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Antibacterial Activities of Red Colored Radish Types (*Raphanus sativus* L.)

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HALUK CAGLAR KAYMAK[1], SUZAN OZTURK YILMAZ[2], SEZAI ERCISLI[1*], ISMAIL GUVENC[3]

[1]*Department of Horticulture, Faculty of Agriculture, Ataturk University, 25240 Erzurum, Turkey*
[2] *Sakarya University Engineering Faculty Department of Food Engineering Sakarya, Turkey*
[3]*Department of Horticulture, Faculty of Agriculture, Kahramanmaraş Sütçü İmam University, 46000 Kahramanmaraş, Turkey*
***Address for correspondence to:** sercisli@atauni.edu.tr*

Abstract

The root parts of red skin colored radish cultivars cv. Antep and cv. Cherry Belle which are belongs to Raphanus sativus L. were harvested from Erzurum region of Turkey and their methanol extracts were evaluated for antibacterial activity against 52 food borne bacteria by using disc diffusion assay. Extracts had a broad-spectrum antibacterial activity against food borne bacteria in broth micro dilution bioassays. Maximum antibacterial activity was observed against Arthrobacter ilicis, Corynebacterium ammoniagenes, Enterobacter hormaechei, Kocuria rosea, Neisseria subflava, Pantoea agglomerans, Proteus vulgaris, Psychrobacter immobilis and Shigella dysenteriae. However, cv. Antep showed antibacterial activity against Bacillus sphaericus and Corynebacterium flavescens and cv. Cherry Belle showed antibacterial activity against Arthrobacter atrocyaneus as well as. These inhibitory effects are interesting in relation to the prevention of microbial contamination in many foods.

Keywords: antibacterial activity; red radishes, *Raphanus sativus* L.

1. Introduction

Horticultural plants are indispensable part of human nutrition used for ages. Fruits, leaves, roots, stems, flowers etc. of horticultural plants have been used not only for nutrition purposes but also to meet personal and social needs such as curing diseases, beautifying the planet etc. They include lots of phytochemicals that important for human health (TOSUN & al. [1]; DOGAN & al. [2], ALP & al. [3]; BOZOKALFA & al. [4]; HRICOVA & al. [5]).

Radish (*Raphanus sativus* L.) takes many forms and has a large economic and commercial value in many regions of the world. Different parts of radish including roots, seeds and leaves are used for medicinal purposes (NADKARNI [6]). Radishes have been ethnically used as laxative, stimulant, digestive aid, and appetizer and in other disorders of stomach (KAPOOR [7]). The radish is also one of the richest sources of iron, calcium and sodium of all the common vegetables (KAYMAK & al. [8]). It contains a variety of chemicals that has antifungal and antiurolithiatic activity on human digestive flora (DE SAMBLANX & al. [9]; VARGAS & al. [10]). More recently many studies report a strong inverse relationship between the intake of crucifer vegetables, including radish and the risk for many cancers (VERHOEVEN & al. [11]). This association has been found to be stronger than the association between cancer risk and fruit and vegetable intake in general (MICHAUD & al. [12]). Epidemiologic studies have demonstrated inverse associations between crucifer intake and the incidence of lung, pancreas, bladder, prostate, skin, stomach, and colon cancer (VERHOEVEN & al. [11]).

The radish is one of the most commonly used vegetables in Turkey. There are many varieties of radish in Turkey, differing in size and color. The red and white colored are most familiar (KAYMAK & al. [8]). In Eastern part of Turkey, radishes has been used as folk remedies to treat various ailments such as stomach pains, indigestion and infectious diseases. They are also used as diuretics and expectorant (YALCIN [13]). However, so far there have been no attempts to study the potential bacterial activity of radishes, in particular red skin colored radish types against a wide range of food-associated microorganisms.

The increasing global incidence of food poisoning cases originating from food contaminated by pathogens has great social and economic costs and causes major concern, both to the general public and to the food industry. The epidemiology of food-borne diseases is rapidly changing. The increased interest in bio preservation of food systems has recently led to the development of new natural antimicrobial compounds having different origin. Therefore, researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (SRINIVASAN & al. [14]; BENKEBLIA [15]). The present study was conducted to investigate antibacterial properties of different root sized red radish cvs. Antep and Cherry Belle against several food-associated bacteria, which have not been evaluated in previous studies.

2. Materials and Methods

2.1. Plant material and extraction. Seeds of two red skin colored radishes (cv. Antep and cv. Cherry Belle) belong to *Raphanus sativus* L. were sown and cultivated in Erzurum region of Turkey in 2003 year at a distance between rows 40 cm and plants 20 cm for cv. Antep and 30x20 cm for cv. Cherry Belle, respectively. 100 kg N/ha and 80 kg P_2O_5/ha applied on all plots. For cv. Antep, All the P_2O_5 and half of nitrogen fertilizer were broadcasted uniformly prior to planting onto the soil surface and incorporated. The remaining half of nitrogen was given 20 days after emergence. For cv. Cherry Belle, all the P_2O_5 and nitrogen fertilizer were broadcasted uniformly prior to planting onto the soil surface and incorporated KAYMAK & al. [8]. During the experiment, the plants were irrigated with furrow irrigation and the other cultural practices have been irrespectively applied on the each plant. After 80 days for cv. Antep (average root weight 521 g at harvest) and 45 days for cv. Cherry Belle (average root weight 23.8 g at harvest) from sowing, plant materials were harvested in fresh condition and root parts were cut into small pieces and then dried in the shade and ground in a grinder. The dried and powdered plant materials (500 g) were extracted successively with methanol by using Soxhlet extractor for 72 h (LIN & al. [16]). The extracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuo at 40°C using a Rotary evaporator. The residues obtained were stored in a freezer at − 80°C until further tests.

2.2. Biological materials. Total 52 microbial (bacteria) strains which are listed in Table 1 were used for antibacterial screening. The microorganisms, maintained on Nutrient Agar (Merck, Darmstadt, Germany) and bacteria were provided by Microbiology Laboratory of Agricultural Faculty of Ataturk University, Erzurum, Turkey. The bacteria were selected because they are frequently reported in food (OZTURK & ERCISLI [17]). Identity of the microorganisms used in this study was confirmed by Microbial Identification System in Biotechnology Application and Research Center at Ataturk University.

2.3. Antibacterial activity. The antibacterial activity of the methanol extracts of red colored radish cvs. Antep and Cherry Belle were evaluated by means of the disc diffusion method (MURAY & al. [18]) using 100 µl of suspension containing 10^8 CFU/ml of bacteria

spread on nutrient agar (NA) medium. The discs (6 mm in diameter) were impregnated with 10 µl of the 30 mg/ml extracts (300 µg /disc) placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ofloxacin (5µg/disc), sulbactam (30µg) + cefoperazona (75 µg) (105 µg/disc) and/or netilmicin (30µg/disc) were used as positive reference standards to determine the sensitivity of one strain/isolate in each bacteria species tested. The inoculated plates were incubated at 27 °C for 24 h. The antibacterial activity was measured as the diameter (mm) of clear zone of growth inhibition. Five disc per plate and three plates were used, and each test was run in triplicate (OZTURK & ERCISLI [17]).

2.4. *In vitro* antibacterial bioassays. In order to quantify the antibacterial activity of red skin colored radish cvs, Antep and Cherry Belle, minimum inhibitory concentration (MIC) values were also studied for the bacteria which were determined as sensitive to the extracts in disc diffusion assay. The inocula of microorganisms were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The radish extracts investigated were dissolved in 0.5 % dimethyl sulphoxide (DMSO) were first diluted to the highest concentration (500 µg/ml) to be tested, and then serial two-fold dilutions were made in a concentration range from 7.8 to 500 µg/ml in 10 ml sterile test tubes containing nutrient broth. MIC values of radish extracts against bacterial strains were determined based on a microwell dilution method (ZGODA & PORTER [19]). The 96-well plates were prepared by dispensing into each well 95 µl of nutrient broth and 5 µl of the inoculum. A 100 µl from radish extracts initially prepared at the concentration of 500 µg /ml was added into the first wells. Then, 100 µl from their serial dilutions was transferred into six consecutive wells. The last well containing 195 µl of nutrient broth without compound and 5 µl of the inoculum on each strip was used as negative control. The final volume in each well was 200 µl. Maxipime (Bristol-Myers Squibb) at the concentration range of 500-7.8 µg /ml was prepared in nutrient broth and used as standard drug for positive control. The plate was covered with a sterile plate sealer. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by absorbance at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc., Highland Park, Vermont, USA) and confirmed by plating 5 µl samples from clear wells on nutrient agar medium. The extract tested in this study was screened two times against each organism. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms.

3. Results and discussion

The crude extracts of red radishes cv. Antep and Cherry Belle belonging to *Raphanus sativus* L. were screened for their antibacterial activity against 52 bacteria species. In general, methanol extracts of red radish cultivars exhibited similar inhibition levels against 52 bacteria as shown in Table 1.

The results showed that the methanol extract of both red radish cultivars showed inhibition effect on 9 of 52 bacteria species including *Arthobacter ilicis*, *Corynebacterium ammoniagenes, Enterobacter hormaechei, Kocuria rosea, Neisseria subflava, Pantoea agglomerans, Proteus vulgaris, Psychrobacter immobilis* and *Shigella dysenteriae* (Table 1). In addition cv. Cherry Belle showed antibacterial activity against *Arthrobacter atrocyaneus* and cv. Antep showed antibacterial activity against *Bacillus sphaericus* and *Corynebacterium flavescens* as well as.

The maximal inhibition zones and MIC values for bacterial strains which were sensitive to methanol extract were in the range of 7-11 mm, and 250-500 µg/ml (Table 2). Based on results, it is possible to conclude that red radishes had stronger and broaden spectrum of antibacterial activity.

Table 1. Antibacterial activity of radish extracts against the bacteria tested based on disc diffusion method

Bacterial species	Number of Strains/origins	Inhibition zone in diameter (mm/sensitive strains)[a]			Positive Controls (mm)[b]
		Raphanus sativus extracts (300µg/disc)		Negative control	
		cv. Cherry Belle	cv. Antep	MeOH	Standart Antibiotic disc
Acidovorax facilis	1/food	-	-	-	28 (OFX)
Arthrobacter agilis	1/food	-	-	-	31 (OFX)
Arthrobacter atrocyaneus	2/food	7-8/2	-	-	15 (OFX)
Arthrobacter ilicis	4/food	7-8/4	7-9/4	-	20 (OFX)
Arthrobacter protophormiae	1/food	-	-	-	27 (OFX)
Bacillus cereus	1/food	-	-	-	21 (OFX)
Bacillus dipsauri	1/food	-	-	-	26 (OFX)
Bacillus flexus	3/food	-	-	-	30 (OFX)
Bacillus lentimorbus	1/food	-	-	-	33 (OFX)
Bacillus lichemiformis	2/food	-	-	-	29 (OFX)
Bacillus marinus	2/food	-	-	-	14 (OFX)
Bacillus megaterium	1/food	-	-	-	26 (OFX)
Bacillus psychrosaccharolyticus	5/food	-	-	-	15 (OFX)
Bacillus pumilus	6/food	-	-	-	30 (OFX)
Bacillus sphaericus	1/food	-	8/1	-	21 (OFX)
Bacillus sp	1/food	-	-	-	25 (OFX)
Bacillus subtilis	4/food	-	-	-	29 (OFX)
Brevibacillus agri	3/food	-	-	-	28 (OFX)
Brevibacillus brevis	4/food	-	-	-	36 (OFX)
Brevibacterium linen	1/food	-	-	-	22 (OFX)
Chryseomonas luteola	1/food	-	-	-	30 (OFX)
Citrobacter amalonaticus	1/food	-	-	-	28 (OFX)
Corynebacterium ammoniagenes	3/food	7-8/3	7-10/3	-	20 (OFX)
Corynebacterium cystitidis	4/food	-	-	-	18 (OFX)
Corynebacterium flavescens	4/food	-	7-8/4	-	24 (OFX)
Enterococcus faecalis	1/food	-	-	-	10 (OFX)
Enterobacter hormaechei	2/food	7-8/2	8/2	-	22 (OFX)
Enterobacter intermedius	3/food	-	-	-	27 (OFX)
Enterobacter sakazakii	4/food	-	-	-	28 (OFX)
Erwinia carotovora	1/food	-	-	-	30 (OFX)
Erwinia chrysanthemi	1/food	-	-	-	29 (OFX)
Exiguobacterium acetylicum	1/food	-	-	-	20 (OFX)
Flavimonas oryzihabitans	2/food	-	-	-	30 (OFX)
Kocuria kristinae	1/food	-	-	-	24 (OFX)
Kocuria rosea	1/food	8/1	8/1	-	15 (OFX)
Micrococcus luteus	2/food	-	-	-	28 (OFX)
Micrococcus lylae	1/food	-	-	-	30 (OFX)
Moraxella catarrhali	3/food	-	-	-	18 (OFX)
Neisseria subflava	1/food	7-9/2	7-9/2	-	24 (OFX)
Paenibacillus apiarius	1/food	-	-	-	30 (OFX)
Paenibacillus macerans	1/food	-	-	-	30 (OFX)
Paenibacillus polymyxa	1/food	-	-	-	10 (OFX)
Pantoea agglomerans	1/food	7/1	7/1	-	30 (OFX)
Proteus vulgaris	1/food	7/1	8/1	-	20 (OFX)
Pseudomonas putida	1/food	-	-	-	17 (OFX)
Pseudomonas syringae syringae	1/food	-	-	-	15 (OFX)
Psychrobacter immobilis	3/food	7-8/3	8-9/3	-	20 (OFX)
Salmonella typhimuirum	3/food	-	-	-	28 (OFX)
Serratia liquefaciens	2/food	-	-	-	30 (OFX)
Shigella dysenteriae	1/food	9/1	8/1	-	26 (OFX)
Staphylococcus cohnii-cohnii	1/food	-	-	-	12 (OFX)
Xanthomonas arb. corylina	1/food	-	-	-	22 (OFX)
Total 52 bacterial species	100	7-11/16	7-11/21		

[a] MEOH, methanol extract. [b] OFX, ofloxacin (5 µg/disc); SCF, sulbactam (30 µg) + cefoperazona (75µg) (105 µg/disc); NET, netilmicin (30 µg/disc) were used as positive reference standarts antibiotic discs (oxoid).

Table 2. The MIC values of radish extracts against to microorganisms tested in microdilution assay (MIC in µg/ml)

Bacterial species	Number of Strains/origins	*Raphanus sativus* extracts cv. Cherry Belle MeOH µg/ml	*Raphanus sativus* extracts cv. Antep MeOH µg/ml	Standard drug (Maxipime)(µg/ml)
Arthrobacter atrocyaneus	2/food	500	-	15.60
Arthrobacter ilicis	4/food	250-500	250	31.25-15.60
Corynebacterium ammoniagenes	3/food	250-500	250-500	62.50-31.25
Corynebacterium flavescens	4/food	-	250-500	31.25-15.60
Enterobacter hormaechei	2/food	250-500	250-500	125.0-62.50
Kocuria rosea	1/food	250	250	125
Neisseria subflava	1/food	500	500	31.25
Pantoea agglomerans	1/food	500	500	7.81
Proteus vulgaris	1/food	250	250	125
Psychrobacter immobilis	3/food	250	250	31.25-15.60
Shigella-dysenteriae	1/food	500	250	15.60

4. Conclusion

Findings in this study supported the observation of some other researchers about radish containing some substances with antifungal and antibacterial properties (YALCIN [12]; NEHRASH [20]). Based on these results, it is possible to conclude that red radishes has stronger and broader spectrum of antibacterial activity. This is the first study to provide data that the extracts of red radishes evaluated against a wide range of bacteria possess potential antibacterial activities.

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