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Evaluation of Antioxidant Properties, Trace Element and Mineral Composition of *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski (Orchidaceae)

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ABSTRACT: In this study, antioxidant properties, trace element and mineral composition of *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski (Orchidaceae) grown in the East Anatolia Region of Turkey was determined. The concentrations of trace element and mineral were determined by using inductive paired plasma-optical emission spectrometer (ICP-OES). It has founded that the quantities of important trace elements Mn, Cu, Co and Se were high and also the quantity of the Mg mineral was quite high. *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski plant's element quantities were determined to be Sr > Mg > V > Mn > Li > Ti > Cu > Se > Cr > Pb > Co > As > Be > Zn > Cd respectively. Total antioxidant activity, total phenolic and flavonoid contents were 12.44 ± 0.20 mM ascorbic acid g^{-1}, 4.97 ± 0.48 mg gallic acid g^{-1} and 4.51 ± 0.29 mg quarcetin g^{-1} respectively in methanol extracts of *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski plant. The presence of high levels of some trace elements, antioxidant activity, total phenolic and flavonoid contents in the methanol extract may have important to the showed antioxidant properties.

Key words: *Dactylorhiza umbrosa*, antioxidant, trace element

Dactylorhiza umbrosa (Kar. & Kir.) Nevski (Orchidaceae)'nin Antioksidan Özellikleri, İz Element ve Mineral Kompozisyonunun Değerlendirilmesi

ÖZET: Bu çalışmada, Türkiye'nin Doğu Anadolu Bölgesi'nde yetişen *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski (Orchidaceae)'in antioksidan özellikleri, iz elementleri ve mineral bileşimleri belirlenmiştir. İz element ve mineral konsantrasyonları, indüktif eşleşmiş plazma-optik emisyon spektrometresi (ICP-OES) kullanılarak belirlenmiştir. Önemli eser elementler olan Mn, Cu, Co ve Se miktarlarının yüksek olduğu ve Mg mineralinin miktarının da oldukça yüksek olduğu tespit edilmiştir. *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski bitkisinin element miktarlarının sırasıyla Sr> Mg> V> Mn> Li> Ti> Cu> Se> Cr> Pb> Co> As> Be> Zn> Cd olarak sıralandığı belirlenmiştir. *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski'nin metanol ekstraktındaki toplam antioksidan aktivite, toplam fenolik ve flavonoid içerikleri sırasıyla 12.44 ± 0.20 mM askorbik asit g^{-1}, 4.97 ± 0.48 mg gallik asit g^{-1} ve 4.51 ± 0.29 mg quarcetin g^{-1} olarak belirlendi. Metanol ekstraktında bazı eser elementlerin yüksek seviyelerinin bulunması ve antioksidan aktivite, toplam fenolik ve flavonoid içeriklerinin varlığı, gösterilen antioksidan özellikler açısından önemli olabilir.

Anahtar kelimeler: *Dactylorhiza umbrosa*, antioksidan, iz element

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INTRODUCTION

Throughout history, plants have been used to heal people and protect them from disease. In recent years their use has increased (Esetlili et al., 2014). The medicinal plants have positive effects on human health as they are rich in trace elements and minerals (Agay et al., 2005; Esetlili et al., 2014). They are important nutritional resources for minerals (Özcan, 2004). Mn, Cu, Fe, Zn, Se, Ca, Mg, N, P, K, Na, B and Mo are significant for plants and are vital for growth and development of all living bodies. However, Co, Cr, Ni, Pb and Cd are toxic heavy metals and they could be harmful to health according to international references (Agay et al., 2005; Esetlili et al., 2014). Medical and aromatic plants may be dangerous for health due to the high content of heavy metals they contain. As a result, the analysis of the measurement of heavy metal biomass due to environmental pollution in medical and aromatic plants has increased in recent years (Esetlili et al., 2014).

There are about 75 species of *Dactylorhiza* (Orchidaceae) in the World that are mainly distributed in the Northern Hemisphere. Species of this genus occupy a wide range of open habitats, from dune valleys to alpine meadows and including swamps and peat bogs (Shapoo, et al., 1904). 204 orchid species belonging to 23 genuses are grown in our country (Sandal, 2009). 40-50 million orchid plants are collected annually in Turkey, according to estimates. Collecting this excessive amount leads to a reduction of the wild resources of the plant (Ghorbani et al., 2014).

Orchid species have been used for many years for diarrhea, dysentery, internal diseases, cough, colds and tuberculosis treatment. Many orchid species are also used as food supplements for children and newborns and also as aphrodisiacs in the adults (Ghorbani et al.,2014). In Turkey Orchidaceae species are being used to obtain salep and is known as a drug used for aphrodisiac since the ancient times. It is also known to be beneficial in the defense of mucous membrane and in the removal of respiratory and bronchial and gastric ulcer diseases (Sandal, 2009).

The aim of the present study were to determine trace element and mineral levels, total phenolic and flavonoid contents, antioxidant activity of methanolic extract of *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski (Orchidaceae).

MATERIAL AND METHODS

Plant Material and Extraction

Dactylorhiza umbrosa (Kar. & Kir.) Nevski (Orchidaceae) leaf was collected in Van, Gevaş. Identification of the plant species was implemented by Dr. Süleyman Mesut PINAR from Y.Y.U. Witness plant sample is kept in Herbarium of Van Y.Y.U. with the code of VANF 164132.

The plant sample was ground to fine powder using a Kenwood Multi-Mill (Kenwood Ltd., UK). Methanol extraction of *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski was done to the method of described by Gulcin et al., (2004) with slight modifications. For methanol extraction, 20 g *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski sample was ground into a fine powder in a mill and mixed with 0.4 L methanol. The residue was re-extracted under the same condition until the extraction solvent became colorless. The combined extracts were filtered over whatman No. 1 paper. Extracts were evaporated to dryness in a vacuum by a rotary evaporator and crude methanol extract was obtained by lyophilization with a freeze-drier. The crude extracts were placed in a dark bottle and stored at -20°C prior to further use.

Mineral Determination

Determination of the amount of minerals was determined using dry burning the method of described by Zurera (et. al. 1987). Analyses of Mg, As, Be, Co, Cd, Cu, Cr, Mn, Li, Se, Pb, V, Sr, Ti and Zn elements were performed using inductive paired plasma-optical emission spectrometer (ICP-OES).

Antioxidan Properties

Total antioxidant activity

Evaluation of TAC of *Dactylorhiza umbrosa* (KAR. & KİR.) Nevski plant was assessed by the method of described by Prieto (et.al. 1999). 0.2 ml of sample solutions were combined in a tube with 2 ml of reagent solution; 4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulfuric acid. The tubes were capped and incubated at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Antioxidant capacity was expressed as equivalent of ascorbic acid (AAE) per gram of sample.

Total phenolic assay

The TPC was measured by using Folin–Ciocalteu reagent described by Gamez-Meza (et. al. 1999); Yi (et. al. 1997). Total phenolic contents was expressed as mg of gallic acid equivalent (GAE) per gram of sample.

Total flavonoids

The total flavonoid content was measured by the aluminium chloride colorimetric assay method described by Lamaison, (et. al. 1990); Ürgeova ve Polivkau, (2009) by reading the absorbance at 394 nm. Total flavonoid content was expressed as mg of quercetin equivalent (QE) per gram of sample.

RESULTS AND DISCUSSION

Mineral Levels

Table 1 demonstrates trace elements (As, Be, Cr, Cd, Mn, Co, Se, Cu, V, Li, Pb, Zn, Ti, Sr) and mineral (Mg) levels of *Dactylorhiza umbrosa* (KAR. & KIR.) Nevski plant.

It was determined that the *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski plants element quantities were ordered Sr > Mg > V > Mn > Li > Ti > Cu > Se > Cr > Pb > Co > As > Be > Zn > Cd respectively, in our study.

Table 1. Trace Element and Mineral levels of *Dactylorhiza umbrosa* (KAR. & KIR.) Nevski plant

Minerals	*D. umbrosa* (KAR. & KIR.) Nevski $\bar{x} \pm$ SEM
As (μmol kg^{-1})	2.054 ± 1.37
Cu (mmol kg^{-1})	0.099 ± 0.015
Cr (μmol kg^{-1})	14.54 ± 2.83
Co (μmol kg^{-1})	3.21 ± 0.83
Mg (mmol kg^{-1})	112.55 ± 1.68
Mn (mmol kg^{-1})	0.58 ± 0.089
Li (mmol kg^{-1})	0.22 ±0.059
Be (μmol kg^{-1})	1.23 ± 0.17
Cd (μmol kg^{-1})	0.21 ± 0.0075
Pb (μmol kg^{-1})	6.67 ± 1.089
Se (μmol kg^{-1})	1.0005 ± 0.81
Sr (mmol kg^{-1})	662.41 ±25.11
Ti (mmol kg^{-1})	0.12 ± 0.0077
V (μmol kg^{-1})	71.058 ± 28.43
Zn (mmol kg^{-1})	0.32 ± 0.095

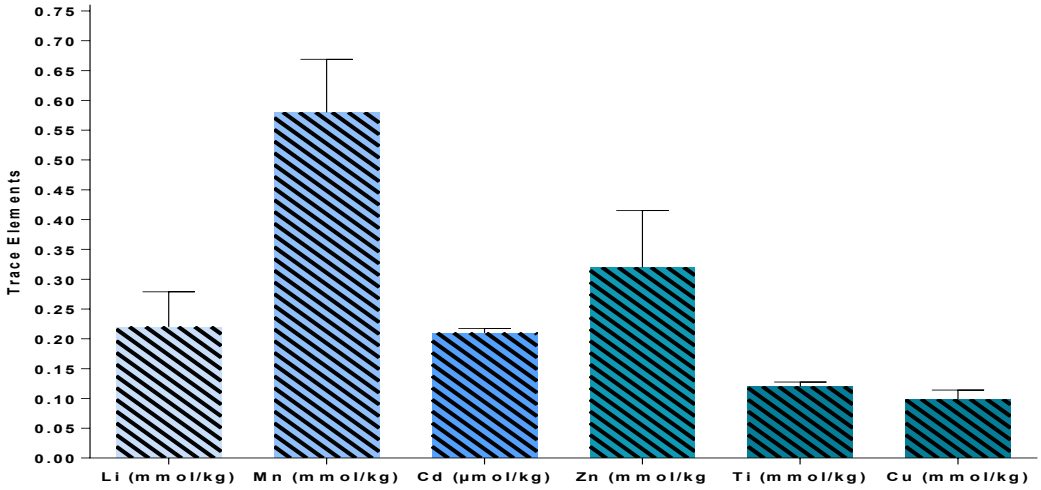

Figure 1: Trace element (Li, Mn, Cd, Zn, Ti, Cu) levels of *Dactylorhiza umbrosa* (KAR. & KİR.) Nevski plant

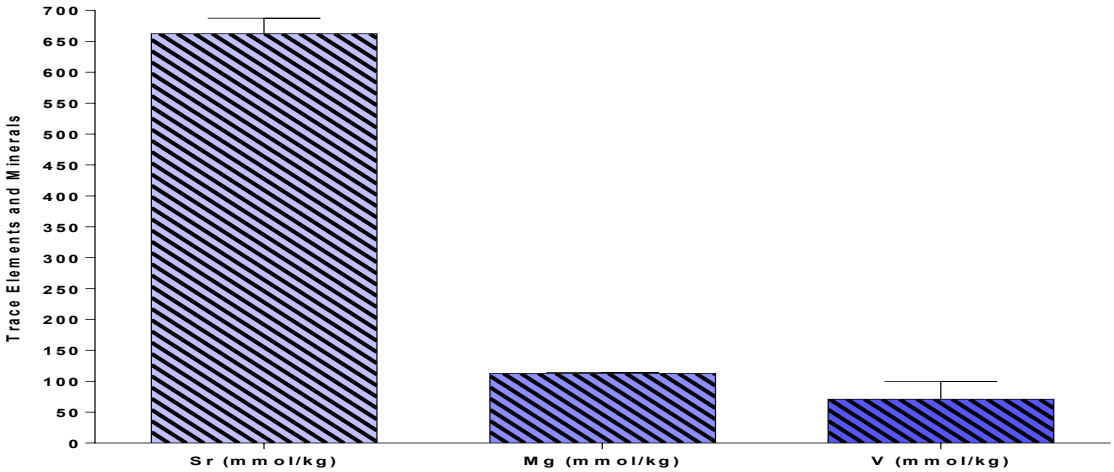

Figure 2: Trace element (Sr, V) and mineral (Mg) levels of *Dactylorhiza umbrosa* (KAR. & KIR.) Nevski plant

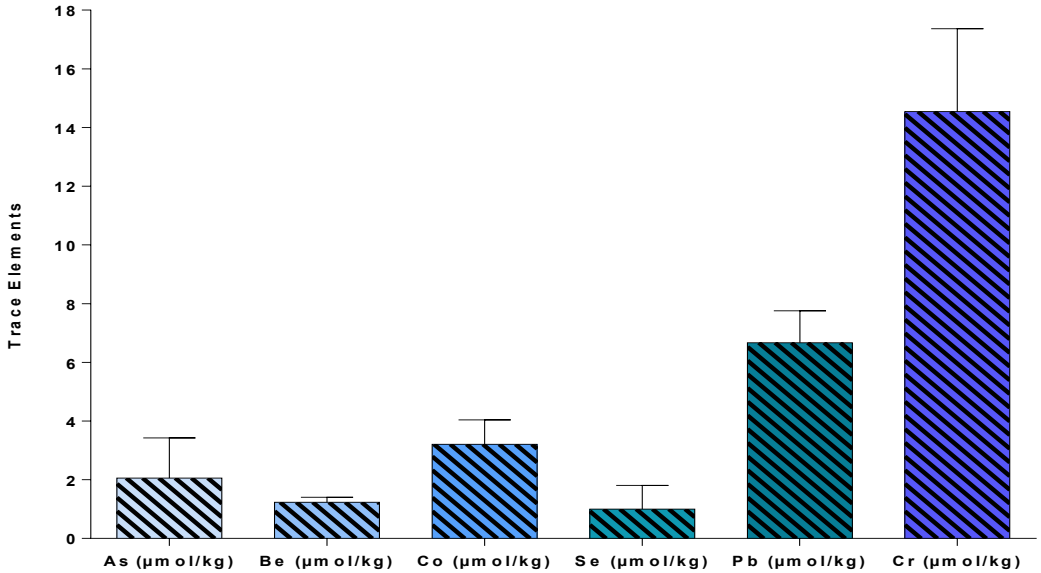

Figure 3: Trace element (As, Be, Co, Se, Pb, Cr) levels of *Dactylorhiza umbrosa* (KAR. & KİR.) Nevski plant

Antioxidant Levels

Total phenolic, flavonoid content and antioxidant activity levels of methanol extracts of *Dactylorhiza umbrosa* (KAR.& KIR.) Nevski plant were determined as follows; 12.44 ± 0.20 mM ascorbic acid g^{-1}; 4.97 ± 0.48 mg gallic acid g^{-1}; 4.51 ± 0.29 mg quadrant g^{-1}.

The present study was performed to investigate trace element and mineral levels, total phenolic and flavonoid contents, and antioxidant activity of *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski plant's methanolic extract. The *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski's element quantities determined as Sr > Mg > V > Mn > Li > Ti > Cu > Se > Cr > Pb > Co > As > Be > Zn > Cd respectively. The quantities of Mn, Cu, Zn, and Se which are important trace elements were high and also the quantity of the Mg, mineral was quite high. The levels of As, Co, Cd and Pb from toxic heavy metals were also lower than international references.

Table. 2. Levels of Total Antioxidant Activity, Total Phenolic and Flavonoid Content In Methanol Extracts of *Dactylorhiza umbrosa* (KAR.& KIR.) Nevski plant

Parameters	*D. umbrosa* (KAR. & KIR.) Nevski	$\overline{x}$ ± SEM
Total antioxidant activity (mM ascorbic acidg[1])		12.44 ± 0.20
Total phenolic content (mg gallic acid g^{-1})		4.97 ± 0.48
Total flavonoid content (mg quarcetin g^{-1})		4.51 ± 0.29

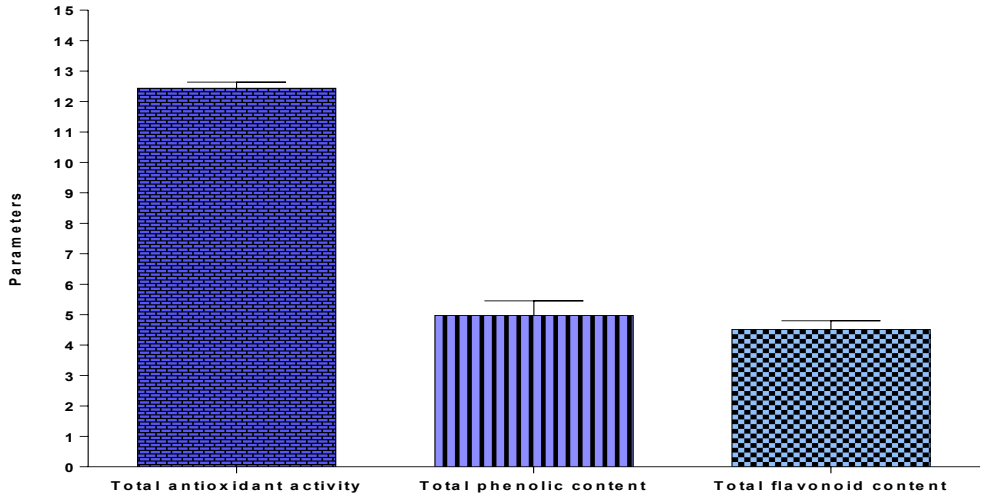

Figure 4: Total Antioxidant Activity, Total Phenolic and Flavonoid Contents In Methanol Extracts of *Dactylorhiza umbrosa* (KAR.& KIR.) Nevski plant

Pb, Cd, As, Co, and Cr are toxic heavy metals and they could be harmful to health according to international references (Agay et al., 2005; Esetlili et al., 2014; Jan et al., 2015). The concentrations of Pb and Cd reported by WHO (1999) for medicinal and aromatic plants are 10 mg kg^{-1} (48.26 µmol kg^{-1}) and 0.3 mg kg^{-1} (2.67 µmol kg^{-1}), respectively (Esetlili et al., 2014). In our study the concentration of Pb was 6.67 ± 1.089 µmol kg^{-1} and also the concentration of Cd was found as 0.21 ± 0.0075 µmol kg^{-1}. So in our study, Pb and Cd concentrations were found to be much lower than these values. Lead is the most toxic heavy metal in the environment. Lead poisoning occurs as a result of food or water lead contamination. Lead is poisonous enough to affect any organ, adversely affecting the central nervous system, the cardiovascular system, the kidneys and the immune system. Lead affects most of the nervous system in all organs (Wani et

al., 2015). In humans, Cd intoxication is caused by the inhalation of cigarette smoke and also by water, food and air. Intoxication of Cd leads to liver, lung, testis and kidney damages also it leads to metabolism and immune system disorders (Vukivećević, T., 2012). The safe concentrations of arsenic for white rice was reported by WHO as 0.2 mg kg^{-1} (2.66 µmol kg^{-1}) (Jan et al., 2015).

In our study the level of As was as 2.054 ± 1.37 µmol kg^{-1} concentration was found to be lower than safe concentrations. Arsenic plays a role in the metabolism of methyl compounds and inadequacy leads to problems in the reproductive system and in cardiac function (Singh, et al., 2010). In this study *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski was found Co ve Cr content 3.21 ± 0.83 µmol kg^{-1} and 14.54 ± 2.83 µmol kg^{-1} respectively. Co is an element that participates in the structure of vitamin B_{12} (Esetlili et al., 2014). The recommended daily intake is 0.05 to 1 mg day^{-1} (Kumar et al., 2007). Thus the range of Cobalt in *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski falls within the safety limit in this study. Chromium acts as a glucose tolerance factor and is used at diabetes metabolism (Singh, et al., 2010). Cr concentration of wheat flour is 5-10 µg kg^{-1} (96.16 - 192.32 µmol kg^{-1}). Recommended daily intake for people with chromium is 60 µg (Esetlili et al., 2014). The quantities of Cr was found to be higher than international reference values, in our study.

In this study Li content in *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski was obtained as 0.22 ± 0.05 mmol kg^{-1}. The amount above 2 mmol l^{-1} is toxic to humans. Lithium is an alkali metal and it is a highly reactive. The human body usually has lithium deficiency. It is used in the treatment of manic depressive psychosis (Tudosie et al., 2012). In our study, Li concentration was found to be much lower than toxic values.

In this study, contents of Ti, Be and Sr in *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski were determined 5.67 ± 0.366; 0.011 and 58040 ± 2200 mg kg^{-1} respectively. Titanium is harmful to the body and its function is not known precisely (Singh, et al., 2010). Beryllium in the soil can easily be taken by plants. Beryllium may be toxic when inhaled or ingested by humans. Therefore, it is important to study beryllium toxicity in plants (Shah, et al., 2016). Contamination with strontium occurs via air, drinking water, soil and nutrients. It is mostly taken with water and food to the body. If taken at high doses, it can cause malignancy at bone and also, it can cause leukemia (Özdemir ve Esen., 2004).

It was seen in our study that copper content was 0.099 ± 0.015 mmol kg^{-1}, in *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski. The amount found in plants generally ranges from 16 to 20 mg kg^{-1} (0.2515 to 0.3147 mmol kg^{-1}). Cu is vital for plants, animals and humans, but excessive intake can cause health problems. Daily doses of 2.5 mg Cu meet the daily needs of adults (Esetlili et al., 2014). Copper is the third most abundant trace element in the body. Cu is included in the oxidation of Fe^{+2} to Fe^{+3} during hemoglobin formation (Singh, et al., 2010). The quantities of Cu was found to be lower than international reference values, in our study.

In this study Zn content in *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski was obtained as 0.32 ± 0.095 mmol kg^{-1}. Zn is essence for all living cells. The daily amount to be taken for humans is 4.5 mg (Esetlili et al., 2014). Zn is essential for all organisms and has a role in metabolism, growth and development. It is involved in the structure of more than 200 enzymes. Zn deficiency leads to recurrent infections and immune system failure. Low intake of Zn leads to coronary artery disease (Singh, et al., 2010). In this study the quantities of Zn was found to be higher than international reference values.

Dactylorhiza umbrosa (Kar. & Kir.) Nevski was found Mg content as 112.55 ± 1.68 mmol kg^{-1}. Mg plays a role as a cofactor in more than 600 enzymatic reactions which are very important in metabolic pathways. Mg is a control factor in

nervous system, smooth muscle contraction, cardiac excitability, and in the blood pressure (Nielsen and Jhonson., 2017). The recommended dosage of Mg in adults is 300-400 mg (Esetlili et al., 2014). The quantities of Mg was found to be higher than recommended dosages, in our study.

In this study the contents of Manganese, Selenium and Vanadium in *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski were respectively 0.58 ± 0.089 mmol kg^{-1}; 1.0005 ± 0.81 µmol kg^{-1}; 71.058 ± 28.43 µmol kg^{-1}. Manganese is essential for many enzymes and organisms. Daily intake requirements of the Mn is 2.5 - 5.0 mg day^{-1} (Singh, et al., 2010). Selenium is involved in the structure of glutathione peroxidase and some other enzymes. It features antioxidant properties and its deficiency leads to muscle and pancreatic degeneration. (Singh, et al., 2010). It is recomendd that Se intake of 50-200 µgday^{-1} is safe and adequate by the U.S. National Academy of Sciences (McLaughlin, et al., 1999). In our study, Se concentration was found to be within the recomanded values. Vanadium is involved in the protection system against tissue damage. It also participates in enzyme formation. Vanadium deficiency leads to developmental retardation, problems in the reproductive system and tooth decay (Singh, et al., 2010).

Orchidaceae is one of the largest members of the flowering plant family. Most of its members are traded worldwide. Many medicinal orchid species contain many phytochemicals such as phenol, flavonoids, alkaloids and stereoids (Nguyen et al., 2018). The aim of this study is contribute to the search for uses of this plant as a natural antioxidant and for medicinal purposes. In our study aerial parts of *Dactylorhiza umbrosa* (KAR.& KİR.) Nevski plant investigated for, total phenolic, flavonoid content and antioxidant activity of its. The levels of total antioxidant activity, total phenolic and flavonoid content in methanol extracts of *Dactylorhiza Umbrosa* (KAR.& KIR.) Nevski were ordered 12.44 ± 0.20 (mM ascorbic acid g^{-1}); 4.97 ± 0.48 (mg gallic acid g^{-1}); 4.51 ± 0.29 (mg quarcetin g^{-1}) respectively. Minh et al., (2016) investigated total phenolic and flavonoid contents and total antioxidant activity of Phalaenopsis orchid hybrids in their studies. The ethanol extracts of the leaves and roots of the hybrid Chien Xen Queen have the highest amount of total phenol with 11.52 ± 0.43 mg gallic acid aquavalent per g dry weight and the highest total flavonoid with 4.98 ± 0.27 mg rutin aquivalent per g dry weight. Nguyen et al., (2018) studied white, yellow and purple flowering species of Phalaenopsis (Phal.) Orchids and found that the purple orchid flavonoid content was the highest with amount 188.70 ± 3.03 mg QE g^{-1} DW.

The amount of polyphenols determined in the flowers and leaves of the yellow flowering orchids was higher than the others. The determined amounts of polyphenols of its in the flowers and leaves were as 446. 22 ± 60.03 mg gallic acid g^{-1} DW and 244. 23 ± 51.39 mg gallic acid g^{-1} DW, respectively. Štajner et al., (2010) has invastigated the flavonoid content of anacampitis pyrimidalis L. which is an orchid species. Flavonoid content was determined to be 30.38 ± 3.45 (mg g^{-1}) and 13.85 ± 2.04 (mg g^{-1}) respectively in the flower and the above ground part. *Dendrobium signatum* belongs to the genus Dendrobium, which is the largest genus of orchid species in Thailand (Rattana and Sangchanjiradet, 2017). Chimsook, T, (2016) worked with *Dendrobium signatum* and they determinated the total phenol content of *Dendrobium signatum* ethanol extract at 5.52 + 0.28 g GAE. $(100\ g)^{-1}$.

CONCLUSION

The results showed that the *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski contains high content of total phenolic, flavonoid content and total antioxidant activity. Presented results indicated that extract of *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski exhibited antioxidant abilities in above ground parts. Therefore the above ground part of the *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski could be used as a source of

natural antioxidant. Our investigation could be starting point for further phytochemical investigations of this plant.

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