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In vitro antioxidant and antimicrobial activity of some Lamiaceae species

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Abstract

The methanolic extracts, infusions, decoctions and hydrosols of six plants were investigated for their total phenolic contents, antioxidant and antimicrobial activities: *Mentha piperita* (Peppermint), *Thymus vulgaris* (thyme), *Melissa officinalis* (lemon balm), *Ocimum basilicum* (basil), *Rosmarinus officinalis* (rosemary) and *Salvia officinalis* (sage) (Lamiaceae). Total phenolic contents were determined by Folin-Ciocalteu procedure and ranged from 111.03 ± 0.6 (sage methanolic extract) to 19.07 ± 0.0 mg (basil methanolic extract) gallic acid equivalent /g extract. The antioxidant activity was evaluated by two methods, DPPH and phosphomolybdenum assays. The decoction of rosemary showed the highest DPPH radical scavenging activity (IC_{50} = 8.36 µg/mL). The methanolic extract of Peppermint showed the highest total antioxidant activity (241.85 ± 1.9 mg ascorbic acid equivalent /g extract) measured by phosphomolybdenum assay. The antimicrobial activities of herbs were tested against eight bacteria and two yeasts.

Keywords*:* Antimicrobial activity; antioxidant activity; DPPH; Lamiaceae; phenolics

1. Introduction

Reactive oxygen species (ROS) and free radicals are continuously generated by normal metabolic actions such as oxygen metabolism. When there is an over-production of these species or failure in the defense mechanisms including antioxidant compounds and enzymes, these cause oxidative stress [1]. Oxidative stress has been associated with an increased risk of a great number of pathological disturbances, such as atherosclerosis, brain disfunction, cardiovascular disease, cancer, other chronic diseases and death [2-4]. Natural antioxidants present in the diet prevent oxidative damages and they may have a substantial impact on human health. The current interest is toward natural antioxidants, especially plant polyphenolics. Tea and herbal infusions are an important source of antioxidant phenolic compounds in our diet [5]. Herbal supplements are sold in many forms such as capsules, pills, liquids and creams, but the current trend has been to consume these herbal supplements as tea or infusion, especially those traditionally used in folk medicine [6].

Several studies have been conducted for the presence and the activity of antioxidants in tea and herbs but emphasis has been given to organic solvent extracts isolated from dried leaves. Little is known about the phenolic profiles and antioxidant activity in infusions of herbs [7]. The aim of the present study was to examine the total phenolic contents and related total antioxidant and antimicrobial activities of methanolic extracts, infusions and decoctions of six plants (Peppermint, thyme, lemon balm, basil, rosemary and sage) that are consumed as herbal teas and spices in Turkey.

2. Material and methods

2. 1. Chemicals

Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, gallic acid, ascorbic acid, nutrient agar, nutrient broth, malt extract agar and malt extract broth were purchased from Merck. The other chemicals and solvents used were of analytical grade, and were purchased from Merck.

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2. 2. Preparation of herbal extracts

Plants were purchased from local markets at Kayseri, Turkey, as dried material. They were identified botanically by Prof. Dr. Ahmet Aksoy, Biology Department of Erciyes University, Kayseri. The aerial parts of each plant were cleaned and cut into small pieces before extractions. Plants used in this study belong to Lamiaceae family: *Mentha piperita* (Peppermint), *Thymus vulgaris* (thyme), *Melissa officinalis* (lemon balm), *Ocimum basilicum* (basil), *Rosmarinus officinalis* (rosemary) and *Salvia officinalis* (sage).

2. 3. Preparation of methanolic extracts

The plants were ground to a fine powder with a grinder. Then the powdered plant material
(10 g) was extracted using a Soxhlet type extractor with 100 mL methanol (MeOH) at 60 ^{o}C for 6 h. Thereafter, the extract was filtered and evaporated (Rotavator, Buchi, Switzerland) to dryness under vacuum at 40 ^{o}C with a rotary evaporator. After determining the yield, the extract was stored at 4 ^{o}C until further analyses [8].

2. 4. Preparation of infusions

Plant extracts were prepared as tea. The procedure adopted was as follows: 100 mL of boiling water was added to 5 g dried plant minced material. Infusion was left to stay at room temperature without additional heating and was incubated for 5 min (Turkish medicinal plants, following the ethnic use). Supernatants were then filtered Whatman No. 1 paper. Prepared extracts were stored at 4 ^{o}C until further analyses [9].

2. 5. Preparation of hydrosols

Hydrosols of the plants were produced by the Clevenger hydrodistillation method. Plant materials (10 g), cut into small pieces, were placed in a flask with 100 mL of double distilled water and hydrodistilled for 1 h. After hydrodistillation, the oil was collected in cooling vapour to separate the essential oil of the plant. The mixture without essential oil in the flask was identified as hydrosol. The hydrosol was then filtered and preserved in sterile dark bottles at 4 ^{o}C until further analysis [10].

2. 6. Preparation of decoctions

Plant materials (10 g), cut into small pieces, were boiled in water (100 mL) by using an apparatus for re-cooling for 1 h and were filtered, cooled and stored at 4 ^{o}C until use. This mixture is called a decoction [11].

2. 7. Determination of total phenolics in the plant extracts

The total phenolic contents in the plant extracts were estimated by a colorimetric assay based on procedures described by Singleton & Rossi [12]. Briefly, a 40 µL aliquot of plant extract dissolved in the same solvent was pipetted into a test tube containing 2.4 ml of distilled water. After mixing the contents, 200 µL of the Folin and Ciocalteu's phenol reagent, 600 µl of a saturated sodium carbonate solution and 760 µL of distilled water were added. The contents were vortexed for 15 s and then left to stand at room temperature for 2 h. Absorbance measurements were recorded at 765 nm using a spectrophotometer (Shimadzu, UV–Vis Spectrophotometer, Model 1240) and gallic acid was used in the construction of the standard curve. Estimation of the phenolic compounds was carried out in triplicate. The results are mean values and expressed as mg of gallic acid equivalents/g extract.

2. 8. Determination of antioxidant capacity by the phosphomolybdenum method

The antioxidant activity of the plant extracts was evaluated by the phosphomolybdenum method of Prieto, Pineda, & Aguilar [13]. An aliquot of 0.4 mL of sample solution (1 mg/mL) was combined with 4 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). In the case of the blank, 0.4 mL of the solvent was used in place of sample. The tubes were capped and incubated in water bath at 95 ^{o}C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm. The antioxidant capacity was expressed as an equivalent of ascorbic acid (mg ascorbic acid/g dried extract).

2. 9. DPPH radical scavenging activity

The free radical scavenging activity was determined by the DPPH assay described by Lee et al [14]. 50 µL aliquots of the extract dilution at a concentration range of 0.1–2 mg/mL was mixed with 450 µL Tris–HCl buffer (pH=7.4) and 1 mL of the methanolic DPPH solution (0.1 mM). The solvent was used as a control instead of extract. The mixtures were left for 30 min at room temperature in the dark and the absorbance at 517 nm was measured using methanol as blank. Extract concentration providing 50% inhibition (IC_{50}) was calculated using the graph by plotting inhibition percentage against extract concentration. Synthetic

antioxidant reagent butylated hyroxytoluene (BHT) was used as a positive control. The measurements were performed in triplicate and the results were averaged. Radical scavenging activity was expressed as percentage inhibition of DPPH radical and was calculated by the following equation:
% Inhibition=(Absorbance of control-Absorbance of sample /Absorbance of control) x 100

2. 10. Determination of antimicrobial activity

Strains were obtained from type culture collections of the Food Engineering Department, Engineering Faculty at Erciyes University, Turkey. The microorganism strains used in this study were *Bacillus cereus* RSKK 863, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 27736, *Morganella morganii, Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Yersinia enterocolitica* ATCC 1501, *Candida albicans* ATCC 1223, *Saccharomyces cerevisiae* BC 5461.

The agar-well diffusion method was applied to detect antimicrobial activity [8]. All microorganisms were grown at 37 ^{o}C for 18 h in nutrient broth, except *C. albicans*, *S. cerevisiae* were grown in malt extract broth at 27 ^{o}C. Suspensions of microorganisms, adjusted to 10^{6}-10^{7} colony-forming units (cfu)/mL, were placed in flasks containing 25 mL sterile nutrient broth or malt extract broth at 45 ^{o}C and poured into Petri dishes (9 cm in diameter). When the agar was solidified, the equidistant wells (4 mm in diameter) were cut from the agar. The dried plant extracts were dissolved in the same solvent to a final concentration of 50 mg/mL and sterilized by filtration by 0.45 µm Millipore filters. 50 µL of solution of each extract was transferred to the wells. Absolute methanol and water without extract was used as a control. The inoculated plates were incubated at 37 ^{o}C (27 ^{o}C for yeasts) for 18-24 h for bacterial strains, 24-48 h for yeast in the inverted position. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. The diameters of these zones were measured in millimeters. Standard antibacterial agents, Streptomycin (S-10 µg) and Tetracycline (TE-30 µg) were used as positive control.

2. 11. Statistical analysis

Data from the experiments were subjected to analysis of variance (ANOVA) using SPSS [15] for Windows. Percentage data were transformed using arcsine √x before ANOVA. Means were separated at the 5% significance level by the least significant difference (LSD) test. Bivariate correlations were analyzed by Pearson's test using SPSS 10.0 on Windows.

3. Results and discussions

In this study, total extract yields, total phenolic contents, antioxidant and antimicrobial activities of methanolic extracts, infusions and decoctions obtained from *M. piperita* (Peppermint), *T. vulgaris* (thyme), *M. officinalis* (lemon balm), *O. basilicum* (basil), *R. officinalis* (rosemary) and *S. officinalis* (sage) were determined. The methanolic extract yields were determined as 16.0%, 9.0%, 11.0%, 12.0%, 23.0% and 26.0% (w/w) for Peppermint, thyme, lemon balm, basil, rosemary and sage, respectively.

Phenolic (phenolic acids and flavonoids) substances have been shown to be responsible for the antioxidant activity of plant materials [16, 17]. Therefore, the amount of total phenolic contents in the extracts was investigated by the Folin-Ciocalteu method. The content of total phenols is expressed as gallic acid equivalents (mg GAE/g extract). Total phenolic contents in the methanolic extracts, infusions and decoctions of all plants tested are presented in Table 1. These results demonstrate the presence of natural antioxidant phenolic compounds in all these extracts. Generally, total phenolic contents of the methanolic extracts were higher than the infusions and decoctions. Significantly, the highest results for the methanolic extracts were found in the order sage>lemon balm>Peppermint>thyme>rosemary>basil ($p<0.05$). A high content of total phenolics for infusions was observed in lemon balm in comparison with other plant infusions followed by rosemary, sage, basil, Peppermint and thyme, which had the lowest ($p<0.05$). The phenolic contents in the decoctions studied decreased in the order of rosemary>lemon balm>thyme>sage>basil>Peppermint ($p<0.05$).

Table 1. Phenolic contents of the methanolic extracts, infusions and decoctions of each plant tested

Plants	Total phenolic content (mg GAE/g dry extract)		
	Methanolic extract	Infusion	Decoction
Peppermint	91.42±0.6^{b}	22.55±0.1^{e}	20.06±0.1^{f}
Thyme	69.44±2.1^{c}	22. 40±0.2^{e}	39.68±0.1^{c}
Lemon balm	93.40±2.1^{b}	51.08±0.6^{a}	50.25±0.6^{b}
Basil	19.07±0.0^{e}	23.15±0.3^{d}	28.89±0.3^{e}
Rosemary	64.71± .7^{d}	49.80±0.2^{b}	59.23±0.1^{a}
Sage	111.03±0.6^{a}	34.40±0.2^{c}	37.34±0.2^{d}

In each column, means of three independent experiments (± SD) with different superscript letters are significantly different ($p<0.05$). Total phenolic content expressed as gallic acid equivalent (GAE).

The total antioxidant activities of methanolic extracts, infusions and decoctions of six plant species were investigated using the phosphomolybdenum assay and are expressed as ascorbic acid equivalents (mg AAE/g extract). As can be seen from Table 2, the methanolic extracts, infusions and decoctions obtained from plants showed various degrees of antioxidant activity. It can be concluded from the Table 2 that significant differences ($p<0.05$) were found in the antioxidant indices of the tested plants. Significantly, the highest antioxidant activities were determined in the methanolic extracts followed by infusions and decoctions for all of the plants. Peppermint had the highest antioxidant activity, followed by rosemary, thyme, lemon balm and sage, while basil exhibited the weakest activity for the methanolic extracts. Total antioxidant activities of infusions followed the order: lemon balm > rosemary > sage > basil > thyme > Peppermint ($p<0.05$). But total antioxidant activities in the decoctions studied decreased in the order of rosemary > lemon balm > thyme > sage > basil > Peppermint ($p<0.05$).

Table 2. Total antioxidant activities of the methanolic extracts, infusions and decoctions of each plant tested in phosphomolybdenum assay

Plants	Total antioxidant activitiy (mg AAE/g extract)		
	Methanolic extract	Infusion	Decoction
Peppermint	241.85±1.9^{a}	57.34±0.1^{f}	28.14±0.1^{f}
Thyme	228.91±0.3^{b}	58.02±0.1^{e}	48.58±0.4^{c}
Lemon balm	190.19±1.5^{c}	123.02±0.0^{a}	73.46±0.7^{b}
Basil	143.19±0.1^{e}	58.94±0.2^{d}	39.23±0.2^{e}
Rosemary	229.03±1.4^{b}	119.20±0.2^{b}	77.13±0.5^{a}
Sage	156.43±1.3 ^{d}	80.06±0.3^{c}	43.02±0.3^{d}

In each column, means of three independent experiments (± SD) with different superscript letters are significantly different ($p<0.05$). Total antioxidant activity expressed as ascorbic acid equivalent (AAE).

The antioxidant activities of methanol extracts, infusions and decoctions of each plant were assayed by using two test systems. Recent investigations showed differences between the test systems used for the determination of antioxidant activity. Thus, using at least two methods has been recommended. The hydrogen-radical scavenging action is known as an important mechanism of antioxidation. DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of various samples [18]. The free radical scavenging activities of six plants were determined using stable DPHH free radical and the results are summarized in Table 3. As shown in Fig. 1 and Table 3, all methanolic extracts, infusions and decoctions of herbs studied have potent free radical scavenging activities. The decoctions of herbs studied almost completely inhibited DPPH radical (Peppermint-90.26%, thyme-88.99%, lemon balm-92.32%, basil-92.43%, rosemary-94.24% and sage-93.64%) at 2 mg/mL ($p<0.05$). These values of lemon balm, basil, rosemary and sage are higher than that found with the antioxidant standard, BHT at 2 mg/mL. Also, the methanolic extracts and infusions of lemon balm, rosemary and sage showed a high inhibitory effect compared to the BHT. The methanolic extracts and infusions were considerably effective radical scavengers, as well. IC_{50} values (the amount of extract providing 50% inhibition of DPPH radical) are given Table 3. Lower IC_{50} value reflects better protective action of the methanolic extracts, infusions and decoctions. Decoctions were the most effective DPPH radical scavengers compared to methanolic extracts and infusions. The best results were obtained with decoction of rosemary (IC_{50} =8.36 µg/mL). Decoction of rosemary (IC_{50} = 8.36 µg/mL) was more active than the infusion (IC_{50} =11.14 µg/mL) and methanolic extract (IC_{50} =15.15 µg/mL). Also, the decoctions of thyme, lemon balm, basil and sage (IC_{50} = 11.69, 9.07, 20.46 and 14.83 µg/mL, respectiely) were the most active compared to infusions (IC_{50} =39.20, 10.16, 26.37 and 16.45 µg/mL) and methanol extracts (IC_{50} =21.91, 20.16, 41.80 and 15.04 µg/mL). The results show that water was the best solvent for extracting the DPPH radical scavenging components from the plant samples. Thus, it can be suggested that polar compounds present in the herb are mainly responsible for its free radical scavenging activity. The radical scavenging activities of Peppermint varied in order of methanolic extract (IC_{50} = 17.91 µg/mL) > decoction (IC_{50} = 27.58 µg/mL) and infusion (IC_{50} = 35.75 µg/mL). Among the plants tested, rosemary exhibited the highest total antioxidant activity and DPPH radical scavenging activity, while basil exhibited the least activity.

Table 3. Free radical scavenging activities, represented by IC_{50} (µg/ml) of the methanolic extracts, infusions and decoctions of each plant tested in DPPH assay

Plants	IC_{50} (µg/mL)		
	Methanolic extract	Infusion	Decoction
Peppermint	17.91^{d}	35.75^{b}	27.58^{a}
Thyme	21.91^{b}	39.20^{a}	11.69^{d}
Lemon balm	20.16^{c}	10.16^{f}	9.07^{e}
Basil	41.80^{a}	26.37^{c}	20.46^{b}
Rosemary	15.15^{e}	11.14^{e}	8.36^{f}
Sage	15.04^{f}	16.45^{d}	14.83^{c}

In each column, means of three independent experiments (± SD) with different superscript letters are significantly different ($p<0.05$).

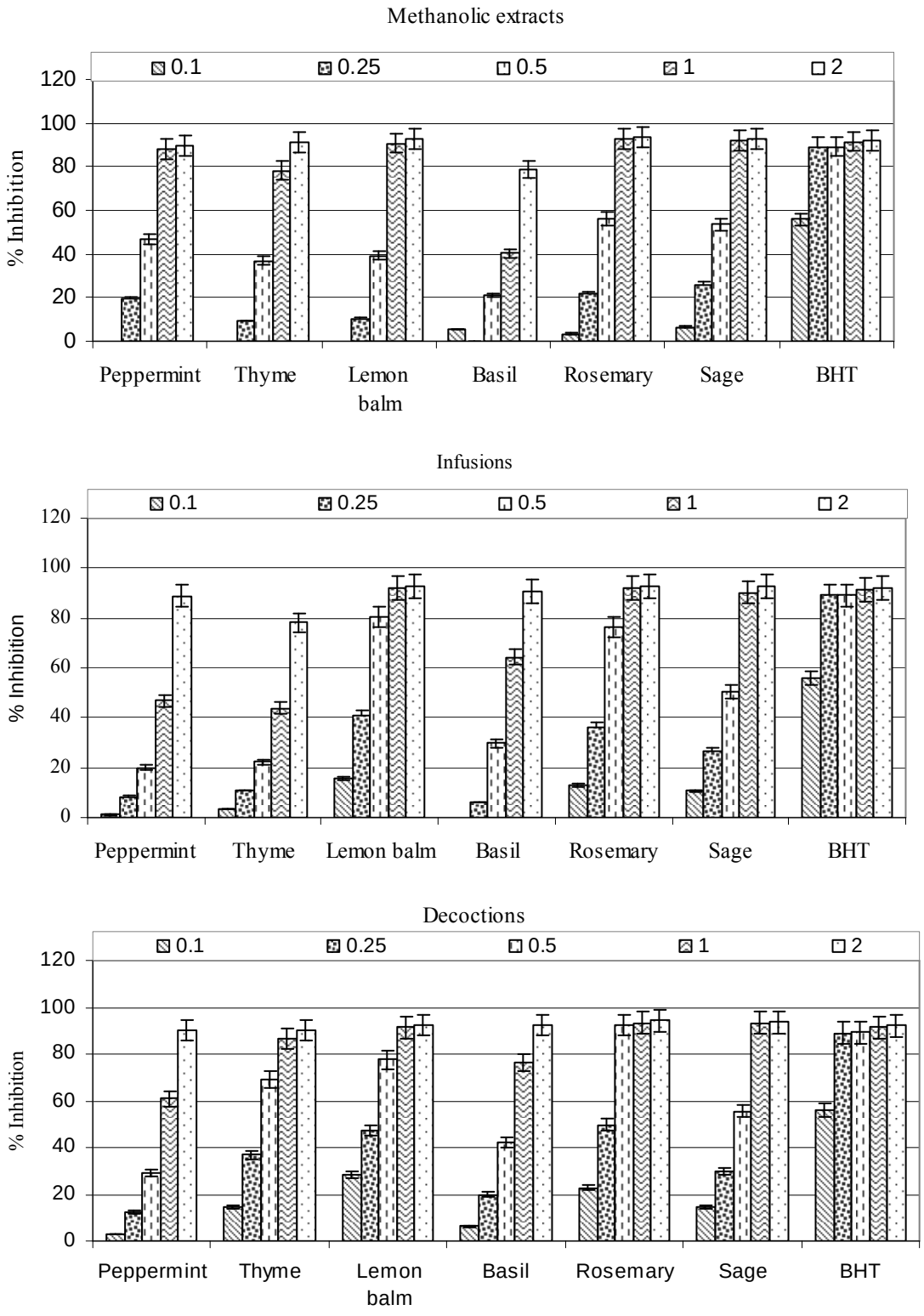

Fig. DPPH absorption inhibition (%) of methanolic extracts, infusions and decoctions of six plants tested

In addition, DPPH radical scavenging activities of the methanolic extracts of the herbs tested correlated significantly with the phenolic contents (r^2=-0.856) and total antioxidant activities (r^2=-0.562). The correlation of the total phenolic contents of infusions with their DPPH radical scavenging activities and total antioxidant activities gave linear curves with correlation coefficients of 0.915 and 0.998, respectively. The content of total phenolics of decoctions showed a good correlation with DPPH radical scavenging activities (r^2=0.943) and total antioxidant activities (r^2=0.971). Also, the total antioxidant assay results of infusions and decoctions correlated with their DPPH radical scavenging assay results (r^2=0.897 and r^2=0.892, respectively).

Tea and herbal infusions are an important source of antioxidant phenolic compounds in our diet but research has focused mainly on black and green tea infusions. Recently, attention was given to other herbal water extracts, which are now investigated for phenolic antioxidants. Most of the traditional herb extracts are prepared from parts of the Lamiaceae family plants [5]. Also, essential oils and extracts obtained by organic solvents of aromatic herbs have been extensively studied for their antioxidant activity in lipid substrates. Very little is known about the possible presence of antioxidants in polar extracts from herbs used in preparation of infusions and decoctions [19]. Thus, the antioxidant activities of infusions and decoctions of Peppermint, thyme, lemon balm, basil, rosemary and sage were examined in the current study.

It was reported that Peppermint has significant antimicrobial and antiviral activities, strong antioxidant and antitumor actions, and some antiallergenic potential [20]. McKay & Blumberg [20] reported previously that the phenolic constituents of the peppermint leave include rosmarinic acid and several flavonoids, primarily eriocitrin, luteolin and hesperidin. It was previously reported that lemon balm and peppermint had a very high content of total phenolics and showed very high DPPH radical scavenging activities [21]. The total phenolic content of basil and lemon balm infusions were determined as 1.10 and 1.04 mmoL trolox /g [22]. Katalinic et al. [9] determined the total phenolic contents (as catechin equivalent) and DPPH radical scavenging capacities of *Melissae*, *Thymus*, *M. piperita*, *Salviae* infusions. The total phenolic content (200 mg/L GAE) and DPPH radical scavenging activity (IC_{50} = 0.30 g/L) of infusion of *T. vulgaris* were determined by Kulišić, Dragović-Uzelac & Miloš [23]. Hinneburg et al. [17] found for hydrodistilled extract obtained from basil there is a lower DPPH radical scavenging activity, but a higher total phenolic content than that of basil decoction tested in this study. The reported gallic acid equivalent capacities of 106 mg/240 mL for mint infusion and 124 mg /240 mL for sage infusion [7] are generally higher than the values given (Table 1). Infusion of *M. officinalis* demonstrated antioxidant activity on ABTS (2,2–azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical decolorization assay with high phenolic content [24]. Mencherini, Picerno, Scesa & Aquino [25] demonstrated that the major component of the ethanol extract of *M. officinalis* and rosmarinic acid had free radical scavenging and antimicrobial activities. The DPPH radical scavenging activity of the methanolic extracts of *O. basilicum* was demonstrated by Jayasinghe, Gotoh, Aoki & Wada [26]. In a previous study, the total polyphenolic contents (1.81, 2.29, 2.08, 2.63 mM GAE, respectively) and DPPH radical scavenging activities of the infusions of salvia, rosemary, thyme and Peppermint were determined. However, these researchers used only herbal tea extracts diluted two hundred times by 50% (v/v) aqueous ethanol solution in DPPH assay [27]. DPPH radical scavenging activities found for infusions of rosemary, thyme, mint and basil were significantly higher than those reported by du Toit, Volsteedt & Apostolides [28], who showed that IC_{50} values of these infusions were 300, 900, 1200 and 1300 µg/mL, respectively.

The antimicrobial activities of the methanolic extracts, infusions, decoctions and hydrosols of the six plants studied are shown in Table 4 and compared with standard antibiotic discs. The extracts, infusions, decoctions and hydrosols obtained from all the plants studied had no effect against *E. coli*. Comparing the six plants studied, it is clear that sage is the most effective. The methanolic extracts, infusions, decoctions and hydrosols of basil had no inhibitory effect against the microorganisms tested. *K. pneumoniae*, *M. morganii* and *S. aureus* were only inhibited by the methanolic extract of sage. Also, hydrosol of thyme was only effective against *Y. enterocolitica*. Among the hydrosols, only thyme hydrosol was effective against *B. subtilis* and *Y. enterocolitica*. Infusions and decoctions of the herbs tested were not effective against bacteria except for sage, and its infusion and decoction showed antibacterial activity against *B. cereus* and *P. aeruginosa. C. albicans* and *S. cerevisiae* were not inhibited by the plants tested.

Table 4. Antimicrobial activities of the methanolic extracts, infusions and decoctions of each plant tested (diameter of the inhibition zone measured in mm)

		Bc	Bs	Kp	Mm	Pa	Sa	Ye
Peppermint	Methanol	-	-	-	-	7.0	-	-
	Infusion	-	-	-	-	-	-	-
	Decoction	-	-	-	-	-	-	-
	Hidrosol	-	-	-	-	-	-	-
Thyme	Methanol	13.0*	-	-	-	10.0	-	-
	Infusion	-	-	-	-	-	-	-
	Decoction	-	-	-	-	-	-	-
	Hidrosol	-	6.0	-	-	-	-	7.0
Lemon balm	Methanol	12.0	-	-	-	8.0	-	-
	Infusion	-	-	-	-	-	-	-
	Decoction	-	-	-	-	-	-	-
	Hidrosol	-	-	-	-	-	-	-
Rosemary	Methanol	-	19.0	-	-	10.5	-	-
	Infusion	-	-	-	-	-	-	-
	Decoction	-	-	-	-	-	-	-
	Hidrosol	-	-	-	-	-		-
Sage	Methanol	15.0	-	10.0	10.0	12.0	8.0	-
	Infusion	7.0	-	-	-	7.0	-	-
	Decoction	8.0	-	-	-	7.0	-	-
	Hidrosol	-	-	-	-	-	-	-
Streptomycin		15.0	16.0	8.0	6.5	8.5	8.0	13.0
Tetracycline		25.0	27.0	15.0	6.5	7.0	15.0	22.0

*: inhibition zones include diameter of hole (4 mm) and disc (6 mm).
Bc: *B. cereus*; Bs: *B. subtilis*; Kp: *K. pneumoniae*; Mm: *M. morganii*; Pa: *P. aeruginosa*; Sa: *S. aureus*; Ye: *Y. enterocolitica*. Streptomycin: S-10 µg; Tetracycline: TE-30 µg-: Not detected

Yadegarinia, Gachkar, Rezaei, Taghizadeh, Astaneh, & Rasooli [29] observed that the oil of *M. piperita* had excellent antimicrobial activities against *E. coli*, *S. aureus* and *C. albicans*. In a previous study, the aqueous and organic extracts of *M. piperita* leaves were found to possess strong antibacterial activity against *B. subtilis, P. aureus, P. aerogenosa, Serratia marcesens* and *Streptococcus aureus* [30]. Our results are not in agreement with this report. However, similar to our results, Gulluce et al. [31] showed that the methanolic extract of *M. longifolia* ssp. *longifolia* almost remained inactive against 30 microorganisms including *B. subtilis*, *K. pneumoniae*, *E. coli*, *S. aureus*, *P. aeruginosa* and *C. albicans*. These differences may be attributed to the genotypic variation, climatic conditions, using different bacteria, different activity assay and different extract concentrations. Similar to our results, Adıgüzel, Güllüce, Şengül, Öğütçü, Şahin, & Karaman [32] showed that the methanolic extract of *O. basilicum* was not effective against *B. cereus*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa*, but was effective against *S. aureus* and *C. albicans*. Conversely, in the present study the methanolic extract of basil was not effective against *S. aureus* and *C. albicans*. Similar results for antimicrobial activity of *R. officinalis* have been found by Celiktas, Kocabas, Bedir, Sukan, Ozek, & Baser [33], who reported that the methanolic extract of this plant exhibited low antimicrobial activity against *S. aureus* and had no activity against *P. vulgaris*, *P. aeruginosa*, *K. pneumoniae*, *Enterococcus fecalis*, *E. coli*, *S. epidermidis*, *B. subtilis* and *C. albicans*. In another report, the main bioactive antimicrobial compounds present in rosemary extracts were determined as carnosic acid and rosmarinic acid, and it was reported that the methanolic extract of rosemary was effective against Gram (+), Gram (-) bacteria and yeasts, in contrast the water extract of rosemary showed narrow activity [34]. Oskay & Sarı [35] reported that ethanolic extract of *R. officinalis* showed broad spectrum antimicrobial activity against Gram (+) and Gram (-) bacteria. There are numerous studies on the antimicrobial potential of essential oil and organic solvent extracts of various herbs and spices. However, there has been no attempt to study the antibacterial activities of the infusions and decoctions from herbs tested in the present study according to our literature survey.

To conclude, the methanolic extracts, infusions and decoctions of Peppermint, thyme, lemon balm, basil, rosemary and sage contain a considerable amount of phenolic compounds and showed strong total antioxidant activities and DPPH radical scavenging activities when compared to standards such as BHT. Good correlation was obtained between DPHH radical scavenging and phosphomolybdenum assays. The amount of total phenolic contents in investigated plants correlated with their antioxidant activity. The results of this study show that the mehanolic extracts, infusions and decoctions of Peppermint, thyme, lemon balm, basil, rosemary and sage can be used as natural sources in food and the pharmaceutical industry due to their strong antimicrobial and antioxidant activities. They can be used in stabilizing food against oxidative deterioration. However, *in vivo* studies are needed to confirm the health-promoting potential of these plants.

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