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ORIGINAL RESEARCH ARTICLE

Chemical Composition of Essential Oils of *Centella asiatica* (L.) Urban from Different Habitats of Nepal

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ABSTRACT

The chemical composition of the essential oil obtained by hydro-distillation of *Centella asiatica* (Apiaceae) collected in three different habitats (shady grassland, open grassland, and open agricultural land) of Nepal were analyzed by GC/FID and GC/MS.. The oil had pale yellow color and intensive odor. Fifty two compounds were identified from the three different oils, accounting from 94.01 % to 95.98 % of the oil contents. The oils were characterized by a high amount of sesquiterpenoid hydrocarbons ranging from 70.25 to 82.09% among which γ-caryophyllene (9.24-32.3%), β-caryophyllene (7.5–24.2%), β-farnesene (1.7–18.89%), α-humulene (0.05–17.09%). Oxygenated sesquiterpenoids such as caryophyllene oxide (0.56-8.46%) were also present as well as monoterpenoids even though in very low amount (0.50-2.34%). The quali-quantitative differences in the chemical composition of the essential oils grown in different habitats of Nepal confirm the importance of the geographic and climatic conditions for the production of essential oils of *Centella asiatica*.

Key words: Apiaceae, *Centella asiatica*, Essential oil, Spathulenol, α-Terpineol, Caryophyllene oxide α-Pinene.

INTRODUCTION

Centella asiatica (L) Urban (Apiaceae) commonly called pennywort or gotukola, is a perennial creeping herb, commonly found in moist places. The plant is widely distributed in Nepal up to 2200 m above sea level [1]. In the traditional medicine it is used as a tonic, for treating leprosy, as a blood purifier, as diuretic, and for indigestion. Leaves are taken to improve memory, for skin diseases, syphilis, and rheumatism [1] .The plant contains a large variety of compounds, mainly triterpenoids, flavonoids, alkaloids and steroids [2]. From the plant was also obtained the essential oil in which terpenoidal constituents such as β-caryophyllene, β-cymene, trans-β-farnesene, germacrene-D, α-terpineol, limonene, and linalool were identified [2]. Among these components, α-terpineol is known for myorelaxant and antispasmodic effects [3]. Linalool, the main compound of this oil, is a very important substance used in foodstuffs as additive [4,5] and in pharmacology [6,7] for its different properties. Another terpenoid, farnesol, have shown anti-cancer effects and antibacterial activity in several different studies [8,9]. Among the hydrocarbon, some compounds such as β-elemene, limonene and β-cymene are important compounds finding application in fragrance, pharmaceutical and agrochemical fields [10-13]. Limonene has also been studied for its possible anti-cancer activity [14].

A number of phytochemical and biological studies have been reported on *Centella asiatica* but, to our knowledge, the chemical composition of the essential oil of *C. asiatica* growing in Nepal has been poorly investigated. For this reason, in this paper we aim to examine the chemical composition of the *C.asiatica* essential oils growing in different habitats of Nepal.

MATERIAL AND METHODS

Experimental

Plant Materials

Fresh mature plants of *Centella asiatica* were collected in June 2008 from different habitats (85°14.34’E Long.; 27°40.54’N Lat.) of Nepal

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(grassland, open grassland, and open agricultural lands) and shade dried. Voucher specimens were prepared according to standard method and numbered as CA-EO1 to CA-EO3.

Extraction of the essential oil

200 g of dried plant from the three different habitats were hydrodistilled with a Clevenger apparatus for 4 h. Pale yellow color oils were collected and stored at 4°C in dark containers until the analysis.

GC-MS analysis

Centella asiatica essential oil was analyzed using an Agilent 6840N gas chromatograph coupled with mass spectrometry (GC-MS) (Varian Saturn 2000 GC-MS series), and a fused-silica- capillary column, DB-5(30 m × 0.25 mm × 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA). GC-MS analysis was carried out using the following conditions: carrier gas He; flow rate 0.8 mL/min; split 1:10; injection volume 1µL; injection temperature 250°C; oven temperature 45°C for 5 min and progress from 45°C to 220°C at 3°C/min, from 220°C to 250 °C at 10°C/min and holding at 250 °C for 5 min. The ionization mode used was electronic impact at 70 eV. Identification of the components was achieved by comparison of their mass spectral fragmentation patterns with those stored in the data bank (Wiley library) and by their retention indices.

GC-FID analyses were carried out on Agilent 6840 N gas chromatograph equipped with a FID detector. The analytical conditions were the same as described for GC-MS analysis. The FID temperature was at 220°C. The percentage composition of the oil was computed by the normalization method from the GC peak areas, without using correction factors.

RESULTS

The yield of essential oil across the habitats ranged from 0.09 to 0.12% (shady grassland = 0.12%, open grassland = 0.1%, open agricultural land = 0.09%) (**Table 1**). As shown in (**Table 2)**, over 94% of the constituents of the *Centella asiatica* essential oil were determined in the collected samples, and the number of identified compounds were different depending on the habitat: 39 in the essential oil from plants grown in shady grassland, 34 in open grassland, and 36 in open agricultural land). GC/FID analysis showed that the oils were characterized by a high amount of sesquiterpenoid hydrocarbons ranging from 70.25 to 82.09%, mainly γ-caryophyllene (9.24-32.3%), β-caryophyllene (7.5–24.2%), β-farnesene (1.7–18.89%), α-humulene (0.05–17.09%). The oxygenated sesquiterpenoid fraction (3.75-10.53%) was mainly composed of caryophyllene oxide (0.56-8.46%), whereas the monoterpenoid fraction was very low in all three oil samples (0.50-2.34%). Among all the compounds, γ-caryophyllene, β-caryophyllene and caryophyllene oxide were present in higher amounts in plants grown in open agricultural land, whereas α-humulene and β-farnesene in plants grown in open grassland.

Table 1: Yield of essential oil and soil characters of habitat relative to the analyzed samples of *Centella asiatica*

Attributes	Partially shaded grassland (Under shrub)	Open Grassland	Open agricultural land
Soil pH	6.01	5.81	5.71
Soil nitrogen (%)	0.28	0.16	0.18
Soil Organic carbon (%)	3.8	1.28	2.25
Soil Organic matter (%)	6.58	2.21	3.89
Oil yield (%)	0.12	0.10	0.09
Oil colour	Pale yellow	Pale yellow	Pale yellow
No of identified chemical components	39	34	36

Table 2: Chemical composition (%) of *Centella asiatica* essential oils from different habitats

S.No	Compound [a]	Kovat Index [b]	Shady grassland [c]	Open grassland [c]	Open Agricultural land[c]
1	Thujopsene	926	-	0.34	0.48
2	α-Thujene	931	0.64	-	1.12
3	α-Pinene	939	0.02	0.06	-
4	Camphene	953	-	-	0.05
5	β-Pinene	980	-	0.02	0.42
6	3,6-Heptadiene-2-ol	995	0.15	-	-
7	β-Cymene	1030	-	0.08	0.27
8	Eucalyptol	1033	1.57	-	-
9	3-Nonen-2-one	1065	0.49	0.06	0.80
10	β-Linalool	1098	0.13	1.36	-
11	L-Camphor	1143	-	-	0.59
12	trans-Borneol	1169	0.11	-	0.08
13	4 –Terpinenol	1177	0.18	-	0.11
14	α –Terpeneol	1189	0.12	-	0.07
15	Cis-Geraniol	1255	-	0.98	1.24
16	Isobornyl acetate	1286	0.06	-	-
17	4,8a-Dimethyloctahydro-4a(2H)-naphthalenol	1373	-	5.24	0.23

18	α –Copaene	1377	0.54	0.37	0.39
19	7-Tetradecene	1378	-	0.08	-
20	β-Cubebene	1390	-	1.24	0.79
21	β-Elemene	1391	5.01	3.93	2.34
22	γ- Caryophyllene	1420	26.45	9.24	32.30
23	β-Caryophyllene	1428	24.20	7.50	24.50
24	β-Gurjunene	1433	0.55	0.13	0.20
25	γ-Elemene	1437	1.05	-	0.81
26	Isocaryophyllene	1438	3.01	5.24	-
27	Aromadendrene	1441	0.78	0.43	-
28	Cis-β-Guainene	1440	-	-	0.58
29	α –Humulene	1455	0.05	17.09	6.86
30	β-Farnesene	1456	12.36	18.89	1.70
31	Alloromadendrine	1460	-	0.73	0.55
32	β-Acoradiene	1470	0.34	0.09	-
33	γ-Murrocene	1476	-	-	0.15
34	Germacrene-D	1485	1.82	2.5	0.08
35	β-Selinene	1490	3.8	-	-
36	α-Selinene	1493	0.75	-	-
37	α-Chamigrene	1499	0.98	0.60	1.09
38	γ-Cadinene	1514	0.17	-	-
39	β –Candinene	1519	0.17	2.13	0.37
40	α-Panasinsen	1535	0.06	0.06	0.75
41	Selina-3,7(11)-diene	1543	1.22	-	-
42	Caryophyllene oxide	1581	0.56	7.68	8.46
43	-(-)Spathulenol	1578	0.47	0.80	0.41
44	Viridiflorol	1593	-	0.56	0.81
45	Valeranone	1675	1.20	0.03	-
46	Isoaromadendrene epoxide	1740	0.20	1.46	-
47	Aristolene epoxide	1763	0.83	-	0.75
48	1-Naphthalenol	2248	0.30	2.7	1.35
49	3,7,11,15-Tetramethyl α- hexadecane-1-ol	2282	1.18	0.06	0.88
50	1R,4s,7s,11R-2,2,4,8-Tetramethytricyclone		1.33	0.51	1.23
51	1H-Cyclopropa[a]naphthalene, decahydro-1		1.64	0.95	3.08
52	Tricyclo[5.2.2.0(1,6)undecan-3-ol,2-me		1.34	0.90	0.29
	Total		94.70	94.01	95.98

[a] Compounds are listed in order of elution time on DB-5 column.
[b] Retention indices calculated on DB-5 capillary column.
[c] Relative area percentage: peak area relative to total peak area percent, calculated on DB-5 column by GC-FID analysis.

DISCUSSION

This is the first report aimed at studying the impact of the growing habitat of Nepal on the composition of the essential oil of *Centella asiatica*. Essential oil content was the highest from plants grown in partially shaded environment where biomass production was also relatively high (Table 1). There is often positive correlation between biomass production and essential oil content in plants [15]. The amount of essential oil obtained from plant grown in Nepal was higher than that reported from *C. asiatica* grown in South Africa (0.06%) [16].

The quali-quantitative composition of the essential oils varied greatly among the samples grown in the three different habitats (Table 2) and only 18 compounds, such as γ-caryophyllene, caryophyllene oxide, β-caryophyllene, and spathulenol, were in common. In the essential oil of *Centella asiatica* from South Africa, the germacrane derivatives were among the predominat class of constituents (21.78%) [16], whereas in our samples from Nepal, sesquiterpenoid hydrocarbons are the most abundant. In essential oil samples extracted in present study, 24 constituents out of 52 (Table 2) were identified in amount higher than 1%. Variation in essential oil constituents across the habitats could be due to variation in light condition. Accumulation of essential oil in herbs directly or indirectly depends upon light [17]. Although we did not evaluate the light intensity perceived by the plants, higher levels of canopy cover above plants generally result in higher levels of shading. In open conditions *C. asiatica* produced high amount of α-humulene and caryophyllene oxide, while in shaded area these compounds were produced in low amount. Studies on essential oil yield conditioned by shade levels have shown that each species responds differently to light intensity. In peppermint (*Mentha piperita*), highest oil yields, including the production of limonene, resulted from high photon flux density [18]. Quantitative differences in production of particular oil components are also seen in *Pinus monticola* where southern-facing branches have higher *α*-pinene, *β*- pinene, and limonene content than the more shaded northern-facing branches [19]. The total oil concentration in

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Pothomorphe umbellata was the highest in the plants subjected to 30% shade [20]. Shading effects would also be compounded by differences in leaf temperature [17], but this aspect was not explored in present study.

In species such as *Thymus vulgaris* [21] and *Matricaria chamomila* [22], essential oil content increased when grown under intense light. But in species such as *Anethum graveolens* [23], *Salvia officinalis* [21] and *Pothomorphe umbellata* [20], higher essential oil yield was obtained when cultivated under shade. Variation in the amount of essential oil from different habitats could also be due to variation in soil nutrients and pH (Table 1) [24]. In species such as *Thymus vulgaris* [25] and *Satureja hortensis* [26] increased the yield and influenced the essential oil components were obtained by the application of nitrogen and phosphorous fertilizers.

Some of the compounds such as Thujopsene, α-terpineol, α- Pinene and camphene were detected in the oils in very small amounts (<1%) (Table 2). Constituents of *Centella asiatica* has wide use in traditional medicine. Geraniol has high relative ovicidal activity against human lice [27]. Linalool is very important substance used in foodstuffs as a food additive [4,5] and for various uses in pharmacology [6,7]. In conclusion, *C. asiatica* can be an important source of essential oils useful for the pharmaceutical, cosmetic and food industries. The oils were characterized by a high amount of sesquiterpenoid hydrocarbons mainly γ-caryophyllene, β-caryophyllene, β-farnesene and α-humulene

We also found and confirm that shade affects the production of the essential oil of *C. asiatica*, as well as the essential oil composition. For this reason, to favor the maximum production of essential oil, it is recommended to cultivate *C. asiatica* in shaded environments. However, the maximum relative proportion of the essential oil should be evaluated depending on the interest in a specific type of chemical constituent.

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