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An Investigation of Antibacterial and Antioxidant Activity of Nettle *(Urtica dioica L.),* Mint *(Mentha piperita),* Thyme *(Thyme serpyllum)* and *Chenopodium album L.* Plants from Yaylacık Plateau, Giresun, Turkey

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ABSTRACT

Ethanol, chloroform and hexane extracts from plants namely *Urtica dioica L., Mentha piperita, Thyme serpyllum and Chenopodium album L.* were evaluated for their total phenolic and total flavonoid contents, antioxidant and antibacterial efficiencies. The antioxidant activities were screened utilizing DPPH radical scavenging activity, ABTS scavenging activity, CUPRAC activity and total antioxidant capacity. Antibacterial activity of the tested extracts was determined by disc diffusion and broth dilution methods. *U. dioica* and *C. album* extracts showed varying activities against the test bacteria. The hexane extracts of *T. serpyllum* and *C. album* showed the weakest copper reducing antioxidant capacity (CUPRAC) activity. 2,2-diphenyl-1picrylhydrazyl (DPPH) activity of the solvents are increased in the following order: Ethanol>Chloroform>Hexane. Our results revealed that all of the tested plants might be an alternative to synthetic antioxidant and antibacterial agents.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi 7(1): 73-80, 2019

Isırgan (Urtica dioica L.), Nane (Mentha piperita), Kekik (Thyme serpyllum) and Chenopodium album L. Bitkilerinin Antimikrobiyal ve Antioksidan Aktivitesinin Araştırılması, Yaylacık Yaylası, Giresun, Türkiye

MAKALE BİLGİSİ

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ÖZ

Urtica dioica L., Mentha piperita, Thyme serpyllum ve *Chenopodium album L.* gibi bitkilerden elde edilen etanol, kloroform ve heksan ekstraktları, toplam fenolik ve toplam flavonoid içerikleri, antioksidan ve antibakteriyel verimleri açısından değerlendirilmiştir. Antioksidan aktiviteler DPPH radikal giderme aktivitesi, ABTS giderme aktivitesi, CUPRAC aktivitesi ve toplam antioksidan kapasite kullanılarak tarandı. Ekstraktların antibakteriyel özelliği disk difüzyon ve broth dilüsyon yöntemleri ile yapıldı. *U. dioica* ve *C. album* ekstreleri, test bakterilerine karşı çeşitli aktiviteler gösterdi. *T. serpyllum* ve *C. album*'ün heksan ekstraktları, en zayıf bakır azaltıcı antioksidan kapasite (CUPRAC) aktivitesini gösterdi. Çözücülerin 2,2-difenil-1-pirilhidrazil (DPPH) aktivitesi, aşağıdaki sırayla artmaktadır: Etanol> Kloroform> Hekzan. Elde ettiğimiz sonuçlar, test edilen tüm bitkilerin sentetik antioksidan ve antibakteriyel ajanlara alternatif olabileceğini gösterdi.

Introduction

Antibiotics are certainly one of the most crucial therapeutic discoveries of the 20th century. On the other hand, only one third of the infectious illnesses have been cured from these synthetic medicines. Because of widespread indiscriminate and misuse of antibiotics, antibiotic resistance has raised in the recent years. One of the ways to decrease the resistance to antibiotics is by utilizing antibiotic resistance inhibitors obtained from plants. Plants generate many compounds to defend themselves against the pathogens (Sen and Batra, 2012).

Antioxidants are substances that can preserve cells from the harmful effect by unstable molecules such as reactive oxygen species (ROS) and free radicals. These unstable molecules are responsible for many health problems such as cancer (Tarlovsky, 2013; Thyagarajan et al., 2018) cardiovascular diseases (Leopold, 2015; Mangge et al., 2014), heart diseases (Nuttall and Kendall, 1999; Chen and Alpert, 2016), inflammation (Arulselvan et al., 2016; Grimble, 1994) and gastric problems (Tandon et al., 2004; Saxena et al., 2012)

Urtica dioica has been used for hundreds of years to treat rheumatism, eczema, urinary tract infections, kidney stones and early stages of an enlarged prostate. It is also used traditionally for the treatment of nose bleeding, arthritis, anemia, hay fever, diuretic, astringents, blood builders, skin complaints, gout, sciatica, neuralgia, hemorrhoids and hair problems (Ahmed and Parsuraman, 2014).

Mentha piperita L, is an aromatic plant and produces rich essential oils which finds different applications in industry. Moreover, peppermint possesses antiviral, anti-aging, antioxidant and antimicrobial properties (Okmen et al., 2017).

Thyme serpyllum is generally utilized for many functions such as carminative antiseptic, anthelmintic, tonic, sedative, and expectorant. Moreover, ice produced from *Thyme serpyllum* hydrosol has proved preservative property against fish storage (Rehman et al., 2007).

Chenopodium album uses as anthelmintic, diuretic, tonic, laxative, abdominal pain and eye problems. Moreover, it also improves appetite (Yadav et al., 2007).

This study aims to antioxidant and antibacterial activities of the extracts of *Urtica dioica*, *Mentha piperita*, *Thyme serpyllum* and *Chenopodium album L*. from Yaylacık plateau, Giresun, Turkey.

Materials and Methods

Collection of Plant Samples

Nettle (*Urtica dioica*), mint (*Mentha piperita*), thyme (*Thyme vulgaris*) and lamb's quarters (*Chenopodium album)* were collected in 2017 from Giresun. The collected plant materials were dried in the room conditions for three weeks.

Test Microorganisms

Ten bacteria were used in this work. *Staphylococcus aureus* subsp. *aureus* ATCC 25923, *Salmonella enterica* serovar *typhimirium* ATCC 14028, and *Listeria monocytogenes* ATCC 7644 were obtained from Giresun Province Control Laboratory; *Enterobacter aerogenes* CCM 2531, *Bacillus subtilis* IMG 22 and *Proteus vulgaris* FMC 1 were obtained from Department of Biology at Fırat University; *Enterococcus faecalis* ATCC 29212 and *Bacillus cereus* 702 ROMA were obtained from Department of Molecular Biology at Rize University; *Escherichia coli* ATCC 35218 was acquired from Giresun University Faculty of Education; *Gordonia rubripertincta* (lab isolate) was obtained from Department of Genetic and Bioenginneering at Yeditepe University.

Chemicals

Butylated hydroxytoluen (BHT), 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were purchased from Fluka Chemical Co. (Buchs, Switzerland). 2,2-diphenyl-1-picryl-hydrazyl (DPPH), gallic acid, catechin, neocuproine, $CuCl_2$, ammonium acetate, sulfuric acid, sodium phosphate, ammonium molybdate, ascorbic acid, rutin, Dimethyl sulphoxide (DMSO), Mhüller Hinton Agar and Mhüller Hinton Broth were obtained from Sigma Chemical Co. (St. Louis, MO, USA). was purchased from Sigma

Obtaining of the Crude Extracts

Fifteen grams of the ground nettle, mint, thyme and *C. album* were extracted in each of 150 mL ethanol, chloroform and hexane solvents, separately for 24 h at room temperature with shaking at 150 rpm. The extraction process was followed by filtration using Whatman filter paper no. 1. The filtered extracts were concentrated using a rotary evaporator (Heidolph G3 Rotary Evaporator, America) (Obeidat et al., 2012).

Antimicrobial Activity

The antibacterial activity of the extracts was identified by disc diffusion assay and Minimum Inhibition Concentration (MIC) methods. The extracts were dissolved in DMSO at 30 mg/mL concentration. Dissolved extracts were sterilized through 0.45 µm Millipore filters (Murray et al., 1995). Tetracycline and ciprofloxacin were used as standard antibacterial agents. The turbidity of bacterial suspensions were adjusted 0.5 Mc Farland standard (10^8 CFU/mL), then, the bacterial suspension inoculated into MHA plates and allowed to dry. Discs (6 mm diameter) were put onto inoculated agar. 25 µL ethanol extract of ground nettle, 25 µL chloroform extract of ground nettle, 25 µL hexane extract of ground nettle, 25 µL ethanol extract of mint, 25 µL chloroform extract of mint, 25 µL hexane extract of mint, 25 µL ethanol extract of thyme, 25 µL chloroform extract of thyme, 25 µL hexane extract of thyme, 25 µL ethanol extract of *C. album*, 25 µL chloroform extract of *C. album*, 25 µL hexane extract of *C. album* and 25 µL DMSO were added to discs, separately (Saric et al., 2009). The inoculated plates were standed in refrigerator for one hour then plates were incubated at 37°C overnight. Diameter of zones were measured.

The 96 well plates were prepared by dispensing into each well 95 µL of Mhüller Hinton Broth and 5 µL of the inoculum. 100 µL plant extracts initially prepared at the concentration of 1 mg/ mL was added into the first wells. Then, 100 µL from their serial dilutions were added into seven consecutive wells. This 96 well plate was incubated

at 37°C for bacteria overnight The MIC was expressed as the lowest concentration of the test compounds to inhibit the growth of microorganisms (Yiğit et al., 2009).

Total Phenolic Content

Amount of total phenolic content in the extracts were evaluated using the Folin-Ciocalteu solution by the procedure of Slinkard and Singleton (1977). The dried plant extracts were dissolved in DMSO to obtain 1 mg/mL concentration. Gallic acid was used as a standard in the study. The content of total phenolic compounds was calculated from the calibration curve of gallic acid standard solution and denoted as µg GAE/mL. The tests were performed in triplicate.

Total Flavonoid Content

Total flavanoid contents in the extracts was studied according to Zhishen et al. (1999). The dried plant extracts were dissolved in DMSO to get 1 mg/mL concentration. The content of total flavonoid compounds was calculated from the calibration curve of catechin standard solution and expressed as µg CE/mL. The analyses were performed in triplicate.

Total Antioxidant Capacity

Total antioxidant capacity of the extracts were defined by the method of Prieto et al. (1999). The dried plant extracts were dissolved in DMSO to get 1 mg/mL concentration. Absorbance was measured at 695 nm. Ascorbic acid used as the standard. The total antioxidant capacity expressed as µg ascorbic acid equivalent (AAE)/mL. The tests were performed in triplicate (Prieto et al. 1999).

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of the extracts was studied using the method of Brand Williams et al. (1995). Ethanol extracts of the plants were dissolved in DMSO to get 25-100 µg/mL concentration. Chloroform extracts of the plants were dissolved in DMSO to get 150-300 µg/mL concentration. Hexane extracts of the plants were dissolved in DMSO to get 250-1000 µg/mL concentration. The tests were carried out three times. The following formula is used to calculate the percent inhibition:

$$\% \text{ Inhibition} = [1 - (A/B)] \times 100$$

A : Absorbance of control;

B : The absorbance of sample

Copper Reducing Antioxidant Capacity (CUPRAC)

0.5 mL extract (prepared in 250-1000 µg/mL), 1.0 mL $CuCl_2$ solution (1×10^{-2}), 1.0 mL neocuproine solution (7.5×10^{-3} M) and 1.0 mL ammonium acetate buffer (1.0 M, pH: 7.0) were mixed in a test tube. Then, the tube was vortexed and stored in a dark place for 30 min. absorbance was read at 450 nm. Butylated hydroxytoluene (BHT) was used as standard antioxidant agent (Özyürek et al., 2009).

Statistical Analysis

Data were analyzed using One way ANOVA and Duncan's post hoc tests by using IBM SPSS 24 software (SPSS Inc., Chicago, IL, USA) with a significance level of 5%. Antioxidant tests were carried out three times.

Results and Discussion

Antibacterial Activity

Antibacterial activity of the plants was demonstrated in Table 1. Inhibition zone values ≤8 mm were considered as not active against microorganisms (Bhalodia and Shukla 2011). The results revealed that all the extracts are potent antimicrobials against all the microorganisms studied except for extracts of thyme and mint. Inhibition zones of the studied extracts ranged from 6-15 mm. The highest inhibition zone was found in the ethanol and chloroform extracts of nettle. The lowest inhibition zone was observed in the hexane extract of thyme. Tetracycline and ciprofloxacin exhibited higher zones when compared with the extracts. DMSO which used as negative control had no inhibitory effect against the test bacteria.

The different sensitivity levels of the bacterial species could be originated from the intrinsic tolerance of the bacteria and combinations of phytochemicals available in the plants (Al-Sum and Al-Arfaj 2013).

MIC values of the extracts demonstrated in Table 2. MIC values of the extracts of ranges from 256 µg/mL to >1024 µg/mL for nettle and it ranges from 128 µg/mL to 1024 µg/mL for *C. album.*

There are different studies about antimicrobial activity of nettle, mint, thyme and *C. album.* For example, Kukric et al. (2012) found that methanol extract of nettle were active against *B. subtilis, E., Pseudomonas aeruginosa* and *Lactobacillus plantarum*. Likewise, we found activity in ethanol extract of nettle against *B. subtilis* but the activity was higher than Kukric's study.

Mzid et al. (2017) found that ethanol extract of nettle possessed activity against *B. subtilis, S. aureus* and *Salmonella enteritis* but no activity against *E. coli* and *E. faecalis*. In agreement with this survey, we found activity against *B. subtilis* but we found weak activity against *S. aureus* and *S. typhimirium.*

Al-Sum and Al-Arfaj (2013) investigated antibacterial activity of aqueous extract of mint against *Bacillus fastidious, S. aureus, Proteus mirabilis, P. vulgaris, Salmonella choleraesuis, E. coli, P. aeruginosa, Klebsiella pneumoniae* and *Serratia odorifera*. It was found activity against *S. aureus* and *S. choleraesuis.* In aggrement with this study, we found activity in chloroform extract of mint against *S. aureus*, but no activity in hexane and ethanol extracts against *S. aureus*. Moreover, all the tested extracts showed no activity towards *E. coli, P. vulgaris*. Different results might be associated with using different solvents and collecting plant materials from different locations.

Pramila et al. (2012) revealed that methanol extract of peppermint had antibacterial activity against *E. coli, S. aureus* and *Acinetobacter*. In our study, chloroform extract of peppermint exhibited very low activity against *S. aureus* and ethanol, whereas hexane and ethanol extracts did not show any activity to *S. aureus*.

Rota et al. (2008) searched antibacterial action of some Thyme species such as *Thyme vulgaris, Thyme zygis* subsp. *gracilis* and *Thyme hyemalis.* Maksimovic et al. (2008) demonstrated that essential oil of *Thyme pannonicus* possessed antimicrobial activity against the test microorganisms.

Table 1 Inhibition zones of the extracts (mm)

Plant Extracts	1	2	2	4	5	6	7	8	9	10
EEN	8	15	7	9	7	10	8	NA	8	7
CEN	11	15	12	13	7	10	10	NA	8	7
HEN	11	7	7	12	7	8	10	NA	8	10
EET	7	8	7	8	NA	NA	NA	NA	NA	NA
CET	8	7	8	7	NA	NA	NA	NA	NA	NA
HET	8	8	6	8	NA	NA	NA	NA	NA	NA
EEM	8	8	NA	NA	NA	NA	NA	NA	NA	NA
CEM	8	NA	7	7	NA	NA	NA	NA	NA	NA
HEM	8	8	NA	NA	NA	NA	NA	NA	NA	NA
EEC	8	10	9	NA	7	8	NA	NA	8	NA
CEC	7	11	12	10	7	8	NA	NA	NA	NA
HEC	10	10	15	12	7	8	NA	NA	NA	NA
Tetracycline	14	10	17	13	18	15	10	18	17	20
Ciprofloxacin	36	22	24	32	32	26	30	34	28	18

1: S. Typhimirium, 2: E. Aerogenes, 2: S. Aureus, 4: B. subtilis, 5: B. cereus, 6: L. Monocytogenes, 7: P. Vulgaris, 8: E. Coli, 9: G. rubripertinca, 10: E. Faecalis; NA: No Activity: Tetracycline (10µg/disc); Ciproflaxacin (5 µg/disc); EEN: Ethanol extract of nettle; CEN: Chloroform extract of nettle; HEN: Hexane extract of nettle; EET: Ethanol extract of thyme; CET: Chloroform extract of thyme; HET: Hexane extract of thyme; EEM: Ethanol extract of mint; CEM: Chloroform extract of mint; HEM: Hexane extract of mint; EEC: Ethanol extract of *C. album*; CEN: Chloroform extract of *C. album*; HEC: Hexane extract

Table 2 MIC values of the extracts (µg/mL)

Plant Extracts	1	2	3	4	6	7	10
EEN	NT	256	NT	256	512	NT	NT
CEN	256	256	512	256	512	1024	NT
HEN	0.512	NT	NT	1024	NT	1024	>1024
EEC	NT	128	512	NT	NT	NT	NT
CEC	NT	512	1024	512	NT	NT	NT
HEC	512	512	512	512	NT	NT	NT

1: S. Typhimirium, 2: E. Aerogenes, 2: S. Aureus, 4: B. subtilis, 6: L. Monocytogenes, 7: P. Vulgaris, 10: E. Faecalis; NT: Not tested

Table 3 Total phenolic and flavonoid contents of the extracts

Plant Extract	µg GAE/mL	µg CE/mL
EEN	29.20 ± 0.56^{d}	142.19 ± 0.83^{d}
CEN	14.77 ± 0.61^{bc}	27.16 ± 0.29^{a}
HEN	8.73 ± 0.86^{ab}	42.56 ± 0.05^{a}
EEM	155.79 ± 0.53^{f}	300.06 ± 1.65^{f}
CEM	18.53 ± 0.34^{bc}	122.33 ± 0.39^{c}
HEM	11.48 ± 0.39^{abc}	209.08 ± 0.83^{e}
EET	137.69 ± 1.64^{e}	146.48 ± 5.42^{d}
CET	17.71 ± 1.40^{c}	90.75 ± 0.55^{b}
HET	11.17 ± 0.44^{ab}	49.43 ± 0.33^{a}
EEC	18.36 ± 0.90^{bc}	24.46 ± 0.50^{a}
CEC	9.23 ± 0.93^{ab}	15.55 ± 0.62^{a}
HEC	3.48 ± 2.34^{a}	25.18 ± 0.02^{a}

Different letters in the column represent significant differences (P<0.05) for each assay, individually.

Table 4 Total antioxidant capacity of the extracts

Plant Extract	µg AAE/mL
EEN	71.27 ± 0.96^{abc}
CEN	68.19 ± 0.15^{abc}
HEN	57.05 ± 0.83^{abc}
EEM	149.56 ± 0.47^{c}
CEM	82.72 ± 0.36^{bc}
HEM	79.05 ± 0.34^{abc}
EET	104.84 ± 0.94^{abc}
CET	81.30 ± 0.02^{bc}
HET	76.76 ± 0.50^{abc}
EEC	37.60 ± 0.41^{a}
CEC	55.99 ± 0.64^{abc}
HEC	41.62 ± 0.50^{ab}

Different letters in the column represent significant differences (P<0.05) for each assay, individually.

Table 5 CUPRAC and DPPH radical the scavenging activity of extracts

Plant Extract	Concentration	CUPRAC Activity (Absorbance)*	Concentration	DPPH Scavenging Activity (% inhibition)
EEN	250	0.557±0.027[a]	25	9.37±0.54[a]
	500	0.823±0.030[a]	50	12.32±0.30[a]
	750	1.159±0.012[a]	75	17.19±0.62[a]
	1000	2.172±0.019[b]	100	25.29±0.43[a]
CEN	250	0.328±0.043[a]	150	5.87±0.21[a]
	500	0.333±0.054[ab]	200	8.91±0.83[a]
	750	0.389±0.002[ab]	250	11.14±0.03[a]
	1000	0.633±0.024[b]	300	17.01±0.19[a]
HEN	250	0.241±0.042[a]	250	2.55±0.01[a]
	500	0.263±0.023[a]	500	4.71±0.13[a]
	750	0.333±0.032[a]	750	7.66±0,24[a]
	1000	0.421±0.004[a]	1000	8.76±0.04[a]
EEM	250	1.317±0.011[a]	25	62.14±0.75[a]
	500	1.470±0.042[a]	50	69.28±0.82[a]
	750	1.941±0.036[a]	75	74.16±0.94[a]
	1000	2.254±0.046[a]	100	75.21±0.70[a]
CEM	250	0.317±0.002[a]	150	36.07±1.57[a]
	500	0.458±0.029[ab]	200	38.05±0.67[a]
	750	0.554±0.007[b]	250	40.65±0.23[a]
	1000	0.840±0.030[c]	300	41.56±1.08[a]
HEM	250	0.154±0.002[a]	250	21.18±0.64[a]
	500	0.171±0.004[a]	500	21.62±0.31[a]
	750	0.213±0.020[a]	750	32.13±0.74[a]
	1000	0.317±0.001[a]	1000	38.26±0.03[a]
EET	250	1.331±0.008[a]	25	60.37±0.20[a]
	500	2.052±0.128[a]	50	68.45±0.40[a]
	750	2.518±0.071[a]	75	71.78±0.69[a]
	1000	2.560±0.122[a]	100	73.82±1.16[a]
CET	250	0.235±0.008[a]	150	30.43±0.47[a]
	500	0.360±0.005[ab]	200	31.54±0.23[a]
	750	0.594±0.003[b]	250	32.82±0.25[a]
	1000	0.630±0.007[b]	300	34.86±0.90[a]
HET	250	0.125±0.002[a]	250	25±0.73[a]
	500	0.153±0.007[a]	500	26.27±0.34[a]
	750	0.396±0.010[a]	750	28.69±0.89[a]
	1000	0.503±0.013[a]	1000	28.58±0.16[a]
EEC	250	0.160±0.001[a]	25	21.48±1.46[a]
	500	0.360±0.003[ab]	50	23.07±0.33[a]
	750	0.474±0.010[ab]	75	24.26±0.92[a]
	1000	0.587±0.005[b]	100	24.90±0.78[a]
CEC	250	0.223±0.011[a]	150	16.25±1.25[a]
	500	0.413±0.012[ab]	200	16.48±0.34[a]
	750	0.483±0.004[ab]	250	17.41±1.00[a]
	1000	0.659±0.016[b]	300	17.47±0.37[a]
HEC	250	0.140±0.002[a]	250	9.28±0.34[a]
	500	0.212±0.003[a]	500	9.82±0.49[a]
	750	0.387±0.010[a]	750	10.92±0.47[a]
	1000	0.421±0.014[a]	1000	13.10±0.28[a]
BHT	250	0.658±0.015	250	86.47±0.14
	500	0.790±0.005	500	87.47±0.40
	750	0.850±0.009	750	90.76±0.41
	1000	0.972±0.017	1000	92.08±0.32
Rutin	250	NT	250	89.37±0.54
	500	NT	500	90.45±0.85
	750	NT	750	92.73±0.24
	1000	NT	1000	92.27±2.78

Different letters in the column represent significant differences (P<0.05) for each assay, individually. NT: Not tested

Varga et al. (2015) demonstrated that *Thymus vulgaris* and *Thymus seryllum* have the highest activity against the microorganisms studied in their work with essential oils, which is related to thymol content. The researchers also stated that essential oils of *Thymus* strains are highly active disinfectants so they can be used as evaporators, which can be applied against different human pathogenic bacteria and yeasts.

Lone et al. (2017) showed that methanol extract showed maximum antibacterial action towards *S. aureus*, while as mild inhibitory effect was revealed against *E. coli* among the tested strains.

The antimicrobial property of plants depends on the species of plant, the amount which utilized in the test, locations where the plant collected, the type of solvent and the type of tested microorganism (Obeidat et al., 2012).

Antioxidant Activity

Phenolics possess redox characteristics, which could behave as antioxidants. Because of hydroxyl groups in the phenolics, the total phenolic content might be utilized for quick screening of antioxidant activity (Baba and Malik, 2015). Table 3 illustrates the total phenolic content in the plant extracts. Among the tested plants, the highest phenolic content was found in ethanol extract of mint as 155.79±0.53 µg GAE/mL, followed by ethanol extract of thyme as 137.69±1.64 µg GAE/mL and ethanol extract of nettle as 29.20±0.56 µg GAE/mL.

Flavonoids are plant compounds which the antioxidant capacity of them is linked with the availability of free OH groups (Baba and Malik, 2015). Table 3 shows total flavonoid content of the extracts. The total flavonoid content of the plant extracts expressed as µg CE/mL. The highest and the lowest contents were found in ethanol extract of the mint as 300.06±1.65 µg CE/mL and found in chloroform extract of *C. album* as 15.55±0.62 µg CE/mL, respectively.

The total antioxidant capacity of the extracts was studied by the phosphomolybdate method. The total antioxidant capacity expressed as µg AAE/mL. Total antioxidant capacity (TAC) method involve single electron transfer (SET) and hydrogen atom transfer (HAT) (Agbo et al., 2015). The results obtained for all the extracts were in the range of 37.60±0.41 to 149.56±0.47 µg AAE/mL (Table 4).

The CUPRAC assay was performed to evaluate the ability of the antioxidants in the extracts to reduce cupric copper to the cuprous form (Rico et al., 2013). When CUPRAC activity of the plant extracts were compared, ethanol extract of mint and ethanol extract of thyme revealed the highest values at 1000 µg/mL concentration (Table 5). The hexane extracts of mint and *C. album* gave the weakest activity. Ethanol extracts of thyme and mint exhibited higher antioxidant activities than BHT.

The inhibition percentage of DPPH radical scavenging activities is summarized in Table 5. DPPH radical scavenging activities of the plant varied from 2.55% to 75.21%. Ethanol extracts of the plants showed higher activity than other extracts. DPPH activity of the solvents are increased in the following order: Ethanol>Chloroform>Hexane. Ethanol extract of mint showed the highest DPPH radical scavenging activity and hexane extract of nettle exhibited the lowest DPPH radical scavenging activity.Many researchers have examined the antioxidant properties of nettle, peppermint, thyme and *C. album.* Gülçin et al. (2004) found that nettle possessed strong antioxidant activity so neetle might be use natural antioxidants and food supplement in the pharmaceutical industry. Similarly, we found moderate antioxidant activity in the nettle in our study.

Kukric et al. (2012) found that fresh nettle leaves contained small amounts of chlorophyll, carotenoids and proteins, and an increase in soluble peroxidase activity. The researchers reported that total phenolic content of the ethanol extract of nettle was high while the content of total flavonoids and flavonols was relatively low. In contrast this, we found that total phenolic content of nettle was lower when compared to the total flavonoid content of nettle.

Stajonevic et al. (2013) declared that total phenolic content of aqueous extract of thyme was found as 2008.33 ± 10.6 mg GAE/mL. Moreover, the ferric reducing antioxidant power and antioxidant capacity analysis revealed the strong antioxidative properties of thyme extract. We also found strong CUPRAC activity and DPPH scavenging activity in the ethanol extracts of thyme.

Mierina et al. (2017) revealed that peppermint extracts were found as the highest free radical scavengers among all studied plant extracts. The highest phenol content was obtained utilizing 40% or 70% ethanol for extraction. In accordance with this study, we determined high total phenol and flavonoid contents, antioxidant activity in the ethanol extract of peppermint.

In the study of Lone et al. (2017), DPPH radical scavenging activity of the methanol extracts of *C. album* ranged from 45% to 73% at concentrations varying from 50 to 300 µg/mL, whereas DPPH radical scavenging activity of the aqueous extracts of *C. album* ranged from 84% to 96% at concentrations between 50-300 µg/mL (Lone et al., 2017). In our study, DPPH radical scavenging activity of the extracts of *C. album* ranged from 9.28% to 24.90%.

Conclusion

According the data obtained from this study the highest total phenol and flavonoid contents were found in the ethanol extract of mint. The highest total antioxidant capacity was found in the ethanol extract of thymus. Moreover, the highest antibacterial activity was found in nettle and *C. album*. The results of the present study show that ethanol, chloroform and hexane extracts of nettle, thyme, peppermint and *C. album* possess antioxidant activity so they might be utilized both in the pharmaceutical and the food industry. The extracts of nettle and *C. album* also exhibited antibacterial activity. Additional works were needed required to isolate and determine active compounds, and understand the action mechanism of pharmacological agents.

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