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Investigations of *in vitro* Antioxidant Activities, Elemental Compositions and Lipid Constituents of *Acanthus dioscoridis* L. var. *dioscoridis* L. as a Medicinal Plant

Cumali KESKİN[1]

ABSTRACT: In our study, some *in vitro* biological activities of hexane (HEG), ethylacetate (EtOAc) and methanol (MeOH) extracts of *Acanthus dioscoridis* L. var. *dioscoridis* L. aerial parts have been investigated in details. Plant extracts were tested in view of its *in vitro* antioxidant activities as total phenolic and total flavonoid contents, DPPH free radical-scavenging, metal chelating and reducing power activity. Total phenolic content of MeOH extract has been found as 71.18 µg GAE/mg. The highest amount of flavonoids has been detected in EtOAc extract. The highest DPPH radical-scavenging activity and reducing power activity has been determined in MeOH extract. HEG extracts of *Acanthus dioscoridis* has showed clearly higher metal chelating activity when compared to other extracts. ΣPUFA percentage was meaningfully higher than ΣMUFA and ΣSFA. By considering the high PUFA content and strong antioxidant activities it could be concluded that *A. dioscoridis* could be recommended to possible use in medical science, cosmetic and food industry.

Keywords: *Acanthus dioscoridis* L. var. *dioscoridis* L., biological activity, fatty acids, medicinal plants, trace elements

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Tıbbi Bir Bitki Olarak *Acanthus dioscoridis* L. var. *dioscoridis*' in *in vitro* Antioksidan Aktiviteleri, Elementel Bileşimi ve Lipit Bileşenlerinin Araştırılması

ÖZET: Bu çalışmamızda *Acanthus dioscoridis* L. var. *dioscoridis* L.' in toprak üstü kısımlarının hekzan (HEG), etilasetat (EtOAc) ve metanol özütlerinin bazı *in vitro* biyolojik aktiviteleri detaylı olarak incelenmiştir. Bitki özütlerinin *in vitro* antioksidan aktiviteleri total fenolik ve flavonoid içerikleri, DPPH serbest radikal söndürücü, metal şelatlama ve indirgeme gücü aktiviteleri bakımından test edildi. MeOH özütünün toplam fenolik madde içeriği 71.18 µg GAE/mg olarak tespit edilmiştir. En yüksek flavonoid miktarı EtOAc özütünde tespit edilmiştir. En yüksek DPPH radikali söndürme aktivitesi ve indirgeme gücü aktivitesi MeOH özütünde belirlenmiştir. *Acanthus dioscoridis* L. var. *dioscoridis* L.' nin HEG özütü diğer özütler ile karşılaştırıldığında açık bir şekilde daha yüksek metal şelatlama aktivitesi göstermiştir.ΣPUFA yüzdesi anlamlı olarak ΣMUFA ve ΣSFA' dan daha yüksekti. Yüksek PUFA içeriği ve güçlü antioksidan aktiviteleri göz önüne alındığında *A. dioscoridis'* in tıbbi bilimlerde, kozmetik ve gıda endüstrisinde olası kullanımı önerilebilir.

Anahtar Kelimeler: *Acanthus dioscoridis* L. var. *dioscoridis* L., biyolojik aktivite, iz elementler, tıbbi bitkiler, yağ asitleri

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[1] Mardin Artuklu Üniversitesi, Sağlık Yüksekokulu, Beslenme ve Diyetetik, Mardin, Türkiye
Sorumlu yazar/Corresponding Author: Cumali KESKİN,ckeskinoo@gmail.com

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INTRODUCTION

Plants are rich sources of natural antioxidant and they play critical protective roles in human health. This protective effects are related to plant antioxidant compounds such as carotenoids, vitamins, and phenols found in different parts of plants (Pietta, 2000;Saura and Goni, 2006).

Plants synthesize a large range of fatty acids and most plants store the lipids in the form of triglycerides (Murphy, 1990). Recent studies show that polyunsaturated fatty acids, especially ω-3 fatty acids are very important for human health (Amiguet et al., 2012).

Metals like cobalt, magnesium, selenium, iron, zinc and copper are essential nutrients for various biochemical and physiological pathways. However, excessive intake of these micronutrients results in a variety of deficiency diseases or syndromes in organisms, and they limit the use of medicinal plants. It is a known fact that metals accumulate in plants through their root system and demonstrate their dispersion in the different parts of plants such as root, leaf, and fruit. So, plants can also be used as bio-accumulator (WHO, 1996).

Acanthus is a genus of about 30 species of flowering plants in the family *Acanthaceae*. It is represented by eight taxa in Turkey. *Acanthus* and Bear's breeches (Davis, 1993) are the most common names of the genus.

Acanthus species are widely used as folk medicines for treatments of rheumatism, lymph node inflammations, snakebite, palsy, hepatic disorders, asthma attack, and bellyache in different countries (Hai et al., 2009). Moreover, studies show that many species of genus *Acanthus* include different secondary metabolites, especially benzoxazinoides, phenylethanoides (protochatecuic acid), lignans (shikimic acid, pinoresinol), flavongycoside (linaroside, homoplantagenin, acetocide, homoplantagonenin), megastigmanes, fatty acids (palmitic acid) and aliphatic alcohol and sterol glycosides (sitosterol-3-O-β-Dglucoside) (Elham et al., 2014;Huang et al., 2014).

According to various medical studies, some *Acanthus* species show different pharmacological and biological activities such as antioxidant, hepatoprotective, antimicrobial, antiulcer, osteoblastic, antileishmanial, anti-inflammatory and anticancer activity (Singh and Aeri, 2013).

This study aims to evaluate the antioxidant ability, trace metals contents and lipid constituents of aerial parts of *A. dioscoridis* L. var. *dioscoridis* L.

MATERIAL AND METHODS

Instrumentation

Concentrations of Cd, Ni, Cu, Pb, Cr, Fe, B and Al were measured by a inductively coupled plasma-optical emission spectrometry (ICP-OES) with instrumental conditions reported in literature (Keskin et al., 2014).

Fatty acid methylesters (FAMEs) were separated and quantified by capillary GC using a Hewlett Packard (Wilmington, DE) GC (model 6890), a BPX-70 capillary column (30 m x 320 μm (i.d) x 0.250 μm film thickness and bonded 70% cyanopropyl) (J & W Scientific, Folsom, CA), a flame ionization detector (FID) and Hewlett-Packard ChemStation software. The injection port temperature was 270ºC, the detector temperature 280ºC. The split ratio was 1:20. The flow rates of compressed air and hydrogen were 300 mL/min, 30 mL/min, respectively. Helium was used as the carrier gas (1.0 mL/min). The initial oven temperature was set to 130 °C and the oven was kept in this temperature for a minute. Later on, the temperature was increased to 170 °C at a rate of 6.5 °C/min, then again, it was increased to 215 °C at a rate of 2.75 °C/min and the oven was kept in this temperature as well for 12 minutes. Finally, the oven was heated to 230 °Cat a rate of 40 °C/min and it was kept at 230 °C for 3 minutes. Thus, total amount of time spent for analysis was 38.8 minutes

Collection of plant samples

Plant materials were collected from Mardin city, located in the South East region of Turkey, in the period of April and May, and stored in polyethylene bags according to their species and transferred to the laboratory for preparation and experiment. The aerial parts (the flower, leaf, and stem) of the plants were used in the experiments. Subspecies of the collected plant was identified by Prof. Dr. Selçuk Ertekin from Biology Department of Faculty of Science of Dicle University.

Transmethylation of lipids and extraction of methyl derivatives

The samples were analysed according to the procedure described by Garches and Mancha (1993). TLC (0.25 mm silica gel 60 F254, Merck) was used to separate phospholipids (PL) and triacylglycerol (TG) by the help of a mixture of petroleum ether, diethyl ether and acetic acid (80:20:1; v/v) as the mobile phase. TLC plates were air-dried and sprayed with a solution of 0.2% (w/v) 2,7-dichlorofluorescein (Supelco, Supelco Park, Bellefonte, PA, USA) in metanol and bands were visualised under UV light (254 and 366 nm). PL and TG fractions were recovered from the TLC plates by scraping off the appropriate bands. Acidified methanol was used to transesterified to samples and the FAMEs were extracted by hexane.

Statistical analysis

SPSS 15.0 was used for statistical calculations. Statistical comparisons of the fatty acid percentages were carried out by an analysis of variance (ANOVA) and comparisons between means were performed by using Tukey's test. *P* values less than 0.05 were considered statistically significant.

Digestion of plant species

Firstly, plants were washed under running water to remove foreign substances, and then washed again with deionised water. The samples were cleaned twice by distilled water to remove contaminants and finally dried at room temperature for 48 hours. Plant samples were also dried in an oven at 75 °C for 48 hours before the digestion procedure. Then, they were grounded in a porcelain mortar to obtain a fine powder. Approximately, 3.0 g of powdered plant samples were inserted in porcelain crucible. It was kept at 100°C in an oven for a day. They were heated at 525 °C for 5hours. A 3.0 mL portion of concentrated HNO_3 was added to the samples and heated on a heater until dried, and then it was left at 525 °C for 2 hours to complete the ashing residues. Finally, a 3.0 mL mixture of HNO_3 and HCl (3:1, v/v) was added to crucibles and heated until dried. The ashed samples were acidified by adding 10.0 mL portion of 1.0 M of HNO_3 before transferred to PTFE tubes. Concentrations of metal ions were measured by ICP-OES. Certified tea sample (NCSZC 73014) was also digested in same procedure to check the accuracy of the method.

***In vitro* activity measurement methods**

Total phenolic contents of samples were determined by the well-known Folin-Ciocalteu colorimetric method using gallic acid as a standard at 765 nm by UV-VIS (Slinkard and Singleton, 1997). Gallic acid was used as standard to construct a linear calibration curve. The equation was A = 0.002×[GAE] - 0.051, R^2 = 0.995. Total flavonoid content of extracts were determined based on the formation of flavonoid-aluminium complex by using quercetin as the standard described by Moreno et al., 2000. The equation was as A = 0.010×[QE]-0.009, R^2 = 0.999. Absorbances were measured as 415 nm by UV-VIS. The free radical scavenging activities were quantitatively tested using a 2.2-diphenyl-1-picrylhydrazyl (DPPH) based on Shimada et al., (1992) method. UV-VIS measurements were performed at 515 nm and results were calculated from the equation given in literature (Dorman and Hiltunen, 2004). Ferric reducing antioxidant powers for different extracts were investigated according to the method of Oyaizu (1988). BHA and BHT were used as the standard for free radical scavenging and reducing power activities respectively. Metal chelating activities of extracts were determined by modified Dinis method (Singh et al., 2007).

RESULTS AND DISCUSSION

***In vitro* biological activities**

HEG, EtOAc and MeOH extracts were prepared to examine the total phenolic content, flavonoid concentration and antioxidant activity. The yields of solid residue after extraction and evaporation from 30 grams dried plant parts for HEG, EtOAc and MeOH extracts were 0.687, 0.539 and 10.816 gr respectively.

The highest total phenolics were determined in MeOH extracts as 71.18 µg GAE/mg extract, whereas the lowest was determined for HEG extracts as 28.16 µg GAE/mg extract (Table 1). The high contents of phenolic compounds indicated that these compounds contribute to the antioxidant activity (Stankovic´, 2011).

Total flavonoid content of HEG extract was found as two times higher than MeOH extract. The highest amount of flavonoid was detected in EtOAc (153.54 µg QE/mg extract) extract which was 9.11

higher than MeOH and 4.45 times higher than HEG extracts (Table 1).

It is reported that flavonoids and phenolics are biosynthesized through several pathways (Gharibi et al., 2015). It was highlighted that, content of flavonoid in plants depends on the type of solvents (Tohidi et al. 2017).

In addition, the amount and composition of them is highly affected by composition of soil, climatic and environmental factors (Rahimmalek et al., 2009).

Table 1: Total phenolic and flavonoid contents of hexane, ethyl acetate and methanol extracts of *Acanthus dioscoridis var. dioscoridis*.

Plant	Total phenolic content (µg GAE/mg extract)			Total flavonoid content (µg QE/mg extract		
	HEG	EtOAc	MeOH	HEG	EtOAc	MeOH
A. dioscoridis	28.16±1.15*	33.89±0.35	71.18±2.71	34.45±0.53	153.54±1.02	16.85±0.23

*Data are presented as mean values; ±standard deviation (SD) of triplicate values.

The radical-scavenging activities of the extracts were investigated in the ranges of 25-500 µg/mL of extract while BHA and BHT are at standard level. Results show that DPPH radical-scavenging activity (Table 2) of HEG extract increased when concentration of extract increased. The highest activity was observed for EtOAc extract as 60.06% while 350 µg/mL of the extract was used. A plateau was observed for MeOH extract over the concentration of 150 µg/mL extract (87.57%). The measured DPPH radical-scavenging activity of MeOH at this concentration was higher than BHT (63.64%) while it was lower than BHA (95.99%).

The highest DPPH radical-scavenging activity was determined for MeOH extract whereas the lowest activity was observed for the HEG extract. Our study establishes that the crude methanol extract of *A. dioscoridis* have strong anti-oxidant activity. These activities of plant extracts are mainly attributed to the presence of phenolic compounds.

Table 2: Effect of different solvent extract of *Acanthus dioscoridis var. dioscoridis* on the inhibition of DPPH free radical.

		% İnhibition of DPPH free radical (Concentration µg/mL)					
		25	50	150	250	350	500
Stan-dards	BHA	74.98±0.08*	89.95±0.53	95.99±0.38	96.60±0.08	96.67±0.00	96.67±0.00
	BHT	20.71±1.59	39.53±2.57	63.64±2.04	80.57±1.44	90.10±0.08	94.10±0.45
Extract of *A. dioscoridis*	HEG	0.23±0.78	1.17±0.16	4.97±0.54	8.62±0.62	9.32±0.23	14.37±0.16
	EtOAc	4.58±1.09	4.90±0.47	34.19±0.23	51.90±0.08	60.06±0.00	52.06±0.08
	MeOH	20.59±0.31	45.07±2.25	87.57±0.00	86.25±0.23	85.86±0.00	85.08±0.16

*Data are presented as mean values; ±standard deviation (SD) of triplicate values.

Figure 1, shows the data for the reducing power of the extract. The reducing power of the extract increased with increase in extract concentration from 25 to 350 µg/mL and exhibited lower reducing power than BHA and BHT as standards. No activity was observed for 12.5 µg/mL concentration for all extracts.

The highest reducing activity was determined for MeOH extract whereas the lowest activity was observed for HEG extract.

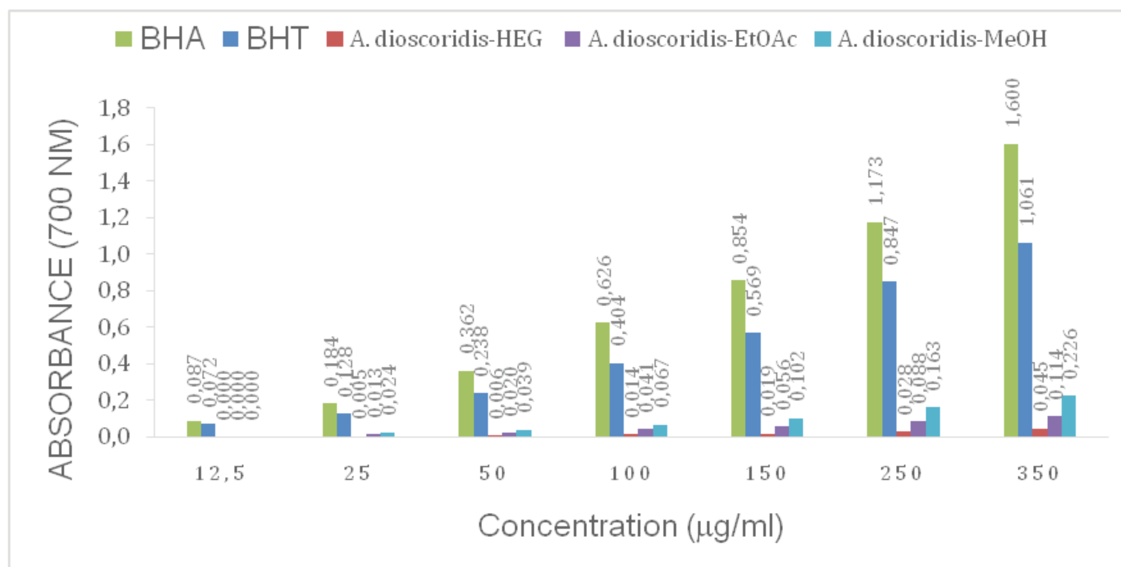

Figure 1. Reducing power activity of different solvent extract of *Acanthus dioscoridis* var. *dioscoridis* as compared to standards BHA and BHT. The values are the mean for set of tree values.

Metal chelating capacities of HEG, EtoAc, MeOH extracts of *A. dioscoridis* in the range of 12.5-300 µg/mL extract were investigated and results were presented in Figure 2, and EDTA was used as standard. The chelating effect of EDTA over 50 µg/mL concentration was approximately 100%. No activity was observed for 12.5 µg/mL extract for all extracts. As shown in Figure 2, HEG extracts of *Acanthus dioscoridis* L. showed clearly higher activity when compared to MeOH and EtOAc extracts.

The chelating capacity of HEG extract was found concentration-dependent. Linear increasing in the metal chelating capacity was observed with increase in concentration of extract. Metal chelating capacities of MeOH and EtOAc were found close to each other.

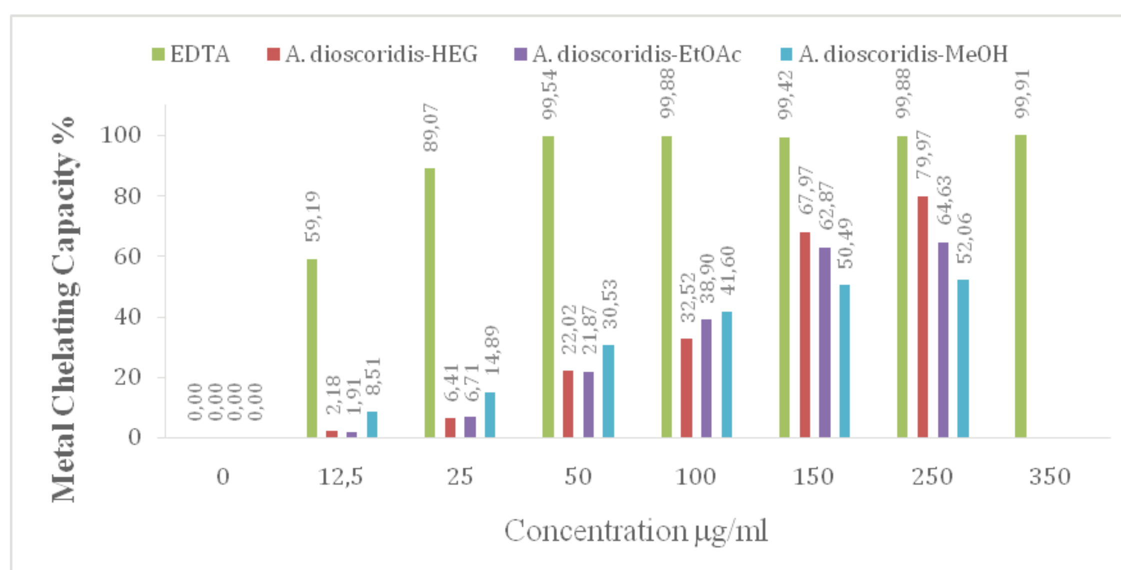

Figure 2. Metal chelating capacity of different solvent extract of *Acanthus dioscoridis* var. *dioscoridis* as compared to standard EDTA. The values are the mean for set of tree values.

Trace metals determinations by ICP-OES

Accuracy of the ICP-OES method was controlled by analysing certified reference tea leaves (NCSZC 73014) (Table 3). Certified value of Al was not given, because it was not approved by producer. As a result, it has been understood that approved values are in accordance with each other. As shown in Table 3, error values were in the ranges of 90-107%. Thus, high precision and accuracy values showed the applicability of the method to real plant samples too. Cd concentration in *A. dioscoridis* was found lower than LOQ. Concentrations of toxic metals; Ni, Cu, Pb and Cr were determined as 2.1±0.061, 16.0±0.94, 0.32±0.031 and 1.45±0.115 mg/kg, respectively. It was reported that plants require nickel (Ni) at lower (0.01–5.0 g/g dry weight) concentrations (da Silva et al., 2012). WHO recommends that nutrients should contain less than 150 µg Ni (FAO, 1996). A permitted daily exposure (PDE) of 50 µg Cu/kg/day in a 50 kg subject is considered suitable for both subchronic and chronic ingestion (Stern, 2010). In humans and animals, exposure to Pb (above 5 µg/day) may create nervous system disorders, paralysis, developmental delay, sensitivity reactions, peripheral vascular disease, nephritic and reproductive system disorders (abnormal spermatogenesis) (Flora et al., 2012;Lisa et al., 2014). Low consumption of chromium causes changes in the metabolic pathway of glucose and lipids and it is possibly related with monogenic diabetes, circulatory system disorders, and nervous system disorders (Anderson, 1993;1995). It has been recommended that daily consumption of Cr should not exceed 0.77 mg (FSA, 2003).Concentrations of Fe, B and Al elements were determined as 345±15.8, 27.9±1.48 and 568±27.4 mg/kg, respectively. Fe is an essential human and animal nutrient with different biochemical and physiological roles including those, which are associated with hemoglobin, myoglobin, ferritin, Fe-containing enzymes, and oxidation of macromolecules. Fe, B and Al concentrations were determined in the 59–689, 5.4-67.3 and 3-2087 mg/kg ranges in 18 different species of medicinal plants by Esetlili et al., 2014.

Table 3: Metal determinations in *Acanthus dioscoridis* var. *dioscoridis* and certified reference tea sample (NCS ZC 73014), n=3

Parameter	Cd µg g^{-1}	Ni µg g^{-1}	Cu µg g^{-1}	Pb µg g^{-1}	Cr µg g^{-1}	Fe µg g^{-1}	B µg g^{-1}	Al µg g^{-1}
Certified value	0.062	3.40	18.6	1.50	0.45	242	14.0	-
Founded value	0.060	3.52	19.9	1.53	0.44	235.5	12.6	597.2
SD[1]	0.005	0.152	0.028	0.007	0.014	24.6	0.155	22.7
RSD[2], %	8.4	4.3	0.14	0.46	3.2	10.4	2.0	3.8
Error, %	96.8	103	107	102	97.8	97.3	90.0	-
A. dioscoridis	<LOQ[3]	2,1±0,061 (2,9)	16,0±0,94 (5,9)	0,32±0,031 (9,7)	1,45±0,115 (7,9)	345±15,8 (4,6)	27,9±1,48 (5,3)	568±27,4 (4,8)

[1]Standard deviation, [2]Relative standard deviation, <LOQ: Under the limit of quantification, [3]RSD values were given in parenthesis

FA, TG, PL determinations by GC

Total fatty acids (ΣFA), triacylglycerol (TG) and phospholipids (PL) concentrations as percentage were measured by GC. It is clear to see in tables that ΣPUFA percentages (51.51%) were meaningfully higher than ΣMUFA (10.25%) and ΣSFA (38.22%). In triacylglycerol fraction, the MUFA percentage (40.00%) was the highest whereas PUFA (25.06%) was the lowest. The ΣSFA (42.66%) percentage was found higher than ΣMUFA (21.88%) and ΣPUFA (35.45%) in phospholipids fraction. Myristic acid is one of the saturated fatty acids. In ΣFA, TG and PL fractions were determined as 4.09%, 5.75% and 3.66%, respectively. Palmitic acid was the dominant fatty acid for ΣFA (26.04%), TG (22.27%) and PL (25.99%) in SFAs. In addition, a small amount of odd numbered fatty acids, namely pentadecyclic acid (from 0.45 to 0.82%)

and margaric acid (from 0.44 to 0.78%) were also detected in small quantities (<1%) in the ΣFA, TG and PL fractions. Oleic acid was the major fatty acid for MUFAs, ranging from 8.54% to 26.33%. Palmitoleic acid (13.67%) was prominently higher than ΣFA (1.71%) and PL (2.66%).

Table 4: ΣFA, TG andPL compositions of *Acanthus dioscoridis* var. *dioscoridis* (%), (Mean±SD, n=3).

Fatty Acids	Total fatty acids (ΣFA)	Triacylglycerol (TG)	Phospholipids (PL)
C14:0[§]	4.09±0.52[a]	5.75±0.89[a]	3.66±0.68[a]
C15:0	0.47±0.14[a]	0.82±0.20[b]	0.45±0.21[a]
C16:0	26.04±2.02[a]	22.27±1.99[a]	25.99±2.43[a]
C17:0	0.78±0.20[a]	0.44±0.50[b]	0.71±0.16[a]
C18:0	6.83±1.05[a]	5.63±1.82[a]	11.82±1.11[b]
ΣSFA	**38.22**	**34.93**	**42.66**
C16:1 n-7	1.71±0.87[a]	13.67±1.17[b]	2.66±0.99[a]
C18:1 n-9	8.54±1.16[a]	26.33±2.33[c]	19.22±1.98[b]
ΣMUFA	**10.25**	**40.00**	**21.88**
C18:2 n-6	16.26±2.03[a]	13.79±1.44[ab]	10.82±1.00[b]
C18:3 n-3	35.25±2.98[a]	11.26±1.90[c]	24.62±2.65[b]
ΣPUFA	**51.51**	**25.06**	**35.45**

[a, b, c] means followed by different letters in same line are significantly different ($P< 0.05$) by Tukey's test.

CONCLUSION

To sum up, the results presented here can be considered as the first information on the *in vitro* biological properties and FA, TG, PL contents of *A. dioscoridis* as a medicinal plant. The study provides the evidence that the methanolic extract of *A. dioscoridis* shows reducing potential and strong antioxidant activity. It may originate the high total phenolic contents. Therefore, further studies may recommend more such as anti-cancer, cytotoxic potential and industrial application of the *A. dioscoridis*.

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