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Intraspecific chemical variability in essential oil of *Cymbopogon distans* (Nees ex Steud.) W.Watson from Uttarakhand Himalaya (India)

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The essential oil composition of ten populations of *Cymbopogon distans* (Nees ex Steud.) W. Watson (Family Poaceae) growing wild in Himalayan region of Uttarakhand (India) has been studied to find out possible chemotypes. Oils were analyzed using gas chromatography and gas chromatography–mass spectrometry techniques to determine the intraspecific chemical variability. Altogether, 42 constituents were identified, representing 85.54-95.72 % of the oil composition. The main constituents in the essential oils of most of the populations were piperitone (1.05-48.78 %), cis- and trans -p-menth-2-en-1-ol (0.26-39.52 %), α-terpinene (0.52-24.86 %), cis and trans -piperitol (0.52-23.19 %), limonene (2.44-12.59 %) and camphene (0.01-6.22 %). Bornyl acetate (15.52-27.87 %), β-selinene (0.24-11.45 %), β-terpineol (5.45-6.16 %) and borneol (4.78-5.72 %) were also detected in some of the populations. The results from the chemical analysis of the essential oils were subjected to chemometric cluster analysis in order to identify the marker compounds, which differentiate the groups of individuals. Based on major compounds, the populations were represented by p-menth-2-en-1-ol, piperitol, α-terpinene (chemotype I), bornyl acetate, limonene, borneol (chemotype II), piperitone and α-terpinene (chemotype III). The present analysis revealed a new chemotype, containing bornyl acetate as the major component in the essential oil of *C. distans* from Uttarakhand, which was not reported earlier as a chief constituent.

Keywords: *Cymbopogon distans*, Essential oil, Piperitone, p-Menth-2-en-1-ol, Bornyl acetate, GC-MS.
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Introduction

Cymbopogon distans (Nees ex Steud.) W. Watson belongs to the Family Poaceae and is an aromatic perennial grass, distributed in tropical and sub-tropical zones of North-Western Himalaya from 1220-2744 m[(Ref. 1,2)]. The leaves are very narrow and tufted at their bases, spikes solitary or few together scattered and sessile spikelets[1]. In Uttarakhand, the species is locally known as Ganiya Grass[3]. The essential oil of *C. distans* has been traditionally used to treat inflammation and coughs, cold, asthma, chronic bronchitis and also shown antibacterial activity[4,5]. The oil of the species has immense industrial potential for their subsequent uses in aromatherapy, fragrances and natural isolates[3]. Several studies have been carried out on chemical composition of *C. distans* and reported nerol (36.7 %), cadinol (4.3 %) and palustrol (3.9 %) from Hubei Province of China[6]. The main components of the essential oil from Yunnan Province of China included trans-Geraniol (16.54 %), (R)-citronellal (15.44 %), (+)-citronellol (11.51 %), α-elemol (9.06 %), β-eudesmol (5.71 %) and limonene (5.05 %)[(Ref. 5)]. In another study, α-terpinene (27.8 %), piperitone (18.2 %), intermedeol (14.4 %) and geranyl acetate (7.3 %) were detected as the main components in the essential oil of *C. distans*[7]. The chemical composition of the essential oil of *C. distans* has been analyzed previously in India and a distinct composition was found[8-10]. In the present investigation, a detailed study to provide more information on intraspecific variability in the essential oil of wild growing populations of *C. distans* has been carried out to find out other possible chemotypes in terms of new aroma chemicals from abundant wild sources of Uttarakhand Himalaya.

Materials and Methods

Plant materials

The leaves of *C. distans* (flowering stage) were collected in the month of August 2012 from ten wild populations growing in different regions of Uttarakhand Himalaya, viz. P1 (Thal; altitude 1000 m), P2 (Munsyari; 2320 m), P3 (Raisera; 1480 m), P4

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(Chakrata; 1900 m), P5 (Narayan Ashram; 2734 m), P6 (Gaithia; 1000 m), P7 (Betalghat; 380 m), P8 (Nathuwakhan; 1900 m), P9 (Dhanolti; 2280 m) and P10 (Jabarkhet; 2000 m). The samples were duly identified by Dr Sunil Sah, Scientist C (Taxonomy), Centre for Aromatic Plants (CAP), Dehradun and voucher specimens from each population was deposited in the herbarium of CAP, Dehradun (Acc. no. CAP/CD201-210).

Isolation of essential oils

The essential oils from semi-dried leaves of *C. distans* were isolated by hydro-distillation for 4 h using a Clevenger apparatus. The oils yield varied from 1.1-3.2 % (v/w) in all the populations. The oil samples obtained were dehydrated over anhydrous sodium sulfate and kept in cool and dark place until analyses.

Gas chromatography and gas chromatography–mass spectrometry analyses

The gas chromatography (GC) analyses of the essential oils were carried out by Agilent (model 6890 N) gas chromatograph equipped with Flame Ionization Detector (FID) using N_2 as the carrier gas. The column was HP-5 fused silica capillary column (30 m × 0.32 mm, 0.25 µm film thickness) and temperature program was used as follows: initial temperature of 60 °C (hold: 2 min) programmed at a rate of 3 °C/min to a final temperature of 220 °C (hold: 5 min). Temperatures of the injector and FID were maintained at 210 and 250 °C, respectively. The injection volume of oil was 0.1µL.

The gas chromatography-mass spectrometry (GC-MS) analyses of the oils were performed with a Perkin Elmer Clarus 500 gas chromatograph equipped with a split/splitless injector (split ratio 1:50) data handling system. The column was Rtx-5 capillary columns (30 m × 0.32 mm, 0.25 µm film thickness). Helium was the carrier gas at a flow rate 1.0 mL/min. The GC was interfaced with (Perkin Elmer Clarus 500) mass detector operating in the EI^+ mode. Temperature program used was the same as described above for GC analyses. The temperatures of the injector, transfer line and ion source were maintained at 210, 210 and 200 °C, respectively. Mass spectra were taken over m/z 40-500 amu that revealed the total ion current, using an ionizing voltage of 70 eV.

Identification of compounds: The identification of constituents was performed on the basis of retention index, determined with reference to the homologous series of n-alkanes, C_8-C_{24} with co-injection of standards (Sigma Aldrich USA) under same analytical conditions and by matching their recorded mass spectra with installed MS library (NIST/Pfleger/Wiley) and available literature[11].

Cluster analysis

The percentage compositions of the essential oils were used to determine the relationship between the different populations of *C. distans* by cluster analysis using the SPSS software (ver 13.0). Euclidean distance was selected as a measure of similarity and the nearest-neighbor method was used for cluster definition.

Results and Discussion

The chemical constituents of ten populations of *C. distans* with their retention indices and percent contents (%) in the essential oils are listed in the Table 1, according to their elution order. Altogether, 42 constituents were identified, representing 85.54-95.72 %

Table 1–Chemical constituents of the essential oils from 10 wild growing populations of *Cymbopogon distans*

Compounds	RI	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Tricyclene	927	0.01	0.22	0.02	0.24	0.03	0.06	0.04	0.03	1.13	1.72
α-Pinene	939	0.02	0.03	0.06	0.40	0.03	0.1	0.04	0.03	2.10	3.27
Camphene	954	0.02	0.10	0.09	1.62	0.01	0.42	0.23	0.01	6.22	1.82
β- Myrcene	991	1.19	0.25	0.04	0.18	0.16	0.19	0.12	0.17	0.26	0.38
L-Phellandrene	1003	0.04	0.09	1.93	0.35	0.54	0.39	0.19	0.32	1.09	0.16
α-Terpinene	1017	24.86	23.63	11.91	23.25	22.41	6.78	5.00	21.90	3.37	0.52
p-Cymene	1025	3.54	1.88	5.09	3.02	5.40	0.49	0.24	4.95	2.08	0.70
Limonene	1029	3.66	2.81	6.13	5.28	5.70	3.91	2.44	5.14	11.11	12.59
β-Ocimene	1032	0.34	-	0.22	0.36	0.18	0.17	0.03	0.18	0.10	0.10
γ-Terpinene	1060	0.40	0.31	-	-	-	-	-	-	0.20	0.17

(Contd.)

Table 1–Chemical constituents of the essential oils from 10 wild growing populations of *Cymbopogon distans*—*(Contd.)*

Compounds	RI	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
cis-p-Menth-2-en-1-ol	1118	1.54	0.26	21.13	18.22	22.71	18.62	22.50	23.87	0.73	1.29
trans-p-Menth-2-en-1-ol	1136	-	-	18.39	7.87	10.76	6.94	7.85	11.58	0.87	0.95
Camphor	1141	-	-	-	-	-	-	-	-	1.32	1.74
Eucarvone	1146	-	-	0.1	0.37	0.53	0.44	3.68	-	-	-
iso-Borneol	1162	-	-	-	-	-	-	-	-	2.55	2.95
β-Terpineol	1163	-	-	-	-	-	-	-	-	5.45	6.16
Borneol	1169	-	-	-	-	-	-	-	-	5.72	4.78
Umbellulone	1171	-	-	1.38	1.27	-	4.24	-	0.44	-	-
p-Mentha-1,5-dien-8-ol	1172	1.03	0.6	1.14	1.46	1.33	0.27	0.10	0.83	-	-
Terpinen-4-ol	1177	-	-	-	-	-	-	-	-	2.52	2.11
p-Cymen-8-ol	1183	0.40	0.83	-	-	-	-	-	-	1.25	1.04
α-Terpineol	1189	1.48	1.08	-	-	-	-	-	-	5.93	2.77
cis-Piperitol	1196	-	-	7.43	14.58	12.95	18.34	18.94	13.23	0.48	0.30
trans-Piperitol	1208	0.52	-	14.69	4.33	5.59	4.85	3.95	5.69	0.80	0.92
2,3-Epoxycarane	1213	0.31	0.2	0.07	0.92	0.62	0.81	0.73	0.64	-	-
Carveol	1229	0.37	0.55	0.20	0.60	0.60	0.33	0.72	0.72	-	-
Carvone	1243	-	-	-	-	-	-	-	-	1.32	1.54
Piperitone	1253	45.34	48.78	3.73	1.62	4.47	4.24	4.62	4.44	1.05	1.18
Bornyl acetate	1289	-	-	-	-	-	-	-	-	15.52	27.87
2-Undecanone	1294	-	-	-	0.66	0.27	-	-	0.26	-	-
E-Citral	1341	0.89	0.56	-	-	-	-	-	-	-	-
Neryl acetate	1362	0.48	0.46	-	0.30	0.23	-	-	0.23	6.59	3.72
β-Elemene	1391	0.46	1.61	-	0.74	0.34	0.22	1.11	0.68	2.43	2.17
α-Gurjunene	1410	1.05	0.37	-	-	-	0.70	-	-	-	-
trans-Caryophyllene	1419	-	-	0.18	0.74	0.42	0.54	0.33	0.38	1.85	2.44
Calarene	1431	0.86	-	-	-	-	-	-	-	-	-
γ-Curcumene	1483	-	-	-	-	-	0.96	0.88	-	-	-
β-Selinene	1490	-	-	-	0.24	-	10.82	11.45	-	-	-
β-Cadinene	1518	0.57	0.32	-	-	-	1.36	-	-	-	-
Caryophyllene oxide	1583	1.48	1.07	-	-	-	1.18	1.07	-	1.50	2.15
α-Eudesmol	1654	-	-	-	0.53	-	-	-	-	-	-
Bisabolone	1748	-	-	-	-	-	4.28	4.13	-	-	-
MONOTERPENE HYDROCARBONS		34.08	29.32	25.49	34.7	34.46	12.51	8.33	32.73	27.66	21.43
OXYGENATED MONOTERPENE		52.36	53.32	68.26	52.2	60.06	59.08	63.09	61.93	52.1	59.32
SESQUITERPENE HYDROCARBONS		2.94	2.3	0.18	1.72	0.76	14.6	13.77	1.06	4.28	4.61
OXYGENATED SESQUITERPENE		1.48	1.07	-	0.53	-	5.46	5.2	-	1.5	2.15
Total identified (%)		**90.86**	**86.01**	**93.93**	**89.15**	**95.28**	**91.65**	**90.39**	**95.72**	**85.54**	**87.51**

of the oil composition. The main constituents in the essential oils of most of the populations were piperitone (1.05-48.78 %), cis and trans- p-menth-2-en-1-ol (0.26-39.52 %), α-terpinene (0.52-24.86 %), cis and trans- piperitol (0.52-23.19 %), limonene (2.44-12.59 %) and camphene (0.01-6.22 %). Bornyl acetate (15.52-27.87 %), β-selinene (0.24-11.45 %), β-terpineol (5.45-6.16 %) and borneol (4.78-5.72 %) were also detected in some of the populations. To characterize and verify the variation of the essential oils and to identify the different possible chemotypes in the populations of *C. distans*, their compositions were analyzed by cluster analysis. As shown in Fig. 1, cluster analysis allowed differentiation of *C. distans*

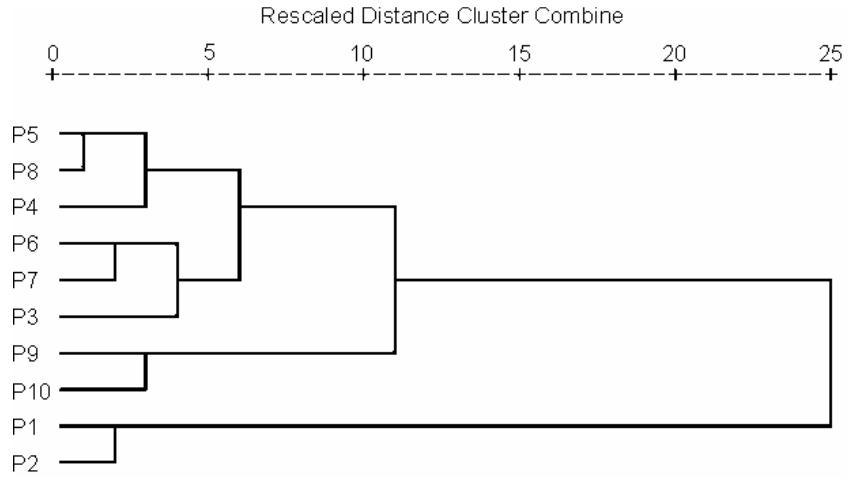

Fig. 1—Dendrogram obtained from the cluster analysis of essential oil components of *Cymbopogon distans* populations using nearest neighbor method with Euclidean distance measure

populations into three groups, each representing a chemotype. The dendrogram exhibited a distinct separation of the P3, P4, P5, P6, P7 and P8 populations as chemotype I, having p-menth-2-en-1-ol, piperitol, α-terpinene. In these oils the composition of marker compounds such as p-mentha-2-en-1-ol ranged from 25.56-39.52 %, followed by piperitol (18.54-23.19 %) and α-terpinene (5.00-23.25 %). Limonene and piperitone ranged from 2.44-6.13 % and 1.62-4.62 %, respectively in the oils. Bornyl acetate and borneol were not detected in these oils. The essential oils from two populations (P6 and P7) contained β-selinene (10.82-11.45 %) and bisabolone (4.13-4.28 %), which were absent in other populations. The chemotype II was composed of populations P9 and P10 with bornyl acetate, limonene and borneol as the major constituents. In the oils, bornyl acetate (15.52-27.87 %) and limonene (11.11-12.59 %) were found as the major constituents, followed by β-terpineol (5.45-6.16 %) and borneol (4.78-5.72 %). The chemotype is distinct, as the major compounds reported apart from limonene, were not detected in the essential oils of rest of the populations. In the oil, α-terpinene (0.52-3.37 %), piperitone (1.05-1.18 %), p-menth-2-en-1-ol (1.60-2.24 %) and piperitol (1.22-1.28 %) were found in minor quantities. In a previous report on *C. distans* essential oil, limonene (29 %) and methyl eugenol (13 %) were reported as the major constituents, while constitution of bornyl acetate was only 4.8 %[(Ref. 12)]. Two populations (P1 and P2) belonged to chemotype III, were characterized by high contents of piperitone and α-terpinene. Piperitone concentration ranged from 45.34-48.78 %, followed by α-terpinene (23.63-24.86 %). Limonene ranged between 2.81-3.66 %, while p-menth-2-en-1-ol and piperitol were found in traces only. Bornyl acetate, borneol and β-terpineol were not detected in these oils. Earlier, researchers have taken keen interest to explore the diverse chemical profiling of *C. distans* from Uttarakhand Himalaya and various chemotypes were noted as α-oxobisabolene (19.1 %); cital (35.0 %) and geranyl acetate (15.0 %); piperitone (60.0 %); eudesmanediol (34.4 %) and p-menthenol (66 %)[(Ref. 8,9)].

Conclusion

The present study revealed the presence of three chemotypes, viz. p-menth-2-en-1-ol, piperitol and α-terpinene (chemotype I), bornyl acetate, limonene and borneol (chemotype II) and piperitone and α-terpinene (chemotype III). The chemotype II, containing bornyl acetate as the major constituent in the essential oil of *C. distans* is a new chemotype from Uttarakhand, which was not reported earlier as a chief constituent. Considering the variability that exists among the chemical composition of investigated populations, selection of chemotype with a unique aroma is possible which could be further exploited for use in relevant perfumery, fragrance industries or as germplasm for the breeding programs of superior chemotype.

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