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# Evaluation of Antioxidant and Antimicrobial Activities of *Potentilla* recta L.

Gökçe ŞEKER KARATOPRAK[1], Başak ÖKDEM[2], Selen İLGÜN[3], Müberra KOŞAR[4]
[1,2]Department of Pharmacognosy, Faculty of Pharmacy, Erciyes University, 38039 Kayseri, Turkey, [3]Department of Pharmaceutical Botany, Faculty of Pharmacy, Erciyes University, 38039 Kayseri, Turkey, [4]Department of Pharmacognosy, Faculty of Pharmacy, Eastern Mediterranean University, 99628, Famagusta, North Cyprus,
[1]https://orcid.org/0000-0001-5829-6914, [2]https://orcid.org/0000-0003-1482-5938, [3]https://orcid.org/0000-0002-8544-0683
[4]https://orcid.org/0000-0002-1559-998X
✉: gskaratoprak@erciyes.edu.tr

## ABSTRACT
The objective of this study was to determine the effectiveness and the antioxidant activity of *Potentilla recta* L. (Rosaceae), which is traditionally used in the treatment of many diseases, against pathogenic microorganisms in the skin. The antioxidant activity was determined using 1,1-Diphenyl-2-picrylhydrazyl (DPPH·) and 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS·+) radical scavenging activities, inhibition of ß-carotene bleaching, protection of 2-deoxyribose and bovine brain-derived phospholipids against hydroxyl radical-mediated degradation assay, at different concentrations ranging from 0.001 to 2 mg $mL^{-1}$. Antimicrobial activity was evaluated against *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 3699), *Pseudomonas aeruginosa* (ATCC 27853) *and Candida albicans* (ATCC 90028) by microdilution method. Besides, viability enhancing effects on murine fibroblast cells (L929) were determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. The key parameters for the extract included the following: DPPH· $IC_{50}$: 0.19 mg $mL^{-1}$, TEAC: 0.76 mmol $L^{-1}$ Trolox, reduction: 0.87 mmol $g^{-1}$ AsscE, and protection of lipid peroxidation $IC_{50}$: 0.07 mg $mL^{-1}$. A strong effect on *S. epidermidis* was observed with 79% inhibition at a concentration of 125 mg $mL^{-1}$ but did not show toxicity to L929 cells below 250 mg $mL^{-1}$ concentration. The present findings highlight the need for research of plants for traditional medicinal uses and the importance of scientific evaluations.

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## *Potentilla recta* L.'nin Antioksidan ve Antimikrobiyal Aktivitelerinin Değerlendirilmesi

## ÖZET
Bu çalışmada geleneksel olarak birçok hastalığın tedavisinde kullanılan *Potentilla recta* L. (Rosaceae) 'nin ciltteki patojen mikroorganizmalara karşı etkinliği ve antioksidan aktivitesi araştırılmıştır. Antioksidan aktivite 1,1-Difenil-2-pikrilhidrazil (DPPH·) ve 2,2-Azinobis (3-etilbenzotiyazolin-6-sülfonat) (ABTS·+) radikal temizleme, ß-karoten ağartmanın inhibisyonu, 2-deoksiriboz ve sığır beyninden türetilmiş fosfolipidlerin hidroksil radikal aracılı degradasyonuna karşı koruma etkileri deneyleri ile 0.001 - 2 mg $mL^{-1}$ arasında değişen farklı konsantrasyonlarda belirlenmiştir. Antimikrobiyal aktivite, mikrodilüsyon yöntemi ile *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 3699), *Pseudomonas aeruginosa* (ATCC 27853) *Candida albicans* (ATCC 90028) üzerinde değerlendirilmiştir. Ayrıca fare fibroblast hücreleri (L929) üzerindeki canlılığı artırıcı etkiler MTT (3- (4,5-dimetiltiyazol-2-il)-2,5-difeniltetrazolyum bromür) yöntemi ile değerlendirilmiştir. Ekstreler için temel parametreler: DPPH • $IC_{50}$: 0.19 mg $mL^{-1}$, TEAC: 0.76 mmol $L^{-1}$ Trolox, redüksiyon: 0.87 mmol $g^{-1}$ AsscE ve lipid peroksidasyonun korunması $IC_{50}$: 0.07 mg $mL^{-1}$ olarak belirlenmiştir. 125 mg $mL^{-1}$ konsantrasyonda % 79 inhibisyonla *S. epidermidis*

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üzerinde güçlü bir etki gözlenirken 250 mg $mL^{-1}$ konsantrasyonunun altında L929 hücrelerine toksisite gözlenmemiştir. Mevcut bulgular, geleneksel tıbbi kullanımı olan bitkilerin araştırılması gerektiğine ve bilimsel değerlendirmenin önemli olduğuna ışık tutmaktadır.

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## INTRODUCTION

The Rosaceae family, which includes the *Potentilla* genus, is the 19th largest family in the plant kingdom. It has approximately 100 genera and 2830-3100 species (Folta and Gardiner 2009). Sixty *Potentilla* species are widely found in Turkish Flora (Güner and Aslan 2012). *Potentilla* species are known as "Beşparmak otu, İncibar kökü, Sarı kabusluk" in Turkey and are popularly used as antipyretic and tonic agents and also for constipation since ancient times (Baytop 1999). Underground and aerial parts of species have traditionally been used to treat inflammations, some types of cancer, wounds, infections, diarrhea, diabetes, and some other diseases. Greek physician Dioscorides recommended the use of decoction prepared from underground parts of *Potentilla erecta* in inflammatory facial eczema and ulcerations of oral cavities. In the Middle Ages, physicians and botanists defined the *Potentilla* species in their botanical books for Europe. Extracts prepared with different solvents such as water, milk, honey, and ethanol have been used as an ailment for toothache, sore throats, wound healing, jaundice, mouth ulcers, dysentery, and homeostatic (Tomczyk and Latté 2009). The herb parts of species have been studied in many phytochemical studies. *P. erecta* is the first species with the highest number of identified components among the *Potentilla* species, (a total of 68 compounds in the aerial and underground parts), followed by *P. anserina* (37 compounds in the aerial and underground parts). The main groups of these compounds are flavonoids, condensed tannins, organic and phenolic carboxylic acids, coumarins, and sterols (Tomczyk and Latté 2009). In a study for investigating the polyphenols *of P. recta;* 10 components; 1 neolignan glycoside and 9 flavonoids, were identified and their structures were elucidated (Şöhretoğlu and Kırmızıbekmez 2011).

Due to the known toxic effects of synthetic drugs, people's tendency to herbal medicines has increased, especially in the treatment of simple diseases. For this reason, it is important to determine the biological effects and possible toxic effects of plants traditionally used in treatment. One of the current examples of these herbal medicines for Anatolia is *Potentilla recta.* This plant, which grows naturally in Turkey, is traditionally used in the treatment of respiratory diseases, various skin diseases, gastrointestinal and neurological disorders, as well as for its wound healing properties (Tuzlacı, 2006). To better understand the traditional usage of *Potentilla recta*, the antioxidant activity of the plant has been investigated in detail, and its effectiveness on the microorganisms that cause infection in the skin has been evaluated. The toxicity of the plant extract was evaluated using the mouse fibroblast (L929) cell line, which is frequently used in toxicity studies.

## MATERIAL and METHOD

### Plant material, reagents, and extraction

*Potentilla recta* was collected from Pınarbaşı-Kayseri/Turkey, in June 2013. The herbarium material from the plant was kept in the Herbarium (ERCH) of Erciyes University Faculty of Science, Department of Biology. All the chemicals are analytical grade and purchased from Sigma Chemical Company (St. Louis, MO).

Aerial parts of the 150 g dried plant material were macerated with an appropriate volume of 70% methanol (MeOH) in 24 hrs in the shaking water bath (3 times repeats). Methanol phase concentrated under vacuum (37° C). The obtained concentrated extract was lyophilized and maintained at -18 C until analysis.

### Reduction of iron (III) to iron (II)

The reduction of iron (III) to iron (II) was assessed by (Oyaizu 1986) method. The solutions, 0.2 M phosphate buffer (pH 6.6), 1% (w/v) potassium hexacyanoferrate solution, 10% w/v trichloroacetic acid (TCA,), and 0.1% (w/v) $FeCl_3$, were used. Extract dissolved in 70% MeOH and then phosphate buffer and potassium hexacyanoferrate solutions (2.5 mL each) were added. TCA (2.5 mL) was added after incubation at 50°C for 30 min. The reaction mixture solution was centrifuged (1000 g, 10 min). Separated upper layer (2.5 mL) was added in $H_2O$ (2.5 mL) and mixed with 0.5 mL of $FeCl_3$. The absorbance of all samples was read at 700 nm. The results were evaluated as ascorbic acid equivalents (AscAE, mmol ascorbic acid/g sample). Triplicate analyses were done and then mean values are given.

### DPPH● Scavenging Assay

DPPH• radical scavenging effects of the extract were evaluated by Gyamfi, Yonamine et al. (1999) method.

1 mL of DPPH• methanolic solution (0.1 mM) was mixed with 50 µL of extract solution and 450 µL of Tris-HCl buffer. The absorbance at 517 nm against the blind, which is composed of MeOH, was recorded after incubation of the reaction solutions at room temperature for 30 min. The inhibition of samples was calculated with Eq. 1 and the mean $IC_{50}$ values are given after triplicate analyses.

$$\%\ inhibition = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100 \quad \text{(Eq. 1)}$$

### Inhibition of ß-carotene bleaching

In this assay, the antioxidant capacity of the extract and standards were–evaluated using the method of Velioglu et al. (1998). After mixing, 1.2 mL of *ß*-carotene solution (1 mg $mL^{-1}$ in chloroform), Tween 20, and linoleic acid, chloroform removed from media via rotary evaporator. The emulsion was prepared by adding distilled water to this mixture and mixing gently. For the blanks of the control and samples, the same procedure was performed without using ß-carotene. After the samples were put into tubes together with the emulsion, these samples were kept in a water bath for 2 hours at 50 °C for autoxidation and the fading level was measured at 470 nm every 15 minutes. Inhibition of ß-carotene bleaching was calculated using Eq. 2.

$$AA\% = [1-(Abs0\ sample - Abs120\ sample)/(Abs0\ control - Abs120\ control)] \times 100 \quad \text{(Eq. 2)}$$

### $ABTS^{+\bullet}$ Radical Scavenging Activity

The concentrate $ABTS^{+\bullet}$ solution was prepared using 2.45 mM $K_2S_2O_8$ and after 12-16 hours' incubation in dark. This prepared $ABTS^{+\bullet}$ radical solution was measured at 734 nm and diluted with ethyl alcohol until an absorbance of 0.700 (± 0.030) was reached. For the measurement, standard/sample (10 µL) and diluted $ABTS^{+\bullet}$ solution (990 µL) were mixed and then the kinetic profiles were recorded at 734 nm for 30 minutes (1 min interval). Inhibitions (%) were calculated using Trolox (TEAC) calibration equations. Results are given in mean values (n=3) (Re et al. 1999).

### Ascorbate-Iron (III)-Catalyzed Phospholipid Peroxidation

Folch VII type bovine brain extract, 10 mM phosphate buffer saline (PBS) (pH 7.4), $FeCl_3$ (1 mM), 2% (w/v) butylated hydroxytoluene (BHT), TCA (2.8%, w/v), and 2-thiobarbituric acid (TBA) (1%, w/v) were used in the experiment. Liposome was prepared with bovine brain extract and PBS. Liposomes (0.2 mL), PBS buffer (0.5 mL), $FeCl_3$ (0.1 mL) and extract/standard (0.1 mL) were mixed. Ascorbate solution (0.1 mL) was mixed to speed up the peroxidation reaction. After incubation (37 °C, 60 min), BHT (50 µL) of, TCA (1 mL) of and TBA (1 mL) were added. After the heating process (100 °C) was done in a water bath for 20 minutes, chromogens formed by malondialdehyde and thiobarbituric acid (MDA- (TBA) 2) were extracted with 2 mL of n-butanol. The absorbance was measured at 532 nm and Equation 1 was used for calculations. After triplicate analyses, $IC_{50}$ values are given as mean values (Aruoma et al. 1997).

### Iron (II) Chelate Activity

200 µL extract, 100 µL aqueous $FeCl_2$ (2.0 mM) and 900 µL methanol were added in a tube. After 5 minutes of incubation, the reaction was accelerated by ferrozine (400 µL, 5.0 mM) addition. The absorbance was recorded at 562 nm after 10 minutes. $Na_2EDTA$ equivalent iron-chelating activity ($mg_{Na2EDTA}/g_{sample}$) is given as the average of triplicate analyses (Carter 1971).

### Non-site Specific Hydroxyl Radical (●OH) Mediated 2-deoxy-D-ribose Degradation

2-deoxyribose (5.6 mM, pH 7.4), $FeCl_3$/ EDTA (1: 1, v /v), $H_2O_2$ (1.0 mM), aqueous ascorbic acid (1.0 mM), 2.8% TCA, and 1.0% TBA were used in the experiment. 100 µL of extract, 500 µL of 2-deoxyribose, 200 µL of premixed $FeCl_3$/ EDTA, $H_2O_2$ (100 µL), and ascorbic acid (100 µL) were mixed. After incubation at 50 °C for 30 minutes, TCA (1 mL) and TBA (1 mL) were added and incubated again at 50 ° C for 30 minutes. The oxidation was calculated as % inhibition after measuring the absorbance at 532 nm. Equation 1 was used for $IC_{50}$ (n=3) (Halliwell, Gutteridge et al.1987).

### Antimicrobial activity

The study to determine the minimum inhibition concentration was performed according to the Clinical and Laboratory Standards Institute with microdilution method (Wayne 2009). Gram (+) strains *Staphylococcus aureus* ATCC29213, *Staphylococcus epidermidis* ATCC 3699, Gr-(-) strain *Pseudomonas aeruginosa* ATCC27853 and fungal culture *Candida albicans* ATCC 90028) were used in the study. Mueller Hinton Agar and Mueller Hinton Broth were used for bacterial strains and Sabouraud Dextrose Broth and Sabouraud Dextrose Agar were used for the culture of *C. albicans*

Bacterial cultures were transferred to agar medium to obtain isolated colonies, after 24 hours' incubation at 37 ° C, 4-5 well-isolated colonies were taken into 0.9% saline. The turbidity of the prepared bacterial suspension was set to 0.5 ($1 \times 10^8$ CFU/mL) McFarland for bacteria and $5 \times 10^6$ CFU/mL for yeast in the turbidimeter. In a 96-well plate, 5% of bacteria were cultivated on 0.2 mL nutrient broth containing different concentrations of plant extract and left for 24 hours at 37 ° incubation. At the end of incubation, the concentration without turbidity was determined as Minimum inhibitory concentration (MIC). All analyzes

were carried out in three parallel.

### Cell viability assay on L929 cells

The L929 (mouse fibroblast) cells were cultivated in EMEM medium containing 1% penicillin/streptomycin mixture and horse serum (10%, 37 °C, in 5% CO2 and 95% air). The cell line was purchased from American Type Culture Collection (CCL-1 Manassas, VA, USA).

Using MTT colorimetric method, the toxicity, and viability enhancing effect of the extract on the L929 cell line was determined. The cultured cells were counted 24 hours before the experiment and distributed on a 96-well microplate at a number of 1 x $10^4$ cell/well. After 24 hours, the supernatant of cells adhering to the plate base was discarded and in 100 µL medium, 15.6; 31.25; 62.5; 125; 250; 500; 1000 and 2000 µg $mL^{-1}$ concentrated extract was added. After 24 hours' incubation, the wells were drained and MTT solution (100 µL, in PBS (5 mg/mL)) was mixed. After 2 hours' in the incubator, the wells were emptied and dimethyl sulfoxide (100 µL, DMSO) was dispensed into the wells. The absorbance was recorded at 570 nm wavelength using ELISA (Biotek Synergy HT). Results are given as mean ± standard error after triplicate analyses.

### Statistical analyses

Variance homogeneity was tested with the Levene test. One-way analysis of variance was used for comparisons between more than two groups. Dunnett's test and Tukey test were used for multiple comparisons. The data were evaluated with SPSS Version 11.0 statistic software package. The significance level was accepted as $p < 0.05$.

## RESULTS

### Iron (III) to iron (II) reduction activity

The capabilities of the samples on the reduction of iron were evaluated and given in Table 1 as ascorbic acid equivalent. The reducing capacity of the extract was 0.87 mmol $g^{-1}$ AscAE. The extract was not found to be as active as ascorbic acid (AA), BHT, butylated hydroxyanisole (BHA), rosmarinic acid (RA) and gallic acid (GA) used as positive controls for reducing iron (III). The activity of the extract was statistically significant ($P < 0.001$) than the activity of the standards.

### DPPH• Radical Scavenging Activity

The $IC_{50}$ value is defined as the necessary amount to scavenge 50% of the radical. The decrease in absorbance is indicative of high free radical scavenging ability. The $IC_{50}$ value of the extract and the standards are given in Table 1. *P. recta* extract scavenged the DPPH· radical depending on the concentration at physiological pH and the $IC_{50}$ value was found 0.19±0.00 mg $mL^{-1}$. However, it has been determined that the scavenging effect of the AA, BHT, BHA, GA, and RA were more active than the extract ($P < 0.001$).

### Inhibition of β-carotene bleaching

Free radicals formed by the emergence of hydrogen from linoleic acid attacks β-carotene and the resultant color changes. The degree of inhibition of oxidation with *P. recta* extract was given in Table 1. AAC was compared to the different groups and statistically significant variances were identified in the intergroup comparisons. According to the results, the activity of the extract was significantly higher ($P < 0.001$) than the GA and RA groups and lower than the BHT and BHA groups ($P < 0.001$).

### ABTS+●Radical Scavenging Activity

This radical scavenging test measures the amount of antiradical spectroscopically using the $ABTS^{+\bullet}$ radical. In this method, hydrophilic and lipophilic antioxidants such as flavonoids, hydroxycinnamic acids, and carotenoids can be measured. $ABTS^{+\bullet}$ scavenging ability of the *P. recta* was evaluated and given in Table 1. The extract and all standards were analyzed at 2 different concentrations. After comparison of the TEAC values, TEAC variance was found statistically significant in the intergroup comparisons. The activity of the extract was significantly found lower than GA, RA, AA, BHT, BHA groups at 0.2 mg $mL^{-1}$ concentration ($P<0.001$) and significantly lower than the BHT, BHA, AA, GA groups at 0.1 mg $mL^{-1}$ concentration ($P<0.001$) whereas significantly higher than RA group ($P<0.001$). The TEAC value of the extract and the most active GA standard were as follows respectively: 0.76±0.04 mmol $L^{-1}$ Trolox and 2.49±0.01 mmol $L^{-1}$ Trolox.

### Ascorbate-Iron (III)-Catalyzed Phospholipid Peroxidation

Biologically important phospholipids in which contain a high amount of polyunsaturated fatty acids susceptible to degradation due to hydroxyl radicals (·OH). In the experiment, the phospholipid liposomes obtained from the bovine brain were rapidly subjected to ·OH induced peroxidation, resulting in the formation of MDA and similar aldehydes. These reactive aldehydes formed were reacted with TBA and the resulting pink color was measured. Antioxidants, which act by removing ·OH and preventing the formation of TBARS, show increasing color intensity with increasing concentration. As seen in Table 1, the extract showed higher activity than AA used as standard. The IC50 value of the extract was 0.07±0.00 mg $mL^{-1}$ and the IC50 value of the AA was 0.90±0.13 mg $mL^{-1}$. $IC_{50}$ (mg/mL) values were compared between the groups and found statistically significant in the intergroup comparisons. As a result, the activity of the

extract was significantly lower than the AA group ($P < 0.001$), and there were no significant differences from BHT, BHA, GA, and RA.

### Iron (II) Chelate Activity

The metal chelating activity is based on the principle that ferrozine quantitatively complexes with $Fe^{+2}$. To bind $Fe^{+2}$ ions, ferrozine reagent, a strong iron chelator, the metal-binding compounds present in the medium compete with the reactive. If the chelating power is high, the production of $Fe^{+2}$/ferrozine complex is prevented. The effect of the extract on the chelation of iron (II) ions was investigated and the activity of the extract was found to be greater than 10 mg $mL^{-1}$ and the $IC_{50}$ value was not calculated.

### Non-site Specific Hydroxyl Radical (•OH) Mediated 2-deoxy-D-ribose Degradation

The experiment was based on the principle that the ·OH produced by the Fenton reaction causes deoxyribose attack and degradation products resulting from fragmentation react with TBA with low pH and temperature and constitute pink color. Only AA was used as a standard in this experiment. The $IC_{50}$ value of the ascorbic acid was 0.81±0.12 mg $mL^{-1}$ and of the extract was 0.62±0.03 mg $mL^{-1}$. IC50 values were compared between the groups. The extract was not significantly different from the AA group ($P>0.05$).

Table 1 The antioxidant activity results of P. recta extract and standards
*Çizelge 1. P. recta ekstresinin ve standartların antioksidan aktivitesi*

| Sample[A] | AscAe[B](mmol $g^{-1}$) | DPPH[C] IC50 (mg $mL^{-1}$) | TBAD IC50 (mg $mL^{-1}$) | AAC[E] | TEAC[F] (mmol $L^{-1}$ Trolox) | |
|---|---|---|---|---|---|---|
| | | | | | 0,1 mg $mL^{-1}$ | 0.2 mg $mL^{-1}$ |
| **BHT** | 2.26±0.005* | 0.12±0.01* | 0.09±0.00 | 933.23±1.25* | 0.55±0.01* | 1.45±0.01* |
| **BHA** | 1.93 ±0.006* | 0.07±0.00* | 0.02±0.00 | 987.55±1.33* | 0.86±0.01* | 1.87±0.01* |
| **RA** | 3.10±0.03* | 0.04±0.0* | 0.05±0.01 | 661.42±1.10* | 0.37±0.01* | 1.32±0.00* |
| **GA** | 4.08±0.01* | 0.02±0.00* | 0.16±0.06 | 639.13±0.88* | 2.09±0.02* | 2.49±0.01* |
| **AscAs** | 5.72±0.1* | 0.13±0.00* | 0.90±0.13* | - | 1.18±0.01* | 1.91±0.00* |
| ***P. recta*** | 0.87±0.00 | 0.19±0.00 | 0.07±0.00 | 857.62±1.14 | 0.41±0.02 | 0.76±0.04 |

[A] BHT: butylated hydroxytoluene; BHA: butylated hydroxyanisole; RA: rosmarinic acid; GA: gallic acid; AA: ascorbic acid; Pr 70: 70% methanol extract. [B] Iron (III) reduction. CDPPH radical scavenging. [D] Inhibiton of malondialdehyde formation. [E] Determination of Inhibition of ß-Carotene/Linoleic acid Co-oxidation. [F] TEAC is defined as the concentration of Trolox (mmol/L) having the $ABTS^{+\bullet}$ radical scavenging activity. * expresses difference with Pr70 extract ($P<0.05$). Data are expressed as mean ± standard error.

### Antimicrobial activity

The extracts were studied at a concentration of 0.0625-8 mg $mL^{-1}$ in the antimicrobial activity test. Inhibition of viability at a concentration of 0.0625 mg $mL^{-1}$ was found as follows: 44.18% for *S. aureus* (ATCC 29213), 78.29% for *S. epidermidis* (ATCC3699), 24.05% for *P. aeruginosa* (ATCC 27853), and 32.05%, for *C. albicans* (ATCC 90028). The extract had the strongest effect *on S. epidermidis* and the viability could not exceed 22%. The weakest effect was on *P. aeruginosa*, with inhibition of 71.78% at 8 mg $mL^{-1}$. Results are presented in Figures 1-4.

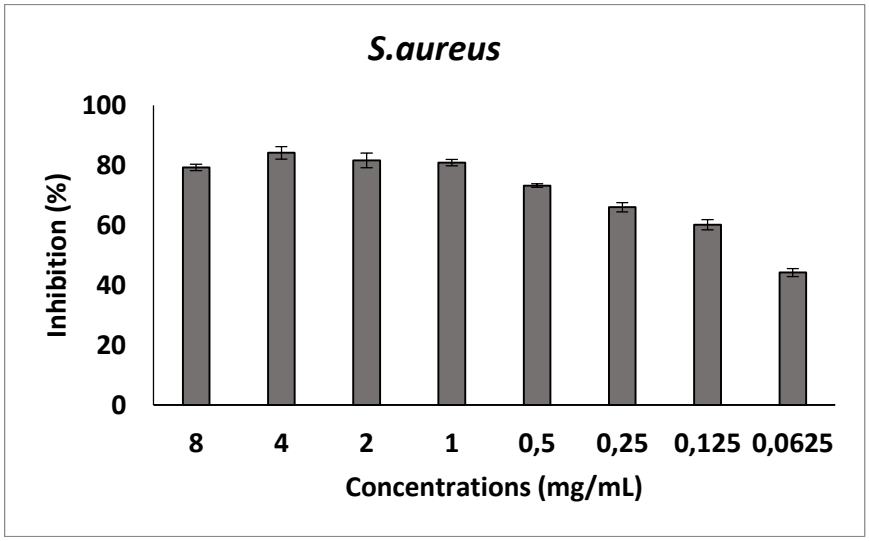


Figure 1. Inhibitory effects of P. recta extract on S. aureus. Values given as mean ± standard error were specified in the ±95% confidence interval (n = 3)

*Şekil 1. P. recta ekstresinin S. aureus üzerindeki inhibe edici etkileri. Ortalama ± standart hata olarak verilen değerler ±% 95 güven aralığında (n = 3) belirtildi*

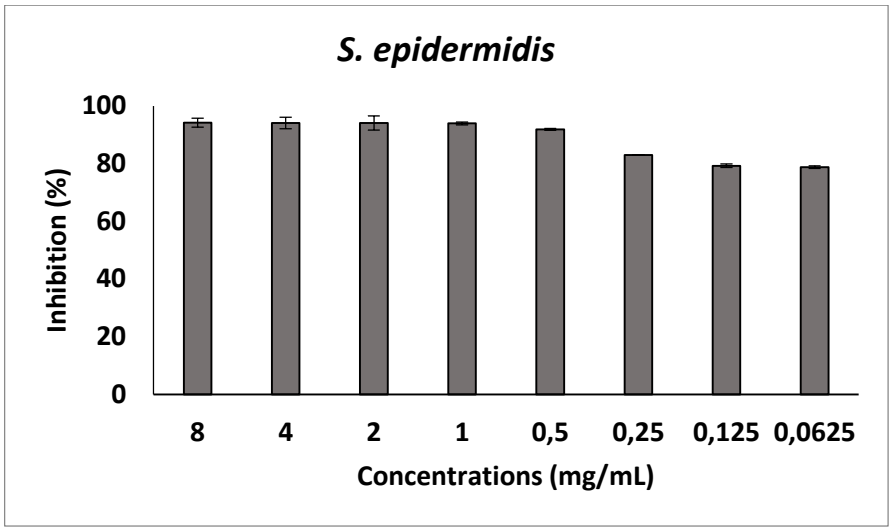


Figure 2. Inhibitory effects of P. recta extract on S. epidermidis. Values given as mean ± standard error were specified in the ±95% confidence interval (n = 3)

*Şekil 2. P. recta ekstresinin S. epidermidis üzerindeki inhibe edici etkileri. Ortalama ± standart hata olarak verilen değerler ±% 95 güven aralığında (n = 3) belirtildi*

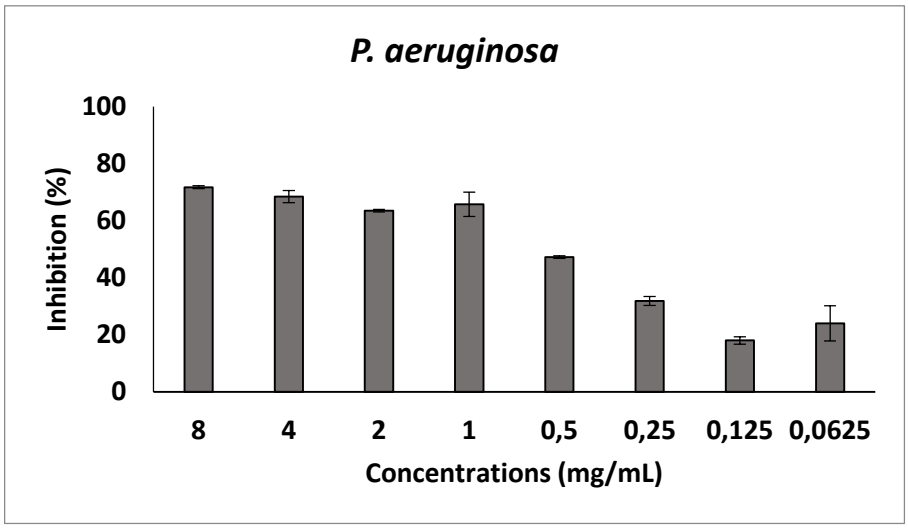


Figure 3. Inhibitory effects of P. recta extract on P. aeruginosa. Values given as mean ± standard error were specified in the ±95% confidence interval (n = 3)

*Şekil 3. P. recta ekstresinin P. aeruginosa üzerindeki inhibe edici etkileri. Ortalama ± standart hata olarak verilen değerler ±% 95 güven aralığında (n = 3) belirtildi*

### Cell viability assay on L929 cells

The effects of the extract studied in the 7.8-2000 µg $mL^{-1}$ concentration range on cell viability were evaluated by the MTT method. Experimental data was presented in Figure 5. The extract has decreased the viability below 50% between the concentrations of 500-2000 µg/mL at $P<0.001$ significant comparisons with the control. At 250 µg $mL^{-1}$, the viability was found to be 73% ($P<0.05$), while at 7.8 -62.5 µg $mL^{-1}$ concentrations, the viability exceeded 100%.

## DISCUSSION

In this study, antioxidant capacity, antimicrobial potential, and vitality enhancing effects on L929 cells were evaluated to better understand the ethnomedical applications of *P. recta*. In our previous study, *P. recta* was found to have a high amount of total phenolics (185.85 ± 6,51 $mg_{GAE}$ $g^{-1}_{extract}$). In phytochemical analyzes, kinic acid, ellagic acid, caffeic acid derivative, kaempferol glycoside, quercetin glucuronide, quercetin derivative, Bis-HHDP glucose, and potentillin was found (Ökdem et al. 2018). The fact that the content is rich in phenolic compounds has enabled *P. recta* to have high antioxidant capacity. Phenolic acids and flavonoids exhibit antioxidant activity by donating electrons (Kang et al. 1996), (Leopoldini et al. 2004). Antioxidant activity increased in proportion to the tannin content and the increase in the tannin concentration has been reported to increase the iron (III) reducing power in the literature (Zhang and Lin 2009). From recently published articles

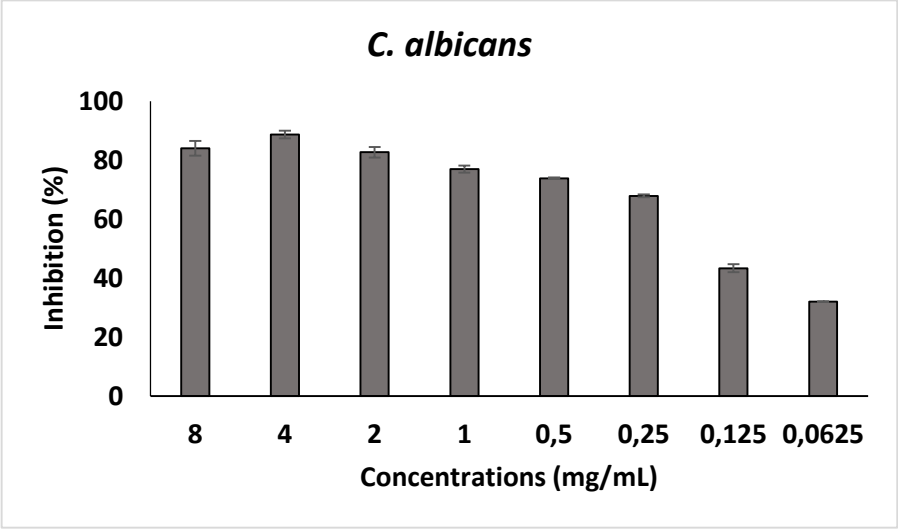


Figure 4. Inhibitory effects of P. recta extract on C. albicans. Values given as mean ± standard error were specified in the ±95% confidence interval (n = 3)

*Şekil 4. P. recta ekstresinin C. albicans üzerindeki inhibe edici etkileri. Ortalama ± standart hata olarak verilen değerler ±% 95 güven aralığında (n = 3) belirtildi*

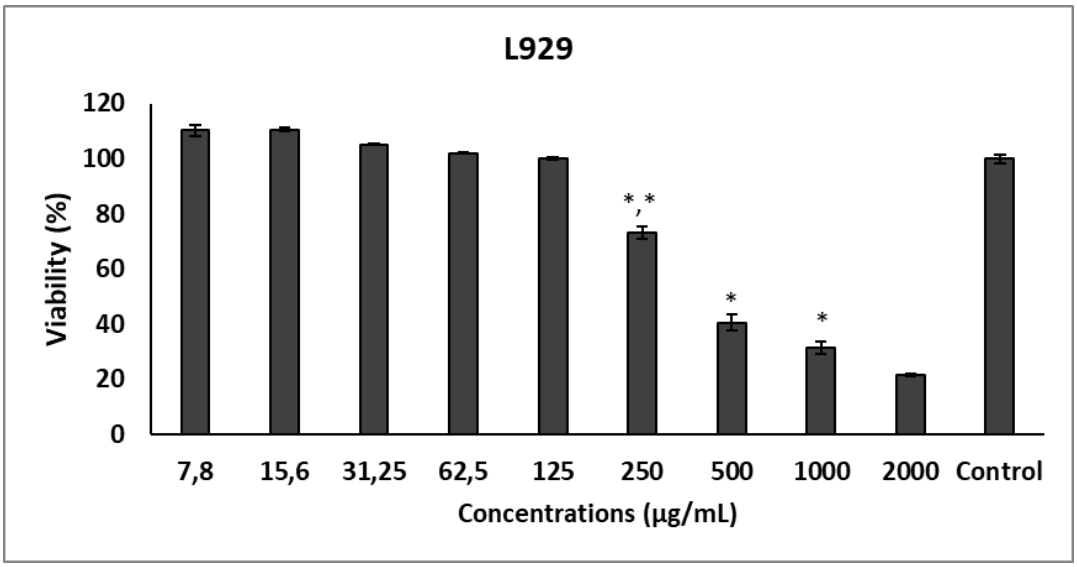


Figure 5. Effect of P. recta extract on L929 cell line viability. Values given as mean ± standard error were specified in the ±95% confidence interval (n = 3) * P < 0.01; ** P < 0.05.

*Şekil 5. P. recta ekstresinin L929 hücre hattı canlılığı üzerindeki etkisi. Ortalama ± standart hata olarak verilen değerler ±% 95 güven aralığında (n = 3) belirtildi, * P < 0.01; ** P < 0.05.*

(Sut et al. 2019), *P. recta* methanol extract has been reported to exhibit 175.60 mg TE $g^{-1}$ extract iron (III) reducing activity as measured by Trolox equivalent. In a different study conducted with *Potentilla reptans* and *Potentilla speciosa*, the iron reduction activities of extracts were found to be 81.59 to 219.97 mg TE $g^{-1}$ extract for *P. reptans* and 56.97-214.49 mg TE $g^{-1}$ extract for *P. speciosa* (Uysal et al. 2017).

The DPPH• radical scavenging effect of *P. recta* was not as high as AA, BHT, BHA, GA, and RA used as positive controls, but comparing with the literature we set a lower $IC_{50}$ (0.19±0.00 mg $mL^{-1}$) value from the results found by Şöhretoğlu et al (2015).

In the DPPH radical scavenging activity with *P. reptans*, the $IC_{50}$ value of the aerial part extract was found to be 12.11 µg $mL^{-1}$ and the $IC_{50}$ value of root was found to be 2.57 µg $mL^{-1}$. It has been found that the radical scavenging effect of the root was higher than that of the standard BHT (Tomovic et al. 2015). The biological activities of aqueous extracts of *Potentilla fruticosa*, *P. norvegica*, *P. pensylvanica*, *P. thuringiaca*, *P. crantzii,* and *P. nepalensis* were analyzed and their antiradical activities were found to be dose-dependently (Tomczyk et al. 2013).

While antioxidant activity is being investigated, it is essential to use lipid oxidation assay media. The *β-*

carotene/linoleic acid method, which is one of the most widely used methods for this purpose, is based on the principle that the degradation products resulting from linoleic acid oxidation will turn on the color of the *β*-carotene molecules (Liyana-Pathirana and Shahidi 2006). It has been observed that the *P. recta* extract has higher activity than GA and RA standards. Although there is no carotene bleach test of *P. recta* in the literature, the activity of *P. fruticosa* was evaluated and it was reported to exhibit strong antioxidant activity (Miliauskas et al. 2004).

$ABTS^{\cdot+}$ radical removal effect of extract and standards were evaluated at two different concentrations of 0.1 and 0.2 mg $mL^{-1}$. It has been determined that the scavenging effect of the extract is not as high as AA, BHT, BHA, GA, and RA used as positive controls at a concentration of 0.2 mg $mL^{-1}$. The scavenging effect of the extract was found to be higher than RA at the concentration of 0.1 mg $mL^{-1}$. It has been observed that extracts and all standards were more active in the high concentration in terms of $ABTS^{\cdot+}$ radical scavenging effect. In Sut et al.'s (2019) study, it was reported that *P. recta* water extract was more active than methanol and ethyl acetate extracts with a value of 3.85 mmol TE $g^{-1}_{extract}$ in $ABTS^{\cdot+}$ test system (Sut et al. 2019). Similar results were reported in the study conducted with *P. fruticosa*, *Potentilla glabra,* and *Potentilla parvifolia*, with activity results of 2140.22-2763.48 µmol Trolox $g^{-1}$ at a concentration of 0.2 mg $mL^{-1}$ (Wang et al. 2013).

The most important effects of free oxygen radicals are on the lipid systems. This phenomenon is known as lipid peroxidation and is briefly defined as the conversion of membrane phospholipids in cells into oxidized peroxide derivatives (Yarsan 2014). In this experiment, phospholipidic liposomes were rapidly produced MDA with iron (III) and ascorbic acid after $^{\cdot}OH$- induced peroxidation. Strong antioxidant effect was obtained from *P. recta* extract by preventing the formation of TBARs. The extract had higher activity than the ascorbic acid standard and did not show a significant difference in activity according to BHA, BHT, RA, and GA standards. Leaf, flower, and stem extracts of *P. fruticosa,* in the experiment using egg yolk, have been declared to demonstrate strong peroxidation inhibition (Yu et al. 2016). In alloxan-induced diabetic rats *P. fulgens* at 250 mg $kg^{-1}$ bw dose caused a reduction (75%) in TBARS levels in liver tissue (Saio et al. 2012). Strong antioxidant properties of *Potentilla* species are also prominent with their lipid peroxidation inhibitory activities, and in our study, strong inhibition of *P. recta* on lipid peroxidation was reported by us for the first time.

Ferrozine test is frequently used in determining the chelating activity of iron ions. In the test, ferrozine complexes with $Fe^{+2}$ ions, and these complexes are evaluated quantitatively. Chelating agents in the environment inhibit the formation of $Fe^{+2}$ complexes with ferrozine (Sarikurkcu et al. 2015). The iron (II) chelating activity of *P. recta* extract was found to be greater than 10 mg/mL. When we compare the iron (II) chelating activity of extract with free radical scavenging activity results, any correlation is not observed. This can be attributed to the fact that the radical scavenging activity experimentation mechanisms are different. Contrary to our results, (Grochowski et al. 2017) state that *P. thuringiaca* showed strong metal chelating activity and they associated this with the compound 1-*o*-beta-D glucopyranosyl synapate. In a different study evaluating the metal chelating activity of methanol, water, and ethyl acetate extracts of *Potentilla anatolica,* the results are given between the range of 27.44- 32 mgEDTA $g^{-1}$ (Uysal and Aktumsek 2015).

The $^{\cdot}OH$ is one of the most active radical that damages endogen components within the living organisms. In *in vitro* Fenton reaction, the $^{\cdot}OH$ bind to deoxyribose and converts to reactive TBA products by directing non-specific ($Fe^{+2}$ + $H_2O_2$ + EDTA) and specific ($Fe^{+2}$ + $H_2O_2$) $^{\cdot}OH$ radicals. Phenolics in the extracellular composition are liable for the scavenging of the $^{\cdot}OH$ in the $^{\cdot}OH$ -induced 2-deoxyribose degradation assay (Halliwell et al. 1987). The ability to inhibit 2-deoxyribose degradation directed by the non-specific $^{\cdot}OH$ of the extract was observed to be no significant ($P>0.05$) difference when compared to the AA used as a positive control.

The role of microorganisms in chronic wound pathology is quite important, and the combination of topical antimicrobial agents is used in the treatment. Wounds provide the microorganisms from the skin surface that make up the skin microbiota, a combined opportunity to gain access to the underlying tissues and to find optimal conditions for colonization and growth. *S. aureus, S. epidermidis, P. aeruginosa and C. albicans* are microorganisms found in wounds (Tomic-Canic et al. 2020; Wolcott et al. 2016). In a study evaluating the antimicrobial activity of extracts of *P. recta* against streptococci mutants, the visibility of inhibition zones was evaluated by measuring the zone diameters. The results presented that 50% methanol extract and subfractions (chloroform, diethyl ether, ethyl acetate, and n-butanol) inhibited the growth of all the tested oral *Streptococci* (Tomczyk et al. 2011). In another study, the MIC value of *P. recta* for *S. aureus* was stated as 25 mg $mL^{-1}$, and for *P. aeruginosa* as >100 mg $mL^{-1}$ (Tomczyk et al. 2008). The low effect of *P. recta* against *P. aeruginosa* in our study is similar to the literature. Our result that *P. recta* is effective against *C. albicans* is also supported by the research of Tosun et al. (2006). According to their results, *P. recta* showed 21 mm inhibition zone diameter in the disk diffusion method against *C. albicans*.

L929 mouse fibroblast cells are a reference cell often used in many studies to determine the cytotoxicity of samples (Karatoprak et al. 2020; Pitz et al. 2016; Talekar et al. 2017). The extract does not decrease the viability below 250 µg $mL^{-1}$ concentration compared to the control and even increases the viability in the concentration range of 7.8-62.5 µg $mL^{-1}$ compared to the control. These results prove that the extract showed both antioxidant and antimicrobial activity in the concentration range where it is not toxic to fibroblast cells and increases proliferation.

## CONCLUSION

In this study, antioxidant, antimicrobial and toxic effects of *P. recta* on fibroblast cell line were evaluated. *P. recta* has been found to have potent antioxidant capacity and moderate antimicrobial activity. At effective concentrations evaluated in both antioxidant and antimicrobial activity tests, the extract did not show toxicity to fibroblast cell lines and even increased viability. The results of the study provided preliminary information to understand the use of *P. recta* as a traditional herbal remedy, but *in vivo* studies are needed to fully prove efficacy.

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### Author's Contributions

The contribution of the authors is equal.

### Statement of Conflict of Interest

Authors have declared no conflict of interest.

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