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Antimicrobial Activities of Water and Methanol Extracts of Bitter Apricot Seeds

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The aim of this study was to test the antimicrobial activity of the methanolic extract of bitter apricot seeds. Bitter apricot seeds used in folk medicine in the treatment of skin diseases and parasitic diseases. It been traditionally used to treat parasitic infections and skin diseases. Water and methanol extracts of bitter apricot seeds were screened against some bacterial strains. Seeds were extracted by percolation method. Aliquots of the extracts at variable concentrations were then incubated with different bacterial strains and the antimicrobial activities of the water and methanolic extracts from bitter apricot seeds were determined by MIC. Three antibiotics were used as reference compounds for antibacterial activities. Bitter apricot seeds extract inhibited significantly the growth of the tested bacterial strains. Among the bacterial strains tested, *Staphylococcus aureus* was most susceptibility. The highest antibacterial was exhibited by water extract. Results from these findings suggest that this bitter apricot seeds extract may be used as natural antibacterial for treatment of some of diseases, especially local skin diseases.

Key words: Bitter apricot seeds, antimicrobial activity, minimum inhibitory concentration (MIC)

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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 (Diallo *et al*., 1999). 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 (Cai *et al*., 2004).

Bitter apricot (*Prunes armeniuca*) seeds (kernels) are by-products of the apricot processing industry. They are used as a substitute for bitter almonds to produce persipan for the bakery industry. The oil (53% in the seed) is used, in e.g., cosmetics (Alpaslan and Hayta, 2006), as a cheaper substitute for bitter almond oil. The seeds can also be of interest as a food or feed ingredient because of their high crude protein content. It contained approx. 21% (w/w) crude protein, 52% (w/w) crude fat, 1.5% (w/w) crude fibre and 25.5% (w/w) carbohydrates based on dry matter. Bitter apricot seeds originate from the variety *Prunus armeniaca* var. *amar* (El-Badawy *et al*., 1994).

In general, bitter apricot seeds used in folk medicine in the treatment of skin diseases and parasitic diseases.

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 (Erdogrul, 2002). Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use. This study reports the results of a survey that was done based on folk uses by traditional practitioners in Iran for antimicrobial activity.

To our knowledge, antimicrobial activities of methanolic extract of bitter apricot seeds have not been reported to date. In this study, we examined the antimicrobial activity of bitter apricot seeds which have long been used as a medicinal source in Iran.

MATERIALS AND METHODS

Fresh bitter apricot seeds were collected from the Arak villages, Iran in June 2007.

Bitter apricot seeds extract was prepared by percolation method. The plant materials were dried under shade and ground into fine powder using electric blender. 50 g of dried powder was soaked in 50 mL methanol or water for 2 h with intermittent shaking.

It was extracted with 80% methanol by percolation method. The extract was collected and evaporated to dryness in a rotary evaporator. The antimicrobial activity of water and methanolic extract were individually tested against a panel of microorganisms, including *Escherichia coli* (PTCC 1330), *Staphylococcus aureus* (PTCC 1431), *Salmonella typhi* (PTCC 1639), *Salmonella para typhi* A (PTCC 1230) and *Salmonella para typhi* B (PTCC 1231) (Gram+ and Gram-). All microorganisms were obtained from Pasture Institution, Tehran, Iran. Prior to the experiment, working cultures were prepared by subculturing 100 μL of each stock culture in 9 mL of Brain Heart Infusion agar (BHI, Merck) and incubated 37°C for 24 h in order to obtain inoculate containing cultures in the exponential growth phase of approximately 1×10^{6} cfu mL^{-1}.

The Minimum Inhibitory Concentrations (MICs) of extract determined by tube broth dilution. Briefly, geometric dilutions, ranging from 2 to 512 μg mL^{-1} of the methanolic extract, were prepared in tubes, volume being 1 mL. Then 1 mL of BHI, was added onto tubes. Finally, 1 mL of 10^{6} colony forming units (cfu mL^{-1}) (according to Mc Farland turbidity standards) of standardised microorganism suspensions were inoculated onto tubes and the test was performed in a volume of 2 mL. Tubes were incubated at 37°C for 24 h. The same tests were performed simultaneously for sterility control (BHI + test extract). Gentamycin, Oxacillin 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. The MICs were considered to be the lowest antibiotic concentrations (in micrograms per milliliter) at which there was no visible growth in the wells. All tubes with no visible growth were subcultured by transferring, in duplicate, 10 μL to sheep blood agar.

Dilution ranges for the reference method were from 1 to 512 μg mL^{-1}. The tubes containing 1 mL antibiotic dilutions were inoculated with 0.1 mL of a bacterial suspension in broth containing 10^{6} cfu mL^{-1}. The inoculum was injected below the broth surface with a 1 mL pipette first into the antibiotic-free growth control and then, by using the same tip, into the tubes in sequence, starting with the tube containing the lowest concentration of antibiotic and ending with the tube containing the highest concentration of antibiotic. All tests were performed in duplicate (National Committee for Clinical Laboratory Standards, 1985).

RESULTS AND DISCUSSION

Table 1 shows the minimum concentration of extract required to completely inhibit the growth of five bacterial strains. The relative growth of each microorganism after

Table 1: MIC of against different bacterial strains

Test organism MICs ($\mu g\ mL^{-1}$)	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*	*Salmonella para typhi* A	*Salmonella para typhi* B
Water extract	64	16	32	32	64
Methanol extract	128	32	64	32	128
Gentamycin	256	4	16	32	8
Oxacillin	512	128	256	512	512
Chloramphenicol	32	256	128	256	512

72 h of incubation in the presence of different concentrations of bitter apricot seeds extract was compared to the control. The MIC of the methanol extract of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella para typhi* A and *Salmonella para typhi* B were 128, 32, 64, 32 and 128 $\mu g\ mL^{-1}$, respectively. The MIC of the water extract of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella para typhi* A and *Salmonella para typhi* B were 64, 16, 32, 32 and 64 $\mu g\ mL^{-1}$, respectively.

Water extracts exhibited a higher degree of antimicrobial activity as compared with methanol extracts.

In 1830, Robiquet and Boutron-Chalard discovered the structure of the HCN-liberating compound in bitter almonds (Lechtenberg and Nahrstedt, 1999).

Because the compound was isolated from *Prunus amygdalus* (synonym *Prunus dulcis*), it was named amygdalin. Amygdalin has subsequently been found widespread in seeds of other members of the *Rosaceae* like in apples (*Malus* spp.), peaches (*Prunus persica*), apricots (*Prunus armeniaca*), black cherries (*Prunus serotina*) and plums (*Prunus* spp.) (Franks *et al*., 2005; Conn, 1980; Frehner *et al*., 1990). The diglucoside amygdalin was the first member to be isolated of a new class of natural products now known as cyanogenic glucosides. Cyanogenic glucosides are present in more than 2,500 different plant species, including many important crop plants (Seigler and Brinker, 1993; Bak *et al*., 2006). Upon disruption of plant tissue containing cyanogenic glucosides, these are typically hydrolyzed by β-glucosidases with concomitant release of Glc, an aldehyde or ketone and HCN. This two-component system, of which each of the separate components is chemically inert, provides plants with an immediate chemical defense against attacking herbivores and pathogens (Conn, 1969; Nahrestedt, 1985; Jones, 1988; Morant *et al*., 2003; Nielsen *et al*., 2006). In addition to their possible defense function, accumulation of cyanogenic glucosides in certain angiosperm seeds may provide a storage deposit of reduced nitrogen and sugar for the developing seedlings (Lieberei *et al*., 1985; Selmar *et al*., 1988).

In this study, the water and methanol extracts from bitter apricot seeds exhibited significant inhibitory effect on bacterial growth.

The most sensitive bacterium was *Staphylococcus aureus*, which was inhibited by water extract. On the other hand extracts showed only slight activity against *Escherichia coli* and *Salmonella para typhi* B. *Staphylococcus aureus* have a single layer wall as compared to *Escherichia coli*, *Salmonella typhi*, *Salmonella para typhi* A and *Salmonella para typhi* B which have a multi-layered structure.

All the same, given that water extract was effective then methanol extract. Extract had lower antibacterial activity than gentamycin.

The active compounds present in bitter apricot seeds 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 (Femenia *et al*., 1995; Brewer *et al*., 1994).

This study showed that bitter apricot seeds could be potential source of new antibacterial agents and may be used as natural antibacterial for treatment of some of diseases, especially local skin diseases.

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