Research article

Mercimek Takcı, H.A., et al., i*n vitro* Antibacterial, Antioxidant and DNA Damage Protective Activity *of* Blackberry (*Rubus fruticosus* L.*)* Root Extracts. International Journal of Life Sciences and Biotechnology, 2022. 5(2): p. 225-234. DOI: 10.38001/ijlsb.1085539

in vitro Antibacterial, Antioxidant and DNA Damage Protective Activity *of* Blackberry (*Rubus fruticosus* L.*)* Root Extracts

Hatice Aysun Mercimek Takcı[1]*, Sema Genç[2], Ayşenur Yalçın[3], Eda Özdemir[4]

ABSTRACT

The aim of this study is to explore the antibacterial, antioxidant, and DNA damage protection potentials of blackberry (*Rubus fruticosus* L) root extracts. Antioxidant activity of root extracts was researched by using DPPH˙ free radical scavenging and reducing capacity analysis. Methanolic extract of blackberry root system showed the maximum activity for TPC, TFC, DPPH˙, and ferric reducing capacity. Antibacterial activity of blackberry root extracts was screened against clinic isolates (*Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp. and *Staphylococcus aureus*) by the Kirby Bauer method. Although the metanolic extract possessed a significant antibacterial activity at 100 mg/mL concentration, distilled water extract did not show any inhibition to clinic isolates. The protective effects of root extracts on pBR322 plasmid DNA against the mutagenic effect of UV photolysis of H_2O_2 were tested. All concentrations of methanolic and distilled water extracts were observed to protect DNA damage in the presence of H_2O_2 and UV. These results indicated that the presence of antioxidant substances of root system extract of blackberry (*Rubus fruticosus* L) can be effective against harmful effects of free radicals.

ARTICLE HISTORY
Received
10 March 2022
Accepted
17 May 2022

KEYWORDS
Antioxidant activity, antibacterial activity, blackberry, DNA damage protective, root extract

Introduction

Blackberry (*Rubus fruticosus* L.) wild-grown and belongs to the genus *Rubus* of the Rosaceae family, is a perennial shrub [1, 2]. This bushy plant is lasting for three seasons or more. The fruit-bearing species of are naturalized throughout the world from sea level to heights up to 1100 [3]. Its edible fruits, known as blackberries and extensively used for the production of candy, dietary supplements, ice cream, jam, marmalade, and wine, are

[1] Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Kilis 7 Aralık University, Kilis, Turkey
[2] Institute of Science and Technology, Kilis 7 Aralık University, Kilis, Turkey.
[3] The Faculty of Medicine, Van Yuzuncu Yıl University, Van, Turkey
[4] Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Kilis 7 Aralık University, Kilis, Turkey
* Corresponding Author**:** Hatice Aysun Mercimek Takcı, E-mail: mersimek@hotmail.com

functional foods [2]. Blackberries including dietary fiber, vitamin C, vitamin K, and the essential mineral substances possess a high nutritional profile. The roots also contain saponins and tannins [4]. The gastroprotective and cardioprotective properties and pharmacological activities such as antioxidant, anti-carcinogenic, anti-inflammatory, antimicrobial, anti-diabetic, anti-diarrheal, and antiviral of these bioactive components in different parts of the plant are noted [1, 3]. Blackberry leaves and roots are traditionally used in herbal medicine as a regulatory remedy for menses, enteritis, chronic appendicitis, leukorrhea, diarrhea, dysentery, and anemia [4, 5]. Decoction of root bark and leaves being strongly astringent, depurative, diuretic and vulnerary are useful medicinally [6].

Despite the expressed activity of its various parts, very few published studies are on the biological activity of blackberry root extracts. Therefore, we aimed to screen phytochemical properties and pharmacological activities such as antioxidant, antimicrobial, and DNA damage protective of methanolic and aqueous root extracts of *R. fruticosus*.

Materials and Methods

Sample preparation

Rubus fruticosus root parts were extracted by methanol and distilled water (1:10 (w/v)) at room temperature for 3 days under shaking conditions Following filtered by Wattman No. 4 paper, solvents were evaporated, and then samples were suspended in methanol at the 100 mg/mL final concentration. The extracts were kept at +4ºC for antioxidant, antimicrobial, DNA protective activity, and phytochemical analysis. Phytochemical and antioxidant analyses were continued by using 0.5, 1.0, 2.5, 5.0, and 10.0 mg/mL concentrations of blackberry root extracts.

Total phenolic (TPC) and flavonoid content (TFC)

For the total phenolic content in all samples, Folin-Ciocalteu colorimetric method was performed. The total phenolic content was expressed as mg of gallic acid equivalents (mg GAE)/g of extract using a standard curve [7]. The total flavonoid content of samples was determined by a method predicated by Sharm and Vig (2013) [8]. The **results were** calculated according to a standard curve prepared by using rutin and expressed as mg of rutin equivalent (mg RE)/g.

Antioxidant activities of blackberry root extracts

DPPH˙ radical scavenging capacity

Free radical scavenger method by declared Blois (1958) is based on turning colorless of the 1,1-diphenyl-2-picryl-hydrazil (DPPH) reagent solution depending on the electron or proton-transfer ability of samples [9]. For this analysis, 100 µL of the extracts was added to 3.9 mL of DPPH˙ in reagent solution prepared in methanol (0.1 mM). For allowing the chemical reaction, this mixture was incubated at room temperature in the dark for 120 min. Then incubation, the absorbance of the mixture was measured at 517 nm against methanol blank. The absorbance of control was determined by using 1 mL of methanol instead of the sample. DPPH˙ scavenging activity expressed as % inhibition was calculated by the following equation. Butylated hydroxytoluene (BHT) at 200-1000 µg/mL concentrations was used as a standard antioxidant substance.

Inhibition (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

Reducing capacity assay

In this assay, the reducing Fe^{3+} to Fe^{2+} capability of antioxidant substances in extracts was tested [10]. The absorbance of the Prussian blue color formed by adding $FeCl_3$ in the reaction mixture was measured. A high absorbance value is indicated a high reducing capacity of samples. In brief, blackberry root extract (1 mL) was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium $K_3Fe(CN)_6$. This reaction solution was incubated at 50ºC for 20 min. To terminate the activity, 10% TCA was added and the mixture was centrifuged at 2500 rpm for 10 min. The equal volume of distilled water and 0.5 mL $FeCl_3$ (0.1%) were added to 2.5 mL of supernatant. The absorbance of the reaction mixture was measured at 700 nm. A beta-hydroxy acid (BHA) was evaluated as standard.

Statistical analysis

In order to determine significant differences between the samples, the software SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) was performed variance analysis (ANOVA) and Tukey multiple comparison tests. Each spectrophotometric analysis was repeated at least three times.

Antibacterial activity

The antibacterial activity of extracts was researched by using the Kirby-Bauer disk diffusion susceptibility test [11]. For this method, 5 clinical strains obtained from Kilis State Hospital (*E. coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp. and *S. aureus*) were tested. The density of cultures in growth Luria-Bertani (LB) broth for 24 h was adjusted to 0.5 McFarland turbidity. The antibacterial activity was performed on Mueller Hinton Agar. The sterile antimicrobial blank discs impregnated with 20 µL of the extracts were strategically placed away from each other. Methanol solvent and standard antibiotics were used as the negative and positive control, respectively. Following the incubation at 37ºC for 24 h, the clear zones around the discs were evaluated as antibacterial activity. Minimal Inhibition Concentration (MIC) of the extracts that showed antibacterial activity against test microorganisms was determined. This analysis was performed based on fact that the lowest inhibitory concentration is determined to be effective on test microorganisms. In this test, the 10, 25, 50, 75, and 100 mg/mL concentrations of extracts were investigated for their inhibitory effects against various microorganisms.

DNA damage protective activity

DNA damage inhibition activity of *Rubus fruticosus* root extracts was tested by using pBR322 plasmid DNA (vivantis). Oxidative DNA damage was induced by hydroxyl radicals generated from the ultraviolet (UV)/H_2O_2-radical system [12]. For this analysis, blackberry root extracts at different concentrations (50, 40, 20, 10, and 5 mg/mL) were prepared. The reaction mixture contained 3 µL pBR322 plasmid DNA, 5 µL of root extract, and 2 µL of 30% H_2O_2 in a microfuge tube. The tubes were UV irradiated at a wavelength of 230 nm using a UV lamp for 5 min at room temperature. The control tube contained only untreated DNA and DNA and H_2O_2 without root extract as an internal and negative control, respectively. Subsequently UV irradiation, all samples were analyzed by gel electrophoresis performed on 1% agarose gel.

Result and Discussion

As can be seen in Table 1, the range of the total phenolic content (TPC) in blackberry root extracts was very extensive: from 0.02±0.00 and to 0.63±0.01 mg GAE/g. High TPC was

calculated as 0.63±0.01 mg GAE/g in the methanolic root extract at 10 mg/mL concentration.

Table 1. Total phenolic contents of blackberry root extracts (mg GAE/g)

	Methanol	Distilled water
0.5 mg/mL	0.14±0.01^{e}	0.02±0.00^{e}
1 mg/mL	0.24±0.00^{d}	0.02±0.00^{d}
2.5 mg/mL	0.50±0.01^{c}	0.05±0.00^{c}
5 mg/mL	0.59±0.01^{b}	0.12±0.00^{b}
10 mg/mL	0.63±0.01^{a}	0.22±0.00^{a}

*(The presented datas are mean of triplicate determinations (n=3), ± standard deviation. The difference between the values expressed by the different symbols in table (a-e) is significant ($p<0.05$)).

The lower TPC values ranging from 0.02±0.00 to 0.22±0.00 mg GAE/g were obtained from aqueous extracts. The highest total flavonoid content was identified in blackberry methanolic root extract, 0.37±0.02 mg RE/g (Table 2).

Table 2. Total flavonoid compounds of blackberry root extracts (mg RE/g)

	Methanol	Distilled water
0.5 mg/mL	0.01±0.00^{d}	0.00±0.00^{c}
1 mg/mL	0.02±0.00^{d}	0.00±0.00^{c}
2.5 mg/mL	0.05±0.00^{c}	0.01±0.00^{bc}
5 mg/mL	0.09±0.01^{b}	0.01±0.00^{b}
10 mg/mL	0.37±0.02^{a}	0.02±0.01^{a}

*(The presented datas are mean of triplicate determinations (n=3), ± standard deviation. The difference between the values expressed by the different symbols in table (a-c and a-d) is significant ($p<0.05$)).

The total flavonoid contents noted minimum in aqueous root extracts (ranging from 0.00±0.00 to 0.02±0.01 mg RE/g) stated in terms of rutin equivalents (RE). For both methanolic and deionized water extracts, TPC and TFC values in root extracts increased depending upon the increase in concentration. In this present study, all quantitative examinations of phytochemical analysis were found statistically significant ($P<0.05$). Četojević-Simin et al. (2017) predicated that the highest TPC and TFC values of blackberry extracts were 0.089±3.48 mg GAE/g and 0.045±2.16 mg RE/g [13]. Those are quite lower than our methanolic extract results. Whereas 79.1 mg GAE/g value of methanolic blackberry extract reported by Najda and Labuda (2013) [14] was dramatically higher than ours. For both methods applied as shown in Tables 3 and 4, the highest antioxidant activities were recorded in methanolic root extract at 10 mg/mL concentration. DPPH$^{\cdot}$ radical scavenging activity was evaluated compared to standard antioxidant BHT.

Table 3. Antioxidant activity (DPPH˙ scavenging) of blackberry root extracts (%)

	0.5 mg/mL	1 mg/mL	2.5 mg/mL	5 mg/mL	10 mg/mL
Methanol	81.25±1.91^{c}	87.12±0.38^{b}	88.46±0.06^{ab}	89.35±0.06^{ab}	89.60±0.06^{a}
Distilled water	48.85±0.13^{b}	50.83±0.19^{b}	54.97±5.74^{b}	78.13±3.00^{a}	85.08±0.26^{a}
Standard	**200 mg/mL**	**400 mg/mL**	**600 mg/mL**	**800 mg/mL**	**1000 mg/mL**
BHT	26.29±0.18^{e}	42.53±1.31^{d}	51.99±0.54^{c}	63.53±1.61^{b}	70.67±0.30^{a}

*(The presented data are mean of triplicate determinations (n=3), ± standard deviation. The difference between the values expressed by the different symbols in the table (a-b, a-c, and a-e) is significant ($p<0.05$)).

Table 4. Reducing Capacity of blackberry root extracts (absorbance)

	0.5 mg/mL	1 mg/mL	2.5 mg/mL	5 mg/mL	10 mg/mL
Methanol	0.21±0.01^{e}	0.44±0.03^{d}	0.83±0.04^{c}	1.02±0.05^{b}	1.18±0.02^{a}
Distilled water	0.03±0.00^{d}	0.04±0.00^{d}	0.12±0.02^{c}	0.22±0.01^{b}	0.51±0.01^{a}
Standard	**20 mg/mL**	**50 mg/mL**	**100 mg/mL**	**200 mg/mL**	**400 mg/mL**
BHA	0.22±0.01^{c}	0.24±0.01^{c}	0.25±0.02^{c}	0.32±0.03^{b}	0.51±0.01^{a}

*(The presented data are mean of triplicate determinations (n=3), ± standard deviation. The difference between the values expressed by the different symbols in the table (a-c, a-d, and a-e) is significant ($p<0.05$)).

According to results (Table 3), % inhibition values ranged from 48.85±0.13 to 89.60±0.06. Methanol root extract showed comparable % DPPH˙ activity with BHT. However, root methanol and distilled water extracts showed statistically significant antioxidant activity ($P<0.05$). % DPPH˙ removal activity (87.12±0.38%) value observed at 1000 µg/mL extract concentration was higher than this exhibited by BHT (70.67±0.30%). % DPPH˙ radical scavenging activity of distilled water extract at the same concentration was 50.83±0.19% and this value lower than BHT and methanolic extract. In terms of reducing capacity activity, BHA showed higher antioxidant activity than plant extracts. The tested extract revealed a variable value of reducing capacity activity (0.03±0.00-1.18±0.02). The highest reducing capacity among extracts was 1.18±0.02 and recorded for methanolic extract at 10 mg/mL concentration. But this value was rather lower than BHT showing a significant antioxidant activity (0.51±0.01) at 0.4 mg/mL concentration. On the other hand, 0.51±0.01 reducing capacity value for aqueous root extract could be calculated at 10 mg/mL concentration. The results obtained in our study can be discussed with other blackberry studies, Begam et al. (2018) noted as 82.42% and 0.25 abs. of the maximum DPPH radical scavenging and reducing power activity of blackberry ethanol extract, values lower than

our result [15]. Similar results were achieved by other researchers such as Stajčić et al. (2012) [16] who expressed that the DPPH˙ free radical scavenging activity of the extracts increased based on increasing concentration.

The antioxidant activity of the herbal extract is dependent on the solubility of phenolic substances in tested solvents. This is indicated that soluble phenolic compounds at a high amount are present in methanolic extract achieving high DPPH˙ radical scavenging and reducing capacity activities. Our results also expressed the effect of extraction solvents on antioxidant activity and phytochemical contents in bioactivity studies of plants.

The antibacterial activity of both extracts is represented on clinic strains isolated from patients in Table 5.

Table 5. MIC values of methanol extract (in terms of mm)

	Methanol extract				
	100 mg/mL	**75 mg/mL**	**50 mg/mL**	**25 mg/mL**	**10 mg/mL**
***Pseudomonas* spp.**	12.00±0.00	11.00±0.00	9.50±0.50	-*	-*
***Klebsiella* spp.**	11.00±0.00	9.00±0.00	8.00±0.00	-*	-*
***Proteus* spp.**	11.00±1.00	9.50±0.50	9.50±0.50	-*	-*
S. aureus	11.00±1.00	9.30±1.53	9.50±0.50	-*	-*
E. coli	8.50±0.50	8.00±0.00	6.50±0.50	-*	-*

-*: Any inhibition zone was not observed on MHA plates.

Distilled water root extract demonstrated no antibacterial activity against test bacteria. Negative control (methanol) showed no inhibitory effect on strains. Methanolic extract of *R. fruticosus* roots exhibited any inhibition at minimum extract level (10 and 25 mg/mL) tested against clinic strains but had an antibacterial activity of the other concentrations. The methanolic root extract of *R. fruticosus* was active to test bacteria with average zone diameters ranging from 6.50±0.50 to 12.00±0.00 mm. Methanolic root extract was given a greater zone of inhibition (12.00±0.00 mm) to *Pseudomonas* spp. This extract showed the lowest inhibition with 6.50±0.50 and 8.50±0.50 mm zone diameters against *E. coli* for all concentrations performed. Inhibition diameters of positive controls were ranging to 25±0.00 mm for Polymyxin B, 27.5±0.05 mm for Methicilin, and 16.5±0.05 mm for Tetracycline tested on *Pseudomonas* spp., *S. aureus,* and *Klebsiella* spp., respectively. The inhibitory effect recorded on *E.coli* was 9.00±0.00 mm for Tetracycline. These zones had

the most dramatic effect compared to methanolic root extracts. But the antibacterial effect of methanolic root extract observed against *Proteus* spp. (11.00±1.00 mm) had higher than that of standard tetracycline applied (9.00±0.00 mm). So, the remarkable antibacterial activity of methanolic root extract was observed on *Proteus* spp. MIC for *R. fruticosus* was observed starting at 50 mg/mL concentration against tested all clinic strains. Similar results were acquired by Yigit and Yigit (2014) who stated that the methanol extract of blackberry leaves had antibacterial potential on *S. aureus*, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* [17]. Similar to our result, Riaz et al. (2011) [6] declared that blackberry root extract showed comparable antibacterial activity to the standard Ampicillin used at micron dose level.

For DNA damage protective analysis, blackberry roots dried after evaporation of methanol were used by suspending with distilled water to the preparation of concentrations needed. The electrophoretic pattern of pBR322 run following UV-photolysis of H_2O_2 in presence of different concentrations of root extracts is represented in Figure 1. Three sharp bright bands having different molecular weights belongs to the untreated pBR322 plasmid DNA digested with Alu I, Bsn I, and Hind III enzyme showed on agarose gel electrophoresis (lane A1). OH produced after UV photolysis of H_2O_2 without extract is caused the breakage of DNA strand and smears are ascertained on the gel in lane A2. As can be shown in Fig. 1, methanolic and distilled root extracts at tested all concentrations were revealed to protect all of the bands in the presence of H_2O_2 and UV. Protective effect seen against mutagenicity of UV-photolysis of H_2O_2 on pBR322 plasmid DNA is associated with the highest phytochemical content of the extract. Similarly, the DNA protective activity of blackberry extracts against ultraviolet-B (UVB) radiation was declared by other studies [18]. Our findings revealed that natural antioxidant resources such as *Rubus fruticosus* L. extracts have the efficiency to preserve DNA from adverse effects of UV radiations.

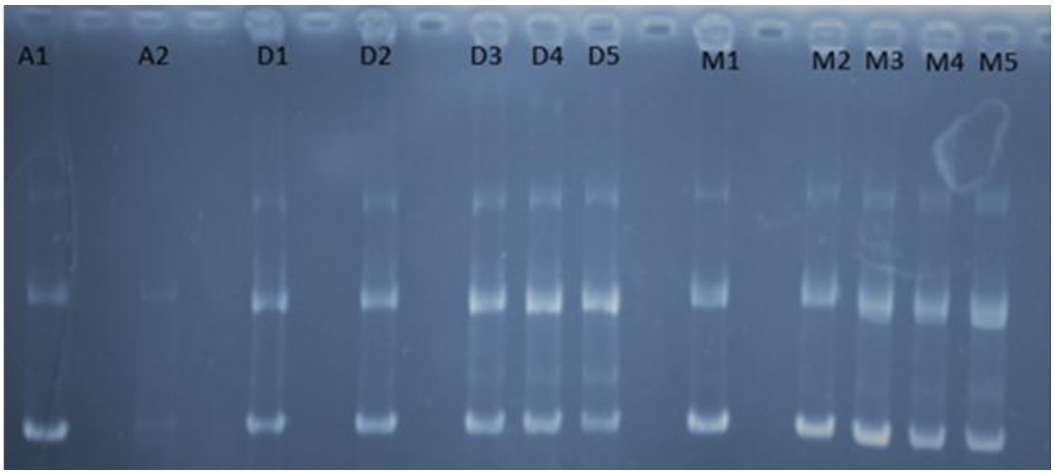

Fig 1 DNA protective activity of the *Rubus fruticosus* L root extracts
A1 lane: pBR322 plasmid DNA+dH_2O, A2 lane: pBR322 plasmid DNA+dH_2O+ 5 min. UV+1 µl H_2O_2, D1 lane: pBR322 plasmid DNA + distilled water root extract (50 mg/mL) + 5 min. UV+ 1 µl H_2O_2, D2 lane: pBR322 plasmid DNA + distilled water root extract (40 mg/mL) + 5 min. UV+ 1 µl H_2O_2, D3 lane: pBR322 plasmid DNA + distilled water root extract (20 mg/mL) + 5 min. UV+ 1 µl H_2O_2, D4 lane: pBR322 plasmid DNA + distilled water root extract (10 mg/mL) + 5 min. UV+ 1 µl H_2O_2, D5 lane: pBR322 plasmid DNA + distilled water root extract (5 mg/mL) + 5 min. UV+ 1 µl H_2O_2, M1 lane: pBR322 plasmid DNA + methanolic root extract (50 mg/mL) + 5 min. UV+ 1 µl H_2O_2, M2 lane: pBR322 plasmid DNA + methanolic root extract (40 mg/mL) + 5 min. UV+ 1 µl H_2O_2, M3 lane: pBR322 plasmid DNA + methanolic root extract (20 mg/mL) + 5 min. UV+ 1 µl H_2O_2, M4 lane: pBR322 plasmid DNA + methanolic root extract (10 mg/mL) + 5 min. UV+ 1 µl H_2O_2, M5 lane: pBR322 plasmid DNA + methanolic root extract (5 mg/mL) + 5 min. UV+ 1 µl H_2O_2

Conclusion

Recently, major researchers have exhibited the biological activity potential of substances obtained by extracting different parts of various plant spices. So, we screened phytochemical properties and pharmacological activities such as antioxidant, antimicrobial, and DNA damage protective of methanolic and aqueous root extracts of *R. fruticosus.* By this study, the methanolic extract exhibited higher activity than those of the standard, BHT and tetracycline for antioxidant and antibacterial analysis. Especially, it was revealed that both extracts possessed significant protective activity of root extracts against UV/H_2O_2 at all tested concentrations in the DNA damage protective test system. Now, the next step is to clarify the chemical structures of health-beneficial bioactive compounds in methanolic root extracts

References

1. Zia-Ul-Haq, M., et al., *Rubus fruticosus L.*: constituents, biological activities and health related uses. Molecules, 2014. 19(8): p. 10998-11029.

2. Ryu, J., et al., Comparison of phytochemicals and antioxidant activity in blackberry (*Rubus fruticosus L.*) fruits of mutant lines at the different harvest time. Plant Breed Biotech, 2016. 4(2): p. 242-251.
3. Espada-Bellido, E., et al., Extraction of antioxidants from blackberry (*Rubus ulmifolius L.*)*:* comparison between ultrasound- and microwave-assisted extraction techniques. Agronomy, 2019. 9(745): p. 2-18.
4. Verma, R., et al., *Rubus fruticosus* (blackberry) use as an herbal medicine. Pharmacognosy Reviews, 2014. 8(16): p. 101-104.
5. Vlad, I. A., et al., Consuming blackberry as a traditional nutraceutical resource from an area with high anthropogenic impact. Journal of Forest Science, 2019. 10(3), p. 246.
6. Riaz, M., M. Ahmad, and N. Rahman, Antimicrobial screening of fruit, leaves, root and stem of *Rubus fruticosus.* Journal of Medicinal Plant Research, 2011. 5(24): p. 5920-5924.
7. Stankovic, M. S., Total Phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. Kragujevac Journal of Science, 2011. 33: p. 63-72.
8. Sharm, S. and P. A. Vig, Evaluation of in vitro antioxidant properties of methanol and aqueous extracts of *Parkinsonia aculeata* L. leaves. The Scientific World Journal, 2013. p. 1-7.
9. Blois, M. S., Antioxidant determinations by the use of a stable free radical. *Nature,* 1958. 26: p. 1199-1200.
10. Oyaizu, M., Studies on product of browing reaction prepared from glucose amine. Japan of Nutrition, 1986. 44: p. 307-315.
11. Bauer, A. W., et al., Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 1966. 45(4): p. 493-496.
12. Russo, A., et al., Free radical scavenging capacity and protective effect of *Bacopa monniera* L. on DNA damage. Phytotherapy Research, 2003. 17: p. 870-875.
13. Četojević-Simin, D. D., et al., Bioactivity of blackberry (*Rubus fruticosus L.*) pomace: polyphenol content, radical scavenging, antimicrobial and antitumor activity. Acta Periodica Technologica, 2017. 48(48): p. 63-76.
14. Najda, A. and H. Łabuda, Content of phenolic compounds and antioxidant properties of fruits of selected orchard shrub species. Modern Phytomorphology, 2013. 3: p. 105-109.
15. Begam, M. S., et al., Assessment of bioactive activity of blackberry seed extract. International Journal of Trend in Scientific Research and Development, 2018. 2(5): p. 1597-1605.
16. Stajčić, S. M., et al., Chemical composition and antioxidant activity of berry fruits. Acta Periodica Technologica, 2012. 43(43): p. 93-105.
17. Yigit, N. and D. Yigit, Antibacterial properties of blackberry (*Rubus fruticocus*). Erzincan University Journal of Science and Technology, 2014. 7(2): p. 267-274.
18. Kyzy, A. R., I. H. Kilic, and S. B. Oguzkan, The antibacterial and DNA-protective effects of blackberry extract. Academia Journal of Medicinal Plants, 2018. 6(11): p. 379-384.