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Research Article

First report on determination of bioelement, vitamin content and antioxidant properties of *Verbascum golawanense*

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Abstract: This study was designed to investigate the antioxidant, antiradical potential and element and vitamin (A, E, K and C) levels in leaf extracts of *Verbascum golawanense* belonging to Scrophulariaceae family. For this purpose, DPPH, ABTS, anti-haemolytic activity, total flavonoid, phenolic content, total antioxidant capacity and vitamin C content were determined and measured spectrophotometrically. Vitamin A, E and K were analysed by HPLC and elemental analysis was performed by ICP-OES method. Phenolic, flavonoid and antioxidant capacity of the plant were determined as 5.861 ± 0.212 mg GA/g, 26.422 ± 0.613 mg QE/g and 124.359 ± 2.562 mM AA/g, respectively. K, Na, Fe, V, retinol, α-tocopherol, phylloquinone and vitamin C were determined as 3.086 ± 0.0131 mmol/kg, 2.260 ± 0.0266 mmol/kg, 1.1465 ± 0.0731 mmol/kg, 29.326 ± 3.072 µmol/kg, 0.1107 ± 0.013 µmol/kg, 0.4002 ± 0.16 µmol/kg, 1.101 ± 0.118 µmol/kg and 2552.0126 ± 187.056 mg/100g, respectively. The IC_{50} values related to the in vitro antioxidant properties of DPPH, ABTS and anti-haemolytic activity assays were 19.170 ± 0.615 µg/mL, 26.877 ± 0.461 µg/mL and 64.87 ± 2.24 µg/mL. In vitro studies revealed that the plant has a good antioxidant potential. It was determined that the vitamin and element content was high and total phenol, flavonoid and antioxidant potential were at reasonable levels. ABTS and DPPH tests showed a promising antioxidant power. These results indicated that the extract could be a potential alternative and could be investigated for the discovery and development of new chemical compounds for the treatment of various diseases.

1. INTRODUCTION

Plants have provided countless benefits for humans since ancient times. In particular, it is known that medicinal plants are only safely consumed for human civilisation, but the main reason why the effectiveness of these plants against any disease has not been confirmed is the limited technology and scientific advances (Zengin *et al.*, 2021). However, with the technological advances, medicinal plants have found more usage areas with the determination of the active substances in the contents of the plants. The proof that this area of use is very wide is understood from the fact that many different plant species are subject to experimental studies (Hazman *et al.*, 2024; Bukhibkh, 2024).

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The family Scrophulariaceae includes the genera mimulus, pensteman, digitalis, veronica and verbascum (Grieve, 1981). The genus *Verbascum* L. [(sığırkuyruğu (in Turkish)] of this family is represented by 228 species (185 of which are endemic) in Turkey. It has been determined that the species belonging to the genus *Verbascum* are used therapeutically in traditional medicine in various diseases such as rheumatic diseases, haemorrhoids, fungal infections, skin disorders, respiratory disorders (Kahraman *et al*., 2022). In the Turkish tradition, the above-ground parts (stem, flower, leaf) of *Verbascum* species are widely used for skin diseases and eczema, and also for the drying effect for wounds (Zengin *et al.*, 2023). *V. golawanense*, an endemic species of the genus *Verbascum* grows wild in wastelands, clearings and roadsides, which are mostly linear in structure (Firat, 2017).

Phenolic compounds in plants are an important group of molecules that show many biological effects against free radicals (Selseleh *et al.*, 2020). Studies are increasing to show that these compounds pose less of a threat to human health, and new ones are added to these plants every day, creating an alternative to existing synthetic drugs (Bakır *et al*., 2023; Boztaş *et al*., 2021). Antioxidants are defined as substances that prevent, reduce or delay the oxidation of cell components that may be exposed to oxidation in living cells. It has been confirmed that non-enzymatic antioxidants are compounds forming numerous groups such as some minerals, vitamins (A, C, E and K), carotenoids, flavonoids (Yılmaz, 2010). Therefore, the investigation of naturally occurring compounds in plants is an important area of interest for the pharmaceutical industry. On the other hand, Turkey is one of the leading countries in the world in terms of its rich biodiversity and endemic species. Therefore, identification of indigenous plant species and knowledge of antioxidant and antiradical properties of the plant may help to identify new natural agents.

The first step after understanding the metabolic profile and biological properties of a plant is to know its antioxidant properties and to determine the plant content. There are studies on phytochemical, biological, antioxidant and antibacterial properties of some *Verbascum* species. However, this is the first report of *V. golawanense* and therefore it will be an important reference for future studies. The aim of this study was to determine the total phenol, flavonoid and antioxidant capacity, vitamin A, E, K and C content, DPPH, ABTS and phenylhydrazine radical scavenging capacity in terms of antioxidant activity, bioelements levels of the methanol extract of the leaf of *V. golawanense* by various methods.

2. MATERIAL and METHODS

2.1. Plant Material and Conditions of Preparation

Location of *V. golawanense* species; Fırat: Türkiye. B9 Van: On the way from Van to Muradiye, near Bendimahi, roadside 1655 m, 38°57'42", 43°37'50", 1 July 2022 *M. Fırat* 36149 & *A. Bakır* (VHLF). This plant species was discovered by Biologist Dr. Mehmet FIRAT during field studies and was introduced to the literature with the name *Verbascum golawanense* (Firat, 2017).

Care was taken to ensure that the freshly collected leaves were healthy, clean and pest-free. To prevent the leaves from sweating and darkening, they were placed in open, breathable cloth sacks. The leaf samples were spread on unprinted paper and left to dry in a shady, airy place. After drying, the leaves were ground to a fine powder in a plant grinder and placed in glass bottles for analyses.

2.2. Preparation of Plant Leaf Extract

For the preparation of MeOH extract of the plant, the method of Cai *et al.*, (2004) was adapted and used in this study. An appropriate amount (20 g) of the plant leaf, previously powdered and stored in glass bottles, was weighed and transferred to a coloured bottle and MeOH at 80% concentration was added. The plant leaf, which was kept away from light, was subjected to extraction at 30 ˚C for 36 hours in a water bath with stirrer. Then centrifuged at 4500 rpm for

15 min and filtered using suitable filter paper (Whatman No. 1). MeOH in the filtrate was extracted under reduced pressure at 40°C using a rotary evaporator and removed. Finally, the crude extracts were lyophilised in a -65˚C refrigerator until dry and stored in the dark at +4°C for further analysis. Calculations showed that the methanolic extraction of the plant leaf produced a yield of 16%.

2.3. Total Phenol Content Experiment

Folin reagent solution (FCR) indicator was used to determine the total phenol content of the plant leaf (Gamez-Meza *et al.*, 1999; Yi *et al.*, 1997). To the leaf extract samples prepared by dilution with MeOH, 300 µl of 2% sodium carbonate, 100 µl of FCR marker were added and incubated at room temperature for 3 hours. The absorbances of the prepared samples were read at 765 nm. A standard curve was prepared using different concentrations of gallic acid solutions. The values were reported in milligrams of gallic acid equivalent (GAE) per gram of dried extract (mg GAE/g).

2.4. Total Flavanoid Content Experiment

In this study, to determine the flavonoid content of *V. golawanense*, 100 µl CH_3COOK was added to 500 µL stock solution of the previously prepared extract diluted with methanol and 100 µL $Al(NO_3)_3$ and 4600 µL EtOH were added. The solutions were then vortexed and incubated at room temperature for 45 minutes. Finally, the plant absorbance value was read against the control sample at the appropriate wavelength. (Park *et al*, 1997; Lamasion *et al.*, 1990). Flavonoid concentration was calculated according to the values of quercetin (QE) compound as a reference, and the flavonoid status of the plant samples was reported as quercetin equivalent (mg QE/g).

2.5. Total Antioxidant Capacity Experiment

The main objective of this method is based on the reduction of acidic Mo-VI to Mo-V to form the green coloured phosphate/Mo(V) compound at acidic pH (Prieto *et al.*, 1999). 200 µL of different concentrations of plant MeOH extracts were taken and 0.2 L of marker solution [600 mM H_2SO_4 + 28 mM Na_2HPO_4 + 4 mM $(NH_4)_2MoO_4$)] was added and kept in a water bath at 100ºC for 1.30 hours. The samples were cooled in a cold water bath and at room temperature. 695 nm wavelength was read against the control sample. Total antioxidant capacity was calculated by drawing ascorbic acid standard graph and total antioxidant capacity of the samples was given as mM ascorbic acid/g.

2.6. Determination of Bio-elements Content

The bio-element content of *V. golawanense*'s dry leaves crushed in a plant grinder was determined by the dry burning method (Mester *et al.*, 2003). According to this method, 1 g of plant leaf sample was taken into porcelain crucibles (care was taken to have 3 repetitions) and 2 ml of alcohol was added to them. The electric muffle furnace was set to a maximum of 550˚C starting from 250˚C. The purpose of doing this was to heat the samples in a controlled manner and not to damage the elements in it suddenly.

The prepared samples were placed in the muffle furnace (with the condition of increasing 50˚C every hour) reaching a temperature of 250˚C. When the temperature of the furnace reached 550˚C, the samples were kept inside until the next day. 5 mL of HCI was added to the samples that were taken to cool. Finally, it was distilled with a blue band filter paper. Finally, the samples were removed from the muffle furnace and subjected to a series of processes and analyzed using ICP-OES and ICP-MS.

2.7. Determination of DPPH Radical Scavenging Activity

In the study, free radical scavenging activities of MeOH extracts of the plant were determined using DPPH (Kirby & Scmidt, 1997; Cuendet *et al.*, 1997). In order to determine the free radical scavenging activity of the prepared extracts by using DPPH ‘total antioxidant potential’

measurement method, 2 mg/ml stock extract solution was diluted with MeOH and prepared in different concentrations. DPPH system follows a stable radical generating procedure. In order to determine the scavenging activity of this radical, 4000 µL of 4/1000% DPPH solution was added to the solutions of different concentrations prepared by dilution with MeOH and incubated at appropriate temperature for 45 min. Absorbance readings were made at 517 nm. Absorbance values of the prepared samples were expressed against the control. BHT, a synthetic antioxidant, was used as a control against the sample. Antiradical activity was determined from the formula below (Duh & Yen, 1997).

$$\text{Inhibition (\%)} = \left\{ \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \right\} X\ 100$$

Using the above % inhibition formula, the plant extraction concentrations were calculated as IC_{50} (The micromolar concentration required to inhibit half of the radical formed in any given situation is µg/mL.) (Burits *et al*., 2001; Smith *et al.*, 1987).

2.8. Determination of ABTS Radical Scavenging Activity

$ABTS^+$ reagent was initially prepared by mixing (v/v) ABTS (0.002 M) and $K_2S_2O_8$ (2.45 mM) solutions. The mixture was incubated for 16 hours in the dark at room temperature. 1800 µL of $ABTS^+$ reagent was added to 200 µL of test sample and incubated in a dark room for 2 h before measuring absorbance at 734 nm. (Almusallam *et al*., 2021; Walker *et al*., 2009).

ABTS + Potasyum persülfat $\longrightarrow$ $ABTS^{\cdot+}$

Methanol extract of *V. golawanense* leaves was carried out by modifying the method determined by Re *et al*., 1999 and Miller *et al*., 1993. Trolox was used as a synthetic antioxidant. The active power of $ABTS^{\cdot+}$, a redox radical, was calculated from the formula below.

$$\text{Inhibition (\%)} = \left\{ \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \right\} X\ 100$$

2.9. Phenylhydrazine ($PhNHNH_2$) Activity Determination

Phenylhydrazine (Phd) easily causes acute hemolytic anemia with erythrocytes. According to this method, the radical scavenging power of *V. golawanense* was determined in erythrocytes chemolyzed with Phd (Valenzuela, 1977). Briefly, 1 mL Phd, 0.1 mL 20% erythrocyte and buffer mixture, 2000 mL buffer were added to samples prepared from different concentrations of methanol extract of *V. golawanense* leaves. Incubated at 34 ºC for 1.5 hour and centrifuged at 6000 rpm for 15 minutes. After transferring the supernatant to another tube, the absorbance at 540 nm was read against the control sample. The results were compared with trolox.

2.10. Determining Vitamin C Content

Vitamin C content in this study was performed spectrophotometrically at a wavelength of 521 nm. To determine the vitamin C content of plant leaves, 2 mL of HPO_3 acid and 0.5% oxalic acid were added to 500 mg sample and centrifuged at 5000 rpm for 5 min. After centrifugation, 2000 µL of the filtrate was taken and 1 drop of thiourea and 500 µL of 2,4-DNFH were added and kept in a water bath at 100°C. After the hot water bath, H_2SO_4 was slowly added to the solutions prepared at different concentrations. Sample tubes prepared at 25 degrees were vortexed for 5 minutes. At the end of the final run, measurements were recorded in a spectrophotometer. Absorbic acid concentrations of the samples were calculated using the calibration graph obtained (Golubkina *et al*., 1989; Brewster, 1984).

2.11. Vitamin A, E and K Analyses

2.11.1. *Standard solution and calibration*

Retinol, α-tocopherol and phylloquinone stock solutions were prepared at 500 µg/mL. To prepare the standard solution, the stock solutions were diluted with methanol accordingly.

Linear regression analysis of the peak area versus standard solution concentrations was used to calculate the calibration.

2.11.2. *Extraction process of vitamins*

The amounts of retinol, α-tocopherol and phylloquinone in *V. golawanense* leaves were determined by modifying the studies of Al-Saleh *et al*., 2006 and Şahin *et al*., 2005. Weighed 4000 mg of dried and shade ground plant leaf samples were extracted with n-hexane and EtOH. BHT (0.02%) was added to the prepared samples, mixed at the appropriate time and then kept in the dark for 30 hours. Then centrifuged at +4°C and 5000 rpm for 15 min. The clear solution in the resulting phase was filtered using filter paper and 500 µL of n-hexane was added. Finally, it was left to dry under nitrogen gas (at 37°C). Immediately after the drying process was completed, the remaining part was dissolved in 250 µL of MeOH+C_4H_8O (98%) and made ready for analysis.

2.11.3. *Chromatographic condition*

Vitamin A, E and K analyses were performed on a Gl Science C_{18} reversed phase high performance liquid chromatography column (250 x 4.6 mm ID), MeOH (80 ml) + tetrahydrofuran (20 ml) mobile phase, 1500 µL/min flow rate at 24°C. HPLC - applications were performed at 325 nm retinol, 290 nm α-tocopherol and 248 nm phylloquinone in 0.1 mL volumes in dark coloured vials in a tray autosampler (-10°C) using a PDA array detector. Chromatographic analysis measurements were performed by isocratic elution (40ºC), a separation technique of HPLC (Al-Saleh *et al*., 2006; Şahin *et al*., 2005).

2.12. Statistics Data

The data of the measurement results are expressed as mean and mean standard error (X ± SEM). Group graphs are also expressed in the same way (X ± SEM). Nonlinear regression analysis was used to find the IC_{50} measurement data. The measurements are presented in three replicates.

3. RESULTS

Antioxidant capacity, total phenolic content, flavonoid content, DPPH, ABTS and anti-haemolytic activity levels were measured to determine the antioxidant and antiradical properties of the MeOH extract of *V. golawanense* leaves. In addition, the levels of vitamins A, E, K and C and elements (Al, K, Na, Fe, Sr, Mn, Ti, Ba, Zn, V, Co, Cu, Cr, Se, Mo, As, Pb and Cd) were determined and the results are shown in Table 1 and Table 2.

Table 1. Comparison of % inhibition and IC_{50} values of *Verbascum golawanense* with positive controls.

	Control	% Inhibition ($\bar{X}$ ± SEM)	IC_{50} (µg/mL) ($\bar{X}$ ± SEM)
DPPH	BHT	87.813 ± 0.156	19.170 ± 0.615
		76.528 ± 0.694	18.508 ± 1.157
ABTS	Trolox	95.514 ± 0.109	26.877 ± 0.461
		81.569 ± 0.446	22.177 ± 0.849
$PhNHNH_2$	Trolox	65.770 ± 0.620	64.870 ± 2.240
		69.410 ± 0.078	62.680 ± 1.270

Values are expressed as mean ± standard error of the mean ($\bar{X}$ ± SEM). Samples were carried out in triplicate. DPPH: 2,2-difenil-1-pikrilhidrazil; ABTS: 2,2'-azinobis (3-etilbenzotiazolin-6-sülfanot); $PhNHNH_2$: Fenilhidrazin

Total phenol, flavonoid and antioxidant capacities of the methanol extract of the plant leaf are shown in Figure 1 and vitamin C content is shown in Figure 2.

Table 2. Vitamins, total phenolic and flavonoid content, total antioxidant capacity and element levels of *Verbascum golawanense*.

Parameters	*Verbascum golawanense* $\bar{X}$ ± SEM
Retinol (µmol/kg)	0.1107 ± 0.013
α-tocopherol (µmol/kg)	0.4002 ± 0.16
Phylloquinone (µmol/kg)	1.101 ± 0.118
Vitamin C (mg 100/g)	2552.0126 ± 187.056
Total phenolic content (mg GA/g)	5.861 ± 0.212
Total flavonoid content (mg QE/g)	26.422 ± 0.613
Total antioxidant capacity (mM A.A/g)	124.359 ± 2.562
Ba (µmol/kg)	41.536 ± 0.761
V (µmol/kg)	29.326 ± 3.072
Ti (µmol/kg)	45.838 ± 4.106
Cr (µmol/kg)	7.885 ± 0.270
Cu (µmol/kg)	12.157 ± 1.088
Sr (mmol/kg)	0.110 ± 0.0008
As (µmol/kg)	0.801 ± 0.136
Se (µmol/kg)	1.375 ± 0.1561
Cd (µmol/kg)	0.0557 ± 0.00378
Pb (µmol/kg)	0.757 ± 0.0535
Mo (µmol/kg)	0.0575 ± 0.0218
Fe (mmol/kg)	1.1465 ± 0.0731
Mn (µmol/kg)	66.025 ± 2.626
Al (µmol/kg)	14.594 ± 0.167
Zn (µmol/kg)	37.171 ± 2.78
Co (µmol/kg)	17.092 ± 0.0226
K (mmol/kg)	3.086 ± 0.0131
Na (mmol/kg)	2.260 ± 0.0266

Values are expressed as mean ± standard error of the mean ($\bar{X}$ ± SEM). Samples were carried out in triplicate.

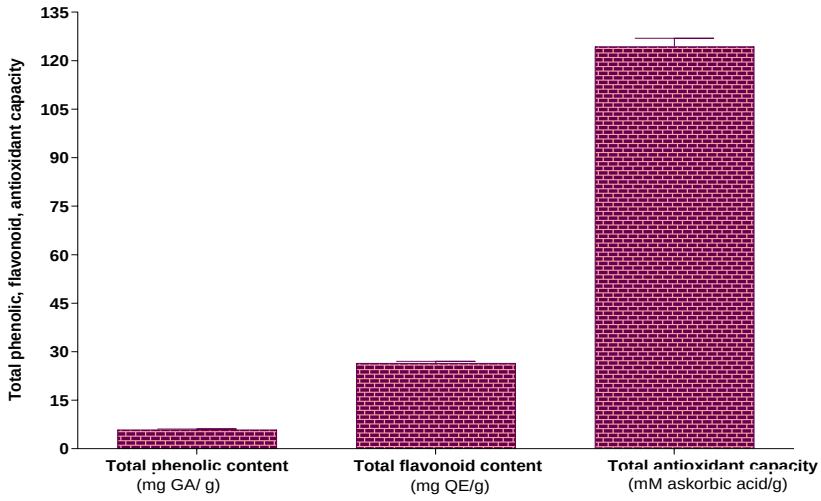

Figure 1. Graph showing the total phenol, flavonoid and antioxidant capacity of methanol extract of *V. golawanense*.

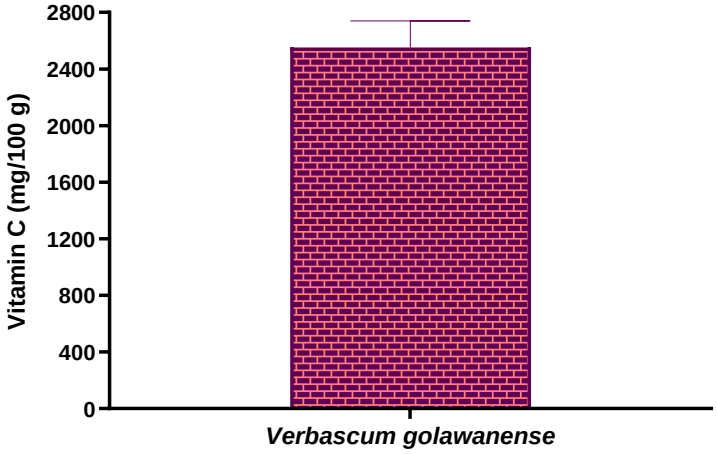

Figure 2. Vitamin C content of dry leaves of *V. golawanense*.

The % inhibition, haemolysis and IC_{50} values of phenylhydrazine, which we used to determine DPPH and ABTS radicals and anti-haemolytic activity, after comparison with synthetic antioxidants BHT and trolox are shown in Figure 3.

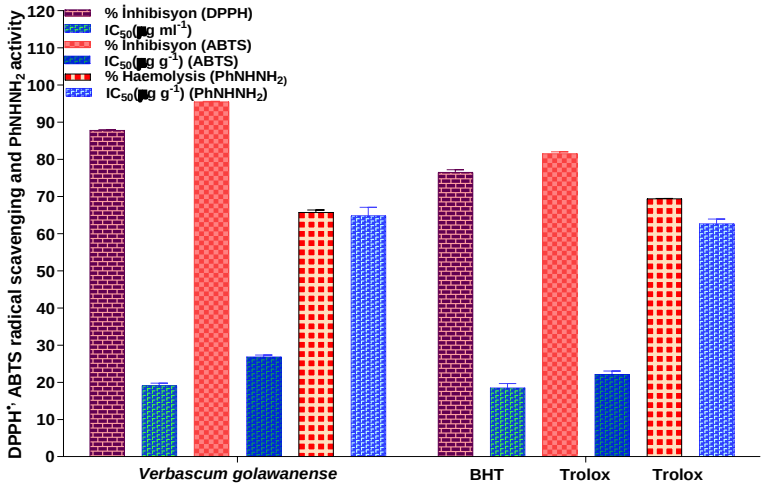

Figure 3. % inhibition, % haemolysis and IC_{50} values for DPPH/BHT, ABTS/trolox and $PhNHNH_2$/trolox showing positive control of *V. golawanense* with radicals.

The mineral and trace element levels of the leaf part of *V. golawanense* are given in Figure 4, Figure 5 and Figure 6.

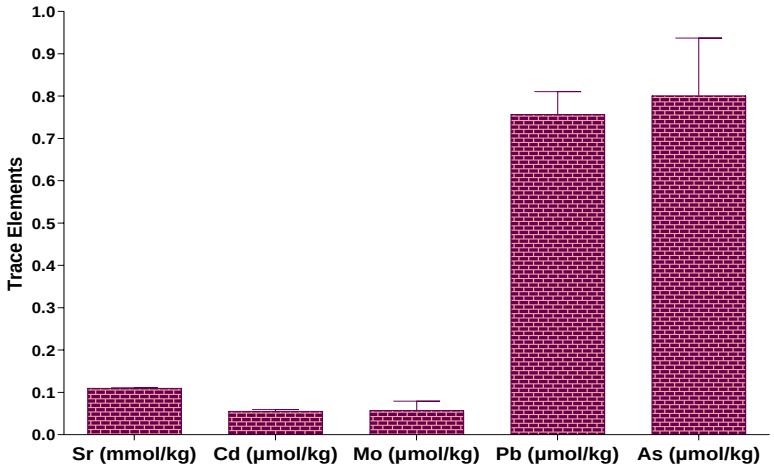

Figure 4. Trace element (Sr, Cd, Mo, Pb and As) levels of *V. golawanense* leaf parts.

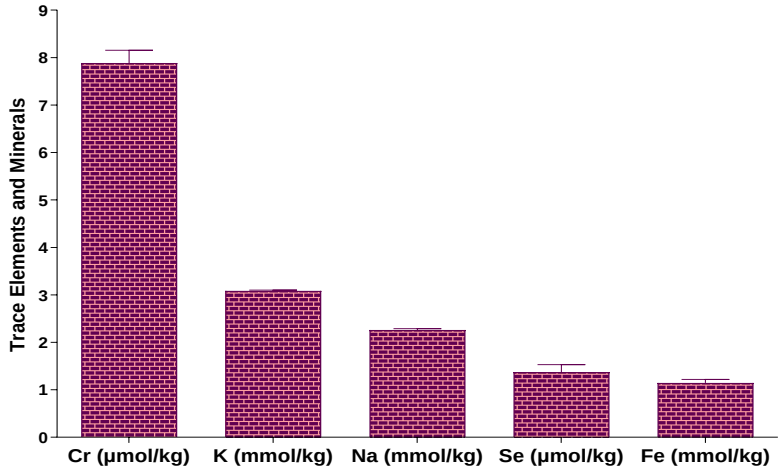

Figure 5. Trace element (Cr, Se and Fe) and mineral (K and Na) levels of *V. golawanense* leaf parts.

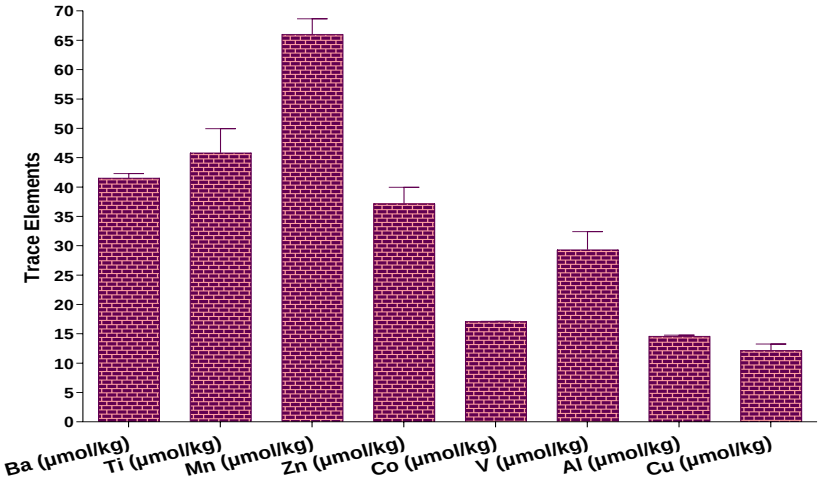

Figure 6. Trace element (Ba, Ti, Mn, Zn, Co, V, Al and Cu) levels of *V. golawanense* leaf parts.

4. DISCUSSION and CONCLUSION

Vitamins A, E and K, which are taken into the organism in the diet to be reused when needed, can be stored in low concentrations in living tissue. However, vitamin C, a type of water-soluble vitamin, lacks the ability to be stored in the organism and is usually taken into the organism through daily food supplements (Stevens, 2021). Vitamin A has a very important role in the organism, which has a strong antioxidant property, facilitating the repair of wounds, strengthening immunity against diseases and infections, especially affecting the lungs (Huang *et al*., 2018). On the other hand, during cell renewal, vitamin E protects the cell against the formation of reactive oxygen species (ROS) thanks to its antioxidant defence ability (Rizvi *et al.*, 2014). Vitamin K, which is synthesised by plants, is met in humans with the help of diet. In addition, the important role of vitamin K in the blood clotting factor is well known. In addition to the antioxidant properties of vitamin C, studies on the positive correlation between the absorption of dietary iron in case of its rich presence in the organism remain current. (Andersen, 2005).

Within the scope of the study, the amounts of vitamins A, E, K and C in the content of *V. golawanense* were determined. The vitamin C level included in the study was 2552.0126 ± 187.056 mg/100g; vitamin A, E and K levels were determined as 0.1107 ± 0.013 µmol/kg, 0.4002 ± 0.16 µmol/kg and 1.101 ± 0.118 µmol/kg, respectively and shown in Table 2. When the vitamin values found in *V. golawanense* leaves are examined, it is seen that the presence of vitamins A, E and K is at a reasonable level, while vitamin C is rich and has a high value, indicating that the plant is a good source of vitamins. In the literature, no studies on vitamin A,

E, K and C values in the plant content have been found. There is more information about the physical properties and habitat of the plant.

The term polyphenol is used to describe secondary metabolites in plants. The most talked about feature of polyphenols and plant phenolics in general is their ability to scavenge or reduce the effects of ROS (Quideau *et al*., 2011). Therefore, plants constitute the most important source of natural antioxidant compounds and phenolics comprise the most fundamental group of natural antioxidants. Bioactive metabolite compounds such as chlorogenic acid, iridoids and verbascoside in *Verbascum* plant genus provide important defence against free radicals (Zengin *et al*., 2023; Bender *et al*, 2021). It has been shown that chlorogenic acid positively activates endogenous antioxidant systems and thus has effects against undesirable states of ROS (Hazman *et al*., 2021). It is also thought that the wound healing effect of some Verbascum species is partly related to the polysaccharides contained in the plant (Süntar *et al.,* 2010). From this point of view, the determination of the antioxidant content of *Verbascum golawanense*, a species of the genus *Verbascum L.* with an important distribution area in Turkey, will provide important parameters for further studies.

In this study, total phenol, flavonoid content and antioxidant capacity were determined in the leaf methanol extract of *V. golawanense.* The measurements were calculated based on gallic acid standard curve for total phenol content, quercetin curve for total flavonoid content and ascorbic acid standard graph for antioxidant capacity. Total phenol, flavonoid and antioxidant amounts were determined as 5.861 ± 0.212 mg GA/g, 26.422 ± 0.613 mg QE/g and 124.359 ± 2.562 mM AA/g, respectively and shown in Table 2. There is no data on *V. golawanense* in previous studies. However, Karamian and Ghasemlou (2013) determined the total phenol amounts as 79.95 ± 0.33, 118.2 ± 2.46 and 95.83 ± 1.93 mg GAE/g and total flavonoid amounts as 4.83 ± 0.13, 4.87 ± 0.06 and 5.77 ± 0.23 mg QE/g in *V. nudicaule*, *V. sinuatum* and *V. speciosum* species, respectively. When the study was evaluated, it was concluded that the total phenol content of the three plants was higher than *V. golawanense*, but the total flavonoid content was very low.

Macro and micro elements are inorganic substances that must be present for the continuity of many important physiological functions in the organism. Therefore, the presence of elements in the plant content is an important cause of antioxidant indicators. These elements function as cofactors that are dependent on enzymes, hormones and vitamins in the cell (Ferrier, 2019). Their main functions such as participation in the structure of bones and teeth, protection of acid-base balance in metabolic reactions, transport processes of gases during immunity and respiration, participation in membrane structures have been defined (Lukaski, 2004).

In this study, when the biyo-element level of *V. golawanense* was analysed, it was found to be 41.536 ± 0.761 µmol/kg Ba, 29.326 ± 3.072 µmol/kg V, 45.838 ± 4.106 µmol/kg Ti, 7.885 ± 0.270 µmol/kg Cr, 12.157 ± 1.088 µmol/kg Cu, 0.110 ± 0.0008 mmol/kg Sr, 0.801 ± 0.136 µmol/kg As, 1.375 ± 0.1561 µmol/kg Se, 0.0557 ± 0.00378 µmol/kg Cd, 0.757 ± 0.0535 µmol/kg Pb, 0.0575 ± 0.0218 µmol/kg Mo, 1.1465 ± 0.0731 mmol/kg Fe, 66.025 ± 2.626 µmol/kg Mn, 14.594 ± 0.167 µmol/kg Al, 37.171 ± 2.78 µmol/kg Zn, 17.092 ± 0.0226 µmol/kg Co, 3.086 ± 0.0131 mmol/kg K ve 2.260 ± 0.0266 mmol/kg Na and shown in Table 2.

The bio-element content of *V. golawanense* plants evaluated within the scope of the study was determined as K > Na > Fe > Sr > Mn > Ti > Ba > Zn > V > Co > Al > Cu > Cr > Se > As > Pb > Mo > Cd. According to these results, it can be said that the presence of critical and important trace bio-elements such as Fe, Mn, Zn, Co, V, Cu, Se, Cr and Mo in addition to macro elements such as K and Na may increase the antioxidant capacity of the plant. Considering the previous studies, it can be concluded that the presence of some trace elements in *V. lasianthum Boiss. Ex Bentham*, which is close to the plant, some elemental analyses were found as follows; 446.98 ± 94.63 ppm Fe, 6.94 ± 0.91 ppm Cu, 38.12 ± 2.39 ppm Mn, 20.67 ± 1.08 ppm Zn, 0.72 ± 0.11 ppm Cr, 0.14 ± 0.01 ppm B, 78.38 ± 10.08 ppm Na, 4409.43 ± 447.33 ppm Ca, 1386.76

± 142.82 ppm Mg, 9.45 ± 0.79 ppm Ba, 1.72 ± 0.09 ppm Ni, 8.66 ± 0.42 ppm Bi, 0.24 ± 0.05 ppm Ga ve 3.08 ± 0.78 ppm Pb (Hazman *et al*., 2021). When the units in the studies were equivalised using the international system of units, although some different elements were measured, it can be said that the bio-element diversity of *V. golawanense* is better when looking at the common elements. *V. golawanense* and many other similar plant species grow naturally on roadsides, close to settlements. Therefore, heavy metals such as Pb, Cd, As, Sr from the exhaust of vehicles or as a result of other human-induced factors are mixed into the soil and then transferred to the plant (Hazman *et al*., 2021). Considering these data, verbascum species such as *V. golawanense* and similar *V. lasianthum* may contribute to the reduction of heavy metal pollution in the soil.

DPPH and ABTS radical scavenging assays are the most widely used methods to measure the total antioxidant capacity in natural compounds. In order to accurately assess the antioxidant capacity measured in the methanol extract of the leaf of *V. golawanense*, determining an IC_{50} value free of DPPH and ABTS concentrations provided an important parameter evaluation (Bakır *et al*., 2023; Martinez-morales *et al*., 2020). The highest % inhibition value for DPPH radical of *V. golawanense* was 87.813 ± 0.156 and 76.528 ± 0.694 for BHT. IC_{50} values of 19.170 ± 0.615 μg/mL for the plant and 18.508 ± 1.157 μg/mL for BHT were recorded and given in Table 1. There is no study in the literature on the antioxidant properties of *V. golawanense*. However, the DPPH radical scavenging effect of the MeOH extract of *V. lasianthum*, the closest species to the plant, was investigated (Hazman *et al*., 2021). The mixture of the plant's leaves, flowers and stem stem was extracted and the DPPH values were found to be 6.44 for the plant and 18.508 mg/mL for BHT. In this study, the leaf part of the plant and the DPPH unit were measured as μg/mL and it was determined that *V. golawanense* showed better antioxidant power.

On the other hand, the highest % inhibition value of the plant on ABTS radical was 95.514 ± 0.109 and 81.569 ± 0.446 for trolox. IC_{50} values were calculated and 50% inhibition of ABTS radical was measured as 26.877 ± 0.461 μg/mL for the plant and 22.177 ± 0.849 μg/mL for trolox and shown in Table 1. Knowing the IC_{50} value is an important parameter in order to make a healthy comparison between plant and synthetic antioxidants. Namely, the lower the IC_{50} value, the higher the radical reducing activity. When the results are analysed, it can be concluded that both DPPH and ABTS assay results and plant values are close to each other and these tests show a promising antioxidant power. Thus, it can be said that DPPH and ABTS radicals show maximum inhibitor when the IC_{50} value expressing a stance against ROS is considered.

Phenylhydrazine is a highly reactive substance that readily reacts with carbonyl groups (-C=O) of biologically important molecules. The substance interacts with haemoglobin and causes oxidative reaction via cytochrome P450, leading to the formation of free radicals and haemolysis (WHO, 2000; Walenzuela *et al*., 1977).

In this study, the scavenging effect of methanol extract of *V. golawanense* on free radicals produced by haemolysis of erythrocytes with phenylhydrazine was evaluated. The highest haemolysis inhibition percentages of anti-haemolytic activity were 65.77 ± 0.62 for phenylhydrazine and 69.41 ± 0.078 for trolox. The concentration values (IC_{50}) that inhibit 50% of the anti-haemolytic activity were measured as 64.87 ± 2.24 and 62.68 ± 1.27 μg/mL for phenylhydrazine and trolox, respectively, and given in Table 1. When the results were evaluated, it was determined that the plant had a lower percentage inhibition value than the positive control, trolox, and in terms of IC_{50} value, the plant had a value close to the positive control, trolox. It can be said that the anti-haemolytic activity of the plant is at a reasonable level.

Considering the experimental results in general, these data are important in terms of being the first report of *V. golawanense* and being included in the literature. In addition, it is thought that

the data obtained in terms of antioxidant properties will be important in future in vivo studies to investigate the protective effect of the plant against diseases caused by oxidative stress. The high levels of vitamin K and vitamin C and total antioxidant capacity of the plant indicate that it can contribute to food products and pharmaceutical industry.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Ahmet Bakır: Study design, Investigation, Methodology, Resources, Visualization, Software, Validation, Formal Analysis, Writing-original draft and collecting plant material. **Suat Ekin**: Study design, Statistical analysis, Visualization and Validation. **Mehmet Fırat**: Identification and collection of plant material. All authors read and approved the final manuscript before submission.

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