure of Cyclotriaconta-1,7,16,22,-tetraone present in *Cassia angustifolia* with RT= 23.159 using GC-MS analysis.

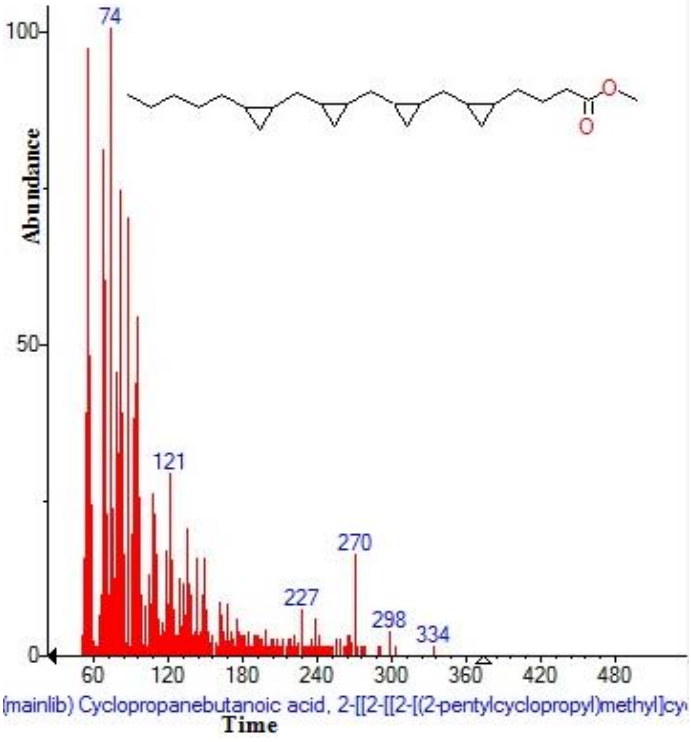


Figure 35. Structure of Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclo present in *Cassia angustifolia* with RT= 22.845 using GC-MS analysis.

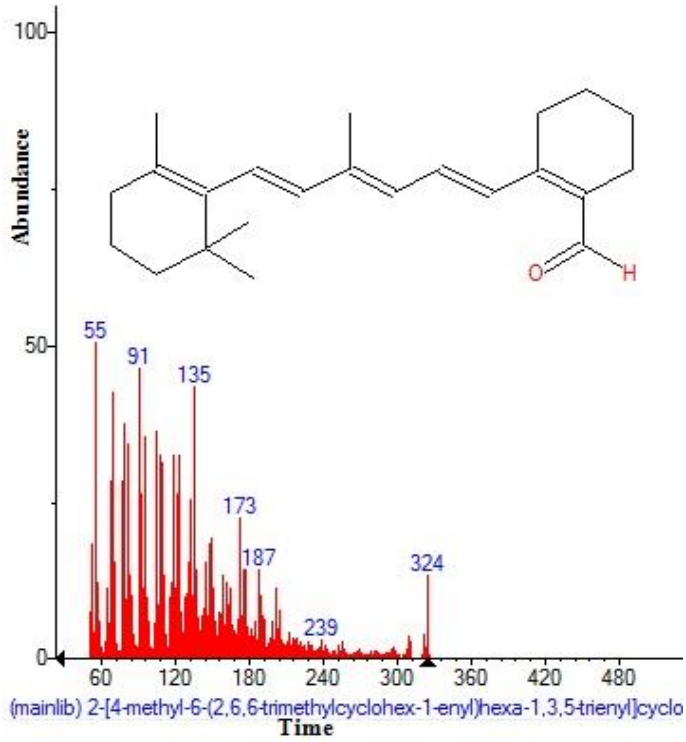


Figure 37. Structure of 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclo present in *Cassia angustifolia* with RT= 23.451using GC-MS analysis.

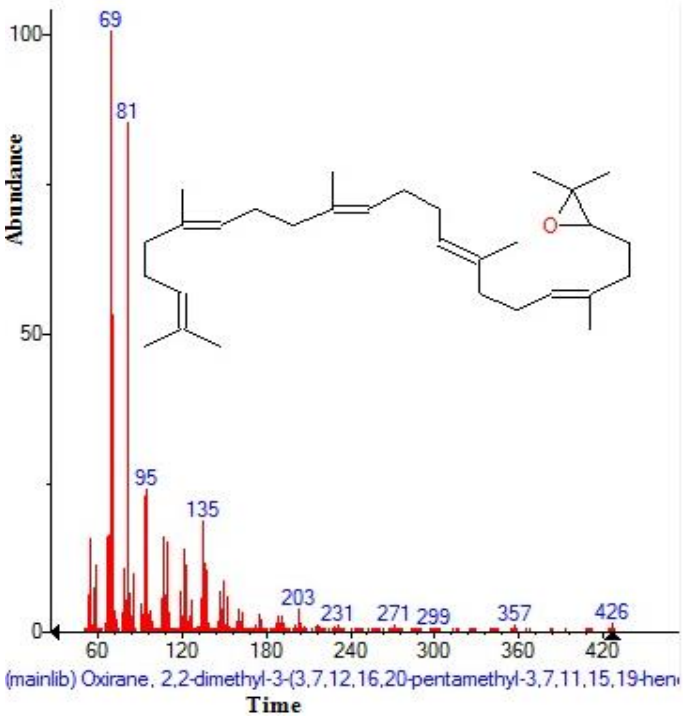


Figure 38. Structure of Oxirane ,2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19,-hen present in *Cassia angustifolia* with RT= 23.657 using GC-MS analysis.

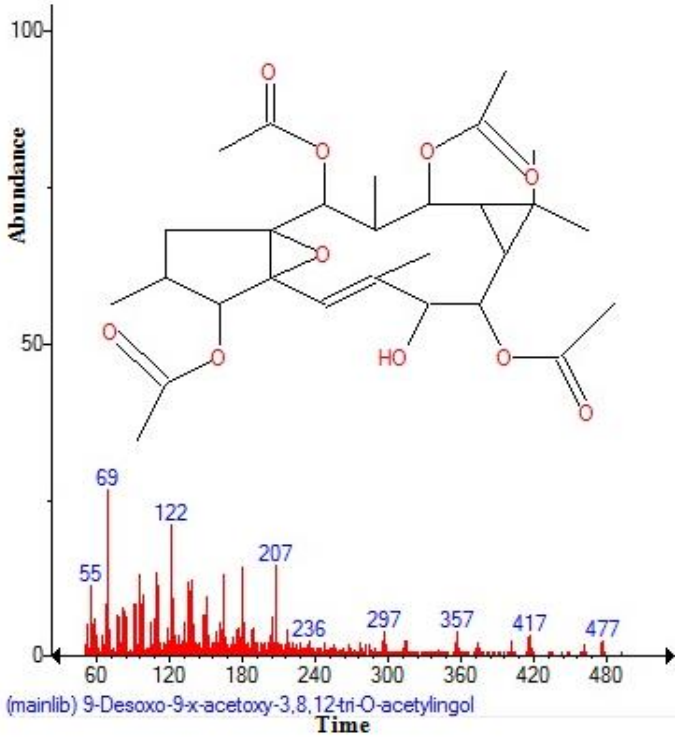


Figure 40. Structure of 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol present in *Cassia angustifolia* with RT= 25.025 using GC-MS analysis.

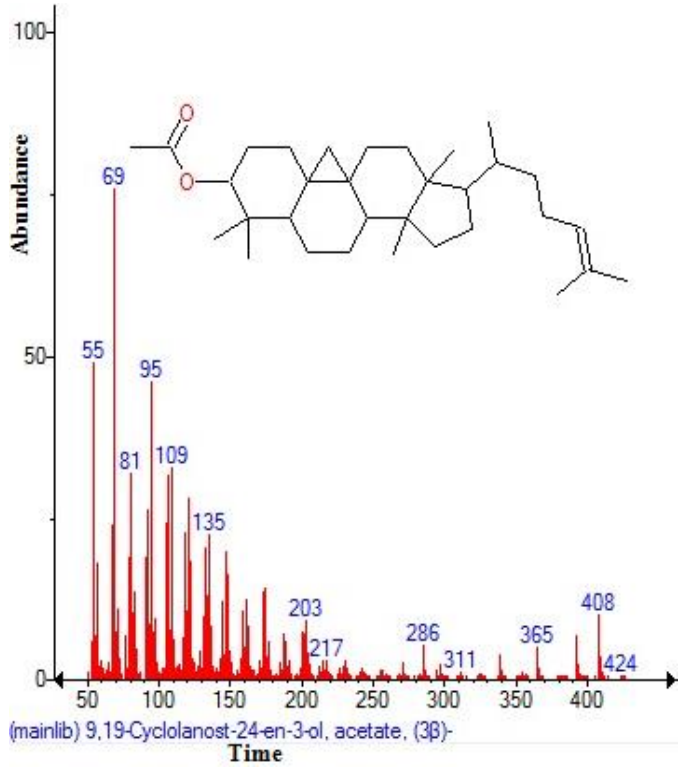


Figure 39. Structure of 9,19-Cyclolanost-24-en-3-ol,acetate , (3ß) present in *Cassia angustifolia* with RT= 23.686 using GC-MS analysis.

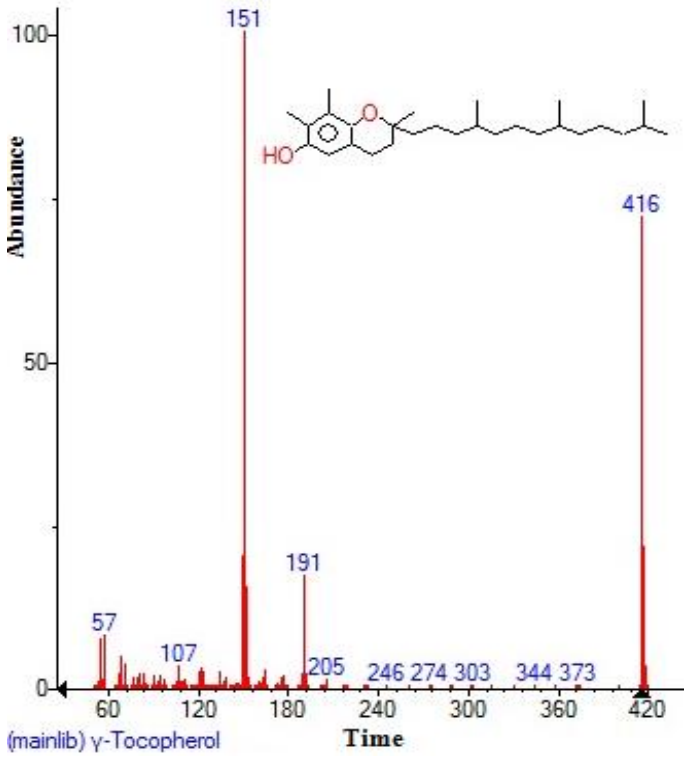


Figure 41. Structure of y-Tocopherol present in *Cassia angustifolia* with RT= 25.236 using GC-MS analysis.

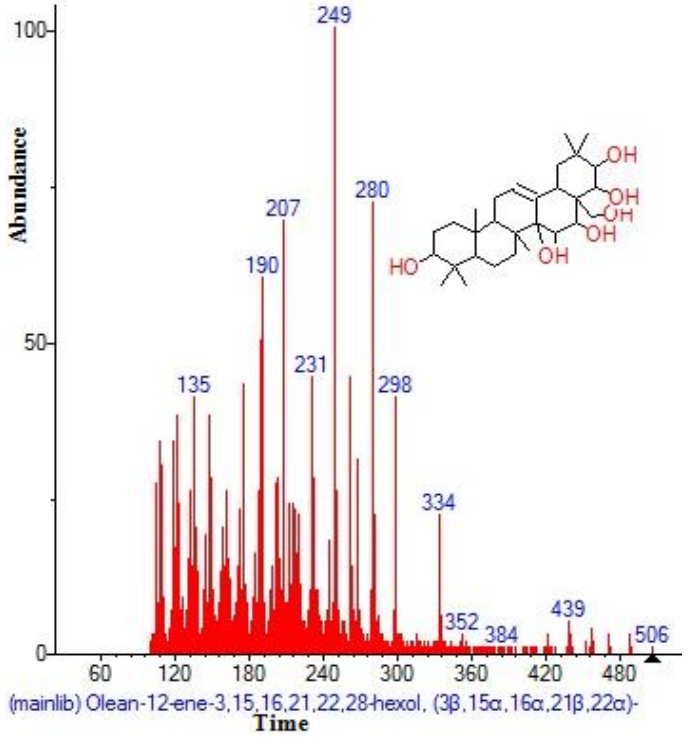


Figure 42. Structure of Olean-12-ene-3,15,16,21,22,28-hexol,(3ß,15α,16α,21ß,22α) present in *Cassia angustifolia* with RT= 25.683 using GC-MS analysis.

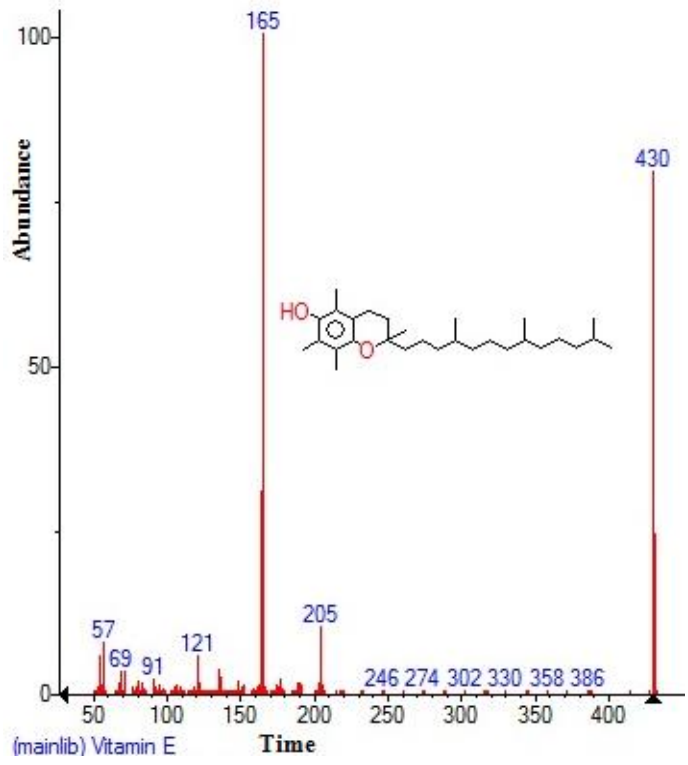


Figure 43. Structure of Vitamin E present in *Cassia angustifolia* with RT= 26.581 using GC-MS analysis.

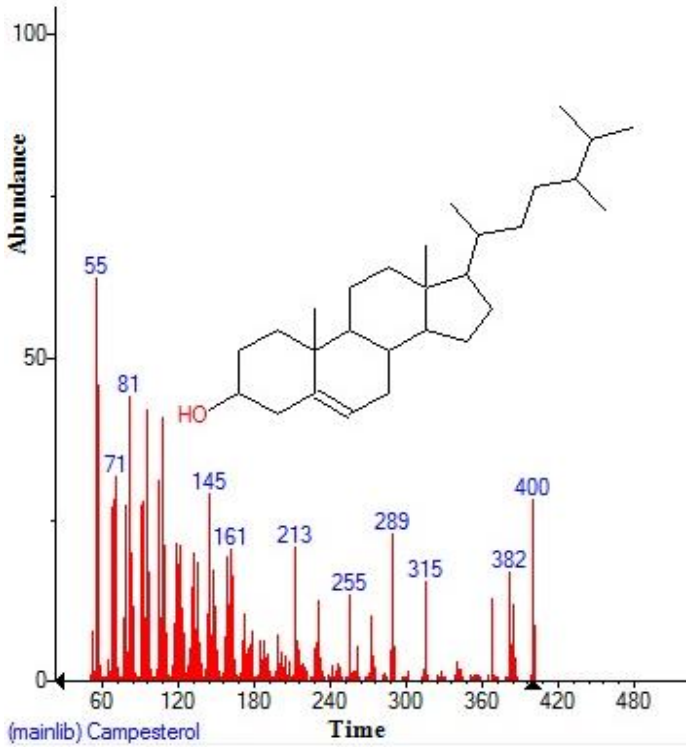


Figure 44. Structure of Campesterol present in *Cassia angustifolia* with RT= 28.315 using GC-MS analysis.

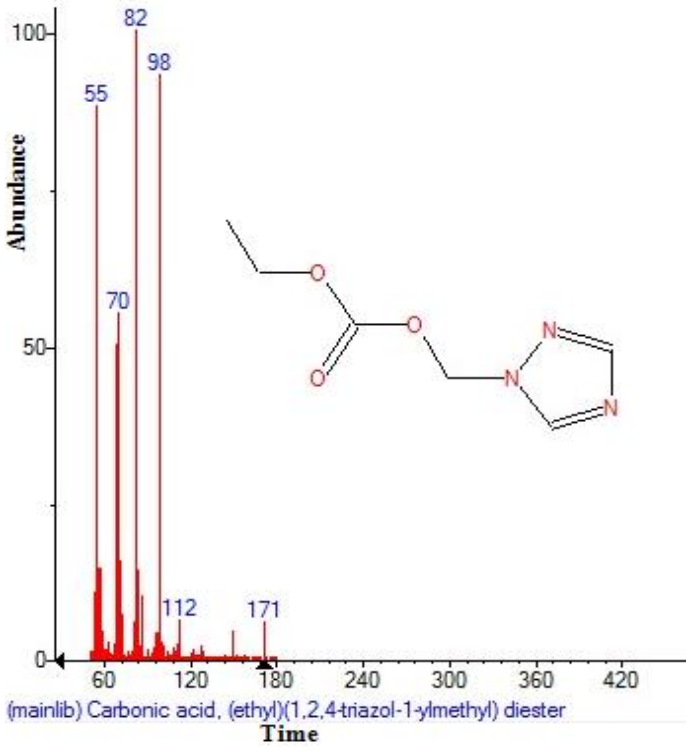


Figure 45. Structure of Carbonic acid, (ethyl)(1,2,4-triazol-1-ylmethyl)diester present in *Cassia angustifolia* with RT= 3.224 using GC-MS analysis.

ACKNOWLEDGEMENT

The authors thank Dr. Abdul-Kareem Al-Bermani, Lecturer, Department of Biology, for valuable suggestions and encouragement.

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