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Original Article

Total Phenolic Content, Antioxidant and Antibacterial Activities of Three *Verbascum* Species from Iran

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Abstract

The genus *Verbascum* L. belongs to the family Scrophulariaceae and includes plants that have been used widely in traditional medicine for a long time. Methanolic extracts of three *Verbascum* species from flora of Iran were *in vitro* screened for their possible antioxidant activities by three complementary test systems, namely DPPH free radical-scavenging, metal chelating activity and β-carotene/linoleic acid. The total phenol and flavonoid contents of the methanolic extracts from the aerial parts were measured by Folin Ciocalteu and $AlCl_3$ assays, respectively. In addition, antibacterial activities of the methanolic extracts were studied by disc diffusion method against 3 Gram positive and Gram negative bacteria. Results showed that the methanolic extract of *V. sinuatum* contains the highest amount of phenolic compounds and of *V. speciosum* represents the highest flavonoid content. Results from antioxidant activity assays showed that the studied extracts are more active than ascorbic acid as a synthetic antioxidant in DPPH radical scavenging assay, but represent lower activity in metal-chelating assay. In β-carotene/linoleic acid system, oxidation of linoleic acid was effectively inhibited by *V. speciosum* extract (58.4±18.1mg/g), followed by *V. sinuatum* (51.41±2.28 mg/g). In addition, methanolic extracts of three *Verbascum* species showed strong antibacterial activity against all tested bacteria.

Key words: Antibacterial activity, Antioxidant property, Flavonoids, Phenols, *Verbascum*

Introduction

In recent years, it has been established that free radicals and oxidative stress are involved in the pathophysiology of a variety of disorders including atherosclerosis, chronic renal failure, diabetes mellitus, cancer, immune dysfunction and aging [1-5]. The antioxidant activity of several plant constituents, beyond the vitamins, in the form of crude extract or isolated compound has been put widely into consideration [6-8]. Antioxidant activity of many phenolic compounds, including flavonoids, have attracted considerable attention and reported to be more powerful antioxidants than vitamins C, E and β-carotene which are largely in routine use [9]. Consumption of the flavonoids and their potential significance as antagonists of oxidative stress has been the interesting subject.

The genus *Verbascum* L. belongs to the tribe Verbasceae of the family Scrophulariaceae and has the main centers of its diversity in Turkey, Iran and Pakistan [10-12]. With about 42 species in Iran [13] and 360 species worldwide [14], it is the largest genus within the large family of Scrophulariaceae. Among the species distributed in Iran, 15 species are endemic [13]. Infusions from the leaves and flowers of different *Verbascum* species are still used for their expectorant and demulcent properties to treat respiratory problems such as irritating coughs with bronchial congestion [15]. Because of the presence of mucilaginous constituents and saponins, aerial parts of *Verbascum* species have soothing action on mucous membranes and expectorant action [16]. The plant is reported to be mildly diuretic and to have a soothing and anti-inflammatory effect on the urinary tract [17]. The leaves, roots and flowers are also used as anodyne, antiseptic, antispasmodic, astringent,

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emollient, nervine, analgesic, antihistaminic, anticancer and antioxidant [18].

The aim of this study is *in vitro* assessment of total phenol and flavonoid contents and antioxidant and antibacterial activities of the methanolic extracts of three *Verbascum* species (*V. nudicaule* Wydl.) Takht., *V. sinuatum* L. and *V. speciosum* Schrad.). A literature search did not reveal reference to previous spacious work on the phenol and flavonoid contents and antioxidant activity of the species studied here.

Material and Methods

Chemicals

1, 1- Diphenyl-2-picrylhydrazyl (DPPH), quercetin, linoleic acid and β-carotene were purchased from Sigma (St. Louis, MO, USA). Gallic acid, Folin-Ciocalteu reagent, Tween 40, chloroform, sodium bicarbonate, aluminum chloride, potassium acetate, butylated hydroxytoluene (BHT), ascorbic acid, Muller-Hinton agar, dimethyl sulfoxide (DMSO), ethanol and methanol were purchased from Merck (Darmstadt, Germany).

Plant Materials

Verbascum plants were collected from Hamedan province, W Iran in July to August 2012. Voucher specimens were deposited in the herbarium of Bu-Ali Sina University (BASU), Hamedan, Iran. Plant materials were dried at room temperature and ground in a mortar. For preparation of methanolic extract, 20 g of plant powder was extracted in 250 ml of methanol by Soxhlet. The solvent was removed under the vacuum at temperature below 70 °C to yield a waxy material. Finally, the extracts were lyophilized and kept in 4 °C until tested.

Determination of Total Phenol

Determination of total phenol content of the extracts was carried out following the Folin-Ciocalteu method by Singleton and Rossi [19]. Briefly, 100 µl of the methanolic extract (1 mg/ml) was mixed with 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min; 0.75 ml of sodium bicarbonate (60 g/l) solution was added and mixed thoroughly. The samples were measured spectrophotometrically (UV-Spectrophotometer, Perkin Elmer, USA) at 765 nm after 90 min at 22 °C. The amount of total phenolics was determined as gallic acid equivalent (GAE) and expressed as mg GAE/g dry weight.

Determination of Total Flavonoids

The flavonoids content was determined by aluminium trichloride method using quercetin as a reference compound [20]. This method is based on the formation of a complex flavonoid-aluminum having the absorptivity maximum at 415 nm, after remaining reaction at room temperature for 30 min. Briefly, 0.5 ml of each extract (1:10 g/ml) was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The calibration curve was prepared by preparing quercetin solution in methanol at different concentrations from 12.5 to 100 g/ml.

DPPH Free Radical Scavenging Activity

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of the purple-coloured methanol solution of DPPH. The stable 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts. In order to determine the radical scavenging ability, the method reported by Mensor et al. [21] was used. Briefly, methanolic solution of 0.5 ml of DPPH (0.3 mM) was added to 2.5 ml of the different concentrations of plant extracts (0.2, 0.4, 0.6, 0.8, and 1 mg/ml). The samples were first kept in the dark and allowed to react at room temperature and then their absorbance was read at 517 nm after 30 min. The antiradical activity (I) was determined using the following formula:

$$I\% = 1- (A_s – A_b)/A_c \times 100$$

Blank samples (A_b) contained 1 ml methanol and 2.5 ml of various concentrations of extract; control sample (A_c) containing 1 ml of 0.3 mM DPPH and 2.5 ml methanol. The optical density of the samples, the control and the empty samples were measured in comparison with methanol. The discoloration was plotted against the sample concentration in order to calculate the IC_{50} value, which is the amount of sample necessary to decrease the absorbance of DPPH by 50% [22].

Metal Chelating Activity

The chelating activity for ferrous ions was measured by the method of Dinis et al. [23]. The reaction mixture contained 0.5 ml of various concentrations of test compounds, 1.6 ml of deionized water and 0.05 ml of 2 mM of $FeCl^{2+}$ solution. After 30 s, 0.1 ml of 5 mM ferrozine solution was added. Fe^{2+}-Ferrozine magenta complex was very soluble and stable in water. After 10 min at room temperature, the absorbance at 562 nm was measured. The relative activities of test compounds to chelate ferrous iron

were expressed as percentage (%) of absorbance disappearance as follows:

Metal-chelating activity (%) = $(A_{control} - A_{sample})/ A_{control} \times 100$

Where A_s is the reaction mixture absorbance in the presence of the plant extract; A_c is the reaction mixture absorbance in the absence of the plant extract.

Antioxidant Activity in β-carotene/linoleic Acid Model System

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [24]. A stock solution of β-carotene/linoleic acid was prepared as follows: first, 0.5 mg of β-carotene was dissolved in 1 ml of chloroform, then 25 µl of linoleic acid and 200 mg of Tween 40 were added. The chloroform was subsequently evaporated using a vacuum evaporator. Then 100 ml of distilled water saturated with oxygen (30 min at 100 ml/min) were added with vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were transferred to test tubes, and 350 µl portions of the extracts (2 g/l in ethanol) were added before incubating for 48 h at room temperature. The same procedure was repeated with BHT at the same concentration and a blank containing only 350 µL of ethanol. After the incubation period, the absorbances of the mixtures were measured at 490 nm. Antioxidant capacities of the samples were compared with those of BHT and the blank.

Antibacterial Activity

Extracts were dissolved in DMSO to a final concentration of 2 mg/ml. Antibacterial activities of the methanolic extracts were evaluated against 2 gram positive (*Haemophilus influenzae* PTCC 1623 and *Bacillus cereus* PTCC 1247) and one gram negative (*Pseudomonas aeruginosa* PTCC 1430) bacteria, by disc diffusion method [25] at 4 different concentrations (100, 50, 25 and 12.5 mg/ml of extracts). The extracts were dissolved in DMSO to make a 100 mg/ml solution and other concentrations make from this concentration, and then apply on the blank sterile paper discs. Dried discs were placed onto Muller-Hinton agar medium that previously inoculated with a bacterial suspension (1.5×10^8 bacteria/ml). The cultures were incubated at 37 ^oC for 24 h. The antibacterial activity against each test organism was quantified by determining mean zone of inhibition. Gentamycin, penicillin, tetracycline, ampicillin and neomycin were also used as positive controls.

Statistical Analysis

The data were recorded as means ± standard deviation. Analysis of variance was performed by Excel and SAS (VER, 9.2) procedures. Statistical analysis was performed using P value < 0.05 was considered significant.

Results and Discussion

Total Phenol and Flavonoid Contents

Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals [26,27]. As shown in Table 1, the content of total phenols was measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: $y = 0.0041x - 0.022$, $R^2 = 0.9982$) The phenol content of *V. sinuatum* extract (118.2 ± 2.46 mg/g dry weight) was higher than the extracts of *V. speciosum* (95.83 ± 1.39 mg GAE/g DW) and *V. nudicaule* (72.95 ± 0.33 mg GAE/g DW). The results showed that there are significant differences between three *Verbascum* species in the content of total phenols. The flavonoid content in terms of quercetin equivalent (the standard curve equation: $y = 0.0067x + 0.0132$, $R^2 = 0.999$) showed that *V. speciosum* (5.77 ± 0.23 mg QE/g DW) was superior to the other species extracts studied. However, there are no significant differences between *V. sinuatum* and *V. nudicaule* in the content of total flavonoids (Table 1, Fig. 1). The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect [28,29]. It is well known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores [30].

Antioxidant Activity

In the light of the differences among the wide number of test systems available, the results of a single-assay can give only a reductive suggestion of the antioxidant properties of extracts toward food matrices and must be interpreted with some caution. Moreover, the chemical complexity of extracts, often a mixture of dozens of compounds with different functional groups, polarity and chemical behavior,

Table 1 Total phenol and flavonoid contents of three *Verbascum* species.

Species	Total phenol (mg GAE/g DW[a])	Total flavonoid (mg QE/g DW[a])
V. nudicaule	72.95 ± 0.33 [c]	4.83 ± 0.13 [b]
V. sinuatum	118.2 ± 2.46 [a]	4.87 ± 0.06 [b]
V. speciosum	95.83 ± 1.39 [b]	5.77 ± 0.23 [a]

[a]DW: Dry Weight
Experiment was performed in triplicate and expressed as mean ± SD. Values in each row with different superscripts are significantly different ($P < 0.05$).

Table 2 DPPH radical scavenging activity of three *Verbascum* species and ascorbic acid.

Species	DPPH concentration (mg/ml)					Average
	0.2	0.4	0.6	0.8	1	
V. nudicaule	89.33 ± 2.04[c]	93.15 ± 0.67[b]	93.28 ± 0.27[b]	94.03 ± 0.59[b]	96.73 ±1.89[a]	93.3
V. sinuatum	88.62 ± 0.78[b]	89.89 ± 1.19[ab]	90.38 ± 0.95[ab]	90.55 ± 0.33[a]	90.7 ± 1.13[a]	89.95
V. speciosum	91.36 ± 8.14[a]	93.513 ± 0.46[a]	94.36 ± 0.18[a]	94.95 ± 0.23[a]	95.08 ± 0.44[a]	91.98
Ascorbic acid	80.00 ± 0.80[a]	80.84 ± 1.70[a]	81.21 ± 1.40[a]	80.84 ± 2.30[a]	80.16 ± 1.9[a]	80.61

Experiment was performed in triplicate and expressed as mean ± SD. Values in each row with different superscripts are significantly different ($P < 0.05$).

Table 3 Metal chelating effect on ferrous ions by three *Verbascum* species and ascorbic acid.

Species	Fe-Chelate concentration (mg/ml)					Average
	0.2	0.4	0.6	0.8	1	
V. nudicaule	0.85 ± 0.30 [b]	0.96 ± 0.30[b]	0.84 ± 0.58[b]	3.50 ± 0.50[b]	19.06 ± 8.80[a]	5.04
V. sinuatum	8.49 ± 0.05 [a]	9.05 ± 0.73[a]	10.66 ±4.92[a]	11.74 ± 2.17[a]	14.44 ± 4.89[a]	10.88
V. speciosum	3.94 ± 2.74 [a]	3.19 ± 0.03[a]	3.38 ± 0.09[a]	4.25 ± 2.46[a]	5.420± 0.72 [a]	4.04
Ascorbic acid	4.02 ± 2.00 [a]	7.48 ± 3.80 [a]	10.70 ± 1.20[a]	17.40 ± 2.50[b]	21.80 ± 3.30[b]	12.3

Experiment was performed in triplicate and expressed as mean ± SD. Values in each row with different superscripts are significantly different ($P < 0.05$).

could lead to scattered results, depending on the test employed. Therefore, an approach with multiple assays in screening work is highly advisable. Among the plethora of methods that can be used for the evaluation of the antioxidant activity, very few of them are useful for determining the activity of both hydrophilic and liphophilic species, thus ensuring a better comparison of the results and covering a wider range of possible applications [31].

Free radical-scavenging capacities of the corresponding extracts were measured by DPPH assay and the results are shown in Table 2. All of the species extracts at different concentrations exhibited more than 80% scavenging activity (Fig. 2). The IC_{50} value of Ascorbic acid as the standard compound was 0.125; while the IC_{50} value of *V.sinuatum* and *V. nudicaule* extracts were 0.11 (mg/ml) represents high antioxidant activity of the *V. speciosum* and Ascorbic acid. The radical scavenging activity in the plant extracts decreased in the following order: *V. nudicaule* = *V. sinuatum* (IC_{50} = 0.11 mg/ml) > *V. speciosum* (IC_{50} = 0.125 mg/ml) (Fig. 3).

Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases [32]. DPPH radical was used as a stable free radical to determined antioxidant activity of natural compounds [33]. The effects of phenolic compounds on DPPH radical scavenging are thought to be due to their hydrogen donating ability [34]. It is reported that the decrease in the absorbance of DPPH radical caused by phenolic compounds is due to the reaction between antioxidant molecules and radicals, resulting in the scavenging of the radical by hydrogen donation and is visualized as a discoloration from purple to yellow [35].

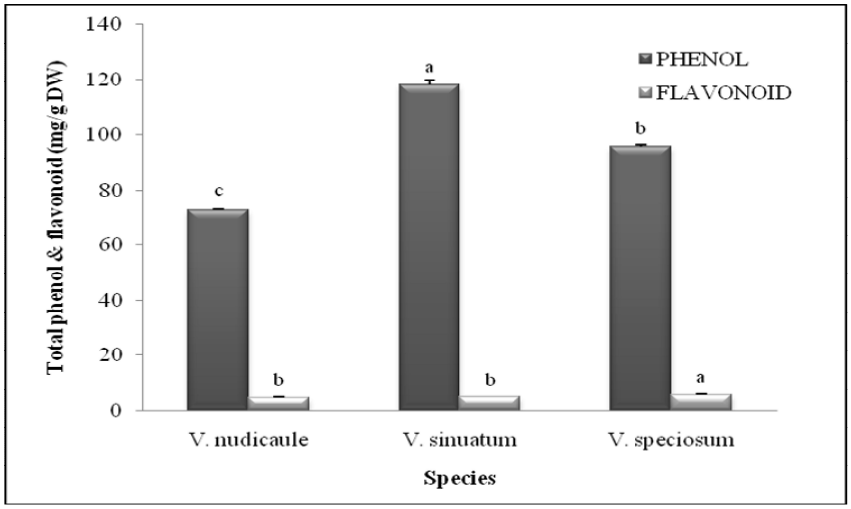

Fig. 1 Comparison of total phenol and flavonoid contents between three *Verbascum* species.

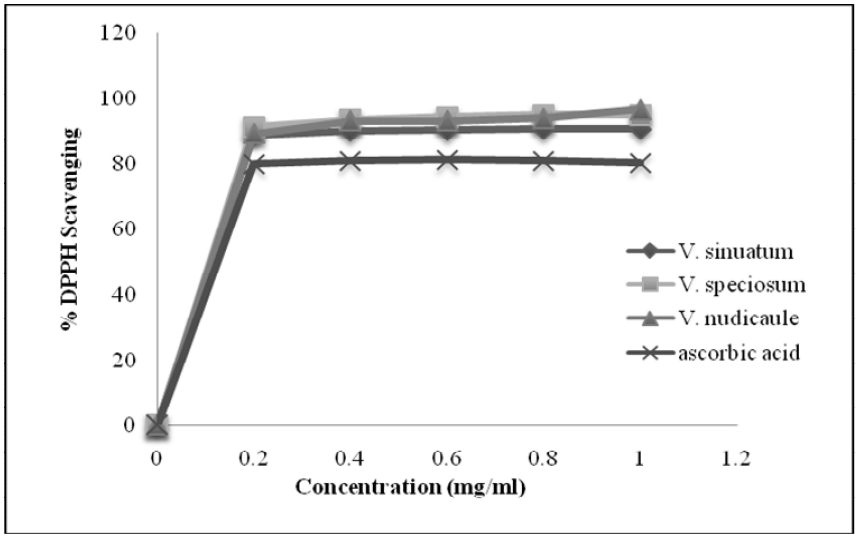

Fig. 2 DPPH radical scavenging activity of three *Verbascum* species at different concentrations.

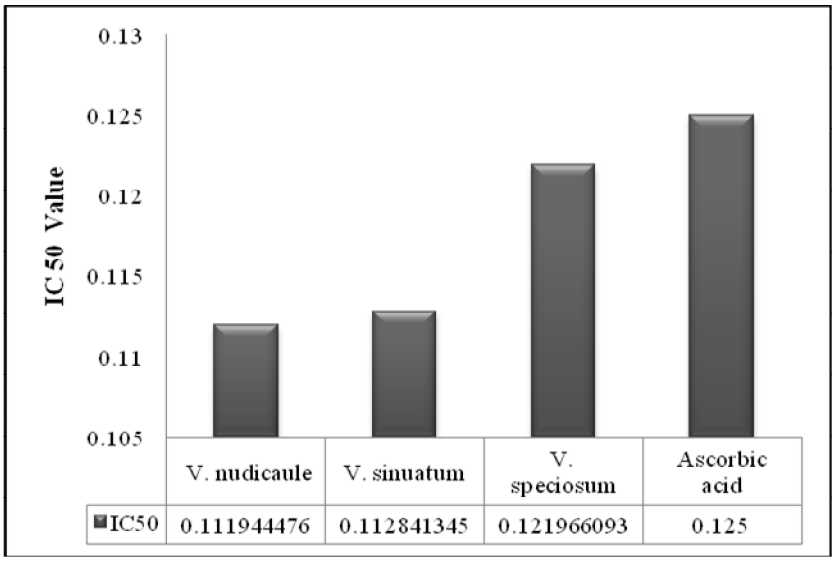

Fig. 3 IC_{50} values of DPPH scavenging activity of three *Verbascum* species and ascorbic acid.

Metal Chelating Activity

Chelating agents may inhibit radical generations by stabilizing transition metals consequently reducing free radical damage. In addition, some phenolic compounds exhibit antioxidant activity through the chelating of metal ions [12]. In the metal chelating assay, ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with consequent decrease in the intensity of the red color of the complex [36].

Analysis of metal-chelating properties showed that all the extracts studied were capable of chelating iron and did so in a concentration-dependent manner but average them weaker than ascorbic acid as synthetic antioxidant. The methanolic extracts of three *Verbascum* species showed that metal chelating activity was enhanced with increasing concentration (Table 3). The highest metal chelating activity average was showed by *V. sinuatum*. Analysis of metal chelating properties showed that all extracts studied have weaker activity than ascorbic acid as a synthetic antioxidant (Fig. 4).

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components [37].

In the β-carotene/linoleic acid system (Table 4), oxidation of linoleic acid was effectively inhibited by *V. speciosum* extract (58.42% ± 18.1), followed by *V. sinuatum* (51.41% ± 2.28) and *V. nudicaule* (48.51% ± 0.58). Results from present study showed that methanolic extracts in three species *Verbascum* have not significantly different in inhibition oxidation of linoleic acid abilities (Fig. 5). Analysis of β-carotene/linoleic acid system showed that all extracts studied have weaker activity than BHT as a synthetic antioxidant. In the β-carotene bleaching assay, some medical plant extracts showed moderate to high antioxidant capacity. Tepe et al. [38] in a study on the antioxidant properties of some endemic species to Turkey showed that the highest antioxidant capacity was related to *Pelargonium endlicherianum* extract (72.6% ± 2.96), followed by *Hieracium cappadocicum* (55.1% ± 2.33), *Verbascum wiedemannianum* (52.5% ± 3.11), *Sideritis libanotica* Labill. subsp. *linearis* (38.5% ± 2.33) and *Centaurea mucronifera* (35.2% ± 3.04).

Antibacterial Activity Assay

Antibacterial activities of methanolic extracts from the aerial parts of *Verbascum* were evaluated against 3 Gram negative and Gram positive bacteria, namely *Haemophilus influenzae*, *Pseudomonas aeruginosa* and *Bacillus cereus* by disc diffusion method [25] at 4 different concentrations. Methanol extracts of three *Verbascum* species showed antibacterial activity against all bacteria tested. *H. influenzae* was the most sensitive bacteria to *V. speciosum* extract.

Table 4 Inhibition percentage of the linoleic acid oxidation by three *Verbascum* species and BHT

Extracts	Inhibition (%)
V. nudicaule	48.51 ± 0.58^{b}
V .sinuatum	51.41 ± 2.28^{b}
V. speciosum	58.42 ± 18.1^{b}
BHT	100.0 ± 3.15^{a}
Control^{-}	26.88 ± 0.22^{c}

Experiment was performed in triplicate and expressed as mean ± SD. Values in each row with different superscripts are significantly different ($P < 0.05$).

Senatore et al. [39] showed that the methanolic extract of *V. sinuatum* showed inhibition against all bacterial strains tested (MIC between 15.5 and 250 µg/ml). Generally the gram positive bacteria were the most sensitive to the extract; among these, however *Staphylococcus epidermidis* showed the lowest MIC (15.5 µg/ml). The gram negative bacteria were less sensitive and the extract showed an antibacterial activity (MIC 62 µg/ml) only against *Proteus vulgaris*, *Proteus mirabilis* and *Citrobacter diversus.* *Pseudomonas aeruginosa* was the sensitive bacterium to *V. sinuatum* and *V. nudicaule* extracts only at 100 mg/ml. Antibacterial activity of aqueous and alcohol extracts obtained from flowers of *Verbascum speciosum* which were investigated considering its *in vitro* antibacterial effect against *Bacillus subtilis*, *Bacillus cereus* and *Escherichia coli*. The results show that ethanol extract was more effective than aqueous against three microorganisms in all tested doses. In case of both aqueous and ethanol extracts, the maximum antibacterial activity was shown against *B. cereus* followed by *B. subtilis* and *E. coli* was most resistant strain [40].

Results of antibacterial activity from all extracts are presented in Tables 5, 6 and 7. Our results showed that extracts are a great source of phenolic compounds and represents the antibacterial activity against gram-positive and negative bacteria.

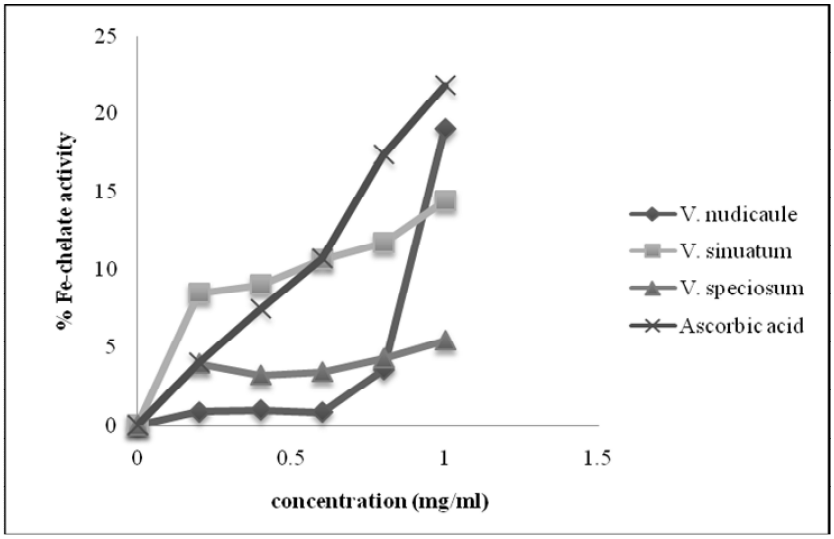

Fig. 4 Metal-chelating activity on ferrous ions by methanolic extracts of three *Verbascum* species and ascorbic acid.β-Carotene-linoleic acid assay

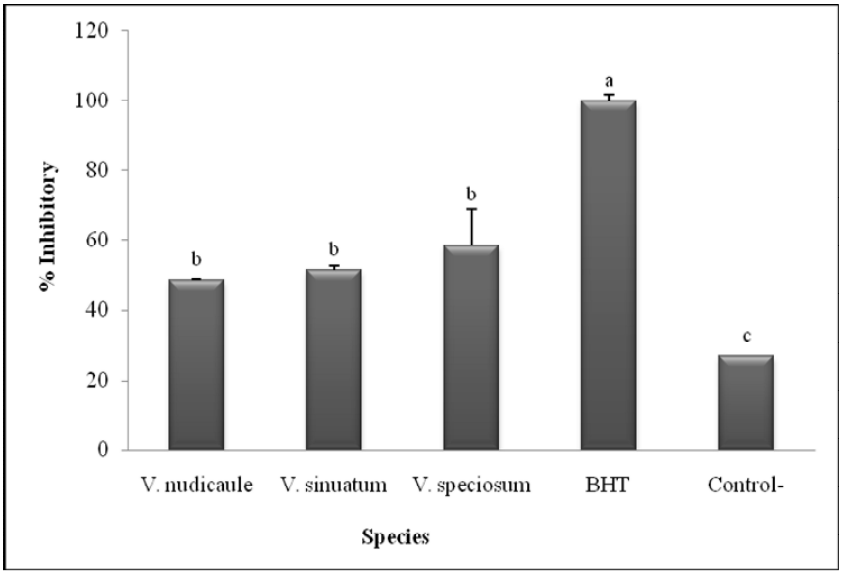

Fig. 5 Inhibition of bleaching of ß-carotene/linoleic acid emulsion by three *Verbascum* species.

Table 5 Average inhibition zone of evaluated strains against methanolic extract of *V. nudicaule.*

Bacteria	Inhibition zone				
	Concentration (mg/ml)				
	100	50	25	12.5	DMSO
Haemophilus influenzae	NA	10 ± 0.00 [a]	NA	NA	NA
Pseudomonas aeruginosa	14.67 ± 4.62 [a]	NA	NA	NA	NA
Bacillus cereus	11.3 ± 1.15 [a]	NA	NA	NA	NA

NA: No Active

Experiment was performed in triplicate and expressed as mean ± SD. Values in each row with different superscripts are significantly different ($P < 0.05$).

Table 6 Average inhibition zone of evaluated strains against methanolic extract of *V. sinuatum.*

Bacteria	Inhibition zone				
	Concentration (mg/ml)				
	100	50	25	12.5	DMSO
Haemophilus influenzae	NA	10 ± 0.00 [a]	NA	NA	NA
Pseudomonas aeruginosa	14.67 ± 4.62 [a]	NA	NA	NA	NA
Bacillus cereus	11.3 ± 1.15 [a]	NA	NA	NA	NA

NA: No Active

Experiment was performed in triplicate and expressed as mean ± SD. Values in each row with different superscripts are significantly different ($P < 0.05$).

Table 7 Average inhibition zone of evaluated strains against methanolic extract of *V. speciosum.*

Bacteria	Inhibition zone				
	Concentration (mg/ml)				
	100	50	25	12.5	DMSO
Haemophilus influenzae	15.3± 4.16 [a]	12.67 ± 1.15 [ab]	10.67 ± 1.15 [b]	10.25 ± 3.2 [b]	NA
Pseudomonas aeruginosa	20.0 ± 0.0 [a]	13.30 ± 3.06 [b]	NA	NA	NA
Bacillus cereus	14.6 ± 3.06 [a]	10.66 ± 2.31 [a]	NA	NA	NA

NA: No Active

Experiment was performed in triplicate and expressed as mean ± SD. Values in each row with different superscripts are significantly different ($P < 0.05$).

Conclusion

Results from the present study showed that the methanolic extract of *V. sinuatum*, which contain the highest amount of phenolic compounds exhibited the greatest antioxidant activity. Three *Verbascum* species showed a higher potency than ascorbic acid in scavenging of DPPH free radical. Our data indicate that all three *Verbascum* species are potential sources of secondary metabolites and methanolic extracts possess a good antioxidant activity. Further studies are needed to evaluate the *in vivo* potential of these extracts in animal models and also isolation and characterization of the active antioxidant compounds. Determination of the antioxidant compounds of plant extracts and essential oils will help to develop new drug supplement for antioxidant therapy. From this point of view, the results presented here could be considered as the first information on the antioxidant activities of the plant species studied.

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References

1. Halliwell B. The antioxidant paradox. Lancet 2000;355: 1179-1180.
2. Heinecke JW. Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis. The Am J Cardiol. 2003;91:12A-16A.
3. Metodiewa D, Koska C. Reactive oxygen species and reactive nitrogen species: relevance to cyto(neuro) toxic events and neurologic disorders. An overview. Neurotox Res. 1999;1:197-233.
4. Maxwell SRJ. Prospects for the use of antioxidant therapies. Drugs 1995;49:345-361.
5. Noguchi N, Niki E. Phenolic antioxidants: A rationale for design and evaluation of novel antioxidant drug for atherosclerosis. Free Rad Biol and Med. 2000;28:1538-1546.
6. Gazzani G, Papetti A, Massolini G, Daglia M. Anti and Prooxidants activity of water soluble components of some common diet vegetables and the effect of thermal treatment. J Agric Food Chem. 1998; 46: 4118-4122.
7. Larson RA. The antioxidants of higher plants. Phytochemistry 1988;27:969-978.
8. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agric Food Chem. 1998;46:4113-4117.
9. Vinson JA, Hao Y, Su X, Zubik L. Phenol antioxidant quantity and quality in foods: vegetables. J Agric Food Chem. 1998;46:3630-34.

10. Huber-Morath A. *Verbascum* L. In: Davis PH [ed.], Flora of Turkey and the East Aegean Islands, Edinburgh: Edinburgh Univ Press, 1978; 6: 461-603.
11. Valdes B. Scrophulariaceae. In: Valdes B, Talvera S, Fernandez Galiano E [eds.], Flora vascular De Andalucia Occidental, Barcelona: Ketres, 1987;2:486-547.
12. Zohary M. Geobotanical Foundations of Middle East (Fisher, Stuttgart) Jerusalem: Israel, Hebrew University, 1973, pp. 307-329.
13. Sharifnia F. Notes on the distribution and taxonomy of *Verbascum* in Iran. Iranian J Bot. 2007;31: 30-32.
14. Judd WS, Campbell CS. Kellogg EA, Stevens PF. Plant Systematic: A Phylogenetic Approach. Sinauer, Sunderland, 1999, pp. 576.
15. Hoffman D. The Herbal Handbook: A User's Guide to Medicinal Herbalism. Healing Arts Press Rochester, 1988, pp. 67.
16. Berk SA. The Naturalist's Herb Guide. New York: Black Dog and Leventhal Publishers, 1996, pp. 162.
17. Mabey R. The new age herbalist. New York: Macmillan Publishing Company, 1988, pp. 113.
18. Grieve M. A Modern Herbal (Vol. II). The Medicinal, Culinary, Cosmetic, and Economic Properties, Cultivation and Folklore of Herbs, Grasses, Fungi, Shrubs and Trees with all their Modern Scientific Uses. New York: Dover Publications, 1981, pp. 562-566.
19. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16:144-158.
20. Chang C, Yang M, Wen H, Chern J. Estimation of Total Flavonoid Content in *Propolis* by Two Complementary Colorimetric Methods. J Food Drug Anal. 2002;10:178-182.
21. Mensor LL, Menezes FS, Leitao GG, Reis AS, Santos TS, Coube CS. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Photother Res. 2001; 15:127-130.
22. Koleva II, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity, a comparative study on three testing methods. Phytochem Anal. 2002;13: 8-17.
23. Dinis TCP, Madeira VMC, Almeida MLM. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys**.** 1994; 315: 161-169.
24. Barriere C, Centeno D, Lebert A, Leroy-Setrin S, Berdague JL, Talon R, Talhouk SN. Roles of superoxide dismutase and catalase of Staphylococcus xylosus in the inhibition of linoleic acid oxidation. FEMS Microbiol Lett. 2001; 201: 181–185.
25. Bauer AW, Kirby WM, Sherries JC, Turck M. Antibiotic susceptibility testing by single disk method. American J Clin Pathol. 1966; 45: 493–496.
26. Decker EA. Phenolics: Prooxidants or Antioxidants? Nutr Rev. 1997; 55: 396-407.
27. Shahidi F, Janitha PK, Wanasundara PD. Crit Rev Food Sci Nutr. 1992; 32: 67-103.
28. Das NP, Pereira TA. Effect of flavonoids on thermal auto oxidation of Palm oil: structure- activity relationship. J Am Oil Chem Soc. 1990; 44:255-258.
29. Younes M. Inhibitory action of some flavonoids on enhanced spontaneous lipid peroxidation following glutathione depletion. Planta Med. 1981; 43:240-245.
30. Vaya J, Belinky PA, Aviram M. Antioxidant constituents from licorice roots: Isolation, structure elucidation and antioxidative capacity toward LDL oxidation. Free Rad Biol Med. 1997; 23(2): 302-313.
31. Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem. 2005; 91: 621–632.
32. Gyamfi MA, Yonamine M, Aniya Y. Free-radical scavenging action of medicinal herbs from Ghana Thonningia sanguinea on experimentally- induced liver injuries. Gen Pharmacol. 1999; 32(6): 661- 667.
33. Ozturk M, Ozturk FA, Duru ME, Topcu G. Antioxidant activity of stem and root extracts of *Rhubarb (Rheum ribes):* An edible Med plant. Food Chem. 2007; 103: 623-630.
34. Siddaraju MN, Dharmesh SM. Inhibition of gastric H^+, K^+-ATPase and *Helicobacter pylori* growth by phenolic antioxidants of *Curcuma amada*. J Agric Food Chem. 2007; 55: 7377-7386.
35. Meir S, Kanner J, Akiri B, Philosof-Hadas S. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J Agric Food Chem. 1995; 43: 1813-1817.
36. Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H. Antioxidative and Anti-glycation activity of garcinol from *Garcinia indica* fruit rind. J Agric Food Chem. 2000; 48(2):180-185.
37. Smith C, Mitchinson MJ, Arouma OI, Halliwell B. Stimulation of lipid peroxidation and hydroxyl radical generation by the contents of human atherosclerosis lesions. Biochem J. 1992; 15: 905- 910.
38. Tepe B, Sokmen M, Akpulat HA, Yumrutas O, Sokmen A. Screening of antioxidative properties of the methanolic extracts of *Pelargonium endlicherianum* Fenzl., *Verbascum wiedemannianum* Fisch. & Mey., *Sideritis libanotica* Labill. subsp. *linearis* (Bentham) Borm., *Centaurea mucronifera* DC. and *Hieracium cappadocicum* Freyn from Turkish flora. Food Chem. 2006; 98: 9–13.
39. Senatore F, Rigano D, Formisano C, Grassia A, Basile A, Sorbo S. Phytogrowth-inhibitory and antibacterial activity of *Verbascum sinuatum*. Fitoterapia 2007; 78: 244–247.
40. Amirnia R, Khoshnoud H, Alahyary P, Ghiyasi M, Tajbakhsh M, Valizadegan O. Antimicrobial activity of *Verbascum speciosum* against three bacteria strains. Fresenius Environ Bull. 2011; 20: 690-693.