ANTIBACTERIAL ACTIVTY OF BLACK MULBERRY (*Morus nigra*) FRUITS AND LEAVES

KARADUT (*Morus nigra*) MEYVE VE YAPRAKLARININ ANTİBAKTERİYEL AKTİVİTESİ

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Geliş Tarihi: 27 Şubat 2008 **Kabul Edilme Tarihi:** 18 Mart 2009

ABSTRACT

Antibacterial activities of the methanol and water extracts obtained from fruits and leaves of black mulberry (*Morus nigra*) were tested against 77 clinical strains of 5 bacteria species (*Enterobacter aerogenes, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa,* and *Staphylococcus aureus*) by disc-diffusion method and minimal inhibitory concentration (MIC) values of each active extract were determined. The black mulberry extracts proved to be active against 4 of the 5 bacteria species tested in this study. The highest antibacterial activity was exhibited by methanol extract of mulberry leaves against *S. aureus* with 18 mm inhibition zone and 0.156 mg/ml MIC value. Also good antibacterial potentials were detected against *E. aerogenes, E. coli,* and *P. aeruginosa* with these extracts. Black mulberry extracts did not show any antibacterial activity against *P. mirabilis.*

Keywords: Antibacterial activity, Black mulberry ((*Morus nigra*)), Disc diffusion method.

ÖZET

Bu çalışmada, Karadut'un (*Morus nigra*) yaprak ve meyvelerinden elde edilen metanol ve su ekstrelerinin antibakteriyel aktivitesi, 5 bakteri türünün (*Enterobacter aerogenes, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus*) 77 klinik kökeni üzerinde disk difüzyon yöntemi kullanılarak belirlenmiştir. Disk difüzyon yöntemiyle aktivite tespit

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edilen bakteriler için minimum inhibitör konsantrasyonları (MİK) belirlenmiştir. Karadut ekstreleri, çalışmada kullanılan 5 bakteri türünden 4'ü üzerinde aktivite göstermiştir. En yüksek aktivite, *S. aureus* bakterisine karşı 18 mm inhibisyon zonu ve 0.156 mg/ml MİK değeriyle, karadut yapraklarının metanol ekstresi tarafından sergilenmiştir. Yine *E. aerogenes, E. coli ve P. aeruginosa* bakterilerine karşı da iyi antibakteriyel etkiler tespit edilmiştir. Karadut ekstreleri, *P. mirabilis* bakterisi üzerinde her hangi bir antibakteriyel aktivite göstermemiştir.

Anahtar Kelimeler: Antibakteriyel etki, Disk difüzyon yöntemi, Karadut (*Morus nigra*),

1. INTRODUCTION

During the past century, research on the role of fruits and vegetables in human nutrition has focused on 'essential' nutrients. Evidence is accumulating from recent research has focused on the role of fruits and vegetables in human nutrition contain various phytochemicals possess health beneficial properties. Supporting evidence is accruing from *in vitro* and *in vivo* epidemiological studies, in some cases human clinical trials (Kalt *et. al*., 1999). The properties of phytochemical groups are believed to benefit health and reduce disease risk. Phenolics are a major group of antioxidant phytochemicals in berry crops. In addition to their antioxidant characteristics, new evidence suggests that phenolics also possess anti-inflammatory and antimicrobial properties (Hodges and Kalt, 2003). In particular, the antimicrobial activity of fruit extracts has formed the basis of many applications, including pharmaceuticals, alternative medicine and natural therapies (Hammer *et. al*., 1999).

The mulberry belongs to the genus *Morus* of the family Moraceae. There are 24 species of *Morus* and one subspecies, with at least 100 known varieties. Mulberry is widely spread through-out all regions from the tropics to the sub-arctic and from sea level to altitudes as high as 4000 m (Ercişli and Orhan, 2007). It is native to southwestern Asia, where it has been cultivated for so long that its precise natural range is unknown. It is a small deciduous tree growing to 10-13 m tall. The edible fruit is dark purple, almost black, when ripe, 2-3 cm long, a compound cluster of several small drupes; it is richly flavored, similar to the red mulberry (*Morus rubra*) but unlike

the more insipid fruit of the white mulberry (*Morus alba*) (Davis, 1982).

In Turkey, and other countries, black mulberries are not only consumed fresh but also used to produce jam, sweeten fruit marmalade (a locally dried fruit pulp product), syrup several types of soft drinks and traditional products such as mulberry pekmez, mulberry pestil (Şengül *et al*., 2005).

Mulberries have been reported to have several biological actions such as anti-diabetic (Asano *et al*., 2001), anti-oxidative (Kim *et al*., 1998), anti-inflammatory (Kim *et al*., 1999) activities. Mulberries are a good source of vitamins and minerals; especially contain a high amount of anthocyanin (Gerasopoulos and Stavorulakis, 1997). Besides its use as food, the plant fruits, leaves and barks are well known as traditional medicine in Turkey and have been used for many years as an anti-fever, as a laxative, anti-helmintic, antimicrobial, expectorant and facilitate discharge of urine and lower blood pressure (Baytop, 1999).

There is one report on flavor, dry matter, total sugar, PH and anthocyanin content of some mulberry species (Ercişli and Orhan, 2007). Recently, it has gained an important position in the local soft drink market, although its biological and pharmacological effects are still poorly defined. However, we recently detected strong anti-candidal activities of black mulberry fruits against some *Candida* spp. (Yiğit *et al*., 2007). However the antibacterial activities of black mulberry have been poorly studied and the lack of references on antibacterial activity of it stimulated this research (Krisch *et al*., 2008). The aim of the present work was to investigate the antibacterial activities of methanol and water extracts of black mulberry fruits and leaves on some human pathogen clinical isolates.

2. MATERIALS AND METHODS

Preparation of extracts

The black mulberry (*Morus nigra*) fruits were collected at their optimum commercial maturity in Uzumlü Town, Erzincan, Turkey. The fresh fruit samples were packed on ice while being transported to the laboratory. Fruits samples were frozen at –20^{o}C until extraction.

The leaves of black mulberry were dried in shade and powdered with a blender. The fruits and powdered leaves were extracted with methanol in a Soxhlet apparatus for 24 h. Then methanol was evaporated with rotary evaporator. Water extracts were also prepared by adding boiling water to 20 g of powdered material in a glass flask and incubated at room temperature for 2 hours on a rotating shaker (200 rpm). Mixture was filtered using Whatman (No.1) filter paper and then filtrate was lyophilized. All extracts were stored in freezer at –24^{o}C until use.

Test Microorganisms

Antimicrobial activity tests were carried out against total 77 clinical strains of 5 bacteria species (*E. aerogenes* n:18, *E. coli* n:17, *P. mirabilis* n:10, *P. aeruginosa* n:12, *S. aureus* n:20). Microorganisms were provided by Department of Clinical Microbiology, Medicine Faculty, Erzurum. The bacteria species were identified using standard taxonomic procedures. The Gram-negative bacteria were submitted to tests including the indol test, motility, methyl red, hydrogen sulfide production, urease and phenylalanine decarboxylase production, growth in Simmons citrate and fermentation of glucose, lactose, saccharose, and mannitol. The colonies with a Gram-positive coccus morphology isolated in blood agar were submitted to tests of colony morphology, pigment production, Gram stain, and catalase and coagulase production. Bacteria species, isolation origins and numbers were showed in Table 1.

Antibacterial Activity

Disc-diffusion assay

The methanol and water extracts were dissolved in the extraction solvent (methanol and sterile distilled water). Final concentration was 30 mg/ml. Antibacterial test were than carried out by disc-diffusion method (Murray *et. al.,* 1995) using suspension containing 10^8 colony forming unit (CFU)/ml of bacteria on nutrient agar (NA; Oxoid). The disc (6mm in diameter) were impregnated with extracts and placed on the inoculated NA. Negative controls were prepared using the same solvents employed to obtain extracts. Ofloxacin (Oxoid) for Gram-positive bacteria, Cefaperazone–sulbactam (Oxoid) for Gram-negative bacteria were used as positive controls. The inocu-

lated plates were incubated at 37°C for 24 h for clinical bacterial strains. Then antimicrobial activity was evaluated by measuring the inhibition zone against test microorganisms.

Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) values were also studied for the microorganisms which were determined as sensitive to the methanol and/or water extracts of plant parts (leaf and fruits) in disc-diffusion assay. MIC values of the extracts against microbial strains were determined based on a micro-well dilution method (Zgoda and Porter, 2001). The inoculations of microorganisms were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Firstly, the extracts dissolved in extraction solvent (methanol and sterile distilled water) were diluted to 10 mg/ml and then serial two fold dilutions were made in a concentration range (0.078-10 mg/ml) in a sterile test tube containing nutrient broth (NB). The 96-well plates were prepared by dispensing into each well 95 µl NB and 5 µl of the inoculums. A 100 µl of extracts solutions initially prepared at the concentration of highest concentration was added the first well, then 100 µl from serial dilutions was transferred into other consecutive wells. The plates covered with a sterile plate sealer and then incubated for 24 h. The MIC was defined as the lowest concentration of the extracts to inhibit the growth of microorganisms.

3. RESULTS AND DISCUSSION

Plant extracts have been used for a wide variety of purposes for many thousands of years. In particular, the antimicrobial activity of some parts of plants has formed the basis of many areas such as alternative medicine and natural therapies.

Mulberries are good sources of sugars, acids and anthocyanin contents, which are also responsible for their color, and taste and presumably also their antioxidant properties. The plants have also been used medicinally in Turkey. Local people traditionally believe that deep-colored fruits, especially black and red mulberry fruits, are healthier for human body (Ercişli and Orhan, 2007).

The antimicrobial activity of water and methanol extracts of black mulberry fruits and leaves were measured by the disc-diffusion method. Totally 77 clinic isolates of human pathogenic microorganism belonging to 5 bacteria species were used in these investigations (Table 1). The methanol and water negative controls showed no inhibiting effect. The inhibition diameters and MIC values of positive controls were ranging to 18–20 mm; 0,12–1µg/mL for Ofloxacin, 19–22 mm; 0,5–1 µg/mL for Cefaperazone and 12–15 mm, respectively.

Table 1. The bacteria species and isolation origins and numbers.

Bacteria	Blood	Urine	Wound	Ear	Throat	Mouth	
Enterobacter aerogenes	–	10	8	–	–	–	18
Escherichia coli	2	12	2	1	–	–	17
Proteus mirabilis	1	7	1	1	–	–	10
Pseudomonas aeruginosa	1	3	7	1	–	–	12
Staphylococcus aureus	10	4	4	1	1	–	20

Our findings showed that the methanol and water extracts from both fruits and leaves were active against Gram-positive and Gram-negative bacteria. The black mulberry extracts proved to be active against 4 of the 5 bacteria species of human pathogenic clinic isolates. The highest antibacterial activity was shown by methanol extract of mulberry leaves against *S. aureus* with 18 mm inhibition zone and 0.156 mg/ml MIC value (Table 2 and 3). Water and methanol extracts of mulberry leaves have activity against *E. aerogenes* and *S. aureus* with 15 mm inhibition zone. A 10 mm inhibition zone diameter and 0.625 mg/ml MIC values were detected against *E. coli*. The methanol and water extracts of leaves have antibacterial ability against *P. aeruginosa* with 10 mm inhibition zone and 0.625 mg/ml MIC values, but not fruits. *P. mirabilis* was not inhibited by black mulberry extracts.

Kuwanon C, mulberrofuran G, and albanol B, which were isolated from *Morus* root bark showed strong antibacterial activity with 5–30 mg/ml of MICs. Morusin, sanggenon B and D, were effective to only gram positive bacteria (Sohn *et. al*., 2004). On the other hand, different authors (Fukai *et. al*., 2005) reported that chalcomoracin, a leaf

phytoalexine of mulberry tree, exhibited considerable antibacterial activity against methicillin-resistant *S. aureus* (MICs 0.78 µg/ml). Our results are agreement with these findings that we detected strong antibacterial activity from mulberry extracts against *S. aureus*.

Table 2. Antibacterial activity of methanol and water extracts of black mulberry fruits and leaves against some clinic isolates.

	Inhibition Zone Diameter (mm)			
	Methanol Extracts		Water Extracts	
Bacteria	Fruit	Leaf	Fruit	Leaf
Enterobacter aerogenes	–	15	–	15
Escherichia coli	10	10	10	10
Proteus miribalis	–	–	–	–
Pseudomonas aeroginosa	–	10	–	10
Staphylococcus aureus	10	18	10	15

–: Not active

Table 3. The MIC values (mg/ml) of methanol and water extract of black mulberry fruits and leaves against some bacteria tested in the microdilution assay.

	Minimum Inhibitory Concentration (MIC) mg/Ml			
	Methanol Extracts		Water Extracts	
Bacteria	Fruit	Leaf	Fruit	Leaf
Escherichia coli	0.625	0.625	0.625	0.625
Enterobacter aerogenes	_	0,312	_	0.625
Staphylococcus aureus	0.625	0.156	1.25	0.312
Pseudomonas aeruginosa	_	0.625	_	0.625

–: extract not tested.

Kuwanon G, isolated from *Morus alba*, showed strong antibacterial activity against *Streptococcus mutans* causing dental caries and also significantly inhibited the growth of other cariogenic bacteria such as *Streptococcus sobrinus* and *Streptococcus san*guis , and *Porphyromonas gingivalis* causing periodontitis (Park *et. al*., 2003).

In this study, we found that black mulberry fruits and leaves have strong antibacterial activity against some clinical isolate pathogens. Methanol and water extracts of mulberry exhibited specific ac-

tivity against the bacteria such as *E. aerogenes, E. coli, P. aeruginosa,* and *S. aureus*. In conclusion, in Turkey, local peoples use black mulberry fruits and leaves as medicine. Our study results support this idea.

4. REFERENCES

Asono, N., Yamashita, T., Yasuda, K., Ikeda, K., Kizu, H., Kameda, Y. (2001). Polyhydroxylated alkoloids isolated from mulberry trees and silkworms. *J Agric. Food Chem*. 49, 4280–4213.

Baytop, T. (1999). *Türkiyede bitkilerle tedavi*. İstanbul Eczacılık Fakültesi Yayınları, İstanbul, pp. 444 (In Türkish).

Davis, P.H. (1982). *Flora of Turkey and the East Agean Island*. Edinburgh University Pres, Edinburgh, pp.642.

Ercişli S., Orhan E. (2007). Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. *Food Chem*. 103, 1380-1384.

Fukai, T., Kiyoshi, K., Terada S. (2005). Antimicrobial activity of 2-arylbenzofurans from *Morus* species against methicillin-resistant *Staphylococcus aureus*. *Fitoterapia*, 76, 708–711.

Gerasopoulos, D., Stavorulakis, G. (1997). Quality characteristics of four mulberry (*Morus* sp.) cultivars in the area of China. *J. Sci. Food Agric*. 73, 261–264

Hammer, K.A., Carson, C.F., Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. *J Appl. Microbiol*. 86, 985–990.

Hodges, D.M., Kalt, W. (2003). Health functionality of small fruit. *Acta Hort*. 626, 17–23.

Kalt, W., Forney, C.F., Martin, A., Prior, R.L. (1999). Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *J. Agric. Food Chem*. 47, 4638–4644.

Kim, S.Y., Park, K.J., Lee, W.C. (1998). Antiinflamatory and antioxidative effects of *Morus* spp. fruit extracts. *Korean J Med. Crop Sci*. 41, 123–128.

Kim, H.B., Bang, H.S., Lee, H.W., Seuk, Y.S., Sung, G.B. (1999). Chemical characteristics of mulberry syncarp. *Korean J Med. Crop Sci*. 47, 3206–3209.

Krisch, J., Galgoczy, L., Tölgyesi, M., Papp, T., Vagvölgyi, C. (2008). Effect of fruit juices and pomace extracts on the growth of Gram-positive and Gram-negative bacteria. *Acta Biol. Szeg*. 52, 267–270.

Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C, Yolke, R.H. (1995). *Manual Clinical Microbiology.* ASM ,Washington , DC., pp.1356.

Park, K.M., You, J.S., Lee, H.Y., Baek, N.I., Hwang, N.I. (2003). Kuwanon G: an antibacterial agent from the root bark of *Morus alba* against oral pathogens. *J. Ethnopharmacol*. 84, 181–185.

Sohn, H.Y., Son, K.H., Kwon S., Kwon, G.S., Kang, S.S. (2004). Antimicrobial and cytotoxic activity of 18 prenylated favonoids isolated from medicinal plants: *Morus alba* L., *Morus mongolica* Schneider, *Broussnetia papyrifera* (L.) Vent, *Sophora flavescens* Ait and *Echinosophora koreensis* Nakai. *Phytomedicine*. 11, 666–672.

Şengül, M., Ertugay, M.F., Şengül, M. (2005). Rheological, physical and chemical characteristics of mulberry pekmez. *Food Control* 16, 73–76.

Yiğit, N., Yiğit, D., Özgen, U., Aktaş, A. e. (2007). Anticandidal activity of black mulberry (*Morus nigra*). *Türk Mikrobiyoloji Cemiyeti Dergisi* 37, 169–173.

Zgoda, J.R., Porter, J.R. (2001). A convenient microdilition method for screening natural products against bacteria and fungi. *Pharm. Biol*. 39, 221–225.

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