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Compositional changes in sesquiterpene constituents of *Blumea mollis* Merr.

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ABSTRACT

Blumea mollis collected from different regions of Uttarakhand (India). GC and GC-MS analysis of the essential oil of these samples showed the dominance of sesquiterpenoids *viz.* (*E*)-caryophyllene, caryophyllene oxide**,** *δ*-cadinene, bisabolene, germacrene D and *α*-humulene beside 2,5-dimethoxy -*p*-cymene.

Keywords:*Blumea*, Asteraceae, (*E*)-caryophyllene, caryophyllene oxide, germacrene D, δ-cadinene, 2, 5-dimethoxy-p-cymene.

INTRODUCTION

The genus *Blumea* (Asteraceae) consists of about 80 species distributed in the tropics of Asia, Africa and Australia [1] of which 35 species occur in India. A number of these are aromatic and medicinal [2]. *Blumea mollis* Merr. an erect, annual biennial herb which possesses high content of essential oil [3, 4].*Blumea* species are known to cure bronchitis, blood disease, fever, anti-inflammatory and to alleviate burning sensation. It is used as febrifuge, antipyretic and diuretic in indigenous medicine [5, 6].In an earlier report, the volatile constituents of *Blumea mollis* collected from Pithoragarh region shows the dominance of *(E)*-caryophyllene and *(δ)*-cadinene[7]. The oil has been reported to possess antibacterial and antifungal activities [8].

MATERIALS AND METHODS

Plant material

The fresh flowering aerial parts of *Blumea mollis* were collected from, Pithoragarh (1800 m), Nainital (1900 m) and Almora (1700 m) districts of Uttarakhand. Plant herbaria were identified from the Botanical Survey of India, Dehradun and voucher specimen (Voucher No. CSM/UCoST/BM) were deposited in the Phytochemistry Laboratory, Chemistry Department, Kumaun University, Nainital.

Extraction of the oil

The fresh plant material (2 kg each) was subjected to steam distillation. The distillates were extracted with *n*-hexane and dichloromethane. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was distilled off. The major compounds were isolated by fractionation of the essential oil on silica gel CC (230-400 mesh, Merck, 600 × 25 cm column) packed with hexane, using Et_2O-hexane as mobile phase with gradually increasing concentration of ether (2-10%).

GC and GC-MS analysis

The oil was analyzed by using Nucon 5765 gas chromatograph equipped with Rtx-5 non-polar fused silica capillary column (30 m × 0.32 mm, 0.25 µm film coating). The oven temperature (60-210º C) was programmed at 3º C min^{-1} using N_2 as carrier gas at 4 Kg cm^{-2}. The injector temperature was 210º C, detector temperature 210º C and the injection volume 0.5 µL, using a 10% solution of the oil in *n*-hexane. GC-MS was conducted on a Thermo Quest Trace GC 2000 interfaced with a Finnigan MAT PolarisQ ion trap mass spectrometer equipped with Rtx-5 non-polar fused silica capillary column (30 m × 0.25 mm, 0.25 µm film coating). The oven temperature (60-210º C) was programmed at 3ºC min^{-1} using helium as carrier gas at 1.0 min^{-1}. The injection, ion source and MS transfer line temperatures were 210º C, 220º C and 275ºC, respectively, the injection volume was 0.1 µL, and the split ratio was

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1:40. MS were taken at 70 eV with mass range of 40-450 amu.

Isolation and identification of constituents

The essential oil was fractionated by column chromatography on silica gel CC (230-400 mesh, Merck, 600 x 25 cm column) packed with n-hexane, and eluted with hexane followed by gradient elution by Et_2O/hexane (5-20%). Characterization of constituents was done on the basis of Linear Retention Index (LRI, determined with reference to homologous series of *n*-alkanes C_9-C_{24}) under identical experimental condition, co-injection with available compounds, MS Library search (NIST and WILLEY) and by comparing with the MS literature data [9]. The relative contents of individual components were calculated GC response on FID without using correction factor.

RESULTS

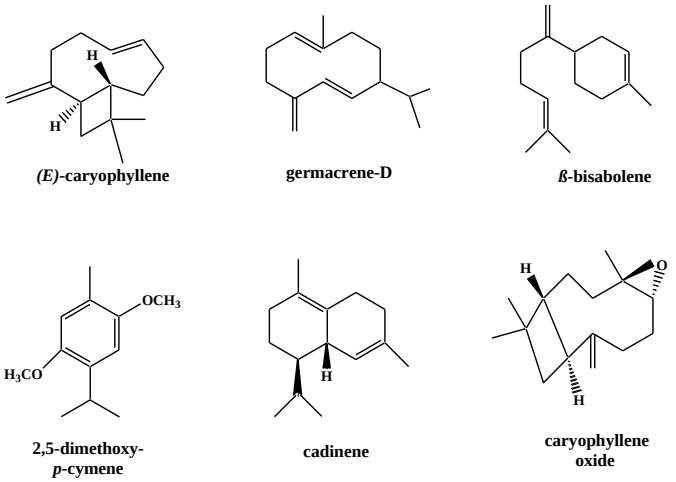
The essential oil of *Blumea* sps. has been found to possess antibacterial and antifungal activities. Itis known to cure bronchitis, blood disease, fever, anti-inflammatory and to alleviate burning sensation [8]. Methanolic extract of *Blumea mollis* was also evaluated for its antioxidant and anticancer properties which showed good inhibitory effect on cancer cells with the increased concentration and duration [10].

The chemical composition of the volatile constituents obtained from the fresh part of *Blumea mollis* from different regions have been given in Table 1. Twenty-two constituents were identified by GC-FID and GC-MS on Rtx-5 non-polar fused silica capillary column. All the samples collected from different areas are subjected to investigate separately for their flowers and leaves. The major constituents obtained from the flowers of *B. mollis* collected from Pithoragarh district of Uttarakhand (BM_P: FO) were *(E)*-caryophyllene (43.9%), *δ*-cadinene (14.6%), *α*-humulene (8.3%), caryophyllene oxide (7.3%) and *β*-bisabolene (5.4%) whereas leaves contains *(E)*-caryophyllene (39.5%), *(δ)*-cadinene (17.0%), *α*-humulene (7.7%), *β*-bisabolene (5.4%) and caryophyllene oxide (5.2%) as a major constituents. The plant material collected from Nainitalcontains germacrene D (21.5%), *(E)*-caryophyllene (20.4%), *α*-humulene (5.6%) and 2, 5-dimethoxy-*p*-cymene (4.7%) as major constituents in its flower oil (BM_N: FO). On the other hand leaves oil (BM_N: LO) contain *(E)*-caryophyllene (30.9%), germacrene D (19.5%), 2, 5-dimethoxy-*p*-cymene (17.0%) and *α*-humulene (6.4%). The flower oil of the sample collected from Almora district of Uttarakhand (BM_A: FO) was rich in *(E)*-caryophyllene (25.6%), germacrene D (11.3%), *δ*-cadinene (8.7%), caryophyllene oxide (6.6%), 2, 5-dimethoxy-*p*-cymene (5.3%) and 1,8-cineole (5.7%). Leaf oil of the sample collected from Almora was also dominated by the presence of *(E)*-caryophyllene (42.4%), caryophyllene oxide (12.6%), germacrene D (10.4%), *δ*-cadinene (5.9%) and 2, 5-dimethoxy-*p*-cymene (4.9%).

Table 1: Terpenoid constituents of *Blumea mollis* Merr.

S. No.	Compounds	LRI	% (FID) BM_P (FO)	% (FID) BM_P (LO)	%(FID) BM_N(FO)	% (FID) BM_N(LO)	% (FID) BM_A(FO)	% (FID) BM_A(LO)
1.	*α*-pinene	941	2.5	1.8	0.4	1.7	0.5	1.8
2.	camphene	954	0.4	1.4	0.2	0.9	0.3	0.5
3.	*β*-pinene	981	0.2	0.9	1.3	t	2.3	1.7
4.	*α*-terpinene	1019	0.6	0.8	0.5	1.1	1.4	-
5.	*p*-cymene	1029	1.3	2.1	1.6	0.1	1.2	0.6
6.	1,8-cineole	1037	1.8	0.9	0.1	0.2	5.7	3.4
7.	linalool	1100	2.9	1.3	-	0.9	-	0.9
8.	isoborneol	1165	1.5	1.2	1.6	-	0.5	1.2
9.	linalyl acetate	1257	0.4	0.7	0.3	0.4	1.2	-
10.	eugenol	1359	-	0.9	t	-	-	0.7
11.	*(E)*-caryophyllene	1418	43.9	39.5	20.4	30.9	25.6	42.4
12.	2,5-dimethoxy *p*-cymene	1428	1.8	2.9	4.7	17.0	5.3	4.9
13.	*α*-humulene	1454	8.3	7.7	5.6	6.4	9.5	1.2
14.	germacrene-D	1481	-	-	21.5	9.6	11.3	10.4
15.	ar-curcumene	1485	-	-	4.6	t	-	-
16.	*α*-zingeberene	1503	-	2.1	3.4	2.5	1.9	-
17.	*δ*-cadinene	1524	14.6	17.0	10.6	5.4	8.7	5.9
18.	*β*-bisabolene	1528	5.4	6.8	5.3	8.3	5.1	2.6
19.	germacrene-D-4-ol	1576	-	-	5.6	3.1	2.1	t
20.	*γ*-asarone	1572	1.2	1.6	0.3	1.5	0.7	t
21.	caryophyllene oxide	1584	7.3	5.2	7.8	4.5	6.6	12.1
22.	*β*-eudesmol	1651	-	-	-	-	4.3	1.6
	Total identified		94.1%	94.8%	95.8%	94.5 %	94.2%	91.9%
	Monoterpene hydrocarbons		5.0%	7.0%	4.0%	3.8%	5.7%	4.6%
	Sesquiterpenehydrcabons		72.2%	73.1%	71.4%	63.1%	62.1%	62.5%
	Oxygenated monotepenes		8.4%	7.9%	6.7%	18.5%	12.7%	11.1%
	Oxygenated sesquitepenes		8.5%	6.8%	13.7%	9.1%	13.7%	13.7%

FO = Flower oil, LO = Leaves oil, BMP = Sample collected from Pithoragarh, BMN = Sample collected from Nainital and BMA = Sample collected from Almora, t = trace < 0.1%



(E)-caryophyllene | germacrene-D | ß-bisabolene

2,5-dimethoxy-*p*-cymene | cadinene | caryophyllene oxide

Major constituents

It is noticeable that the flower and leaves oil of the sample collected from Pithoragarh district of Uttarakhand were fully devoid of germacrene D which was present in the flower and leaf oil of other two samples in a significant amount. The flower oil of the sample collected from Nainital contains maximum amount of 2, 5-dimethoxy-*p*-cymene in comparison with other samples. *β*-eudesmol was only found in the sample collected from Almora which was not present in the samples collected from Pithoragarh and Nainital region even in trace. The amount of the compound 1,8-cineole was higher in the sample collected from Almora in comparison with the flower and leaf oil of the samples collected from Pithoragarh and Nainital.

The sesquiterpene contents in all these samples were much larger in number and quantitative content as compared to the results reported by the earlier workers. Previously linalool, elemene, copaene and estragole were found as major constituents in the essential oil of *B. mollis*[11] but in present investigation *(E)*-caryophyllene (20.4 to 43.9%), caryophyllene oxide (4.5 to 12.1%), germacrene D (9.6 to 21.5%), *(δ)*-cadinene (5.4 to 17.0%) and 2, 5-dimethoxy-*p*-cymene (1.8 to 17.0%) were found as major constituents while copaene and estragole were not detected even in traces in the either of the sample studied under the present investigation.

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