



Chemical compositions and antifungal activity of *Corymbia citriodora*, *Cupressus macrocarpa*, and *Syzygium cumini* extracts: GC–MS and HPLC analysis of essential oils and phenolic compounds

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Abstract

This study was undertaken to explore the content and the chemical constituents of the essential oils (EOs) and methanol leaf extracts (MLEs) from *Corymbia citriodora*, *Cupressus macrocarpa*, and *Syzygium cumini*. Chromatographic analyses of GC–MS and HPLC were used. The EOs and MLEs were prepared at concentrations of 0, 6, 12, 25, and 50 mg/L, while the extract was prepared at concentrations of 0, 500, 1000, 2000, and 4000 mg/L. Sapwood blocks of *Pinus sylvestris* in the dimension of 0.5 × 2 × 2 cm were prepared and autoclaved at 121 °C, and each wood block received 100 µL of the prepared concentrations from the EOs and MEs. The bioactivities of wood-treated EOs or MEs were measured against the growth of *Fusarium solani* MW947256. By GC–MS, the main compounds in the EOs from *C. citriodora* were citronellal, citronellol, p-cymene, spathulenol, and isopulegol with values of 23.95, 9.80, 9.32, 9.29, and 5.38%, respectively, in *Cup. macrocarpa* leaves were sabinene (11.94%), 4-terpinenol (11.34%), citronellol (9.59%), citronellal (9.85%), p-cymene (7.67%), spathulenol (5.24%), γ -terpinene (5.05%), camphor (4.31%), and limonene (3.2%), and in *S. cumini* leaves were trans- β -ocimene (19.11%), α -pinene (18.79%), caryophyllene (9.30%), (Z)- β -ocimene (8.16%), and limonene (6%). By HPLC, the most abundant phenolic compounds in the methanol extract from *C. citriodora* benzoic acid (8.11 µg/g), and gallic acid (7.96 µg/g), from *Cup. macrocarpa* were syringic acid (7.59 µg/g), catechol (6.85 µg/g), and gallic acid (6.78 µg/g), and from *S. cumini* were cinnamic acid (10.66 µg/g), caffeic acid (9.87 µg/g), and ellagic acid (8.76 µg/g). The highest percentages of inhibition (65.71% and 35.71%) against the growth of *F. solani* were seen in the wood treated with *Cup. macrocarpa* EOs at 50 and 25 mg/L, respectively. The maximum level of inhibition was seen (92.85%) when *S. cumini* MLEs at a dose of 4000 mg/L was applied to wood samples, followed by *Cup. macrocarpa* MLEs (70.00%) compared to the positive control of azoxystrobin + difenoconazole (1000 mg/L), which caused 100% inhibition to *F. solani*. The findings indicated that bioactive chemicals present in the extracts and EOs from these trees have strong antifungal properties.

Keywords *Corymbia citriodora* · *Cupressus macrocarpa* · *Syzygium cumini* · Biofungicide · Essential oils · Extracts

1 Introduction

Plant essential oils (EOs) and extracts play a potential role as alternatives to fungicidal agents that are currently used because of their contact inhibition, penetration, and volatile effect. Several EOs have the potential to control

different fungal species and can exert strong antifungal activities [1–5].

Corymbia citriodora (Hook.) K.D.Hill & L.A.S.Johnson or *Eucalyptus citriodora* Hook., the lemon-scented gum, its EOs, and phenolic and flavonoid compounds have great potential uses as antimicrobial and antioxidant activities [6–9]. A qualitative phytochemical analysis was performed for the detection of alkaloids, cardiac glycosides, flavonoids, saponins, sterols, tannins, and phenols [10]. Phytochemical studies on *E. citriodora* kino have described the isolation of triterpenoids, phenolics, and flavonoids [11, 12]. Leaf extract showed promising values against *Staphylococcus aureus* [13]. Citronellal, citronellyl acetate, and

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trans-caryophyllene were the major compounds in the leaf EO and showed the highest antibacterial activity towards *Staphylococcus aureus* and *Bacillus subtilis* and antifungal activity against *Aspergillus fumigatus* and *Saccharomyces cerevisiae* [14]. *C. citriodora* possesses a slight cytotoxic effect which is observed in their study. Leaf extract of plants can delay the loss of climbing ability in drosophila flies [15].

Cupressus macrocarpa Hartweg. ex Gordon (*Callitropsis macrocarpa*) (family Cupressaceae), or Monterey Cypress [16, 17], is traditionally used in rheumatism, whooping cough, stytic problem, improve bladder tone, and coadjuvant [18, 19]. Various phytoconstituents revealed the presence of carbohydrates, anthraquinone glycosides, cardiac glycosides, phenolics, EOs, flavonoids, saponin, protein, amino acid, and sterols in different extracts [20]. This plant contains diterpenes, monoterpenes, biflavonoids, and sesquiterpenes [9, 21–23]. The biflavonoid isolated from *Cup. macrocarpa* was shown a hepato- and nephro-protective activity in mice against carbon tetrachloride-induced toxicity [23].

Syzygium cumini (L.) Skeels (jambolan) or *Eugenia jambolana*, common plum and java plum, is one of the widely used medicinal plants [24]. The leaf EO of *S. cumini* was found to be good antibacterial activity [25]. The EO showed significant activity against *Leishmania amazonensis* [26]. Leaf EO from *S. cumini* with its main α -caryophyllene, β -caryophyllene, and terpineol showed antimycobacterial activity [27].

Extracts and EOs from several medicinal and higher plants have been used to protect wood and other wood-based products and objects from mold infestations [28]. In this context, the inhibitory effects of eighteen EOs isolated from Egyptian plants were evaluated against two wood decay fungi, *Ganoderma lucidum* and *Hexagonia apiaria*. The EOs revealed various mycelial growth inhibitions against the tested fungi depending on fungal species and tested EOs [29]. The use of natural substances that do not have undesirable side effects in place of harmful ways and the implementation of safe, unique substances have been the main topics of new and alternative research [30]. Many plants have natural chemical components such as EOs, phenols, flavonoids, alkaloids, coumarins, and tannins that are employed as nutrition, medicines, and bioactive molecules [31]. Plant-derived natural products may be the ideal solution for safely halting the biodegradation of wood [32–35].

In order to produce a useful practical approach for preventing the growth of fungi on wood, this work seeks to compile the use of pure essential oils and extracts from the trees *Corymbia citriodora*, *Cupressus macrocarpa*, and *Syzygium cumini*. This was accomplished by applying extracts and essential oils to Scots pine sapwood (*Pinus sylvestris*) and testing them against the growth of the fungus *Fusarium solani*. For additional analytical techniques,

GC–MS and HPLC were employed to identify chemical components.

2 Materials and methods

2.1 Plant material

Intact fresh leaves of three trees namely *Corymbia citriodora*, *Cupressus macrocarpa*, and *Syzygium cumini* were collected from Alexandria, Egypt, where *C. macrocarpa* and *S. cumini* were collected from the garden of the Faculty of Agriculture, Alexandria University, and the leaves from *C. citriodora* were collected from a plantation located at Alexandria-Cairo desert road (Albostan area), Alexandria, Egypt. The collected trees have been identified at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt, with their voucher numbers Z007, Z008, and Z009, for *C. citriodora*, *Cup. macrocarpa*, and *S. cumini*, respectively.

2.2 Extraction of the essential oil

The extraction process of the essential oils (EOs) from the fresh leaves of three trees *C. citriodora*, *Cup. macrocarpa*, and *S. cumini*. Plant material of about 100 g was washed using tap water and then cut into small pieces using scissors and put into a 2-L flask containing 1000 mL of distilled water (DW) and extracted by the hydrodistillation method using a Clevenger-type apparatus for 3 h [36]. After obtaining the EOs, they were over anhydrous sodium sulfate and kept dry in sealed Eppendorf tubes stored at 4 °C until chemical and bioassay analyses. The yields of EOs were 4.5, 2.5, and 0.08 mL/100 g plant fresh leaves from *C. citriodora*, *Cup. macrocarpa*, and *S. cumini*, respectively.

2.3 Gas chromatography–mass spectrometry (GC–MS) analysis of the essential oil

The chemical composition of the EOs was performed using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m film thickness). The column oven temperature was initially held at 50 °C and then increased by 5 °C/min to 250 °C held for 2 min and increased to the final temperature of 300 °C by 30 °C/min and held for 2 min. The injector and MS transfer line temperatures were kept at 270 and 260 °C, respectively. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The solvent delay was 2 min and diluted samples of 1 μ L were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at

70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their mass spectra with those of WILEY 09 and NIST 14 mass spectral database [37]. Xcalibur 3.0 data system in the GC–MS with its threshold values was used to confirm that all the mass spectra of the identified compounds were attached to the library. Furthermore, the measurement match factor (MF) with values ≥ 650 was used to confirm the identified compounds [38].

All analyses of studied samples have been done using Thermo Fisher GC–MS, which is supported by a number of mass spectral libraries, and all separated compounds have been identified and all data have been reported using NIST014, which is already supported by retention index (RI) library for 82,868 compounds (<https://www.nist.gov/system/files/documents/srd/NIST1aVer22Man.pdf>) [39, 40].

2.4 Methanol extracts

Leaves from the three tree species (*C. citriodora*, *Cup. macrocarpa*, and *S. cumini*) were air-dried at room temperature for 1 week, thin cut to small sizes or particles using a small laboratory mill. Approximately 50 g of each leaf sample was soaked in about 100 mL of methanol solvent in a conical flask for 1 week and then filtered through a cotton plug and Whatman no. 1 filter paper. The solvent evaporated as the extract was poured into Petri dishes to complete the dryness and then concentrated [41]; then, the methanol leaf extracts (MLEs) were obtained. The yields of the methanol extracts were 8.2, 6.6, and 9.12 g/100 g air-dry leaves from *C. citriodora*, *Cup. macrocarpa*, and *S. cumini*, respectively.

2.5 HPLC analysis of phenolic compounds

For the phytochemical analysis, the phenolic compounds from the MEs of *C. citriodora*, *Cup. macrocarpa*, and *S. cumini* leaves were identified by HPLC (Agilent 1100). The instrument was composed of a binary LC pump, a UV/Vis detector, and a C18 column (125 mm $\times$ 4.60 mm, 5- μ m particle size) [42]. The Agilent Chem-Station was used to acquire and analyze chromatograms. A gradient mobile phase of two solvents—Solvent A (MeOH) and Solvent B [acetic acid in H₂O (1:25)]—was used to separate phenolic acids. The gradient program began with 100% B and remained there for 3 min. This was followed by 5 min of 50% eluent A, 2 min of 80% eluent A, 5 min of 50% eluent A, 2 min of 80% eluent A, 5 min of 50% eluent A, 5 min of 50% eluent A, and 5 min of detection wavelength at 250 nm. As a result, the phenolic components were arranged in order to authenticate standard components by this mobile phase.

2.6 Antifungal activity of wood-treated essential oils and leaf methanol extracts

Air-dried Scots pine (*Pinus sylvestris* L.) sapwood was used for the treatment with the EOs and the MEs. The identification of wood was performed by academics staff in the Forestry and Wood Technology Department, Faculty of Agriculture, Alexandria University using softwood key identification [43]. Microscopic cross section was examined (Fig. 1).

The EOs were prepared at the concentrations of 0, 6, 12, 25, and 50 mg/L, while the MEs were prepared at 0, 500, 1000, and 4000 mg/L by dissolving the respective amount in dimethylsulfoxide (DMSO 10%), and a few drops of Tween 80 was added for the concentrated EOs. Air-dried Scots pine sapwood blocks with dimensions of 0.5 cm $\times$ 2 cm $\times$ 2 cm were autoclaved at 121 °C for 20 min, then treated with the prepared concentrations of EOs and MEs (each block has received a 100 μ L). The fungus *Fusarium solani* MW947256 was assayed for linear growths as incubated with the treated woods in the incubator chamber at 20–25 °C with relative humidity 85–92% for 7 days; then, the percentage of mycelial growth inhibition was measured with the following formula [44]: $MGI = [(Ac - At) / Ac] \times 100$; where MGI is mycelial growth inhibition and AC and AT are average diameters of the fungal colony of the control and treatment, respectively. Wood samples soaked only with 10% DMSO were used as the negative control. Positive control of azoxystrobin + difenoconazole (1000 mg/L) was also employed.

2.7 Statistical analysis

The obtained data from the antifungal activity of essential oils and methanol extracts from the leaves of the three trees with

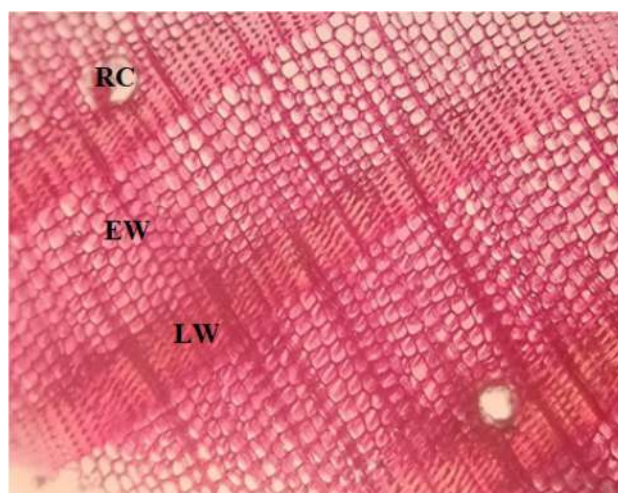


Fig. 1 Cross section of *Pinus sylvestris* wood sample. RC resin canal, EW earlywood tracheids, LW latewood tracheids

their concentrations were analyzed using CoStat software version 6.303 [45], in split-plot design. The factor concentrations were set as the main plot, while the factor EOs or MEs as well as the interactions between the source and concentrations of EOs or MEs. The least significant difference at 0.05 level of probability was used for comparison between the means [46].

3 Results

3.1 Chemical compositions of the essential oils

Table 1 and Fig. 2 present the chemical compounds identified in the essential oil (EO) from *Corymbia citriodora*. The main compounds were citronellal (23.95%), citronellol (9.80%), p-cymene (9.32%), spathulenol (9.29%), isopulegol (5.38%), crypton (4.65%), citronellol epoxide (4.45%), neo-isopulegol (3.67%), citronellic acid (2.96%), eucalyptol (2.7%), 4-terpinenol (2.36%), citronellol acetate (2%), trans-3-nonen-2-one (1.48%), and cuminaldehyde (1.21%).

The chemical constituents of the EO from *Cupressus macrocarpa* leaves are shown in Table 2. The main abundant compounds were sabinene (11.94%), 4-terpinenol (11.34%), citronellol (9.59%), citronellal (9.85%), p-cymene (7.67%), spathulenol (5.24%), γ -terpinene (5.05%), camphor (4.31%), limonene (3.2%), α -terpinene (2.89%), β -myrcene (2.64%), 15-kaurene (2.4%), terpinolene (2.32%), sclareol (1.86%), linalool (1.61%), α -terpineol (1.53%), farnesol (1.48%), crypton (1.32%) and α -thujene (1.03%).

Table 3 shows the chemical compounds of the EO from *Syzygium cumini* leaves. The main compounds were *trans*- β -ocimene (19.11%), α -pinene (18.79%), caryophyllene (9.30%), (*Z*)- β -ocimene (8.16%), limonene (6.00%), humulene (4.69%), β -pinene (4.01%), α -terpineol (3.79%), bornyl acetate (2.88%), myrcene (2.26%), citronellal (1.79%), 2,2,4a,7a-tetramethyl-decahydro-1H-cyclobuta[e]inden-5-ol (1.76%), caryophyllenyl alcohol (1.63%), globulol (1.62%), terpinen-4-ol (1.62%), caryophyllene oxide (1.54%), and citronellol (1.33%).

3.2 Phenolic compounds

Results from Table 4 and Fig. 3 show the identified phenolic compounds in the methanol leaf extracts (MLEs) of *C. citriodora* (Fig. 2a), *Cup. macrocarpa* (Fig. 2b), and *S. cumini* (Fig. 2c). Benzoic acid (8.11 μ g/g), gallic acid (7.96 μ g/g), ellagic acid (5.78 μ g/g), caffeic acid (4.65 μ g/g), and catechol (3.75 μ g/g) were the most abundant compounds in the MLE of *C. citriodora*. The abundant phenolic compounds in the MLE from *Cup. macrocarpa* were syringic acid (7.59 μ g/g), catechol (6.85 μ g/g), gallic acid (6.78 μ g/g), and chlorogenic acid (4.62 μ g/g). In the MLE of *S. cumini*, the most abundant compounds were cinnamic acid (10.66 μ g/g), caffeic acid (9.87 μ g/g), and ellagic acid (8.76 μ g/g).

3.3 Antifungal activity of essential oils and methanol extracts against *Fusarium solani*

The antifungal activity of Scots pine treated with EOs and MLE against the growth of *Fusarium solani* is visually observed in Figs. 4, 5, and 6. The growth of the fungus mycelium was inhibited as EO or MLE concentrations were increased, compared to the positive control of azoxystrobin + difenoconazole (1000 mg/L), which caused 100% inhibition to *F. solani*.

Figure 7 illustrates the main impacts of EO concentration and source on *F. solani* development. The percentage of fungal mycelial growth inhibition increased as the concentrations rose. Additionally, *Cup. macrocarpa* EO was shown to have the highest overall impacts of any EO source. Table 5 shows the proportion of *F. solani* mycelial growth that was inhibited (%) when wood was treated with different EO doses. The wood-treated *Cup. macrocarpa* EO at 50 and 25 mg/L, respectively, showed the highest levels of inhibition (65.71% and 35.71%) against the growth of *F. solani*. Additionally, *S. cumini* EO and *C. citriodora* EO both inhibited *F. solani* at 50 mg/L (35.71% and 35.23%, respectively). Additionally, moderate activity was found when the EO from *Cup. macrocarpa* EO was applied at 12 mg/L with an inhibition value of 28.57%, and from *C. citriodora* EO with a value of 22.85% at 25 mg/L.

The main impacts of MLE concentration and their sources on *F. solani* growth were displayed in Fig. 8. It is evident that fungal inhibition (%) increased as MLE concentration rose. Furthermore, out of all the examined extracts, *S. cumini* MLE displayed the highest overall inhibition. Table 6 displays how MLEs applied to wood inhibited the fungal mycelium growth of *F. solani*. It is interesting to note that *S. cumini* MLE at a concentration of 4000 mg/L when applied to wood samples demonstrated the highest level of inhibition (92.85%), followed by *Cup. macrocarpa* MLE (70%). The wood samples treated with *C. citriodora* MLE at 4000 mg/L (49.52%), 2000 mg/L (41.90%), *Cup. macrocarpa* MLE (37.14%), and *S. cumini* MLE (35.71%) at 2000 mg/L showed good efficacy against the growth of *F. solani* as well.

4 Discussion

The EOs and phenolic compound profiles from the leaves of three Egyptian-grown trees *Corymbia citriodora*, *Cupressus macrocarpa*, and *Syzygium cumini* were examined in the current study using chromatographic equipment for GC–MS and HPLC. When applied to wood samples, the bioactivities against the growth of *Fusarium oxysporum* were likewise documented with varying degrees of inhibition.

Table 1 Chemical constituents of the essential oil from *Corymbia citriodora*

RT*	Area %	Compounds	RI**	MF***	MS fragmentations
3.50	0.51	1-Methyl-3-isopropylcyclopentane	876	932	
4.52	0.17	α -Thujene	964	939	
4.64	0.20	α -Pinene	937	932	
6.41	9.32	p-Cymene	1011	931	
6.56	2.70	Eucalyptol	1023	925	

Table 1 (continued)

6.95	0.71	Melonal	1036	924	
7.30	0.16	γ -Terpinene	1332	925	
8.27	0.66	Linalool	1081	930	
8.46	0.37	trans-5-Caranol	1125	832	
8.76	0.39	cis-2-Menthenol	1123	914	
9.37	5.38	Isopulegol	1159	894	

Table 1 (continued)

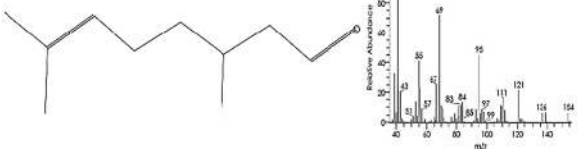
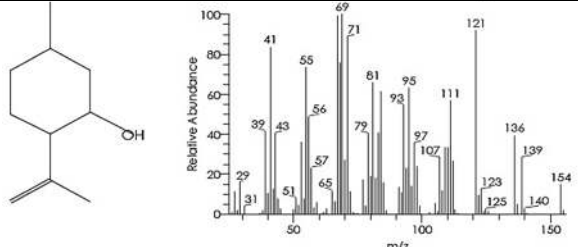
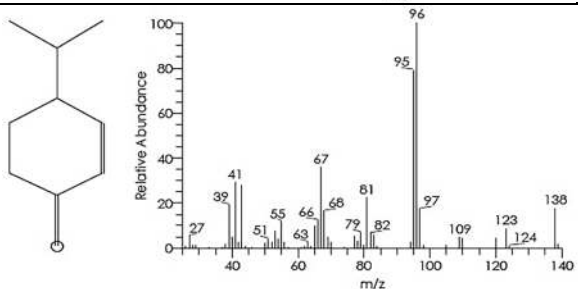
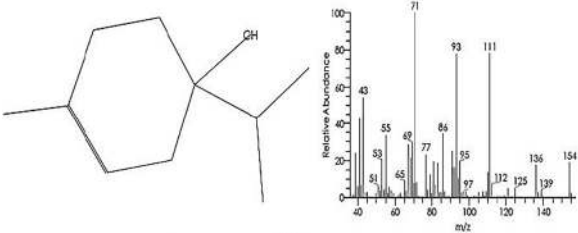
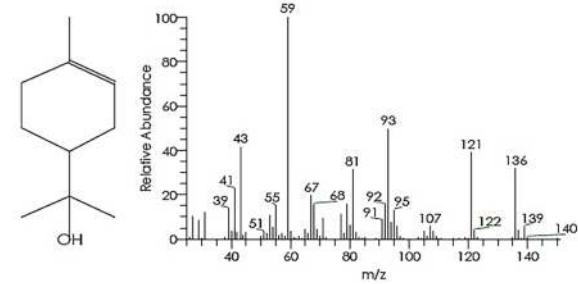
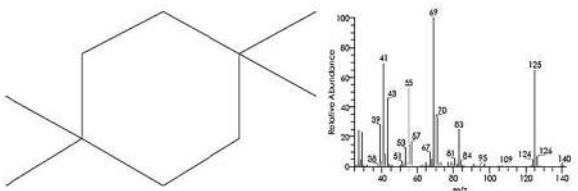
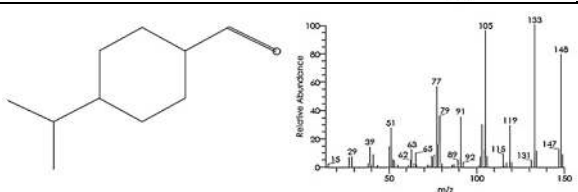
9.52	23.95	Citronellal	1132	942	
9.70	3.67	Neoisopulegol	1196	899	
10.00	4.65	Cryptone	1153	4.65	
10.18	2.36	α -Terpinen	1161	937	
10.49	0.64	α -Terpineol	1271	900	
11.32	0.89	1,1,4,4-Tetramethylcyclohexane	989	761	
11.47	1.21	Cuminaldehyde	1214	955	

Table 1 (continued)

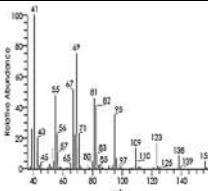
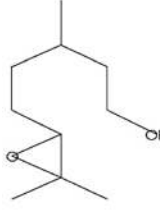
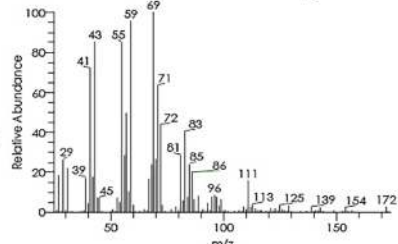
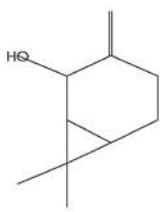
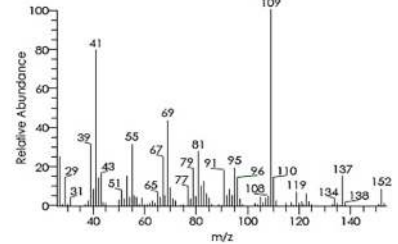
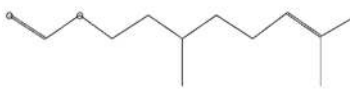
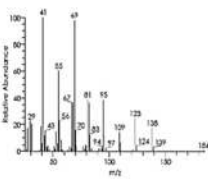
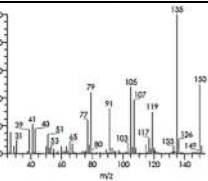
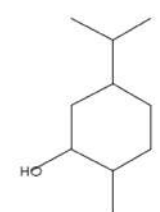
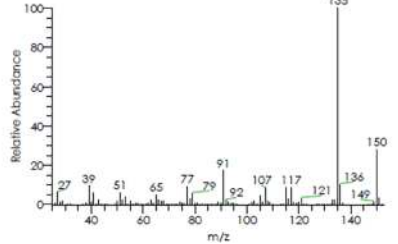
11.65	9.80	Citronellol	1208	930	 
11.98	4.45	Citronellol epoxide	1220	692	 
12.40	0.39	trans-3(10)-Caren-2-ol	1194	888	 
12.78	0.49	Citronellyl formate	1262	914	 
12.96	0.42	Cuminy alcohol	1230	884	 
13.52	0.29	Carvone	1190	862	 

Table 1 (continued)

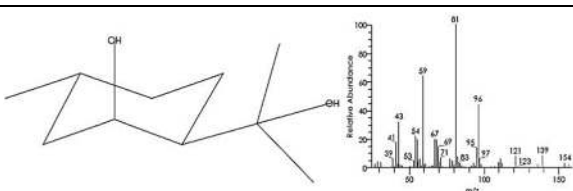
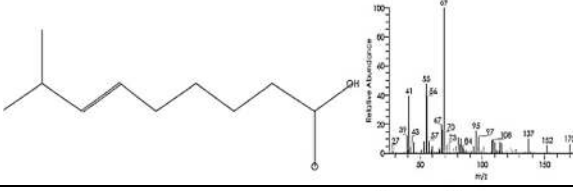
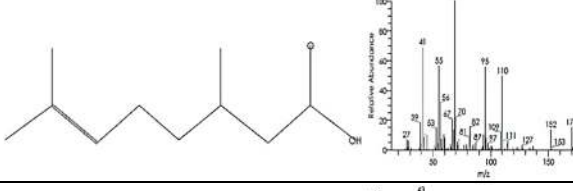
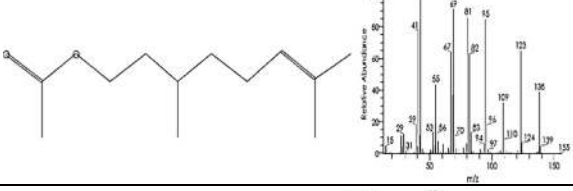
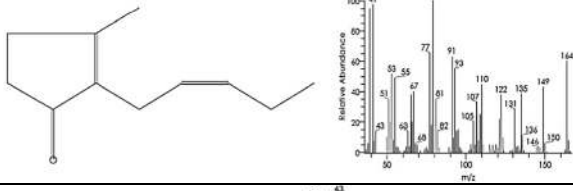
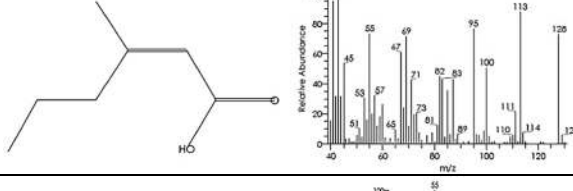
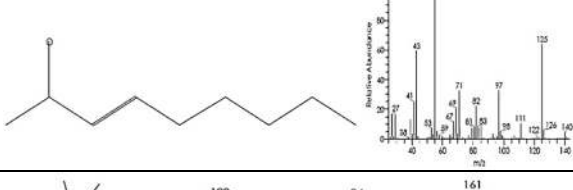
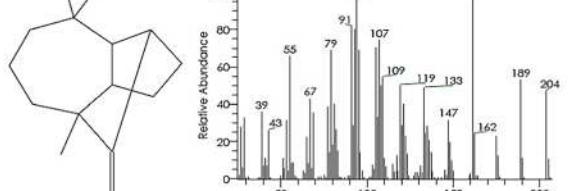
14.02	0.97	8-Hydroxyneomenthol	1320	919	
14.21	0.96	8-Methyl-6-nonenic acid	1372	750	
14.35	2.96	Citronellic acid	1287	811	
14.80	2.00	Citronellol acetate	1331	916	
15.47	0.72	(Z)-Jasmone	1359	813	
16.62	0.57	Z-3-Methyl-2-hexenoic acid	1059	813	
17.52	1.48	trans-3-Nonen-2-one	1114	675	
19.46	0.20	Longifolene	1432	822	

Table 1 (continued)

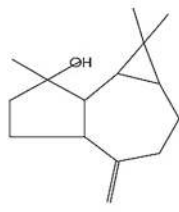
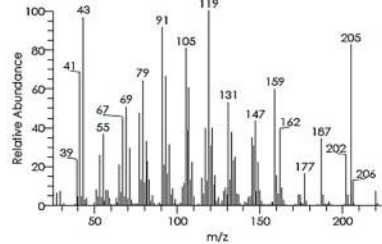
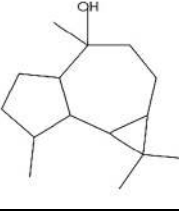
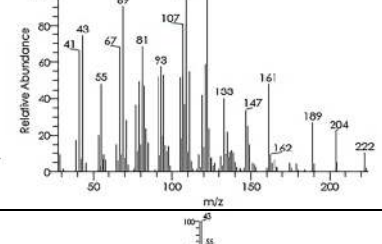
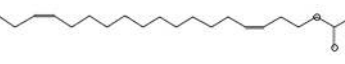
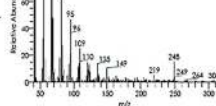
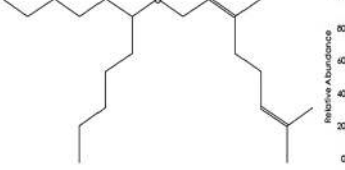
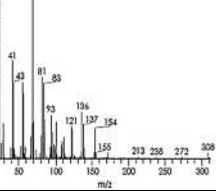
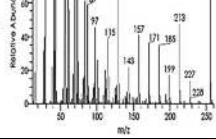
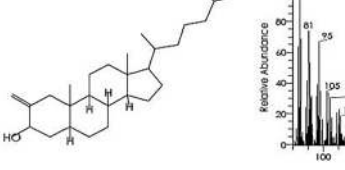
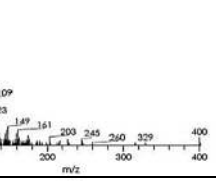
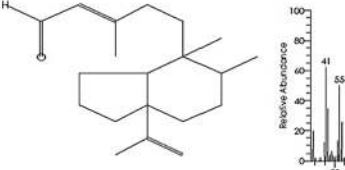
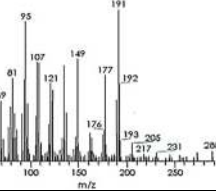
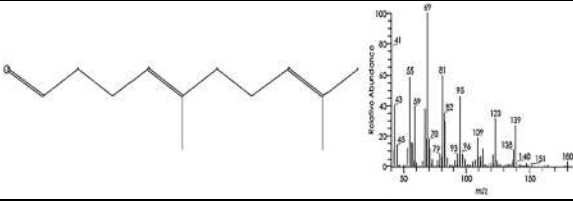
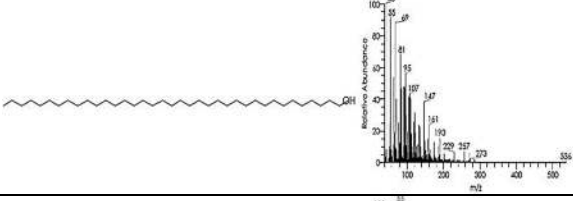
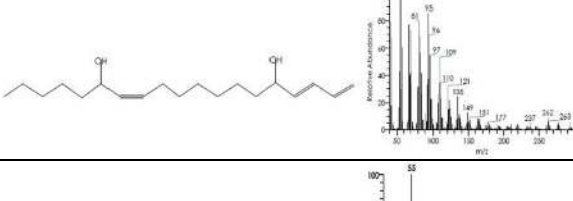
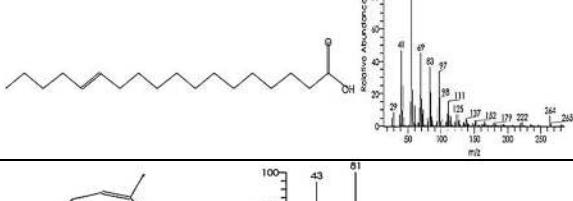
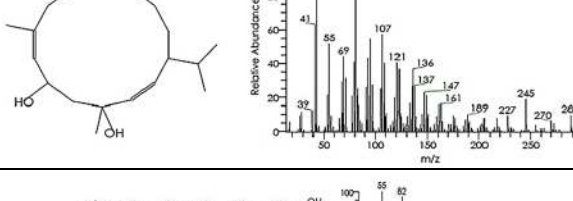
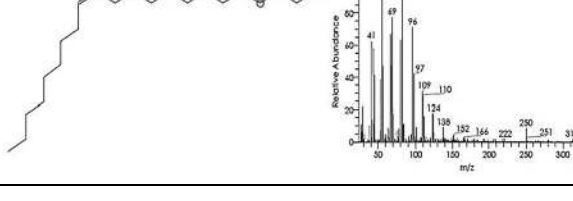
20.14	9.29	Spathulenol	1569	944	 
20.75	0.19	Ledol	1597	915	 
27.57	0.38	(3E,13Z)-Octadecadien-1-yl acetate	2193	747	 
27.80	0.28	3,7-dimethyl-2,6-octadienyl 1-pentylhexyl ether	2140	746	 
28.45	0.48	Palmitic acid	1942	900	 
28.52	0.28	2-Methylenecholestan-3-ol	2652	783	 
28.71	0.50	5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-enal	2149	795	 

Table 1 (continued)

29.82	0.25	Geraldehyde	2048	823	
30.24	0.65	1-Heptatriacontanol	3942	719	
30.44	0.35	(3E,12Z)-1,3,12-Nonadecatriene-5,14-diol	2241	734	
31.62	0.85	trans-13-Octadecenoic acid	2163	912	
35.14	0.38	4,8,13-duvatriene-1,3-diol	2955	825	
40.78	0.30	2-(Octadec-9-enyloxy)ethanol	1702	739	

*RT retention time (min), **RI retention index, ***MF match factor

C. citriodora EO demonstrated the existence of citronellal, citronellol, p-cymene, spathulenol, isopulegol, crypton, citronellol epoxide, neoisopulegol, citronellic acid, eucalyptol, 4-terpinenol, citronellol acetate, trans-3-nonen-2-one, and cuminaldehyde as major components. Citronellal (78%) was the main component for *C. citriodora* EO grown in Portugal [47], while citronellal (83.5%) was found as the predominant compound in the EO from the collected plant from Benin [48]. The EO of *E. citriodora*, which yielded 1.73%, showed strong inhibition for the radial growth of *F. oxysporum* f. sp. *lycopersici*, *Alternaria trititina*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Colletotrichum*

lindemuthianum, *Helminthosporium oryzae*, and *Alternaria solani* [49]. The Algerian *E. citriodora* leaf EO showed the presence of dominant compounds citronellal (69.77%), citronellol (10.63%), and isopulegol (4.66%) with potent anti-fungal activity against *Microsporum canis*, *Trichophyton mentagrophytes*, and *T. rubrum* [50].

E. citriodora were citronellal (40%), isopulegol (14.6%), and citronellol (13%) grown in Colombia [51]. With percentages of 61.78%, 15.54%, and 7.90%, citronellal, isopulegol, and -citronellol were the principal components of the EO of *C. citriodora*. The EO and citronellal had a significant negative impact on the growth of *Pyricularia grisea*,

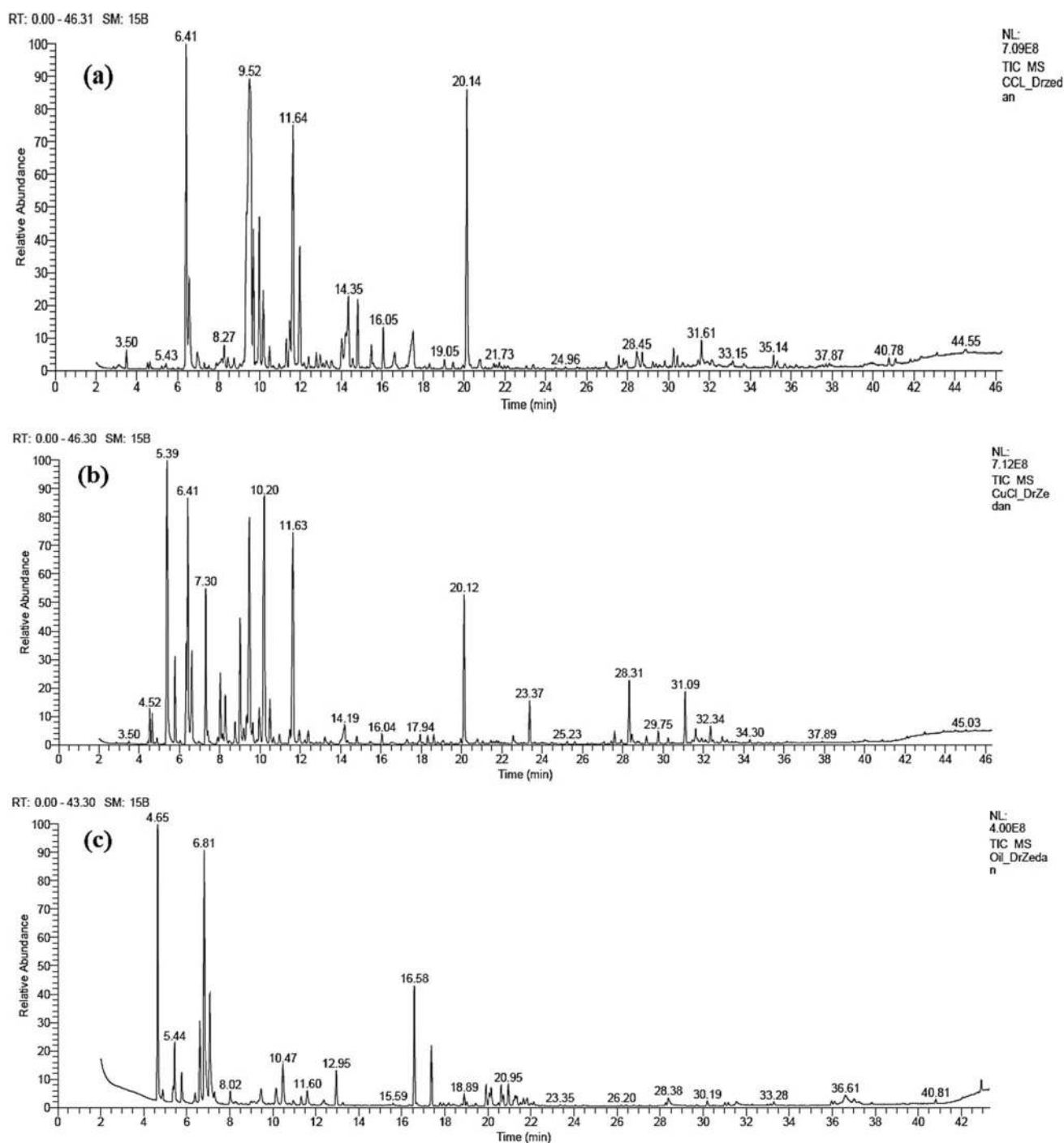


Fig. 2 GC–MS chromatograms of the identified essential oils from leaves of **a** *C. citriodora*; **b** *Cup. macrocarpa*; **c** *S. cumini*

Aspergillus spp., and *Colletotrichum musae* [52]. Citronellal (52.2%), citronellol (12.3%), and isopulegol (11.9%) were the three main compounds of the 0.6% EO obtained from the leaves of the lemon-scented eucalyptus (*Eucalyptus citriodora*) collected from the campus of the Anjab University in Chandigarh, India [53]. Citronellal (67.5%), citronellol

(6.9%), and menthol (6.1%), according to a chromatographic examination of *C. citriodora* EO from Brazil, were the main constituents [54].

Methanolic extract of *E. citriodora* had the highest antimycotic efficacy against four *Aspergillus* spp., with a value of 14.5% followed by EO (12.9%), chloroform extract

Table 2 Chemical constituents of the essential oil from *C. macrocarpa*

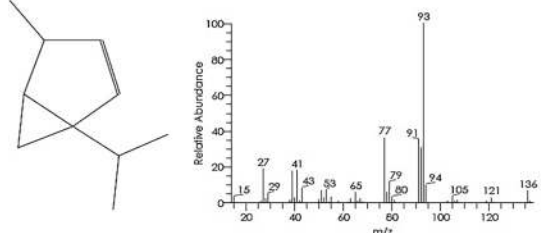
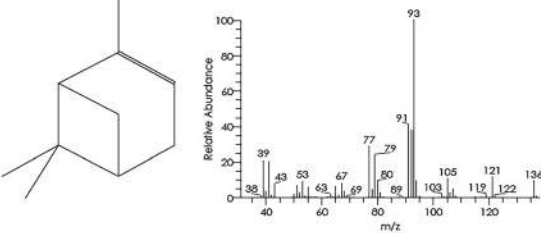
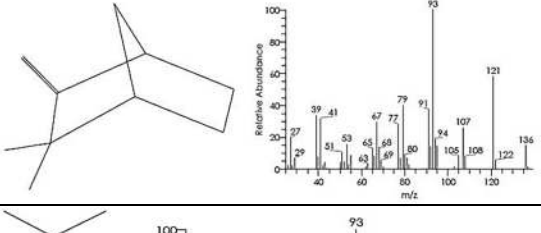
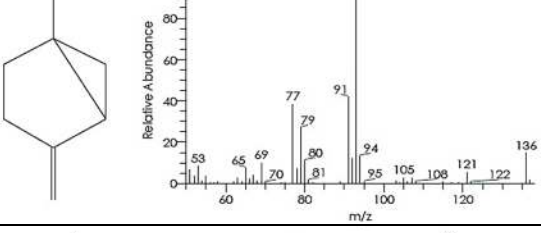
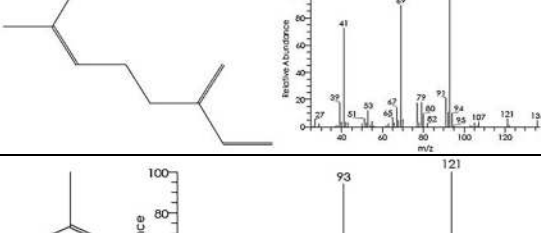
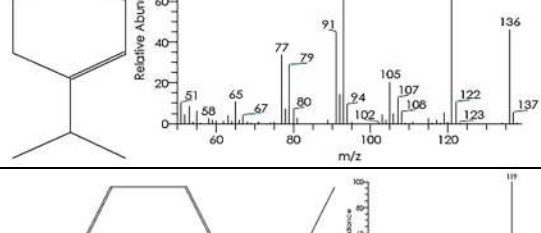
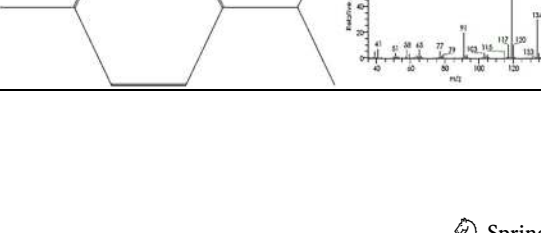
RT*	Area %	Compounds	RI**	MF***	MS fragmentations
4.52	1.03	α -Thujene	964	938	
4.64	0.89	α -Pinene	937	940	
4.88	0.19	Camphene	935	966	
5.38	11.94	Sabinene	964	945	
5.77	2.64	β -Myrcene	979	945	
6.33	2.89	α -Terpinene	1332	941	
6.41	7.67	p-Cymene	1011	931	

Table 2 (continued)

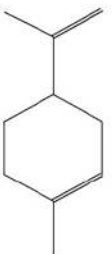
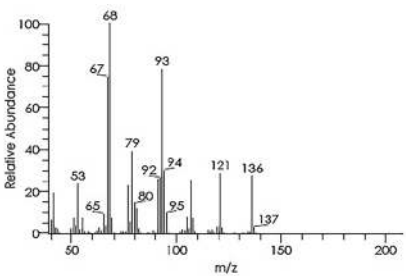
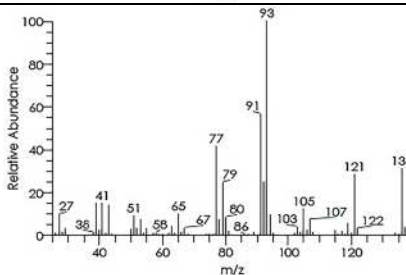
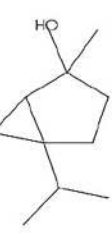
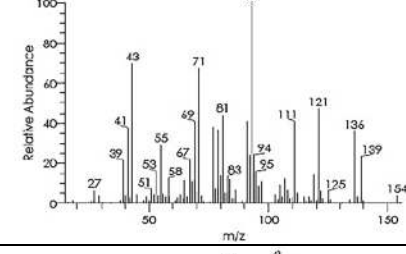
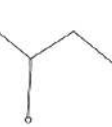
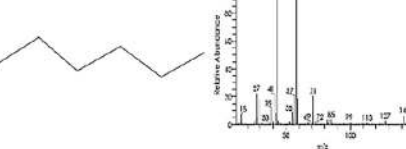
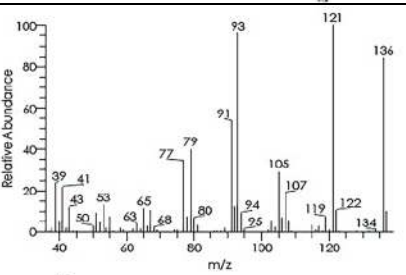
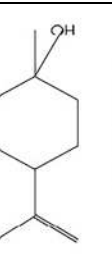
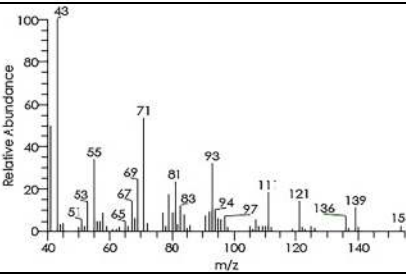
6.61	3.20	Limonene	1018	931	 
7.30	5.05	γ -Terpinene	1332	944	 
7.41	0.37	cis-4-Thujanol	1054	934	 
7.89	0.18	2-Nonanone	1070	729	 
8.03	2.32	Terpinolene	1078	953	 
8.14	0.27	cis- β -Terpineol	2636	932	 

Table 2 (continued)

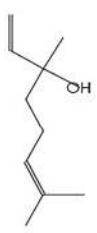
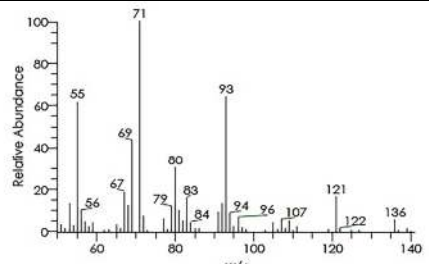
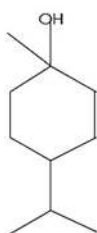
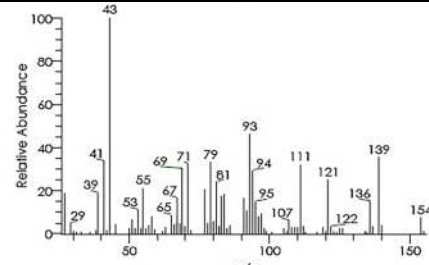
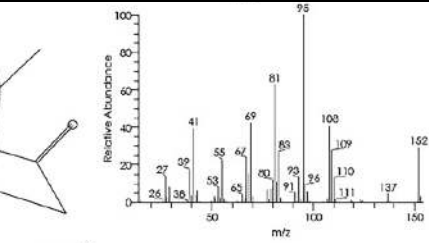
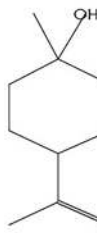
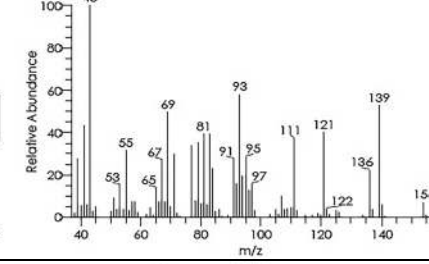
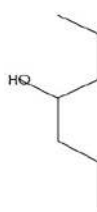
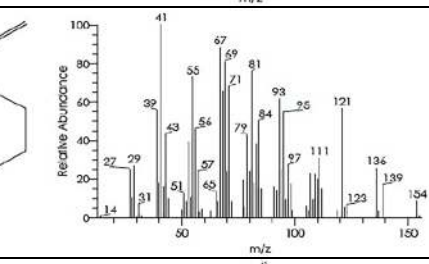
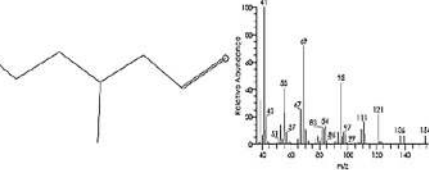
8.26	1.61	Linalool	1081	949	 
8.76	0.81	trans-para-2-Menthen-1-ol	1123	929	 
9.00	4.31	Camphor	1141	951	 
9.19	0.45	3-Terpinen-1-ol	1120	914	 
9.32	0.88	Isopulegol	1159	946	 
9.48	9.85	Citronellal	1132	941	 

Table 2 (continued)

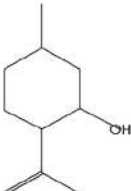
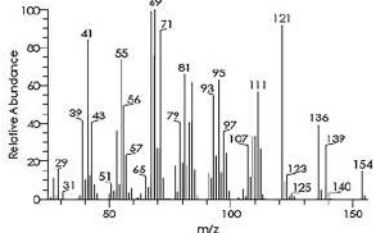
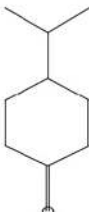
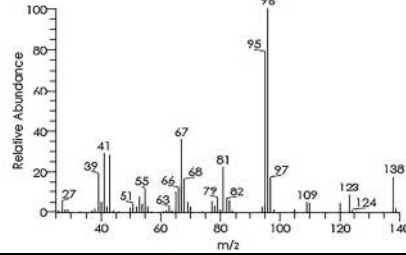
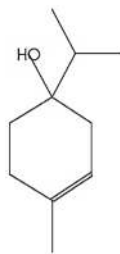
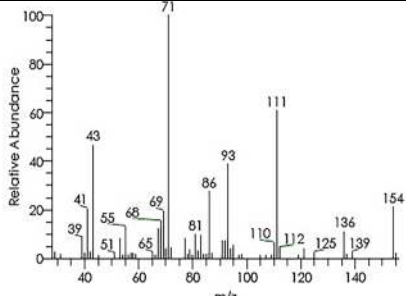
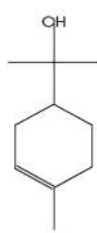
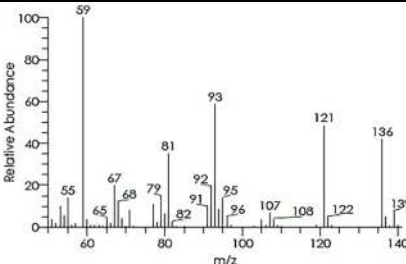
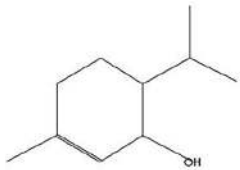
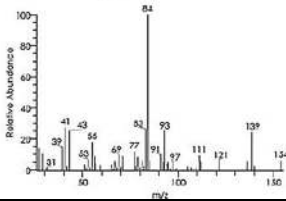
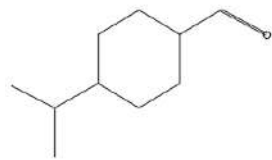
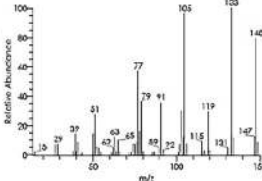
9.63	0.50	Neoisopulegol	1196	903	 
9.96	1.32	Cryptone	1153	949	 
10.21	11.34	4-Terpinenol	1161	921	 
10.48	1.53	α -Terpineol	1143	928	 
10.96	0.35	(Z)-Piperitol	1179	923	 
11.46	0.38	Cuminaldehyde	1214	958	 

Table 2 (continued)

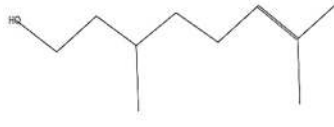
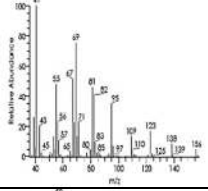
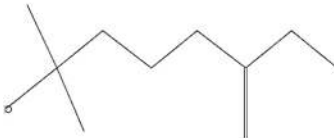
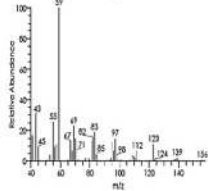
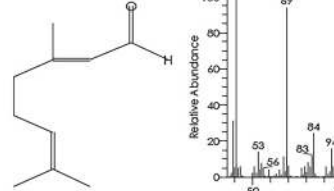
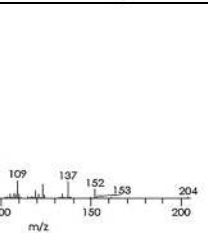
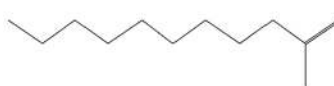
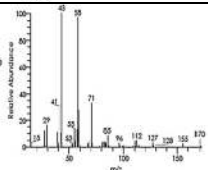
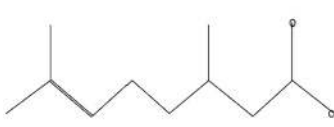
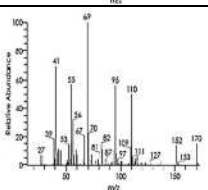
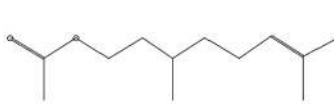
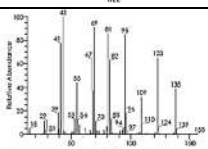
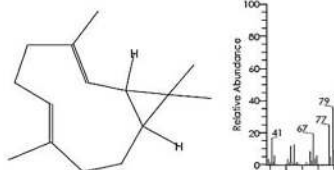
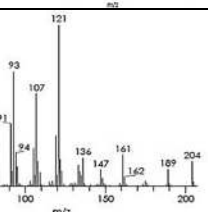
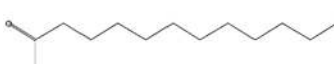
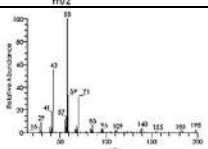
11.64	9.59	Citronellol	1208	928	 
11.94	0.56	Dihydro myrcenol	1074	733	 
12.38	0.54	α -Citral Geranial	1249	885	 
13.21	0.30	2-Undecanone	1274	946	 
14.20	0.74	Citronellic acid	1287	904	 
14.79	0.24	Citronellyl acetate	1331	909	 
17.94	0.37	Bicyclogermacr ene	1496	817	 
18.31	0.28	2-Tridecanone	1481	878	 

Table 2 (continued)

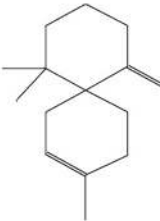
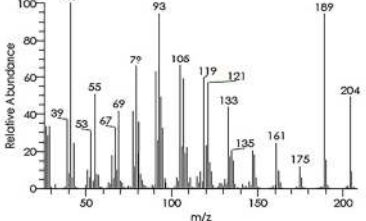
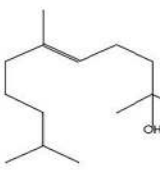
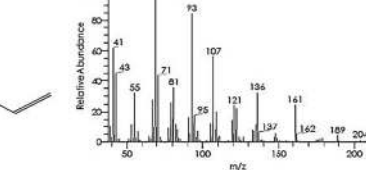
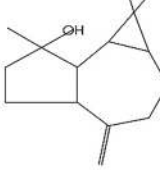
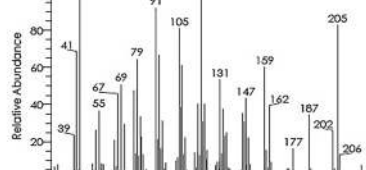
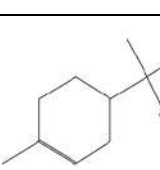
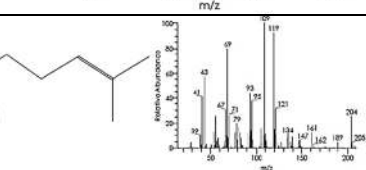
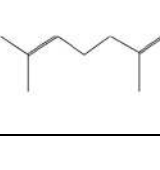
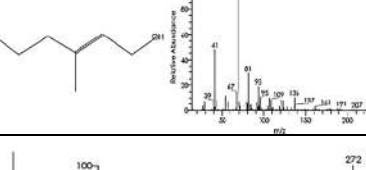
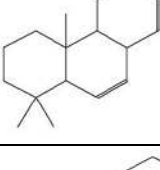
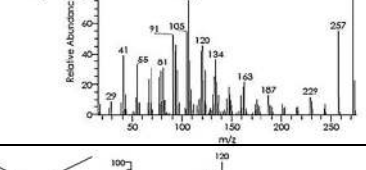
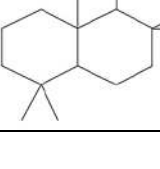
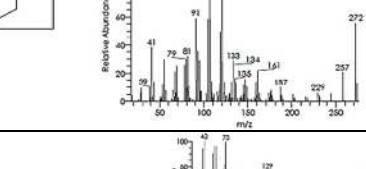
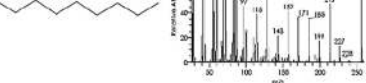
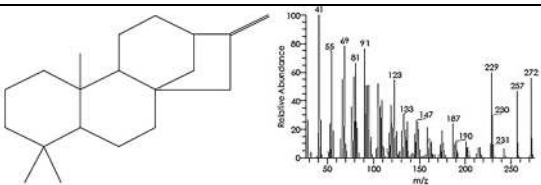
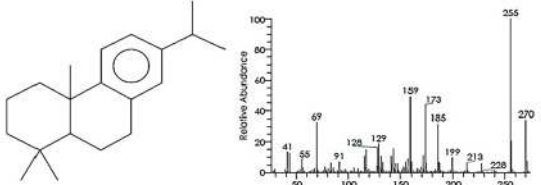
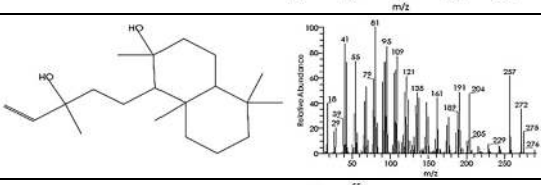
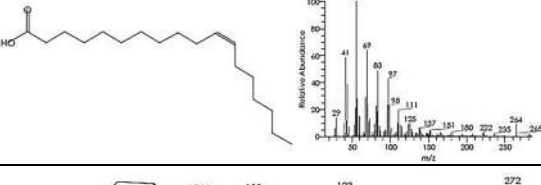
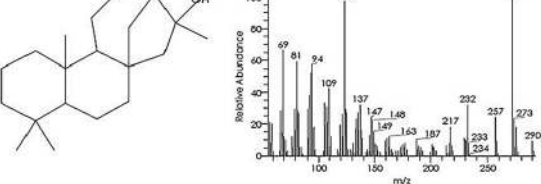
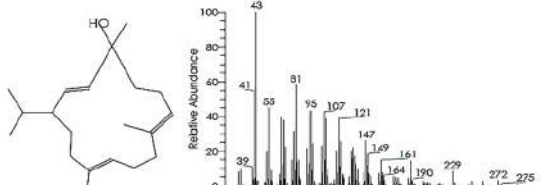
18.62	0.32	β -Chamigrene	1474	850	 
19.96	0.19	Nerolidol	1545	860	 
20.12	5.24	Spathulenol	1569	948	 
22.55	0.38	α -Bisabolol	1683	908	 
23.37	1.48	Farnesol	1658	930	 
27.59	0.44	Cupressene	1880	923	 
28.32	2.40	15-Kaurene	2018	935	 
28.45	0.35	Palmitic acid	1942	917	 

Table 2 (continued)

29.17	0.27	Dacrene	2160	928	
29.75	0.43	ar-Abietatriene	2039	905	
31.09	1.86	Sclareol	2209	803	
31.61	0.50	cis-Vaccenic acid	2116	918	
32.34	0.60	Kauran-16-ol	1934	822	
32.93	0.21	Thunbergol	2032	853	

*RT retention time (min), **RI retention index, ***MF match factor

(10.15%), and aqueous extract (10%) [10]. Flavonoid glycoside, citrioidside C, kaempferol-3-*O*- β -D-glucopyranosyl (12)- α -L-rhamnoside, kaempferol-3-*O*- α -L-rhamnoside, and quercetin-3-*O*- α -L-rhamnoside were isolated from leaves of *E. citriodora* [55]. 8-[1-(*p*-hydroxyphenyl)ethyl]rhamnocitrin, cinnamic acid, *p*-coumaric acid, caffeic acid (4), and gallic acid were isolated from the kino of *E. citriodora* [56]. The flavonoids sakuranetin, narigenin, aromadendrin 7-methyl ether, aromadendrin, kaempferol 7-methyl ether, gallic acid, the glycosides 1-*O*-cinnamoyl-6-*O*-(*p*-coumaroyl)- β -D-glucopyranoside, 7-methylaromadendrin-4'-*O*-(6''-trans-*p*-coumaroyl)- β -D-glucopyranoside, 6-*O*-(*p*-coumaroyl)-D-glucopyranoside,

and the tannin 1-*O*-2,6-digalloyl-6-*O*-(*p*-coumaroyl)- β -D-glucopyranoside were isolated from *E. citriodora* [11].

The main compounds found in *Cupressus macrocarpa* leaves' EO included sabinene (11.94%), 4-terpinenol (11.34%), citronellol (9.59%), citronellal (9.85%), *p*-cymene (7.67%), spathulenol (5.24%), γ -terpinene (5.05%), camphor (4.31%), and limonene (3.2%). Neral (31–35%), hydroxy citronellal (12–16%), geraniol (3–4%), trans-piperitol (7–8%), isobornyl isobutrate (0.7–6.61%), linalool (0.6–5.21%), terpinyl acetate (0.10–3.27%), and myrcene (0.22–2.60%) were the major components in *Cup. macrocarpa* EOs of fresh and dried leaves [57].

From the extract of *Cup. macrocarpa*, several bioactive substances were isolated, including gallic acid,

Table 3 Chemical constituents of the essential oil from *S. cumini* leaves

RT*	Area %	Compound	RI**	MF***	MS fragmentation
4.65	18.79	α -Pinene	937	941	
4.89	0.75	Camphene	935	943	
5.36	0.95	Sabinene	964	925	
5.44	4.01	β -Pinene	970	949	
5.77	2.26	Myrcene	979	932	
6.39	0.76	p-Cymene	1011	871	
6.61	6.00	Limonene	1018	921	

Table 3 (continued)

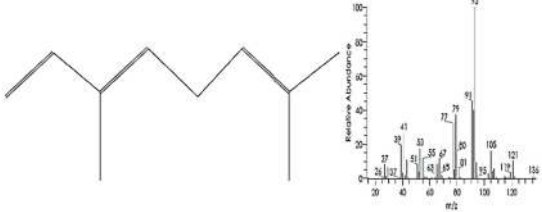
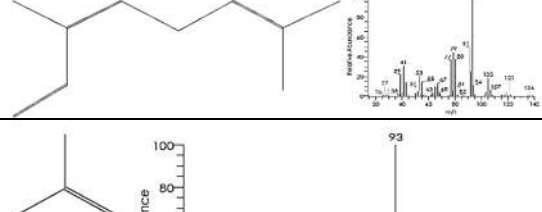
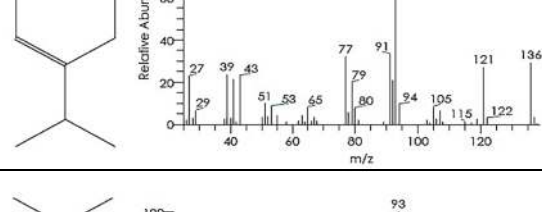
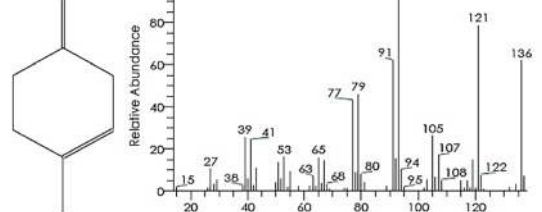
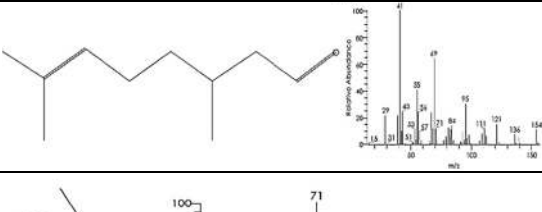
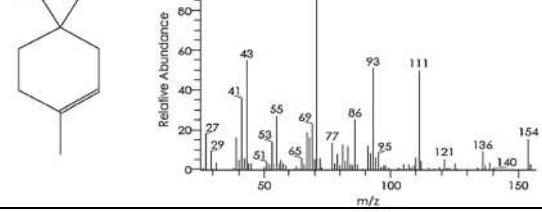
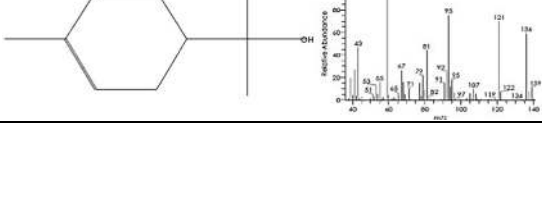
6.81	19.11	trans- β -Ocimene	1034	954	
7.07	8.16	cis- β -Ocimene	1024	935	
7.29	0.52	γ -Terpinene	1332	930	
8.02	1.12	Terpinolene	1078	946	
9.45	1.79	Citronellal	1132	924	
10.15	1.62	Terpinen-4-ol	1160	918	
10.47	3.79	α -Terpineol	1143	938	

Table 3 (continued)

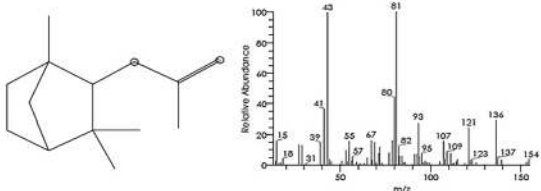
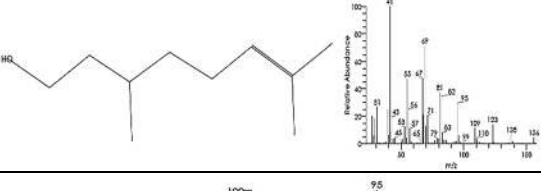
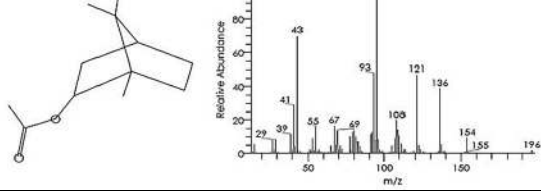
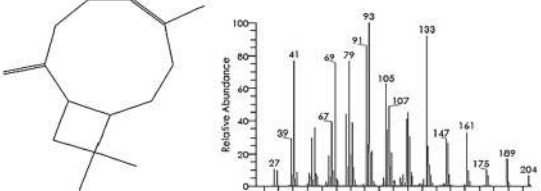
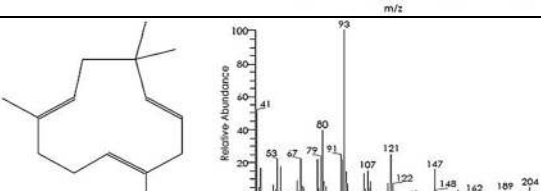
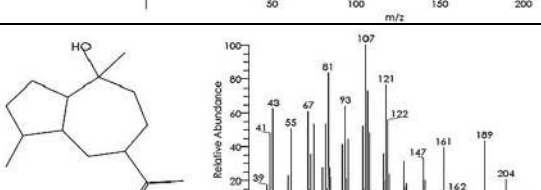
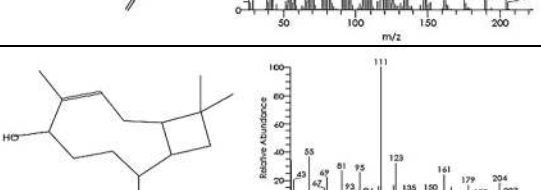
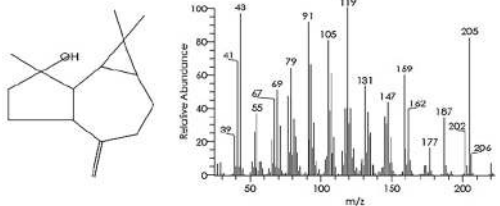
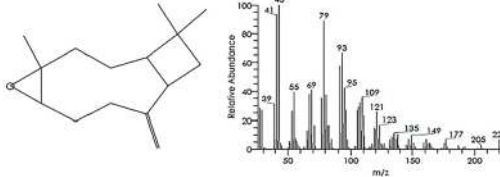
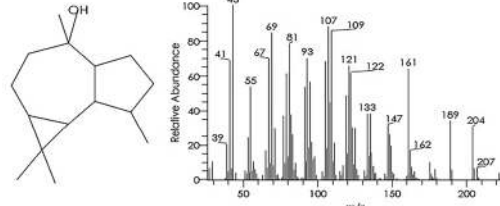
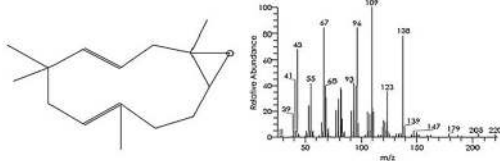
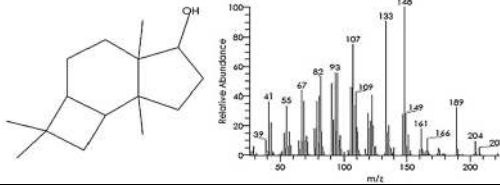
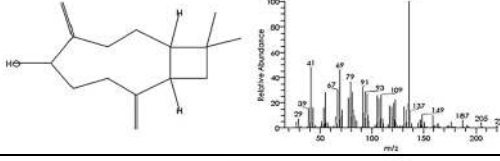
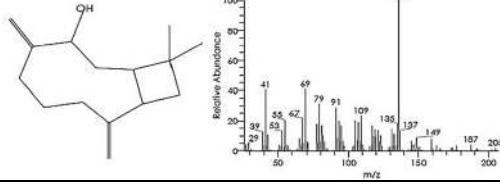
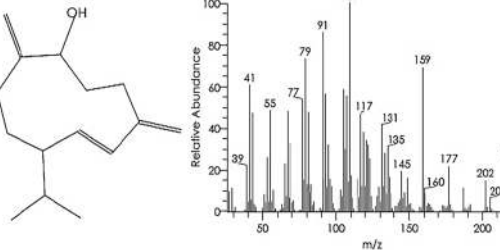
11.32	0.73	Fenchyl acetate	1207	952	
11.59	1.33	Citronellol	1208	878	
12.95	2.88	Bornyl acetate	1273	912	
16.58	9.30	Caryophyllene	1424	945	
17.37	4.69	Humulene	1456	945	
18.89	0.98	Pogostole	1655	802	
19.92	1.63	Caryophyllenyl alcohol	1677	906	

Table 3 (continued)

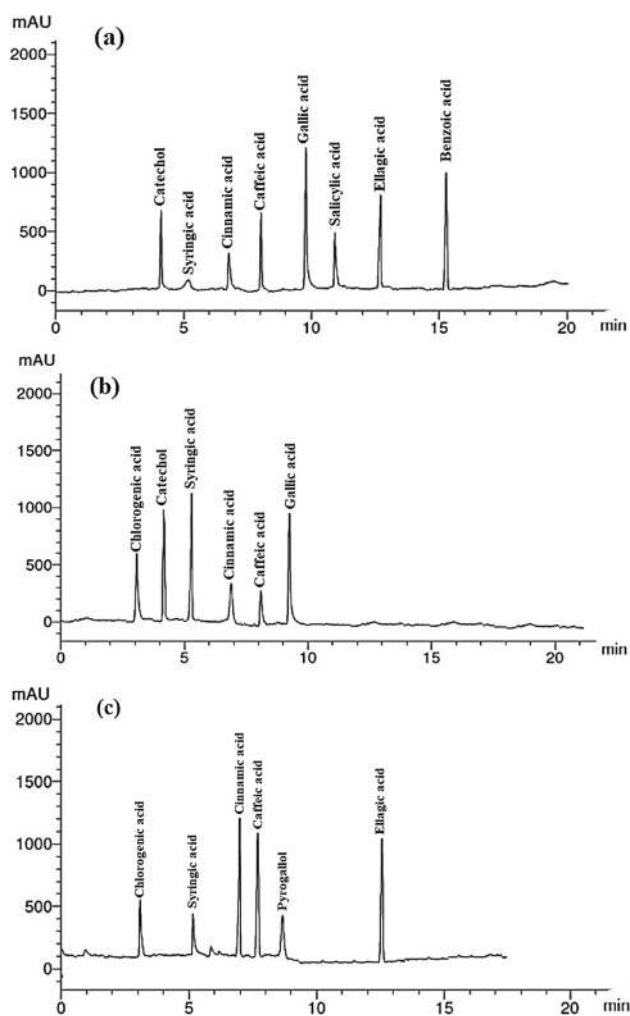
20.06	0.93	Spathulenol	1569	890	
20.14	1.54	Caryophyllene oxide	1576	926	
20.61	1.62	Globulol	1578	879	
20.71	0.85	humulene oxide II	1601	911	
20.95	1.76	2,2,4 α ,7 α -Tetramethyldecahydro-1H-cyclobuta[e]inden-5-ol	1593	815	
21.27	0.75	Caryophylla-4(12),8(13)-dien-5 α -ol	1624	807	
21.34	0.66	11,11-Dimethyl-4,8-dimethylenecyclo[7.2.0]undecan-3-ol	1645	929	
21.82	0.70	Germacre-4(15),5,10(14)-trien-1 α -ol	2228	849	

*RT retention time (min), **RI retention index, ***MF match factor

Table 4 Phenolic compounds ($\mu\text{g/g}$) by HPLC in the methanol leaf extracts from *C. citriodora*, *Cup. macrocarpa* and *S. cumini*

RT	Compound	<i>C. citriodora</i> ($\mu\text{g/g}$)	<i>Cup. macrocarpa</i> ($\mu\text{g/g}$)	<i>S. cumini</i> ($\mu\text{g/g}$)
3.0	Chlorogenic acid	ND	4.62	4.22
4.0	Catechol	3.75	6.85	ND
5.0	Syringic acid	0.56	7.59	3.15
7.0	Cinnamic acid	2.89	2.11	10.66
8.0	Caffeic acid	4.65	1.54	9.87
8.7	Pyrogallol	ND	ND	3.64
10.0	Gallic acid	7.96	6.78	ND
11.0	Salicylic acid	2.17	ND	ND
12.8	Ellagic acid	5.78	ND	8.76
15.3	Benzoic acid	8.11	ND	ND

RT retention time (min), ND not detected

**Fig. 3** HPLC chromatograms of the identified phenolic compounds in the methanol leaf extracts from *C. citriodora* (a), *Cup. macrocarpa* (b), and *S. cumini* (c)

cupressuflavone, sandaracopimaric acid, blumenol-C-glucoside, β -sitosterol, 3-methylbut-3-en-1-ol-O- β -D-glucopyranoside, 3'-demethoxy-dihydrodehydrodiconiferyl alcohol-9'-O- α -L-rhamnopyranoside, agathadiol, protocatechuic acid, and shikimic acid, which they observed antibacterial activity against *Staphylococcus aureus* [58]. A leaf extract from *Cup. macrocarpa* was analyzed using LC-ESI-MS/MS, which revealed 49 compounds, including (-)-shikimic acid, D-(-)-quinic acid, myricetin, p-coumaric acid, rhoifolin, quercetin, kaempferol derivatives, naringenin-7-O-glucoside, and luteolin-3,7-di-O-glucoside [59]. The extract from *Cup. macrocarpa* contained the polyphenolic substances gallic acid, catechol, p-hydroxy benzoic acid, caffeine, chlorogenic, vanillic acid, caffeic acid, syringic acid, vanillin, p-coumaric acid, ferulic acid, benzoic acid, hyperoside rose, ellagic acid, O-coumaric acid, salicylic acid, myricetin, and quercitin [60].

Trans- β -ocimene (19.11%), α -pinene (18.79%), caryophyllene (9.30%), (Z)- β -ocimene (8.16%), and limonene (6.00%) were the primary constituents of the EO from *Syzygium cumini* leaf EO. Pinocarveol, α -terpeneol, myrtenol, eucarvone, muurolol, myrtenal, geranylacetone, α -cadinol, and pinocarvone were previously revealed by *S. cumini* EO [61]. α -Pinene, β -pinene, *trans*-caryophyllene, 1,3,6-octatriene, Δ -3-carene, α -caryophyllene, and α -limonene were presented as the main compounds in EO from leaves of *S. cumini* [62]. While another study showed that α -pinene, (Z)- β -ocimene, and (E)- β -ocimene were the main compounds [26]. The majority of the sesquiterpene components, including terpeneol, α -caryophyllene, and β -caryophyllene, were identified in *S. cumini* EO [27].

It was demonstrated that the content of β -pinene and β -caryophyllene in the EO was associated with its anti-inflammatory properties [63]. It was shown that *S. cumini* EO has antihyperlipidemic, antiallergic, and good antibacterial activity against bacteria, fungi, and protozoa parasites of the species *Leishmania* and *Trypanosoma* [64]. In comparison to methylene chloride extract and EO, the methanol extract showed more action against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Bacillus subtilis*, and *Enterococcus faecalis* [62]. These might be connected to the methanolic extract's increased total phenolic and flavonoid concentration when compared to other extracts. The EO of leaves from *S. cumini* showed moderate activity against *E. coli* and the main component was α -pinene (53.21%) [65].

The primary ingredient concentrations in the EOs of *S. cumini*, *C. citriodora*, and *C. macrocarpa* were different from those described in the literature. These variations in EO compositions could result from a variety of environmental (climatic, seasonal, or geographical) and genetic variations [66–69]. Additionally, it should be taken into

Fig. 4 *Cup. macrocarpa* leaf EO (Ca) and leaf extract (Cb) treated Scots pine sapwood blocks against the growth of *F. solani*. Control: 10% DMSO; positive control: azoxystrobin + difenoconazole (1000 mg/L)

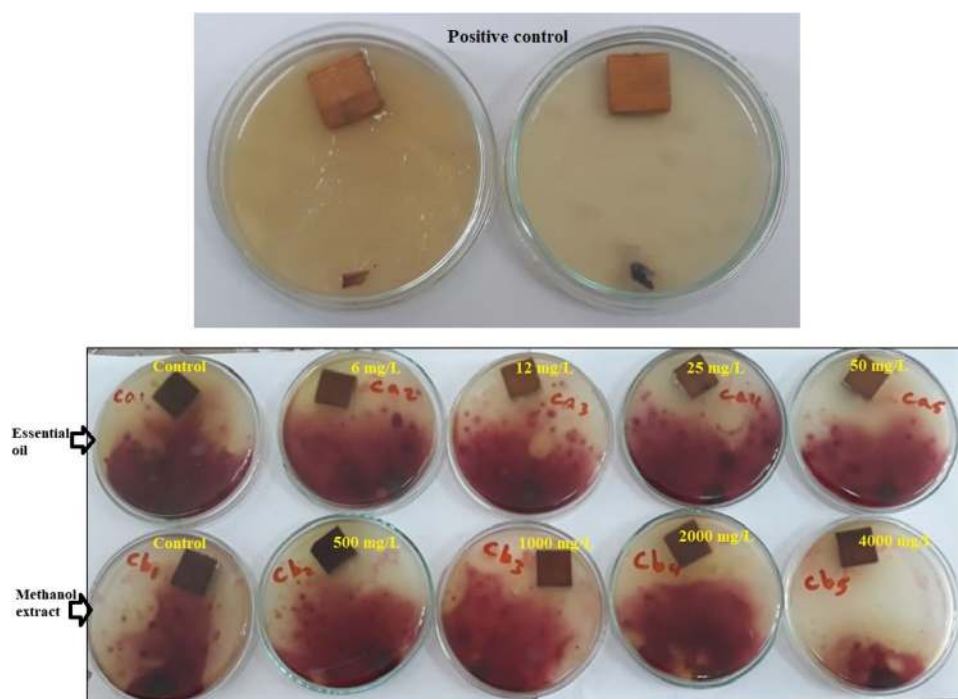


Fig. 5 *C. citriodora* leaf essential oil (Ea) and leaf extract (Eb) treated Scots pine sapwood blocks against the growth of *F. solani*

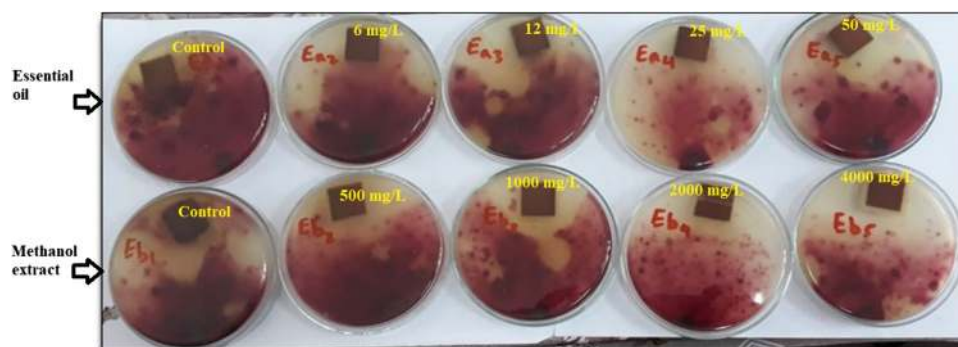
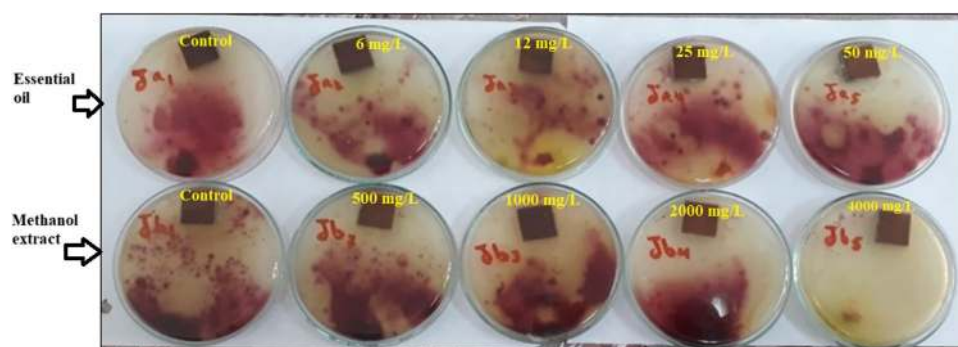


Fig. 6 *S. cumini* leaf essential oil (Ja) and leaf extract (Jb) treated Scots pine sapwood blocks against the growth of *F. solani*



account that the synergistic interactions between the various EO components may contribute to the activity of them. Previously, it was shown that the citronellal and linalool in *Cymbopogon nardus* EO synergized to provide a potent antifungal action [70].

The major chemical constituent citronellal may be responsible for the activity against fungal growth. Citronellal is reported to show activities against fungi from *Aspergillus* [70]. Citronellal was observed to have a strong effect on the growth of *A. niger* [71], while other

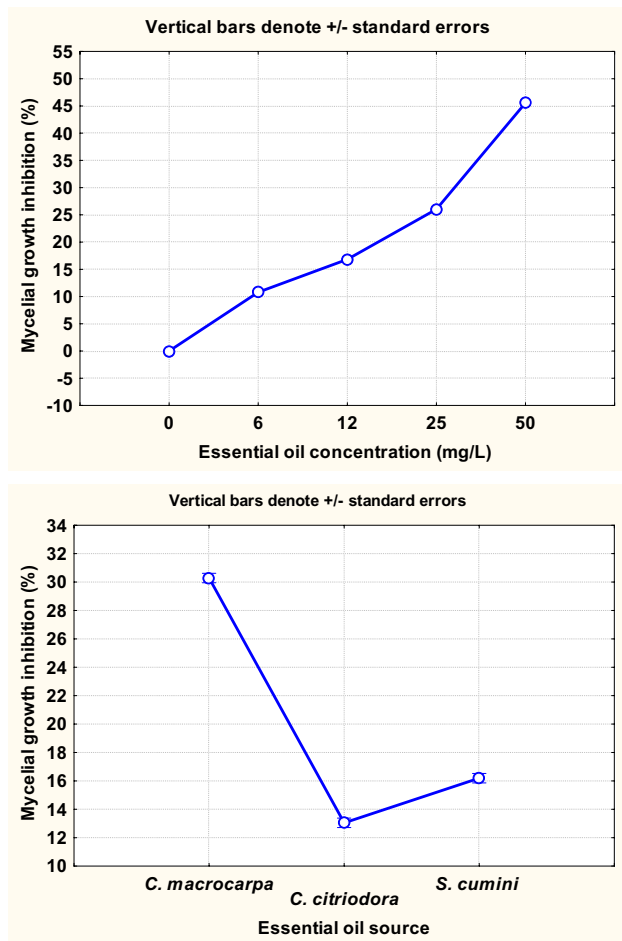


Fig. 7 The main effects of essential oil concentration and source on the growth of *F. solani*

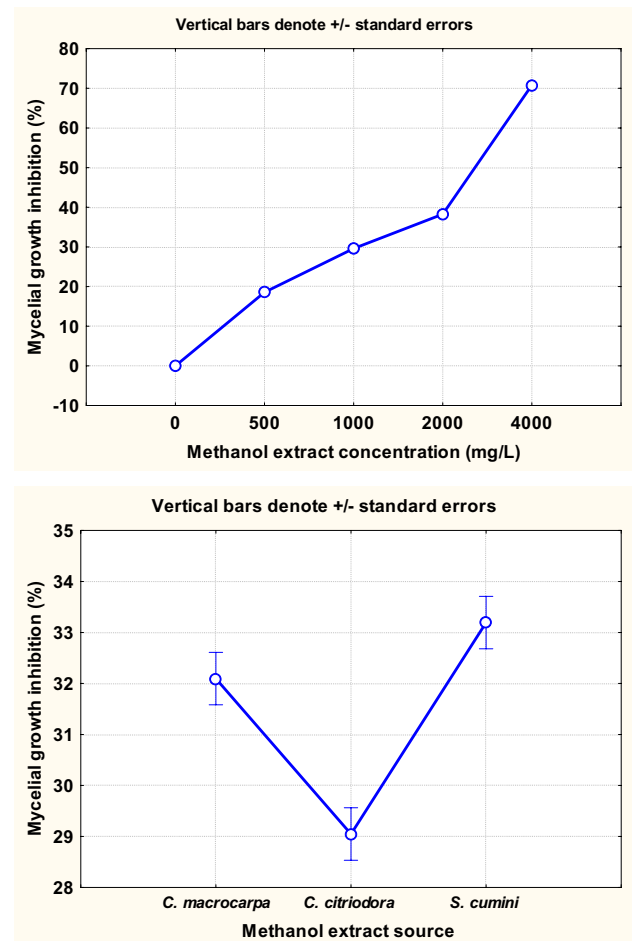


Fig. 8 The main effects of methanol extract concentration and source on the growth of *F. solani*

compounds like α -pinene, β -pinene, myrcene, geraniol, and linalool have antifungal activity [70].

Ferulic and p-coumaric acids showed significantly enhanced inhibitory potential in a synthetic media; ferulic acid remained active against *Monilinia fructicola* and *Alternaria alternata*, and it surpassed p-coumaric acid in suppressing *Botrytis cinerea* [72]. The most effective antifungal agents were caffeic, 2,3,4-trihydroxybenzoic, p-coumaric,

and protocatechuic acids, which virtually completely suppressed *A. alternata* on both early- and late-ripening sweet cherry [73]. Additionally, p-coumaric acid completely stopped *Fusarium* from growing. Conversely, at the same dose of 1000 μ g/g, ferulic acid inhibited 64% of *Fusarium* mycelial development and was especially effective against *B. cinerea* growth [74, 75].

Table 5 Fungal mycelia inhibition percentages of *Cup. macrocarpa*, *C. citriodora*, and *S. cumini* leaf essential oils

Treatment	Inhibition percentage of <i>F. solani</i> mycelial growth (%)				
	Control	6 mg/L	12 mg/L	25 mg/L	50 mg/L
<i>Cup. macrocarpa</i> EO	0.00 h*	21.42 $\pm$ 1.42d	28.57 $\pm$ 1.42c	35.71 $\pm$ 1.42b	65.71 $\pm$ 1.42a
<i>C. citriodora</i> EO	0.00 h	0.00 h	7.14 $\pm$ 1.42 g	22.85 $\pm$ 1.42d	35.23 $\pm$ 0.82b
<i>S. cumini</i> EO	0.00 h	10.95 $\pm$ 0.82f	14.76 $\pm$ 2.18e	19.52 $\pm$ 2.18e	35.71 $\pm$ 1.42b

*Values are mean $\pm$ SD. Means with the same letter are not significantly different according to LSD at 0.05 level of probability

Table 6 Fungal mycelia inhibition percentages of *Cup. macrocarpa*, *C. citriodora*, and *S. cumini* leaf extracts

Treatment	Inhibition percentage of <i>Fusarium solani</i> mycelial growth (%)				
MLE concentration	Control	500 mg/L	1000 mg/L	2000 mg/L	4000 mg/L
<i>Cup. macrocarpa</i> MLE	0.00i*	23.33 ± 2.97 g	30 ± 2.85f	37.14 ± 2.85e	70.00 ± 1.42b
<i>C. citriodora</i> MLE	0.00i	23.33 ± 0.82 g	30.47 ± 3.59f	41.90 ± 1.64d	49.52 ± 2.18c
<i>S. cumini</i> MLE	0.00i	9.04 ± 1.64 h	28.35 ± 2.53f	35.71 ± 1.42e	92.85 ± 0.00a

*Values are means ± SD. Means with the same letter are not significantly different according to LSD at 0.05 level of probability

5 Conclusion

This study looked into the chemical composition and antifungal properties of methanol extracts (MEs) and essential oils (EOs) derived from the leaves of three trees grown in Egypt: *Corymbia citriodora*, *Cupressus macrocarpa*, and *Syzygium cumini*. GC–MS was used to identify the chemical components of EOs, while HPLC was used to identify the chemical components of MEs. Trials evaluating the obtained EOs and MEs' antifungal efficacy against *Fusarium solani* were conducted when applied to wood samples. For the studied EOs and MEs, distinct and significant inhibitory effects were seen. However, additional research is needed to assess the toxicity and the efficacy of treatment for long-term as antifungal agents before the acquired EOs and MLEs can be used as substitutes for synthetic fungicides.

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Data availability Not applicable.

Declarations

Ethics approval Not applicable.

Conflict of interest The authors declare no competing interests.

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