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Altitudinal variation in the volatile constituents of *Cymbopogon flexuosus* from Uttarakhand region of Himalaya

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Abstract
The essential oils of *Cymbopogon flexuosus* (lemon grass) of the family Poaceae collected from different altitudes of the Uttarakhand region of Himalaya were subjected to detailed GC/MS analysis in order to determine the variation of concentration in their constituents. The GC/MS analysis led to the identification of 29 constituents forming 90.62 to 93.58% of their total oil content. The major constituents were citral, geraniol, citronellol, citranellal, linalool, borneol, β-myrcene limonene, β-caryophyllene, camphene, γ-cadinene, α-terpineol, neryl acetate and heptenone. Plants collected from 450 m altitude afforded citral (74.58 %) [a isomeric mixture of geranial and nearl] as a major constituents whereas only a less percentage of citral (64.21%, 68.29%) was found in the plants collected from two other altitudes i.e 250m and 1000m respectively. The geraniol, camphene and neryl acetate were obtained in a high concentration form the plants collected at 250 m altitude but in the plant from two other altitudes, it was found only in less proportion. Similarly, γ-cadinene, α-terpineol and camphene were the major constituents of plants at 1000m altitude but in other plants it was detected in very low concentration. Since, the concentration of plant constituents affected by altitude and season, medicinal properties of such plants and their use in biological application would vary accordingly.

Keywords: Altitudes, Citral, *Cymbopogon flexuosus*, Monterpene, Poacease

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INTRODUCTION

Cymbopogon is the one of most important essential oil yielding genera of family Poaceae. Lemongrass, palmarosa and citronella are the important essential oil yielding members. Essential oils have played an important role in human life, so much so that they have become indispensable in the production of perfumes, cosmetics, medicines, food preparation and as a starting material for the synthesis of various other compounds. (Badoni *et al*., 2010). The essential oils are concentrated, hydrophobic liquid containing volatile aroma compounds of plants, which are called aromatic herbs or aromatic plants. They are also known as volatile or ethereal oils, or simply as the oil of the plants from which they are derived, such as camphor oil, peppermint oil, lemon grass oil, etc.
About 8000 flowering plant species grow in western Himalaya rich in genetic diversity of medicinal and aromatic plants (Rao, 1994). The Genera Cymbopogon comprises about 140 species worldwide, out of which 45 species have been reported to occur in India. The members of the genus Cymbopogon occur abundantly in tropics and sub tropics regions of Asia, Africa and America with a regular distribution ranging from mountains and grassland to arid zones (Soenarko,1997; Bor,1960, Khanuja *et al*.,2005, Padalia *et al*.,2011). Cymbopogon species display wide variation in morphological attributes and essential oil composition at inter and intra specific level over the years (Sangwan *et al*.,2001) The most common economic species viz., *C. winterianus, C. flexuosus, C. martinii var. motia and sofia, C. nardus var. nardus, C. citratus, C. pendulus, C. warancusa, C. khasianus* produce different types of essential oils such as palmarosa oil, lemongrass oil, citronella oil, ginger grass or rusa oil of commercial interest (Gupta and Jain, 1978;Mathela, *et al*.,1986; Kumar, *et al*.,2000).
C. flexuosus is tall, fast growing, lemon scented, perennial grass reaching a height of 1.5 m. It has distinct, dark green foliage and also produces seed. Lemon grass prefers tropical and subtropical climate, it grows well at a temperature range of 10 ^{O}C to 33^{O}C and needs enough sunshine for the development of oil in the plant. The grass is sensitive to cold weather and cannot withstand in frost. Essential part of plant is stalks and leaves. The essential oil is extracted from fresh plant material by means of hydrodistillation (Lawrence, 1988).
The essential oils from Cymbopogon species such as lemongrass, palmarosa and citronella contains

a wide variety of cyclic and acylic monoterpene such like citral(3,7-dimethyl 2,6-octadienal; a mixture of two isomer geranial and neral)is used in vitamin A and ionone synthesis.. Other constituent like geraniol and its ester, citronellol and citronellal are important perfume materials. Several Cymbopogon species possessed significant anthelmintic, anti inflammatory, analgesic, anti-ageing, pesticidal, antimicrobial, mosquito repellant and larvicidal activities and thus, are used in native medicine for curing a number of diseases (Rao, 1997). Studies on the oil composition of various Cymbopogon species have been carried out time to time, who reports geraniol, geranyl acetate, citral, limonene, elemecin, monoterpene alcohols and sesquiterpenes as the major constituents in their essential oils (Kulkarni, *et al*., 1992; Khanuja, *et al*., 2005; Rao,*et al*., 2009; Verma, *et al*.,2009).The commercial aspects of the essential oils of these aromatic grasses and their cultivars prompted us to carry out detailed comparative terpenoid composition of cultivated species *C. flexuosus* in Northern part of India.

MATERIALS AND METHODS

Plant material: The aerial parts of *C.flexuosus* were collected at CIMAP Centre, Pantnagar; CAP Centre, Selaqui Dehradun and Horticulture College, Tehri having altitude 250m , 450m and above 1000m above msl respectively were collected from different altitudes of the Uttarakhand in September, 2016 Himalayan region and duly identified by Botany Department, Forest Research Institute, Dehradun. After identification the voucher specimens of *C. flexuosus* (A-1 to A-3) were kept in the herbarium of D.B.S (P.G) College Dehradun, Uttarakhand (India).

Essential oil extraction: The essential oil was extracted from 100 gms if fresh plant material in 3 replicates by hydro-distillation method using Clevenger apparatus for 3 hours. The essential oil evaporated together with vapors and collected as oil drop after condensation into a closed tube attached to Clevenger apparatus. The essential oil was separated from an aqueous phase using a separating funnel. The essential oil was dried over anhydrous $Na_2SO_4$and stored in sealed vials under refrigeration for further analysis. Oil content in terms of oil percentage was calculated as the mean of 3 samples. The oil yield was calculated on the basis of fresh weight of the material (v/w). (Kulkarni, *et al*., 2003; Padalia, *et al*.,2011)

GC-MS analysis: The GC-MS analysis of the oils were carried out by GC Perkin Elmer Clarus 500 with a mass spectrometer PerkinElmer Clarus 500 fitted with an RTX-5 capillary column (60 m × 0.32 mm i.d., film thickness 0.25 µm). The oven column temperature vary from 60°C–210°C, programmed at 3°C/min, with initial and final hold time of 2 min, using He as carrier gas at 10 psi constant pressure, a split ratio of 1:50, an injection size of 0.02 µL neat, injector, transfer line and source temperatures were 210°C; ionization energy 70 eV; mass scan range 40-450 amu.

Identification of constituents: The Identification of constituents was done on the basis of retention time, Retention Index [RI, determined with reference to homologous series of n-alkanes (C_9-C_{26}, Polyscience Corp., Niles IL) under identical experimental condition], co-injection with standards referencecatalogue (Aldrich and Fluka), mass spectra library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data 7th edition) and by comparing with the mass spectral literature database (Adams,1995; Davies, 1990). The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

RESULTS

The essential oil extracted from fresh aerial parts of three cultivars of *C.flexuosus* species viz. Cauvery, Krishna and Chirharit by hydrodistillation method, using a Clevenger apparatus. The distilled oils were dried over anhydrous sodium sulphate and keep in vial at 4^0 C for further analysis. The composition of essential oils were analyzed by GC-MS. The GC/MS analysis led to the identification of 28-29 constituents forming 92.1 to 97.5% of their total oil composition with monoterpenoids (78.3 to 88.9 %) as most exclusive constituents. The comparative results showed considerable variation in qualitative and quantitative compositions of major constituent of essential oils form three different cultivars of the studied *C.flexuosus* (Table1).

The cultivars of *C. flexuosus* were rich in citrals (comprised of neral and geranial) with highest proportion in cv.Krishna (74.58%) followed by chirharit (71.00 %) and Cauvery (68.60 %). The essential oil extracted from *C. flexuosus* var. Krishna is taken for study of altitudinal variation. The yield of essential oil of *C. flexuosus* harvested in month of September was 0.5 - 0.7% for fresh herbage weight. The yield of oil fluctuates greatly with the season, the condition of plant material, its moisture content and age of planting.

The GC/MS analysis of the essential oil led to the identification of 29 constituents. The major constituents present in *C.flexuosus* plant collected in September month from 250m altitude were camphene (3.89%), limonene (1.04%),citral{(isomeric mixture of geranial (34.65%) and neral (29.56%)}, borneol (1.07%), geraniol (3.82%), neryl acetate (1.75%),Caryophyllene oxide (1.03%). The plants collected from 450 m altitude contained linalool (1.65%),geranial (40.29%), neral (34.29%) β-Caryophyllene(1.14%), and Caryophyllene oxide (1.10%) as a major constituents. γ-Cadinene (2.01%), mentha (1.02%), α-terpineol(2.45%), and

Table 1. Major components of different cultivars of *C.flexuosus.*

S.N.	Major compounds	Concentration (%)		
		Cauvery	Krishna	Chirharit
1.	Neral	31.2	34.29	31.4
2.	Geranial	37.4	40.29	39.6
3.	Geraniol	1.9	1.47	2.7
4.	Limonene	0.5	0.92	0.9

Table 2. Chemical composition of essential oil of *C.flexuosus* harvested in September, 2016 from three altitudes of Uttarakhand.

S.N.	Retention Indices	Name of constituents	Area , %		
			Pantnagar 250 m	Dehradun 450 m	Ranichuri <1000 m
1.	10.00	Tricyclene	0.52	--	0.33
2	10.48	α –Pinene	0.83	0.74	0.42
3.	10.52	β-myrcene	0.39	0.42	0.33
4.	11.13	Camphene	3.89	2.01	2.47
5.	12.70	Hept-5-en-2 one	2.58	1.98	0.85
6.	14.42	Limonene	1.04	0.92	0.51
7.	14.44	β-ocimene	0.01	0.05	0.06
8.	14.52	Eucalyptol	0.83	--	--
9.	14.77	α –Pinene	0.71	0.63	0.35
10	15.18	Carven	0.04	0.14	--
11	16.19	Pentyl propyl ketone	0.9	1.29	1.27
12	16.97	Citronellal	0.31	0.44	0.22
13	17.29	Linalool	1.69	1.65	1.41
14	19.10	Chrysanthemol	0.67	--	0.53
15	19.92	Borneol	1.07	1.01	0.33
16	20.33	Verbenol	1.49	1.7	1.59
17	20.85	α-terpineol	0.34	0.35	2.45
18	21.99	Mentha	--	0.44	1.02
19	22.55	Neral	29.56	34.29	31.1
20.	22.96	Geraniol	3.82	1.47	2.32
21	23.64	Geranial	34.65	40.29	37.19
22	27.21	Neryl acetate	1.75	0.82	0.96
23	28.51	β-Caryophyllene	1.12	1.14	1.21
24	29.36	Isoeugenol	--	0.01	0.01
25	30.01	Citronellol	0.02	0.02	--
26.	31.43	γ-Cadinene	1.09	0.62	2.01
27.	31.45	Nerol	0.21	0.05	0.11
28.	31.58	δ-Cadinene	0.50	--	0.49
29.	33.46	Caryophyllene oxide	1.03	1.10	1.08
		Oil content (%)*	91.06	93.58	90.62

On fresh wt. basis , t= traces(<0.10%), (-)= absent.

camphene(2.47%) were also detected as major constituents of the plants from 1000m. The geraniol(3.82%) and nerylacetate(1.75%) detected as a major component in 250m altitude plants was found low concentration in other altitude plants (1.47,0.82 ; 2.32,0.96). Similarly, citral (isomeric mixture of geranial (40.29%), and neral (34.29%)) were the major constituents of 450m altitude plant, but these were detected in smaller quantities in 250m and 1000m altitude plants (34.65,29.56 ; 37.19,31.10). The concentration of various chemical constituents of*C.flexuosus* from different altitudes is given in Table 2.

DISCUSSION

Essential oils are generally complex mixtures of compounds, and potential synergistic and antagonistic effects should be taken into account when evaluating their biological activities (Padalia, *et al*., 2011, Ganjewala, 2009). The essential oils showed a seasonal and altitudinal discrepancy in the concentrations of their constituents. Therefore, an optimum season and altitude is required for harvesting the essential oil-bearing plants.

As indicated above, the essential oils obtained from *C.flexuosus* of different altitudes showed significant variation in their chemical composition. The citral content in *C. flexuosus* plant of mid altitude (450 m), was found in more concentration than that of the other two altitudes. The essential oil obtained in low altitude (250 m) contained geraniol, camphene and neryl acetate were detected more in concentration than the oil of high altitude plants, while concentration of γ-cadinene and α-

terpineol were increased as the altitude increased. The essential oil of *C.flexuosus* was evaluated for various biological activities. and showed significant results (Ganjewala and Gupta ,2013). The bioactive potential of essential oil has been attributed to its one or more major chemical constituents namely citral and geraniol. Various chemical constituents are responsible for such activities either individually or synergistically. Since the oil collected from the plants of mid altitude contained linalool, geranial, neral, β-Caryophyllene, and Caryophyllene oxide constituents in high concentrations, such oils may demonstrated more potent activities .i.e. antioxidant activity, antifungal and antibacterial etc. Therefore, the plant of *C.flexuosus* cultivated at mid altitude may be used for the production of essential oil for commercial purposes.

Conclusion

The essential of *C. flexuous* owing to their specific aromatic and medicinal properties are used in flavor, fragrance and pharmaceuticals. The pharmacological and medicinal significance of essential oil of this species is due to its constituents, which are mainly monoterpenes with large proportion of citral and geraniol. Despite the immense commercial significance of essential oil, only little work has been done so far towards altitudinal variation of constituents inquantitative and qualitatively .Therefore , we conclude that the *C. flexuous* cultivated at mid altitude should be used for the production of citral for commercial purposes.

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