

Antioxidant and Phenolic Characterization with HPLC of Various Extract of *Verbascum glomeratum* Linneus

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Summary: *Verbascum* L. species have been used since ancient times in traditional medicine thanks to their bioactive compounds. In this study, we evaluated the antioxidant activity and phenolic contents of ethanolic and acetic extract of *Verbascum glomeratum* that collected from Denizli, Turkey. The antioxidant activity were evaluated by using various methods (β -carotene/linoleic acid, ferric reducing power assays (FRAP), radical scavenging assays of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS), phosphomolibdenum methods) and total phenolic, flavonoid and tannin contents in the extracts were determined. Antioxidant activity was determined as β -carotene/linoleic acid (58.5 ± 0.021), DPPH (IC₅₀: 1.220 ± 0.025) and ABTS (IC₅₀: 1.552 ± 0.021), ferric reducing antioxidant power (FRAP) (4.151 ± 0.056 mgTE/g), phosphomolibdenum assay (8.218 ± 0.015 mgAE/g) total phenolic, total flavonoid and tannin contents was found as (4.41 ± 0.007 mgGAE/mL), (87.142 ± 0.009 μ QE/g), and (30.178 ± 0.008 mgCEs/g) respectively, and then 14 different phenolic compounds in ethanol extracts was carried out by HPLC. Epicatechin (2742.09 μ g/g) and 2,5dihidroksi (2544.96 μ g/g) were the most abundant phenolic constituents in the extracts. According the results, the extract of *V. glomeratum* may be considered as a potential source of biological agents.

Key words: *Verbascum glomeratum*, Antioxidant, HPLC.

Introduction

Verbascum L. is the largest genus of the family Scrophulariaceae, with about 2500 species worldwide. The generic name of *Verbascum* is believed to be a corruption of *barbascum*, from the Latin *barba*, meaning a beard, referring to the shaggy appearance of the genus. The genus *Verbascum* is represented by 232 species, 196 of which are endemic, in the flora of Turkey [1].

Verbascum species have numerous medicinal properties. Various preparations of some species of this genus have been used as expectorant and mucolytic, as well as sudorific, sedative and constipate in traditional Turkish medicine [2]. *Verbascum* species are also used externally for desiccating wounds, anal fistula and pruritic conditions in urogenital organs [3]. Additionally, in Europe, Asia and Northern America, several *Verbascum* species have been reported as antiseptic, astringent, demulcent, emollient, expectorant, sedative, narcotic, diuretic and antimalarial and as a treatment for tumors, inflammations, migraine, asthma and spasmodic coughs [4].

The species are also used to treat haemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhoea, and have inhibitory activities against the murine lymphocytic leukaemia and influenza viruses A2 and B. *Verbascum* species have been used since ancient times in traditional

medicine in therapy including that for scabies, eczema, tumors, and various inflammatory affections [5]. The importance of phytochemicals is due to their numerous biological activities such as antioxidant, anti-inflammatory, anti-carcinogenic and others. The traditional medicines always remain the primary source of health care in developing countries [6].

The oil made from the flowers is used to help soothe earache and can be applied externally for eczema and other types of inflammatory skin conditions. For example, the leaves, roots, and flowers have also anodyne, antispasmodic, nervine, vulnerary, analgesic, antihistaminic, anticancer, antioxidant, antiviral, bactericide, cardiodepressant, oestrogenic, fungicide and hypnotic activities [7]. Many wild edible plants are traditionally used in the human nutrition and consumed as vegetable in Turkey [8].

This study aimed to evaluate the several biological activity tests, such as antioxidant (β -carotene/linoleic acid, ferric reducing power assays (FRAP), free radical scavenging assays of 2,2-diphenyl-1-picrylhydrazyl (DPPH) phosphomolibdenum test and 2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS)), total phenolic and flavonoid contents) and quantification phenolic contents of *V. glomeratum* by HPLC method.

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Experimental

Plant material

Verbascum glomeratum (Scrophulariaceae) species were collected from Denizli, at Pamukkale University's around in Spring 2017. The species identified by the Laboratory of Botanic of Pamukkale University. The plant samples were cleaned and dried in the shadow for extraction. The voucher specimen was deposited at the herbarium of Pamukkale University the Laboratory of botanic under the "PAU2015-2023" number.

Extraction preparation

Dried plant samples were pulverized. Each ground sample (10 gr) was transferred into a beaker. Ethanol and acetone (100 mL respectively) were added and they were put in water bath at 55°C for 6 h. The extraction mixture was separated from the residue by filtration through Whatman No:1 filter paper. The plant residue was re-extracted triplicates with ethanol and acetone. After the filtration two extracts were combined. The residual solvent of ethanol and acetone extracts of samples were removed under reduced pressure at 48–49°C using a rotary evaporator (rotavapor IKA VB 10, Germany). The water in the extract was lyophilized using a freeze dryer (Thermosavant Modulyo D, USA). The yields of these fractions were 3.6 and 2.7 g respectively.

Analysis of phenolic contents by HPLC

Phenolic compounds were analyzed by high performance liquid chromatography (HPLC) according to the method of Caponio *et al.*, with some modification [9]. Detection and quantification were performed with a diode array detector (SPD-M20A), a LC-20AT pump, a CTO-10ASVp column heater, SIL-20ACHT auto sampler, SCL-10Avp system controller and DGU-14A degasser. Separations were carried out at 30°C. The eluates were identified at 280 nm. The mobile phases were A: 3.0% formic acid in distilled water and B: methanol. The elution gradient applied at a flow rate of 1 ml min⁻¹ was: 95% A/5% B for 5 min, 80%A/20%B in 15 min, 60%A/40%B in 10 min, 50%A/50%B, in 10 min, 40%A/60%B in 10 min, 30%A/70%B 10 min, 100%B in 10 min until the end of the run. Methanol was used to dissolve samples, and then 20 µL of this solution was injected into the column. Gallic acid, 3,4- hydroxybenzoic acid, 4-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, cinnamic acid (Sigma-

Aldrich Co., USA) were used as standards. The differentiation and quantitative analysis were made by comparing the standards. The quantity of each phenolic compound was expressed as µg per gram of the extract.

Determination of Total Antioxidant Activity

Phosphomolybdenum method

Antioxidant activities of acetone and ethanol extracts were evaluated by phosphomolybdenum method according to Prieto *et al.*, (1999) [10]. In phosphomolybdenum method, different concentrations of extracts (0.3 mL) were combined with 3 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was placed in test tubes and the tubes were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm against a blank.

Ferric reducing power methods

The reducing power of the extracts was determined according to the method of Oyaizu [11]. Extracts (0.10 mg) in phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) were added to potassium ferricyanide (2.5 mL, 10 g/L) and the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 mL, 100.0 g/L) was added to the mixture, which was then centrifuged at 650 *x g* for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 1.0 g/L), and then the absorbance was read spectrophotometrically at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

Total phenolic content

The concentrations of total phenolic content in the crude extracts were expressed as gallic acid equivalents (mg/gGAE extract) were determined with Folin-Ciocalteu reagent (FCR) according to the method of Slinkard and Singleton [12]. Briefly, 1 ml of the solution extracts (1 mg) was added to 46 ml of distilled water and 1 ml of FCR and was mixed thoroughly. After 3 min, the mixture was added to 3 ml of sodium carbonate (2%) and shaken intermittently for 2 h. The absorbance was measured at 760 nm with spectrophotometer. Gallic acid was used for calibration and the results were expressed as mg of gallic acid equivalents dry weight.

Total flavonoid content

Total flavonoid content was determined as described by Arvouet Grand *et al.*, with some modifications [13]. 1 milliliters of 2% aluminum chloride (AlCl₃) in methanol were mixed with the same volume of each sample solution of plant extract (1mgmL⁻¹). Absorption readings at 415 nm were taken after 10 min against a blank solution. The mean of three readings was used and expressed as mg quercetine equivalent (QE) per gram of extract.

Determination of DPPH free radical scavenging activity

Free radical scavenging activity of the extracts was determined using method of Wu *et al.* [14]. In this method, 0.1 mM solution of DPPH in methanol was prepared and 4 mL of this solution was added to 1 mL (0.2 - 1.0 mg) of extracts in, ethanol, acetone and dH₂O at different concentrations. 30 minutes later, the absorbance was measured at 517 nm. Synthetic antioxidant BHT (butylated hydroxytoluene) was used as a positive control. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Free radical scavenging activity was measured using the equation below:

$$\text{Scavenging activity} = [(A_0 - A_1 / A_0) \times 100]$$

where A₀ is the absorbance of the control (blank, without extract) and A₁ is the absorbance in the presence of the extract. The results were expressed as IC₅₀ (the concentration required to inhibit 50% of the DPPH)

β-Carotene–linoleic Acid Assay

The antioxidant activity of the crude extracts was evaluated using the β-carotene-linoleic acid test system with slight modifications [15]. β-Carotene (0.2 mg) in 1 mL of chloroform was added to 20 μL of linoleic acid and 200 mg of Tween-20 emulsifier mixture. The mixture was then evaporated at 40°C for 10 min by means of a rotary evaporator to remove chloroform. After evaporation of chloroform, 100 mL of distilled water saturated with oxygen, 4.8 mL of this emulsion was placed into test tubes which had 0.2 mg of the sample and 0.2 of the extract in them. For control, 0.2 mL of solvent (ethanol and acetone) was placed in test tubes instead of the extract. As soon as the emulsion was added into the test tubes, initial absorbance was measured with a spectrophotometer at 470 nm. The measurement was carried out at 30 min intervals for 2 h. All samples

were assayed in triplicate. BHT was used as standard. The antioxidant activity was measured in terms of successful bleaching of β-carotene by using the following equation. The measurements were made using the equation below:

$$[1 - (A_0 - A_t / A_0^\circ - A_t^\circ)] \times 100$$

where A₀ and A₀[°] are the absorbance values measured at the initial incubation time for samples and control, respectively, While A_t and A_t[°] are the absorbance values measured in the samples or standards and control at t/2 h.

ABTS free radical scavenging assay

Experiments were performed according to Re *et al.*, 1999 [16] with small modifications. ABTS and potassium persulfate were dissolved in distilled water to a final concentration of 7 mM and 2.45 mM respectively. These two solutions were mixed and the mixture allowed to stand in the dark at room temperature for 16 h before use in order to produce ABTS radical (ABTS^{•+}). For the study of phenolic compounds the ABTS radical solution was diluted with distilled water to an absorbance of 0.70 (±0.02) at 734 nm. After the addition of 10 μL of sample to 4 mL of diluted ABTS solution, the absorbance was measured at 30 min. All samples were analyzed in triplicate. The results were expressed as IC₅₀ values.

Determination of tannin content components

Tannin content was determined by the vanillin method of Bekir *et al.*, (2013) [17] with slight modification. The extracts (0.5 mL) were mixed with 1.5 mL vanillin reagent (1% vanillin in 7M H₂SO₄) in test tubes that are placed in an ice bath. Then reaction mixture was left standing for 15 min at room temperature. Absorbance of the solution was measured at 500 nm. Tannin content was expressed as equivalents of catechin (mgCEs/g)

Results and Discussion

In this research, there are some methods for determining the antioxidant activity and phenolic compounds of *V. glomeratum*. The content of common phenolic compounds of *V. glomeratum* were quantified with fifteen standards by HPLC analysis. The phenolic compound were separately by comparing the retention time of their peaks with HPLC method. The results (Fig. 1, Fig. 2 and Table-1). According this data epicatechin (2742.09 μg/g) and 2,5-dihydroxy benzoic acid (2544.96 μg/g) were the most abundant phenolic constituents in ethanol

extract of *V. glomeratum* while p-coumaric acid (0.34 µg/g) is the minor compound. Phenolic compounds are the largest group of phytochemicals and responsible for antioxidant activity of plants or plant products [18]. In a reference survey, only two studies have been found relevant for the evaluation of the effect of *Verbascum* species on inflammatory conditions. Previous investigations on Turkish *Verbascum* species by our research group led to the isolation and characterization of a number of secondary metabolites such as iridoids, monoterpene glucoside, saponins, phenylethanoid, neolignan, and flavonoid glycosides [19]. Phenolic, flavonoid and tannin compounds derived from plants are small molecules which play important roles as antioxidants. Phenolic compounds are widely distributed in plants and possess ultraviolet protection, pigmentation, and disease resistance [20]. Free radical scavenging and cell-aggregation inhibitory activities of 36 secondary metabolites isolated from the methanolic extracts of *V. cilicicum* Boiss., *V. lasianthum* Boiss. ex Benth., *V. pterocalycinum* var. *mutense* Hub.-Mor., and *V. salviifolium* Boiss. (Scrophulariaceae) were investigated [21]. Total phenolic, total flavonoid contents and total tannin contents were identified as gallic acid, quercetin and catechin equivalents (Table-2). Acetone extracts had the highest amount of total phenolic compounds while ethanol extracts have the lowest amount. According to this data, the highest flavanoid amount was determined in acetone extracts. Ethanol extracts had the highest amount of total tannin compounds while acetone extracts have the lowest amount. Dalar *et al.*, [22] were found phytochemical composition, antioxidant and enzyme inhibitory activities of extracts obtained from stem and flower of *V. cheiranthifolium* var. *cheiranthifolium*. In this work flower extract (5073.3±71.6 µmol Trolox Equivalent/gDW) had high levels of ORAC assay and effectively suppressed activity of pancreatic lipase enzyme, which was rich in verbascoside compound. Luteolin hexoside rich stem extract had pronounced FCR (76.5±2.0mg/g GAE), FRAP (1110.4±36.1 µmol Fe²⁺/g) and α-glucosidase inhibitory activities. Phenolic compounds and volatile compounds present in the extracts might be the main contributors of antioxidant capacity and enzyme inhibitory activities of the stem and flower extracts. Also, antioxidant properties of ethanol and acetone extracts of *V. glomeratum* were determined by five methods, namely, β-Carotene/Linoleic Acid Assay, DPPH, Reducing power, ABTS and phosphomolybdenum method (Table-3 and Fig 2.). The highest antioxidant activity that were used with β-carotene assay were determined in acetone extract (58.5±0.021%) and the lowest antioxidant activity was found in ethanol

extract (36.3±0.032%). The ferric reducing antioxidant power of extracts were expressed as mg/g of trolox equivalent. The acetone fraction had the highest reducing antioxidant power of 4.151±0.056 mg TE/g trolox equivalent. In this work, ethanol extract of *V. glomeratum* had higher scavenging activity (IC₅₀:1.220±0.025) with DPPH assay. The highest DPPH free radical scavenging activities were displayed by the ethanol extract (90.57%) (Fig. 3). In another free radical scavenging assay, we were determined acetone extract of *V. glomeratum* had (IC₅₀: 1.552±0.021) the most ABTS scavenging activity. The total antioxidant activities of various solvent extracts were measured by using phosphomolybdenum assay. The assay was reported by Prieto *et al.*, (1999) [10] based on the reduction of Mo (VI) to Mo (V) by antioxidant and subsequent formation of a green phosphate/Mo(V) complex at acidic pH. The acetone extract (8.218±0.015 mg AE/g) showed a much higher antioxidant potential than ethanol extract (3.224±0.011 mg AE/g).

Table-1: HPLC analysis of ethanolic extracts for phenolic acids contents.

Phenolic Compounds	µg/g	Retention Time (min)
Gallic acid	18.11	6.8
3,4-dihydroxy benzoic acid	52.23	10.7
Chlorogenic acid	66.19	18.2
4-hydroxy benzoic acid	337.65	15.7
2,5-dihydroxy benzoic acid	2544.96	17.2
Vanilic acid	1276.14	19.2
Cafeic acid	17029.78	22.7
p-coumaric acid	0.34	26.1
Ferulic acid	30.73	30.1
Rutin	97.88	45.6
Ellagic acid	182.94	47.7
Cinnamic acid	359.21	71.1
Quercetin	60.45	70.4
Epicatechin	2742.09	21.3
Naringin	-	49.7

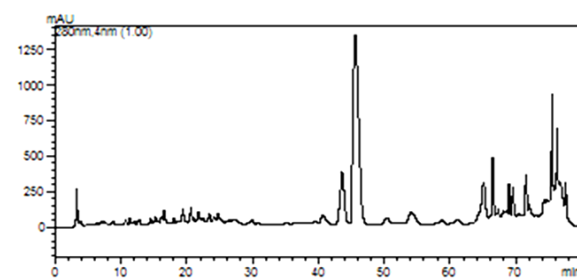


Fig. 1: HPLC Chromatogram of *V. glomeratum* ethanolic extracts.

Table-2: Total phenolic and flavonoid contents of the extract *Verbascum glomeratum*.

Samples	Total Flavonoids content (µQE/g)	Total phenolic content (mg GAE/mL extract)	Total tannins content (mg CES/g)
Ethanol	53.198±0.011	4.22±0.005	30.178±0.008
Acetone	87.142±0.009	4.41±0.007	17.337±0.003

Table-3: Antioxidant activities of ethanol and acetone extracts of *V. glomeratum*.

Samples	IC ₅₀ ,mg/mL		FRAP assay	β- caroten/linoleic acid assay	Phosphomolybdenum method
	DPPH	ABTS	(mg TE/g)	(%)	(mg AE/g)
Ethanol	1.220±0.025	2.223±0.014	4.085±0.074	36.3±0.032	3.224±0.011
Acetone	2.698±0.013	1.552±0.021	4.151±0.056	58.5±0.021	8.218±0.015
BHT	0.010±0.04	0.012±0.02	75.03±0.06	96.08±0.05	-

In each column, means followed by different letters are significantly different ($p < 0.05$).

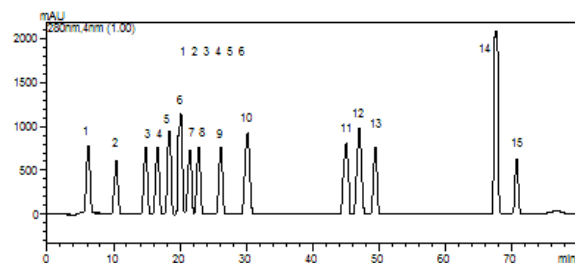


Fig. 2: HPLC Chromatogram of the standards (¹gallic acid; ^{2,3-4} hydroxybenzoic acid; ^{3,4}-hydroxybenzoic acid; ^{4,2,5}-dihydroxy benzoic acid; ⁵chlorogenic acid; ⁶vanillic acid; ⁷ epicatechin; ⁸caffeic acid; ⁹p-coumaric acid; ¹⁰ferulic acid; ¹¹rutin; ¹²ellagic acid; ¹³naringin; ¹⁴cinnamic acid; ¹⁵quercetin)

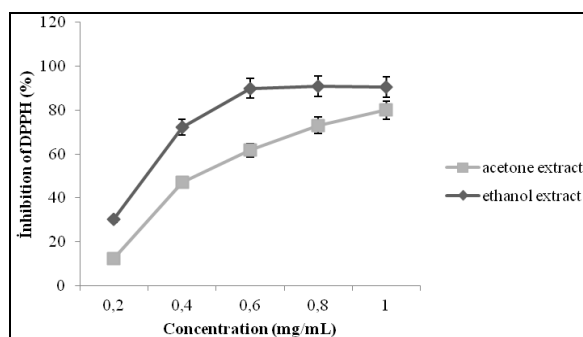


Fig. 3: DPPH free radical scavenging activity of extracts.

Several *Verbascum* species are known as antiseptic, antimalarial, astringent, demulcent, emollient, sedative, and narcotic and have been used for treatment of tumors, inflammations, migraine, asthma and spasmodic coughs in Europe, Asia and Northern America [23].

Sen-Utsukarcı *et al.*, [24] were investigated the antioxidant and cytotoxic activities of various extracts from the aerial parts of five *Verbascum* species and also the antibiofilm activities of the extracts, which showed antimicrobial activity. The methanolic and hydrophilic extracts of *Verbascum* species showed antioxidant activity. Thusly, *Verbascum* species in this study indicate an important antioxidant activity.

Marian *et al.*, [25] were found chemical, antimicrobial, antioxidant and antitumor activities of extracts obtained from *V. phlomoides*. Their study showed that *V. phlomoides* extract had total polyphenolic and flavonoid content (471.33 ± 0.13 mgGAE/100gDW and 5.36 ± 0.22 mgQE/100g DW). The antioxidant activities were investigated by FRAP, DPPH and ABTS methods. The results show that extract had strong antioxidant activities.

Pharmaceutical forms, such as capsules, tablets, a dried form as in a tea, a diluent or any delivery system prepared from the extract of *V. thapsus*, are used for the treatment of lung conditions or other degenerative conditions due to aging because of their essential antioxidant ingredients [26].

Conclusion

In conclusion, such detailed studies on antioxidant activity and phenolic compounds of *V. glomeratum* have been performed with spectroscopic methods. Thus, their phenolic compounds was determined indicating significant amounts. For antioxidant activities, β-Carotene/Linoleic Acid Assay, FRAP, ABTS, DPPH and phosphomolybdenum were used. Total phenolic, flavonoids and tannin contents were determined. Also, phenolic components of *V. glomeratum* were revealed by HPLC. Our all results show that *V. glomeratum* may be used as potential antioxidant source for pharmaceutical studies. *Verbascum* plants could serve as attractive mines of powerful antioxidants for the food, cosmetics, and pharmaceutical industries.

References

1. A. Huber-Morath, In: Flora of Turkey and the East Aegean Islands, University Press, Edinburgh Vol. 6 (Davis P. H., ed.), p. 461. (1978)
2. E. Tuzlacı, M.K. Erol, Turkish folk medicinal plants. Part II: Egirdir (Isparta), *Fitoterapia*, **70**, 593 (1999).
3. T. Baytop, Therapy with Medicinal Plants in Turkey (Past and Present) Nobel Medical Bookstores Ltd., İstanbul, 334 (1999).

4. E. Sezik, E. Yeflilada, G. Honda, Y. Takaishi, Y. Takeda, T. Tanaka, Traditional medicine in Turkey X. Folk medicine in Central Anatolia, *J. Ethnopharmacol*, **75**, 95 (2001).
5. A. Turker, N.D. Camper, Biological activity of common mullein, a medicinal plant, *J Ethnopharmacol*, **82**, 117 (2002).
6. R. Taj, S. Ali, M.A. Siddiqui, M. Yaqoob, Z. Hussain, R.B. Tareen, Phytochemical Composition, Antioxidant and Antifungal Activities of *Jaubertia aucheri* Guill, *J.Chem.Soc.Pak.*, **40**, 568 (2018).
7. A.U. Turker, E. Gurel, Common mullein (*Verbascum thapsus* L.): Recent advances in research, *Phytotherapy Research* **19**, 733 (2005).
8. O. Kılınçarslan, R. Mammadov, HPLC analysis and Antioxidant, Antibacterial and Cytotoxicity Activities of Various Solvent Extracts of *Erysimum kotschyianum* Gay. (Brassicaceae), *J.Chem.Soc.Pak.*, **40**, 707 (2018).
9. F. Caponio, V. Alloggio, T. Gomes, Phenolic compounds of virgin olive oil: influence of paste preparation techniques. *Food Chem*, **64**, 203 (1999).
10. P. Prieto, M. Pineda, M. Aguilar, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E, *Anal Biochem*, **269**, 337 (1999).
11. M. Oyaizu Studies on products of browning reaction: Antioxidative activity of product of browning reaction prepared from glucosamine, *Japan J Nutr*, **44**, 307 (1986).
12. K. Slinkard, V.L. Singleton, Total phenol analyses: automation and comparison with manual methods, *Am J Enol Viticult*, **28**, 49 (1977).
13. A. Arvouet-Grand, B. Vennat, A. Pourrat, P. Legret, Standardisation d'un extrait de propolis et identification des principaux constituants, *J. Pharma Belg*, **49**, 462 (1994).
14. L. Wu, H. Hsu, Y. Chen, C. Chiu, Y. Lin, J. Ho, Antioxidant and antiproliferative activities of red pitaya, *Food Chemistry* **95**, 319 (2006).
15. G.K. Jayaprakasha, R.P. Singh, K.K. Sakariah, Antioxidant activity of grape seed (*Vitis vinefera*) extracts on peroxidation models in vitro, *Food Chemistry*, **73**, 285 (2001).
16. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, **26**, 1231 (1999).
17. J. Bekir, M. Mars, J.P. Souchard, J. Bouajila, Assessment of antioxidant, antiinflammatory, anti-cholinesterase and cytotoxic activities of pomegranate (*Punica granatum*) leaves, *Food and Chemical Toxicology*, **55**, 470 (2013).
18. L. Ji, J.Wu, W. Gao, J. Wei, J. Yang, C.Guo, Antioxidant capacity of different fractions of vegetables and correlation with the contents of ascorbic acid, phenolics and flavonoids, *J. Food Sci*, **76**, 1257 (2011).
19. I.I. Tatli, Z.S. Akdemir, E. Yesilada, E. Kupeli, Anti-inflammatory and antinociceptive potential of major phenolics from *Verbascum salviifolium* Boiss. *Z Naturforsch C*, **63**, 196 (2008).
20. G. Zengin, S. Uysal, R. Ceylan, Phenolic constituent, antioxidative and tyrosinase inhibitory activity of *Ornithogalum narbonense* L. from Turkey: A phytochemical study, *Industrial Crops and Products* **70**, 1 (2015).
21. I. I. Tatli, S. Takamatsu, I.A. Khan, Z.S. Akdemir, Screening for free radical scavenging and cell aggregation inhibitory activities on secondary metabolites from Turkish *Verbascum* species, *Z Naturforsch C*, **62**, 673 (2007).
22. A. Dalar, A.S. Bengu, O. Allahverdiyev, Analysis of Phytochemical Composition and Biological Activities of *Verbascum cheiranthifolium* var. *cheiranthifolium* stem and flowers, *IJSM*, **5**, 233 (2018).
23. A.R. Shakeri, A. Farokh, Phytochemical evaluation and antioxidant activity of *Verbascum sublobatum* Murb. Leaves *RJP*, **2**, 43 (2015).
24. B. Sen-Utsukarçı, S. Dosler, T. Taskın, M. Abudayyak, G. Ozhan, A. Mat, An Evaluation of Antioxidant, Antimicrobial, Antibiofilm and Cytotoxic Activities of Five *Verbascum* Species in Turkey, *Farmacia*, **66**, 1014 (2018).
25. E. Marian, L. G. Vıcas, T. Jurca, M. Muresan, A. Pallag, R.L. Stan, B. Sevestre, Z. Diaconeasa, C. Ionescu, A. C. Hangan, *Salvia officinalis* L. and *Verbascum phlomoides* L. Chemical, Antimicrobial, Antioxidant and Antitumor Investigations, *Rev.Chim.(Bucharest)*, **69**, 365 (2018).
26. J. Intelisano, Food supplement/herbal composition for health enhancement, *Appl*. **39**, 416 (2002).