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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Evaluation of Persimmon (*Diospyros kaki*), Cherry laurel (*Prunus laurocerasus*) and Blueberry (*Vaccinium arctostaphylos*) Fruits as a Natural Antioxidant Source[]

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Abstract: Antioxidants are substances that can delay or prevent the damage caused by oxygen in living environments, although they exist in low concentrations. Epidemiological studies report that an antioxidant-rich diet is beneficial and can have a great impact on disease prevention. For this reason, a lot of research should be done on natural antioxidants. For this purpose, persimmon (*Diospyros kaki*), cherry laurel (*Prunus laurocerasus*), blueberry (*Vaccinium arctostaphylos*) fruits are dried at 60°C and then mixed with ethanol (40°C), hot water (75°C) and warm water (40°C). were subjected to the extraction process and extracts were obtained at the rates of 2.5% and 5%. The DPPH method was used to determine the antioxidant effect. According to the results obtained, the highest antioxidant activity was determined in the hot water extract of the blueberry fruit (85.24%), and the lowest in the ethanol extract of the cherry laurel fruit (3.49%). It was observed that increasing the extract concentration (2.5%→5%) slightly increased the antioxidant activity.

Keywords: Antioxidant, blueberry, cherry laurel, herbal extract, persimmon, ROS.

Hurma (*Diospyros kaki*), Karayemiş (*Prunus laurocerasus*) ve Mavi Yemiş (*Vaccinium arctostaphylos*) Meyvelerinin Doğal Antioksidan Kaynağı Olarak Değerlendirilmesi

***Sorumlu yazar:**
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Öz: Canlı ortamlarda oksijenin sebep olduğu zararı düşük konsantrasyonda var olduğunda bile geciktirebilen ya da önleyen maddelere antioksidanlar denir. Epidemiyolojik çalışmalar antioksidanca zengin beslenmenin yararlı olduğunu ve hastalıkların önlenmesinde büyük bir etkisi olabileceğini bildirmektedir. Bu sebeple doğal antioksidanlar üzerine birçok araştırma yapılmalıdır. Bu çalışma Rize ve çevresinde yetişen hurma, karayemiş ve mavi yemiş meyvelerinin farklı yöntemler ile elde edilen ekstraktların antioksidan kapasitesini belirlemek amacıyla yapılmıştır. Bu amaçla *Diospyros kaki* (hurma), karayemiş (*Prunus laurocerasus*) ve mavi yemiş (*Vaccinium arctostaphylos*) meyveleri 60°C'de kurutulduktan sonra ethanol (40°C), sıcak su (75°C) ve ılık su (40°C) ile ekstraktsiyon işlemine tabi tutulup %2,5 ve %5 oranlarında ekstraktlar elde edilmiştir. Antioksidan etkisinin belirlenmesinde DPPH yöntemi kullanılmıştır. Elde edilen sonuçlara göre antioksidan aktivitesi en fazla mavi yemiş meyvesinin sıcak su ekstraktında (85,24), en düşük karayemiş meyvesinin ethanol ile yapılan ekstraktında (%3,49) belirlenmiştir. Ekstrakt konsantrasyonunun arttırılmasının (%2,5→%5) az bir miktarda antioksidan aktiviteyi arttırdığı belirlenmiştir.

Anahtar kelimeler: Antioksidan, bitkisel ekstrakt, hurma, karayemiş, mavi yemiş, ROS.

INTRODUCTION

Health expenditures of the countries increase as time progresses. With the development of technology, the habits of human beings are changing, and new products produced can have a negative impact on health. For this reason, states allocate resources for research on preventive and therapeutic issues (Okan et al., 2013).

Oxygen, which living things use to obtain energy, is an indispensable element for the continuation of life. In organisms where oxygen is used and energy is produced, reactive oxygen species called free radicals and reactive nitrogen species are produced. These high-energy atoms or molecules, which have one or more unpaired electrons in their outer orbitals, can easily react with substances such as lipids, proteins and nucleic acids in their environment and cause changes in their structure (Karabulut & Gülay, 2016b). Free radicals can be formed as a result of metabolism or can be taken from the outside. The presence of high levels of free radicals in the organism causes cell damage and various health problems. It is linked to many ailments such as gastrointestinal and cardiovascular diseases associated with free radicals, infertility, and respiratory and excretory disorders. In order to prevent these diseases, oxidant substances and antioxidant substances in the organism must be balanced.

Substances that can prevent cell damage and scavenge free radicals in the body are called antioxidants. Antioxidants, which can be produced by the living organism and taken from the outside through food, react quickly with free radicals and prevent the progression of the autoxidation/peroxidation reaction. Antioxidants, which play a role in neutralizing excess free radicals in the body and reducing their toxic effects, protect cells against toxic effects and strengthen the defense mechanism against diseases (Karabulut & Gülay, 2016a).

Phenolic compounds, which are secondary metabolites found naturally in plants and have antioxidant properties, are among the most important water-soluble antioxidants. It is high in vegetables and fruits. Antioxidant, antimicrobial and other pharmacological effects of phenolic components are utilized (Kobya et al., 2019). Phenolic compounds are basically divided into two as phenolic acids and flavonoids. Phenolic compounds have started to be used as a natural antioxidant source in food development studies. Studies have shown that these compounds reduce the amount of cholesterol in the blood, have osteoporotic and anticarcinogenic effects, and inhibit unwanted bacterial infections (Meral et al., 2012; Karsli et al., 2021). Epidemiological studies have shown that consumption of phenolic antioxidants reduces the occurrence of cardiovascular diseases and some cancer types (Carpes et al., 2007; Cook & Samman, 1996).

As in the world and in Turkey, the tendency to eat plant foods rich in antioxidants and anthionines is increasing (Kaya et al., 2017; Scheerens, 2001). Considering the plant biodiversity of Turkey, it is obvious that it has a great source of antioxidants. The Eastern Black Sea region is one of the regions where this potential is highest (Okan et al., 2013).

Persimmon (*Diospyros kaki*), whose homeland is China, is an orange-colored fruit with its own unique color and odor, and its content is rich in antioxidants and phenolic compounds. In studies, it has been reported that it lowers cholesterol and blood pressure, strengthens the immune system, and has an important role in preventing diseases such as digestive problems and cancer (Chen et al., 1998; Gu et al., 2008; Kaya et al., 2017; Wright & Kader, 1997). *Laurocerasus officinalis* (cherry laurel) belongs to the Rosaceae (Rosaceae) family, grows in damp and shady areas and is evergreen, and is distributed in the provinces of Rize, Artvin, Trabzon, Giresun and Ordu (Anşin et al., 1994; Okan et al., 2013). Used in making jam, marmalade, fruit juice and liquor, cherry laurel leaves are also utilized by making tea (Okan et al., 2013). Thanks to many aromatic substances and phenolic acids in its structure, it is a good source of antioxidants (Okan et al., 2013; Orhan & Akkol, 2011). Blueberry (*Vaccinium spp*.), which grows naturally in the Eastern Black Sea region and is an important non-wood forest product, is known by different names in the region such as likapa, bearberry, tea currant and lifor. Even though this and many other non-wood forest products are used for many purposes in the Eastern Black Sea region, billions of Turkish lira are lost every year due to infrastructure deficiencies such as unconscious collecting, technological inadequacy in the processing of these plants, inability to make the necessary inventory and not keeping statistical records. Contrary to the land conditions of the region that are not suitable for industry, the use of herbal products will provide many economic, social and environmental contributions to the local people and will provide many benefits to the food industry as a natural antioxidant source (Okan et al., 2013).

In this study, it was aimed to determine the antioxidant capacity of persimmon, cherry laurel and blueberry fruits, which are widely grown in Rize and its surroundings, in order to use them as a food-based natural antioxidant source.

MATERIAL AND METHOD

Collection of Fruits and Extraction: Persimmon (*Diospyros kaki*, Figure 1), cherry laurel (*Laurocerasus officinalis*, Figure 2) and blueberry (*Vaccinium myrtillus*,

Figure 3) fruits were collected during their ripening period in Rize / Turkey (41°08′92.9′′N; 40°72′10.7′E, Figure 4) and brought to the laboratory. The skin of the persimmon fruits was peeled off, the blueberry and cherry laurel fruits were used in their whole form. All fruits washed, cleaned of woody parts, seeds removed from persimmon and blueberries and all fruits were dried in an oven at 60°C and turned into powder.

Figure 1a. Persimmon.

Figure 1b. Persimmon endocarps.

Figure 2. Cherry laurel

Figure 3. Blueberry.

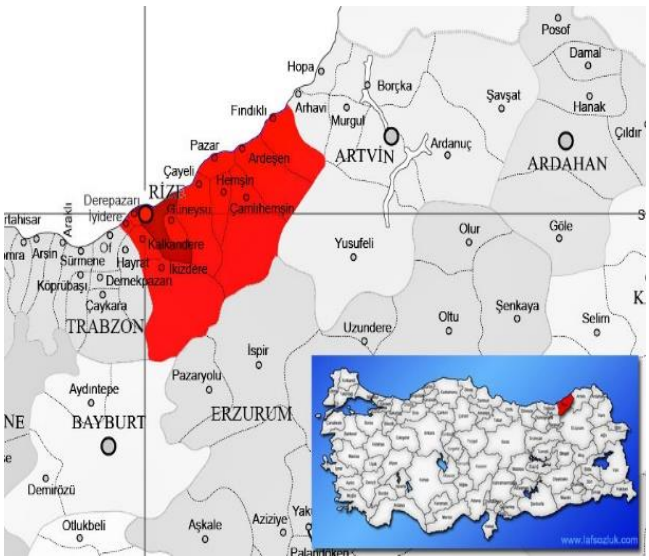

Figure 4. The region where the fruits used in the analysis were collected.

Herbal materials are pulverized in a laboratory type mill (waring blender 8011es) and turned into a solution using distilled water and ethanol (40°C), hot water (75°C) and warm water (40°C) at concentrations of 2.5% and 5%, Then, it was allowed to be extracted by shaking at 110rpm in a water bath for 24 hours The extracts were filtered through coarse filter paper in the next step. The extract obtained with ethanol was kept in a water bath for 4 hours by adding the same amount of distilled water after evaporation of the ethanol in the rotary evaporator. The herbal extract was allowed to pass into the distilled water at the same rate as hot water and warm water.

DPPH (2.2.-diphenyl-1-picrihydrazil) Radical Scavenging Capacity Method: Antioxidant capacity of prepared herbal extracts was determined according to Brand-Williams et al., (1995) method. In this method, an antioxidant substance is added to the environment where the free radical is present, and a color change is observed as a result of the reversal of the DPPH radical formation reaction. DPPH antioxidant activity was determined as % inhibition by spectrophotometric measurement at 515nm wavelength according to the equation below.

$$\% \text{ Inhibition} = [(A_{Control} - A_{Sample} / A_{Control})] \times 100$$

$A_{Control}$ = Absorbance of the control reaction

A_{Sample} = For example Absorbance of the sample

RESULTS

In this study, extracts were obtained with 2 different concentrations (2.5% and 5%) and 3 different extract methods (ethanol, hot water and warm water) by using the fruit of 3 different plant species (persimmon, cherry laurel and blueberry). The antioxidant values of the obtained extracts are shown in Table 1.

Table 1. Antioxidant values of fruit extracts.

Plant	Concentration (%)	Solvent	Inhibition (%)
Persimmon	2.5	Ethanol	5.26
Persimmon	5	Ethanol	17.23
Persimmon	2.5	Hot water	8.81
Persimmon	5	Hot water	22.88
Persimmon	2.5	Warm water	4.91
Persimmon	5	Warm water	8.91
Cherry laurel	2.5	Ethanol	3.49
Cherry laurel	5	Ethanol	10.18
Cherry laurel	2.5	Hot water	19.33
Cherry laurel	5	Hot water	30.87
Cherry laurel	2.5	Warm water	12.37
Cherry laurel	5	Warm water	17.32
Blueberry	2.5	Ethanol	47.37
Blueberry	5	Ethanol	55.03
Blueberry	2.5	Hot water	83.26
Blueberry	5	Hot water	85.24
Blueberry	2.5	Warm water	38.25
Blueberry	5	Warm water	80.26

The highest and lowest antioxidant activity was found to be 85.24% in the hot water extract prepared at 5% concentration of blueberry fruit, and 3.49% at the 2.5% concentration of the ethanol extract prepared with cherry laurel fruit, respectively.

Antioxidant values of ethanol, hot water and warm water extracts of persimmon fruits are given in Figure 5. The highest antioxidant value was found in the hot water extract (22.88%) prepared at 5% concentration, and the lowest antioxidant value was found in the warm water extract prepared at 2.5% concentration. When the antioxidant activities of herbal extracts prepared with persimmon fruit are evaluated according to solvents, it is seen that the highest and lowest antioxidant activity is in hot water and warm water extract, respectively. When sorting according to the solvent type, it was seen that the % inhibition values were sorted as warm water>ethanol>hot water.

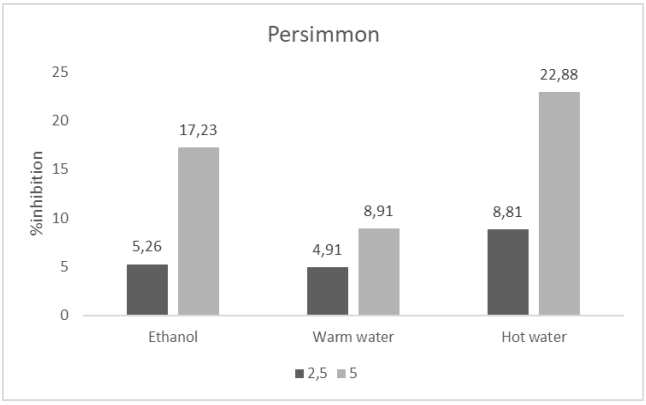

Figure 5. Antioxidant values of persimmon fruit (%).

Antioxidant values of ethanol, hot water and warm water extracts of cherry laurel fruit are given in Figure 6. The highest and lowest antioxidant values were found in hot water prepared with 5% concentration and ethanol extract prepared with 2.5%, respectively. It is seen that the antioxidant values are significantly affected by the solvent type, and the antioxidant activity of the extracts increased depending on the concentration. It was determined that antioxidant values of cherry laurel fruit were ranked as ethanol > warm water > hot water according to solvent type.

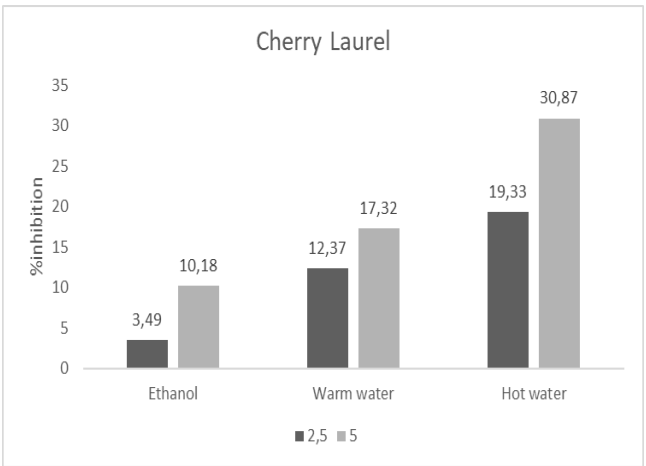

Figure 6. Antioxidant values of cherry laurel fruit (%).

Antioxidant values of ethanol, hot water and warm water extracts of blue berry fruit are given in Figure 7. The highest and lowest antioxidant values were found in hot water extract prepared with 5% and warm water extract with 2.5%, respectively. The antioxidant activity of the hot water and ethanol extracts from blue berry fruit were not affected by the extract concentrate, it while the antioxidant activity of the 5% warm water extract was approximately doubled compared to the 2.5% extract.

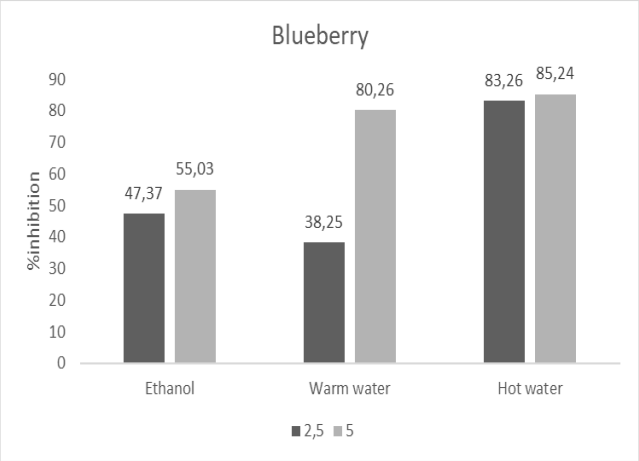

Figure 7. Antioxidant values of blueberry fruit (%).

The antioxidant values obtained in the study according to their concentrations is given in Table 1. It was observed that the highest antioxidant value in both concentrations was in the hot water extract of the blueberry fruit, and the doubling of the extract concentration (2.5%→5%) did not significantly affect the %inhibition value except for the cherry laurel fruit. While the lowest %inhibition value was seen in cherry laurel fruit at a concentration of 2.5%, it was determined in persimmon fruit at a concentration of 5%. The highest %inhibition at 5% concentration was determined as blueberry fruit in all 3 solvent types. Considering the solvent type, it was determined that warm and hot water extracts exhibited higher %inhibition value than ethanol extracts.

Also, it was determined that the antioxidant activity value changed according to the fruit and solvent type and the highest %inhibition value in the persimmon fruit extract was in the extract made with hot water. If we look at the %inhibition order of the persimmon fruit according to the solvent type, it is seen that hot water> warm water> ethanol and %inhibition order of the cherry laurel fruit according to the solvent type, it is seen that it was hot water> warm water> ethanol and % inhibition order of the blueberry fruit according to the solvent type, it is seen that it was hot water> warm water> ethanol in Figure 8.

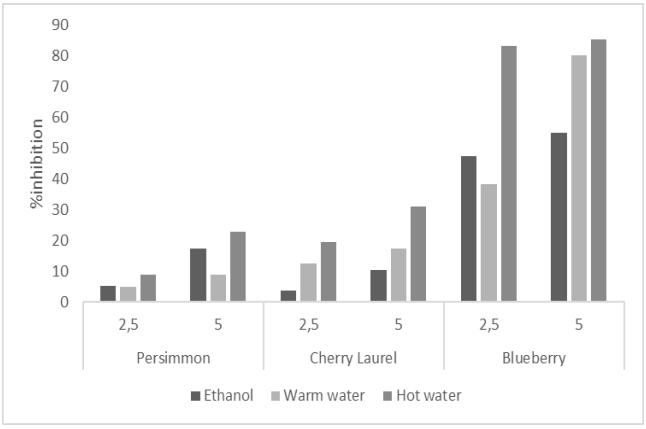

Figure 8. Antioxidant values according to solvent properties (%).

DISCUSSION

Considering the results obtained from the present study, the highest antioxidant activity was determined in the hot water extract (85.24%) of 5% concentration prepared with blueberry fruit, while the lowest antioxidant capacity was seen in the ethanol extract of the cherry laurel fruit. Sakanaka et al., (2005) prepared extracts with persimmon leaves using distilled water and methanol at 80°C and they reported that the pure water extract with a concentration of 0.1% had a higher inhibition value of more than 90% than the methanol extract. Jang et al., (2010) obtained 5% herbal extracts from different parts of the persimmon (core, sepal, peel and fleshy part) using different solvents (methanol, acetone, ethanol and water) and they stated that the extract prepared with water showed more antioxidant activity than methanol, ethanol and acetone extract with an inhibition value of 43.51%. Chen et al., (2008) determined the antioxidant capacity of 10% ethanol extracts prepared by using the skins of persimmon, grapes, apples and tomatoes fruits. According to their results, persimmon fruits showed more antioxidant properties than other fruits and they reported that grapes have more significant radical scavenging activities than regularly consumed grapes, apples and tomatoes due to their well-known and widely documented antioxidant properties. Akter et al., (2010) prepared extracts using acetone, methanol, ethanol and water in his study with persimmon kernels. Stated that the aqueous extract showed less antioxidant activity but had higher phenolic and flavonoid content, antioxidant activity is not related to phenolic and flavonoid content, and that antioxidant activity can sometimes change due to synergistic effects. When we look at the results obtained from in this study, it is seen that the % inhibition order of the persimmon fruit was warm water>ethanol>hot water and shows similar results with the studies in the literature. Karslı, (2020) reported that the leaves and peel of the feijoa fruit grown in the Eastern Black Sea region can be used as a natural antibacterial and antioxidant source for functional foods. In

addition, Karslı, (2020) stated that the antioxidant and antimicrobial effects of plants vary depending on various factors such as different parts of the plant, type of solvent, extract concentration and extraction method. The data obtained in our study also confirms these results, and it was determined that the antioxidant activity of the plants varies according to the extract concentration and solvent type.

According to the results obtained in the present study, the order of % inhibition values of cherry laurel extracts was determined as ethanol>warm water>hot water. Celik et al. (2020) prepared extracts of cherry laurel fruits with water and methanol at 2% concentration. They determined the antioxidant capacity of the extracts they prepared. They reported that the antioxidant activities of cherry laurel fruits made with methanol were higher. Celep et al., (2012) determined the antioxidant activity of cranberry, cherry laurel and persimmon fruits prepared using methanol at 80% concentration, they stated that the persimmon fruits showed the highest antioxidant activity and followed by cherry laurel fruits, and cranberry fruits. In general, cherry laurel fruits showed slightly higher antioxidant activity than persimmon fruits. It can be said that this difference was due to the fact that methanol dissolves substances with antioxidant properties better than ethanol and that antioxidant activity can be increased with the synergistic effect of phenolic and flavonoid substances. In this respect, the data of this study show similar results with the literature studies. Bilgin et al., (2011) determined the antioxidant activity of the blue berry fruits and leaves extracts prepared at 5% concentration of ethyl acetate, ethanol and water. They reported that the most suitable solvent was ethanol and blueberry have the potential to be used in industry. Ceylan et al., (2017) investigated antioxidant activity of blue berry (bilberry) leaves extracts prepared with acetone, ethanol, diethyl ether and water. And they reported that the antioxidant activity of extracts prepared from the dried samples showed higher antioxidant activity than the flesh ones. The highest activity was detected in the ethanol extract of dried samples. In this study, it was determined that ethanol extract of blue berry showed less antioxidant activity than hot water and warm water extracts. Many factors such as the drying temperatures of the obtained plants, the regions where they were collected, the time of harvest, the altitude above sea level and the soil structure in which they are grown can affect the amount and action mechanism of substances with antioxidant properties such as flavonoid and phenolic compounds can affect the antioxidant activity of the plant and fruit. The flavonoid and phenolic components found in plant sources can show their antioxidant properties alone, or they can come together to increase these capacities with a synergistic effect, and the polarity of the solvents that can be used in obtaining the substances with antioxidant properties and the polarity of their concentrations may vary according to the source used. In addition, there was an opinion that the responses of these components to solvents may change due to the physiological structure of the plants. Bilgin et al., (2011) determined the antioxidant activity of the extracts they prepared with 5% concentration of ethyl acetate, ethanol and water obtained from blueberry fruits and leaves. According to their results, they reported that the most suitable solvent was ethanol, and that blueberry plants and fruits have the potential to be used in industry. Ceylan et al., (2017) used different antioxidant activity determination methods in blueberry leaf extracts made with acetone, ethanol, diethyl ether and water in their study and reported that the extract made in the wet state showed higher activity than the dried form. The highest activity was detected in the ethanol extract of dry leaves. In this study, it was determined that ethanol extract of blueberry fruit showed less antioxidant activity than hot water and warm water extract. Many factors such as the drying temperatures of the obtained plants, the regions where they were collected, the time of harvest, the altitude above sea level and the soil structure in which they are grown can affect the amount and mechanism of action of substances with antioxidant properties such as flavonoid and phenolic compounds. The flavonoid and phenolic components found in plant sources can show their antioxidant properties alone, or they can come together to increase these capacities with a synergistic effect, and the polarity of the solvents that can be used in obtaining the substances with antioxidant properties and the polarity of their concentrations may vary according to the source used. In addition, there was an opinion that the responses of these components to solvents may change due to the physiological structure of the plants.

CONCLUSION

In this study, it was seen that the use of hot water, one of the solvents used in the extraction of cherry laurel, persimmon and blueberry fruit, was more effective in terms of antioxidant capacity and the plant concentration did not affect the antioxidant capacity much in terms of efficiency, but increased the antioxidant values, albeit in a small amount. These results show that such extract applications are practical and applicable for industrial use. However, in order to determine the synergistic effect capacities of the substances/extracts to be obtained, it is important and similar studies should be studied more specifically and directly on the active substance. In addition, the high antioxidant activity results from aqueous extracts in this study revealed important results in terms of these products being more natural, environmentally friendly, free of chemical additives and ease of use in the industry.

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