S.Ü. Ziraat Fakültesi Dergisi 17 (32):2003, 53 -56

ANTIBACTERIAL EFFECT OF NARINCE GRAPE (VITIS VINIFERA L.) POMACE EXTRACT

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ABSTRACT

In the study, total phenolic content and antibacterial activity of pomace extract of Narince grape variety widely used wine-making in Turkey were determined. The amount of total phenolic in Narince pomace extract was found as 218.54 mg gallic acid equivalents (GAE) g^{-1}. This extract at 1, 2.5, 5, 10 and 20% concentrations was tested for its antibacterial effect by using the agar diffusion method against Aeromonas hydrophila, Bacillus cereus, Enterobacter aerogenes, Enterococcus feacalis, Escherichia coli, Escherichia coli O157:H7, Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella enteritidis, Salmonella typhimurium, Staphylococcus aureus and Yersinia enterocolitica. All of concentrations of the pomace extract had inhibited the growth of all the bacteria. The most sensitive and resistant bacteria were E. coli (24.67 mm) and E. feacalis (13.67 mm), respectively.

Key words: *Grape, pomace extract, antibacterial activity, total phenolic content*

NARINCE ÜZÜM (Vitis vinifera L.) CIBRESI EXTRAKTININ ANTIBAKTERIYAL ETKISI

ÖZET

Bu çalismada, Türkiye'de sarap yapiminda yaygin olarak kullanilan Narince üzüm çesidine ait cibrelerin ekstrakti hazirlanmis ve bu ektraktin antibakteriyal etkisi ile toplam fenolik madde içerigi belirlenmistir. Ekstraktin toplam fenolik madde miktari gallik asit esdegeri olarak 218.54 mg g^{-1} bulunmustur. Bu ekstraktlarin 1, 2.5, 5, 10 ve 20% konsantrasyonlari hazirlanarak, antibakteriyal etkiyi belirlemek amaciyla Aeromonas hydrophila, Bacillus cereus, Enterobacter aerogenes, Enterococcus feacalis, Escherichia coli, Escherichia coli O157:H7, Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella enteritidis, Salmonella typhimurium, Staphylococcus aureus ve Yersinia enterocolitica'dan olusan bakterilere karsi agar difüzyon metodu kullanilarak denenmistir. Cibre ekstraktinin bütün konsantrasyonlari, tüm bakterilerin gelisimini engellemistir. En hassas bakteri E. coli (24.67 mm) ve en dayanikli bakteri E. feacalis (13.67 mm) olarak saptanmistir.

Anahtar sözcükler: *Üzüm, cibre ekstrakti, antibakteriyal etki, toplam fenolik içerigi*

INTRODUCTION

In recent years, many researches focused on natural alternative preservatives rather than chemical origin. In nature there are a large number of different types of antimicrobial compounds and the most common of them are herbs and spices extracts for this aim (Kivanç and Akgül 1986, Deans and Svoboda 1990, Dorman and Deans 2000, Özcan and Boyraz 2000, Basim et al. 2000, Özcan and Erkmen 2001, Sagdiç et al. 2002, Sagdiç and Özcan 2003, Özkan et al. 2003, Baydar et al. 2003). The other antimicrobial compound and natural antioxidant is also grape pomace consisting of seeds, skins and stems extract (Revilla and Ryan 2000, Wang et al. 2000, Jayaprakasha et al. 2001, Murthy et al. 2002, Jayaprakasha et al. 2003, Ahn et al. 2002).

Grape has been extensively investigated, because of its large amounts of phenolic compound (Somers and Ziemelis 1985, Oszmianski and Lee 1990, Ricardo da Silva et al. 1990). The grape pomace, an important by-product after winemaking and traditional food production such as molasses, vinegar, köfter in Turkey, can consider as an important industrial waste. However, grape seed and pomace extracts have many favourable effects on human health (Frankel et al. 1993, Teissedre et al. 1996, Mayer et al. 1997, Waterhouse 1994) because of their large quantity of monomeric phenolic compounds and dimeric, trimeric and tetrameric procyanidins (Saito et al. 1998). But there is a limited number of research on the inhibitory effects of grape seed extracts (Murthy et al. 2002, Shoko et al. 1999, Göktürk Baydar et al. 2003) and no any study on antimicrobial effects of grape pomace, a rich sources of polyphenols.

The purpose of this work is to determine the total phenolic compound and the antimicrobial activity of Narince pomace extract against some bacteria.

MATERIALS AND METHODS

Clusters of the most popular wine-making grape cultivar Narince grown in Turkey were collected at optimal maturity from the experimental vineyard of the Agricultural Faculty of Ankara University (Ankara, Turkey). After processing wine, the pomace as by-product was used the following steps of the research.

Extraction

The grape pomace was dried at 70 ^{o}C for 72 h, after pressing and ground to fine powder by grinder. Then the powdered pomace was extracted in a Soxhlet

extractor with petroleum ether (60 °C for 6 h) to remove the fatty materials and re-extracted in a Soxhlet apparatus for 8 h with 200 ml acetone: water: acetic acid (90:9.5:0.5) as described by Jayaprakash et al. (2003). After that, all of the extracts were concentrated by using rotary evaporator under vacuum at 70°C and the crude extract was stored in a desiccator until use.

Determination of total phenolic compounds

The concentration of phenolic compounds in the pomace extract was determined by the Folin-Ciocalteu colorimetric method (Singleton and Rossi 1965). The estimation of phenolic compounds in the extract was carried out in triplicate and calculated by a calibration curve obtained with gallic acid. Total phenolic was expressed as gallic acid equivalents (mg GAE g^{-1} extract).

Bacterial cultures

Aeromonas hydrophila ATCC 7965, Bacillus cereus FMC 19, Enterobacter aerogenes CCM 2531, Enterococcus feacalis ATCC 15753, Escherichia coli ATCC 25922, Escherichia coli O157:H7 KUEN 1461, Mycobacterium smegmatis RUT, Proteus vulgaris FMC 1, Pseudomonas aeruginosa ATCC 27853, Pseudomonas fluorescens EU, Salmonella enteritidis, Salmonella typhimurium, Staphylococcus aureus Cowan 1 and Yersinia enterocolitica EU were used as test organisms. These bacteria were supplied by Department of Biology, Sütçü Imam University, Kahramanmaras-Turkey.

Determination of antibacterial effect

The stock cultures of *P. vulgaris*, *P. aeruginosa*, *P. fluorescens* and *Y. enterocolitica* were grown in nutrient broth (Acumedia Manufacturers, Inc., Maryland) at 25 °C for 22 h. The other bacteria were grown in the same medium at 37 °C for 22 h (Ilçim et al. 1998). Of all test bacteria in nutrient broth were enumerated by using serial dilution method. Final cell concentrations were 10^9-10^8 cfu ml^{-1}. 250 µl of the each bacterium adjusted to 10^6-10^7 cfu ml^{-1} final cell concentrations was added into flask containing 25 ml sterile nutrient agar at 43-45 °C. The prepared bacterial cultures were poured into petri plates (9 cm diameter), and then the agar was allowed to solidify. The agar well diffusion method was used to detect the antibacterial activity of the pomace extracts. Four equidistant holes were made in the agar using sterile cork borers (∅ = 4 mm). 50 µl of 1, 2.5, 5, 10 and 20% volume each pomace extract solution with pure methanol (Merck-Darmstadt, Germany) were added to the holes using a pipettor and absolute methanol was used as control. The *P. vulgaris*, *P. aeruginosa*, *P. fluorescens* and *Y. enterocolitica* in test plates were refrigerated at 8 °C for 1 h and then incubated at 37 °C for 18-24 h in the inverted position. The other bacteria were grown in the same media at 35 °C for 18-24 h (Sagdiç et al. 2002, Sagdiç and Özcan 2003, Aurelli et al. 1992). At the end of the period, inhibition zones formed on the medium were measured in millimeter (mm). All the tests were triplicate.

Statistical analyses

Results of the research were tested for statistical significance by one-way ANOVA. Differences were considered statistically significant at the $p < 0.05$ level (Özdamar 1999). All analysis was performed as triplicate.

RESULTS AND DISCUSSION

The % yield of grape pomace was found to be 9.82% of Narince. Murthy et al. (2002) reported that yields of grape pomace were 5.6% in methanol, 3.9% in ethyl acetate and 1.1% in water extracts. These findings are similar to our results. The content of total phenolic compound was also found to be 218.54 mg GAE g^{-1}. The studies focused on grape pomace were limited. The study of Murthy et al. (2002) is one of them. Our values are similar their findings, 35.7 % in phenolic methanol extracts of pomace, 27.9 % in ethyl acetate and 6.1 % in water extracts, respectively.

Table 1. Antibacterial effects of Narince grape pomace extract (zone size, mm)

Test bacteria	Different concentrations of the extract (%)				
	20	10	5.0	2.5	1.0
A. hydrophila	22.00	20.33	13.00	08.67	6.67
B. cereus	22.00	20.67	13.67	12.00	8.00
E. aerogenes	23.83	19.67	13.00	08.17	6.00
E. feacalis	13.67	11.67	09.67	07.00	5.50
E. coli	24.67	20.67	17.00	12.00	9.00
E. coli O157:H7	18.50	17.67	10.00	09.33	8.00
M. smegmatis	22.00	20.00	13.00	11.00	8.33
P. vulgaris	22.00	18.00	11.67	11.00	8.00
P. aeruginosa	24.00	17.17	14.00	09.50	6.00
P. fluorescens	20.00	18.67	15.00	07.00	5.50
S. enteritidis	23.83	22.50	16.33	11.67	8.00
S. typhimurium	24.00	18.00	13.50	11.00	6.67
S. aureus	21.67	12.00	08.00	06.67	5.67
Y. enterocolitica	22.67	20.00	12.33	07.67	5.00

Antibacterial effects of pomace extract on bacteria are given in Table 1. Pure methanol as control had no inhibitory effect on the fourteen bacteria tested. There were statistically significant differences among the bacteria and pomace extracts' concentration ($p<0.05$). The effectiveness of the extracts' concentrations followed the sequence: 20 > 10 > 5 > 2.5 > 1%. The most effective concentrations were 20 and 10%, respectively (Figure 1).The grape pomace extract at all concentrations was effective on all tested bacteria. The most sensitive bacteria were *E. coli* (24.67 mm) and the most resistant bacteria were *E. feacalis* (13.67 mm). Jayaprakasha et al. (2003) and Göktürk-Baydar et al. (2003) were obtained similar results at grape

seed extracts. The inhibitory effect of the extract could be attributed to their phenolic composition, especially gallic acid as the most active compound for inhibition of bacteria (Shoko et al. 1999).

As a result, the pomace at all concentrations extracted from grapes has antibacterial effect on important food-borne bacteria. Pomace is used commonly as grape by-products and wastes and it may suggest at low concentration as natural additive to prevent the deterioration of stored foods and feeds by bacteria if they have any acceptable organoleptic effects.

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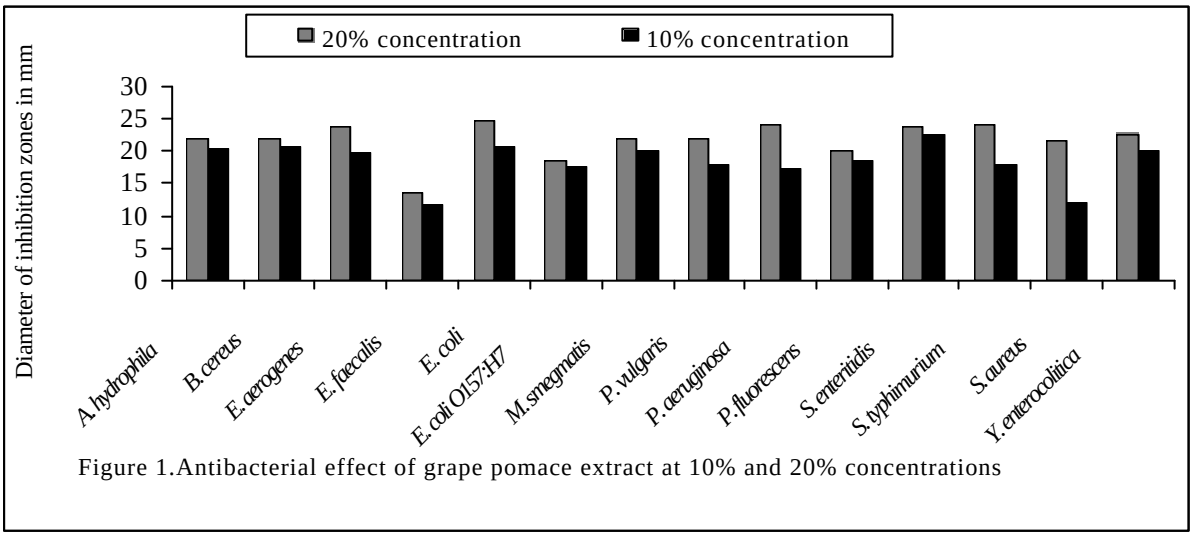

Figure 1.Antibacterial effect of grape pomace extract at 10% and 20% concentrations