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Chemical composition and antimicrobial activity of *Opuntia stricta* F. essential oil

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Abstract

Due to biological activities of *Opuntia* sp. and use of this plant in traditional medicine, chemical composition and antimicrobial activity of the essential oil of *Opuntia stricta* F. were studied. The essential oil of the plant was extracted using hydrodistillation method and analyzed by GC and GC/MS. Nineteen compounds were identified, with thymol (42.7%) as the dominant component. The antimicrobial activity of the oil was evaluated using disc diffusion method against standard strains of *Bacillus cereus*, *Bacillus licheniformis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. All of the microorganisms were sensitive in 20 mg/ml concentration of the oil. MIC values about *B. cereus*, *B. licheniformis*, *E. coli*, *P. aeruginosa* and *C. albicans* were 1.25, 1.25, 5, 20 and 2.5 mg/ml, respectively. It could be concluded that *Opuntia stricta* has a potent antimicrobial activity and its effect may be attributed to high content of thymol which was proved in this study. Consequently, the essential oil of the plant can introduce to develop new drug candidate for antimicrobial therapy and food preservative as well.

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Introduction

Plant oils and extracts have been used for a wide variety of purposes from many years ago (Hayek and Ibrahim 2012). In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many processed food preservation, pharmaceuticals applications, and natural therapies (Saenz, 2002). *Opuntia sp.* belongs to the Cactaceae family (Dib *et al.*, 2013). The most common species of *Opuntia* are *O. humifusa*, *O. stricta*, *O. cubensis*, *O. humifusa*, *O. pusilla,* and *O. triacantha* (Benson and Walkington, 1965; Griffith, 2004). *Opuntia stricta* can grow up to 2 meters in height and produce lemon yellow flowers followed by purplish-red fruits (Abd El-Razek and Hassan, 2011). It is drought resistant because of its succulent nature, lack of leaves and thick, succulent shrubs (Jana, 2012). It uses the majority of its internal tissues for water storage (Nobel, 1980; Obon *et al.*, 2009). Common terms of *Opuntia stricta* are Erect Prickly Pear, prickly cactus pear, Haw and Nepal Estricto (Esquivel *et al.*, 2011). The term 'prickly pear' also relates to the fruits which are often spiny and pear-shaped. Stems are divided into segments (pads or joints) that are flat and often incorrectly called leaves (Fig. 1).

Fig 1. Stem and fruits of *Opuntia stricta*

Opuntia stricta has been introduced to many parts of the world, including Africa, Southern Europe, Australia and Southern Asia (Reyes-Agueroa *et al.*, 2006; Hosking *et al.*, 1988). In Iran, *Opuntia sp.* are grown in green houses. The high sugar and acid content gives cactus fruit a sweet acidic taste (Galati *et al.*, 2003). It has been used in traditional folk medicine and industrial uses (Sáenz, 2000) including anti-inflammatory effects (Park *et al.*, 2001), hypoglycemic properties (Frati *et al.*, 1990; Hassan Abd El-Razek and Hassan, 2011) anti-hyperglycemia and regulator of blood cholesterol (Tesoriere *et al.*, 2004), control of peptic ulceration (Galati *et al.*, 2002), neuroprotective and calming the nervous system effects (Dok-Go *et al.*, 2003), antioxidant activities and also used to treatment of burns and asthma (Kim *et al.,* 2006). Cactus pear fruit, generally consumed fresh or in processed form such as drinks (Joubert, 1993), syrups, candies, jellies, barbecue sauces (Mohamed-Yasseen *et al.*, 1996), natural sweeteners (Moßhammer *et al.*, 2006) and nectars (Kuti, 2004). The aim of this study was chemical components determination and *In vitro* evaluation of the antimicrobial activity of prickly cactus pear fruit oil.

Materials and methods

Plant material and isolation of the oil

The fresh cactus pear fruit of *Opuntia stricta* (500 g) were obtained from local green house in March 2012 in Keman, Iran. Three hundred grams of powdered dried fruit were hydrodistilled in a Clevenger apparatus for 3 hours to obtained essential oil (Scheffer, 1997). The oil was dried over anhydrous sodium sulfate and stored in a tightly closed dark vial at 4˚C until analyses.

Tested microorganisms

Microorganisms were as follows: *Bacillus cereus* (PTCC 1015), *Bacillus licheniformis* (PTCC 1525), *Escherichia coli* (PTCC 1339), *Pseudomonas aeruginosa* (PTCC 1074), and *Candida albicans* (PTCC 5027).These cultures supplied by technological and scientific research center in Tehran, Iran.

GC and GC/MS analysis

GC analysis of the essential oil was performed by a Hewlett-Packard 6890 instrument coupled to a flame ionization detector (FID). Compounds were separated on a HP-5 capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Helium was used as the carrier

gas at a constant flow of 1 mL/min. The column temperature was kept at 60˚C for 3 min and programmed to 220˚C at a rate of 5˚C/min. Injector and detector temperatures were kept at 250˚C and 270˚C, respectively. A mixture of aliphatic hydrocarbons (C_6–C_{23}) in hexane was directly injected into the GC injector under the above temperature program in order to calculate the retention indices of each compound. GC/MS analysis was performed using an Agilent 5975C mass spectrometer coupled to an Agilent 7890A gas chromatograph equipped with a HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). The carrier gas was helium, and the chromatographic conditions were as above. Spectrometer was scanned over the 40-400 amu range with an ionization voltage of 70 eV and an ionization current of 150 µA.

Identification of components

Identification of compounds was made by comparison of their retention indices with those of pure components, matching mass spectral data with those from the Wiley and NIST libraries or with the published mass spectra (Adams, 2004; Massada, 1976). The percentage composition of the individual components was computed from the GC-FID peak areas without the use of correction factors.

Antimicrobial investigation

Antimicrobial activity of the essential oil was accessed in presence of different concentrations. For this purpose, obtained essential oil was diluted by using serial dilution method with dimethyl sulfoxide/methanol (1:1 v/v) solvent (Shahidi-Bonjar *et al*., 2003). In this study, the antimicrobial activity was investigated using agar disc diffusion method (Klančnik *et al*., 2010). The bacteria/yeast suspension equal 1.5×10^8 cells/ml in sterile normal saline (adjusted to 0.5 McFarland standard) was prepared and inoculated on Muller-Hinton agar medium (Merck Company) by sterile cotton swab (Nalubega *et al*., 2011).

Every essential oil was assayed for antimicrobial activity in triplicate. Each microbial inoculum was spread evenly on to the surface of Muller-Hinton agar (Merck Company) plate with sterile swab and strilled blank discs were positioned in the center of inoculated agar plate. 0.2 ml of essential oil was coated on filter paper discs with 6 mm in size (Klančnik *et al*., 2010). Each essential oil was assayed in triplicate. Dimethyl sulfoxide/methanol (1:1 v/v) was used as negative control (Shahidi Bonjar *et al*., 2004), while broad-spectrum antibiotics such as tetracycline for bacteria and clotrimazole were used as positive control for obtaining comparative results. All plates were incubated for 24 h at 37˚C. Following incubation, antimicrobial activity was determined by measuring the inhibition zones around discs in mm.

Determination of MIC

To determine Minimum Inhibitory Concentration (MIC), Two fold dilution series (40, 20, 10, 5, 2.5 and 1.25 mg/ml) of essential oil of cactus pear fruit in the solvent of DMSO/methanol (1:1 v/v) were prepared and bio assayed in disc diffusion assay as mentioned above (Shakibaa *et al*., 2011) .

Results

The results of the chemical analysis of the essential oil have been presented in Table 1, in which the percentage and retention indices (RI) of the components are given. The yield of *Opuntia stricta* oil was 1% (w/w). Nineteen compounds were identified in the essential oil of the plant, representing 98.7 % of the total oil. The main components were thymol (42.7%) and *n*-octane (18.6%).

Table 1. Identified compounds in the essential oil of *Opuntia stricta*

Compound	RI	Percent (%)	Compound	RI	Percent (%)
3-Methyl heptane	762	1.4	γ-Cadinene	1511	1.4
1-Ethyl-3-methyl cyclopentane	785	0.7	δ-Cadinene	1521	1.3
n-Octane	798	18.6	Caryophyllene oxide	1581	3.8
n-Decane	997	1.4	*epi*-α-Cadinol	1639	4.8
Thymol, methyl ether	1232	0.9	α-Cadinol	1653	0.9
Carvacrol, methyl ether	1242	0.7	Dibutyl phthalate	1868	1.0
Thymol	1291	42.7	*n*-Nonadecane	1900	0.8
β-Caryophyllene	1416	9.2	Plamitic acid	1968	4.3
Geranyl propanoate	1472	1.1	*n*-Eicosane	1998	1.6
Germacrene D	1480	2.1	Total percentage	_	98.7

Antimicrobial activities findings of the essential oil of *Opuntia stricta* have presented in Table 2. The oil tested in the disc-diffusion method showed antimicrobial activity in concentration of 40 and 20 mg/ml. The essential oil inhibited Gram-positive bacteria and *Candida albicans* in very small concentrations with MIC value 1.25 mg/ml and 2.5 mg/ml, respectively. Two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* were inhibited by the concentration of the oil 5 mg/ml and 20 mg/ml as MIC value, respectively.

Table 2. Dose response and MIC value of the essential oil of *Opuntia stricta* on 5 microorganisms, +: effectiveness of oil

Oil c (mg/ml) / Microorganism	40	20	10	5	2.5	1.25	Negative control	Positive control
B. cereus	+	+	+	+	+	+	_	+
B. licheniformis	+	+	+	+	+	+	_	+
E. coli	+	+	+	+	_	_	_	+
P. aeruginosa	+	+	_	_	_	_	_	+
C. albicans	+	+	+	+	+	_	_	+

Discussion

Historically, many plant oils and extracts have been used as topical antiseptics, or have been reported to have antimicrobial properties (Alzoreky and Nakahara, 2003). It is important to examine scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Prabuseenivasan *et al*., 2006). Based on GC/MS analysis, the dominant compound in the essential oil of *Opuntia stricta* was thymol. Thymol (isopropyl-*m*-cresol) is only slightly soluble in water at neutral pH, but it is extremely soluble in alcohols and other organic solvents. It is also soluble in strongly alkaline aqueous solutions due to deprotonation of the phenol. Thymol has antimicrobial activity because of its phenolic structure, and has shown antibacterial activity against bacterial strains including *Aeromoans hydrophila* and *Staphylococcus aureus* (Dorman and Deans, 2000). This antibacterial activity is caused by inhibiting growth and lactate production, and by decreasing cellular glucose uptake (Evans and Martin, 2000). It is also used as a preservative, and as active antiseptic ingredient in some toothpastes when used to reduce plaque and gingivitis. Thymol has been found to be more effective when used in combination with chlorhexidine than used purely by itself (Filoche *et al*., 2005). Derivatives of thymol and carvacrol with increased antimicrobial activities have been developed (Mathela *et al*., 2010). The *In vitro*-obtained results from another study suggested that thymol may be effectively used as an alternative preservative to increase the lag time as well as to decrease the maximum cell load reached in the

stationary phase of growth cycle for some bacteria (Falcone *et al*., 2007). Antimicrobial properties of different medicinal and traditional plants have been described worldwide by many investigators (El Abed *et al*., 2014; Abouhosseini Tabari *et al*., 2012; Vatľák *et al.,* 2014). A variety of essential oils such as *Cinnamomum zeylancium*, *Thymus broussonetii*, *Rosmarinus officinalis*, *Origanum vulgare*, *Syzygium aromaticum*, *Artemisa arbrescens*, *Carum carvic*, *Cymbopogon citratus* and *Salvia officinalis* have been screened for their antimicrobial activity (Akthar *et al*., 2014). In this study, essential oil obtained from fruit of *Opuntia stricta* showed antimicrobial activity. Totally, all of the tested microorganisms were susceptible to the essential oil. Gram-positive bacteria were more sensitive than gram-negative ones due to the differences in their cell wall structure. Gram-negative organisms are considered to be more resistant due to their outer membrane proteins acting as a barrier to many environmental substances, including antimicrobial agents (Vukovic *et al*., 2007). In similar study, antimicrobial activity of methanol fruit extract of *Opuntia stricta* was investigated and the findings showed that the methanol extract of fruits of the plant had antimicrobial effects against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Shafiei *et al*., 2013). In another investigation, antibacterial activity of methanol extract of *Opuntia stricta, Trachyspermum ammi, Terminalia chebula*, and *Terminalia citrina* against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Klebsiella pneumonia* have been studied by agar well diffusion method as well and the MIC value of methanol extract *of Opuntia stricta* against *Staphylococcus aureus* was 1.25 mg/ml (Salehi *et al*., 2013).

Conclusion

Given the results in this study indicated that *Opuntia stricta* possess good antimicrobial activity against tested microorganisms. Further studies are needed to evaluate the *In vivo* potential of the oil in animal models. Identification of thymol as the dominant compound in this essential oil will help to develop new drug candidates for antimicrobial therapy and food preservatives.

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