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Phytochemical composition and antibacterial activity of *Opuntia Ficus Indica* cladodes extracts

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Abstract

Nowadays, plants with strong antimicrobial agents are becoming important alternatives to treat several infectious diseases. The aim of this study was to determine the phytochemical composition and antibacterial activity of *Opuntia ficus indica* cladodes extracts against some selected bacterial isolates. Qualitative analysis of phytochemicals in the extracts was done using methanol, ethanol and chloroform solvents. Antibacterial activity of the extracts against bacteria isolates *Escherichia coli* (ATCC2592), *Staphylococcus pneumonia* (ATCC63), *Salmonella typhi* (B2836) and *Bacillus subtilis* (S456) were evaluated using agar well diffusion method. The extracts showed the presence of phenolic substances, tannins, glycosides, alkaloids, flavonoids, saponins, steroids, alkaloids and amino acids. The highest inhibition zone recorded was by methanol extract against *S. pneumonia* (ATCC63) (10.29±0.96) followed by chloroform extract against *B.subtilis* (10.23±0.66). This finding indicated that *Opuntia ficus indica* cladodes have great antibacterial effects against bacteria isolates and needs further investigations to unveil their antimicrobial potential against other multidrug resistant microbial species.

Keywords: *Opuntia ficus indica*, cladodes, phytochemicals, antibacterial activity

1. Introduction

Nowadays, medicinal plants with strong antimicrobial action are becoming important antimicrobial agents for treatment of several human diseases. This antimicrobial importance of medicinal plants stems from the fact that green medicine is easily accessible, safe and with less side effects [1, 3]. *Opuntia ficus indica* is one among the medicinal plants that has been used in traditional folk medicine because of its role in treating a number of diseases and conditions, including antimicrobial, antioxidant actions and anti-inflammatory effects [5]. *Opuntia ficus indica* belongs to the *Cactaceae* family and grows in arid and semiarid regions of the world [7]. The plant has different parts and the major ones include cladodes, stem, vegetative part, flower and fruit. Studies demonstrate that the various parts of the cactus plant have interesting biological constituents and activities [9, 12, 14]. Recently, many bacterial pathogens are becoming resistant to the currently used antibiotics due to their indiscriminant use in treatment of infectious diseases and favor of the antibiotics for the survival and spread of pathogens [11]. Hence, there is an urgent interest to discover new and strong antimicrobial agents from plants. Therefore, the aim of the present study was to determine the phytochemical composition and antibacterial activity of *Opuntia ficus indica* cladodes extracts against some selected bacterial pathogens.

2. Materials and Methods

2.1. Plant Material Collection

Fresh samples of cactus cladodes (*Opuntia ficus-indica*) were collected from Adigrat district, Eastern zone of Tigray Region, Northern Ethiopia (14°16'34" N latitude and 39°27 '52' E longitude). The collected samples were washed thoroughly with tap water and rinsed with distilled water. The cleaned samples were cut into small pieces and dehydrated at 60 °C for two days. Dried samples were grinded in a domestic grinder and the powders were sieved at 1 mm. The fine powders obtained were stored in dark plastic bags at room temperature till used for further studies.

2.2. Culture Media and Chemicals

Dehydrated media, standard antimicrobial drugs (disc), and chemicals from Hi-media 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.

2.3. Extraction and Screening of Phytochemicals

Each fine powder samples of cladodes (100 g) were mixed with ethanol, methanol (Abron chemicals) and chloroform (BDH chemicals ltd, England) in a 1:4 ratio (w/v) ratio in different Erlenmeyer flasks and the flasks were covered with aluminum foil. The mixtures were refluxed at 60 °C for 7 hr. 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 [10]. After doing this, the presence of phenolic substances, tannins, glycosides, alkaloids, flavonoids, steroids, alkaloids, reducing sugars and amino acids were carried out according to phytochemicals screening methods described by others [13].

2.4. Bacterial Pathogens

For testing the antibacterial activity, bacteria isolates namely *Escherichia coli* (ATCC2592), *Streptococcus pneumoniae* (ATCC63), *Salmonella typhi* (B2836) and *Bacillus subtilis* (S456) were kindly obtained from University of Gondar, Department of Microbiology and Immunology and transported to Department of Biology, Adigrat University, using ice box. In the beginning, the bacteria isolates were cultured in Muller-Hinton agar (MHA-pH 7.2) at 37 °C.

2.5. Antibacterial Activity of Cladodes Extracts

The antibacterial activity of the cladodes extracts were evaluated using agar well diffusion methods as done by [8]. 0.1 ml of freshly cultured of test bacterial isolates were aseptically transferred and spread on surface of sterile Muller Hilton agar plates. Wells of 6 mm diameter was made in agar plate. Sterile distilled water (SDW) was used as negative control while antimicrobial agents (Tetracycline 10 µg and Vancomycin 10 µg) were as used as positive controls for the test bacteria. Plates were left for some time at 4 °C till the extracts diffuses in the medium with the lid closed and the test bacterial plates were incubated at 37 °C for 24 hr. Then, the antibacterial activities of the extracts were evaluated by measuring the diameter of inhibition zone formed.

2.6. Data Analysis

All data were statistically analyzed and treated with Duncan test using SPSS (version 16) at 95% confidence. All data were expressed as mean ± standard deviation.

3. Results

3.1. Phytochemicals Analysis

The qualitative analysis of phytochemicals in cladodes extracts of *Opuntia ficus-indica* revealed the presence of phenolic substances, tannins, glycosides, alkaloids, flavonoids, saponins, steroids, alkaloids and amino acids. The phytochemicals determined in cladodes extracts of *Opuntia ficus-indica* are summarized in Table 1

Table 1: Phytochemicals determination in Cladodes extracts of *Opuntia ficus-indica*

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

+ = Presence of phytochemical, - = absence of phytochemical

3.2. Antibacterial activity of cladodes extracts of *Opuntia ficus-indica* against test bacteria

The inhibition zone of methanol, ethanol and chloroform cladodes extracts of *Opuntia ficus indica* against test bacteria is given on Table 2. The results of this study indicated that methanol, ethanol and chloroform cladodes extracts of *Opuntia ficus indica* showed considerable antibacterial activity. Highest inhibition zone was recorded by methanol

extract against *S. pneumonia* (ATCC63) (10.29±0.96) followed by chloroform extract against *B.subtilis* (10.23±0.66). In contrast, minimum inhibition zone was shown by methanol extracts against *S.typhi* (S456) (4.50±0.89). Moreover, the inhibition zones of chloroform extracts against *E.coli* (ATCC2592) and *B.subtilis* (B2836) were greater than methanol and ethanol extracts against these organisms.

Table 2: Antibacterial activity of cladodes extracts of *Opuntia ficus-indica* against test bacteria

Test bacteria	Solvents used for extraction	Inhibition zone of extracts (in mm)
<i>E.coli</i> (ATCC2592)	Methanol	5.06±0.84
	Chloroform	6.80±0.00
	Ethanol	5.78±0.51
<i>S.typhi</i> (S456)	Methanol	4.50±0.89
	Chloroform	5.23±0.80
	Ethanol	6.12±0.23
<i>B.subtilis</i> (B2836)	Methanol	10.00±0.33
	Chloroform	10.23±0.66
	Ethanol	9.04±0.21
<i>S. pneumonia</i> (ATCC63)	Methanol	10.29±0.96
	Chloroform	9.60±0.16
	Ethanol	9.12±0.23

3.3. Comparison of inhibition zone of Cladodes extracts of *Opuntia ficus-indica* and commercial antibiotic discs against test bacteria

The inhibition zone of methanol, ethanol and chloroform cladodes extracts of *Opuntia ficus indica* and commercial antibiotic discs (tetracycline and vancomycin) against the tested bacteria is given on Table 3. The inhibition zone of the antibiotic discs Tetracylin (10 µg) and Vancomycin (10 µg)

against the organisms *B.subtilis* (B2836) and *S. pneumonia* (ATCC63) were less than the inhibition zone of all solvents of cladodes extracts of *Opuntia ficus indica*. Also, the inhibition zone of the antibiotic discs Tetracylin (10 µg) and Vancomycin (10 µg) against *S.typhi* (S456) were less than the inhibition zone of cladodes ethanol extracts of *Opuntia ficus indica*.

Table 3: Comparison of inhibition zone of Cladodes extracts of *Opuntia ficus-indica* and commercial antibiotic discs against test bacteria

Test bacteria	Solvents	Inhibition Zone (mm)			
		Cladodes extracts	Commercial antibiotics		SDW
			Tet	Van	
<i>E.coli</i> (ATCC2592)	Methanol	5.06±0.84	(15.83±0.76)	(21.87±0.23)	0.00±0.00
	Chloroform	6.80±0.00	(14.93±0.45)	(21.81±0.59)	0.00±0.00
	Ethanol	5.78±0.51	(15.19±0.96)	(20.32±0.73)	0.00±0.00
<i>S.typhi</i> (S456)	Methanol	4.50±0.89	(5.76±0.08)	(9.50±0.07)	0.00±0.00
	Chloroform	5.23±0.80	(6.53±0.64)	(6.80±0.01)	0.00±0.00
	Ethanol	6.12±0.23	(0.00±0.00)	(1.00±0.84)	0.00±0.00
<i>B.subtilis</i> (B2836)	Methanol	10.00±0.33	(6.01±0.20)	(4.03±0.30)	0.00±0.00
	Chloroform	10.23±0.66	(2.24±0.11)	(7.56±0.08)	0.00±0.00
	Ethanol	9.04±0.21	(1.01±0.00)	(7.04±0.89)	0.00±0.00
<i>S. pneumonia</i> (ATCC63)	Methanol	10.29±0.96	(0.00±0.00)	(0.00±0.00)	0.00±0.00
	Chloroform	9.60±0.16	(0.00±0.00)	(0.00±0.00)	0.00±0.00
	Ethanol	9.12±0.23	(0.00±0.00)	(0.00±0.00)	0.00±0.00

Discussion

These days, microbial resistance to the currently used antimicrobial agents is becoming a serious global health problem [8, 11]. A large number of bacterial species are becoming resistant to the currently used antibiotics and causing several infectious diseases. Hence, finding new antimicrobial agents with novel mechanism of action is one the alternatives to overcome these problems. Plants are among the potential sources of different phytochemicals that could be used for the prevention and treatment of various infectious diseases. *Opuntia ficus indica* is one the important medicinal plants that have been used traditionally for the treatment of different infectious diseases in several countries [4, 10]. *Opuntia ficus indica* extracts are believed to be potential source of phytochemicals [9, 12, 14]. The results of the present study revealed the presence of phenolic substances, tannins, glycosides, alkaloids, flavonoids, saponins, steroids, alkaloids and amino acids in the cladodes extracts of *Opuntia ficus indica*. The observed considerable antibacterial activity of the *Opuntia ficus indica* cladodes extracts could be attributed to the presence of different phytochemicals.

In the present study, the cladodes extracts of *Opuntia ficus indica* have shown great antibacterial activity against both gram positive and gram negative bacteria. This suggests that the cladodes extracts of *Opuntia ficus indica* have great potential of a broad spectrum of inhibitory activity against both gram positive and gram negative bacteria [2]. But the inhibitory activity of the extracts of *Opuntia ficus indica* against gram positive bacteria was by far greater than the gram negative bacterial, which is in agreement with antibacterial activity of *Opuntia ficus indica* reported by other studies [2, 6, 7]. This might be because of having only an outer peptidoglycan layer in gram positive bacteria, which is not an effective and strong permeable barrier.

The antibacterial activity of the cladodes extracts of *Opuntia ficus indica* against particularly the bacterial species *S. pneumonia* (ATCC63) and *B.subtilis* (B2836) was significantly greater than the currently used antimicrobial agents tetracycline and vancomycin against these organisms.

Cladodes extracts of *Opuntia ficus indica* are excellent sources of phytochemicals like phenolic substances, tannins, glycosides, alkaloids, flavonoids, saponins, steroids and alkaloids [7], which could be responsible for the observed inhibitory activity against the tested bacteria. Thus, with the ever growing resistant strains of microbes to the currently used antimicrobial agents, the naturally available cladodes of *Opuntia ficus indica* could be a promising alternative.

Conclusion

The result of this finding revealed that the cladodes extracts of *Opuntia ficus-indica* have potential antibacterial effects against both gram positive and gram negative bacteria. This is a promising finding and needs further studies to unveil the antimicrobial potential of the cladodes extracts of *Opuntia ficus-indica* against other multidrug resistant microbial species.

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