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Investigation of Essential Oil Composition, Polyphenol Content, and Antioxidant Activity of *Myrtus communis* L. from Turkey

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Abstract: In this study, it is aimed to investigate the chemical composition of the essential oil, polyphenol content, and antioxidant activity of *Myrtus communis* L. from Turkey. The plant, *Myrtus communis* L., was collected from Yalova, in the Marmara region of Turkey. The essential oil was prepared with 0.5% by hydrodistillation of its leaves in a Clevenger-type apparatus. The chemical composition of the essential oil was analyzed by GC and GC-MS, using two columns with stationary phases of different polarity (polar ZB-WaxMS/apolar ZB-5MS). On both columns, monoterpenes were determined to be the dominant compounds. The myrtenyl acetate, α -pinene, 1,8-cineole, linalool, and limonene were the remarkable substances. As polyphenolic compounds, the flavanoids and anthocyanidins in leaves and berries were detected by HPLC. The antioxidant activity was studied with DPPH, Cuprac and Folin-Ciocalteu methods.

Keywords: *Myrtus communis* L.; anthocyanidins; flavonoids; essential oils; antioxidant activity.

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INTRODUCTION

Myrtus communis L., growing in several regions all over the world (1-3) is found in pine forests and all coastal zones in Turkey. Myrtle (*Myrtus communis* L.) is called as "hambeles", "mersin" or "murt" in Turkish.

Myrtus communis L., especially its leaves and berries, is traditionally used in folk medicine as antiseptic, antidiaretic, antibacterial, disinfectant drug, hypoglycaemic, and stomachic (4-6). The essential oils from leaves of this plant also have a private role in treatment of lung diseases (7), perfumery, beverages, candies, ice cream and bakery products (8-9) and show antimicrobial (10), antibacterial (11), and antioxidant activities (12-14). Isolation of some compounds from the leaves (15-16), the essential oil (10,17) and berries (18) has been reported in the literature. Some researchers indicated that the chemical composition of *Myrtus communis* L. essential oils showed a difference depending on the harvesting time, origin, and extraction method (12,19-21). Fatty acid composition of the plant from Erzurum and Muğla region in Turkey was determined in addition to nutritional and physical properties of myrtle fruits from Mersin in the previous studies (22-23).

The human body has a complex system and free radicals produced from biochemical reactions in our body are responsible for chronic diseases. The antioxidant substances can prevent the damaging effects of these radicals to quench them and detoxify the organism (24). The studies in this field emphasize the importance of antioxidant nutrients for protection diseases.

According to the literature, there is no study based on the antioxidant activity of the essential oil and polyphenolic contents of Turkish *Myrtus communis* L. leaves and berries.

This work aims at analyzing the components of the essential oil of *Myrtus communis* L. leaves, determining as the anthocyanidin and flavonoid compounds and evaluating the antioxidant properties of methanolic extracts of *Myrtus communis* L. leaves and berries.

MATERIALS AND METHODS

Reagents and Standards

HPLC grade methanol, acetonitrile, trifluoroacetic acid and Folin - Ciocalteu reagent were purchased from Merck. Anthocyanidin standards of (delphinidin, peonidin, pelargonidin, malvidin, cyanidin chloride) and flavonoids (myricetin, quercetin, p-coumaric acid) and DPPH (1,1-diphenyl-2-picrylhydrazyl), NDGA (Nordihydroguaiaretic acid), ascorbic acid, pyrocatechol were obtained from Sigma-Aldrich.

Standard stock solutions (1 mg mL⁻¹ in methanol) of each of anthocyanidin and flavonoid (**1-8**) were prepared (Figure 1a and 1b).

Plant Material

Leaves and berries of *Myrtus communis* L. from Yalova region of Turkey were dried, powdered, and stored at -20°C.

Hydrodistillation

The leaves of plant were hydrodistilled for 2h with a simple Clevenger- type apparatus. Yield of the oil was 0.5% based on dry plant weight.

GC/MS Analysis of the Essential Oils

Thermo Finnigan trace DSQ GC-instrument (FID) combined with MS system was used to analyze the chemical components. The capillary columns, ZB-WaxMS (polyethylene glycol, 60 m × 0.25 mm i.d., 0.25 µm film thickness) and ZB-5MS (5% polysilarylene, 95% polydimethyl siloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness) were used. Operating conditions were chosen as injection temperature 250 °C, helium carrier gas flow (1,5 mL min⁻¹), split flow: 10 mL min⁻¹. Temperature programming 30-100 °C (2 °C min⁻¹) (5 min)-240 °C at 5 °C min⁻¹, hold 30 min.

The results were determined by looking at the retention indices in the literature (10,12,17,25-26) and comparing with their MS spectra.

Qualitative Analysis of Phenolic Compounds by HPLC

Preparation of *Myrtus communis* L. Extracts for HPLC Analysis: Dried leaves and berries (1 g) were extracted in flasks immersed into an ultrasonic bath firstly with 70% MeOH (15 mL) for 45 min, then 5 mL of the same solvent for second 45 min and minimum 5 mL more of the solvent for last 15 min were added, the total extraction time was 105 min (27). The plant extracts were filtered with a GF/PET 1.0 / 0.45 μm microfiber, and analyzed (Figures 2 and 3).

HPLC / UV-vis Analysis

The HPLC equipment employed in this study was a Shimadzu / DGU-20 A₅ HPLC apparatus fitted with intertsil O DS-3 C₁₈ column (4,6 \times 150 mm, 5 μm particle size) according to the procedure described by literature (13). The elution program was used in the reversed- phase HPLC analysis.

Compounds **1-7** could be detected between 350 and 520 nm, characteristics for flavonoids and anthocyanidin, respectively, according to the chromatographic conditions described in the literature above. The chromatograms can be seen in Figures 2-5.

Hydrolysis of plant extracts have been done according to the literature (27). These extracts were also analyzed (Figures 4, 5a and 5b).

In addition, all flavonoids and anthocyanidins were identified from their spectroscopic properties and their range of retention times.

Antioxidant Activity

DPPH Radical Scavenging Activity: The DPPH radical scavenging activity of the *Myrtus communis* L. extracts were defined by the procedure of Brand-Williams *et al.*(28). An appropriate dilution series (20 to 100 $\mu\text{g mL}^{-1}$) were made for each methanolic extract in methanol, afterwards 0.1 mL of each sample was mixed with 3.9 mL of a 6×10^{-5} M methanolic solution of DPPH. The mixture was flattered fiercely and left in the darkness at room temperature (30 min) and then the absorbance was read at 517 nm against methanol. The equation given below represents the calculation of capability to scavenge the DPPH radical.

$$\text{DPPH radical scavenging activity (\%)} = (A_0 - A_1 / A_0) \times 100 \quad (\text{Eq.1})$$

A_0 and A_1 is the absorbance of control (without sample) and test sample, respectively.

Determination of Total Phenolic Contents

Determination of total phenolic content in *Myrtus communis* L. extracts was predicted using Folin - Ciocalteu method (29). The measured wavelength with maximum absorbance in a spectrophotometer was 760 nm. The total phenolic compound amount in the extract was calculated as mg of pyrocatechol equivalent from the calibration curve and as mg pyrocatechol equivalents per mg of extract.

CUPRAC Method

The proposed Cuprac method by Apak (30) was applied as follows: 1 mL of 1.0×10^{-2} M copper(II) chloride, 1 mL of 7.5×10^{-3} M neocuproine solution and 1 mL of 1 M ammonium acetate buffer at pH 7.0, x mL sample solution and (1- x) mL distilled water (total volume: 4.0 mL) were added to a test tube and mixed well. This mixture was stoppered and let stand at room temperature (30 min). The absorbance was recorded at 450 nm against a reagent blank.

RESULTS AND DISCUSSION

Yield and Chemical Composition of *Myrtus communis* L. Essential Oil

The essential oil of the plant leaves was prepared by hydrodistillation in a Clevenger-type apparatus in 0.5%. The plant collected from Yalova, Turkey was analyzed qualitatively and quantitatively via GC and GC / MS on two different columns, on a polar column ZB-Wax MS and on a apolar column ZB-5MS (Table 1). The Relative Retention Indices (RRI) for all the compounds were calculated using a homologous series of n-alkanes. All components were identified by comparing their relative retention indices and also their mass spectras with the literature data.

Thirty one and eighteen compounds were identified on the polar column and on the apolar column, respectively. The major components were myrtenyl acetate (22.26 %), α -pinene (15.51%), linalool (14.91%), 1,8-cineole (14.30%) and limonene (13.63%) on

ZB-Wax MS. On the ZB-5MS, the main components were myrtenyl acetate (21.42%), 1,8-cineole (16.78%), linalool (15.66%), limonene (15.59%) and α -pinene (15.07%).

HPLC / UV-vis Analysis of *Myrtus communis* L. Leaves and Berries Extracts

Myrtus communis L. leaves and berries were extracted according to the literature (27) for HPLC analysis. The hydrolysis of flavonoid glycosides in plant extracts to their parent aglycone was also identified using standards (quercetin and kaempferol).

Figures 1a and 1b show the chromatogram of synthetic mixture flavonoids and anthocyanidins, respectively. The chromatogram of 70% methanolic extract of *Myrtus communis* L. leaves and the chromatogram of the same extract after 4h hydrolysis are given in Figures 2 and 4, respectively. When Figure 3 represents the chromatogram of 70% methanolic extract of *Myrtus communis* L. berries, Figures 5a and 5b give the chromatogram of the same extract after 4h hydrolysis.

P.coumaric acid, myricetin, and quercetin in the *Myrtus communis* L. leaves and berries, cyanidin, malvidin, pelargonidin, and peonidin in the *Myrtus communis* L. berries have been specified according to their retention times and the spectral characteristics of their peaks against those of standards.

Major peak in the flavonoid chromatogram was identified as myricetin **2** and minor peaks were characterized as compounds **1** and **3**, respectively, p.coumaric acid and quercetin at 350 nm (Figures 4 and 5a).

Major peak in the anthocyanidin chromatogram corresponded to compound **5** was defined as cyanidin and minor peaks were obtained as compounds **6**, **7** and **8**, respectively, malvidin, pelargonidin and peonidin at 520 nm (Figure 5b).

DPPH Assay

DPPH assay is an antioxidant method based on a color change of the solution from violet to yellow upon reduction and this method supplies an easy and rapid way to determine antioxidants by spectrometry.

Figure 6 shows the concentration-inhibition(%) curves for the DPPH radical scavenging activity of the methanolic extracts from *Myrtus communis* L. leaves and berries and standards. The scavenging activity of these extracts from *Myrtus communis* L. berries and leaves, tocopherol, ascorbic acid, butylated hydroxyanisole (BHA), nordihydroguaiaretic acid (NDGA) on DPPH radicals increased between 20-100 $\mu\text{g mL}^{-1}$ and were 82.20 ± 1.237 , 95.09 ± 0.305 , 95.71 ± 0.3010 , 96.93 ± 0.178 , 94.48 ± 0.352 , 96.63 ± 0.178 at a concentration of 100 $\mu\text{g mL}^{-1}$, respectively. Methanolic extract of leaves, BHA, tocopherol, ascorbic acid and NDGA displayed similar DPPH scavenging activities, while the effective DPPH scavenging activity of methanolic extract of berries was quite low (Figure 6).

Total Phenolic Contents

Phenols are good scavengers because of having hydroxyl groups of components found in plants. These antioxidants also exhibit different biological activities like antiatherosclerotic, antiinflammatory, and anticarcinogenic effects (31).

The results of total phenolic content for the methanolic extracts from *Myrtus communis* L. berries and leaves, expressed as pyrocatechol equivalents, are presented in Table 2. Methanolic extract of leaves contains more phenolic compounds than the berry extract.

CUPRAC Method

The reducing power of antioxidant compounds was determined by the CUPRAC assay, which is a useful method and Cu^{2+} is reduced to Cu^{+} with antioxidants in the presence of neocuproin. In this method, a higher absorbance means a higher cupric ion reduction ability. The method is also cheap, and is suitable for a lot of antioxidants (32).

The cupric ion (Cu^{2+}) reducing abilities of the methanolic extracts from *Myrtus communis* L. leaves and berries are given in Table 3. Methanolic extract of leaves exhibited more reducing capacity than that of berries.

CONCLUSION

The chemical composition of *Myrtus communis* L. essential oil could be divided into subgroups according to the relative ratio of α -pinene and myrtenyl acetate or α -pinene and 1,8-cineole (19). Myrtenyl acetate content is high (22.26-21.42%) as Spanish, Moroccan, Portuguese, French, Albanian and Yugoslavian myrtle oils (20,33-34) in the essential oil of *Myrtus communis* L. leaves from Yalova region, Turkey.

Phenolic compounds, flavonoids and anthocyanidins of *Myrtus communis* L. leaves and berries were determined qualitatively by HPLC method at 350 nm and 520 nm. The major component of flavonoids in leaves and berries was myricetin. The main compound of anthocyanidins in berries was cyanidin. However, the anthocyanidins have not been detected in the leaves.

The findings of this study demonstrated that methanolic extracts of *Myrtus communis* L. leaves and berries possess antioxidant activity. The antioxidant activity of these extracts is similar to standards of BHA, tocopherol, ascorbic acid and NDGA.

The plant, *Myrtus communis* L. from Turkey together with its leaves and the berries is a rich antioxidant source due to its essential oil composition (Table 1) and flavonoid content (Figures 4, 5a and 5b) and can be used in food, pharmaceutical, cosmetic industries and against diseases with its good antioxidant ability tested in this study.

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Türkçe Öz ve Anahtar Kelimeler
**Türkiye'den *Myrtus communis* L.'nin Esansiyel Yağ Bileşimi,
Polifenol İçeriği ve Antioksidan Aktivitesinin İncelenmesi**

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Öz: Bu çalışmada, Türkiye'den toplanmış *Myrtus communis* L.'nin esansiyel yağının kimyasal bileşimi, polifenol içeriği ve antioksidan aktivitesinin incelenmesi amaçlanmıştır. Bitki Türkiye'nin Marmara bölgesinde yer alan Yalova ilinden toplanmıştır. Esansiyel yağ, Clevenger tipi cihazda yaprakların hidrodestilasyonu ile %0,5 verimle hazırlanmıştır. Esansiyel yağın kimyasal bileşimi GC ve GC-MS ile analiz edilmiştir, farklı polariteye sahip durağan fazlar içeren iki kolon kullanılmıştır (polar ZB-WaxMS/apolar ZM-5MS). Her iki kolonda da monoterpenlerin baskın olduğu görülmüştür. Mirtenil asetat, α -pinen, 1,8-sineol, linalool ve limonenin belirgin bileşikler olduğu tespit edilmiştir. Polifenolik bileşikler olarak, yapraklarda ve tanelerde flavanoidlerin ve antosiyanidinlerin varlığı HPLC ile tespit edilmiştir. Antioksidan aktivitesi DPPH, Cuprac ve Folin-Ciocalteu yöntemleriyle çalışılmıştır.

Anahtar kelimeler: *Myrtus communis* L.; antosiyanidin; flavanoidler; esansiyel yağlar; antioksidan aktivitesi.

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