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Characterization of the Essential Oil Composition of *Isodon rugosus* (Wall. ex Benth.) Codd. from Garhwal region of Western Himalaya

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Abstract

Background: *Isodon rugosus* belongs to the family Lamiaceae and is an important aromatic shrub, distributed widely throughout the northern temperate regions of the Himalaya. Regardless of the various traditional usage and diverse biological activities, little data exists on the essential oil composition of *I. rugosus* populations. This study aimed to characterize the essential oil of *I. rugosus* collected from two locations (Ghangaria and Tapovan) of Garhwal region of western Himalaya for occurrence of new composition.

Methods: The shade dried plant materials were hydrodistilled in a Clevenger's type apparatus for 3 h for isolation of their essential oils. The resulting essential oils were analysed using gas chromatography-flame ionization detector (GC-FID) and GC-mass spectrometry (GC-MS).

Result: Altogether, 92 constituents, comprising 83.9-92.6% of the total oil compositions were identified. Major constituents of the oil were α-pinene (0.8-19.3%), *trans*-ferruginol (3.0-17.1%), α-phellandrene (2.6-10.5%), (*E*)-caryophyllene (4.0-9.3%), germacrene D (1.9-8.8%), abietatriene (1.9-5.3%), β-phellandrene (3.1-4.4%), δ-cadinene (2.9-3.6%), limonene (1.3-3.6%), myrcene (0.2-3.6%) and *p*-cymene (2.9-3.5%). The examined oil was considerably different in respect of their qualitative and quantitative compositions compared to earlier studies. Moreover, to the best of our knowledge, 52 constituents, including *trans*-ferruginol, abietatriene, totarene, phyllocladene, 4-*epi*-abietal, caryophyllene acetate, cubenol, allo-hedycaryol, δ-amorphene, *trans*-cadina^{-1} (6),4-diene, *cis*-cadina^{-1} (6),4-diene, α-muurolol, amorpha-4,9-dien-2-ol, (*Z*)-nuciferol acetate and viridiflorol were reported for the first time in *I. rugosus* essential oil.

Conclusion: The dominant presence of sesquiterpenoids and diterpenoids in the 'Ghangaria' population and monoterpenoids and sesquiterpenoids in the 'Tapovan' population made them novel compositions. The newly identified constituents, especially diterpenoids can be of chemotaxonomic significance.

Keywords: *Isodon rugosus*; Lamiaceae; Essential oil composition; α-Pinene; *trans*-Ferruginol

Introduction

Isodon rugosus (Wall. ex Benth.) Codd (syn. *Plectranthus rugosus* Wall. ex. Benth.) belongs to the family Lamiaceae and is an aromatic branched shrub, with erect stem possessing ovate and opposite leaves of notched margin and covered with dense small hairs on ventral side. It is distributed widely throughout the northern temperate regions of the Himalaya at the elevation of 1500-2500 m [1,2]. The plant flowers from July to September and the seeds ripen from August to October [3]. The extracts of fresh and dried leaves have shown to be efficacious against worm infestations. It emanates a characteristic aroma and enjoys a reputation of being an antiseptic, germicidal and cardiac stimulant in folk medicine [4]. The plant is used in Pakistani traditional medicine for toothache and is claimed to be effective as an antiseptic, a hypoglycaemic, an antidiarrheal and a bronchodilator [5,6]. A topical administration of fresh leaf extract is used to treat scabies for its immediate effect, while 1-2 drops of this extract are used to treat earache [7]. An extract of the leaves is also used to treat hypertension, fevers, rheumatism and toothache. Branches are used for making dusters [8,9]. The plant extracts and fractions of different solvents exhibited antifungal [3], antibacterial, phytotoxic [10], antioxidant, and lipoxygenase inhibitory activities [11,12]. Besides various medicinal properties, it is also be used for phytoremediation [13]. Phytochemical studies carried out on the plant revealed the presence of steroids, flavonoids, terpenoids, saponin, tannins, cardiac glycosides, coumarins, reducing sugars and β-cyanin. Diterpenoids, namely, rugosinin, effusanin-A, effusanin-B, effusanin-E, lasiokaurin and oridonin have been isolated from the plant [14,15]. In addition to these, triterpenoids, namely, plectranthoic acid A and B, acetyl plectranthoic acid and plectranthadiol have also been isolated from the plant [16,17]. Moreover, studies on the essential oil composition indicate the presence of sesquiterpene hydrocarbons, including β-caryophyllene, germacrene-D and α-humulene as the major constituents [18,19]. Despite the multipurpose usage, there exists merger data on the chemical composition of *I. rugosus* populations growing in temperate Himalayan region. Therefore, in continuation of our research on the essential oil constituents of underexplored aromatic species of Indian flora, the present study was planned to carry out detailed GC-FID and GC-MS investigation of *I. rugosus* essential oils extracted from the populations growing at higher altitudes of Garhwal region of Uttarakhand, India. This study also aimed to characterize several new

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constituents for *I. rugosus* essential oils in addition to earlier reported constituents. The newly identified constituents, especially diterpenoids of the essential oil can be of immense chemotaxonomic value.

Materials and Methods

Plant material and isolation of essential oil

Plant materials (*I. rugosus*) were collected from Ghangaria (30.681851° N and 79.589786° E; altitude 2687.26 m) and Tapovan (30.491288° N and 79.628392° E; altitude: 1917.67 m) regions of Chamoli, Uttarakhand during the third week of August, 2012. The populations growing in Ghangaria and Tapovan regions were in flowering and vegetative phases, respectively during collection time. The plant materials were authenticated at Botany Department of CSIR-CIMAP Research Centre Pantnagar by one of the authors (AC). The shade dried plant materials were hydrodistilled in a Clevenger's apparatus for 3 h to collect their essential oils. The essential oil content (% v/w) was estimated on dry weight basis. The oils obtained were dehydrated over anhydrous sodium sulphate and kept in a cool and dark place until further analyses.

Gas chromatography (GC/FID)

GC analysis of the essential oils was carried out on a PerkinElmer AutoSystem XL gas chromatograph, equipped with DB-5 capillary column (60 m × 0.32 mm i.d., film thickness 0.25 µm) and flame ionization detector (FID). The oven column temperature ranged from 70-250°C, programmed at 3°C $minute^{-1}$, with initial and final hold time of 2 minute, using H_2 as carrier gas at 10 psi constant pressure, a split ratio of 1:35, an injection size of 0.03 µL neat, and injector and detector temperatures were maintained at 250°C and 280°C, respectively.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis of the essential oil samples was carried out on a Clarus 680 GC interfaced with a Clarus SQ 8C mass spectrometer of PerkinElmer fitted with Elite-5 MS fused-silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature program was from 60-240°C, at 3°C $minute^{-1}$, and programmed to 270°C at 5°C $minute^{-1}$; injector temperature was 250°C; transfer line and source temperatures were 220°C; injection size 0.03 µL neat; split ratio 1:50; carrier gas He at 1.0 mL $minute^{-1}$; ionization energy 70 eV; mass scan range 40-450 amu.

Identification of essential oil constituents

Identification of the essential oil constituents was done on the basis of retention index (RI) determined using a homologous series of *n*-alkanes (C_8-C_{30}, Supelco Bellefonte, PA, USA) under identical experimental conditions, co-injection with known essential oil constituents, mass spectra library search (NIST/EPA/NIH, version 2.0 g, and Wiley registry of mass spectral data 9th edition) and by comparing the mass spectral and retention data with literature [20]. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

Results and Discussion

The essential oil yield was observed to be 0.15% and 0.25% (v/w) on dry weight basis in 'Ghangaria' and 'Tapovan' populations of *I. rugosus*, respectively. These variations were probably due to the variations of plant stages and geographical conditions. The resulting essential oils were subjected to GC-FID and GC-MS analyses. A total of 81 constituents, forming 92.6% of the total oil composition of 'Ghangaria' population and 80 constituents, comprising 83.9% of total oil composition of 'Tapovan' population were identified using retention index (RI) and mass spectrum of the individual components (Table 1). Major constituents of the oils were α-pinene (0.8-19.3%), *trans*-ferruginol (3.0-17.1%), α-phellandrene (2.6-10.5%), (*E*)-caryophyllene (4.0-9.3%), germacrene D (1.9-8.8%), abietatriene (1.9-

S. no.	Compound	RI	Content (%)		Identification methods[c]
			I	II	
1	α-Thujene†	926	t	0.1	RI, MS
2	α-Pinene	932	0.8	19.3	RI, MS
3	Camphene	948	t	0.2	RI, MS
4	Sabinene	974	-	0.3	RI, MS
5	1-Octen-3-ol† + β-pinene	978	0.1	0.4	RI, MS
6	Myrcene	988	0.2	3.6	RI, MS
7	α-Phellandrene	1005	2.6	10.5	RI, MS
8	*δ*-3-Carene	1010	0.3	1.0	RI, MS
9	α-Terpinene	1016	0.2	0.3	RI, MS
10	p-Cymene	1022	3.5	2.9	RI, MS
11	o-Cymene†	1024	t	t	RI, MS
12	Limonene	1027	1.3	3.6	RI, MS
13	β-Phellandrene	1028	3.1	4.4	RI, MS
14	1,8-Cineole	1030	0.2	0.2	RI, MS
15	(*Z*)-β-Ocimene	1034	-	0.1	RI, MS
16	(*E*)-β-Ocimene	1045	t	0.4	RI, MS
17	γ-Terpinene	1053	0.3	0.5	RI, MS
18	*cis*-Sabinene hydrate†	1063	0.4	0.3	RI, MS
19	Terpinolene	1089	0.6	1.3	RI, MS
20	p-Cymenene†	1091	t	-	RI, MS
21	Linalool	1097	0.3	0.2	RI, MS
22	*trans*-Sabinene hydrate†	1099	t	-	RI, MS
23	*n*-Undecane†	1102	-	t	RI, MS
24	*cis*-Thujone	1104	t	-	RI, MS
25	*cis*-p-Menth-2-en-1-ol	1122	t	t	RI, MS
26	*trans*-p-Menth-2-en-1-ol	1139	t	t	RI, MS
27	Camphor†	1145	0.1	-	RI, MS
28	Terpinen-4-ol	1177	0.6	0.3	RI, MS
29	p-Cymen-8-ol†	1182	t	t	RI, MS
30	α-Terpineol	1190	t	t	RI, MS
31	*trans*-p-Mentha-1(7),8-dien-2-ol†	1191	t	t	RI, MS
32	γ-Terpineol†	1197	t	t	RI, MS
33	β-Cyclocitral†	1215	0.2	t	RI, MS
34	Carvacrol methyl ether†	1245	t	-	RI, MS
35	Piperitone†	1255	t	-	RI, MS
36	Bornyl acetate	1286	t	t	RI, MS
37	Thymol†	1288	t	t	RI, MS
38	Carvacrol†	1300	t	t	RI, MS
39	α-Cubebene	1353	0.3	0.1	RI, MS
40	α-Copaene	1378	0.5	0.3	RI, MS
41	β-Bourbonene	1390	0.7	0.2	RI, MS
42	Isolongifolene†	1393	0.2	0.3	RI, MS
43	β-Elemene	1394	0.2	0.3	RI, MS
44	(*Z*)-Caryophyllene†	1409	-	0.1	RI, MS
45	α-Gurjunene†	1411	0.3	0.6	RI, MS
46	β-Funebrene†	1415	-	0.1	RI, MS
47	(*E*)-Caryophyllene	1420	9.3	4.0	RI, MS
48	β-Cedrene†	1421	-	t	RI, MS
49	β-Gurjunene	1435	1.7	0.2	RI, MS
50	*cis*-Muurola-3,5-diene†	1452	-	t	RI, MS
51	α-Humulene	1454	1.2	1.0	RI, MS

52	*allo*-Aromadendrene†	1461	0.3	-	RI, MS
53	cis-Cadina-1(6),4-diene†	1465	0.9	1.2	RI, MS
54	*trans*-Cadina-1(6),4-diene†	1478	2.2	1.2	RI, MS
55	Germacrene D	1483	8.8	1.9	RI, MS
56	β-Selinene†	1494	0.2	0.1	RI, MS
57	γ-Amorphene†	1500	0.6	0.3	RI, MS
58	α-Muurolene	1502	2.8	0.7	RI, MS
59	*trans*-β-Guaiene†	1504	0.7	0.5	RI, MS
60	Germacrene A†	1508	-	t	RI, MS
61	α-Bulnesene†	1509	-	t	RI, MS
62	δ-Amorphene†	1513	1.5	0.8	RI, MS
63	γ-Cadinene	1515	1.0	0.2	RI, MS
64	Cubebol	1518	1.7	2.8	RI, MS
65	δ-Cadinene	1524	3.6	2.9	RI, MS
66	α-Cadinene†	1542	0.1	0.2	RI, MS
67	Germacrene-D-4-ol	1578	0.7	1.0	RI, MS
68	Spathulenol	1582	1.1	0.1	RI, MS
69	Caryophyllene oxide	1587	0.1	0.2	RI, MS
70	*allo*-Hedycaryol†	1589	1.4	-	RI, MS
71	Globulol†	1594	0.1	-	RI, MS
72	Viridiflorol†	1596	0.4	0.7	RI, MS
73	1,10-di-*epi*-Cubenol†	1620	0.4	0.1	RI, MS
74	*epi*-α-Cadinol	1642	0.9	0.5	RI, MS
75	α-Muurolol†	1650	0.5	0.9	RI, MS
76	Cubenol†	1651	1.4	0.2	RI, MS
77	α-Cadinol	1656	1.0	1.0	RI, MS
78	Amorpha-4,9-dien-2-ol†	1698	0.8	-	RI, MS
79	Caryophyllene acetate†	1703	1.0	1.2	RI, MS
80	(*Z*)-Nuciferol†	1728	0.1	t	RI, MS
81	(*E*)-Nuciferol†	1760	t	-	RI, MS
82	(*Z*)-Nuciferol acetate†	1835	0.8	-	RI, MS
83	Totarene†	1925	2.0	0.6	RI, MS
84	Sandaracopimara-8(14),15-diene†	1970	0.3	0.2	RI, MS
85	13-*epi*-Dolabradiene†	2003	0.3	0.2	RI, MS
86	Phyllocladene†	2019	1.9	1.2	RI, MS
87	Abietatriene†	2060	5.3	1.9	RI, MS
88	Abietadiene†	2090	0.4	0.3	RI, MS
89	Nezukol†	2137	-	0.4	RI, MS
90	Abieta-8(14),13(15)-diene†	2151	-	0.1	RI, MS
91	4-*epi*-Abietal†	2302	1.0	0.2	RI, MS
92	*trans*-Ferruginol†	2336	17.1	3.0	RI, MS
Class composition					
	Monoterpene hydrocarbons		9.4	45.6	
	Oxygenated monoterpenes		1.8	1.0	
	Sesquiterpene hydrocarbons		37.1	17.2	
	Oxygenated sesquiterpenes		12.4	8.7	
	Diterpene hydrocarbons		10.2	4.5	
	Oxygenated diterpenes		18.1	3.6	
	Benzenoid compounds		3.5	2.9	
	Aliphatic compounds		0.1	0.4	
	Total identified		92.6	83.9	

RI: Retention Index determined on DB-5 gas chromatography column (30 m × 0.25 mm) using a homologous series of *n*-alkanes; I: Ghangaria; II: Tapovan; Identification of the compound based on RI (Retention Index), MS (Mass Spectrometry); †Reported for the first time in *Isodon rugosus* essential oil; t: Trace (<0.05%).

Table 1: Essential oil composition of *Isodon rugosus* collected from Garhwal region of western-Himalaya, India.

5.3%), β-phellandrene (3.1-4.4%), δ-cadinene (2.9-3.6%), limonene (1.3-3.6%), myrcene (0.2-3.6%), p-cymene (2.9-3.5%), cubebol (1.7-2.8%), α-muurolene (0.7-2.8%), *trans*-cadina-1 (6),4-diene (1.2-2.2%), totarene (0.6-2.0%), phyllocladene (1.2-1.9%), β-gurjunene (0.2-1.7%), and δ-amorphene (0.8-1.5%). The oil of 'Ghangaria' population was dominated by sesquiterpenoids (sesquiterpene hydrocarbons: 37.1%; oxygenated sesquiterpenes: 12.4%), followed by diterpenoids (sesquiterpene hydrocarbons: 10.2%; oxygenated sesquiterpenes: 18.1%). However, the oil of 'Tapovan' population was characterised by mainly monoterpenoids (monoterpene hydrocarbons: 45.6%; oxygenated monoterpenes: 1.0%) and sesquiterpenoids (sesquiterpene hydrocarbons: 17.2%; oxygenated sesquiterpenes: 8.7%). Major constituents of the oil of 'Ghangaria' population were *trans*-ferruginol (17.1%), (*E*)-caryophyllene (9.3%), germacrene D (8.8%), abietatriene (5.3%), δ-cadinene (3.6%), p-cymene (3.5%), β-phellandrene (3.1%), α-muurolene (2.8%), α-phellandrene (2.6%), *trans*-cadina-1 (6),4-diene (2.2%), totarene (2.0%), phyllocladene (1.9%), β-gurjunene (1.7%), cubebol (1.7%), δ-amorphene (1.5%), allo-hedycaryol (1.4%), cubenol (1.4%), limonene (1.3%) and α-humulene (1.2%). On the other hand, the characteristic constituents of 'Tapovan' population were α-pinene (19.3%), α-phellandrene (10.5%), β-phellandrene (4.4%), (*E*)-caryophyllene (4.0%), myrcene (3.6%), limonene (3.6%), *trans*-ferruginol (3.0%), p-cymene (2.9%), δ-cadinene (2.9%), cubebol (2.8%), germacrene D (1.9%), abietatriene (1.9%), terpinolene (1.3%), *cis*-cadina-1 (6),4-diene (1.2%), *trans*-cadina-1 (6),4-diene (1.2%), caryophyllene acetate (1.2%) and phyllocladene (1.2%). Thus, comparison of the results clearly showed that *I. rugosus* populations growing in two different locations of Himalaya had considerable quantitative variation in their essential oil compositions.

The essential oil composition of *I. rugosus* have been studied in past by few researchers. All the reported compositions of *I. rugosus* (syn *P. rugosus*) were characterised by higher amounts of sesquiterpenoids. Weyerstahl et al (1983) reported caryophyllene (22.0%) as main constituents along with germacrene D, α-phellandrene, α-pinene, caryophyllene oxide, α-cadinol, δ-cadinene, limonene, β-phellandrene, myrcene and *p*-cymene [21]. Tiwari et al reported β-caryophyllene (38.4%), germacrene D (23.8%), spathulenol (3.2%), α-cadinol (2.2%), p-cymene (3.6%), γ-terpinene (2.8%) and limonene (2.7%) as major constituents of the oil [18]. Similarly, the essential oil of the population collected from Nainital (Kumaon region, western Himalaya) was dominated by sesquiterpene hydrocarbons, represented by β-caryophyllene (29.8-36.2%), germacrene D (25.2-28.2%), α-humulene (6.6-8.6%) and (*E*)-β-farnesene (2.3-3.8%) as the major constituents [19]. However, recently Irshad et al reported spathulenol (21.0%), β-caryophyllene (10.6%), germacrene D (20.0%), 3-carene (4.8%), myrcene (4.0%), α-myrecene (4.0%) and limonene (2.7%) as major constituents of *I. rugosus* essential oil [22]. Thus, the chemical compositions of *I. rugosus* examined in this study showed remarkable qualitative and quantitative differences as compared to the compositions reported earlier from other countries / locations. These variations are likely to be due to both biotic and abiotic factors affecting plant growth and biosynthesis.

Ferruginol (meroterpene), an oxidized abietane diterpene, has led to several synthetic and biological studies due to their interesting structure and potent biological profile [23,24]. The α-pinene, a monoterpene hydrocarbon, possesses antimalarial activity [25]. It is used as an important substance in the manufacture of a variety of synthetic aroma chemicals and its epoxide is isomerised to produce campholenic aldehyde, which is an intermediate for the sandalwood fragrance, santalol [26]. Beside the presence of several common terpenoids, *I.*

rugosus growing in Garhwal region of Himalayan can be a good source of rarely occurring terpenoids such as *trans*-ferruginol and therefore can be further explored for sustainable utilization. According to the literature review, a total of 52 constituents are reported in *I. rugosus* essential oil till now [18,19,21,22]. However, this study identified a total of 92 constituents, which included 40 previously reported constituents. Thus, to the best of our knowledge, about 52 constituents were being reported for the first time for *I. rugosus* essential oil (marked in Table 1). The newly identified constituents of the oil can play an important role in oil authentication and chemotaxonomic studies of this Himalayan *Isodon* species. Moreover, the dominant presence of sesquiterpenoids and diterpenoids in the 'Ghangaria' population and monoterpenoids and sesquiterpenoids in the 'Tapovan' population made them novel compositions of *I. rugosus*.

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