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Original Article

Total phenolic, total flavonoid contents, and *in vitro* biological activities of *Cephalaria procera* Fisch. & Ave-Lall.

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ABSTRACT

Background and Aims: This study aims to determine total phenolic, total flavonoid contents and *in vitro* biological activities of methanol (CEP-1), *n*-butanol (CEP-2), water (CEP-3), *n*-hexane (CEP-4) extracts obtained from *Cephalaria procera*.
Methods: The total phenolic and flavonoid content analysis, *in vitro* DPPH radical scavenging activities, cholinesterase, and tyrosinase inhibitory properties of the extracts were evaluated using spectrophotometric assays. DNA-damage and DNA-damage protective effects of the extracts were examined using agarose gel electrophoresis method. Antimicrobial activities of the extracts were determined by microdilution method.
Results: CEP-3 had the best total phenolic content (79.64±1.11 mg GAE/g dry weight), and CEP-1 had the highest total flavonoid content (15.33±0.27 mg QEE/g dry weight) among tested extracts. CEP-1 showed the highest radical scavenging activity with 83.21±3.20 µg/mL of IC_{50} value. CEP-3 exerted the highest AChE and BuChE inhibitory action with 134.63±4.49 µg/mL and 62.76±0.63 µg/mL of IC_{50} values, respectively. CEP-3 showed significant tyrosinase inhibitory action with 51.95±0.35 µg/mL IC_{50} value compared to kojic acid (58.26±0.25 µg/mL). CEP-1 and CEP-3 were tested, and the both extracts did not damage supercoiled DNA at studied concentrations. Incidentally, results indicated that CEP-1 and CEP-3 protected supercoiled DNA against Fenton's reagents. CEP-4 exhibited the highest antimicrobial activity on *C. tropicalis* with the MIC value of 156.2 µg/mL.
Conclusion: The results showed that crude and subextracts of *C. procera* exerted several moderate activities on tested systems. It suggested that the species might be a promising medicinal plant for the treatment or prevention of several diseases associated with skin damage and wounds.

Keywords: Antioxidant, antimicrobial, anticholinesterase, *Cephalaria procera*, DNA protective, tyrosinase

INTRODUCTION

Natural products are used extensively in drug research, and it is known that many active substances of herbal origin are used use today in modern pharmacotherapy directly or indirectly. According to the World Health Organization, approximately 20.000 plants are still used for treatment today, and approximately 80% of the world population primarily resorts to herbal drugs to eliminate their health problems. In addition, 1881 compounds of natural origin have been approved by the FDA for medical use since 1981, and 25% of pharmaceutical preparations contain active ingredients of plant origin (Faydaoğlu & Sürücüoğlu, 2011; Newman & Cragg, 2020).

The genus *Cephalaria* Schrad. ex Roem. & Schult. is a member of the Caprifoliaceae family. South Africa and the Holarctic Kingdom (from Balkans to West China and from South Ukraine to Middle East) are the main centers of distribution of the genus. It has been

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determined that 94 species are grown in the world and 39 species in Turkey (Göktürk and Sümbül, 2014). Fresh stems of *Cephalaria procera* Fisch. & Avé-Lall. are used for wound healing and as antihemorrhagic, traditionally. The species is called as Ganteper, Gulinga, Cevrük and Cipreş in Turkey (Özgen, Kaya, & Houghton, 2012; Kahraman et al., 2019).

It has been reported that *Cephalaria* is a rich genus in terms of saponins (Boke Sarikahya, Goren, Sumer Okkali, & Kirmizigul, 2021), phenolic compounds (Chrzaszcz, Krzeminska, Celinski, & Szewczyk, 2021), flavonoids (Godjevac et al., 2004), lignans (Pasi, Aligiannis, Skaltsounis, & Chinou, 2002), triterpene glycosides (Top, Sarikahya, Nalbantsoy, & Kirmizigul, 2017; Böke Sarıkahya & Kirmizigul, 2010), iridoid glycosides (Mustafaeva et al., 2011) as phytochemicals. Studies about biological activities showed that *Cephalaria* species have antioxidant (Böke Sarıkahya, Pekmez, Arda, Kayce, Karabay Yavaşoğlu, & Kirmizigul, 2011; Godjevac et al., 2004), antimicrobial (Böke Sarıkahya, Pekmez, Arda, Kayce, Karabay Yavaşoğlu, & Kirmizigul, 2011, Böke Sarıkahya, & Kirmizigul S, 2010), hemolytic (Top, Sarikahya, Nalbantsoy, & Kirmizigul, 2017), immunomodulatory (Celenk, Boke Sarikahya, & Kirmizigul, 2020; Top, Sarikahya, Nalbantsoy, & Kirmizigul, 2017), and cytotoxic activities (Celenk, Boke Sarikahya, & Kirmizigul, 2020; Pasi, Aligiannis, Skaltsounis, & Chinou, 2002).

In this study, it was aimed to test total phenolic, total flavonoid contents and investigate *in vitro* biological activities of *Cephalaria procera* extracts. To the best of our knowledge, there has not been any study conducted to investigate the cholinesterase and tyrosinase inhibitory activities, antimicrobial activity on yeasts, and supercoiled DNA damage and damage protective effects of *Cephalaria procera*.

MATERIALS AND METHODS

Plant material

The aerial parts of *Cephalaria procera* were collected from Erzurum (Eastern Turkey) by Dr. Yeter Yeşil, Nurdan Yazıcı Bektaş and Burak Bektaş in July 2017. Voucher specimens were authenticated by Dr. Yeter Yeşil. These specimens were deposited at the Herbarium of İstanbul University (ISTE 115 326, ISTE 115 327).

Extraction

Air dried and powdered aerial parts of *Cephalaria procera* were extracted at room temperature with methanol for overnight three times. The methanol extract was concentrated to dryness under reduced pressure. The crude methanol extract (CP-1) dissolved with distilled water and extracted with *n*-butanol using partition method. By this way water extract (CP-3) was obtained. Then the *n*-butanol phase was concentrated and extracted with *n*-hexane. Finally, *n*-butanol (CP-2) and *n*-hexane (CP-4) were obtained, concentrated to dryness, and stored at refrigerator (Top, Sarikahya, Nalbantsoy, & Kirmizigul, 2017).

Total phenolic content analysis

The total phenolic content analyses of the extracts were evaluated utilizing the Folin-Ciocalteu colorimetric assay according to study of Barut & Şöhretoğlu (Barut & Şöhretoğlu, 2020). The results were expressed as mg gallic acid equivalent (GAE) per g of dry weight of the extracts.

Total flavonoid content analysis

The total flavonoid content analyses of the extracts were evaluated aluminium nitrate colorimetric assay (Barut et al., 2017). The results were expressed as mg quercetin equivalent (QEE) per g of dry weight of the extracts. The extracts, 10% aluminium nitrate and 1 M ammonium acetate were added to a tube. The mixtures were incubated for 40 min at room temperature. Afterwards, the absorbance was measured at 415 nm.

In vitro Biological activities

DPPH radical scavenging effects of the extracts

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging effects of the extracts were determined using spectrophotometric assay according to previous study conducted by (Barut & Şöhretoğlu, 2020). The results were expressed as IC_{50} (Half-maximal inhibitory concentration) values. Gallic acid (GA) was used as a positive control.

AChE/BuChE inhibitory effects of the extracts

The acetylcholinesterase (AChE) and buthyrylcholinesterase (BuChE) inhibitory effects of the extracts were evaluated using the previously reported method (Barut & Şöhretoğlu, 2020). The results were expressed as IC_{50} values. Galantamine was used as a positive control.

Tyrosinase inhibitory effects of the extracts

The tyrosinase (Tyr) (Sigma, T3824) inhibitory effects of the extracts were evaluated using previous reported method (Barut & Şöhretoğlu, 2020). The results were expressed as IC_{50} values. Kojic acid was used as a positive control.

DNA damage effects of CEP-1 and CEP-3

The supercoiled pBR322 plasmid DNA damage effects of CEP-1 and CEP-3 was determined using agarose gel electrophoresis according to the previous study (Şöhretoğlu, Barut, Sari, Özel, & Arroo, 2020). In this study, Tris-HCl (50 mM, pH 7), plasmid DNA, extracts at various concentrations (50, 100, and 200 µg/mL) was mixed at 37 ºC for 1 h. Afterwards, loading buffer (bromophenol blue, sodium dodecyl sulphate, xylene cyanol, glycerol) was added and the mixtures were loaded on gel (0.8% (m/v)) with ethidium bromide staining for 90 min at 100 V in Tris-acetic acid-EDTA (TAE) buffer. After electrophoresis, gel was visualized and calculated using BioRad Gel Doc XR system and Image Lab Version 5.0.1 software.

DNA damage protective effects of CEP-1 and CEP-3 on Fenton reagents

The supercoiled pBR322 plasmid DNA damage protective actions of CEP-1 and CEP-3 on Fenton's reagents were evaluated using agarose gel electrophoresis (Şöhretoğlu, Barut, Sari, Özel, & Arroo, 2020). In this study, Tris-HCl (50 mM, pH 7), plasmid DNA, H_2O_2 (2%), $FeSO_4$ (1 mM), extracts at various concentrations (50, 100, and 200 µg/mL) was mixed at 37 ºC for 1 h. The electrophoresis studies were performed according to the above method.

Antimicrobial effects of the extracts

The antimicrobial activities of CEP-1, CEP-2, and CEP-3 extracts were determined against a set of microorganisms including *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750 using the broth microdilution technique approved by Clinical Laboratory Standards Institute (CLSI) (CLSI, 1997, 2020)

Cefuroxime, cefuroxime-Na, amikacin, ceftazidime and fluconazole were used as positive control; RPMI-1640 medium for the yeast strain and Mueller-Hinton broth for bacteria were used as negative control.

Statistical analysis

The results were expressed as the mean±SD and were analysed using GraphPad Prism 5.0. In this work, two-way analysis of variance (ANOVA) followed by Bonferroni tests were used as statistical analysis.

RESULTS

Total phenolic and total flavonoid contents of the extracts

In this work, total phenolic and total flavonoid contents of the extracts were investigated and the results were given in Table 1. CEP-3 had the highest total phenolic content with 79.64±1.11 mg GAE/g dry weight among tested extracts. Also, the total phenolic contents of the extracts (CEP-1, CEP-2, and CEP-4) were 68.81±4.11, 13.38±0.82, and 50.05±5.14 mg GAE/g dry weight, respectively. On the other hand, the total flavonoid contents of CEP-1, CEP-2, CEP-3, and CEP-4 were calculated as 15.33±0.27 mg QEE/g dry weight, 2.14±0.50 mg QEE/g dry weight, 11.27±2.21 mg QEE/g dry weight, and 5.25±0.88 mg QEE/g dry weight, respectively.

DPPH radical scavenging effects of the extracts

In this study, DPPH radical scavenging effects of the extracts were determined using a spectrophotometric method. The results were presented in Table 1. CEP-1 showed the highest radical scavenging effect with 83.21±3.20 µg/mL of IC_{50} value among the tested extracts as shown in Table 1. However, CEP-1 found to have less scavenging properties than gallic acid (GA) (IC_{50}=68.25±0.35 µg/mL) which used as a positive control. In addition, the IC_{50} values of CEP-2, CEP-3, and CEP-4 were determined as 264.05±6.52 µg/mL, 89.91±0.13 µg/mL, and 179.02±0.23 µg/mL, respectively.

AChE and BuChE inhibitory effects of the extracts

In this paper, the AChE obtained from *Electrophorus electricus* (electric eel) and BuChE from equine serum inhibitory properties of the extracts were investigated, and the results were shown in Table 2. The IC_{50} value of CEP-3 was 134.63±4.49 µg/mL on AChE, as shown in Table 2. Other extracts have IC_{50}

Table 1. Total phenolic and flavonoid content, and DPPH radical scavenging effects of *C. procera* extracts.

Extracts	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QEE/g extract)	DPPH (µg/mL, IC_{50})
CEP-1	68.81±4.11	15.33±0.27	83.21±3.20
CEP-2	13.38±0.82	2.14±0.50	264.05±6.52
CEP-3	79.64± 1.11	11.27±2.21	89.91±0.13
CEP-4	50.05± 5.14	5.25±0.88	179.02±0.23
GA	-	-	68.25±0.35

GAE: Gallic acid equivalent, QEE: Quercetin equivalent, DPPH: 2,2-Diphenyl-1-picrylhydrazyl, GA: Gallic acid, CEP-1: Methanol extract of *C. procera*, CEP-2: *N*- butanol extract of *C. procera*, CEP-3: Water extract of *C. procera*, CEP-4: *N*-hexane extract of *C. procera*

Table 2. AChE, BuChE, and Tyr inhibitory effects of *C. procera* extracts.

Extracts	AChE (µg/mL, IC_{50})	BuChE (µg/mL, IC_{50})	Tyr (µg/mL, IC_{50})
CEP-1	>200	73.16±1.94	$56.13±1.17^{ns}$
CEP-2	>200	87.07±1.88	100.19±2.00
CEP-3	134.63±4.49	62.76±0.63	51.95±0.35**
CEP-4	>200	78.32±3.58	63.55±2.75
Galantamine	20.30±0.25	36.05±0.18	-
Kojic acid	-	-	58.26±0.25

AChE: Acetylcholinesterase, BuChE: Buthyrylcholinesterase, Tyr: Tyrosinase, CEP-1: Methanol extract of *C. procera*, CEP-2: *N*- butanol extract of *C. procera*, CEP-3: Water extract of *C. procera*, CEP-4: *N*-hexane extract of *C. procera*
*Values expressed are means±standard deviation of three parallel measurements, **p<0.001
ns: not significant vs positive control.

values higher than 200 µg/mL. On the other hand, CEP-3 had the highest BuChE inhibition with 62.76±0.63 µg/mL of IC_{50} value followed by CEP-1 with 73.16±1.94 µg/mL. However, galantamine (IC_{50}=20.30±0.25 for AChE, 36.05±0.18 for BuChE) which was used as a positive control, had higher inhibition than CEP-3 on AChE and BuChE.

Tyrosinase inhibitory effects of the extracts

In this study, the *in vitro* tyrosinase inhibitory properties of the extracts were determined using a spectrophotometric assay. The results were tabulated in Table 2. The IC_{50} value of CEP-3 was 51.95±0.35 µg/mL on Tyr. CEP-3 showed significant inhibitory action when compared to kojic acid (58.26±0.25 µg/mL) against Tyr ($p<0.001$). CEP-1 had similar inhibitory effect with kojic acid according to the their IC_{50} values.

Supercoiled DNA damage effects of CEP-1 and CEP-3

Supercoiled pBR322 plasmid DNA damage effects of CEP-1 and CEP-3 which were the most potent radical scavenging extracts, were evaluated using agarose gel electrophoresis. The results were given in Figure 1. It is well-known that plasmid DNA has three forms on gel: form I (supercoiled form moves the fastest); form II (nicked form); form III (linear form moves the slowest). As shown in Figure 1 (lane 1), the percentage of form I was about 75%. At increasing concentrations CEP-1 and CEP-3, the amounts of form I did not change significantly, and they were determined as about 70-75%. These results showed that both extracts did not show any damage effects on supercoiled pBR322 plasmid DNA at studied concentrations.

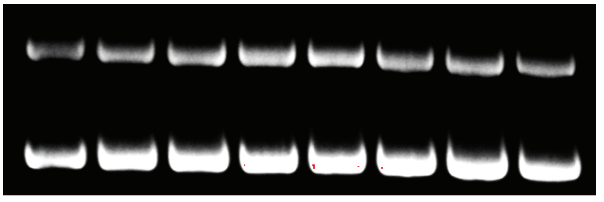

Figure 1. DNA damage effects of the extracts. Lane 1: DNA control; lane 2-4: DNA+ (50, 100, and 200 µg/mL of CEP-1); lane 5-7: DNA+ (50, 100, and 200 µg/mL of CEP-3).

Supercoiled DNA damage protective effects of CEP-1 and CEP-3 on Fenton's reagents

Supercoiled pBR322 plasmid DNA damage protective effects of CEP-1 and CEP-3 against Fenton's reagents were investigat-

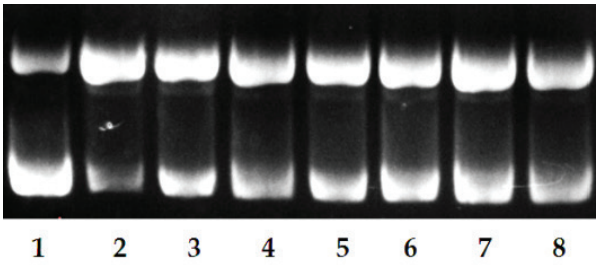

Figure 2. DNA damage protecitve effects of the extracts against Fenton reagents. Lane 1: DNA control; lane 2: DNA+$FeSO_4$+H_2O_2; lane 3-5: DNA+$FeSO_4$+H_2O_2+ (50, 100, and 200 µg/mL of CEP-1); lane 6-8: DNA+$FeSO_4$+H_2O_2+ (50, 100, and 200 µg/mL of CEP-3).

Table 3. The antimicrobial activities of the extracts (MIC values µg/mL).

	Microorganisms											
	Gram-negative Bacteria					Gram-positive Bacteria				Fungi		
	P. aeruginosa	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*E. faecalis*	*S. epidermidis*	*S. aureus*	*C. albicans*	*C. parapsilosis*	*C. tropicalis*
CEP-1	-	-	-	-	-	-	-	-	-	-
CEP-2	-	-	-	-	-	-	-	-	-	-
CEP-3	-	312.5	-	-	-	-	-	-	-	-
CEP-4	-	312.5	-	-	-	-	-	-	-	156.2
Reference antimicrobials	2.4 Ceftazidime	4.9 Cefuroxime-Na	4.9 Cefuroxime-Na	2.4 Cefuroxime-Na	128 Amikacin	9.8 Cefuroxime	1.2 Cefuroxime-Na	1 Fluconazole	2 Fluconazole	4 Fluconazole

CEP-1: Methanol extract of *C. procera*, CEP-2: *N*- butanol extract of *C. procera*, CEP-3: Water extract of *C. procera*, CEP-4: *N*-hexane extract of *C. procera*, *P. aeruginosa*: *Pseudomonas aeruginosa* ATCC 27853, *E. coli*: *Escherichia coli* ATCC 25922, *K. pneumoniae*: *Klebsiella pneumoniae* ATCC 4352, *P. mirabilis*: *Proteus mirabilis* ATCC 14153, *E. faecalis*: *Enterococcus faecalis* ATCC 29212, *S. epidermidis*: *Staphylococcus epidermidis* ATCC 12228, *S. aureus*: *Staphylococcus aureus* ATCC 29213, *C. albicans*: *Candida albicans* ATCC 10231, *C. parapsilosis*: *Candida parapsilosis* ATCC 22019, *C. tropicalis*: *Candida tropicalis* ATCC 750.
-: No Activity

ed using agarose gel electrophoresis. The results were presented in Figure 2. Supercoiled pBR322 plasmid DNA in buffer (including %0.1 DMSO) was used as a negative control and forms I and II was determined as 77.40% and 22.60%, respectively (Figure 2, lane 1). As shown in Figure 2, when Fenton's reagent was added, supercoiled DNA was damaged (Form I: %74.80; Form II: %25.20). When increasing concentrations of CEP-1 and CEP-3 are added into mixture, supercoiled pBR322 plasmid appears to be preserved. Addition of 200 µg/mL of CEP-1 and CEP-3, the amounts of Form I increased from 22.90% to 43.80% and 45.60%, respectively (Figure 2, lanes 5 and 8). These results indicated that CEP-1 and CEP-3 protected supercoiled pBR322 plasmid DNA on Fenton's reagents.

Antimicrobial activities of the extracts

All the *in vitro* antimicrobial activity results of the tested extracts are given in Table 3. Different concentrations from 1.22 to 2500 µg/mL concentrations of CEP-1 and CEP-2 were tested and none of them showed any activity. However, CEP-3 and CEP-4 exhibited moderate *in vitro* antibacterial activity against *E. coli*. Moreover, CEP-4 displayed intense antifungal activity against *C. tropicalis*. According to antifungal screening results, only CEP-4 was active extract against tested *Candida* species.

DISCUSSION

In recent years, natural antioxidants, especially polyphenols, have been notable agents for the treatment of many chronic diseases such as cancer, cardiovascular diseases, diabetes mellitus etc (AlFaris et al., 2021). To the best of our knowledge, there has not been any study conducted to investigate the anti-cholinesterases, anti-tyrosinase, DNA damage, and DNA damage protective activities of *C. procera*. In this paper, total phenolic contents of the extracts ranged from 79.64±1.11 to 13.38±0.82 mg GAE/g dry weight. The results showed that *n*-butanol extract has the highest total phenolic content among the tested extracts. Sarikahya et al. reported that the total phenolic content of *n*-hexane extract was found to be 1.561±0.042 mg GAE/g extract (Sarikahya et al. 2015). These results showed that extracts of this study had higher total phenolic contents than Boke Sarikahya's reports. On the other hand, CEP-1 had the best total flavonoid content than other extracts according to the Table 1.

The DPPH assay is a low cost, short time, and simple spectrophotometric method to understand scavenging effects of natural or synthetic compounds (Akar, Küçük & Doğan, 2017). This assay is based on single electron transfer and hydrogen atom transfer that produces a violet solution (Liang & Kitts, 2014). In this work, CEP-1 had the best radical scavenging properties following by CEP-3, shown as Table 1. The results of total phenolic/flavonoid contents and DPPH radical scavenging studies were found to be compatible. Godjevac and co-authors reported the DPPH radical scavenging activity of the flavonoids isolated from the flowers of *C. pastricensis* (Godjevac et al., 2004). Sarikahya et al. reported that the *n*-hexane extract of DPPH radical scavenging from *C. procera* determined as 6.938±2.56 mg/mL of IC_{50} value (Sarikahya et al., 2015). According to the literatures, *C. procera* contains kaempferol, astragalin, tiliroside, quercimeritrin, gigantoside A, hyperoside, quercitrin, apigenin, luteolin, cynaroside, cyanidin-3-O-glucoside (Sarıkahya & Kirmizigul, 2012; Sarıkahya, Goren & Kirmizigul, 2019). These compounds can be responsible for the antioxidant activities of this plant.

Alzheimer's disease (AD), a type of dementia, is the most common form of neurodegenerative disease. Although the pathophysiology of AD has not been clearly established, the cholinergic hypothesis is one of the most accepted causes. According to the cholinergic hypothesis, AD is associated with alterations of cholinergic markers such as cholinesterases (Tuğrak, Gül & Gülçin, 2020; Kahraman et al., 2019). IC_{50} values of the extracts were above 200 µg/mL on AChE for all extracts, while IC_{50} values for BuChE were determined as below 100 µg/mL. CEP-3 showed the highest AChE and BuChE inhibitory effects. The results demonstrated that the extracts showed moderate inhibition on BuChE and low inhibition against AChE.

Tyrosinase contains two copper atoms in its active site, and it is a metalloenzyme belonging to the oxidoreductase. It commonly is found in mammals, plants, insects, fungi, and bacteria (Şöhretoğlu, Sari, Barut & Özel, 2018). Tyrosinase forms melanin pigment from monophenols with many reactions. The excessive formation of melanin pigment causes various problems such as hyperpigmentation, age spots, melanoma etc. (Şöhretoğlu, Sari, Barut & Özel, 2018). CEP-3 showed more significant tyrosinase inhibitory effects than kojic acid as a positive control ($p<0.001$) in this study. Studies in the literature show that antioxidant compounds have tyrosinase inhibitory properties (Wang et al., 2018; Morais et al., 2018; Sun, Guo, Zhang & Zhuang, 2017). In this study, we determined that extracts with a high antioxidant effect showed high tyrosinase inhibition.

The supercoiled DNA damage actions of CEP-1 and CEP-3 were determined by agarose gel electrophoresis. In this paper, CEP-1 and CEP-3 extracts were used due to their antioxidant potentials. As presented in Figure 1, the amounts of form I were similar percentages. The results showed that both extracts did not damage supercoiled plasmid DNA at increasing concentrations.

When $FeSO_4$ and H_2O_2 are mixed, a hydroxyl radical is formed, and the resulting hydroxyl radical could trigger biological damage such as DNA damage (Barut, Barut, Engin, Özel & Sezen, 2019). In this work, when Fenton's reagent was added to supercoiled DNA, the amount of form II was determined as 74.80%. On the increasing concentrations of extracts, the amount of form II decreased, and form I increased. The obtained results pointed that both extracts preserved supercoiled pBR322 plasmid DNA against Fenton's reagents.

In antimicrobial activity studies, the antimicrobial potential of the tested extract was examined by MIC method. While CEP-3 and CEP-4 showed antibacterial activity against *E. coli*, CEP-1 and CEP-2 did not show *in vitro* activity against all the studied strains. In contrast to our results, Sarıkahya et al. (Böke Sarıkahya, Pekmez, Arda, Kayce, Karabay Yavaşoğlu, & Kirmizigul, 2011) found strong *in vitro* antibacterial activity against a panel of bacteria including *S. aureus*, *S. epidermidis*, *E. coli*, *E. fae-*

calis, *P. aeruginosa* and *K. pneumoniae* with the pure chemical constituents of *Cephalaria* species in Anatolia. The differences between these results could be explained by using different *Cephalaria* species and using total or pure contents of the prepared different extracts. On the other hand, although only CEP-4 exhibited excellent antifungal activity against *Candida tropicalis*, this is the first report on the *in vitro* antifungal activity of the *Cephalaria* procera total extracts.

CONCLUSION

Cephalaria procera is used for wound healing, and as antihemorrhagic in Anatolia, traditionally. This study investigated total phenolic and flavonoid content, DPPH radical scavenging, supercoiled DNA damage/damage protective effects, AChE/BuChE, tyrosinase inhibitory, antimicrobial activities of CEP-1, CEP-2, CEP-3 and CEP-4 obtained from *Cephalaria procera*. The investigations showed that CEP-1 and CEP-3 had better activities on DPPH radical scavenging, tyrosinase enzyme inhibition, DNA damage/DNA damage protection test systems, while they had higher contents of total phenolic, and flavonoid compared to other extracts. These results suggested that crude methanol and water extracts of *C. procera* might have a promising potential for the treatment of several disorders associated with skin damage, and further studies are required to confirm these used test systems and mechanisms of action.

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