GIDA (2015) 40 (4): 193-199 *Research* / Araştırma
doi: 10.15237/gida.GD15035

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *MYRTUS COMMUNIS* L. GROWING WILD IN MARMARIS

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Received / Geliş tarihi: 19.05.2015
Received in revised form / Düzeltilerek Geliş tarihi: 30.05.2015
Accepted / Kabul tarihi: 03.06.2015

Abstract

Three myrtus fruit samples collected from different regions of Marmaris were dried, grinded and extracted with n-hexane, CH_2Cl_2 and MeOH respectively. The extracts of *Myrtus communis* L. were screened in vitro for their antimicrobial activities using disc diffusion method against four test bacteria. The antimicrobial test results showed that the inhibition zones have been measured between 7 to 16 mm. *S. aureus* was the most sensitive one to all concentrations of all *M. communis* L. samples. *P. aeruginosa* was the most resistant one to all concentrations of *M. communis* L. sample from Yeşil Belde. The antioxidant activity of MeOH extracts has also been determined by DPPH assay. This is the first report of comparative antimicrobial and antioxidant study for *M. communis* L. samples collected from different regions from Marmaris/ Mugla/ Turkey.

Keywords: *M. communis* L., disc diffusion method, pathogen bacteria, DPPH method, Marmaris

MARMARİSTE YABANİ OLARAK YETİŞEN *MYRTUS COMMUNIS* L.'NİN ANTİBAKTERİYEL VE ANTİOKSİDAN AKTİVİTESİ

Özet

Marmaris'in üç farklı bölgesinden toplanan *Myrtus* (Mersin ağacı) meyveleri kurutulmuş, öğütülmüş ve sırasıyla, n-heksan, CH_2CI_2 ve MeOH ile ekstrakte edilmiştir. Elde edilen kurutulmuş, ekstraktlar dört farklı test bakterisine karşı disk difüzyon yöntemi kullanılarak in vitro olarak taranmıştır. Antimikrobiyel test sonuçları inhibisyon zonlarının 7-16 mm çapında oluklarını göstermiştir. *S. aureus, M. communis* L. örneklerinin tüm konsantrasyonlarına karşı en duyarlı olan bakteri iken, *P. aeruginosa* ise Yeşil Belde'den toplanan *M. communis* L. örneklerinin bütün konsantrasyonlarına karşı en dirençli bakteri türü olarak belirlenmiştir. MeOH ekstraktlarının antioksidan aktivitesi, DPPH metodu kullanılarak ölçülmüştür. Bu çalışmada, ilk kez Marmaris'in farklı bölgelerinden toplanan *M. communis* L. örneklerinin karşılaştırmalı olarak antimikrobiyel ve antioksidan aktiviteleri saptanmıştır.

Anahtar kelimeler: *M. communis* L., disk diffüzyon metodu, patojen bakteri, DPPH metodu, Marmaris

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INTRODUCTION

Myrtaceae (*Myrtus communis* L.) is an evergreen shrub growing spontaneously throughout the Mediterranean area. It is a typical annual shrub of the Mediterranean countries including Turkey, Greece, Italy, Algeria, Tunisia, and Morocco. In Turkey, myrtle plants are found within the natural pine forests and riversides in the Mediterranean region, particularly in the Taurus Mountains, 500 to 600 m above sea level (1).

In folk medicine, a decoction of leaves and fruits or infusion of myrtle are used for stomachic, hypoglycemic, cough and oral diseases, antimicrobic, for constipation, appetizing, antihaemorrhagic and externally for wound healing (2, 3). In Turkish folk medicine, the leaves and fruits have been used as an antiseptic and for healing wounds as well as in the treatment of urinary diseases (4). Different parts of the plant find various uses in the food industry, such as for flavoring meat and sauces, and in the cosmetic industry (5).

Over the past few years, liqueurs prepared from the berries of myrtle have become popular, (6) while its leaves have been used as a hop substitute in beer and as a cosmetic ingredient in products against hair dandruff (7).

Until now, the majority of studies on myrtle have focused on its volatile fraction (5, 8-18) and of phenolic compounds in leaves and berries (19-27). The leaves contain tannins, flavonoids such as quercetin, catechin and myricetin derivatives and volatile oils (4, 28). The fruits of this plant are mostly composed of volatile oils, tannins, sugars, flavonoids and organic acids such as citric and malic acids (4, 21).

The volatile oil in leaves of *M. communis* L. growing in Turkey contains 1,8-cineole, linalool, myrtenyl acetate and myrtenol as major components (3). In addition, Mansouri et al. (28) reported that a crude methanol extract of *M. communis* L. leaves had potent antibacterial activity against 10 microorganisms, including 6 Gram positive and 4 Gram negative bacteria. A few researches have undertaken the antioxidant activity of myrtle leaf essential oil (28) and extract (30-33).

The main objectives of this study were to investigate the antimicrobial activity of the extracts obtained from *Myrtus communis* L. berries by disc diffusion method against some pathogen bacteria.

As far as our literature survey could ascertain, our study is different from the previous reports on this plant in terms of plant material collected from different region to evaluate regional variety and the extracts obtained by using different solvents.

MATERIAL AND METHODS

Collection of plant material

M. communis L. berries were collected from Bozburun, Çetibeli and Yeşil Belde; Marmaris, Turkey.

Extraction of *M. communis* L. berries

The dried and grinded berries (25 g of samples) were extracted with n-Hexane (500ml), CH_2Cl_2 (500ml) and MeOH (500ml) using soxlet apparatus respectively. The extracts of berries were evaporated to dryness in vacuum at 50 oC. The yields (%, w/w dry plant material) of dry extracts are presented in Table 1.

Table 1: The yields of the crude dry extracts

Locality	Extract yield (%, w/w)		
	n-Hexane	CH_2Cl_2	MeOH
Bozburun	1.51	0.71	77.93
Çetibeli	2.24	1.47	73.67
Yeşil Belde	2.01	0.68	91.88

Antimicrobial assay

Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli and *Pseudomonas aeruginosa* were used as test bacteria. Bacteria inoculates were prepared by growing cells in Nutrient broth (Merck) for 24 h at 37 °C (34). These cells suspensions were diluted with peptone water to provide initial cell counts of about 10^5- 10^6 cfu/ml. The extracts (all extracts were filter-sterilized using a 0.45 µm membrane filter) were prepared at 1%; 2.5%; 5% and 10% concentrations in correspond solvent. 17 ml sterile Mueller–Hinton agar at 45 °C and poured into Petri dishes (9 cm in diameter). Then the agars were allowed to solidify at 4 °C for 1 h. Test bacteria were spread on Muller Hinton Agar. Sterile paper disk of 6 mm diameter were impregnated with this solutions (30 µl) (35). These impregnated disks were applied on solid agar medium in Petri dishes. The treated Petri dishes were left 10-15 minutes at room temperature and then incubated 37± 0.1 °C for 24-48 hours. After the incubation period inhibition zones were measured in millimeters. These experiments were carried out in duplicate (36).

Antioxidant activity

DPPH assays/TLC autographic assay

After developing and drying the TLC plates (samples ranging from 0.1 to 100μg) were sprayed with 0.2% (2mg/ml) of DPPH solution in methanol. The plates were examined half an hour after spraying. Active compounds appeared as yellow spots against a purple background. (37-41).

Antioxitant capacity

One ml of 500 μM (0.2 mg/ml) DPPH in methanol was mixed with equal volumes of test compounds at various concentrations, mixed well and kept in the dark for 30 min. The absorbance at 517 nm was monitored in the presence of different concentrations of the samples. Blank experiment was also carried out, with just solvent and DPPH (i.e., 2 ml of 500 μM in methanol), to determine the absorbance of DPPH before interacting with the compounds. The amount of sample in μg/ml at which the absorbance at 517 nm decreases to half its initial value was used as the IC_{50} value of the MeOH extracts (36, 42, 43).

The samples were done in triplicate and the mean value of three was recorded.

RESULTS AND DISCUSSION

The antibacterial activities of *M. communis* L extracts at different concentrations in vitro test against different pathogenic bacteria were shown in Table 2, 3 and 4.

The inhibition zones were varied related to different concentrations of *M. communis* L extracts. Our results have shown remarkable antimicrobial activity for the n-hexane extract of *M. communis* L (especially BH and ÇH) against all microorganisms tested with inhibition zones.

Table 2: The antimicrobial activity (diameters of growth inhibition zones) of the crude extracts of three *M. communis* L. samples from Bozburun

Microorganisms	Extracts											
	n-Hexane (BH)				CH_2Cl_2 (BC)				MeOH (BM)			
	10%	5%	2.5%	1%	10%	5%	2.5%	1%	10%	5%	2.5%	1%
S. aureus	14	14	14	12	14	12	12	12	12	11	7	7
P. aeruginosa	12	10	8	8	–	–	–	–	–	–	–	–
E. coli	10	8	7	7	7	–	–	–	8	8	8	8
K. pneumonia	12	–	–	–	–	–	10	8	–	–	–	–

Table 3: The antimicrobial activity (diameters of growth inhibition zones) of the crude extracts of three *M. communis* L. samples from Çetibeli

Microorganisms	Extracts											
	n-Hexane (ÇH)				CH_2Cl_2 (ÇC)				MeOH (ÇM)			
	10%	5%	2.5%	1%	10%	5%	2.5%	1%	10%	5%	2.5%	1%
S. aureus	14	14	14	12	12	–	–	–	10	8	7	–
P. aeruginosa	12	10	8	8	–	–	–	–	–	–	–	–
E. coli	10	8	7	7	7	–	–	–	–	–	–	–
K. pneumonia	12	–	–	–	–	–	–	–	–	–	–	–

Table 4: The antimicrobial activity (diameters of growth inhibition zones) of the crude extracts of three *M.communis* L. samples from Yeşil Belde

Microorganisms	Extracts											
	n-Hexane (YH)				CH_2Cl_2 (YC)				MeOH (YM)			
	10%	5%	2.5%	1%	10%	5%	2.5%	1%	10%	5%	2.5%	1%
S. aureus	10	10	10	8	16	16	14	14	10	8	–	–
P. aeruginosa	–	–	–	–	–	–	–	–	–	–	–	–
E. coli	7	–	–	–	7	–	–	–	–	–	–	–
K. pneumonia	15	14	14	12	–	–	–	–	–	–	–	–

The inhibition zones of *M. communis* L. collected from Bozburun and Çetibeli have shown a similarity. The diameters of growth inhibition zones ranged from 7 to 16 mm, with the highest inhibition zone values observed against the medically important pathogens *S. aureus* (16 mm), *K. pneumoniae* (15 mm) and *P. aeruginosa* (12 mm) at 10% concentrations. Though, dichloromethane extracts (YC) were found to have strong activity against *S. aureus* (16 mm) at 10% concentrations, dichloromethane extracts (ÇC) were exhibited moderate activity against *S. aureus* (12 mm) and *E. coli* (7 mm) at 10% concentrations. Except for *S. aureus*, the MeOH extract of *M. communis* L. collected from Çetibeli, showed no antimicrobial activity against the other microorganisms.

In the case of the MeOH extract of *M. communis* L. from Bozburun, the diameters of growth inhibition zones ranged from 7 to 12 mm, with the highest inhibition zone values observed against the medically important pathogens *S. aureus* (12 mm at 10% concentrations) and *E. coli* (8 mm for all concentrations). As can be seen in Table 4 all extracts from Yeşil Belde did not exhibit antimicrobial activity against *P. aeruginosa.* According to the results of this study, *S. aureus* (ranged from 14 to 16 mm) was the most sensitive one to all concentrations for YC and BC. All extracts of M. communis L. samples from Yeşil Belde showed no activity against *P. aeruginosa* at all concentrations.

As a result of the present study, all samples from different regions, showed significant activity against *S. aureus*, weak activity against *P. aeruginosa.* The least active region against *E. coli* was Yeşil Belde.

The free radical scavenging activity of the methanolic extracts of Myrtus communis L. tested were determined through the DPPH method and results are presented in Table 5. DPPH is a useful reagent for investigating the free radical scavenging activities of compounds. In the DPPH test, the extracts were able to reduce the stable radical DPPH to the yellow coloured diphenylpicrylhydrazine. The method is based on the reduction of an alcoholic DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH– H by the reaction (44).

The methanolic extracts of Myrtus berries collected from Bozburun and Çetibeli (IC_{50}=1.22 mg/ml) showed slightly lower scavenging ability on DPPH radicals than the methanolic extract of Yeşil Belde Myrtus berries (IC_{50}= 1.24). However, when compared to quercetin (IC_{50}= 0.007), all the tested extracts showed significantly lower antioxidant activity.

Gortzi et all.(8, 45), has also studied the methanolic extract of *M. communis* leaves for its antimicrobial activity, they have found 14 mm diameter against *S. aureus* (14 mm) and 12 mm diameter against *P. aeruginosa, K. pneumoniae, E. coli.* We found also same result for n-hexane extracts of M. communis L. berries from Bozburun and Çetibeli.

Cherrat et all. (9), have reported that *S. aureus* (24.2 mm) and *Escherichia coli* (7.4-10.8 mm) for essential oil of *M. communis* L. leaves whereas our n-hexane extracts have less active against *S. aureus* (8-14 mm) and they have similar activity for *Escherichia coli* (7-10 mm).

Messaoud et all (46) have studied antioxidant activity of mature dark blue and white berries from two Tunisian Myrtus communis. They obtained their essential oil and they made GC and GC/MS analyses. The total phenol, flavonoid, and flavonol contents and the concentration of the eight anthocyanins, identified by HPLC analysis, were significantly higher in the dark blue fruits. All extracts showed a substantial antioxidant activity, assessed by the free radical scavenging activity and the ferric reducing power, with the dark blue fruit extracts being more effective which we reported our methanol extract of *M. communis* L. berries exhibited strong DPPH scavenging activity, with IC50 values of 1.22 mg/mL.

Table 5: The antioxidant activity (mg/ml) of the MeOH extracts of three *M.communis* L. samples from different regions

Extracts	MeOH			Control
Plant Samples	Bozburun (BM)	Çetibeli (ÇM)	Yeşil Belde (YM)	Quercetin
IC_{50} (mg/ml)	1.22	1.22	1.24	0.007

As for as our literature survey, we could reach a report that showed that the IC_{50} values of the methanol extract of myrtle fruit, sampled from Tunisia, was 2.1 mg/ml, (46) which supported our results.

Previous studies on the antibacterial and antioxidant activity of *M. communis* L. involved its' leaves, berries, seeds, essential oil, flower. However, it is difficult to compare the results of different studies on *M. communis* L. Because our samples have been collected from Marmaris-Mugla.

We hope that our results will provide a starting point for the investigations to exploit new natural food additive and ingredient substances present in the extracts of the plant studied.

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