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Total Phenolic Content and Antioxidant Properties of Various Extracts of Myrtle (*Myrtus communis* L.) Berries

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Abstract

Myrtle (*Myrtus communis* L.) leaves and berries are consumed in therapeutic diet mainly in Mediterranean basin. In this study effect of water (W), hot water (W60), boiling water (W100) ethanol (E), methanol (M), ethanol/water (EW) and methanol/water (MW) mixture on the extraction of total phenolic content (TPC) and ferric reducing antioxidant power (FRAP) of myrtle berries were determined. The highest phenolic content was extracted by EW solvent mixture with 41370 mg/kg on dry basis while the highest ferric reducing antioxidant power was measured on M extract. Fresh or processed myrtle berries can be recommended for diet because of its high phenolic content and ferric reducing antioxidant.

Keywords: Myrtle, *Myrtus communis* L., Total phenolic content (TPC), Ferric reducing antioxidant power (FRAP)

Murt (*Myrtus communis* L.) Meyvesinin Farklı Ekstraktlarının Toplam Fenolik Madde Miktarı ve Antioksidan Özellikleri

Özet

Murt (*Myrtus communis* L.) meyvesi ve yaprağı Akdeniz havzası içinde terapötik diyette tüketilmektedir. Bu çalışmada, üzerine su (W), sıcak suW(60), kaynar su (W100), etanol (E), metanol (M), etanol/su (EW), ve methanol/su (MW) çözgenlerinin demir indirgeme antioksidan kapasitesi (FRAP) ve toplam fenolik madde (TPC) ekstraksiyonu üzerine etkisi incelenmiştir. En yüksek toplam fenolik madde ekstraksiyonu 41370 mg/kg kuru madde olarak EW çözgen karışımında elde edilirken, en yüksek demir indirgeme antioksidan kapasitesi methanol ektraktında gözlenmiştir. Yüksek fenolik madde içeriği ve antioksidan kapasitesine sahip olduğundan murt meyvelerinin taze veya işlenmiş olarak tüketilmesi önerilir.

Anahtar kelimeler: Murt, , *Myrtus communis* L., Toplam fenolik madde (TPC), Demir indirgeme antioksidan kapasite (FRAP)

Introduction

Myrtle (*Myrtus communis* L.) is an aromatic medicinal and evergreen shrub belonging to the family of Myrtaceae and is distributed in Mediterranean basin, Asia and and America (Karamanoğlu, (1977): Baytop, T. (1999); Özek et al. (2000); Wannes et al. (2010)). It grows spontaneously throughout the Mediterranean area. Myrtle is an annual endemic plant in the Mediterranean basin and has been used for medicinal purposes and as food and spice since ancient times (Mimica-Dukić et al. (2010); Ghnaya et al. (2013); Yıldırım et al. (2013)). Myrtle is known as “Mersin” or “Murt” or “Hambeles” in Mediterranean Region of Turkey (Aydın ve Özcan, (2007)). The plant is

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traditionally used for treatment of some infections, digestive and bronchial problems, sinusitis, and dry coughs. The leaves have good aromatic, balsamic, hemostatic and tonic properties and are used for flavoring in preparing some foods. The fruit is carminative and is used in the treatment of dysentery, diarrhea, hemorrhoids, internal ulceration, and rheumatism and also to flavor sauces, syrups, etc (Amensour et al. (2009)).

In literature, many studies have indicated that myrtle plant could be used as a source of antioxidant and antibacterial properties. Ghnaya et al. (2013) has reported myrtle was also used as raw material for the cosmetic, pharmaceutical and food industries. Generally, these studies were mainly focused on the Myrtle leaves extracts (Özek et al. (2000); Hayder et al. (2004); Wannes et al. (2010)). The leaves contain tannins, flavonoids such as quercetin, catechin and myricetin derivatives and volatile oils. Myrtle berries are mostly composed of volatile oils, tannins, carbohydrates, flavonoids and organic acids such as citric cid and malic acid. Recently there is a great interest about myrtle fruit in various scientific field (Çakır, (2004); Aydın and Özcan, (2007); Tuberoso, et al. (2010)).

A considerable feature of medicinal and aromatic plants is excellent sources of phenolic compounds. The presence of phenolic compounds (phenolic acids, polyphenols and flavonoids) in plants, besides essential oils, is gaining increasing attention because of their antioxidant activity in food products and in therapeutic treatments (Chryssavgi et al. (2008)). Phenolic compounds might act as reducing substances, blocking free radicals, chelating metal ions, inhibitors of oxidation reaction, inhibitors of the activity of enzymes contributing to free radical creation. In addition, they may reduce reactive oxygen species to more stable forms (Kobus-Cisowska et al. (2013)). Thus antioxidant components like phenolic compounds can delay or inhibit oxidation reaction (Güngör ve Şengül (2008); Thabti et al. (2011)). and extend the shelf-life of food products.

In recent years, the increasing interest for human health, nutrition and the prevention from disease has driven the demand of the consumer to foods and quality raw material with high nutraceutical value (Contessa et al. (2013)). In epidemiological studies, diets rich in fruits and vegetables were correlated positively with reduced risk of heart disease, cancer and other chronic diseases (Koca (2008); Wang and Hu (2011)). It is generally believed that physiological function of fruits may be partly attributed to their antioxidant activity of phenolic compounds.

Antioxidant rich components of foods play a vital role in both food systems as well as in the human body to reduce stress-related diseases such as cancer and cardiovascular diseases. In food systems, retarding lipid peroxidation and formation of secondary lipid peroxidation product can be prevented by the use of nutritional antioxidants thereby helping to maintain flavour, texture, and the colour of the food product during storage. Use of dietary antioxidants has been recognized as potentially effective to promote human health. Dietary antioxidant supplements and functional foods containing antioxidants like a-tocopherol, vitamin C, or plant derived phytochemicals such as phenolics, lycopene, lutein, isoflavones, green tea extract, and grape seed extracts find a huge demand in the current marketplace (Sindhi (2013)).

In the extraction of phenolic compounds that contribute to antioxidant potential ethanol, methanol, aceton, ethyl acetate, water and various combination of these solvents have been used. To increase extraction yield sonication, enzymation, acidification and heating processes have been used. Extraction of phenolic compounds is also influenced by solvent polarity, extraction temperature, extraction time, sample/solvent ratio and sample particle size (Naczk and Shahidi (2006)).

Recent researches are generally focused on chemical composition, antioxidative properties of extracts from *Myrtus communis* leaves (Özek et al. (2000); Hayder et al. (2004); Wannes et al. (2010)). However, there is a few number of reports available on the antioxidant properties of myrtle berries antioxidant activity and total phenolic content (Tümen (2012); Yadegarinia (2006)). The aim of this research was to evaluate the antioxidant activity and total phenolic content of different extracts (methanol, ethanol, water, methanol/water, ethanol/water) of *Myrtus communis* L. berries as potential sources of natural antioxidants.

Materials and Methods

Chemicals

All solvents used in the experiments (ethanol and methanol with highest available purity) were purchased from Merck (Darmstadt, Germany). Gallic acid, ferrous sulphate, 2,4,6-tripyridyl- S-triazine (TPTZ) and Folin-Ciocalteau's reactive was obtained from Sigma-Aldrich, Fluka (Milan, Italy). Glacial acetic acid, sodium carbonate, ferric chloride, sodium acetate and HCl were supplied by Carlo Erba (Milan, Italy). All chemicals and reagents used in this research were analytical grade.

Myrtle Fruits and Samples Preparation

White myrtle berries were purchased from local market in Mersin, Turkey. The berries were carried to the laboratory and cleaned manually from impurities and dried by a freeze drier (Telstar Crydos -50, Terresa, Spain) at -50 ^{0}C condenser temperature for 24 hours and then the berries of myrtle were separated and ground in a grinder.

Dried powders of myrtle berries were extracted with methanol (M), ethanol (E), water(W) and solvent mixtures methanol:water (MW, 50:50 volume ratio), ethanol:water (EW, 50:50 volume ratio). To determine effect of temperature on phenolic extraction, 2 g of dried sample was extracted by 100 ml of hot water at 60 °C (W60) and by 100 mL of boiling water (W100) by using a hot plate. For all extraction, an aliquot (2 g) of dried powder sample was extracted using 100 ml of solvent or solvent mixture (fruits:solvent ratio was 1:50) for 2 hours. All extractions were replicated two times. At the end of extraction time, extracts were filtered through a filter paper Whatman No. 1 and were stored at 4 ^{o}C in the dark until use.

Total Phenolic Content

Total phenolic content was measured according to Velioğlu et al. (1998), a method based on a colorimetric oxidation/reduction reaction. Folin-Ciocalteu reagent was used as an oxidizing agent. A 0.2 ml of extract, 1.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) was added. After 5 min, 1.5 ml of Na_2CO_3 (60 g/L) were added. The sample was incubated for 90 min at room temperature. After incubation period the absorbance was read at 725 nm against a blank. For a control sample, 0.2 ml of solvents were used. For quantitative measurements standard calibration curve of gallic acid in methanol was prepared. The mean results of samples analysed were expressed as mg/kg of gallic acid equivalents (GAE) in dry matter.

Ferric reducing antioxidant power

The ferric reducing antioxidant power activity was conducted according to Benzie and Strain (1996) method. Acetate buffer (0.3M, pH 3.6) was prepared by dissolving 40.8 g $C_2H_3O_2Na3H_2O$ in 1 L of distilled water and pH was adjusted 3.6 by acetic acid. 23.4 mg of TPTZ (2,4,6-tripyridyl-S-triazine) was dissolved in 7.5 ml of 40 mM HCl for preparing triazine solution. Ferric solution (20 mM) was prepared using $FeCl_36H_2O$. Acetate buffer, TPTZ and ferric solutions at a ratio of 10:1:1 were used for final FRAP reagent. FRAP reagent was prepared freshly and was warmed to 37 ^{o}C before use in a water bath. 200 µL of each extract and 1.8 ml of FRAP reagent were mixed and incubated at 37 ^{o}C for 10 minutes before measuring the absorbance of the reaction mixture at 593 nm. Aqueous standard solutions of $FeSO_47H_2O$ (0-100 ppm) were used for the calibration curve. The results were expressed as ppm $FeSO_47H_2O$.

Results and Discussions

Total Phenolic Content

Figure 1 shows the total phenolic contents of myrtle berries as gallic acid equivalent. Extraction of total phenolic from myrtle berries is depended of solvent type and extraction temperature. EW extraction has higher in phenolic content with 41370±2906 mg/kg than the others while the lowest phenolic content was obtained by water extraction with 17763±3064 and 18174±2422 mg/kg dry basis at 60 °C and ambient temperature, respectively. While M and MW extracts have similar phenolic content about 38000 mg/kg.

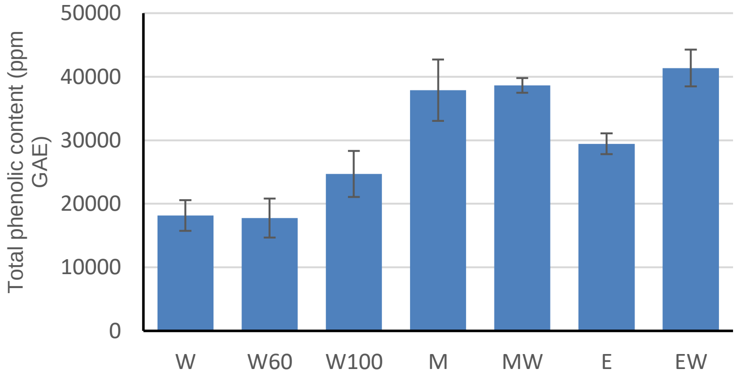

Figure 1. Total phenolic content of myrtle extracts (W:Water, W60: Water at 60 °C, W100:Boiling water, M:Methanol, MW:Methanol/Water E:Ethanol, EW:Etahnol/Water).

Tuberoso et al. (2010). reported that the highest dry matter content (99 g/L) and total phenolic obtained by ethanol extract compare to water and ethyl acetate. Amensour et al. (2009) has found that berry extracts had been showed higher phenolic content in methanol>ethanol>water extracts. Phenolic content of While MW and EW solvent mixtures had similar phenolic content EW was slightly higher than MW. EW can be recommended for extraction of phenolic substances from myrtle berries.

EW extract have higher phenolic content than the phenolic content of myrtle leaf methanol extract with 33670 mg/kg dry leaves (Wannes et al. 2010). Phenolic content of EW extract of myrtle berries on dry basis is pretty much with compare to berry fruits like blackberry (4170-5550 mg/kg) and chocoberry (6625-6900 mg/kg) (Szajdek and Borowsk (2008)) and some astringent fruits like pomegranate (Tezcan et al. (2009)) and persimmon (Akyıldız et al. (2008)) with 2602-10086 mg/L and 467-7332 mg/kg phenolic content respectively.

Ferric Reducing Antioxidant Power

Ferric reducing antioxidant power of different myrtle extracts are shown in Figure 2. The highest antioxidant potentials were obtained M, MW and EW extracts of myrtle about $175x10^3$ mg/kg. The lowest antioxidant potential was obtained by W60 extract and W at ambient temperature. W100 extract had higher ferric reducing power than W and W60 but lower than E, EW, M and MW extracts.

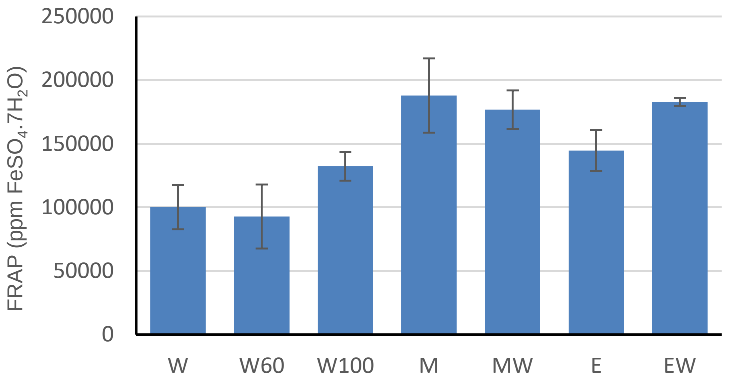

Figure 2. Ferric reducing antioxidant power of myrtle extracts (W:Water, W60: Water at 60 °C, W100:Boiling water, M:Methanol, MW:Methanol/Water E:Ethanol, EW:Etahnol/Water).

Figure 3. shows that there was a good correlation (R2, 0,826) between phenolic content and antioxidant activity. While the highest phenolic content was obtained by EW extract, the highest antioxidant potential was extracted by M extract. Amensour et al. (2009) has reported similar results that methanol extracts of myrtle leaf had higher antioxidant activity than water and ethanol extracts. The ferric reducing power is strongly contributed to the structure of phenolic constituents such as condenced tannins, anthocyanins and flavonoids (Juranic and Zizak (2005)). It is recommended to measure antioxidant potential by two or more methods such as free radical scavenging (DPPH) or Trolox equivalent antioxidant capacity (TEAC) additional to ferric reducing antioxidant power (FRAP).

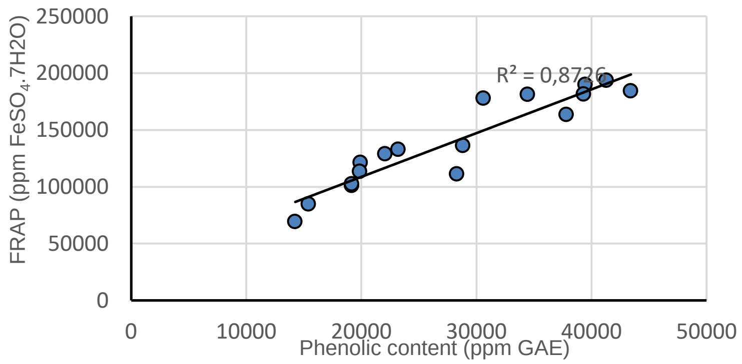

Figure 3. Correlation between total phenolic content and ferric reducing antioxidant power of myrtle extracts.

Conclusion

Myrtle berries are good source of phenolic compounds with reasonable ferric reducing antioxidant power compare to other berry fruits and stringent fruits. The highest phenolic content was obtained by solvent mixture of ethanol and water while the highest ferric reducing antioxidant power was measured in methanol extract of myrtle berries. Total phenolic content and FRAP values showed a good correlation but it is recommended to determine the structure of berry phenolics and to measure antioxidant potential by other methods. Because of its high phenolic content and ferric reducing antioxidant power fresh or processed myrtle berries can be recommended for diet. Myrtle berries can be a good source of phenolic rich constituents or enrichment additives for foods.

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