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Research Article

SEASONAL VARIATION IN THE ESSENTIAL OIL COMPOSITION OF *MAJORANA HORTENSIS* MOENCH FROM WESTERN GHATS REGION OF SOUTH INDIA.

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ABSTRACT

Objective: To evaluate the seasonal variation in the composition of essential oil of *Majorana hortensis* Moench from Western Ghats region of South India during summer and winter seasons and to find out the chemo type of oil.

Method: The essential oil from the plant is extracted by hydro distillation method using Clevenger type apparatus. The compounds are analysed by GC-MS method and identified by NIST library verification.

Results: Winter season oil contains trans-sabinene hydrate along with other constituents in considerable quantity whereas in summer season oil, terpinen-4-ol is the predominant constituent.

Conclusion: Winter season is suitable for oil production in Western Ghats region of South India. The winter seasonal oil belongs to sabinene hydrate/terpinen-4-ol chemo type which is more fragrant than summer seasonal oil. On comparison with the literature datas, it has been found that the chemo type of the oil is similar to that of Egyptian oil in both summer and winter seasons.

Keywords: : *Majorana hortensis* Moench, GC-MS, Trans-Sabinene hydrate, Terpene-4-ol.

INTRODUCTION

Phytotherapy is one of the oldest practices in the medicinal world. The medicinal plants contain wide range of phyto constituents which are used to treat chronic as well as infectious diseases [1]. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha[2]. Aromatic plants represent a renewable source of flavouring substances which can be used in the food and pharmaceutical industries. The pharmaceutical characteristics of aromatic plants are contributed by the essential oil at the major level. The family Lamiaceae includes large number of volatile oil plants and one of the most important plants is Marjoram [3]. The plant *Majorana hortensis* Moench is an evergreen aromatic herb. It is very much recognised by its common name-'Sweet Marjoram' [4-6]. The genus Origanum houses around 900 different species and many species are extensively used for the flavouring of alcoholic beverages, food products and in perfumery owing to their spicy fragrance [7]. Marjoram was popular as a medicine and a culinary herb in England during 16th century. It is native to Cyprus and Eastern Mediterranean and now it is grown in central Hungary, USA, Western Asia, South and North America. Morocco, China and India.

For many years both Marjoram and Oregano were known as Origanum Majorana. Today Marjoram is identified as *Majorana Hortensis* Moench –a member of mint family [8]. The leaves are light greyish, green and oblate to broadly elliptical reaching 21mm in length [9]. The essential oil is very strong and of very pleasant fragrance. The high percentage of oil is found in the leaves, whereas only traces are found on flowers and stalks. Long periods of blooming encourage the accumulation of oil in seeds [10]. The leaves have traditional usage in curing many diseases like diabetes, insomnia, asthma and nervousness [11]. The essential oil has many uses in food, perfumery and pharmaceutical Industries. In food industry, it is mainly used as a spice in sausages, baked foods, processed vegetables, condiments, soups and gravies[12].In addition to this, Marjoram is well known for its medicinal[13], insecticidal[14], antifungal[15], anti-cancer[16] and anti- oxidant properties[17].Even in holy Qura'n, its importance in medicinal field is specified[18].

The essential oil analysis of Marjoram has been investigated by many researchers from various countries. But the investigation of essential oil composition was limited in India[19]. It was investigated from midhills of Northern India[19], Kumaeon region of Himalaya[20,21,22] and South India[23]. However an elaborate research is yet to be done in Western Ghats region of South India [24]. Western Ghats is richly credited with varied kind of vegetation and unimaginable topographical features. The heavy precipitation from southwest monsoon and edapic factors favour to luxuriant growth of plant life. The Western Ghats, Anaimalai sub-cluster including the Anaimalai hills is under consideration by the UNESCO World heritage committee for the selection as a World heritage site. The review of literature shows that a detailed analysis is needed in evaluating the changes in composition of essential oil during seasonal variation and chemo type of oil of Marjoram obtained from Western Ghats region of South India [24].

The essential oil composition is the most important characteristics in determining the economic value of Marjoram. The essential oil content and its composition of aromatic plant are influenced by several factors like plant ontogeny, origin, nutritional relationships, plant density, moisture, salinity, harvesting methods, climate and seasonal variation. The origin and climatic factors are important among them. Based on that, the present work is aimed to evaluate the composition variation in the essential oil obtained during summer and winter seasons. The reports are also compared with that of oils obtained from North India, and other countries [25-27].

Methods and Materials

Plant material and essential oil Extraction:

The fresh leaves of *M.hortensis* Moench were collected from Anaimalai hills region of Western Ghats, South India during the month of January which is the winter season in that area. The authentication of the plant was done by Botany department, Karpagam University, Coimbatore and voucher specimen preservation was done there with specimen number: 10CH004.The fresh aerial part was subjected to hydro distillation using Clevenger type apparatus for 3h. After decanting and drying over anhydrous sodium sulphate, slight yellowish oil was recovered with a yield of 0.76 %(v/w).

GC-MS analysis

GC-MS analysis was done using Agilent 6890N GC instrument with 5975 inert MSD (E.I Quadra pole) equipped with DB-5 capillary column (30mmX0.25mmwith the thickness 250microns). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. The carrier gas is Helium which is supplied at a flow rate of 1.0 ml /min. The injector and MS transfer line temperature were set at 250°C &280°C respectively. The programme used was 90-300°C at a rate of 5°C/min, held isothermal for 4 minutes. Accurately100 µl sample was dissolved in 1ml of hexane and 1µl of this sample was injected manually in the split less mode. The identification of compounds were based on the comparison of their relative retention times & mass spectra with those of standard NIST library version 2.0and literature data. The relative amounts of the individual components were calculated based on GC peak areas without using correction factors.

RESULTS

The composition of the essential oil obtained during the winter season is shown in table:1.

Table: 1 Composition of essential oil obtained during winter season

S.No	Compound	Kovat index	% Quantity
1.	α-Terpinene	998	5.75
2.	Terpenoline	1052	2.387
3.	γ-terpinene	998	13.203
4.	O-cymine	1042	3.732
5.	D-Limonene	1018	3.363
6.	t-Sabinene Hydrate	1014	20.308
7.	1,4-Isopropyl-1-methyl 2-cyclo hexan-1-ol	1109	2.183
8	Terpinen-4-ol	1137	32.647
9.	α-Terpineol	1143	5.432
10.	Linalool acetate	1272	2.949
11.	2,3-Epoxy carane-E	961	1.606
12.	Cis-P-menth-2,8-dien-ol	1140	0.619
13.	β-Caryophyllene	1494	3.143
14.	1,6-(Hydroxy methyl)-1,4,4-trimethyl bicycle-[3.1.0]hexan-2-ol	1322	0.964
15.	Elixene	1431	1.917
16.	(-) Spathulenol	1536	0.342
17.	Caryophyllene oxide	1507	0.642
18.	7-Tetra Cyclo-6.2.1.0(3.8)0(3.9) Undecanol4,4,11,11-tetra methyl	1385	0.453
	Yield of the oil (v/w)		0.76%

The table: 2 revealed the comparison of seasonal variation in composition changes of essential oil of south Indian Marjoram and Egyptian Marjoram [25] since the Egypt was one of the country producing Marjoram oil in large scale commercially. Comparison of essential oil composition was also done with that of oil obtained from other parts of India and it was shown in table: 3.Comparison of essential oil composition obtained in the present study was compared with that of oil obtained from other countries like Albania, Egypt, Germany and Turkey where Marjoram oil is produced commercially. It was tabulated in table:4.

Table 2: Comparison of essential oil composition of Marjoram of South India and that of Egypt obtained during summer and winter seasons

Class	% Composition of Marjoram oil			
	Egypt[25]		South India (our results)	
	Summer	Winter	Summer	Winter
Mono Terpenes	45.6	16.1	23.48	37.97
Sesqui-Terpenes	4.7	1.4	3.21	6.42
Oxygenated mono terpenes	49.1	81.7	62.19	50.86
Oxygenated Sesqui terpenes	--	--	0.64	--
Yield of the oil	2.5%	2.8%	0.25%	0.76%

Table 3: Comparison of Marjoram oil composition of Western ghats region with other countries.

Compound	% of the constituents in various countries					
	Western ghats region (our results)	Albania[27]	Egypt-Sinai peninsula desert[14]	Egypt-Giza[25]	Germany[27]	Turkey[26]
α- Terpinene	5.75	8.88	6.77	2.8	4.9	1.0-1.3
γ- Terpinene	13.203	14.0	11.34	5.3	8.3	3.7-4.8
Terpinene-4-ol	32.647	21.33	29.96	16.3	16.4	0.2-03
α- Terpineol	5.432	3.26	0.54	4.1	3.8	0.6-1.4
Linalool	-	1.35	-		3.3	0.2-0.7
Sabinene	-	8.27	3.87		5.3	0.03-0.04
Trans-Sabinene hydate	20.308	15.53	10.81	5.6	13.8	2.0
Cis-Sabinene hydrate	-	3.8	-	54.4	-	-
p-cymene	3.732	1.29	2.61	-	2.2	4.3-4.7
Limonene	3.363	2.03	-	-	-	0.2-0.3
Terpenoline	2.387	3.2	2.81	0.3	-	-
β-Caryophyllene	3.143	2.28	1.72	0.9	-	-
Cis-p-menth-2,8,dienol	0.619	-	-	-	-	-
Cis-P-menthen-1-ol	-	-	1.87	-	-	-

Trans-P-menthen-1-ol	-	-	-	-	-	-
P-Menth-2-en-1-ol	-	2.3	-	0.5	-	-
Spauleneol	0.342	-	0.78	-	-	-
Caryophyllene oxide	0.642	0.06	-	-	-	-
Veridiflorene	1.943	-	-	-	-	-
Veridiflorol	-	-	0.19	-	-	-
α-Thujene	-	-	1.08	-	-	-
α-Myrecene	-	2.13	0.98	-	-	-
α-Phellandrene	-	0.42	0.98	-	-	-
Trans-Piperitol	-	0.46	0.52	-	-	-
Nerol	-	--	0.20	-	-	-
Linalyl acetate	-	2.23	0.17	0.9	-	-
Trans-Sabinene hydrate acetate	-	0.08	1.11	-	-	-
Thymol	-	-	0.14	-	-	-
δ-elemene	-	-	0.13	-	-	-
Neryl acetate	-	-	0.19	-	-	-
Geranyl acetate	-	-	0.26	-	-	`-`
α-trans-Bergamotene	-	-	0.12	-	-	-
α-Cadinol	-	-	0.16	-	-	-
Globulol	-	-	0.11	-	-	-

Table 4: Essential oil composition of Marjoram from different parts of India.

Compound	% composition of Marjoram oil		
	Western Ghats region, south India (Our Results)	Lower region of Kumaon Himalaya,Uttrakand[20]	Mid hills of North India[20]
α-Terpinene	5.75	1.09	2.67
Terpenoline	2.387	0.85	
Gamma-terpinene	13.203	3.43	6.37
O-cymine	3.732	-	-
p-Cymine	-	5.15	5.83
D-Limonene	3.363	1.30	-
Trans-Sabinene hydrate	20.308	9.16	3.63
Cis-Sabinene hydrate	-	30.81	16.86
1,4-Isopropyl-1-methyl 2-cyclo hexan-1-ol	2.183	-	-
Terpinen4-ol	32.647	22.02	30.55
α-Terpineol	5.432	3.56	3.71
Linalool acetate	2.949	-	-
2,3-Epoxy carane-E	1.606	-	-
4-Terpineolacetate	0.619	-	-
Cis-P-menth-2,8-dien-ol	0.619	-	-
β-Caryophyllene	3.143	1.41	-
1,6-(Hydroxy methyl)-1,4,4-trimethyl bicycle-[3.1.0]hexan-2-ol	14.646	-	-
Elixene	1.917	-	-
(-) Spathulenol	0.342	0.56	-
Caryophyllene oxide	0.642	0.30	-
7-Tetra Cyclo-6.2.1.0(3.8)0(3.9) Undecanol4,4,11,11-tetra methyl	16.898	-	-
Terpinen-4-yl acetate	0.197	0.26	-
α-Thujene	-	0.31	-
α-Pinene	-	0.56	0.53
β-Pinene	-	0.08	-
β-Myrcene	-	1.77	-
Terpinen-4yl acetate	-	0.26	-

The GC-Ms spectrum obtained in the present study which is winter season oil and that of previous study, obtained in summer season are shown in fig1&2 respectively.

DISCUSSION

Nineteen constituents were identified and the predominant constituents were found to be 4-terpineol (32.647%), t-sabinene hydrate (20.308%) which contribute almost 50% of the oil composition. The composition of the oil obtained during the summer season was reported in earlier study [24] in which the presence of sabinene hydrate was not up to the detectable quantity. The major constituents of the oil obtained during summer season were4-terpineol (44.337), Gamma-terpinene (20.907%), α-Terpinene (6.542%) and α-Terpineol (6.527%)

The t-sabinene hydrate, limonene, p-cymene, Elixene were in considerable amount in winter seasonal oil whereas they were negligible in oil obtained during summer period.

The oxygenated monoterpenes (62.19%)in the oil were predominant in summer season. The monoterpenes (37.97%) and sesqui terpenes (6.423%) were in major proportion in oil obtained in winter season.

Trans sabinene hydrate and terpinen-4-ol were the major components responsible for the characteristics flavour and fragrance [28], and they were present in the oil obtained in winter season. Two chemo types of Marjoram were reported–Terpinen-4-ol/sabinene hydrate rich type and thymol/carvacrol –rich type[29,30]. Our results had showed that South Indian Marjoram oil

belonged to terpinen-4-ol/sabinene chemo type which has more characteristic flavour and fragrance.

On comparison it had revealed that in Indian oil sesqui terpenes and oxygenated mono terpenes were present in large scale in both summer and winter season, whereas in Egyptian oil, they were predominant in winter season. The oil composition obtained in the present study was compared with the Marjoram oil obtained from other countries and other parts of India.

From the Table:3 which summarized the important constituents of Marjoram essential oil obtained from different countries, It was found that our oil composition was matching with the composition of Egyptian oil and Albanian oil. The compositions of Marjoram oils obtained from different part of India were tabulated in table: 4. α – Pinene, p-cymene and cis-sabinene hydrate are absent. The oil is richer in t-sabinene hydrate and α and γ –terpinene when compared with the oil obtained from the other regions of India. It exhibited that Indian oil belongs to sabinene hydrate/terpineol rich chemo type.

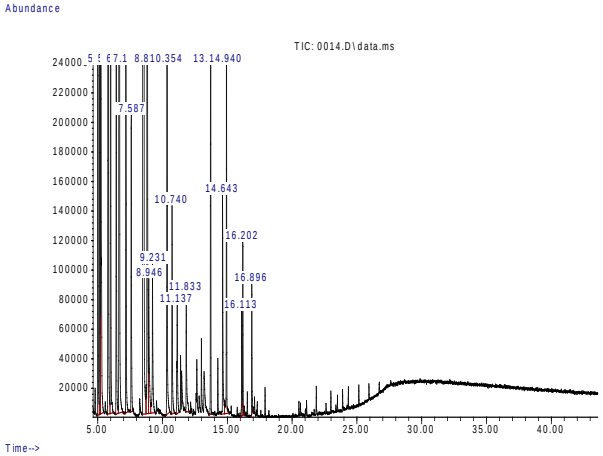

Fig: 1: GC.-MS chromatogram of essential oil of Marjoram in winter season.

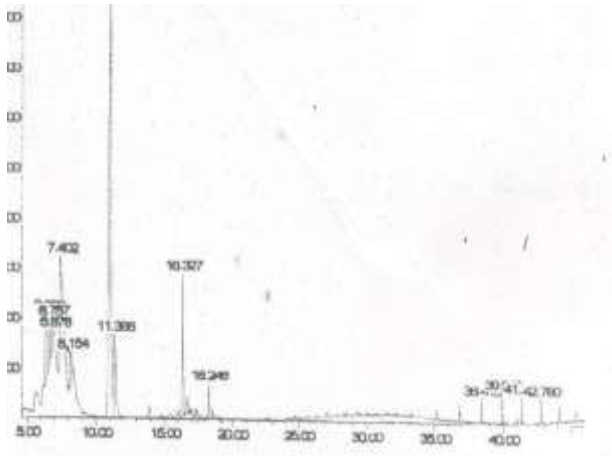

Fig. 2: GC.-MS chromatogram of essential oil of Marjoram in summer season.

CONCLUSION

The present study reveals that oil displays terpinen-4-ol/sabinene hydrate chemo type during winter season with mono-terpene alcohols 37.972% (Table-1). It is concluded that the winter season is the suitable climate for the cultivation of Marjoram in the Western Ghats region of South India to attain higher yield in oil production with unique flavour and fragrance.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest regarding the publication of this paper.

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