Asian Journal of Chemistry; Vol. 34, No. 11 (2022), 2849-2853

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2022.23821

Chemical Composition and Biological Activities of Essential Oil of *Elsholtzia ciliata* obtained at Central Highlands of Vietnam's Two Seasons

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Received: 8 March 2022; *Accepted*: 9 July 2022; *Published online*: 19 October 2022; AJC-20996

From the essential oil of the whole plant from *Elsholtzia ciliata*, obtained by hydrodistillation and then using gas chromatography/mass spectrometry to analyze and identify 42 constituents, carveol (26.89%) and *z*-citral (29.35%) main contents (collected in the dry season time); and 46 constituents, *cis*-ocimene (17.95%), carveol (14.84%), *Z*-citral (17.01%), isocaryophyllene (13.19%) and *trans*-caryophyllene (9.76%) as main contents (collected in the rainy season time). The major essential oils in dry season exhibited good inhibition activity on the *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans* with MIC value of 200 µg/mL; exhibited weak cytotoxic activity on the PC-3 line (IC_{50} 66.34 µg/mL) and A549 line (IC_{50} 93.11 µg/mL). The essential oils in rainy season exhibited good inhibition activity on the *Escherichia coli*, *Bacillus subtillis* and *Saccharomyces cerevisiae* with MIC value of 200 µg/mL and *Candida albicans* with MIC value of 100 µg/mL; displayed good cytotoxic activity on the PC-3 line (IC_{50} 30.62 µg/mL) and Hep-G2 line (IC_{50} 22.78 µg/mL), however have weak cytotoxic activity on the A549 line (IC_{50} 85.35 µg/mL).

Keywords: *Elsholtzia ciliata*, Essential oil, Antimicrobial activity, Anticancer activity.

INTRODUCTION

Elsholtzia Willd. genus is one of the major essential oil bearing genera of Lamiaceae family. Around the world, there are more than 40 species of plants of this genus [1]. According to Ho *et al.* [2] and Ban *et al.* [3], seven species of *Elsholtzia* genus *viz*. *Elsholtzia blanda*, *Elsholtzia communis*, *Elsholtzia penduliflora*, *Elsholtzia pilosa*, *Elsholtzia rugulosa*, *Elsholtzia winitiana* and *Elsholtzia ciliata*) are the flora of Vietnam. It is maily used in cooking and considered as a valuable medicinal ingredient [2,3].

Elsholtzia ciliata (Thumberg) Hylander, also known as the Presidency, Presence or wisdom Marjoram. It is a kind of herb, widely distributed in throughout Vietnam, Japan, China, Korea, European and American countries [4]. Its leaves and stems have long been used in folk medicine to treat diseases *e.g*. colds, fever, flu, headache, dizziness, rheumatism, bone pain, body aches, sore throat, vomiting, measles, itchy sores, boils; treatment of bleeding, bleeding, vomiting blood, nosebleeds, bloody urination, leprosy, *etc*. [4,5].

Fresh *Elsholtzia ciliata* contains 0.3-0.6% essential oil, mainly in leaves and flowers [4]. Korolyuk *et al.* [6] isolated four essential oils *viz*. perillene (2.1-3.9%), *Elsholtzia* ketone (3.3-19.3%), α-dehydro-*Elsholtzi* one (2.0-5.7%), dehydro-*Elsholtzia* ketone (66.1-72.4%) and humulene (1.5-3.8%) from *Elsholtzia ciliata*. Similarly, Pudziuvelyte *et al.* [1] isolated 48 components using dynamic headspace solid phase micro extraction, where the major components were dehydro-*Elsholtzia* (24.94%) and *Elsholtzia* (71.34%). Essential oils showed the antiproliferative activity on three tested cancer cell

lines (human glioblastoma, pancreatic cancer and triple negative breast cancer). The EC_{50} values of essential oil against those cells were in the range of 0.017-0.021%.

Nhan & Huyen [7] identified 26 constituents, which represent 97.5% of the total amount. The main constituents of essential oils were geranial (28.4%), β-*cis*-ocimene (23.0%) and neral (21.7%). The essential oils isolated from the leaves of *Elsholtzia ciliata* exhibited the effective antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The essential oils obtained from the cultivated *Elsholtzia ciliata* from Vinh City and Ho Chi Minh City of Vietnam contain 30 components as analyzed by Dung *et al.* [8]. The main constituents were neral (15.2-20.5%), geranial (19.5-26.5%), limonene (10.9-14.2%) and (*Z*)-β-farnesene (10.8-11.7%).

The difference in chemical compositions and biological activities may be attributed due to the different seasons and/or global climate change conditions of the grown flora [9]. Thus, in this study, we aimed to determine and compare the chemical constituents, antimicrobial and anticancer activities of the essential oils obtained from the whole plant *Elsholtzia ciliata* collected in Buon Ma Thuot city, Dak Lak province, Central highlands of Vietnam's in two different seasons.

EXPERIMENTAL

The plant wisdom Marjoram [*Elsholtzia ciliata* (Thumberg) Hylander] was collected from Dak Lak province, Vietnam during the month of January and July 2021. The samples were scientifically identified by Dr. Nguyen Quoc Binh and the specimen was depoisted at the Department of Chemistry, Tay Nguyen University. The chemicals used were of the highest purities and procured from the reputed commerical sources.

Distillation method: Placed 150 g marjoram leaves into the flask of the Clevenger distillation system containing 550 mL distilled water and then heated system using electric stove. When the mixture boils, the essential oils was collected into the condenser system. After condensing, extract the essential oil with diethyl ether, dried the extracted solution using anhydrous sodium sulphate.

GC-MS analysis: The chemical composition of essential oils was determined by means of gas chromatography-mass spectrometry (GC-MS) using Thermo Trace GC Ultra-ITQ900 GC/MS. TG-SQC chromatographic column with length 30 m, inner diameter (ID) = 0.25 mm, thin film 0.25 µm. He carrier gas, sample injection chamber temperature (Temperature Program Technique-PTV): 250 ºC, detector temperature: 260 ºC. Thermostat chamber temperature program: 60 ºC (2 min), increase 4 ºC/min to 200 ºC, halt at this temperature for 5 min and then increased to 10 ºC/min until 260 ºC.

Antimicrobial activity: The assessment of antimicrobial activity of the extracted sample was conducted according to the method of Berghe & Vlietlinck [10] on eight bacterial strains *viz*. *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus niger*, *Fusarium oxysporum*, *Saccharomyces cerevisiae* and *Candida albicans*. Minimum inhibitory concentrations (MIC) tests were carried out by using microplates (96 wells).

Anticancer activity: The cytotoxic activity was tested by MTT method [3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide] [11,12] on four cancer cell lines *viz*. PC-3 (human prostate adenocarnoma), MCF-7 (human breast adenocarcinoma cells), A549 (human lung adenocarcinoma epithelial cells) and Hep-G2 (hepatocellular carcinoma) were purchased from the American Type Culture Collection (USA).

RESULTS AND DISCUSSION

The water distillation of the collected wisdom Marjoram plants were done during the two seasons *i.e.* dry season (May to October) and rainy season (November to April). The quantity and contents of the wisdom Marjoram essential oils depends on the several factors *i.e*. time of distillation, time of collection, time to wither, harvest season, *etc*. It was observed that the oncentration of essential oil obtained in the dry season (0.40%) is lower than that in the rainy season (0.48%).

Sensory and physical characteristics of *Elsholtzia ciliata* essential oils: The organoleptic properties of *Elsholtzia ciliata* essential oil was obtained as a liquid, pale yellow and has mild fragrance in both seasons all year around. The physical characteristics obtained from the experiment having density of 0.819 (dry season) and 0.818 (rainy season) indicated that *Elsholtzia ciliata* essential oil is lighter than water, high refractive index and optical rotation index of essential oil: +2.98 (dry season) and +3.21 (rainy season). These properties are relative similar when grown in two different seasons.

Chemical composition of wisdom Marjoram essential oil: The GC-MS chromatograms of wisdom Marjoram essential oil collected in January and July 2021 are shown in Fig. 1. The chemical composition of the essential oil is presented in Table-1.

GC-MS analysis of the essential oil obtained from whole wisdom Marjoram plant (leaves and stems) grown in the dry season contains 42 constituents, accounting for 98.89% of the total weight of essential oil, which consist of 12 oxygenated monoterpenes (62.73%), 7 monoterpene hydrocarbons (18.38%), 8 sesquiterpene hydrocarbons (11.16%), 5 oxygenated sesquiterpenes (3.53%), 2 derivatives of benzene (0.38%), 5 derivatives of fatty acid (2.38%) and 3 other compounds (0.33%). The main compositions of the essential oil were determined as carveol (26.89%), *Z*-citral (29.35%) *cis*-ocimene (9.41%), δ-3-carene (5.34%), germacrene-D (5.40%) and *trans*-caryophyllene (4.49%).

In case of rainy season, collected essential oil contains 46 constituents, which accounted 96.43% of the total weight of essential oil. There were 16 oxigenated mono-terpenes (37.21%), 5 monoterpene hydrocarbons (28.21%), 14 sesquiterpene hydrocarbons (26.53%), 5 oxygenated sesquiterpenes (2.93%), 2 derivatives of benzene (0.20%), 3 derivatives of fatty acid (1.32%) and 1 other compounds (0.03%). The main compositions of the essential oil were *cis*-ocimene (17.95%), *z*-citral (17.01%), carveol (14.84%), isocaryophyllene (13.19%), *trans*-caryophyllene (9.76%), δ-3-carene (5.47%), *trans*-β-ocimene (5.22%) and β-pinene (4.90%).

Comparitive studies: The total essential oil obtained from the harvested wisdom Marjoram in two different seasons are relatively similar. However, the major components of essential

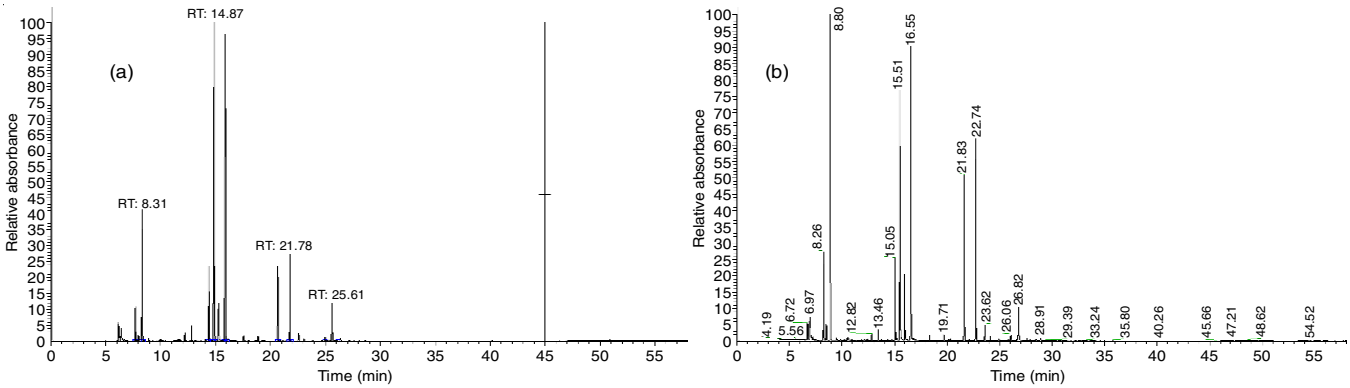

Fig. 1. GC-MS chromatogram of Marjoram essential oil in (a) dry and (b) rainy season

TABLE-1
CHEMICAL COMPOSITION OF MARJORAM ESSENTIAL OIL IN THE DRY AND RAINY SEASON

S. No.	Compound	Molecular formula	Content (%)	
			Dry season	Rainy season
1	Myrtenol	$C_{10}H_{16}O$	0.01	0.06
2	*cis*-Ocimene	$C_{10}H_{16}$	9.41	17.95
3	Panaxydol	$C_{17}H_{24}O_2$	0.11	0.02
4	(-)-Myrtenol	$C_{10}H_{16}O$	0.60	0.07
5	(-)-Isopulegol	$C_{10}H_{18}O$	0.02	0.12
6	β-Pinene	$C_{10}H_{16}$	0.15	4.90
7	δ-3-Carene	$C_{10}H_{16}$	5.34	5.47
8	α-Humulene	$C_{15}H_{24}$	0.18	0.14
9	Carveol	$C_{10}H_{16}O$	26.89	14.84
10	*z*-Zitral	$C_{10}H_{16}O$	29.35	17.01
11	α-Sinensal	$C_{15}H_{22}O$	0.07	0.10
12	*trans*-Caryophyllene	$C_{15}H_{24}$	4.49	9.76
13	Germacrene-d	$C_{15}H_{24}$	5.40	1.08
14	γ-Muurolene	$C_{15}H_{24}$	0.17	0.25
15	δ-Cadinene	$C_{15}H_{24}$	0.05	1.11
16	Copaene	$C_{15}H_{24}$	0.33	0.13
17	Verrucarol	$C_{15}H_{22}O_4$	0.11	0.07
18	*trans*-Valerenyl acetate	$C_{17}H_{26}O_2$	3.10	2.65
19	Dehydroaromadendrene	$C_{15}H_{24}O$	0.19	0.08
20	β-Guaiene	$C_{15}H_{24}$	0.05	0.15
21	(1*r*)-*cis*-Verbenol	$C_{10}H_{16}O$	0.04	0.63
22	Verbenol	$C_{10}H_{16}O$	0.95	0.35
23	*trans*-2-Ethyl-2-hexen-1-ol	$C_8H_{16}O$	2.04	–
24	1-Pentanol, 5-cyclopropyliden-	$C_8H_{14}O$	–	1.20
25	6-Camphenol	$C_{10}H_{16}O$	1.45	–
26	Pinanediol	$C_{10}H_{18}O$	–	3.13
27	Ethyl-2 methyl-3 pentene-1	C_8H_{16}	0.2	–
28	1-Phenyl-pent-4-en-1-one	$C_{11}H_{10}O$	–	0.19
29	Camphene	$C_{10}H_{16}$	2.87	–
30	3-*p*-Menthen-7-al	$C_{10}H_{16}O$	–	0.03
31	β-Phellandrene	$C_{10}H_{16}O$	0.45	–
32	*trans*-d-Dihydrocarveol	$C_{10}H_{18}O$	–	0.04
33	Benzoylformic acid	$C_{15}H_{10}O_5$	0.21	–
34	*trans*-β-Ocimene	$C_{10}H_{16}$	–	5.22
35	Sabinene	$C_{10}H_{16}$	0.27	–
36	*trans-p*-Mentha-1(7),8-dien-2-ol	$C_{10}H_{16}O$	–	0.05
37	1-(2-Propan-2-ylidenecyclopropyl)ethanone	$C_8H_{12}O$	0.08	–
38	Chavicol	$C_9H_{10}O$	0.17	–
39	1,5-Decadiyne	$C_{10}H_{14}$	–	0.03
40	*trans*-2-Caren-4-ol	$C_{10}H_{16}O$	0.17	–
41	*trans*-Chrysanthemal	$C_{10}H_{16}O$	–	0.05
42	α-Terpinolene	$C_{10}H_{16}$	0.07	–
43	γ-Terpinene	$C_{10}H_{16}$	–	0.08

44	Myrtenylformate	$C_{11}H_{16}O_2$	2.44	–
45	(-)-Carvyl acetate	$C_{12}H_{18}O_2$	–	0.06
46	Methyl 4,6-tetradecadiynoate	$C_{15}H_{22}O_2$	0.14	0.10
47	Myrtenyl methyl ether	$C_{11}H_{18}O$	–	0.19
48	Methyl chrysanthemate	$C_{11}H_{18}O_2$	0.36	0.29
49	*trans*-Geranic acid methyl ester	$C_{11}H_{18}O$	–	0.29
50	Methyl 2,4-tridecadiynoate	$C_{14}H_{20}O_2$	0.05	–
51	2-Methyl-3-phenyl-2-propen-1-ol	$C_{10}H_{12}O$	–	0.01
52	α-Pinene	$C_{10}H_{16}$	0.27	–
53	*z*-α-*trans*-Bergamotol	$C_{15}H_{24}O$	–	0.03
54	*trans*-*z*-α-Bisabolene epoxide	$C_{15}H_{24}O$	0.06	–
55	Alloaromadendrene	$C_{15}H_{24}$	–	0.02
56	β-Caryophyllen	$C_{15}H_{24}$	0.49	–
57	Calarene	$C_{15}H_{24}$	–	0.06
58	3β,17β-Dihydroxyestr-4-ene	$C_{18}H_{28}O_2$	0.05	–
59	Isocaryophyllene	$C_{15}H_{24}$	–	13.19
60	Falcarinol	$C_{17}H_{24}O$	0.04	–
61	Isocaryophyllen	$C_{15}H_{24}$	–	0.39
62	δ-Guaijene	$C_{15}H_{24}$	–	0.06
63	β-Longipinene	$C_{15}H_{24}$	–	0.02
64	(-)-Isoledene	$C_{15}H_{24}$	–	0.17
	Monoterpene hydrocarbons		18.38	28.21
	Oxygenated monoterpenes		62.73	37.21
	Sesquiterpene hydrocarbons		11.16	26.53
	Oxygenated sesquiterpenes		3.53	2.93
	Derivatives of benzen (benzenoid)		0.38	0.20
	Derivatives of fatty acids		2.38	1.32
	Other compounds		0.33	0.03
	Total		98.89	96.43

oils in the dry and rainy seasons have major differences, for example, in case of oxygenated monoterpenes (62.73%) predominated with main composition as carveol (26.89%) and *z*-citral (29.35%), where as in case of grown plant in rainy season, oxygenated monoterpenes consist of only 37.21% with main composition as carveol (14.84%) and *Z*-citral (17.01%). Similarly, monoterpene hydrocarbons (18.38% in dry season and 28.21% in rainy season) and sesquiterpene hydrocarbons (11.16% in dry season and 26.53% in rainy season) also differs.

The results of present study are similar to the reported studies [6-8,13], which evidenced that the chemical compositions of the essential oil will be different, if the geographical locations, soil and harvesting conditions are different. Moreover, the difference in the chemical compositions of wisdom Marjoram essential oils collected during the two different seasons will also lead to differences in biological activities.

Antimicrobial activity: The antimicrobial activity of the essential oil of whole plant (leaves and stems) of wisdom Marjoram were tested on eight bacterial strains. The results indicated that the essential oil of wisdom Marjoram in dry season exhibited good inhibition activity on *Escherichia coli*, *Bacillus subtillis*, *Staphylococcus aureus* and *Candida albicans* with MIC value of 200 µg/mL, whereas The wisdom Marjoram essential oil in rainy season exhibited good inhibition activity on *Escherichia coli*, *Bacillus subtillis* and *Saccharomyces cerevisiae* with MIC value of 200 µg/mL and *Candida albicans* with MIC value of 100 µg/mL. The remaining bacterial strains did not exhibit any activity.

Anticancer activity: The anticancer activity of wisdom Marjoram essential oil obtained by water distillation were evaluated of cytotoxic activity on four cancer cell lines (PC-3, MCF-7, Hep-G2 and A549). The results showed that the wisdom Marjoram essential oil obtained by dry season exhibited weak cytotoxic activity on the PC-3 cell line (IC_{50} value of 66.34 µg/mL) and A549 cell line (IC_{50} value of 93.11 µg/mL), however, the essential oil obtained during rainy season displayed good cytotoxic activity on the PC-3 cell line (IC_{50}value of 30.62 µg/mL) and Hep-G2 cell line (IC_{50} value of 22.78 µg/mL) but exhibited weak cytotoxic activity on the A549 line (IC_{50} value of 85.35 µg/mL). Unfortuntely, the essential oils obtained from grown wisdom Marjoram plant in different conditions was not effective against MCF-7 cell lines (Table-2).

TABLE-2
ANTICANCER ACTIVITY OF THE MARJORAM ESSENTIAL OIL AGAINST FOUR CANCER CELL LINES

Marjoram essential oil in:	IC_{50} (µg/mL)			
	PC-3	MCF-7	Hep-G2	A549
Dry season	66.34	> 100	> 100	93.11
Rainy season	30.62	> 100	22.78	85.35
Positive control (paclitaxel)	3.51 ng/mL	4.34 ng/mL	4.12 ng/mL	3.85 ng/mL

Conclusion

The chemical components of the essential oil obtained using hydrodistillation from the wisdom Marjoram [*Elsholtzia ciliata* (Thumberg) Hylander] whole plant harvested at the Central highlands of Vietnam at two different climatic seasons were characterized by GC-MS technique. The results indicated that collected essential oil from *Elsholtzia ciliata* grown in rainy season contain more effective bioactive compounds than the oil procured from the plant harvested in dry season. Similarly, collected *Elsholtzia ciliata* essential oil in rainy season had good inhibition activity on the tested bacterial organisms as compared to obtained essential oil acquired in dry season plant. Likewise, the obtained essential oil from rainy season plant displayed better cytotoxic activity on the PC-3 and Hep-G2 cell lines as compared to dry season plant.

ACKNOWLEDGEMENTS

This research is funded by Tay Nguyen University under grant No. T2022-44CBTĐ.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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