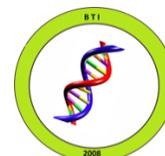




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Research article

ANTIBACTERIAL ACTIVITY OF *OPUNTIA FICUS INDICA* SKIN FRUIT EXTRACTS

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ABSTRACT

Antimicrobial resistance is one of the most serious health problems in the twenty first century. To overcome such problems, plants with novel antimicrobial action play an important role for treatment and prevention of various microbial diseases. Fresh skin fruits of *Opuntia ficus indica* were collected; processed and bioactive constituents were extracted using ethanol, methanol and chloroform solvents (1:4 w/v). Antibacterial potential of the extracts against *Escherichia coli* (ATCC2592), *Streptococcus pneumoniae* (ATCC63), *Salmonella typhi* (B2836) and *Bacillus subtilis* (S456) were evaluated using agar well diffusion method. The skin fruit extracts of *Opuntia ficus indica* demonstrated significant ($P < 0.01$) antibacterial activity against the four bacterial isolates with growth inhibition (9.40 mm - 23.52 mm). Their inhibitory activity against *S. typhi* (S456), *B. subtilis* and *S. pneumoniae* (ATCC63) were significantly ($P = 0.00-0.1$) greater than the tetracycline (10 μ g/disc) and vancomycin (10 μ g/disc). The antibacterial activity against the test Gram positive bacteria were greater than the test Gram negative bacteria. Moreover, the antibacterial activity against *S. typhi* (S456), *B. subtilis* (B2836) and *S. pneumoniae* (ATCC63) were greater than the presently used antibiotics (Tetracycline and Vancomycin). The skin fruit extracts of *Opuntia ficus indica* possess effective antibacterial constituents against multi-drug resistant bacteria and could be used for prevention and treatment of different bacterial diseases.

Keywords: *Opuntia ficus indica*, antibacterial activity, skin fruit.

INTRODUCTION

Antimicrobial resistance is one of the most serious health problems in the twenty first century that causing various infectious

diseases. This antimicrobial resistance has been caused by indiscriminate use of antimicrobial drugs for treatment of different microbial diseases as well as due to the favor of the antimicrobial drugs for the

survival and spread of microorganisms (Somaie *et al.*, 2013). To conquer such problems, nowadays investigators have begun to assess the therapeutic value of plants beyond their basic nutritional benefits (Puangpronpitag and Sittiwet, 2009). Traditionally, plants have been used in different countries for the treatment of different diseases due to belief that green medicine is safe, easily accessible and with fewer side effects (Burda and Oleszek, 2001; Gebreselema *et al.*, 2013).

Opuntia ficus indica that belongs to *Cactaceae* family is one of the extremely drought resistant plants that grow wild in arid and semi-arid regions. Like other flowering plants, *Opuntia ficus indica* has cladodes, stem and pear. *Opuntia ficus indica* is originated from central and southern Mexico. This drought resistant plant was introduced to Ethiopia at the end of 19th century and widely distributed in the northern arid and semi arid regions of the country. In Ethiopia, particularly in Tigray region, the prickly pears of *Opuntia ficus indica* serve as a source of food for human and feed for livestock (Mulugeta *et al.*, 2010).

It was reported that cactus pears are used in folk medicine to treat various diseases like diabetes mellitus, cancer, allergies, high blood glucose levels, obesity and gastrointestinal disorders (Jean *et al.*, 2006). Also, studies have shown that the fruit skin extract of the cactus plant have analgesic, anti-inflammatory, anti-microbial, and anti-oxidant actions (Monia *et al.*, 2014).

Despite wide spreading of the cactus plant throughout the northern part of

Ethiopia, its medicinal value is still hardly recognized due to insufficient scientific investigations. Therefore, this study was intended to determine the presence of different bioactive constituents in the skin fruits of *Opuntia ficus indica* and to evaluate the antibacterial potential of the skin fruit extracts of *Opuntia ficus indica* against some selected bacteria isolates.

MATERIALS AND METHODS

Description of Study Area

The study was conducted in Adigrat district, Eastern Zone of Tigray Region, Northern Ethiopia. Adigrat district is found about 918 km far from Addis Ababa, capital city of Ethiopia. It is located at altitudinal ranges from 2000-3000 meter above sea level and geographically located 14°16'34' N latitude and 39°27'52' E longitudes. It has a unimodal rainfall distribution with the highest rain falling from June to early September. Annual average rainfall of this zone ranges from 450mm-600mm and the minimum and maximum temperature is 6 °C and 21°C.

Plant Material Collection

Fresh skin fruits (peels) of cactus pear (*Opuntia ficus indica*) were collected in the month of July 2015 from nearby area of Adigrat district. The sample of the skin fruit were washed thoroughly with tap water and rinsed with distilled water and the cleaned samples were cut into small pieces (with a thickness of about 3-4 mm) and dehydrated at 60°C for two days. The dried samples were grinded in a domestic coffee grinder and the powders were sieved at 1mm. The fine powder obtained were stored in amber bottles or dark plastic bags at room temperature for further investigations.

Preparation of Culture Media

Dehydrated media, standard antimicrobial drugs (Tetracycline and Vancomycin), sterile discs, and chemicals from HiMedia laboratories were used for culturing of the test bacteria. All the media were prepared in sterilized glass petri-plates according to the manufacturer's instructions.

Collection and Maintenance of Microorganisms

Clinically isolated bacteria namely *Escherichia coli* (ATCC2592), *Streptococcus pneumoniae* (ATCC63), *Salmonella typhi* (B2836) and *Bacillus subtilis* (S456) were kindly obtained from the Department of Microbiology and Immunology, University of Gondar and transported to Department of Biology, Adigrat University. The bacteria strains were cultured in Muller-Hinton agar (MHA - pH 7.2) at 37°C. The stock culture slants were maintained at 4°C.

Preparation of Inoculums

The bacteria isolates were cultured in Muller-Hinton broth at 37°C for 4 to 6 hours. The turbidity of the broth culture was adjusted 0.5 ($1-2 \times 10^8$ CFU/ml) and used as standard inoculums for the antibacterial studies (Mackie and MacCartney, 1996).

Extraction of Bioactive Constituents

Each fine powder sample of skin fruit (100g) were mixed with ethanol, methanol (Abron chemicals, batch No. AB 130507) and chloroform (BDH chemicals Ltd, Poole, lot No.27710, England) in a 1:4 (w/v) ratio in different Erlenmeyer flasks. The flasks were covered with aluminum foil to avoid light exposition. The mixtures were

refluxed at 60°C for 7 h. After this, the extracts were filtered using Whatman filter paper no. 4. The solvents were removed using a rotary evaporator (Yamato RE540) using a temperature below 60°C (Elham *et al.*, 2014). Next, the samples were stored at 4°C in refrigerator for further studies.

Determination of Bioactive Constituents (Qualitative Analysis)

The presence of different bioactive compounds in the skin fruit extracts of *Opuntia ficus indica* were qualitatively determined using the standard methods or with slight modifications (Sofowora, 1993).

Test for phlobatannins

Two ml of aqueous extract were mixed with 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

Test for Phenols

Crude extracts were mixed with 2ml of 2% solution of FeCl₃. A blue green or black coloration indicated the presence of phenols.

Test for Flavonoids

Crude extracts were mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of dilute acid which indicated the presence of flavonoids.

Test for Tannin

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution were added. No color change was observed which indicates the absence of tannins.

Test for Steroid

Ten mg of extracts were dissolved in chloroform. Few drops of acetic anhydride were added followed by 1 ml of concentrated sulphuric acid. Blue color in chloroform layer, which changes to green, shows the presence of steroids.

Test for Saponins

Two ml of the extract was added to distilled water and shaken vigorously. Froth (foam) that persisted for more than 10 minutes indicated the presence of saponins.

Test for Alkaloid

The extracts of the skin fruit were heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixtures were filtered and treated with a few drops of Meyer's reagent. The samples were then indicated the presence of turbidity or yellow precipitation, which is indicated the presence of alkaloids.

Test for Glycoside

To the solution of extract in glacial acetic acid few drops of ferric chloride and conc. H₂SO₄ was added and observed for reddish brown coloration at the junction of two layers and bluish green color in upper layer.

Test for Reducing Sugar

Two ml of the extract was diluted in 2ml of distilled water and Fehling's solutions (A+B) added to the mixture. A brick red precipitate after standing in the heat or water bath indicated the presence of reducing sugars.

Evaluation of the Antibacterial Potential of Skin Fruit Extracts of *Opuntia ficus indica*

The antibacterial potentials of the skin fruit extracts of *Opuntia ficus indica* were studied using agar well diffusion method as described by Monia *et al.* (2014). 0.1 ml of freshly grown culture of test bacterial pathogens (10⁶CFU/ml) was aseptically introduced and spreaded on the surface of sterile Muller Hilton agar plates. Wells of 6 mm diameter was made in agar plate with the help of sterile cork-borer. Sterile distilled water (SDW) was used as negative control while commercial antibiotics (Tetracycline 10µg and Vancomycin 10µg) were used as positive controls. Plates were left for some time at 4°C till the extract diffuses in the medium with the lid closed and the bacteria plates were allowed to incubate at 37⁰C for 24 hr. After this, the antibacterial potentials of the extracts were evaluated and compared by measuring the diameter of the zone of inhibition against the tested bacteria isolates.

Determination of Minimum Inhibitory Concentration

The MIC for the test bacteria isolates was determined by using agar diffusion (Shahidi *et al.*, 2004).

Data Analysis

All data were statistically analyzed and treated with a Duncan test using SPSS (version 16) to determine any significant differences between the average values at 95% confidence. Values of the different parameters were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Determination of Bioactive Constituents

The qualitative analysis of ethanolic, methanolic and chloroform skin fruit extracts of *Opuntia ficus indica* showed the presence of phenolic substances, glycosides, alkaloids, flavonoids, steroids, alkaloids, reducing sugars and amino acids (Table 1). The presence of the bioactive constituents in

skin fruit extracts of *Opuntia ficus indica* indicates how the fruit of *Opuntia ficus indica* is nutritionally rich and its potential use as an excellent source of food supplement. The nutritional composition of skin fruit of *Opuntia ficus indica* also reveals its beneficial to use for the prevention and treatment of different diseases.

Table 1. Bioactive constituents analysis of *Opuntia ficus indica* skin fruit extracts.

Bioactive constituents	Solvents		
	Ethanol	Methanol	Chloroform
Phenols	+	+	+
Flavonoids	+	+	+
Tannins	-	-	-
Steroid	+	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Glycoside	+	+	+
Reducing Sugar	+	+	+
Phlobatannis	-	-	-
Amino acids	+	+	+

Evaluation of Antibacterial Potential

The antibacterial potential of the skin fruit extracts of ethanol, methanol and chloroform against the bacteria isolates were done by measuring the inhibitory activity of the extracts (Table 2). The skin fruit extracts of *Opuntia ficus indica* demonstrated good antibacterial activity against the four bacterial isolates with zone of growth inhibition ranging from 9.40 mm to 23.52 mm, which was significant ($P < 0.01$). The mean inhibitory activity of chloroform skin fruit extracts of *Opuntia ficus indica* (9.60 mm) against *E.coli* (ATCC2592) was

significantly ($P=0.04$) less than the inhibitory activity of ethanol and methanol extracts; while the inhibitory activity of the skin fruit extracts of ethanol (10.46 mm) against *E.coli* (ATCC2592) was significantly ($P=0.03$) greater than the inhibitory activity of the other extracts. The inhibitory activity of ethanol skin fruit extract against *S.typhi* (S456) was significantly ($P=0.02$) greater than the inhibitory activity of methanol and chloroform skin fruit extracts. Also, there were no statistically significant differences between the inhibitory activity of ethanol,

methanol and chloroform skin fruit extracts against *B.subtilis* (B2836). However, the inhibitory activity of the ethanol skin fruit extract (21.90 mm) against *B.subtilis* (B2836) was relatively greater than the inhibitory activity of methanol and chloroform extracts. Moreover, the inhibitory activity of ethanol skin fruit

extract against *S.pneumonia* (ATCC63) (23.52 mm) was significantly ($p=0.04$) greater than the inhibitory activity of methanol and chloroform skin fruit extracts. The inhibitory activity of ethanol skin fruit extract against the bacteria isolates were significantly ($P=0.04$) greater than the extracts.

Table 2. Inhibition zone of skin fruit extracts of *Opuntia ficus indica* against bacteria isolates.

Bacteria isolates	Solvent extraction	Inhibition zone of extracts (mm diameter)
<i>E.coli</i> (ATCC2592)	Ethanol	(10.46 ± 0.45) ^a
	Methanol	(9.89 ± 0.86) ^a
	Chloroform	(9.60 ± 0.60) ^a
<i>S.typhi</i> (S456)	Ethanol	(11.42 ± 0.33) ^a
	Methanol	(10.39 ± 0.44) ^a
	Chloroform	(9.90 ± 0.65) ^a
<i>B.subtilis</i> (B2836)	Ethanol	(21.90 ± 0.10) ^{bc}
	Methanol	(21.18 ± 0.64) ^{bc}
	Chloroform	(21.20 ± 0.64) ^{bc}
<i>S.pneumoniae</i> (ATCC63)	Ethanol	(23.52 ± 0.11) ^b
	Methanol	(19.98 ± 0.78) ^b
	Chloroform	(19.14 ± 0.01) ^b

Values were mean triplicate determinations. ^{a-c}, indicate letters with significantly different at ($P \leq 0.05$).

Antibacterial resistance has become one of the most serious global health problems in twenty first century (Somaie *et al.*, 2013). A large number of bacterial species have become resistant to antibacterial drugs and causing a number of infectious diseases. Thus, it is vital to develop new antibiotics with novel mechanism of action to overcome these problems. Plants have been used traditionally for the prevention and treatment of different microbial diseases (Burda and Oleszek, 2001; Gebreselema *et al.*, 2013). The use of plant extracts with

known antimicrobial properties can be of great importance for therapeutic treatments. *Opuntia ficus indica* is one of the medicinal plants that have been used traditionally for controlling many different pathogenic bacterial infections (Jean *et al.*, 2006).

In the present study, the skin fruit extracts of *Opuntia ficus indica* have revealed good antibacterial activity against both Gram positive and Gram negative bacteria isolates. This indicates that the skin fruit extracts of *Opuntia ficus indica* have a broad spectrum of antibacterial potential. This might be due the combined effect of the

bioactive constituents present in skin fruit of *Opuntia ficus indica* and this result was in agreement with results reported by Durgesh and Tumane (2016). The Gram positive bacteria isolates were found to be more susceptible to the inhibitory action of the skin fruit extracts than the Gram negative bacteria isolates. This was due to the presence of an extra outer membrane in Gram negative bacteria, which contains lipopolysaccharide that make the cell wall of the bacteria impermeable to extracts. Similar inhibitory activity of extracts against Gram positive bacteria were reported by (Durgesh and Tumane, 2016). Also, the skin fruit extracts have shown varied inhibitory activity against the test bacteria isolates. This varied inhibitory activity against the bacteria isolates suggesting that the varying efficiency and combined effect of the different constituents present in the extracts as well as due to difference in bacteria isolates used in the present study (Parekh and Chanda, 2008).

Comparison of antibacterial activity of ethanol, methanol and chloroform skin fruit extracts of *Opuntia ficus indica* and antibacterial agents (antibiotics) against test bacteria isolates

The inhibitory action of the antibacterial agents i.e. tetracycline (10µg/disc) and vancomycin (10µg/disc) against *S.typhi* (S456), *B.subtilis* and *S.pneumoniae* (ATCC63) were significantly (P=0.00-0.01) less than the inhibitory activity the ethanol, methanol, chloroform skin fruit extracts of *Opuntia ficus indica* against these bacterial isolates. This shows that these bacterial isolates were resistant to some of the currently used antibacterial agents. On the other hand, the inhibitory activity of antibacterial agents i.e. tetracycline (10µg/disc) and vancomycin (10µg/disc) against *E.coli* (ATCC2592) were greater than the inhibitory activity of ethanol, methanol and chloroform skin fruit extracts. (Table 3). Comparisons of the inhibitory activity the skin fruit extracts of *Opuntia ficus indica* with previous findings have shown similar results (Somaie *et al.*, 2013). Hence, with the ever growing resistant bacteria species to the already available antibacterial agents, the naturally available skin fruit extracts of *Opuntia ficus indica* could be alternative choices to make antibacterial agents for treating various bacteria diseases.

Table 3. Inhibition zone skin fruit extracts of *Opuntia ficus indica* and antibacterial discs (antibiotics) against bacteria isolates.

Bacteria isolates	Solvents	Inhibition zone diameter (mm)			
		skin fruit extracts	Commercial antibiotics		DW
			Tet	Van	
<i>E.coli</i> (ATCC2592)	Ethanol	(10.46 ± 0.45) ^a	(15.83±0.76) ^b	(21.87±0.23) ^{bc}	0.00±0.00
	Methanol	(9.89 ± 0.86) ^a	(14.93±0.45) ^b	(21.81±0.59) ^{bc}	0.00±0.00
	Chloroform	(9.60 ± 0.60) ^a	(15.19±0.96) ^b	(20.32±0.73) ^{bc}	0.00±0.00
<i>S.typhi</i> (S456)	Ethanol	(11.42 ± 0.33) ^a	(5.76±0.08) ^a	(9.50±0.07) ^a	0.00±0.00
	Methanol	(10.39 ± 0.44) ^a	(3.53±0.64) ^a	(6.80±0.01) ^a	0.00±0.00

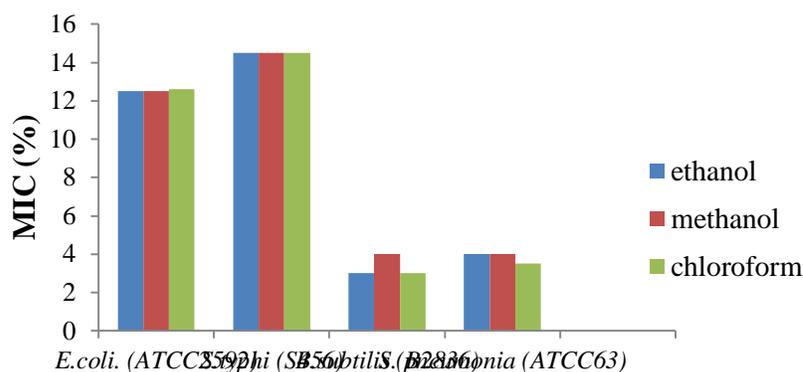
<i>B.subtilis</i> (B2836)	Chloroform	(9.90 ± 0.65) ^a	(0.00±0.00) ^a	(1.00±0.84) ^a	0.00±0.00
	Ethanol	(21.90 ± 0.10) ^{bc}	(6.01±0.20) ^a	(4.03±0.30) ^a	0.00±0.00
	Methanol	(21.18 ± 0.64) ^{bc}	(2.24±0.11) ^a	(7.56±0.08) ^a	0.00±0.00
<i>S. pneumoniae</i> (ATCC63)	Chloroform	(21.20 ± 0.64) ^{bc}	(1.01±0.00) ^a	(7.04±0.89) ^a	0.00±0.00
	Ethanol	(23.52 ± 0.11) ^b	(0.00±0.00) ^a	(0.00±0.00) ^a	0.00±0.00
	Methanol	(19.98 ± 0.78) ^b	(0.00±0.00) ^a	(0.00±0.00) ^a	0.00±0.00
	Chloroform	(19.14 ± 0.01) ^b	(0.00±0.00) ^a	(0.00±0.00) ^a	0.00±0.00

Values were mean triplicate determinations. ^{a-c}, indicate letters with significantly different at (P<=0.05), Tet: tetracycline (10µg/disc), Van: Vancomycin (10µg/disc), DW: Sterile distilled water.

Determination of Minimum Inhibition Concentration (MIC)

As shown in Figure 1, the minimum inhibitory concentrations of ethanol, methanol and chloroform skin fruit extracts of *Opuntia ficus indica* against *E. coli* (ATCC2592) were 12.5%. Whereas the MIC of all solvents of skin fruit extracts of *Opuntia ficus indica* against *S.typhi* (S456) was 14.125%. The MIC of ethanol and methanol skin fruit extracts of *Opuntia ficus indica* against *B.subtilis* (B2836) was 2.75% while the MIC of methanol of extract against *B.subtilis* (B2836) was 3.75% and the MIC of *Opuntia ficus indica* extracts of ethanol and methanol against *S. pneumoniae*

(ATCC63) were 3.35%. But, the MIC of *Opuntia ficus indica* extracts of chloroform against this bacterium was 3.125%. According to this study, the MIC of skin fruit extracts varied, suggesting that the ability of the pathogenic bacteria to resist to the extracts and the test bacteria was not easily killed at lowest concentration (highest dilution) by the extracts of *Opuntia ficus indica*. The Gram positive bacteria isolates were easily inhibited at the lowest concentration. This might be due to their sensitive for any antibacterial agents. This result was in line with the results other reports (Adomi and Umukoro, 2010; Somaie *et al.*, 2013).



Bacterial Isolates

Figure 1. MIC of skin fruit extracts *Opuntia ficus indica* against bacterial isolates.

CONCLUSION

The findings of the current study have shown that the skin fruit extracts of *Opuntia ficus indica* has potential antibacterial activity against all tested pathogenic bacteria. The antibacterial activity of the skin fruit extracts of *Opuntia ficus indica* against the test Gram positive pathogenic bacteria were far greater than the test Gram negative pathogenic bacteria. In addition, the antibacterial activity of *Opuntia ficus indica* skin fruit extracts against *S.typhi* (S456), *B.subtilis* (B2836) and *S. pneumoniae* (ATCC63) were greater than the activity of the currently used antibiotics (Tetracycline and Vancomycin). The skin fruit extracts of *Opuntia ficus indica* possess effective antibacterial bioactive constituents against multi-drug resistant bacteria and can be used for prevention of different infectious diseases. Further studies should be conducted to investigate the antibacterial potential of *Opuntia ficus indica* skin fruit extracts against other multidrug resistant bacteria species and to unveil its various therapeutic application for preventing and treating of various microbial diseases.

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