

Short Communication

Antimicrobial activities of ethanol extract of black grape

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There is considerable interest in alternative/adjuvant approaches for the eradication of infections using biologically active compounds, especially antioxidants from plants. Phenolic compounds in grape products play important roles for antioxidant, anti-inflammatory, and antimicrobial effects. Grapes are rich sources of potentially bioactive polyphenols. This study was conducted to measure the antibacterial activity of ethanol extract of total grape (*Vitis vinifera* L., Vitaceae) against several pathogenic bacteria. Grape was extracted by percolation method. Aliquots of the extracts at variable concentrations were then incubated with different bacterial strains, and the antimicrobial activities of the ethanolic extracts from total grapes were determined by the minimum inhibitory concentration (MIC). Total grapes extract inhibited significantly, the growth of the tested bacterial strains. Among the bacterial strains tested, *Staphylococcus aureus* was the most susceptible. Results from these findings suggest that total grapes extract may be used as natural antibacterial for treatment of some diseases, especially local skin diseases.

Key words: Antimicrobial activity, grape, minimum inhibitory concentrations (MIC).

INTRODUCTION

In the past decade interest on the topic of antimicrobial plant extracts has been growing. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Abtahi et al., 2008).

Grape (*Vitis* spp.) is one of the most widely grown fruit crops in the world. Grape juice jams and raisins are also important commodities in the market of the whole world.

Numerous studies focused on the health-promoting and antioxidant effects of grapes. Interest in the health benefits of muscadines has increased due to their high phenolics contents. Most phenolics in muscadines are located in the seeds (Poudel et al., 2008). Gallic acid, catechin and epicatechin are the main phenolics found in muscadine seeds, while ellagic acid and myricetin are the major ones in the skins. Muscadines and Grapes (*Vitis vinifera*), well known for their high levels of antioxidants and polyphenols, have also shown promise as novel antimicrobial agents (Brown et al., 2009), anti-cancer properties (Mertens-Talcott et al., 2006), anti-inflammatory activity (Greenspan et al., 2006) and antimicrobial activity against *Escherichia coli* O157:H7 (Kim et al., 2009).

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (Duraipandiyar et al., 2006). Screening of medicinal plants for antimicrobial activities and photochemical is important for finding potential new compounds for therapeutic use. This paper

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Table 1. MIC of ethanol extracts of black grage against different bacterial strains.

Test organism MICs ($\mu\text{g/ml}$)	<i>E. coli</i>	<i>S. aureus</i>	<i>S. Typhi</i>	<i>P. aeruginosa</i>
Ethanol extract	125	32	125	250
Gentamicin	32	8	16	8
Ciprofloxacin	16	64	32	64
Chloramphenicol	512	128	128	512

reports the results of a survey that was done based on folk uses by traditional practitioners in Iran for antimicrobial activity.

Few studies have already reported the anti-*H. pylori*, *E. sakazakii* activities of grape seed including an active chemical constituent (Brown et al., 2009). However, no effort has been made to evaluate the ethanol extract grape. In this study, we examined the antimicrobial activity of ethanol extract total grape. The objectives of this study were to investigate the effects of ethanolic grape extract against different bacterial strains and the antimicrobial activities determined by minimum inhibitory concentrations (MIC).

MATERIALS AND METHODS

Fresh grapes were purchased from a local supermarket. Skins from red, white, and black table grapes were dried at 70°C for 3 days in a gravity convection oven (Fisher Scientific, Gaithersburg, MD). Total grape extract was prepared by percolation method. 50 g of dried powder was soaked in 50 ml ethanol for 2 h with intermittent shaking. It was extracted with 70% ethanol by percolation method. The extract was collected and evaporated to dryness in a rotary evaporator, and then filter and sterilized (0.2 μm ; VWR). All extracts were stored at -20°C in the dark and used within 1 week.

The antimicrobial activity ethanolic extract were individually tested against a panel of microorganisms, including *E. coli* (PTCC 1330), *Staphylococcus aureus* (PTCC 1431), *Salmonella typhi* (PTCC 1639), *Pseudomonas aeruginosa* (PTCC 1310). All microorganisms were obtained from Pasture Institution, Tehran, Iran.

The Muller – Hinton broth supplemented with magnesium and calcium cations were used for test culture. Bacteria were grown in Muller – Hinton broth (Difco) at 37°C for 18 h. The MIC were determined by a liquid micro dilution method using inoculums of 10^5 CFU/ ml in Mueller Hinton broth (Difco). Serial dilutions of the compounds analysed, previously dissolved in ethanol, analysed were prepared to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 $\mu\text{g/ml}$. To each well was added 50 μl of 18 h old inoculums. The MIC, defined as the lowest concentration of the test compounds which inhibited visible growth after 18 h, were visually determined after incubation for 18 h at 37°C.

Ciprofloxacin, Gentamicin and Chloramphenicol were used as reference compound for antibacterial activities. Antibiotics were reconstituted according to the manufacturers' directions, filtered through a sterile 0.45 mm-pore-size polysulfone membrane and used the same day. Dilution ranges for the reference method were from 1 to 512 $\mu\text{g/ml}$. The tubes containing 50 μl antibiotic dilutions were inoculated with 50 μl of a bacterial suspension in broth containing 10^5 CFU/ml. The MIC, defined as the lowest concentration of the test compounds which inhibited visible growth after 18 h, were visually determined after incubation for 18 h at

37°C.

RESULTS AND DISCUSSION

Table 1 indicated the minimum concentration of extract required to completely inhibit the growth of four bacterial strains. The relative growth of each microorganism after 72 h of incubation in the presence of different concentrations of ethanolic black grape extract was compared to the control. The MIC of the ethanol extract of *E. coli*, *S. aureus*, *S. typhi*, *P. aeruginosa* were 125, 32, 125 and 250 $\mu\text{g/ml}$, respectively.

In this study, ethanol extract from total grape extract exhibited significant inhibitory effect on bacterial growth. The most sensitive bacteria were *S. aureus* and *S. typhi*, which were inhibited. The extract of grape was effective against Gram-negative bacteria. The results are similar to those obtained from the extracts of grape seeds (Jayaprakasha et al. 2006). They reported that grape seed extracts inhibited Gram-positive bacteria, such as *S. aureus*, *B. cereus* and *B. subtilis* more easily than Gram-negative ones, such as *P. aeruginosa* and *E. coli*. It was also reported that phenolics are the most important compounds in grape extracts active against bacteria.

The active compounds present in grape had a stronger and a broader spectrum of antimicrobial activity. The antibacterial activity may be indicative of the presence of some metabolic toxins or broad-spectrum antibiotic compounds. Among those antimicrobial compounds, phenolic compounds, terpenoids, and alkaloids are very important components in antimicrobial effects (Yi et al., 2009).

The antimicrobial effects of plant extracts have been well studied (Smith et al., 2004; Cowan, 1999). These natural products are classified as generally recognized as safe (GRAS). Hence, the use of such naturally occurring plant extracts may provide a potential additional barrier to inhibit the growth of food borne pathogens in meat and poultry products. However, using high concentrations of these extracts to achieve inhibitory effects can affect the flavor of the food products. Grape seed extract is generally regarded as safe for food additive and known for its antioxidant and antimicrobial activities (Rababah et al., 2004). The partial hydrophobic nature of their phenolic compounds is responsible for the antimicrobial activity. Accumulation and attachment of these phenolics to the bacterial cytoplasmic membrane eventually lead to

cell death (Pyla et al., 2010). This study showed that bitter apricot seeds could be potential source of new anti-bacterial agents and may be used as natural antibacterial for treatment of some of diseases.

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