Romanian Biotechnological Letters *Vol. 16, No. 4, 2011*

ORIGINAL PAPER

Antibacterial and antioxidant activity of fruits of some rose species from Turkey

Received for publication, February 8, 2011
Accepted, August 3, 2011

SUZAN OZTURK YILMAZ[1], SEZAI ERCISLI[2*]
[1]*Sakarya University, Faculty of Engineering, Department of Food Engineering, Esentepe 54187 Sakarya, Turkey*
[2]*Ataturk University, Faculty of Agriculture, Department of Horticulture, 25240 Erzurum, Turkey*
*Corresponding author: E-mail: sercisli@hotmail.com

Abstract

The rose hips from plants native to Turkey's flora are well known for their aromatic and medicinal properties.The country has a very rich rose hip germplasm. In the present study the antibacterial and antioxidant activities characteristic to hips from four rose taxa (Rosa pisiformis, Rosa canina, Rosa villosa and Rosa dumalis subsp. antalyensis) were determined. Among bacteria tested, Bacillus cereus was the most susceptible against rose hip fruit extracts. All Rosa taxa fruit extracts inhibited growth of B. cereus with 9-14 mm inhibition zones. Rosa canina was found particularly a more effective antibacterial agent, being capable of inhibiting the growth of the majority of bacteria tested. Total phenolics, vitamin C and antioxidant activity varied from 78 to 102 mg GAE/g DW; 681-840 mg/100 g and 83.8-91.4% in β-Carotene method among taxa species.

Key words: Antibacterial activity, *Rosa*, antioxidant activity, food borne bacteria.

Introduction

In recent years, bacterial resistance against the present used antibiotics is becoming a concern to public health [1]. In addition, due to development of bacterial super resistant strains, in many instances the antibiotics failed to solve the bacterial infections. Therefore, in many parts of world, numerous researchers are trying to obtain new antimicrobial agents, either by the design and synthesis of new agents, or through the search of natural sources for as yet undiscovered antimicrobial agents [2].

Among the most important new antimicrobial agents are those used in folk medicines in most countries. Systematic screening of them may result in the discovery of novel effective compounds [3]. The use of medicinal plants in folk medicine is very common particularly in Near East and Asian countries. In fact, medicinal plants have been used as sources for illness relief since early civilization in China, India, in the Near East and in the New World [4].

Recent studies showed that horticultural crops, fruit and vegetables, are very rich in bioactive compounds and they are important sources to prevent several diseases, including cancer. It is well known that horticultural crops are the main sources of natural antioxidants [5].

The genus *Rosa* contains over 100 species that are widely distributed in Europe, Asia, the Middle East and North America. These deciduous shrubs are widely grown in gardens for their flowers and fruits. The plants show strong resistance to hard environmental conditions (rocky, inclined places, poor soils and limiting water). Turkey is one of the most important germplasm centres for rose species. Twenty five rose species (about 25% of all rose species) have so far been reported to grow in Turkey [6].

Members of the Rosaceae family, including rose hips, have long been used for food and medicinal purposes. Rose hips are well known for their efficacy in strengthening the body defense against infections, and particularly the common cold. The fruits, leaves and even roots are boiled in water and used as diuretics and as ingredients in common cold remedies in

Turkey [7]. Rose hips are also well known to have the highest vitamin C content (300–4000 mg/100 g) among horticultural crops fruits and vegetables. In addition, rose hips contain other vitamins and minerals, carotenoids, tocopherol, bioflavonoids, fruit acids, tannins, pectin, sugars, organic acids, amino acids and essential oils [8, 9].

The medicinal properties of rose hips may be partly attributed to their abundance in phenolics. Phenolics possess a wide spectrum of biochemical activities, such as antioxidant, antimutagenic, anticargionogenic effects, as well as ability to modify gene expression [10].

In this study, fruits of four *Rosa* taxa (*Rosa pisiformis, Rosa canina, Rosa villosa* and *Rosa dumalis subsp. antalyensis*) which had been described in herbal books and folklore medicine of Turkey were investigated for their antibacterial and antioxidant activity.

Materials and Methods

Plant material

Plant material (fruits of *Rosa pisiformis, Rosa canina, Rosa villosa* and *Rosa dumalis subsp. antalyensis*) were sampled from Erzurum region in Eastern Anatolia region in Turkey.

Antimicrobial activity

The air-dried and powdered fruit materials (400 g) were extracted in a Soxhlet apparatus with methanol (MeOH) for 72 h [11]. The extracts were filtered using Whatman filter paper (No:1) and then concentrated in vacuum at 40 °C using a Rotary evaporator. The residues obtained were stored in a freezer at − 80 °C until further tests. A total 8 foods related bacteria species (*Bacillus subtilis, Yersinia enterocolitica, Escherichia coli, Enterecoccus faecalis, Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium* and *Klebsiella pneumoniae*) were used in this study. Identity of the bacteria used in this study was confirmed by Microbial Identification System in Biotechnology Application and Research Center at Ataturk University. The antibacterial activity of the extracts was carried out by disc diffusion test [12] using 100 µl of suspension containing 10^8 CFU/ml of bacteria spread on nutrient agar (NA) medium. Sterile 6 mm diameter filter paper discs were impregnated with 300µg of extract/disc and placed onto nutrient 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 in each bacterial species tested. The inoculated plates with food-associated bacteria were incubated at 35 °C for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its antibacterial activity. Five discs per plate used and each test was run in triplicate [13]. The inocula of bacteria were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The Minimal Inhibitory Concentration (MIC) of *Rosa* fruit (rose hip) extracts against bacterial strains was determined based on a micro-well dilution method [14]. 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 rose hip 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. Contents of each well were mixed on a 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 of each extracts was taken as the former one of the lowest concentration that showed no growth [15].

Antioxidant activity

For each species, 50 fruits were thawed together as 5 replicate at room temperature and homogenized in a standard food blender. Homogenates were assayed for Vitamin C determination. Ascorbic acid (Vitamin C) of samples was quantified with the reflectometer set of Merck Co (Merck RQflex). For extraction, fruit homogenates obtained with a blender were extracted with a buffer containing acetone, water, and acetic acid (70:29.5:0.5, v/v/v) for 1 h in darkness [16]. This extract was filtered and used for phytochemical analysis. Total phenolics were determined colorimetrically using Folin-Ciocalteu reagent as described by Slinkard and Singleton [17]. The results were expressed as mg GAE/g DW (dry weight). Total antioxidant capacity of samples was determined by β-carotene bleaching assays. In the β-carotene bleaching assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [18]. Antioxidant capacities of the samples were compared with those of the synthetic antioxidant butylated hydroxyanisole (BHA) and the blank.

Results and Discussions

Antibacterial activity

The antimicrobial activities of different *Rosa* taxa fruit extracts against food associated microorganisms examined in the present study and their potency were assessed by the presence or absence of inhibition zones and zone diameter, and MIC values. The results are given in Tables 1.

The data of the study clearly indicated that the methanolic extract of different *Rosa* taxa fruits has antibacterial activity against a number of bacteria and antibacterial activity was differed among taxa tested against bacteria. Regarding the taxa tested, *Rosa pisiformis* extract inhibited the growth of *Yersinia enterocolitica, Streptococcus aureus, Bacillus cereus* and *Salmonella typhimurium* with 0-13 mm inhibition zone.

Table 1. Antibacterial activity (The value of MIC and inhibition zone) of *Rosa* taxa methanol extracts against the bacteria species

Food Borne Bacteria	1 Inhibition zone (mm) MIC values (µg/ml)	2 Inhibition zone (mm) MIC values (µg/ml)	3 Inhibition zone (mm) MIC values (µg/ml)	4 Inhibition zone (mm) MIC values (µg/ml)	*MIC values (µg/ml)	Negative control MeOH	Positive control standard antibiotic disc
B. subtilis	- -	- -	- -	- -	31.25	-	25 (NET)
Y. enterocolitica	10 >500	11 >500	- -	10 >500	NT	-	14 (OFX)
E. coli	- -	- -	- -	- -	250	-	11 (SCF)
E. faecalis	- -	10 >500	12 250	- -	7.81	-	13 (SCF)
S. aureus	10 >500	- -	- -	- -	125	-	11 (NET)
B. cereus	10 31.25	9 62.50	10 31.25	14 125	7.81	-	19 (OFX)
S. typhimurium	13 62.50	- -	- -	- -	125	-	13 (NET)
K. pneumoniae	- -	- -	- -	12 >500	125	-	15 (SCF)

1:*Rosa pisiformis*; 2:*Rosa canina*; 3:*Rosa villosa*; 4:*Rosa dumalis subsp. antalyensis*
OFX: ofloxacin (5µg/disc), SCF: sulbactam (30µg) + cefoperazona (75 µg) (105 µg/disc), NET: netilmicin (30µg/disc)
*: standart drug maxipime; NT: Not tested

Rosa canina extract inhibited the growth of *Yersinia enterocolitica, Enterococcus faecalis* and *Bacillus cereus* with 9-11 mm inhibition zone. *Rosa villosa* extract inhibited the growth of *Enterococcus faecalis* and *Bacillus cereus* with 10-12 mm inhibition zone. *Rosa dumalis subsp. antalyensis* extract inhibited the growth of *Yersinia enterocolitica, Bacillus cereus* and *Klebsiella pneumoniae* with 10-14 mm inhibition zone. According to these results, it can be said that among *Rosa* taxa, *Rosa canina* were found to be more active on microorganisms (Table 1). The maximal inhibition zones and MIC values for food borne bacterial strains, which were sensitive to the methanol extracts of *Rosa* taxa were in the range of 31.25–500 μg/ml and the MIC values of control, standard drug Maxipime was between 7.81-250 μg/ml (Table 1). In general, we did not find any specific effect of extracts on gram-positive and gram-negative bacteria. However, previous works showed that the gram-positive bacteria are more sensitive to plant extracts than the gram-negative ones [19].

Previously several studies have been performed concerning the antimicrobial activity of extracts of other *Rosa* taxa and demonstrated that the members of the genus *Rosa* show a high antimicrobial activity due to the presence of alkanes and phenolics [20, 21, 22].

Antioxidant activity

The total phenolic contents of the fruits of *Rosa* taxa varied from 78 mg GAE/ g DW in *Rosa villosa* to 102 mg GAE/100 g DW in *Rosa canina* (Table 2).

Total antioxidant capacity of *Rosa* taxa is shown in Table 2. The genotype seemed to influence the extent of antioxidant activity in *Rosa* taxa.

In β-carotene linoleic acid assay, antioxidant capacity was in order of 91.4% (*Rosa canina*)>90.3% (BHA)>89.6% (*Rosa pisiformis*)> 87.3% (*Rosa dumalis* subsp. *antalyensis*)>83.8% (*Rosa villosa*) (Table 2).

These results indicated that rose hips are important natural antioxidant sources. These results are also in agreement with those previously reported for rose hips as having good antioxidant capacity [22, 23]. It was previously reported that the genotype affects the antioxidant capacity in different fruit species such as mulberries [23] and currants [5].

Table 2. Bioactive content of *Rosa* taxa

Species	Total phenolic content (mg GAE/g DW)	Vitamin C (mg/100 g FW)	Antioxidant capacity (%)
R. pisiformis	83c	719ab	89.6ab
R. canina	102a	840a	91.4a
R. villosa	78d	681b	83.8c
R. dumalis subsp *antalyensis*	91b	745ab	87.3b
BHA			90.3a

*Values in the same column with different lower-case letters are significantly different at $P<0.05$.
DW: Dry weight; FW: Fresh weight; BHA: Standard antioxidant

Vitamin C contents of the fruits of *Rosa* taxa varied from 681 mg/100 g in *Rosa villosa* to 840 mg/100 g in *Rosa canina* (Table 2).

The results of present investigation clearly indicate that the antibacterial and antioxidant activity vary with the *Rosa* taxa. The variation in antibacterial and antioxidant activity noticed in this investigation is important knowledge for the selection of plant material to be used in food production, health industry, and future breeding programs.

References

1. S. MONROE, R. POLK. Antimicrobial use and bacterial resistance. *Current Opinion in Microbiology*, 3, 496-501 (2000).
2. S. BHAVNANİ, C.H. BALLOW. New agents for Gram-positive bacteria. *Current Opinion in Microbiology*, 3, 528–534 (2000).
3. N. TOMOKO, A. TAKASHI, T. HIROMU, I. YUKA, M. HIROKO, I. MUNEKAZU, T. TOTSHIYUKI, I. TETSURO, A. FUJIO, I. IRIYA, N. TSUTOMU, W. KAZUHITO. Antibacterial activity of extracts preparated from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus*. *Journal of Health Science*, 48, 273-276 (2002).
4. S.C. FERRENCE, G. BENDERSKY. Therapy with saffron and the goddess at thera. *Perspectives in Biology and Medicine*, 47(2), 199-226 (2004).
5. A. HEGEDUS, E. BALOGH, R. ENGEL, B.Z. SIPOS, J. PAPP, A. BLAZOVICS, E. STEFANOVIST-BANYAI. Comparative nutrient element and antioxidant characterization of berry fruit species and cultivars grown in Hungary. *HortScience* , 43, 1711-1715 (2008).
6. S. ERCISLI. Rose (*Rosa* spp.) germplasm resources of Turkey. Genetic Resources and Crop Evolution, 52, 787–795 (2005).
7. S.M. SEN, M. GUNES. Some chemical and physical properties of roses are grown in Tokat provinces in Turkey. In Proceedings of 1st National Rose hip Conference, 4–7 September, Gumushane–Turkey, pp. 231–239 (in Turkish) (1996)..
8. J.T. CHAI, Z.H. Ding. Nutrients composition of *Rosa laevigata* fruits. *Science and Technology of Food Industry*, 3, 26-29 (1995).
9. M. UGGLA, X. GAO, G. WERLEMARK. Variation among and within dog rose taxa (*Rosa sect. caninae*) in fruit weight, percentages of fruit flesh and dry matter, and vitamin C content. *Acta Agriculturae Scandinavica Section B, Soil and Plant Science*, 53, 147–155 (2003)..
10. Y. NAKAMURA, S. WATANABE, N. MIYAKE, H. KOHNO, T. OSAWA. Dihydrochalcones: evaluation as novel radical scavenging antioxidants. *Journal of Agriculture and Food Chemistry*, 51, 3309–3312 (2003).
11. J. LIN, A.R. OPOKU, M. GEHEEB-KELLER, A.D. HUTCHINGS, S.E. TERBLANCHE, A.K. JAGER, J. van STADEN. Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. *Journal Ethnopharmacology*, 68, 267-274 (1999).
12. J. KIM, M.R. MARSHALL, C. WIE. Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agriculture and Food Chemistry*, 43, 2839-2845 (1995).
13. C.D. DJIPA, M. DELMEE, J. QUETIN-LECLERCQ. Antimicrobial activity of bark extracts of *Syzygium jambos* L. *Journal Ethnopharmacology*, 71, 307-313 (2000).
14. K.M.S. SWANSON, F.F. BUSTA, E.H. PETERSON, M.G. JOHANSON. Colony count methods. In: Vanderzant C., Splittstoesser, D.F. (eds.) Compendium of methods for microbiological examination of food. 3rd edn. American Public Health Association, Washington, pp 75-95 (1992)..
15. S. OZTURK, S. ERCISLI. Antibacterial activity and chemical constitutions of *Ziziphora clinopodioides. Food Control*, 18(5), 535-540 (2007).
16. V.L. SINGLETON, J.L. ROSSI. Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. *American Journal Enology and Viticulture,* 16, 144-158 (1965).
17. K. SLINKARD, V.L. SINGLETON. Total phenol analyses: automation and comparison with manual methods. *American Journal Enology and Viticulture*, 28, 49-55 (1977).
18. C. KAUR, H.C. KAPOOR. Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology*, 37, 153-161 (2002).
19. S. COSENTINO, C.I.G. TUBEROSO, B. PISANO, M. SATTA, V. MASCIA, E. ARZEDI, F. PALMAS. *In vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. *Letters in Applied Microbiology*, 29, 130-135 (1999).
20. B.C. ARIDOGAN, H. BAYDAR, S. KAYA, M. DEMIRCI, D. OZBASAR, E. MUMCU. Antimicrobial activity and chemical composition of some essential oils. *Archives of Pharmacal Research*, 25, 860-864 (2002).
21. R. NOWAK, U. GAWLIK-DZIKI. Polyphenols of *Rosa* L. leaves extracts and their radical scavenging activity. *Zeitschrift für Naturforschung A,* 62, 32-38 (2007).
22. O. YI, E.M. JOVEL, G.H.N. TOWERS, T.R. WAHBE, D. CHO. Antioxidant and antimicrobial activities of native *Rosa* sp. from British Columbia, Canada. *International Journal Food Science and Nutrition*, 58 (3), 178-189 (2007).
23. C. CHRUBASIK, B.D. ROUFOGALIS, U.M. LADNER, S. CHRUBASIK. A systematic review on the *Rosa canina*. Effect and Efficacy Profiles. *Phytotheraphy Research.* 22*,* 725-733 (2008).