

Short communication

Evaluation of antimicrobial activity of *Malus domestica* fruit extract from Kashan area

Sara Jelodarian^{1*}, Abdolrasoul Haghiri Ebrahimabadi², Fereshteh Jookar Kashi²

Abstract

Objective: Many species of plants present noticeable potency against human bacterial and fungal pathogens.

Materials and Methods: In the current study, antimicrobial activity of the fresh fruits of 4 cultivars (A to D) of *Malus domestica* cultivated in the Qamsar area of Kashan, Iran was investigated. The disk diffusion and minimal inhibitory concentration (MIC) evaluation antimicrobial activity tests were performed.

Results: The samples showed moderate antimicrobial activities with inhibition zones from 11 to 16 mm in these tests. Cultivar D with inhibition zones of 16, 14, and 12 mm for *E. coli*, *S. epidermidis* and *K. pneumoniae*, respectively exhibited the best results in these tests. Cultivar A also showed a zone of inhibition of 11 mm against *P. aeruginosa*.

Conclusion: Moderate antimicrobial activities were observed for the studied apple cultivars.

Keywords: Apple, Antimicrobial, Extract, *Malus domestica*

1- Department of Horticulture, Faculty of Agriculture and Natural Sources, Science and Research Branch, Islamic Azad University, Tehran, I. R. Iran

2- Essential Oils Research Institute, University of Kashan, Kashan, I. R. Iran

*Corresponding author: Tel: +989131636427

E-mail: sjelodarian@yahoo.com

Introduction

Fruits and vegetables are the main food source for some essential nutrients and also include a series of bioactive components, which might have multiple effects in the fields of health (Yigit *et al.*, 2009). Among fruits, apples are consumed in different forms, i.e., fresh, in juices, and cider.

Apples have several useful aspects for everybody's health in regard to their high capacity in phenolic contents (Alberto, 2006). Phenolic compounds include a significant class of phytochemicals that possess various biological functions such as astringent, antioxidant, anticancer, anti-inflammation, and antibacterial activity (Jeong *et al.*, 2009; Rubnov *et al.*, 2001; Vaya & Mahmood, 2006; Ryu *et al.*, 1998). In addition, incidence or survival/growth of *Listeria monocytogenes*, *Listeria innocua*, *Salmonella serovars*, and *Escherichia coli* O157:H7 in fruit juices and apple cider has been demonstrated (Raybaudi-Massilia *et al.*, 2009; Ceylan *et al.*, 2004; Harris *et al.*, 2003; Ingham *et al.*, 2006; Miller & Kaspar, 1994; Raybaudi-Massilia *et al.*, 2006).

Malus domestica Borkh is a kind of fruit that is consumed all over the world (Shoji *et al.*, 2004). Our samples are ancient with medium size and circular shape. The yellow–pink skins are thin, rather wax-like, and their white flesh is soft, juicy, aromatic, and sweet. Because of staying on the tree, the skin color of these 4 apple cultivars changes gradually and becomes red.

The objective of this study was to measure the antimicrobial potential of these fruit extracts.

Materials and Methods

Fruit collection

Fresh fruit samples from Hossain, Sayyed Babaei, Shekareh, and Golab apple cultivars were collected in the Qamsar area of Kashan, Iran, in the June 2008 when the fruit had just been harvested. They were randomly named as A, B, C and D.

Extraction procedure

Apples were characterized by a plant taxonomist, immediately transported to the laboratory, washed, dried, cut manually with a knife into small pieces, whole fruit except seeds extracts were obtained using a kitchen-type blender (Moulinex, France), and concentrated with a rotary evaporator.

Antimicrobial activity

Microbial strains

The extracts of *Malus domestica* were individually tested against a panel of eleven microorganisms. Following microbial strains were provided by Iranian Research Organization for Science and Technology (IROST) and were used in this research: *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 29737), *Klebsiella pneumonia* (ATCC 10031), *Staphylococcus epidermidis* (ATCC 12228), *Shigella dysenteriae* (Persian Type Culture Collection or PTCC 1188), *Proteus vulgaris* (PTCC 1182), *Salmonella paratyphi-A serotype* (ATCC 5702), *Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 16404).

Bacterial strains were cultured overnight at 37 °C in nutrient agar (NA) and fungi were cultured overnight at 30 °C in sabouraud dextrose agar (SDA).

Bacterial strains were cultured overnight at 37 °C in nutrient agar (NA) and *Candida albicans* and *Aspergillus niger* were cultured overnight at 30 °C in sabouraud dextrose agar (SDA).

Disc diffusion assay

Determination of antimicrobial activity of extracts was accomplished by agar disc diffusion method (NCCLS, 1997). The extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 30 mg/ml and filtered by 0.45 µm Millipore filters for sterilization.

Antimicrobial tests were carried out by the disc diffusion method reported by Murray *et al.*, (1999) using 100 µl of suspension

Antimicrobial activity of *Malus domestica* extract

containing 10^8 CFU/ml of bacteria, 10^6 CFU/ml of yeast and 10^4 spore/ml of fungi spread on the nutrient agar (NA), sabouraud dextrose (SD) agar, and potato dextrose (PD) agar mediums, respectively. The discs (6 mm in diameter) were impregnated with 10 μ l of the essential oil or the extract solution (300 μ g/disc) and DMSO (as negative control) were placed on the inoculated agar.

The inoculated plates were incubated for 24 h at 37 °C for bacterial strains and 48 h and 72 h at 30 °C for yeast and mold isolates, respectively. Gentamicin (10 μ g/disc) and rifampin (5 μ g/disc) were used as positive controls for bacteria and nystatin (100 IU) for fungi.

The diameters of inhibition zones were used as a measure of antimicrobial activity and each assay was repeated twice.

Micro-well dilution assay

Bacterial strains and yeast sensitive to the extracts in disc diffusion assay were studied for their minimal inhibition concentration (MIC) values using micro-well dilution assay method (Güllüce et al., 2004).

The inocula of the bacterial strains and fungi were prepared from 12 h and 18 h broth cultures, respectively and suspensions were adjusted to 0.5 McFarland standard turbidity. The extracts of apples dissolved in 10% DMSO solution were first diluted to the highest concentration (5 mg/ml) to be tested, and then serial two-fold dilutions were made in a concentration range from 0.078 to 5 mg/ml in 10 ml sterile test tubes containing brain heart infusion (BHI) broth for bacterial strains and sabouraud dextrose (SD) broth for yeast. The 96-well plates were prepared by dispensing 95 μ l of the cultures media and 5 μ l of the inoculum into each well.

A 100 μ l aliquot from the stock solutions of the extracts was initially prepared at the concentration of 5 mg/ml

and added into the first wells. Then, 100 μ l volumes from their serial dilutions were transferred into six consecutive wells. The last well containing 195 μ l of the cultures media without the test materials and 5 μ l of the inoculum on each strip was used as the negative control. The final volume in each well was 200 μ l.

Gentamicin and rifampin for bacteria and nystatin for yeast were used as standard drugs for positive control in conditions identical to tests materials. The plates were covered with sterile plate sealers. 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 the presence of a white pellet on the well bottom and confirmed by plating 5 μ l samples from clear wells on NA medium. The MIC value was defined as the lowest concentration of the extracts required for inhibiting the growth of microorganisms. All tests were repeated two times.

Results

The antimicrobial activity of extract of *Malus domestica* was evaluated against a set of eleven microorganisms and their potencies were assessed qualitatively and quantitatively by the presence or absence of inhibition zones, the corresponding zone diameters, and MIC values. The results given in Table 1 indicate that at the tested concentrations, the extract of cultivars D and A have notable antimicrobial activity against tested microorganisms. *P. aeruginosa* was sensitive to cultivar A and inhibition zone of 11 mm was reported.

E. coli, *S. epidermidis*, and *K. pneumoniae* sets were sensitive to cultivar D and Inhibition zone of 16, 14, and 12 mm was reported. However, cultivars B and C did not show any activity against the microorganisms tested.

Table 1. Antimicrobial activities of extracts of four apple cultivars against tested microbial.

	Extracts								Antibiotics					
	A		B		C		D		Rifampin		Gentamicin		Nystatin	
Test microorganisms	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC
Gram-negative bacteria, Gram-positive bacteria and fungi														
<i>P. aeruginosa</i>	11	7.81	-	-	-	-	-	-	-	-	8	500	NA	NA
<i>E. coli</i>	-	-	-	-	-	-	16	500	11	125	20	500	NA	NA
<i>K. pneumoniae</i>	-	-	-	-	-	-	12	500	7	500	22	250	NA	NA
<i>P. vulgaris</i>	-	-	-	-	-	-	-	-	10	250	20	500	NA	NA
<i>S. paratyphi-A serotype</i>	-	-	-	-	-	-	-	-	-	-	20	250	NA	NA
<i>B. subtilis</i>	-	-	-	-	-	-	-	-	13	250	18	7.81	NA	NA
<i>S. aureus</i>	-	-	-	-	-	-	-	-	10	500	24	500	NA	NA
<i>S. epidermidis</i>	-	-	-	-	-	-	14	125	40	125	39	500	NA	NA
<i>S. dysenteriae</i>	-	-	-	-	-	-	-	-	-	-	25	500	NA	NA
<i>C. albicans</i>	-	-	-	-	-	-	-	-	NA	NA	NA	NA	33	125
<i>A. niger</i>	-	-	-	-	-	-	-	-	NA	NA	NA	NA	27	31.2

^a DD (Disc diffusion method), Inhibition zones in diameter (mm) around the impregnated discs.

^b MIC (Minimal Inhibition concentrations as µg/ml).

NA (Not applicable).

Discussion

In general, the Gram-positive strains of bacteria tested appeared to be more sensitive to the extracts. However, this study also records a significant susceptibility of some of the examined Gram-negative bacteria. According to literature, the antimicrobial activity could be influenced by the phenolic compounds (Alberto et al, 2001; Alberto et al, 2002; Alberto et al, 2004). Moreover, Alberto (2006) reported that there is a direct relationship between phenolic content and antibacterial effect in four apple cultivars (Xiangyang et el., 2003) and their polyphenol extracts had stronger inhibition effects on the bacteria. An *in vivo* assay is necessary to confirm the antimicrobial

activities of *Malus domestica*, which could be usefully applied to the food, pharmaceuticals, and cosmetics industries. Isolation of the gene responsible for the antimicrobial activity would be an interesting future study topic aimed at identifying the molecule generating the desirable efficacy. As a whole, these samples had moderate antimicrobial activities against a set of eleven microorganisms.

Many plant species are currently used as sources of nutritional additives because of their antioxidant and antimicrobial properties that increase immunity to some diseases. This work, as the first study on the *in vitro* antimicrobial activity of four cultivars, reports appreciable antimicrobial

potential for these samples. These findings candidate them as a good cases for more in-depth studies. Moreover, these cultivars can be proposed in food industries, as flavor and preservative or in cosmetic-health industries as antimicrobial agents. We wish our future research lead to the identification and structure elucidation of biologically active molecules present in their extracts.

Acknowledgments

The authors appreciate the financial support made by the Essential Oils Research Institute, University of Kashan, Kashan, Iran and Department of Hortiscience, Tehran Science and Research Branch, Islamic Azad University, Iran.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

- Alberto MR, Canavosio MAR, De nadra MCM. 2006. Antimicrobial effect of polyphenols from apple skins on human bacterial pathogens. *Electronic J Biotechnol*, 9: 3.
- Alberto MR, Farías ME, Manca De Nadra, M. C. 2002. Effect of wine phenolic compounds on *Lactobacillus hilgardii* 5w viability. *J Food Prot*, 65: 211-213.
- Alberto MR, Gómez-Cordovés C, Manca De Nadra MC. 2004. Metabolism of gallic acid and catechin by *Lactobacillus hilgardii* from wine. *J Agri Food Chem*, 52: 6465-6469.
- Alberto MR, Farías ME, and Manca De Nadra MC. 2001. Effect of gallic acid and catechin on *Lactobacillus hilgardii* 5w growth and metabolism of organic compounds. *J Agri Food Chem*, 49: 4359-4363.
- Ceylan E, Fung DYC, and Sabah JR. 2004. Antimicrobial activity and synergistic effect of cinnamon with sodium benzoate or potassium sorbate in controlling *Escherichia coli* O157:H7 in apple juice. *J Food Sci*, 69: 102-106.
- Gulluce M, Sokmen M, Sahin F, Sokmen A, Adiguzel A, Ozer H. 2004 Biological activities of the essential oil and methanolic extract of *Micromeria fruticosa* (L) Druce ssp. *Serpyllifolia* (Bieb) PH Davis plants from the eastern Anatolia region of Turkey. *J Sci Food Agric*, 84: 735-741.
- Harris LJ, Farber JN, Beuchat LR, Parish ME, Suslow TV, Garrett EH, et al. 2003. Outbreaks associated with fresh produce: Incidence, growth, and survival of pathogens in fresh and fresh-cut produce. In *Comprehensive reviews in food science and food safety* pp. 78-141. Iowa: Blackwell Publishing Professional.
- Ingham SC, Schoeller EL, and Engel RA. 2006. Pathogen reduction in unpasteurized apple cider: Adding cranberry juice to enhance the lethality of warm hold and freeze-thaw steps. *J Food Prot*, 69: 293-298.
- Jeong MR, Kim HY, and Jeong-Dan Cha JD. 2009. Antimicrobial activity of methanol extract from *Ficus carica* leaves against oral bacteria. *J Bacteriol Virol*, 39: 97 – 102.
- Miller LG, and Kaspar CW. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. *J Food Prot*, 57: 460-464.
- Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH. 1995. *Manual of clinical microbiology*, 7th ed. Washington. DC: ASM. pp: 1773.
- Raybaudi-Massilia R, Mosqueda-Melgar J, and Martín-Belloso O. 2006. Antimicrobial activity of essential oils on *Salmonella Enteritidis*, *Escherichia coli* and *Listeria innocua* in fruit juices. *J Food Prot*. 69: 1579-1586.
- Raybaudi-Massilia R, Osqueda-Melgar J, Martín-Belloso O. 2009. Antimicrobial activity of malic acid against *Listeria monocytogenes*, *Salmonella enteritidis* and *Escherichia coli* O157:H7 in apple, pear and melon juices. *Food Control*, 20: 105-112.
- Rubnov S, Kashman Y, Rabinowitz R, Schlesinger M, Mechoulam R. 2001. Suppressors of cancer cell proliferation from fig (*Ficus carica*) resin: isolation and structure elucidation. *J Nat Prod*, 64: 993-996.
- Ryu SR, Cho H, Jung JS, Jung ST. 1998. The study on the separation and antitumor activity as new substances in fig. *J Applied Chem*, 2: 961-964.
- Shoji T, Akazome Y, Kanda T, Ikeda M, 2004. The toxicology and safety of apple polyphenolic extract. *Food Chem Toxicol*, 42: 959-967.
- NCCLS (National Committee for Clinical Laboratory Standards). 1997. *Performance Standards for Antimicrobial Disk*

- Susceptibility Test, sixth ed. Approved Standard. M2-A6, Wayne, PA.
- Vaya J, Mahmood S. 2006. Flavonoid content in leaf extracts of the fig (*Ficus carica* L.), carob (*Ceratonia siliqua* L.) and pistachio (*Pistacia lentiscus* L.). *Biofactors*, 28. 169-175.
- Yigit D, yigit N, and Mavi A. 2009. Antioxidant and antimicrobial activities of bitter and sweet apricot (*Prunus armeniaca* L.) kernels. *Braz J Med Biol Res*, 42: 346-352.
- Xiangyang Qi. 2003. Study on antibacterial effect of apple-polyphenol extracts. *Food Sci*, 05.