

Antibacterial activity of methanol extracts of 4 plants used in traditional herbal medicine of Kerman, Iran

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ABSTRACT: The present study describes the anti-bacterial activity of methanol extract of *Opuntia stricta* F., *Trachyspermum ammi* S., *Terminalia chebula* F. and *T. citrina* F. against bacteria include *Staphylococcus aureus* (PTCC 1431), *Bacillus cereus* (PTCC 1015), *Escherichia coli* (PTCC 1338), and *Klebsiella pneumoniae* (PTCC 1290). For this purpose extraction was done by maceration method and tested by agar well diffusion method. As a result of this study it was found that all of extracts generally revealed anti-bacterial activity against both gram-positive bacteria and gram-negative bacteria. *Staphylococcus aureus* was the most active bacteria. The MIC value of 4 plant extract *Opuntia stricta* F., *Trachyspermum ammi* S., *Terminalia chebula* F. and *T. citrina* F. against *Staphylococcus aureus* was 1.25, 2.5, 2.5 and 10 mg/ml respectively.

INTRODUCTION

Resistance to antimicrobial agents has become a progressively significant and pressing global problem. Based on World Health Organization, about 80% of world population rely essentially on plant traditional medicine for their primary healthcare need (World Health Organization, 2002). Traditional healing system around the world that utilizes herbal therapies are an important reserve for the discovery of modern remedies (Koehn & Carter, 2005). *Trachyspermum ammi* is a native plant of Egypt and is cultivated in Iran, India, Iraq, Afghanistan and Pakistan (Bairwa, Sodha, & Rajawat, 2012). *Trachyspermum ammi* belongs to Apiaceae family (Singh & Singh, 2000). English and Persian names are Bishop's weed and Zenian respectively. It is widely grown in arid and semi-arid regions with soils contain high levels of salts (Joshi, 2000). *Trachyspermum ammi* with its characteristic aromatic smell and pungent taste is widely used as a spice in curries. Its seeds are used in small quantities for flavoring numerous foods, as preservatives, in medicine and for the manufacture of essential oil in perfumery (Pruthi, 1992). The seeds contain 2–4.4% oil known as Ajwain. The essential component of this oil is thymol, which is used in the treatment of gastro-intestinal complaints, bronchial infections, antioxidant (Umar et al., 2012) and its seeds acts as appetizer, anthelmintic, carminative, laxative, and stomachic (Krishnamoorthy & Madalageri, 1999). The seed extract of *Trachyspermum ammi* has showed the maximum degradation of aflatoxin G1. (Velazhahan et al., 2010). *Terminalia citrina* and *Terminalia chebula* are belong to the family of Combretaceae and commonly known as highly medicinal plant which are distributed in Iran, India and Car Nicobar Island, Myanmar, Bangladesh, Egypt, Turkey and China. English and Persian names of *Terminalia citrina* or *Terminalia multiflora* is Yellow Myrobalan and Helileh Zard respectively. English and Persian names of *Terminalia chebula* are Black myrobalan and Helileh Siah respectively. The fruits of the trees possess diverse health benefits and has been used as traditional medicine for household remedy against numerous human disorders since ancient times. These are used in traditional medicine due to the wide spectrum of pharmacological activities such as the treatment of number of diseases like cancer, paralysis, cardiovascular diseases, ulcers, leprosy, arthritis, gout and epilepsy (Choudhary, 2011). It has been reported as antioxidant (Suchalatha & Devi 2009), anti-diabetic (Rao & Nammi, 2006) and (G. Kumar, Arulselvan, Kumar, & Subramanian, 2006), antibacterial and irregular fevers (Kannan, Ramadevi, & Waheeta, 2009), antiviral (T. Kim et al., 2001), antifungal, anti-cancerous, anti-mutagenic, digestive diseases and wound healing (Surya Prakash, Sree Satya, Avanigadda, & Vangalapati, 2012). The *Opuntia stricta* is a cactus belongs to Cactaceae family and grows mainly in arid and semiarid zones (Hosking, McFadyen, & Murray, 1988). Erect Prickly Pear, prickly pear or tuna, is an oval, elongated berry of 67-216 g weight and have about 85% water, 15% sugar, 0.3% ash and

less than 1% protein (Mohamed-Yasseen, Barringer, & Splittstoesser, 1996). The thick pericarp is covered with small-barbed spines hosting a juicy pulp with 150-300 nonedible seeds. It is used as anti-diabetic agent (Frati, Jiménez, & Ariza, 1990), anti-inflammatory (Park, Kahng, Lee, & Shin, 2001), inhibition of peptic ulceration (Galati et al., 2003), antioxidant actions and also used for treating burns and asthma (J. Kim et al., 2006).

MATERIAL AND METHODS

The plants tested in this study were *Opuntias stricta* F., *Trachyspermum ammii* S., *Terminalia chebula* and *T. citrina* that collected locally and were identified by the department of Agriculture, Shahid Bahonar University, Kerman, Iran.

Preparation and formulation of extract

The collected plants were dried in shade at room temperature and then milled to fine powder and extracted with methanol (Merck, Germany) for 7 days at agitation conditions in a separating funnel. The solvent were removed in rotary evaporator (Heidolph, Germany) in 42° C and the crude extracts were stored at 4° C for anti-bacterial analysis. The different amount of crude extract was suspended in dimethylsulfoxide and methanol 1:1 v/v to get different concentration of suspension.

Bacteria tested

The Bacteria used in this study were provided from Iranian Research Organization for Science and Technology (IROST) which include *Staphylococcus aureus* (PTCC 1431), *Bacillus cereus* (PTCC 1015), *Escherichia coli* (PTCC 1338) and *Klebsiella pneumonia* (PTCC 1290).

Antibacterial activity

The anti-bacterial activity of the methanol extracts was carried by well diffusion method. A suspension of tested bacteria equal turbidity of 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL of bacterial suspension) was spread on Muller-Hinton Agar (Merck, Germany) medium (Shakibaa, Kariminik, & Parsia, 2011). The wells with 6mm in diameter were punctured in the media using sterile cork borers and individually impregnated with different concentration of extracts. The plates were subsequently incubated at 37° C for 24 hours. Following incubation the growth inhibition were quantified by measuring the diameter of the zone of inhibition in mm. All the tests were performed in triplicate. Dimethyl sulfoxide and methanol 1:1 v/v served as negative control (GH Shahidi Bonjar et al., 2003).

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 arranged and evaluated in well diffusion agar assay as mentioned above. The Minimum Inhibitory Concentration (MIC) of the crude extracts on all bacteria were determined (GH Shahidi Bonjar & Kariminik, 2004).

RESULTS

In vitro preliminary screening of the antibacterial activity of the plant extracts were studied against some bacteria using the well diffusion method. The extract of *Opuntias stricta* F. at the concentration of 40 mg/ml had antibacterial activity on the tested bacteria from high to low respectively. *Staphylococcus aureus* PTCC 1431: 18mm, *Bacillus cereus* PTCC 1015: 15 mm, *Escherichia coli* PTCC 1338: 10 mm, and *Klebsiella pneumonia* PTCC 1290: 16 mm. The extract of *T. chebula* at the concentration of 40 mg/ml had antibacterial activity on the tested bacteria from high to low respectively. *Staphylococcus aureus* PTCC 1431: 22 mm, *Bacillus cereus* PTCC 1015: 16mm, *Escherichia coli* PTCC 1338: 10 mm, and *Klebsiella pneumonia* PTCC 1290: 10mm. The extract of *T. citrina* at the concentration of 40 mg/ml had antibacterial activity on the tested bacteria from high to low respectively. *Staphylococcus aureus* PTCC 1431: 25 mm, *Bacillus cereus* PTCC 1015: 20 mm, *Escherichia coli* PTCC 1338: 22 mm, and *Klebsiella pneumonia* PTCC 1290: 12 mm. The extract of *Trachyspermum ammii* at the concentration of 40 mg/ml had antibacterial activity on the tested bacteria from high to low respectively. *Staphylococcus aureus* PTCC 1431: 20mm, *Bacillus cereus* PTCC 1015: 20 mm, *Escherichia coli* PTCC 1338: 17 mm and *Klebsiella pneumonia* PTCC 1290: 13 mm. Minimum Inhibitory Concentration (MIC) was Determined. MIC values of *Opuntias stricta* F. on *Staphylococcus aureus* PTCC 1431, *Bacillus cereus* PTCC 1015, *Escherichia coli* PTCC 1338, and *Klebsiella pneumonia* PTCC 1290 were 10, 10, 40 and 20 mg/ml respectively. MIC values of *T. chebula* on *Staphylococcus aureus* PTCC 1431, *Bacillus cereus* PTCC 1015, *Escherichia coli* PTCC 1338, and *Klebsiella pneumonia* PTCC 1290 were 2.5, 5, 40 and 40 mg/ml respectively. MIC values of *T. citrina* on *Staphylococcus aureus* PTCC 1431, *Bacillus cereus* PTCC

1015, *Escherichia coli* PTCC 1338, and *Klebsiella pneumonia* PTCC 1290 were 1.25, 5, 20 and 20 mg/ml respectively. MIC values of *Trachyspermum ammion* *Staphylococcus aureus* PTCC 1431, *Bacillus cereus* PTCC 1015, *Escherichia coli* PTCC 1338, and *Klebsiella pneumonia* PTCC 1290 were 2.5, 5, 10 and 5 mg/ml respectively.

DISCUSSION

The data indicated that *Staphylococcus aureus* PTCC 1431 was the most sensitive strain of those tested with all of the extracts. The extract concentration of 40 mg/ml also exhibit high antimicrobial activity against all bacteria tested. Many investigations showed different activities of used plants tested in this study. Kumaret al, studied antimicrobial activity of aqueous extracts from *T. chebula* against some microorganisms using the filter paper disc diffusion method and compare to some antibiotics and found that the extract had better activity than tobramycin and cephalixin (M. Kumar, Agarwal, Dey S, Rai, & Johnson, 2009). Malekzadeh et al, studied antibacterial activity of black myrobalan (*Terminalia chebula* Retz) against *Helicobacter pylori* (Malekzadeh, Ehsanifar, Shahamat, Levin, & Colwell, 2001). Studies of Bairwa et al revealed that *Trachyspermum ammi* is a source of medicinally active compounds and have various pharmacological effects (Bairwa et al., 2012). Rajput et al, suggested that the methanol extract of *Trachyspermum ammi* prolonged PT, similar to that of Warfarin suggesting its possible effects on the extrinsic pathway, while a PTT was not altered suggesting that it may have no effects on the intrinsic pathway (Rajput, Khan, Qazi, & Feroz, 2012). Current surveys showed the effectiveness of polysaccharides derived from *Opuntia* spp. in addition to taurine against H₂O₂-induced damage, free radical-scavenging, anti-diabetic, and blood lipid-lowering effects (Huang, Li, Guo, & Yan, 2008).

CONCLUSION

In conclusion it may be said that plant extracts can be used as bactericide against gram positive and gram negative bacteria. However, further studies on the extract are needed to pinpoint the finding. This report may serve as a footstep on this aspect.

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