OKU Fen Bilimleri Enstitüsü Dergisi
6(Ek Sayı): 467-485, 2023

OKU Journal of The Institute of Science and Technology, 6(Special Issue):467-485, 2023

Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi

Osmaniye Korkut Ata University Journal of The Institute of Science and Technology

Antimicrobial and Antioxidant Activities of Some Maquis Species from Mersin, Turkey

Oskay KAHRAMAN[1], Ersan TURUNC[2], Deniz ALKAYA[3], Aylin DÖGEN[4], Rıza BINZET[5*]

[1,5] Department of Biology, Faculty of Science, Mersin University, 33343, Mersin, Turkey
[2] Department of Chemistry and Chemical Processing Technologies, Technical Science Vocational School, Mersin University, Mersin, 33343, Turkey
[3,4] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Mersin, Mersin, Turkey

[1]http://orcid.org/0000-0002-0904-7396
[2]http://orcid.org/0000-0001-6412-9020
[3]http://orcid.org/0000-0001-8580-4152
[4]http://orcid.org/0000-0002-0388-306X
[5]http://orcid.org/0000-0003-0336-8305
*Corresponding author: rbinzet@mersin.edu.tr

Research Article

Article History:
Received: 28.04.2023
Accepted: 11.09.2023
Published online: 20.12.2023

Keywords:
Antioxidant
Antimicrobial activity
Maquis element
Extract
Mersin

ABSTRACT

In this paper, we have investigated the antimicrobial and antioxidant activity of some maquis species from Mersin vil., Turkey. Extracts of *Cistus creticus* L., *Erica manipuliflora* L., *Myrtus communis* L., *Pistacia terebinthus* L. and *Rosmarinus officinalis* L., species, which are the elements of the scrub vegetation, were obtained by using hexane, methanol and petroleum ether solvents, respectively, and the antioxidant and antimicrobial properties of these extracts were determined. The plant samples showed different antimicrobial and antioxidant activity. Specifically, *M. communis* extracts have more effective antimicrobial activity against Gram (+) bacteria species. *M. communis* methanolic extract showed the MIC value of 31.25 µg/mL against *S. aureus*, *S. pneumoniae* and *E. faecalis*. *M. communis* hexane extract showed that the MIC value of 31.25 µg/mL against *E. faecalis*. In the current study, the petroleum ether extract of *M. communis* showed the highest antifungal effect against *C. parapsilosis* with a MIC value of 31.25 µg/mL. The highest and lowest DPPH radical scavenging activity were calculated as 91.25% and 63.3% in the petroleum ether extract of *P. terebinthus* and in the methanolic extract of *C. creticus*, respectively. Furthermore, the highest and lowest hydrogen peroxide (H_2O_2) scavenging activity were found to be 78.87% and 45.8% in the methanolic extract of *M. communis* and in hexane extract of *C. creticus*, respectively.

Mersin, Türkiye'den Bazı Maki Türlerinin Antimikrobiyal ve Antioksidan Aktiviteleri

Araştırma Makalesi

Makale Tarihçesi:
Geliş tarihi: 28.04.2023
Kabul tarihi:11.09.2023
Online Yayınlanma: 20.12.2023

Anahtar Kelimeler:
Antioksidan
Antimicrobiyal aktivite
Maki elemanı
Özüt
Mersin

ÖZ

Bu çalışmada, Mersin, Türkiye'den bazı maki türlerinin antimikrobiyal ve antioksidan aktivitelerini araştırdık. Maki bitki örtüsünün elemanlarından olan *Cistus creticus* L., *Erica manipuliflora* L., *Myrtus communis* L., *Pistacia terebinthus* L. ve *Rosmarinus officinalis* L. türlerinin sırasıyla hekzan, metanol ve petrol eteri çözücüleri kullanılarak özütleri elde edilmiştir. Bitki örnekleri farklı antimikrobiyal ve antioksidan aktivite göstermiştir. Spesifik olarak, *M. communis* özütleri, Gram (+) bakteri türlerine karşı daha etkili antimikrobiyal aktiviteye sahiptir. *M. communis* metanolik ekstraktı, *S. aureus*, *S. pneumoniae* ve *E. faecalis*'e karşı 31,25 µg/mL MİK değeri

gösterdi. *M. communis'*in hekzan özütü, *E. faecalis*'e karşı MİK değerinin 31,25 µg/mL olduğu belirlenmiştir. Mevcut çalışmada, *M. communis*'in petrol eteri özütü, 31,25 µg/mL MİK değeri ile *C. parapsilosis*'e karşı en yüksek antifungal etkiyi göstermiştir. En yüksek ve en düşük DPPH radikal yakalama aktivitesi sırasıyla *P. terebinthus*'un petrol eteri özütünde %91,25 ve *C. creticus*'un metanolik özütünde %63,3 olarak hesaplanmıştır. Ayrıca, en yüksek ve en düşük hidrojen peroksit (H_2O_2) süpürme aktivitesi sırasıyla *M. communis*'in metanollü özütünde %78,87 ve *C. creticus*'un hekzan özütünde %45,8 olarak bulunmuştur.

To Cite: Kahraman O., Turunc E., Aktaş D., Dögen A., Binzet R. Antimicrobial and Antioxidant Activities of Some Maquis Species from Mersin, Turkey. Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi 2023; 6(Ek Sayı): 467-485.

Introduction

Resistance to antimicrobial drugs is one of the most important health problems today. Infections of bacterial origin are a complex problem to solve due to multidrug resistance of microbial pathogens. This multidrug resistance applies not only to bacteria but also to other microorganisms such as fungi and viruses. The rapid development of resistance to antibacterial, antiviral and antifungals has prompted scientists to seek new phytocompounds with antimicrobial action (Levy and Marshall, 2004; Leal et al., 2021). However, the undesirable side effects of some antibiotics and the emergence of rare infections, it has forced scientists to seek new antimicrobial agents from various complementary sources such as herbs. Phytocomponents obtained from plant extracts showed that plants are a potential source of new anti-infective agents when viewed in terms of antimicrobial activity (Sharma et al., 2009; Elumalai et al., 2011; Peixoto et al., 2011).

Plants contain various phytochemicals like alkaloids, flavonoids, tannins, essential oils and these phytochemicals have might potential against multi-drug resistance bacterial diseases. Essential oils and phytochemicals have shown a wide spectrum of biological activity and are used in the food industry and medicine (Barchuk et al., 2021; Micić et al., 2021; Napoli and Di Vito, 2021; Nie et al., 2021; Aćimović et al., 2022; Leyane et al., 2022; Li and Xu, 2022).

Reactive oxygen species (ROS) including free radicals like superoxide ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$) etc. These free radicals are involved in various processes in the body, and oxidative stress occurs as a result of free radical excess. Oxidative stress has main role in the various disease such as Alzheimer's disorders, cancer, cardiovascular diseases, atherosclerosis, inflammation, hypertension and ageing (Qi et al., 2005; Conforti et al., 2008; Ighodaro and Akinloye, 2008). Therefore, it is very important to destroy these free radicals in the body or to keep them at a certain level. Antioxidant molecules can keep the balance of the free radical in the body or destroy free radicals. Several synthetic antioxidant molecules like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) are consumed to prevent oxidative stress-related diseases but they cause various adverse effects like side effects problems (Ndhlala et al., 2010; Lourenço et al., 2019). For instance, BHT and BHA cause hepatotoxicity and have been demonstrated to be carcinogenic in the studies carried out. Also, synthetic antioxidants are difficult and expensive to obtain this limits their use. Therefore, it is very important to use alternative antioxidants that are cheap, easy to access and have no side effects instead

of synthetic antioxidants. Due to the plants containing active biomolecules such as flavonoids, phenolic compounds, alkaloids have the effective potential antioxidant molecules. It is known that the antioxidants properties of plants play a protective role in the body against wide spectrum diseases like the risk of cancer, heart disease, hypertension, dementia, and stroke (Havsteen, 2002; Hodgson and Croft, 2010; Szymanska et al., 2016). In the literature review, it was stated that phytochemicals showed antioxidant and antimicrobial activities (Ivanović et al., 2021; Mohammed et al., 2021; Idris and Mohd Nadzir, 2021; Mokhtar et al., 2021; Vidana Gamage et al., 2021; Malada et al., 2022; Mohammed et al., 2022; Paudel et al., 2022; Tavan et al., 2022).

All taxa examined within the scope of this study are members of the maquis vegetation and were collected from the same region.

Cistus creticus L. belongs to the Cistaceae family. *C. creticus* is generally distributed in the Mediterranean phytogeographical region (Davis et al., 1965). The species of *Cistus* genus have been utilized for folk medicine (Baytop, 1999; Polat and Satıl, 2012; Sargın et al., 2013; Sargın et al., 2014).

Erica manipuliflora L. belongs to the Ericaceae family. This family includes more than 800 species around the world especially in the coastal sea of the Mediterranean. *E. manipuliflora* is more widespread in Southwest Anatolia. The people of Turkey have used it for medical purposes for a long time (Akkol et al., 2008; Mitic et al., 2018; Kuş et al., 2019).

Myrtus communis L. is aromatic and economical species that belongs to the Myrtaceae family (Hennia et al., 2019). *M. communis* is known as Myrtle. Myrtle is largely distributed in tropical and subtropical areas in the world. This species has solitary axillary white or rosy flowers, followed by spherical and dark red to violet berries. The fruits are dark and white in color and are widely consumed as snacks in Turkey (Aleksic and Knezevic, 2014).

Pistacia terebinthus L. is an economically valuable species belonging to the Anacardiaceae family. This species is commonly known as terebinth or turpentine tree. *P. terebinthus* is native to the Mediterranean phytogeographic region and is distributed in arid areas, forest clearings and calcareous rocky areas (Tutin et al., 1968). *P. terebinthus* is widely consumed in traditional medicine, coffee and food in Turkey.

Rosmarinus officinalis L. is a medicinal and aromatic plant and belongs to the Lamiaceae family. This species is known as Rosemary in Turkey. One of the natural elements of maquis vegetation, this species is an evergreen shrub native to the Mediterranean region. Due to this feature, it is widely used as a hedge plant in Mediterranean coastal cities in Turkey. In addition to its medicinal and cosmetic use all over the world, it is also consumed as a spice and tea (González-Trujano et al., 2007; Özcan and Chalchat, 2008).

The natural maquis vegetation in Mersin University Çiftlikköy campus is being destroyed due to building and anthropogenic interventions, and it is predicted that it will disappear in a very short time if necessary precautions are not taken. Considering this situation, in this study, it was aimed to prepare

various extracts of five different natural maquis species distributed in the same soil properties and at the same altitude and to determine the antioxidant and antimicrobial properties of these extracts.

Material and Methods

Chemicals

All chemicals are of analytical grade. 2,2-diphenyl-1-picryl hydrazyl (DPPH), and hydrogen peroxide (H_2O_2) were purchased from Merck. Hexane, methanol and petroleum ether were purchased from Sigma Aldrich.

Plant Materials

In this study, *C. creticus* belonging to the Cistaceae family, *E. manipuliflora* belonging to the Ericaceae family, *M. communis* belonging to the Myrtaceae family, *P. terebinthus* belonging to the Anacardiaceae family and, *R. officinalis* belonging to the Lamiaceae family, which are naturally distributed in Ciftlikköy Campus of Mersin University, were collected (Turkey C5: Mersin, Southern of Mersin, Mersin University, Ciftlikköy Campus, 36°47'23"N 34°31'11"E, 90-110 m, 15 v 2021, Binzet 202141-202145). The collected samples were identified by Rıza Binzet using the flora of Turkey (Riedl, 1978) and a doublet of the identified samples was taken under protection in Mersin University herbarium (MERA) (Fig. 1).

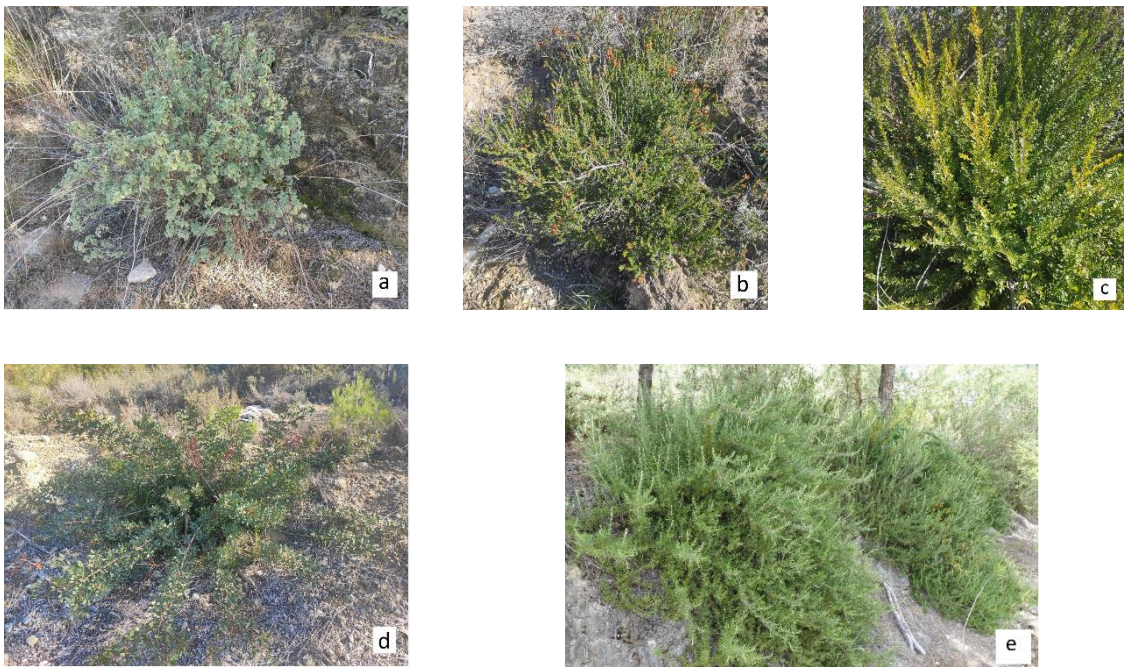


Fig. 1. The general habitus of examined species. a) *C. creticus*, b) *E. manipuliflora*, c) *M. communis*, d) *P. terebinthus*, e) *R. officinalis*

Preparation of Plant Samples

Five different plant samples collected from the natural/disturbed maquis areas of Mersin University campus were washed with tap water and left to dry in the shaded area for about a month. Then, the leaves of the dried samples were removed and the dried leaves were ground with the help of a mill (Blender 8011ES Model HGB2WTS3 400 W). The powdered samples were taken into dark colored bottles to be used in extraction and stored at room temperature.

Extraction

Each plant powders (1 gr) were mixed with 50 mL of different solvents (methanol, petroleum ether and hexane) in the sterile bottles and the mixtures were stirred at 37 °C for 24 hours. After the 24 hours, the extracts were filtered using filter paper (0.11 μm) and organic solvents were evaporated with the help of rotary evaporator. The obtained plant extract samples were stored at 4 °C for analysis.

Antimicrobial Activity

The antimicrobial susceptibility test was evaluated using the modification microdilution broth method (Albayrak et al., 2022). Six reference bacterial strains and two fungal strains were used: *Staphylococcus aureus* (ATCC 29213), *Streptococcus pneumoniae* (ATCC 49619), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 1951), *Pseudomonas aeruginosa* (ATCC 27853), *Candida metapsilosis* (CBS 2916), *Candida parapsilosis* (CBS 2916). The fungal and bacterial cell inoculum were prepared from the stock culture grown in Tryptic Soy Agar (TSA, Merck, Darmstadt, Germany) at 28 °C for 24 h and Mueller-Hinton Agar (MHA, Merck, Darmstadt, Germany) 37 °C for 24 h, respectively. The microorganisms suspension concentrations were adjusted according to McFarland 0.5 turbidity tubes using sterilized saline. Stock solution of plant extracts and reference drugs were prepared in DMSO at 1000 μg/mL. A modified microdilution test was applied for antimicrobial activity and the experiments were run in duplicates independently.

To determine antimicrobial activity tests, 100 μL of tryptic soy broth and Mueller-Hinton water were added to each well for fungi and bacteria, respectively. A 100 μL aliquot of the plant extracts solution was added to the first well, and two-fold dilutions were prepared for subsequent wells. Then, 5 μL of fungal or bacterial suspension was added to each well except the last ones, which acted as control wells for each group. Control tubes containing 5 μL of each of the fungal or bacterial suspensions alone without the plant extracts solution were also prepared. All plates were incubated at 28 °C (for fungi) and at 37 °C (for bacteria) for 24 h. After the incubation, the minimal inhibitory concentrations (MIC) were noted by controlling the growth inhibition for plant extracts. Fluconazole and ampicillin were used as reference drug. The results were read visually and by measuring optical density for 24 h.

Antioxidant Activity

DPPH Radical Scavenging Assay

The radical scavenging activity of the samples was investigated method by used Mensor et al with using some modifications (Mensor et al., 2001). 3 mL of 0.1 mM solution of DPPH (in methanol) was added to 1 mL (100 µg/mL - 1000 µg/mL) extract or standard in sterile vials and allowed to react in the dark at room temperature for 30 min. The absorbance was measured against a corresponding blank at 517 nm. Methanol (1 mL) plus extract solution (0.5 mL) was used as the blank. Ascorbic acid was used as a positive control. All measurements were done in triplicate. All solutions were freshly prepared. Inhibition (%) of free radical DPPH was calculated as in Equation 1.

Inhibition (%) = [(Ac – As)/Ac]*100 **Eq. 1**

Ac and As are the absorbances of the control and solutions, respectively.

H_2O_2 scavenging assay

The ability of the samples to the H_2O_2 scavenge was determined by modification to the method of Ruch et al. (1989). 1 mL of extract samples (100-400 µg/mL) was transferred into the bottles and their volumes were made up to 2 mL with 40 mM phosphate buffer solution (pH 7.4) followed by the additions of 0.6 mL of H_2O_2 solutions (40 mM). The reaction mixture was stirred for a few minutes and after 10 min of incubation time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. Phosphate buffers were used as blank. All measurements were done in triplicate. All solutions wereas freshly prepared. The ability of the extracts to scavenge the H_2O_2 was calculated using the Equation 1.

Results and Discussion

Extraction Yield

The percentage of extraction yield was calculated by following equations.

Yield of extract (%) = (R/S)*100 **Eq. 2**

R refers to the dried samples weight after to the evaporation of solvent, and S: refers to the dry weight before extraction process (raw plant powder).

In this study, the highest extraction yield in methanol extract was obtained in *R. officinalis*, while the lowest extraction yield was obtained in hexane extract of *P. terebinthus* (Table 1).

Table 1. Extract yield percentage of examined samples

Samples	Solvent	Extract Yield (%)
	Methanol	1.63
C. creticus	Petroleum ether	1.70
	Hexane	1.29
	Methanol	1.38
E. manipuliflora	Petroleum ether	1.06
	Hexane	1.32
	Methanol	1.74
M. communis	Petroleum ether	1.29
	Hexane	1.64
	Methanol	1.94
P. terebinthus	Petroleum ether	1.56
	Hexane	1.02
	Methanol	1.94
R. officinalis	Petroleum ether	1.56
	Hexane	1.02

Antimicrobial Activity

MIC values of different solvent plant extract of *C. creticus, E. maniupuiliflora*, *M. communis, P. terebinthus* and *R. officinalis* displayed strong antimicrobial activity against Gram (+) and Gram (-) bacteria and fungal species (Table 2.).

In this study, especially the methanol and petroleum ether extract of *M. communis* showed more antimicrobial effect against Gram (+) bacteria than Gram (-) bacteria strains. Results of this study showed that the *M. communis* extracts more effective antimicrobial activity against Gram (+) bacteria species. *M. communis* methanolic extract showed the MIC value at 31.25 µg/mL against *S. aureus, S. pneumoniae, E. faecalis.*

Candida species are among the important factors of invasive fungal diseases. In intensive care units, *C. parapsilosis* takes the second or third place as a candidemia agent. The second most common cause of candidemia in our country is *C. parapsilosis*, and its incidence is increasing day by day. *C. parapsilosis* species complex includes *C. parapsilosis, C. metapsilosis* species. Our results show, petroleum ether extract of *M. communis* showed the MIC value at 31.25 µg/mL against *C. parapsilosis*. Also, *M. communis* petroleum ether extract showed MIC value at 62.5 µg/mL against *S. aureus, S. pneumoniae, E. facealis. M. communis* hexane extract showed a MIC value of 31.25 µg/mL against *E. faecalis. E. manipuiliflora* hexane extract showed MIC value at 62.5 µg/mL against *S. pneumoniae E. faecalis* and *E. coli.* The methanolic extract of *C. creticus, R. officinalis and P. terebinthus* showed MIC value at 62.5 µg/mL against *C. metapsilosis and C. parapsilosis*. Methanolic and petroleum ether extract of *P. terebinthus* and *C. creticus* showed the MIC value at 62.5 µg/mL against *C. parapsilosis*. Also, all plant samples (extracts) showed the MIC values at 125 µg/mL against *K. pneumoniae* strains (Table 2).

The previous studies reported that the MIC values below 100 µg/mL correspond to strong antimicrobial properties for plant extracts and the MIC values between 100 µg/mL and 625 µg/mL correspond to moderate antimicrobial activity (Kuete, 2010). The plant extracts used in this study showed different inhibition effects against bacterial and fungal species (Table 2). In this study, methanolic and petroleum ether extract of *M. communis* and hexane extract of *E. maniupuiliflora* showed strong inhibition against antibacterial activities. In addition, in the results of the study, it was observed that all plant extract samples showed a strong inhibition against *Candida* species, which is an important fungal pathogen.

Table 2. MIC values (µg/mL) of different extracts and standard drugs Ampicillin and Fluconazole against selected bacteria and fungi

	Gram(+) bacteria			**Gram(-) bacteria**			**Fungal species**	
Samples	*S. aureus*	*S. pneumoniae*	*E. faecalis*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*C. metapsilosis*	*C. parapsilosis*
C. creticus (Methanol)	125	125	125	125	125	125	**62.5**	**62.5**
C. creticus (P. ether)	250	250	125	125	125	125	125	125
C. creticus (Hexane)	250	250	125	125	125	125	125	**62.5**
E. manipuliflora (Methanol)	250	125	125	125	250	125	125	125
E. manipuliflora (P. ether)	250	250	125	125	125	125	125	125
E. manipuliflora (Hexane)	125	**62.5**	**62.5**	**62.5**	125	125	125	125
M. communis (Methanol)	**31.25**	**31.25**	**31.25**	125	125	125	125	125
M. communis (P. ether)	**62.5**	**62.5**	**62.5**	125	125	125	125	**31.25**
M. communis (Hexane)	125	125	**31.25**	125	125	125	125	125
P. terebinthus (Methanol)	125	125	250	125	125	125	**62.5**	**62.5**
P. terebinthus (P. ether)	125	250	250	125	125	125	125	**62.5**
P. terebinthus (Hexane)	125	250	125	125	125	125	125	125
R. officinalis (Methanol)	125	125	125	125	125	125	**62.5**	125

R. officinalis (P. ether)	125	250	250	125	125	125	125	125
R. officinalis (Hexane)	125	125	125	125	125	125	125	125
Fluconazole	*	*	*	*	*	*	-	-
Ampicillin	-	-	-	**31.25**	**31.25**	**31.25**	*	*

(–) All tested concentrations are active; * Not tested; Bold numbers are more effective

Antioxidant activity

DPPH radical scavenging assay activity

Phytochemicals found in plants can reduce or prevent the harmful effects of oxidative stress. Phytochemicals in plants show free radical scavenging properties.

DPPH is a stable nitrogen-centered free radical commonly used for testing radical scavenging activity of the compound or plant extracts. When the stable DPPH radical accepts an electron from the antioxidant compound, the purple color of the DPPH radical was transformed to light yellow or colorless (Fig. 2) and DPPH structure transform to the non-stabile DPPH-H structure (Scheme 1).

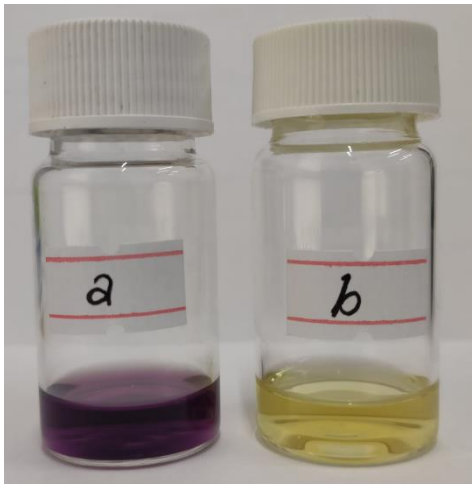
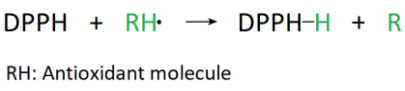


Fig. 2. DPPH radical (a) and after the incubation of DPPH radical with plant extract (b)



Scheme 1. The chemical transformation mechanism of DPPH with antioxidant molecules.

In our study, different antioxidant activities were obtained depending on the types of phytochemicals released during the extraction process. Hydrogen or electron donating properties of phytochemicals released as a result of extraction are directly related to their antioxidant properties.

In this work, we have evaluated the free radical scavenger activity of prepared extracts with different solvents of *C. creticus*, *E. manipuliflora*, *M. communis*, *P. terebinthus* and *R. officinalis*. According to

the results of the DPPH radical scavenging assay, different plant extracts have shown different free radical scavenging activity at the highest concentration (1000 µg/mL) (Fig. 3). According to the result of our study, the highest DPPH radical scavenging activity was observed as 91.25% in the petroleum ether extract of *P. terebinthus.* Furthermore, the lowest DPPH radical scavenging activity was observed as 63.30% in the methanolic extract of *C. creticus.* Ascorbic acid was used as a positive control and showed 92.25% inhibition at DPPH radical scavenging activity.

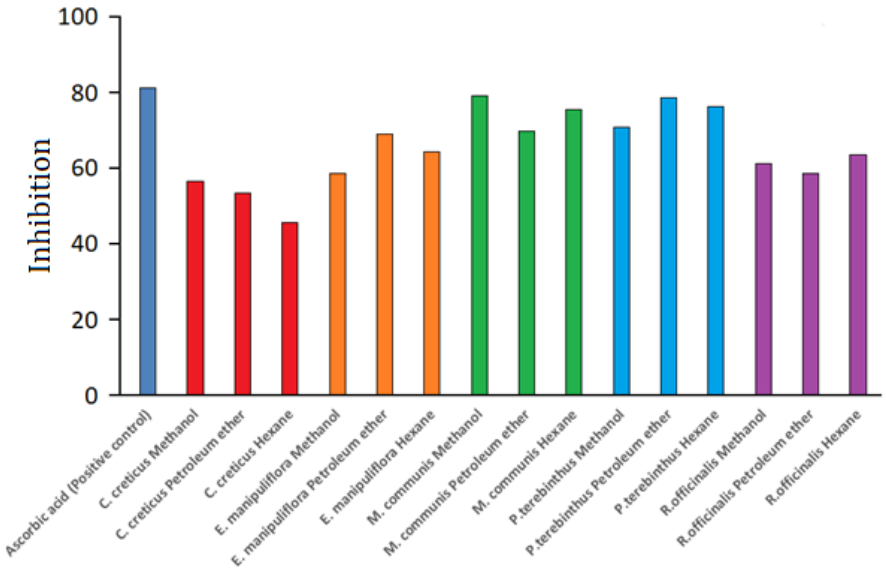


Fig. 3. The DPPH scavenging assay of different plan samples

H_2O_2 scavenging assay activity

H_2O_2 has oxidizing properties. It can penetrate membranes and may oxidize a various compounds (Gülçin et al., 2010). In addition, it can be harmful to the cell as it can transform into hydroxyl radicals with toxic potential in cells. Therefore, it is important to destroy H_2O_2 from cell metabolism. Plants are good electron donors because of the phytochemicals they contain. Phytochemicals donates electrons to H_2O_2, and neutralize to H_2O (Ebrahimzadeh et al., 2009) (Scheme 2).

Scheme 2. Convert the H_2O_2 to H_2O

Fig. 4 shows the H_2O_2 scavenging activity of different plant extract samples at the highest concentration (400 µg/mL). As a result of our study, the highest H_2O_2 scavenging activity was observed at 78.87% in the methanolic extract of *M. communis*. The lowest H_2O_2 scavenging activity was observed in hexane extract of *C. creticus* with 45.80%.

The IC_{50} values of plant extract samples showed at Table 3. According to the DPPH test results, the highest IC_{50} value of 262.34 µg/mL was observed in the methanolic extract of *C. creticus*, while the

lowest IC_{50} value was 71.14 µg/mL in the petroleum ether extract of *P. terebinthus*. The IC_{50} value of the petroleum ether extract of the *P. terebinthus* in the antioxidant activity was 71.14 µg/mL and it was found very close to the ascorbic acid value of 70.66 µg/mL. Also, according to the H_2O_2 scavenging assay test results the highest IC_{50} value at 247.21 µg/mL was observed in the hexane extract of *C. creticus*, the lowest IC_{50} value was observed in the petroleum ether extract of *P. terebinthus* with 152.1 µg/mL.

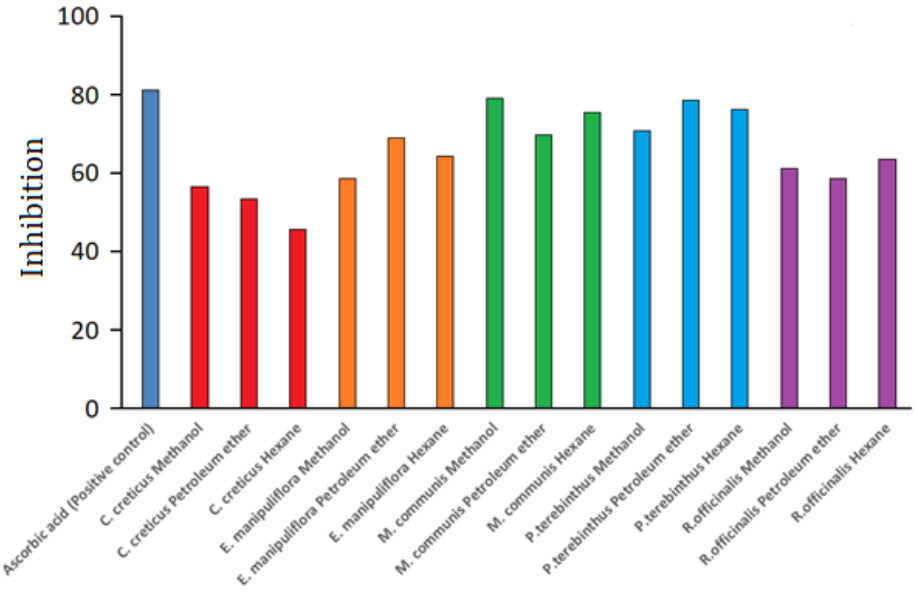


Fig. 4. The H_2O_2 scavenging assay of different plant samples

In our work, IC_{50} value of the all samples except *C. creticus* methanol extract observed to be in the range of 71.14 µg/mL - 86.16 µg/mL at DPPH radical scavenging assay (Table 3). Based on the IC_{50} values, all samples except *C. creticus* methanolic extract showed moderate DPPH activity.

Table 3. The IC_{50} values of antioxidant tests on the examined extract samples

Samples/Solvent	**IC_{50} value for DPPH Radical Scavenging Assay**	**IC_{50} value for H_2O_2 Radical Scavenging Assay**
C. creticus (Methanol)	262.34	214.44
C. creticus (Petroleum ether)	82.41	221.33
C. creticus (Hexane)	77.16	247.21
E. manipuliflora (Methanol)	74.68	176.34
E. manipuliflora (Petroleum ether)	80.14	189.93
E. manipuliflora (Hexane)	75.78	197.24
M. communis (Methanol)	73.22	154.33
M. communis (Petroleum ether)	75.64	161.20
M. communis (Hexane)	78.21	157.42
P. terebinthus (Methanol)	75.88	160.83

P. terebinthus (Petroleum ether)	71.14	152.10
P. terebinthus (Hexane)	83.05	154.45
R. officinalis (Methanol)	75.34	201.37
R. officinalis (Petroleum ether)	82.46	211.62
R. officinalis (Hexane)	86.16	199.05

Mastino et al. (2018) reported that the antimicrobial activity of the butanol and ethyl acetate extracts of *C. creticus* showed the MIC values at 500 μg/mL and 125 μg/mL against *E. coli* and *S. aureus* strains. In our study, we determined that all extracts of *C. creticus* had a range of 125 μg/mL against *E. coli* and 125 - 250 μg/mL against *S. aureus*. In the study by Owlia et al. (2009), essential oil of *M. communis* showed a MIC value of 64 μg/mL against *P. aeurouginosa*. A study by Amensour et al. (2010) reported that different solvent extracts of *M. communis* showed the MIC value at 625 μg/mL against *P. aeruginosa* and did not show inhibition against *S. aureus* and *E. coli*. Taheri et al. (2013) reported that the hydroalcoholic extract of *M. communis* showed antimicrobial activity against *S. aureus* and *E. coli* with MIC values at 200 μg/mL and 800 μg/mL. Besukefad et al. (2017) reported that hexane extract of *M. communis* showed MIC value of 31.25 μg/mL and 125 μg/mL against *E. coli* and *S. aureus*. In our study, all extracts of *M. communis* exhibited the MIC values of 125 μg/mL against *E. coli* and *P. aeruginosa* and a MIC value in the range of 31.25 - 125 μg/mL against *S. aureus.* A study by Kavak et al. (2010) reported that antimicrobial activity of *P. terebinthus* leaf extract displayed a MIC value of 1.56 mg/mL against *S. aureus,* and crude extract exhibited no antimicrobial activity on *E. coli*. In our study, all extracts of the *R. officinalis* exhibited a MIC value of 125 μg/mL against *E. coli* and *S. aureus*. Manilal et al. (2021) reported that antimicrobial activity of ethanolic extract of *R. officinalis* displayed MIC value at 4000 μg/mL for *S. aureus* and 8000 μg/mL for *E. coli* and *Salmonella* spp. Jarrar et al. (2010) reported that antimicrobial activity of the ethanolic extract of *R. officinalis* displayed 125 μg/mL against *S. aureus*. In this study, all extracts of the *R. officinalis* exhibited a MIC value of 125 μg/mL against *E. coli* and *S. aureus*.

Lachen et al. (2020) reported the antioxidant activity of *C. creticus* leaves content. It was stated that IC_{50} values of 12.53 μg/mL, 10.91 μg/mL, 26.64 μg/mL and 30.27 μg/mL correspond to flavonoids, saponin, crude extracts and alkaloids, respectively. Kilic et al. (2019) ethanol, dichloromethane (DCM) and hexane extractions of *C. creticus* leaves were studied and the IC_{50} values calculated for these were reported as 165.10 μg/mL, 189.71 μg/mL and 397.29 μg/mL, respectively. In our study, the IC_{50} values of all extracts of *C. creticus* were found to be in the range of 77.16 - 262.34 μg/mL. In another study, essential oils of *Myrtus* black and *Mrytus* white samples of *M. communis* species were obtained and the DPPH scavenging activities of these samples at 1000 μg/mL were determined as 95.26 ± 0.074 μg/mL and 92.80 ± 0.097 μg/mL, respectively (Ibrahim et al., 2021). In the study of Abdulhadi et al. (2020), the antioxidant activity of *M. communis* extract at 1000 μg/mL was found to be 90.17%. In our study, it was determined that the antioxidant activities of *M. communis* extracts

obtained in all three solvents at 1000 µg/mL ranged between 76.8% and 89.4%. Kadri et al. (2011) obtained the essential oils of the cultivated *R. officinalis* plant and determined that its antioxidant activity was 61.00 ± 2.30% DPPH radical scavenging effect at 300 µg/mL. In the same study, it was also reported that an IC_{50} inhibitor concentration of 110.20 µg/ml was obtained from the DPPH test. In this study, all extracts of *R. officinalis* exhibited DPPH scavenging activity in the range of 80.2 to 88.3% at 1000 µg/mL.

As a result, the reason for the significant differences between the antioxidant and antimicrobial activities data obtained in our study and other similar studies is thought to be the distribution areas of the plants, collection periods, different drying methods, edaphic properties, different solvents used in extraction and different methods.

Conclusion

The problem of drug resistance of bacteria and fungi is increasing and it is predicted that the use of antimicrobial drugs will pose a serious problem in the future. Therefore, it is important the uses new and alternative antimicrobial agents. Previously reported studies have shown that the extract of the medicinal plants are alternative antimicrobial agents against bacteria and fungi. In general, plant extracts show a strong inhibitory effect against *Candida* species. Especially, all extracts of *M. communis* showed stronger inhibition activity against Gram (+) bacteria than other plant extracts in our study. As a result, it can be suggested that some plant extracts handled in this study can be evaluated as antimicrobial agents against drug-resistant bacteria and fungi. In addition, the plant extracts used in our study have a high antioxidant potential, and therefore they may have the potential to be used in the possible elimination of free radicals in the body and the prevention of diseases such as cancer and cardiovascular disease. Moreover, the plants used in this study offer alternative antioxidant potentials compared to synthetic antioxidant substances that of are cheap, easily accessible and have no side effects.

Acknowledgement

We would like to thank Prof. Dr. Sybren de Hoog for providing the *Candida parapsilosis* and *Candida metapsilosis* strain used in this study (Radboud University, Medical Center, Nijmegen, Netherlands)

Disclosure statement Conflict of interests:

The authors declare that they have no conflict of interest.

Author contributions: All authors contributed equally to this work.

Ethical approval: All ethical guidelines have been adhered.

Sample availability: Samples of the species are available from the last author.

References

Abdulhadi SM., Shami AM., Saleh MM. Antibacterial effect, antioxidant potential and total phenolic content of polyphenol extracts of *Myrtus communis* leaves. Plant Archives 2020; 20(2): 286-291.

Aćimović MG., Cvetković MT., Stanković Jeremić JM., Pezo LL., Varga AO., Čabarkapa IS., Kiprovski B. Biological activity and profiling of *Salvia sclarea* essential oil obtained by steam and hydrodistillation extraction methods via chemometrics tools. Flavour and Fragrance Journal 2022; 37(1): 20-32. https://doi.org/10.1002/ffj.3684

Akkol KE., Yeşilada E., Güvenç A. Valuation of anti-inflammatory and antinociceptive activities of *Erica* species native to Turkey. Journal of Ethnopharmacology 2008; 116(2): 251-257. https://doi.org/10.1016/j.jep.2007.11.023

Albayrak F., Çiçek M., Alkaya D., Kulu I. Design, synthesis and biological evaluation of 8-aminoquinoline-1,2,3-triazole hybrid derivatives as potential antimicrobial agents. Medicinal Chemistry Research 2022; 31: 652-665. https://doi.org/10.1007/s00044-022-02866-2

Aleksic V., Knezevic P. Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L. Microbiological Research 2014; 169(4): 240-254. https://doi.org/10.1016/j.micres.2013.10.003

Amensour M., Bouhdid S., Fernández-Lopez J., Idaomar M., Senhaji NS., Abrini J. Antibacterial activity of extracts of *Myrtus communis* against food-borne pathogenic and spoilage bacteria. International Journal of Food Properties 2010; 13(6): 1215-1224. https://doi.org/10.1080/10942910903013399

Barchuk O., Pryshlyak A., Shanaida M. Chemical compositions and sedative activities of the *Dracocephalum maldavica* L. and *Ocimum americanum* L. essential oils. Pharmacology OnLine 2021; 2: 179-187.

Baytop T. Türkiye'de Bitkiler ile Tedavi (Geçmişte ve Bugün). İstanbul: Nobel Tıp Kitabevleri. Ankara, 1999; 480 p.

Besufekad SY., Mekdes M., Abebech M., Delesa D., Tekalign D., Demitu K., Birtukan B. The antimicrobial activity of leaf extracts of *Myrtus communis*. Journal of Microbial & Biochemical Technology 2017; 9(6): 290-292. https://doi.org/10.4172/1948-5948.1000380

Conforti F., Sosa S., Marrelli M., Menichini F., Statti GA., Uzunov D., Tubaro A., Menichini F., Loggia RD. In vivo anti-inflammatory and in vitro antioxidant activities of Mediterranean dietary plants. Journal of Ethnopharmacology 2008; 116(1): 144-151. https://doi.org/10.1016/j.jep.2007.11.015

Davis PH., Cullen J., Coode MJE. (Ed.). Cistaceae. Flora of Turkey and the East Aegean Islands, vol 1, Edinburgh University Press. Edinburgh, UK, 1965; 506p.

Ebrahimzadeh MA., Nabavi SF., Nabavi SM. Antioxidant activities of methanol extract of *Sambucus ebulus* L. flower. Pakistan Journal of Biological Sciences 2009; 12(5): 447-450. https://doi.org/10.3923/pjbs.2009.447.450

Elumalai EK., Ramachandran M., Thirumalai T., Vinothkumar P. Antibacterial activity of various leaf extracts of *Merremia emarginata*. Asian Pacific Journal of Tropical Biomedicine 2011; 1(5): 406-408 https://doi:10.1016/S2221-1691(11)60089-0

González-Trujano ME., Peña EI., Martínez AL., Moreno J., Guevara-Fefer P., Deciga-Campos M., López-Muñoz FJ. Evaluation of the antinociceptive effect of *Rosmarinus officinalis* L. using three different experimental models in rodents. Journal of Ethnopharmacology 2007; 111(3): 476-482. https://doi.org/10.1016/j.jep.2006.12.011

Gülçin İ., Huyut Z, Elmastaş M., Aboul-Enein HY. Radical scavenging and antioxidant activity of tannic acid. Arabian Journal of Chemistry 2010; 3(1): 43-53. https://doi.org/10.1016/j.arabjc.2009.12.008

Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics 2002: 96(2-3): 67-202. https://doi.org/10.1016/S0163-7258(02)00298-X.

Hennia A., Nemmiche S., Dandlen S., Miguel MG. *Myrtus communis* essential oils: insecticidal, antioxidant and antimicrobial activities: a review. Journal of Essential Oil Research 2019; 31(6): 487-545. https://doi.org/10.1080/10412905.2019.1611672

Hodgson JM., Croft KD. Tea flavonoids and cardiovascular health. Molecular Aspects of Medicine 2010; 31(6): 495-502. https://doi.org/10.1016/j.mam.2010.09.004

Ibrahim FM., Fouad R., EL-Hallouty S., Hendawy SF., Omer EA., Mohammed RS. Egyptian *Myrtus communis* L. essential oil potential role as invitro antioxidant, cytotoxic and α-amylase inhibitor. Egyptian Journal of Chemistry 2021; 64(6): 3005-3017. https://doi.org/10.21608/EJCHEM.2021.57354.3245

Idris FN., Mohd Nadzir M. Comparative studies on different extraction methods of *Centella asiatica* and extracts bioactive compounds effects on antimicrobial activities. Antibiotics 2021; 10(4): 457. https://doi.org/10.3390/antibiotics10040457

Ighodaro OM., Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine 2018; 54(4): 287-293. https://doi.org/10.1016/j.ajme.2017.09.001

Ivanović M., Makoter K., Islamčević Razboršek M. Comparative study of chemical composition and antioxidant activity of essential oils and crude extracts of four characteristic Zingiberaceae herbs. Plants 2021; 10(3): 501. https://doi.org/10.3390/plants10030501

Jarrar N., Abu-Hijleh A., Adwan K. Antibacterial activity of *Rosmarinus officinalis* L. alone and in combination with cefuroxime against methicillin–resistant *Staphylococcus aureus*. Asian Pacific

Journal of Tropical Medicine 2010; 3(2):121-123. https://doi.org/10.1016/S1995-7645(10)60049-1

Kadri A., Zarai Z., Chobba IB., Békir A., Gharsallah N., Damak M., Gdoura R. Chemical constituents and antioxidant properties of *Rosmarinus officinalis* L. essential oil cultivated from the South-Western of Tunisia. Journal of Medicinal Plants Research 2011; 5(29): 6502-6508. https://doi.org/10.5897/JMPR11.423

Kavak DD., Altıok E., Bayraktar O., Ülkü S. *Pistacia terebinthus* extract: As a potential antioxidant, antimicrobial and possible β-glucuronidase inhibitor. Journal of Molecular Catalysis B: Enzymatic 2010; 64(3-4): 167-171. https://doi.org/10.1016/j.molcatb.2010.01.029

Kilic DD., Siriken B., Erturk O., Tanrikulu G., Gül M., Başkan C. Antibacterial, antioxidant and DNA interaction properties of *Cistus creticus* L. extracts. Journal of International Environmental Application and Science 2019; 14(3): 110-115.

Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Medica 2010; 76(14): 1479-1491. https://doi.org/10.1055/s-0030-1250027

Kuş C., Taş M., Küçükaydın S., Tel-Çayan G., Duru ME. Chemical analysis and in vitro antioxidant and anticholinesterase activities of essential oils and extracts from different parts of *Erica manipuliflora*. Journal of Research in Pharmacy 2019; 23(6): 1098-1105. https://doi.org/10.35333/jrp.2019.74

Lahcen SA., El Hattabi L., Benkaddour R., Chahboun N., Ghanmi M., Satrani B., Tabyaoui M., Zarrouk A. Chemical composition, antioxidant, antimicrobial and antifungal activity of Moroccan *Cistus creticus* leaves. Chemical Data Collections 2020; 26: 100346. https://doi.org/10.1016/j.cdc.2020.100346

Leal ALAB., da Silva PT., da Rocha MN., Marinho EM., Marinho ES., Marinho MM., Bandeira PN., Nogueira CES., Barreto HM., Teixeira AMR., dos Santos HS. Potentiating activity of Norfloxacin by synthetic chalcones against NorA overproducing *Staphylococcus aureus*. Microbial Pathogenesis 2021; 155: 104894 https://doi.org/10.1016/j.micpath.2021.104894

Levy SB., Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine 2004; 10(12): S122-S129. https://doi:10.1038/nm1145

Leyane TS., Jere SW., Houreld NN. Oxidative stress in ageing and chronic degenerative pathologies: molecular mechanisms involved in counteracting oxidative stress and chronic inflammation. International Journal of Molecular Sciences 2022; 23(13): 7273. https://doi.org/10.3390/ijms23137273

Li J., Xu S. Tilianin attenuates MPP^+-induced oxidative stress and apoptosis of dopaminergic neurons in a cellular model of Parkinson's disease. Experimental and Therapeutic Medicine 2022; 23(4): 293. https://doi.org/10.3892/etm.2022.11223

Lourenço SC., Moldão-Martins M., Alves VD. Antioxidants of natural plant origins: From sources to food industry applications. Molecules 2019; 24(22): 4132. https://doi:10.3390/molecules24224132

Malada PM., Mogashoa MM., Masoko P. The evaluation of cytotoxic effects, antimicrobial activity, antioxidant activity and combination effect of *Viscum rotundifolium* and *Mystroxylon aethiopicum*. South African Journal of Botany 2022; 147: 790-798. https://doi.org/10.1016/j.sajb.2022.03.025

Manilal A., Sabu KR., Woldemariam M., Aklilu A., Biresaw G., Yohanes T., Seid M., Merdekios B. Antibacterial activity of *Rosmarinus officinalis* against multidrug-resistant clinical isolates and meat-borne pathogens. Evidence-Based Complementary and Alternative Medicine 2021; 2021: 6677420. https://doi.org/10.1155/2021/6677420

Mastino PM., Mauro M., Jean C., Juliano C., Marianna U. Analysis and potential antimicrobial activity of phenolic compounds in the extracts of *Cistus creticus* subspecies from Sardinia. The Natural Products Journal 2018; 8(3): 166-174. https://doi.org/10.2174/2210315508666180327151318

Mensor LL., Menezes FS., Leitão GG., Reis AS., dos Santos TC., Coube CS., Leitão SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research 2001; 15(2): 127-130. https://doi.org/10.1002/ptr.687

Micić D., Đurović S., Riabov P., Tomić A., Šovljanski O., Filip S., Tosti T., Dojčinović D., Božović R., Jovanović D., Blagojević S. Rosemary essential oils as a promising source of bioactive compounds: chemical composition, thermal properties, biological activity, and gastronomical perspectives. Foods 2021; 10(11): 2734. https://doi.org/10.3390/foods10112734

Mitic VD., Ilic MD., Stankov-Jovanovic VP., Stojanovic GS., Dimitrijevic MV. Essential oil composition of *Erica spiculifolia* Salisb-first report. Natural Product Research 2018; 32(2): 222-224. https://doi.org/10.1080/14786419.2017.1340287

Mohammed FS., Kına E., Sevindik M., Doğan M., Pehlivan M. Antioxidant and antimicrobial activities of ethanol extract of *Helianthemum salicifolium* (Cistaceae). Indian Journal of Natural Products and Resources 2021; 12(3): 459-462. https://doi.org 10.56042/ijnpr.v12i3.46635

Mohammed FS., Kına E., Uysal İ., Mencik K., Dogan M., Pehlivan M., Sevindik M. Antioxidant and antimicrobial activities of ethanol extract of *Lepidium spinosum*. Turkish Journal of Agriculture – Food Science and Technology 2022: 10(6): 1116-1119. https://doi.org/10.24925/turjaf.v10i6.1116-1119.5207

Mokhtar M., Bouamar S., Di Lorenzo A., Temporini C., Daglia M., Riazi A. The influence of ripeness on the phenolic content, antioxidant and antimicrobial activities of pumpkins (*Cucurbita moschata* Duchesne). Molecules 2021; 26(12): 3623. https://doi.org/10.3390/molecules26123623

Napoli E., Di Vito M. Toward a new future for essential oils. Antibiotics (2021) 10(2): 207.

Ndhlala AR., Moyo M., Van Staden J. Natural antioxidants: fascinating or mythical biomolecules? Molecules 2010; 15(10): 6905-6930. https://doi:10.3390/molecules15106905

Nie L., Li R., Huang J., Wang L., Ma M., Huang C., Wu T., Yan R., Hu X. Abietane diterpenoids from *Dracocephalum moldavica* L. and their anti-inflammatory activities in vitro. Phytochemistry 2021; 184: 112680. https://doi.org/10.1016/j.phytochem.2021.112680

Owlia P., Saderi H., Rasooli I., Sefidkon F. Antimicrobial characteristics of some herbal oils on *Pseudomonas aeruginosa* with special reference to their chemical compositions. Iranian Journal of Pharmaceutical Research 2009; 8(2): 107-114.

Özcan MM., Chalchat JC. Chemical composition and antifungal activity of rosemary (*Rosmarinus officinalis* L.) oil from Turkey. International Journal of Food Sciences and Nutrition 2008; 59(7-8): 691-698. https://doi.org/10.1080/09637480701777944

Paudel PN., Satyal P., Satyal R., Setzer WN., Gyawali R. Chemical composition, enantiomeric distribution, antimicrobial and antioxidant activities of *Origanum majorana* L. essential oil from Nepal. Molecules 2022; 27(18): 6136. https://doi.org/10.3390/molecules27186136

Peixoto JRO., Silva GC., Costa RA., Fontenelle JLS., Vieira GHF., Filho AAF., Vieira RHSF. In vitro antibacterial effect of aqueous and ethanolic *Moringa* leaf extracts. Asian Pacific Journal of Tropical Medicine 2011; 4: 201-204.

Polat R., Satıl F. An ethnobotanical survey of medicinal plants in Edremit Gulf (Balıkesir-Turkey). Journal of Ethnopharmacology 2012; 139(2): 626-641. https://doi.org/10.1016/j.jep.2011.12.004

Riedl H. — *Onosma* L., in Davis P. H. (ed.), Flora of Turkey and the East Aegean Islands. Vol. 6. Edinburgh University Press, Edinburgh 1978; 237-437.

Qi H., Zhang Q., Zhao T., Chen R., Zhang H., Niu X., Li Z. Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) in vitro. International Journal of Biological Macromolecules 2005; 37(4): 195-199. https://doi.org/10.1016/j.ijbiomac.2005.10.008.

Ruch RJ., Cheng SJ., Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989; 10(6): 1003-1008. https://doi.org/ 10.1093/carcin/10.6.1003

Sargın SA., Akçicek E., Selvi S. An ethnobotanical study of medicinal plants used by the local people of Alaşehir (Manisa) in Turkey. Journal of Ethnopharmacology 2013; 150(3): 860-874. https://doi.org/10.1016/j.jep.2013.09.040.

Sargın SA., Selvi S., Açar M. Türkiye'de yayılış gösteren *Cistus* L. (Cistaceae) cinsi üzerinde taksonomik ve morfolojik araştırmalar. Ulusal Botanik Bitki Bilimi Kongresi, 2014; p.121.

Sharma A., Verma R., Ramteke P. Antibacterial activity of some medicinal plants used by tribals against UTI causing pathogens. World Applied Sciences Journal 2009; 7(3): 332-339.

Szymanska R., Pospisil P., Kruk J. Plant-derived antioxidants in disease prevention. Oxidative Medicine and Cellular Longevity 2016; 2016: 1920208. https://doi.org/10.1155/2016/1920208

Taheri A., Seyfan A., Jalalinezhad S., Nasery F. Antibacterial effect of *Myrtus communis* hydro-alcoholic extract on pathogenic bacteria. Zahedan Journal of Research in Medical Sciences 2013; 15(6): 19-24.

Tavan M., Azizi A., Sarikhani H., Mirjalili MH., Rigano MM. Phenolics diversity among wild populations of *Salvia multicaulis*: as a precious source for antimicrobial and antioxidant applications. Natural Product Research 2022; 36(5): 1332-1336. https://doi.org/10.1080/14786419.2020.1864369

Tutin TG., Heywood VH., Burges NA., Moore DN., Valentine DH., Walters SM., Webb DA. Flora Europaea Vol 2. University Press, Cambridge, 1968; 206-211.

Vidana Gamage GC., Lim YY., Choo WS. Anthocyanins from *Clitoria ternatea* flower: biosynthesis, extraction, stability, antioxidant activity, and applications. Frontiers in Plant Science 2021; 12: 792303. https://doi.org/10.3389/fpls.2021.792303