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Research Article

Evaluation of Insecticidal Activity of Bioactive Compounds from *Eucalyptus citriodora* Against *Tribolium castaneum*

Nebras M Sahi

College of Science for Women, Babylon University, Iraq

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ABSTRACT

Methanolic extract of bioactive compounds of *Eucalyptus citriodora* was assayed for in vitro anti-insect activity against *Tribolium castaneum* (Herbst). GC-MS analysis of *Eucalyptus citriodora* revealed the existence of the α-Pinene , Eucalyptol , 2,4-Dimethylstyrene , Isopinocarveol , Cis-p- menthe-1(7),8-dien-2-ol , Bicyclo[3.1.1] heptan-3-ol,6,6-dimethyl-2-methylene-,[1S-(1α , 5-Caranol,(1S,3R,5S,6R)-(-)- , Terpinen-4-ol , Thymol , α-Terpineol , 7-epi-trans-sesquisabinene hydrate , γ-Elemene , β-copaene , Azulene ,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methyl) , Alloaromadendrene , β-Guaiene , Epiglobulol , Globulol, 2-Naphthalenemethanol , decahydro-α,α, 4a-trimethyl-8-methyl , 8-epi-gama-eudesmol , α-acorenol , Perhydrocyclopropa[e]azulene-4,5,6-triol , 1,1,4,6-tetramethyl , Phenylalanine , 4-amino-N-t-butyloxycarbonyl-,t-butyl ester , 1,4-Naphthoquinone , 6-acetyl-2,5,7-trihydroxy- , 1-Glyceryl ricinoleate , Decanedioic acid , dibutyl ester , Curan-17-oic acid ,2,16-didehydro-19-hydroxy-,methyl ester, 1,4-Naphthoquinone , 2-acetyl-5,8-dihydroxy-3-methoxy- , 9-Octadecenamide , (Z)- , 9-Octadecenamide, 12-hydroxy-,[R-(Z)]- , Tertbutyloxyformamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl, 17-Pentatriacontene, Vitamin E and γ-Sitosterol. The FTIR analysis of *Eucalyptus citriodora* leaves proved the presence of Alkenes, alkyl halides and alkanes. *Eucalyptus citriodora* was highly active on accumulative mortality of *Tribolium castaneum* (Herbst) (adult).

Keywords: GC/MS, Bioactive compounds, FT-IR, *Eucalyptus citriodora*, *Tribolium castaneum*.

INTRODUCTION

Tribolium castaneum (Herbst) is considered as a major pest of stored grains[1]. Annual post-harvest losses resulting from insect damages, microbial deterioration and others factors are estimated to be 10- 25% of worldwide production[2]. Control of these insects relies heavily on the use of synthetic insecticides and fumigants. But their widespread use has led to some serious problems including development of insect strains resistant to insecticides[3-4], toxic residues on stored grain, toxicity to consumers and increasing costs of application. However, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally friendly. Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides. To avoid pollution of the environment, deterrent and repellent substances have been searched for pest control during recent times[6,7]. Plant products have been successfully exploited as insecticides, insect repellents and insect antifeedants[8]. Higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control[9]. Insecticidal activity of many plants against several insect pests has been demonstrated. The deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant growth inhibitor, suppression of reproductive behaviour and eduction of fecundity and fertility. However, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally friendly[10,11]. Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides. The aim of our study is to evaluate the insecticidal activity of the methanol extracts from *E. citriodora* against larvae and adults of *Tribolium castaneum*.

MATERIALS AND METHODS

Extraction and isolation of E. citriodora

The fresh leaves (1.8 kg) of *E. citriodora* (purchased from local market in Hilla city, middle of Iraq) were hydro distilled in all glass clevenger apparatus. *Eucalyptus citriodora* was stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use. Methanolic extract of *Eucalyptus citriodora* powdered were soaked in 1000 mL methanol for ten hours in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis[12-14].

Determination of anti-insect activity

Tribolium castaneum was obtained from laboratory cultures Maintained in the dark in incubators at 26 ± 1°C. This insect was reared on wheat flour mixed with yeast (10:1, w: w). Adults of 1-week old were used for the study of plant effects. A control was prepared in the same

*Author for Correspondence: imad_dna@yahoo.com

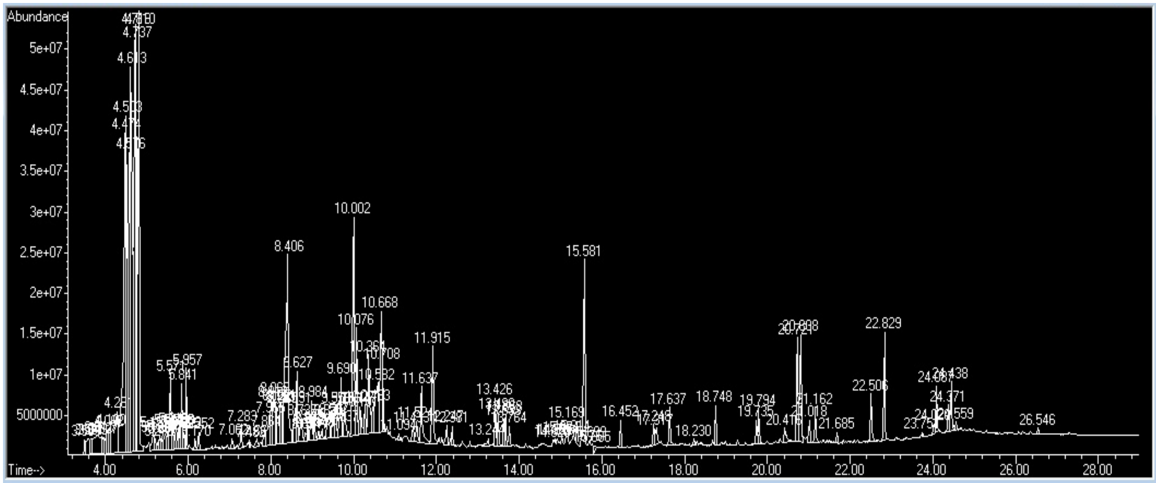


Figure 1: GC-MS chromatogram of methanolic extract of Eucalyptus citriodora

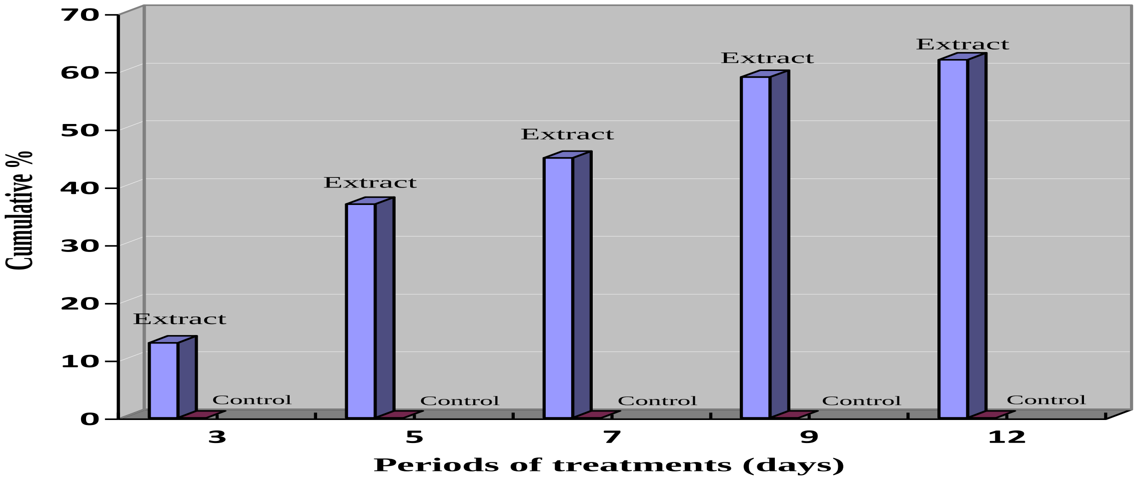


Figure 2: Effect of methanolic leaves extract of Eucalyptus citriodora on accumulative mortality of Tribolium castaneum (Herbst) (larvae).

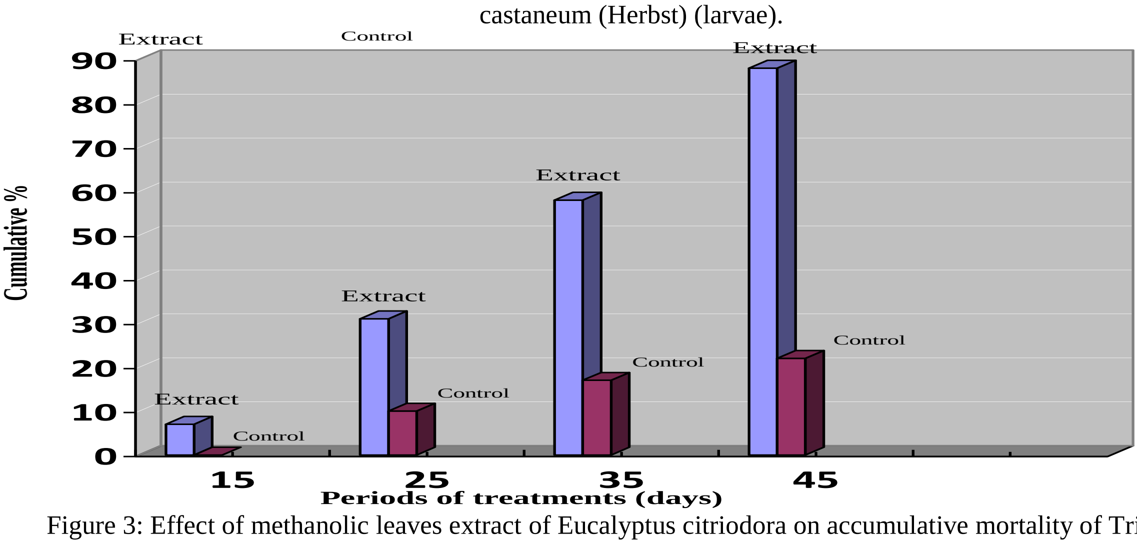


Figure 3: Effect of methanolic leaves extract of Eucalyptus citriodora on accumulative mortality of Tribolium castaneum (Herbst) (adult).

way but extract application was omitted. Five replicates were set up for the treated[15-17]. To assess the effects of different extracts on progeny production (F1), 30 adults were added to each glass vial containing a culture medium treated as above. After 48 h, the adults were removed and the glass vials were returned to the incubator until F1 adult emergence.

Gas chromatography – mass spectrum analysis

GC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. The GC-MS analysis of the plant extract was made in a (Agilent 789 A) instrument under computer control at 70 eV. About 1µL of the methanol extract was

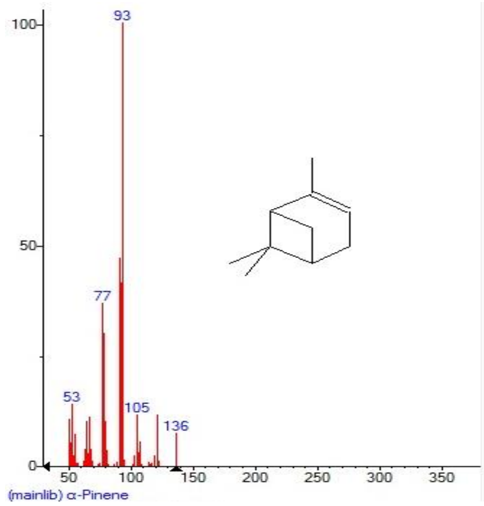


Figure 4: Mass spectrum of α-Pinene with Retention Time (RT)= 3.499

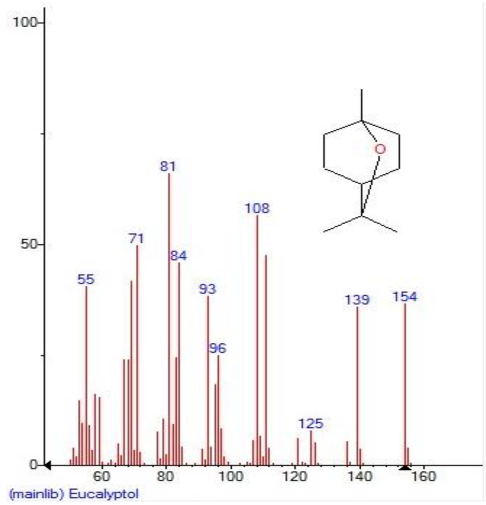


Figure 5: Mass spectrum of Eucalyptol with Retention Time (RT)= 4.632

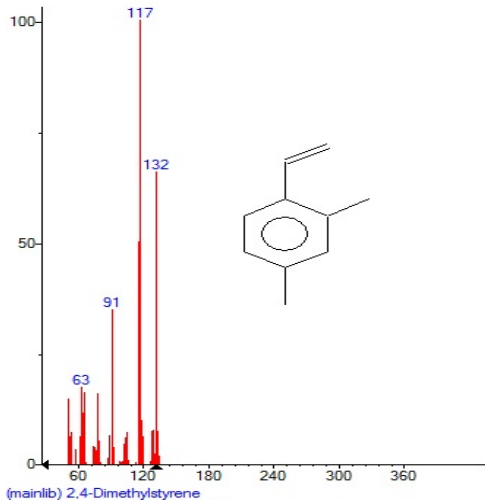


Figure 6: Mass spectrum of 2,4-Dimethylstyrene with Retention Time (RT)= 5.158

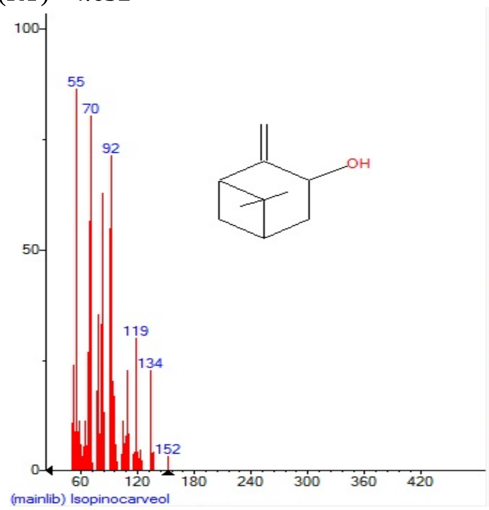


Figure 7: Mass spectrum of Isopinocarveol with Retention Time (RT)= 5.267

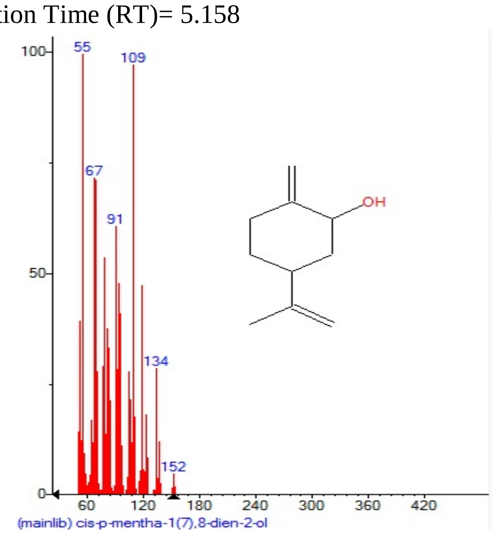


Figure 8: Mass spectrum of Cis-p- menthe-1(7),8-dien-2-ol with Retention Time (RT)= 5.370

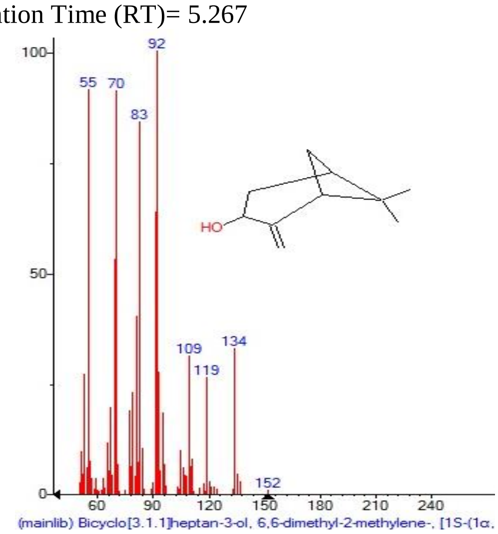


Figure 9: Mass spectrum of Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl-2-methylene-,[1S-(1α with Retention Time (RT)= 5.570

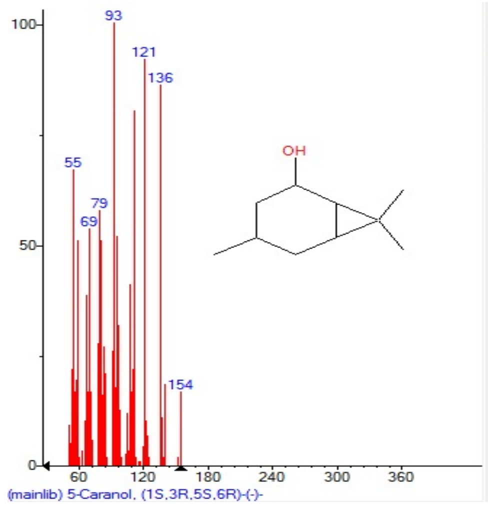


Figure 10: Mass spectrum of 5-Caranol,(1S,3R,5S,6R)-(-)- with Retention Time (RT)= 5.628

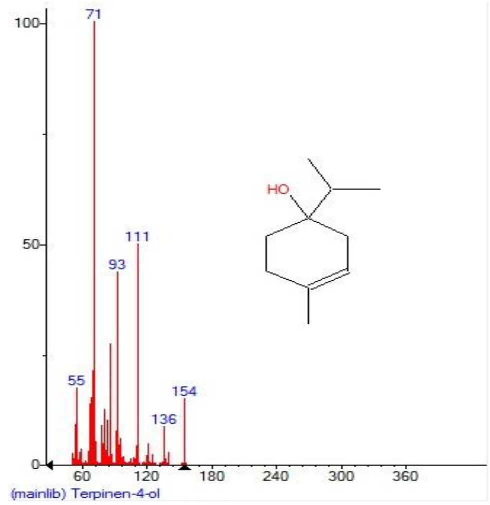


Figure 11: Mass spectrum of Terpinen-4-ol with Retention Time (RT)= 5.851

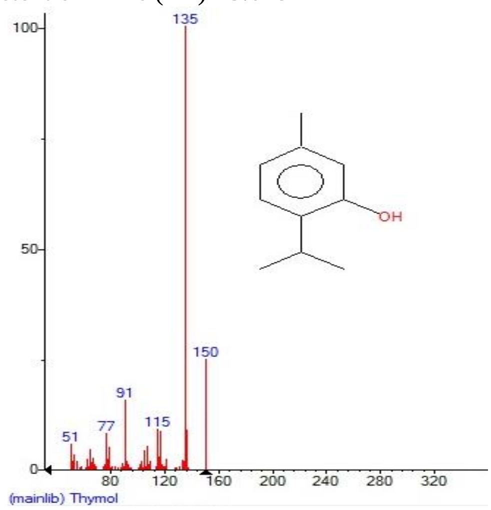


Figure 12: Mass spectrum of Thymol with Retention Time (RT)= 5.891

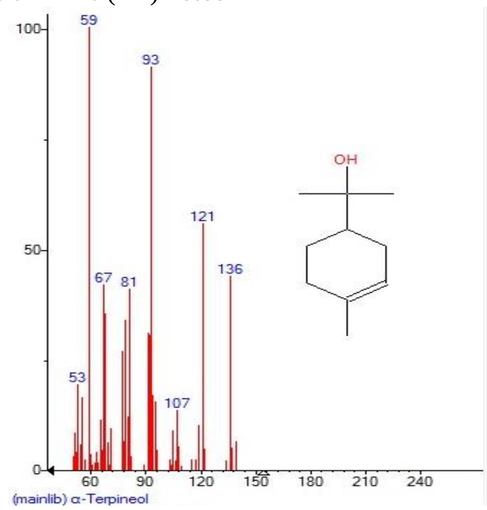


Figure 13: Mass spectrum of α-Terpineol with Retention Time (RT)= 5.948

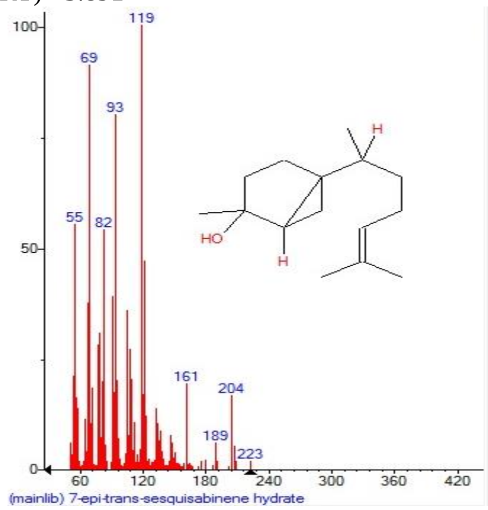


Figure 14: Mass spectrum of 7-epi-trans-sesquisabinene hydrate with Retention Time (RT)= 7.190

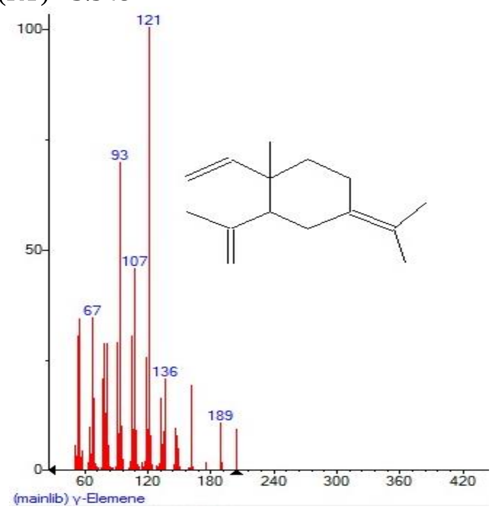


Figure 15: Mass spectrum of γ-Elemene with Retention Time (RT)= 7.287

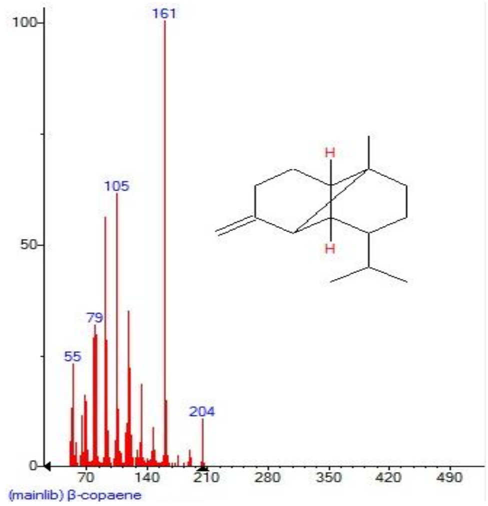


Figure 16: Mass spectrum of β-copaene with Retention Time (RT)= 7.665

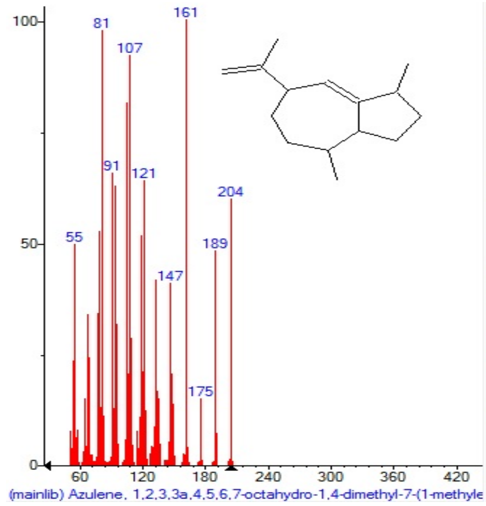


Figure 17: Mass spectrum of Azulene ,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methyl) with Retention Time (RT)= 8.054

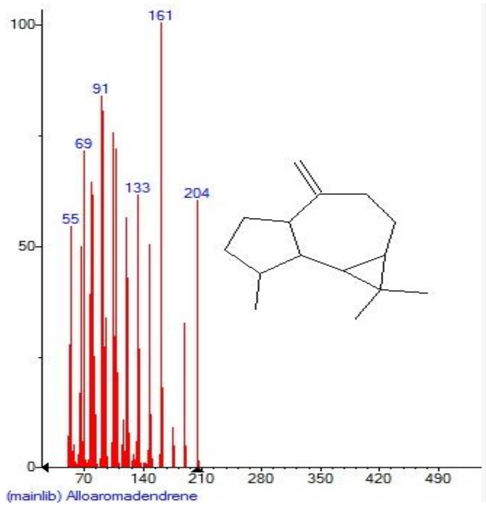


Figure 18: Mass spectrum of Alloaromadendrene with Retention Time (RT)= 8.242

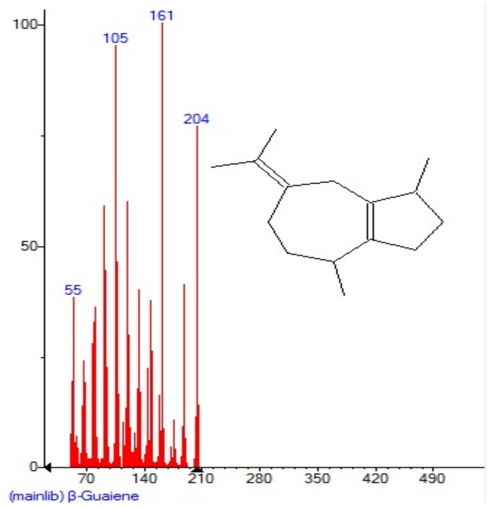


Figure 19: Mass spectrum of β-Guaiene with Retention Time (RT)= 8.740

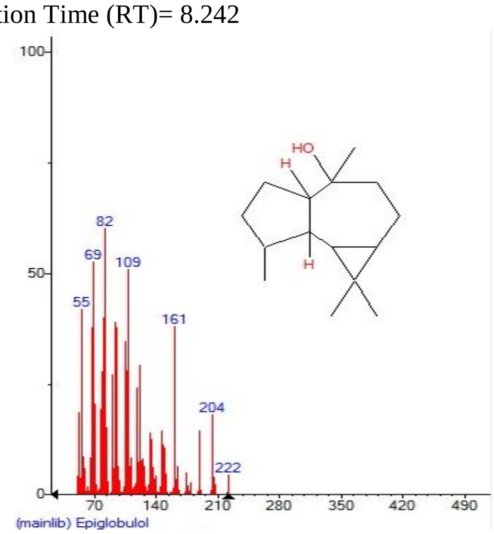


Figure 20: Mass spectrum of Epiglobulol with Retention Time (RT)= 9.427

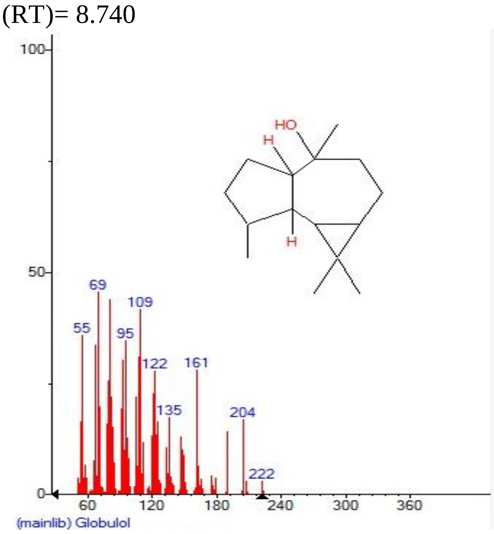


Figure 21: Mass spectrum of Globulol with Retention Time (RT)= 9.982

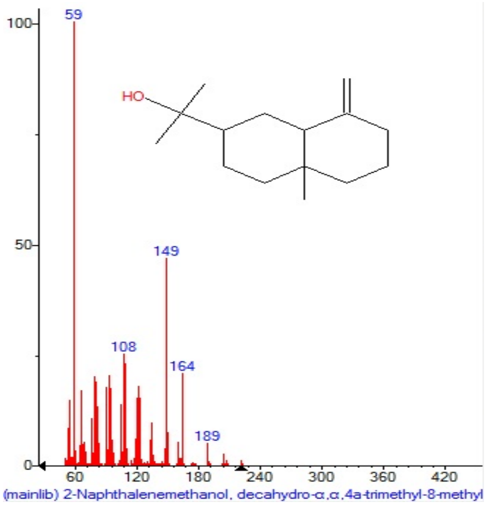


Figure 22: Mass spectrum of 2-Naphthalenemethanol , decahydro-α,α,4a-trimethyl-8-methyl with Retention Time (RT)= 10.142

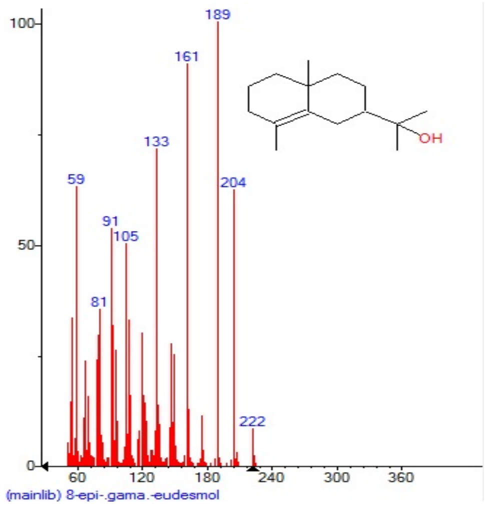


Figure 23: Mass spectrum of 8-epi-gama-eudesmol with Retention Time (RT)= 10.669

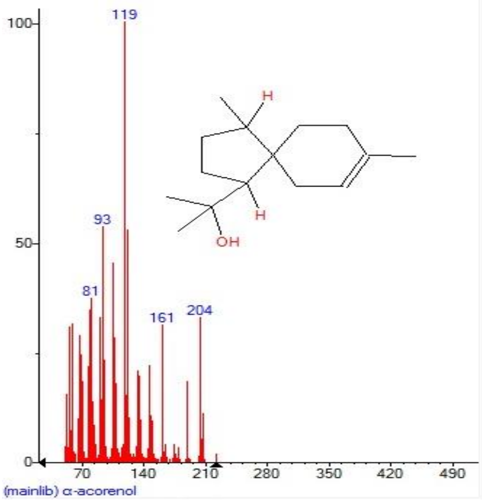


Figure 24: Mass spectrum of α-acorenol with Retention Time (RT)= 10.720

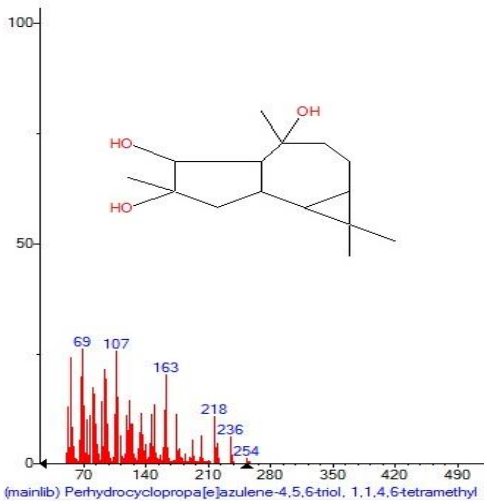


Figure 25: Mass spectrum of Perhydrocyclopropa[e]azulene-4,5,6-triol , 1,1,4,6-tetramethyl with Retention Time (RT)= 11.041

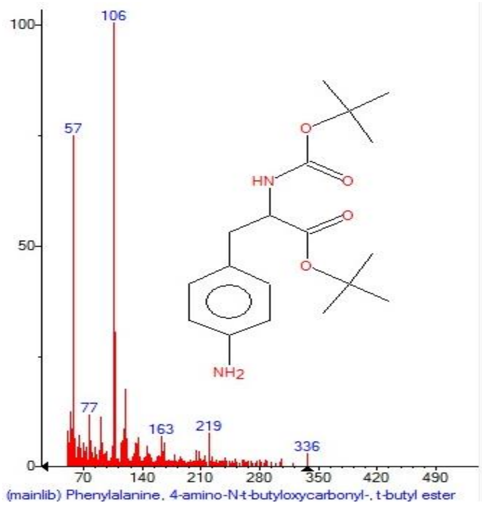


Figure 26: Mass spectrum of Phenylalanine , 4-amino-N-t-butyloxycarbonyl-,t-butyl ester with Retention Time (RT)= 14.182

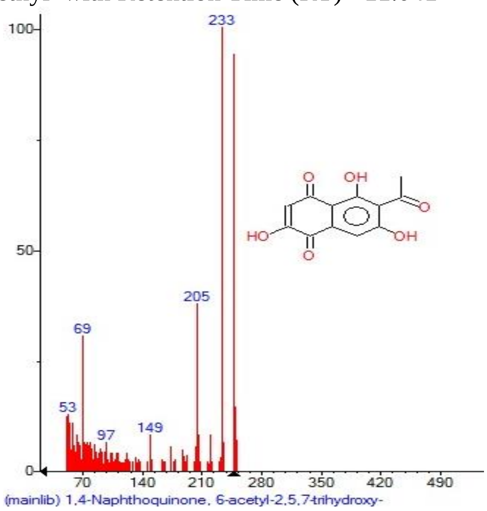


Figure 27: Mass spectrum of 1,4-Naphthoquinone , 6-acetyl-2,5,7-trihydroxy- with Retention Time (RT)= 13.415

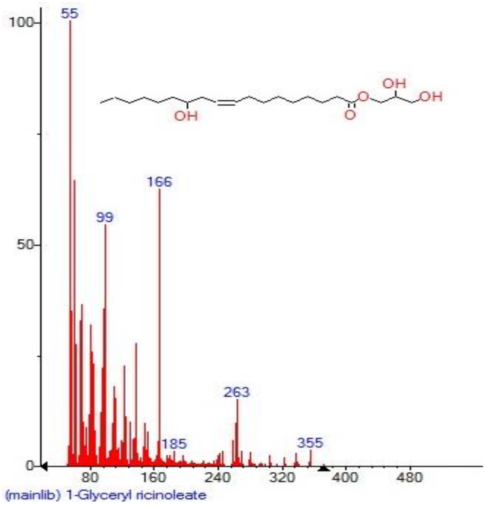


Figure 28: Mass spectrum of 1-Glyceryl ricinoleate with Retention Time (RT)= 15.315

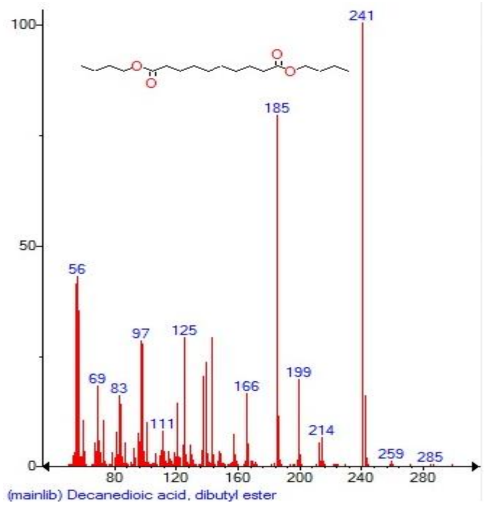


Figure 29: Mass spectrum of Decanedioic acid , dibutyl ester with Retention Time (RT)= 15.555

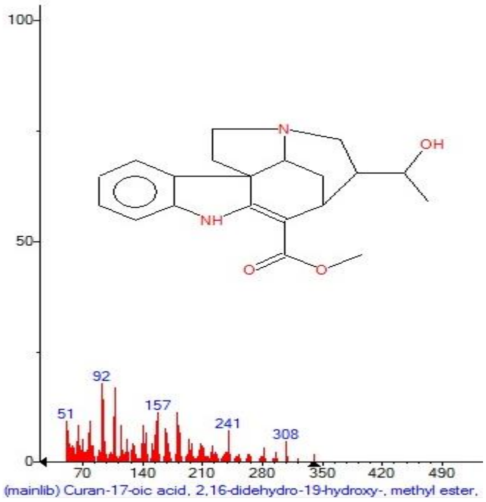


Figure 30: Mass spectrum of Curan-17-oic acid ,2,16-didehydro-19-hydroxy-,methyl ester, with Retention Time (RT)= 15.783

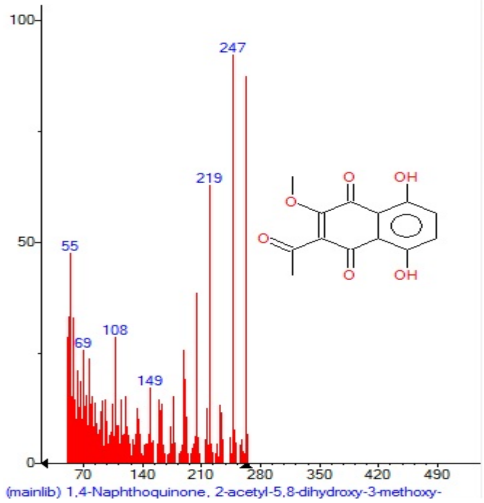


Figure 31: Mass spectrum of 1,4-Naphthoquinone , 2-acetyl-5,8-dihydroxy-3-methoxy- with Retention Time (RT)= 16.082

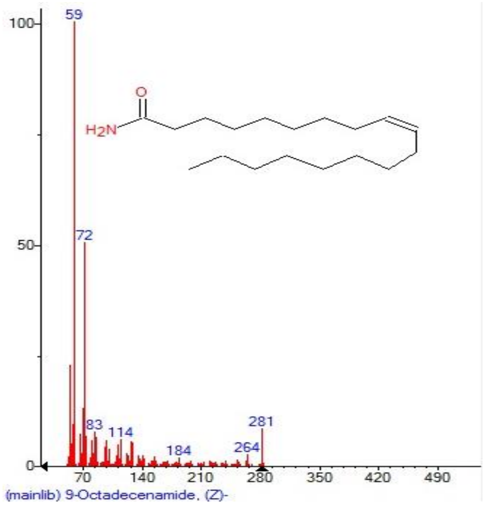


Figure 32: Mass spectrum of 9-Octadecenamide , (Z)- with Retention Time (RT)= 17.243

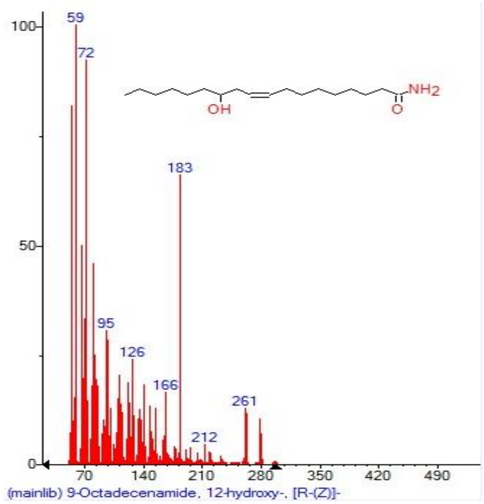


Figure 33: Mass spectrum of 9-Octadecenamide , 12-hydroxy-,[R-(Z)]- with Retention Time (RT)= 17.295

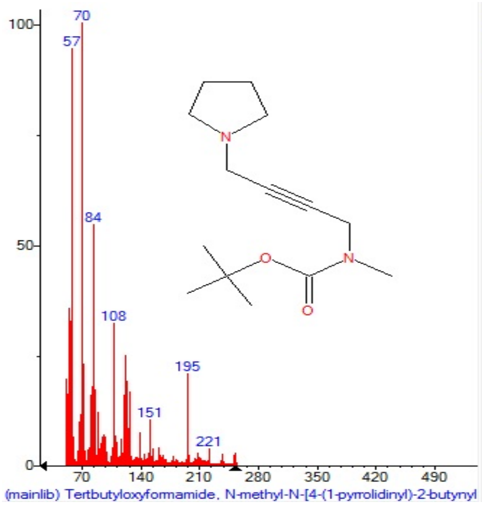


Figure 34: Mass spectrum of Tertbutyloxyformamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl with Retention Time (RT)= 18.010

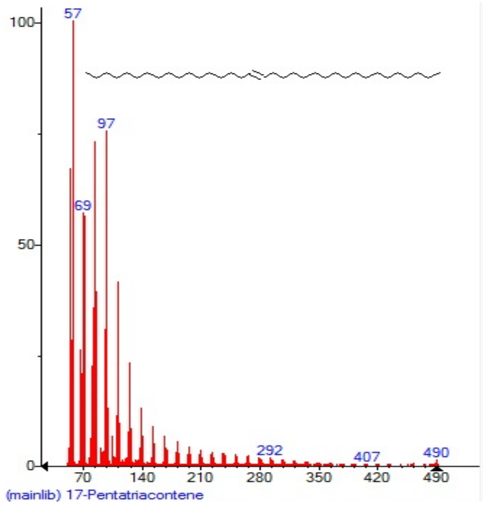


Figure 35: Mass spectrum of 17-Pentatriacontene with Retention Time (RT)= 21.174

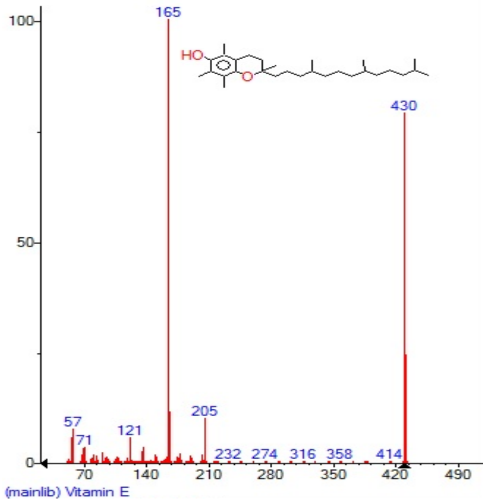


Figure 36: Mass spectrum of Vitamin E with Retention Time (RT)= 22.839

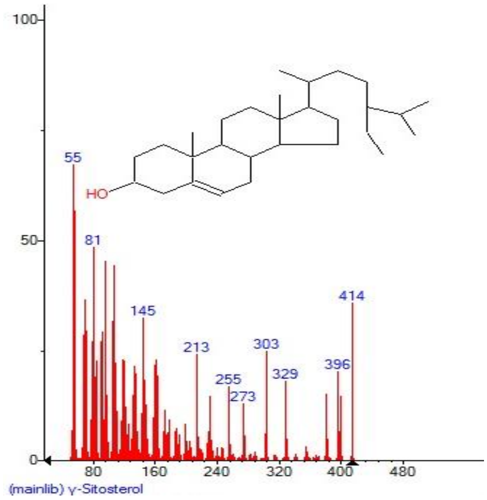


Figure 37: Mass spectrum of γ-Sitosterol with Retention Time (RT)= 24.064

injected into the GC-MS using a micro syringe and the scanning was done for 45 minutes. The time from when the injection was made (Initial time) to when elution occurred referred to as the Retention time (RT). Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1ml per minute. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries[18,19].

Statistical analysis

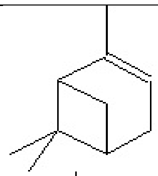
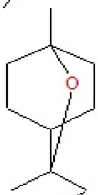
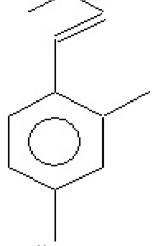
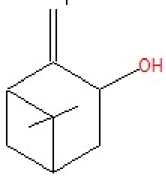
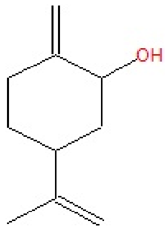
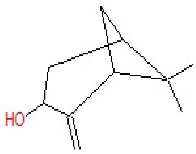
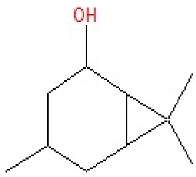
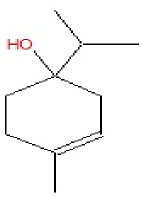
Results of the study were based on analysis of variance (ANOVA) using Statistica Software[20]. A significance level of 0.05 was used for all statistical tests.

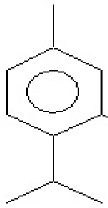
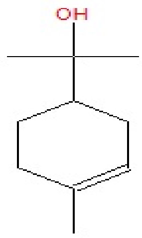
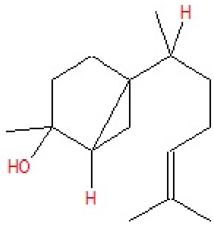
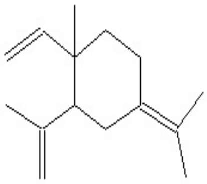
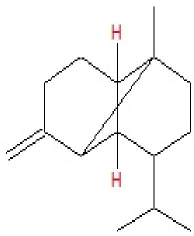
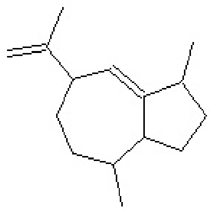
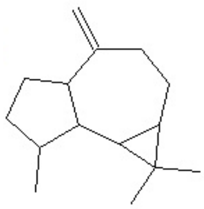
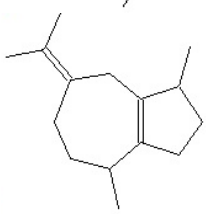
RESULTS AND DISCUSSION

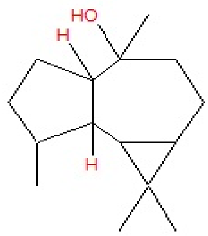
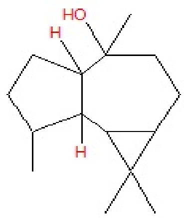
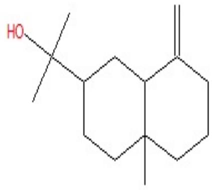
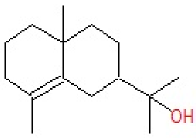
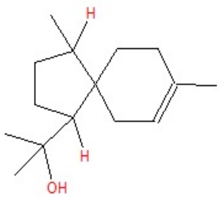
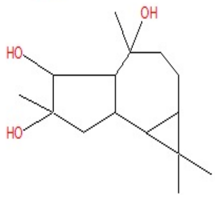
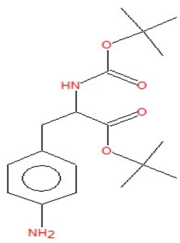
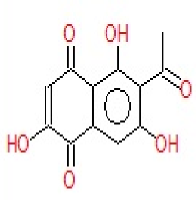
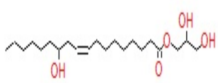
Identification of phytochemical compounds

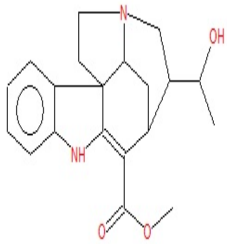
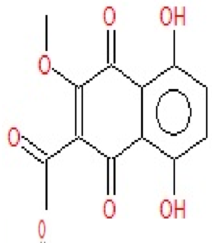
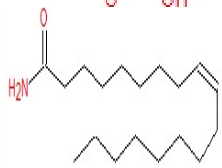
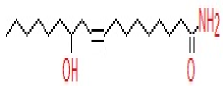
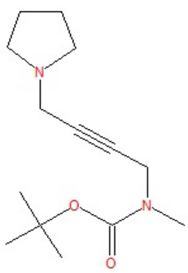
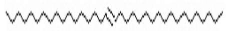
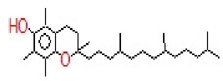
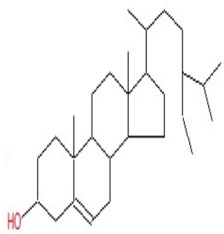
Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic leaves extract of *Eucalyptus citriodora*, shown in Table 1. The GC-MS chromatogram of the 31 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Eucalyptus citriodora* showed the presence of thirty-one major peaks and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be α-Pinene, Eucalyptol, , Isopinocarveol, Cis-p- menthe-1(7),8-dien-2-ol, Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl-2-methylene-,[1S-(1α, 5-Caranol,(1S,3R,5S,6R), Terpinen-4-ol, α-Terpineol, 7-epi-trans-sesquisabinene hydrate, γ-Elemene, β-copaene, Azulene ,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methyl), Alloaromadendrene, β-Guaiene, Epiglobulol, Globulol, 2-Naphthalenemethanol , decahydro-α,α,4a-trimethyl-8-methyl, 8-epi-gama-eudesmol, α-acorenol, Perhydrocyclopropa[e]azulene-4,5,6-triol , 1,1,4,6-tetramethyl, Phenylalanine , 4-amino-N-t-butyloxycarbonyl-,t-butyl ester, 1,4-Naphthoquinone , 6-acetyl-2,5,7-trihydroxy, 1-Glyceryl ricinoleate, Decanedioic acid , dibutyl ester, Curan-17-oic acid ,2,16-didehydro-19-hydroxy-,methyl ester, 1,4-Naphthoquinone , 2-acetyl-5,8-dihydroxy-3-methoxy, 9-Octadecenamide, (Z), 9-Octadecenamide, 12-hydroxy-,[R-(Z)], Tertbutyloxyformamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl, 17-Pentatriacontene, Vitamin E and γ-Sitosterol, (Figure 4-37). The FTIR analysis of

Table 1: Major phytochemical compounds identified in methanolic extract of Eucalyptus citriodora.

S. No.	Phytochemical compound	RT (min)	Molecular Weight	Exact Mass	Chemical structure	MS Fragment-ions	Pharmacological actions
1.	α-Pinene	3.499	136	136.1252		53,77,93,105,136	Anti-Bacterial Agents
2.	Eucalyptol	4.632	154	154.135765		55,71,81,84,93,96,108,125,139,154	Anti-Inflammatory
3.	2,4-Dimethylstyrene	5.158	132	132.093901		63,91,117,132	antioxidant activity
4.	Isopinocarveol	5.267	152	152.120115		55,70,92,119,134,152	antioxidant, *anti*-inflammation and antimicrobial
5.	Cis-p-menthe-1(7),8-dien-2-ol	5.370	152	152.120115		55,67,91,109,134,152	antiplasmodial and antitrypanosomal activities
6.	Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl-2-methylene-,[1S-(1α	5.570	152	152.120115		55,70,8392,109,119,134,152	antimicrobial and antioxydant activities
7.	5-Caranol,(1S,3R,5S,6R)-(-)-	5.628	154	154.135765		55,69,79,93,121,136,154	antimicrobial, anti-inflammatory, and antioxidative
8.	Terpinen-4-ol	5.851	154	154.135765		55,71,93,111,136,154	anti-inflammatory action

9.	Thymol	5.891	150	150.1044655		51,77,91,115,135,150	antimicrobial agent
10.	α-Terpineol	5.948	154	154.135765		53,59,67,81,93,107,121,136	anti-ulcer *activity*
11.	7-epi-trans-sesquisabinene hydrate	7.190	222	222.198365		55,69,82,93,119,161,189,204,223	Anti-oxidative activities
12.	γ-Elemene	7.287	204	204.1878		67,93,107,121,136,189	anti-tumor effects
13.	β-copaene	7.665	204	204.1878		55,79,105,161,204	anti-inflammatory
14.	Azulene ,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methyl)	8.054	204	204.1878		55,81,91,107,121,147,161,175,189,204	Unknown
15.	Alloaromadendrene	8.242	204	204.1878		55,69,91,133,161,204	antibacterial, anti- fungal
16.	β-Guaiene	8.740	204	204.1878		55,105,161,204	anti-inflammatory

17.	Epiglobulol	9.427	222	222,198365		55,69,82,109,161,204,222	anti-inflammatory
18.	Globulol	9.982	222	222.198365		55,69,95,109,122,135,161,204,222	Anti-Bacterial Agents
19.	2-Naphthalenemethanol , decahydro-α,α,4a-trimethyl-8-methyl	10.142	222	222.198365		59,108,149,164,189	anti-micro-organism
20.	8-epi-gama-eudesmol	10.669	222	222.198365		59,81,91,105,133,161,189,204,222	anti-inflammatory activity
21.	α-acorenol	10.720	222	222.198365		81,93,119,161,204	Unknown
22.	Perhydrocyclopropa[e]azulene-4,5,6-triol , 1,1,4,6-tetramethyl	11.041	254	254.188194		69,107,163,218,236,254	anti-inflammatory activity
23.	Phenylalanine , 4-amino-N-t-butyloxycarbonyl-,t-butyl ester	14.182	336	336.204906		57,77,106,163,219,336	Anti-cancer activity
24.	1,4-Naphthoquinone , 6-acetyl-2,5,7-trihydroxy-	13.415	248	248.032088		53,69,97,149,205,233	antimicrobial, larvicidal, anti-inflammatory and antioxidant activities
25.	1-Glyceryl ricinoleate	15.315	372	372.287575		55,99,166,185,263,355	anti-inflammatory effects

26.	Decanedioic acid , dibutyl ester	15.555	314	314.24571		56,69,83,97,111, 125,166,185,199, 214,241,259,285	antimicrobial, antispasmodic and anti-inflammatory effects
27.	Curan-17-oic acid ,2,16-didehydro-19-hydroxy- ,methyl ester,	15.738	340	340.178692		51,92,157,241,308	Anti-diabetic activity
28	1,4-Naphthoquinone , 2-acetyl-5,8-dihydroxy-3-methoxy	16.082	262	262.047737		55,69,108,149,219,247	antimicrobial, larvicidal, anti-inflammatory and antioxidant activities
29	9-Octadecenamide , (Z)-	17.243	281	281.271864		59,72,83,114,184,264,281	anti-inflammatory activity
30.	9-Octadecenamide , 12-hydroxy- ,[R-(Z)]-	17.295	297	297.266779		59,72,95,126,166,183,212,261	anti-inflammatory actions
31.	Tertbutyloxyformamide , N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl	18.010	252	252.183778		57,70,84,108,151,195,221	anti-histaminc properties
32.	17-Pentatriacontene	21.174	490	490.547752		57,69,97,292,407,490	anti-microbial
33.	Vitamin E	22.839	430	430.38108		57,71,121,165,205,232,274,316,358,414,430	antioxidants
34.	γ-Sitosterol	24.064	414	414.386166		55,81,145,213,255,273,303,329,396,414	anti-inflammatory activity

Eucalyptus citriodora leaves proved the presence of Alkenes, alkyl halides and alkanes which shows major peaks at 678.94, 873.75, 1016.49, 1143.79, 1236.37, 1319.31, 1747.51, 2330.01, 2358.94, 2358.94, 2854.65 and 2924.09 (Table 2).

Evaluation of anti-insect activity

In the current study, the anti-insect activity of the methanolic extract was evaluated. Figure 2 showed that no mortality occurred in larvae fed with control diet.

Table 2: FT-IR peak values of Eucalyptus citriodora

S. No	Peak (Wave number cm^{-1})	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	678.94	66.824	0.654	684.73	673.16	2.006	0.029	Broad, Strong	C-H	Bending	Alkenes	610-700
2.	873.75	76.346	2.616	883.40	854.47	3.086	0.162	Strong	=C–H	Bending	Alkenes	650-1000
3.	1016.49	58.548	19.485	1128.36	885.33	40.212	14.093	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1143.79	76.777	2.245	1192.01	1130.29	6.149	0.437	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1236.37	81.991	1.670	1255.66	1201.65	4.369	0.230	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1319.31	81.932	1.949	1330.88	1301.95	2.315	0.127	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1747.51	78.179	7.967	1762.94	1737.86	2.029	0.473	-	-	-	Unknown	-
8.	2330.01	70.295	2.018	2333.87	2281.79	4.463	0.130	-	-	-	Unknown	-
9.	2358.94	61.534	16.751	2391.73	2349.30	5.476	2.004	Medium	C-H	Stretch	alkanes	2850-3000
10.	2854.65	82.423	5.366	2875.86	2800.64	4.394	0.477	Medium	C-H	Stretch	alkanes	2850-3000
11.	2924.09	78.332	7.476	2949.16	2877.79	5.740	1.076	Medium	C-H	Stretch	alkanes	2850-3000

Extract of *Eucalyptus citriodora* caused 58% mortality during the 10 days after treatment. The methanol extracts of *Eucalyptus citriodora* significantly affected survival of adult with 92%, during 32 days after treatment (Figure 3). The relation between exposure period and treatment was very significant $p < 0.01$. Significant insecticidal activity against *T. castaneum* larvae and adults was observed with crude methanol extract from *Eucalyptus citriodora*. Adults were more susceptible than larvae to extract of *Eucalyptus citriodora*. Sadek (2003)[22] showed that the time of pupation of *Spodoptera littoralis* (Boisduval) of larvae increased by the extract of *Adhatoda vasica* \(Nees). Jeyabalan et al. (2003)[23] have reported that extract of *Pelargonium citrosa* (Van Leenii), prolonged the duration of larval instars and the total developmental time of *Anopheles stephensi* (Liston). Zhong et al. (2001)[23] have also highlighted that extract from *Rhododendron molle* (G. Dorn) flowers extend the duration of development of *Pieris rapae* L. Scott et al. (2003)[24] have reported that pupal stage of *Leptinotarsa decemlineata* (Say) was less sensitive to the *Piper nigrum* L. extracts. These results suggest that there may be different compounds in extracts possessing different bioactivities. Methanol extracts of *Eucalyptus citriodora*, significant insecticidal effect and could be a potential grain protectant against *T. castaneum*. More recently, a study of Ramsewak et al. (2003)[25] showed that 1,8-cineol extracted from *E. citriodora*, presented anti-inflammatory activity and caused significant inhibition of the production of leukotriene B4 and thromboxane B2. Beside antimicrobial activity, the essential oil and its constituents have also been used for their herbicidal[26], insecticidal, and anti-leech[27] properties, as well as in integrated disease management against phytopathogenic fungi, nonspecific skin infections[28-38].

CONCLUSION

Medicinal property of plant extract is due to presence of secondary metabolites identified by GC-MS analysis. In the present study determined that thirty-one phytoconstituents were identified from methanol extract of the whole plant of *Eucalyptus citriodora*. This plant was highly active on accumulative mortality of *Tribolium castaneum* (adult).

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