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DETERMINATION OF BIOACTIVE CHEMICALS AND ANTIOXIDANT CAPACITY IN DIFFERENT PLANT PARTS OF CORIANDER (CORIANDRUM SATIVUM L.)

F. Ergün

Kırşehir Ahi Evran University, Faculty of Health Sciences Kirsehir/Türkiye Corresponding Author's E-mail: fatma.ergun@ahievran.edu.tr

ABSTRACT

Substances with antioxidant activity protect the metabolism against oxidative damage caused by free radical molecules. The most important sources of these substances are herbal natural nutrients. Coriander (*Coriandrum sativum* L.) is a plant used both as a nutrient and as a medicine. Leaf, stem and seed parts of the plant are widely used as individual flavors. In this study, total phenolic and flavonoid content of hexane and methanol extracts obtained from leaf, stem and seeds of Coriander (*C. sativum*) plant were determined. The amount of phenolic substance was determined as 250 μg GAE / mL in the highest hexane seed extract and the lowest amount as 50 μg GAE / mL in methanol stem extract. The flavonoid substance was obtained in the highest hexane leaf extract with 535.71 μg QE/mL and the lowest in the hexane seed extract with 20.408 μg QE/mL. Although total phenolic and flavonoid content differed in leaf, stem and seed extracts, it was found to be higher in hexane extracts compared to the methanol extracts. In addition, IC₅₀ values of both extracts were calculated using DPPH• radical removal activity method, which is among the antioxidant determination methods. Although the amount of phenolic and flavonoid content of methanol extract of coriander plant was low, DPPH• radical removal activity was found to be higher. It is considered that dietary incorporation of fresh leaf and seed parts of the coriander plant, whose phenolic substance and antioxidant capacity have been determined, might be significant with respect to the partial provision of the antioxidants needed by the organism.

Key words: Coriander, Phenolic substance, Flavonoid substance, Antioxidant, DPPH

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INTRODUCTION

Antioxidants are present in all parts of plants. Antioxidants of natural origin are abundant in cereals and legumes, fruits and medicinal plants (Cao *et al.*, 1996; Foo and Porter, 1981). Antioxidants found in these sources; tocopherols, flavonoids, phenolic compounds, alkaloid, chlorophyll, protein, nitrogenous compounds containing amino groups, polyfunctional organic acids and carotenes (Larson, 1988).

In general, aromatic plants produce some extracts to reproduce, to survive and to protect themselves against certain pests. These extracts, which are of essential oil nature extracted from plants by different methods, are mostly phenol compounds. Essential oils that are responsible for the aroma of the plant are also used as disinfectants (Özkan and Açıkgöz, 2007).

Many aromatic plants, seeds, fruits, leaves or roots due to their active chemical compounds are used in various fields due to different modes of action. The effects of these plants vary depending on the active substances. In addition, the essential oils they contain have positive effects on the digestive and excretory system. (Maksimovic *et al.*, 2005; Zahid *et al.*, 2018).

Essential oils contain antioxidative properties of phenolic hydroxyl groups in the structure of the components they contain (Vekiari *et al.*, 1993).

Phenolic compounds have great structural differences. It is thought that the inhibitory effect is caused by the hydroxyl groups in their structure. Because it is thought that hydroxyl groups cause disruption of bacterial cellular integrity. (Gyawali and Ibrahim, 2014; Xue et al., 2013). The antimicrobial properties of plants or their parts depend on the quinones, flavonoids, alkaloids, organic acids and phenolic compounds found in their composition (Lai and Roy, 2004). Allspice, almond, laurel, black pepper, cinnamon, clove, coriander, cumin, garlic, grapefruit, lemon, mandarin, onion, orange, thyme, rosehip, sage and marjoramn have antimicrobial effects due to their essential oil contents (Göncü and Akın, 2017).

The *C. sativum* plant, which is grown as a spice in many regions of the World, has a medicinal plant feature. (Momin *et al.*, 2012) and is used both as a food and as a medicine. Coriander, known as a spice for many years, is used as a flavoring agent in cuisine and medicine. Coriander also has a memory-enhancing effect and is thought to be caused by antioxidant compounds and essential oil (Ulutas *et al.*, 2018). In the illustrated manuscript books on medieval midwifery, coriander was

used to accelerate birth (Early 13th century) (Burdock and Carabin, 2019). It was determined, in a study, coriander has a protective role against colon cancer due to its effects on lipid metabolism (Momin *et al.*, 2012; Chithra and Leelamma, 2000). In a study by Wong and Kitts, phenolic compounds obtained from coriander were shown to be partly responsible for their antioxidant activity (Wong and Kitts, 2006).

Parts of the coriander plant containing essential oil such as leaf and seeds are used in traditional medicine systems of different civilizations. In this studies, the leaf of coriander had not examined in detail as much as the seeds. However, compounds such as essential oil, flavonoids, some phenolic acids, vitamin C, carotene, calcium, capric acid have been detected in leaves (Ulutas *et al.*, 2018; Sahib *et al.*, 2013; Javanova and Panovska, 2019).

In the study, the total phenolic and flavonoid content of coriander (*C. sativum*) leaf, stems and seed extracts were determined. Also, DPPH• free radical removal potentials were examined and determined which part of the plant had more antioxidant capacity.

MATERIALS AND METHODS

Supply of plant samples: The Coriander plant to be used in this research was sown from seeds obtained from local farmers in Kırşehir Turkey, producing locally and grown without using any chemical drugs. When some of the plants reached 20-30 cm in length, they were collected to obtain the leaves and stem parts. The rest of the plant was collected after the seeds matured.

Plants to be used in the study were washed with distilled water to remove physical contamination. The leaves, stems and seeds were dried at +25 °C and in the shade and hid at +4 °C until use.

Preparation of methanol extracts: Methanol extraction was carried out in leaves, stems and seeds of coriander plant according to literature (Gulcin *et al.*, 2005). 15 g of dried plant specimens was ground in a grinder and placed in a 1 liter closed flask. Twenty times of the plant sample methanol (300 mL) was then added and stirred in the magnetic stirrer. The methanol extract obtained was filtered. This procedure was repeated several times at regular intervals. The filtered extracts were combined and methanol was removed at 45 °C in the evaporator. The extracts were hid at + 4 °C for the studies.

Preparation of hexane extracts: Hexane extraction of coriander was conducted according to Gulcin *et al.* (2005). 15 g of plant specimens were ground in grinder. Twenty times of the plant sample (w/v) hexane (300 mL) was, then, added and stirred in the magnetic stirrer. The hexane extract obtained was separeted. This procedure was repeated several times and the extracts were

combined. Then hexane was removed at 40 $^{\circ}$ C in the evaporator. The extracts were hid at + 4 $^{\circ}$ C for the studies.

Determination of total phenolic substances: Total phenolic content was found according to Folin-Ciocaltaeu method in all extracts obtained from leaves, stems and seeds of coriander plant (Slinkard and Singleton, 1977). Standard graphic was prepared using gallic acid. Solutions of plant methanol and hexane extracts at 1000 ppm concentration were prepared. 40 µL was taken from stock solutions, completed to 1840 µL with distilled water. 40 µL of Folin-Ciocalteu reagent (FCR) was added to the mixture and incubated for 3 minutes at ambient temperature. Then, 120 µL of 2% (w / v) Na₂CO₃ solution was added. The mixture was kept in ambient temperature for 2 hours. The absorbance of the samples was read at 760 nm against the blind containing distilled water instead of sample. The total phenolic contents of the extracts were found as equivalent to gallic acid using the equation obtained from the standard graph (µg GAE /mL).

Determination of total flavonoid substance: The total flavonoid contents of the extracts prepared were found by aluminum nitrate method as equivalent to quercetin (μ g QE /mL) (Moreno *et al.*, 2000). Standard graphic was prepared using quercetin. 1000 ppm solution was prepared from methanol and hexane extracts obtained from coriander plant. 40 μ L of this stock solution was taken and the volumes were completed to 1920 μ L with methanol. 40 μ L of 1 M potassium acetate was added and after one minute 40 μ L of 10% aluminum nitrate was added. It was incubated for 40 minutes. Absorbance at 415 nm against the blind prepared with pure water was measured.

Determination of DPPH• free radical removal activity: Free radical removal activities of the extracts were found by using Blois method (Blois, 1958). 1,1-Diphenyl-2-picrylhydrazyl (DPPH•) solution was used as free radical. 1000 ppm stock solution was prepared from hexane and methanol extracts of coriander plant. In addition, 1000 ppm stock solution was prepared in 2,6-dit-butyl-1-hydroxytoluene (BHT) which was used as standard. 20, 50, 100 and 200 µL were taken from these stock solutions and their volumes were completed to 400 µL with methanol. Then, 1600 μL DPPH• solution (0.1 mM) was added. The prepared solutions were incubated for 30 minutes at ambient temperature in the dark. The absorbance changes at 517 nm were measured against methanol. Control solution was prepared under the same conditions using methanol instead of sample and standard material. Decreasing absorbances yielded the amount of free DPPH• solution remaining, ie free radical removal activity (Magsood et al., 2019)

% DPPH• radical removal activity was calculated by the following formula:

% DPPH• radical removal activity = $[(A_0-A_1)/A_0]\times 100$ A₀: Absorbance of control reaction, A₁: Absorbance of plant extracts and standard solutions

RESULTS AND DISCUSSION

Phenolic compounds in plants are of great interest due to their antioxidant activities. Many studies have been conducted on this subject. Antioxidants are used remove radicals formed by normal means in the metabolism. Antioxidants fall into two groups as natural and synthetic (Wong and Kitts, 2006). Although synthetic antioxidants have a wide range of uses, they have recently been restricted for their undesirable side effects (Gulcin *et al.*, 2003). Therefore, the importance of plants containing antioxidants and used in nutrition has increased.

In this study, the determination of total phenolic content and, total flavanoid content, DPPH• radical removal activities were determined in methanol and hexane extracts of coriander plant which was used as a spice by drying both fresh and seeds in the cuisine of many countries in the world. The activity results obtained were compared with BHT.

In hexane and methanol extracts of coriander plant, total phenolic contents were calculated as equivalent to gallic acid by using gallic acid standard graph given in figure 1.

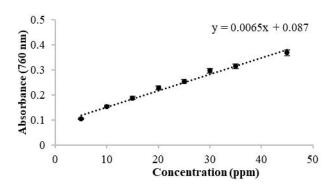


Figure 1. Calibration curve graph of gallic acid

Hexane extracts obtained from the leaves, stems and seeds of coriander plant yielded higher amount of total phenolic substances than those of methanol extracts (Table 1).

In addition, when considered for both extracts, the most phenolic substances were obtained from seeds, $250~\mu g$ GAE/mL in hexane extract and $138.46~\mu g$ GAE/mL in methanol extract.

Previous studies reported that the phenolic content of ethanol extracts from some plant species was

higher than that of aqueous extracts (Gulcin *et al.*, 2003; Yildirim *et al.*, 2001). The total amount of flavonoids, a large group of phenolic compounds was calculated as equivalent to quercetin using the standard graph of quercetin given in Figure 2.

The total amount of flavonoids was relatively higher in the hexane extract than in the methanol extract, regardless of whether they come from leaf, stem or seed. The maximum amount of flavonoid substance was obtained from leaf parts of coriander plant with 535.71 μg QE/mL in hexane extract and 454 μg QE/mL in methanol extract (Table 2).

Table 1. Total phenolic content in extracts of coriander plant equivalent to gallic acid.

	Plant parts	Total phenolics (μg of GAE/mL)
Methanol	Coriander leaf	100
Extract	Coriander stem	50
	Coriander seed	138.46
Hexane	Coriander leaf	215.38
Extract	Coriander stem	192.307
	Coriander seed	250

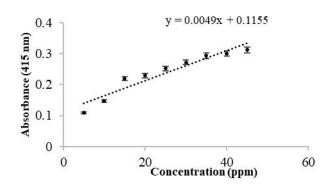


Figure 2. Calibration curve graph of quercetin

Table 2. Total amounts of flavonoid substances equivalent to quercetin of hexane and methanol extracts of coriander plant.

	Plant parts	Total flavonoids (μg of QE/mL)
Methanol Extract	Coriander leaf	454
	Coriander stem	56.122
	Coriander seed	81.625
Hexane Extract	Coriander leaf	535.71
	Coriander stem	76.53
	Coriander seed	20.408

Less polar solvents such as hexane can extract non-polar extraneous compounds found in plants (Stalikas 2007). The amounts of polyphenol substance relatively high in the hexane extract may be due to the presence of undesirable phenolic compounds such as wax, oil and chlorophyll. DPPH• is an organic structured radical that gives absorbance at 517 nm. By measuring the absorbance reduction of the DPPH• radical at 517 nm, the amount of free DPPH• solution remaining, i.e., free radical removal activity, was determined. BHT was used as standard in activity studies.

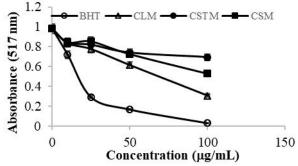


Figure 3. The DPPH· radical removal activity of methanol extracts and BHT at different concentrations (BHT: Butylated hydroxytoluene, CLM: Coriander leaf methanol extracts, CSTM: Coriander stem methanol extracts, CSM: Coriander seed methanol extracts).

In the study, parallel to the increase in concentration (10-100 μg / mL) of hexane and methanol extracts prepared from coriander, an increase in DPPH• radical removal activities was observed (Figure 3, Figure 4).

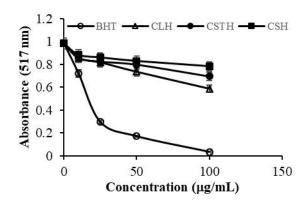


Figure 4. The DPPH• radical removal activity of hexane extracts and BHT at different concentrations (BHT: Butylated hydroxytoluene, CLH: Coriander leaf hexane extracts, CSTH: Coriander stem hexane extracts, CSH: Coriander seed hexane extracts)

DPPH• radical removal activities of the extracts and BHT were calculated (Table 3). In the antioxidant analysis, when the DPPH radical removal activities were compared at $100~\mu g$ / mL concentration, it was observed that the activity of methanol extracts was lower than the standard used, but it was higher than hexane extracts. The highest radical removal activity was determined in methanol extracts of coriander leaves (Figure 5).

Table 3. DPPH• radical removal activities of coriander hexane and methanol extracts and BHT.

Extracts	10 μg/mL	$25 \mu g/mL$	$50 \mu g/mL$	100 μg/mL
CLM	14.96	20.87	37.37	68.94
CSTM	14.46	16.49	24.64	29.42
CSM	13.84	13.93	25.96	46.02
CLH	12.93	16.80	25.15	40.32
CSTH	12.93	16.19	18.83	29.32
CSH	10.18	12.52	15.68	20.46
BHT	26.78	70.06	82.6	96.6

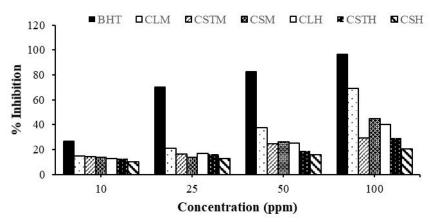


Figure 5. Comparison of DPPH radical removal activities in methanol and hexane extracts of coriander plant with BHT (10-100 µg/mL).

The concentration of extract and standard substance was determined as IC₅₀, which inhibited 50% of DPPH• radical removal. This value was calculated using graph of % DPPH• radical removal activity values versus studied concentrations (Wei *et al.*, 2010).

Table 4. Coriander extracts and IC₅₀ values of BHT (BHT: Butylated hydroxytoluene, CLM: Coriander leaf methanol extracts, CSTM: Coriander stem methanol extracts, CSM: Coriander seed methanol extracts CLH: Coriander leaf hexane extracts, CSTH: Coriander stem hexane extracts, CSH: Coriander seed hexane extracts).

Extracts / standard	IC ₅₀ (µg/mL)	
CLM	69.92	
CSTM	214.15	
CSM	112.23	
CLH	131.45	
CSTH	218.040	
CSH	361.065	
BHT	17.095	

Since the IC₅₀ value is inversely proportional to DPPH• radical removal activity, the activity ranking is BHT> CLM> CSM> CLH> CSTM> CSTH> CSH. In addition, it was determined that those except methanol leaf extract could not decrease below 50% inhibition value.

There are different compounds that show polarity and antioxidant properties in plants. These compounds affect each other as they are present in the plant together. Therefore, radical trapping activity may not be estimated by the total amount of phenolic substance (Parejo *et al.*, 2002). Although coriander plant had more phenolic and flavonoid substances in hexane extracts, radical removal activity was low.

Conclusion: Due to the abundance of bioactive phytochemicals, interest in the use of culinary plants as natural antioxidants is increasing. Therefore, the number

of studies on antioxidants is increasing day by day. In our study, total phenolic and flavonoid content of methanol and hexane extracts of coriander plant were calculated. The phenolic and flavonoid contents of hexane extract was found higher than those of methanol extract. On the basis of plant parts, it was determined that there were more phenolic and flavonoid substances in leaves than other parts. According to the results of antioxidant activity determined using DPPH• radical removal method, antioxidant activity of methanol extract was determined to be more active than hexane extract. According to the results, there was no linear connection between the amount of phenolic substance and antioxidant activity. Considering that differences in phenolic content of plants may affect antioxidant activity, it is important to determine phenolic content especially in plant studies. Variable amounts of phenolic substances may be present in the tissues in response to the environmental conditions in which plants are present. Therefore, such studies are important in terms of determining which part of the plant is preferred in nutrition and herbal medicine use.

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