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Volatile oil composition and antioxidant activity of leaf of *Chaerophyllum villosum* Wall. ex DC from Uttrakhand, India

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Abstract

The genus *Chaerophyllum*, belonging to Apiaceae family, comprised of about 110 species which includes annual and perennial herbal plants widely distributed in temperate and sub temperate zones of Asia, Africa and Europe. *Chaerophyllum villosum* Wall. ex DC. was widely distributed in E. Asia Himalayas from India to Bhutan, Nepal and China and widely grows in moist shady places, road sides or open grassy places at elevations of 2100-3500 m. In high altitude tribes of Uttarakhand Himalaya (India) it was commonly known and sold in the name of 'Ganjari' widely used by people in food, spice and also as medicine. The volatile oil composition of leaf of *Chaerophyllum villosum* Wall. ex DC. (family: Apiaceae) were analyzed and compared using capillary GC and GC-MS. The leaf essential oil of *C. villosum* was dominated by monoterpene hydrocarbons (91.34%) represented by γ-terpinene (74.93%) as single major constituent followed by p-cymene (10.00%), terpinolene (2.93%) and β-pinene (2.54%), the antioxidant activity of leaf essential oil also evaluated.

Keywords: *Chaerophyllum villosum*, Apiaceae, essential oils, γ-terpinene, antioxidant activity.

INTRODUCTION

The genus *Chaerophyllum*, belonging to Apiaceae family, comprised of about 110 species which includes annual and perennial herbal plants widely distributed in temperate and sub temperate zones of Asia, Africa and Europe [1-3]. *Chaerophyllum villosum* Wall. ex DC. was widely distributed in E. Asia Himalayas from India to Bhutan, Nepal and China and widely grows in moist shady places, road sides or open grassy places at elevations of 2100-3500 m. In high altitude tribes of Uttarakhand Himalaya (India) it was commonly known and sold in the name of 'Ganjari' widely used by people in food, spice and also as medicine [4-5]. Although the various species in the genus *Chaerophyllum* were known to possess toxin chaerophyllin capable of causing diarrhea and incoordination, yet these were also used as antimicrobial, antioxidant, stimulant and expectorant. Literature survey revealed that there were few reports on the essential oils of *Chaerophyllum* species which report wide variety of terpenoid constituents. Sabinene (30.0%), *p*-cymen-8-ol (16.0%) and terpinolene (11.5%) were reported as major constituents from essential oil of *C. byzantinum.* The hydro distilled essential oils from flower, leaf and stem of *C. macropodum* were characterized by myristicin (15.7%-42.5%) and *trans*-β-ocimene (24.9%-54.2%) as major constituents. The principal constituents identified in *C. libanoticum*, used as a food plant in Turkey, were β-phellandrene (17.6%), limonene (15.9%), β-pinene (8.8%), and sabinene (8.5%). While the essential oil isolated from aerial parts of *C. macrospermum* showed (E)-β-ocimene (55.9%), terpinolene (9.8%), α-pinene (7.5%), β-phellandrene (4.3%) and β-pinene (4.2%) [6-10].

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MATERIALS AND METHODS

Plant collection and identification

The fresh leaves of *C. villosum* were collected from Milam glacier (Uttarakhand, India) at an altitude of 3600 m in the month of August at mature stage. The identification was done from Botany Department, Kumaun University, Nainital and BSI, Dehradun. The voucher specimens (No.Chem/DST/CV/08) have been deposited in the Phytochemistry lab of the Chemistry Department, Kumaun University, Nainital.

Isolation of essential oil

The fresh planting materials (2 kg each) were subjected to steam distillation using a copper electric still, fitted with spiral glass condensers. The distillates were saturated with NaCl and extracted with *n*-hexane and dichloromethane. The organic phase was dried over anhydrous sodium sulfate and the solvents were distilled off in a rotary vacuum evaporator at 30^{0}C and the percentage oil content was calculated on the basis of fresh weight of plant materials.

GC and GC-MS analysis

The oils were analyzed by using a Nucon 5765 gas chromatograph (Rtx-5 column, 30 m X 0.32 mm, FID), split ratio 1: 48, N_2 flow of 4 kg/cm^2 and on Thermo Quest Trace GC 2000 interfaced with MAT Polaris Q Ion Trap Mass spectrometer fitted with a Rtx-5 (Restek Corp.) fused silica capillary column (30 m x 0.25 mm; 0.25 µm film coating). The column temperature was programmed 60^0 -210^{0}C at 3^{0}C/min using He as carrier gas at 1.0 mL/min. The injector temperature was 210^{0}C, injection size 0.1µL prepared in hexane, split ratio 1:40. MS were taken at 70 eV with a mass range of 40-450 amu.

Identification of the components

Identification of constituents shown in table 1 were done on the basis of Retention Index (RI, determined with reference to homologous series of n-alkanes (C_9-C_{24}, Polyscience Corp., Niles IL) under identical experimental condition), co injection with standards (Sigma and known essential oil constituents (standard isolates), MS Library search (NIST and WILEY), by comparing with the MS literature data [11]. The relative amounts of individual components were calculated based on GC peak area (FID response) without using correction factor.

Reducing power activity

The reductive potential of essential oil was determined according to the method of Oyaizu [12]. The different concentrations of essential oil (5, 10, 15, 20 mg/ml) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)6$] (2.5 ml, 1%). The mixture was incubated at 50^0 C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifugation for 10 min at 1000g (MSE Mistral 2000, UK). The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reductive potential.

RESULTS

The essential oils composition of leaves of *C. villosum* were analyzed and compared by using capillary GC and GC-MS. The oil yields were 0.40% (v/w). The analysis led to the identification of 31 constituents forming 98.50% of the total oil compositions in leaves. The identified constituents with their respective percentages and class composition are given in table 1. The leaf essential oil of *C. villosum* was dominated by monoterpene hydrocarbons (91.34%) and represented by γ-terpinene (74.93%) as single major constituent followed by p-cymene (10.00%), terpinolene (2.93%) and β-pinene (2.54%). Other constituent present in significant amount was myristicin (4.77%).

Table 1. Volatile oil composition of leaves of *Chaerophyllum villosum*

Compounds*	RI[a]	RI[b]	*Chaerophyllum villosum*
			Leaf oil %
α-Thujene	932	931	t
α-Pinene	941	939	0.30
β-Pinene	982	980	2.54
Myrcene	994	991	0.53
α-Phellandrene	1009	1005	t
α-Terpinene	1019	1018	0.11
p-Cymene	1029	1026	10.00
Limonene	1034	1031	-
β-Phellandrene	1037	1031	-
γ-Terpinene	1065	1062	74.93
Terpinolene	1089	1088	2.93
			91.34
1,8-Cineole	1038	1033	t
Linalool	1101	1098	t
Borneol	1167	1165	0.12
Terpinen-4-ol	1180	1177	t
Bornyl acetate	1285	1285	0.13
			0.25
β-Caryophyllene	1418	1418	0.31
α-Humulene	1457	1454	0.11
Germacrene D	1481	1480	0.39
Bicyclogermacrene	1497	1495	0.30
			1.11
Germacren D-4-ol	1578	1574	t
Caryophyllene oxide	1584	1581	t
Humulene epoxide-II	1606	1606	t
β-Eudesmol	1652	1649	t
			t
Thymol methyl ether	1192	1189	0.34
Carvacrol methyl ether	1195	1194	0.69
Thymoquinone	1198	1195	t
p-Anisaldehyde	1209	1205	-
Thymol	1340	1339	t
Carvacrol	1355	1351	t
Myristicin	1524	1520	4.77
			5.80
Total			**98. 50**

*Mode of identification: Retention Index (LRI, Based on homologous series of n-alkanes; C_8-C_{24}), coinjection with Standards/Peak enrichment with known oil constituents, MS (GC-MS), t= trace (<0.1%); (-) = not detected,

[a]RI: Retention index on Rtx-5 column (30 m × 0.25 mm; 0.25 µm film coating); [b]RI: Literature value (Adams, 1995)

Table 2. Reducing power activity shown by leaf volatile oil of *C. villosum*

oil/standerd	(mean) 5mg	10 mg	15 mg	20 mg
Chv	0.424	0.508	0.598	0.607
BHT	2.303	2.413	2.456	2.497
Gallic acid	4.000	4.000	4.000	4.000
Linolic acid	0.373	0.379	0.395	0.411
Catechin	4.000	4.000	4.000	4.000

DISCUSSION

The essential oil composition of *C. macrospermum* of Azerbaijan origin was reported to be predominated by 1,8 cineole, linalool, terpineol, δ-3-carene and farnesol, while the oil of an Iranian collection was reported to contain (E-β-ocimene (40%), tricyclene (19.4%), δ-3-carene (18.3%) and myrcene (10.1%) [13]. Analysis of Turkish origin *C. aksekiense* oil by GC/MS produced heptacosane (10.1%), humulene epoxide II (7.8%), (E)-β-farnesene (6.2%), caryophelene oxide (6.0%), α-humulene (5.5%), terpinolene (5.5%), nonacosane (5.3%), and terpinen-4-ol (4.6%) [14] while *C. azoricum* oil endemic to the Azores Archipelago was dominated by the monoterpene fraction (82-91%), with terpinolene (44-62%) and γ-terpinene (9-13%) being the major components of the oil [15-16]. The oil obtained from the whole plant of wild grown *C. reflexum* from Pakistan contained santene (1.2%), γ-tepenene (3.1%), p-cymene (8.6%), myristicin (17.3%), 1,8-cineole (2.5%), α-terpineol (4.1%), andhydroxymyristic acid (27.4%) Kudrzycka-Bieloszabska *et al*. [17] reported that the oils of different organs of *C. hirsutum* grown in Poland contained α-fenchyl acetate (50%), eugenol (2.8-22.5%), and 1,8-cineol (3.9-21%). Similarly, Kudrzycka-Bieloszabska and Glowniak [18] reported α-pinene (0.02-2.2%), limonene (1.1-3.6%), cineole (0.02-2.9%), fenchone (0.13-0.88%), terpineol (0.64-3.79%), and eugenol (16.3-18.8%) for Polish grown *C. hirsutum* ssp. cucutaria var. glabrum [19].

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