Original Article: Chemical Composition of Essential Oil in *Anthemis austroiranica* in the Six Regions of Hormozgan Province

Kamkar Jaimand[1*], Mohammad Amin Soltanypour[2], AbdolHamide Hajebee[2], Fatemeh Sefidkon[1], Mohammad Bager Rezaee[1], Razieh Azimi[1], Mahmood Nadery[1], Someyeh Fekry[1], Shahrokh Karimi[1] and Firoozeh Hatami[1]

[1*]Department of Medicinal Plants and Byproducts Research, Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
[2]Hormozgan Research Center Agricultural and Natural Resources Research Center, Agricultural Research Education and Extension Organization (AREEO), Hormozgan Province , Iran

Received: 15 November 2020
Accepted: 16 April 2021

Abstract

The genus Anthemis L. belongs to the family Asteraceae, is the second-largest genus in the Compositae family, consists of 39 annual and perennial species distributed all over Iran. According to literature, this species was not the subject of research up to now and therefore its chemical composition is not well known. In this research six samples of *Anthemis austroiranica* were collected from Hormozgan province, in 2019. The specimen is deposited in the Central Herbarium of the Research Institute of Forests and Rangelands. The volatile constituents of *A. austroiranica* were isolated by water distillation (Clevenger apparatus) and analyzed by GC and GC/MS. The major components of the aerial partof samples collected from Bandar-e-Abass, Geno Mountain, altitude 2200 meters, were geranyl benzoate 17.94%, γ-eudesmol 16.33%, phenyl ethyl hexanoate 15.33% and n-heptadecane 14.84%. The major components of the aerial part of the second samples from Bandar-e-Abbas, Tang-e-Zagh, altitude of 1000 meters, were linoleic acid 25.24%, geranyl benzoate 23.66%, 7-hydroxy coumarin 10.37%. The major components of the aerial part of the third sample from Bandar-e-Abbas, Geno mountain, altitude 1385 meters, were geranyl benzoate 28.68%, 7-hydroxy coumarin 21.10%, (E)-nuciferol 7.66%. The major components of the aerial part of the fourth sample from Bastak, altitude of 450 meters, were hexadecanoic acid 31.17%, 7-hydroxy coumarin 8.39%, and phenyl ethyl hexanoate 7.44%.The major components of the aerial part of the fifth sample from Bandar-e-Abbas, Geno Mountain, altitude 1600 meters, were geranyl benzoate 24.35%, γ-eudesmol 15.50%, 7-hydroxy coumarin 7.65%. The major components of the aerial part of the last sixth samples from Hajiabad, Sirmand, Tel Mara, altitude 112 meters, were nonanal, dimethyl acetal 33.08%, hexadecanoic acid 6.16%, phenyl ethyl hexanoate 4.01%. Differences in the chemical composition of essential oils of *A. austroiranica* six regions can be due to differences in altitude in Geno Mountain and its distribution in Hormozgan province, as well as soil type and genetic issues.This research is performed on *A. austroiranica,* endemic species for the first time in Iran.

Keywords: Anthemis, Asteraceae, Compositae, Essential oil, Hormozgan

Introduction

The genus *Anthemis* L. belongs to the family Asteraceae and was the second-largest genus in the Compositae family, tribe Anthemideae, nearly 130 species of genus Anthemis occur throughout the world consisting of 39 annual and perennial species distributed all over Iran (Rechinger, 1986). According to available sources, not much research has been done on *A. austroiranica.* However, other componentssuch as flavonoids, polyphenolic acids, terpenes, and sesquiterpenes have been reported in other species (Klimes *et al*., 1981). The species of the Anthemis genus are widely used in pharmaceutics,

* Corresponding author: Department of Medicinal Plants and Byproducts Research, Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
E-mail: camcarjaimand@yahoo.com

cosmetics and food craft. The flowers of the genus have well-documented use as disinfectants and healing herbs, the main components being natural flavonoids and essential oils (Uzel *et al*., 1981). Some *Anthemis* spp. essential oils contain anti-aging activity. While *A. cotula* essences has been proved to possess antioxidant confidants and essential oil from *A. nobilis* flowers is generally used for pharmaceuticals, food additives, as well as the main source in aromatic and cosmetic industries (Saroglou *et al*., 2006).

A. gayana Boiss.existentan annual endemic plant of the Asteraceae family. This plant grows in Isfahan, the west region of Iran. First,leaves essential oil were investigated by Sonboli and more than 34 compounds of leaves representing 92.4% of total oil were identified, germacrene-D (30.2%), geranylisovalerate (7.4%), bicyclogermacrene (6.7%) and α–caryophyllene (5.5%) were detected as the major compounds (Sonboli *et al*., 2005).

Amjad reported that methanolic extract of *A. gayana* flowers and leaves were not active against Candida glabrata CBS 2175 and Candida albicans ATCC 62061, ATCC 1677. Flowers and leaves methanolic extract had more effect against Candida parapsilosis (Amjad *et al*., 2013), Thus, leaves methanol extract had more effect against Candida albicans ATCC 3153 (Amjad, and *et al*., 2012).

Rezaee and Jaimand reported the volatile constituents of *A. altissima* L. var. *altissima* which were extracted essential oilby hydrodistillation and analyzed by GC and GC/MS. The major constituents of *A. altissima* flowers oil were spathulenol (18.7%), caryophyllene oxide (9.3%), 1-eicosene (7%) and sabinene (6.2%), while the leaves oil contained spathulenol (18.2%), caryophyllene oxide (9.5%), methyl hexadecanoate (8%) and isocaryophyllene (7.4%) (Rezaee and Jaimand, 2006). They also showed volatile constituents of *A. triumfettii* (L.) subsp. *Triumfettii* which were extracted essential oil by hydrodistillation and analyzed by GC and GC/MS. The major constituents of *A. triumfettii* flowers oil were elemol (15.8%), α-copaene (8.6%), elemicin (7.9%), and humulene oxide II (8.0%), while the leaves oil was rich in α-copaen-8-ol (7.6%), β-eudesmol (7.2%), α-fenchene (5%), ledol (4.2%) and elemol (4%) (Rezaee and Jaimand, 2008).

Rezaee and Jaimand in 2007, announced chemical componentsof essential oil from leaves and flowers in *A. cotula*L. from Gilan Province. The major compositionof flowers oil was n-nonadecane (10.8%), cedrane (9.2%) and (E,E)-α-farnesene (6%), while the leaves oil was contained 1-eicosane (11%), benzyl salicylate (8.9%) and aromadendrene (7.1%) (Rezaee and Jaimand, 2007).

Rezaeeinformed the volatile constituents of *Anthemis kotschyana* Boiss. Var. *discoides* (Borron.) Grierson extracted essential oilby hydro-distillation and analyzed it by GC and GC/MS. In this study, samples were collected from west Azarbaijan province between Orumieh and Shahpur on May 2003. The major constituents of *A. kolschyana Boiss.*var. *discoides*(Borron.) Grierson from flowers head were β-acorenol (11.9%), artemisia alcohol (9.4%), ethyl hexanoate (8.8%) and n-nonadecane (5.6%) (Rezaee *et al*., 2007).

Rezaee *et al*. (2007) reported the volatile constituents of *A. coelopoda* Boiss. which were extracted essential oil by hydrodistillation and analyzed by GC and GC/MS. In this study, samples were collected from Gilan province in Rodbar in late May 2003. The major constituents of *A. coelopoda* Boiss. flowers oil were cis-chrysanthenyl acetate (27.3%), hexyl butanoate (16%), and myrcene (7%), while the leaves oil contained isobornylformate (30.6%), trans-ethyl chrysanthemumate (15%) and *p*-mentha-1,5-diene-8-ol (13.7.4%) (Rezaee, *et al*., 2008).

Rezaee *et al*, (2010) reported on volatile constituentsof *A. hyalina* DC. which were extracted essential oil by hydrodistallation and analyzed by GC and GC/MS. In this study samples were collected from Qazvin province in 2010. The major constituents of *A. hyaline* DC. were α-terpinene 58.5%, trans-chrysanthenyl acetate 5.3%, β-calacorene 4% (Rezaee *et al*., 2010).

The purpose of this study is to investigate the chemical composition of *A. austroiranica* essential oil from six regions in Hormozgan province. This is the first study on *A. austroiranica*, which is endemic species.

Material and Methods

Sample Collection

In this research, six samples of *A. austroiranica* were collected from Hormozgan province, (Bandar-e-Abass, Geno Mountain, altitude 2200 meters; Bandar-e-Abbas, Tang-e-Zagh, altitude 1000 meters; Bandar-e-Abbas, Geno Mountain, altitude 1385

meters; Bastak, altitude 450 meters; Bandar-e-Abbas, Geno Mountain, altitude 1600 meters; HajiabadSirmand, Tel Mara, altitude 112 meters), in 2019. The specimen is deposited in the herbarium of the botanical garden of Iran88275 (TARI), in the Research Institute of Forests and Ranglands with a coded. No. IMP. H. A0208.

Essential Oil Preparation

Samples of *A. austroiranica* were collected from six locations in Hormozgan province, and essential oil was extracted by water distillation. The essential oil yields from Bandar-e-Abass, GenoMountain, altitude 2200 meters were 0.10%, for the second samples from Bandar-e-Abbas, Tang-e-Zagh, altitude 1000 meters, were 1.63 %, for the third samples from Bandar-e-Abbas, Geno Mountain, altitude 1385 meters, were 0.17%, for the fourth samples from Bastak, altitude 450 meters, were 0.19%, for the fifth samples from Bandar-e-Abbas, Geno Mountain, altitude 1600 meters, were 0.21%, and for the last sixth samples from Hajiabad, Sirmand, Tel Mara, altitude 112 meters, were 0.41%. The chemical composition of *A. austroIranica* essential oil was measured and identified by GC and GC/MS.

Gas Chromatography

GC analyses were performed using gas chromatography, Ultra-Fast Module-GC, made in Italia. Profile column machine brand Ph-5 capillary column, with 10 m, ID 0.1 mm, final temperature 0.4, oven: 60-285°C/min., Rate: 40°C/min., Hold time: 3 min., Run time: 8.63 min. Detector: FID, 280°C, injector: 280°C, carrier Gas: He, 0.5 ml/min.

Gas Chromatography-Mass Spectrometry

The GC/MS unit consisted of a Varian Model 3400 gas chromatography coupled to a Saturn II ion trap detector was used. Mass spectrometer conditions were: ionization potential 70 eV; electron multiplier energy 2000 V.

The identification of oil components was established from their GC retention indices, relative to C7-C25 n-alkanes standards mixture, and by comparison of mass spectra and retention indices with those reported in the literature (Shibamoto, 1987, Davies,1990A, dams, 2017), and by computer matching with the Wiley 5 and NIST mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

Results

As you can see in Table 1, six samples of *A. austroiranica* were collected from Hormozgan province, in 2019. The major components of aerial parts ofsamples collected from Bandar-e-Abass, GenoMountain, and altitude 2200 meters were geranyl benzoate 17.94%, γ-eudesmol 16.33%, phenyl ethyl hexanoate 15.33% and n-heptadecane 14.84%. The major components of the aerial partof the second samples from Bandar-e-Abbas, Tang-e-Zagh, altitude 1000 meters, were Linoleic acid 25.24%, Geranyl benzoate 23.66%, and 7-hydroxy coumarin 10.37%. The major components of the aerial partof the third sample from Bandar-e-Abbas, Geno Mountain, altitude of 1385 meters were geranyl benzoate 28.68%, 7-hydroxy coumarin 21.10%, (E)-nuciferol 7.66%. The major components of the aerial partof the fourth sample from Bastak, altitude 450 meters were hexadecanoic acid 31.17%, 7-hydroxy coumarin 8.39%, and phenyl ethyl hexanoate 7.44%.The major components of the aerial partof the fifth sample from Bandar-e-Abbas, Geno Mountain, altitude of 1600 meters, were geranyl benzoate 24.35%, γ-eudesmol 15.50%, and 7-hydroxy coumarin 7.65%. The major components of the aerial partof the last sixth samples from Hajiabad, Sirmand, Tel Mara, altitude 112 meters, were nonanal, dimethyl acetal 33.08%, hexadecanoic acid 6.16%, phenyl ethyl hexanoate 4.01%. Differences in the results of the amount of chemical compounds detected in the essential oil of *A. austroiranica* in six regions can be due to differences in altitude in Geno Mountain and its distribution in Hormozgan province, as well as soil type and genetic issues.This research is performed for the first time on *A. austroiranica*,the endemic species in Iran.

extraction have effects on the effective composition of the plant. Therefore, based on the studies performed on a decomposed sample of essential oil in *A. austroiranica*, there is a difference in the amount of active ingredients of the collected species. As you can see in the table, *A. austroiranica* species have been collected from Genu Mountain in Bandar Abbas from 2000, 1600 and 1385 meters, and γ-eudesmol composition decreased from 16.33% to 15.50% and 5.06%, respectively. Also, for samples collected from Tang-e-Zagh with a height of 1000, Bastak with 450 and Hajiabad, Sirmand, Tel Mara, it was decreased by 3.22%, 6.96% and 2.85%

respectively.γ-eudesmol, is a member of the class of compounds known as eudesmane, isoeudesmane or cycloeudesmane sesquiterpenoids. Eudesmane, isoeudesmaneorcycloeudesmane sesquiterpenoids are sesquiterpenoids with a structure based on the eudesmane skeleton. γ-eudesmol is practically insoluble (in water) and an extremely weak acidic compound (based on its pKa). γ-eudesmol is a sweet and waxy tasting compound and can be foun d in a number of food items such as rosemary, ginkgo nuts, mango, and common thyme, which makes γ-eudesmol a potential biomarker for the consumption of these food products (Gamma-Eudesmol, PubChem).

Discussion

According to the articles, it can be stated that changing the height, collection area, time and method of essential oil extraction have effects on the effective composition of the plant. Therefore, based on the studies performed on a decomposed sample of essential oil in *A. austroIranica*, there is a difference in the amount of active ingredients of the collected species.

As you can see in the table, *A. austroIranica* species have been collected from Genu Mountain in Bandar Abbas from 2000, 1600 and 1385 meters, and γ-eudesmol composition decreased from 16.33% to 15.50% and 5.06%, respectively. Also, for samples collected from Tang-e-Zagh with a height of 1000, Bastak with 450 and Hajiabad, Sirmand, Tel Mara, it was decreased by 3.22%, 6.96% and 2.85% respectively.γ-eudesmol, is a member of the class of compounds known as eudesmane, isoeudesmane or cycloeudesmane sesquiterpenoids. Eudesmane, isoeudesmaneorcycloeudesmane sesquiterpenoids are sesquiterpenoids with a structure based on the eudesmane skeleton. γ-eudesmol is practically insoluble (in water) and an extremely weak acidic compound (based on its pKa). γ-eudesmol is a sweet and waxy tasting compound and can be found in a number of food items such as rosemary, ginkgo nuts, mango, and common thyme, which makes γ-eudesmol a potential biomarker for the consumption of these food products (Gamma-Eudesmol, PubChem).

Regarding the composition of Phenyl ethyl hexanoate, the results have been variable from the heights of 2000, 1600, 1385, 1000, 450 and 112 meters, with the amount of 15.33%, 4.47%, 6.35%, 2.96%, 44/7%. 4.01% respectively.Phenyl ethyl hexanoate, also known as 2-phenylethyl caproate, belongs to the class of organic compounds known as fatty acid esters. These are carboxylic ester derivatives of a fatty acid. Phenethyl hexanoate is considered to be a practically insoluble (in water) and relatively neutral molecule. Phenethyl hexanoate has been primarily detected in urine. Within the cell, phenethyl hexanoate is primarily located in the membrane (predicted from log P) and cytoplasm. Phenethyl hexanoate exists in all eukaryotes, ranging from yeast to humans. Phenethyl hexanoate is a sweet, banana, and floral tasting compound that can be found in alcoholic beverages, citrus, and fruits. This makes phenethyl hexanoate a potential biomarker for the consumption of these food products (Phenylethyl hexanoate, PubChem).

In relation to the n-heptadecane combination, only at an altitude of 2000 m, the composition is 14.84%.Heptadecane is an organic compound, an alkanehydrocarbon with the chemical formula C17H36. The unbranched isomer is normal or n-heptadecane, CH3(CH2)15CH3. In the IUPAC nomenclature, the name of this compound is simply heptadecane, since the other isomers are viewed and named as alkyl-substituted versions of smaller alkanes (*DeSilva* and *Goodman*, 2005).

The composition of 7-hydroxy coumarin also varies according to the height from the heights of 2000, 1600, 1385, 1000, 450 and 112 meters, with the amount of 9.69%, 7.65%, 21.10%, 10.10%, 8.39% and 1.35%, respectively. Umbelliferone, also known as 7-hydroxycoumarin, hydrangine, skimmetine, and beta-umbelliferone, is a natural product of the coumarin family. It absorbs ultraviolet light strongly at several wavelengths. Despite several indications that this chemical is photomutagenic, it is used in sunscreens(Lupei, 2008) [20]. Umbelliferone has been reported to have antioxidant properties (Sim et al., 2015). It is a yellowish-white crystalline solid that has a slight solubility in hot water, but high solubility in ethanol.

Table 1 Chemical composition of essential oil in *A. austroiranica*

	R.I.	*A. austroiranica*					
		Bandar-e-Abass, Geno mountain			Tang-e-Zagh	Bastak	Hajiabad Sirmand – Tel Mara
altitude meters		2200	1600	1385	1000	450	112
Oil %		0.10	0.21	0.17	1.63	0.19	0.41
Compounds name							
Terpinolene	1086	-	-	-	-	-	0.33
1,3,8-p-menthatriene	1108	-	-	-	-	1.60	-
allo-ocimene	1127	-	-	-	-	-	0.39
Trans-β-terpineol	1159	-	-	-	1.28	-	6.36
p-cymen-8-ol	1178	-	-	-	1.63	-	9.43
Methyl nonanoate	1222	-	-	-	-	-	7.65
trans-sabinene hydrate acetate	1254	-	-	-	-	-	1.24
Nonanal, dimethyl acetal	1276	-	-	-	7.85	-	33.08
Prenylhexanoate	1292	-	-	-	-	-	2.02
n-tridecane	1300	-	-	-	-	-	3.26
n-nonanyl acetate	1310	2.64	1.22	-	2.59	0.96	1.72
Methyl decanoate	1320	-	-	-	-	-	0.38
α-copaene	1373	-	-	-	-	-	4.31
Geranyl acetate	1380	-	-	-	-	-	0.48
Methyl eugenol	1404	-	1.51	-	-	0.50	-
Linalool butanoate	1421	3.04	-	-	-	-	1.19
(E)-β-farnesene	1454	-	-	-	-	-	1.28
9-epi-(E)-caryophyllene	1462	-	-	-	-	-	1.93
Germacrene D	1485	-	-	-	-	-	0.37
Nerylisobutanoate	1493	-	2.95	-	-	-	0.77
(E,E)-α-farensene	1506	-		-	-	0.44	-
Trans-calamenene	1519	-	1.30	-	-	0.34	0.36
Cis-cadinene ether	1553	3.90		-	-	1.80	-
Elemicin	1554	-	14.72	1.12	-	3.31	-
(E)-nerolidol	1562	-	-	-	-	1.50	-
Caryophyllenyl alcohol	1571	-	-	-	-	0.79	-
Caryophyllene oxide	1582	-	2.62	1.78	1.34	0.62	-
Ledol	1603	-	-	-	1.11	-	2.35
Hexyl phenyl acetate	1627	-	0.94	-	-	1.37	-
γ-eudesmol	1632	16.33	15.50	5.06	3.22	6.96	2.85
Phenyl ethyl hexanoate	1642	15.33	4.47	6.35	2.96	7.44	4.01
β-eudesmol	1652	-	1.22	-	-	0.47	-
α-bsabolol oxide B	1657	-	-	-	-	0.44	-
7-epi-α-eudesmol	1664	2.03	-	-	-	1.32	-
n-tetradecanol	1671	2.47	1.79	2.35	1.56	1.57	0.56
(2Z, 6Z)-farnesal	1683	-	-	-	1.11	-	1.83
(Z)-α-trans-bergamotol	1690	-	-	-	-	0.81	-
2-pentadecanone	1696	-	-	-	-	5.21	-
n-heptadecane	1700	14.84	-	-	-	0.30	-
(2E, 6Z)-farnesal	1714	1.94	3.00	3.58	3.28	1.42	1.27
Methyl tetradecanoate	1719	-	1.79			0.36	-
(E)-nuciferol	1752	4.85	5.41	7.66	2.53	5.42	0.60
Cedryl acetate	1766	-	-	-	-	0.53	-
(2Z, 6E)-farnesyl acetate	1824	-	0.82	-	-	0.47	-
7-hydroxy coumarin	1835	9.69	7.65	21.10	10.37	8.39	1.35
(Z, Z)-farnesyl acetone	1859	-	1.79	1.87		1.18	-
(5E, 9Z)-farnesyl acetone	1883	1.11	1.08	1.80	1.61	2.05	-
(5E, 9E)-farnesyl acetone	1914	-	-			0.90	-
Geranyl benzoate	1954	17.94	24.35	28.68	23.66	31.17	6.16
Occidol acetate	1974	2.01	2.05	1.39	-	3.32	0.41
(E)-methyl-isoprenylcinnamate	2063	-	-	3.74	-	-	-
n-heneicosane	2100	-	-	5.48	-	-	-
Methyl octadecanoate	2123	1.79	2.36	2.96	3.01	1.90	0.56
Linoleic acid	2135	-	-	-	25.24	-	-
n-docosane	2206	-	-	-	-	1.49	-
Methyl sandaracopimarate	2259	-	1.34	4.97	3.50	3.46	0.47

Major Applications of 7-hydroxy coumarin isfluorescent brighteners, fungicides, cosmetics, anti-trypanosomal, anti-leishmanial activities, antifungal, antibacteria l, anti-inflammatory, anti-HIV, detection of microorganisms, treatment of impaired neurotransmission disorders, Alzheimer's disease, vascular and lymphatic edema cancer, early diagnosis of stroke, dental material, drug-coated coronary stent system.

Geranyl benzoate composition also varies based on the height from the height of 2000, 1600, 1385, 1000, 450 and 112 meters, with the amount of 17.94%, 24.35%, 28.68%, 23.66%, 31.17% and 16.16%, respectively, in which the highest amount was at an altitude of 450 meters.Geranyl benzoate, belongs to the class of organic compounds known as aromatic monoterpenoids. These are monoterpenoids containing at least one aromatic ring. Geranyl benzoate is an extremely weak basic (essentially neutral) compound (based on its pKa) (Geranyl benzoate, PubChem).

Acknowledgement

This research has been done in the Research Institute of Forest and Rangeland (RIFR) in the department of medicinal plants research.Thanks to everyone who contributed to the research.Special thanks to our provincial colleagues Dr. Amin Soltanipour and Dr. Abdul Hamid Hajebi from the Hormozgan Research Center for collecting samples and I would also like to thank the Medicinal Plants Research Department for assisting in this research.

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