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ANTIOXIDATIVE CAPACITIES AND PHENOLIC COMPOUNDS OF VARIOUS EXTRACTS OF *AUBRIETA DELTOIDEA*

Abstract *This study was designed to examine the phenolic compounds and antioxidant capacities of various extracts (ethanol, acetone and water) of Aubrieta deltoidea. The antioxidant activities of these extracts were evaluated by phosphomolybdenum and reducing power methods. In addition, total phenolic, flavonoid and tannin contents were also determined. In phosphomolybdenum method, among the three different extracts of A. deltoidea evaluated, acetone extract (52.76 ± 0.57 µg/mg), showed the highest activity. Ethanol extract showed the highest amount of power reducing activity (0.137 ± 0.005 mg/mL). The total phenolic and flavonoid content of A. deltoidea extracts ranged from 15.21 ± 2.29 to 58.08 ± 8.10 mgGAE/g and 27 ± 0.87 to 93.18 ± 0.36 mg QEs/g respectively. Total tannins content of A. deltoidea extracts varied from 2.20 ± 0.38 to 56.94 ± 9.89 mgCE/g.*
Key words: *Aubrieta deltoidea, Phosphomolybdenum, Phenolic compounds, Antioxidant.*

Introduction. Use of plants as medicines has been known for centuries. Nowadays plants are basic food source and a way to prevent illnesses especially in developing country for the vast majority of the world population (Pezzuto, 1997). Large number of plants has been investigated for their antioxidant properties since natural antioxidants are very effective to prevent the destructive processes caused by oxidative stress (Zengin et al., 2011). Brassicaceae family has number of biological active compounds such as phenolic acids, flavonoids and vitamins which are associated with antioxidant and anticancer properties. Therefore, Brassicaceae family possess both antioxidant and anticarcinogenic properties (Vaughn and Berhow, 2005; Cohen et al., 2000; Chu et al., 2002). Aubrieta genus, in the Brassicaceae family, consist of about 12 species (Al-Shehbaz et al., 2006) and genus Aubrieta comprises perennial herbs distributed in Southwest Asia (mostly Anatolia) and South and Southeast Europe. Aubrieta deltoidea is a species of flowering plant in the Aubrieta genus and it has an Anatolian-Balkan-Appennines area of distribution (Ancev and Goranova, 2009). Many papers have been published in which antioxidant properties and phenolic compounds of different plant species in Brassicaceae family especially Brassica vegetables are studied but as far as our literature survey could ascertain, there are no reports on the biological activities of *Aubrieta deltoidea*. Therefore, the present study is the first in this area.

Objective. This study was designed to evaluate antioxidant properties, phenolic, flavonoid and tannin content of extracts isolated from *Aubrieta deltoidea* by different polarity solvents, such as ethanol, acetone and water.

Materials and methods: Plant material and plant extract. *A. deltoidea* was collected in the flowering stage from Honaz Mountain in Denizli (1300-1400 m) and identified in our laboratory. The aerial parts were air-dried and powdered. The extracts of *A. deltoidea* were prepared according to Ozay et al. (2015) and all extracts were lyophilized (Labconco FreeZone, Kansas City, MO) and stored at -20 °C until use.

Determination of total antioxidant activity. Phosphomolybdenum method.

Antioxidant activities of acetone, ethanol and water extracts were evaluated by phosphomolybdenum method according to Prieto et al. (1999). In phosphomolybdenum method, different concentration 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 ^{o}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 activity carried out with slight modifications of the method of Oyaizu (1996). Different concentration of extracts were mixed with 0.2M phosphate buffer (pH:6.6) and 1% potassium ferricyanide. The mixture was incubated at 50^{o}C for 20 min and then 10% trichloroacetic acid was added. Reaction mixture (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 ml of 0.1% ferric chloride. The solution absorbance was measured at 700 nm.

Determination of bioactive components. Tannin content. Tannin content was determined by the vanillin method of Bekir et. al. (2013) with slight modification. The extracts (0.5 mL) were mixed with vanillin reagent (1% in 7M H_2SO_4) in test tubes that are placed in an ice bath. Absorbance of the solution was measured at 500 nm after 15 min incubation at room temperature.

Total phenolic content. Total phenolic content was determined with Folin-Ciocalteu method (Slinkard and Singleton 1977). In this method, extract (1 mg/mL) was mixed with Folin-Ciocalteu reagent (1 mL) and distilled water (46 mL). After 3 min, 2% sodium carbonate (Na_2CO_3) solution was added. The mixture was incubated in dark for 2 h at room temperature and absorbance measured at 760 nm. Gallic acid was used for calibration and the results were expressed as mg of gallic acid equivalents (mg GAE g $^{-1}$ extract).

Total flavonoid content. Total flavonoid content of extracts was determined according to Arvouet-Grand et al. (1994). Briefly, 1mL of 2% $AlCl_3$ was mixed with the extract solution (2 mg/mL). After 10 min incubation at room temperature absorbance of the reaction mixtures were measured at 415 nm. The flavonoid content was calculated from a quercetin standard curve (mg QEs/g extract).

Statistical analysis. All analyses were performed in triplicate and The results obtained were analysed by using MINITAB Statistical Package program. The differences between the different extracts were tested with Analyses of Variance (ANOVA) and to see which groups are different from the others tested with Tukey ($P<0.05$).

Results and discussion. Phosphomolybdenum and reducing power activity. There is no standard method for determining the antioxidant activity of a compound due to antioxidant activity determination methods depend upon several parameters such as the reaction condition in the system studied and the structure of the compound to be analyzed. Thus, it is recommended that the antioxidant measurements should be evaluated using a several methods, at least two methods (Brand-Williams et al., 1995; Zengin and Aktümsek, 2014). Consequently, we applied two antioxidant methods (phosphomolybdenum and reducing power method) to evaluate true antioxidant potential of the extracts. In this study, phosphomolybdenum activity of acetone, ethanol and water extract of *A. deltoidea* were 52.76 ± 0.57, 40.52 ± 4.37 and 34.40 ± 2.61 µg/mg respectively. There was a statistically significant difference between acetone, ethanol and water extracts (Table 1). The higher activity in acetone extracts were may due to high contents of antioxidant components. In previously studies phosphomolybdenum activity were determined from different species of Brassicaceae family (Ozay and Mammadov 2016; Savran et al., 2016). Savran et al. (2016) extracted *Pseudosempervivum sempervivum* using different solvents (acetone, metanol and water) and they found that phosphomolybdenum activity were found to be different according to the solvents used and this results were in aggrement with our results.

As can be seen from Table 1, the highest reducing power activity (0.137 ± 0.005 mg/mL) was observed in ethanol extract of A. deltoidea also the lowest reducing power activity (0.13 ± 0.003 mg/mL) was observed in water extract. In previously study, total reducing power activity was determined from *Zilla macroptera* of Brassicaceae family (Keffous et al., 2016). In compared results of this study and our study, reducing power activity of water extract of *A. deltoidea* is higher than water extract of *Z. macroptera.*

Total phenolic, flavonoid and tannin contents. Phenolic acids are phenolic compounds that have been extensively studied over the last years

Table 1

Antioxidative potentials of the extracts of A. deltoidea

Sample	Phospho-molydenum (µg/mg)	Power reducing (mg/mL)
Acetone	52.76 ± 0.57 [a]	0.132 ± 0.004[ab]
Ethanol	40.52 ± 4.37 [b]	0.137 ± 0.005 [a]
Water	34.40 ± 2.61 [c]	0,13 ± 0.003[b]

**Values are mean of three replicate determinations (n=3) ± standard deviation.*

Mean values follwed by different superscripts in a column are significantly different (p<0.05).

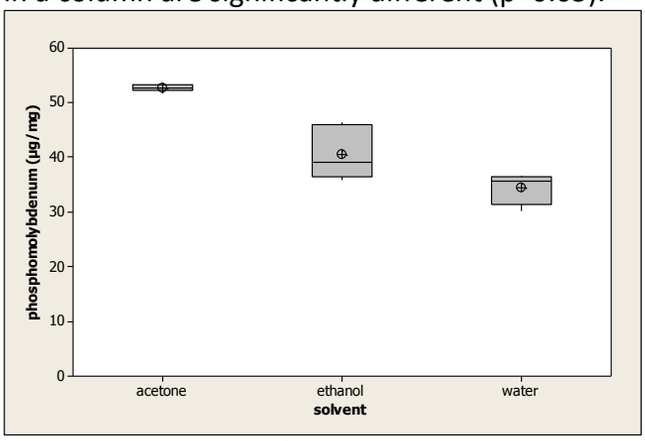

Figure 1. Comparison of phosphomolybdenum activity of different extracts of A. deltoidea

because of their potency in protecting against various diseases such as cancer, dermatological and coronary heart diseases. (Piasek et al., 2009; Mammadov 2014). Phenolic compounds are good indicators of antioxidant activity because of their hydrogen donor and radical scavenging properties (Miguel, 2010) and chelating ability (Michalak, 2006). In present study, total phenolic content of A. deltoidea extracts ranged from 15.21 ± 2.29 to 58.08 ± 8.10 mgGAE/g. Acetone extract contained the highest total phenolic content (58.08 ± 8.10 mgGAE/g) followed by ethanol extract (22.34 ± 2.22 mgGAE/g), and water extract (15.21 ± 2.29 mgGAE/g) (Table 2). Previous reports have demonstrated that water extracts from several plants possess lowest phenolic contents, which is in agreement the present results (Zengin et al., 2015, Savran et al., 2016).

Flavonoids are secondary metabolite that are abundant and commonly found in plant foods. Flavonoids also have significant antioxidant properties and they can act as free radical scavenger and metal chelators (Aktümsek et al., 2013; Carocho and Ferreira, 2013). In the present study, acetone extract (93.18 ± 0.36 mgQEs/g) possessed highest content of flavonoid compared to ethanol and water extracts with 77.59 ± 1.29 and 27 ± 0.87 mg QEs/g respectively (Table 2). In previously studies total flavonoid content were determined from different species of Brassicaceae family (Ozay and Mammadov, 2016; Savran et al., 2016). Savran et al. (2016) indicated that total flavonoid content of acetone, metanol and water extracts of *Pseudosempervivum sempervivum* were 30.9 ± 0.6, 41.5 ± 0.2 and 13.2 ± 0.4mg QEs/g respectively. In compared results of this study and our study, flavonoid content of acetone and water extracts of *A. deltoidea* (93.18 ± 0.36 and 27 ± 0.87 mg QEs/g) are higher than acetone and water extracts of *P. sempervivum* and flavonoid contents were found to be different according to the solvents used.

In the present study, total tannins content of A. deltoidea extracts was presented in Table 2 and varied from 2.2 ± 0.38 to 56.94 ± 9.89 mgCE/g. Acetone extract contained the highest value of total tannins content (56.94 ± 9.89 mgCE/g) followed by ethanol (22.76 ± 4.26 mgCE/g), and water (2.20 ± 0.38 mgCE/g). There was a statistically significant difference between acetone, ethanol and water extracts (Table 2). Bekir et al., (2013) extracted *Punica granatum* using different solvents and they found that tannin contents were found to be different according to the solvents used. This was in aggrement with our

Table 2.

Total flavonoid, phenolic and tannin content of A. deltoidea extracts

Sample	Total flavonoid content (mgQEs/g)	Total phenolic content (mgGAEs/g)	Total tannin content (mgCEs/g)
Acetone	93.18 ± 0.36[a]	58.08 ± 8.10 [a]	56.94 ± 9.89 [a]
Ethanol	77.59 ± 1.29[b]	22.34 ± 2.22 [b]	22.76 ± 4.26 [b]
Water	27 ± 0.87[c]	15.21 ± 2.29 [c]	2.2 ± 0.38 [c]

**Values are mean of three replicate determinations (n=3) ± standard deviation. Mean values followed by different superscripts in a column are significantly different (p<0.05).*

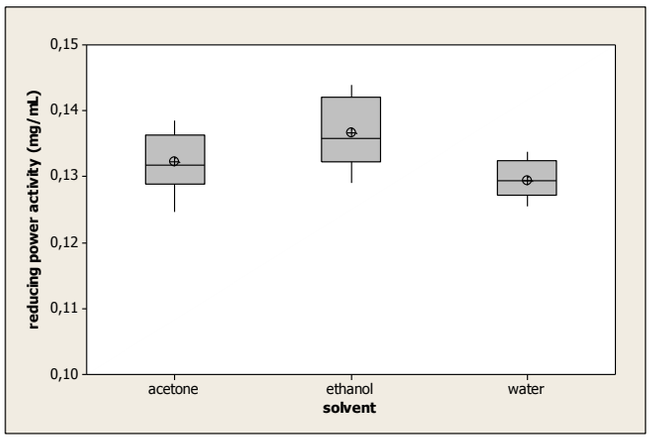

Figure 2. Comparison of reducing power activity of different extracts of A. deltoidea

results, tannin content varied according to the solvent used.

Conclusions. Interest in antioxidants have also increased considerably in recent years because the use of antioxidants is an effective way of protecting against free radical-related diseases (Babu et al., 2013). In the present study, antioxidant capacities, total phenolic, flavonoid and tannin content of ethanol, acetone and water extracts of *A. deltoidea* were evaluated. To the best our knowledge, this study is the first undertaken on the antioxidant properties and phenolic compunds of *A. deltoidea*. We think that the results showed here will supply new information for further studies in this species.

Prospects of further studies. The results presented here will help us to understand the antioxidant capacity and phenolic compounds of *A. deltoidea* and might provide additional information for the further studies about evaluate biological activities of this species and also help us to understand the importance of A. deltoidea. However, further indepth studies, such as the study of additional antioxidant capacities, phenolic compound and also study of anthelmintic, antimicrobial activities are needed.

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