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Chemical analysis of volatile oils in Eucalyptus species by GC mass

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Abstract

Essential oils have many possible applications and are thus commonly employed as a functional element. Hydrosols, byproducts of plant distillation, have been used in the food and cosmetics sectors as well as biological agriculture; yet, little is known about the volatile content of hydrosols. To determine the chemical content of the essential oils of *Eucalyptus* leaves. The yields of leaves essential oils from the hydrodistillation of *Eucalyptus* species were 0.6- 1% (based on fresh leaves). Gas chromatography-mass spectrometry (GC-MS) methods were employed for Oils found in the leaves of 20 types of *Eucalyptus* trees in Baghdad, Iraq. Terpenoids and alkaloids are abundant in both types of oils. The terpenoid contains mostly monoterpene hydrocarbons, sesquiterpenoids, and only six species contain the diterpenoid. Essential leaf oils exhibited greater activity, probably due to the higher p-cymene concentration in leaves. *Eucalyptus* constitutions contain high levels of terpene diversity, led by monoterpenes and sesquiterpenoids, with 17 out of 20 species containing alkaloids.Different types of constitutions demand different methods to use the species medically or commercially.

Keywords: *Eucalyptus*, Terpenoid, Alkaloid, GC/Mass.

1. Introduction

One of the most significant and commonly planted plant families in the world is the Australian native genus *Eucalyptus*, which is part of the family of Myrtaceae and with more than 900 species and subspecies. (1- 4). For its lumber, pulp, and essential oils, it is primarily grown, all of which have medicinal characteristics and therapeutic purposes (5). It has been widely spread over the globe. Essential oils and plant components, which are used to make natural goods, have gained a lot of attention in recent decades (6). James Cook, an explorer, and Sir Joseph Banks, a renowned

botanist, travelled to Australia in 1770 and discovered the species *Eucalyptus* (Myrtaceae) (7).
The presence of a specific oil ingredient gives eucalyptus oil its therapeutic effect. (Eucalyptol) 1, 8-cineole (8). Traditional remedies for respiratory infections like the common cold, the flu, and sinus congestion often include hot water extracts of *Eucalyptus* citriodora's dried leaves (9), *Eucalyptus camaldulensis* and *Eucalyptus urophylla* are also recognized for their bioactive components, which have demonstrated antimicrobial (10), antifungal (11), analgesic (9), anti-inflammatory, antioxidative, and antiradical activity (12). In addition, the volume and content of leaf oil may change seasonally and diurnally for particular plants within the *Eucalyptus* genus, depending on environmental circumstances. This is the case in several studies (13).
The volatile aromatic oil (essential oil, EO) extracted by steam distillation from the plant's leaves is one of the most extensively traded essential oils in the world by volume. Significant attention has been paid to the study of EO's anti-microbial, antibacterial, antiseptic, fungicidal, and nematocidal characteristics. (2, 14- 16). The essential oil has a long history of usage as a remedy for the symptoms of a variety of respiratory illnesses, including the common cold, influenza, sinusitis, and rhinitis (17). According to the results of several in vitro tests, the essential oil obtained from the leaves of the *E. globulus* plant has the potential to be employed as a natural antibiotic for the treatment of some infectious diseases brought on by Staphylococcus aureus and Escherichia coli (18). The use of EO to treat pork that had been stored in the refrigerator resulted in a considerable reduction in the number of *Pseudomonas* spp. and an increase in consumer acceptability (19). It was shown that the 1,8-cineol was not only responsible for the antifungal activity but for the full phytocomplex when it was tested against *A. flavus* and *A. parasitics*. The testing was done using EO from *E. globulus* and its main constituent 1, 8-cineole (20).
There is a strong demand for natural extracts that not only have a pleasant taste and/or scent but also can preserve products by inhibiting lipid deterioration, fungal growth, oxidation, and microbial spoilage. Because of their low toxicity, wide consumer acceptance, and various chances for exploitation, essential oils are gaining appeal as functional components in the food, beverage, and cosmetics sectors. This is a trend that is expected to continue.
This study aimed to analyze the chemical compounds of volatile oils in *Eucalyptus* species by using the GC mass device.

2. Materials and Methods

2.1. Samples collection

Two hundred and thirteen samples were collected from different regions in Baghdad between November 2020 and April 2021, from this number only 20 samples appeared variations in morphological characters. Diagnosis of this species by the sequencing genome of ITS of *Eucalyptus* using 18s ribosomal RNA gene (molecular method. A 150-gram sample was subjected to hydrodistillation using a Clevenger apparatus for 24 hours to extract essential oils. The resulting distillate was separated into two layers: the lower aqueous layer was removed, and the aromatic violet layer was dissolved in methanol. The extracted oils ranged from 0.2 to 1 ml in volume. The complex was then analyzed using gas chromatography-mass spectrometry (GC-MS) under the

following conditions: The Gas Chromatograph was an Agilent 7820A, while the analytical column was an Agilent HP-5ms Ultra Inert (30 m length x 250 µm inner diameter x 0.25 µm film thickness). The injection volume was 1 µl, with a pressure of 11.933 psi, and the GC inlet line temperature was set to 250°C. The carrier gas was HE 99.99%, and the injector temperature was set to 250°C for splitless injection. The mass spectrometer was scanned over the m/z range of 25-1000. The oven temperature program consisted of ramp1 from 60°C to 180°C (held for 3 min), ramp 2 from 180°C to 280°C (at a rate of 70°C/min), ramp 3 from 280°C to 380°C (at a rate of 80°C/min), at last, ramp 4 was held at 380°C for 3 min.

According to Cheng *et al*. (21), the Clevenger method was employed to extract volatile oil from the *Eucalyptus* plant, with certain modifications. A round bottom flask with a volume of two litres was charged with 150 grams of plant material, and 1850 ml of distilled water that has been acidified was added. The mixture was subjected to the Clevenger apparatus under heating, and the aqueous distillate was obtained. During distillation, oil was separated from water through the condensation of water vapor, and the distilled water was collected via a funnel connected to a glass continuer. The distillation process continued until the disappearance of color, after which the oil was separated from the water layer using a separating funnel. Neglecting the water layer yielded 3.5 ml of the brown oily layer.

2.2. The Use of Gas Chromatography (GC)

The essential oil was evaluated using an HP 6890 GC that has a flame ionization detector (FID) and an HP-5ms capillary column (30 m 0.25 mm i.e., film thickness 0.25 m). As described below, the column and analytical conditions were identical to those used in GC-MS. Without using any correction factors, to determine the essential oils' percentage composition, GC-FID peak regions were employed.

2.3. The analysis of oils was carried out using the gas chromatography-mass spectrometry (GC-MS) technique.

A Hewlett Packard 6890 gas chromatograph connected to a 5973-mass spectrometer equipped with an HP-5ms capillary column (30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness) was employed. The oven temperature was programmed to increase from 70 to 240°C at a rate of 5°C per minute. The ion source temperature and electron ionization energy were set at 240°C and 70 eV, respectively. The scanning range was set between 35 amu and 425 amu, with helium being employed as the carrier gas at a flow rate of 1 mL/min. A volume of 1.0 L of oil diluted in n-hexane (Merck) was injected into the GC-MS. The retention index (RI) was calculated by co-injection of an analogous sequence of n-alkanes (C8-C25) (22) under comparable experimental conditions and was used to identify the components (23- 27). The components were further identified by comparing their mass spectra to those present in the NIST 98 Libraries (on Chem Station HP) and the Wiley 7th Edition. Without utilizing any correction factors, the relative amounts of the different components were determined using the GC peak area (FID response).

3. Results

Essential oils from *Eucalyptus erytrocorys* have the following chemical makeup: Hydrodistillation of fresh *E. erythrocytes* L. leaves yielded an essential oil that was light yellow, had a strong odour, and had a density of 0.95 at room temperature. The percentage of oil extracted was 0.8% [(v/w), volume/dry weight]. Chromatographic examination revealed a complicated variety of components, with monoterpenes and sesquiterpenes consistently making up a substantial portion of the total. Table 1 contains the quantitative results and a list of the chemicals in elution order. Only 3% of the oil was not able to be pinned down to a specific chemical, while the remaining 97% was broken down into 20 different compounds. The chemical and analytic results of the *Eucalyptus* plant species and its active groups are shown in Table (1). The results show that the plant *Eucalyptus contains* 20 species. The more significant species found were *Eucalyptus erytrocorys*, *Eucalyptus macarthurii*, *Eucalyptus leucoxylon*, *Eucalyptus camadulensis* and *E. camadulensis var. obtuse* , with active oil groups Alkaloid & Terpenoid.

The most active group in the *Eucalyptus* species is the Terpenoid. Moreover, the most active subgroup of Terpenoid is the sesquiterpenoid followed by the monoterpenoid, and diterpenoid (if it is found), respectively. The Monoterpenoid contains β- pinene, α- phellandrene, 2- careen, Terpinen- 4- ol, Eucalyptol, Thymol, and p-cymene-7- ol. While then Sesquiterpenoid contains Ledol, Solavetivone, Viridifloral, α- santalol, Caryophyllene, and Aromandendrene. In addition, the Diterpenoid contains Heptadecane, trans- Geranylgeranyiol.

Table (1): Chemical structure of essential oil of the species of Eucalyptus.

Plant species	Types of active group		Area %	R.T
E. alba		Alkaloid	3.18	8.452, 12.930
	Terpenoid	monoterpenoid	47.97	4.040- 8.323, 8.582- 10.891, 13.512, 15.638
		sesquiterpenoid	48.84	11.872, 13.059,13.620- 15.390, 15.756- 17.504
		diterpenoid	-	-
E. alba var. alba		Alkaloid	5.31	8.981, 14.095, 16.253, 18.626
	Terpenoid	monoterpenoid	38.85	6.068- 8.873, 9.154- 10.600, 15.810
		sesquiterpenoid	48.22	13.005- 13.620, 15.519- 15.638, 15.983,16.490- 18.033, 20.120- 27.753
		diterpenoid	7.62	28.261- 28.757
E. botryoides		Alkaloid	2.19	16.879
	Terpenoid	monoterpenoid	57.49	4.202- 9.132, 10.751- 12.121, 17.127,19.177
		sesquiterpenoid	40.32	10.103, 13.836- 16.728,17.267- 18.896
		diterpenoid	-	-
E. camadulensis		Alkaloid	-	-
	Terpenoid	monoterpenoid	41.32	4.191- 8.582, 16.177
		sesquiterpenoid	56.29	11.894- 12.736, 13.599- 15.972, 17.364- 17.623
		diterpenoid	2.41	13.059
E. camadulensis var. obtuse		Alkaloid	1.32	16.221
	Terpenoid	monoterpenoid	68.93	4.385- 11.776, 15.983
		sesquiterpenoid	29.75	13.329- 15.832, 16.328- 29.987
		diterpenoid	-	-

Plant species	Types of active group		Area %	R.T
E. curtisii		Alkaloid	-	-
	Terpenoid	monoterpenoid	87.2	4.008- 8.593, 15.821
		sesquiterpenoid	12.8	14.775- 15.390, 16.080- 22.672
		diterpenoid	-	-
E. delegatensis		Alkaloid	6.77	9.305, 10.902
	Terpenoid	monoterpenoid	44.94	3.975- 9.042, 9.575- 10.384, 11.161- 11.333, 14.786
		sesquiterpenoid	48.28	13.869- 14.484, 14.915- 17.084, 17.494- 19.360
		diterpenoid	-	-
E. erythrocorys		Alkaloid	1.40	8.517
	Terpenoid	monoterpenoid	87.23	4.029- 8.345, 8.658- 11.010, 15.228- 15.379
		sesquiterpenoid	11.36	12.747- 15.088, 15.659- 22.219
		diterpenoid	-	-
E. globoidea		Alkaloid	6.7	8.593, 16.393
	Terpenoid	monoterpenoid	72.07	4.191- 8.399, 8.744- 10.438, 13.534- 14.020, 15.800,17.127, 17.612- 19.586
		sesquiterpenoid	21.22	11.689- 13.103, 14.311- 15.627,15.951- 16.123
		diterpenoid	-	-
E. leucoxylon		Alkaloid	16.06	19.586 – 20.665
	Terpenoid	monoterpenoid	34.48	4.213- 9.111, 16.868- 17.127
		sesquiterpenoid	49.46	13.825- 16.652, 17.310- 18.216, 21.992- 24.754
		diterpenoid	-	-
E. macarthurii		Alkaloid	1.58	13.512, 15.228
	Terpenoid	monoterpenoid	81.83	4.191- 11.031, 15.616
		sesquiterpenoid	16.58	12.736- 13.059, 14.688- 15.098, 15.400, 15.756- 21.647
		diterpenoid	-	-
E. nicholii		Alkaloid	0.50	9.003
	Terpenoid	monoterpenoid	54.39	4.385- 8.895, 9.154- 11.020, 11.732, 14.343, 16.026, 16.911,18.087
		sesquiterpenoid	45.11	11.527, 12.800- 14.160, 14.526- 15.789, 16.285- 16.717, 17.450- 17.806, 22.381
		diterpenoid	-	-
E. pauciflora		Alkaloid	1.14	14.257, 16.253
	Terpenoid	monoterpenoid	51.58	4.094- 6.068, 15.595
		sesquiterpenoid	47.27	11.862- 13.027, 13.610, 14.581- 15.336, 15.724- 16.091, 16.371- 17.483
		diterpenoid	-	-
E. siderophloia		Alkaloid	16.47	16.555 – 18.162
	Terpenoid	monoterpenoid	53.46	3.975- 8.561, 15.368
		sesquiterpenoid	30.07	12.714- 15.185, 15.576- 16.134, 18.918- 22.100
		diterpenoid	-	-
		Alkaloid	5.40	17.008
	Terpenoid	monoterpenoid	11.35	4.169- 5.842, 11. 743
		sesquiterpenoid	80.28	10.092, 12.887- 16.814, 17.278- 19.295
		diterpenoid	2.96	18.659

Plant species	Types of active group		Area %	R.T
E. tereticornis	Alkaloid		-	-
	Terpenoid	monoterpenoid	62.35	4.418- 8.776, 9.154- 11.732, 18.076
		sesquiterpenoid	37.20	13.329- 15.929, 16.350- 16.911, 17.795
		diterpenoid	0.45	17.461
E. tereticornis var. rotunda	Alkaloid		12.84	15.195, 16.555 – 18.918
	Terpenoid	monoterpenoid	64.65	3.954- 9.931, 15.379- 15.595
		sesquiterpenoid	22.51	12.714- 15.066, 15.759- 16.145
		diterpenoid	-	-
	Alkaloid		13.66	4.148, 6.565, 7.266 – 11.874 16.717 – 16.965, 19.015 – 27.333
	Terpenoid	monterpenoid	11.07	5.939, 14.699- 15.703, 18.065, 13.163
		sesquiterpenoid	72.57	6.155, 14.505, 20.169- 25.326, 28.088
		diterpenoid	2.7	6.899, 27.689
E. vicina	Alkaloid		1.61	9.078
	Terpenoid	monoterpenoid	45.85	4.234- 8.884, 9.240- 11.344, 13.081, 17.310- 17.515
		sesquiterpenoid	52.55	12.606- 12.930, 13.254- 16.760, 17.742- 20.762
		diterpenoid	-	
	Alkaloid		2.52	9.003
	Terpenoid	monoterpenoid	67.98	4.569- 8.884, 9.165- 12.639, 14.106, 15.983, 18.626- 19.176
		sesquiterpenoid	29.2	13.653, 15.336- 15.821, 16.253- 18.022, 20.158, 22.338
		diterpenoid	0.30	20.277

Figure 1. shows the Eucalyptus plant chemical pounds for oils compositions of the alkaloid, and **Figure 2.** shows the terpenoids.

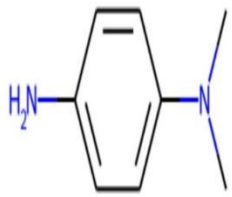
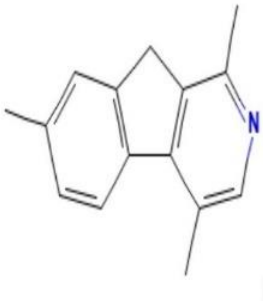
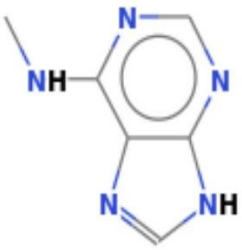
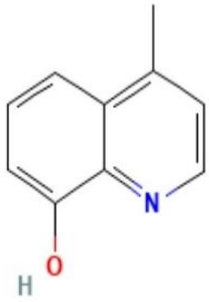
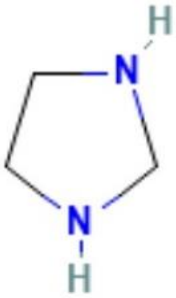
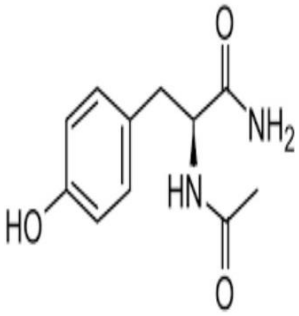
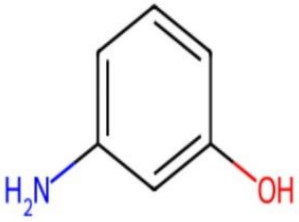
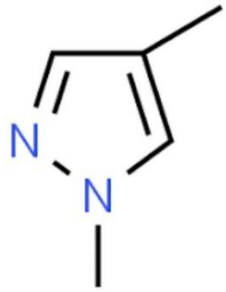
			
1,4 Benzendiamine,N,N-diethyl	1,4,7- trimethyl-2-azafluorenone	1H-purin-6- amine, N-methyl	8- quinolinol, 4-methyl
			
Imadazolidine,1,3- diphenyl -2- propyl	N, Acetyl-L- tyrosiamide	Phenol, 3- amine	Pyrazol,1,4- dimethyl

Figure 1. Chemical compositions of alkaloids in the *Eucalyptus* plant.

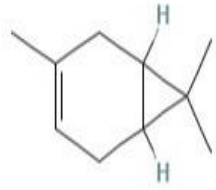
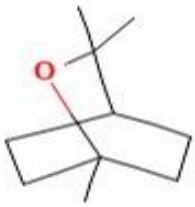
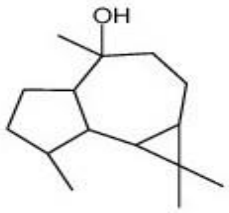
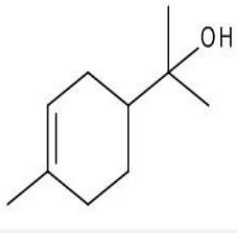
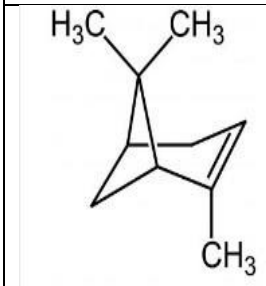
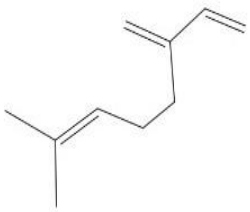
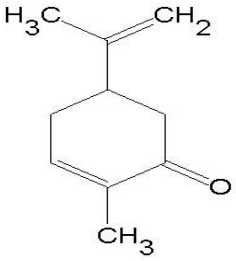
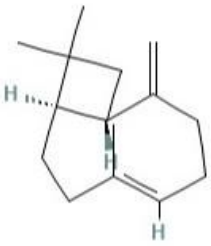
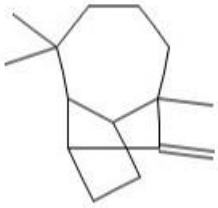
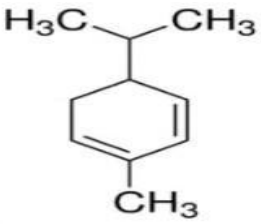
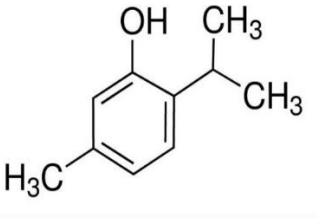
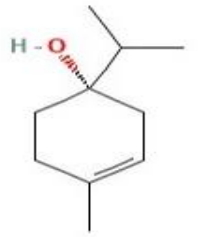
			
3- Carene	Eucalyptol	Viridiflorol	α- Terpenoil
			
α - pinene	β- myrcene	Carvone	Caryophyllene
			
Longifolene	Phellandrene	Thymol	Terpenine- 4- ol

Figure 2. Chemical compositions of terpenoids in E*ucalyptus* plant

The findings of the GC/MS analysis revealed that *Eucalyptus erytrocorys*, *E. macarthurii*, *E. leucoxylon*, and *E. camadulensis* diving essential oils mainly contained volatile compounds. The *Eucalyptus erytrocorys* dives essential oils were primarily composed of volatile compounds. At the first 17 minutes, as shown in Figure (3). The volatile oils of *Eucalyptus macarthurii* show a higher peak in the first 7 minutes, while the other compounds appear until 22 minutes, as shown in Figure (4). The volatile oils of *Eucalyptus leucoxylon* show a higher peak in the first 9 minutes, and no volatile was found from 9 until 14 minutes to 25 minutes, as shown in Figure (5). As depicted in Figure (6), *Eucalyptus camadulensis* volatile oils demonstrate a greater magnitude of peak within the initial 18-minute timeframe.

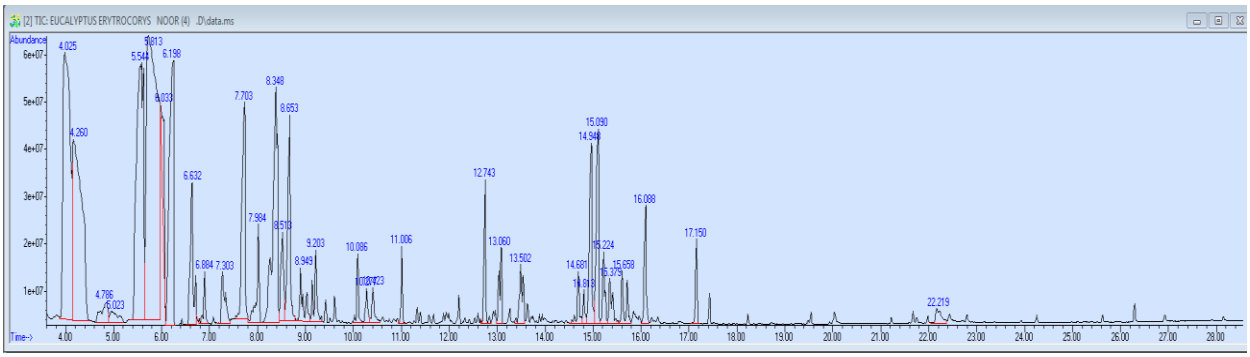


Figure 3. The results of GC/Mass of *Eucalyptus erytrocorys*

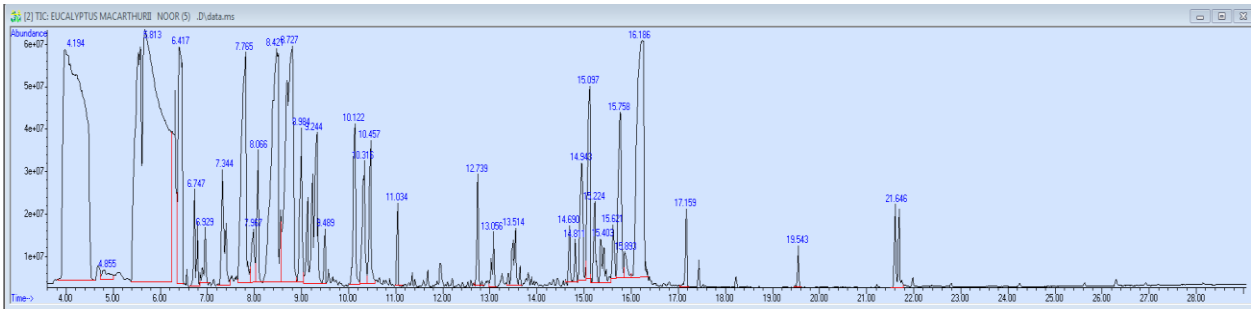


Figure 4. The results of GC/Mass of *Eucalyptus macarthurii*

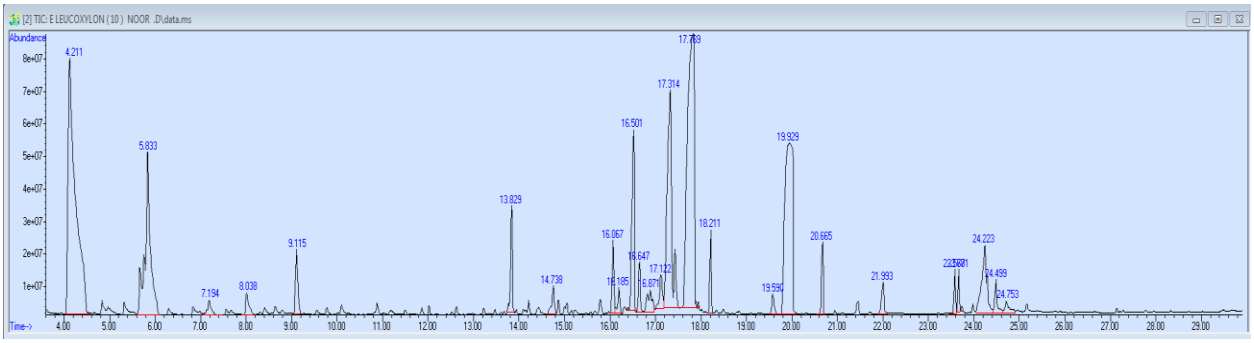


Figure 5. The results of GC/Mass of *Eucalyptus leucoxylon*

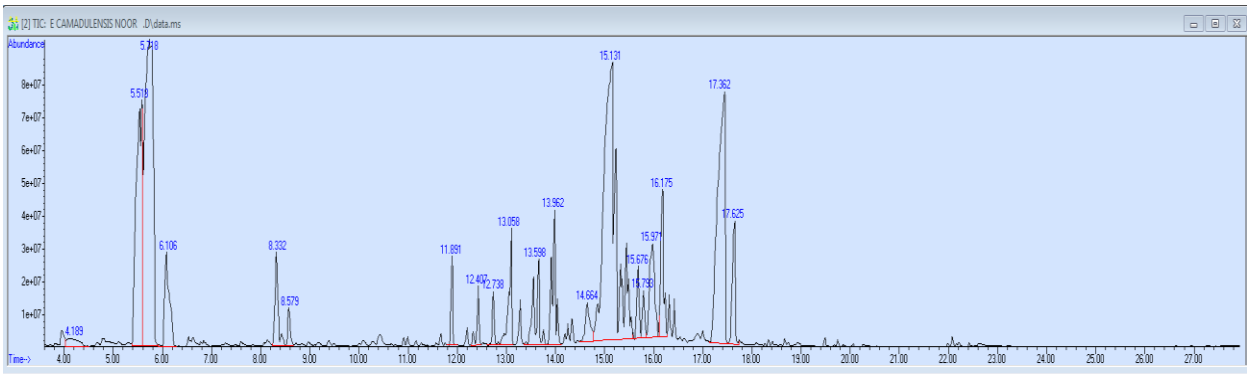


Figure 6. The results of GC/Mass of *Eucalyptus camadulensis*

4. Discussion

In the literature, very little is known about the chemical makeup of *Eucalyptus* essential oils. Traore, *et al.* (28) noticed that a South African *E. dives* essential oil's heavy end included a lot of piperitone whereas the light end included significant quantities of a-phellandrene, a-thujene, and a-terpinolene along with other minor volatiles (29).

Our results disagreed with Rahimi-Nasrabadi *et al.* (30) that found that the piperitone (42.9%) and aphellandrene (30%) were the predominant components of *E. dives* essential oil extracted from the plant's Australian leaves. The final result for piperitone (40.5%) was comparable to ours, however, the number for a-phellandrene (30.0%) was much higher than in this study.

The diversity of terpenes found in different species of *Eucalyptus* trees in Australia is vast and well-known. *Eucalyptus* is particularly renowned for its foliar terpenes. Our study of two *Eucalyptus* genomes has revealed that the most extensive gene family of terpene synthases known to date is responsible for this remarkable variety. Terpenes in Eucalyptus have been extensively researched over the last century, with over 2,000 publications documenting their links to numerous plant processes, the ecosystem, and their global impact on the environment due to their effect on forest fires and atmospheric concentrations. Recent studies into how quantitative variation is controlled and recent evolutionary analyses of the Myrtaceae, terpenes, along with the discovery of the diverse TPS gene family in the *Eucalyptus* genome, present a unique opportunity to comprehend the origin of variation in *Eucalyptus* terpenes and their manipulability in the world's most commonly planted hardwood tree (25).

Larger gene families are linked to species like *Eucalyptus* and grape that have evolved specialized storage organs for terpenes (18). Terpene synthases genes (TPS) in *Eucalypts* are thought to take place in the secretory cavity cells, where the terpenes and other non-volatile ingredients like oleuropeyl glucose esters are kept (28, 29), however, this has not been confirmed by any investigations.

Two terpene chemotypes being present in *E. grandis* is suggested by the fact that the monoterpene fraction is dominated by either -pinene or 1,8-cineole. These two monoterpenes are likely the results of separate TPSs because of their distinct carbocation origins. None of the TPS genes described by Külheim *et al*. (26), however, were effective in generating significant quantities of either chemical.

The oil of certain plants contains numerous sesquiterpenes, and previous studies have shown that the oil can be composed of up to 30 different sesquiterpenes, with bicyclo germacrene and spathulenol being the most prominent. We have discovered a second sesquiterpene synthase that is capable of producing 15 sesquiterpenes and a bicyclo germacrene synthase that can generate an additional four molecules. It may not be necessary for Sesquiterpene synthases will be expressed in abundance in mature leaves to account for the diverse oil profiles previously observed. The expression of just a few sesquiterpene synthases may be sufficient to produce the observed sesquiterpenes. Therefore, our findings expand our understanding of the mechanisms underlying the production of sesquiterpenes in plants, which may have practical implications for the development of new plant-based products.

Several studies have investigated the chemical composition of essential oils extracted from different *Eucalyptus* species. For instance, Elaissi *et al.* (27) analyzed the essential oil of 15 *Eucalyptus* species and found that 1, 8-cineole and spathulenol were the most abundant compounds. Similarly, Traore *et al.* (28) reported that the essential oil of *E. camaldulensis* from Mali contained 1, 8-cineole, p-cymene, a-pinene, limonene, a-terpinene, and trans-pinocarveol as primary constituents. Three different species of Eucalyptus's essential oils have been identified in another study of their leaves 1, 8-cineole, -pinene, terpinene-4-ol, -terpineol, aromadendrene, and viridiflorol as the most common compounds (29).

In addition, the chemical content of essential oils from *E. procera* grown in central Iran was studied by Rahimi-Nasrabadi *et al.* (30), who identified 1,8-cineole, -pinene, and viridiflorol as the primary components of the oil. Similarly, a study of essential oils from the aerial portions of *E. loxophleba* isolated 1, 8-cineole, methyl amyl acetate, aromadendrene, viridiflorol, and -pinene as the major compounds (31). Our findings align with these previous investigations, particularly for the most significant components identified in *Eucalyptus* essential oils. These studies collectively demonstrate the complex and varied chemical profiles of *Eucalyptus* essential oils.

5. Conclusion

In this study, we have analyzed the *Eucalyptus* constitutions and we observed that it primarily contained 20 species' significant terpene synthase gene families. We found out that the greatest amount of TPS genes are present in most species, which is indicative of high levels of terpene diversity. Followed especially by the monoterpenes by the sesquiterpenoid. The diterpenoid of Terpenoid was found in six *Eucalyptus* species only. Seventeen out of 20 species of *Eucalyptus* were found to contain alkaloids. Resolution and identification of the wide range of eucalypt terpenoid compositions place high demands on analytical methods. The presence of chemical variants highlights the significance of selective culture in meeting niche industrial and commercial needs.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

The research was not subject to research ethics as it included conducting a survey of the species of the *Eucalyptus* genus in Baghdad and studying the types of active compounds present in the studied plant without using laboratory animals or patients.

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