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Chemical Composition, Antioxidant and Antimicrobial Activities of Essential Oil from *Leutea kurdistanica* mozaff

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Abstract

Background and Aim: *Leutea kurdistanica* mozaff. (Apiaceae) is a perennial herb endemic to Kurdistan province of Iran. The plants of this family are known for diversity in essential oils and economic importance. The goals of the present study were to evaluate chemical composition, antioxidant and antimicrobial activities of essential oils of *L. kurdistanica*.

Materials and Methods: GC-FID and GC-MS were used for compositional analysis of the essential oils.Antioxidant activities of the oil were assessed using DPPH free radical test. Antibacterial and antifungal activities of the oils were assessed by disc diffusion and agar dilution methods.

Results: A total of 33 compounds representing 97.3-94.2% of the essential oils were identified. Limonene (25.3%) was the main compound of the oil followed by γ–terpinene (18.1%), elemicin (15.4%) and Δ-3-carene (8.2%). In DPPH assay, the oil has radical scavenging activity with IC_{50} value of 51.4±2.3. The results of antimicrobial tests showed that the oil had remarkable inhibitory effects on the growth of Gram-positive bacteria and fungal strains.

Conclusions: Regarding to significant antioxidants and antimicrobial activities of the oil of *L. kurdistanica*, these oils could be consider as a natural preservative in food and other industries. Although more investigation is needed to clarify the exact compounds responsible for observed biological activities.

Keywords: Antimicrobial, Antioxidant, Essential oil, *Leutea kurdistanica* mozaff

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Introduction

Apiaceae (formerly Umbelliferae) family has been considered to be the most important families of plants comprising about 455 genera and 3600 species (1). The plants of this family are known for diversity in essential oil and economic importance, especially in perfume, food and pharmaceutical industry (2). In the

last decade more and more studies have been published on the antimicrobial and antioxidant properties of essential oils from this family (3-5).The genus *Leutea*, belong to the Apiaceae family, consist of at least 70 endemic species (6).*Leutea kurdistanica* Mozaff. is one of endemic herbs of this family growing wild in Sarala mountain of Kurdistan province of Iran. The essential oil compositions of some *Leutea* species have been reported (7, 8). The main constitutes of *L. albursensis* are α-pinene (37.3%), β-pinene (36.1%) and limonene (4.8%). Compositional analysis of volatile compounds of *L. glaucopruinosa* revealed α-pinene (31.5%), sabinene (9.7%), β-pinene (9.2%) and exo-fenchyl acetate (4.5% w/w) as major constituents. The essential oil compositions of *L. kurdistanica* have been previously studied (9). The main compounds at the flowering stage were reported as α-asarone (62.5%) and elemicin (22.5%). To the best of our knowledge, there were no reports regarding biological activities of *L. kurdistanica* Mozaff. essential oil. Therefore, the aim of the current survey was to provide new information on the chemical composition of the essential oil gained from aerial parts of *L. kurdistanica* and assess its antioxidant and antimicrobial activities.

Materials and Methods

Plant material

The aerial parts of *L. kurdistanica* were collected during the flowering stages on the 7th of June, 2014 from Sarala mountain of Kurdistan province, Sanandaj, Iran. A voucher specimen (8793) has been deposited at the herbarium of Research Center of Agriculture and Natural Resources, Sanandaj, Iran.

Essential oil isolation

Air-dried aerial parts of the plant were crushed to ensure maximum yield. One hundred grams of the plant materials were subjected to hydro distillation using a Clevenger-type apparatus for 4 hours at normal pressure. The oil was dehydrated by anhydrous sodium sulfate and kept in the refrigerator (2-8°C) in sealed glass vial for further analysis.

GC and GC/MS analysis

The essential oils were explored with a Thermoquest-Finnigan Trace (Thermo Fisher Scientific, USA) with a flame ionization detector (FID). Analysis was carried out on a fused silica capillary DB-5 column (60m×0.25mm, 0.25µm film thickness). The constant flow (1.1 ml/min) of nitrogen was used as the carrier gas and injection volume was 1 µL. Injector temperature 250 ºC, detector temperature (FID) 280 ºC, Column temperature was elevated from 60ºC (hold: 2 min) to 250ºC (hold: 5 min) at a rate of 4ºC/min. GC-MS analysis was carried out in the electron impact (EI) mode at 70 eV using a Thermoquest-Finnigan Trace instrument. The column and temperature programming were the same as mentioned for GC-FID analysis with helium (He) used as the carrier gas (1.1 ml/min). The essential oil components of *L. kurdistanica* were identified from calculations of their retention indices (RIs) for a series of n-alkanes C_6–C_{28} under the identical chromatographic circumstances and by comparison of their mass spectra with Wiley 7.0 library or with authentic compounds (10).

DPPH radical scavenging assay

Radical scavenging activity of the essential oil was assessed using DPPH (2.2-diphenyl-1-picrylhydrazyl) free radical as a reagent with some alteration.[11,12] Briefly, 4 mL of a freshly prepared solution of DPPH (0.1 mM) radical in methanol was mixed with 1 mL of different methanolic dilutions of essential oils. The reaction mixture were vigorously shaken and incubated for 30 min in a dark. The absorbance for each sample was read three times by use of a spectrophotometer (Shimadzu UV-1601) at a 517 nm and the percentage inhibition was calculated using the following equation:

$$\% \text{ inhibition} = [(A_{blank} - A_{Sample}) / A_{blank}] \times 100$$

Where A_{blank} and A_{Sample} are the absorbance of control reaction mixture except the oils and the oils, respectively.

Assessment of antimicrobial activity

Microbial strains

The essential oils of *L. kurdistanica* were tested against two Gram-positive bacteria, *Staphylococcus aureus* (ATCC 29737) and *Micrococcus luteus* (PTCC 1110), three Gram-negative bacteria, *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhi* (PTCC 1609). Two fungi, *Aspergillus niger* (ATCC 16404) and *Candida*

albicans (ATCC 10231) were also used for the determination of antifungal activity.

Disc diffusion assay

The antimicrobial activity of *L. kurdistanica* essential oil was evaluated by agar disc diffusion assay (13, 14). The essential oils were dissolved in dimethylsulfoxide (DMSO) and filtered for sterilization. Briefly, 0.1 mL of suspension of tested microorganisms comprising 10^8 colony-forming units (CFU)/mL of bacteria and 10^4 CFU/mL spores of fungi spread on nutrient agar and potato dextrose agar medium, respectively. Sterile paper discs were positioned on the surface of plates and were infused with 15 µL of oils. The plates were placed at room temperature for 30 min to permit the diffusion of the oil and then incubated at 37±0.1°C for 24 hours for bacterial and at 30±0.1°C for 48 hours for fungi. Antimicrobial activity was assessed by calculating the zones of inhibition (in millimeters) around the discs after the incubation period. Ampicillin, gentamicin (10µg/disc) and ketoconazole (20µg/disc) were used as a positive control for Gram-positive, Gram-negative bacteria and fungal strains, respectively.

MIC agar dilution assay

Minimum inhibitory concentration (MIC) represents the lowest concentration of the compounds which entirely inhibit the visible growth of microorganisms. The MIC value of *L. kurdistanica* essential oil against bacterial and fungal strains was assessed according to a conventional agar dilution method (15,16). The appropriate volumes of obtained essential oil were added aseptically to sterile Luria–Bertani media comprising Tween 80 (0.5%, v/v) to yield different concentration (7.8-2000 µg/ml) of the oils. Then, 0.1mL of standard microorganism suspension was mixed and transfer into the microplates for incubation. Finally, the lowest concentration which inhibits the growth of the bacteria and fungi strains was consider as the MIC value of the oils.

Statistical analysis

The experiments were done in triplicate. Data are expressed as means±SD. Data were analysed by SPSS 19.0 statistical software and student t-test was used for copmarision between groups. $P< 0.05$ was considered to be statistically significant.

Results and Discussion

Table 1: Chemical composition of the essential oil of *L. kurdistanica*.

No.	Compounds	RI	%
1	α-Thuiene	930	0.4
2	α-Pinene	939	7.1
3	Camphene	954	0.2
4	Verbenene	968	0.1
5	Sabinene	975	1.3
6	β-Pinene	979	0.6
7	Myrcene	991	2
8	α-Phellandrene	1003	2.5
9	α-Terpinene	1017	0.7
10	Limonene	1029	25.3
11	Δ- 3- Carene	1031	8.2
12	(Z)-β -Ocimene	1037	0.6
13	γ - Terpinene	1060	18.1
14	Terpinolene	1089	0.6
15	Linalool	1097	0.1
16	Allocimene	1132	0.2
17	1- Terpineol	1134	tr
18	4- Terpineol	1177	0.3
19	Bronyl acetate	1289	0.1
20	Thymol	1290	tr
21	Carvacrol	1299	tr
22	Cyclosativene	1371	0.1
23	α – Copaene	1377	0.1
24	β-Elemene	1391	tr
25	β-Caryophyllene	1419	tr
26	Croweacin	1460	1.1
27	Germacrene D	1485	1
28	Bicyclogermacrene	1500	2.6
29	Myristicin	1519	4.8
30	Elemicin	1557	15.4
31	Germacrene B	1561	0.3
32	Spathulenol	1578	0.4
33	Apiol	1678	3.1
	Total identified		97.3

tr = trace (<0.1%)

[a]RI = Retention index with respect to a homologous series of n-alkanes (C_6–C_{28}) on the DB-5 column.

Essential oils composition

The volatile compound identified by GC and GC/MS analysis in the oils of aerial parts of *L. kurdistanica*has been reported in Table 1. A total of 33 compounds representing 97.3-of the essential oil were identified. The major constitutes found in the aerial parts were limonene (25.3%), γ–terpinene (18.1%), elemicin (15.4%), Δ-3-carene (8.2%), α-pinene (7.1%) and myristicin (4.8%). Monoterpene hydrocarbons (67.9%) were found as the most plentiful representatives. The results of essential oil composition were different from previous study, which refers to α-asarone as the main compound of the oil (9). This variation in the main component of the oil could be due to different plant collection times and stress caused by insets or microorganisms (17).

Antioxidant and antimicrobial activities

The essential oils of this plant also exhibited a moderated antioxidant activity in DPPH free radical scavenging test (IC_{50} 51.4 ± 2.3), compared to vitamin E (IC_{50} 18.6 µg/ml). The observed antioxidant activity may be due to the existence of the different chemical components especially monoterpenes (67.9%) found in this oil. It has been demonstrated that the essential oils which contain monoterpene hydrocarbons, have considerable antioxidative properties and can act as radical scavenging agents. These activities could be ascribed to the presence of limonene, γ–terpinene, Δ-3-carene and α–pinene found in the essential oils of *L. kurdistanica*(18-20). The essential oils of *L. kurdistanica* were tested against five bacterial and two fungal strains (Table 2). The results showed that the oils had an inhibitory effect against tested bacteria and fungi. The obtained results from disc diffusion and MIC assays showed that *S. aureus* and *M. luteus* were the most sensitive bacteria showing biggest zones of inhibition (18.3±1.7 mm), (17.2±0.7mm) and lowest MIC values (250µg/mL), respectively. On the other hand, less activity was observed against Gram-negative bacteria with the smallest zone of inhibition (ranged from 6 to 9mm) and highest MIC value (>2000 µg/mL). Although the exact mechanism responsible for observed antimicrobial activity of these oils is still unclear, but it has been shown that monoterpene could cease ion transport processes in bacteria cell membrane and finally destroy cellular integrity.[21] Moreover, strong antimicrobial and antioxidant activity of the main compounds limonene, γ–terpinene and elemicin have been demonstrated (22-25). The results of antimicrobial assay showed that the oil had the better activity against Gram-positive bacteria than Gram-negative bacteria this could be due to existence of lipopolysaccharide layer on the cell wall of Gram-negative bacteria which make them

Table 2: Antimicrobial activity of the essential oil of *L. kurdistanica*.

	Essential oil		Antibiotics		
Microbial strains	**DD[a]**	**MIC[b]**	**Amp[c]**	**Gen[d]**	**Ket[e]**
Gram-positive bacteria					
Staphylococcus aureus	18.3 ± 1.7	250	18.8	NT[f]	NT
Micrococcus luteus	17.2 ± 0.7	250	19.1	NT	NT
Gram-negative bacteria					
Escherichia coli	6.8 ± 0.9	>2000	NT	17.2	NT
Pseudomonas aeruginosa	7.3 ± 0.2	>2000	NT	17.7	NT
Salmonella typhi	8.2 ± 1.6	>2000	NT	17.4	NT
Fungi					
Aspergillus niger	22.1 ± 0.2	250	NT	NT	27
Candida albicans	24.2 ± 0.1	250	NT	NT	28

[a]DD, diameter of inhibition zone in mm including disc diameter (6 mm); [b]MIC, minimal inhibition concentrations expressed in µg/ml; [c]Ampicillin (10 µg/disc); [d]Gentamicin (10 µg/disc); [e]Ketoconazole (20 µg/disc); [f]NT, not tested.

more resistant to the effect of essential oils. Lack of this layer in Gram-positive bacteria makes them more susceptible to the oils (26). The results of antifungal assays indicated that the essential oils of *L. kurdistanica* has inhibitory effects on the growth of *A. niger* and *C. albicans* (Table 2). The plant essential oils comprise a complex mixture of different components and the major or minor compound(s) might have an antifungal activity (27).Probable synergistic and antagonistic properties of compounds also play a chief role in antifungal activities (28). Although the mixture of the compounds exhibited antifungal activity, fungitoxic activity of the main compounds limonene, γ–terpinene and α–pinene on studied species have been reported (29-32).

Conclusion

The result of chemical composition in essential oil of the *L. kurdistanica* announces them as a source of monoterpene hydrocarbons. Regarding to significant antioxidants and antimicrobial activities of the oil from aerial parts of *L. kurdistanica*, this oil could be consider as a natural preservative in food and other industries. Although more in-depth investigation is recommended to clarify the exact compounds responsible for observed biological activities.

Conflict of Interest

The authors declare that they have no conflict of interest.

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