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Chemical Variation in Volatiles of *Craniotome furcata*: Correlation with Soil Properties

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Abstract: The essential oils from the aerial parts of ten wild growing *Craniotome furcata* (Link.) O. Kuntze of the family Lamiaceae collected from different locations in Uttarakhand, India, was analyzed by capillary GC and GC/MS. The oils were rich in sesquiterpenoids. The cluster analysis showed the presence of four groups. Group-I was significantly rich in δ-elemene and germacrene D while group-II had germacrene D in abundant quantity. α-Bisabolol oxide A, α-cadinol and germacrene D-4-ol were major constituents of group-III. Group-IV showed the presence of α-muurolol and germacrene D as major constituents. Germacrene D was found to be present in all the samples of *C. furcata* collected from different locations and was positively correlated with the altitude of the collection regions. Chemical variation in the volatiles was statistically correlated with altitude and soil properties.

Keywords: *Craniotome furcata*; Lamiaceae; essential oil composition; germacrene D; chemical variation

1. Introduction

Craniotome furcata (Link.) O. Kuntze of the family Lamiaceae is a soft hairy herb with white pink or yellow flowers. The plant is distributed in temperate region from Shimla to Bhutan at a height 1500-2300m [1]. *C. furcata* is the only species of the genus in India and is reported to be used in folk medicine [2,3]. Craniosiden A and B, mussaeniside and ningpogenin were isolated from the ethyl acetate fraction of *C. furcata* for the first time. Among them, cranioside A and B were identified as new compounds [4]. Essential oil composition and antimicrobial activity of *C. furcata* have been reported earlier from India [5-7]. Joshi (2010) has investigated antimicrobial and antioxidant activities of the essential oil of *C. furcata* from Karnataka [8]. A preliminary assay *in vitro* was applied to evaluate their cytotoxicity against several tumor cell-lines [9]. As there are very few reports on the essential oil composition of this genus, the aim of the present work is to study the variability in essential oil composition of *C. furcata* collected from different locations of western Himalaya and correlate them with soil properties.

2. Materials and Methods

2.1. Plant Collection and Identification

Fresh plant material of *C. furcata* (Link.) O. Kuntze along with its soil samples (0-20 cm) were collected in September to November, 2010 from ten locations in western Himalaya (Uttarakhand, India) (Table 1). The plants were in full blooming stage. The botanical identification of the specimen was done at Botany Department, Kumaun University, Nainital and submitted in Botany Department, Nainital and in Botanical Survey of India, Dehradun.

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Table 1. Sites of collection of *C. furcata* along with voucher record number

S. No.	Locations	Latitude and longitude	Voucher record number
A	Binsar	29°37'N: 79º40'E	34806
B	Munsiyari	30°04'37''N: 80°23'04''E	34806 S01
C	Jeolikot	29º23'N: 79º30'E	34806 S02
D	Mussoorie	30º 27' N: 78º 06' E	34806 S03
E	Mukteshwar	29°28'N: 79°39'E	34806 S04
F	Kilbury	29º23'N: 79º30'E	34806 S05
G	Nainital	29º23'N: 79º30'E	34806 S06
H	Rushi village	29º23'N: 79º30'E	34806 S07
I	Bhowali	29º23'N: 79º31'E	34806 S08
J	Ramgarh	29º23'N: 79º30'E	34806 S09

2.2. Physicochemical Properties of Soil

Soil pH and EC was determined in 1:2 soil/water suspension, soil organic carbon content was determined by Walkley and Black method and texture was determined by hydrometer method [10, 11]. Cation exchange capacity was measured by the NH_4^+ saturation method given by Chapman (1969) [12]. The total metal content of the soils was determined in aqua regia digests [13]. Water holding capacity of the soils was determined by using Hilgard apparatus. Total nitrogen was determined by kjeldahl method [14]**.** Available Phosphorus in soil was determined by Olsen's method and Potassium estimation was done by using flame photometer [15]. Soil samples were extracted for DTPA extractable metals following the procedure developed by Lindsay and Norvell (1978) [16]. The properties of soil samples taken for this study are given in Table 2 and climatic and some other properties are given in Table 3.

2.3. Preparation of Plant Samples for Micronutrient Analysis

The collected plant material were washed sequentially with tap water, 0.1N HCl solution and finally with distilled water. Dry plant tissue was finely ground and wet ashed using HNO_3: H_2SO_4: $HClO_4$ (10:1:4 v/v) (Piper, 1942) [17].

2.4. Heavy Metal Analysis in Soil and Plant Samples

Concentration of Zn, Cu, Mn and Fe in prepared plant and soil samples were analyzed by atomic absorption spectrophotometer (GBC-902 and Avanta sigma Models).

2.5. Isolation of Essential Oil

One kilogram of fresh aerial plant material was steam distilled for 3 h using a copper still fitted with spiral glass condensers and extracted with 80 mL n-hexane and 40 mL dichloromethane. The organic phase was dried over anhydrous sodium sulfate and the solvent removed by distillation using a thin film rotary vacuum evaporator at 25º-30ºC. The oil yield was 0.2-0.3% (v/w).

2.6. Analysis of the Essential Oil

The oil was analyzed on a Perkin Elmer Autosystem XL GC using Equity-5 column (60m x 0.32mm x 0.25µm film thickness). The oven temperature (70º-290ºC) was programmed at 3ºC/min and hydrogen was used as the carrier gas at 0.689 bar column head pressure. The injector temperature was 280ºC, detector (FID) temperature 290ºC and the injector volume 0.05µL neat with split ration 1:50. The GC/MS used was an Autosystem XL GC with Equity-5 column (60 m x 0.32 mm x 0.25µm film thickness) and interfaced with Perkin Elmer Turbomass Quadrupole mass spectrometer. The oven temperature (70º-290ºC) was programmed at 3ºC /min using helium as carrier gas at 0.689 bar column head pressure. The injection volume was 0.05 μL neat with a split ratio of 1:50. The 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: NIH version 2.1 and WILEY: 7th edition), by comparing with the MS literature data [18]. The relative amounts of individual components were calculated based on GC peak area (FID response) without using correction factor.

2.7. Statistical Analysis

Experimental data were processed using Microsoft Excel XP. Correlation coefficients were calculated among the major constituents of oil, micro and macronutrients, microclimatic conditions and physical properties of soil. Significance level of correlation coefficient was checked on probability level of $p \leq 0.05$ and $p \leq 0.01$. Ward's hierarchical clustering analysis of major constituents of essential oils was conducted in order to discriminate chemical groups. All statistical analyses were performed using SPSS 16.0.

3. Results and Discussion

3.1. Essential Oil Composition

The essential oils of *C. furcata* (Link.) O. Kuntze collected from ten sites was analyzed by GC and GC/MS. Ward's hierarchical clustering analysis of major constituents of essential oils was conducted in order to classify groups. The result of cluster analysis revealed the presence of four groups on the basis of difference in their main chemical constituents On the basis of hierarchical cluster analysis four groups were clearly discriminated (Table 4, Figure 1). Group one (A & B) was significantly rich in δ-elemene (9.9-11.1%) and germacrene D (52.8-59.8%) while the second group (C, D, E & F) was further divided in to two subgroups on the basis of their similarity in dendrogram. The main components of the first subgroup (group IIa) (C & D) were δ-elemene (3.4-7.9%), germacrene D (36.7-36.8%), α-zingiberene (3.5-12.8%) and α-cadinol (1.1-9.3%) while the components of second subgroup (group IIb) (E & F) was germacrene D (42.9-46.2%), α-zingiberene (5.5-5.8%), germacrene B (1.6-11.9%) and α-muurolol (8.5-10.5%). Group III (G & H) showed the presence of γ-cadinene (6.6-9.6%), germacrene D-4-ol (10.0-24.8%), α-muurolol (2.4-5.2%), α-cadinol (9.2-12.0%), oplopanon (5.2-6.2%) and α-bisabolol oxide A (6.1-10.6%). The plants having high content of δ-elemene (3.0-6.8), germacrene D (13.3-17.5%), α-zingiberene (5.1-14.0%), germacrene B (3.5-15.6%), α-muurolol (8.1-15.2%) and α-cadinol (2.3-8.6 %) belong to group fourth (I & J).

Group-I: δ-elemene and germacrene D

Group-IIa: δ-elemene, germacrene D, α-zingiberene and α-cadinol

Group-IIb: germacrene D, α-zingiberene, germacrene B and α-muurolol

Group-III: γ-cadinene, germacrene D-4-ol, α-muurolol, α-cadinol, oplopanon and α-bisabolol oxide

Group-IV: δ-elemene, germacrene D, α-zingiberene, germacrene B, α-muurolol and α-cadinol

Table 2. Physicochemical properties of soils

Sites		A	B	C	D	E	F	G	H	I	J
General soil properties	Sand (%)	66	70	78	68	82	84	78	76	80	70
	Silt (%)	20	26	15	26	16	14	12	18	15	22
	Clay (%)	16	8	4	6	4	2	8	6	5	8
	Texture	Sandy loam	Sandy loam	Loamy sand	Sandy loam	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Sandy loam
Other soil properties	pH (1:2)	5.83±0.27	5.42±0.010	7.85±0.02	7.62±0.33	6.1±0.80	6.83±0.06	6.84±0.69	7.44±0.04	6.32±0.03	6.41±0.07
	O.C. %	1.71±0.10	4.17±0.04	1.68±0.03	3.04±0.14	3.85±0.04	1.2±0.00	3.23±0.20	2.65±0.35	2.69±0.06	3.15±0.04
	EC	0.111±0.000	0.11±0.001	0.42±0.07	0.156±0.011	0.054±0.022	0.23±0.040	0.074±0.001	0.34±0.02	0.78±0.01	0.195±0.01
	CEC	10.26±0.030	27.28±0.070	10.78±0.010	18.13±0.060	16.3±0.100	31.97±0.040	25.76±0.080	38.11±0.010	13.64±0.100	15.06±0.130
	W HC	37.92±0.070	40.36±0.010	43.52±0.030	58.51±0.100	38.24±0.080	49.69±0.010	39.57±0.050	46.08±0.010	42.88±0.070	35.11±0.040
Total content (mg kg^{-1})	Zn	57.663±0.028	38.67±0.002	25.05±0.16	54.281±0.05	26.875±0.10	62.657±0.03	47.377±0.26	91.677±0.01	42.007±0.11	41.718±0.05
	Fe	561.849±0.228	519.33±0.230	521.664±0.01	522.604±0.01	516.859±0.26	566.618±0.04	542.206±0.03	559.248±0.05	556.288±0.03	528.632±0.06
	Mn	15.5±0.130	24.63±0.090	10.70±0.10	19.825±0.29	10.7±0.010	25.783±0.01	14.67±0.06	15.55±0.08	11.484±0.05	10.825±0.01
	Cu	158.336±0.02	165±0.73	192.105±0.08	220.897±0.09	182.15±0.02	275.888±0.07	303.703±0.09	348.023±0.03	255.251±0.12	221.418±0.05
Available content (mg kg^{-1})	Zn	2.710±0.05	0.784±0.03	3.954±0.03	0.984±0.05	1.706±0.01	9.452±0.01	7.3680.03	12.262±0.05	1.486±0.03	1.104±0.01
	Fe	22.65±0.04	36.68±0.07	32.53±0.04	91.70±1.03	29.00±0.08	29.94±0.11	28.77±0.06	57.98±0.30	35.38±0.40	33.28±0.08
	Mn	7.08±0.06	14.28±0.11	6.68±0.05	17.11±0.88	3.00±0.73	14.56±0.21	15.43±0.42	17.39±0.20	9.58±0.07	10.00±0.23
	Cu	0.150±0.03	1.119±0.01	0.96±0.02	1.820±0.03	0.320±0.04	2.256±0.11	1.830±0.02	7.328±0.08	0.610±0.07	0.400±0.00
Macronutrient content (%)	N (av)	0.005±0.10	0.008±0.03	0.009±0.05	0.012±0.34	0.012±0.71	0.011±0.69	0.010±0.63	0.014±0.01	0.009±0.25	0.012±0.09
	N(tot)	0.20±0.01	0.28±0.01	0.20±0.04	0.25±0.03	0.24±0.01	0.21±0.04	0.18±0.05	0.35±0.03	0.13±0.02	0.30±0.06
	P (av)	0.0026±0.00	0.0011±0.00	0.0014±0.00	0.0009±0.00	0.0007±0.00	0.0037±0.00	0.0019±0.001	0.0024±0.01	0.0006±0.00	0.0033±0.00
	K (av)	0.0163±0.00	0.016±0.001	0.0076±0.00	0.0176±0.004	0.0046±0.001	0.0255±0.00	0.0132±0.00	0.009±0.002	0.0242±0.001	0.0193±0.001

*(av)=Available, (tot)= Total, EC= Electrical conductivity (dS cm^{-1}), WHC= Water holding capacity, CEC= Cation exchange capacity (c mol kg^{-1}), O.C.%= Organic carbon %

Table 3. Geographic and oil properties

Microclimatic and other properties	A	B	C	D	E	F	G	H	I	J
Altitude (m)	2300	2386	1490	2000	2265	2200	2100	1600	1706	1789
Temperature (^{0}C)	23	18	30	25	20	22	23	28	28	23
Plant height (cm)	138.9 ±1.53	95.7 ±2.52	116.8 ±2.65	89.7 ±3.51	87.2 ±2.52	67.7 ±1.53	69.4 ±1.53	99.9 ±3.51	66.0 ±2.66	86.4±2.00
Oil colour	yellow	yellow	Yellow	yellow	yellow	light green	light green	yellow	yellow	yellow
Oil yield (%)	0.43	0.45	0.30	0.52	0.38	0.36	0.34	0.35	0.32	0.32

These four groups showed the chemical variability in essential oil composition of *C. furcata* collected from ten regions. Earlier reports showed the presence of germacrene D (49.2%), germacrene D-4-ol (8.8%), *epi*-α-cadinol (5.9%) and 10-*epi*-γ-eudesmol (4.2%) as major constituents from the flowering aerial parts of the plants [5] while germacrene D (30.9%), germacrene D-4-ol (12.1%), α-cadinol (6.4%), 3-octanone (3.1%), germacrene A (5.8%) and *epi*-α-cadinol (4.0%) have been reported from the stems and leaves of the essential oil of *C. furcata* [6].

It has been investigated earlier that germacrene D plays an important role as a precursor of various sesquiterpenes such as cadinenes and selinenes [19, 20]. Plant terpenes have been found to show anti-herbivore defenses [21]. Germacrene D has also been reported to have deterrent effects against herbivores and insecticidal activity against mosquitoes as well as repellent activity against aphids and ticks [22, 23, 24].

Germacrene D varied quantitatively in these essential oils, showing regular increase in its percentage with the altitude of the ten regions.

3.2. Correlation Among Major Constituents

δ-Elemene was positively correlated with germacrene D. Germacrene D-4-ol was positively correlated with α-cadinol and α-bisabolol oxide A while α-cadinol correlated with α-bisabolol oxide A (Table 5).

3.3. Macronutrient, Micronutrients and Essential Oil Composition

Correlation analysis revealed that micronutrients in soil and plant affected essential oil composition. Available nitrogen was negatively correlated with δ-elemene. Total and available zinc, available copper and total manganese in soil was positively correlated with α-bisabolol oxide A, suggesting the role of nitrogen, zinc, copper and iron in their biosynthesis in *C. furcata* (Table 6-7).

3.4. Plant Characteristics, Microclimatic Conditions and Essential Oil Composition

Altitude is positively correlated with germacrene D ($r=0.644$, $P\leq0.05$) and negatively with temperature ($r=-0.909$, $P\leq0.01$) while plant height is positively correlated with δ-elemene ($r=0.723$, $P\leq0.05$) and negatively correlated with α-muurolol ($r=-0.759$, $P\leq0.05$) (Table 8).

Composition of volatile constituents of *C. furcata* from ten locations indicates the existence of four groups, which may be due to some environmental factors such as climate, habitat, harvesting time, water stress and altitude [25, 26]. To the best of our knowledge it is the first report on the chemosystematics of *C. furcata.*

Table 4. Essential oil composition of *C. furcata* collected from different sites

No	Compounds[a]	RI[b]	RI[c]	Group I		Group II				Group III		Group IV	
				(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)	(J)
1	sabinene	965	969	-	-	-	-	-	0.4	-	-	-	-
2	1-octene-3-ol	979	974	0.4	0.2	0.8	3.2	1.7	3.1	0.7	0.2	1.4	0.3
3	(*Z*)- β-ocimene	1037	1032	-	-	1.4	1.3	1.7	-	-	-	-	0.3
4	(*E*)- β-ocimene	1050	1044	0.9	0.9	-	-	-	-	1.6	0.4	-	-
5	δ-elemene	1338	1335	**11.1**	**9.9**	**7.9**	3.4	2.7	3.3	2.2	1.7	3.0	**6.8**
6	α-cubebene	1348	1345	1.9	0.9	1.1	1.3	3.2	1.5	3.3	0.5	3.3	**5.4**
7	α-copaene	1376	1374	2.0	1.7	-	-	-	-	-	-	1.7	-
8	β-bourbonene	1388	1387	3.5	4.5	-	-	-	-	-	-	-	-
9	β–elemene	1389	1388	4.2	3.2	1.4	1.6	0.5	-	3.8	2.5	0.5	1.1
10	(*E*)-caryophyllene	1419	1417	3.3	2.7	1.0	0.7	1.2	1.0	4.2	1.6	1.9	0.5
11	γ-elemene	1436	1434	1.9	2.1	-	0.3	-	-	-	-	-	-
12	α-guaiene	1439	1437	-	0.8	-	0.7	0.7	-	-	-	-	1.3
13	(*Z*)-β-farnesene	1442	1440	-	-	0.9	-	-	-	-	-	-	-
14	9-*epi*-(*E*)-caryophyllene	1466	1464	-	-	-	1.2	-	-	-	-	-	-
15	ar-curcumene	1480	1479	-	-	1.7	3.5	-	-	-	-	-	-
16	germacrene D	1485	1484	**52.8**	**59.8**	**36.7**	**36.8**	**46.2**	**42.9**	**4.0**	**4.5**	**13.3**	**17.5**
17	α-zingiberene	1493	1493	-	-	**12.8**	3.5	**5.5**	**5.8**	3.7	1.8	**5.1**	**14.0**
18	*epi*-cubebol	1494	1493	1.7	-	-	-	-	-	-	-	-	-

19	Bicyclogermacrene	1500	1500	-	-	-	-	-	0.9	-	-	-	-
20	α-muurolene	1500	1500	-	-	-	0.8	-	3.3	0.7	0.7	-	-
21	(*E*)-β-guaiene	1502	1502	-	-	-	-	-	1.4	-	-	-	-
22	α-farnesene	1505	1505	-	-	1.3	0.6	-	1.1	2.8	1.0	-	-
23	(*Z*)-α-bisabolene	1507	1506	-	-	-	-	-	**10.3**	-	-	-	-
24	Germacrene A	1509	1508	2.8	0.3	-	-	-	-	-	-	2.8	-
25	δ-amorphene	1512	1511	-	-	2.1	3.8	-	-	-	-	-	-
26	γ-cadinene	1513	1513	-	-	0.9	0.9	-	**6.8**	**6.6**	**9.6**	**8.8**	-
27	β-sesquiphellandrene	1522	1521	-	-	3.0	2.3	1.0	1.1	2.6	0.9	4.5	3.1
28	δ-cadinene	1523	1522	1.4	0.6	1.0	1.3	-	-	-	-	2.5	-
29	Hedycaryol	1548	1546	-	-	-	0.3	-	-	-	-	-	-
30	Germacrene B	1561	1559	1.5	1.7	1.0	4.1	**11.9**	1.6	4.8	**6.9**	3.5	**15.6**
31	Germacrene D-4-ol	1575	1574	-	-	-	4.4	1.5		**10.0**	**24.8**	2.9	2.2
32	1,10-di-*epi*-cubenol	1619	1618	-	-	-	-	-	0.8	-	-	-	-
33	α-muurolol	1646	1644	-	-	2.7	3.8	**8.5**	**10.5**	**5.2**	2.4	**15.2**	**8.1**
34	β -eudesmol	1650	1649	-	-	2.6	-	-	-	-	-	-	-
35	α-cadinol	1654	1652	0.7	-	1.1	**9.3**	1.5	0.9	**9.2**	**12.0**	**8.6**	2.3
36	ar-turmerone	1669	1668	-	-	-	0.6	3.2	-	4.6	1.3	3.3	1.9
37	*epi*-β-bisabolol	1671	1670	-	-	-	1.6	1.2	-	3.7	1.9	3.6	2.2
38	Khusinol	1680	1679	-		-	1.2	-	-	-	-	-	-
39	(*Z*)-α-*trans*-bergamotol	1690	1690	1.1	0.6	-	-	-	-	-	-	-	-
40	Oplopanone	1740	1739	-	-	-	0.7	2.7	-	**5.2**	**6.2**	1.7	3.3
41	α-bisabolol oxide A	1749	1748	-	-	-	-	-	-	**6.1**	**10.6**	-	-
				91.2	**89.9**	**81.4**	**93.2**	**94.9**	**96.7**	**81.4**	**93.2**	**94.9**	**96.7**

[a] Order of elution and percentages of individual components are given on a Equity-5 capillary column. Identification was made on the basis of their RI and MS (GC/MS); Bold type indicates major components. t = trace (<0.1%), n.i.= not identified, RI[b] = Calculated, RI[c]= Published [18]

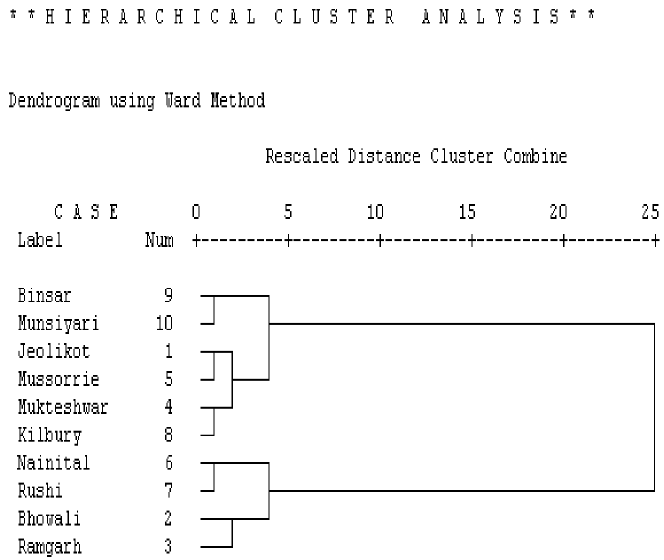

Figure 1. Agglomerative hierarchical clustering analysis by SPSS 16.0 for the chemical abundances of 12 essential oil components in the 10 populations of *C. furcata***.**

Table5. Simple correlation matrix (r) among major constituents.

S.N	1 δ-elemene	2 α-cubebene	3 germacrene D	4 α-zingiberene	5 (*Z*)-α-bisabolene	6 γ-cadinene	7 germacrene B	8 germacrene D-4-ol	9 α-muurolol	10 α-cadinol	11 oplopanon	12 α-bisabolol oxide A
1	1.00	0.137	0.641*	-0.018	-0.195	-0.678*	-0.275	-0.549	-0.564	-0.702*	-0.648*	-0.488
2		1.00	-0.184	0.335	-0.206	-0.240	0.581	-0.308	0.362	-0.177	-0.059	-0.289
3			1.00	-0.235	0.199	-0.695*	-0.335	-0.682*	-0.364	-0.789**	-0.733*	-0.678*
4				1.00	0.043	-0.198	0.396	-0.264	0.360	-0.234	-0.223	-0.287
5					1.00	0.298	-0.255	-0.208	0.354	-0.288	-0.249	-0.160
6						1.00	-0.183	0.635*	0.441	0.675*	0.627	0.640*
7							1.00	0.143	0.276	0.041	0.292	0.046
8								1.00	-0.172	0.780**	0.866**	0.966**
9									1.00	0.163	0.032	-0.226
10										1.00	0.694*	0.702*
11											1.00	0.906**
12												1.00

Table 6. Correlation matrix (r) between macronutrients and major constituents of essential oil

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
S. N.	N (av.)	N(total) %	P_2O_5 %(av)	K_2O %(av)	δ-elemene	α-cubebene	germacrene D	α-zingiberene	(*Z*)-α-bisabolene	γ-cadinene	germacrene B	germacrene D-4-ol	α-muurolol	α-cadinol	oplopanon	α-bisabolol oxide A
1	1.00	0.588	0.093	-0.141	-0.732*	-0.159	-0.479	0.277	0.112	0.307	0.602	0.552	0.308	0.477	0.456	0.416
2		1.00	0.278	-0.326	0.009	-0.247	-0.031	-0.026	-0.131	-0.149	0.416	0.518	-0.457	0.085	0.238	0.421
3			1.00	0.349	0.110	0.221	-0.111	0.208	0.586	0.139	0.041	0.095	-0.020	-0.234	-0.031	0.164
4				1.00	0.032	0.205	-0.014	-0.032	0.521	0.269	-0.314	-0.341	0.493	-0.44	-0.427	-0.353

Av = available

Table 7. Correlation matrix (r) among zinc (Zn), Iron (Fe), copper (Cu) and manganese (Mn) in soil and plant with major constituents of essential oil

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
S.N.		Total Metal	DTPA Extractable metal	Metal in plant	δ-elemene	α-cubebene	germacrene D	α-zingiberene	(Z)-α-bisabolene	γ-cadinene	germacrene B	germacrene D-4-ol	α-muurolol	α-cadinol	Oplopanon	α-bisabolol oxide A
Zn	1	1.00	0.728*	0.937**	-0.306	-0.311	-0.355	-0.463	0.251	0.594	-0.175	0.735*	-0.167	0.525	0.541	0.695*
	2		1.00	0.767**	-0.477	-0.411	-0.452	-0.157	0.454	0.722*	-0.185	0.703*	-0.044	0.380	0.387	0.778**
	3			1.00	-0.458	-0.254	-0.349	-0.288	0.513	0.642*	-0.096	0.611	0.091	0.449	0.461	0.561
Fe	1	1.00	-0.209	-0.055	-0.175	0.036	-0.294	-0.324	0.479	0.699*	-0.363	0.293	0.248	0.234	0.230	0.343
	2		1.00	0.776**	-0.339	-0.462	-0.135	-0.169	-0.169	0.048	-0.053	0.363	-0.163	0.587	0.033	0.185
	3			1.00	-0.340	-0.233	-0.442	0.186	-0.269	0.242	0.134	0.443	0.135	0.613	0.113	0.232
Cu	1	1.00	0.202	0.588	0.105	-0.537	0.449	-0.516	0.607	0.065	-0.517	-0.100	-0.228	-0.175	-0.292	-0.066
	2		1.00	0.862**	-0.483	-0.581	-0.480	-0.280	0.096	0.639*	-0.073	0.912**	-0.201	0.622	0.664*	0.871**
	3			1.00	-0.383	-0.578	-0.277	-0.444	0.328	0.591	-0.164	0.705*	-0.170	0.423	0.505	0.712*
Mn	1	1.00	0.633*	0.662*	-0.766**	-0.203	-0.825**	-0.042	0.243	0.886**	0.019	0.791**	0.296	0.754*	0.748*	0.800**
	2		1.00	0.750*	-0.336	-0.480	-0.348	-0.363	0.217	0.481	-0.334	0.533	-0.165	0.560	0.285	0.528
	3			1.00	-0.280	-0.353	-0.505	-0.351	0.069	0.716*	-0.374	0.521	0.042	0.513	0.468	0.592

Table 8. Correlation matrix (r) between microclimatic conditions and major constituents of essential oil

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Altitude	Oil %	Temp	Plant height	δ-elemene	α-cubebene	germacrene D	α-Zingiberene	(Z)-α-bisabolene	γ-cadinene	germacrene B	germacrene D-4-ol	α-muurolol	α-cadinol	oplopanon	α-Bisabolol oxide A
1	1.00	0.588	-0.909**	0.001	0.262	0.115	0.644*	-0.574	0.239	-0.372	-0.039	-0.395	-0.203	-0.437	-0.243	-0.319
2		1.00	-0.437	0.233	0.212	-0.274	0.553	-0.615	-0.086	-0.418	-0.207	-0.137	-0.461	-0.004	-0.335	-0.223
3			1.00	0.131	-0.234	-0.197	-0.548	0.348	-0.186	0.434	-0.244	0.350	0.143	0.486	0.188	0.291
4				1.00	0.723*	-0.068	0.440	-0.128	-0.369	-0.369	-0.211	-0.070	-0.759*	-0.354	-0.262	-0.066

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

4. Conclusions

The comparative study of the essential oils of *C. furcata* showed variation in the essential oil composition. On the basis of their major constituents, the oils were divided into four groups, which highlight the chemosystematics of this genus. Essential oil composition of *Craniotome furcata was* affected by variation in soil properties and microclimatic conditions. Germacrene D was found as a common constituent in all the essential oils of *C. furcata,* which varied remarkably among different regions. In our study, altitude seems one of the important factors influencing the percent variation of germacrene D. Zinc, copper and manganese in soil were found to affect α-bisabolol oxide A percentage.

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