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# DPPH Radical Scavenging, Phenolic and Antimicrobial Activity of Momordica charantia and Rheum ribes.

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# ABSTRACT

In this study was to investigate total phenolic contents, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>), antimicrobial and antifungal activity by using different extract of *Momordica charantia* and *Rheum ribes*. The extraction applied to different part of *Momordica charantia* as husk and fruit and *Rheum ribes* as blossom and stem. Three different were used extraction solvent such as hexane, ethanol and water/acetic acid (1%). The highest radical scavenging activity was observed in ethanol extract of rhubarb blossom, acetic acid (1%) extract of rhubarb showed remarkable radical scavenging activity and the highest phenolic content value was observed acetic acid (1%) extracts of bitter melon. The highest antimicrobial efficacy against *C. albicans* was provided by the ethanol extract of the part of crust of *Momordica charantia* and acetic acid-water extraction of the blossom of *Rheum ribes*.

Keywords: DPPH<sup>•</sup>, Phenolic Content, Antimicrobial activity, Momordica charantia and Rheum ribes



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#### INTRODUCTION

It is known that free radicals are the main cause of various chronic and degenerative diseases such as aging, coronary heart disease, inflammation, cancer and others [1,2]. Many epidemiologic results suggest that there is a relationship between people who are rich in fresh fruits and vegetables and those who have a reduced risk of cardiovascular diseases and some types of cancer [3]. Fruits, vegetables and herbal medicines are concentrated in various types of antioxidant activities, such as various phenolic compounds, natural ingredients [4]. It has been reported that these antioxidant activities correlate with the total phenolic content. Research in recent years has shown that some of the plant-derived therapeutic benefits result from antioxidant activities [5-7].

Momordicacharantia, such a plant commonly used as a medicine [8,9]. The fruit is a long and small cucumber, emerald green that turns into orange-yellow when young fruit matures. Topically used internally and externally in the treatment of wounds, respiratory and parasites. It is also used as an antiviral for emmenagog, measles and hepatitis. In Turkish folk medicine, ripe fruits of Momordicacharantia are used externally and internally for peptic ulcer treatment for rapid healing of wounds [10].

Rheum ribes belongs to the family Polygonaceae. It is used for medical purposes and fresh stems and leaves are consumed as vegetables. It is widely available in Iran, eastern Turkey and Lebanon. R. ribes is the only Rheum species growing in Turkey [11]. Rhubarb roots have been used as laxatives and antipsychiatric drugs in Iran [12].Origins of the species are also used in the treatment of diabetes, hypertension, obesity and diarrhea [13,14]. The young R. ribes are used for daily and antiemetic treatment [11].

For this reason, the aim of this study is to investigate the total phenolic content, 1, 1-Diphenyl-2picrylhydrazyl (DPPH\*), antimicrobial and antifungal activity by using different extract of Momordicacharantia and Rheum ribes. The extraction applied to different part of Momordicacharantia as husk and fruit and Rheum ribesas blossom and stem. Three different were used extraction solvent such as hexane, ethanol and water/acetic acid (1%).

# **EXPERIMENTAL**

#### **Materials, Chemicals and Reagents**

Momordicacharantia were collected south Anatolian area from Turkey. The ripe fruits were harvested as fully yellow, raw fruits were harvested as fully green. Rheum ribes were collected Southeast Anatolia from Turkey. The whole consumable part of the plant is green. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH\*), Folin-Ciocalteu reagent, ethanol, hexane were obtained from Sigma-Aldrich. Gallic Acid, Dimethyl Sulfoxide, acetic acid were purchased from Merck.

# Extraction

The plant of Momordicacharantia and Rheum ribes was extracted with acetic acid (%1) [15]), ethanol and hexane. The extraction process has been continued for 24 h at a room temperature and using an orbital shaker. Extraction of plants three times with each solvent. The extracts of each solvent were combined and filtered, respectively. The filtrates were evaporated to dryness. Each extract was dissolved in DMSO (Dimethyl sulfoxide) for using antimicrobial and antifungal activity assays.

# **DPPH**<sup>•</sup> Radical Scavenging Activity Assay

The free radical scavenging activity of acetic acid (1%), ethanol and hexane extracts of Momordicacharantia and Rheum ribes fruits and stems was determined by DPPH<sup>•</sup> assay described by Blois [16] with minor modifications. Briefly, 1 mM solution of DPPH<sup>•</sup> in methanol was prepared. Then, five separate test tubes were added to 600 µL of DPPH<sup>•</sup> and extraction solutions at specific concentrations (20, 40, 60, 80, 100  $\mu$ L) were added and complete with methanol as volume 6 mL.

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#### Determination of total flavonoid content

Total flavonoid contents were performed according to Singleton and Rossi [17]. The reaction mixture contained 1 ml of samples fraction extract, 5 mL of the Folin–Ciocalteu reagent, 10 mL of sodium carbonate (saturated) and 75 mL of distilled water complete with distilled water as volume 100 mL. It was then allowed to stand for 2 hours with intermittent agitation, the absorbance at 720 nm was measured and was used to calculate the phenolic content using gallic acid as standard.

#### Preparation of microorganism cultures

Listeria monocytogenes, Staphylococcus aureus (ATCC 25923), Escherichia coli Tip 1, Vibrio parahaemolyticus (ATCC 17802), Bacillus cereus, Salmonella typhimurium, Staphylococcus epidermis, Yersinia pseudotuberculosis, Enterococcus faecalis, Klebsiellapneumoniae, Proteus vulgaris, Aspergillus niger and Candida albicans were used in this study as test microorganism. Each colony grown on selective media was inoculated into 5 ml Nutrient Broth (Merck) medium for bacterial cultures and Sabouraud Dextrose Broth (Merck) medium for yeast and mold cultures. Cultures left incubation overnight at 37 °C for bacterial cultures and at 25 °C for yeast and mold samples. After incubation, bacterial and yeast-mold stock solutions were prepared which the cultures containing 1-2x108 CFU/mL cells corresponding to 0.5 McFarland standard[18,19].

#### **Determination of Antimicrobial Activity**

Antimicrobial activities of plant extracts were determined according to the Clinical Laboratory Standards Institute (CLSI, 2012) standards using the disk diffusion method against pathogen strains. Bacterial, yeast and mold stock solutions were inoculated with 100  $\mu$ L of appropriate media (Mueller Hinton Agar (Merck) for bacteria, Sabouraud Dextrose Agar (Merck) for *Aspergillus niger and Candida albicans*) and spread to the surface of the media with sterile glass baguette and then left for 15-20 minutes to dry. For disk diffusion test; the obtained dry and/or waxy plant extracts were dissolved in dimethyl sulfoxide (99.9%; DMSO). Sterile empty antibiotic discs with a diameter of 6 mm (Schleicher &Schül, Nr. 2668, Germany) were placed on media, which inoculated with stock cultures at sterile conditions. Sterile empty antibiotic discs were impregnated with plant extracts at 10, 20 and 30  $\mu$ L concentrations according to aseptic conditions. The media prepared were allowed to incubate for 18-24 hours at 37 °C for bacterial cultures and at 25 °C for 18-24 hours for yeast and molds. The inhibition zone diameter that occurred after the incubation was recorded by measuring as millimeter. For comparison, empty antimicrobial discs containing no antimicrobial agent were used as controls [19-21]

#### **Statistical analysis**

The samples were analyzed individually in triplicate, and the data were reported as the mean  $\pm$  standard deviation. One-way analysis of variance (ANOVA) with the post hoc Duncan test was used to compare any significant differences between the extracts and the control at the 5% significance level (p < 0.05).

#### **RESULTS AND DISCUSSION**

#### **DPPH**<sup>•</sup> Radical Scavenging Activity

DPPH<sup>•</sup>, a stable free radical with characteristic absorption at 517 nm, was used to study the radical scavenging effects of the extracts. Antioxidants donate proton to these radicals while absorption decreases. The decrease in absorbance is taken as a measure of the degree of radical sweep [22]. The scavenging effects of DPPH<sup>•</sup> was investigated of acetic acid (1%), ethanol and hexane extract. The highest radical scavenging activity was observed in ethanol extract of rhubarb blossom, acetic acid (1%) extract of rhubarb showed remarkable radical scavenging activity. The different solvents extracts from bitter melon and bitter melon crust observed close activity to BHA. Rhubarb hexane extract was observed very low activity.

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# Figure 2. Radical scavenging activities of Bitter melon and Bitter melon crust

# Determination of total phenolic compounds

The concentration of total phenolic compounds in the bitter melon (*Momordicacharantia*) and rhubarb (*Rheum ribes*)was extracted with acetic acid (%1), ethanol and hexane extract, determined as micrograms of gallic acid equivalents (Table 1), by using an equation that was obtained from the standard gallic acid graph, is given as:

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	Acetic Acid (1%) Extract	Ethanol Extract	Hexane Extract
Bitter Melon	188.4±0.08ª	123.3±0.01 <sup>a</sup>	36.5±0.04ª
Bitter Melon Crust	70.7±0.02 <sup>b</sup>	121.1±0.06 <sup>b</sup>	171.8±0.4 <sup>b</sup>
Rhubarb	123.8±0.1 <sup>c</sup>	161.15±0.26 <sup>c</sup>	131.05±0.35°
Rhubarb Blossom	26.49±0.1 <sup>d</sup>	17.72±0.007 <sup>d</sup>	0.162±0.001 <sup>d</sup>

#### Table 1: Total phenolic content of plant extracts (mg gallic acid/g extract)

a-d: followed by the same letters are not significantly different according Duncan's multiple range test at  $P \le 0.05$  for each plant

Phenolic compounds are prevalent in plants that are very noticeable due to their antioxidant activity, potentially positive for human health, and their ability to clear free radicals [23-27]. Total phenol content was determined in comparison with standard gallic acid and the results expressed in terms of mg GAE/g dry sample. The highest phenolic content value was observed acetic acid (%1) extracts of bitter melon, the lowest phenolic content was observed hexane extracts of rhubarb blossom.

# **Antimicrobial Activity**

In the study, antimicrobial activities against some microorganisms known to be human and food pathogens were determined for flowers and edible parts of *Momordicacharantia* and *Rheum ribes* extracted with hexane, acetic acid-water and ethanol. *Listeria monocytogenes, Staphylococcus aureus* (ATCC 25923), *Escherichia coli* Tip 1, *Vibrio parahaemolyticus*(ATCC 17802), *Bacillus cereus, Salmonella typhimurium, Staphylococcus epidermis, Yersinia pseudotuberculosis, Enterococcus faecalis, Klebsiella pneumoniae, Proteus vulgaris, Aspergillus niger* and *Candida albicans* were used in this antimicrobial study as test microorganism. Each extract were added to the discs at different concentrations (10, 20 and 30  $\mu$ l) according to disc diffusion method. After the incubation, antimicrobial effect was determined by measuring the zone diameters. For determining the antimicrobial effect, according to the disc diffusion method, 5.5 - 9 mm is too low inhibition diameter, 9 - 12 mm is the low inhibition diameter, 12-15 mm is the mean inhibition diameter and 15 mm and above is high inhibition diameter as evaluated [28].

The antimicrobial effect of Momordicacharantia extracts against test microorganisms was shown in Tables 2 and 3. When Table 2 and Table 3 were examined, all extracts, which were used against the strongest antimicrobial effect against Candida albicans, while the weakest antimicrobial effect against Bacillus cereus (p≤0.05). As shown in Table 2, while BM-1 extract showed the strongest antimicrobial effect against Aspergillus niger, it did not show any antimicrobial effect against B. cereus, Escherichia coli type 1, Enterococcus faecalis, Salmonella Typhimurium and Candida albicans. While BM-2 extract showed the strongest antimicrobial effect against Vibrio parahaemolyticus ATCC 17802, it did not show any antimicrobial effect against B. cereus and Klebsiella pneumoniae. While the BM-3 extract was the strongest antimicrobial effect against Candida albicans, it did not show any antimicrobial effect against B. cereus and Staphylococcus epidermis. As seen in Table 3, the BMC-1, BMC-2 and BMC-3 extracts showed the strongest antimicrobial effect against Candida albicans. The BMC-1 extract did not show any antimicrobial activity against Klebsiella pneumoniae. Similarly, BMC-2 extract did not show any antimicrobial activity against B. cereus, E. coli Type 1 and Klebsiella pneumoniae, while BMC-3 extract did not show any antimicrobial activity against B. cereus and Klebsiella pneumoniae. Thiruvengasam et al. (2016), in a similar study, was determined antimicrobial effect of different extracts of Momordicacharantia against some food pathogens by using the disk diffusion method and measured zone diameters. According to the results of this study, zone diameters were obtained for S. aureus  $20.5 \pm 0.2$  to  $25.1 \pm 0.2$  mm; for *E. coli*  $19.2 \pm 0.5$  to  $23.0 \pm 0.5$  mm; for *A. niger*  $18.5 \pm 0.4 - 22.0 \pm 1.0$  mm and it was determined that the extracts showed antimicrobial properties.

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					Extracto					
		BM-1*			BM-2*		 BM-3*			
	Inhibition zone diameter (mm)**									
Microorganisms	10µl	20µl	30µl	10µl	20µl	30µl	10µl	20µl	30µl	
Bacillus cereus	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	
Escherichia coli	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	8±1.528 <sup>b</sup>	NI <sup>a</sup>	NI <sup>a</sup>	11±2.00 <sup>b</sup>	
Staphylococcus aureus	7±1.155ª	9±1.000 <sup>b</sup>	12±0.577 <sup>c</sup>	7±1.155ª	10±1.528 <sup>ab</sup>	12±1.528 <sup>b</sup>	7±0.577ª	10±1.528 <sup>b</sup>	13±2.000 <sup>c</sup>	
Staphylococcus epidermis	7±1.155ª	11±1.000 <sup>b</sup>	15±2.082 <sup>c</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	
Vibrio parahemolyticus	NI <sup>a</sup>	9±0.577 <sup>b</sup>	12±1.000 <sup>c</sup>	8±0.000ª	11±1.528 <sup>b</sup>	13±0.577 <sup>b</sup>	8±0.000ª	11±0.577 <sup>b</sup>	13±1.528 <sup>c</sup>	
Yersinia pseudotuberculosis	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	7±1.732 <sup>b</sup>	8±0.577ª	10±1.000 <sup>b</sup>	12±1.000 <sup>c</sup>	
Listeria monocytogenes	NI <sup>a</sup>	8±1.155 <sup>b</sup>	10±1.000 <sup>c</sup>	NI <sup>a</sup>	7±1.155 <sup>b</sup>	11±0.577 <sup>c</sup>	NI <sup>a</sup>	8±0.577 <sup>b</sup>	12±1.528 <sup>c</sup>	
Enterococcus faecalis	NIª	NI <sup>a</sup>	NIa	NI <sup>a</sup>	NI <sup>a</sup>	7±1.155 <sup>b</sup>	6±0.577ª	10±1.000 <sup>b</sup>	12±1.000 <sup>c</sup>	
Salmonella Typhimurium	NIª	NI <sup>a</sup>	NIa	NI <sup>a</sup>	NI <sup>a</sup>	9±1.155 <sup>b</sup>	6±0.000ª	8±0.577 <sup>b</sup>	11±0.577 <sup>c</sup>	
Klebsiella pneumoniae	NIª	NI <sup>a</sup>	6±0.577 <sup>b</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NIa	NI <sup>a</sup>	NIª	10±1.000 <sup>b</sup>	
Proteus vulgaris	7±1.000ª	10±0.577 <sup>b</sup>	12±0.577 <sup>c</sup>	NI <sup>a</sup>	9±1.000 <sup>b</sup>	12±1.155°	7±1.155ª	10±0.577 <sup>b</sup>	13±0.577 <sup>c</sup>	
Candida albicans	NI <sup>a</sup>	NI <sup>a</sup>	NIª	6±0.000ª	8±1.528 <sup>b</sup>	11±0.577 <sup>c</sup>	7±1.155ª	13±0.577 <sup>b</sup>	14±1.155 <sup>b</sup>	
Aspergillus niger	8±0.577ª	12±1.155 <sup>b</sup>	16±0.577 <sup>c</sup>	NI <sup>a</sup>	NI <sup>a</sup>	6±0.577 <sup>b</sup>	NI <sup>a</sup>	7±1.155 <sup>b</sup>	13±2.082 <sup>c</sup>	

#### Table 2: Antimicrobial activity of fruit part of *M. charantia* acetic acid-water, ethanol and hexane extracts against test microorganisms

\*BM-1: The acetic acid- water extract of fruit part of *M. charantia*;BM-2:The ethanol extract of fruit part of *M. charantia*;BM-3: The hexane extract of fruit part of *M. charantia*.

\*\* Values, including the diameter of the well (6 mm), Means  $\pm$  standart deviation of three replicates (n = 3); a-c: followed by the same letters are not significantly different according Duncan's multiple range test at P  $\leq$  0.05 for each row and for each microorganism; NI: No inhibition.



					Extracts						
		BMC-1*			BMC-2*			BMC-3*			
	Inhibition zone diameter (mm)**										
Microorganisms	10µl	20µl	30µl	10µl	20µl	30µl	10µl	20µl	30µl		
Bacillus cereus	NI <sup>a</sup>	13±1.528 <sup>b</sup>	16±1.155 <sup>c</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>		
Escherichia coli	NI <sup>a</sup>	7±0.577 <sup>b</sup>	10±1.732 <sup>c</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	6±0.000 <sup>a</sup>	9±0.577 <sup>b</sup>	11±1.155 <sup>c</sup>		
Staphylococcus aureus	NI <sup>a</sup>	10±2.000 <sup>b</sup>	13±0.577 <sup>c</sup>	8±1.000 <sup>a</sup>	10±0.577 <sup>b</sup>	14±0.577 <sup>c</sup>	NI <sup>a</sup>	9±0.577 <sup>b</sup>	13±1.000 <sup>c</sup>		
Staphylococcus epidermis	7±0.000 <sup>a</sup>	9±0.577 <sup>b</sup>	12±1.155 <sup>c</sup>	6±0.577 <sup>a</sup>	9±0.000 <sup>b</sup>	11±1.528 <sup>c</sup>	NI <sup>a</sup>	10±0.577 <sup>b</sup>	12±0.577 <sup>c</sup>		
Vibrio parahemolyticus	NI <sup>a</sup>	7±0.577 <sup>b</sup>	11±1.000 <sup>c</sup>	6±0.577 <sup>a</sup>	11±1.000 <sup>b</sup>	14±1.732 <sup>c</sup>	NI <sup>a</sup>	8±0.577 <sup>b</sup>	10±1.000 <sup>c</sup>		
Yersinia pseudotuberculosis	NI <sup>a</sup>	NI <sup>a</sup>	11±0.577 <sup>b</sup>	6±0.577 <sup>a</sup>	9±0.577ª	12±2.082 <sup>b</sup>	NI <sup>a</sup>	9±0.577 <sup>b</sup>	12±1.155 <sup>c</sup>		
Listeria monocytogenes	NI <sup>a</sup>	NI <sup>a</sup>	8±1.155 <sup>b</sup>	NI <sup>a</sup>	8±0.577 <sup>b</sup>	12±1.732 <sup>c</sup>	NI <sup>a</sup>	NI <sup>a</sup>	8±1.155 <sup>b</sup>		
Enterococcus faecalis	NI <sup>a</sup>	7±1.000 <sup>b</sup>	10±1.155 <sup>c</sup>	7±0.577 <sup>a</sup>	9±0.577 <sup>b</sup>	12±1.528 <sup>c</sup>	NI <sup>a</sup>	9±0.577 <sup>b</sup>	11±1.528 <sup>c</sup>		
Salmonella Typhimurium	9±0.577ª	10±0.000 <sup>b</sup>	11±0.577 <sup>c</sup>	7±1.155ª	11±1.000 <sup>b</sup>	14±0.577 <sup>c</sup>	NI <sup>a</sup>	NI <sup>a</sup>	6±0.000 <sup>b</sup>		
Klebsiella pneumoniae	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>		
Proteus vulgaris	7±1.155ª	10±1.155 <sup>b</sup>	14±1.155 <sup>c</sup>	8±0.577ª	12±1.155 <sup>b</sup>	14±1.155 <sup>c</sup>	NI <sup>a</sup>	10±0.577 <sup>b</sup>	12±1.155°		
Candida albicans	7±1.155ª	17±1.528 <sup>b</sup>	31±2.082 <sup>c</sup>	12±0.577ª	20±2.082 <sup>b</sup>	21±1.528 <sup>b</sup>	11±1.000ª	18±0.577 <sup>b</sup>	29±1.528°		
Aspergillus niger	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NIª	8±0.577 <sup>b</sup>	10±1.000 <sup>c</sup>	NIª	NIª	NIª		

Table 3: Antimicrobial activity of crust part of M. charantia acetic acid-water, ethanol and hexane extracts against test microorganisms

\*BMC-1: The acetic acid- water extract of crust part of *M. charantia*;BMC-2:The ethanol extract of crust part of *M. charantia*;BMC-3: The hexane extract of crust part of *M. charantia*;BMC

\*\* Values, including the diameter of the well (6 mm), Means  $\pm$  standart deviation of three replicates (n = 3); a-c: followed by the same letters are not significantly different according Duncan's multiple range test at P  $\leq$  0.05 for each row and for each microorganism; NI: No inhibition.



					Extracts				
		RB-1*			RB-2*			RB-3*	
	Inhibition zone diameter (mm)**								
Microorganisms	10µl	20µl	30µl	10µl	20µl	30µl	10µl	20µl	30µl
Bacillus cereus	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	7±1.155ª	11±1.155 <sup>b</sup>	14±1.000 <sup>c</sup>	6±0.000 <sup>a</sup>	9±1.000 <sup>b</sup>	11±1.000 <sup>c</sup>
Escherichia coli	7±1.155ª	10±1.528 <sup>b</sup>	13±1.528 <sup>c</sup>	NI <sup>a</sup>	8±0.577 <sup>b</sup>	13±2.082 <sup>c</sup>	NI <sup>a</sup>	8±0.577 <sup>b</sup>	12±0.577 <sup>c</sup>
Staphylococcus aureus	6±0.577ª	10±0.577 <sup>b</sup>	12±0.000 <sup>c</sup>	12±1.000 <sup>a</sup>	19±1.528 <sup>b</sup>	23±1.155 <sup>c</sup>	9±1.155ª	11±0.577ª	15±1.258 <sup>b</sup>
Staphylococcus epidermis	8±0.577 <sup>a</sup>	10±0.577 <sup>b</sup>	14±1.155 <sup>c</sup>	9±1.155ª	18±0.577 <sup>b</sup>	23±1.155 <sup>c</sup>	8±0.577 <sup>a</sup>	10±0.577 <sup>a</sup>	12±0.577 <sup>b</sup>
Vibrio parahemolyticus	6±0.577ª	10±0.577 <sup>b</sup>	14±1.528 <sup>c</sup>	NI <sup>a</sup>	8±0.577 <sup>b</sup>	14±0.577 <sup>c</sup>	NI <sup>a</sup>	9±1.155 <sup>b</sup>	12±1.000 <sup>c</sup>
Yersinia pseudotuberculosis	8±0.577ª	12±1.528 <sup>b</sup>	27±1.155 <sup>c</sup>	10±0.577ª	15±0.577 <sup>b</sup>	19±0.577 <sup>c</sup>	NI <sup>a</sup>	9±1.155 <sup>b</sup>	13±2.646 <sup>c</sup>
Listeria monocytogenes	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	6±0.577ª	10±0.577 <sup>b</sup>	12±1.732 <sup>c</sup>	NI <sup>a</sup>	10±0.577 <sup>b</sup>	13±2.000 <sup>c</sup>
Enterococcus faecalis	7±0.577 <sup>a</sup>	9±0.000 <sup>b</sup>	12±0.577 <sup>c</sup>	7±0.577ª	10±0.577 <sup>b</sup>	13±2.000 <sup>c</sup>	8±0.577 <sup>a</sup>	11±1.155 <sup>b</sup>	15±1.732 <sup>c</sup>
Salmonella Typhimurium	NI <sup>a</sup>	NI <sup>a</sup>	9±1.732 <sup>b</sup>	9±0.577ª	12±0.577 <sup>b</sup>	15±0.577 <sup>c</sup>	7±1.155ª	10±1.528 <sup>ab</sup>	12±1.732 <sup>b</sup>
Klebsiella pneumoniae	7±1.155ª	9±2.000 <sup>ab</sup>	12±2.646 <sup>b</sup>	NI <sup>a</sup>	10±0.577 <sup>b</sup>	13±1.000 <sup>c</sup>	7±1.155ª	9±1.155 <sup>b</sup>	12±2.000 <sup>c</sup>
Proteus vulgaris	7±0.577ª	10±0.577 <sup>b</sup>	14±1.000 <sup>c</sup>	12±1.155ª	15±1.155 <sup>b</sup>	20±1.528°	7±1.155ª	11±1.155 <sup>b</sup>	13±0.577 <sup>c</sup>
Candida albicans	10±0.577ª	24±2.082 <sup>b</sup>	30±0.577 <sup>c</sup>	8±0.577ª	11±1.155 <sup>b</sup>	13±1.155°	9±0.577ª	11±1.000 <sup>b</sup>	14±0.000 <sup>c</sup>
Aspergillus niger	8±1.155ª	11±2.309 <sup>ab</sup>	13±2.082 <sup>b</sup>	NI <sup>a</sup>	8±0.577 <sup>b</sup>	12±0.577 <sup>c</sup>	NI <sup>a</sup>	6±0.000 <sup>ab</sup>	8±2.000 <sup>b</sup>

Table 4: Antimicrobial activity of blossom part of Rheum ribesacetic acid-water, ethanol and hexane extracts against test microorganisms

\*R-1: The acetic acid- water extract of blossom part of *Rheum ribes*;R-2: The ethanol extract of blossom part of *Rheum ribes*;R-3: The hexane extract of blossom part of *Rheum ribes* 

\*\* Values, including the diameter of the well (6 mm), Means ± standart deviation of three replicates (n = 3); a-c: followed by the same letters are not significantly different according Duncan's multiple range test at P ≤ 0.05 for each row and for each microorganism; NI: No inhibition.



					Extracts				
		R-1*			R-2*			R-3*	
				Inhibition zo	one diameter				
Microorganisms	10µl	20µl	30µl	10µl	20µl	30µl	10µl	20µl	30µl
Bacillus cereus	6±0.577ª	8±0.000 <sup>b</sup>	12±1.000 <sup>c</sup>	9±1.155ª	12±0.577 <sup>b</sup>	13±0.577 <sup>c</sup>	NI <sup>a</sup>	8±1.155 <sup>b</sup>	12±0.577 <sup>c</sup>
Escherichia coli	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	7±1.155ª	11±1.000 <sup>b</sup>	13±1.155°	NI <sup>a</sup>	9±1.155 <sup>b</sup>	13±1.000 <sup>c</sup>
Staphylococcus aureus	11±0.577ª	14±1.000 <sup>b</sup>	17±1.155 <sup>c</sup>	9±1.155ª	14±0.577 <sup>b</sup>	21±1.155 <sup>c</sup>	8±0.577 <sup>a</sup>	11±0.577 <sup>b</sup>	14±0.577 <sup>c</sup>
Staphylococcus epidermis	9±1.155ª	12±2.082 <sup>b</sup>	14±2.517 <sup>c</sup>	7±1.000 <sup>a</sup>	9±1.155 <sup>b</sup>	12±1.528 <sup>c</sup>	NI <sup>a</sup>	8±0.577 <sup>b</sup>	11±0.577 <sup>c</sup>
Vibrio parahemolyticus	NI <sup>a</sup>	12±2.082 <sup>b</sup>	15±2.309 <sup>c</sup>	7±1.155ª	10±0.577 <sup>b</sup>	12±1.528 <sup>b</sup>	6±0.000 <sup>a</sup>	10±0.577 <sup>b</sup>	12±0.577 <sup>c</sup>
Yersinia pseudotuberculosis	11±0.577ª	13±1.000 <sup>b</sup>	17±1.000 <sup>c</sup>	6±0.577 <sup>a</sup>	12±0.577 <sup>b</sup>	14±0.577 <sup>c</sup>	6±0.577 <sup>a</sup>	10±0.577 <sup>b</sup>	13±1.155 <sup>c</sup>
Listeria monocytogenes	NI <sup>a</sup>	7±1.155 <sup>b</sup>	11±1.155 <sup>c</sup>	7±0.577 <sup>a</sup>	10±0.577 <sup>b</sup>	12±1.155 <sup>c</sup>	6±0.000 <sup>a</sup>	7±1.000 <sup>a</sup>	10±0.577 <sup>b</sup>
Enterococcus faecalis	7±1.155ª	9±0.577 <sup>b</sup>	12±1.155 <sup>c</sup>	7±1.155ª	10±0.577 <sup>b</sup>	12±1.155 <sup>c</sup>	6±0.000 <sup>a</sup>	$10 \pm 1.000^{b}$	13±1.155 <sup>c</sup>
Salmonella Typhimurium	NI <sup>a</sup>	9±1.000 <sup>b</sup>	12±1.000 <sup>c</sup>	8±.000 <sup>a</sup>	11±1.000 <sup>b</sup>	16±0.577 <sup>c</sup>	NI <sup>a</sup>	8±0.577 <sup>b</sup>	12±1.155 <sup>c</sup>
Klebsiella pneumoniae	7±1.155ª	10±2.082 <sup>b</sup>	14±0.577 <sup>c</sup>	NI <sup>a</sup>	10±0.577 <sup>b</sup>	14±1.000 <sup>c</sup>	6±0.000 <sup>a</sup>	9±1.000 <sup>b</sup>	12±1.528°
Proteus vulgaris	9±1.155ª	13±1.155 <sup>b</sup>	16±0.577 <sup>c</sup>	7±1.155ª	11±1.155 <sup>b</sup>	18±0.577°	NI <sup>a</sup>	10±0.577 <sup>b</sup>	12±0.577 <sup>c</sup>
Candida albicans	8±0.577ª	12±2.082ª	29±3.215 <sup>b</sup>	7±1.155ª	16±2.000 <sup>b</sup>	21±3.606 <sup>c</sup>	7±1.155ª	10±2.000ª	15±3.055 <sup>b</sup>
Aspergillus niger	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>	7±1.155ª	9±2.000 <sup>b</sup>	12±1.000 <sup>c</sup>	NI <sup>a</sup>	NI <sup>a</sup>	NI <sup>a</sup>

Table 5: Antimicrobial activity of fruit part of Rheum ribes blossom acetic acid-water, ethanol and methanol extracts against test microorganisms

\*RB-1: The acetic acid- water extract of fruit part of *Rheum ribes*; RB-2: The ethanol extract of fruit part of *Rheum ribes*; RB-3: The hexane extract of fruit part of *Rheum ribes*; RB-2: The ethanol extract of fruit part of *Rheum ribes*; RB-3: The hexane extract of fruit part of *Rheum ribes*; RB-2: The ethanol extract of fruit part of *Rheum ribes*; RB-3: The hexane extract of fruit part of *Rheum ribes*; RB-2: The ethanol extract of fruit part of *Rheum ribes*; RB-3: The hexane extract of fruit part of *Rheum ribes*; RB-2: The ethanol extract of fruit part of *Rheum ribes*; RB-3: The hexane extract of fruit part of *Rheum ribes*; RB-2: The ethanol extract of fruit part of *Rheum ribes*; RB-3: The hexane extract of fruit part of *Rheum ribes*; RB-2: The ethanol extract of fruit part of *Rheum ribes*; RB-3: The hexane extract of fr



In another study by Jabeen and Khanum (2017), zone diameters were obtained  $3.66 \pm 0.577$  to  $11.66 \pm 0.577$  mm for *S. aureus*;  $6.66 \pm 1.000$  to  $12.00 \pm 1.150$  mm for *E. coli*;  $4.33 \pm 0.577 - 9.00 \pm 0.577$  mm for *Salmonella Typi* by the different concentrations of extracts of *Momordicacharantia* extracts with different extraction methods. Shoba et al. (2014), zone diameter were obtained 7 mm for *E. coli*; 8 mm for *S. aureus*; 6 mm for *C. albicans* and 5 mm for *A. niger* and the antimicrobial effect were achieved by using the methanol extracts of *Momordicacharantia*. Roopashree et al. (2008)were investigated the antimicrobial activity of *Momordicacharantia* of water, petroleum ether, methanol and ethanol extract against *S. aureus*, *E. coli* and *P. aeruginosa*[29]. The results of this study showed that the aqueous extract of *Momordicacharantia* has a higher antibacterial activity against *S. aureus*, with a zone diameter of 22 ± 0.64 mm. In another comprehensive study, which conducted by Ozusaglam and Karakoca (2013), ethanol extracts of *Momordicacharantia* had shown antimicrobial activity with zone diameters of 10.73 ± 0.34 - 21.68 ± 0.23 mm for *B. cereus* RSKK 863;0.30±0.36 - 14.58±0.13 mm for *E. coli* O157: H7; 0.42±0.63 - 20.52±0.02 for *S. aureus* ATCC 25923; 10.31±0.10 - 18.98±0.05 mm for *E. coli* ATCC 35218; 11.21±0.40 - 25.22±0.43 mm for *C. albicans* ATCC 10231 and 10.68±0.21 - 16.38±0.36 mm for *L. monocytogenes* ATCC 7644[30].

The antimicrobial activity of the extracts of *Rheum ribes* was shown in Table 4 and 5. When were examined Table 4 and Table 5, it was found that all used extracts of *Rheum ribes* showed the strongest antimicrobial effect against *Candida albicans* and the weakest antimicrobial effect against *Bacillus cereus*, similar to extracts from *Momordicacharantia*. The increase in the concentration of the extracts added to the discs also resulted in an increase in the diameters of the antimicrobial zones ( $p \le 0.05$ ). It was determined that the highest antimicrobial effect of R-1 extract was against *Candida albicans*, but it did not show any antimicrobial effect *against B. cereus* and *L. monocytogenes*. The R-2 extract was shown the highest inhibition effect against *Staphylococcus aureus*, while the lowest inhibitory effect was detected against *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Salmonella Typhimurium*, *Klebsiella pneumoniae* and *Candida albicans* by using R-2 extract. Similarly, the highest antimicrobial effect of the R-3 extract was found to be against *Staphylococcus aureus* while the lowest inhibitory effect was against *Aspergillus niger*.

The low inhibition effect was determined against Bacillus cereus, Escherichia coli, Staphylococcus epidermis, Vibrio parahemolyticus, Yersinia pseudotuberculosis, Listeria monocytogenes, Salmonella typhimurium, Klebsiella pneumoniae, Proteus vulgaris and Candida albicans, while the mean inhibition effect against Enterococcus faecalis and Staphylococcus aureus. The RB-1, RB-2 and RB-3 extracts showed the highest antimicrobial activity against C. albicans while RB-1 extract had the lowest antimicrobial activity against E. coli and A. niger. It was determined that the RB-2 extract showed the lowest antimicrobial activity against S. epidermidis and L. monocytogenes, whereas the RB-3 extract was shown against A. niger. In a similar study conducted by Darsanaki and Lisar (2014) by using methanol extract of Rheum ribes was determined antimicrobial activity with a zone diameter  $8.25\pm0.35 - 11.75\pm0.21$  mm for *S. aureus*;  $9.5\pm0.70 - 12.5\pm0.70$ for E. coli;  $10.5 \pm 0.70 - 13.5 \pm 0.35$  mm for K. pneumoniae. Tartik et al. reported that the root ethanol extract of Rheum ribes showed high antimicrobial activity on E. aerogenes, S. aureus and E. coli[31]. Alan et al. were detected the antimicrobial effect by measuring the zone diameter with using ethanol and methanol extracts of Rheum ribes and 16-22 mm for B. subtilis ATCC 6633, 21 - 23 mm for Enterobacteraerogenes ATCC 13048 zone diameter was reported[32]. In a similar study by conducted Amiriet al.[33], the antimicrobial activity of hexane extract of *Rheum ribes* was determined with zone diameters and zone diameter was reported as  $15.0 \pm 0.1$ mm for S. epidermidis; 7.8  $\pm$  0.1 mm for S. pneumoniae; 6.5  $\pm$  0.1 mm for K. pneumoniae; 8.0  $\pm$  0.1 mm for S. Typhimurium.

# CONCLUSION

The results of this study indicate that the *Momordicacharantia and Rheum ribes* different solvent extracts remarkable total phenolic content and DPPH<sup>•</sup> radical scavenging activity.. When the results of antimicrobial activity of plant extracts are evaluated, the highest antimicrobial efficacy against *C. albicans* was provided by the ethanol extract of the part of crust of *Momordicacharantia*. It has been determined that *B. cereus* is the most resistant to all extracts of the same plant. Similarly, the highest antimicrobial activity was found against *C. albicans* with the acetic acid-water extraction of the blossom of *Rheum ribes*, while *B. cereus* 



was found to be the most resistant to all extracts of Rheum ribes. In forward studies, isolation from crude extracts may provide high antimicrobial and antioxidant fractions.

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