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 subtropical zones of North-Western Himalaya from 1220-2744 m\cite{Ref. 1,2}. The leaves are very narrow and tufted at their bases, spikes solitary or few together scattered and sessile spikelets\cite{1}. In Uttarakhand, the species is locally known as Ganiya Grass\cite{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\cite{4,5}. The oil of the species has immense industrial potential for their subsequent uses in aromatherapy, fragrances and natural isolates\cite{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\cite{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 %)\cite{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\cite{7}. The chemical composition of the essential oil of C. distans has been analyzed previously in India and a distinct composition was found\cite{8,9,10}. 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...
(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 N₂ 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, C₅-C₂₄ 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 literature¹¹.

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>
<thead>
<tr>
<th>Compounds</th>
<th>R1</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
<th>P8</th>
<th>P9</th>
<th>P10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tricyclene</td>
<td>927</td>
<td>0.01</td>
<td>0.22</td>
<td>0.02</td>
<td>0.24</td>
<td>0.03</td>
<td>0.06</td>
<td>0.04</td>
<td>0.03</td>
<td>1.13</td>
<td>1.72</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>939</td>
<td>0.02</td>
<td>0.03</td>
<td>0.06</td>
<td>0.40</td>
<td>0.03</td>
<td>0.1</td>
<td>0.04</td>
<td>0.03</td>
<td>2.10</td>
<td>3.27</td>
</tr>
<tr>
<td>Camphene</td>
<td>954</td>
<td>0.02</td>
<td>0.10</td>
<td>0.09</td>
<td>1.62</td>
<td>0.01</td>
<td>0.42</td>
<td>0.23</td>
<td>0.01</td>
<td>6.22</td>
<td>1.82</td>
</tr>
<tr>
<td>β-Myrcene</td>
<td>991</td>
<td>1.19</td>
<td>0.25</td>
<td>0.04</td>
<td>0.18</td>
<td>0.16</td>
<td>0.19</td>
<td>0.12</td>
<td>0.17</td>
<td>0.26</td>
<td>0.38</td>
</tr>
<tr>
<td>L-Phellandrene</td>
<td>1003</td>
<td>0.04</td>
<td>0.09</td>
<td>1.93</td>
<td>0.35</td>
<td>0.54</td>
<td>0.39</td>
<td>0.19</td>
<td>0.32</td>
<td>1.09</td>
<td>0.16</td>
</tr>
<tr>
<td>α-Terpinene</td>
<td>1017</td>
<td>24.86</td>
<td>33.63</td>
<td>11.91</td>
<td>23.25</td>
<td>22.41</td>
<td>6.78</td>
<td>5.00</td>
<td>21.90</td>
<td>3.37</td>
<td>0.52</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>1025</td>
<td>3.54</td>
<td>1.88</td>
<td>5.09</td>
<td>3.02</td>
<td>5.40</td>
<td>0.49</td>
<td>0.24</td>
<td>4.95</td>
<td>2.08</td>
<td>0.70</td>
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<tr>
<td>Limonene</td>
<td>1029</td>
<td>3.66</td>
<td>2.81</td>
<td>6.13</td>
<td>5.28</td>
<td>5.70</td>
<td>3.91</td>
<td>2.44</td>
<td>5.14</td>
<td>11.11</td>
<td>12.59</td>
</tr>
<tr>
<td>β-Ocimene</td>
<td>1032</td>
<td>0.34</td>
<td>-</td>
<td>0.22</td>
<td>0.36</td>
<td>0.18</td>
<td>0.17</td>
<td>0.03</td>
<td>0.18</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>
| γ-Terpinene     | 1060| 0.40| 0.31| -   | -   | -   | -   | 0.20| 0.17| (Contd.)
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*.
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. 8,9). 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-mentheneol (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.

**References**


6 Chen L and Lu H, Analysis of the chemical constituents of essential oil from Hubei *Cymbopogon distans* by GC-MS, *Chinese J Hospital Pharm*, 2009, 15, 1290-1291.


