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ANALYSIS OF ESSENTIAL OIL OF *ORIGANUM VULGARE* LINN. BY GC AND GC-MS

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ABSTRACT

The aim of present study to analyze the essential oil components of whole plant of *Origanum vulgare* Linn. (Lamiaceae) by gas chromatography and gas chromatography/mass spectrometry (GC/MS). The essential oil obtained by hydrodistillation method from the whole plant of *O. vulgare* and analyzed by GC and GC-MS method. The individual constituents were identified by GC and GC-MS analysis by comparison with spectrometer database of NBS 54 K.L, WILEY8 libraries and published literature. The yield of the essential oil was 0.45 % (v/w). Sixty-seven compounds representing 99.03 % of the oil were characterized. The volatile oil was found rich in monoterpenes (69 %) and sesquiterpenes (23.42 %). Carvacrol acetate (66.01 %) was predominant in monoterpenes identified in essential oil of *O. vulgare*. Diterpenes and aliphatic components are also present in essential oil of Oregano in lesser quantity. The current study investigated the composition of essential oil of *O. vulgare* for making foundation for quality control and clinical treatments.

INTRODUCTION

The small genus *Origanum*, (Lamiaceae), is an annual, perennial, and shrubby herb that is native to the Mediterranean, Euro-Siberian, and Irano-Siberian regions.[1] *Oregano* is the common name for the aroma and taste that comes primarily from more than 60 species of plants used worldwide as a spice. *Oregano* characterized by the presence of glandular trichomes covering the aerial organs. The glandular trichomes secrete essential oils with a unique flavor, which is mainly due to its major compounds such as carvacrol acetate and α-farnesene.[2] *Origanum vulgare* L. (Figure 1) is a perennial herbaceous plant belonging to the family Lamiaceae. It is widely known as a very versatile plant with many therapeutic properties such as diaphoretic, carminative, antispasmodic,

antiseptic, tonic and being applied in traditional medicine systems in many countries [3]. It has been widely used in agricultural, and perfumery for its spicy fragrance.[4] The aim of this paper is to identify the chemical constituents of the essential oil of whole plant of *O. vulgare* by GC and GC-MS analysis.

Figure 1: Aerial parts of *Origanum vulgare*

MATERIAL AND METHODS

Plant material and authentication: Fresh whole plant of *Origanum vulgare* Linn. was purchased from Samsi Dawakhana, Ballimaran, Delhi and authenticated by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Voucher specimen of drug was deposited in the Raw Materials Herbarium and Museum, NISCAIR, New Delhi, with reference number Ref. NISCAIR/RHMD/consult/-2010-11/1705/05.

Essential oil isolation: The fresh undamaged *O. vulgare* whole plant (flowers, stems, and leaves) 500 g were submitted to hydro-distillation [5] in a Clevenger-type apparatus for 6 h. At the end of distillation, the oil was collected, dried with anhydrous sodium sulfate (Na_2SO_4) prior to analyses, measured, and transferred to glass vials and stored at 4°C.

GC Analysis: The gas chromatographic analysis of the volatile oils was carried out on Shimadzu 2010 Gas Chromatograph (Japan) equipped with a flame ionization detector (FID) and AB-Innowax 7031428 WCOT fused capillary column (60 m x 0.25 mm x 0.25 µm). The injector and detector (FID) temperatures were maintain at 250 and 270 ºC, respectively. The carrier gas used was nitrogen at a flow rate of 1.21 ml/min with column pressure of 155.1 kPa. The sample (0.2 µl) was injected into the column with a split ratio of 80:1. Component separation was achieved following a linear temperature programmed from 60-230 ºC at a rate of 3 ºC/min and then held at 230 ºC for 9 min, with a total run time of 55.14 min. Percentage of the constituents were calculated by electronic integration of FID peak areas.

GC-MS Analysis: The analysis of the volatile constituent was run on a Shimadzu QP-2010 GC-MS system equipped with AB-Innowax 7031428 WCOT column (60 m x 0.25 mm x 0.25 µm) directly coupled to the MS. The carrier gas was helium with a flow rate of 1.21 ml/min. Oven temperature was programmed as 50 ºC for 1 min and subsequently held isothermal for 2 min. injector port: 250 ºC, detector: 280 ºC, split ratio 1:50, volume injected: 1 µl of the oil. The recording was performed at 70 eV, scan time 1.5 sec; mass range 40-750 amu. Software adopted to handle mass spectra and chromatograph was a Chem station.

Identification: The individual peaks/constituents were identified by gas chromatography by comparison of their retention indices (R.I.) either with those of authentic compounds available in author's laboratory or with those of literature in close agreement to R.I. Further identification was made by comparison of fragmentation pattern of mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K.L, WILEY8 libraries and published literature.[6-12] Retention indices of the components were determined relative to the retention times of a series of *n*-alkanes

relative to C_9-C_{20} on HPS and HP-20M columns.

RESULTS AND DISCUSSION

O. vulgare is an important aromatic and medicinal plant. It is used as a culinary condiment and largely employed as antimicrobial, antifungal, antioxidant, antibacterial, anti-mutagenic, cytotoxic and anticancer agent. *O. vulgare* gave light brown colour volatile oil with 0.45 % (v/w) yield. Volatile oil of *O. vulgare* was characterised (Table 1) by GC and GC-MS methods (Figure 2, 3). The results of GC-MS analysis showed the presence of larger amount of monoterpenes (69 %), sesquiterpenes (23.42 %) and lesser amount of diterpenes (2.34 %) and aliphatic components. Among fifteen monoterpenes (69 %), eleven were monoterpene hydrocarbons (0.88 %), two monoterpene alcohols (2.05 %), one each monoterpene oxide (0.06 %) and one monoterpene ester (66.01 %). The monoterpene ester, carvacrol acetate (66.01 %) was predominant in monoterpenes. The monoterpene hydrocarbons were consisted of *α*-pinene (0.05 %), sabinene (0.12 %), *β*-pinene (0.06 %), *β*-myrtene (0.16 %), limonene (0.24 %), *γ*-terpinene (0.04 %), *β*-ocimene (0.04 %), *cis*-sabinene hydrate, terpineolene, *β*-elemene. The major monoterpene alcohol was found to be 1,8-cineole (1.56 %) and other was linalool (0.49 %). Among thirty-four sesquiterpenes there were nine sesquiterpene hydrocarbons (13.46 %), twenty alcohols (8.4 %), two sesquiterpene ketones (0.31 %) and one each oxide (1.02 %), epoxide (0.09 %) and sesquiterpene aldehyde (0.14 %). The dominant sesquiterpene hydrocarbon was found *α*-farnesene (11.62 %). The sesquiterpene hydrocarbons were caryophyllene (0.39 %), *β*-gurjulene (0.11 %), (*E*)-*β*-farnesene (0.29 %), germacrene D (0.44 %), germacrene B (0.12 %), *β*-calacorene (0.25 %), epi-*α*-selinene (0.74 %) and isoledene (0.28 %). There were two sesquiterpene ketones namely *α*-atlantone (0.22 %) and farnesyl acetone A (0.09 %). There were one each sesquiterpene oxide, epoxide and aldehyde, caryophyllene oxide (1.02 %), *α*-cedrane epoxide (0.09 %) and viridifloral (0.14 %). Among three diterpenes (2.34 %), there were diterpene hydrocarbon, *R*-(-)-cambrene; diterpene alcohol, phytol (2.06 %); and diterpene oxide, *epi*-13-manoyl oxide (0.22 %); respectively. Among fifteen aliphatic components, five were aliphatic hydrocarbons (0.93 %), four aliphatic alcohols (1.82 %) and two each aliphatic ketone (0.26 %), aliphatic acids (0.36 %) and aliphatic esters (1.69 %). The aliphatic hydrocarbons were consisting of heptadecane (0.37 %), 9-octadecane (0.20 %), *n*-eicosane (0.10 %), *n*-heneicosane (0.12 %) and dehydronaphthalene (0.14 %). The aliphatic alcohols were consisting of tetradecanol (1.35 %), *β*-ionol (0.06 %), pentadecanol (0.19 %) and *n*-octanol (0.22 %). The aliphatic ketones were namely 2-pentadecanone (1.76 %), nonan-2-one (0.04 %), aliphatic acids, *n*-tetradecanoic acid (0.23 %) and palmitic acid (0.13 %) and aliphatic esters, ethyl hexadecanoate (1.59 %) and ethyl palmitate (0.10 %).

CONCLUSION

In this study we do not find thymol as reported by earlier researchers.[1] In the present study carvacrol acetate and *α*-farnesene were found as major constituents of essential oil of *O. vulgare*. Variation in the composition of essential oils depends on their genetic variations, geography, time of collection, stages of plant growth and seasonal and environmental factors. Variations in the traded herbal composition occurs on account of geo-climatic conditions of their growth, maturity at the time of collection, species variation at times, substitutability on the basis of perceived efficacy and dubious trade practices.[13]

Table 1: Chemical constituents of essential oil of *O. vulgare*

S. No.	Components	Kovats index	Percent (%)
1	*α*-pinene	939	0.05
2	Sabinene	973	0.12
3	*β*-pinene	980	0.06
4	*β*-myrtene	991	0.16
5	Limonene	1030	0.26
6	1,8-cineol	1031	1.56
7	*γ*-terpinene	1037	0.04
8	*β*-ocimene	1040	0.04
9	*cis*-Sabinene hydrate	1044	0.05
10	*cis*-Linalool oxide	1047	0.06
11	Terpinolene	1067	0.03
12	*p*-Cymen-8-ol	1070	0.03
13	*n*-Octanol	1072	0.22
14	Nonan-2-one	1092	0.04
15	Dehydronaphthalene	1094	0.14
16	Linalool	1098	0.49
17	*β*-elemene	1391	0.04
18	Caryophyllene	1405	0.39
19	*β*-Gurjunene	1413	0.11
20	(*E*)-*β*-Farnesene	1456	0.29
21	Germacrene D	1480	0.44
22	Cubenol	1498	0.22
23	*δ*-Cadinene	1501	0.45
24	Elemol	1524	0.37
25	Ledol	1544	0.16
26	Caryophyllene oxide	1550	1.02
27	Germacrene B	1556	0.12
28	*β*-Calacorene	1560	0.25
29	Nerolidol	1562	0.18
30	*Epi*-*α*-Selinene	1563	0.74
31	Palustrol	1565	0.37
32	(-)-Spathulenol	1580	0.72
33	Carvacrol acetate	1582	66.01 %
34	*α*-Farnesene	1584	11.62
35	Viridifloral	1590	0.14
36	*β*-Eudesmol	1630	0.27
37	Isospathulenol	1634	1.44
38	*t*-Cadinol	1640	0.23
39	*t*-Muurolol	1642	0.21
40	*α*-Eudesmol	1650	0.25
41	Caryophylla-4,8-dien-5-ol	1661	0.51
42	Cadinadienol	1672	0.55
43	*β*-Bisabolol	1674	0.30
44	Tetradecanol	1675	1.35
45	Eudesma-3,5-dien-1-ol	1689	0.23

46	2-Pentadecanone	1697	1.76
47	(2*Z*,6*E*)-Farnesol	1722	0.20
48	Heptadecane	1746	0.37
49	Isoledene	1748	0.28
50	*n*-Tetradecanoic acid	1751	0.23
51	*β*-Bisabolen-12-ol	1760	1.24
52	*α*-Atlantone	1773	0.22
53	Dehydrovomifoliol	1828	0.36
54	9-Octadecanal	1831	0.20
55	Vomifoliol	1838	0.14
56	*α*-Cedrane epoxide	1941	0.09
57	*R*(-)-Cembrene	1945	0.06
58	Methyl palmitate	1950	0.10
59	*β*-Ionol	1953	0.06
60	Palmitic acid	1960	0.13
61	Ethyl hexadecanoate	1969	1.59
62	*n*-Eicosane	1993	0.10
63	Farnesyl acetone A	2009	0.09
64	*Epi*-13-Manoyl oxide	2018	0.22
65	*n*-Heneicosane	2102	0.12
66	Tetradecanol	2105	0.19
67	Phytol	2111	2.03

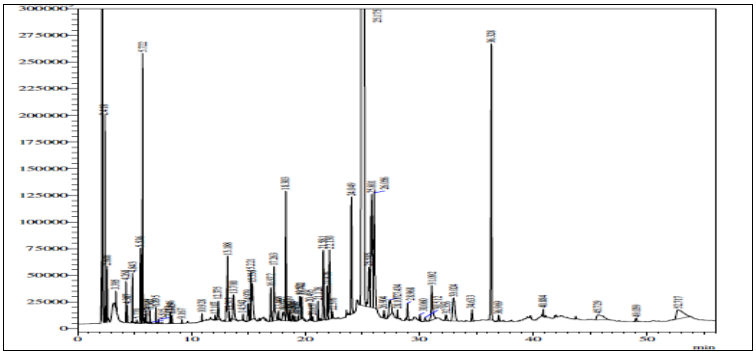

Figure 2: GC spectrum of *O. vulgare* essential oil

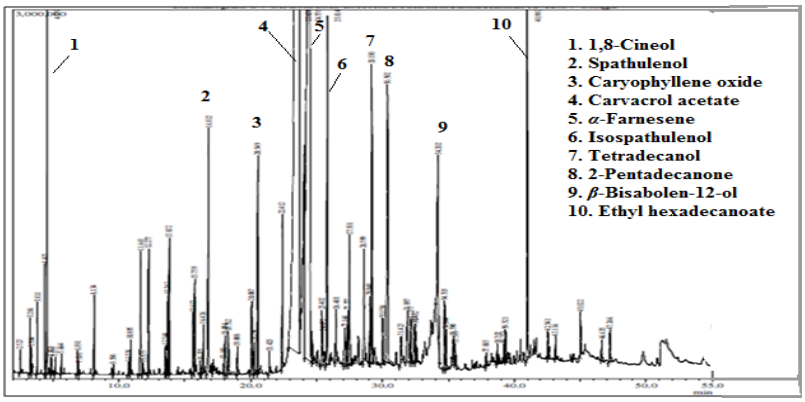

Figure 3: GC-MS spectrum of *O. vulgare* essential oil

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