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Aroma Profile of *Majorana hortensis* as Influenced by Harvesting Height in Northern India

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Abstract: An experiment was conducted to observe the influence of harvest cut height on essential oil yield and composition of sweet marjoram (*Majorana hortensis* Moench) cultivated in Kumaon region of western Himalaya. Three different cuts viz. top 1/3; top 2/3 and whole plant were carried out in respective plants. Essential oil yield was highest in the top-1/3 (0.47 %) followed top-2/3 (0.38 %) and whole herb (0.33 %). A total of 35 constituents representing 94.56 % - 98.31 % of the total oils were identified by GC and GC-MS. The major components were (*Z*)-sabinene hydrate (27.09 % - 36.0 %), terpinen-4-ol (17.16 % - 21.65 %), γ-terpinene (5.42 % - 10.71 %), sabinene (7.34 % - 7.89 %), (*E*)-sabinene hydrate (5.79 % - 6.40 %), α-terpinene (4.65 % - 6.66 %), and α-terpineol (3.46 % - 3.88 %). The concentration of oxygenated monoterpenes (63.01 % - 69.79 %) was decrease with the inclusion of lower portion of the plant, whilst its reverse was true for monoterpene hydrocarbons (26.86 % - 33.56 %).

Keywords: *Majorana hortensis*, harvest cut height, essential oil, GC-MS, (*Z*)-sabinene hydrate, terpinen-4-ol

1. Introduction

Majorana hortensis Moench (Syn. *Origanum majorana* L.) commonly known as 'sweet marjoram' is a perennial aromatic herb of family Lamiaceae. It is native to Cyprus and eastern Mediterranean countries [1]. The aerial parts of the plant are used for isolation of essential oil, which has a lot of uses in flavour, perfumery and pharmaceutical industry. The essential oil is employed for external application in bruises, sprain, stiffs and paralytic limbs and toothache and for hot fomentation in acute diarrhea. In food industry, it is mainly used as a spice in sausages, but its use in baked goods, processed vegetables, condiments, soups, snack foods and gravies is also reported [2]. The plant is also reported to possess anticancer [3], antioxidant [4] and antifungal properties [5, 6]. The essential oil composition of marjoram has been investigated by number of workers from different countries [7-15].

The essential oil content and composition of aromatic plants is influenced significantly by several factors and leave position on plant is one of them [16-18]. Consequently, it is imperative to be familiar with the distribution pattern of chemical constituents in plant to get good yield and quality as well. Sweet marjoram is a commerial aromatic crop cultivated in India as well as in other countries for dry herb or essential oil and due to lack of proper scientific knowledge there is no standard height of harvesting the crop resulting in the huge variation of the percentage of major and minor constituents in essential oil. Therefore, in present communication essential oils obtained from top 1/3, top 2/3 and whole plant of sweet marjoram cultivated in Kumaon region of Uttarakhand have been compared for yield and composition.

2. Experimental

2.1. Plant material

Sweet marjoram (*M. hortensis*) crop was raised following normal agricultural practices in the experimental farm of Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Purara, Uttarakhand. The site is located between the coordinates 28^0 60' to 31^0 29' N, 77^0 49' to 80^0 60' E and at a height of 1250 m in Kattyur valley. Climatologically, the site falls in temperate zone of western Himalaya, with the monsoon usually breaking in June and continuing up to September. The harvesting was done in three different modes viz., top third (1/3), top 2/3 and whole herb (5 cm above the ground). The random samples of each kind were collected, pooled with silmilar harvest cuts and used for study.

2.2. Essential oil extraction

Freshly harvested samples immediately subjected to hydro-distillation in a Clevenger apparatus for 3 hrs for extraction of essential oil. The oils were collected, measured, dehydrated by anhydrous sodium sulphate and kept in a cool and dark prior to analysis.

2.3. Gas chromatography analysis (GC-FID)

GC analyses of the oil samples was carried out on a Nucon gas chromatograph model 5765 and Perkin-Elmer Auto XL GC equipped with Flame Ionisation Detector (FID) and two different stationary phases, BP-20 (30 m x 0.25 mm x 0.25 µm film thickness) and PE-5 (50 m x 0.32 mm; 0.25 µm film coating) fused silica capillary columns, respectively. Hydrogen was the carrier gas at 1.0 mL/min.

Temperature programming was done from 70 ^{0}C – 230 ^{0}C at 4 ^{0}C/min (for BP-20) and 100 ^{0}C – 280 ^{0}C at 3 ^{0}C/min (for PE-5). The injector and detector temperatures were 200 ^{0}C and 230 ^{0}C on BP-20 and 220 ^{0}C and 300 ^{0}C on PE-5 column, respectively. The injection volume was 0.02 µL neat and Split ratio was 1: 30.

2.4. Gas chromatography - mass spectrometry (GC-MS)

GC-MS recorded on a Perkin Elmer Auto System XL GC and Turbo Mass Spectrometer fitted with fused silica capillary column, PE-5 (50 m x 0.32 mm, film thickness 0.25 µm). The column temperature was programmed 100^{0}C - 280^{0}C at 3^{0}C /min, using helium as carrier gas at constant pressure of 10 psi. MS conditions were: EI mode 70 eV, ion source temperature 250^{0}C.

2.5. Idenntification of components

The identification was done on the basis of retention time, retention indices, MS Library search (NIST & WILEY), *n*-alkane (C_9-C_{22}) hydrocarbons pattern (Nile, Italy) and by comparing mass spectra with the MS literature data [19, 20]. The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

3. Results and Discussion

The essential oil yield and terpenoids composition were found to vary with respect to harvest cut height in sweet marjoram (Table 1 & Fig 1). The top 1/3 portion of *M. hortensis* produced higher yield of essential oil (0.47 %) as compared to top 2/3 (0.38 %) and whole herb (0.33 %). This could be due to variation in essential contents of upper, middle and lower leaves and basipetal increase of stem percentage. Similar variations have also been reported in other aromatic plants of Lamiaceae [21, 22].

Thirty five compounds comprising up to 98.31 % of the total composition were identified in the volatile oils of *M. hortensis* obtained from different harvest cuts. Although the qualitative composition of the oils was same but there were considerable variation in quantitative compositions. The major components of these oils were (*Z*)-sabinene hydrate, terpinen-4-ol, (*E*)-sabinene hydrate, sabinene, α-terpinene, γ-terpinene and α-terpineol. The amount of (*Z*)-sabinene hydrate , (*E*)-sabinene hydrate, β-caryphyllene, camphor, (*E*)-piperitol and geraniol was found to vary from 27.09 % to 36.00 %, 5.79 % to 6.40 %, 1.26 % to 1.66 %, trace to 0.31 %, 0.17 % to 0.24 % and 0.15 % to 0.65 %, respectively with the maximum in top 1/3 portion. However, the percentage of α-pinene (1.19 % - 1.38 %), sabinene (7.34 % - 7.89 %), 1,8 cineole (0.16 % - 0.67 %) and (*Z*)-*p*-menth-2-en-1-ol (0.48 % - 3.23 %) was found to be higher in top 2/3 portion of marjoram plant. Further, the percentage of terpinen-4-ol, γ-terpinene, α-terpinene, β-myrcene, α-terpineol, linalyl acetate, β-pinene, limonene, α-terpinolene and 1-octen-3-ol is ranged from 17.16 % to 21.65 %, 5.42 % to 10.71 %, 4.65 % to 6.66 %, 1.93 % to 2.29 %, 3.46 % to 3.88 %, 0.98 % - 1.16 %, 0.10 % to 0.13 %, 1.29 % to 1.53 %, 1.67 % to 2.61 % and trace to 0.97 %, respectively in different harvesting mode. The concentration of these compounds was higher in whole herb as compared to top 1/3 and top 2/3 portions. A variability of oil compounds is known to occur in the family Lamiaceae even in different leaf verticals [21, 23]. This knowledge is confirmed by our experiments with sweet marjoram (*M. hortensis*).

TABLE 1. Essential oil yield and composition of *Majorana hortensis* under different methods of harvesting

Compound (%)	RI[a]	RI[b]	A	B	C
α-Pinene	1026	935	1.19	1.38	1.27
Camphene	1065	954	t	t	t
β-Pinene	1105	980	0.12	0.10	0.13
Sabinene	1119	974	7.34	7.89	7.40
Myrcene	1163	989	1.93	2.22	2.29
α-Terpinene	1176	1021	4.65	5.97	6.66
Limonene	1194	1030	1.29	1.42	1.53
1,8 Cineole	1204	1035	0.16	0.67	0.60
(*Z*)-β-Ocimene	1210	1042	t	-	t
γ-Terpinene	1238	1062	7.88	5.42	10.71
(*E*)-β-Ocimene	1244	1047	t	t	t
p-Cymene	1271	1025	0.79	0.47	0.96
α-Terpinolene	1278	1090	1.67	2.36	2.61
3-Octanol	1394	1001	t	t	t
1-Octen-3-ol	1411	978	t	t	0.97
(*E*)-Sabinene hydrate	1463	1068	6.40	6.28	5.79
α-Bourbonene	1525	-	t	t	t
Camphor	1527	1146	0.31	t	t
(*Z*)-Sabinene hydrate	1556	1097	36.00	33.12	27.09
Linalyl acetate	1561	1257	0.98	0.84	1.16
(*E*)-*p*-Menth -2-en-1-ol	1568	1143	t	t	t
Bornyl acetate	1588	1285	t	t	t
β-Caryophyllene	1598	1419	1.66	1.26	1.65
Terpinen-4-ol	1601	1177	18.19	17.16	21.65
4-Terpenenyl acetate	1627	-	0.10	t	t
(*Z*)-*p*-Menth -2-en-1-ol	1638	1124	2.71	3.23	0.48
(*Z*)-β-Terpineol	1641	-	t	0.17	0.10
(*E*)-Sabinyl acetate	1658	-	0.24	0.17	0.23
α-Humulene	1669	1462	-	t	t
(*E*)-Piperitol	1672	1210	0.24	0.17	0.22
α-Terpineol	1702	1188	3.46	3.54	3.88
(*Z*)-Piperitol	1743	1194	0.23	0.21	0.30
Geranyl acetate	1750	1373	0.12	0.36	0.38
Geraniol	1830	1237	0.65	0.15	0.16
Caryophyllene oxide	1995	1584	t	t	t
Class composition					
Momoterpene hydrocarbons			26.86	27.23	33.56
Oxygenated monoterpenes			69.79	66.07	63.01
Sesquiterepene hydrocarbons			1.66	1.26	1.65
Oxygenated sesquiterpenes			t	t	t
Total identified			98.31	94.56	98.22
Essential oil yield (%)			*0.47*	*0.38*	*0.33*

A = Top 1/3; B = Top 2/3; C = Whole herb; [a] Retention indices on polar column (BP-20); [b] Retention indices on non-polar column (PE-5); t=trace (<0.1%)

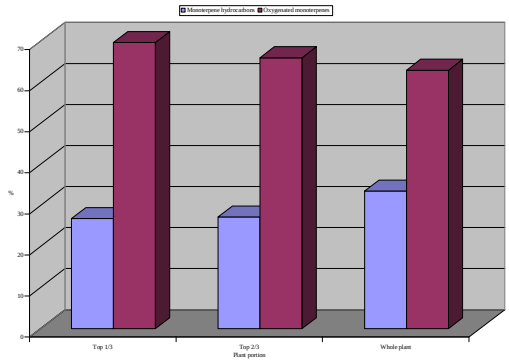

Figure 1. Distribution of terpenoids in different portions of *M. hortensis*

The oxygenated monoterpenes which constituted major portion of the marjoram oil was decreased towards base of the plant, while monoterpene hydrocarbons, the second major group of compounds were found to increase in same direction (Fig. 1). The flavor compounds of marjoram consist mainly of light oxygenated compounds; the reports about this oil always refer to (*Z*)-sabinene hydrate [24, 25]. Therefore, it is concluded that a good yield of high quality essential oil can be obtained from sweet marjoram by harvesting the top 1/3 portion instead of harvesting whole herb which is the common practice of growers in most of countries. Further, harvesting of top 1/3 portion of *M. hortensis* could also facilitate to cultivate it as a multi harvest crop which will produce younger leafy herb rich in oxygenated monoterpenes. Alternatively, upper and lower portions of the plant could be processed separately to get essential oils of two different qualities.

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REFERENCES

1. Ietswarrt J.H., A taxonomic revision of the genus Origanum (Labiatae). Leiden University Press, Leiden, **1980**.
2. Burdock G.A., Fenaroli's handbook of flavour ingredients. 3rd edn Vol.1: Natural flavours, CRC Press, Boca Raton FL, **1995**.
3. Hartweel J.L., *Lioydia*, 32(3), **1969,** 247-296.
4. EI-Ghorab A.H., Mansour A.F. and El-massry K.F., *Flav. Fragr. J.*, 19, **2004**, 54-61.
5. Afifi A.F. and Dowidar A.E., *J. Physiol. Sci.*, 3, **1976**, 81.
6. Pruthi J.S., Spice and condiments: Chemistry, Microbiology, Technology. Academic Press, New York, **1980**.
7. Sarer E., Scheffer J.J.C. and Svedsen A.B., *Planta Med*., 46, **1982**, 236-239.
8. Lawrence B.M., Progress in essential oils. *Perf. Flav*., 4, **1989**, 29-41.
9. Komaitis M.E., Infanti-Papatragianni N. and Melissari-Panagiotou E., *Food Chem*., 45, **1992**, 117-118.
10. Baser K.H.C., Kirimer N. and Tumen G., *J. Essent. Oil Res*., 5, **1993**, 577-579.
11. Vera R.R. and Chan Ming J., *Food Chem*., 66, **1999**, 143-145.
12. Barazandeh M.M., *J. Essent. Oil Res*. 13, **2001**, 76-77.
13. Mishra A.C., Negi K.S., Suneja P. and Maheshwari M.L., *Ind. Perf.*, 48(1), **2004**, 41-45.
14. Verma R.S, Verma R.K, Chauhan A. and Yadav A.K., *J. Serb. Chem. Soc*., 75(4), **2010**, 441-447.
15. Verma R.S., Sashidhara K.V., Yadav A. and Naqvi A.A., *Acta Pharma. Sci*., 52(1), **2010**, 19-22.
16. Adzet T., Ponz R., Wolf E. and Schulte E., *Planta Med*., 58, **1992**, 562-564.
17. Rao B.R.R., Bhattacharya A.K., Kaul P.N., Chand S. and Ramesh S., *J. Essent. Oil Res.* 5, **1993**, 301-304.
18. Hose S., Zanglein A., Van den Berg T., Schultze W., Kubeczka K.H. and Czygan F.C., *Pharmazie*, 52, **1997**, 247-53.
19. Davies N.W., *J. Chromatogr. A*, 503, **1990**, 1-24.
20. Adams R.P., Identification of essential oil components by Gas Chromatograph/Quadrupole Mass Spectrometery. Allured Publishing Corp, Carol Stream, IL, **2001**.
21. Mrlianova M., Tekelova D., Felklova M., Reinohl V. and Toth J., *Planta Med*. 68, **2002**, 178-180.
22. Kurkin V.A., Kurkina T.V., Zapesochnaya G.G., Avdeeva E.V., bogolyubova Z.V., Vandychev V.V. and Tchikina I.Y., *Khimiya Prirodnykh Soedinenii*, 2, **1995**, 318-20.
23. Felkolva M. and Marlianova M., *Ceskoslovenska Farmacie*, 33, **1984,** 149-155.
24. Nitz S., Kollmannsberger H. and M. Punkert, *Chem. Mikrobiol. Technol. Lebensm*, 14, **1992**, 108-116.
25. Franz C. and Novak J., In: Padulosi S. (ed) Proc. IPGRI Int. Workshop on Oregano. CIHEAM, 8-12 May, Valenzano (Bari), Italy, **1996**, pp 49-56.

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