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Research Article

Antimicrobial and Antioxidant Effects of Spice Extracts[&]

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Abstract

The in vitro antimicrobial activities of total 50 extracts from spices were investigated by using the disc diffusion and agar dilution method, against seven foodborne bacteria and two kinds of fungi. Their antioxidant activities were also evaluated. Many spices contained high levels of phenolics and showed antimicrobial activity against foodborne pathogens. Gram (+) bacteria were more tolerant to the tested extracts than Gram (-) ones. *S. typhimurium* was the most sensitive, while *P. aeruginosa* was the most resistant. This study offers that active compounds present in having high activity species could play a big role in naturally preservation against diseases.

Key words: Antimicrobial, medicinal herbs, alternative medicine.

Baharat Ekstraktlarının Antimikrobiyal ve Antioksidan Etkileri

Özet

Çalışmada 50 adet baharat bitkisinden elde edilen ekstratların in vitro antimikrobiyal aktiviteleri, yedi adet gıda kaynaklı bakteri ve iki adet mantar türüne karşı disk difüzyon ve agar seyreltme metodu kullanılarak araştırılmıştır. Araştırmada ekstraktların ayrıca antioksidan aktiviteleri de değerlendirilmiştir. Birçok ekstraktın yüksek düzeyde fenolik içerdiği ve gıda kaynaklı patojenlere karşı antimikrobiyal aktivite gösterdiği tespit edilmiştir. Gram (+) bakterilerin, test edilen ekstraktlara Gram (-) bakterilerden daha toleranslı olduğu görülmüştür. Çalışmada *S. typhimurium* en hassas, *B. cereus* ise en dirençli mikroorganizma olarak belirlenmiştir. Bu çalışma, yüksek aktivite gösteren türlerdeki aktif bileşiklerin, hastalıklara karşı doğal olarak korunmada büyük bir rol oynayabileceğini göstermektedir.

Anahtar kelimeler: Antimikrobiyal, tıbbi bitkiler, alternatif tıp.

Introduction

Spices have been used for diseases for decades. Although pharmacological industries have provided drugs and herbicides, resistance to these has increased by microorganisms. Chemical protectives have been consumed in daily life for years. However, an accelerating perception by people that chemicals may caused to health problems has led to a decreased acceptance for them to use. Nowadays, there is a growing attention in additives as potential natural antioxidants (Moure et al., 2001; Gulcin et al., 2002; Gulcin et al., 2003; Oktay et al., 2003). The industry is looking for nature alternatives that exhibit strong anti-microbial/oxidant properties in order to please consumer's requests in the reliable products (Zhang et al., 2009; Nimsha et al., 2010, Mulaudzi et al., 2011; Ahmad et al., 2015; Aziz and Karboune, 2018). The extracts of spices are capable of being alternatives to chemical antimicrobial agents to improve the shelf-life of products or using as natural antioxidant agents in order to inhibit lipid oxidation (Brewer, 2011; Ahmad et al., 2015). Some natural anti-oxidants/microbials were found not only to be able to elongate the shelf-life of food products but also to be useful as protective medicine against diseases (Irkin and Esmer, 2015; Aziz and Karboune, 2018).

Turkey is very rich in terms of spice species. The using of spice is very common, thus, spice consumption has very importance in terms of gastronomy of Turkey. Turkey has considerable export potential for medical and aromatic herbs. However, the exact number and amount of exported herbs are unclear (Akbulut and Bayramoglu, 2013).

The main objective of this study was to present the in vitro antimicrobial and antioxidant activity of spices. Although the antimicrobial and antioxidant activities of some spices have been well reported, nevertheless, there is such insufficient report about many spices concerning the investigation of antimicrobial and antioxidant activity against main pathogens.

Materials and Methods

Plant materials

Fifty Turkish medicinal spices were obtained from a well-known market for Turkish spices in Gaziantep, Turkey. The identification of the spices was defined by using Flora of Turkey (Davis, 1966). The species are listed in Table 1.

Table 1. The species used in the study

Scientific name	Parts tested	Local name	Scientific name	Parts tested	Local name
Artemisia dracunculus L.	Flower	Tarhın	*Nigella sativa L.*	Seed	Çörek otu
Anethum graveolens L.	Flover	Dere otu	*Ocimum basilicum L.*	Leaf	Reyhan
Achillea millefolium L.	Leaf	Civanperçemi	*Pimpinella anisum L.*	Fruit	Anason
Alpinia officinarum H.	Rhizome	Havlıcan	*Piper cubeba L.*	Fruit	Kebabe
Allium sativum L.	Root	Sarımsak	*Peganum harmala L.*	Flower	Üzerlik
Brassica nigra L.	Seed	Hardal	*Piper longum L.*	Fruit	Darı fülfül
Cassia angustifolia L.	Flower	Sinameki	*Prunus mahleb L.*	Seed	Mahlep
Capsicum annuum L.	Fruit	İsot	*Piper nigrum L.*	Fruit	Karabiber
Cuminum cyminum L.	Flower	Kimyon	*Pimenta officinalis L.*	Fruit	Yenibahar
Cannabis indica L.	Seed	Kendir	*Papaver somniferum L.*	Seed	Haşhaş
Curcuma longa L.	Rhizome	Zerdeçal	*Rosa canina L.*	Fruit	Kuşburnu
Cocos nucifera L.	Fruit	Hind. cevizi	*Rhus coriaria L.*	Fruit	Sumak
Coriandrum sativum L.	Seed	Kişniş	*Rosmarinus officinalis L.*	Flower	Biberiye
Crocus sativus L.	Flower	Safran	*Syzygium aromaticum L.*	Flower	Karanfil
*Capsicum tetragonum*M.	Fruit	Kırmızı biber	*Sesamum indicum L.*	Seed	Susam
Carthamus tinctorius L.	Flower	Aspir	*Salvia officinalis L*	Flower	Adaçayı
Cinnamomun zeylanicum L.	Bark	Tarçın	*Trigonella foenum-graecum L.*	Seed	Çemen
Elettaria cardamomum L.	Seed	Kakule	*Theobroma cacao L*	Fruit	Kakao
Foeniculum vulgare M.	Flower	Rezene	*Terminalia citrina R.*	Flower	Sarı halile
Glycyrrhiza glabra L.	Fruit	Meyan kökü	*Terebenthina communis L.*	Seed	Çam sakızı
Gummi myrrhe L	Resin	Mirsafi	*Thymbra spicata L.*	Flower	Zahter
Laurus nobilis L.	Leaf	Defne	*Thymus vulgaris L.*	Leaf	Kekik
Lepidium sativum L.	Leaf	Tere	*Urtica dioica L.*	Leaf	Isırgan
Linum usitatissimum L.	Seed	Keten	*Ziziphus zizyphus L.*	Leaf	Hünnap
Mentha piperita L.	Leaf	Nane	*Zingiber officinale R.*	Rhizome	Zencefil

Preparation of extract

The extracts of dried samples were prepared with the methods described by Holopainen et al. (1988) and Alkofahi et al. (1990) with little modification. In the method, dried plants were extracted with ethanol at room temperature . The extracts were kept at 4°C for five days, and they were filtered through 0.45µm membrane filter. And then the solvent was evaporated. The crude extracts were stored at -20°C until used.

Tested microorganisms

Bacillus cereus, *Listeria monocytogenes*, *Staphylococcus aureus*, *Esherichia coli*, *Salmonella typhimurium*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* were tested with the extracts.

Antibacterial assay

The activity was determined using the disc diffusion method (Ronald, 1990). Tested bacterial suspension were adjusted to 10^8 cfu/ml As positive control Ampicillin and Cephazolin 10 µl were used and as negative control 70% ethanol was used. Inhibition diameters were determined after incubation at 37°C for 24 h. All tests were made in triplicate.

Antifungal assay

The activity was determined using the disc diffusion method (Ronald, 1990). Tested fungal suspension were adjusted to 10^7 cfu/ml. One hundred units of nystatin was used as positive control and ethanol as a negative control. Inhibition

zones were determined after incubation at 27°C for 48 h. All tests were made in triplicate.

Minimum inhibition concentration

The agar dilution method was used with little modifications (Vanden Berghe and Vietinck, 1991) with the doses of 10, 5, 2.5, 1.25 and 0.625 mg/ml. After the incubation period, the growths were assessed by a stereo microscope.

Determination of total phenolic contents

Total phenolic contents were determined in plant extracts by the Folin-Ciocalteau procedure (Slinkard and Singleton, 1977). Briefly, 0.1 mL of various concentrations of gallic acid and methanolic samples (1 mg/ml) were diluted with 5.0 ml distilled water. 0.5 mL of 0.2 N Folin-Ciocalteu reagents was added and the contents were vortexed. After 3 min incubation, 1.5 mL of Na_2CO_3 (2%) solution was added and after vortexing, the mixture was incubated for 2 h at 20 ºC with intermittent shaking. The absorbance was measured at 760 nm at the end of the incubation period. The concentration of total phenolic compounds was calculated as mg of gallic acid equivalents per g of 100 g FW, by using a standard graph (Figure 1).

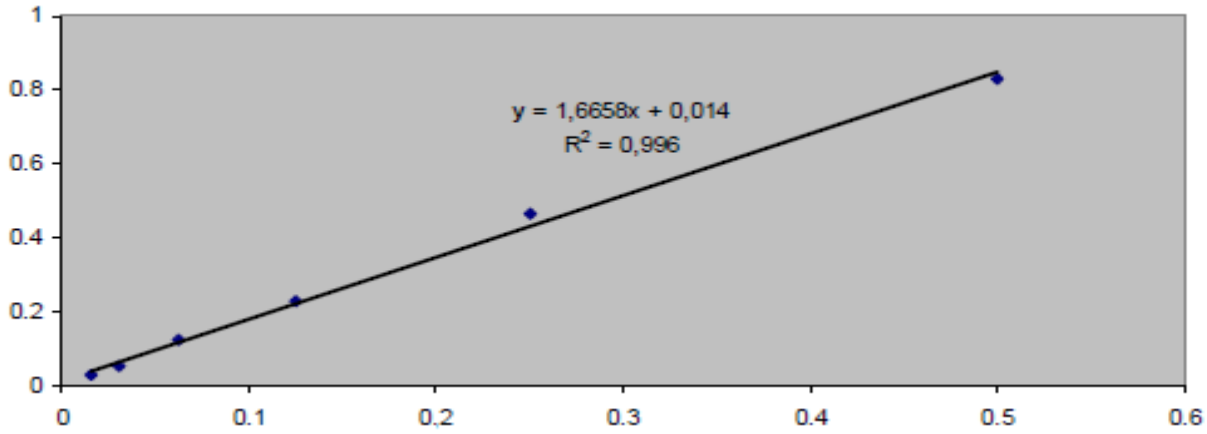

Figure 1. Standart curve of gallic acid.

Determination antioxidant capacity

The cupric reducing antioxidant capacity (CUPRAC) of the methanolic extracts was determined according to method of Apak et al. (2004). 1.0 mL of CuCl2 (1.0x10-2 M), 1.0 mL ethanolic neocuproine solution(7.5x10-3 M) and 1 mL NH_4AC (1M) buffer solution in a test tube were added to a test tube and mixed (0.1 ml) with methanolic extracts followed by water additing up to 4.1 ml and mixed well. Absorbance against a regeant blank was measured at 450 nm after 30 min incubation. Trolox® equivalent antioxidant capacity (TEAC) values were given as millimoles of Trolox® equivalent per gram of sample.

Statistical analysis

The experiment were designed in randomized plots with three replications. The results are evaluated in the confidence limit of 0.05. All calculations were performed with SPSS (v. 17.0) software. All tests were made in triplicate.

Results and Discussion

In the study, three of the bacteria (*L. monocytogenes, B. cereus* and *S. aureus*) were Gram-positive, four of the bacteria (*P. vulgaris, E. coli, S. typhimurium,* and *P. aeruginosa*) were Gram-negative and two were fungi (*C. albicans, A. niger*). There was a significant difference in the antibacterial and antifungal activities of 50 extracts (Table 2). For *Proteus vulgaris*, the DIZ values of 14 extracts (accounting for 28% of the 50 tested extracts) were between 15.33 mm and 24.00 mm and those of 34 extracts (68%) were between 8.00 mm and 14 mm. However, 2 extracts (4%) had no inhibitory activity (6.00 mm). *C. angustifolia* exhibited the strongest activity (DIZ: 24 mm), followed by *T. spicata* (23 mm) and *S. indicum* (22 mm). For *E. coli*, the DIZ values of 20 extracts (accounting for 40% of the 50 tested extracts) were between 15.33 mm and 25.00 mm and those of 28 extracts (56%) were between 9.33 mm and 14.00 mm. However, 2 extracts (4%) had no inhibitory activity (6.00 mm). *A. officinarum* exhibited the strongest antibacterial activity (DIZ: 25.00 mm), followed by *A. graveolens* and *P. anisum* (21.33 mm). For *B. cereus*, the DIZ values of 23 extracts (accounting for 46% of the 50 tested extracts) were between 15.33 mm and 32.67 mm and those of 26 extracts (52%) were between 7.00 mm and 14.00 mm. However, one extract (2%) had no inhibitory activity (6.00 mm). *A. officinarum* showed the strongest antibacterial activity (DIZ: 32.67 mm), followed by *A. graveolens* (28.00 mm) and *T.*

communis (26.00 mm). For *S. aureus*, the DIZ values of 24 extracts (accounting for 48% of the 50 tested extracts) were between 15.33 mm and 28.00 mm and those of 25 extracts (50%) were between 8.00 mm and 14.00 mm. However, 1 extract (2%) had no inhibitory activity (6.00 mm). *T. citrina* showed the strongest antibacterial activity (DIZ: 28.00 mm), followed by *A. dracunculus* (25.00 mm). For *S. typhimurium*, the DIZ values of 34 extracts (accounting for 68% of the 50 tested extracts) were between 15.33 mm and 33.00 mm and those of 14 extracts (28%) were between 7.00 mm and 14.00 mm. However, two extracts (4%) had no inhibitory activity(6.00 mm). *A. officinarum* showed the strongest antibacterial activity (DIZ: 33.00 mm), followed by *A. officinarum, R. officinalis* and *T. communis* (30.67mm). For *L. monocytogenes*, the DIZ values of 12 extracts (accounting for 24 % of the 50 tested extracts) were between 15.33 mm and 28.00 mm and those of 36 extracts (72 %) were between 7.00 mm and 14.00 mm. However, 2 extracts (4%) had no inhibitory activity (6.00 mm). *A. officinarum* exhibited the strongest antibacterial activity (DIZ: 28.00 mm), followed by *T. communis* (22.00 mm), *A.officinarum* and *A. millefolium* (20.67 mm). For *P. aeruginosa*, the DIZ values of 4 extracts (accounting for 8 % of the 50 tested extracts) were between 15.33 mm and 23.00 mm and those of 42 extracts (84 %) were between 7.00 mm and 14.00 mm. However, 4 extracts (8%) had no inhibitory activity (6.00 mm). *T. communis* exhibited the strongest antibacterial activity (DIZ: 23.00 mm), followed by *C. longa* (20.67 mm) For *C. albicans*, the DIZ values of 6 extracts (accounting for 12 % of the 50 tested extracts) were between 15.33 mm and 17.00 mm and those of 41 extracts (82 %) were between 7.00 mm and 14.00 mm. However, 3 extracts (6 %) had no inhibitory activity(6.00 mm). *C. zeylanicum* exhibited the strongest antibacterial activity (DIZ: 17.00 mm) followed by *P. nigrum* (16.67 mm). For *A. niger*, the DIZ values of 9 extracts (accounting for 18 % of the 50 tested extracts) were between 15.33 mm and 23.00 mm and those of 37 extracts (74 %) were between 7.00 mm and 14.00 mm. However, 4 extracts (8 %) had no inhibitory activity(6.00 mm). *T. foenum-graecum* and *Z. officinale* exhibited the strongest antibacterial activity (DIZ: 23.00 mm), followed by *P. nigrum* (22.00 mm). Results obtained that the extract from *A. officinarum* showed the highest antibacterial activity against all of the bacteria. Among the 50 plants screened, highest inhibitory zones were observed in the extract of *C. angustifolia* (33.00 mm) against *S. typhimurium* followed by *A. officinarum* and *C. angustifolia* (32.67 mm) against *B. cereus*. In the study, Gram (+) bacteria were more tolerant to the tested extracts than Gram (-) ones. It is also true for many spices (Cai et al., 2004). In the study, *S. typhimurium* was the most sensitive, while *P. aeruginosa* was the most resistant.

Determination of the MIC method (Tables 3) showed that many plant extracts with low concentration exhibited an antimicrobial effect against some of the tested nine microorganisms. According to MIC values, the spice extracts with the highest antimicrobial values inhibited the sensitive microorganisms in the concentration of >0.625 mg/ml (Table 3).

Some plants previously screened by other investigators were included in this study. But the concentration of active compounds in extracts depend on the plant variety, origin, time of harvest, conditions of processing and storage (Deans and Ritchie, 1987). In the present study, the results of the antimicrobial activity and minimum inhibition concentration are agree with Ceylan and Fung (2004), Erturk (2006), Tajkarimi et al. (2010), Ababutain (2011). The activity of some of the crude extracts tested in this study was similar to that of the antibacterial standarts Ampicillin and Cefazolin against *S. typhimurium*, *S. aureus*, *P. vulgaris*, *P. aeruginosa, B.cereus*, *L. monocytogenes* and *E. coli*. In addition, the antifungal activity of the crude extracts was similar to that of the standard antifungal Nystatin against *C. albicans* and *A. niger*.

From the results in the present work it can be concluded that many of the extracts which showed high antimicrobial activity could be used in the treatment of infectious diseases caused by resistant microorganisms

The strong effects of the spices are mainly caused by the presence of bioactive compounds, including phenolics, terpenes, aldehydes, isoflavonoids and acids etc. The substances of the spices having antimicrobial activity may affected microbial cells by a number of mechanisms, including charging the phospholipid bilayer of the cell membrane, enzyme systems and genetic material of the microorganism.

In the present study, according to phenolic and antioxidant capacity results, it is concluded that *C. zeylanicum*, *C. longa*, *B. nigra*, *S. aromaticum*, *S. officinalis*, *T. spicata*, *R. officinalis*, *Z. officinale*, *A. officinarum*, *T. citrina*, *R. coriaria*, *P. officinalis*, *P. cubeba*, *C. angustifolia*, *M. piperita*, *T. vulgaris* and *L. nobilis* showed high activity. In the study, the correlation coefficient between TPC (Total Phenolic Content) and TEAC (Trolox Equivalent Antioxidant Capacity) was found to be 0.83. The results of the phenolic content and antioxidant activity are agree with Shobana and Naidu (2000), Hinneburg et al. (2006), Suhaj (2006), Khalaf et al. (2008)..

Table 3. Results of minimum inhibitory concentration (MIC mg/ml)

Samples	*P.v.*	*E.c.*	*B.c.*	*S.a.*	*S.t.*	*L.m.*	*P.a.*	*C.a.*	*A.n.*
A. dracunculus	>5	>5	>10	>0.625	>5	>10	>10	>5	>1.25
A. graveolens	>5	>0.625	>0.625	>1.25	>1.25	>10	-	>10	>10
A. millefolium	>1.25	>2.5	>1.25	>5	>5	>1.25	>1.25	>2.5	>2.5
A. officinarum	>2.5	>0.625	>0.625	>0.625	>0.625	>0.625	>5	>10	>10
A. sativum	>2.5	>5	>2.5	>2.5	>0.625	>5	>10	>10	>10
B. nigra	>5	>5	>5	>1.25	>1.25	>10	>10	>10	>5
C. angustifolia	>0.625	>5	>0.625	>1.25	>0.625	>0.625	>5	>2.5	>2.5
C. annuum	>2.5	>2.5	>2.5	>2.5	>5	>10	>10	>5	>2.5
C. cyminum	>2.5	>2.5	>5	>2.5	>5	>10	>10	>5	>2.5
C. indica	>2.5	>5	>1.25	>2.5	>10	>2.5	>10	>10	>10
C. longa	>1.25	>2.5	>1.25	>1.25	>0.625	>2.5	>1.25	>1.25	>1.25
C. nucifera	>5	>1.25	>5	>5	>10	>10	-	>10	>10
C. sativum	>1.25	>1.25	>2.5	>5	-	>2.5	>5	>10	>5
C. sativus	>2.5	>5	>10	>0.625	>5	>10	>10	>2.5	>2.5
C. tetragonum	>5	>2.5	>10	>10	>10	>10	-	>2.5	>2.5
C. tinctorius	>5	>2.5	>10	>1.25	>1.25	>10	>10	>10	>10
C. zeylanicum	>2.5	>5	>1.25	>2.5	>0.625	>5	>5	>1.25	>2.5
E. cardamomum	>5	>1.25	>2.5	>2.5	>0.625	>2.5	>10	>5	>5
F. vulgare	>10	>1.25	>1.25	>1.25	>0.625	>2.5	>10	>5	>5
G. glabra	>5	>1.25	>1.25	>1.25	>0.625	>1.25	>10	>10	>5
G. myrrhe	>2.5	>10	>2.5	>1.25	>0.625	>1.25	>10	>10	>5
L. nobilis	>1.25	>0.625	>1.25	>1.25	>0.625	>10	>10	>10	>5
L. sativum	>5	>1.25	>1.25	>5	>1.25	>10	>10	>5	>10
L. usitatissimum	>1.25	>10	>1.25	>1.25	>0.625	>10	>5	>10	>10
M. piperita	>1.25	>2.5	>5	>1.25	>0.625	-	>10	>5	>10
N. sativa	>5	>5	>5	>2.5	>5	>10	>10	>10	>10
O. basilicum	>5	>1.25	>5	>5	>2.5	>5	>5	>5	>5
P. anisum	>2.5	>0.625	>2.5	>5	>10	>10	>10	>10	>10
P. cubeba	>1.25	>2.5	>5	>2.5	>0.625	>5	>2.5	>5	>2.5
P. harmala	>1.25	>1.25	>5	>5	>0.625	>2.5	>5	>10	>5
P. longum	>5	>5	>2.5	>10	>5	>10	>10	>10	-
P. mahleb	>2.5	-	>10	>5	>2.5	>10	>10	>10	>2.5
P. nigrum	>2.5	>5	>10	>1.25	>0.625	>10	>5	>1.25	>0.625
P. officinalis	>5	>1.25	>5	>2.5	>2.5	>2.5	>10	>10	>10
P. somniferum	>5	>2.5	>5	>2.5	>1.25	>10	>10	>5	>5
R. canina	>5	>5	>5	>10	>5	>10	>10	>2.5	>1.25
R. coriaria	>1.25	>1.25	>0.625	>2.5	>0.625	>1.25	>2.5	>2.5	>5
R. officinalis	>2.5	>1.25	>1.25	>5	>0.625	>5	>10	>5	>10
S. aromaticum	>5	>2.5	>0.625	>2.5	>0.625	>1.25	>1.25	>5	>5
S. indicum	>0.625	>2.5	>5	>5	>2.5	>10	>10	>10	>10
S. officinalis	>0.625	>2.5	>1.25	>2.5	>1.25	>1.25	>2.5	>10	>10
T. foenum-graecum	>5	>5	>10	>5	>2.5	>10	>10	>2.5	>0.625
T. cacao	>10	>5	>10	>1.25	>0.625	>2.5	>10	>10	>5
T. citrina	>10	>5	>5	>0.625	>0.625	>10	>10	>10	>10
T. communis	>1.25	>5	>0.625	>1.25	>0.625	>0.625	>0.625	>5	>2.5
T. spicata	>0.625	>2.5	>1.25	>2.5	>0.625	>1.25	>10	>5	>2.5
T. vulgaris	>5	>1.25	>1.25	>2.5	>10	>10	>10	>2.5	>2.5
U. dioica	>10	>1.25	>10	>5	>10	>10	>10	>5	-
Z. zizyphus	>2.5	>5	>10	>10	>1.25	>10	>10	>10	>1.25
Z. officinale	>5	>5	>2.5	>0.625	>1.25	>10	>10	>2.5	>10
Ampicillin	NT	NT	NT	NT	NT	NT	NT	NT	NT
Cephazolin	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nystatin	NT	NT	NT	NT	NT	NT	NT	NT	NT
Solvent (Ethanol)	NT	NT	NT	NT	NT	NT	NT	NT	NT

Microorganisms; P.v.: *Proteus vulgaris,* E.c.: *Escherichia coli,* B.c.: *Bacillus cereus,* S.a.: *Staphylococcus aureus,* S.t.: *Salmonella typhimurium,* L.m.: *Listeria monocytogenes,*P.a.: *Pseudomonas aeruginosa,* C.a.: *Candida albicans,* A.n.: *Aspergillus niger*. -: No inhibition, NT: not tested.

Conclusion

Many previous studies have reported the antimicrobial activity, phenolic content or antioxidant activities of spices and herbs. But it was not easy to compare directly the results of different studies and to establish reasonable relationships between antimicrobial activity, phenolic content and antioxidant activity because of the low number of spice and herb samples tested, different determination methods and different microorganism strains used.

As a consequence the extracts which showed antimicrobial and antioxidant activity could be used in natural preservation. This study is capable of to get concious consumer perception for spices using in Turkey. In addition, to the best of our knowledge, this is the first report regarding the antimicrobial and antioxidant activity of *Prunus mahleb*, *Gummi myrrhe*, *Terminalia citrina* and *Terebenthina communis*.

[&]: The data in the study has taken from master's thesis

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Table 2. Antimicrobial activity, antioxidant capacity and total phenolic content of 50 extracts from spice species

Scientific name	TPC	TEAC	Antimicrobial activity (mm)									
			P.v.	*E.c.*	*B.c.*	*S.a.*	*S.t.*	*L.m.*	*P.a.*	*C.a.*	*A.n.*	*Mean*
A. dracunculus	0.0408	524.17	8.00±0.42	10.00±0.45	10.00±0.45	25.00±0.44	13.33±0.44	8.00±0.42	7.00±0.34	13. 33±0.44	15.33±0.44	12.22
A. graveolens	0.0312	281.83	9.33±0.42	21.33±0.34	28.00±0.34	16.67±0.42	19.67±0.42	10.00±0.45	6*.00±0.00	12.00±0.42	10.00±0.45	14.77
A. millefolium	0.0410	274.50	15.33±0.44	16.67±0.42	19.67±0.42	15.33±0.44	13.33±0.44	20.67±0.42	19.67±0.42	15.33±0.44	15.33±0.44	16.81
A. officinarum	0.1183	1863.75	12.00±0.42	25.00±0.44	32.67±0.42	22.00±0.44	30.67±0.42	28.00±0.34	13.33±0.44	9.33±0.42	10.00±0.45	20.33
A. sativum	0.0046	67.50	12.00±0.42	10.00±0.45	15.33±0.44	14.00±0.44	20.33±0.42	12.00±0.42	10.00±0.45	8.00±0.42	6*.00±0.00	11.96
B. nigra	0.0614	1002.71	13.33±0.44	9.33±0.42	14.00±0.44	18.67±0.42	16.67±0.42	7.00±0.34	8.00±0.42	9.33±0.42	12.00±0.42	12.03
C. angustifolia	0.0953	797.92	24.00±0.00	9.33±0.42	32.67±0.42	18.67±0.42	33.00±0.34	20.67±0.42	12.00±0.42	13.33±0.43	14.00±0.44	19.74
C. annuum	0.0441	574.17	14.00±0.44	14.00±0.44	15.33±0.44	15.33±0.44	12.00±0.42	10.00±0.45	7.00±0.34	12.00±0.42	13.33±0.44	12.54
C. cyminum	0.0337	364.50	13.33±0.44	14.00±0.44	15.33±0.44	14.00±0.44	12.00±0.42	10.00±0.45	8.00±0.42	12.00±0.42	14.00±0.44	12.51
C. indica	0.0263	362.50	14.00±0.44	14.00±0.44	19.67±0.42	15.33±0.44	6*.00±0.00	13.33±0.44	11.67±0.42	10.00±0.45	8.00±0.42	12.44
C. longa	0.0964	711.39	18.67±0.42	13.33±0.44	19.67±0.42	16.67±0.42	24.00±0.00	16.67±0.42	20.67±0.42	15.33±0.44	17.00±0.44	18.00
C. nucifera	0.0181	261.17	10.00±0.45	15.33±0.44	14.00±0.44	12.00±0.42	9.33±0.42	6*.00±0.00	6*.00±0.00	9.33±0.42	7.00±0.34	9.88
C. sativum	0.0227	298.17	18.67±0.42	18.67±0.42	16.67±0.42	10.00±0.45	6*.00±0.00	13.33±0.44	12.00±0.42	8.00±0.42	12.00±0.42	12.81
C. sativus	0.0455	204.50	14.00±0.44	12.00±0.42	8.00±0.42	22.00±0.44	12.00±0.42	7.00±0.34	8.00±0.42	14.00±0.44	14.00±0.44	12.33
C. tetragonum	0.0470	372.17	8.00±0.42	14.00±0.44	8.00±0.42	8.00±0.42	9.33±0.42	8.00±0.42	6*.00±0.00	15.33±0.44	14.00±0.44	10.07
C. tinctorius	0.0250	126.17	12.00±0.42	15.33±0.44	10.00±0.45	16.67±0.42	18.67±0.42	9.33±0.42	10.00±0.45	8.00±0.42	9.33±0.42	12.14
C. zeylanicum .	0.0998	1325.28	14.00±0.44	10.00±0.45	20.67±0.42	13.33±0.44	19.67±0.42	12.00±0.42	12.00±0.42	17.00±0.44	14.00±0.44	14.66
E. cardamomum	0.0145	157.50	12.00±0.42	16.67±0.42	17.00±0.44	13.33±0.44	22.00±0.44	14.00±0.44	8.00±0.42	12.00±0.42	11.67±0.42	14.07
F. vulgare	0.0223	239.17	6*.00±0.00	18.67±0.42	18.67±0.42	16.67±0.42	22.00±0.44	13.33±0.44	8.00±0.42	11.67±0.42	11.67±0.42	14.07
G. glabra	0.0935	241.17	12.00±0.42	18.67±0.42	18.67±0.42	19.67±0.42	20.67±0.42	18.67±0.42	11.67±0.42	10.00±0.45	12.00±0.42	15.78
G. myrrhe	0.0476	245.58	14.00±0.44	6*.00±0.00	13.33±0.44	16.67±0.42	21.33±0.34	18.67±0.42	8.00±0.42	10.00±0.45	11.67±0.42	13.29
L. nobilis	0.0819	790.42	14.00±0.44	17.00±0.44	14.00±0.44	14.00±0.44	20.67±0.42	7.00±0.34	6*.00±0.00	6*.00±0.00	10.00±0.45	12.07
L. sativum	0.0500	614.83	9.33±0.42	18.67±0.42	17.00±0.44	13.33±0.44	17.00±0.44	8.00±0.42	9.33±0.42	14.00±0.44	6*.00±0.00	12.51
L. usitatissimum	0.0088	141.50	18.67±0.42	18.67±0.42	17.00±0.44	16.67±0.42	22.00±0.44	9.33±0.42	13.33±0.44	7.00±0.34	8.00±0.42	14.51
M. piperita	0.0971	1071.25	18.67±0.42	14.00±0.44	12.00±0.42	18.67±0.42	21.33±0.34	6*.00±0.00	8.00±0.42	12.00±0.42	11.67±0.42	13.59
N. sativa	0.0548	415.17	8.00±0.42	10.00±0.45	11.67±0.42	14.00±0.44	16.67±0.42	9.33±0.42	8.00±0.42	9.33±0.42	8.00±0.42	10.55
O. basilicum	0.0426	472.83	12.00±0.42	15.33±0.44	11.67±0.42	12.00±0.42	15.33±0.44	13.33±0.44	12.00±0.42	11.67±0.42	12.00±0.42	12.81
P. anisum	0.0563	751.25	13.33±0.44	21.33±0.34	14.00±0.44	12.00±0.42	8.00±0.42	7.00±0.34	9.33±0.42	8.00±0.42	7.00±0.34	11.11
P. cubeba	0.0989	1098.75	15.33±0.44	13.33±0.44	9.33±0.42	14.00±0.44	29.00±0.34	12.00±0.42	13.33±0.44	11.67±0.42	14.00±0.44	12.96
P. harmala	0.0638	460.50	18.67±0.42	17.00±0.44	12.00±0.42	13.33±0.44	25.00±0.42	14.00±0.44	12.00±0.42	6*.00±0.00	12.00±0.42	14.44
P. longum	0.0374	547.50	10.00±0.45	10.00±0.45	14.00±0.44	9.33±0.42	13.33±0.44	9.33±0.42	10.00±0.45	6*.00±0.00	6*.00±0.00	9.77
P. mahleb	0.0127	128.50	13.33±0.44	6*.00±0.00	10.00±0.45	13.33±0.44	15.33±0.44	10.00±0.45	8.00±0.42	10.00±0.45	14.00±0.44	11.11
P. nigrum	0.0708	761.25	13.33±0.44	12.00±0.42	7.00±0.34	18.67±0.42	20.67±0.42	8.00±0.42	11.67±0.42	16.67±0.42	22.00±0.44	14.44
P. officinalis	0.1483	1925.42	10.00±0.45	18.67±0.42	12.00±0.42	14.00±0.44	15.33±0.44	15.33±0.44	8.00±0.42	10.00±0.45	9.33±0.42	12.51
P. somniferum	0.0071	99.50	11.67±0.42	14.00±0.44	12.00±0.42	14.00±0.44	17.00±0.44	10.00±0.45	8.00±0.42	12.00±0.42	12.00±0.42	12.29
R. canina	0.0292	346.46	8.00±0.42	9.33±0.42	13.33±0.44	6*.00±0.00	10.00±0.45	8.00±0.42	8.00±0.42	15.33±0.44	16.67±0.42	10.51
R. coriaria	0.1493	1492.92	17.00±0.44	18.67±0.42	21.33±0.34	15.33±0.44	21.33±0.34	18.67±0.42	14.00±0.44	14.00±0.44	13.33±0.44	17.07
R. officinalis	0.1292	1432.92	13.33±0.44	16.67±0.42	18.67±0.42	12.00±0.42	30.67±0.42	12.00±0.42	9.33±0.42	11.67±0.42	10.00±0.45	14.92

Table 2. Antimicrobial activity, antioxidant capacity and total phenolic content of 50 extracts from spice species (continue)

Scientific name	TPC	TEAC	Antimicrobial activity (mm)									
			P.v.	***E.c.***	***B.c.***	***S.a.***	***S.t.***	***L.m.***	***P.a.***	***C.a.***	***A.n.***	***Mean***
S. aromaticum	0.1502	1472.08	12.00±0.42	14.00±0.44	20.67±0.42	15.33±0.44	27.00±0.34	16.67±0.42	19.67±0.42	12.00±0.42	12.00±0.42	16.59
S. indicum	0.0111	50.50	22.00±0.44	13.33±0.44	11.67±0.42	13.33±0.44	15.33±0.44	11.67±0.42	9.33±0.42	7.00±0.34	8.00±0.42	12.40
S. officinalis	0.0764	939.17	19.67±0.42	14.00±0.44	17.00±0.44	16.67±0.42	18.67±0.42	18.67±0.42	13.33±0.44	10.00±0.45	11.67±0.42	15.52
T. f.-graecum	0.0191	47.17	10.00±0.45	13.33±0.44	7.00±0.34	12.00±0.42	13.33±0.44	8.00±0.42	10.00±0.45	14.00±0.44	23.00±0.00	12.29
T. cacao	0.0119	457.5	9.33±0.42	12.00±0.42	8.00±0.42	16.67±0.42	29.00±0.34	14.00±0.44	10.00±0.45	10.00±0.45	11.67±0.42	13.40
T. citrina	0.0747	784.38	6*.00±0.00	12.00±0.42	11.67±0.42	28.00±0.34	26.67±0.44	11.67±0.42	8.00±0.42	7.00±0.34	10.00±0.45	13.44
T. communis	0.0857	59.72	16.67±0.42	9.33±0.42	26.00±0.34	19.67±0.42	30.67±0.42	22.00±0.44	23.00±0.0	12.00±0.42	16.67±0.42	19.55
T. spicata	0.0917	511.39	23.00±0.00	14.00±0.44	17.00±0.44	15.33±0.44	22.00±0.44	19.67±0.42	11.67±0.42	12.00±0.42	14.00±0.44	16.51
T. vulgaris	0.0989	583.75	10.00±0.45	17.00±0.44	19.67±0.42	14.00±0.44	7.00±0.34	7.00±0.34	8.00±0.42	13.33±0.44	13.33±0.44	12.14
U. dioica	0.0332	373.5	8.00±0.42	16.67±0.42	10.00±0.45	11.67±0.42	8.00±0.42	10.00±0.45	11.67±0.42	12.00±0.42	6*.00±0.00	10.44
Z. zizyphus	0.052	151.83	16.67±0.42	11.67±0.42	6*.00±0.00	10.00±0.45	16.67±0.42	8.00±0.42	8.00±0.42	10.00±0.45	19.67±0.42	11.85
Z. officinale	0.1087	2035.42	10.00±0.45	13.33±0.44	7.00±0.34	12.00±0.42	13.33±0.44	8.00±0.42	10.00±0.45	14.00±0.44	23.00±0.00	12.29
Sign.			***	***	***	**	***	***	***	***	***	
Mean of 50 spices			13.25	14.27	15.12	15.10	18.16	12.18	10.48	11.19	12.26	
Ampicillin			28.00±0.34	15.33±0.44	27.00±0.34	10.00±0.45	28.00±0.34	25.00±0.42	28.00±0.34	NT	NT	23.04
Cephazolin			6*.00±0.00	15.33±0.44	23.00±0.0	6*.00±0.00	22.00±0.44	32.67±0.42	24.00±0.00	NT	NT	18.42
Nystatin			NT	NT	NT	NT	NT	NT	NT	16.67±0.42	15.33±0.44	16.00
Solvent (Ethanol)			6*.00±0.00	6*.00±0.00	6*.00±0.00	6*.00±0.00	6*.00±0.00	6*.00±0.00	6*.00±0.00	6*.00±0.00	6*.00±0.00	6.00

[a]TEAC expressed as millimoles of trolox equivalent per 100 g dry weight. [b]TPC expressed as grams of gallic acid equivalents (GAE) per 100 mg dry weight.

[c]The zone diameter of disk is 6 mm and the diameter of inhibition zone (DIZ) of negative control for each bacterium is also 6 mm.

If the DIZ value is 6 mm (*), that means the extract has not inhibitory effect against tested microorganism.

The differences of the TPC and TEAC values are statistically significant ($p<0.05$); The differences between the means in the same column are statistically significant, $p<0.05$; NT: not tested P.v.: *Proteus vulgaris,* E.c.: *Escherichia coli,* B.c.: *Bacillus cereus,* S.a.: *Staphylococcus aureus,* S.t.: *Salmonella typhimurium,* L.m.: *Listeria monocytogenes,*P.a.: *Pseudomonas aeruginosa,* C.a.: *Candida albicans,* A.n.: *Aspergillus niger.*