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Antimicrobial and antioxidant activities of *Momordica charantia* from Turkey

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***Momordica charantia* L. has long been regarded as a food and medicinal plant. Although it is not a native plant in Turkey, it is frequently used in folk medicine, especially in west and southwest parts of Anatolia. In the present study, unripe/ripe seed and fruit ethanol extracts of *M. charantia* from Turkey were screened for their potential antimicrobial and antioxidant activities. The antimicrobial activities of the extract were determined against four gram positive bacteria, seven gram negative bacteria, and one yeast with disc diffusion and microdilution broth methods. The extracts were also tested *in vitro* against four different fish pathogens. The unripe fruit extract was the most active against the tested microorganisms in the study with higher inhibition zones and lower minimal bactericidal or fungicidal activities (MBC or MFC) than the other extracts. Antioxidant capacity of the extracts was investigated by different assay, namely, total antioxidant activity, free radical scavenging activity (DPPH assay), iron (III) and cupric ion reduction assay. The total phenolic content was only determined from ripe fruit and seed extracts as 23.45 and 9.36 mg GAE/g, respectively. The results show that the ripe fruit extract has the strongest antioxidant capacity compared with other extracts. The findings indicate the potential use of unripe fruit and ripe fruit extracts as biopreservatives as they demonstrated high antimicrobial and antioxidant activities, respectively.**

Key words: Fruit, seed, ethanol extract, food borne and clinical pathogens, fish pathogens.

INTRODUCTION

For thousands of years, mankind has been benefitting from the drugs from nature. Plant extracts were highly regarded by ancient civilizations for the treatment of various ailments (Grabley and Thiericke, 1999). Even today, plant materials remain as an important resource to combat illnesses, including infectious diseases, and many of these plants have been investigated for novel drugs or templates for the development of new therapeutic agents (Konig, 1992). The treatment of infectious diseases with antimicrobial agents continues to present problems in modern-day medicine as many studies show a significant increase in the incidence of bacterial resistance to several antibiotics (Finch, 1998; Kunin, 1993). Therefore, there is a need to search for new infection-fighting strategies to control microbial infections (Sieradzki et al., 1999). Numerous studies have been carried out on various natural products by screening their antimicrobial activity (Nitta et al., 2002; Ates and Erdogrul, 2003;

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Bhattacharjee et al., 2006; Parekh and Chanda, 2006, 2007a; Vaghasiya et al., 2008). Recently, researches have been initiated to evaluate the feasibility of using herbal medicines in fish disease management (Abutbul et al., 2005). Moreover, the bacterial infections are considered as the major cause of mortality in aquaculture (Grisez and Ollevier, 1995). Due to the growing bacterial resistance against commercial standard and reserve antibiotics, the search for new active substances with antibacterial activity against pathogenic bacteria is of increasing importance (Service, 1995; Mundt et al., 2003). The medicinal plants may be used as potential and promising drugs against fish pathogens in the organic aquaculture (Turker et al., 2009a). Apart from antimicrobials, plants are also known to contain additives like preservatives and antioxidants as secondary metabolites of importance (Abalaka et al., 2011). Antioxidant effects of various medicinal plants used in traditional therapeutics are associated with their antioxidant properties (Sathishsekar and Subramanian, 2005; Aiyegoro and Okoh, 2009)

Momordica charantia L. (bitter melon), a member of the Cucurbitaceae family, has long been used as a food and medicine (El Batran et al., 2006). Bitter melon is known as bitter gourd, balsam pear, karela, and pare since it grows in tropical regions such as India, Malaya, China, tropical Africa, Middle East, America (Kirtikar and Basu, 1993) and Thailand. Antioxidant, anti-diabetes, anti-inflammatory, anti-bacterial and anti-cancer effects of *M. charantia* have been reported (Grover and Yadav, 2004; Budrat and Shotipruk, 2009). Fruits and seeds of *M. charantia* possess medicinal properties such as anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial and antitumor (Taylor, 2002). The plant was generally used to investigate for immunostimulant activity, chemotaxis stimulation, treating ulcers, antihyperglycemic and hypoglycemic activity and antioxidant enzyme activities in Turkey (Basaran et al., 1997; Cakıcı et al., 1994; Semiz and Sen, 2007). In addition, it was reported to exhibit diverse biological activities such as being antimicrobial. However, a few previous studies have evaluated the antimicrobial activity of the plant for a limited number of microorganisms, especially for the treatment of ulcer against *Helicobacter pylori* in Turkey (Yeşilada et al., 1999; Basaran et al., 1996).

The present study was carried out to evaluate the antimicrobial efficacy of fruit and seed ethanol extracts of unripe/ripe *M. charantia* L., grown in Adana-Turkey, against food borne and clinical pathogen microorganisms. The plant extracts were also investigated for their herbal potentials as an alternative to commonly used antibiotics in aquaculture; particularly against bacterial disease. The antioxidant activity of these extracts was characterized by various methods. Furthermore, the phenolic content in these extracts was also measured in this study. These useful data might help in the development of alternative controls in pharmaceutical and food/feed industry for human and/or fish.

MATERIALS AND METHODS

Plant materials and extraction procedure

The ripe and unripe fruits of *M. charantia* were obtained from Adana, Turkey. The *M. charantia* fruits were washed with distilled water, and the seeds were separated. The fruits were then sliced into small pieces and dried in drying oven at 50°C. The dried plant materials were then blended into powder using an electric blender for extraction. The powdered seeds and fruits of *M. charantia* were separately extracted with ethanol by using Soxhlet apparatus for 24 h. The extracts were concentrated by using a rotary evaporator and used for further test.

Determination of extraction yield

The yield of evaporated dried extracts based on dry weight was calculated from the equation shown below:

Yield (%) = (*W1* * 100) / *W2*

Where, *W*1 is the weight of extract after evaporation of solvent and *W*2 was the dry weight of the sample.

Microbial strains

In vitro antimicrobial studies were carried out on four gram-positive bacteria (*Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* RSKK 863, *Micrococcus luteus* NRRL B-4375), seven gram-negative bacteria (*Escherichia coli* ATCC 11229, *Escherichia coli* ATCC 35218, *Escherichia coli* O157:H7, *Salmonella enteritidis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27853, *Shigella sonnei* Mu:57, *Yersinia enterocolitica* NCTC 11175), and one yeast (*Candida albicans* ATCC 10231). Nutrient agar (NA) and Tryptic Soy Agar (TSA) were used for the cultivation of bacteria while YPD medium was used to culture yeast. All bacterial cultures were incubated at 37°C for 24 h whereas yeast cultures were incubated at 30°C for 48 h.

The following pathogen bacteria isolated from patient fish were used in the screening of antibacterial activity: one gram positive bacterium *Lactococcus garvieae* and three gram negative bacteria *Yersinia ruckeri*, *Vibrio anguillarum* (M1 and A4 strains, from two different companies) and *Vibrio alginolyticus*. *V. anguillarum* and *V. alginolyticus* were cultured in TSA supplemented with 2% NaCl. *Y. ruckeri* and *L. garviae* were grown on TSA without additional NaCl. All bacterial cultures were incubated at 25°C for 24 h.

Inhibitory effect with the disc diffusion method

The disc diffusion method was employed for the determination of the antimicrobial activity (Murray et al., 1995). The culture suspensions were adjusted by comparing with 0.5 McFarland. 100 µl of suspension of the test microorganisms were spread on solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 10 µl of the extracts, and then placed on the inoculated plates. Afterwards, they were kept for 2 h in refrigerator to enable prediffusion of the extracts into the agar. Then, the inoculated plates were incubated for 24 and 48 h for bacterial and yeast strains, respectively. Antibiotic discs of Ampicillin (Amp, 10 µg/disc), Gentamicin (CN, 10 µg/disc), and Amikacin (AK, 30 µg/disc) were also used as positive controls. Absolute ethanol was

used as negative control. The diameters of inhibition zones (mm) were used as a measure of antimicrobial activity, and each assay was repeated twice.

Determination of minimal bactericidal (MBC) or fungicidal (MFC) concentrations

The MBC/MFC values of the extracts were determined by the microdilution method using serially diluted (two folds) plant extracts according to Chandrasekaran and Venkatesalu (2004). Some modifications were made to the method. The extracts were studied for microorganisms which are sensitive to the extracts in the disc diffusion assay. Inocula of the microorganisms were prepared using 12 h cultures, and the suspensions were adjusted to 0.5 McFarland standard turbidity.

The test samples were added to growth broth medium to get a final concentration of 90.00 mg/ml, and serially diluted to reach 45.00, 22.50, 11.25, 5.63, 2.82, 1.41 and 0.71 mg/ml. The final volume in each tube was 100 µl. 2.5 µl of standardized suspension of each tested microorganism was transferred to each tube. A positive control (containing 2.5 µl inoculum and 100 µl growth medium) and a negative control (con-taining 2.5 µl of extract, 100 µl growth medium without inoculum) were included on each microtube. The contents of the tubes were mixed by pipetting, and they were incubated for 24 h.

The MIC was defined as the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism (Andrews, 2001). However, the tested plant extracts in the study were colored, and the visible growth could not be observed and so, 5 µl samples from all tubes were plated on solid growth medium to confirm microbial growth (Şahin et al., 2003). The MBC and MFC were recorded as the lowest concentration of the extract that did not permit any visible bacteria and fungal colony growth on the appropriate agar plate after the period of incubation (Chandrasekaran and Venkatesalu, 2004). Therefore, the concentrations of the extracts that prevent the growth of a microorganism on the solid media were evaluated as MBC or MFC values in this study. Each test was repeated twice.

Determination of antioxidant capacities

Assays for total phenolic content

The total phenolic content in the extracts was estimated with the Folin–Ciocalteu method, based on the procedure of Slinkard and Singleton (1977). The extracts were dissolved in methanol, and 0.2 ml of extract solution (1 mg/ml) was introduced into the test tube containing 1 ml of Folin-Ciocalteu's reagent and 2 ml of Na_2CO_3 (7.5%). The final volume was adjusted to 7 ml with deionized water. After incubation for 2 h at room temperature, the absorbance against blank was measured at 765 nm with an UV-Vis Spectrophotometer (HITACHI U-2000). The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry extract.

Determination of total antioxidant capacity

The total antioxidant capacity of extract was evaluated with the phosphomolybdenum method according to Prieto et al. (1999). The extracts were dissolved in methanol (2 mg/ml), and 0.3 ml of each extract was added to 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm against a blank. The antioxidant activity was expressed as mg ascorbic acid equivalent/g dry extract.

1,1-Diphenyl-2-picrylhydrazy (DPPH) radical-scavenging activity

The free radical scavenging activity of the plant extract was determined through slight modifications of the method described by Sarikurkcu et al. (2008). 0.5 ml of test samples at different concentrations was mixed with 3 ml 6.10^{-5} M of a methanol solution of DPPH. The reaction mixture was incubated in the dark at room temperature. The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm after 30 min. The inhibition activity was calculated from the equation:

$$I(\%)= 100 \times (A_0-A_1)/A_0$$

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. BHT (butylated hydroxytoluene) was used as positive control.

Ferric ion reducing power

The reducing power was determined according to the method of Oyaizu (1986) with slight modifications. Different concentrations of extracts were mixed with 2.5 ml of 0.2 M phosphate buffer and potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. After the incubation, 2.5 ml of 10% trichloroacetic acid was added. 2.5 ml of the reaction mixture was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%). The solution absorbance was measured at 700 nm. The increasing absorbance of the reaction mixture indicates an increasing in reducing power. The same procedure was applied with BHT.

Cupric ion reducing antioxidant capacity (CUPRAC assay)

The cupric ion reducing capacity of extracts was determined according to the method of Apak et al. (2006). 1 ml each of 10 mM $CuCl_2$, 7.5 mM neocuproine, and NH_4Ac buffer (1 M, pH 7.0) solutions were added into a test tube. Then, 0.5 ml of different concentrations of the extract was mixed, and the total volume was brought up to 4.1 ml with deionized water. After 30 min incubation at room temperature, the mixture absorbance at 450 nm was recorded against a blank. The same procedure was applied with BHT.

RESULTS AND DISCUSSION

Antimicrobial activity

The extractive yield of the unripe/ripe seed and fruit ethanol extracts of *M. charantia* are presented in Table 1. Maximum yield was obtained with ripe fruit extract of *M. charantia* (63.22%) while minimum was with the unripe fruit extract (5.05%). The differences between the yields of extracts might be attributed to the availability of different extractable components (Oke and Aslim, 2010) and ripeness of the fruit.

In the present study, the antimicrobial activities of *M. charantia*, obtained from Adana in Turkey, were determined against a wide range of microorganisms on the basis of disc diffusion and microdilution assays. The antimicrobial activities of unripe/ripe *M. charantia* seed and fruit ethanol extracts were examined in the present study, and their potencies were quantitatively assessed

Table 1. The yield, total phenolics and total antioxidant capacities of *M. charantia* ethanol extracts.

Plant	Part	Extract yield (% (w/w))	TPC (mg GAE/g dry extract)	TAC (mg AAE/g dry extract)
Unripe	Fruit	5.05	-	39.92±0.54
	Seed	23.40	-	19.15±2.72
Ripe	Fruit	63.22	23.45±2.25	81.46±7.07
	Seed	30.92	9.36±0.32	32.62±5.44

TPC, Total Phenolic Content; GAE, Galllic Acid Equivalent; TAC, Total Antioxidant Capacity; AAE, Ascorbic Acid Equivalent. Values are reported as means ± S.D. of two separate experiments.

with the presence or absence of inhibition zones, zone diameters, and MBC or MFC values (Tables 2 and 3). As stated by Cowan (1999), nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds and are most often obtained through initial ethanol or methanol extraction. Therefore, we used ethanol as solvent to extract almost all of the proposed antimicrobial and antioxidant agents in order to prepare the basis to monitor different antimicrobial and antioxidant agents. The inhibition zone diameters of antibiotics are presented in Tables 2 and 3.

The disc diffusion assay results of *M. charantia* extracts are given in Table 2 and indicate that the highest inhibitory activities were determined against *Y. enterocolitica* NCTC 11175 (14.66 mm) and *C. albicans* ATCC 10231 (25.22 mm) in the unripe seed and fruit part, respectively. The weakest inhibitory activities were determined against *E. coli* ATCC 11229 (9.68 mm) and *Y. enterocolitica* NCTC 11175 (13.74 mm) from the extracts of the same part. As a result, the inhibition zones of unripe *M. charantia* extracts, obtained against all test microorganisms, were in the range of 9.68 to 25.22 mm. It was found that ripe seed and fruit extracts of the plant showed inhibitory activities against the tested microorganisms in the range of 8.79 to 14.46 mm and 8.05 to 14.82 mm, respectively. The results obtained from the disc diffusion method indicate that unripe fruit ethanol extract of *M. charantia* exhibited a stronger antimicrobial activity in comparison with the ripe fruit ethanol extract (Table 2).

S. aureus owes its clinical significance due to the fact that it causes a variety of suppurative (pusforming) infections and toxinoses in the humans. *S. aureus* causes food poisoning and toxic shock syndrome by the release of superantigens into the blood stream (Michael et al., 1999). *P. aeruginosa* owes its clinical significance to the fact that it is aetiology of a good number of infections such as septic burns and wounds, conjunctivitis, endocarditis, meningitis, and urinary tract infections. In the study, the unripe fruit ethanol extract of *M. charantia* showed high antimicrobial activity against *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853; 20.52 mm and 19.02 mm, respectively.

The unripe fruit extract has shown better antibacterial activities against 10 out of 12 bacteria when compared with standard Gentamicin (Table 2). Chattopadhyay et al. (2009) reported that the test strains were sensitive to the ethanol extract of *T. chebula* and *A*. *marmelos*. The ethanol extracts of both *T. chebula* and *A. marmelos* also showed a stronger and wider spectrum of antibacterial activity against all the test strains with respect to gentamicin, which is a result similar with our study (Table 2).

MBC or MFC values for the microorganisms which were sensitive to unripe seed and fruit extracts of *M. charantia* were in the range of 22.50 to 45.00 mg/ml and 1.41 to 22.50 mg/ml, respectively (Table 2). The results of the MBC or MFC show that the unripe fruit extracts seemed to be more effective than the unripe seed extracts against the tested microorganisms used in this study. MBC or MFC values of ripe seed and fruit extracts were in the range of 5.63 to 45.00 mg/ml. The results of the MBC or MFC show that unripe/ripe fruit extracts seemed to be more effective than unripe/ripe seed extracts against all tested microorganisms used in this study. Also, higher antimicrobial activities were observed from unripe fruit extracts and ripe seed extracts in comparison with ripe fruit and unripe seed extracts (Table 2).

In this study, *M. charantia* extracts were also screened for antibacterial activity against four fish pathogens. Those bacterial pathogens are the ones that commonly occur in aquaculture sector and cause serious infectious diseases and mortality in fish (Buller, 2004) (Table 3). The extracts of *M. charantia* exhibited broad spectrum activity against the fish pathogenic bacteria in the range of 10.28 to 21.68 mm. Generally, gram negative bacteria are more resistant than gram positive bacteria (Rabe and van Staden, 1997; Kelmanson et al., 2000; Parekh et al., 2005), but the result of this present work is different. Here, the maximum antibacterial activity was seen against gram negative bacteria (Table 3), which is a result similar with that of Parekh and Chanda (2007b) and Türker et al. (2009b). The best antibacterial activity was determined against *V. anguillarum* A4 (21.68 mm) among the tested pathogenic bacteria. Türker et al.

Table 2. Antimicrobial activity of *M. charantia* ethanol extracts against test microorganisms.

Test microorganism	MBC or MFC (mg/ml)				Inhibition zone diameter[a] (mm)				Inhibition zone diameter[a] (mm)		
	Unripe		Ripe		Unripe		Ripe		Antibiotic		
	Seed	Fruit	Seed	Fruit	Seed	Fruit	Seed	Fruit	Amp	CN	AK
B.cereus RSKK 863	45.00	1.41	22.50	5.63	10.93±0.02	21.68±0.23	10.73±0.34	14.82±0.71	38.11±0.02	13.39±0.16	22.28± 0.05
E. coli O157:H7	45.00	5.63	45.00	45.00	10.30±0.36	14.58±0.13	14.46±0.38	11.32±0.72	27.51±0.13	12.22±0.03	22.31±0.10
S. sonnei Mu:57	22.50	11.25	22.50	11.25	12.05±0.55	19.30±0.18	10.72±0.41	10.94±0.70	33.54±0.08	11.51±0.01	13.57±0.02
M. luteus NRRL B-4375	45.00	22.50	5.63	11.25	11.45±0.54	22.00±0.26	12.61±0.35	13.62±0.54	34.68±0.07	11.06±0.05	13.24±0.12
Y. enterocolitica NCTC 11175	45.00	11.25	45.00	22.50	14.66±0.59	13.74±0.10	10.52±0.06	11.03±0.43	13.90±0.05	16.96±0.01	24.38±0.15
E. coli ATCC 11229	45.00	11.25	45.00	22.50	9.68±0.30	22.84±0.21	12.42±0.25	8.05±0.48	25.27±0.03	10.78±0.07	20.82±0.09
P. aeruginosa ATCC 27853	45.00	1.41	22.50	22.50	14.36±0.10	19.02±0.32	11.25±0.70	13.91±0.97	-	16.60±0.09	19.55±0.05
S. aureus ATCC 25923	45.00	11.25	45.00	22.50	10.42±0.63	20.52±0.02	11.72±0.98	12.24±0.07	36.76±0.30	15.40±0.07	17.35±0.09
E. coli ATCC 35218	45.00	5.63	45.00	22.50	13.79±0.32	18.98±0.05	12.74±0.05	10.31±0.10	21.56±0.13	11.33±0.16	15.27±0.10
S. enteritidis ATCC 13076	22.50	5.63	22.50	11.25	11.94±0.38	21.72±0.01	8.79±0.75	10.65±0.64	25.96±0.38	10.53±0.05	14.62±0.04
C. albicans ATCC 10231	45.00	11.25	45.00	22.50	14.12±0.28	25.22±0.43	11.21±0.40	13.16±0.08	-	-	-
L. monocytogenes ATCC 7644	45.00	5.63	45.00	22.50	10.68±0.21	16.38±0.36	11.64±0.6	11.94±0.33	32.21±0.41	20.06±0.15	19.14±0.06

MBC, Minimal bactericidal concentration; MFC, minimal fungicidal concentration; [a]diameter of the inhibition zone including disc diameter. Values are reported as means ± SD of two separate experiments. Amp, Ampicillin; CN, Gentamicin; AK, Amikacin.

Table 3. Antibacterial activity of *M. charantia* ethanol extracts against different bacterial fish pathogens.

Test microorganism	MBC (mg/ml)				Inhibition zone diameter[a] (mm)				Inhibition zone diameter[a] (mm)		
	Unripe		Ripe		Unripe		Ripe		Antibiotic		
	Seed	Fruit	Seed	Fruit	Seed	Fruit	Seed	Fruit	Amp	CN	AK
L. garviae	45.00	22.50	45.00	22.50	13.53±0.16	18.64±0.18	13.48±0.18	16.52±0.15	33.10±0.12	15.19± 0.10	10.30± 0.08
Y. ruckeri	45.00	11.25	22.50	45.00	12.50±0.12	17.30±0.14	12.18±0.07	10.28±0.06	32.30±0.15	18.85±0.05	18.69±0.12
V. anguillarum M1	22.50	2.82	22.50	22.50	10.67±0.14	19.60±0.19	15.15±0.16	13.91±0.09	9.02±0.04	12.38±0.09	9.46±0.12
V. anguillarum A4	22.50	2.82	22.50	22.50	10.98±0.09	21.68±0.14	14.51±0.17	15.10±0.32	9.40±0.11	15.13±0.15	12.07±0.13
V. alginolyticus	-	-	-	11.25	-	-	-	10.68±0.26	13.57±0.09	15.06±0.07	15.03±0.03

MBC, Minimal bactericidal concentration; [a]diameter of the inhibition zone including disc diameter. Values are reported as means ± SD of two separate experiments. Amp, Ampicillin; CN, Gentamicin; AK, Amikacin.

(2009b) reported that *Aeromonas hydrophila*, a gram negative fish pathogenic bacterium, appeared to be more susceptible to the plant extracts used in their experiments. *V. alginolyticus* was only inhibited by the ripe fruit extract of *M. charantia* (10.68 mm). The results obtained from the disc diffusion method indicated that unripe fruit ethanol extract of *M. charantia* exhibited a stronger antibacterial activity against the tested fish pathogenic

bacteria in comparison with the other extracts (Table 3), which is a result similar with antimicrobial activity against the other test microorganisms used in this study (Table 2). The unripe fruit extract of *M. chrantia* exhibited better antibac-terial activities against *Y. ruckeri* and *L. garviae* when compared to *Trifolium pannonicum* sub sp. *elongatum* ethanol and methanol extracts (Türker et al., 2009b).

The unripe fruit extract showed better antibacterial activities against *V. anguillarum* M1 and A4 when compared with standard Amp, CN and AK (Table 3). The wide and frequent use of antibiotics in aquaculture has resulted in the development and spread of antibiotic resistance. Because of the health risks associated with the use of antibiotics in animal production, there is a growing awareness that antibiotics should be used with more care. For a sustainable further development of the aquaculture industry, novel strategies to control bacterial infections are needed (Defoirdt et al., 2011). Extracts of *M. charantia*, especially unripe fruit extract, may be used as an alternative to antibiotics in fisheries. Toxicity studies should also be done to determine their safety.

MBC values for bacterial strains, sensitive to the extracts of *M. caharantia*, were in the range of 2.82 to 45.00 mg/ml (Table 3). The two highest inhibitory activities for the unripe extract were against *V. anguillarum* (M1 and A4 strains) which showed the lowest MBC (2.82 mg/ml). The results of the MBC show that the unripe fruit extract was the most effective among other extracts against all the tested pathogenic bacteria used in this study. The study results of Laith et al. (2012) who recorded the MBC values for the methanol extracts of *Sonneratia caseolaris* and *Rhizophora apiculata*, medicinal plants, are 12.5 and 25 mg/ml, respectively. Compared to these results, unripe fruit extract showed lower MBC values for *V. anguillarum* in this study. Direkbusarakom et al. (1998) reported that among 16 Thai traditional herbs, ethanol extracts of *M. charantia* and *P. gaujawa* displayed the highest activity against *Vibrio harveyi* and *V. parahaemolyticus*, and MIC of *M. charantia* was found to be 1.25 mg/ml. Similarly, Muniruzzaman and Chowdhury (2004) indicated that MIC values of *M. charantia* leaves ethanol extracts were 1.2 mg/ml against *A. hydrophila* and *Pseudomonas flourescens*, and 2.5 mg/ml against *Edwardsiella tarda*, which were fish pathogenic bacteria.

Previous studies have also demonstrated that *M. charantia* is very rich in triterpenes, proteins, and steroids. Those of major interest include momordin, alpha- and betamomorcharin, cucurbitacin B1, and oleanolic acid (Oliff, 2007; Kohlert et al., 2000). It is speculated that the antimicrobial activities of triterpenes depend on interactions between their lipid components and the net surface charge of microbial membranes. Furthermore, the drugs might cross the cell membranes, penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity (Trombetta et al., 2005). It is noticeable that the results of the antimicrobial investigation on unripe/ripe *M. charantia* fruit and seed ethanol extracts showed better antibacterial and antifungal activities for some microorganisms with less MBCs or MFCs than the other studies of *M. charantia* (Roopashree et al., 2008; Laith et al., 2012).

M. charantia grows generally in tropical regions (Kirtikar and Basu, 1993). Although it is not a native plant in Turkey, it is frequently used in folk medicine, especially in west and southwest parts of Anatolia (Semiz and Sen, 2007). Therefore, for the first time to our knowledge, we report the antimicrobial activity of *M. charantia* grown in Adana-Turkey against 11 test bacteria; *E. coli* ATCC 11229, *E. coli* ATCC 35218, *S. aureus* ATCC 25923, *L. monocytogenes* ATCC 7644, *E. coli* O157:H7, *B.cereus* RSKK 863, *P. aeruginosa* ATCC 27853, *M. luteus* NRRL B-4375, *S. sonnei* Mu:57, *Y. enterocolitica* NCTC 11175, *S. enteritidis* ATCC 13076, one yeast; *C. albicans* ATCC 10231 and four fish pathogenic bacteria; *Y. ruckeri*, *L. garviae, V. alginolyticus*, *V. anguillarum* (from two different companies). The unripe seed and fruit extracts of *M. charantia* were also compared with ripe seed and fruit extracts. According to Buwa and Van Staden (2007), various factors including internal biochemical factors, extracted plant part, and external environmental factors such as climate, location, season, and growth conditions all influence the effectiveness of medicinal plants. The results show that all the extracts exhibited varying degrees of antimicrobial activity on the microorganisms tested. Some extracts of *M. charantia* were more effective than traditional antibiotics to combat the pathogenic microorganisms studied.

Antioxidant capacity

Several methods have been used to determine antioxidant activity of plants. Our present study, therefore, involved four various established methods to evaluate antioxidative activity of *M. charantia*, namely, total antioxidant capacity, DPPH radical-scavenging activity, ferric ion reducing power and CUPRAC assay. The total phenolic content of the extracts was also determined.

Total phenolic content

Phenolics or polyphenols have received considerable attention because of their physiological function, including antioxidant, antimutagenic, and antitumour activities (Othman et al., 2007). Phenolic compounds are widely distributed in plants (Li et al., 2006), which have gained much attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health (Govindarajan et al., 2007; Imeh and Khokhar, 2002; Li et al., 2006; Ross and Kasum, 2002). The total phenolic content in the extract was determined spectrometrically according to the Folin-Ciocalteu procedure and was calculated as gallic acid

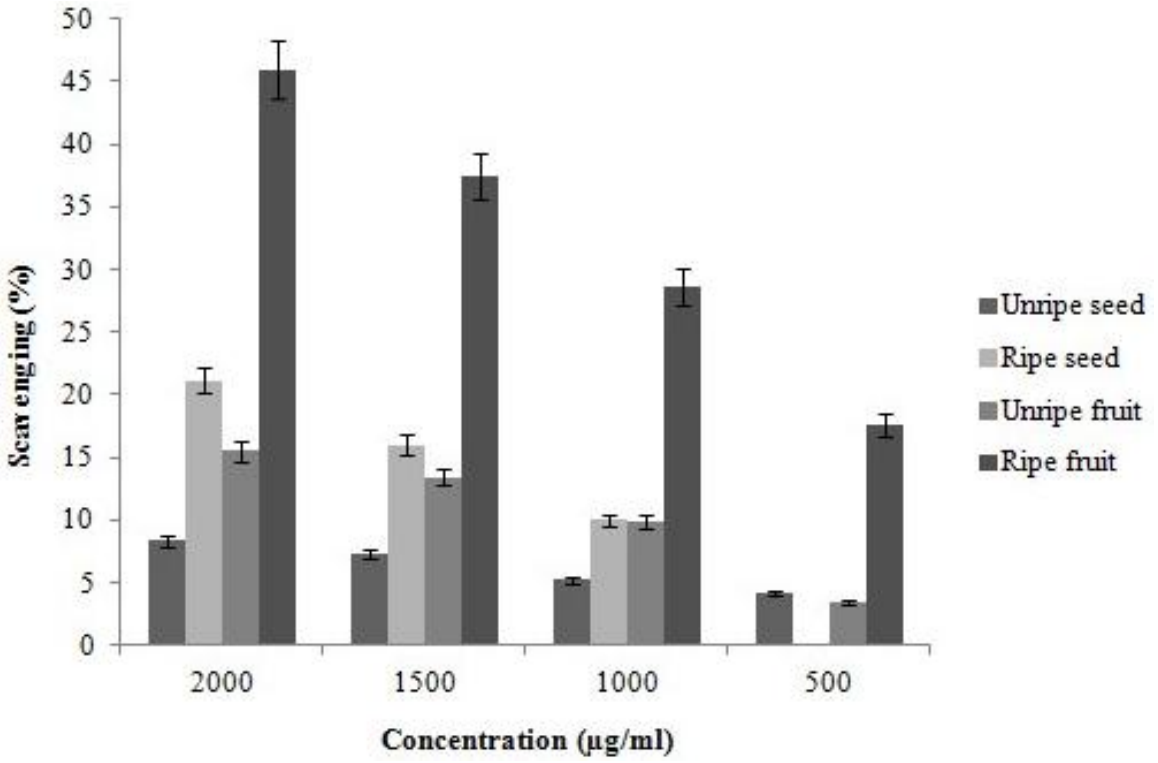

Figure 1. DPPH scavenging activity of *M. charantia* ethanol extracts.

equivalents (GAE). The total phenolic contents of ethanolic extracts of *M. charantia* are presented in Table 1. The total phenolic content (TPC) of ripe fruit and seed extracts were determined as 23.45 and 9.36 mg GAE/g, respectively whereas TPC was not determined from unripe fruit and seed extracts as gallic acid equivalent. It may be due to the physiological changes that accompany ripening that brings about changes in pigments, which may have caused an increase in the total phenol content (Oboh et al., 2007; Materska and Perucka, 2005). Aminah and Anna (2011) also reported that more ripened stages of bitter melon fruit resulted in higher total phenolic content. Kubola and Siriamornpun (2008) reported the total phenolics content of green fruit and ripe fruit of Thai bitter gourd (*M. charantia*), as 324 and 224 mg GAE/g dry sample, respectively. Budrat and Shotipruk (2008) reported total phenolic compound on bitter melon (using soxhlet extraction, methanol as solvent) as 4.992 mg GAE/g dry weight and (using solvent extraction, methanol as solvent) as 7.743 mg GAE/g dry weight.

Determination of total antioxidant capacity

The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) with the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. The phosphomolybdenum method is quantitative since the antioxidant activity is expressed as the number of equivalents of ascorbic acid (AAE) (Prieto et al., 1999). Unripe and ripe fruit extracts of *M. charantia* showed higher capacities than their seed extracts (Table 1). Ripe fruit extract had the highest capacity (81.46 mg AAE/g) in comparison with the other parts extracts. The extracts were found to have different levels of antioxidant activity in the systems tested. The total antioxidant activities of the parts were: ripe fruit > unripe fruit > ripe seed > unripe seed. Kubola and Siriamornpun (2008) reported that water extract of green fruit *M. charantia* had a higher capacity than ripe fruit water extract. The results of their study indicate that the water extract of ripe fruit had lower capacity (0.061 mg AAE/mg) than ethanol extract of ripe fruit used in our study. In their investigation, however, a higher capacity from unripe fruit water extract was reported than the for ethanol extract of unripe fruit.

DPPH radical scavenging activity

DPPH is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples (Çakmak et al., 2012; Sakanaka et al., 2005). To evaluate the scavenging effects of DPPH of ethanol extract of ripe/unripe seed and fruit, DPPH inhibition was investigated. These results are shown as relative activities against BHT, the synthetic antioxidants used in food industry (Figure 1). The investigation showed that the radical scavenging activity increased with the increase of the concentration of all the extracts. The radical scaven-

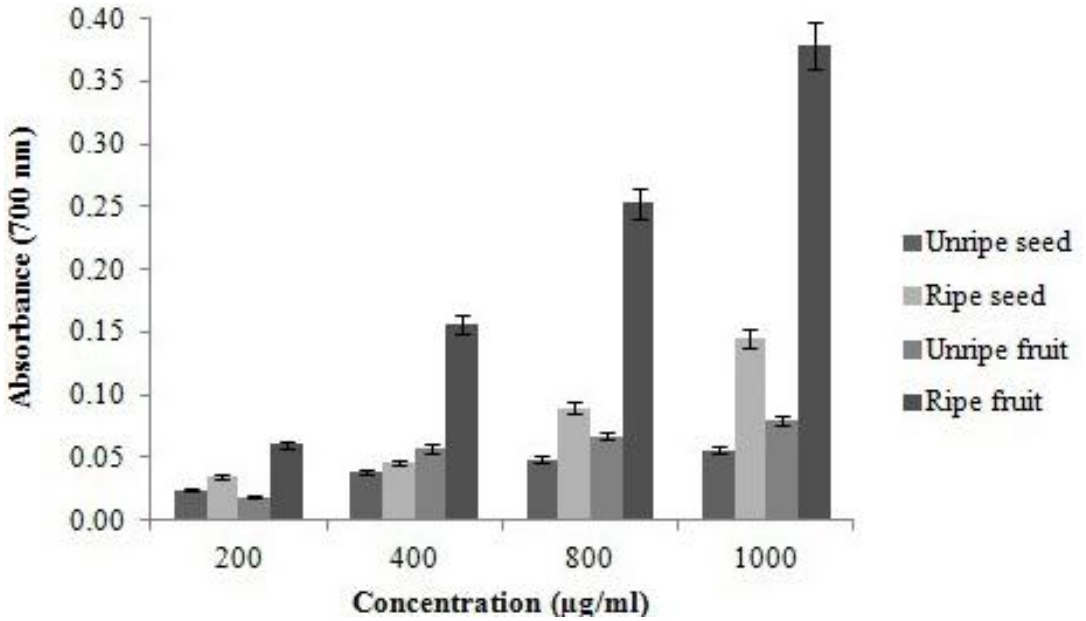

Figure 2. Ferric ion reducing power of *M. charantia* ethanol extracts.

ging of BHT at 200 µg/ml concentration (91.19%) was higher than the ethanol extracts of *M. charantia* at concentrations ranging from 500 to 2000 µg/ml. The activity of ripe fruit (45.95%) was the highest, followed by ripe seed (21.14%), unripe fruit (15.49%), and unripe seed (8.32%) at 2000 µg/ml concentration. Kubola and Siriamornpun (2008) reported that DPPH radical scavenging activity of Thai *M. charantia* water extract was 81.4 and 55.5% for green fruit and ripe fruit, respectively, at 1.6 mg/ml concentration. Rezaeizadeh et al. (2011) found that DPPH radical scavenging activity was higher for fresh whole fruit of *M. charantia* methanolic and chloroformic extracts when compared to our results at 500 µg/ml concentration. The radical scavenging values, however, depend on locality and polarity of extraction solvents.

Ferric ion reducing power

The reducing capacity of compound Fe^{3+}/ferrecyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity, and it is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Yildirim et al., 2001). Figure 2 shows the dose response curves for the reducing power of the extracts from *M. charantia* ethanol extracts. The reducing power of *M. charantia* extracts are in the order of ripe fruit> ripe seed > unripe fruit > unripe seed. The ripe seed ethanol extract exhibited a bit lower reducing power at 1000 µg/ml concentration that was compared with seed water extract of *M. charantia* from Pakistan (Saeed at al., 2010). However, ripe fruit ethanol extract showed a higher reducing power than peel water extract of *M. charantia* at the same concentration (1000 µg/ml) in this study. BHT, at 31.25 µg/ml the concentration, exhibited remarkably higher reducing power (A_{700nm} 0.61) than the extracts. The reducing power of an extract is often used as an indicator of electron-donating ability which is an important mechanism of antioxidant compounds (Dorman et al., 2003).

CUPRAC assay

The CUPRAC assay used the copper (II)-neocuproine reagent as the chromogenic oxidizing agent. The method is based on the measurement of absorbance at 450 nm with the formation of stable complex between neocuproine and copper (I) (Özyürek et al., 2011). The cupric ion reducing power of extracts was dependent on the concentration of extract (Figure 3). The results of the CUPRAC assay show that among the ethanol extracts of *M. charantia*, ripe fruit extract was the most effective, followed by ripe seed, unripe fruit, and unripe seed, at 800 µg/ml concentration. BHT at 31.25 µg/ml the concentration, exhibited remarkably higher cupric ion reducing power (A_{450nm} 0.39) than the extracts. According to our knowledge, there is no data on the reducing power of *M. charantia* extracts.

Conclusion

It can be concluded from the results of the present study that different fruit and seed extracts of *M. charantia* have variable antimicrobial and antioxidant activities. Due to the undesirable problems and side effects from the

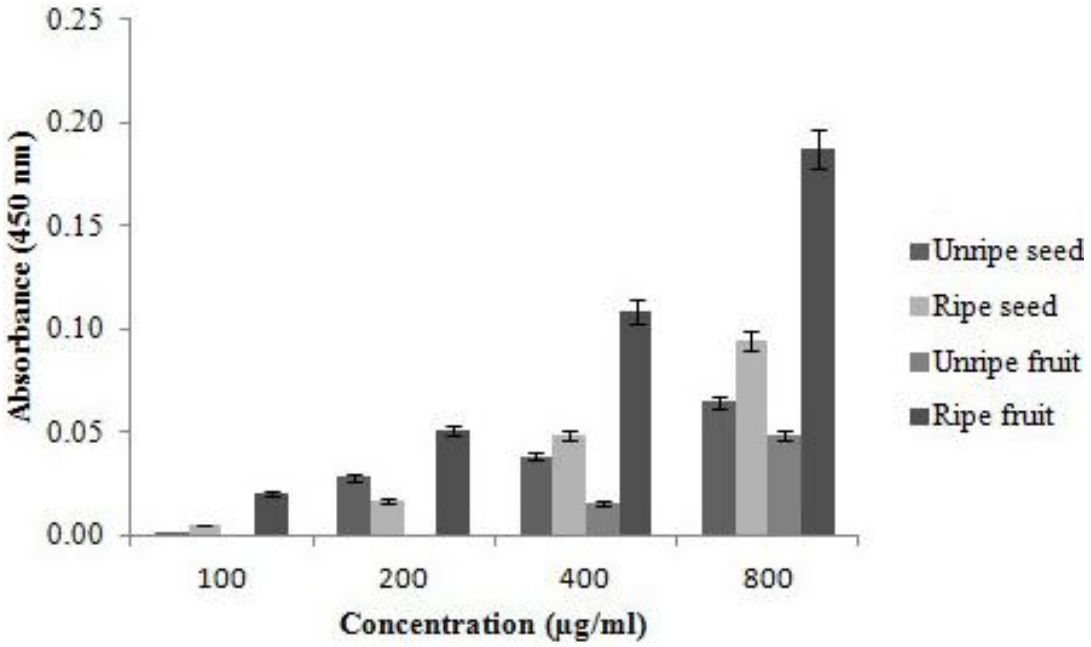

Figure 3. Cupric ion reducing power of *M. charantia* ethanol extracts.

consumption of artificial chemical compounds, extracts from various plant species, especially edible and medicinal ones, have attained appreciable interest among the research community. The results presented here may suggest that various extracts of *M. charantia* possess antimicrobial and antioxidant properties, and therefore, they may be a new potential source of a natural preservative in pharmaceutical and food/feed industry.

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