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Root essential oil composition of *Chaerophyllum villosum* Wall. ex DC. from Uttarakhand, India

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ABSTRACT

Chaerophyllum villosum Wall. ex DC (*C. villosum*) is widely distributed in east 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. The present study investigates the chemical composition of essential oil isolated from the roots of *C. villosum*. The essential oil was hydro distilled and isolated essential oil was analyzed by Gas Chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS).Chemical composition analysis of root essential oil led to the identification of 31 constituents accounting for 91.49% of the total oil composition. The carvacrol methyl ether (31.12 %), myristicin (19.06 %), thymol methyl ether (18.60 %), γ-terpinene (11.69 %), were the principle components.

Keywords: Apiaceae, Essential oils, GC, GC-MS.

1. 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, 2]. *Chaerophyllum villosum* Wall. ex DC. is widely distributed in Himalayas from India to Bhutan, Nepal and China and widely grows in moist shady places, road sides or open grassy places. In high altitude tribes of Uttarakhand Himalaya (India) it was commonly known and sold in the name of 'Ganjari or Ganjiadi' widely used by people in food, spice and also as medicine [3, 4]. Previous reports on the essential oils of *Chaerophyllum* species from different regions showed variety of terpenoids and aliphatic volatile compounds. The composition of the essential oil from the leaves and fruits of *Chaerophyllum aromaticum* L. collected in two consecutive years showed, that the leaf oil was dominated by γ-terpinene, followed by *p*-cymenene and *p*-cymene, as well as germacrene D. The fruit oil had γ-terpinene and β-phellandrene as main compounds, with β-pinene, α-pinene and 2,6-dimethyl-1,3,5,7-octatetraene as further major constituents [5] and the essential oils of the aerial parts and fruits of *Chaerophyllum aureum* L., collected from two mountains in Serbia, contains the sabinene (18.5-31.6%), *p*-cymene (7.9-25.4%) and limonene (1.9-10.9%) were characterized as the main constit [6]. Distillation of the fresh flowering tops of *Chaerophyllum prescottii* DC collected from Altai region of Siberia showed major components consisting of (*E*)-β-ocimene (35.6%), (*Z*)-β-ocimene (19.4%), γ-terpinene (18.8%), myrcene (10.6%) and terpinolene (4.6%), representing 89.0% [7]. The oil of *C. macropodum* was found to contain α-pinene (23.0%), β-pinene (17.3%) and fenchyl acetate (13.8%) as the major constituents. The essential oil of *C. crinitum* was characterized by a higher amount of (*E*)-β-ocimene (50.5%). both oils were richer in monoterpene than sesquiterpene hydrocarbons [8]. As a part of our work on phytochemical analysis on genus *Chaerophyllum*, we have previously reported the leaf essential oil composition, antimicrobial and antioxidant activity of *C. villosum* [9-11]. To explore it in commercial scale which is presently used in many purposes in Uttarakhand tribes of High altitudes, now presenting the root oil is evaluated for chemical composition.

2. Material and methods

2.1 Collection of Plant Material

The roots of *C. villosum* were collected at mature stage in August 2008 from Western Himalayan region of Uttarakhand at the attitude of 3000 m. The identification was done from Botany Department, Kumaun University, Nainital and BSI, Dehradun. The voucher specimens (DST/CV/08) have been deposited in the laboratory of the Chemistry Department, Kumaun University, Nainital.

2.2 Isolation of the Essential oils

The roots (1 kg) were subjected to steam distillation in a copper electric still fitted with spiral glass condensers for two hours obtaining 2 L water distillate. The distillates were saturated with NaCl and the oils were extracted with hexane and dichloromethane. The organic phase was then dried over anhydrous Na_2SO_4 and the solvent distilled in a thin film rotary vacuum evaporator at 30 °C. The oil yields 0.3 % (v/w).

Table 1: Chemical Constituents of Root of Essential oil of *Chaerophyllum villosum*

S. No.	Compounds	RI	%
1.	α-Thujene	932	-
2.	α-Pinene	941	-
3.	β-Pinene	982	*0.21*
4.	Myrcene	994	*0.36*
5.	α-Phellandrene	1009	*0.33*
6.	α-Terpinene	1019	*0.28*
7.	*p*-Cymene	1029	*3.47*
8.	Limonene	1034	*0.39*
9.	β-Phellandrene	1037	*0.10*
10.	γ-Terpinene	1065	*11.69*
11.	Terpinolene	1089	*0.44*
12.	1,8-Cineole	1038	*0.24*
13.	Linalool	1101	*0.60*
14.	Borneol	1167	*0.36*
15.	Terpinen-4-ol	1180	*0.56*
16.	Bornyl acetate	1285	*1.18*
17.	β-Caryophyllene	1418	*0.13*
18.	α-Humulene	1457	*0.14*
19.	Germacrene D	1481	-
20.	Bicyclogermacrene	1497	*0.46*
21.	Germacren D-4-ol	1578	*0.19*
22.	Caryophyllene oxide	1584	-
23.	Humulene epoxide-II	1606	*0.13*
24.	β-Eudesmol	1652	*t*
25.	Thymol methyl ether	1192	*18.60*
26.	Carvacrol methyl ether	1195	*31.12*
27.	Thymoquinone	1198	*0.32*
28.	*p*-Anisaldehyde	1209	*0.22*
29.	Thymol	1340	*0.17*
30.	Carvacrol	1355	*0.74*
31.	Myristicin	1524	*19.06*
	Monoterpene hydrocarbons		***17.27%***
	Oxygenated monoterpenes		***2.94%***
	Sesquiterpene hydrocarbons		***0.73%***
	Oxygenated sesquiterpenes		***0.30%***
	Phenolic compounds		***70.23%***
	Total		***91.49%***

*Mode of identification: Retention Index (LRI, Based on homologous series of n-alkanes; C8-C24), coinjection with Standards/Peak enrichment with known oil constituents, MS (GC-MS), t= trace (<0.1%);(-) = not detected, RI: Retention index on Rtx-5 column (30 m × 0.25 mm; 0.25 µm film coating);

2.3 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 °C -210 °C at 3 °C/min using He as carrier gas at 1.0 mL/min. The injector temperature was 210 °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 constituents 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 [12]. The relative amounts of individual components were calculated based on GC peak area (FID response)

without using correction factor.

3. Results

The identified constituents with their respective percentages and class composition are given in table 1. The root essential oil showed the dominant presence of phenolic compounds (70.23%) with carvacrol methyl ether (31.12%), thymol methyl ether (18.60%) and myristicin (19.06%) as major marker constituents. Other constituents identified in significant amount in rhizome essential oil of *C. villosum* were γ-terpinene (11.69%), p-cymene (3.47%) and bornyl acetate (1.18%).

4. Discussion

The previous report on 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%) [11].The essential oil of ripe fruits and umbels of *C. coloratum* from Yugoslavia showed high content of (*E*)-β-farnesene (79.2%, 68.4%, respectively) [12].

We can see here that the essential oil composition of leaf and root was totally different. In comparison to these reports on *Chaerophyllum* species from different geographic regions; in present analysis γ-terpinene (74.93%) and p-cymene (10.00%) were found as major constituents of leaf oil; whereas carvacrol methyl ether (31.12%) and thymol methyl ether (18.60%) were noticed as the major constituents in rhizome essential oil *C. villosum.* Monoterpenes viz. γ-terpinene, p-cymene were earlier reports as major or in significant amount in essential oil of other studied *Chaerophyllum* species; but the phenolics viz. carvacrol methyl ether, and thymol methyl ether were not noticed as major constituents in their essential oils Further, the comparison of the present findings on the essential oil composition on *C. villosum* for the first time showed significant qualitative and quantitative variations.

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