NPC Natural Product Communications

2017
Vol. 12
No. 3
445 - 448

Composition and Antimicrobial Properties of Essential Oils of *Laser trilobum* Rhizomes and Fruits

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Received: September 27th, 2016; Accepted: December 20th, 2016

The compositions of hydrodistillated essential oils of *Laser trilobum* (L.) Borkh. rhizomes and fruits from Serbia, were investigated using GC and GC/MS. In the dark-blue rhizome oil forty-six compounds (93.1% of the total oil) were identified, with α-pinene (31.5%), γ-terpinene (9.0%), *p*-cymene (7.9%), β-pinene (6.1%) and 1,4-dimethylazulene (6.0%) as the major components. In the colorless fruits oil, twenty components (96.8% of the total oil) were identified, and the main constituents were limonene (51.6%) and perillaldehyde (26.8%). The antimicrobial activity of the oils was tested using the broth microdilution method against nine bacterial and two fungal strains. The oils revealed significant antimicrobial effect, mainly better than that of thymol, used as a reference compound. The strongest activity was recorded for the rhizome oil against *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans* (MICs=25 μg/mL), and the fruit oil against *C. albicans* ATCC 10259 (MIC=12.5 μg/mL).

Keywords: *Laser trilobum*, Rhizome, Fruits, Essential oils, Antimicrobial activity.

Laser trilobum (L.) Borkh. (Apiaceae) is a perennial, herbaceous, aromatic plant, up to 120 cm tall, with pinnate leaves and broadly ovate leaflets [1,2]. This plant, known as Kefe cumin, horse caraway, gladich or three-lobed sermountain, grows in Iran, Southeast Asia, the Balkan Peninsula, and Central and Eastern Europe [2-6]. It prefers dry and light woods and scrubs on rocky ground, but also can be found on steppe pastures [1]. The shoots of this plant were eaten as a vegetable [7] and dried, ground mature fruits are occasionally used as a spice in some meat products, in the same way as cumin [3]. The demonstrated significant antimicrobial effect of the ground fruits justified its use as a condiment [8]. Also, the fruit methanol extract exhibited an inhibitory effect on a series of pathogenic bacteria [3].

Laser trilobum rhizome contains a whole range of sesquiterpene lactones, the most abundant being trilobolide, which possess deterrent activity against some storage pests [9], and immunostimulatory properties [5,10,11]. The presence of trilobolide was also confirmed for the other plant organs, particularly the fruits [5]. A hydroalcoholic leaf extract exhibited antioxidant and antihaemolytic activities [12]. The chemical compositions of the essential oils obtained from fruits, leaves and aerial parts of *L. trilobum* have been published [3,7,13,14], while data concerning the antimicrobial activity of these essential oils are scarce [3,15].

The aim of this study was to analyse the essential oils of wild growing *L. trilobum* from Serbia, in order to establish the composition and antimicrobial activity of the rhizome oil for the first time, as well as to compare the composition and antimicrobial activity of the fruit oil with data previously obtained for samples of different origin. In the dark-blue rhizome oil (sample 1) forty-six compounds (93.1% of the total oil) were identified. Monoterpenes constituted 71.5% of oil, with hydrocarbons prevailing (66.4%). Among sesquiterpenes (15.3%), hydrocarbons were the most abundant (14.0%). The major components of the rhizome oil were α-pinene (31.5%), γ-terpinene (9.0%), *p*-cymene (7.9%), and β-pinene (6.1%), as well as 1,4-dimethylazulene (6.0%), which gives the intensive blue colour to this oil.

In the colorless fruit oil (sample 2) twenty compounds (96.8% of the total oil) were identified, among them sixteen monoterpenes. Sesquiterpenes were present in traces only. The main constituents of this oil were limonene (51.6%), perillaldehyde (26.8%), α-pinene (7.4%) and *cis*-limonene oxide (4.8%) (Table 1).

The chemical composition of the fruit essential oils of wild growing *L. trilobum* from Turkey [3,13] and Austria [7] was previously investigated, along with commercial samples from India and Germany [3]. In these studies, limonene and perillaldehyde in varying proportions were also reported as the major compounds of the oils (26.7-91.0% and 4.2-79.7%, respectively). These compounds also characterised the fruit oil of the closely related *Laserpitium siler* L. [16].

Previously, in the essential oil from leaves of *L. trilobum* bornyl angelate (67.3-73.5%) and α-pinene (3.4-12.6%) were determined as the main compounds [7], whereas in the oil from aerial flowering parts β-caryophyllene (22.4%), myrcene (21.7%) and β-sesquiphellandrene (19.2%) were detected as the dominant constituents [14]. Based on our results and literature data it can be concluded that the essential oils of the various plant organs of *L. trilobum* differ considerably in terms of their chemical composition.

Table 1: Chemical composition of *Laser trilobum* essential oils.

RI exp[a]	Compound[b]	Sample 1 (rhizome oil) (%[c])	Sample 2 (fruit oil) (%)
938	α-Pinene	31.5	7.4
952	Camphene	0.6	-
973	Sabinene	4.4	-
977	β-Pinene	6.1	0.9
989	Myrcene	3.1	0.8
996	*n*-Octanal	0.3	-
1015	α-Terpinene	0.3	-
1023	*p*-Cymene	7.9	-
1026	Limonene	2.3	51.6
1028	1,8-Cineole	0.2	-
1035	*(Z)*-β-Ocimene	0.4	tr[d]
1048	*(E)*-β-Ocimene	0.6	tr
1057	γ-Terpinene	9.0	-
1087	Terpinolene	0.2	-
1094	6-Camphenone	tr	-
1101	*cis*-Thujone	0.7	-
1107	1,3,8-*p*-Menthatriene	-	0.3
1112	*trans*-Thujone	tr	-
1120	*trans*-*p*-Mentha-2,8-dien-1-ol	-	tr
1123	α-Campholenal	tr	-
1135	*cis*-Limonene oxide	-	4.8
1136	*trans*-Pinocarveol	tr	-
1139	*trans*-Limonene oxide	-	2.3
1143	Camphor	0.6	-
1166	Borneol	tr	-
1174	Terpinen-4-ol	0.3	-
1185	α-Terpineol	tr	-
1193	(4R,8R)-8,9-Epoxy-*p*-menth-1-ene	-	0.5
1196	(4R,8S)-8,9-Epoxy-*p*-menth-1-ene	-	0.5
1214	*trans*-Carveol	-	0.2
1231	Thymol, methyl ether	2.9	-
1240	Carvone	-	0.5
1268	Perillaldehyde	-	26.8
1283	Isobornyl acetate	tr	-
1291	Perillalcohol	-	0.2
1342	Bicycloelemene	0.5	-
1348	α-Cubebene	1.5	-
1374	α-Copaene	1.5	tr
1385	β-Cubebene	0.9	tr
1387	β-Elemene	0.9	tr
1415	*(E)*-Caryophyllene	0.7	-
1424	2,5-Dimetoxy-*p*-cymene	0.4	-
1451	*α*-Humulene	0.4	-
1454	*(E)*-β-Farnesene	tr	-
1476	γ-Muurolene	0.8	-
1481	Germacrene D	2.2	-
1486	β-Selinene	0.2	-
1496	Bicyclogermacrene	4.0	-
1502	β-Bisabolene	tr	-
1518	7-*epi*-α-Selinene	0.4	-
1520	*δ*-Cadinene	0.7	-
1531	*trans*-Cadina-1,(2),4-diene	tr	-
1557	1,4-Dimethylazulene	6.0	-
1574	Spathulenol	1.1	tr
1580	Caryophyllene oxide	0.2	-
1633	Isospathulenol	tr	-
	Monoterpene hydrocarbons	66.4	35.8
	Oxygenated monoterpenes	5.1	61.0
	Sesquiterpene hydrocarbons	14.0	tr
	Oxygenated sesquiterpenes	1.3	tr
	Others	6.3	-
	Identified	**93.1**	**96.8**

[a]RI exp - Retention indices on HP-5MS column relative to C_9-C_{24} *n*-alkanes; [b]Constituents listed in the order of elution on HP-5MS column; % - Relative area percentage of the compounds obtained from FID area percent data; [d]tr - Trace (<0.1%).

The results of the antimicrobial activity are presented in Table 2. *L. trilobum* rhizome and fruit essential oils exhibited significant antimicrobial properties (MICs against all tested strains were ≤ 100 μg/mL) [17,18]. The best activity was showed by rhizome oil against *E. coli*, *K. pneumoniae* and both strains of *C. albicans* (MICs=25 μg/mL), and by fruit oil against *C. albicans* ATCC 10259 (MIC=12.5 μg/mL). The tested Gram-negative bacteria were more sensitive to the rhizome oil than to the fruit oil. In addition, it is interesting that rhizome oil exerted a more pronounced effect against Gram-negative than Gram-positive bacteria, since Gram-negative bacteria are generally more resistant to essential oils [19]. The antimicrobial property of the investigated *L. trilobum* essential oils was compared with thymol, a well known antimicrobial agent. While the effect of the rhizome oil against *S. aureus* and fruit oil against *S. epidermidis* was comparable with the effect of thymol, the activity of both oils against all other tested microorganisms was even better than that of the reference compound.

Table 2: Minimum inhibitory concentrations (MICs) (μg/mL) of investigated *Laser trilobum* essential oils.

Microorganism	Sample 1 (rhizome oil) MIC	Sample 2 (fruit oil) MIC	Thymol MIC
S. aureus	100	25	93
S. epidermidis	50	100	93
M. luteus	nt	50	93
M. flavus	100	nt	nt
E. faecalis	100	100	465
B. subtilis	nt	50	nt
E. coli	25	100	186
P. aeruginosa	50	100	>558
K. pneumoniae	25	100	279
C. albicans ATCC 10259	25	12.5	93
C. albicans ATCC 24433	25	50	93

nt, not tested.

It should be noted that our findings differ considerably from the results of earlier studies referring to the antimicrobial effect of *L. trilobum* fruit oil. Namely, in previous investigations on its activity against some of the bacterial strains used in our study, but conducted using different methods (agar diffusion and other dilution methods), it was concluded that the oil either had no significant effect [3], or was fairly active [15].

Antimicrobial activity of α-pinene, limonene and perillaldehyde, the most abundant constituents of the analyzed oils, as well as that of some other major and minor components, such as *p*-cymene, β-pinene, sabinene and germacrene D, was shown previously [20-22]. In some cases, the activity of isolated components was weaker than that of the *L. trilobum* oils analyzed in this study, suggesting that the antimicrobial effect of these oils may be due to a synergistic action of their constituents, a well-known phenomenon for essential oils [23].

Despite the fact that *L. trilobum* is rich in sesquiterpene lactones, of which the most characteristic and most substantial is trilobolide, closely related to the extensively studied thapsigargin, this plant remained less attractive for proper phytotherapeutic study [5]. The antimicrobial (and particularly antifungal) activity of essential oils of *L. trilobum* rhizome and fruits demonstrated in this work is an additional reason why this plant might be a good natural source of biologically active isolates, with potential use in both the pharmaceutical and food industries. It is important to point out that in spite of being a common mountain plant it should be cultivated in order to achieve its rational exploitation as an industrial raw material.

Experimental

Plant material: The rhizome and mature fruits of *L. trilobum* were collected in south-eastern Serbia (Sićevačka gorge), in April and

August 2007, respectively. A voucher specimen with overground parts (No 20070401 BEO) is deposited at the Herbarium of the Natural History Museum, Belgrade (BEO).

Isolation of the essential oils: The essential oils were isolated from the air-dried and finely ground rhizomes (sample 1) and mature fruits (sample 2) by hydrodistillation using a Clevenger-type apparatus, according to the Ph. Eur. Essential oil yields for samples 1 and 2 were 0.5% and 6.9%, w/w, respectively.

Essential oils analysis: GC analysis was carried out using a SRI 8610C GC/FID system, equipped with DB-5 capillary column (30 m × 0.32 mm; film thickness 0.25 μm) and connected to a FID detector. The injector and detector temperature was 280ºC. The carrier gas was He, at flow rate of 1.2 mL/min. The thermal program was 60-280ºC at a rate of 3ºC/min. GC/MS analysis was performed on a Hewlett Packard 6890-5973 GC/MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (200ºC). The transfer line temperature was 250ºC. He was used as carrier gas (1 mL/min) and the capillary column used was an HP 5MS (30 m × 0.25 mm; film thickness 0.25 μm). The temperature program was the same as that used for the GC analyses; split ratio 1:10. The identification of the compounds was based on comparison of their linear retention indices (RI), their retention times (RT) and mass spectra with those obtained from the NIST/NBS, Wiley libraries and literature [24]. The linear retention indices (RI) were determined in relation to a homologous series of *n*-alkanes (C_9-C_{24}) under the same operating conditions [25]. Relative percentages of compounds were calculated based on the peak areas from the FID data.

Antimicrobial activity: Antimicrobial activity of isolated oils was assayed according to the recommendation of the Clinical and Laboratory Standards Institute [26]. Nine bacterial strains were tested: Gram-positive *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Micrococcus flavus* ATCC 10240, *M. luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633, and *Enterococcus faecalis* ATCC 29212, and Gram-negative *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* NCIMB 9111, along with two standard strains of *Candida albicans* (ATCC 10259 and 24433). Microorganisms were provided by the Institute for Immunology and Virology, Torlak, Belgrade.

The broth microdilution assay was used for the determination of minimum inhibitory concentrations (MICs) [27]. Müller-Hinton and Sabouraud dextrose broth supplemented with Tween 80 detergent (final concentration of 0.5%, v/v), were used for bacteria and fungi, respectively. Microbial strains were suspended in broth to give a final density of 5×10^5 CFU/mL. Dilutions of essential oils solutions in DMSO (15 mg/mL) were prepared in concentrations ranging from 12.5 to 250 μg/mL.The positive growth of the microorganisms was indicated by the presence of a pellet on the bottom of the well after incubation at 37°C (18 h for bacteria, and 48 h for *Candida albicans*). Thymol (Fluka, Germany) was used as the reference compound. The MIC value was defined as the lowest concentration of essential oil/thymol at which the microorganism did not demonstrate visible growth. The test was performed 2 times in duplicate against each microorganism.

Acknowledgments - This research was supported by the Ministry of Education and Science of Republic of Serbia (Grant No. 173021).

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