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ANTIBACTERIAL PROPERTIES OF BLACKBERRY (*RUBUS FRUTICOSUS*)

BÖĞÜRTLENİN (*RUBUS FRUTICOSUS*) ANTİBAKTERİYAL ÖZELLİKLERİ

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ABSTRACT

Antibacterial activities of methanol and water extracts of blackberry (*Rubus fruticosus*) were tested against 63 clinical isolates of bacteria strains (*Enterobacter aerogenes, Escherichia coli, Proteusmirabilis, Pseudomonas aeruginosa,* and *Staphylococcus aureus*) by disc-diffusion method and minimal inhibition concentration (MIC) values of each active extract were determined. The blackberry extracts proved to be active against all bacteria species tested in this study. The highest antibacterial activity was expressed by methanol and aquouse extracts of blackberry fruits against *S. aureus* with 20 mm inhibition zone and 0.312 mg/ml MIC value. Also good antibacterial potentials were detected against *Enterobacter aerogenes, Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa* with methanol and water extracts of blackberry leaves ranging with 10-18mm inhibition zones.

Keywords: Blackberry (*Rubus fruticosus),* Antibacterial activity, Disc diffusion method.

ÖZET

Bu çalışmada, böğürtlenin (*Rubus fruticosus*) antibakteriyal aktivitesi, 63 klinik izolat bakteri suşu (*Enterobacter aerogenes, Escherichia coli, Proteusmirabilis, Pseudomonas aeruginosa, Staphylococcus aureus*) üzerinde disk diffüzyon yöntemi kullanılarak belirlenmiştir. Disk diffüzyon yöntemiyle aktivite tespit edilen bakteriler için minimal inhibisyon konsantrasyonları (MİK) da belirlenmiştir. Böğürtlen ekstraktları, çalışmada kullanılan bütün bakteritürleri üzerinde aktivite göstermiştir. En yüksek aktivite, böğürtlen meyvesinin metanol ve su ekstraktlarından,*S. aureus* türüne karşı 20 mm inhibisyon alanı ve 0.312 mg/ml MİK değeriyle elde edilmiştir. Yine böğürtlen yapraklarından elde edilen metanol ve su ekstraksiyonlarından *Enterobacter aerogenes, Escherichia coli, Proteus mirabilis ve Pseudomonas aeruginosa* bakterilerine karşı da 10-18 mm inhibisyon zonlarıyla iyi

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antibakteriyal etkiler tespit edilmiştir. **Anahtar Kelimeler**: Böğürtlen (*Rubus fruticosus*), Antibakteriyal etki, Disk diffüzyon yöntemi

1. INTRODUCTION

Blackberry (*Rubus fruticosus*) is a perennial shrub. It has sprawling, woody and thorny stems. They can reach the height of about 5 meters. It has dark green, hairy leaves, toothed along the margins. Flowers are white to pale pink, appearing from late summer until autumn. Fruits are the well-known fleshy, black berries (Kim,2012).Today, rubus fruit are considered a healthy and nutritions food, containing phenolics, vitamin C, dietary fibre, α-tocopherol, tocotrienol, calcium, potassium, magnesium, carotenoids, linoleic acid (Lee *et al*.,2012). Berries contain a variety of phenolic compounds (phenolic acids, flavonoids, lignansand polymeric tannins) located in plant tissues.The main phenolic compounds in raspberries are flavonoid anthocyanins, ellagic acid and ellagitannins, complex water-soluble phenolic polymers. From the phenolic acids, hydroxycinnamic or hydroxybenzoic acid derivatives are the most common in berries(Oksman-Caldenteyetand Puupponen-pimia, 2006).Due to a high content and wide diversity of phenolic compounds and their health-promoting properties, berries are often regarded as natural functional products properties (Bobinaite *et al*., 2012). It was usually used to treat dysentery, diarrhea, hemorrhoids and cystitis. Nowadays Blackberry is widely recognized as a powerful antioxidant, and it can be used as prevention against heart disease, cancer and eye degenerationsNot only the fruit, but also the raspberry leaves and roots have long tradition as medicinal agents (Baytop,1999).Infusion from the leaves are traditionally used for easing childbirth-related muscle spasms, morning sickness, for colds,sour throats, diarrhoea, threat wounds, colic pain, uterin relaxant, etc. (Anonymous,2014).

Berries have been used as natural antimicrobial pharmaceuticals (Krisch *et al*.,2009; Riaz *et al*,,2011; Abahi *et al*.,2013).Since the plant has potential medicinal properties as antioxidative and antimicrobial, therefore, we conducted current work toscreen antibacterial activities of methanol and water extracts of blackberry fruits and leaves on some human pathogen clinical isolates.

2. MATERIALS AND METHODS

2.1. Preparation of extracts

The blackberry (*Rubus fruticosus*) fruits (at their optimum commercial maturity) and leaves were collected in Bayırbağ Town, Erzincan, Turkey. A voucher specimen number FEF 1651 was deposited in herbarium at Biology Department of Science and Art Faculty, University of Erzincan. The fresh fruit samples were packed on ice while being transported to the laboratory. Fruits samples were frozen at –20°C until extraction. The leaves of blackberry were dried in shade and powdered with a blender. The fruits and powdered leaves were extracted with methanol in a Soxhlet apparatus for 24 h. Then methanol was evaporated with rotary evaporator. Water extracts were also prepared by adding boiling water to 20 g of powdered material in a glass flask and incubated at room temperature for 2 hours on a rotating shaker (200 rpm). Mixture was filtered using Whatman (No.1) filter paper and then filtrate was lyophilized. All extracts were stored in freezer at –24°C until use.

2.2. Test Microorganisms

Antimicrobial activity tests were carried out against clinical isolates of 63 bacterial strains. Microorganisms were provided by Department of Clinical Microbiology, Medicine Faculty, Erzurum. Microorganism species, isolation origins and numbers were showed for bacteria in Table 1.

Table 1.The bacteria species and isolation origins and numbers.

Microorganisms	Blood	Urine	Wound	Ear	Total
Enterobacter aerogenes	–	8	6	–	14
Escherichia coli	2	8	2	1	13
Proteusmirabilis	_	7	2	1	10
Pseudomonas aeruginosa	_	3	8	1	12
Staphylococcus aureus	8	2	3	1	14

2.3.Antibacterial Activity

2.3.1.Disc-diffusion assay

The dried methanol and water extracts were dissolved in the extraction solvent (methanol and sterile distilled water). Final concentration was 30 mg/ml. Antibacterial test were than carried out by disc-diffusion method (Murray *et. al.,* 1995) using suspension containing 10^8 colony forming unit (CFU)/ml of bacteria on nutrient agar (NA; Oxoid). The disc (6mm in diameter) were impregnated with extracts and placed on the inoculated NA. Negative controls were prepared using the same solvents employed to obtain extracts. Ofloxacin (Oxoid) for Gram-positive bacteria, Cefaperazone–sulbactam (Oxoid) for Gram-negative bacteriawere used as positive controls. The inoculated plates were incubated at 37°C for 24 h for clinical bacterial strains. Then antimicrobial activity was evaluated by measuring the inhibition zone against test microorganisms.

2.3.2.Minimal Inhibition Concentration (MIC)

The minimal inhibition concentration (MIC) values were also studied for the microorganisms which were determined as sensitive to the methanol and/or water extracts of plant parts (leaf and fruits) in disc-diffusion assay. MIC values of extracts against microbial strains were determined based on a micro-well dilution method (Zgoda and Porter, 2001). The inoculations of microorganisms were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Firstly, the extracts dissolved in 10% dimethyl sulfoxide (DMSO) were diluted to 10 mg/ml and then serial two fold dilutions were made in a concentration range (0.078-10 mg/ml) in a sterile test tube containing nutrient broth (NB). The 96-well plates were prepared by dispensing into each well 95 □l NB and 5 □l of the inoculums. A 100 □l of extracts initially prepared at the concentration of highest concentration was added the first well, then 100 □l from serial dilutions was transferred into other consecutive wells. The plates covered with a sterile plate sealer and then incubated for 24 h. The MIC was defined as the lowest concentration of the extracts to inhibit the growth of microorganisms.

3. RESULTS AND DISCUSSION

Plant extracts have been used for a wide variety of purposes for many thousands of years. In particular, the antimicrobial activity of some parts of plants has formed the basis of many areas such as alternative medicine and natural therapies.

Rubus fruit have long been collected and consumed worldwide, regardless of whether they were recognised for their possible health benefits from their natural phytochemicals or simply because they tasted good. The plant has also been used medicinally in Turkey. Local people traditionally beleive that deep-coloured fruits, especially black red fruits, are healthier for human body.

The antimicrobial activity of water and methanol extracts of blacklberry fruits and leaves were measured by the disc-diffusion method totally 63 clinic isolates of human pathogenic microorganism belonging to 5 bacteria were used in these investigations (Table 1). The methanol and water negative control showed no inhibiting effect. The inhibition diameters and MIC values of positive controls were ranging to 18–20 mm; 0,12–1□g/mL for Ofloxacin, 19–22 mm; 0,5–1 □g/mL for Cefaperazone, respectively.

Our findings showed that the methanol and water extracts from both fruits and leaves had antibacterial activity against Gram-positive and Gram-negative bacteria. The highest antibacterial activity was expressed by methanol and water extracts of blackberry fruits against *S. aureus* with 20 mm inhibition zone and 0.156 mg/ml MIC value (Table 2 and 3). Both water and methanol extracts of blackberry leaves have activity against all bacteria species ranging between 10-18mm inhibition zone and 0.312-1.25mg/mL MIC values. Water and metanol extracts of fruits have antibacterial ability against only *S. aureus* with 20mm inhibition zone and0.312 mg/mL MIC values but not have any activity against the other species.

Not active, inhibition zone was no greater than 6mm; (7–12 mm), moderately active; (> 12), highly active. Negative controls (Methanol and Water) showed no inhibiting effect. İnhibition diameters of positive controls were ranging to 18–20 mm for Ofloxacin, 19–22 mm for Cefaperazone, respectively.

Table 2.Antibacterial activity of methanol and water extracts of blackberry fruits and leaves against some clinic isolates.

	Inhibition Zone Diameter (mm)			
	Methanol Extracts		Water Extracts	
Microorganisim	Fruit	Leaf	Fruit	Leaf
Enterobacter aerogenes	_	10	_	10
Escherichia coli	_	18	_	18
Proteusmirabilis	_	10	_	10
Pseudomonas aeruginosa	_	10	_	10
Staphylococcus aureus	20	18	20	15

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Table 3.The MIC values (mg/ml) of methanol and water extracts of blackberry fruits and leaves against some bacteria tested in the microdilution assay.

	Minimal Inhibition Concentration (MIC) mg/mL			
	Methanol Extracts		Water Extracts	
Microorganisim	Fruit	Leaf	Fruit	Leaf
Proteus mirabilis	_	1.25	_	1.25
Escherichia coli	_	0.625	_	0.625
Enterobacter aerugenes	_	1.25	_	1.25
Staphylococcus aureus	0.312	0.312	0.312	0.625
Pseudomonas aeroginosa	_	1.25	_	1.25

–: exract not tested. MIC values of positive controls were ranging to 0,12–1 □g/mL for Ofloxacin, 0,12–0,5 □g/mL for Cefaperazone, respectively.

Riaz et. al (2011) reported that methanolic extract of *R. fruticosus* leaves showed zone ofinhibition at minimum dose level especially against *E.coli*, *B. subtilis*, *S. aureus* and *P. mirabilis.* On the other hand different authors (Krisch*et. al*., 2009) reported that both aqueous and methanol extracts of blackberry fruits exhibited considerable antibacterial activityon Gram-positive and Gram-negative bacteria (*Bacillus cereus, B. subtilis, Campylobacter jejuni, E. coli, Salmonella typhimurium*and *Serratia marcescens)* Our results are agreement with above reports that we detected strong antibacterial activity from aqueous and methanol extracts of blackberry fruits and leaves against Gram-positive *S. aureus*and Gram-negative *E. coli*.

Phenolic compounds affected the growth of different bacterial species by differentmechanisms, yet not well understood. It is assumed that not only phenolic compounds are responsible for the antimicrobial activity. The fruits of the genus *Rubus* are rich in ellagitannins, which can permeate the outer cell membrane of Gram-negative bacteria (Ördögh, 2010). So, antimicrobial activity of berries is likely to be caused by multiple mechanisms and synergies because they contain various compounds, for example, weak organic acids, phenolic acids, and tannins and their mixtures of different chemical forms (Velicanski *et al*., 2012).

In this study, we successfully detected strong antibacterial activities from blackberry fruits and leaves against some clinical isolate pathogens. Methanol and water extracts of blackberry exhibited specific activity against the bacteria such as *Enterobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus*. In conclusion, in Turkey, local peoples use blackberry fruits and leaves as medicine. Our study results support this idea. Also we can suggest the plant fungicidal activity on clinic isolate of *Candida* species and antioxidant activityfor future studies using various extracts or fractions.

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