Chemical Composition and Antimicrobial Activities of the Essential Oils from Flower and Leaves of *Lagochilus kotschyanus* Boiss. A New Species from Iran

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Abstract

Background: The genus *lagochilus*, family Lamiaceae consists of 44 species, 33 of which grow in central Asia. Only one report on the analysis of essential oil of *Lagochilus* species has been published.

Objective: Aim of this study is to determine the chemical composition and antimicrobial activities of the hydrodistilled oil of flower and leaves of *Lagochilus kotschyanus* Boiss. of Iranian origin for the first time.

Methods: The essential oils obtained by hydrodistillation from the flower and leaves of *L. kotschyanus* were analyzed by GC and GC/MS. The antimicrobial activities were determined by measuring the growth inhibitory zones (well diffusion assay).

Results: The major constituents of the flower and leaf oils appeared to be myrcene (28.2% and 36.6%), α-pinene (25.9% and 29.8%) and β-caryophyllene (10.3% and 9.4%), respectively.

Conclusion: In *L. kotschyanus* we identified 34 components representing 95.2% and 18 constituents representing 96.3% of the flower and leaf oils, respectively. Both oils were rich in regard to monoterpene hydrocarbons (72.7% and 79.7%) respectively. The sesquiterpene fraction was relatively small, representing 19.4% and 14.1%, respectively. Antibacterial activity was measured using the growth inhibitory zones. It was found that oil from leaves and flowers of *L. kotschyanus,* and especially that of flowers, exhibited interesting antibacterial activity.

Keywords: *Lagochilus kotschyanus*, Lamiaceae, Essential oil composition, Myrcene, α-pinene, β-caryophyllene, Antimicrobial activity

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Introduction

The genus *lagochilus*, family Lamiacea consists of 44 species, 33 of which grow in central Asia. The flora of Iran comprises five species, including four endemics: *L. alutaceus* Bunge., *L. aucheri* Boiss., *L. kotschyanus* Boiss. and *L. macranthus* Fisch. & C.A. Mey. [1, 2]. Chemical studies on some *Lagochilus* species have resulted: diterpenoids from *L. hirsutissimus* [3-5] and *L. inebrians* [6-8], flavonoids from *L. proskorjacovi* [9] and *L. platycalyx* [10] and polysaccharides from *L. usunachmaticus* [11] and *L. zeravschanicus* [12]. Only one report on the analysis of essential oil of *Lagochilus* species has been published [13]. The present work presents the chemical composition and antimicrobial activities of the hydrodistilled oil of flower and leaves of *Lagochilus kotschyanus* Boiss. of Iranian origin for the first time.

Material and Methods

Plant material

The flowers and leaves of *L. kotschyanus*, which is endemic to Iran, were collected from Saveh, Province of Markazii, Central of Iran, in July 2008 during the flowering stage. Plant materials were dried at ambient temperature and shade condition. Voucher specimens have been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Preparation of oil

Flowers (50 g) and Leaves (40 g) of *L. kotschyanus* were subjected to separate hydrodistillation using a Clevenger-type apparatus for 3h. After decanting and drying of the oils over anhydrous sodium sulfate, the corresponding oils were isolated in yields of 0.1 and 0.1 (w/w), respectively.

GC-MS analysis

The oil was analyzed using a Hewlett-Packard 5973 with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm). The column temperature was kept at 60°C for 3 min and programmed to 220°C at a rate of 5°C/min and kept constant at 220°C for 5 min. The flow rate of Helium as carrier gas was (1 mL/min). MS were taken at 70 eV. Identification of the constituents of oil was made by comparison of their mass spectra and retention indices (RRI) with those given in the literature and those authentic samples [14]. GC analysis was performed on a Shimadzu 15A gas chromatograph equipped with a capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 pin). Spilt/spiltless injector and a flame ionization detector were heated at (250°C). N2 was used as carrier gas (1 mL/min). The oven temperature was kept at 60°C for 3 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac integrator without the use of correction Factors.

Antimicrobial Assay

The antimicrobial activities of the flower and leaf oils of *L. kotschyanus* were determined by measuring the growth inhibitory zones (well diffusion assay) against four Gram-Positive and two Gram-negative bacteria. The Gram-Positive bacteria included *Staphylococcus aureus* PTCC 1885, *Streptococcus agalactia* PTCC 1913, *Streptococcus pyogenes* PTCC 1940 and *Bacillus anthracis* PTCC 1036, and Gram-negative bacteria included *kelebsiella pneumoniae* PTCC 1249 and *Pseudomonas aeruginosa* PTCC 1547. The microorganisms

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were obtained from the Research Center of Science and Industry, Tehran, Iran.

Microorganisms (obtained from enrichment culture of the microorganisms in 1ml. Muller-Hilton broth, incubated at 37 ^{0}C for 12h) were cultured on Muller-Hilton agar medium. The inhibitory activity was compared with that of standard antibiotics, such as gentamicine (10 µg), which where obtained from the Iran Daru Company. After drilling wells on medium using a 6mm cork bower 100 l of oils obtained from flowers and leaves of *L. kotschyanus* were poured into each well. The plates were incubated at 37 ^{0}C overnight; the diameter of the inhibition zone was measured to the nearest millimeter. Each test was carried out in triplicate and the average was calculated for inhibition zone diameters. The antibacterial activity was recorded as the radial extent of the area cleared of bacterial growth around the well. The micro-dilation broth susceptibility assay was used for the evolution of minimal inhibitory concentration (MIC). After incubation at 37 ^{0}C for 24 h, the first well without turbidity was determined as minimal inhibitory concentration.

Results and Discussion

Chemical composition identified in the flower and leaf oils of *L. kotschyanus* and their percentage composition are listed in Table 1. In *L. kotschyanus* we identified 34 components representing 95.2% and 18 constituents representing 96.3% of the flower and leaf oils, respectively. The main components in both oils were myrcene (28.2% and 36.6%), α-pinene (25.9% and 29.8%) and β-caryophyllene (10.3% and 9.4%), respectively. Other notable constituents in flower and leaf oil of the plant was limonene (7.3% and 6.0%) respectively.

Table 1- Chemical composition (%) of the essential oil from flowers and leaves of *L. kotschyanus*

Compound	RI[a]	Flower	Leaves
Tricyclene	926	0.2	-
α-Thujene	931	2.9	2.2
α-Pinene	939	25.9	29.8
Camphene	953	0.3	-
Thuja-2,4(10)-diene	957	0.1	-
Sabinene	976	2.6	1.6
β-Pinene	980	1.8	1.4
Myrcene	991	28.2	36.6
α-Phellandrene	1005	0.3	-
δ-3-Carene	1011	0.7	-
α-Terpinene	1018	0.2	-
P-Cymene	1026	0.2	-
Limonene	1031	7.3	6.0
(Z)-β-Ocimene	1040	0.5	0.6
(E)-β-Ocimene	1050	0.7	0.6
γ-Terpinene	1062	0.3	0.3

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Continue Table 1- Chemical composition (%) of the essential oil from flowers and leaves of *L. kotschyanus*

Compound	RI[a]	Flower	Leaves
Terpinolene	1088	0.5	0.3
Perillene	1099	1.0	0.8
α-Champholenal	1125	0.4	-
cis-Verbenol	1140	0.3	-
trans-Verbenol	1144	0.5	-
Pinocarvone	1162	0.2	-
Terpinen-4-ol	1177	0.3	0.4
Myrtenal	1193	0.2	-
β-Caryophyllene	1418	10.3	9.4
α-Humulene	1454	1.5	1.3
Germacrene D	1480	2.0	-
(E)-β-Ionone	1485	-	1.1
Bicyclogermacrene	1494	0.2	-
(E,E)-α-Farnesene	1508	0.2	-
Caryophyllene oxide	1518	4.2	3.1
Viridiflorol	1590	0.3	-
Humulene epoxide II	1606	0.4	0.3
Khusinol	1680	0.3	-
6',10,14,-trimethyl-2-Pentadecanone	1849	0.2	0.5
Monoterpene hydrocarbons		72.7	79.4
Oxygenated monoterpenes		2.9	1.2
Sesquiterpene hydrocarbons		14.2	10.7
Oxygenated sesquiterpenes		5.2	3.4
Non-terpenoid compounds		0.2	1.6
Total		**95.2**	**96.3**

Retention indices as determined on a DB-5 column using the homologous series of n-alkane

as can be seen from the above information, the composition of the flower and leaf of *L. kotschyanus* were quite similar. Both oils were rich in regard to monoterpene hydrocarbons (72.7% and 79.7%) respectively. The sesquiterpene fraction was relatively small, representing 19.4% and 14.1%, respectively. The main components in the essential oil of *L. diacanthophyllus* from Kazakhstan were α-pinene (8.7%) and dillapole (3.1%).

Antimicrobial activities

The antimicrobial activities of flowers and leaves of *L. kotschyanus* oils were assayed against four Gram-positive and two Gram-negative bacteria and results presented in Table 2, were compared with standard antibiotics, such as gentamicine. The present study revealed that the oil of flowers indicated significant activity against all Gram-positive bacterial. This oil showed strong inhibitory

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Table 2- Antimicrobial activities of the flowers and leaves of *L. kotschyanus*

Microorganisms	Gram +/	Flowers		Leaves		Gm
		IZ	MIC	IZ	MIC	
Staphylococcus aureus PTCC 1885	+	20	12.5	_	_	20
Streptococcus agalactia PTCC 1913	+	40	3.125	_	_	20
Streptococcus pyogenes PTCC 1940	+	45	3.125	20	12.5	_
Bacillus anthracis PTCC 1036	+	40	3.125	_	_	32
kelebsiella pneumoniae PTCC 1249	_	30	6.25	_	_	_
Pseudomonas aeruginosa PTCC 1547	_	_	_	_	_	16

activity against *Streptococcus pyogenes*, *Streptococcus agalactia* and *Bacillus anthracis*. *Staphylococcus aureus* showed a smaller zone of inhibitory by the oil of Flower. This oil also showed inhibitory activity against Gram-negative bacteria, *Kelebsiella pneumoniae*. *Pseudomonas aeruginosa* was insensitive to the oil of flowers. The leaves oil only showed inhibitory activity against *Streptococcus pyogenes*.

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