Antimicrobial activity of methanol extract of Opuntia stricta F.

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ABSTRACT: Over the past century, many attempts have been made to treat various diseases especially infectious diseases. Today, antimicrobial resistance has become a major global problem which is caused by the indiscriminate use of antimicrobial drugs. In a way, the use of medicinal plants, which provide a rich source of novel antimicrobial agents in human history because many infectious diseases have traditionally been treated with herbal medicines. In many developing countries, herbal medicine is one of the primary health care systems as well. In this regard, the antimicrobial effects of methanol extracts of fruit of Opuntia stricta were investigated. Fruit methanol extract prepared by maceration method and its inhibitory effect on four bacteria Staphylococcus aureus, Gram-negative Escherichia coli (two strains) and one fungi named Candida albicans were investigated. MIC of the extract was determined as well. Results showed that the methanol extract of fruits of Opuntia stricta have antibacterial effects against tested microorganisms. MIC values about Staphylococcus aureus (PTCC 1764), Escherichia coli (PTCC 1270), Escherichia coli (PTCC 1330) and Candida albicans (PTCC 5027) were 10, 40, 20 and 40 mg/ml respectively. The present study reveals the antimicrobial activities of fruit of Opuntia stricta.

INTRODUCTION

Opuntia stricta belongs to the Cactaceae family that can grow up to 2 meters in height and produce lemon yellow flowers in the spring and summer, followed by purplish-red fruits (Abd El-Razek & Hassan, 2011). Its habitats are rocky slopes, river banks and urban areas and is drought resistant because of its succulent nature, lack of leaves and thick, tough skins (Jana, 2012). These features result in plants that use the majority of its internal tissues for water storage and its outer parts to reduce water loss and damage by grazing and browsing animals (Nobel, 1980). It can remain vigorous in hot, dry conditions that cause most other plants to lose vigor or even die (Obon, Castellar, Alacid, & Fernández-López, 2009). Common names of Opuntia stricta include Erect Prickly Pear, prickly cactus pear, Haw and Nepal Estricto (Esquivel, Moreno, Álvarez, Álvarez, & Giusti, 2011). The term ‘prickly pear’ also relates to the fruit, which is often spiny and pear-shaped. Plants are normally leafless succulent shrubs. Stems are divided into segments (pads or joints) that are flat and often incorrectly called leaves (Fig 1).

It has been introduced to many parts of the world, including Africa, Southern Europe and southern Asia (Reyes-Aguero, Aguirre, & Valiente-Banuet, 2006). It has a wide distribution in Australia. In Australia it has been the subject of one of the first really effective biological control exercises using the moth Cactoblastiscactorum (Hosking, McFadyen, & Murray, 1988). The high sugar and acid content gives cactus fruit a sweet acidic taste. It is also put to different traditional and industrial uses (Sáenz, 2000).

The use of medicinal plants is as old as human life. Opuntia stricta has been used in traditional folk medicine because of its role in treating a number of diseases and conditions, including anti-inflammatory effects (Park, Kahng, Lee, & Shin, 2001), hypoglycemic properties (Frati, Jiménez, & Ariza, 1990), inhibition of peptic ulceration (Galatí et al., 2003), neuroprotective effects (Dok-Go et al., 2003), antioxidant actions and also used for treating burns and asthma (Kim et al., 2006). In the present investigation, the methanol extracts of fruit of Opuntia stricta were investigated through in vitro studies.
Figure 1. Stem and prickly pearfruits of Opuntia stricta

**MATERIAL AND METHOD**

**Plant material and extraction procedure**

The fresh cactus purple oval fruits of Opuntia stricta (500 g) were collected from greenhouse of Kerman in March of 2012. The samples were crushed and poured by an electric mixer and were extracted with methanol by maceration techniques for 7 days in shaking condition at room temperature. The obtained extract was filtered by Whatman paper No. 1. Alcohol was then totally evaporated by the Rotary evaporator (Heidolph, Germany) and concentrated extract was obtained (GH Shahidi Bonjar, 2004).

**Microorganisms tested**

Microorganisms studied in this project are provided from Persian Type Culture Collection Institute, Tehran, Iran and include Staphylococcus aureus (PTCC 1764), Escherichia coli (PTCC 1270), Escherichia coli (PTCC 1330) and Candida albicans (PTCC 5027).

**Antimicrobial activity**

The Muller-Hinton agar (Merck Company) medium was prepared, sterilized and poured into petri dishes kept on a level surface. The depth of the medium was approximately 4 mm. After the medium got solidified, the plates were allowed to dry for one hour by placing them in an incubator about 35°C to 37°C. The bacterial/yeast suspension equal 1.5×10⁸ cells/ml in sterile normal saline (adjusted to 0.5 McFarland standards) was prepared as described by Nalubega et al. and inoculated on Muller-Hinton agar Medium (Merck Company) by sterile cotton swabs (Nalubega, Kabasa, Oilla.D, & Kateregga, 2011). Different concentrations of crude extract in solvent DMSO: Methanol 1:1 V/V (Merck Germany) prepared (Eloff 1998). The first concentration used was 80 mg/ml (GH Shahidi Bonjar & Kariminik, 2004). In the present study well diffusion agar assay was used to evaluate the antimicrobial activities. Wells in 6 mm diameter were punctured in the media using sterile cork borers. These wells were placed in petri dishes allowing a distance of 2 to 4 cm between each well, and filled with 20 μl of the extract. The plates were then incubated at 37°C for 24 hours. Following incubation, bioactivity was determined by measuring the inhibition zones around the crude extract in mm (Shakibaa, Kariminik, & Parsia, 2011). All tests were done in triplicate. DMSO: Methanol (1:1 V/V) solvent is considered as negative control.

**Determination of Minimum inhibitory concentration (MIC)**

To determine Minimum Inhibitory Concentration (MIC), two fold dilution series (80, 40, 20, 10, 5, 2.5 and 1.25 mg/ml) in the solvent of DMSO: Methanol 1:1 V/V were prepared and bio assayed in well diffusion agar assay as mentioned above (GH Shahidi Bonjar et al., 2003).

**RESULTS**

Methanol extract of Opuntia stricta showed antimicrobial activity against all microorganism tested in 80 mg/ml concentration. Minimum Inhibitory Concentration (MIC) was determined. MIC values about Staphylococcus aureus (PTCC 1764), Escherichia coli (PTCC 1270), Escherichia coli (PTCC 1330) and Candida albicans (PTCC 5027) were 10, 40, 20 and 40 mg/ml respectively (Fig 2).
DISCUSSION

Nowadays, the antibiotic resistant microorganisms can cause the most important infectious disease such as nosocomial infections (Abouhosseini et al., 2012). Development of antibiotic resistance pathogens demands new strategies and the native peoples ethno pharmacological knowledge that has received less importance are valuable sources which should be applied to advance health oriented aims (Alzoreky & Nakahara, 2003). To resolve these problem, many investigators noticed the effect of medicinal plants and herbal extracts or essential oil instead of antibiotics (Dastagir, Hussain, & Khan, 2012). Literature Studies showed that there is not enough information in the field of antimicrobial activity of cactusplant. Antimicrobial activity of cactus Opuntia stricta extracts against both gram-positive and gram-negative bacteria and Candida albicans was investigated. It was clarified that Opuntia stricta extract is an effective extract with antibacterial and anti-fungal activity. The most sensitive microorganism was Staphylococcus aureus (PTCC 1764). The findings of present study may form the basis for further investigation to isolate active compounds, elucidate the structure and evaluate it against wide range of antibiotic resistant bacteria with the subject to find new therapeutic principles. Many reports have been showed the effectiveness of traditional herbs against microorganisms (Wei, Musa, Wee, Musa, & Tse Seng, 2007), (Shakeri, Hazeri, Vlizadeh, Ghasemi, & Zaker Tavallaei, 2012).

Shahidi et al., antibacterial and antifungal activity of methanol plant-extracts of 221 species from 98 families which had documented uses in Iranian herbal-medicine were screened for against 11 standard bacterial strains and 3 fungal species at 20 mg/ml. Eighty one samples in 39 families showed antibacterial and/or antifungal activity against at least on one of the tested microorganism (GH Shahidi Bonjar, Aghighi, & Karimini, 2004).

Adomi et al., the antibacterial activity of aqueous and ethanol extracts of the root bark of two plants Alstonia booneiDe wild and Morindalucida against seven bacteria and showed different bioactive components are present in each species (Adomi & Umukoro, 2010). Recent investigations showed that the effectiveness of polysaccharides derived from Opuntia spp. as well as taurine against H2O2-induced damage, free radical scavenging, anti-diabetic, and blood lipid-lowering effects (Huang, Li, Guo, & Yan, 2008). Cactus fruit is regarded as a valuable food that is consumed as fresh fruit, as a vegetable in salads and sauces, as a main ingredient in desserts and an appetizer (Joubert, 1993). It is also processed on a small scale in the food industries as a jelly and jam. Moreover, cactus pear fruit contains betalain pigments which have good potential for use as natural food colorants (Jana, 2012).

CONCLUSION

Thus based on our results that showed antimicrobial properties of Cactus fruit, further studies are suggested to determine which of chemical constituent of this extract carry the best antimicrobial activity and
also we recommended in vivo investigation of the same substance in animal models infected with pathogenic microorganisms. As a result, plants are one of the bedrocks for modern chemotherapy.

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