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Research Article

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GC-MS analysis and Antimicrobial Activity of Sudanese *Eucalyptus camaldulensis* Dehn. (Myrtaceae) Essential Oil

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Abstract Eucalyptus oil has been used traditionally for bronchitis and asthma. The oil which is plentiful in leaves has also been used against diabetes, cystitis, malaria, kidney disorders, leucorrhoea and laryngitis. The anticancer activity of this oil has been studied *in vivo*. The GC-MS analysis revealed the presence of 42 components. Major constituents are : eucalyptol (21.24%), decahydro-1,1,7-trimethyl-4-methylene - 1H-cycloprop[e]azulen-7-ol (12.59 %), p-cymene (12.11 %)**,** decahydro-1,1,4,7-tetramethyl- 1H-cycloprop[e]azulen-4-ol (11.64 %) and β-phellanderene (8.59%). The oil was evaluated for antibacterial activity via the agar diffusion bioassay against: Gram positive: *Staphylococcus aureus* and Gram negative: *Esherichia coli* using ampicilin and gentamycin as positive controls. At a concentration of 100mg/ml the oil showed moderate activity against *Escherichia coli* and *Staphylococcus aureus.*

Keywords *Eucalyptus camaldulensis*, Essenital Oil, GC-MS analysis, Antibacterial Activity

Introduction

The genus Eucalyptus is a large genus (about 900 species) in the family Myrtaceae which is indigenous to Astralia, Tasmania and New Guinea. This genus is now grown worldwide for its economic value .The genus Eucalyptus is widely distributed in Sudan [1-4].

Eucalyptus oil has been used traditionally for bronchitis and asthma. The oil which is plentiful in leaves has also been used against diabetes, cystitis, malaria, kidney disorders, leucorrhoea and laryngitis [5-9]. The anticancer activity of this oil has been studied in vivo [10].

The oil contains, among others, geraniol, β-pinene, camphene, limonene beside some mono- and sesquiterpenes. However, depending on plant origin, it could be dominated by the bioactive constituent 1,8-cineole [11-15].

It has been reported that the genus Eucalyptus contains some secondary metabolites like flavonoids, tannins, terpenoids and cyanogenic glycosides [16].

Eucalyptus camaldulensis **Dehn**. is a perennial medim-sized to tall tree (up to 30m high) in the family Myrtaceae [17]. Among the diverse uses of this species is the production of its bioactive essential oil which is used traditionally and in pharmaceutical preparations [18,19].

Babaya *et.al*. demonstrated [20] that the methanolic extract of *Eucalyptus camaldulensis* leaves inhibited the growth of *Bacillus subtilis* , while the plant crude extract exhibited anticandidal properties. The antinociceptive activity of some constituents of *Eucalyptus camaldulensis* essential oil has been outlined [21]. Also it has been shown that the leaves of *Eucalyptus camaldulensis* exhibited good free radical scavenging capacity [22].

Materials and Methods

Plant material

Eucalyptus camaldulensis leaves were collected from, Khartoum, Sudan. The plant was identified and authenticated by The Institute of Medicinal and Aromatic Plants, Khartoum, Sudan

Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m length; 0.25mm diameter; 0.25 μm, thickness) was used for GC-MS analysis**.**

Test organisms

Test organisms used in this study are: *Staphylococcus aureus* and *Escherichia coli*.

Methods

Extraction of oil

Powdered leaves of *Eucalyptus camaldulensis* (300g) were water distilled in a Clevenger type apparatus for four hours.

GC-MS analysis

The volatile oil from *Eucalyptus camaldulensis* was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument. Oven temperature was held at 150^{o} C for one minute and then programmed from 150 to 300 ^{o}C at 4/minute. Other chromatographic conditions are presented in Table 1.

Table 1: Chromatographic conditions

Injection mode	**Split**
Flow control mode	Linear velocity
Pressure	139.3 KPa
Total flow	50.0 ml/ min
Column flow	1.54 ml/sec.
Linear velocity	47.2 cm/sec.
Purge flow	3.0 ml/min.
Spilt ratio	- 1.0

Antibacterial assay

The cup-plate agar diffusion bioassay was used, with some minor modifications, to assess the antibacterial activity of the oil. Standardized bacterial stock suspension (2 ml) was mixed with 200 ml of sterile molten nutrient agar which was maintained at 45 °C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes.

Cups (6 mm in diameter) were cut using sterile cork borer (No 4). The agar discs were removed and the cups were filled with 0.1 ml samples of test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 °C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured.

Results and Discussion

GC-MS analysis of *Eucalyptus camaldulensis* volatile oil was conducted and the identification of the constituents was based on retention times and computer matching of the MS data with the (NIST) mass spectral library. Excellent matching was observed when comparing the observed mass spectra with the database on the MS library

Constituents of oil

The GC-MS analysis of the studied oil revealed the presence of 42 components (Table 1). The typical total ion chromatograms (TIC) is depicted in Fig.1.

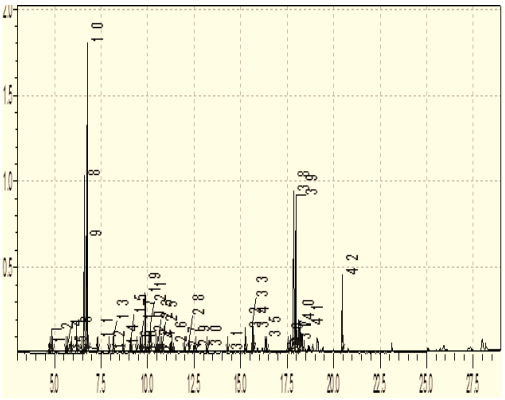


Figure 1: Total ions chromatograms

The major constituents of the oil are briefly discussed below:

Eucalyptol (21.24%)

The EI mass spectrum of eucalyptol is shown in Fig. 2. The peak at m/z 154, which appeared at R.T. 6.765 in total ion chromatogram, corresponds $M^+[C_{10}H_{18}O]^+$.

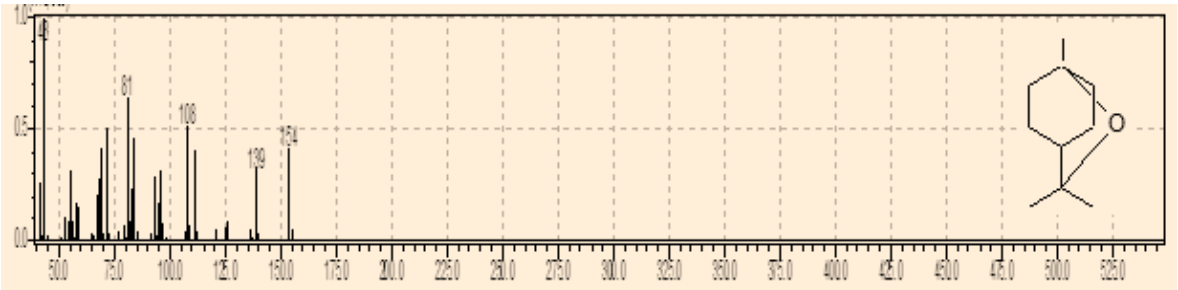


Figure 2: Mass spectrum of eucalyptol

Decahydro-1,1,7-trimethyl-4-methylene - 1H-cycloprop[e]azulen-7-ol (12.59 %)

The mass spectrum of decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulen-7-ol is presented in Fig. 3. The molecular ion- $M^+[C_{15}H_{24}O]^+$ - appeared at m/z 220 (R.T. 17.858)**.**

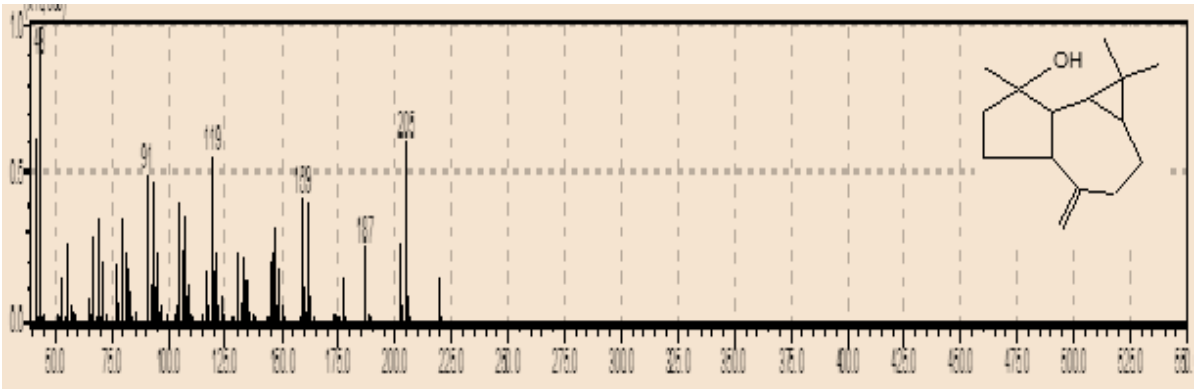


Figure 3: Mass Spectrum of decahydro-1,1,7-trimethyl-4-methylene - 1H-cycloprop[e]azulen-7-ol

p-Cymene (12.11 %)

The mass spectrum of p-cymene is displayed in Fig. 4. The peak at m/z 134, which appeared at R.T. 6.599 is due to the molecular ion $M^+[C_{10}H_{14}]^+$.

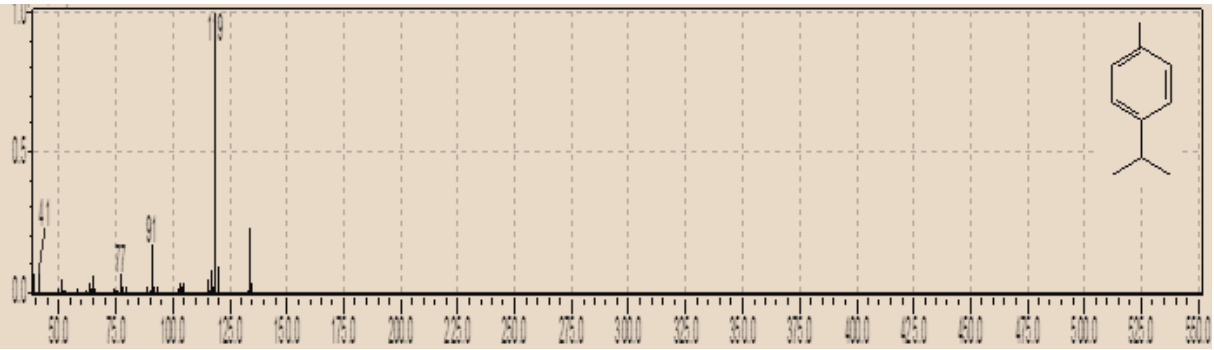


Figure 4: Mass spectrum of p-cymene

Decahydro-1,1,4,7-tetramethyl- 1H-cycloprop[e]azulen-4-ol (11.64 %)

Figure 5 displays the mass spectrum of decahydro-1,1,4,7-tetramethyl- 1H-cycloprop[e]azulen-4-ol(m.wt.220). The peak at m/z 204 is due to loss of a hydroxul function.

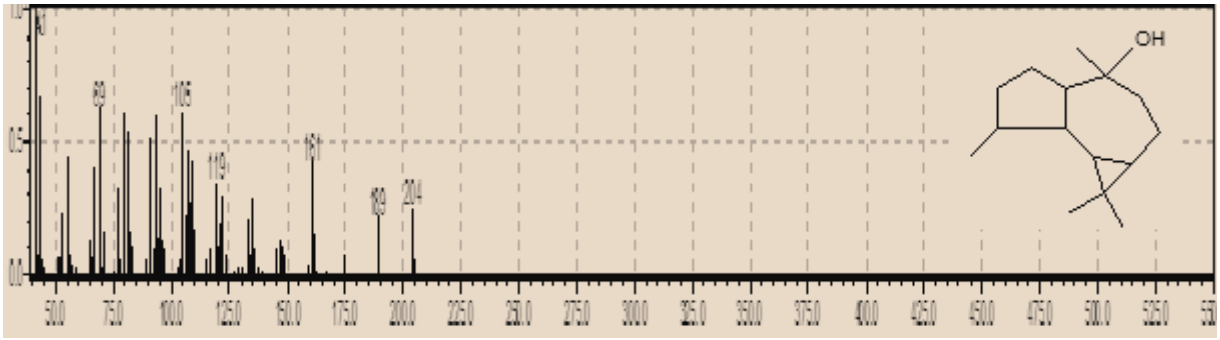


Figure 5: Mass spectrum of decahydro-1,1,4,7-tetramethyl- 1H-cycloprop[e]azulen-4-ol

β-Phellanderene (8.59%)

The mass spectrum of β-phellandrene is shown in Fig. 6. The peak at m/z 136 (R.T. 6.701) is attributed to the molecular ion $M^+[C_{10}H_{14}]^+$.

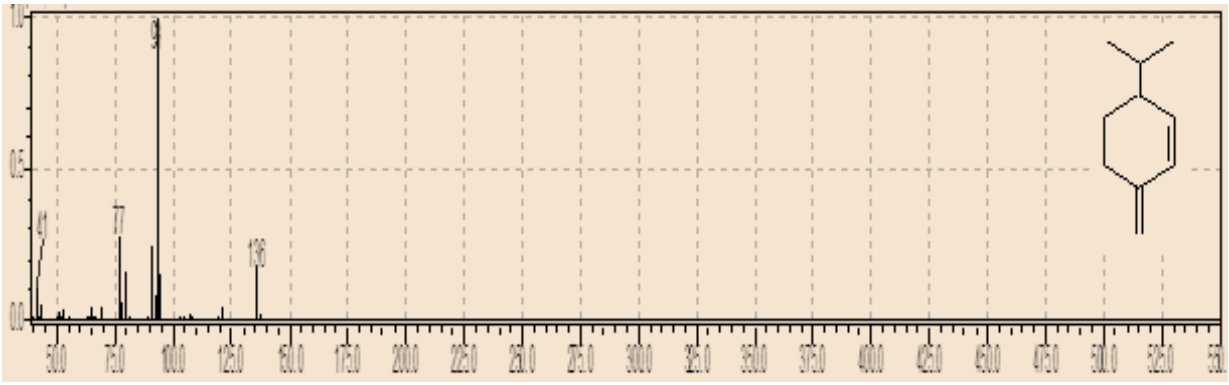


Figure 6: Mass spectrum of β-phellandrene

The constituents of *Eucalyptus camaldulensis* essential oils are presented in Table 3.The composition of *Eucalyptus camaldulensis* leave oil varies according to plant origin. Zrira et al [23] studied the constituents of *Eucalyptus camaldulensis* grown in Morocco and reported p-cymene and spathulenol as major constituents. This results agrees with the findings of Chalchat et al [24] who reported on the constituents of a Jerusalem plant material. However, Pagula et al [25] on studying *Eucalyptus camaldulensis* grown in Mozambiqe claimed the predominance of 1,8-cineole and β-pinene. Dethier et al [26] reported on the constituents of *Eucalyptus camaldulensis* growing in Burindi and stated the dominance of limonene and 1,8-cineole.

Antibacterial activity

In cup plate agar diffusion assay, the oil was assayed for antibacterial activity. The averages of the diameters of the growth inhibition zones are listed in Table 2. Ampicilin and gentamicin were used as positive controls. At a concentration of 100mg/ml the oil showed moderate activity against *Escherichia coli* and *Staphylococcus aureus.*

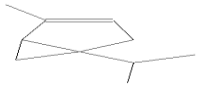
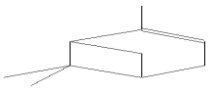
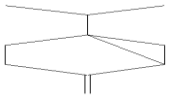
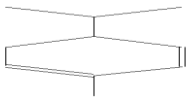
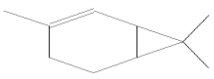
Some reports indicated that Gram negative bacteria are more resistant to essential oils than Gram positive bacteria [27], while many authors claimed that Gram positive bacteria are equally sensitive compared to Gram negative bacteria [27-32]. Some key elements seems to play a crucial role in the antibacterial activity of essential oils including the permeability of the bacterial membrane, intracellular distribution of oil constituents and occurrence of porin proteins in Gram negative bacteria [33].

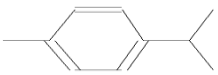
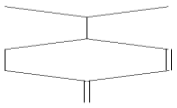
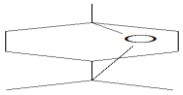
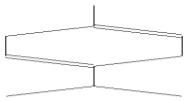
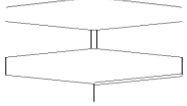
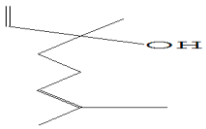
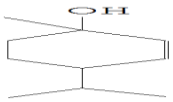
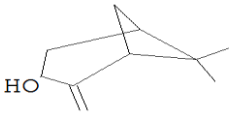
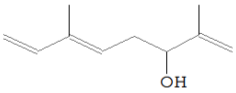
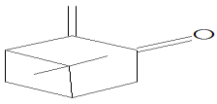
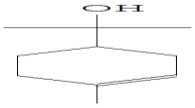
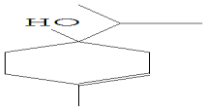
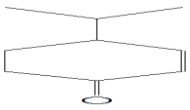
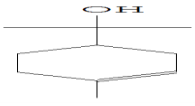
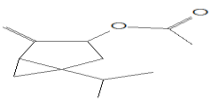
Literature reports on the antibacterial activity of *Eucalyptus camaldulensis* oil reflected diverse results. Though, Akin –Osanaiya et al [34] reported complete inhibition of *E. coli* and *S. aureus* by *Eucalyptus camaldulensis* oil, Cimanga et al [35] presented inhibition zones of 10-20mm and 18-30mm for *E. coli* and *S. aureus* respectively. Oskay and Sari [36] studied the oil from the Turkey material of *Eucalyptus camaldulensis* and concluded that the oil exhibited significant activity against both Gram negative *E. coli* and Gram positive *S. aureus*.

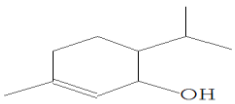
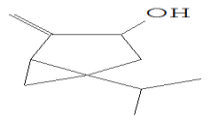
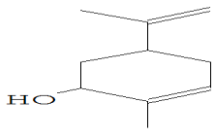
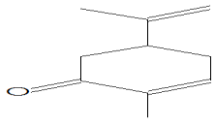
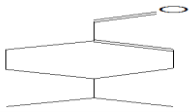
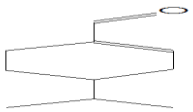
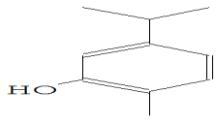
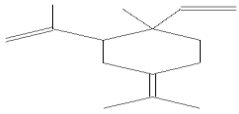
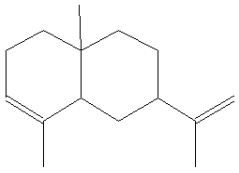
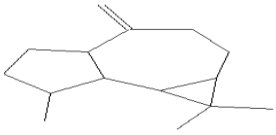
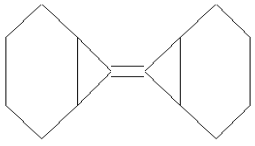
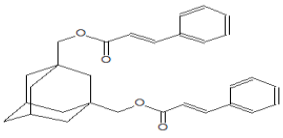
Table 2: Antibacterial activity

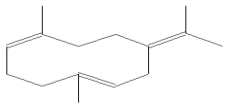
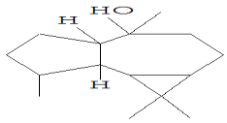
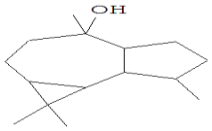
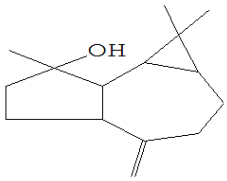
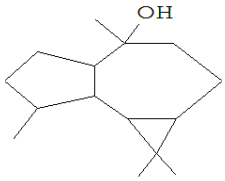
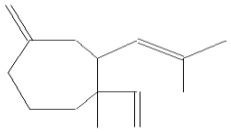
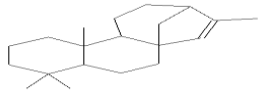
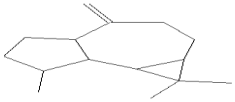
Sample	***E. coli***	***S. aureus***
Eucalyptus camaldulensis (100 mg/ml)	16	14
Ampicilin (40 mg/ml)	-	30
Gentamicin (40 mg/ml)	22	19

Table 3: Constituents of *Eucalyptus camaldulensis* oil

No.	**Name**	**RT**	**Formula**	**Area %**	**structure**
1	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	7.124	$C_{10}H_{16}$	0.55	
2	alpha.-Pinene	4.853	$C_{10}H_{16}$	1.39	
3	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	5.585	$C_{10}H_{16}$		
4	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	5.665	$C_{10}H_{16}$	0.23	
5	beta.-Myrcene	5.874	$C_{10}H_{16}$	0.60	
6	alpha.-Phellandrene	6.181	$C_{10}H_{16}$	1.68	
7	(+)-2-Carene	6.425	$C_{10}H_{16}$	0.13	

8	p-Cymene	6.599	$C_{10}H_{14}$	12.11	
9	beta.-Phellandrene	6.701	$C_{10}H_{16}$	8.59	
10	Eucalyptol	6.765	$C_{10}H_{18}O$	21.24	
11	gamma.-Terpinene	7.291	$C_{10}H_{16}$	0.81	
12	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	7.922	$C_{10}H_{16}$	0.15	
13	1,6-Octadien-3-ol, 3,7-dimethyl-	8.138	$C_{10}H_{18}O$	0.94	
14	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans	8.659	$C_{10}H_{18}O$	0.54	
15	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1.alpha.,3.alpha.,5.alpha.)]-	9.063	$C_{10}H_{16}O$	0.74	
16	1,5,7-Octatrien-3-ol, 2,6-dimethyl-	9.375	$C_{10}H_{16}O$	0.13	
17	Pinocarvone	9.573	$C_{10}H_{14}O$	0.14	
18	L-.alpha.-Terpineol	9.652	$C_{10}H_{18}O$	0.18	
19	Terpinen-4-ol	9.870	$C_{10}H_{18}O$	3.66	
20	2-Cyclohexen-1-one, 4-(1-methylethyl)-	10.085	$C_9H_{14}O$	1.01	
21	alpha.-Terpineol	10.153	$C_{10}H_{18}O$	1.61	
22	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, acetate	10.405	$C_{12}H_{18}O_2$	0.12	

23	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, trans-	10.506	$C_{10}H_{18}O$	0.21	
24	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, (1.alpha.,3.alpha.,5.alpha.)-	10.607	$C_{12}H_{18}O_2$	0.07	
25	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	10.745	$C_{10}H_{16}O$	0.07	
26	(-)-Carvone	11.280	$C_{10}H_{14}O$	0.64	
27	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-	11.948	$C_{10}H_{16}O$	0.37	
28	Safrole	12.209	$C_{10}H_{10}O_2$	0.37	
29	Phenol, 2-methyl-5-(1-methylethyl)-	12.487	$C_{10}H_{14}O$	0.58	
30	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-	13.166	$C_{15}H_{24}$	0.45	
31	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	14.262	$C_{15}H_{24}$	0.17	
32	Aromandendrene	15.242	$C_{15}H_{24}$	1.62	
33	Bicylo[4.1.0]heptane, 7-bicyclo[4.1.0]hept-7-ylidene-	15.625	$C_{14}H_{20}$	1.88	
34	1,3-Bis(cinnamoyloxymethyl)adamantane	15.669	$C_{30}H_{32}O_4$	1.22	

35	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)-	16.326	$C_{15}H_{24}$	1.31	
36	Epiglobulol	17.510	$C_{15}H_{26}O$	0.76	
37	(-)-Globulol	17.665	$C_{15}H_{26}O$	0.93	
38	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	17.858	$C_{15}H_{24}O$	12.59	
39	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	17.974	$C_{15}H_{26}O$	11.64	
40	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	18.118	$C_{15}H_{24}$	1.77	
41	Kaur-15-ene, (5.alpha.,9.alpha.,10.beta.)-	18.205	$C_{20}H_{32}$	1.53	
42	Alloaromadendrene	20.459	$C_{15}H_{24}$	4.93	

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