

Phytochemical Analysis and Antioxidant Activity of Eucalyptus Leaf Essential Oil

Rozhgar Mustafa Ahmed  

Department of Biotechnology and Crop Sciences – College of Agricultural Engineering Sciences –
University of Sulaimani – Sulaimani – Iraq

ABSTRACT

The genus *Eucalyptus* belongs to the Myrtaceae family and is a large tree with several uses due to its content of some useful active components. These phytochemical components have been used for many years to treat various illnesses. The extraction process and identification of these components are essential points to maximize and develop the level of content. The extraction of essential oil from the eucalyptus leaf was investigated using hydrodistillation based on four locations in the Sulaimani region. Furthermore, the antioxidant activity was also studied using a free radical scavenging assay. The highest essential oil content was noticed in Qara Dagh in both fresh and dry leaves (2.33% and 4.67%), while the lowest level of fresh leaves was detected in Sharbazher (1.27%) and dry leaves in Kalar (2.40%). Fifty-eight chemical components were identified in eucalyptus essential oil using GC-MS analysis. Eucalyptol and α -pinene were found to be major components at all locations. The greatest amounts of eucalyptol and α -pinene were observed in Qara Dagh ($52.309 \pm 0.967\%$ at 6.163 min) and ($14.652 \pm 0.194\%$ at 3.703 min), respectively. The essential oil scavenging effect indicated that the Sharbazher location gave a maximum scavenging effect of 60.156%. Compared to the lowest scavenging effect, which was achieved by the Qara Dagh location with a value of 47.613%.

Key words: DPPH assay, *Eucalyptus camaldulensis*, GC-MS analysis, Medicinal plants, Phytochemical constituents.



Copyright© 2025. The Author (s). Published by College of Agricultural Engineering Sciences, University of Baghdad. This is an open-access article distributed under the term of the Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cite.

Received: 16/8/2024, Accepted: 24/11/2024, Published: 30/6/2026

INTRODUCTION

Hundreds of plants that grow wild naturally in Kurdistan belong to different plant families, and they are genera with distinct aromas. These plants contain essential oils that are specific to their organs. Some of these plant species grow in large wild populations, and their chemical components have not been examined yet. Both quantities and qualities of these components change based on the elevation, growth areas, pathogen interaction, and environmental conditions (Dudareva *et al.*, 2004; Ahmed, 2017; Abdulrahman, 2023). Within these, the genus *Eucalyptus* is a large genus belonging to the Myrtaceae family, with over 900 species (Quan *et al.*, 2015; Surbhi *et*

al., 2023). More than 300 species in their leaves contain volatile oil (Brooker, 2000; Dhakad *et al.*, 2018). Researchers discovered that changes in climate conditions, such as humidity, temperature, and rainfall, also affect the eucalyptus bioactive components (essential oils, phenols, and flavonoids) and their pharmacological properties (Oliveira *et al.*, 2014; Heikal, 2017). *Eucalyptus* is native to the south of Australia and Tasmania Island and is known as one of the most important and widely grown species of hardwood forest around the world. It is also naturalized on many different continents (Yost *et al.*, 2021). Globally, medicinal plants, including *Eucalyptus*, are one of the most important

sources of medicine. Traditionally and in modern medicine, most people use these plants for several treatments. They possess antimalarial, nematocidal, antidiabetic, chemotherapeutic, antibacterial, cytotoxic, anti-cancerous, herbicidal, antifungal, antiseptic, antiviral, anti-inflammatory, analgesic, and antioxidant properties. Eucalyptus is also commercially used in the cosmetic and pharmaceutical industries (Dhakad *et al.*, 2018; Surbhi *et al.*, 2023). Eucalyptus also plays an important role as a source of cellulose pulp, energy, and timber, which has become more conscious of using renewable resources (Labate *et al.*, 2009). Additionally, eucalyptus essential oil production is used as a raw material in many countries on a commercial scale in phytotherapy, cosmetics, aromatherapy, beverages, perfume, and food (Vecchio *et al.*, 2016; Arun & Inderjeet, 2022; Cristina *et al.*, 2023). The gas chromatography-mass spectrum (GC-MS) technique is considered an efficient method to determine the bioactive components of terpenoid, flavonoid, and phenol compounds from Eucalyptus plant materials (Bourakna *et al.*, 2022; Nagappan *et al.*, 2024). The extraction methods using different solvents, including water, ethanol, acetone, hexane, and methanol extractions, and extraction methods like Soxhlet, steam distillation, supercritical fluid extractions, and water distillation using the Clevenger apparatus, are also used as a beginning step in the Eucalyptus species study to produce the highest production of plant material extraction used for biochemical analysis (Abed *et al.*, 2015; Kumar *et al.*, 2021; González-Hernández *et al.*, 2024). It was found that the most important bioactive components of eucalyptus leaf essential oil were 1,8-cineole, α -terpineol, p-cymene, limonene, globulol,

and guaiene (Sebei *et al.*, 2015). Eucalyptus essential oils have been used for treating respiratory problems for a long folkloric history. The main terpenoid constituent, such as 1, 8-cineole, is also studied in clinical and preclinical settings (Galan *et al.*, 2020). Moreover, oil extractions like 1,8-cineole and citronellol act as antiseptics and reduce the pain of sore throats, colds, coughs, and many other infections (Mulyaningsih *et al.*, 2011; Pries *et al.*, 2023). Thus, using eucalyptus biotechnological techniques is key to improving, conserving, modifying, and multiplying medicinal plant genotypes (Labate *et al.*, 2009). The study aims to determine a structure for extracting essential oils and a high level of the main bioactive component content found in plant leaves in different areas of the Sulaimani region based on the meters above sea level. Identify and characterize phytochemical component content by the use of GC-MS and its antioxidant capacity.

MATERIALS AND METHODS

Sample collection and preparation:

The identification of chemical components of some wild eucalyptus volatile oils, which are part of the flora of Kurdistan, was performed. Fresh leaves of eucalyptus from the native local flora in Kurdistan were collected in four different areas of the Sulaimani region (Sharbazher, Qara Dagħ, Bakrajo, and Kalar) during autumn 2022, based on the other meters above sea level using the Global Positioning System (GPS) (Table 1). They were freshly chopped, just after collection and extraction, to compare the quantity of oil with dry samples; other samples were dried for 3 days at room temperature in a dark place to avoid the volatilization of essential oils. The dry samples were then milled by an electric blender for distillation.

Table 1. The collection of Eucalyptus leaves at different elevations

Locations	Latitude (North)	Longitude (East)	Altitude (masl)
Sharbazher	35° 52' 44"	45° 35' 8"	1446
Qara Dagħ	35° 20' 1"	45° 23'50"	982
Bakrajo	35° 33' 36"	45° 21'46"	758
Kalar	34° 37' 45"	45° 19' 20"	231

The protocol for Eucalyptus essential oil extraction: The sample extraction was carried out using the Clevenger apparatus. For each

sample extraction, 50g of fresh and dried samples was added to 500 mL of distilled water. All Samples were prepared in triplicate.

After 3 hours of extraction. The essential oil was collected, dried over anhydrous sodium sulfate, and stored at +4 °C until used (Tum *et al.*, 2016).

GC-MS analysis of bioactive compounds:

The analysis was achieved using an Agilent Technologies 7890A gas chromatograph prepared with a column Agilent 190915-433:325 °C (30 m × 250 µm × 0.25 µm) coupled with an Agilent Technologies 5975C inert XL MSD mass spectrometer. The column temperature was 40 °C as an initial temperature, with a 10 °C increase per minute to a temperature of 280 °C. The injector port temperature was 290 °C, the heater temperature was 250 °C, and the carrier gas was helium at a 1 mL/min flow rate. The injection mode was split into a 5:1 ratio. Determined peaks were obtained by comparing mass spectra to a mass spectral database (Anil *et al.*, 2010).

Eucalyptus essential oil antioxidant activity:

Free radical scavenging activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay to assess the antioxidant potential of the essential oil from different locations (James *et al.*, 2009). As a positive control, 100 mg of ascorbic acid was dissolved in methanol as a stock solution. The various

concentrations of 10, 20, 40, 80, and 100 µg/mL Eucalyptus essential oil from each location were prepared by dissolving in Dimethylsulfoxide (DMSO). The same serial concentration of ascorbic acid was prepared in Figure 1. An equal volume of diluted essential oil solution in phosphate buffer (pH 7.4) was mixed with one ml of 100 µM of DPPH in methanol, well mixed, and the test tube was wrapped with aluminum foil, then kept in the dark for 20 minutes. The absorbance measurement was conducted at 517 nm using a spectrophotometer (UV/Vis spectrophotometer UVM6100). The inhibition capacity IC% is calculated as follows (Mishra *et al.*, 2010).

$$IC (\%) = \frac{(A \text{ control} - A \text{ test})}{(A \text{ control})} \times 100$$

Statistical analysis: A one-way ANOVA was performed to assess the differences between locations. The results were presented as the mean values of three replications. Mean comparisons were conducted using the LSD test at significance levels of $p \leq 0.01$ and $p \leq 0.05$. Additionally, the average and standard deviation (Mean ± SD) were calculated for the abundance percentages (Mahmoud & Muhammad, 2000).

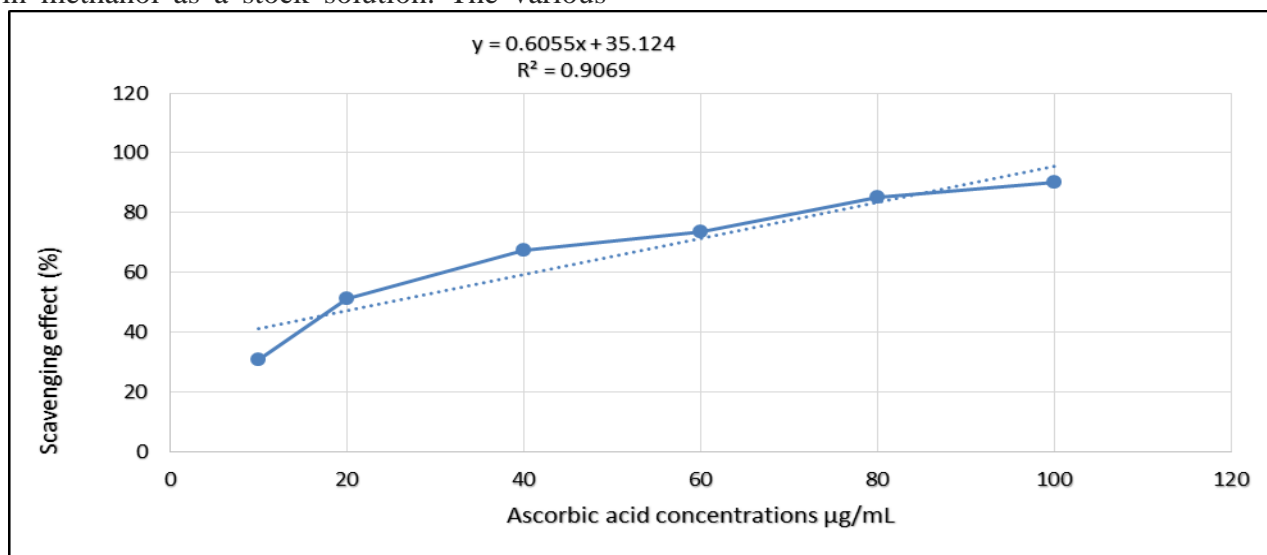


Figure 1. Free radical DPPH scavenging effect of the standard compound (ascorbic acid)

RESULTS AND DISCUSSION

Essential oil extraction: All the results were presented as means. The analysis of variance indicated highly significant differences between plants at different locations. Highly significant effects were detected for fresh and

dry leaves (Table 2). The results show that hydro-distillation was an efficient method using the Clevenger apparatus to extract eucalyptus essential oils. This is in agreement with those reported by Dalal (2017) and Immaroh *et al.* (2021), who showed that

hydro-distillation was a superior method to extract Eucalyptus essential oil and could protect the plants; initial quality compared to others like ultrasound, solvent, microwave, and supercritical fluid, and hydro-distillation extractions that might affect the production of bioactive compound content. In terms of dry samples, the mean of Qara Dagħ showed the greatest amount of essential oil extract (4.67%) compared to other locations; however, the lowest amount was found in Kalar (2.40%) (Table 2 and Figure 2). Similarly, regarding fresh samples, Qara Dagħ revealed the highest

level of extract (2.33%), and Sharbazher offered the lowest level (1.27%). Overall, both dry and fresh samples of Qara Dagħ were exposed to greater levels of essential oil extraction found in Eucalyptus leaves, possibly due to their higher above-sea levels, which causes them to produce and store more bioactive compounds in plant materials with less volatility. In support of this current finding, Nataraj *et al.* (2022) reported that plants' total number of essential oil compounds significantly increased at different elevations .

Table 2. The means of fresh and dry Eucalyptus leaves essential oil extraction at different locations

Locations	Fresh leaves (%)	Dry leaves (%)
Sharbazher	1.27	3.47
Qara Dagħ	2.33	4.67
Bakrajo	2.00	3.13
Kalar	1.50	2.40
LSD ($p \leq 0.05$)	0.37	0.84
LSD ($p \leq 0.01$)	0.54	1.23

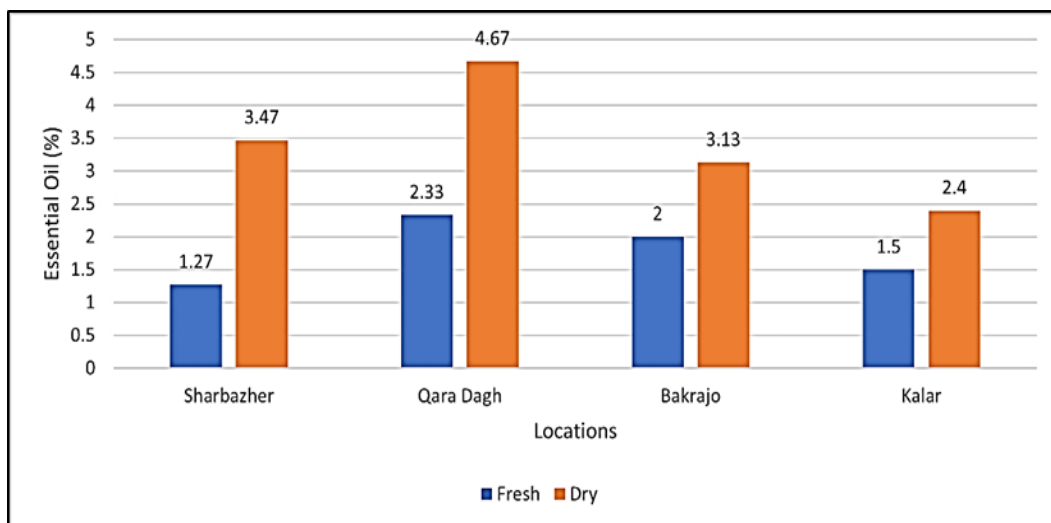


Figure 2. Fresh and dry Eucalyptus leaves essential oil extraction using hydro-distillation based on different locations

Identification of Eucalyptus essential oil components using GC-MS analysis: As a result, fifty-eight chemical components of Eucalyptus essential oil were identified by GC-MS analysis at different areas and retention times based on four studied locations, as shown in Table 3 and Figures 3, 4, 5, and 6. Monoterpenes and sesquiterpenes were indicated as the most common active components. Among all components, only eight were detected in all locations, including d- α -pinene, α -pinene, limonene, eucalyptol, γ -

terpinene, terpinene-4-ol, α -terpineol, and aromadendrene, where eucalyptol was found to be the main monoterpene active component (Maciel *et al.*, 2010; Bachheti, 2015; Cmiková *et al.*, 2023). This is in agreement with those indicated by Zhou *et al.* (2021), who showed that monoterpenes and sesquiterpenes were the main active ingredients found in different Eucalyptus plant species. The highest amount of eucalyptol was observed in Qara Dagħ ($52.309 \pm 0.967\%$ at 6.163 min), while the minimum value was noticed in Bakrajo

(35.99±0.011 % at 6.132 min). α -Pinene was also shown to be the most abundant active component after eucalyptol was isolated from Eucalyptus essential oil. The maximum amount of α -pinene was observed in Qara Dagh (14.652±0.194 at 3.703 min); however, the minimum level was found in Kalar (8.626±0.297 at 3.672). Similarly, Almas *et al.* (2021) revealed that the major components of

Eucalyptus leaf essential oil were eucalyptol and α -pinene 54.29% and 7.78%, respectively. Overall, it was clear that the greatest active components were indicated in Qara Dagh, and the smallest amount was noticed in Kalar. This might be due to the fact that plants from lower altitudes produce lower bioactive compounds compared to those from higher altitudes (Camas *et al.*, 2014).

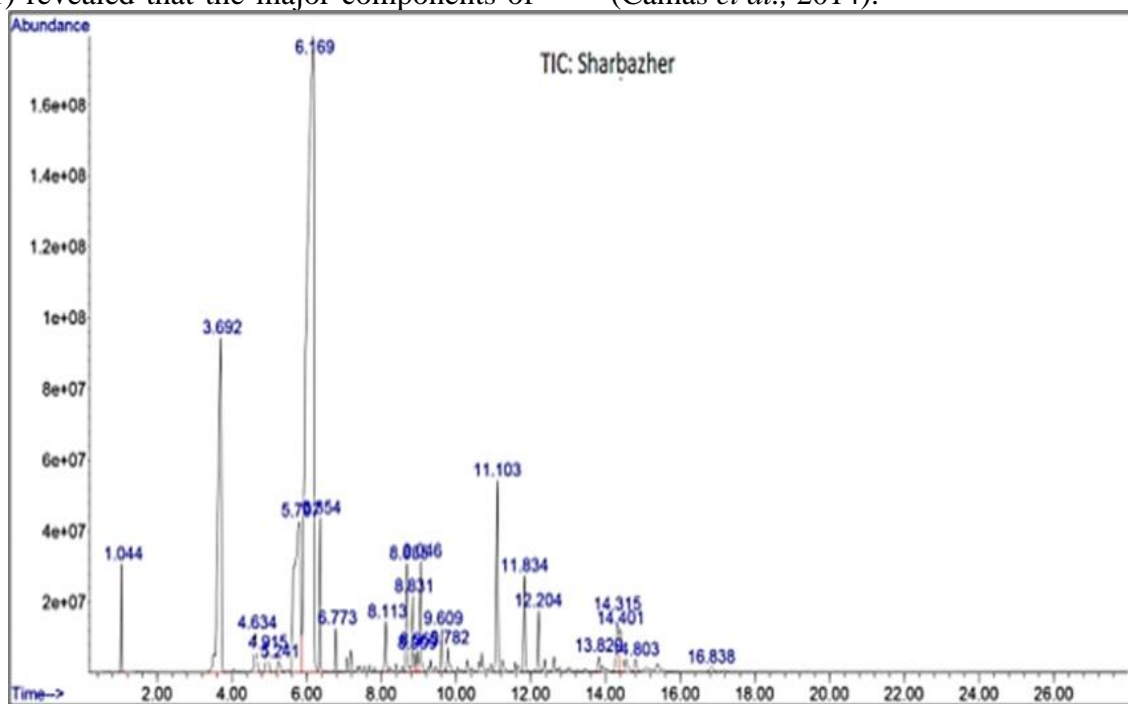


Figure 3. GC-MS analyses of Sharbazher Eucalyptus leaf essential oil

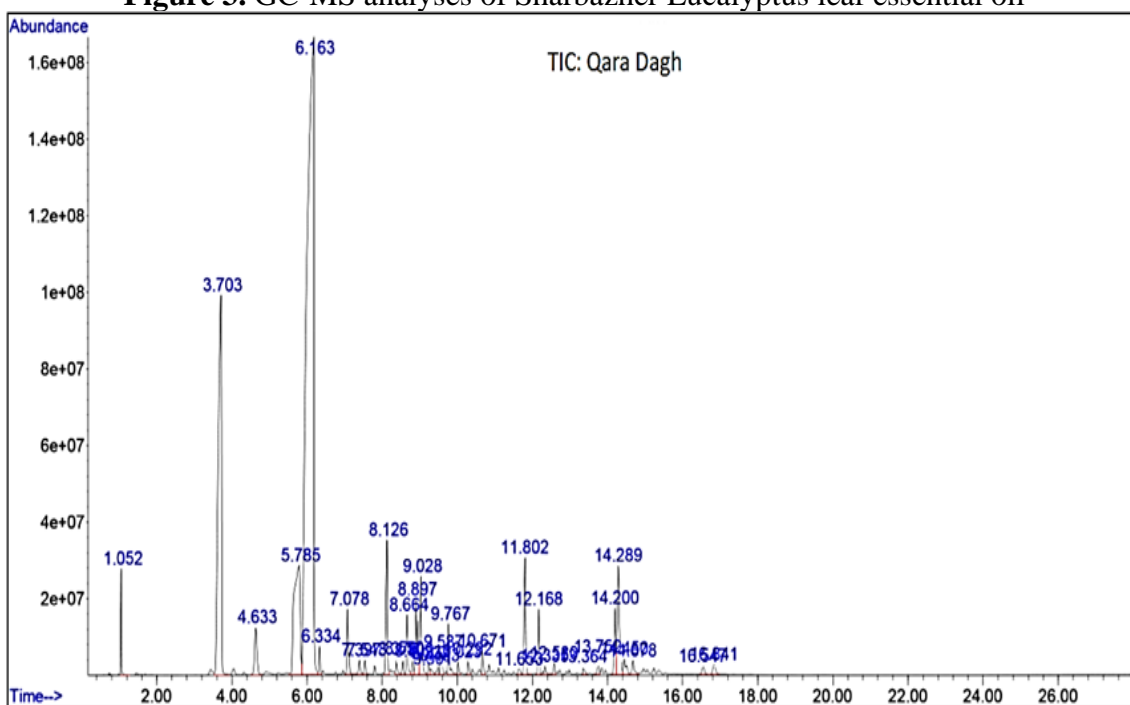


Figure 4. GC-MS analyses of Qara Dagh Eucalyptus leaf essential oil

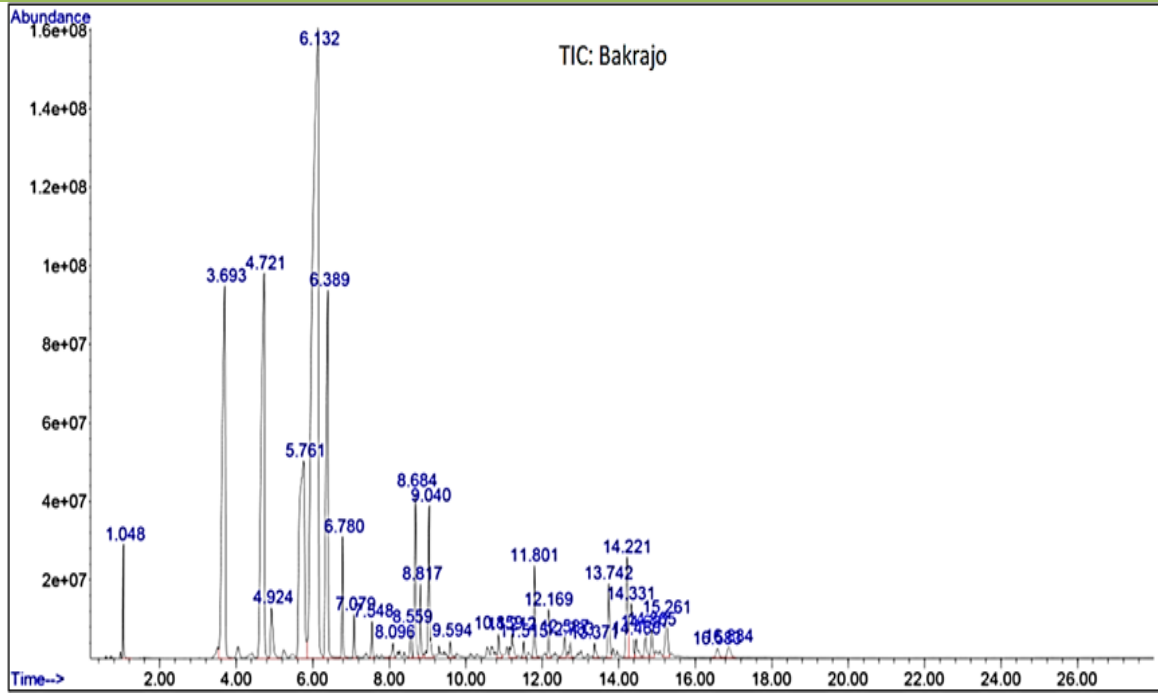


Figure 5. GC-MS analyses of Bakrajo Eucalyptus leaf essential oil

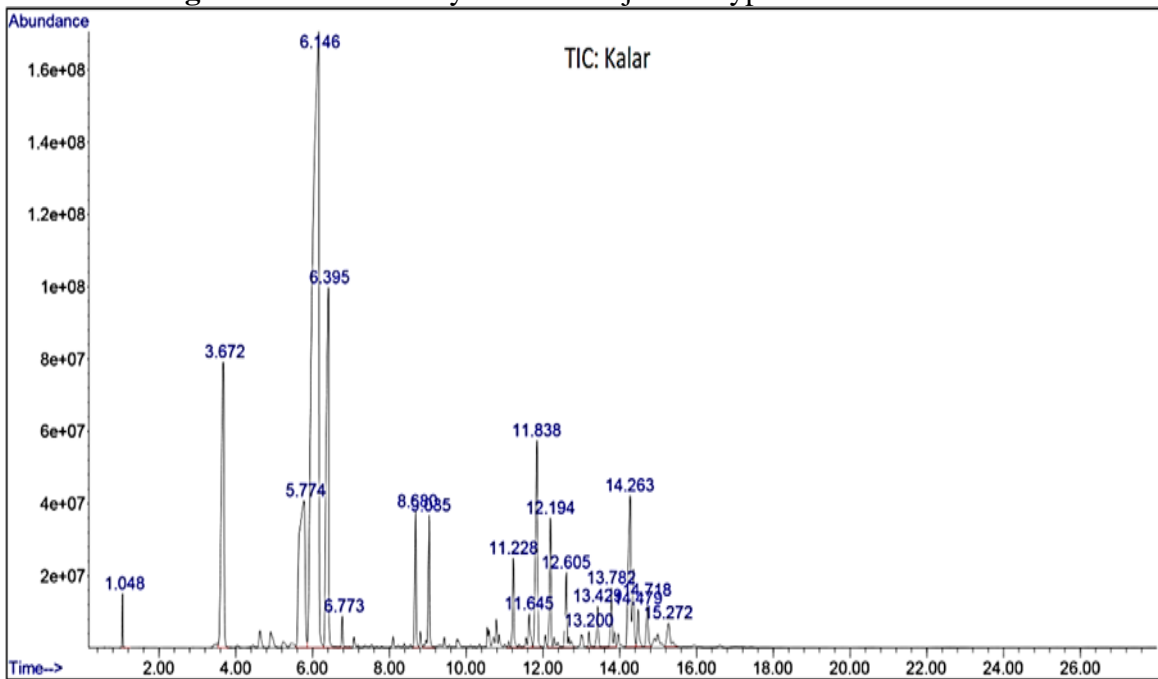


Figure 6. GC-MS analyses of Kalar Eucalyptus leaf essential oil

Table 3. The GC-MS analysis of major bioactive components identified in the essential oil of Eucalyptus leaves

Peaks	Components	Sharbazher (1446)			Qara Dagh (982)			Bakrajo (758)			Kalar (231)		
		RT (min.)	Relative Abundance (%)	Similarity* (%)	RT (min.)	Relative Abundance (%)	Similarity* (%)	RT (min.)	Relative Abundance (%)	Similarity* (%)	RT (min.)	Relative Abundance (%)	Similarity* (%)
1	d- α -Pinene	1.044	1.012 \pm 0.013	79	1.052	0.893 \pm 0.029	78	1.048	0.809 \pm 0.006	80	1.048	0.425 \pm 0.078	80
2	α -Pinene	3.692	12.926 \pm 0.093	93	3.703	14.652 \pm 0.194	88	3.693	11.208 \pm 0.011	82	3.672	8.626 \pm 0.297	91
3	β -Pinene	4.634	1.216 \pm 0.079	84	4.633	1.324 \pm 0.086	84	4.721	12.245 \pm 0.008	80	/	/	/
4	β -Myrcene	4.915	0.74 \pm 0.033	81	/	/	/	4.924	1.134 \pm 0.002	84	/	/	/
5	α -Phellandrene	5.241	0.44 \pm 0.073	90	/	/	/	/	/	/	/	/	/
6	Limonene	5.797	11.221 \pm 0.131	95	5.785	8.02 \pm 0.318	95	5.761	9.951 \pm 0.003	95	5.774	9.737 \pm 0.223	95
7	Eucalyptol	6.169	50.903 \pm 0.011	99	6.163	52.309 \pm 0.967	97	6.132	35.99 \pm 0.011	85	6.146	43.805 \pm 0.739	97
8	γ -Terpinene	6.354	2.185 \pm 0.078	79	6.334	0.365 \pm 0.010	79	6.389	7.911 \pm 0.019	79	6.395	10.126 \pm 0.124	79
9	(+)-2-Carene	6.773	0.603 \pm 0.073	95	/	/	/	6.78	1.442 \pm 0.008	95	6.773	0.459 \pm 0.079	95
10	Valeric acid, 3-methyl butyl ester	/	/	/	7.078	1.162 \pm 0.003	/	7.079	0.53 \pm 0.030	71	/	/	/
11	α -Pinene epoxide	/	/	/	7.397	0.294 \pm 0.005	98	/	/	/	/	/	/
12	Fenchol	/	/	/	7.543	0.267 \pm 0.003	/	7.548	0.468 \pm 0.001	90	/	/	/
13	E)-3(10)-Caren-4-ol	/	/	/	/	/	/	8.096	0.29 \pm 0.017	94	/	/	/
14	trans-p-mentha-1(7),8-dien-2-ol	8.113	0.951 \pm 0.052	87	8.126	2.677 \pm 0.071	86	/	/	/	/	/	/
15	1,7-Octadiene-3,6-diol, 2,6-dimethyl-	/	/	/	8.376	0.285 \pm 0.004	99	/	/	/	/	/	/
16	endo-Borneol	/	/	/	8.55	0.246 \pm 0.024	94	8.559	0.433 \pm 0.014	95	/	/	/
17	Terpinen-4-ol	8.685	1.846 \pm 0.126	86	8.664	0.972 \pm 0.060	97	8.684	2.391 \pm 0.009	86	8.68	2.263 \pm 0.091	86
18	Citral	8.831	1.125 \pm 0.098	98	8.809	0.223 \pm 0.001	98	8.817	0.857 \pm 0.078	98	/	/	/
19	Pinocarvone	/	/	/	8.897	1.841 \pm 0.138	94	/	/	/	/	/	/
20	Berbenone	8.909	0.262 \pm 0.010	93	/	/	/	/	/	/	/	/	/
21	cis-Carveol	8.965	0.349 \pm 0.009	99	/	/	/	/	/	/	/	/	/
22	α -Terpineol	9.046	1.925 \pm 0.070	78	9.028	1.817 \pm 0.090	78	9.04	2.436 \pm 0.038	78	9.035	2.29 \pm 0.155	78
23	(-)-cis-Isopiperitenol	/	/	/	9.237	0.169 \pm 0.026	92	/	/	/	/	/	/
24	p-Cymen-7-ol	/	/	/	9.301	0.167 \pm 0.007	100	/	/	/	/	/	/
25	(-)-Myrtenol	/	/	/	9.483	0.172 \pm 0.003	100	/	/	/	/	/	/
26	Carvomenthene	/	/	/	9.587	0.576 \pm 0.071	95	9.594	0.279 \pm 0.010	98	/	/	/
27	Spiro[4.5]decane	9.609	0.702 \pm 0.014	91	/	/	/	/	/	/	/	/	/
28	cis-Carveol	9.782	0.554 \pm 0.009	99	9.767	0.864 \pm 0.053	99	/	/	/	/	/	/

29	Verbenone	/	/	/	10.023	0.204±0.004	93	/	/	/	/	/	/
30	(-)-Carvone	/	/	/	10.292	0.274±0.005	90	/	/	/	/	/	/
31	Isoneral	/	/	/	10.671	0.391±0.015	98	/	/	/	/	/	/
32	Anethole	/	/	/	/	/	/	10.859	0.383±0.008	97	/	/	/
33	cis-Ocimenol	11.103	3.902±0.081	90	/	/	/	/	/	/	/	/	/
34	α-Gurjunene	/	/	/	/	/	/	11.212	0.601±0.009	95	11.228	1.608±0.076	95
35	Caryophyllene	/	/	/	/	/	/	11.515	0.232±0.009	96	/	/	/
36	γ-Murolene	/	/	/	/	/	/	/	/	/	11.645	0.864±0.068	99
37	Aromadendrene	11.834	1.79±0.078	98	11.802	2.041±0.056	98	11.801	1.261±0.011	98	11.838	4.754±0.046	98/
38	γ-Gurjunene	/	/	/	12.168	0.988±0.075	96	12.169	0.609±0.001	97	12.194	2.427±0.022	96
39	.Isocaryophyllene	12.204	1.016±0.070	96	/	/	/	/	/	/	/	/	/
40	Eudesma-4(15),7-dien-1β -ol	/	/	/	12.335	0.212±0.010	99	/	/	/	/	/	/
41	Eremophilene	/	/	/	12.58	0.23±0.008	97	12.587	0.384±0.016	100	/	/	/
42	Azulene,	/	/	/	/	/	/	/	/	/	12.605	1.7±0.041	96
43	γ-Element	/	/	/	/	/	/	12.733	0.245±0.006	96	/	/	/
44	β-Vatirenene	/	/	/	/	/	/	/	/	/	13.2	0.273±0.006	97
45	Butanoic acid,	/	/	/	13.364	0.17±0.004	82	13.371	0.262±0.008	82	/	/	/
46	Isoamyl phenylacetate	/	/	/	/	/	/	/	/	/	13.429	0.765±0.064	91
47	Viridiflorol	/	/	/	13.752	0.301±0.001	98	13.742	1.298±0.011	98	13.782	1.327±0.084	98
48	Ledol	13.829	0.372±0.022	100	/	/	/	/	/	/	/	/	/
49	Globulol	/	/	/	14.200	1.29±0.009	95	/	/	/	/	/	/
50	Epiglobulol	/	/	/	14.289	2.988±0.024	98	14.221	1.74±0.060	98	14.263	5.804±0.830	97
51	Elemol	14.315	1.789±0.082	98	/	/	/	14.331	1.572±0.084	96	/	/	/
52	Epiglobulol	14.401	1.499±0.081	/	14.45	0.487±0.071	/	14.46	0.412±0.014	84	14.479	0.8±0.082	/
53	Rosifoliol	/	/	/	14.678	0.369±0.057	90	14.697	0.496±0.007	90	/	/	/
54	β-Selinol	/	/	/	/	/	/	/	/	/	14.718	1.018±0.013	90
55	γ-Eudesmol	14.803	0.368±0.008	87	/	/	/	14.865	0.513±0.015	87	/	/	/
56	β-Eudesmol	/	/	/	/	/	/	15.261	1.007±0.007	92	15.272	0.903±0.038	92
57	Ylangenal	/	/	/	16.547	0.248±0.007	97	16.583	0.255±0.015	95	/	/	/
58	Isocaryophyllene	16.838	0.303±0.001	98	16.841	0.349±0.034	89	16.884	0.355±0.010	96	/	/	/
Total Identification%		99.999±0.395			99.827±0.854			99.998±0.310			99.973±0.018		

*% of similarity relative to the reference library in the GC/MS

Antioxidant activity: DPPH is commonly utilized as a reagent to determine free radicals. To form a stable diamagnetic molecule, it must receive an electron or a hydrogen radical. Antioxidant activity was defined as the antioxidants' ability to eliminate DPPH radical absorption at 517 nm. Figure 7 indicates that the result of the scavenging effect of Eucalyptus essential oil among locations was statistically significant ($p \leq 0.01$). The Sharbazher location gave a maximum scavenging effect of 60.156%, and the lowest scavenging effect was achieved by the Qara Dagh location with a value of 47.613%. The antioxidant capacity of Eucalyptus essential oil may be due to the presence of various secondary metabolites, especially hydroxylated compounds (Abbaci *et al.*, 2023). The variation in essential oil constituents is mostly affected by geographical locations. This allows for the choice of essential oils with preferred components to be used in the food industry, perfume, drugs, and

pharmaceuticals (Khalid *et al.*, 2020). Studies have revealed that plants belonging to the same species but growing in different environments have different concentrations of a specific secondary metabolite. This is because, in order to combat environmental stress, the plant must produce secondary metabolites in a specific quantity and quality. As a result, research on each environmental element is crucial to understanding plant availability and adaptability in a given area. Pant *et al.* (2021) and Ben *et al.* (2010) reported that the antioxidant capacity of Eucalyptus essential oil is related to its content of monoterpenoids. Eucalyptus essential oil from all locations contains a higher percentage of eucalyptol and α -pinene. Ciesla *et al.* (2016) reported that the solid antagonistic activity between the binary mixture of p-cymene and eucalyptol causes weak antioxidant activity. This may explain the weak antioxidant activity of the essential oil from the Qara Dagh location.

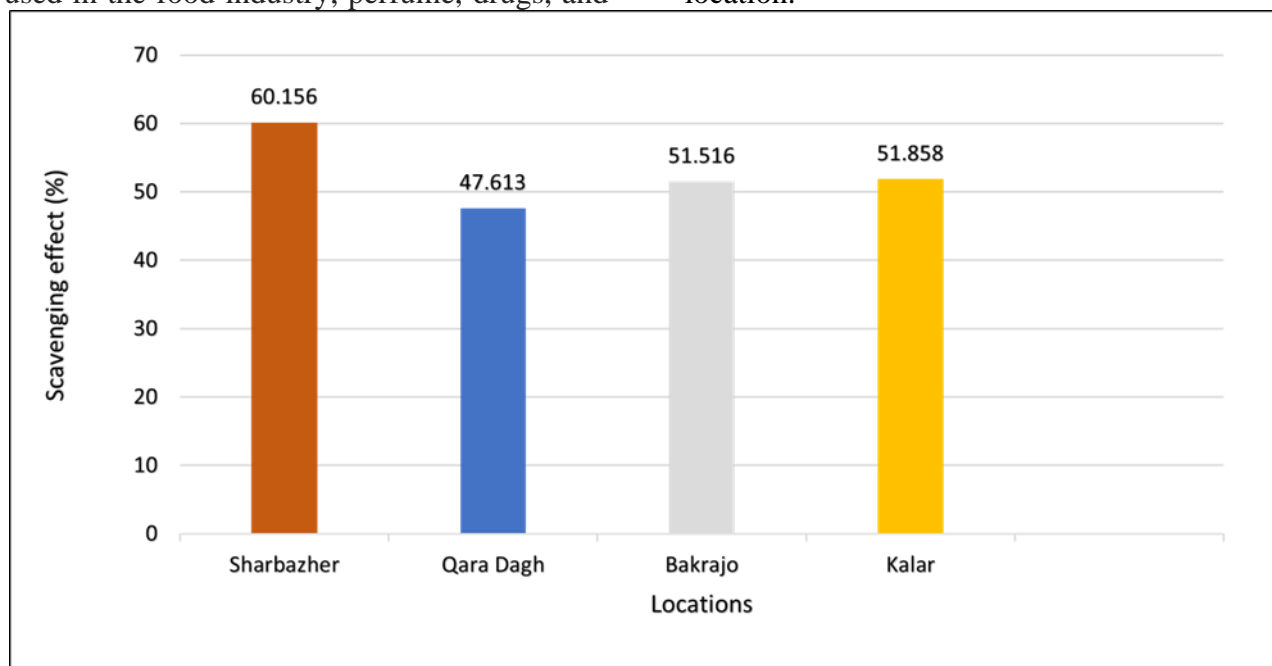


Figure 7. Eucalyptus leaves essential oil scavenging effect (%) based on locations

The data presented in Figure 8 explain the significant differences ($p \leq 0.01$) of the scavenging effect between the essential oil concentration and DPPH radical, which linearly increased as the concentration increased from 10 to 100 $\mu\text{g/mL}$. The eucalyptus essential oil with 100 $\mu\text{g/mL}$

exhibited 74.836 % activity, whereas the 10 $\mu\text{g/mL}$ exhibited 38.389 % inhibition. The same result was obtained by Mishra *et al.* (2010), who reported that by increasing essential oil concentration, the scavenging effect percentage increased.

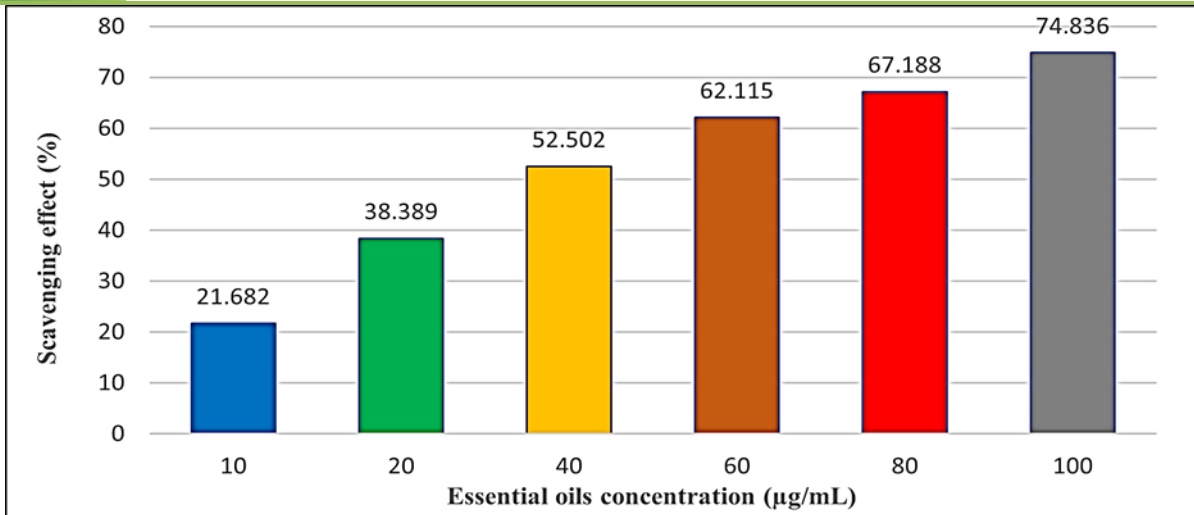


Figure 8. Eucalyptus leaves essential oil scavenging effect (%) based on different concentrations. The interaction between locations and essential oil concentration on the scavenging effect% was illustrated in Figure 9. The result indicated that the interaction effect was statistically significant ($p \leq 0.01$). The highest scavenging effect was recorded by the interaction between essential oil from the Sharbazher location and 100 µg/mL. and the lowest scavenging effect% was obtained by the interaction between Kalar location and 10 µg/mL. The IC₅₀s were 32.666 µg/mL, 56.798 µg/mL, 49.111 µg/mL, 48.473 µg/mL, and 24.568 µg/mL for Sharbazher, Qara Dagh, Bakrajo, Kalar, and ascorbic acid, respectively. The Sharbazher location gained a stronger IC₅₀ than ascorbic acid, and the other locations' essential oils were relatively less than ascorbic acid (Serçe *et al.*, 2016).

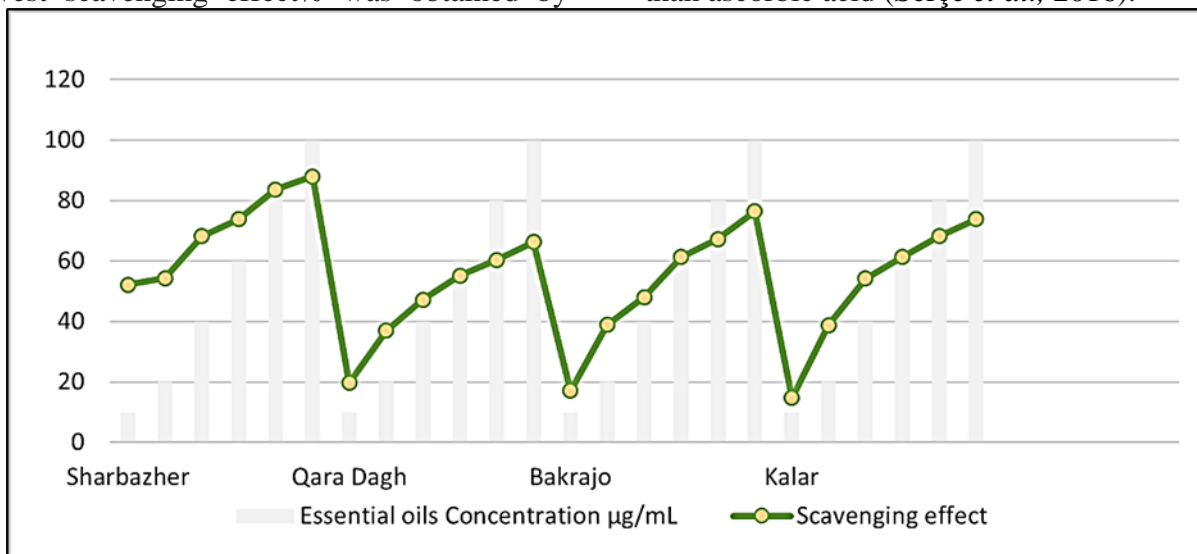


Figure 9. Eucalyptus leaves essential oil scavenging effect (%) based on the interaction between locations and concentration. Sharbazher $y = 0.5342X + 32.55$, $R^2 = 0.9346$, Qara Dagh $y = 0.4654X + 23.566$, $R^2 = 0.9019$, Bakrajo $y = 0.5925X + 20.902$, $R^2 = 0.9212$, and Kalar $y = 0.5821X + 21.748$, $R^2 = 0.8599$

CONCLUSION

This current investigation suggests that the Eucalyptus tree is a source of some naturally active components that act as antioxidants and tend to reduce the risk of some serious human diseases. Thus, selecting the extraction processes and using a sufficient solvent are two important keys that need to be reviewed to

maximize the oil yield. This might be a good chance to increase the active components' content in the essential oil. In addition, the study of GC-MS analysis provided at least an understanding of the qualitative and quantitative analysis of some beneficial components found in different plant materials. Therefore, further research is needed on the

various methods of extraction, such as Soxhlet extraction, and other solvents, including hexane, methanol, and ethanol, to make a comparison with hydro distillation in order to increase the highest yield of Eucalyptus essential oil. The antibacterial activity of Eucalyptus leaf essential oil is also important to consider.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

Author/s signature on Ethical Approval Statement.

Ethical Clearance and Animal Welfare

Funds:

REFERENCES

Abbaci, H., E. H., Nabti, A. M., Al-Bekairi, S. A., Hagra, M. M., Salem-Bekhit, A., Adjaoud, H. A., Alzahrani, L., Bensidhoum, R., Alenazy, A., Piras, & D., Falconieri, (2023). Comparative bioactivity evaluation of chemically characterized essential oils obtained from different aerial parts of *Eucalyptus gunnii* Hook. f. (Myrtaceae). *Molecules*, 28(6): 2638.

<https://doi.org/10.3390/molecules28062638>

Abdulrahman, M. D., (2023). Plants biodiversity utilisation in Bardarash, Kurdistan Region, Iraq. In *IOP Conference Series: Earth and Environmental Science*, 1185(1): 012034. IOP Publishing. <https://doi.org/10.1088/1755-1315/1185/1/012034>

Abed, K. M., B. M., Kurji, & B. A. Abdul-Majeed, (2015). Extraction and modeling of oil from *Eucalyptus camadulensis* by organic solvent. *Journal of Materials Science and Chemical Engineering*, 3: 35-42.

<https://doi.org/10.4236/msce.2015.38006>

Ahmed, H. M., (2017). Traditional uses of Kurdish medicinal plant *Pistacia atlantica* subsp. *kurdica* Zohary in Ranya, Southern Kurdistan. *Genet Resour Crop Evol*, 64: 1473–1484.

<https://doi.org/10.1007/s10722-017-0522-4>

Almas, I., E. Innocent, F. Machumi, & W. Kisinza (2021). Chemical composition of essential oils from *Eucalyptus globulus* and *Eucalyptus maculata* grown in Tanzania. *Scientific African*, 12, e00758.

<https://doi.org/10.1016/j.sciaf.2021.e00758>

Anil P, Manish S, R. S. Garvendra, B. Vijay & K. Tarachand (2010). In vitro antioxidant studies of *Lagerstroemia speciosa* leaves. *Pharmacog Journal*, 2(10): 357-360.

[https://doi.org/10.1016/S0975-3575\(10\)80109-9](https://doi.org/10.1016/S0975-3575(10)80109-9)

Arun, S., & K. Inderjeet, (2022). Extraction Fuel During Hydro-distillation Modifies *Eucalyptus* Essential Oil Yield, Phytochemical Quality, and Capital Cost. *Current Indian Science*, 1(1): e231222212138. <https://doi.org/10.2174/2210299x01666221223091643>

Bachheti, R. K., (2015). Chemical composition and antibacterial activity of the essential oil from the leaves of *Eucalyptus globulus* collected from Haramaya University, Ethiopia. *Der Pharma Chemica*. 7 (2): 209-214.

Ben Marzoug, H. N., J., Bouajila, M. Ennajar, A. Lebrihi, F. Mathieu, F. Couderc, M. Abderraba, M. & Romdhane, (2010). *Eucalyptus* (*gracilis*, *oleosa*, *salubris*, and *salmonophloia*) essential oils: Their chemical composition and antioxidant and antimicrobial activities. *Journal of medicinal food*, 13(4): 1005-1012.

<https://doi.org/10.1089/jmf.2009.0153>

Bourakna, Z., Righi, K., & Assia Righi, F. (2022). GC/MS Analysis of *Eucalyptus globulus* L. (Myrtaceae) leaves essential oil from Algeria and their insecticidal activity against adults of *Bactrocera oleae* (Rossi)(Diptera; Tephritidae). *Journal of Essential Oil-Bearing Plants*, 25(4), 876-887.

<https://doi.org/10.1080/0972060X.2022.2129459>

Brooker, M. I. H. (2000). A new classification of the genus *Eucalyptus* L'Her. (Myrtaceae). *Australian systematic botany*, 13(1), 79-148.

<https://doi.org/10.1071/SB98008>

Camas, N., Radusiene, J., Ivanuskas, L., Jakstas, V., & Cirak C., (2014). Altitudinal changes in the content of bioactive substances in *Hypericum orientale* and *Hypericum pallens*. *Acta Physiol Plant*, (36): 675-686.

<https://doi.org/10.1007/s11738-013-1446-z>

- Ciesla, L. M., Wojtunik-Kulesza, K. A., Oniszczyk, A., & Waksmundzka-Hajnos, M. (2016). Antioxidant synergism and antagonism between selected monoterpenes using the 2, 2-diphenyl-1-picrylhydrazyl method. *Flavour and Fragrance Journal*, 31(6), 412-419. [https://doi.org/ 10.1002/ffj.3330](https://doi.org/10.1002/ffj.3330).
- Čmiková, N., Galovičová, L., Schwarzová, M., Vukic, M. D., Vukovic, N. L., Kowalczewski, P. Ł., & Kačániová, M. (2023). Chemical composition and biological activities of *Eucalyptus globulus* essential oil. *Plants*, 12(5), 1076. [https://doi.org/ 10.3390/plants12051076](https://doi.org/10.3390/plants12051076)
- Cristina, D., P. Malaspina, L. Cornara, A. Smeriglio, D. Trombetta, V. DeFeo, and S. Vanin (2023). *Eucalyptus* essential oils in pest control: A review of chemical composition and applications against insects and mites. *Crop Protection*, 176. [https://doi.org/ 10.1016/j.cropro.2023.106319](https://doi.org/10.1016/j.cropro.2023.106319)
- Dalal, P. 2019. Hydro distillation method extraction of *Eucalyptus* oil & Lemongrass oil. *Social Science Journal*, 4, 36-44.
- Dhakad, A. K., Pandey, V. V., Beg, S., Rawat, J. M., & Singh, A. (2018). Biological, medicinal, and toxicological significance of *Eucalyptus* leaf essential oil: a review. *Journal of the Science of Food and Agriculture*, 98(3), 833-848. [https://doi.org/ 10.1002/jsfa.8600](https://doi.org/10.1002/jsfa.8600)
- Dudareva, N., Pichersky, E., & Gershenzon, J. (2004). Biochemistry of plant volatiles. *Plant physiology*, 135(4), 1893-1902. <https://doi.org/10.1104/pp.104.049981>.
- Galan, D. M., Ezeudu, N. E., Garcia, J., Geronimo, C. A., Berry, N. M., & Malcolm, B. J. (2020). *Eucalyptol* (1, 8-cineole): an underutilized ally in respiratory disorders?. *Journal of essential oil research*, 32(2), 103-110. [https://doi.org/ 10.1080/10412905.2020.1716867](https://doi.org/10.1080/10412905.2020.1716867)
- González-Hernández, R. A., Valdez-Cruz, N. A., & Trujillo-Roldán, M. A. (2024). Factors that influence the extraction methods of terpenes from natural sources. *Chemical Papers*, 78(5), 2783-2810. [https://doi.org/ 10.1007/s11696-024-03339-z](https://doi.org/10.1007/s11696-024-03339-z)
- Heikal, A. A. E. M. (2017). Variation in the essential oil content and its composition in *Eucalyptus cinerea* leaves and its relation to some environmental factors. *Journal of Essential Oil-Bearing Plants*, 20(4), 995-1005. [https://doi.org/ 10.1080/0972060X.2017.1351896](https://doi.org/10.1080/0972060X.2017.1351896)
- Immaroh, N. Z., Kuliahsari, D. E., & Nugraheni, S. D. (2021, April). *Eucalyptus globulus* essential oil extraction method. In *IOP conference series: earth and environmental science* (Vol. 733, No. 1, p. 012103). IOP Publishing. [https://doi.org/ 10.1088/1755-1315/733/1/012103](https://doi.org/10.1088/1755-1315/733/1/012103)
- James, O., Nnacheta, O. P., Wara, H. S., & Aliyu, U. R. (2009). Invitro and invivo studies on the antioxidative activities, membrane stabilization, and cytotoxicity of water spinach (*Ipomoea aquatica* forsk) from Ibaji ponds, Nigeria. *Int. J. PharmTech Res*, 1(3), 474-482.
- Khalid, K. A., Essa, E. F., Ismaiel, H. M., & Elsayed, A. A. (2020). Effects of geographical locations on essential oil composition of navel orange leaves and flowers. *Journal of Essential Oil-Bearing Plants*, 23(1), 139-148. [https://doi.org/ 10.1080/0972060X.2020.1727369](https://doi.org/10.1080/0972060X.2020.1727369)
- Kumar, P., Mishra, A. K., Chaudhari, S. K., Sharma, D. K., Rai, A. K., Singh, K., & Singh, R. (2021). Carbon sequestration and soil carbon build-up under *Eucalyptus* plantation in semi-arid regions of North-West India. *Journal of Sustainable Forestry*, 40(4), 319-331. [https://doi.org/ 10.1080/10549811.2020.1749856](https://doi.org/10.1080/10549811.2020.1749856)
- Labate, C. A., de Assis, T. F., Oda, S., de Mello, E. J., González, E. R., Zauza, E. A. V., & Salvatierra, G. R. (2009). *Eucalyptus*. *Compendium of transgenic crop plants*, 35-108.
- Maciel, M. V., Morais, S. M., Bevilaqua, C. M. L., Silva, R. A., Barros, R. S., Sousa, R. N., & Souza-Neto, M. A. (2010). Chemical composition of *Eucalyptus* spp. essential oils and their insecticidal effects on *Lutzomyia longipalpis*. *Veterinary parasitology*, 167(1), 1-7. [https://doi.org/ 10.1016/j.vetpar.2009.09.053](https://doi.org/10.1016/j.vetpar.2009.09.053)
- Mahmoud, A. R. K., & Muhammad, K. A. A. (2000). Design and analysis of agricultural experiments. University of Mosul-Ministry of Higher Education and Scientific Research, Dar Al Kutub for Printing and Publishing/Iraq.

- Mishra, A. K., Sahu, N., Mishra, A., Ghosh, A. K., Jha, S., & Chattopadhyay, P. (2010). Phytochemical screening and antioxidant activity of essential oil of Eucalyptus leaf. *Pharmacognosy journal*, 2(16), 25-28.
[https://doi.org/10.1016/S0975-3575\(10\)80045-8](https://doi.org/10.1016/S0975-3575(10)80045-8).
- Mulyaningsih, S., Sporer, F., Reichling, J., & Wink, M. (2011). Antibacterial activity of essential oils from Eucalyptus and of selected components against multidrug-resistant bacterial pathogens. *Pharmaceutical biology*, 49(9), 893-899.
<https://doi.org/10.3109/13880209.2011.553625>
- Nagappan, D., Sivaraj, M., Periyasamy, S., Kamalanathan, A., Gnanavel, B., & Chinnappan, B. (2024). Phytochemical Analysis and Evaluation of Antifungal Properties of Eucalyptus Oil against Candida Species. *Shanlax Int. J. Arts Sci. Humanit*, 12, 1-15.
<https://doi.org/10.34293/sijash.v12i1.7699>
- Nataraj, S., Subramanian, M., & Geethalakshmi, V. (2022). Allelopathic effect of Eucalyptus tereticornis smith aqueous leaf extract on Echinochloa crus-galli (L.) P. Beauv. *International Journal of Ecology and Environmental Sciences*, 2(4).
- Oliveira, F. N., Fortes, G. A., Paula, J. R., Ferri, P. H., & Santos, S. C. (2014). Seasonal influence on the essential oil of Eucalyptus microcorys. *Natural product communications*, 9(4), 1934578X1400900439.
<https://doi.org/10.1177/1934578X14009004>
- Pant, P., Pandey, S., & Dall'Acqua, S. (2021). The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review. *Chemistry & biodiversity*, 18(11), e2100345.
<https://doi.org/10.1002/cbdv.202100345>
- Pries, R., Jeschke, S., Leichtle, A., & Bruchhage, K. L. (2023). Modes of action of 1, 8-cineol in infections and inflammation. *Metabolites*, 13(6), 751.
<https://doi.org/10.3390/metabo13060751>
- Quan, V., Chalmers, A. C., Jyoti Bhuyan, D., Bowyer, M. C., & Scarlett, C. J. (2015). Botanical, phytochemical, and anticancer properties of the Eucalyptus species. *Chemistry & biodiversity*, 12(6), 907-924.
<https://doi.org/10.1002/CHIN.201534276>
- Sebei, K., Sakouhi, F., Herchi, W., Khouja, M. L., & Boukhchina, S. (2015). Chemical composition and antibacterial activities of seven Eucalyptus species essential oils leaves. *Biological research*, 48(1), 7.
<https://doi.org/10.1186/0717-6287-48-7>.
- Serçe, A., Toptancı, B. Ç., Tanrikut, S. E., Altaş, S., Kızıllı, G., Kızıllı, S., & Kızıllı, M. (2016). Assessment of the antioxidant activity of Silybum marianum seed extract and its protective effect against DNA oxidation, protein damage and lipid peroxidation. *Food technology and biotechnology*, 54(4), 455-461.
<https://doi.org/10.17113/ftb.54.04.16.4323>
- Surbhi, Kumar, A., Singh, S., Kumari, P., & Rasane, P. (2023). Eucalyptus: phytochemical composition, extraction methods and food and medicinal applications. *Advances in Traditional Medicine*, 23(2), 369-380.
<https://doi.org/10.1007/s13596-021-00582-7>
- Tum, P. K., Kasha, G. M., Kithure, J. G., & Mwazighe, F. M. (2016). Optimization of essential oil extraction from Eucalyptus grandis leaves by Clevenger distillation. *Journal kenya chemical society*, 9(1), 91-102.
- Vecchio, M. G., Loganés, C., & Minto, C. (2016). Beneficial and healthy properties of Eucalyptus plants: A great potential use. *The Open Agriculture Journal*, 10(1).
<https://doi.org/10.2174/1874331501610010052>
- Yost, J. M., Wise, S. L., Love, N. L., Steane, D. A., Jones, R. C., Ritter, M. K., & Potts, B. M. (2021). Origins, diversity, and naturalization of Eucalyptus globulus (Myrtaceae) in California. *Forests*, 12(8), 1129.
<https://doi.org/10.3390/f12081129>.
- Zhou, L., Li, J., Kong, Q., Luo, S., Wang, J., Feng, S., & Ding, C. (2021). Chemical composition, antioxidant, antimicrobial, and phytotoxic potential of Eucalyptus grandis × E. urophylla leaves essential oils. *Molecules*, 26(5), 1450.
<https://doi.org/10.3390/molecules26051450>

التحليل الكيميائي النباتي والنشاط المضاد للأوكسدة لزيت أوراق الأوكالبتوس العطري

روژگار مصطفى أحمد

قسم التقنية الحيوية وعلوم المحاصيل الحقلية - كلية علوم الهندسة الزراعية - جامعة السليمانية - السليمانية - العراق

المستخلص

ينتمي جنس الأوكالبتوس إلى عائلة Myrtaceae وهي شجرة كبيرة لها عدة استخدامات نظراً لمحتواها من بعض المكونات النشطة المفيدة. وقد استخدمت هذه المكونات الكيميائية النباتية لسنوات عديدة لعلاج الأمراض المختلفة. تعتبر عملية الاستخراج وتحديد هذه المكونات من النقاط الأساسية لزيادة وتطوير مستوى هذه المحتويات. تمت دراسة مستخلص الزيت العطري من أوراق الأوكالبتوس باستخدام التقطير المائي من أربعة مواقع في منطقة السليمانية. علاوة على ذلك، تمت دراسة نشاط مضادات الأوكسدة أيضاً باستخدام (مقاييس الجذور الحرة). ولوحظ أعلى محتوى من الزيت العطري في قره داغ في كل من الأوراق الطازجة و الجافة (2.33 جم و 4.67 جم)، في حين تم العثور على أدنى مستوى في الأوراق الطازجة في شهربازير (1.27 جم) والأوراق الجافة في كلار (2.40 جم). تم التعرف على ثمانية وخمسين مكوناً كيميائياً في زيت الأوكالبتوس العطري باستخدام تحليل GC-MS. تم العثور على الأوكالبتوس والألفا بينين كمكونات رئيسية في جميع المواقع. وقد لوحظت أكبر كميات من الأوكالبتوس والألفا بينين في قره داغ ($52.309 \pm 0.967\%$ عند 6.163 دقيقة) و (14.652 ± 0.194 عند 3.703 دقيقة) على التوالي. وأشار تأثير إزالة الزيوت العطرية إلى أن موقع شربازر أعطى أقصى تأثير إزالة بنسبة 60.156%. وبالمقارنة، تم تحقيق أقل تأثير إزالة من خلال موقع قره داغ بقيمة 47.613%.

الكلمات المفتاحية: تحليل DPPH، Eucalyptus camaldulensis، تحليل GC-MS، النباتات الطبية، المكونات الكيميائية النباتية.