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Comparison of different solvent types for determination biological activities of myrtle berries collected from Turkey

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RESEARCH ARTICLE

Abstract

In the present study, we investigated the effects of four extracting solvents (70% acetone:water (v/v), 80% ethanol:water (v/v), 80% methanol:water (v/v) and distilled water) on the total phenolic content and antiradical, antioxidant activity and antimicrobial activities of extracts of myrtle berries (*Myrtus communis*) collected from eight different locations in Turkey. The tested plant extracts were found to contain appreciable amounts of total phenolic contents (39.933 to 207.4 mg GAE/g dry extract) and have 1.1-diphenyl-2- picrylhydrazyl scavenging activity ranging between 6.73 and 65.6%. The antioxidant activity of the myrtle fruits was measured using the phosphomolybdenum spectrophotometric method. The highest antioxidant activity value (241.533 mg ascorbic acid equivalents /g dry extract) was observed in the methanolic extract of the fifth sample. The agar diffusion method was used to determine the antimicrobial activity of the extract samples. It was found that the methanol and acetone extracts were more efficient against six pathogenic bacteria including *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* Typhimurium and *Yersinia enterocolitica*. The solvent systems used for extraction had an important effect on the bioactive compounds extracted. Based on the results, we conclude that myrtle berries can be considered as a good source of natural antioxidant and natural antimicrobial compounds.

Keywords: antimicrobial activity, antioxidant activity, myrtle berry, solvent type, total phenolic content

1. Introduction

There has been increasing public concern and awareness worldwide concerning the quality, safety and geographical origin of food. Furthermore, a growing interest in natural and organic foods especially medicinal foods, including wild edible plants and fruits, has arisen. Medicinal foods have a wide range of health beneficial components such as antioxidants, antimicrobials and phenolics that potentially prevent the risk of many free radical-mediated diseases (Chryssavgi *et al*., 2008; Jaroni and Ravishankar, 2012; Lamien-Meda *et al*., 2008; Montoro *et al*., 2006).

Myrtus communis (myrtle) is an evergreen shrub and belongs to the Myrtaceae family that comprises about 50 species which are naturally and widely distributed throughout the Mediterranean region. *M. communis* L. is 1-3 m high and has white flowers which blossom from June to July (Barboni *et al*., 2010; Ciccarelli *et al*., 2008; Piras *et al*., 2009).

Ripe *M. communis* turns dark blue-red coloured spherical berries, which are approximately 5 mm in diameter, from November to December. The essential oils obtained from the leaves and flowers of the plants are widely used in the perfumery, cosmetic, food and pharmaceutical industries (Barboni *et al*., 2010; Ciccarelli *et al*., 2008; Lamien-Meda *et al*., 2008). In Turkey, myrtle trees mainly grow just above sea level at 500-600 m in pine forests and on riversides. Myrtle is called as 'hambeles', 'mersin' or 'murt' in Turkish (Aydin and Ozcan, 2007). It has been used traditionally as an antiseptic and disinfectant drug because of its

anti-hyperglycemic and anti-inflammatory activities. In addition to being used in the treatment of lung disorders, the essential oil of myrtle leaves also have antibacterial, anti-lousing and antioxidant properties (Hayder *et al*., 2007; Wannes *et al*., 2009). In Turkey, the leaves and fruits of myrtle have also been used for their antiseptic properties such as for healing wounds. Myrtle berries contain volatile oils, tannins, sugars, flavonoids and organic acids such as citric and malic acids (Wannes *et al*., 2010). Linalool, 1,8-cineole, myrtenyl acetate and myrtenol are the major volatile compounds of the essential oil of myrtle which grows in Turkey. The leaves of myrtle contain tannins, flavonoids such as quercetin, catechin and myricetin derivatives and volatile oil (Cakir, 2004).

Some studies related to the composition and bioactivities of myrtle have mainly focused on its bioactive compounds such as anthocyanins, flavonols and phenolics (Barboni *et al*., 2010). In addition the antibacterial activity of *M. communis* against some microorganisms, composition of the essential oil and fatty acids has been investigated (Cakir, 2004). As is known, solvent type used for extraction is rather important for the characterisation of the bioactive properties of plants because solvent type has a significant effect on bioactivity. Although the biological activities of *M. communis* have been investigated in different countries, there are only a few reports on the *Myrtaceae* species belonging to the Turkish flora. In the present study, both the bioactive properties of *M. communis* grow in Turkey and the efficacies of different solvents for determination of bioactive compounds extraction capabilities were investigated and compared.

2. Materials and methods

Materials

The myrtle samples were collected from eight different locations in Mersin, Turkey during autumn and preserved in a deep-freezer (-18 °C) until analysis. The samples were coded as S1, S2, S3, S4, S5, S6, S7 and S8. The colour of the samples was purple except for S8 which was light yellow.

Extraction procedure

The berries were divided into two pieces and dried at 50 °C. The dried fruits were then ground to a fine powder with a grinder. Four different solvent types were used for the extraction: (1) acetone:water (70%, v/v); (2) ethanol:water (80%, v/v); (3) methanol:water (80%, v/v); and (4) distilled water. Two g of myrtle powder was weighed and 50 ml of solvent was added and homogenised by Ultra Turrax (IKA, Staufen, Germany) for 1 min. After homogenisation, the prepared samples were kept for 24 h at 25 °C for cold extraction in dark conditions. The mixtures of fruit and each solvent were centrifuged at 4,100 rpm for 15 min (Nuve, Ankara, Turkey); they were then filtrated and the supernatants were collected. The whole procedure described above was repeated once more for the filter cake using 15 ml fresh solvent for each sample. After filtration, the clear supernatants were evaporated to dryness under vacuum at 50 °C with a rotary evaporator (Buchi, Flawil, Switzerland). After this treatment, the dry extracts were preserved at +4 °C. For determination of bioactive properties, dry extracts were dissolved in acetone:water (70%, v/v), ethanol:water (80%, v/v) methanol:water (80%, v/v) and distilled water at certain concentrations.

Determination of total phenolic content by Folin-Ciocalteu colorimetric method

The total phenolic content of extracts was determined by the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). Briefly, 2,400 µl of distilled water, 40 µl of 2,500 mg/kg myrtle extracts (solvents for control) and 200 µl of Folin-Ciocalteu reagent were added to each tube. After 5 min incubation, 600 µl of sodium carbonate (20%, w/v) and 760 µl of distilled water were added. Then, each tube was homogenised by a vortex and incubated in the dark at room temperature for 2 h. After the incubation, the absorbance of the samples was measured at 765 nm by using spectrophotometer (Varian, Palo Alto, CA, USA). The amount of total phenolic compounds was expressed as gallic acid equivalents (GAE) in mg/g dry fruit extract.

Determination of antiradical activity by DPPH radical scavenging activity

The 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was adjusted by a slight modification of the procedure described by Sagdic *et al*. (2008). Briefly, 50 µl of the 1000 mg/kg of extract solutions (solvents for control) was added to each tube and mixed with 3,500 µl DPPH solution in methanol (1000 mg/kg) followed by homogenisation with a vortex. After 30 min incubation in the dark and at room temperature, absorbance was measured at 517 nm by using spectrophotometer (Varian). Methanol was used as the blank and the radical scavenging activities were calculated by the equation below.

$$\text{Radical scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100 \quad (1)$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

Determination of total antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of the fruit extract was measured as spectrophotometrically according to the procedure described by Silici *et al*. (2010). A 400 µl solution of the extracts (solvents for control) was added

to each tube and mixed with 4 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After homogenisation with a vortex, the mixture was incubated at 95 °C for 90 min and absorbance was measured at 695 nm by using spectrophotometer (Varian). The results were expressed as ascorbic acid equivalents (AAE) in mg/g dry fruit extract.

Determination of antibacterial activity by agar diffusion method

The antibacterial activity of the myrtle extracts was determined by the agar diffusion method according to a protocol described by Sagdic and Ozcan (2003). The six bacteria used as test microorganisms were as follows: *Bacillus cereus* FMC 19, *Escherichia coli* O157:H7 RS-932, *Listeria monocytogenes* 1/2B, *Staphylococcus aureus* ATCC 28213, *Salmonella* Typhimurium NRRLE 4463 and *Yersinia enterocolitica* ATCC 1501. The bacterial cultures were grown in nutrient broth at 35 °C for 18 h and final cell concentrations were measured to be 10^6-10^7 cfu/ml. 250 µl of each microorganism was added to a flask containing 25 ml nutrient agar at 45 °C and poured into Petri dishes (9 cm in diameter). Then, the agars were kept at 4 °C for 1 h for the agar to solidify. Four equidistant holes were made by sterile cork borers and a 50 µl aliquot of each extract was added to each hole (Ø = 4 mm). Dilutions of myrtle extracts (10, 5, 2 and 1%) were used for the determination of the minimum inhibitory concentration of the extracts on the tested pathogenic microorganisms. Following the incubation of plates at 35 °C for 24 h, inhibition zones were measured in mm. All the tests were performed in duplicate and average results were presented.

Statistical analysis

All statistical analyses were carried out using the Statistical Analysis System (SAS, 2000). A two-way analysis of variance (ANOVA) was conducted using the general linear model procedure. Differences among mean values were determined using the Tukey multiple range test, with the significance level of 0.05.

3. Results and discussion

Total phenolic content

The Folin-Ciocalteau method used to determine the total phenolic content of the extracts is based on a chemical reduction process with a chemical mix which includes tungsten and molybdenum oxides. The total phenolic contents of the extracts presented in Table 1 were found to range from 207.440 to 39.933 mg GAE/g dry extract for all the solvent types. In the acetone extract samples, there were no significant differences between samples S1, S3, S8 and S2, S7 with respect to their total phenolic content (TPC) values. However, the TPC values of S4, S5 and S6 were significantly (P<0.05) different from those of the other samples. In the ethanolic extract was S8 had the lowest TPC value (39.93 mg GAE/g dry extract). The TPCs of the ethanolic extracts of S2, S3 and S5 were found to be similar (P>0.05) to each other. In addition, the methanol and water extracts of S8 had the lowest TPC, followed by the acetone extract (185.30 mg GAE/g dry extract). It can be said that acetone was a more suitable extraction solvent to obtain the TPC values for S8 sample. The TPC values of the methanolic extracts were determined to range from 207.44 to 52.333 mg GAE/g dry extract. In general, the lowest TPC values were observed in the aqueous extract samples. These values were found to be between 148.9 and 39.93 mg GAE/g dry extract for the

Table 1. Total phenolic content of myrtle berry extracts for each solvent type.[1]

	Sample no.	Solvent extracts			
		Acetone	Ethanol	Methanol	Water
Total phenolic content (mg GAE/g dry extract)	S1	184.29 ± 2.06^{Ab}	66.80 ± 4.63^{De}	160.69 ± 3.04^{Bb}	108.43 ± 3.57^{Cc}
	S2	130.89 ± 2.15^{Cd}	148.87 ± 3.76^{Ba}	207.44 ± 2.07^{Aa}	84.41 ± 0.94^{Dd}
	S3	184.95 ± 3.07^{Ab}	146.13 ± 6.82^{Ca}	111.04 ± 0.24^{De}	169.80 ± 1.94^{Ba}
	S4	114.00 ± 3.43^{Ae}	85.43 ± 2.00^{Bd}	109.10 ± 4.91^{Ae}	78.08 ± 2.93^{Ce}
	S5	205.33 ± 1.78^{Aa}	143.39 ± 2.49^{Ba}	116.98 ± 5.49^{Dd}	135.97 ± 1.95^{Cb}
	S6	145.91 ± 5.08^{Ac}	94.84 ± 0.76^{Cc}	124.48 ± 1.75^{Bc}	73.04 ± 0.36^{Df}
	S7	128.88 ± 1.60^{Ad}	103.53 ± 3.14^{Cb}	110.05 ± 3.12^{Be}	70.87 ± 0.56^{Df}
	S8	185.30 ± 5.89^{Ab}	39.93 ± 4.61^{Cf}	52.33 ± 1.61^{Bf}	52.24 ± 0.89^{Bg}

[1] Superscript uppercase letters in same row indicate significant differences between effects of the solvent types (P<0.05); superscript lowercase letters in same column indicate significant differences between effects of the samples (P<0.05).
GAE = gallic acid equivalents.

ethanolic extract and between 52.24 and 169.8 mg GAE/g dry extract for the aqueous extract. The effect of solvent type on total phenolic content was found to be significant (P<0.05); this was attributed to the fact that the polarity of solvents is different from each other. As is known, the more polarity solvents have, the more polar phenolic compound can be extracted. Because phenolic composition shows a great variation in each fruit type, no universal solvent system exists for the extraction of phenolic compounds from different kinds of fruits. Therefore, different types of solvent systems need to be tested in studies in which the phenolic composition is investigated. In this study, we found that less polar polyphenolic compounds are dominant in myrtle fruits. The main reason for the differences between the total phenolic content of myrtle samples can be attributed to the variation in geological properties. In addition, other compounds, such as sugars, aromatic amines, ascorbic acid, sulphur dioxide and iron interacting with the Folin-Ciocalteu reagent may be another contributing factor, as well as some inorganic substances interacting with the reagent. Because of such interactions with the above mentioned components, the results of analysis for the determination of the total phenolic content in fruits may be affected (Chryssavgi *et al*., 2008). It has also been reported that the TPCs of the methanolic extract of myrtle collected in February and August were measured to be 307 and 373 mg GAE/g dry plant, respectively (Chryssavgi *et al*., 2008). In another study (Tuberoso *et al*., 2010), the TPCs of myrtle were determined to be 4.57 and 0.52 g GAE/l for ethanolic and aqueous extracts, respectively. According to these findings, the TPCs of myrtle fruits were found to be higher than those reported by Tuberoso *et al*. (2010). The possible reason for the observed differences may be attributed to variations in geological conditions and climatic factors. The observed differences with earlier studies may also be attributed to differences in the methodologies used as well as to experimental and climatic conditions.

Antiradical activity

The DPPH method has been used to determine the antiradical activities of many plant extracts. In this method, the DPPH solution loses its colour when transformed to DPPH-H, thus leading to lower absorbance which shows higher radical-scavenging activity (Serteser *et al*., 2008). The antiradical activity (AA) values of the myrtle extracts are shown in Table 2. The AA values show (P<0.05) significant variations among the samples. It is clear that solvent type affected (P<0.05) the radical scavenging capacity of the myrtle fruit extracts and the highest percentage inhibition values were observed in the ethanolic extracts (Table 2). In the acetone extracts, S5 showed the strongest antiradical activity among the myrtle samples. Also, in the methanolic extracts, the highest antiradical activity was observed in S3 to be 43.92%. Water was not found to be an effective solvent for determining antiradical activity. Among the all samples, the lowest antiradical activity value was found in the water extracts to be 6.73% in S8. According to Pearson correlation analysis results, a significant correlation (P<0.05) was observed between the TPC and AA of the myrtle fruit extracts. Chryssavgi *et al*. (2008) found the IC_{50} (mg/l) values of myrtle to be 17.1±0.78 in the samples collected in February and 9.54±0.93 in the samples collected in August. In another study, Hayder *et al*. (2004) reported that the percentage inhibitions of 1000 mg/kg myrtle extract solution were 41±2.1% and 13.3±3% in aqueous and methanolic extracts, respectively.

Antioxidant activity

Antioxidant activity was determined according to the phospomolybdenum method based on the reduction of molybdenum (Mo) (IV) to Mo(V) by the sample analyte and the subsequent formation of green phosphate/Mo(V) compounds. Table 3 shows the antioxidant activity values of myrtle fruit extracts.

Table 2. Antiradical activities of myrtle berry extracts for each solvent type.[1]

	Sample no.	Solvent extracts			
		Acetone	Ethanol	Methanol	Water
Antiradical activity (% inhibition of 1000 mg/kg dilutions)	S1	21.26±0.60^{Ag}	15.46±0.11^{Cg}	20.14±0.13^{Bg}	19.53±0.35^{Bf}
	S2	29.93±0.66^{Ce}	64.41±0.51^{Ab}	31.95±0.53^{Bd}	20.27±0.41^{De}
	S3	29.68±0.43^{Ce}	65.56±1.20^{Aa}	43.92±0.34^{Ba}	29.92±0.43^{Cb}
	S4	37.84±0.54^{Ab}	27.94±0.75^{Ce}	29.95±0.60^{Be}	21.16±0.47^{Dd}
	S5	46.59±0.24^{Aa}	38.18±0.32^{Bc}	37.81±0.34^{Cc}	34.16±0.34^{Da}
	S6	36.66±0.45^{Ac}	31.70±0.51^{Bd}	28.06±0.69^{Cf}	22.25±0.38^{Dc}
	S7	31.99±1.12^{Bd}	22.44±1.06^{Cf}	39.44±2.2^{Ab}	19.99±0.13^{Df}
	S8	25.06±0.37^{Af}	14.37±0.17^{Ch}	15.05±0.41^{Bh}	6.73±0.04^{Dg}

[1] Superscript uppercase letters in same row indicate significant differences between effects of the solvent types (P<0.05); superscript lowercase letters in same column indicate significant differences between effects of the samples (P<0.05).

In our study, the antioxidant capacity values of the myrtle fruit extracts were found to range from 241.533 to 89.333 mg AAE/g dry extract. Among the all samples, the highest antioxidant capacity value was found in S5 to be 241.53 mg AAE/g dry extract while the lowest one was determined in the ethanolic extract of S3. As can be seen from Table 1, the antioxidant capacity of the samples was found to be significantly (P<0.05) different from each other. Also, solvent type showed differences in the antioxidant capacity values of the samples. The highest antioxidant capacity was determined to be 218.28 mg AAE/g dry extract in the acetone extracts while the lowest was in S4 (148.40 mg AAE/g dry extract). In the methanolic extract, samples had a relatively higher antioxidant capacity than the others. According to the Pearson correlation analysis results, significant correlations (P<0.05) were observed between the TPC and antioxidant activity as well as between the antiradical activity and antioxidant activity of the extracts. Although some studies have been conducted to determine the antioxidant capacity of myrtle fruits, the number of those using the phosphomolybdenum method is still limited.

Antimicrobial activity

Antimicrobial activity assay is a method to measure the diameter of the inhibition zone formed by any antimicrobial compound tested against microbial growth on any agar medium. The antimicrobial activities of myrtle extracts are displayed in Table 4. Generally, the ethanolic extract was more efficient than the others with respect to their antimicrobial effects against *S. aureus*, *E. coli* O157:H7, *Y. enterocolitica*, *B. cereus* while the acetone extract was more efficient against *L. monocytogenes* and *Salmonella*. The water extract of S6 showed the lowest antimicrobial effect against all the microorganism types tested. Against *L. monocytogenes, Salmonella* Typhimurium and *S. aureus*, the acetone extract of S1 had the strongest antibacterial activity and the activity values against these pathogens were detected to be 41.69, 29.71 and 28.22 mm, respectively. The highest inhibition zone values against *B. cereus, E. coli* O157:H7 and *Y. enterocolitica* were observed when the ethanolic extracts of S2, S5 and S3 (27.81, 31.3 and 32.41, respectively) were used. Gortzi *et al*. (2008) stated that the results for the inhibition zones were 14±0.3 mm for *S. aureus*, 12±0.1 mm for *E. coli* and 10±0.2 mm for *L. monocytogenes* in the methanolic myrtle extract. Regarding the effect of extract concentration, the effect of the extracts increased as their application concentrations increased, resulting the increased inhibition zone diameters. Until now myrtle essential oils have been mostly used in antimicrobial activity assays rather than their extracts (Gunduz *et al*., 2009; Rasooli *et al*., 2002). Curini *et al*. (2003) investigated the *in vitro* antifungal activity of the essential oils *Erigeron canadensis* and *M. communis* from France, reporting that *M. communis* had stronger antifungal activity than *E. canadensis*. Ozcan and Erkmen (2001) studied the antimicrobial activity of the essential oils of Turkish plant spices and determined that the essential oils of myrtle leaves did not show any inhibitory effect against the tested microorganisms.

4. Conclusions

Our study showed that there were remarkable variations in the contents of antioxidant compounds of the myrtle fruits collected from different locations. On the other hand, the solvent systems used for extraction had an important effect on the type/amount of the bioactive compounds extracted. In addition, we concluded that myrtle fruit may be considered as a good source of natural antioxidants because of its strong antioxidant activity. It was also found

Table 3. Antioxidant activities of myrtle berry extracts for each solvent type.[1]

	Sample no.	Solvent extracts			
		Acetone	Ethanol	Methanol	Water
Antioxidant capacity (mg AAE/g dry extract)	S1	218.28 ± 0.68^{Aa}	145.50 ± 0.43^{Df}	205.49 ± 0.37^{Bd}	160.98 ± 0.56^{Cd}
	S2	197.00 ± 1.74^{Bb}	200.45 ± 0.52^{Bc}	209.58 ± 3.41^{Ac}	145.94 ± 1.32^{Cf}
	S3	174.18 ± 0.87^{Bc}	56.47 ± 0.69^{Cg}	220.82 ± 3.91^{Ab}	171.19 ± 0.71^{Bc}
	S4	148.40 ± 1.80^{Cf}	154.64 ± 1.82^{Be}	181.25 ± 1.56^{Ae}	133.59 ± 1.61^{Dg}
	S5	180.09 ± 0.69^{Dc}	204.42 ± 1.66^{Cb}	241.53 ± 3.16^{Aa}	223.33 ± 1.15^{Ba}
	S6	155.33 ± 4.93^{Ce}	210.71 ± 0.82^{Aa}	211.48 ± 1.58^{Ac}	190.77 ± 0.47^{Bb}
	S7	150.26 ± 0.56^{Ce}	163.42 ± 1.65^{Ad}	162.30 ± 1.39^{Bg}	89.33 ± 0.63^{Dh}
	S8	158.23 ± 7.87^{Bd}	147.41 ± 0.48^{Cf}	173.45 ± 1.41^{Af}	152.21 ± 0.04^{Be}

[1] Superscript uppercase letters in same row indicate significant differences between effects of the solvent types (P<0.05); superscript lowercase letters in same column indicate significant differences between effects of the samples (P<0.05).
AAE = ascorbic acid equivalents.

Table 4. Antibacterial activities (formed inhibition zones, mm) of the myrtle berry extracts obtained using different solvents.

Microorganisms	Sample no.	Solvent extracts															
		Acetone (%)				Ethanol (%)				Methanol (%)				Water (%)			
		1	2	5	10	1	2	5	10	1	2	5	10	1	2	5	10
Bacillus cereus ATCC 33019	S1	10.71	14.19	16.23	18.34	-[1]	7.86	11.57	24.57	11.40	14.76	17.74	22.49	6.60	7.46	10.87	14.20
	S2	7.71	10.02	14.28	15.61	-	14.95	26.41	27.81	9.53	13.46	18.94	23.19	-	6.35	8.78	13.10
	S3	9.26	13.78	20.87	22.68		13.38	21.07	26.34	10.47	11.66	18.62	19.28	-	9.25	12.38	14.07
	S4	6.89	10.57	17.63	21.17	-	12.33	19.74	23.10	7.49	9.75	9.95	11.95	7.16	8.65	11.70	12.35
	S5	9.22	9.49	19.64	21.66	-	21.03	22.30	25.17	12.85	16.81	24.02	28.26	11.45	13.37	15.12	17.78
	S6	5.81	7.26	13.13	17.71	6.43	6.98	11.53	16.86	8.73	9.81	10.35	14.98	-	-	-	11.18
	S7	8.64	11.48	16.56	18.14	3.58	10.14	21.84	26.06	8.56	10.62	16.52	19.27	-	10.14	14.42	16.63
	S8	-	15.54	18.60	20.95	-	12.98	21.74	27.29	8.61	8.87	12.09	17.07	-	7.41	8.34	12.93
Escherichia coli O157:H7 ATCC 33150	S1	14.34	18.06	22.47	25.33	-	19.70	21.09	25.42	16.12	17.52	21.23	23.61	12.08	18.12	20.97	23.18
	S2	13.73	15.09	20.02	21.98		18.18	21.46	23.08	14.10	14.35	17.79	20.03	-	14.42	17.34	20.35
	S3	15.92	18.53	22.77	28.78	10.15	21.55	25.11	28.65	-	21.10	23.43	29.33	16.38	19.42	21.03	23.40
	S4	11.27-	13.31	18.21	22.05	-	17.24	22.26	24.48	13.70	15.22	19.13	23.13	10.38	13.01	17.43	19.33
	S5	15.06	15.73	18.50	20.04	-	22.96	24.09	31.30	16.00	22.92	25.20	28.32	13.70	15.35	19.04	22.12
	S6	-	18.00	19.98	21.45	-	15.38	24.09	25.12	12.30	16.62	22.94	24.57	-	8.60	13.44	14.75
	S7	16.74	20.34	21.59	22.32	11.10	21.85	24.02	25.76	13.26	19.27	20.56	22.43	9.56	13.14	17.58	20.49
	S8	11.01	18.64	25.67	30.31	8.09	13.82	21.83	22.70	-	14.57	18.5	23.00	11.61	13.46	16.96	19.64
Listeria monocytogenes ATCC 7644	S1	20.27	28.42	33.59	41.69	-	18.32	20.11	25.73	18.08	19.29	26.43	30.49	14.56	16.05	18.70	18.83
	S2	15.59	19.19	22.90	24.98	14.54	16.74	18.89	23.73	10.95	16.07	21.16	24.47	-	11.06	14.21	16.93
	S3	17.73	21.40	21.71	32.90	9.05	14.66	18.35	21.26	16.44	21.05	23.02	26.67	-	5.44	11.92	18.36
	S4	16.43	20.02	23.86	29.47	9.11	17.85	20.90	27.21	16.29	20.49	23.23	26.57	11.71	14.75	15.57	18.94
	S5	15.28	27.42	28.44	33.19	14.06	21.63	27.39	32.10	17.42	22.14	25.61	27.70	16.07	18.44	23.81	26.01
	S6	19.89	22.61	23.68	24.82	13.56	18.97	20.74	24.84	11.73	16.52	18.11	23.58	-	7.96	12.51	15.61
	S7	15.54	21.30	25.71	25.79	10.11	16.28	21.95	26.32	15.03	15.44	17.79	28.13	13.63	14.74	20.12	19.82
	S8	11.88	14.63	20.84	20.37	-	17.89	21.54	21.98	11.03-	17.94	20.51	26.09	11.22	13.16	15.18	14.46
Salmonella Typhimurium ATCC 14028	S1	17.02	20.81	23.98	29.71	10.41	18.04	18.81	24.82	-	17.56	23.25	29.70	-	11.30	12.20	14.76
	S2	13.65	17.10	22.40	28.33	13.59	24.63	24.56	27.84	13.11	18.91	20.02	22.86	-	13.39	15.05	16.66
	S3	15.80	19.48	22.57	23.80	-	14.80	22.11	22.72	16.05	18.91	23.26	23.98	17.25	19.06	22.85	25.25
	S4	13.16	16.32	19.26	29.67	-	-	19.90	22.63	13.17	19.80	22.67	26.52	10.28	12.28	14.33	14.79
	S5	14.79	19.37	21.80	26.62	11.50	20.24	23.08	25.27	17.19	17.44	22.67	25.48	-	11.48	13.57	20.02
	S6	19.55	23.34	27.77	28.11	7.81	21.21	21.47	28.36	11.47	15.82	21.35	24.02	-	8.86	9.58	11.11
	S7	17.32	17.87	21.93	22.73	8.62	17.94	18.54	20.20	13.13	17.31	20.04	22.75	-	12.04	14.19	15.00
	S8	14.71	19.16	23.91	27.89	-	14.69	17.11	24.02	11.34	16.41	18.50	25.06	9.70	10.00	12.50	14.30
Staphylococcus aureus ATCC 25923	S1	15.15	18.59	27.61	28.22	-	14.78	25.14	26.43	12.52	18.38	18.56	24.08	-	11.55	12.38	15.16
	S2	12.43	17.75	22.8	25.42	10.92	22.10	25.13	27.80	11.10	12.21	14.01	17.11	-	10.21	11.14	15.4
	S3	15.8	20.09	23.03	23.01	7.34	11.99	20.36	22.82	11.97	12.32	15.46	23.28		12.53	13.4	19.47
	S4	10.30	17.42	18.21	20.78	09.11	17.89	24.91	27.3	7.77	9.78	10.12	10.15	-	8.43	11.49	13.28
	S5	15.86	17.35	21.75	22.34	8.45	18.29	24.09	25.17	10.48	11.24	18.60	20.94	11.95	14.24	14.60	15.1
	S6	-	10.53	18.51	19.02	-	18.59	22.26	23.31	11.76	13.93	18.67	21.77	-	10.01	12.95	13.22
	S7	8.88	11.34	15.42	15.64	9.68	14.52	25.27	26.42	8.58	11.98	16.79	26.49	7.42	10.45	12.55	15.09
	S8	9.97	14.61	22.24	23.4	-	-	22.41	24.51	11.10	15.96	16.68	19.46	-	7.63	12.12	13.52
Yersinia enterocolitica	S1	20.28	21.82	22.25	26.62	-	15.97	24.45	26.69	12.52	18.38	18.56	23.84	-	11.30	12.20	14.76
	S2	11.92	14.34	17.34	27.56	11.42	23.11	24.13	28.80	11.10	12.21	14.10	17.10	-	10.93	13.36	16.66
	S3	12.74	16.52	20.72	23.21	13.64	25.46	29.24	32.41	11.96	13.04	15.78	23.27	-	14.06	15.47	21.04
	S4	8.70	17.47	19.31	27.43	9.11	17.98	24.87	27.30	7.77	9.14	9.70	10.60	-	10.28	14.33	14.79
	S5	14.85	22.49	30.22	32.12	8.45	18.29	24.09	25.16	10.48	11.66	18.6	22.02	-	11.48	13.57	20.02
	S6	10.87	16.43	18.87	32.43	-	18.58	22.24	23.83	11.76	13.93	18.64	21.76	-	8.60	9.58	11.11
	S7	-	13.87	15.29	19.64	-	14.57	25.25	25.42	8.58	11.97	17.5	26.47	-	12.04	13.71	15.48
	S8	-	15.24	22.35	25.50	-	17.22	22.39	24.48	11.32	16.73	16.85	19.44	9.70	10.00	12.50	14.29

[1] - = ineffective.

that myrtle had considerable antibacterial activity and may be used as a natural antimicrobial agent in the food industry.

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