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Chemical composition in two species of *Verbascum* collected from natural habitats, southern Iran

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ABSTRACT

Background & Aim: In the present research, we investigated phytochemical characteristics of two important species of *Verbascum* genus. Enormous reconnaissance and field surveys were conducted to identify the most important natural habitats of *Verbascum* species in Kohgiluyeh va Buyerahmad province, southern Iran.
Experimental: The aerial parts of *Verbascum songaricum* and *V. cheirantifolium* were collected at flowering stage in July 2011. The extraction was conducted by digestion method, and then to analysis and identify extracts used by GC-MS. Totally, the number of 60 and 52 compositions from different *V. songaricum* and *V. cheirantifolium* extracts identified, respectively. In order to compare different compositions present in parts between both species, first compositions were classified into six functional groups (alcohol, amin, ester, hydrocarbon, ketone, and acid), then these groups were analyzed.
Results & Discussion: Results indicated that there were significant differences among various parts of flowers, leaves, and stems in both species in terms of alcohol, amin, ester, hydrocarbon, and ketone. Given that enhancement of medicinal qualitative and quantitative yields not only depends on ecological conditions, but also varies in respect to various extractions methods, etc. In addition, this is associated o plant genetically architecture. Results obtained from *V. songaricum* and *V. cheirantifolium* showed that although both species grow close together.
Recommended applications/industries: The leaves of species of *Verbascum* have the higher chemical variation than other plant organs such as flower and stem.

1. Introduction

According to WHO reports, nowadays up to eighty percent of people all over the world (nearly 5 billion people) use medicinal plants to cure the diseases. Approximately, one fourth of all world medicines, have herbal origin either extracted directly from plants or synthesized based on plant composition. The term "medicinal plant" does not denote only as people disease

healer, but they frequently are used as baiter, potables, sweeter, natural pigments and color, insecticides as well as ingredients for cosmetics (Omidbeigi, 1997). Ancient humans have been using these plants to treat respirational ailments. Physicians has been used it to treat caught and European emigrants brought these plants into America so that it has been useful to cure caught, cold, throat and tonsils inflammation, diarrhea, emerods and emiction channel infections (Mirhaidar, 2005). Iran serves as resourceful origin of industrially and medicinally important wild plants species. Out of 3000 medicinal plants species identified in the world, the number of 1000 species inhabit in Iran (Karimian *et al*., 2012).

Verbascum is frequently found in Iran so that 42 species are widely distributed in this country of which 14 species are endemic (Kheiri, 2009). Gruhi et al. (2007) while studying on *Verbascum songaricum* Schrenk, concluded that saponins existed in this species is effective to treat hair loss. In addition, Azadshahraki et al. (2008) while evaluating *Verbascum songaricum* Schrenk found that it has great deal of importance in absorption and sequestration of heavy metals. Studies of Tatli et al. (2003) on some *Verbascum* species in Turkey containing saponines have the most strong antifungal activity against fungi *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides*. Tatli and Akdemir (2004), by evaluating chemical compositions in some *Verbascum* species reported that the species have saponin, glycoside iriodid, phenylethanoid, monoterpens, neolignan, flavonoid, stroides, spermine, and other metabolites. Akdemir et al. (2011) according stepwise segregation method, separated four iriodid glycosides including ajugal, lasianthoside, catalpol and aucubin, and two saponines of ilwensisaponin A and C and glycoside of phenylethanoid from *Verbascum mucronantm* and determined their structures through spectrometry approach. In addition, they concluded that verbascoside compositions are so promising to cure wounds, pains andalso act as anti-inflammation without any adverse side effects and impacts on digestive system. Shalabia et al. (2010) studied glycosides of *Verbascum letourneuxii* and its antioxidant properties. They reported that the metanolic extracts of *Verbascum leourneuxii* have five iridoid

The present research, the main aims to evaluate and compare chemical compositions in different aboveground organs (flower, leaf and stem) of two species *Verbascum songaricum* Schrenk and *Verbascum cheirantifolium* BOISS belonged to genus verbascum under similar ecological conditions in rangelands of Kohgiluyeh and Buyerahmd province. So we will identify chemical compositions, as well as variations in both species under the same ecological conditions.

2. Materials and Methods

2.1. Identification and collection of plants specimens

Enormous reconnaissance and field surveys were conducted to identify the most important natural habitats of *verbascum* species in Kohgiluyeh and Buyerahmad province. Finally the most important habitat on which where both species inhabited t together, were selected to sampling in elevation 2900 m in Dena mountain. Aboveground organs of *V. cheirantifolium* and *V. songaricum* were collected in flowering period in July 2011 from abovementioned habitat and transferred into herbarium of Isfahan university technology to be identified by botanists using valid scientific literatures. The samples were shadow dried and ground.

Fig 1. *V. songaricum* and *V. cheirantifolium* in flowering period.

2.2. Extracts preparation

the extraction process was followed according to digestion approach. Amount of 20 g dry powder from every collected sample was mixed separately in 208 cc 96% alcohol ethanol with 184 cc distilled water and stirred for three hours. Then vessels contents was filtered by filter paper. To be better filtered, the specimens were centrifuged in 3000 cycles per second. Then solution was poured into beaker and was placed in evaporator device under vacuum n 45 C in moderate cycle to bring it into 6 cc.

2.3. Essence extraction

The extracts were mixed with 12 cc ethanol, passed through Decanto in which two phases were appeared. This operation was repeated four times until metabolites was entered into bothanolic phase, then it poured into separator vessel and collected. Finally it was stored in darkness under 4 C in refrigerator.

2.4. Specimen's analysis by Gas Chromatography Mass Spectroscopy

In the present research, chemical compositions present in leaves, stems and flowers of both species were distinguished in central laboratory of Isfahan University of technology by device GC/MS model Agilent Technologies. This device was equipped with mass selective detector having a capillary column model HP-5 MS (30 m * 0.25 mm, film thickness of 0.25im) contained 5% polyphenol methyl siloxane. Helium contained gas flow velocity was 1, L/min and column temperature of column 60 by 3 C/min was 270 C . In addition, injection and detector temperature was 260 C, injection volume was 1 microliter and set mass spectrometry parameters characteristics were as following:

Ionic potential 70 ev, ionic flow 2A, ionic subsequent temperature 240 C and resolution was 1000.

to analysis composition variations in both species in rangeland site of Dena the Paired t-Test by software Minitab was done and results are shown in table. Since normal data are required to data analysis, normalization test was carried out before analysis.

3. Results and Discussion

3-1. The ingredients extracted from flower extracts in species V. songaricum and V. cheirantifolium and comparing them

The extracted metabolites from flower extracts of species *V. songaricum* and *V. cheirantifolium* B. and comparing them with the number of ۱۵ compositions in extracts of flower *Verbascum songaricum* showed that it accounts for 99.78% total extracts. The main ingredients in extracts included 9, 12-Octadecadienoic acid (21.53), Butanoic acid, butyl ester (%۱۹/۷۲) and aldol by product(%۱۰/٤٥) . Other ingredients for this extract are shown in table1.

The number of 12 mixtures in extracts of flowers in *V. cheirantifolium* was identified accounting for 99.99T of total extracts. The compositions of Butanoic acid, butyl ester(35.29%), 1-Tert-buthoxy-5-trimethylsilyloxypentane (18.82%) and 8.exo.-cyanobicyclo- nona-3,6-dien-2 (11.16%) were the main ingredients in this extract. Other components are shown in table 2.

3-2. Ingredients extracted from leaves extractions in V. songaricum and V. cheirantifolium and comparison of them

The number of twenty ingredients in extractions of leaves in specimen drived from *V.songaricum* were identified, explaining 99.88% of total extracts. The major ingredients in this extract were Butanoic acid, butyl ester (28/3%), ۲،۳،۳a,8a-Tetrahydro-4,6-dimethoxyfuro- benzofuran(12.30%) and Butane, 1,1-dibutoxy (10.68%) among others. Others components are shown in table 3. The number of fourteen ingredients in leaves extracts from *V. cheirantifolium* was recognized, which covered 99.96% of total extracts. The ingredients Butanoic acid, butyl ester (31.38%), Butane, 1,1-dibutoxy (16.76%) and Propionic acid, 2,2-dimethyl-, octyl ester (10.25%) accounted large proportion of extract content. Others components are shown in table 4.

3-3. The ingredients extracted from stem extracts in species V. songaricum and V. cheirantifolium and comparing them

The number of eleven ingredients was found in extraction of stem in specimens taken from *v. songaricum*, so that accounted for 99.9% of total extracts. The ingredients with high proportion in extracts involved butyl ester, Butanoic acid (36.7%), Benzene, 1-cyclohexen (19.3%) and Propionic acid, 2,2-dimethyl-, heptyl ester (11.45). the others components belonged to this extract are shown in table 5. The number of 14 ingredients was identified in extracts from shoots (stem) in specimen consisted 99.82% of total extracts. The ingredients Butanoic acid, butyl ester (33.2%), Benzenamine, 2,5-difluoro(16.49%) and .-cyanobicyclo -3,6-dien-2-one8.exo.-(9.61%) were the main metabolites found in extracts. others ingredients are shown in table 6.

3.4. Chemical compositions in both species V. songaricum and V. cheirantifolium

To compare chemical compositions in different organs (flower, leaf and stem) between two species, the paired t-Test was used. Results can be seen in table 7.

First, ingredients were classified by phytochemical experts into six functional groups (alcohol, Amin, ester, hydrocarbon, ketone and acid) and then they were analyzed.

Results from table 7 indicate that different aboveground organs of flower, leaf and stem in *v. songaricum* with *v. cheirantifolium* do not vary significantly in probability level of 5%. However, they differed in terms of acidic compositions among different organs of *verbascum songaricum* with *v. cheirantifolium* in significant way.

Table 1. The identified substances in flower extracts of *V. songaricum.*

No	Ingredients	Inhibiting time	Flower (%)
1	2,4-Dipropyl-5-ethyl-1,3-dioxane	3.293	3.61
2	Butanoic acid, butyl ester	3.911	19.72
3	1-Decene, 5-methyl	4.168	٤.٧٤
٤	Nonane, 3,7-dimethyl	4.432	1.5
٥	3-Undecene	4.477	٢.٤١
٦	Decane, 4-methylene	4.54	7.78
٧	4-vinylphenol	5.977	6.9
٨	aldol by product	6.217	10.45
٩	4-vinyl-2-methoxy-phenol	6.755	1.27
١٠	Cinnamamide, N-(benzyloxy)	7.562	1.77
١١	Butanoic acid, 2-methyl-, 1-methylpropyl ester	8.094	9.56
١٢	4,4-Dimethyl-cyclohex-1-enecarbonitrile	9.473	٤.٤١
١٣	Hexadecanoic acid	10.531	1.37
١٤	9,12-Octadecadienoic acid	11.378	21.53
١٥	Linoleic acid ethyl ester	11.51	3.55
-	Total	-	99.78

Having been undergone some technological conversion, medicinal plants have difference substances most of which affect human body. The field dealing with on metabolites, their structure and condition in plant, their variations during plant life span and preparing herbal medicines is called phyto-chemistry. In the present study we shed lights on phytochemical characteristics of two species belonged to verbasum family. Some qualitative and quantitative changes in metabolites were observed in their organs under the same ecological condition. As it can be seen in tables 1-2-3-4-5-6, as a whole, *v. songaricum* is much more diverse than *v. cheirantifolium*. However, compositions in the stem of former species are less than later. In aboveground organs of flower, leaf and stem of *V.songaricum* and *V. cheirantifolium*, the number of 20, 25, 15 and 18, 16, 16 chemical compositions were extracted and identified respectively (tables 1-2-3-4-5-6). Tables indicate that 9, 12-Octadecadienoic acid accounts for highest proportion of ingredients in all of both species organs except flower. In other organs, Butanoic acid, butyl ester serves as the main component, indicating that *verbascum* species have considerable potential to produce this ideal composition. Of course in flowers, Butanoic acid, butyl ester followed by Octadecadienoic acid has great deal of contribution. Given to amount of produced metabolites, it seems that in *V. songaricum*, leaves are the most cost effective organs due to higher production followed by flower and stem

Table 2. the identified ingredients in flower extracts of *V. cheirantifolium.*

No	Ingredients	Inhibiting time	Flower (%)
1	Propanoic acid, 2-methyl-, 2-methylpropyl ester	3.138	1.37
2	4-Heptanone, 3-methyl	3.293	4.32
3	Butanoic acid, butyl ester	3.905	35.29
4	Cyclopentane, hexyl	4.168	5.26
5	1-Hexanol, 2-ethyl	4.231	1.37
6	Nonane, 2-methyl	4.431	2.26
7	5-Undecene	4.477	3.34
8	(3S)-3-Ethyl-2,2-dimethylcyclohexan-1-one	4.54	4.97
٩	4-vinylphenol	5.988	٩.٤١
١٠	1-Tert-buthoxy-5-trimethylsilyloxypentane	6.211	18.82
١١	Benzofuran, 2,3-dihydro	6.76	2.42
١٢	8.exo.-cyanobicyclo[3.2.2]nona-3,6-dien-2-one	8.094	11.16
-	total	-	99.99

. For other species, stems and leaves have much more importance than flowers. Since there was significant difference between extracts of species of interest, it can be stated that metabolite production in medicinal plants are controlled by wide verities of factors.

Given that environmental factors impose some changes in growth of medicinal plant and their metabolite quality like volatile oils (essences) and so on, it is worthy to note that metabolites are cost effective when the primary and secondary metabolites reach desirable level (Beigi 1995). Various plant species defense against pathogens and organisms themselves through production of various secondary chemicals metabolites. Metabolites quality and quantity vary with different species and various extraction methods (Edeoga *et al*., 2005; Albert *et al*., 2011; Krishnaiah *et al*., 2007). Table 7 indicates that metabolites in verbascum species vary considerably, implying that these metabolites are affected by various factors.

Table 3. The identified ingredients in leaves extracts of *V. songaricum.*

No	Ingredients	Inhibiting time	leaf (%)
1	Pentane, 2,3-dimethyl	3.287	2.14
2	2(3H)-Furanone, dihydro-3,3-dimethyl	3.493	0.78
3	Butanoic acid, butyl ester	3.751	28.3
4	1-Decene, 5-methyl	4.031	3.16
5	Cyclobutane, 1,1-dimethyl-2-octyl	4.105	0.88

6	4-Decene, 9-methyl	4.168	1.19
7	Octane, 2,2-dimethyl	4.317	1.09
8	Cyclopropane, 1,2-dibutyl	4.363	1.72
9	2-Undecene	4.432	6.69
10	1-Decene, 2-methyl	4.54	7.27
11	Butane, 1,1-dibutoxy	6.182	10.68
12	aldol by product	6.211	4.14
13	1-(BUTYLTHIO)-2-PROPANOL	7.561	1.56
14	8.exo.-cyanobicyclo[3.2.2]nona-3,6-dien-2-one	8.088	9
15	gamma.-(4-Fluorophenyl)-.gamma.-butyrolactone	9.478	1.14
16	2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester	9.547	1.08
17	Anthracene	9.896	1.55
18	2,3,3a,8a-Tetrahydro-4,6-dimethoxyfuro[2,3-b]benzofuran	10.565	12.03
19	9,10-Anthracenedione	10.834	1.07
20	4-Vinylcyclooctene	11.412	4.41
-	Total	-	99.88

Table 4. The identified ingredients in leaves extracts of *V. cheirantifolium.*

No	Ingredients	Inhibiting time	leaf (%)
1	4-Heptanone, 3-methyl	3.287	3.4
2	Butanoic acid, butyl ester	3.905	31.38
3	1-Decene, 5-methyl	4.168	3.95
4	Nonane, 2-methyl	4.431	1.51
5	4-Undecene	4.477	2.68
6	5-Undecene	4.54	3.86
7	Butane, 1,1-dibutoxy	6.211	16.76
8	4-vinylphenol	6.766	5.36
9	Cycloheptasiloxane, tetradecamethyl	7.876	7.56
10	Propanoic acid, 2,2-dimethyl-, octyl ester	8.093	10.25
11	Stannane, tetraethyl	8.563	1.13
12	Megastigmatrienone	8.86	1.25
13	3-Chloro-1,4-dimethyl-2-quinolone	9.696	2.12
14	Dillapiole	10.605	8.75
-	Total	-	99.96

Table 5. The identified ingredients in extracts from stem in *V. songaricum.*

No	Ingredients	Inhibiting time	Stem (%)
1	4-Heptanone, 3-methyl	3.299	4.42
2	Butanoic acid, butyl ester	3.911	36.7
3	Cyclopropane, 1-butyl-1-methyl-2-propyl	4.174	5.72
4	Undecane	4.437	2.15
5	5-Undecene	4.483	3.4
6	2-Undecene	4.546	4.92
7	Benzene, 1-cyclohexen	6.217	19.3
8	2-Methoxy-4-vinylphenol	6.772	7.35
9	Propanoic acid, 2,2-dimethyl-, heptyl ester	8.094	11.4
10	Phenol	11.395	2.74
11	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl	11.51	1.8
-	Total	-	99.9

Table 6. The identified ingredients in extracts from stem in *V. cheirantifolium*.

No	Ingredients	Inhibiting time	Stem (%)
1	4-Heptanone, 3-methyl	3.287	3.98
2	Cyclotetrasiloxane, octamethyl	3.859	4.72
3	Butanoic acid, butyl ester	3.905	33.2
4	2-Undecene	4.168	5.14
5	Propanoic acid, nonyl ester	4.432	6.68
6	4-Undecene	4.477	2.86
7	Cyclopropane, 1,2-dibutyl	4.54	4.21
8	6-Aza-5,7,12,14-tetrathiapentacene	5.336	2.29
9	Benzenamine, 2,5-difluoro	6.211	16.49
10	Cyclohexasiloxane, dodecamethyl	6.72	1.45
11	1,1,1,3,5,7,9,9,9-Nonamethylpentasiloxane	7.859	4.13
12	8.exo.-cyanobicyclo[3.2.2]nona-3,6-dien-2-one	8.094	9.61
13	1,3,5,7,9-Pentaethylbicyclo[5.3.1]pentasiloxane2.	8.929	2.07
14	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	9.822	2.99
-	Total	-	99.82

Table 7. Difference of chemical compositions mean in *v. songaricum* with *v. cheirantifolium* in rangeland sites of Dena.

	acid	ester	hydrocarbon	alcohol	ketone	Amin
species	Mean	Mean	Mean	Mean	Mean	Mean
A	22.6 [a]	27.1[a]	29.77 [a]	12.42 [a]	11.44[a]	12.42[a]
B	21.6[b]	1.8[a]	29.38 [a]	15.23 [a]	4.94[a]	15.23[a]

- Uncommon letters represent significant difference in probability level of 5% Species A and B indicate *v. cheirantifolium* and *V. songaricum*

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