Variation in Essential Oil Content and Composition (Pimpinella anisum L.)

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Abstract
The essential oils were isolated from dried *Pimpinella anisum* L. seeds by Cleavenger aperture extraction, and fractions were identified by gas chromatography-mass spectrometry (GC-MS). 51 components were detected in aniseed. The components were mainly 19 sesquiterpenes (37.25 %), ten monoterpene (19.60%) , tow diterpene (3.92%) and one hemiterpene (1.96 %). The major components identified in the essential oil of *Pimpinella anisum* was trans-Anethole or Anisole (71.52 %) , benzocycloheptene (6.59 %),and Isoeugenyl acetate (4.63 %). In addition 44 components were present at less than 1%.
Keywords: *Pimpinella anisum*, anise, essential oils, trans-Anethole, GC-MS.

Introduction
Anise (*Pimpinella anisum* Linn.) is one of the common and important medicinal plants belonging to the family of Apiaceae (Umbelliferae) , it is a native of the eastern Mediterranean region and Southwest Asia, As well as anise is found in Central and Southern Europe, Egypt, Russia, Cyprus, Syria and North America. (Buchgraber et al. 1997 ; Hemphill and Hemphill,1988). Aniseed were used as traditional medicine in China as early as in the 5th century ,it is contain 1.5–5% essential oil and used as flavouring, digestive, carminative, relief of gastrointestinal spasms, Consumption of aniseed in lactating women increases milk and also reliefs their infants from gastrointestinal problems. Anise oil is also used in traditional medicine for the therapeutic treatment, including anti-phlogistic, anti-vomiting, analgesic, anti-spasmodic, anticarminative, kidney reinforcement, antiseptic, diuretic, odontalgic, stomachic, tonicardiac (Zargari, 1996 and Hossain *et al*., 2012). In addition to its medicinal value it's have been used in cooking, food processing, candy, toothpaste, pharmacy, perfumery and cosmetics industries (Ross, 2001; El. Nasr and Ottai 2012; Ullah, 2012). It has also been used to treat many diseases such as disorder of digestive systems (Baytop, 1984). Some components of anise are useful as antibacterial (Alhaider *et al* . 2007; Sergio et al., 2013 ; Hashempour et al., 2014 ; Mohamed et al 2015), as well as antifungal (Ivankosalec et al., 2005; Kosalec et al., 2005; El-Said and Goder, 2014), insecticidal and antioxidant effect of some compounds in the *P. anisum* extracts and essential oil on human health (Tunc and Sahinkaya, 1998, Gülcin et al. 2003; Besharati-seidani *et al*., 2005; Özcan and Chalchat 2006, Tepe *et al.* 2006, Tirapelli *et al*. 2007; Haliem and Mohamed , 2011). Volatile compounds of the essential oil obtained from seeds have exhibited in vitro activity against *Saccharomyces cerevisiae* and some clinical yeast isolates (Fujita and Kubo, 2004; Kosalec *et al*., 2005; Kadan *et al*., 2013). Anticancer activity of ethanol extract of anise (*Pimpinella anisum* L.) seed was investigated by (El-Sayed *et al.*, 2015).

(Andarwulan and Shetty, 1999) found that *P. anisum* oil protected rats from aspartame-induced liver histopathological changes.Moreover, Cengiz *et al.*(2008) showed that diethyl ether extract of anise seed could ameliorate carbon tetrachloride (CCl4)-induced liver injury. pharmacological properties and chemical constituents of *Pimpinella anisum* written by (Orav *et al* , 2008; Conforti *et al.,* 2010; Shojaii and Fard, 2012 and Aloghareh et al., 2013).

According to the study by Askari *et al*. (1998) anise seed contains 1.5 - 3.5% mass of volatile oil consisting of trans-Anethole and cis-anethole as major component .This compound ranged from 78.63% - 95.21% (Arslan *et al*., 2004) The major component of anise, trans-Anethole, is largely used as a substrate for synthesis of various pharmaceutical substances (Kosalec *et al.*, 2005).

The number of volatile components varies between the parts of plant, Askari and Sefidkon (2005) studied stems, leaves, inflorescence and seeds of *Pimpinella tragium* Vill, and found eighteen constituents in the stems and leaves oil, twenty six constituents in the inflorescence and twenty-three constituents in the seed oil were identified. Naher *et al*., (2012) mentioned that nine chemical constituents were found by gas chromatography and mass spectrometry (GC-MS) analysis from the essential oil of Bangladeshi Dhaka aniseed, the major constituent was cis-Anethole (69.404%) and D-Limonene (13.273%). The major constituent in the essential oil of the fruits was phenylpropanoid, namely trans-Anethole identified by Ullah, et al., (2013). Haşimi *et al*, (2014) determined that the main components of the anise essential oil were trans-Anethole (52.94%) followed by iso-anethole (13.89%), caryophllene oxide (8.55%) and caryophyllene (2.4).While Mohammed *et al*., (2014) reported that essential oil has trans-Anethole (55.491%) , 2,4-decadienal (11.353%) , P-anisaldehyde (4.769%) and tetrachloroethylene (4.179%), the extracted aniseed oil was tested for antimicrobial and antioxidant properties and showed resistance properties on *E.coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella*. The major constituent of anise oil was *trans*-Anethole (82.1%) followed by γhimachalene (7.0%) (Ullah et al, 2014).In Yeman , Al- Maofari et al.,(2013) showed that 4-allylanisole was the major compound of *Pimpinella anisum* L. with percentages of 76.70% and 85.28% of Moroccan and Yemen, respectively, in addition to other minor compounds such as limonene (9.75% for Moroccan species and 5.53% for Yemen species) and fenchone (6.16% for Moroccan species and 4.12% for Yemen species). Furthermore, both essential oils were evaluated for their antibacterial activity against a panel of pathogenic microorganisms. The oil of aniseed was characterized by higher amounts of trans-Anethole (96.80%) by Acimovic *et al*.,(2015).

present work aimed to investigate the components of Anise essential oils, by using GC-MS Chromatography.

Materials and Methods

Plant material:

This research was carried out at the University of Basrah, College of Science, Department of Biology, Iraq. The GC-MS Chromatography carried at the University of Basrah, College of Agriculture, Iraq.

Isolation of the essential oil:

Essential oil obtained by hydrodistillation method for 4h, using Clevenger-type apparatus from crushed mature seeds.

GC-MS analysis

The oil quality was assessed through analysis by combined gas chromatography and mass spectrometry. GC-MS analysis was performed by using an Shimadzu GC-QP 2010 Ultra gas chromatograph,The GC oven temperature was programmed from 40°C to 250 °C at a rate of 4.3 °C/min. Helium was used as carrier gas; inlet pressure was 100.0 kPa; linear velocity was 48.1 cm/sec. Column flow was 1.78 mL/min, Injector temperature: 250°C; injection mode: split. MS scan conditions: source temperature, 200 °C; interface temperature, 250 °C; Detector Gain, 0.70 kV +0.10 kV; Scan speed, 1666. Start 50 m/z, End 800 m/z. The components of the anise oil were identified by comparing the spectra with those of known compounds stored in the NIST library (2005)(Fig. 2-47).

Results and Discussion

Chemical composition of the essential oil

The essential oils extracted by hydrodistillation from Anise seeds are colorless and present a pungent odor at room temperature. The content of essential oil in seeds of *Pimpinella anisum* was 1.25 %. The composition of the essential oils was determined by gas GC-mass as summarized in (Table 1). The structures of the major compounds are presented in (Figure 1).

Table 1: Compounds identified in the essential oil of *Pimpinella anisum* L. seeds using gas chromatography mass spectrometry (GC-MS).

Peak number	Formula	Retention time	Content (%)	Components
1		5.111	0.01	not identified.
2	C_5H_7NS	6.102	0.43	4-Isothicyanate -1-butene
3	$C_{10}H_{14}$	6.953	0.01	m-Cymene
4		7.042	0.01	not identified.
5	$C_{10}H_{16}$	7.589	0.02	Crithmene
6	$C_6H_8O_4$	7.968	0.03	2,3-Dimethylfumaric acid
7	$C_{10}H_{18}O$	8.354	0.44	linalool
8	$C_{11}H_{16}$	9.032	0.06	Cyclohexene, 3,4-diethenyl-3-methyl-
9	$C_{10}H_{12}O$	10.004	2.25	p-Propenylanisole
10	$C_{10}H_{12}O$	10.692	0.06	Cumaldehyde or Cuminal
11	$C_{10}H_{12}O$	10.858	0.63	p-Propenylanisole
12	$C_8H_8O_2$	11.152	0.19	p-Anisaldehyde
13	$C_{10}H_{12}O$	11.721	71.52	trans-Anethole(Anisole)
14	$C_{15}H_{24}$	12.115	0.29	Delta- Elemene
15	$C_{10}H_{12}O_2$	12.374	3.10	Chavibetol (3-Allyl-6-methoxypheno)
16	$C_{15}H_{24}$	12.577	0.13	Ylangene
17	$C_{12}H_{20}O_2$	12.680	0.15	Geranyl acetate
18	$C_{10}H_{12}O_2$	12.766	0.05	Anisketone
19	$C_{15}H_{24}$	12.849	0.15	delta-Elemene
20	$C_{15}H_{24}$	13.264	1.17	Caryophyllene
21	$C_{15}H_{24}$	13.436	0.12	alpha-Bergamotene
22	$C_{15}H_{24}$	13.671	0.92	1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene,(4aS-cis)
23	C_9H_9NS	13.847	0.22	(Phenylethyl isothiocyanate)
24	$C_{15}H_{24}$	14.077	6.59	benzocycloheptene (cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H))
25	$C_{11}H_{14}O_2$	14.200	0.48	(Eugenyl methyl ether)
26	$C_{15}H_{24}$	14.260	1.02	Zingiberene
27	$C_{15}H_{24}$	14.346	0.50	Beta-Himachale
28	$C_{15}H_{24}$	14.442	0.49	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene- 4-hexenyl)-
29	$C_{12}H_{14}O_3$	14.562	0.91	Eugenol acetate
30	$C_{15}H_{24}$	14.677	0.17	beta-Sesquiphellandrene
31	$C_{15}H_{24}$	15.231	0.11	Germacrene
32	$C_{15}H_{24}O$	15.520	0.13	Caryophyllene oxide
33	$C_{12}H_{18}$	15.589	0.06	6,7-Dimethyl-1,2,3,5,8,8a-hexahydronaphthalene
34	$C_{15}H_{24}$	15.783	0.04	benzocycloheptene (cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H))
35	$C_{15}H_{24}O$	15.861	0.05	.beta.-Himachalen oxide
36	$C_{15}H_{24}O$	16.003	0.27	Spathulenol
37	$C_{14}H_{22}O_2$	16.058	0.09	Menthol, 1'-(butyn-3-one-1-yl)-, (1S,2S,5R)
38	$C_{15}H_{26}O$	16.241	0.20	.alpha.-Cadinol
39	$C_{16}H_{28}O$	16.375	0.12	(-)-Isolongifolol, methyl ether
40	$C_{15}H_{26}O$	16.475	0.10	Humulane-1,6-dien-3-ol
41	$C_{10}H_{16}O$	16.571	0.14	1-(1,2,3-Trimethyl-cyclopent-2-enyl)-ethanone
42	$C_{20}H_{28}N_2O_2$	16.942	0.03	1,2-Bis(4-methoxyphenyl)-N,N,N',N'-tetramethylethane-1,2-diamine
43	$C_{14}H_{12}O_2$	17.067	0.03	Benzyl Benzoate (Ascabin)
44	$C_{16}H_{26}O$	17.172	0.03	cis,cis,cis-7,10,13-Hexadecatrienal
45	$C_{12}H_{14}O_3$	17.433	4.63	Isoeugenyl acetate (Phenol, 2-methoxy-4-(1-propenyl)-, acetate)
46	$C_{12}H_{15}NO_4$	17.709	1.36	3-Hydroxycarbofuran
47	$C_{17}H_{34}O_2$	17.858	0.03	Hexadecanoic acid, methyl ester
48	$C_{38}H_{68}O_8$	18.026	0.16	l-(+)-Ascorbic acid 2,6-dihexadecanoate
49	$C_{19}H_{34}O_2$	18.567	0.05	10,13-Octadecadienoic acid, methyl ester
50	$C_{15}H_{28}O_2$	18.742	0.23	Cyclopentadecanone, 2-hydroxy-
51	$C_{20}H_{36}O_2$	18.817	0.04	Z,Z-4,15-Octadecadien-1-ol acetate

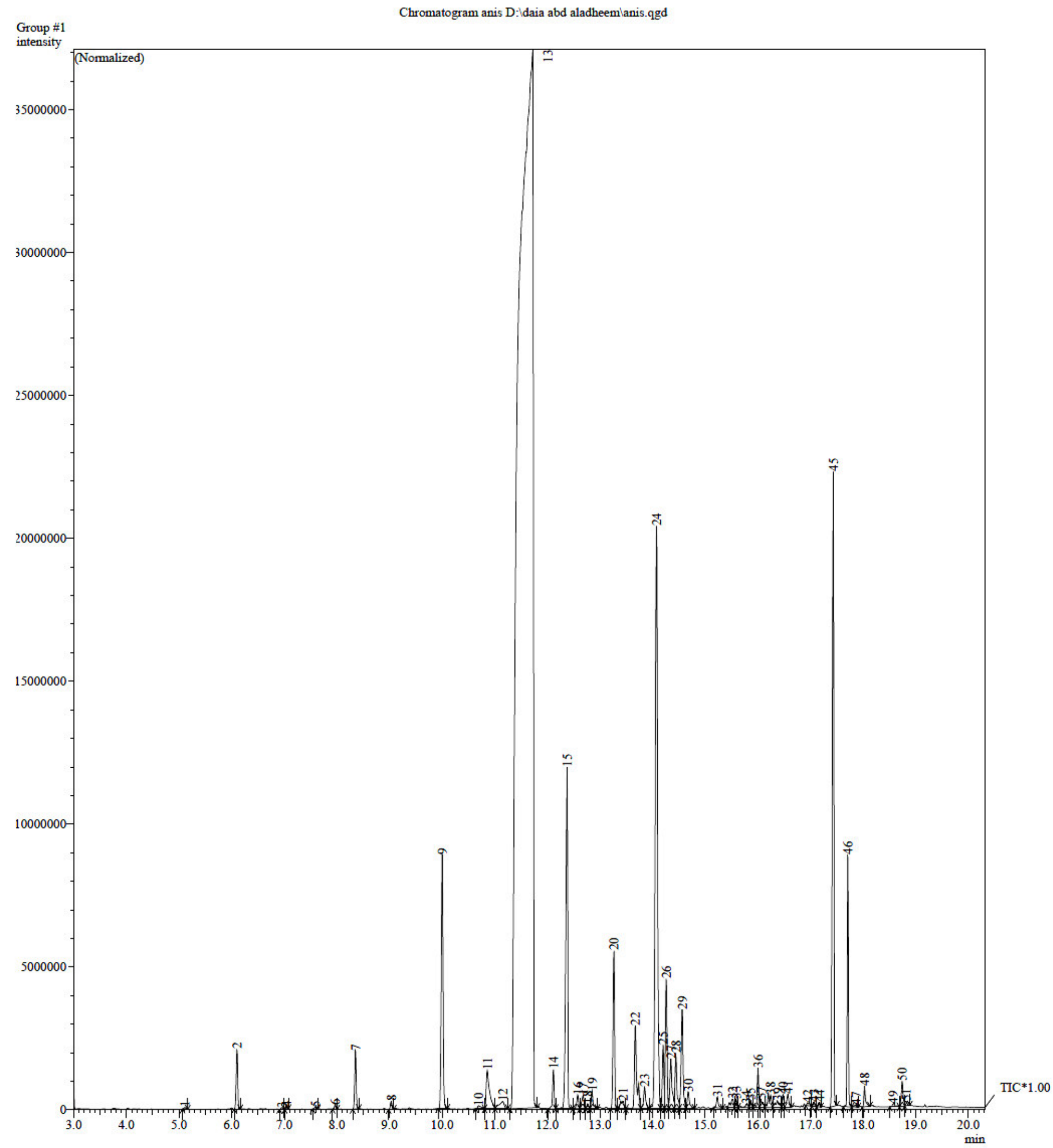

Fig. 1: Chromatogram of essential oil of the leaves of *Pimpinella anisum* L. seeds.

51 components were detected in aniseed. Sesquiterpenoid were represented (37.25%) about 19 components and the most important compounds belonging to this class were: the already mentioned caryophyllene (1.17%), Zingiberene (1.02 %), Beta-Himachale (0.50), Caryophyllene oxide (0.13 %), Ylangene (0.13%) and beta.-Himachalen oxide(0.05%) .The percentage of total monoterpenoid was 19.60 %.The major component as trans-Anethole (71.52%),The percentage of chavibetol is rather important (3.10 %), linalool (0.44 %), Crithmene (0.02%) and m-Cymene (0.01%) were identified. The essential oil also contains two compounds of ditpenes, Z,Z-4,15-Octadecadien-1-ol acetate (0.04%) and 1,2-Bis(4-methoxyphenyl)-N, N', N'-tetramethylethane-1,2-diamine (0.03%) ,As well as one compounds hemiterpene (1.96 %), it was isothiocyanic acid.

There are other compounds was also identified by using GC-MS such as Isoeugenyl acetate (4.63%), p-anisaldehyde (0.19%), Geranyl acetate (0.15%), Phenylethyl isothiocyanate(0.22%), Eugenol acetate (0.91%) and Ascabin (0.03%) .

Essential oils are mixtures of compounds of different molecular weights, from the most volatile hydrocarbons of ten carbon atoms, called monoterpenes, to oxygenated compounds of 15 atoms of carbon, or sesquiterpenes (Francisco et al., 2008). Our results dissimilar with Acimovic *et al*.,(2015), they reported that A part of anise plant contains nine sesquiterpenes and one monoterpene were present in essential oil of *Pimpinella anisum*.

Although the essential oils have a great number of components, only one or two of those compose of major components. The major components identified in the essential oil of *Pimpinella anisum* was Anisole or trans-Anethole (71.52 %), then cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetram (6.59 %) ,and Isoeugenyl acetate (Phenol, 2-methoxy-4-(1-propenyl)-, acetate) with (4.63 %). while 44 components were present at less than 1%, including two components unknown as shown in the (Figure 6 and Table 2).This is in agreement with the previous studies results in Turkey, Bangladesh and Sultanate of Oman (Alma *et al*., 2007; Bhuijan *et al*., 2010; Hossain *et al*., 2012). The composition of essential oils differ in their main components compared to data reported from other sources, such as Askari and Sefidkon(2005), whose main components are germacrene D (34.7 %) and Naher et al.,(2012) mentioned the main components was cis-Anthole (69.404 %) then D-Limonene (13.273 %), In Yeman with 85.28% of 4-allylanisole (Al-Maofari et al., 2013).

trans-Anthole and caryophyllene that available in the essential anise oil components make it potentially useful for the preparation of both herbal and modern medicines because they exhibit antibacterial, antifungal, anti-inflammatory capacities, insecticidal and antioxidant potential, and are also used traditionally as a flavoring agent and antimicrobial material in food industry (Dorman and Deans., 2000 ; Niwano *et al*., 2011).

Acimovic et al.(2015) and Gende *et al.* (2009) found that the oils of *P. anisum* were rich in trans-Anethole,87.85% and 96.8 %, respectively. In our investigation, the concentration of trans-Anethole in aniseed was almost same as in the Gende *et al.* (2009)and Acimovic et al., 2015 study. Moreover, trans-Anethole identified by (Ullah et al.,2013; Ullah et al.,2014; Hasimi et al.,2014; Mohammed et al.,2014). While Gerogiannaki and Masouras(2015) reported that trans-Anethole and isomer cis- anthole are main in aniseed seed. Genetic structures and ecological conditions the main reason affecting on essential oil composition , as well as the weather conditions during the years had a strong influence on the content of essential oil in fruits of anise. In the case of anise, some conditions were unfavorable for the synthesis of essential oil (Acimovic et al., 2014: Aćimović et al., 2015). In a study by Orav *et al.* (2008), the major component of essential oil from *Pimpinella anisum* L. fruits obtained from different geographical areas of Europe, was trans-Anethole. This was similar to our results.

Conclusion

Results from this study have shown that the essential oil of *Pimpinella anisum* seeds contains compounds with proven pharmacological effects. GC-MS analysis revealed that 51 different chemical components were identified in the essential oil of aniseed oil**.** The essential oils from aniseed were characterized by high amounts of trans-Anethole (71.52 **%**). The percentages of monoterpenoid compounds are low. The majority of components of the essential oil are constituents with sesquiterpenoid structure.

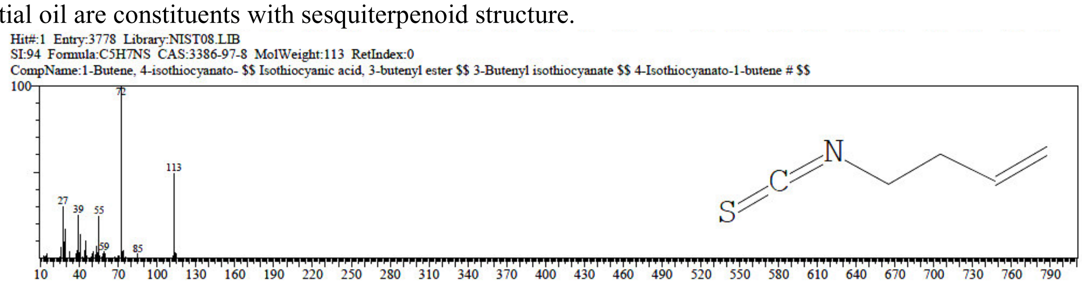

Fig.2 : A typical gas chromatogram of the 4-Isothicyanate -1-butene

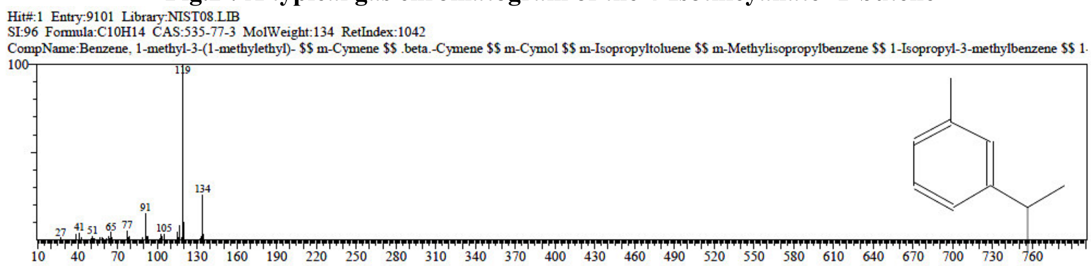

Fig. 3: A typical gas chromatogram of the m-Cymene

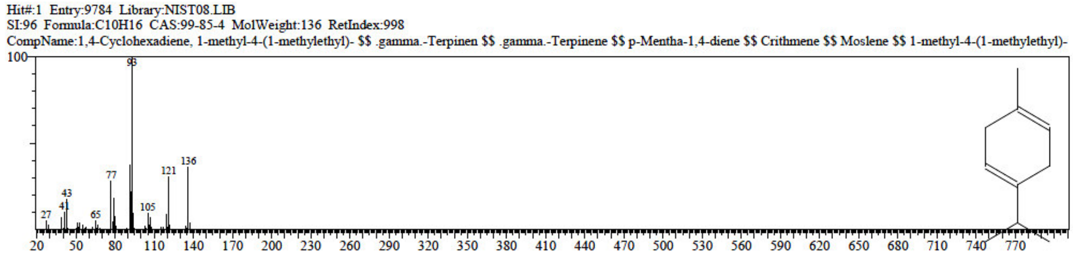

Fig. 4: A typical gas chromatogram of the Crithmene

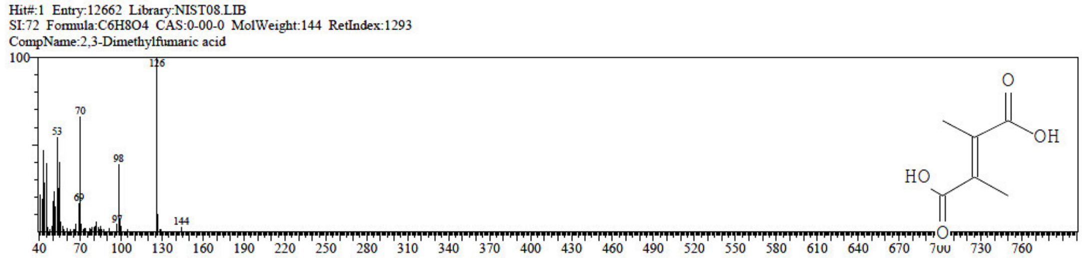

Fig. 5: A typical gas chromatogram of the 2,3-Dimethylfumaric acid

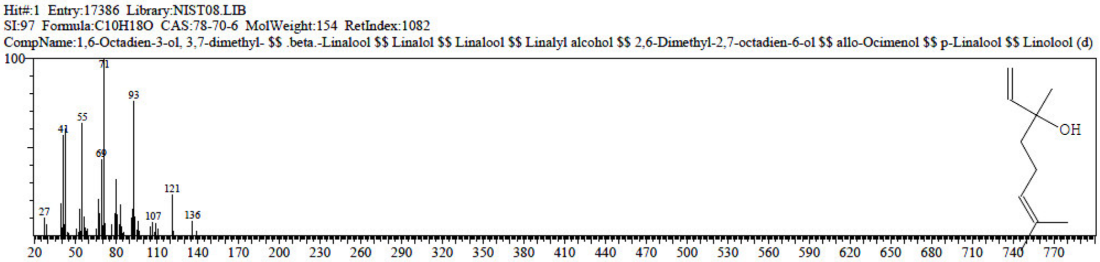

Fig. 6: A typical gas chromatogram of the linalool

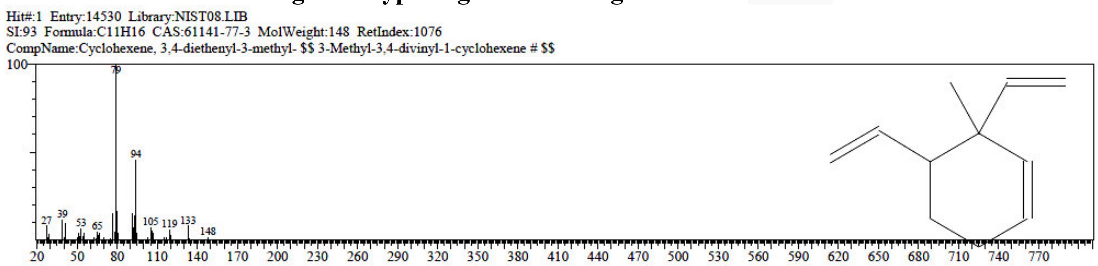

Fig. 7: A typical gas chromatogram of the Cyclohexene, 3,4-diethenyl-3-methyl-

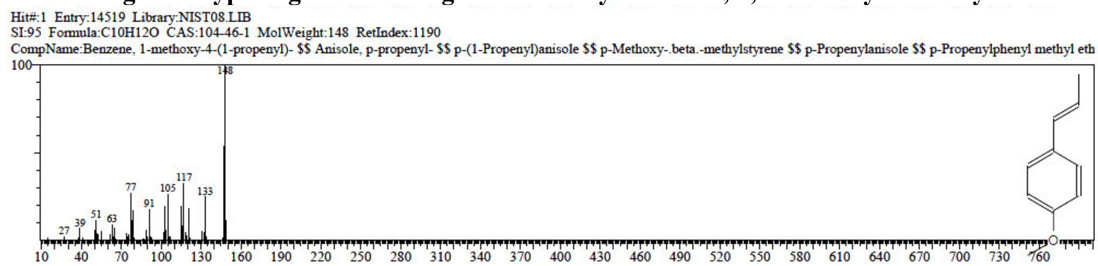

Fig. 8: A typical gas chromatogram of the tran- Anethole(Anisole)

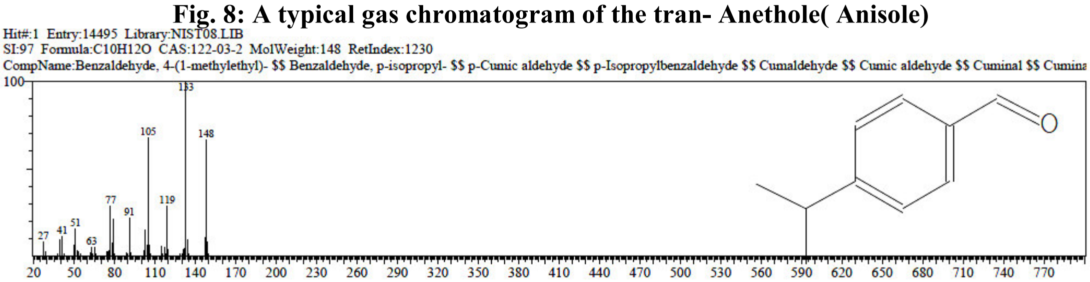

Fig. 9: A typical gas chromatogram of the Cumaldehyde or Cuminal

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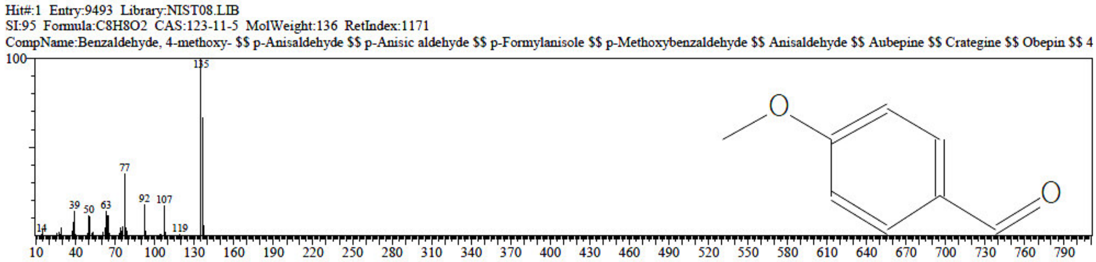

Fig. 10: A typical gas chromatogram of the p-Anisaldehyde

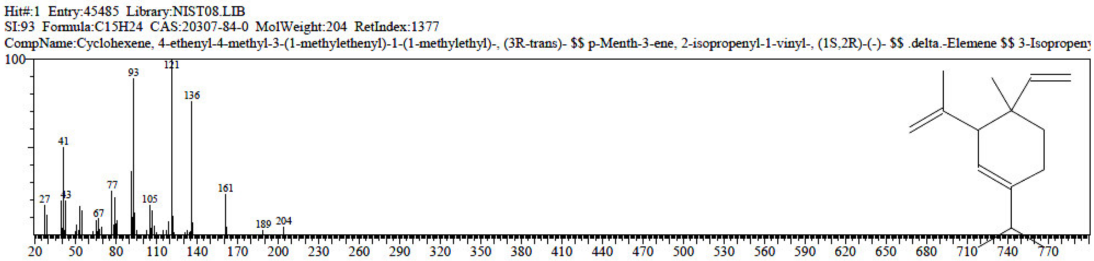

Fig. 11 : A typical gas chromatogram of the Delta- Elemene

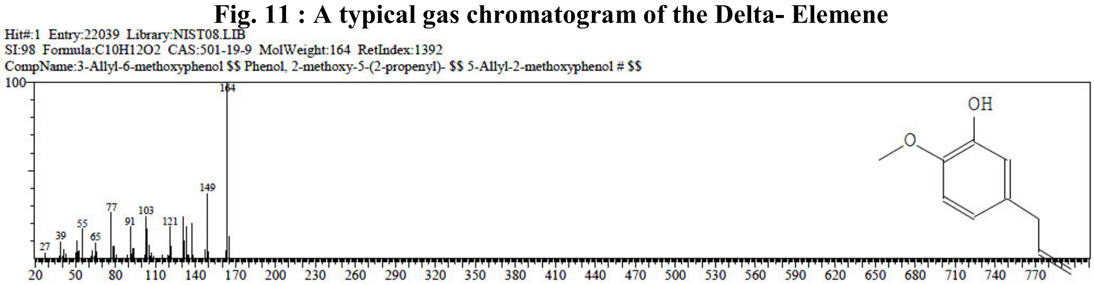

Fig. 12: A typical gas chromatogram of the Chavibetol

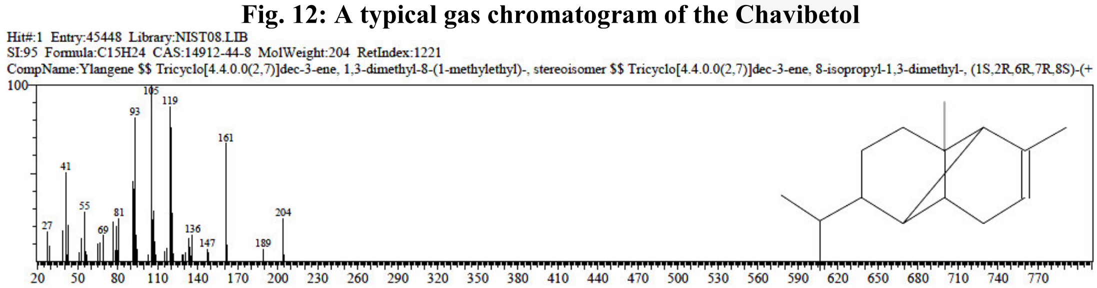

Fig. 13: A typical gas chromatogram of the Ylangene

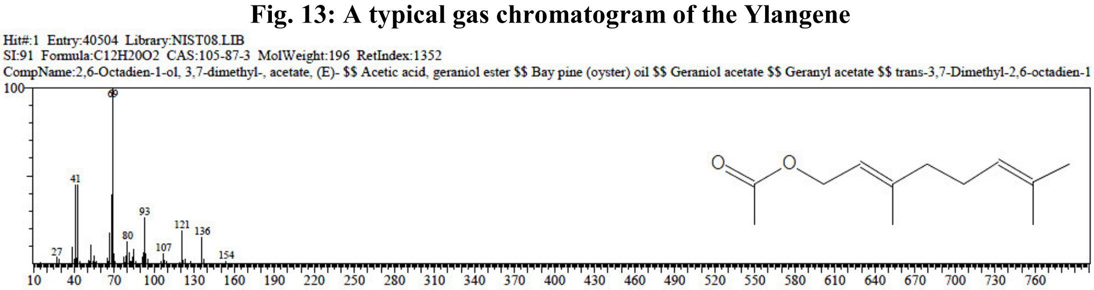

Fig.4 1: A typical gas chromatogram of the Geranyl acetate

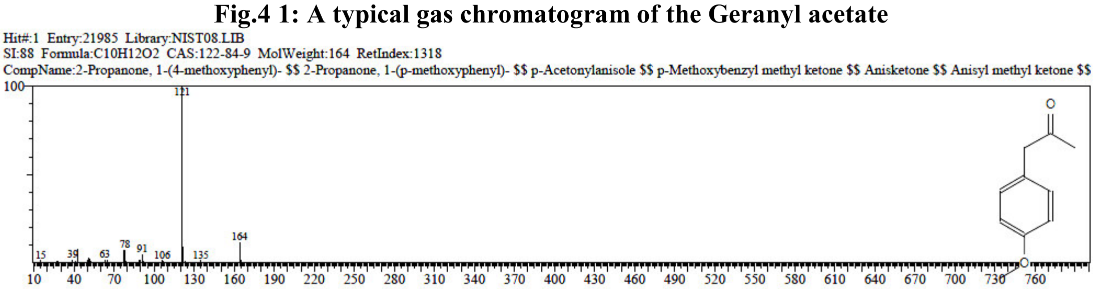

Fig. 15: A typical gas chromatogram of the Anisketone

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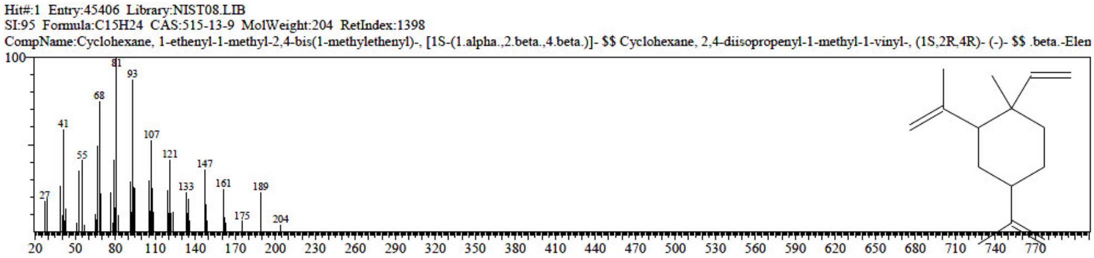

Fig. 16: A typical gas chromatogram of the delta-Elemene

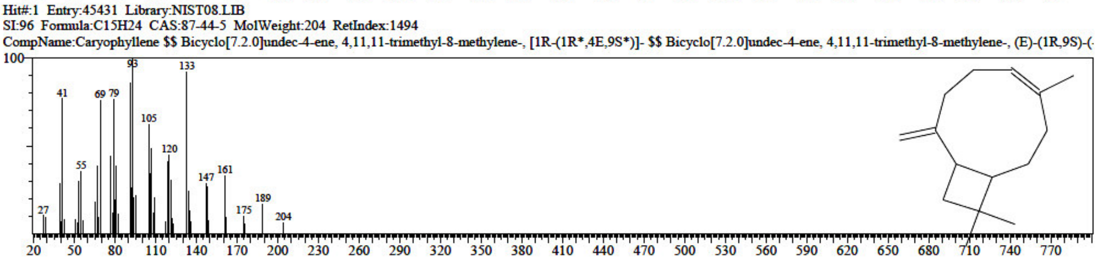

Fig. 17: A typical gas chromatogram of the Caryophyllene

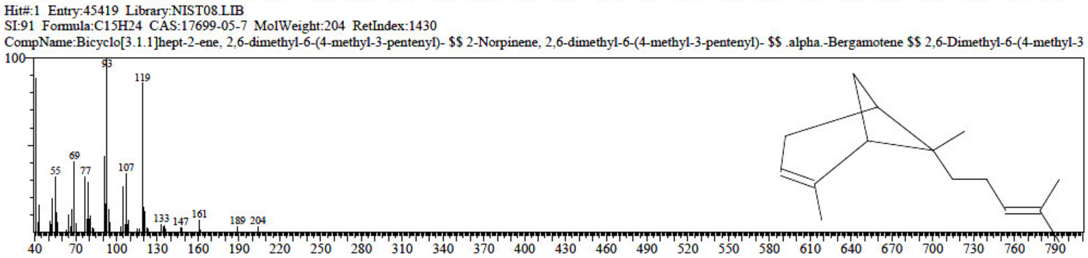

Fig. 18: A typical gas chromatogram of the alpha-Bergamotene

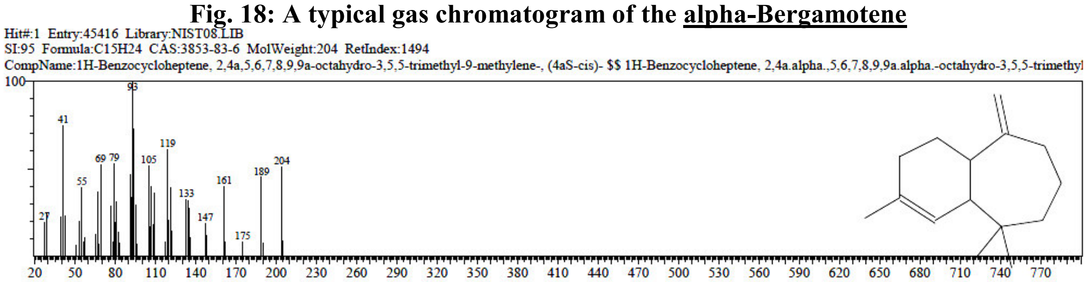

Fig. 19: A typical gas chromatogram of the 1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene,(4aS-cis)

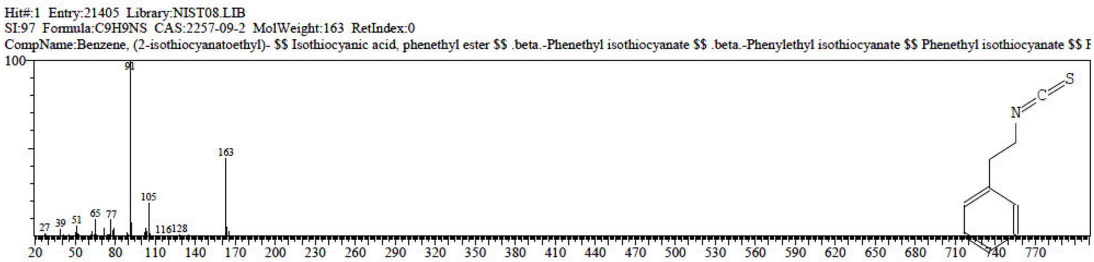

Fig. 20: A typical gas chromatogram of the Phenylethyl isothiocyanate

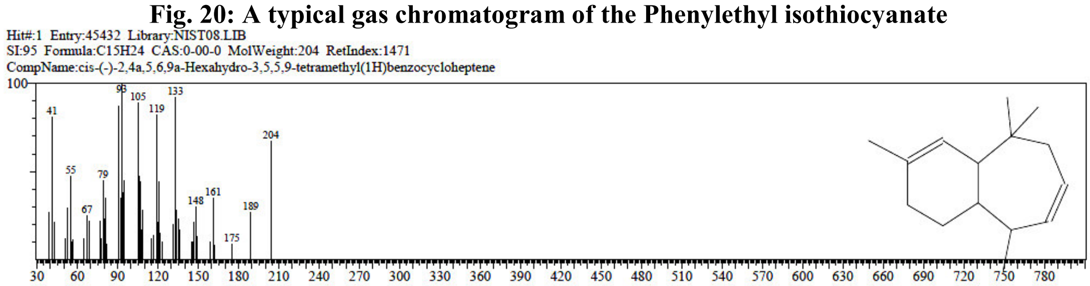

Fig. 21: A typical gas chromatogram of the cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H) benzocycloheptene

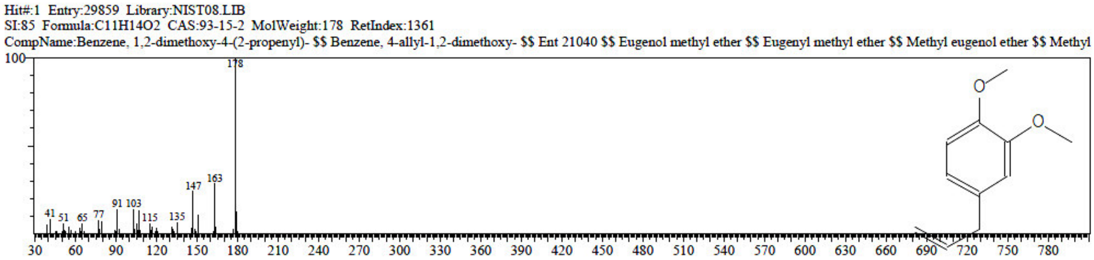

Fig. 22: A typical gas chromatogram of the Eugenyl methyl ether

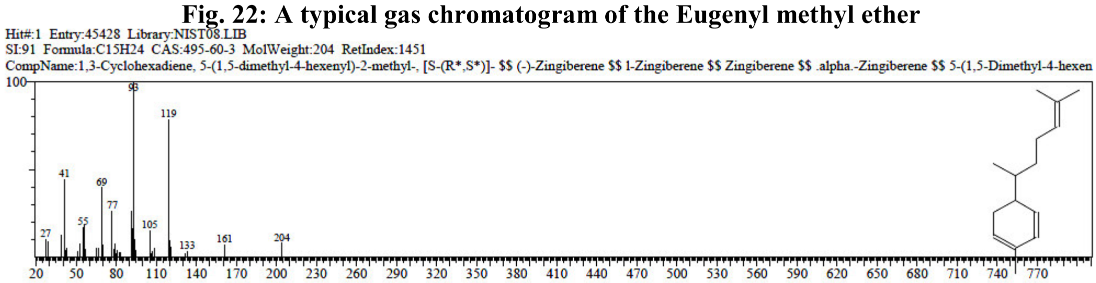

Fig. 23: A typical gas chromatogram of the Zingiberene

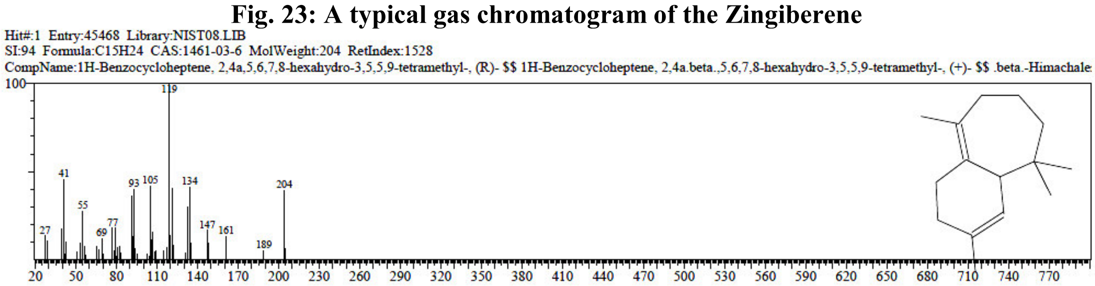

Fig. 24: A typical gas chromatogram of the Beta-Himachale

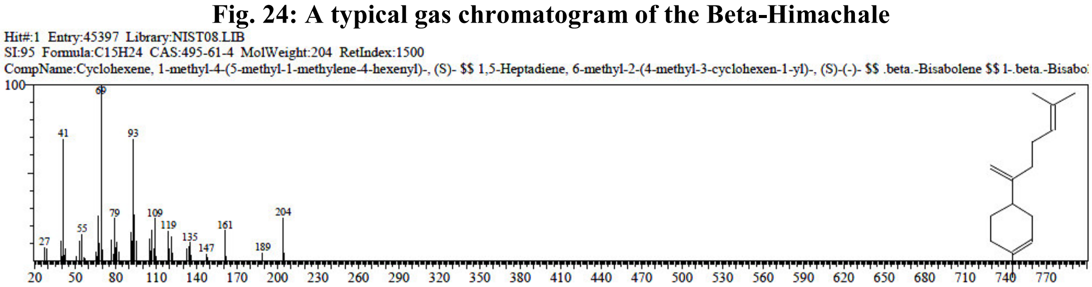

Fig. 25: A typical gas chromatogram of the beta-Bisabolene

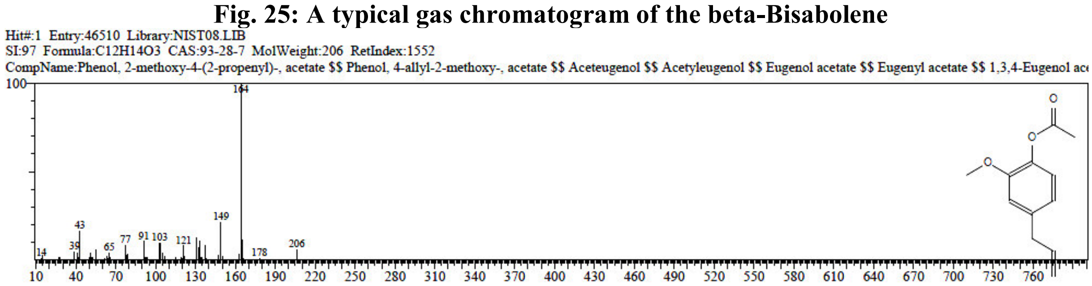

Fig. 26: A typical gas chromatogram of the Eugenol acetate

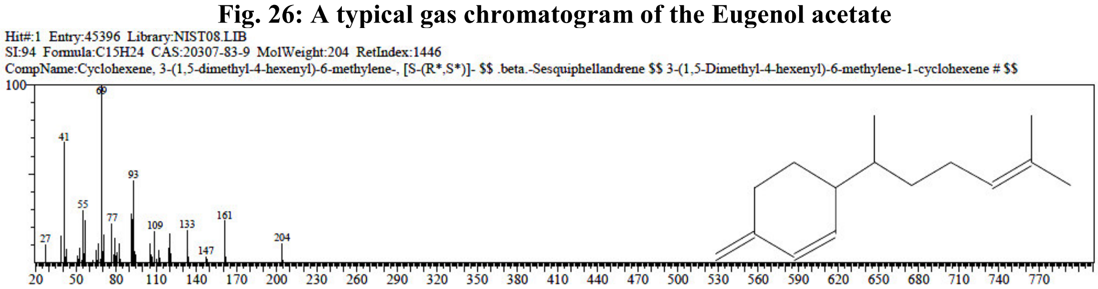

Fig. 27: A typical gas chromatogram of the beta-Sesquiphellandrene

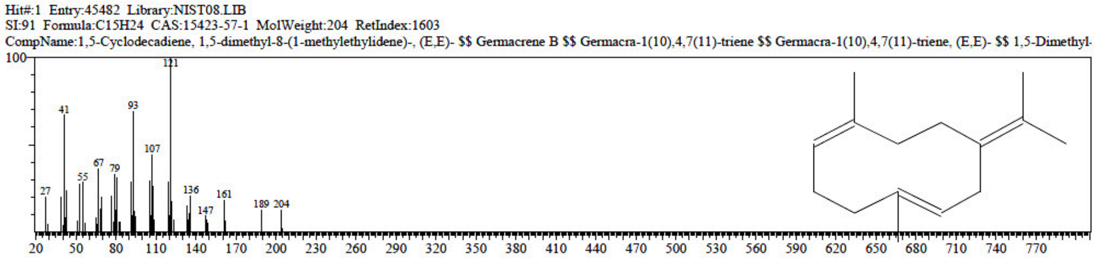

Fig. 28: A typical gas chromatogram of the Germacrene

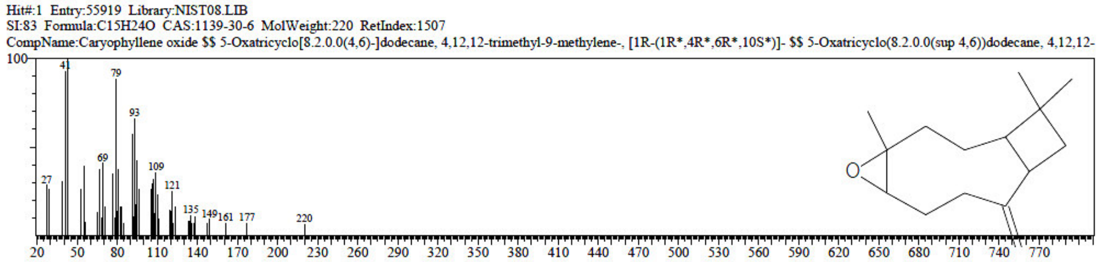

Fig. 29: A typical gas chromatogram of the Caryophyllene oxide

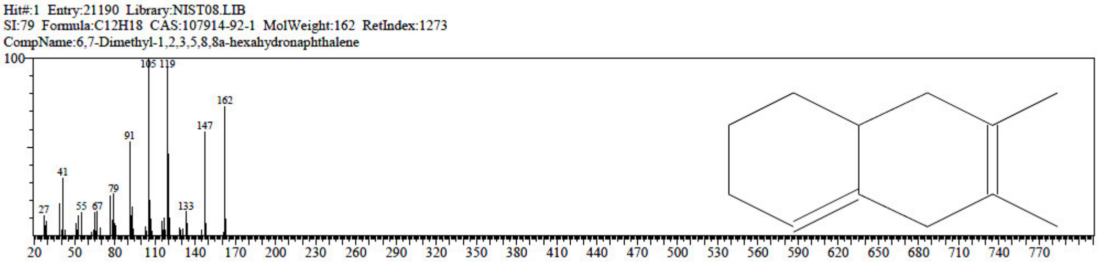

Fig. 30: A typical gas chromatogram of the 6,7-Dimethyl-1,2,3,5,8,8a-hexahydronaphthalene

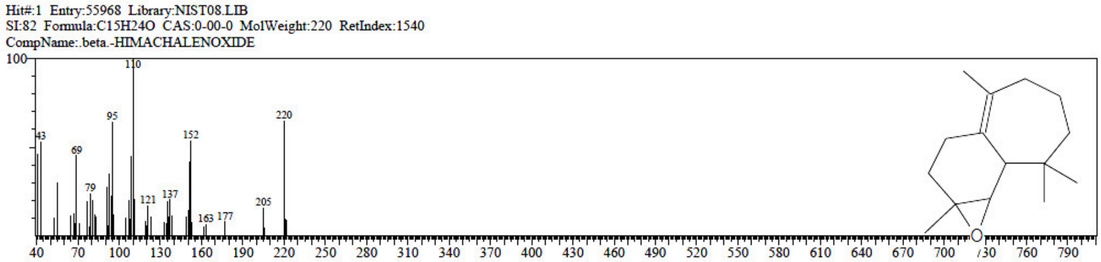

Fig. 31: A typical gas chromatogram of the beta.-Himachalen oxide

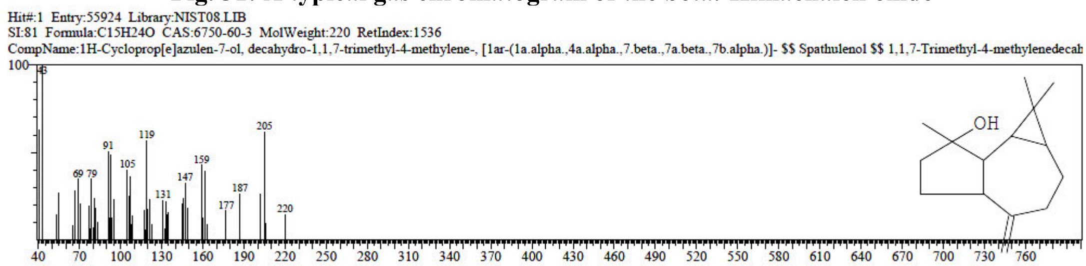

Fig. 32: A typical gas chromatogram of the Spathulenol

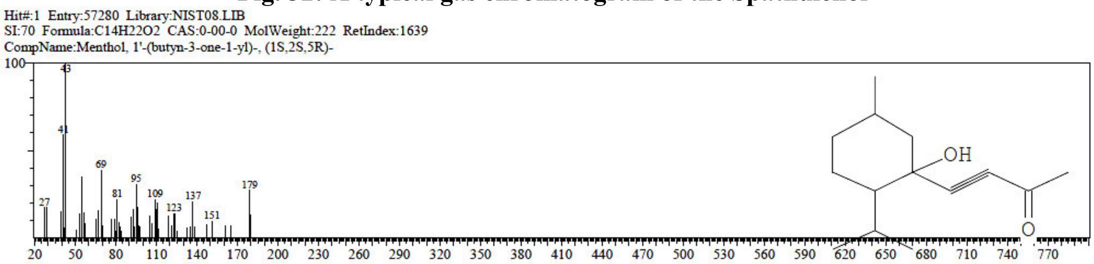

Fig. 33: A typical gas chromatogram of the Menthol, 1'-(butyn-3-one-1-yl)-, (1S,2S,5R)

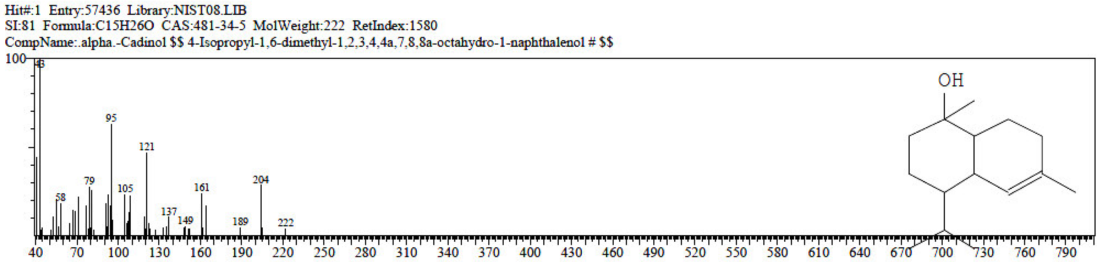

Fig. 34: A typical gas chromatogram of the alpha.-Cadinol

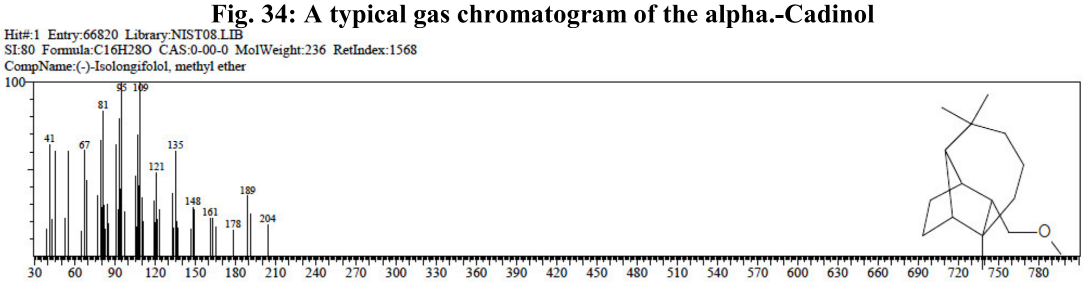

Fig. 35: A typical gas chromatogram of the (-)-Isolongifolol, methyl ether

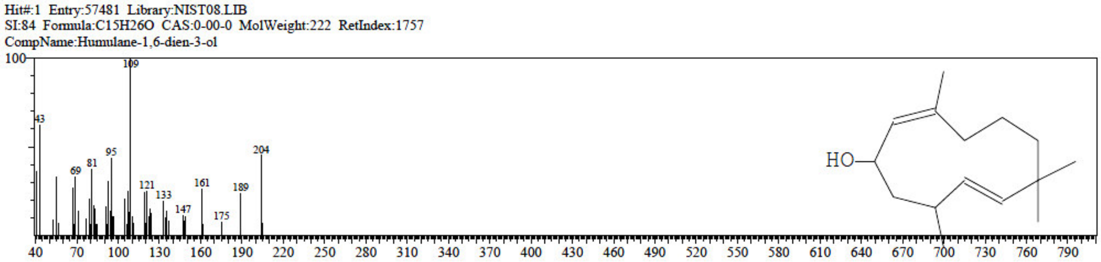

Fig.36: A typical gas chromatogram of the Humulane-1,6-dien-3-ol

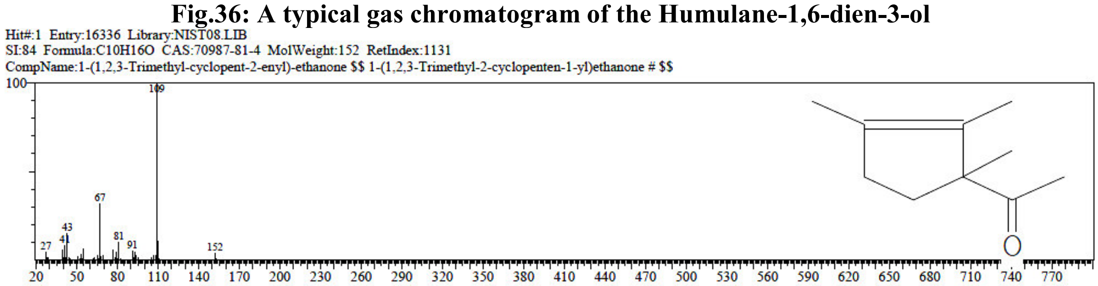

Fig. 37: A typical gas chromatogram of the 1-(1,2,3-Trimethyl-cyclopent-2-enyl)-ethanone

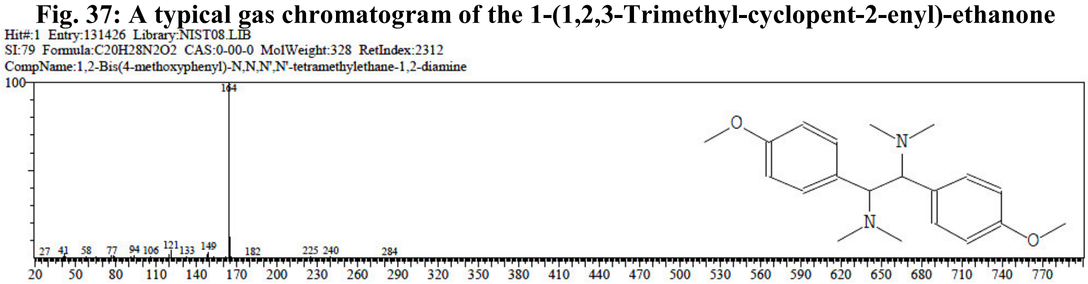

Fig. 38: A typical gas chromatogram of the 1,2-Bis(4-methoxyphenyl)-N,N,N',N'-tetramethylethane-1,2-diamine

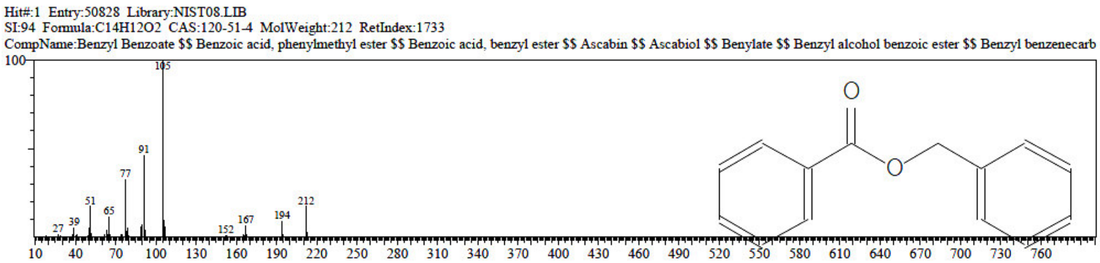

Fig. 39: A typical gas chromatogram of the Ascabin

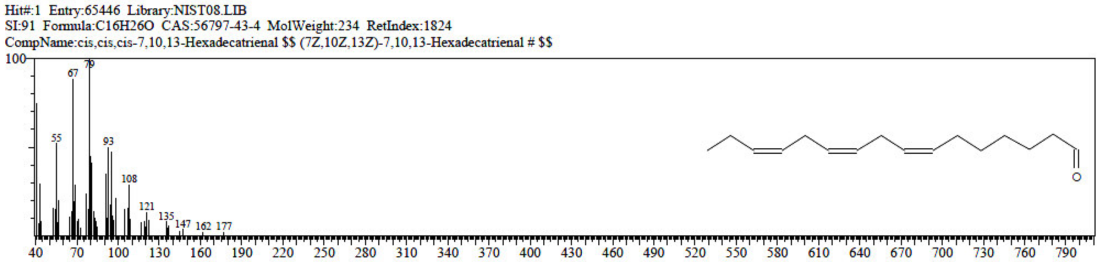

Fig. 40: A typical gas chromatogram of the cis,cis,cis-7,10,13-Hexadecatrienal

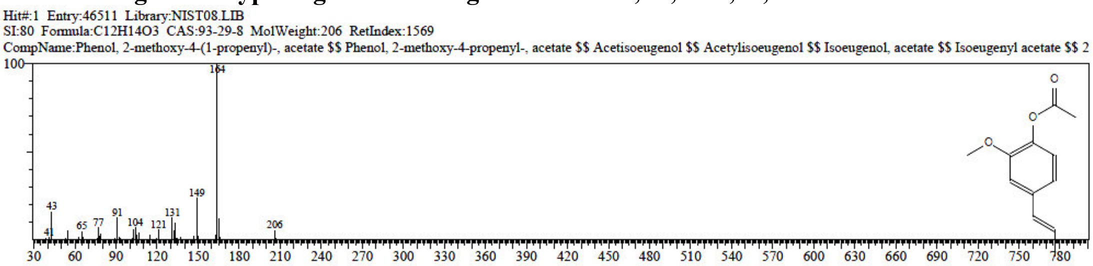

Fig. 41: A typical gas chromatogram of the Isoeugenyl acetate

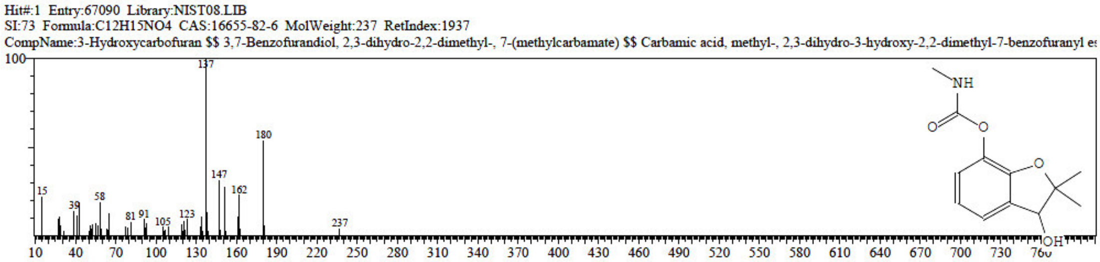

Fig. 42: A typical gas chromatogram of the3-Hydroxycarbofuran

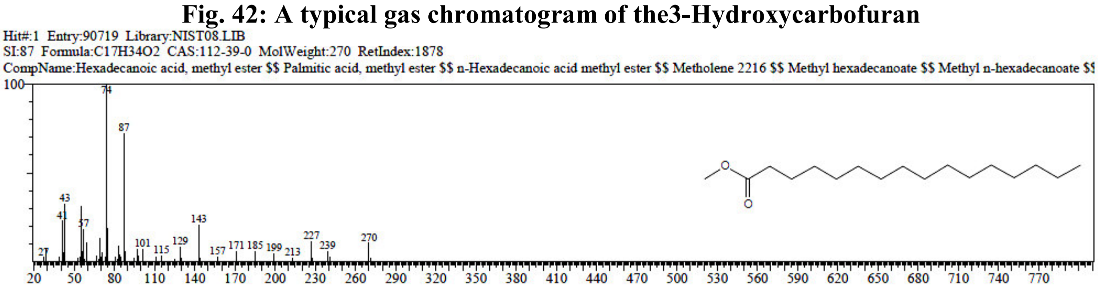

Fig. 43: A typical gas chromatogram of the Hexadecanoic acid, methyl ester

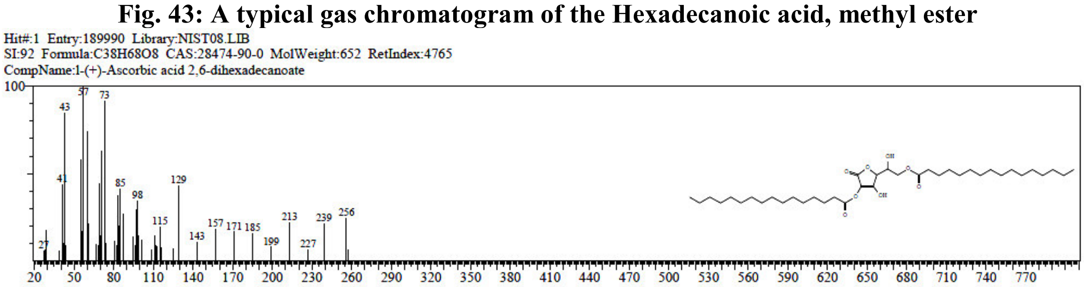

Fig. 44: A typical gas chromatogram of the l-(+)-Ascorbic acid 2,6-dihexadecanoate

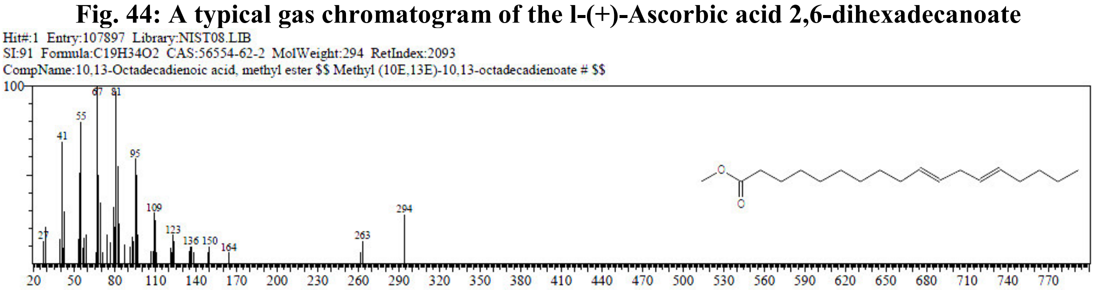

Fig. 45: A typical gas chromatogram of the 10,13-Octadecadienoic acid, methyl ester

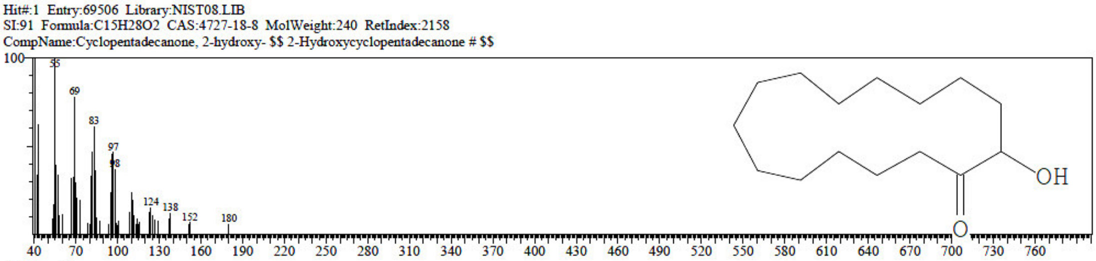

Fig. 46: A typical gas chromatogram of the Cyclopentadecanone, 2-hydroxy-

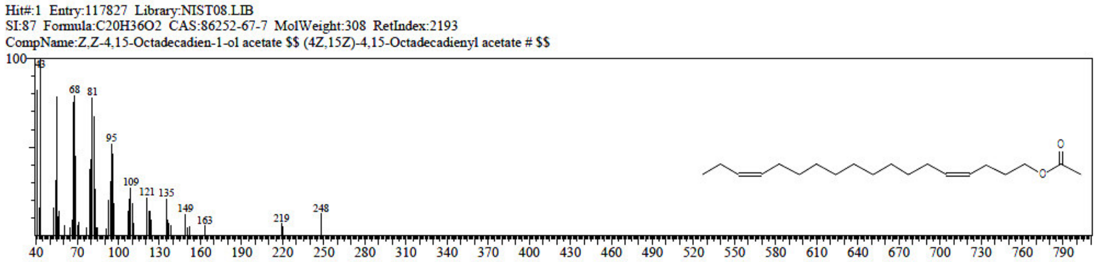

Fig. 47: A typical gas chromatogram of the Z,Z-4,15-Octadecadien-1-ol acetate

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