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Antimicrobial and antioxidant potentials, total phenolic contents of some herbal waters

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ABSTRACT: The aim of the actual study is to evaluate antimicrobial and antioxidant potentials, total phenolic contents of thyme (*Thymus* sp.), myrtle (*Myrtus communis* L.), eucalyptus (*Eucalyptus globulus* L.) and rosemary herbal waters (*Rosmarinus officinalis* L.). They were bought a retailer in Giresun. In the studies, it was determined that only thyme water exhibited antimicrobial activity in all herbal waters. Streptomycine, tetracycline and nystatin which were synthetic antimicrobials demonstrated higher activity than studied herbal waters. Moreover; total flavonoid contents of the tested waters ranges from 50.19±0.0038 µL CE/mL to 126.15±0.004 µL CE/mL. The highest and the lowest total phenolic contents were detected in the thyme water and the eucalyptus water as 688.18±0.009 µL GAE/mL and 24.54±0.0008 µL GAE/mL, respectively. DPPH and ABTS radical scavenging activities of the herbal waters exhibited a dose dependent manner and increased with increasing conentrations. As a result of this study, it was concluded that thyme water could be an alternative to synthetic antimicrobial agents and thyme water, myrtle water, eucalyptus water and rosemary waters might be an alternative to synthetic antioxidative agents. Hence, further and detailed investigations are needed to determine active constituents in the herbal waters.

Keywords: Herbal water; Antimicrobial activity; Bacteria; Fungus; Antioxidant activity; Oxidative stress.

1. INTRODUCTION

Excessive utilization of antibiotic is harmful to human health and ecosystem. It might also leads to increase drug-resistant pathogens. Antibiotic resistance is a worldwide trouble which cause morbidity and mortality. It has been observed that many pathogenic bacteria gain resistance to the antimicrobial drugs quickly. Hence, multiple drug resistant bacteria caused the main failure in the treatment of infectious diseases. It is necessary to investigate and design the alternative drugs to combat resistant bacteria. Bioactive phytochemicals with antibacterial activity could be one of the alternative way to control multiple drug resistant bacteria. Owing to mechanism of action of plants differs from antibiotics, plants might neutralize resistant bacteria [1].

Oxidative stress leads serious diseases such as cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, ulcerative colitis atherosclerosis and aging [2]. Antioxidants can be described as any substance that delays or hinders oxidative damage to a target

molecule. The main property of an antioxidant is its capability to trap free radicals. Antioxidant compounds such as phenols, polyphenols and flavonoids scavenge free radicals such as peroxide, or lipid peroxyl. Plants known as good antioxidant since ancient times [3].

The utilize of plants for the treatment of many illnesses on Earth is an ancient tradition that starts with humans preferring a settled life. Herbal remedies are significant part of the culture of rural communities in developing countries [4].

Herbal water describes the liquid acquired from the distillation of medicinal plants. It can also be known aqueous herbal extract. Different parts of plants, such as flowers, roots, seeds, leaves, fruits use to obtain herbal waters. Herbal waters are commonly utilized as dietary supplements and medicine [5].

The aim of the current study is to evaluate antimicrobial and antioxidant potencies, total phenolic contents of thyme (*Thymus* sp.), myrtle (*Myrtus communis* L.), eucalyptus (*Eucalyptus globulus* L.) and rosemary (*Rosmarinus officinalis* L.) herbal waters.

2. MATERIALS AND METHODS

2.1. Providing of the samples

Myrtle water (commercial hydrosol), thymus water (commercial hydrosol), eucalyptus water (commercial hydrosol) and rosemary water (commercial hydrosol) were bought from a retailer in Giresun, Turkey. The scientific names of the herbs, common name and medicinal functions are detailes in Table 1.

Table 1. Names and medicinal properties of herbal waters.

Scientific name	Use
Thymus sp.	Antimicrobial, appetite stimulant, astringent, anthelmintic and tonic. Thyme also utilizes against intestinal infections like hookworms, bacteria and fungi [6]
Myrtus communis L.	Antimicrobial, antioxidant, antimutagenic, astringent, antiseptic, anti-inflammatory, insecticide activities. Moreover, myrtle leaves are used in sweet liquors which have digestive features [7]
Eucalyptus globulus L.	It can be used as anesthetic, astringent, antiseptic, disinfectant, deodorant, expectorant, hemostat and vermifuge. It is also traditionally used in the treatment of various diseases like arthritis, asthma, bronchitis and wounds [8]
Rosmarinus officinalis L.	Rosemary diterpenes have also antioxidant property which inhibit neuronal cell death [9]

2.2. Microorganisms

Eight bacteria and three yeast species were used in the study. *Staphylococcus aureus* (ATCC 29213) and *Salmonella enterica* (ATCC 14028) were obtained from Giresun Province Control Laboratory. *Enterococcus faecalis* (ATCC 29212) was obtained from were acquired from Rize University Department of Molecular Biology. *Bacillus subtilis* (ATCC 6633), Proteus vulgaris (ATCC 13315), *Enterobacter aerogenes* (CMM 2531), *Candida albicans* (FMC 17) and *Candida tropicalis* (ATCC 13803) were obtained from Fırat University Department of Biology. *Gordonia rubripertincta* (lab isolate) was obtained from Yeditepe University Department of Genetic and Bioenginneering. *Klebsiella pneumoniae* (ATCC 700603) and *Candida parapsilosis* (ATCC 22019) were obtained from Giresun University, Faculty of Education.

2.3. Determination of antimicrobial activities of herbal waters

Disc diffusion method was used to reveal antimicrobial activity of herbal waters. Herbal waters were sterilized by using 0.45 μm pore sized filter. Standards antibiotics and antifungal agents (tetracycline,

gentamycine and nystatin) were used to compare inhibition zones. The turbidity of bacterial suspensions were adjusted 0.5 Mc Farland standard, then, the bacterial suspension inoculated into Müller Hinton Agar plates and allowed to dry. The turbidity of fungal suspensions were adjusted with 0.5 Mc Farland standard (10^7 CFU/mL fungi concentration), then the fungal suspensions spread petri dishes which contain Sabaroud Dextrose Agar and allowed to dry. The discs were put into agar plates. The discs (6 mm diameter) on the petri were impregnated with 20 µL of thymus water, myrtle water, eucalyptus water and rosemary water, separetely. The inoculated plates were standed in refrigerator for one hour. Then, plates were then incubated for 24 h at 37°C for bacteria and 48 h at 30°C for fungi. Diameter of zones were measured with a ruler. The sensitivity of the microorganisms to the studied waters was revealed by measuring the inhibitory zones size on the agar surface around the discs [10-13]. The tests were carried out three times.

2.4. Determination of antioxidant activities of herbal waters

2.4.1. Total phenolic content

Total phenolic compounds of plant waters were determined with Folin–Ciocalteu reagent, according to the method of Slinkard and Singleton [14]. Aliquots (0.1 mL) of the herbal waters were transferred into test tubes and their volumes were made up to 4.6 mL with distilled water. After addition of 0.1 mL Folin–Ciocalteu reagent (previously diluted 3-fold with distilled water) and 0.3 mL 2% Na_2CO_3 solution, tubes were vortexed and the absorbance of the mixture was recorded after 2 h at 760 nm using a spectrophotometer. The quantity of the total phenolic content was denoted as µL of gallic acid equivalent (GAE)/mL. The tests were carried out three times.

2.4.2. Total flavonoid content

Total flavonoids of herbal waters were determined by the procedure of Zhishen et al. [15]. 0.25 mL herbal water was added to 1.25 mL distilled water followed by 75 µL $NaNO_2$ (5%) and incubated for 5 min. Afterwards, 150 µL $AlCl_3.6H_2O$ (10%) was incorporated to the mixture and further incubated for 5 min, the reaction mixture was treated with 0.5 mL NaOH (1 M) and 275 µL distilled water. It was measured spectrometrically at 510 nm. The quantity of the total flavonoid content was denoted as µL of cateschin equivalent (CE)/mL. The tests were carried out three times.

2.4.3. Total antioxidant capacity

The total antioxidant capacity of the herbal waters was evaluated by the phosphomolybdenum method according to the procedure described by Prieto et al. [16]. 0.3 mL herbal water was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer. The quantity of the total antioxidant capacity was denoted as µL of ascorbic acid equivalent (AAE)/mL. The tests were carried out three times.

2.4.4. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

Herbal waters were prepared at 250-1000 µL/mL concentrations. 0.1 mL of each dilution was added to 3.9 mL of a $6x10^{-5}$ M methanolic solution of DPPH followed by vortexing. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. The decrease in absorbance of the resulting solution was measured spectrophotometrically at 517 nm against methanol [17]. The tests were

carried out three times. BHT and Rutin were used as standards. The DPPH radical scavenging activity was calculated using the following equation:

DPPH radical scavenging activity (%) = [(A0 – A1) / A0] x 100

A_0 is the absorbance of the control

A_1 is the absorbance of the sample

2.4.5. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)(ABTS)radical scavenging activity

Herbal waters were prepared at 250-1000 µL/mL concentrations. 150 µL herbal water of each dilution was mixed with 2850 µL of the $ABTS^{\cdot+}$ solution for 2 h in the dark. Then the absorbance was taken at 734 nm using the spectrophotometer [18]. The $ABTS^{\cdot+}$ scavenging activity was calculated using the following equation:

ABTS radical scavenging activity (%) = [(A0 – A1) / A0] x 100

A_0 is the absorbance of the control

A_1 is the absorbance of the sample.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial activity

Disc diffusion method was use to determine the antimicrobial activity of the tested herbal waters. The inhibition zones of studied waters, standard antifungal and antibiotics are presented in Table 2. It was found that the myrtle, eucalyptus and rosemary waters didn't show any antimicrobial activity against tested microorganisms. However, only thyme water inhibited bacteria with inhibition zone diameters ranging from 8-13 mm. *B. subtilis* was resistant to thyme water. Thyme water also had the highest and the lowest antifungal activity against *C. parapsilosis* and *C. tropicalis*, respectively. Streptomycine, tetracycline and nystatin which used standard antibiotic and antifungal agents had higher activity than tested herbal waters.

Table 2. Inhibition zones of tested herbal waters, streptomycine, tetracycline and nystatin.

	Inhibition Zone (mm)						
Microorganisms	**TH**	**MH**	**EH**	**DH**	**Str.**	**Tet.**	**Nys**
B. subtilis	--	--	--	--	20.66±1.15	9.33±0.57	NT
S. aureus	13.33±0.57	--	--	--	14.33±1.15	15.66±0.57	NT
E. faecalis	10.33±0.57	--	--	--	--	17.66±0.57	NT
G. rubripertincta	11.33±0.57	--	--	--	20.66±1.15	15±1.00	NT
E. aerogenes	8.33±0.57	--	--	--	20.66±0.57	12.33±0.57	NT
S. enterica	10.66±0.57	--	--	--	13.33±0.57	15.33±0.57	NT
P. vulgaris	11.66±0.57	--	--	--	21.66±0.57	9.33±1.15	NT
K. pneumoniae	11.33±1.15	--	--	--	18.66±0.57	7.33±0.57	NT
C. albicans	9.33±0.57	--	--	--	NT	NT	22.66±1.15
C. tropicalis	9.33±0.57	--	--	--	NT	NT	23.33±0.57
C. parapsilosis	11.66±0.57	--	--	--	NT	NT	22±1.00

TH: Thymus water, MH: Myrtle water, EH: Eucalyptus water, RH: Rosemary water, Str: Streptomycine, Tet: Tetracycline, Nys: Nystatin, NT: Not Tested; (--): No inhibition. Values are expressed as means of three replicates ± SD.

Herbal waters are commonly utilized in aromatherapy and researchs about their antimicrobial activities in vitro is limited. The antimicrobial effects of thyme, rosemary and mrytle waters have also been determined by other researchers. For example, Sağdıç indicated that two thyme (*Thymus vulgaris* L. and *T. serpyllum* L.) waters possessed a bactericidal action against *Escherichia coli*, *E. coli* O157H:7, *Staphylococcus aureus* and

Yersinia enterocolitica. In accordance with this study, we also found that thyme water had activity against *S. aureus* [19]. Yavuzer and Boğa found that thyme hydrosol had activity against *S. aureus*, *Salmonella paratyphi* and *Klebsiella pneumoniae, Vibrio vulnificus*, *Pseudomonas luteola*. We also found antimicrobial activity against *S. aureus* and *K. penumoniae* [20]. Oral et al. demonstrated that thyme, rosemary and mrytle hydrosols was active against *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. It was revealed also only thyme hydrosol active against *E. coli* [21]. Sağdıç and Özcan revealed that rosemary hydrosol were inactive against microorganisms including *E. coli*. Similarly, we also found no antimicrobial activity of rosemary water [22]. Hay et al. investigated antimicrobial activity of Colombian thyme and rosemary hydrosols against against *E. coli*, *P. aeruginosa*, *S. aureus*, *Candida albicans* and *Aspergillus niger*. Rosemery hydrosol didn't present any activity against the bacteria and fungi up to 500 µL/mL. Thyme hydrosol showed a minimum microbicidal activity of 250 µL/mL against *P. aeruginosa*, *S. aureus*, *C. albicans* and *A. niger*. We also found that thyme water had activity against *C. albicans* and *S. aureus* but rosemary water had no activity against *C. albicans* and *S. aureus* [23].

3.2. Antioxidant activity

Flavonoids are secondary plant metabolites that possess substantial antioxidant and chelating features [24]. Total flavonoid content and total antioxidant capacity of the herbal waters were summarized in Table 3. The maximum total flavonoid content was found in thyme water (126.15±0.004 (µL CE/mL) and the minimum total flavonoid content was found in rosemary water (50.19±0.0038 µL CE/mL). Total antioxidant capacities of the herbal waters increase in the following order: Myrtle water < Thyme water < Rosemary water < Eucalyptus water.

Table 3. Total flavonoid content (TFC) and total antioxidant capacity (TAC) of the herbal waters.

Herbal waters	TFC (µL CE/mL)	TAC (µL AAE/mL)
Thyme water	126.15±0.004	53.75±0.0016
Myrtle water	113.26±0.0004	15.5±0.0024
Eucalyptus water	82.88±0.0002	163.1±0.097
Rosemary water	50.19±0.0038	77.85±0.0075

Values are expressed as means of three replicates ± SD.

DPPH solution exhibits demonstrates a deep purple colour at 517 nm. This purple colour usually discolours when antioxidant molecules scavenges DPPH radicals and turns into them a bleached product [24]. DPPH radical scavenging activities of the herbal waters are given in Table 4. DPPH radical scavenging activity of the tested waters and standards (Rutin and BHT) increased steadily with increasing concentration of samples.

The highest DPPH radical scavenging activity were observed in myrtle water (54.50±0.012) and the lowest DPPH radical scavenging activity were observed eucalyptus water (16.53±0.015) at 1000 µL/mL concentrations. All plant hydrosols have lower activity than standards (BHT and Rutin).

ABTS radical scavenging activities of the tested herbal waters are illustrated in Table 5. ABTS radical scavenging activities of the waters were compared with the standards BHT and Rutin. The strongest antioxidant properties, determined by ABTS radical scavenging assay, were in thyme water even better than standard antioxidants. Activities of the herbal waters increase with the increasing concentration. Rutin and BHT exhibited higher activity than the tested waters except for thyme water. ABTS radical scavenging

activity of the herbal waters and standards increased in the following order: Thyme water > BHT > Rutin > Rosemary water > Eucalptus water > Myrtle water.

Table 4. DPPH scavenging activity of the herbal waters and standards.

Herbal Water	Concentration (µL/mL) (% inhibition)	DPPH scavenging activity
Eucalyptus water	250	2.68±0.005
	500	8.12±0.014
	750	14.08±0.010
	1000	16.53±0.015
Rosemary water	250	12.64±0.008
	500	14.80±0.013
	750	22.03±0.007
	1000	22.72±0.004
Myrtle water	250	40.42±0.012
	500	42.80±0.017
	750	46.35±0.017
	1000	54.50±0.012
Thyme water	250	23.27±0.004
	500	38.02±0.006
	750	50.56±0.007
	1000	50.74±0.012
Rutin	250	88.17±0.005
	500	88.59±0.009
	750	90.21±0.005
	1000	92.25±0.002
BHT	250	84.62±0.007
	500	86.39±0.004
	750	89.49±0.009
	1000	90.50±0.007

Values are expressed as means of three replicates ± SD.

Table 5. ABTS radical scavenging activity of the herbal waters and standards.

Herbal water	Concentration (µL/mL) (% inhibition)	DPPH scavenging activity
Eucalyptus water	250	13.95±0.019
	500	23.66±0.027
	750	28.29±0.013
	1000	31.93±0.019
Rosemary water	250	16.80±0.852
	500	25.63±0.725
	750	29.31±0.684
	1000	35.85±0.619
Myrtle water	250	10.93±0.023
	500	17.36±0.009
	750	21.23±0.019
	1000	23.51±0.010
Thyme water	250	96.66±0.0004
	500	99.04±0.0006
	750	99.54±0.0004
	1000	99.65±0.0002
Rutin	250	78.54±0.048
	500	81.94±0.019
	750	85.26±0.010
	1000	87.63±0.006
BHT	250	93.48±0.011
	500	93.92±0.006
	750	94.43±0.004
	1000	96.65±0.008

Values are expressed as means of three replicates ± SD.

Antioxidant activity of thyme, rosemary and eucalptus hydrosols was searched by other researchers. Hay et al. found that rosemary and thyme hydrosols had ABTS radical scavenging activity [23]. Gharb revealed that *Eucalyptus camaldulonsis* hydrosol had DPPH radical scavenging activity and ferric reducing antioxidant potential (FRAP) [25]. Jeon et al. worked out antioxidant activity of rosemary hydrosols produced in Jeju. It was concluded that rosemary hydrosol had DPPH and ABTS radicals scavenging activity but it hadn't Fe^{++} ion chelating activity [26]. We also found DPPH and ABTS radicals scavenging activities in thyme water, eucalyptus water and rosemary water.

3.3. Total phenolic content

Total phenolic content of herbal waters were presented in Table 6. Total phenolic contents in the examined herbal waters ranged from 24.54±0.0008 µL GAE/mL to 688.18±0.009 µL GAE/mL. The maximum total phenolic content was detected in thyme water and the minimum total phenolic content was found eucalyptus water.

Table 6. Total phenolic contents (TPC) of herbal waters.

Herbal water	TPC (µL GAE/mL)
Thyme water	688.18±0.009
Myrtle water	40.45±0.0003
Eucalyptus water	24.54±0.0008
Rosemary water	105.90±0.0008

Values are expressed as means of three replicates ± SD.

4. CONCLUSION

Synthetic antioxidants and antimicrobials cause side effects to the body and doubts about their reliability cause studies on natural products in recent years. Phenolic compounds which found in plants and plant products is focused studies to plant essential oils and hydrosols. This study demonstrated that thyme water can be an alternative to synthetic antimicrobial agents and thyme water, myrtle water, eucalyptus water, rosemary water can be an alternative to antioxidant agents. The presented results will be the base for future research but it is needed detailed investigations for a better understanding of the active compounds involved in herbal waters.

Authors' Contributions: SA: studied antioxidant activity of the herbal waters and structured the paper. AC: studied antimicrobial activity of the herbal waters and edited the manuscript. Both authors read and approved the final manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

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