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Mass Spectroscopic Analysis, MNDO Quantum Chemical Studies and Antifungal Activity of Essential and Recovered Oil Constituents of Lemon-Scented Gum against Three Common Molds

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Abstract: The present study described the possibility of using wood-treated oil-fungicide of lemon-scented gum (*Corymbia citriodora*) from newly emerged leaves and unripened fruits against the infestation of *Fusarium culmorum*, *Rhizoctonia solani* and *Penicillium chrysogenum*. Air-dried wood samples of *Melia azedarach* were treated with the extracted oils from leaves and unripened fruits from *C. citriodora*. The main chemical constituents identified in the essential oil (EO) from leaves were citronellal (55.31%), citronellol (21.03%) and isopulegol (10.79%), while in unripened fruits were α-pinene (17.86%), eudesmol (13.9%), limonene (9.19%), γ-terpinen (8.21%), and guaiol (7.88%). For recovered oils (ROs), the major components from leaves were *D*-limonene (70.23%), γ-terpinene (13.58%), β-pinene (2.40%) and isopregol (2.23%), while, 4-terpineol (21.35%), *cis*-β-terpineol, (19.33%), *D*-limonene (14.75%), and γ-terpinene (7.42%) represented the main components in fruits. EOs from leaves and fruits at the amounts of 100, 50 and 25 µL showed the highest inhibition percentage (IP) of 100% against *F. culmorum* and *P. chrysogenum* compared to control treatment, while at the amounts of 100, and 50 µL showed 100% IP of *R. solani*. Wood treated with ROs from leaves and fruits showed IPs of 96.66% and 93.33%, respectively, against the growth of *R. solani*. The mass spectra of the main components of *C. citriodora* leaves and fruits' EOs have been recorded in electron ionization mode at 70 eV and fragmentation has been reported and discussed. On the other hand, different quantum parameters such as the heat of formation, ionization energy total energy, binding energy, electronic energy and dipole moment using the modified neglect of diatomic overlap (MNDO) semi-empirical method have been calculated.

Keywords: mass spectrometry; fruit oils; leaf oils; *Corymbia citriodora*; lemon-Scented Gum; MNDO Quantum; wood bio-fungicide

1. Introduction

Essential, fixed and recovered oils as well as the other natural extracts (phenolic, flavonoids, saponins, alkaloids and tannins) from medicinal plants have great effects as antifungal agents against the growth of molds that grow on lignocellulosic materials such as wood, linen, ancient manuscripts, pulp and paper, archaeological artifacts and wood products [1–9].

A number of studies have demonstrated the antimicrobial properties of Eucalyptus essential oils (EOs) and their antimicrobial effects against a wide range of microorganisms have been studied. These species showed potential antifungal and antibacterial activities, especially *E. citriodora* (lemon-scented gum) EO, which has been shown to have a wide spectrum of antifungal activity [10–14].

Corymbia citriodora (*C. citriodora*) (Hook.) K.D. Hill and L.A.S. Johnson (*Eucalyptus citriodora* (Hook.) has been reported to be a good source oil (lemon-scented Eucalyptus), which is used for pharmaceutical, perfumery and other related industries and was reported as non-toxic under the GRAS (Generally Regarded as Safe) category by Food and Drug Administration of the USA [15]. *C. citriodora* leaf EOs have a broad spectrum of uses such as antibacterial [16,17], pesticide [18–20], nematicidal [21], antifungal [13,17], and herbicidal [22] uses, and moderate to strong antioxidant activities [23] being rich in monoterpenoids compounds [23–25].

There are several bioactive compounds such as α-citronellal, citronellol acetate, α-citronellol, isopulegol, eucalyptol that are the main compounds that have been identified in the EOs extracted from *C. citriodora*; from the tree grown in Egypt from air-dried leaves [17]; while *cis*-geraniol, 3-hexen-1-ol, citronellol acetate, 5-hepten-1-ol, 2,6-dimethyl, and citronellal were the major components in the green leaf EO of *C. citriodora* [26]. Citronellal, β-citronellol, and isopulegol were the major monoterpenoids in the leaf EO of the tree planted in India [23,27]. Volatile composition of the leaves of *E. citriodora* grown in the Delhi region showed the presence of reported α-pinene, β-pinene, sabinene and α-thujene as the major compounds [28].

Several studies have reported the antifungal and antibacterial activities of the EOs from *C. citriodora* [10,12,14,17,29,30]. For example, strong antifungal activity against *Macrophomina phaseolina*, *Colletotrichum lindemuthianum*, *Fusarium oxysporum* f. *sp. lycopersici*, *Helminthosporium oryzae*, *Alternaria triticina*, *Rhizoctonia solani,* and *Alternaria solani* was found with the application of *C. citriodora* oil compared with Mancozeb [13]. Filter paper disks impregnated with *C. citriodora* leaf EO at 10 µL showed good activity against *E. coli* and *S. aureus* Elaissi [30]. With the presence of sabinene and terpinen-4-ol as main compounds in the essential oil of *C. citriodora*, the EO displayed potent antifungal activity against *Trichophyton rubrum* [31].

Recovery oil (RO) using *n*-hexane solvent from the distillate of *Matricaria chamomilla* fresh flowers after obtaining the EOs were reported to have potential antifungal activity against *A. niger* and *A. terreus* [6]. Therefore, the present study firstly aimed to extract the essential and recovery oils from *C. citriodora* leaves and unripened fruits; and secondly to explore their bioactivity as wood-biofungicides; and, finally, modified neglect of diatomic overlap (MNDO) quantum chemical studies have been reported.

2. Materials and Methods

2.1. Oil Extraction

C. citriodora plant materials (leaves and unripened fruits) were cut into small pieces then 100 g from each plant were hydrodistilled using the Clevenger apparatus for 3 h [32]. After collecting the essential oils, the recovered oils dissolved in water from the hydrodistillation were isolated using *n*-hexane solvent. The *n*-hexane fraction or layer was separated using a funnel separator [6]. The essential and *n*-hexane oils were stored in glass tubes in the refrigerator at 4 °C until chemical and antifungal analyses.

2.2. Gas Chromatography–Mass Spectrometry (GC–MS) Analysis of the Oils

Essential oils and n-hexane recovered oils were analyzed for their chemical composition using Focus GC-DSQ (Gas Chromatography-Dual Stage Quadrupole) Mass Spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 μm film thickness, Agilent, Palo Alto, CA, USA) apparatus at Atomic and Molecular Physics Unit, Experimental Nuclear Physics Department, Nuclear Research Centre, Egyptian Atomic Energy Authority, Inshas, Cairo, Egypt. The column oven temperatures, injection properties, compound separation and identification can be found in previous works [3,6,33].

2.3. Antifungal Activity of Wood Treated with Oils

Three common molds namely *Fusarium culmorum*, *Rhizoctonia solani* and *Penicillium chrysogenum* with their accession numbers of MH352452, MH352450, and MH352451, respectively, were used for the bioassay [3,7,9]. Oils were applied at the amounts of 0, 25, 50, and 100 μL. Air-dried wood samples of *Melia azedarach* were prepared with the approximate dimension of 0.5 × 1 × 2 cm then autoclaved at 121 °C for 20 min and left to cool. Nine wood samples were treated with each concentration (three for each fungus) from each oil. Wood samples without oil treatments were used as a control. The antifungal effect of treated wood was measured following our previous works with minor modification [3,34–37]

2.4. Statistical Analysis

Data of the antifungal activity were statistically analyzed with three factors (plant part, type of oil and the concentration) using analysis of variance, Statistical Analysis Software (SAS) system [38]. The differences among the mean of treatments were recorded using Fisher's Least Significant Difference $LSD_{0.05}$.

3. Results

3.1. Chemical Composition and Mass Spectrometric Investigations

3.1.1. Chemical Composition of *Corymbia Citriodora* Leaves and Fruits' Essential Oils

The identified chemical composition of *C. citriodora* leaf EO is shown in Table 1 and represented 14 compounds. The main chemical components in *C. citriodora* leaf EO were citronellal (55.31%), citronellol (21.03%), isopulegol (10.79%), citronellol acetate (2.31%), citronellic acid (2.08%), and caryophyllene (1.32%). The identified chemical composition of unripe fruits' EO comprised 27 compounds (Table 2). The main chemical components in *C. citriodora* unripe fruits EO were α-pinene (17.86%), eudesmol (13.9%), limonene (9.19%), γ-terpinen (8.21%), and guaiol (7.88%).

Table 1. Phytochemicals screening of *Corymbia citriodora* leaf essential oil by gas chromatography–mass spectrometry (GC–MS).

No.	RT[1]	compound Name	Molecular Formula	MW[2]	Peak Area %	SI[3]	RSI[4]	Most Fragment ions with RI* (%)
1	4.24	5-Octen-2-one, 6-methyl-	$C_9H_{16}O$	140	0.58	702	705	140(10%),111(50%), 82(60%), 69(65%)and 67(100%)
2	4.65	β-Pinene	$C_{10}H_{16}$	136	0.49	845	920	136(10%),93(100%), 79(60%) and 69(100%)
3	6.49	D-Limonene	$C_{10}H_{16}$	136	0.63	843	963	136(10%), 93(60%), 79(40%) and 68(100%)
4	6.70	Eucalyptol	$C_{10}H_{18}O$	154	1.33	895	913	154(30%), 139(32%), 111(36%),

								108(56%),93(76%), 81(100%),71(80%) and 55(60%)
5	7.57	Sabinene	$C_{10}H_{16}$	136	0.31	796	800	136(25%), 121(22%), 93(100%) and 77(42%)
6	10.27	Melonal	$C_9H_{16}O$	140	0.47	797	879	140(5%),139(10%), 82(100%) and 67(85%)
7	13.63	Citronellal	$C_{10}H_{18}O$	154	55.31	951	951	154(5%),136(10%), 95(60%), 84(20%),69(100%) and 55(60%)
8	15.21	Linalool	$C_{10}H_{18}O$	154	0.71	879	920	154(3%),121(20%), 93(56%), 80(32%),71(100%) and 55(62%)
9	15.77	Isopulegol	$C_{10}H_{18}O$	154	10.79	920	949	154(5%),121(56%), 95(57%), 84(60%),67(100%) and 55(72%)
10	16.45	Caryophyllene	$C_{15}H_{24}$	204	1.32	879	940	204 (3%), 189(12%), 161(22%), 133(46%),105(59%) ,93 (86%) and 69(100%)
11	17.99	Citronellol acetate	$C_{12}H_{22}O_2$	198	2.31	885	926	138(20%),123(35%), 95(60%), 81(100%),67(80%) and 55(42%)
12	18.80	α-Terpineol	$C_{10}H_{18}O$	154	0.71	820	853	154(2%),136(22%), 121(25%), 93(45%),81(40%) and 59(100%)
13	20.39	Citronellol	$C_{10}H_{20}O$	156	21.03	913	922	156(3%),138(12%), 123(15%), 95(43%),81(65%), 69(100%), 67(95%) and 55(62%)
14	30.19	Citronellic acid	$C_{10}H_{18}O_2$	170	2.08	733	780	170(5%),152(12%), 110(36%), 95(50%), 69(100%) and 55(52%)

RI* relative intensities, 1. RT: Retention Time, 2. MW: Molecular Weight (g/mol), 3. SI: Standard Index, 4. RSI: Reverse Standard index.

Table 2. Phytochemicals screening *of Corymbia citriodora* fruit essential oil by GC–MS.

No.	RT[1]	compound Name	Molecular Formula	MW[2]	Peak Area %	SI[3]	RSI[4]	Most Fragment ions with RI* (%)
1	3.47	α-Pinene	$C_{10}H_{16}$	136	17.86	965	966	136 (10%), 93(100%), and 77(40%)
2	3.90	Camphene	$C_{10}H_{16}$	136	0.38	789	916	136 (9%), 93(100%), and 79(82%)

3	4.72	β-Pinene	$C_{10}H_{16}$	136	9.92	951	951	136 (8%), 93 (100%), 79 (25%), and 69 (40%)
4	5.79	β-Myrcene	$C_{10}H_{16}$	136	0.55	892	923	136 (8%), 93(100%), and 69(80%)
5	6.59	Limonene	$C_{10}H_{16}$	136	9.19	937	938	136 (15%), 121(20%), 93(70%), and 68(100%)
6	7.67	γ-Terpinene	$C_{10}H_{16}$	136	8.21	939	948	136 (25%), 121(21%), 93 (100%) and 77 (40%)
7	8.22	β-Cymene	$C_{10}H_{14}$	134	5.35	935	948	134 (23%), 119 (100%) and 91 (40%)
8	8.52	α-Terpinene	$C_{10}H_{16}$	136	1.74	912	918	136 (35%), 121(70%), 93(100%), and 79(32%)
9	13.52	β-Citronellal	$C_{10}H_{18}O$	154	0.84	896	923	154 (2%), 121 (20%), 95 (50%), 69 (100%) and 55 (50%)
10	14.84	α-Gurjunene	$C_{15}H_{24}$	204	0.47	885	900	204 (33%), 189 (30%), 161 (50%), 133(40%), 119(52%), 105(100%), 91(70%) and 81 (40%)
11	15.21	Linalool	$C_{10}H_{18}O$	154	0.18	880	930	154 (2%), 121 (20%), 93 (60%), 71 (100%) and 55 (62%)
12	15.55	Isopulegol	$C_{10}H_{18}O$	154	0.99	891	944	154 (10%), 121 (52%), 93 (60%), 71 (90%) and 67 (100%)
13	16.03	Fenchol	$C_{10}H_{18}O$	154	0.35	898	923	154 (2%), 121 (10%), 93 (20%), 81(100%) and 69 (35%)
14	16.47	Caryophyllene	$C_{15}H_{24}$	204	3.72	934	940	204 (2%), 189(10%), 161(20%), 133(45%),105(60%) ,93 (90%) and 69(100%)
15	16.54	Eucalyptol	$C_{10}H_{18}O$	154	2.06	861	863	154 (12%), 139 (11%), 93 (100%), 81 (52%) and 71 (42%)
16	17.99	(+)-Menthol	$C_{10}H_{20}O$	156	1.08	867	928	156 (2%), 123 (40%), 95(62%), 81 (100%) and 67 (82%)
17	18.82	(-)-β-Fenchol	$C_{10}H_{18}O$	154	4.29	919	929	154 (1%), 136 (20%), 121(42%), 93 (60%) and 59(100%)
18	19.65	Lepidozene	$C_{15}H_{24}$	204	0.57	895	910	204 (5%), 121(85%), 107(60%), 93(100%), 79(60%) and 67(40%)
19	20.37	Citronellol	$C_{10}H_{20}O$	156	1.49	930	936	156(5%), 138(12%), 81(60%), 69(100%), 67(82%) and 55(65%)
20	21.85	Calamenen	$C_{15}H_{22}$	202	0.74	914	941	202(5%), 159(100%), 131(21%), and 105(15%)

21	23.10	Benzyl isovalerate	$C_{12}H_{16}O_2$	192	0.61	885	938	192(15%), 108(80%), 91(100%), and 57(42%)
22	24.22	Caryophyllene oxide	$C_{15}H_{24}O$	220	1.00	899	923	220(5%), 164(34%), 149(40%),122(60%),110(45%),93(60%),79(100%) and 55(60%)
23	26.89	Globulol	$C_{15}H_{26}O$	222	1.11	871	887	222(5%), 161(40%), 121(42%),109(80%),93(62%),81(100%),69(95%) and 55(63%)
24	27.01	Hedycaryol	$C_{15}H_{26}O$	222	3.23	940	951	222(3%), 161(40%), 121(32%),107(43%),93(66%)and 59(100%)
25	27.19	Guaiol	$C_{15}H_{26}O$	222	7.88	908	910	222(3%), 189(20%), 161(100%),119(40%),105(82%), 81(60%)and 59(85%)
26	28.71	Eudesmol	$C_{15}H_{26}O$	222	13.89	915	930	222(3%), 189(65%), 161(100%),133(68%), 107(62%), 91(65%)and 59(83%)
27	32.16	Farnesol	$C_{15}H_{26}O$	222	0.68	830	863	222(3%),161(5%), 93(20%), 81(30%)and 69(100%)

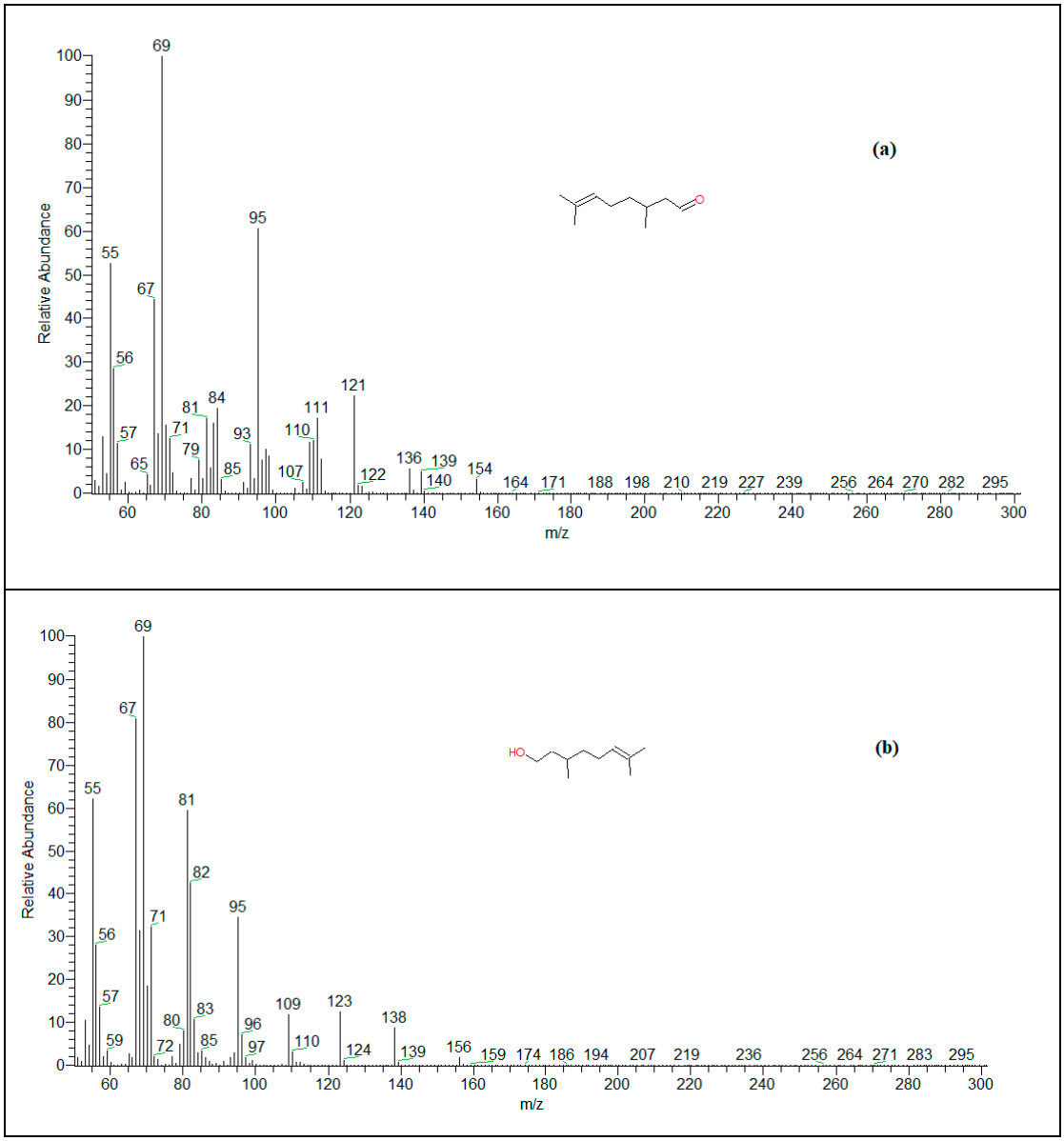
RI* relative intensities, 1. RT: Retention Time, 2. MW: Molecular Weight (g/mol), 3. SI: Standard Index, 4. RSI: Reverse Standard index.

3.1.2. Mass Spectrometric Investigations of the Main Components of *Corymbia Citriodora* Leaves and Fruits' Essential Iils

The 70 eV mass spectra of the major constituents of *C. citriodora* leaf EO are recorded and discussed as shown in Figure 1. The mass spectrum (MS) of the peak at retention time (RT) 13.63 min (Figure 1a) represent the citronellal component suggesting its molecular formula $C_{10}H_{18}O$ (Table 1). The molecular ion peak (MIP) was observed at *m*/*z* 154 with relative intensity (RI) = 5% and the peak at 69 (RI = 100%) representing the base peak (BP). Fragment ion (FI) of *m*/*z* 136 (10%), 95 (60%), 84 (20%) and 55 (60%) were also reported.

The MS of the peak at RT 20.39 min (Figure 1b) represent citronellol component suggesting its molecular formula $C_{10}H_{20}O$ (Table 1). The MIP was observed at *m*/*z* 156 with RI = 3% and the peak at 69 (RI = 100%) representing the BP. Other significant FI observed were *m*/*z* 138 (12%), 123 (15%), 95 (43%), 81 (65%), 67 (95%) and 55 (62%).

The MS of the peak at RT 15.77 min (Figure 1c) represent isopulegol component suggesting its molecular formula $C_{10}H_{18}O$ (Table 1). The MIP was observed at *m*/*z* 154 with RI = 5% and the peak at 67 (RI = 100%) representing the BP. Significant FI with *m*/*z* 121 (56%), 95 (57%), 84 (60%) and 55 (72%) were also observed.


(a)
Relative Abundance
m/z
69
95
55
67
56
81
84
121
111
57
71
93
110
79
65
85
107
122
136
139
140
154
164
171
188
198
210
219
227
239
256
264
270
282
295
(b)
Relative Abundance
m/z
HO
69
67
55
81
82
95
71
56
57
109
123
80
83
138
59
72
85
96
97
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186
194
207
219
236
256
264
271
283
295

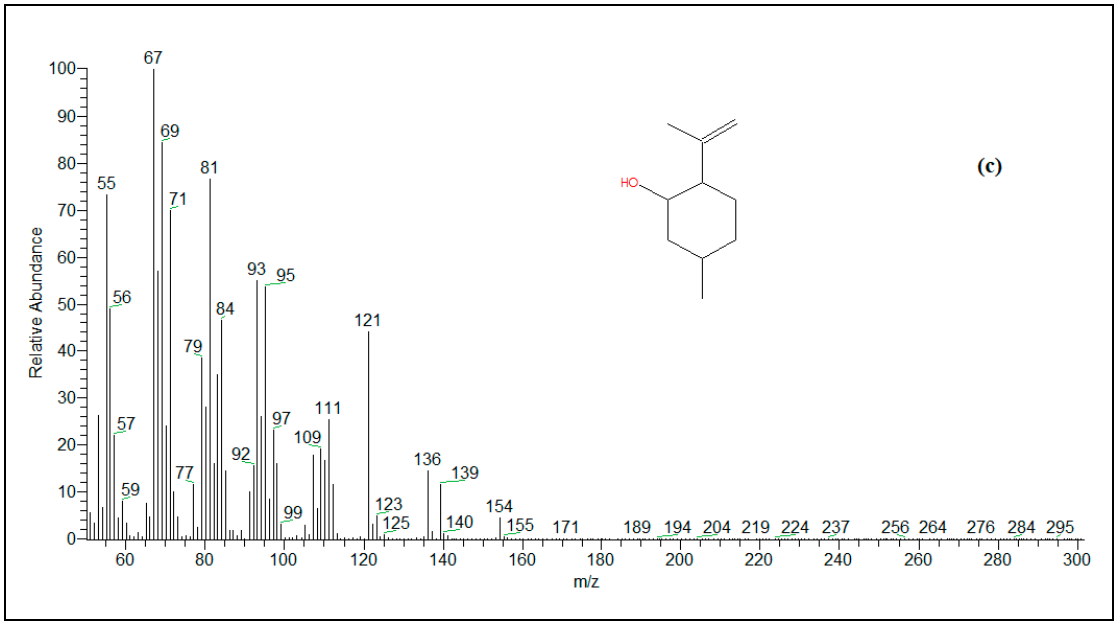


Figure 1. The 70 eV mass spectrum of (a) citronellal, (b) citronellol, and (c) isopulegol in *C. citriodora* leaves' essential oil. M stands for mass and Z stands for charge number of ions.

From the fragmentation pattern of citronellol (citronellal isomer) compound, it is shown that the first fragmentation pathway of the molecular ion (MI) of citronellol is the formation of the FI at *m/z* 138 (Figure 2). This could be could be explained by the formation of the $[M\text{-}H_2O]^{+\bullet}$ ion, which loses the H_2O from the MI. In addition, the ion $[M\text{-}H_2O]^{+\bullet}$ can be fragmented in three ways: first by loss of a $CH_3^{\bullet}$ radical to produce the fragment ion $[M\text{-}H_2O\text{-}CH_3]^{+}$ at *m/z* 123. The second way is by the loss of a $C_3H_7^{\bullet}$ radical to produce $[M\text{-}H_2O\text{-}C_3H_7]^{+}$ at *m/z* 95. The third way is by the loss of $C_4H_9^{\bullet}$ to produce $[M\text{-}H_2O\text{-}C_4H_9]^{+}$ at *m/z* 81 [39]. The second fragmentation pathway of the MI of citronellol is by the simple cleavage to produce directly the FI $C_5H_9^{+}$ with *m/z* 69, which represents the BP in the MS as shown in Figure 1a.

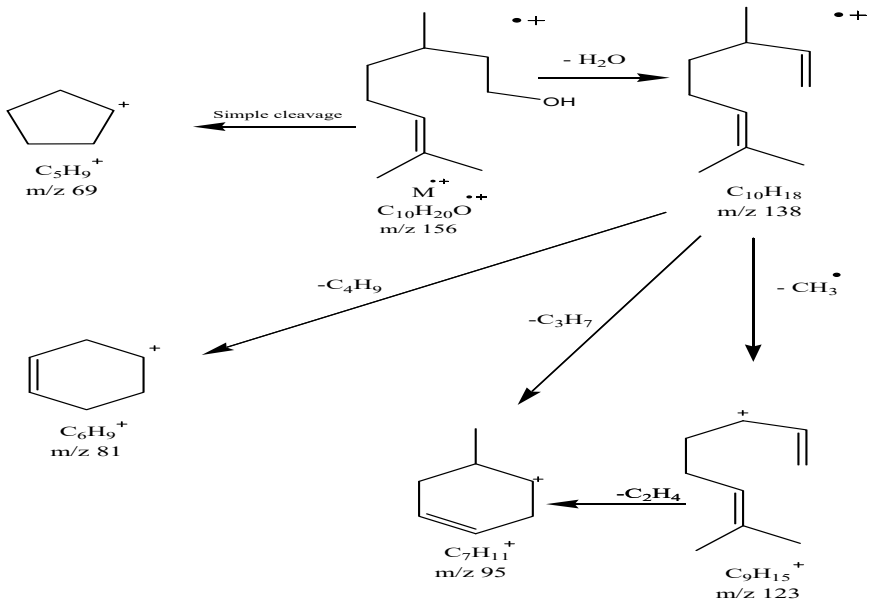
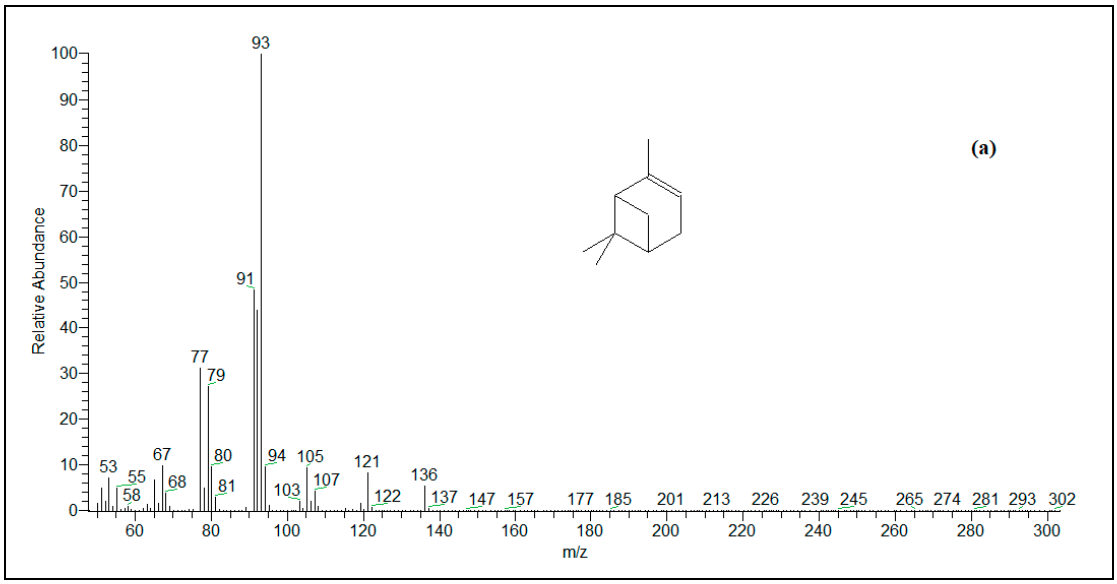


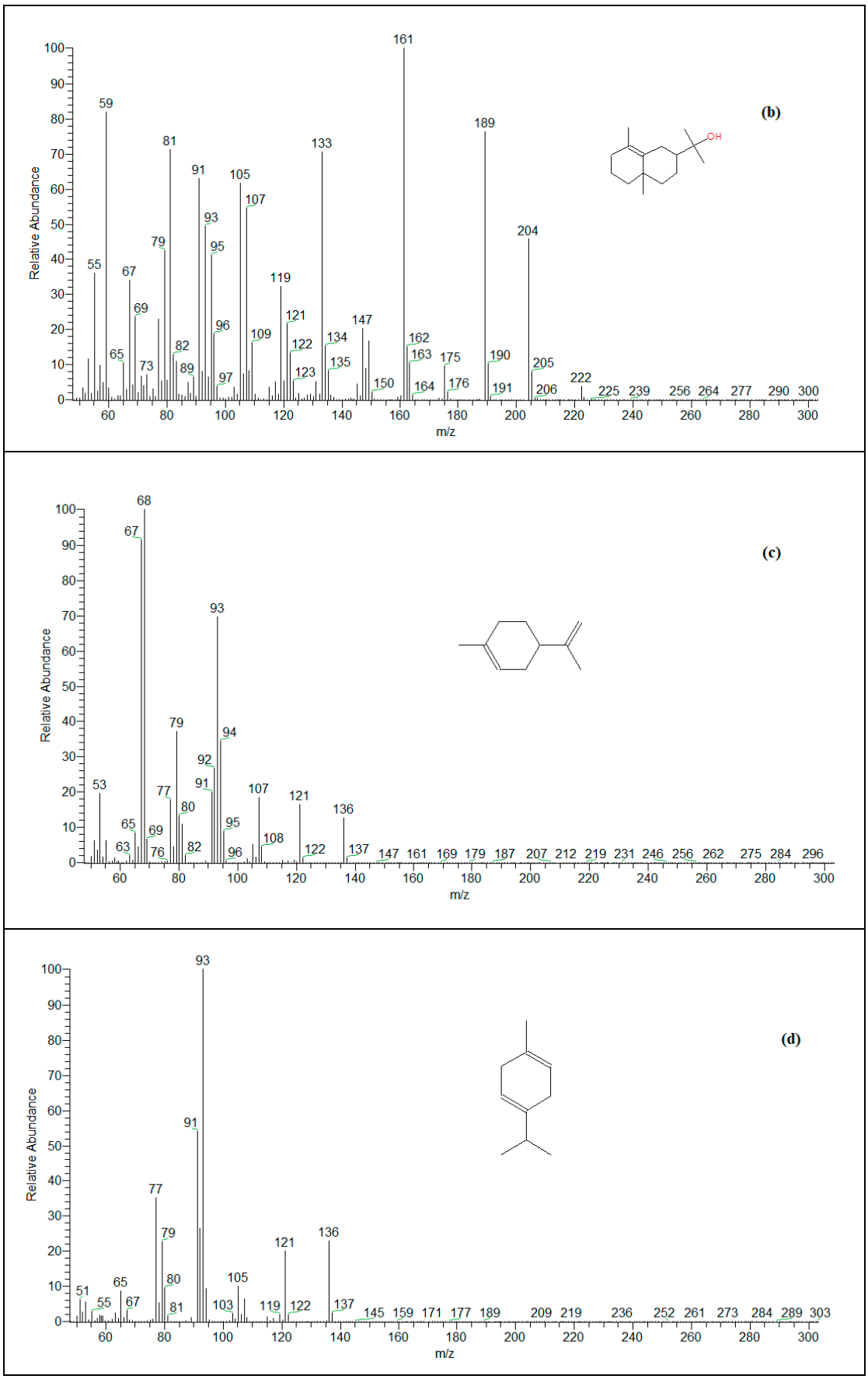
Figure 2. Fragmentation pattern of citronellol under electron ionization 70 eV.

The 70 eV mass spectra of the major constituents of *C. citriodora* fruits' EO are recorded and discussed as shown in Figure 3 and the chemical composition are shown in Table 2.

The MS of the peak at RT 3.47 min (Figure 3a) represent α-pinene component suggesting its molecular formula is $C_{10}H_{16}$ (Table 2). The MIP was observed at *m*/*z* 136 with RI = 10% and the peak at 93 (RI = 100%) represent the BP. Fragment with *m*/*z* 77 (40%) was observed as other significant on.

The MS of eudesmol has been recorded and investigated as shown as in Figure 3b suggesting its molecular formula is $C_{15}H_{26}O$. The MIP was observed at *m*/*z* 222 with RI = 3% and the peak at 161 (RI = 100%) representing the BP. Other significant FIs observed were *m*/*z* 189 (65%), 133 (68%), 107 (62%), 91 (65%) and 59 (83%). Limonene MS has been recorded as shown in Figure 3c suggesting its molecular formula $C_{10}H_{16}$. The MIP was observed at *m*/*z* 136 with RI = 15% and the peak at *m*/*z* 68 (RI = 100%) represent the BP. Other significant FIs observed were *m*/*z* 121 (20%) and 93 (70%). γ-Terpinene MS was as shown in Figure 3d suggesting its molecular formula is $C_{10}H_{16}$. The MIP was observed at *m*/*z* 136 with RI = 25% and the peak at *m*/*z* 93 (RI = 100%) representing the BP. Other significant FIs observed were *m*/*z* 121(21%) and 77(40%). Guaiol MS presented in Figure 3c suggesting its molecular formula is $C_{15}H_{26}O$. The MIP was observed at *m*/*z* 222 with RI = 3% and the peak at *m*/*z* 161 (RI = 100%) representing the BP. Other significant FIs observed were *m*/*z* 189(20%), 119 (40%), 105 (82%), 81 (60%) and 59 (85%).




(b)
Relative Abundance
m/z
OH
(c)
Relative Abundance
m/z
(d)
Relative Abundance
m/z

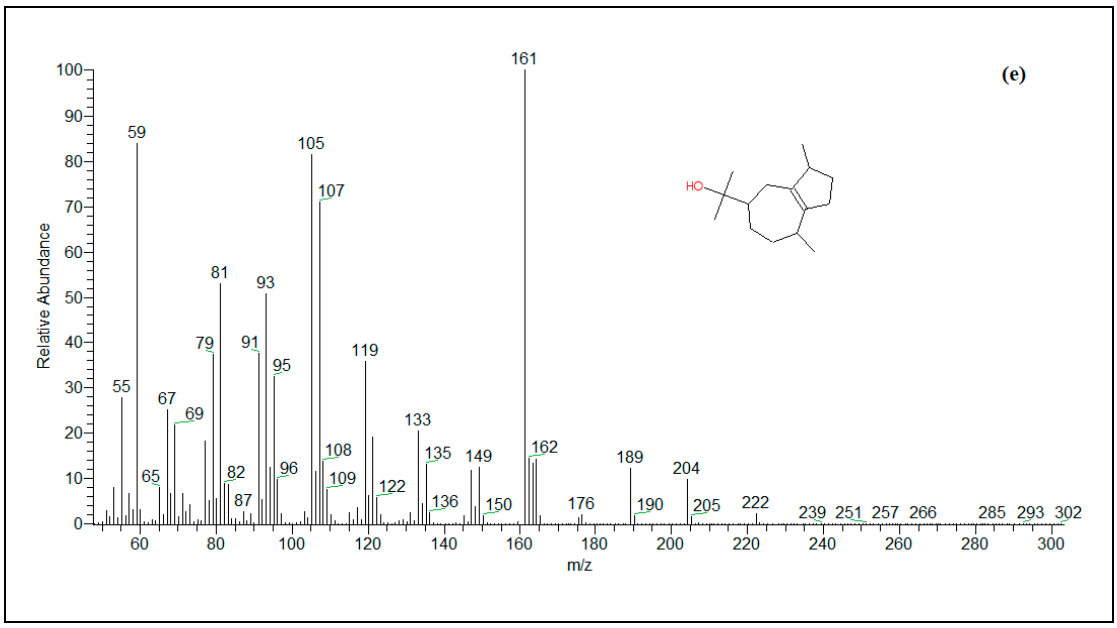


Figure 3. The 70 eV mass spectrum of (a) α-pinene (b) eudesmol (c) limonene, (d) γ-terpinene and (e) guaiol in *C. citriodora* unripe fruits' essential oil. *M* stands for mass and *Z* stands for charge number of ions.

3.1.3. Chemical Composition of *Corymbia Citriodora* Leaves and Fruits' Recovery Oils

The major components of the *C. citriodora* leaf recovery oils (RO, Table 3) were D-limonene (70.23%), γ-terpinene (13.58%), β-pinene (2.40%) and isopregol (2.23%), while, α-terpineol (21.35%), *cis*-β-terpineol (19.33%), D-limonene (14.75%), γ-terpinene (7.42%) and (1α,2β,5α)-bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl) (6.30%) represented the main components of *C. citriodora* fruit RO (Table 4).

The MS of D-limonene component of *C. citriodora* leaf RO at 70 eV (Figure 4a) shows that the MIP at *m*/*z* 136 have RI = 40% (stable molecular ion) and the other FIs at *m*/*z* 121(30%), 93(90%) and 68(100%) represent the BP in D-limonene MS.

The MS of γ-terpinene component of *C. citriodora* leaf RO at 70 eV are recoded as shown in (Figure 4b). Using MS one can note that the MIP at *m*/*z* 136 have RI = 40% and the other FIs are 121 (32%) and 77 (30%). The fragmentation processes of γ-terpinene achieved by mean of their unimolecular dissociation of the parent ion by loss of C_3H_7 radical to produce the main fragment ion (BP) $[M\text{-}C_3H_7]+$ at *m*/*z* = 93

The MS of β-pinene component of *C. citriodora* leaf RO at 70 eV (Figure 4c) shows that the MIP at *m*/*z* 136 have RI = 18% the most intense peak (InP) at *m*/*z* 93 with RI = 100%, which represent the FI $[M\text{-}C_3H_7]+$ due to the loss of C_3H_7 radical from the parent ion, and the other FIs are 79 (25%) and 69 (30%).

The MS of isopregol component of *C. citriodora* leaf RO 70 eV (Figure 4d) shows that the MIP at *m*/*z* 154 have RI = 20% and the other FIs 121 (60%), 79 (45%) and 68 (85%), while the FI at *m*/*z* 93 represent the BP with RI = 100%.

The MS of α-terpineol component of *C. citriodora* fruits RO at 70 eV (Figure 5a) show that the MIP at *m*/*z* 154 have RI = 15%, the most InP at *m*/*z* 71 with RI = 100%, and the other FIs are 111 (50%) representing the FI $[M\text{-}C_3H_7]+$, 93 (58%), 71 (100%) and 55 (25%).

The MS of *cis*-β-terpineol component of *C. citriodora* fruits RO at 70 eV (Figure 5b) shows that the MIP at *m*/*z* 154 have RI = 3%, the most InP at *m*/*z* 71 with RI = 100%, and the other FIs are 121 (35%), 93 (55%) and 81 (65%).

The MS of γ-terpinene component of *C. citriodora* fruits RO at 70 eV (Figure 5c) show that the MIP at *m*/*z* 136 have RI = 30% (stable molecular ion peak), the most InP at *m*/*z* 93 with RI = 100% representing $[M\text{-}C_3H_7]+$ ion, and the other FIs are 121 (25%) and 77(45%).

The MS of 2-methyl-5-(1-methylethyl)-(1α,2β,5α)-bicyclo[3.1.0]hexan-2-ol component of *C. citriodora* fruits RO at 70 eV (Figure 5d) show that the MIP at *m*/*z* 154 have RI = 10%, the most InP at *m*/*z* 71 with RI = 100%, and the other FIs are 121 (65%), 93 (92%) and 55(45%).

Table 3. Phytochemicals screening of *Corymbia citriodora* leaf recovered oil by GC–MS.

No.	RT[1]	compound Name	Molecular Formula	MW[2]	Peak Area %	SI[3]	RSI[4]	Most Fragment ions with RI* (%)
1	5.30	Ethriol	$C_6H_{14}O_3$	134	0.78	661	916	134 (5%), 86 (40%) and 57 (100%)
2	5.58	α-Phellandrene	$C_{10}H_{16}$	136	0.19	707	750	136 (20%), 93 (100%), 77 (38%) and 57 (85%)
3	5.78	α-Pinene	$C_{10}H_{16}$	136	2.76	904	918	136 (15%), 93 (100%), 77 (25%) and 57 (10%)
4	6.83	Ocimene	$C_{10}H_{16}$	136	0.12	692	767	136 (20%), 93 (92%), 77 (40%) and 57 (100%)
5	6.98	β-Pinene	$C_{10}H_{16}$	136	2.40	896	951	136 (18%), 93 (100%) 121(20%), 69(35%) and 57 (15%)
6	7.78	3-Carene	$C_{10}H_{16}$	136	1.33	740	862	136 (22%), 93 (100%), 77 (25%) and 57 (60%)
7	8.40	D-Limonene	$C_{10}H_{16}$	136	70.23	922	923	136 (40%), 121 (35%), 93 (85%), 79 (42%) and 68 (100%)
8	9.25	γ-Terpinen	$C_{10}H_{16}$	136	13.58	934	938	136 (45%), 121 (40%), 93 (100%), and 77(25%)
9	12.43	Isopregol	$C_{10}H_{18}O$	154	2.23	705	755	154 (20%), 121 (60%), 93 (100%), 79 (45%) and 68 (85%)
10	13.65	Grandlure II	$C_{10}H_{18}O$	154	0.50	679	838	154 (5%), 121 (62%), 93 (100%), 78 (40%) and 59 (42%)
11	15.07	2,6,10-Trimethyl-dodecane	$C_{15}H_{32}$	212	1.80	725	786	212 (2%), 133 (35%), 85 (65%), 71 (90%) and 57 (100%)
12	19.24	8,8-dimethyl-2,4-di(propan-2-ylidene)bicyclo[5.1.0]octan-6-one	$C_{16}H_{24}O$	232	0.12	708	775	232 (5%), 189 (40%), 150 (45%), 133 (100%) and 105 (70%)
13	20.34	3-[2-(1,2,3,4-tetrahydronaphthalen-2-ylmethyl)phenyl]propanoic acid	$C_{20}H_{22}O_2$	294	2.12	643	746	294 (6%), 165 (100%), 129 (97%), 105 (90%) and 75 (80%)

14	24.25	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	324	0.25	733	767	324(2%), 150 (55%), 126 (65%), 97 (100%) and 69 (50%)
15	29.33	1-Hexacosene	$C_{26}H_{52}$	364	1.32	770	793	364 (3%), 125 (40%), 111 (45%), 97 (80%), 85 (80%) and 71 (100%)

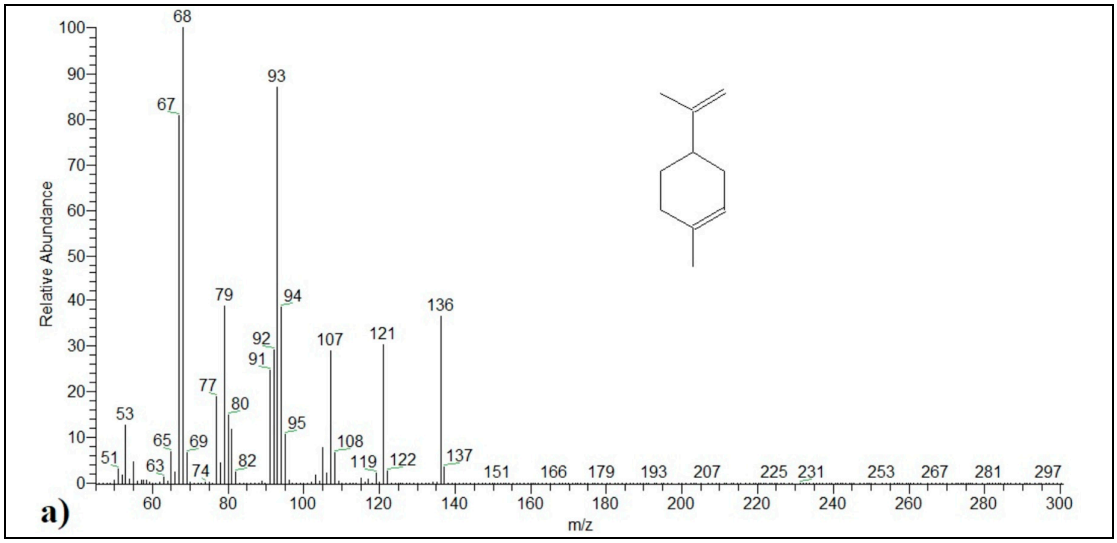
RI* relative intensities, 1. RT: Retention Time 2. MW: Molecular Weight (g/mol), 3. SI: Standard Index, 4. RSI: Reverse Standard index.

Table 4. Phytochemicals screening of *Corymbia citriodora* fruits' recovered oil by GC–MS.

No.	RT[1]	compound Name	Molecular Formula	MW[2]	Peak Area %	SI[3]	RSI[4]	Most Fragment ions with RI* (%)
1	7.26	α-Phellandrene	$C_{10}H_{16}$	136	0.84			136 (13%), 93 (100%), 77 (50%), and 65 (10%)
2	7.57	α-Pinene	$C_{10}H_{16}$	136	1.00			136 (10%), 93(100%), and 77(40%)
3	9.03	Sabinene	$C_{10}H_{16}$	136	4.74	865	896	136 (20%), 93(100%), and 77(45%)
4	10.43	Terpinolene	$C_{10}H_{16}$	136	3.77	813	866	136 (42%), 121(85%), 93(100%), and 77(45%)
5	10.82	D-Limonene	$C_{10}H_{16}$	136	14.75	896	914	136 (40%), 121 (30%), 93(90%), and 68(100%)
6	11.09	Ocimene	$C_{10}H_{16}$	136	0.90	763	870	136 (25%), 121 (15%), 93(100%), and 77(40%)
7	11.22	m-Cymene	$C_{10}H_{14}$	134	3.51	823	937	136 (22%), 119(100%), and 91(40%)
8	11.90	γ-Terpinene	$C_{10}H_{16}$	136	7.42	879	950	136 (30%), 121 (25%), 93(100%), and 77(45%)
9	12.80	*trans*-4-Thujanol	$C_{10}H_{18}O$	154	6.30	827	920	154 (10%), 121 (65%), 93 (92%), 71 (100%) and 55(45%)
10	13.61	Linalool	$C_{10}H_{18}O$	154	1.64	680	789	154 (2%), 121 (25%), 93 (78%), 71 (100%) and 55 (75%)
11	14.01	cis-β-Terpineol	$C_{10}H_{18}O$	154	19.33	913	951	154 (3%), 121 (35%), 93 (55%), 81 (65%) and 71 (100%)

12	16.61	4-Terpineol	$C_{10}H_{18}O$	154	21.35	885	927	154 (15%), 111 (50%), 93 (58%), 71 (100%) and 55 (25%)
13	17.33	α-Terpineol (P-menth-1-en-8-ol)	$C_{10}H_{18}O$	154	4.70	693	834	154 (2%), 121 (55%), 93 (65%), 81 (45%) and 59 (100%)
14	17.74	Linalyl acetate	$C_{12}H_{20}O_2$	196	5.10	716	857	196 (10%), 121 (22%), 93 (100%), 80 (50%) and 69 (25%)
15	18.05	α-Terpinyl propionate	$C_{13}H_{22}O_2$	210	3.09	664	751	210 (2%), 121 (80%), 93 (100%), 81 (35%) and 55 (15%)
16	21.07	2-[4-Methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	324	1.33	604	627	324 (5%), 315 (30%), 273 (25%), 195(100%) and 91 (25%)

RI* relative intensities, 1. RT: Retention Time 2. MW: Molecular Weight (g/mol), 3. SI: Standard Index, 4. RSI: Reverse Standard index.



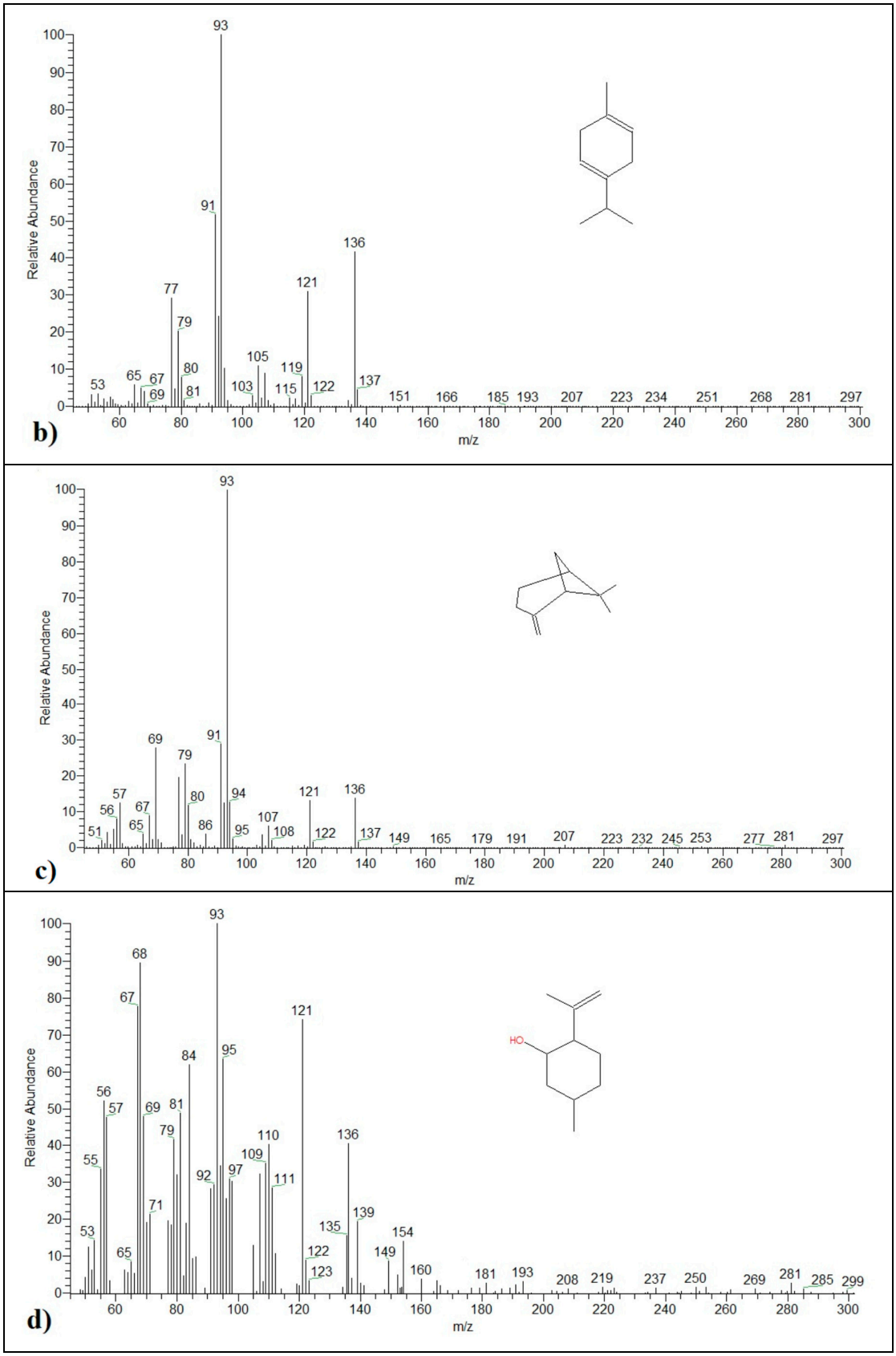
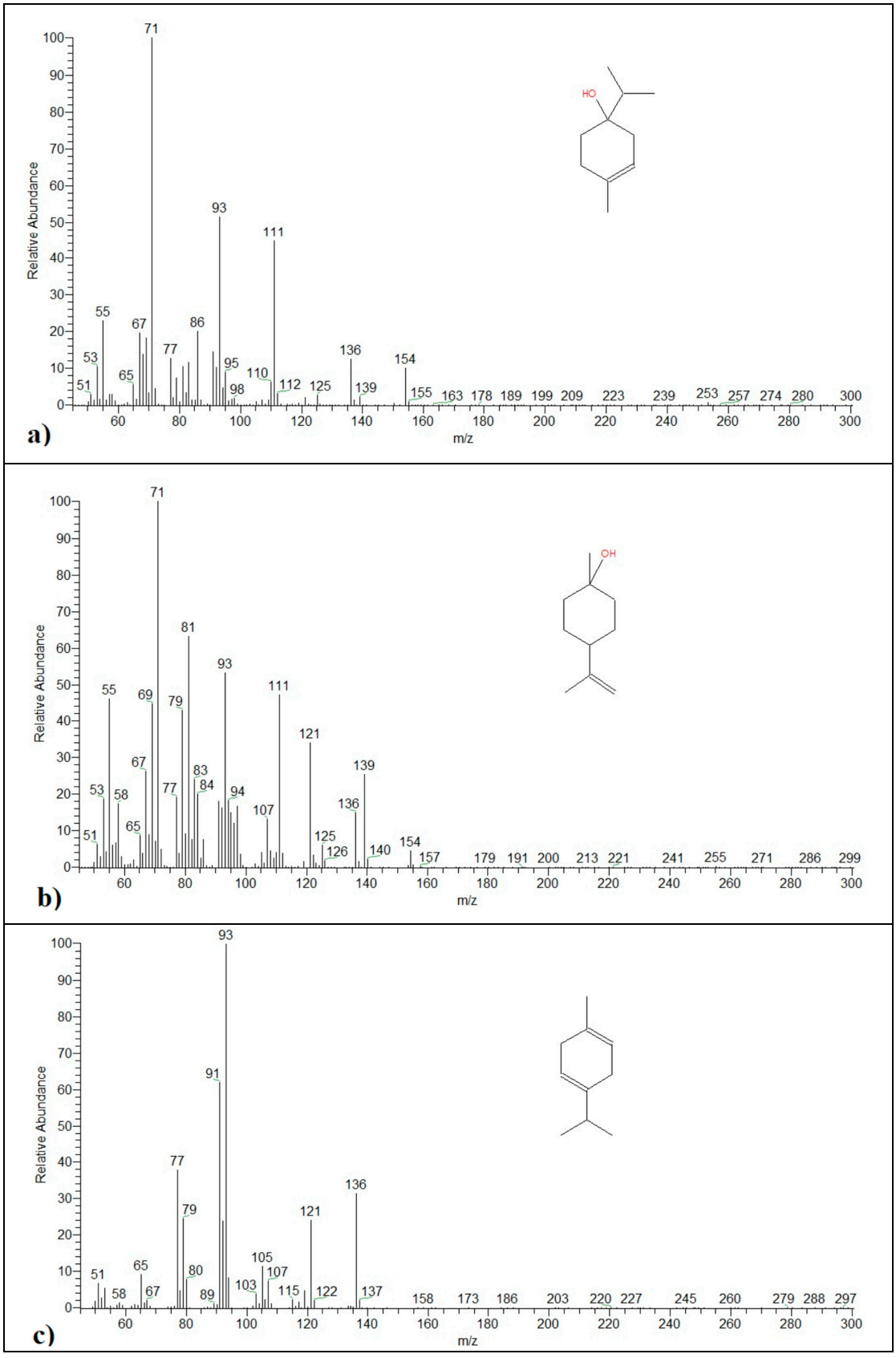


Figure 4. The 70 eV mass spectrum of *D*-limonene (a), γ-terpinene (b), β-pinene (c), and isopregol (d) components of *Corymbia citriodora* leaves' recovery oil.


a)
Relative Abundance
m/z
HO
b)
Relative Abundance
m/z
OH
c)
Relative Abundance
m/z

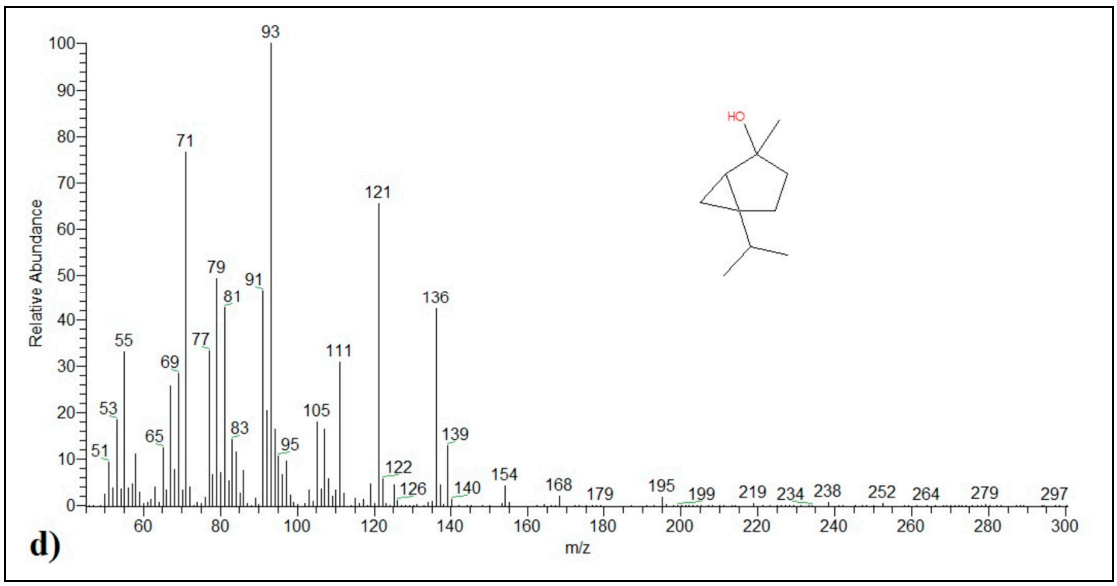


Figure 5. The 70 eV mass spectrum of 4-Terpineol (a), *cis*-β-Terpineol (b), γ-Terpinen (c), and (1α,2β,5α)-Bicyclo[3.1.0]hexan-2-ol,2-methyl-5-(1-methylethyl) (d) components of *Corymbia citriodora* fruits' recovery oil.

3.2. Computation Method

The geometry of the studied molecules has been optimized based on semi-empirical calculations, using the molecular modeling program Hyperchem7.5 (W.Thiel 2003, HyperChemTM, Release 7.5 Pro 2002). Semi-empirical calculations were carried out using the routine MNDO and Polak–Ribiere conjugated gradient algorithm. For the optimized structure of the neutral and cation states, geometry optimization mode was carried out to give the molecular properties including heat of formations, total energy, binding energy, electronic energy and nuclear energy and dipole moment [40]. From the calculated data of the studied compounds (Table 5), values were obtained for heat of formation, ionization, total, binding, electronic energies and dipole moment. These thermochemical data are necessary in the description of the conformational properties of the studied molecules [41].

Table 5. Thermodynamic data of the studied molecules calculated within the modified neglect of diatomic overlap (MNDO) framework.

Name of compound	Δ_F(M) (Kcal/mol)	$\Delta_F(M)^+$ (Kcal/mol)	Ionization Energy (eV)**	Total Energy (Kcal/mol)	Binding Energy (Kcal/mol)	Nuclear Energy (Kcal/mol)	Electronic Energy (Kcal/mol)	Dipole Moment (Debye)
Citronellol	−70	133	8.8	−43491	−2880	191052	−234543	1.401
Isopulegol	−57	146	8.8	−42825	−2763	202592	−245417	1.374
α-Pinene*	17	217	8.7	−34707	−2525	195366	−187656	0.113
Eudesmol	−64	130	8.6	−43586	−2745	189563	−225862	1.353
Limonene*	2	209	9	−34722	−2541	198765	−180021	0.122
Guaiol	−60	130	8.2	−60190	−4038	378879	−439069	1.447
γ-Terpinene	2	198	8.8	−34730	−2548	156724	−191454	0.028
Isopregol	−56	146	8.8	−42824	−2763	202759	−245583	1.227

* Data from Abd El-kareem et al. [42]; ** The value of the ionization energies was calculated with the following equation, IE [M] =ΔH_f [M] $^{+\bullet}$− ΔH_f [M]; where ΔH_f [M] $^{+\bullet}$ and ΔH_f [M] are the heats of formation of the molecular ion and neutral molecule, respectively.

From the calculated data of the studied molecule (Table 5), one can observe that the negative values of the heat of formations Δ_F(M) and total energy for group 1 (citronellol , isopulegol, eudesmol guaiol, and isopregol) neutral molecules have negative values that mean these molecules are stable and the citronellol molecule is the most stable. This is due to the presence of the OH group in their structures, while group 2 α-pinene, limonene and γ-terpinene have the positive values of heat of formations. From these values the second group is relatively less stable than the first group which has OH group in their structures. This is confirmed by the values of dipole moment, hence the first group has approximately the same dipole moment(1.401, 1.374, 1.353, 1.447 and 1.227) in comparison with the second group (0.113, 0.122 and 0.028).

3.3. In Vitro Visual Observations of Dual Fungal Growth Against Oil-Treated Wood

To test the antifungal properties of essential (EO) and recovered (RO) oils from leaves and fruits of *C. citriodora*, oil-treated wood were bio-assayed against the growth of three fungi (*F. culmorum, R. solani* and *P. chrysogenum*) compared to control treatments in Figure 6. Nearly no growth of *F. culmorum*, *R. solani* and *P. chrysogenum* were found over wood treated with *C. citriodora* leaves and fruits oils after 14 days from incubation. On the other hand, the treated wood with EOs showed complete inhibition to the growth of *F. culmorum* and *P. chrysogenum* at all the oil amounts used (100, 50 and 25 µL), and also, at the amount of 100 and 50 µL of both oils, no growth of *R. solani* was observed. By visual observation and compared to control treatment, nearly no inhibition was found around the treated wood with ROs against the growth of *P. chrysogenum* but, also, no growth was observed over the treated wood samples. The extract from the unripened fruit prevents surrounding fungal growth in comparison to the extract from the leaf. Also, when little growth was observed it differed in appearance but it was still stopped by the ROs.

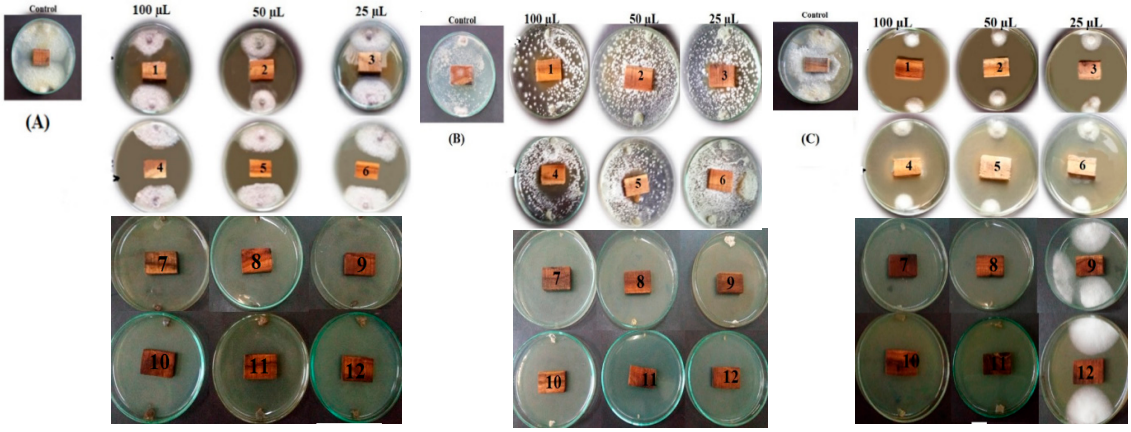


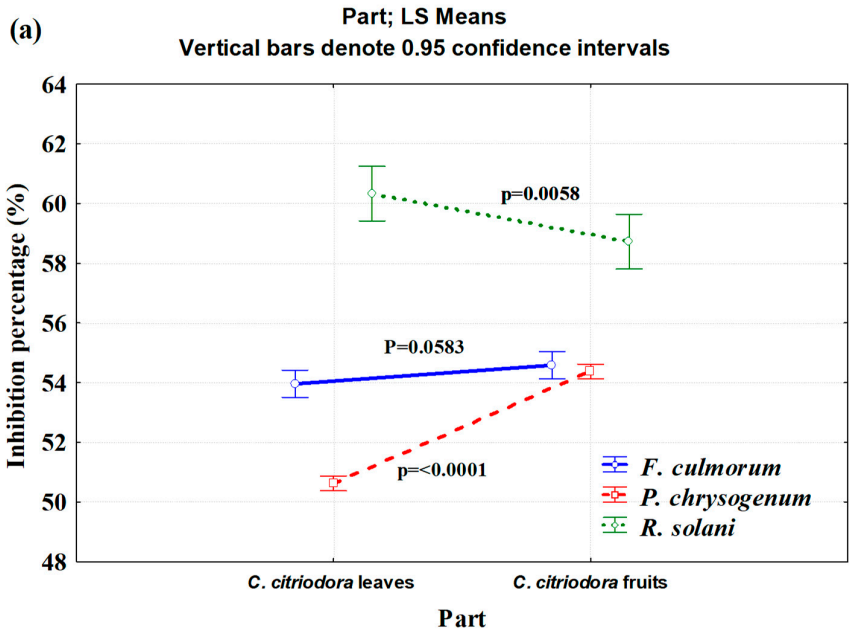
Figure 6. In vitro antifungal bioassay of treated-wood with (1,2,3) *C. citriodora* leaf recovered oil; (4,5,6) *C. citriodora* fruit recovered oil; (7,8,9) *C. citriodora* leaf essential oil and (10,11,12) *C. citriodora* fruit essential oil. (A) *Fusarium culmorum*, (B) *Penicillium chrysogenum*, (C) *Rhizoctonia solani*.

3.4. Antifungal Activity of the Oils

Overall, leaves and fruits of *C. citriodora* showed the highest activity against *F. culmorum* and *P. chrysogenum* (Figure 7a). EOs were observed much higher activity against the studied fungi than ROs (Figure 7b). In addition, with increasing the oil amount, the activity was increased compared to the control (Figure 7c). The antifungal activity values of treated wood with *C. citriodora* leaf and fruits' EOs at the amounts of 100, 50 and 25 µL in Table 6 show that the highest inhibition percentage (IP)

of 100% was observed against *F. culmorum* and *P. chrysogenum* compared to the control treatment. The treated wood with both EOs at the amounts of 100, and 50 µL showed 100% IP of *R. solani*.

The treated wood with ROs from leaves and fruits observed less activity against the growth of *F. culmorum* and *P. chrysogenum*, where IP reached 46.66% against *F. culmorum* on wood treated with *C. citriodora* leaf RO in the amount of 100 µL. Also, IP showed 60% against *P. chrysogenum* with wood treated at 100 µL of *C. citriodora* fruit RO. The ROs from leaves and fruits showed IPs of 96.66% and 93.33% against the growth of *R. solani* in the oil amount of 100 µL.



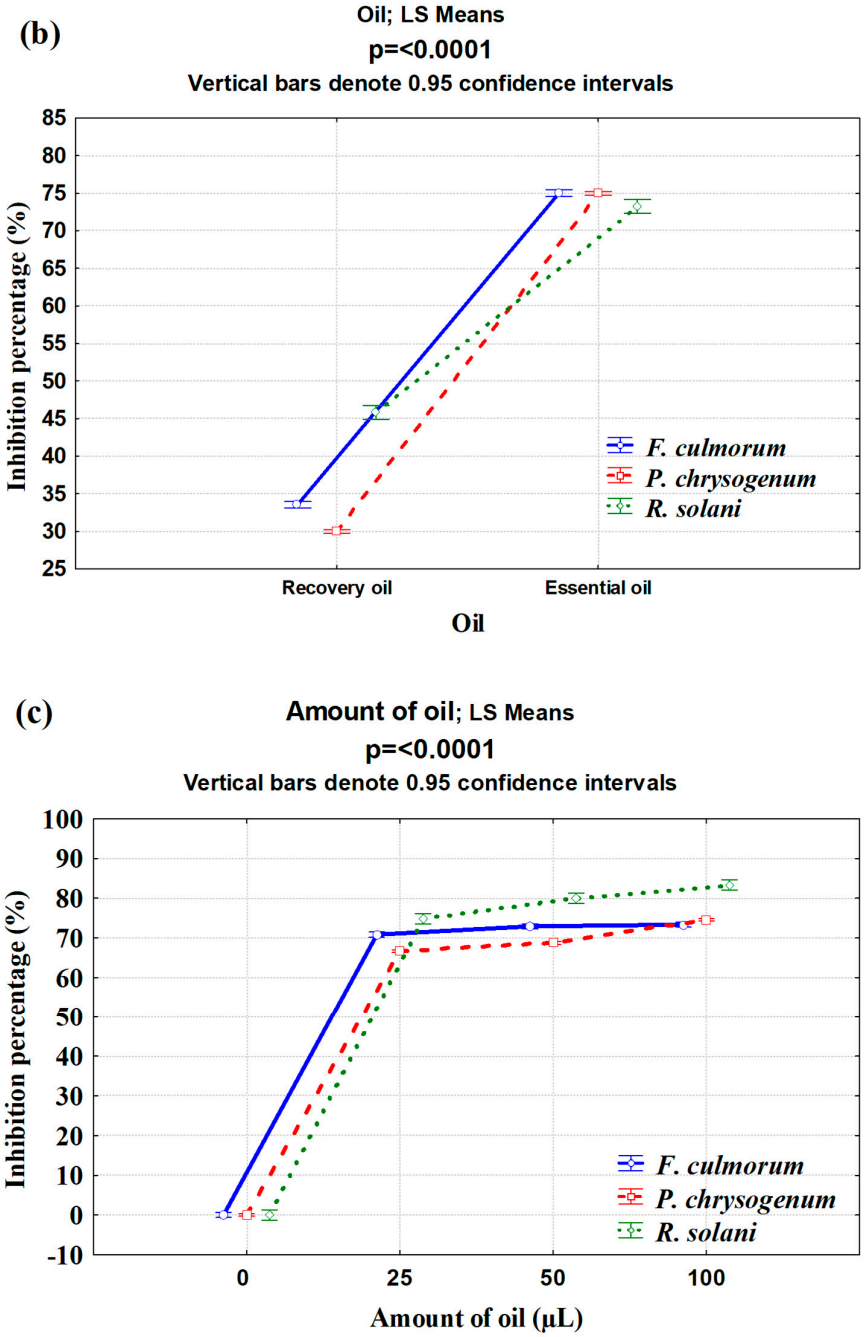


Figure 7. Overall bioactivity of *C. citriodora* (a) part source, (b) oil source and (c) oil amount against fungal linear growth of *F. culmorum*, *P. chrysogenum* and *R. solani.*

Table 6. Inhibition percentages of fungal growth (%) as affected by wood treated with *C. citriodora* oils with different amounts.

Part	Oil type	Oil amount (µL)	*F. culmorum*	*P. chrysogenum*	*R. solani*
C. citriodora leaves	RO	0	0.00	0.00	0.00
		25	38.33 ± 1.66	33.33 ± 0.00	90.00 ± 0.00
		50	46.66 ± 3.33	33.33 ± 0.00	90.33 ± 0.33
		100	46.66 ± 0.00	38.33 ± 1.66	96.66 ± 0.00

	EO	0	0.00	0.00	0.00
		25	100 ± 0.00	100 ± 0.00	62.66 ± 0.00
		50	100 ± 0.00	100 ± 0.00	100 ± 0.00
		100	100 ±0.00	100 ± 0.00	100 ± 0.00
C. citriodora fruits	RO	0	0.00	0.00	0.00
		25	45 ± 1.66	33.33 ± 0.00	86.66 ± 3.33
		50	45 ± 1.66	41.66 ± 1.66	86.66 ± 3.33
		100	46.66 ± 0.00	60 ± 0.00	93.33 ± 3.33
	EO	0	0.00	0.00	0.00
		25	100 ± 0.00	100 ± 0.00	63.11 ± 0.38
		50	100 ± 0.00	100 ± 0.00	100 ± 0.00
		100	100 ± 0.00	100 ± 0.00	100 ± 0.00
P value			0.0003	<.0001	0.00823

RO: Recovred oil; EO: Essential oil.

4. Discussion

In the present study, gas chromatography–mass spectrometry (GC–MS) with some calculations that were reported in the computation method was used for identification of the phytocompounds in EO and RO from *C. citriodora* leaves and unripe fruits [43–45]. For example, the fragmentation pathway of some identified main compounds such as D-limonene has been reported and discussed by Abd El-kareem et al. [42].

The main chemical constituents in EO from *C. citriodora* leaves were citronellal, citronellol and isopulegol, while in the fruits were α-pinene, eudesmol, limonene, γ-terpinene, and guaiol. The main compounds found in the recovery oils from leaves were D-limonene, γ-terpinene, β-pinene and isopregol, while, α-terpineol, *cis*-β-terpineol, D-limonene, γ-terpinene and (1α,2β,5α)-bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl) were found in fruits. These compounds have been identified in the oils from Eucalyptus species, especially *C. citriodora* ,with significant antimicrobial and antioxidant activities.

Our previous work showed that the leaf EO from *C. citriodora* leaves had a potential antimicrobial activity against Listeria monocytogenes, *Bacillus cereus, Micrococcus flavus, Staphylococcus aureus, Dickeya solani, Escherichia coli, Pectobacterium atrosepticum, Pectobacterium carotovorum* subsp. *carotovorum, Pseudomonas aeruginosa, Aspergillus flavus, A. ochraceus, A. niger, Candida albicans, Penicillium funiculosum* and *P. ochrochloron* with the presence of α-citronellal, α-citronellol, citronellol acetate, isopulegol, eucalyptol, and citronellic acid as the main compounds [17].

Other studies reported that the main compounds of the EOs of *C. citriodora* leaves were α-citronellal and isopulegol [46]; citronellal, β-citronellol, and isopulegol [23]; citronellal and β-citronellol [28]; β-citronellal in plants grown in Brazil [47]. Citronellal, citronellol, *Neo*-isopulegol, *E*-caryophyllene, *iso*-isopulegol, and citronellyl acetate were found as the main compounds in the EO of *C. citriodora* from Benin [48]; α-citronellal, citronellol acetate, α-citronellol, and isopulegol were also the main compounds [20]; α-pinene and 1.8-cineole the main compounds in the plant grown in Tunisia [30]. α-pinene and α-terpineol have been shown antimicrobial activity [49], which are found in *C. citriodora* leaf oil. Limonene and other monoterpenes (linalool, linalyl acetate, bergapten, citropten, bergamottin, γ-terpinen, α-pinene and β-pinene) have good biological properties [17,50]. The major components of EO in *E. citriodora* from the zoological garden in Giza, Egypt, were 5-hepten-1-ol, 2,6-dimethyl, 3-hexen-1-ol, cis-geraniol, citronellol acetate, and citronellal [26]. Limonene, *E*-nerolidol, and *E*-farnesol which exhibited high antifungal activity [51,52].

E. citriodora EO also inhibits the growth of phyto- and post-harvest pathogens [12,13,53], and its antifungal activity is attributed to citronellal, the major volatile constituent of this EO [12]. Significant inhibition of growth of *Rhizoctonia solani* was observed in Citronella (83.53%), and Lemon-tulsi (70.39%), Eucalyptus (68.63%), Pepper Mint (55.69%), and Patchauli (52.75%) which also effectively

reduced the growth of the fungus [54]. *E. citriodora* and its major constituent citronellal was effective against rice pathogen *R. solani* and fully inhibited growth by the minimum concentrations [55]. Also, the synergism that occurred between citronellal and linalool showed strong antifungal activity [56]. Recently, the EO from *C. citriodora* leaves which contain α-citronellal (56.55%), α-citronellol (14.89%), and citronellol acetate (13.04%) was found to be highly toxic to the bacterial pathogen *Ralstonia solanacearum* phylotype II, the causal agent of brown rot disease [57]. The recovered compounds from hydrodistillation selected Lamiaceae species showed good antiradical and antioxidant activity [58]. Water-soluble oil was recovered by hexane extraction with 82.7%–83.3% dissolved in hot water and 90.0%–90.5% dissolved cold water from *Tagetes minuta* [59]. Recently, oil was recovered from hydrosol of *Matricaria chamomilla* flowers and showed good antifungal activity against *A. niger* [6]. Other study showed that linalyl acetate and limonene were recovered from from bergamot juice by supercritical and liquid CO_2 [60].

5. Conclusions

In the present study, the treated *Melia azedarach* wood with *C. citriodora* leaf and fruits essential oils (25, 50 and 100 μL) showed the highest antifungal activity (100% inhibition) against *F. culmorum* and *P. chrysogenum*. Treated wood with both essential oils at 50 and 100 μL observed potent activity against the growth of *R. solani* with an inhibition percentage 100%. Recovered oils from leaves and fruits showed good activity against *R. solani*, where the inhibition percentage reached 96.66%, and 93.33%, respectively. Additionally, weak to moderate activity was observed against *F. culmorum* and *P. chrysogenum* as wood treated with recovered oils from leaves and fruits. Therefore, both oils could be used as natural antifungal agents for the treatment of several plant infection diseases and as wood bio-fungicide that can be used for packaging fruits or vegetables. Also, the mass spectra of the major components are recoded and discussed, where the main fragment ions were observed at *m*/*z* 67, 68, 69, 71, 93 and 161 for the main components of the studied samples. From MNDO calculations, citronellol molecule has the most negative values of heat of formations and it is the most stable molecule, while α -pinene is the least stable molecule.

Author Contributions: S.I.B., R.A.N., M.S.M.A.E.-K., H.M.A. and M.Z.M.S. designed the experiments, conducted the laboratory analyses, wrote parts of the manuscript, and interpreted the results; all coauthors contributed to writing and revising the article. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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