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# Radical Scavenging Activity and Chemical Composition of Methanolic Extract from *Arum dioscoridis* SM. var. *dioscoridis* and Determination of Its Mineral and Trace Elements

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**Abstract:** *Arum dioscoridis* SM. var. *dioscoridis* (*A. dioscoridis*) which is a member of Arum L. genus and belonging to Araceae family was extensively analyzed in detail. DPPH free radical scavenging capacity *of A. dioscoridis* was obtained as 0.01091 mg gallic acid (GA) and 0.0929 mg Trolox (Tr) equivalent per mg of extract, respectively, based on DPPH free radical scavenging activity analysis. Chemical composition of *A. dioscoridis* was evaluated and 16 compounds were detected in the methanolic extract using GC-MS. Obtained compounds were assessed for their health benefits according to literature works. 20 elements were obtained in the mineral and trace element analysis by ICP-MS using microwave digestion procedure.

**Keywords:** *Arum dioscoridis SM. var. dioscoridis*; Antioxidant activity; DPPH•; Chemical composition; trace elements.

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## INTRODUCTION

Araceae is a large plant family represented by 3800 species in 118 genera spreading in a wide range of ecological habitats from sea level to 3000 m of height (1). *Arum* L., which is known as a genus of flowering plants of Araceae family, is represented by 26 species that are distributed in Northern Africa, Mediterranean region, Western Asia, and Europe (2). The species of *Arum* L. have been widely used in folk medicine to prevent several chronic diseases such as stomach acidity, atherosclerosis, cancer, and diabetes. In addition, these plants have been used in traditional treatment methods and were consumed as daily nutrients (3-4). As a member of *Arum* L., *A. dioscoridis* is traditionally used in the Mediterranean gastronomy (5). While Asian, European and especially Turkish people are familiar to Arum taxa, very few studies have been detected concerning *A. dioscoridis*.

Free radical species that are formed as a result of metabolic activities threaten human health. Antioxidants can effectively prevent the tissue or cell damages caused by free radicals through scavenging chain between radicals. Thus, antioxidants protect living cells against many chronic diseases, such as cancer, diabetes, and cardiovascular disease (6-8).

Natural food, including traditional herbs, provide antioxidative components such as polyphenols. Various plants contain phenolic compounds, which are known as powerful antioxidant agents that are used to prevent oxidative reactions (9). In addition, phytochemical compounds, involving polyphenols and other antioxidants, have anti-mutagenic and anti-carcinogenic activities, anti-inflammatory, and neuroprotective effects (10). Therefore, we need to know and consume the food or plants containing antioxidative compounds.

2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) free radical scavenging activity method comes to prominence due to its applicability and fastness, comparing various methods used for evaluating antioxidant activity (11) . $DPPH^{\bullet}$ is used to measure the radical scavenging properties of an electron or hydrogen atom donating components, thanks to its colorful nature (12-14). In the present study, *A. dioscoridis* was investigated for determining its total radical scavenging activities in the methanolic extract.

The identification of mineral and trace element composition and chemical composition of *A. dioscoridis* is another point of the investigation that is firstly done within this work. In addition, mineral and trace element composition of herbs is of great importance and draws researchers' attention due to their medical and nutritional benefits (15). Minerals play a key role in the formation of bio-active components which have great importance for metabolic processes such as human development and health (16). Furthermore, mineral and trace elements can positively or adversely affect health due to the insufficient or excessive intake of them (17). Thus, 20

elements and their levels found in *A. dioscoridis* (B, Na, Mg, Al, P, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, Sn, Ba, Pb) as well as 16 chemical components were demonstrated by this study.

## MATERIALS AND METHODS

### Materials and instruments

$HNO_3$, HCl, and $H_2O_2$ were obtained from Merck (Darmstadt, Germany), Tr, GA, $C_7$-$C_{40}$ saturated alkane standard mixture and DPPH were obtained from Sigma-Aldrich (St. Louis, MO), Argon and Helium gases were obtained from Linde Gas (Turkey). Syringe filter (0.45 μL) was supplied by Agilent (Wilmington, DE, USA). Aqueous solutions were prepared using ultra-pure water (Millipore Milli-Q Advantage A10).

Soxhlet apparatus, rotary evaporator (Hei-VAP, Heidolph Instruments, Germany), UV-1601 spectrophotometer (UV-1601, Shimadzu, Japan), GC-MS (GC: 7890A, MS: 5975C, Agilent, USA), ICP-MS equipped with octopole reaction system (Agilent 7500ce, USA), and microwave system (CEM MARS 250/40, CEM Corporation, USA) were used throughout this work.

### Collection and characterization of plant material

Specimens of *A. dioscoridis* were collected from Mersin, Yenişehir, Akkent, open-field, 10-20 m, EY201704 in 15.04.2017 by the author and identified by Assoc. Prof. Dr. Rıza Binzet. The specimens are deposited with a deposit number of 7810 in the research herbarium of the biology department of Mersin University, Turkey.

### Preparation and extraction of the plant sample

*A. dioscoridis* samples were air-dried at room temperature in the dark for 45 days and were milled. 10 grams of crushed and homogenized sample was used in each triplicated Soxhlet extraction experiment. Samples were refluxed with methanol for 4 hours. Each extract was concentrated to 50 mL at 40 °C using a rotary evaporator.

### Radical scavenging activity assay by DPPH radical

Free radical scavenging activity of methanolic extract of *A. dioscoridis* was evaluated according to DPPH method. This method, which we mentioned in our previous work (14) and briefly summarized below, has been previously reported by Dziri *et al.* (18). Firstly, 2 mL of $10^{-3}$ M DPPH solution was added to 1 mL of each concentrated methanolic extract containing 0.5 to 12 mg of dried *A. dioscoridis* per mL of solution. Subsequently, the resulting mixture was kept in the dark for 15 minutes at room temperature to complete the reaction between the components exhibiting antioxidant properties and the DPPH radical. After keeping time, UV absorbance of each sample mixture was recorded at 515 nm. Finally, scavenging activity of samples was calculated in percentages by comparing recorded absorbance of samples and of stock DPPH

solution according to the equation given in our previous work (14). In addition, the $IC_{50}$ value of the methanolic extract of *A. dioscoridis* was determined by using Figure 1 which shows the curves of DPPH free radical scavenging activity against concentration of extracts. Furthermore, the $IC_{50}$ value of the methanolic extract of *A. dioscoridis* was calculated as mg of gallic acid equivalent (GAE) and mg of Trolox equivalent (TrE per mL of the extract, respectively, against $DPPH^{\bullet}$ stock solution according to the previously identified $IC_{50}$ values of GA and Tr, respectively (14).

**Chemical composition assay**

The chemical composition of *A. dioscoridis* was determined by analyzing methanolic extract of *A. dioscoridis* using GC-MS in the scanning range of $M^+$=50-550 m/z. 1 μL of the concentrated extract was filtered through 0.45 μL syringe filter and injected to GC-MS injection port (250 °C) in splitless mode. The extract was eluted using HP5-MS capillary column (30m x 0.25 mm x 0.25 μm) at helium gas flow rate of 1.75 mL $min^{-1}$ under fixed 21.21 psi of pressure. The analysis was held for a total of 70 minutes by applying following temperature program for elution of the sample. The temperature of the oven was gradually enhanced after keeping at 50 °C for 2 minutes. Later on, it was enhanced to 100 °C at 5 °C $min^{-1}$ and held for 5 minutes. Then, it was increased to 150 °C at 5 °C $min^{-1}$ and held for 8 minutes. Finally, increased to 250 °C at 5 °C $min^{-1}$ and kept for 15 minutes. The Kováts index of each compound was determined using C7-C40 Saturated Alkane Mixture, a certified reference material, which contains each C7-C40 component in a concentration of 1000 μg in mL of hexane.

**Mineral and trace element composition assay**

Dried samples of *A. dioscoridis* were acid-digested using microwave system. 12 mL of $HNO_3$-HCl digestion mixture and 12 mL of $H_2O_2$ were mixed with 0.5 g of sample in microwave vessels. Then, the vessels were held in the microwave oven for 20 minutes at 200 °C for the digestion process. Finally, the obtained acidic solution was diluted to 50 mL with ultrapure water. The analysis was repeated three times and the average values of obtained results were given along with their standard deviation values in Table 3.

The metal content was determined by ICP-MS applying the following conditions. RF power was set to 1500 W. Flow rates of plasma gas, carrier gas, and auxiliary gas was fixed to 15 L $min^{-1}$, 1 L $min^{-1}$, and 1 L $min^{-1}$, respectively. Nebulizer pump was set to 0.1 rps, and temperature of spray chamber was set to 2 °C. Samples were introduced at a fixed flow rate of 1 L $min^{-1}$. The determinations were carried out by an external calibration method using internal standard mixture formed of Li, Sc, Ge, Y, In, Tb and Bi and prepared in 2 % $HNO_3$ matrix. Ten-point calibration curves using NIST single element reference standards ($R^2 \geq 0.999$) were employed.

## RESULTS AND DISCUSSION

### Evaluation of extraction method

Since the selection of the method in the extraction process is a crucial, effective, reliable, and reproducible method is required for extraction of *A. dioscoridis*. Herein, Soxhlet extraction method has a wide application area in scientific works and in industrial applications in the analysis of antioxidant potential and total phenolic content of various matrices (18-20). In addition, methanol can provide high extraction of antioxidative and polyphenolic compounds as indicated in previous works (20-22). The relative ease of evaporation when comparing to water provides an advantage and thus provides ease of operation. Based on the features mentioned above, Soxhlet extraction method was applied in the extraction process and methanol was used as a solvent.

### Evaluation of radical scavenging activity assay

Application and popularity of DPPH method has been increasing day by day, as it offers many advantages such as being a cheap, easy, rapid, and effective method. In addition, it does not require a sample preparation step (13, 23-25). Thus, DPPH method was performed to evaluate the radical scavenging activity of *A. dioscoridis*. Figure 1 demonstrates the DPPH free radical scavenging activity percentages (inhibition rates) against the concentration of methanolic extracts. In addition, Figure 1 also demonstrates the $IC_{50}$ value of *A. dioscoridis* as 2.422 mg $mL^{-1}$.

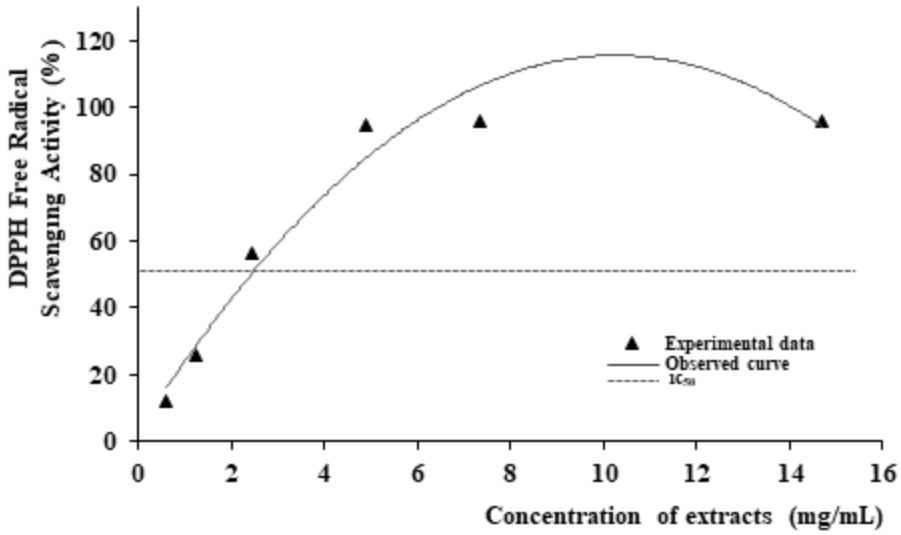


**Figure 1:** DPPH radical scavenging activity of methanolic extract from *A. dioscoridis*.

Furthermore, the radical scavenging capacity of *A. dioscoridis* was evaluated according to GA and Tr standards, which are known as natural and highly antioxidative compounds (26, 27). Thus, the $IC_{50}$ value of *A. dioscoridis* was calculated according to $IC_{50}$ values of GA and Tr, which were given in our previous work (14).

**Table 1:** $IC_{50}$ value of *A. dioscoridis* and radical scavenging activity values of standards and *A. dioscoridis*

| $IC_{50}$ value of *A. dioscoridis* (mg $mL^{-1}$) | $IC_{50}$ values of standards (mg $mL^{-1}$) | | $IC_{50}$ equivalent values of *A. dioscoridis* (mg $mL^{-1}$) | |
|---|---|---|---|---|
| | GA | Tr | GAE | TrE |
| 2.242 ± 8.185x$10^{-3}$ | 0.02642 ± 7.55x$10^{-5}$ | 0.225 ± 5.292x$10^{-3}$ | 0.01091 ± 7x$10^{-5}$ | 0.0929 ± 8.72x$10^{-4}$ |

Table 1 demonstrates the results of radical scavenging activity assay. Results of triplicated experiments were given along with their standard deviations. Radical scavenging activity of *A. dioscoridis* was calculated as 0.01091 ± 7x$10^{-5}$ mg GAE and 0.0929 ± 8.72x$10^{-4}$ mg TrE per mL of extract with the help of the $IC_{50}$ values of GA and Tr of 0.02642 ± 7.55x$10^{-5}$ mg $mL^{-1}$ and 0.225 ± 5.292x$10^{-3}$ mg $mL^{-1}$, respectively.

Kalogeropoulos *et al.* obtained DPPH radical scavenging activity values of ethanolic extracts of 6 propolis samples which are collected from various sites from 0.33 to 1.33 mmol Tr/g ethanolic extract of propolis (28). It was obtained in the present study that *A. dioscoridis* has higher radical scavenging activity than the findings of Kalogeropoulos *et al.*

Oliveira *et al.* analyzed DPPH radical scavenging activity of ethanolic extracts of two different types of commercial tea, and two plants (Brazilian cherry and *Moringa oleifera*) using electrochemical and spectrophotometric methods (29). The lower $IC_{50}$ value, which is known as the concentration of a compound or sample etc. to scavenge DPPH radicals by 50 %, the higher radical scavenging activity. So, it is obvious that *A. dioscoridis* has a reasonably high antioxidative capacity considering these consequences.

**Evaluation of Chemical composition**

16 compounds which are detected in the methanolic extract of *A. dioscoridis* were given in Table 2 along with their retention times ($t_r$), names, chemical formulas, molecular weights (g $mol^{-1}$), peak areas (%), peak qualities (%), Kováts indices (KI), literature retention indices (RIL), and reference numbers.

Accuracy and reliability of gas chromatography method can be increased and standardized by using retention indices. Kováts indices, which can be obtained from the equation developed by Kováts in 1958, are widely used (30-31). A mixture of saturated alkanes (C7-C40) is analyzed in the GC and Kováts indices are calculated by comparing retention times of each compound with the components of the mixture according to the mentioned equation. In this way, though GC conditions, such as pressure and volume of carrier gas, column length, *etc*. affect retention times, they do not affect the KI. In addition, literature retention indices were given for all compounds in Table 2. It is clearly shown that Kováts indices for detected compounds are sufficiently compatible with the literature retention indices.

The identification of obtained compounds was carried out by a computerized procedure. This method is simply based on matching the mass spectrum of the detected compounds with Wiley 7 Nist05.L and NIST05a.L, which are the mass spectral libraries of the GC-MS system.

When Table 2 is examined, it is seen that all compounds are above 74% of the quality ratios. Furthermore, despite the compounds given in Table 2 show antioxidative properties one by one or collectively, their characteristics may vary widely. The detected compounds were comprehensively evaluated from many perspectives below according to previous works.

Jordán *et al.* obtained the concentration of 2-cyclopentene-1-one (C. No. 1) as 0.50 ppm in passion fruit essence (32). Also, KI of 796.12 for 2-cyclopentene-1-one is compatible with their results (802). 2-cyclopentene-1-one was proved to be effective in the treatment of inflammatory diseases by Ianaro *et al.* (33). Valeric aldehyde (pentanal) (C. No. 2) was reported by Warner *et al.* as a volatile flavor of vegetable oil and could be used as indicators of oil quality (35). 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (C. No. 3) was reported by Xu *et al.* to be found in the extracts of fungus *Paecilomyce* sp. isolated from *Panax ginseng*. They pointed out that the obtained extract has a potential of antifungal and antitumor activities (36).

**Table 2:** Components detected by GC-MS obtained from methanolic extract of *A*

| C. No. | $t_r$ | Compound Name | Chemical Formula | Molecular Weight (g/mol) | Peak Area (%) |
|---|---|---|---|---|---|
| 1 | 8.23 | 2-Cyclopentene-1-one | $C_5H_6O$ | 82.10 | 4.4 |
| 2 | 12.66 | Valeric aldehyde | $C_5H_{10}O_2$ | 86.13 | 1.34 |
| 3 | 14.95 | 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | $C_6H_8O_4$ | 144.13 | 3.67 |
| 4 | 22.02 | p-Vinylguaiacol | $C_9H_{10}O_2$ | 150.07 | 0.24 |
| 5 | 30.14 | Formanilide | $C_7H_7NO$ | 121.14 | 0.13 |
| 6 | 44.40 | Neophytadiene | $C_{20}H_{38}$ | 278.52 | 0.16 |
| 7 | 47.15 | Palmitic acid | $C_{16}H_{32}O_2$ | 256.42 | 0.06 |
| 8 | 48.19 | Elaol | $C_{16}H_{22}O_4$ | 278.34 | 0.29 |
| 9 | 49.64 | Stearyl alcohol | $C_{18}H_{38}O$ | 270.50 | 0.15 |
| 10 | 52.89 | Linoleic acid ethyl ester | $C_{20}H_{36}O_2$ | 308.50 | 0.58 |
| 11 | 53.03 | Linolenic acid | $C_{18}H_{30}O_2$ | 278.43 | 1.08 |
| 12 | 56.91 | Oleamide | $C_{18}H_{35}NO$ | 281.48 | 3.80 |
| 13 | 60.83 | 1-Eicosanol | $C_{20}H_{42}O$ | 298.56 | 2.43 |
| 14 | 64.80 | 1-Docosanol | $C_{20}H_{46}O$ | 326.61 | 1.87 |
| 15 | 68.26 | γ-Tocopherol | $C_{28}H_{48}O_2$ | 416.68 | 0.46 |
| 16 | 70.30 | α-Tocopherol | $C_{29}H_{50}O_2$ | 430.71 | 5.57 |

tr: Retention times, C. No: Component number, KI: Kováts index, RIL: Retention Index obtained from literature, nd: n

Asuming *et al.* detected *p*-vinylguaiacol (2-methoxy-4-vinylphenol) (C. No. 4) in their investigation of essential oil composition of four *Lomatium* Raf. species. They defined the percent of *p*-vinylguaiacol and other components in percent of oil from fruits, stems and leaves, and roots of each herb (37). Palic *et al.* obtained neophytadiene (C. No. 6) as one of the major components of the essential oil of the oriental tobacco, Prilep (38). Also, they pointed out that the extract which contains the mentioned component has antimicrobial activity against the microorganisms (38). In addition, KI value of neophytadiene is in good agreement with their findings. Palmitic acid (hexadecanoic acid) (C. No. 7) was reported as a component of the essential oil of *Phlomis ferruginea* Ten. by Formisano *et al.* Their results for KI value of palmitic is in very good agreement with our results (39). In addition, Lalitharani *et al.* pointed out in their work that palmitic acid has an antioxidative activity potential, in which they identified the components of the ethanolic extract of *Pothos scandens* L. leaf using GC-MS (40). Stearyl alcohol (octadecane-1-ol) (C. No. 9) was found in the extract of Mentha spp. Honey and the Bee-Stomach, which was reported by Jerković *et al.* (42). Linoleic acid ethyl ester (Ethyl linoleate) (C. No. 10) was found as a member of the main fraction of volatile constituents of *Ailanthus excelsa* Roxb. by Tzakou *et al.* (43). In addition, Park *et al.* showed the anti-inflammatory activity of linoleic acid ethyl ester detected in the extract of *Allium sativum* (44). Linoleic acid (C. No. 11) was reported to be found in various percentages composition in essential oil from flowers, leaves, and stems of *Wisteria brachybotrys* by Miyazawa *et al.* (45). Additionally, Hu *et al.* pointed out that Linoleic acid (α-Linolenic acid) (C. No. 11) might reduce the risk of arrhythmia (46). Vedernikov and Roshchin identified 1-eicosanol (eicosyl alcohol) (C. No. 13) and 1-docosanol (behenic alcohol) (C. No. 14) along with two unsaturated fatty acids, linoleic acid, and linolenic acid, in the *Betula pendula* Roth. extract (47). Finally, two bio-active compounds, namely γ-tocopherol (Vitamin E) (C. No. 15) and α-tocopherol (Vitamin E) (C. No. 16), types of vitamin E, were detected in the extract of the methanolic extract of *A. dioscoridis* (49-50). These compounds are also known as the endogenous antioxidant and α-tocopherol is known for its peroxyl radical scavenging activity (51).

**Evaluation of mineral and trace element composition**

Mineral and trace elements have crucial roles in various metabolic processes (52,53). Although deficiency or excess levels of trace elements cause certain diseases (53), some are nutritious for humans (54). Thus, the determination of mineral and trace element is of great importance.

**Table 3:** Concentration levels (ppm) of metals in dried *A. dioscoridis*.

| B | Na | Mg | Al | P | K | Ca | Cr |
|---|---|---|---|---|---|---|---|
| nd | 636.0±5.7 | 1301.4±7.3 | 331.1±2.9 | 218.9±3.4 | 4142.0±9.3 | 14406.6±18.8 | 0.3 ±0.0 |
| **Co** | **Ni** | **Cu** | **Zn** | **As** | **Se** | **Cd** | **Sn** |
| 0.7 ±0.01 | 9.1 ±0.9 | 38.9 ±1.2 | 83.2 ±2.4 | nd | nd | 0.004 ±0.00 | 0.68 ±0. |

nd: not dedected

Table 3 demonstrates the concentration values of metals in ppm in the dried mass of *A. dioscoridis*. The highest values were obtained for Ca as 14406.6 ppm and the lowest value was obtained as 0.004 ppm for Cd. However, B, As, and Se were found to remain under detection limits. Comparatively high concentration of Na, Mg, P, K, Ca, Mn, Fe, Cu and Zn, which are known for their nutritional benefits are favorable (54). Nevertheless, trace elements such as Co, Ni, Cd, Sn, Ba, and Pb, which adversely affect human health due to their toxic potential, were obtained in considerably low levels (54, 55).

The soil of planting and the fertilizers used by the plant, as well as the plant structure, are influential in the diversity of the plant's elemental composition (56). However, the presence of Cd and Pb may also be due to exposure of the plant to exhaust fumes or other contaminants, as the plant is collected from the open area within the city.

## CONCLUSION

Being widely used as a food herb and owing medicinal and economic potential, A. dioscoridis has been widely investigated. The overall radical scavenging capacity of *A. dioscoridis* was determined by employing DPPH free radical scavenging activity analysis. $IC_{50}$ values of methanolic extract from *A. dioscoridis* which were determined as GA and Tr equivalents, suggest that *A. dioscoridis* is a functional herb considering its quite high DPPH radical scavenging capacity. Further, the mentioned $IC_{50}$ values put forward the potential of *A. dioscoridis* to be used as a natural antioxidant for the industrial applications. Considering the pharmaceutical and medicinal importance of 16 compounds, which were detected in the methanolic extract of *A. dioscoridis*, they were evaluated separately by comparing literature work. All compounds were shown to be very rich in terms of their diversity and usefulness. In addition, trace and mineral element composition were determined to assess the nutritional value and possible therapeutic or illness effect. Herein, 20 elements were determined by ICP-MS analysis and their concentration values were evaluated. Further studies should be carried out to determine the detailed biochemical and medicinal effect of this herb.

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