RESEARCH ARTICLE

Isolation, Characterization, and Biological Activity of some Fatty Acids and Volatile Oils from Iraqi *Eucalyptus microtheca* Plant

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ABSTRACT

This study aims at investigating the chemical composition, antimicrobial and antioxidant activity of some extracted essential and fixed oils from leaves of *Eucalyptus microtheca* plant grown in Iraq. Analysis of the isolated oils has been achieved by gas chromatography coupled with mass spectrometry gas chromatography–mass spectrometry (GC-MS) Technology. The study reveals existence of sixteen compounds. Camphene (20.60%), 4-carene (18.53%), 1,8-cineole (11.96%), terpin-4-ol (8.70%) and *p*-cymene (8.39%) were the highest components in these essential oils. While nine compounds were obtained as fixed oils, pentadecanoic acid (36.47%) and *cis*-vaccenic acid (30.31%) were the major components. The antimicrobial activity of the leaves extracts was evaluated against six different gram-positive and Gram-negative bacteria using disk diffusion method and exhibited good inhibition activity. Moreover, antioxidant assay (free radical scavenging activity) demonstrated good activity for the extracted oils. The results show that the aerial parts (leaves) of the Iraqi *E. microtheca* plant possess antibacterial and antioxidant properties and may suggest it as a good candidate to use for medicinal purposes.

Keywords: Antibacterial, Antioxidants, *E. microtheca*, Essential and fixed oils, GC-MS analysis.

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INTRODUCTION

Medicinal treatment from natural or herbal sources is a subject of interest among researchers in the past few years.[1] The World Health Organization (WHO), has estimated that 80% of the population in the developing countries depend on traditional medicine, mostly medicinal plants for their initial health care needs.[2] Due to allergy, side effects of synthetic medicine, and the problem of drug-resistant bacteria, the interest in naturally available plants and their constituents for medication has increased worldwide.[1] Essential oils (volatile) and fixed oils (non-volatile) from plant origins have been exploited increasingly in food, cosmetics and medicinal industries.[3,4] The essential oils are aromatic compounds, usually can be extracted from the aerial parts of the plants. These essential oils contain various chemical compounds; oxygenated compounds such as phenols, esters, alcohols, ethers, aldehydes and ketones; hydrocarbons like terpenes, organic sulphur and nitrogenous compounds and derivatives of benzene ring.[5,6] The use of essential oils as food preservatives and flavorings is highly recommended due to its safety and broad spectrum of activity against several bacteria and fungi strains. This can be related to the presence of a high percentage of different compounds such as thymol, eugenol, cinnamic aldehydes, monoterpenes, and polyphenols.[7,8]

Eucalyptus is an evergreen aromatic flowering tree. It is a genus of *Myrtaceae,* found around the globe with nearly 900 species; most of the species are native to Australia. However, they have been cultivated throughout the tropical and subtropical regions, including the Middle East (Iraq).[9] *Eucalyptus* is an easily adaptable and rapid growth tree. It has economic value; it is a valuable source of gum, polyphenols, proteins, tannins, and dyes. *Eucalyptus* has commercial importance globally. It is used in construction, fuel, paper, and furniture industry, as fragrance material in household products and cosmetics such as perfumes, detergents, soaps, and lotions. Also, it can be used as flavor materials in foods, drinks, meat products, ice cream, and confectionaries.[10,11] *Eucalyptus* is well-known globally and has various medicinal uses. Its use to treat or prevent human diseases has been revived and expanded recently. Extracted essential oils from different *Eucalyptus* species exhibit antioxidant, antibacterial, antifungal, antiviral, insecticidal and anti-inflammatory activities. For instance, *Eucalyptus* volatile oils are used to treat rheumatism, diarrhea, skin diseases and as an expectorant in case of chest complaints.[12-19] Species variation and geographical distribution of *Eucalyptus* could affect the chemical constituents, the antioxidant, and the antibacterial properties of the extracted oils significantly.

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This requires more investigations to explore the potential use of this plant. Therefore, the Iraqi *E. microtheca* species has been selected to identify the chemical composition of the extracted essential and fixed oils from leaves, to determine the antibacterial activity against six different bacteria strains and to assess the antioxidant property for the extracted oils.

MATERIALS AND METHODS

Sample Collection

E. microtheca fresh leaves were collected from Mosul city, the northern part of Iraq, in April 2018. The plant was identified by specialists from the college of agriculture and forestry, university of Mosul, Mosul, Iraq.

Sample Preparation

The fresh leaves were washed up with tap water three times to remove the dirt. The plant material then air-dried in the shade for 5 days at lab temperature, chopped to small pieces, and kept in a paper envelop to avoid extra damaging and minimize contamination until further use.

Isolation of Essential and Fixed Oils

The essential oils were extracted from the fresh leaves of *E. microtheca* plant through hydro-distillation for three hours using Clevenger-type apparatus.[20,21] The chopped small pieces of the plant material sample (100 g) was charged to the distillation flask. Pressurized steam was circulated through the sample. The pure essential oils vapor along with steam were collected in a receiver flask after condensation. The flask was kept in ice water to prevent the evaporation of the low boiling point substances. The residual oily layer (5 mL) was dissolved in diethyl ether (75 mL), separated from aqueous layer, concentrated by evaporation, dried over anhydrous Na^2SO_4, and filtered off. The process of extraction was repeated several times to collect about 20 mL of oil. The obtained oil was stored at -6 °C far from light till analysis. The chemical compositions and yields of the obtained essential oils are listed in Tables 1 and 2.

Chemical Composition through GC-MS analysis

Gas chromatography-Mass spectrometry analysis was achieved using Shimadzu GC-9A gas chromatograph supplied with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 µm). Oven temperature was fixed at 40 °C for 5 minutes and then set to 280 °C at a rate of 4 °C/min. Injector and detector (FID) temperature was 290 °C; He was used as carrier gas with a linear velocity of 32 cm/s. The percentage of the compounds was calculated by the area normalization method, without considering response factors. GC-MS analysis was made by a Varian 3400 GC-MS system provided with a DB-5 fused silica column (30 m x 0.25 mm i.d.), and its characteristics were as follows, oven temperature was 40 °C to 250 °C at a rate of 4 °C/min, transfer line temperature 260 °C, carrier gas He with a linear velocity of 31.5 cm/s, split ratio 1/60, ionization energy 70 eV; scan time 1 second and mass range of 40-300 amu. The chemical composition for oil components was identified and confirmed by comparison of their mass spectra with the available standard data, or with authentic compounds and their retention indices values reported in the literature.

Antimicrobial Activity

The antimicrobial effect of extracted essential oils has been tested at various concentrations, 200, 100, 50, 25, and 12.5 mg/mL) against six pathogenic microorganisms. The bacteria used in the current study were *Proteus vulgaris, Escherichia coli*, *Salmonella typhimurium* (gram-negative) and *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis* (gram-positive). The antibacterial activity test was carried out using the disk diffusion method described by Bauer *et al* and compared to that of control samples.[22] All bacterial strains were obtained from the laboratory of the biology department, college of education for pure science, Mosul university, Mosul. Iraq.[23]

Antioxidant Assay

The free radical scavenging activity of leaf extracts was measured using 1,1-diphenyl-1-picrylhydrazyl (DPPH) as described by Blois with slight modifications.[24] The test compound (1.5 mL) of pure antioxidant or essential oil was taken in various concentrations and mixed with 0.2 mM methanolic solution of DPPH (1.5 mL). After 60 min of incubation at room temperature in the dark, the absorbance value of the produced mixture was measured at 520 nm (wavelength of maximum absorbance of purple-colored DPPH, the color will disappear in the presence of antioxidant in the solution) and recorded as a sample. Repeating the same procedure to a solution without the test material (the control solution containing all reagents except the test compound) was also carried out, and the absorbance value was recorded as A (blank). All experiments were repeated three times. The absorbance value of the free radical scavenging (antioxidant) activity was then converted to percentage inhibition and calculated according to the following formula:

$$\% \text{ Inhibition} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{bank}}) \times 100]$$

Moreover, the inhibitory concentration or IC_{50} (the concentration of antioxidant which causes 50% loss in the activity of DPPH) value was also used to evaluate the antioxidant activity of the isolated essential oils compared to the standard gallic acid.

RESULTS AND DISCUSSION

Chemical Composition of Essential Oils

Hydro-distillation technique was used to isolate the oils from the fresh leaves of *E. microtheca* plant. Characterization of the isolated oils was achieved using GC-MS analysis. The obtained essential and fixed oils with their percentages, retention index and retention time are shown in Tables 1 and 2, respectively. The differences in quality and quantity of the chemical compositions of plant oils could be related to several reasons. Geographical location, harvest season, effects of climate, the soil nature, the plant age, the state of plant material used (dried

Table 1: The extracted essential oils from *E. microtheca* leaves.

No	*Compound*	*%*[1]	*RI*[2]	*RT*[3]*(min)*
1	α-Pinene	3.69	1186	4.389
2	Camphene	20.60	1262	4.800
3	4-Carene	18.53	1536	11.472
4	β-Pinene	4.18	1763	14.657
5	Limonene	2.35	1494	15.074
6	*p*-Cymene	8.39	1441	19.179
7	1,8-Cineole	11.96	1859	21.962
8	Terpinolene	1.33	3183	22.170
9	β-Linalool	3.40	1847	22.369
10	Terpin-4-ol	2.78	1549	22.987
11	β-*cis*-Ocimene	3.99	1272	24.330
12	γ-Terpinene	3.70	2453	25.330
13	α- Phellandrene	1.83	1945	25.450
14	α –Campholenal	1.18	2175	25.554
15	Terpin-4-ol	8.70	1400	25.749
16	α-Copaene	3.41	1275	25.919

[1] Compound percentage, [2] Retention index, [3]Retention time.

Table 2: The extracted fixed oils from *Eucalyptus microtheca* leaves.

No	*Compound*	*%*[1]	*RI*[2]	*RT*[3]*(min)*
1	Pentadecanoic acid	36.47	1869	18.429
2	9,9-Dimethoxybicyclo	4.40	1610	18.870
3	Tridecanoic acid	2.12	1670	18.960
4	Azelaic Acid	5.55	1629	19.166
5	Sandaracopimar-15-ene-6. beta.,8.beta.,11.alpha.-triol	4.19	2442	21.667
6	cis-Vaccenic acid	30.31	2175	21.783
7	Octadec-9-enoic acid	3.03	2175	22.050
8	Eicosanoic acid	6.81	:2366	22.267
9	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl	7.11	2788	27.961

[1] Compound percentage, [2] Retention index, [3]Retention time.

or fresh) and time of collection may affect the contents of the oil of the plant [25].

Antibacterial Activity

The antibacterial activity of essential and fixed oils from *E. microtheca* leaves was tested against different gram-positive and Gram-negative bacteria at various concentrations (12.5–200 mg/mL). The results showed that the oils had significant antibacterial activity against all microorganisms. In general, there is a relationship between chemical composition and antimicrobial activity. The antimicrobial properties of the extracted oils could be related to the high contents of oxygenated compounds. The presence of oxygenated compounds may affect the cell membrane of these bacterial strains by disrupting the proton motive force, electron flow, active transport, and causing a coagulation of the cell. All data are presented in Tables 3 and 4, respectively.

Antioxidant Activity

Antioxidant activity of the extracted oils from *E. microtheca* leaves has been determined by the DPPH radical scavenging assay. The results showed that the essential oils of *E. microtheca* leaves have a significant antioxidant activity with an IC_{50} value of 86.5 /mL comparing to the standard material Figure 1.

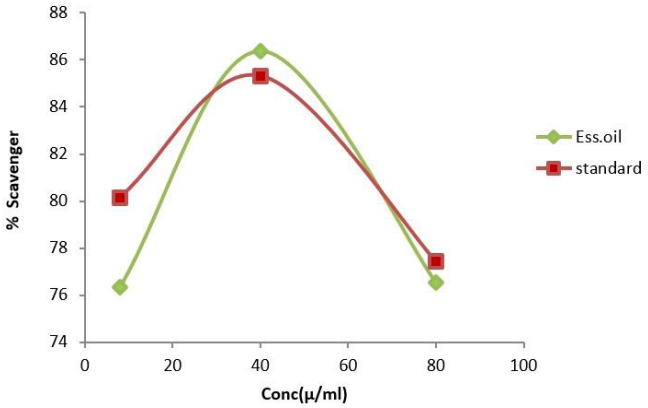

Figure 1: Antioxidant activity of essential oils from *E. microtheca* leaves.

Table 3: Antimicrobial activity of isolated essential oils from *E. microtheca* leaves.

Bacteria (mg/ml)	*Gram-positive bacteria*			*Gram-negative bacteria*		
	S. aureus	*S. epidermidis*	*B. cereus*	*E. coli*	*P. vulgaris*	*S. typhimurium*
200	25	28	27	25	20	27
100	22	23	25	21	16	22
50	18	19	20	18	13	17
25	12	15	16	14	11	14
12.5	11	14	11	10	-	11
			*Antibiotics**			
Amikacin	22	21	24	25	23	21
Gentamycin	25	22	23	22	24	26

*Disc diameter 6 mm

Table 4: Antimicrobial activity of extracted fixed oils from *E. microtheca* leaves.

Bacteria (mg/ml)	*Gram-positive bacteria*			*Gram-negative bacteria*		
	S. aureus	*S. epidermidis*	*B. cereus*	*E. coli*	*P. vulgaris*	*S. typhimurium*
200	24	26	25	20	24	25
100	20	22	20	17	21	23
50	17	17	18	15	18	17
25	14	15	16	10	14	15
12.5	10	12	11	-	9	10
			Antibiotics*			
Amikacin	22	21	24	25	23	21
Gentamycin	25	22	23	22	24	26

*Disc diameter 6 mm.

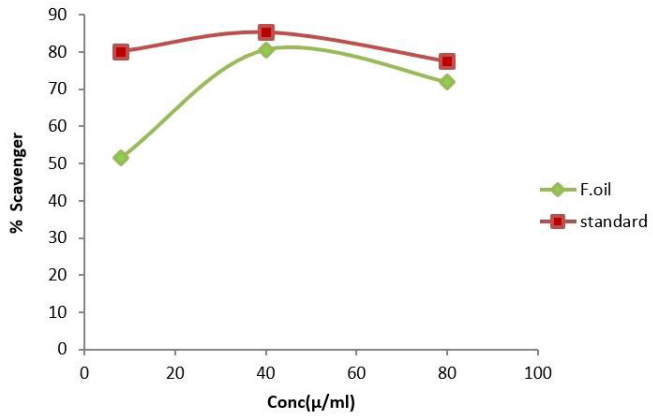

Figure 2: Antioxidant activity of fixed oils from *E. microtheca* leaves.

While the antioxidant activity of the fixed oils extracted from *E. microtheca* leaves exhibited a moderate antioxidant activity with an IC_{50} value of 85.5 /mL comparing to 87.1/mL for the standard substance Figure 2.

The results of the free radical scavenging activity propose a significant relationship between the antioxidant activity of *E. microtheca* oils and the compounds of hydrogen donating free radical scavengers, for example, phenolic compounds [26]. This finding is also supported by another study reporting 81.8% scavenging activity for *E. microtheca* oils [20,27].

CONCLUSION

Different essential and fixed oils have been isolated from the fresh leaves of *E. microtheca* plant located in Mosul city-Iraq. The obtained oils were characterized using GC-MS analysis. The main constituents were Camphene (20.60%), 4-Carene (18.53%), 1,8-Cineole (11.96%), Terpin-4-ol (8.70%) and *p*-Cymene (8.39%) as essential oils. The major fixed oils were Pentadecanoic acid (36.47%) and *cis*-Vaccenic acid (30.31%). The isolated oils showed remarkable antibacterial activity against various Gram-positive and-negative pathogens. The antioxidant activity exhibited the potential of this plant and may suggest it as cheap and new antioxidant source. Thus, the use of naturally available compounds from plant origin against pathogenic bacteria and as antioxidant agents could be considered a suitable alternative to the synthetic medicinal products.

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