Antimicrobial Activity of *Opuntia cochenillifera* (L.) Mill Fruit and Cladode Extracts

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Abstract. In the present study the antimicrobial activity of chloroform and methanolic extracts of *Opuntia cochenillifera* for both cladode and fruits was investigated. Methanolic extract was found to be an effective against the microbes namely, *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* *Candida albican* *C.glabrata* *C.haemulonii* *C.Tropicalis*. Maximum activity was observed against *E. coli*, *B. Subtilis* and, *C.albican* and *C.glabrata* at 40mg/ml. Agar well diffusion assay was used to determine minimum inhibitory concentration of all test microorganisms.

Introduction

Nowadays, antimicrobial resistance has become a major global problem which is caused by the indiscriminate use of antimicrobial drugs. The use of medicinal plants, which provide a rich source of novel antimicrobial agents in human history as many infectious diseases have traditionally been treated with herbal medicines. Plant based antimicrobials represent a vast source for medicines and further exploration of the plant. Antimicrobials of origin have enormous therapeutic potential [1]. Human infections, especially those involving microorganisms i.e. bacteria, fungi, cause serious infections in tropical and subtropical countries of the world. In recent time, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used of such diseases [2, 3]. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents [4]. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts are used to extract as raw drugs which possess different medicinal properties. Medicinal plants are major source of new chemical substances with potential therapeutic effects [5]. There is need to identify novel substance active towards highly resistant pathogens until natural products have been approved as new antimicrobial drugs, [6, 7].

Opuntia species have been used by humans for thousands of years [8] besides being consumed as food or beverages. Most portions of the plants have been used as medicine. This species is free of spines and spine-hairs on maximum portion [9, 10]. This plant has been used to treat rheumatism, diarrhoea and other inflammatory problems as well as a diuretic and analgesic, particularly for ear and tooth aches [11, 15]. The anti-inflammatory principle was related to a b-sitosterol [16]. Despite these biological properties, validation of any antibiotic potential against bacterial and fungal species has not been reported much from India.

Materials and Methods

Plant material

Habit

Inflorescence

Fruit

Collection of raw materials

The plant material collected from Gulbarga University campus located at southern part of India. The Plant species were identified with the reference of the Digital flora of Karnataka, Flora of Presidency of Madras, Flora of Gulbarga district and the Flora of Karnataka. [17, 19] The cladodes and fruits were shade dried, powdered mechanically and stored in airtight containers for extraction.

Preparation of crude extracts

The powdered material of *Opuntia cochenillifera* fruit and cladode were subjected to successive solvent extraction. The powder is taken separately in 1 litre capacity thimble of Soxhlet apparatus and refluxed successively with chloroform and methanol for 48hrs in 8 batches of 500g each. Then it was allowed to evaporate which yield crude extracts used for antimicrobial activity.

Test Microorganisms

Authentic pure cultures of human pathogenic bacteria were obtained from the Medicinal plants and Microbiology laboratory, Department of Botany, Gulbarga University Kalaburagi Karnataka, India. And *Candida* sps were taken from (MTCC) Chandigarh India

Preparation of sample

In the study of antimicrobial activity, the extract was dissolved in Dimethyl sulphoxide (DMSO). The corresponding concentration was expressed in term of mg of extract per ml of solvent (mg/ml).

Antibacterial activity

Antibacterial study (plate hole diffusion or agar well diffusion) assay was used to determine the growth inhibition of bacteria by plant extracts. Total of 25ml of nutrient agar plate was prepared and poured into sterile Petri dishes. The depth of the medium was approximately 4 mm. After the medium got solidified, the plates were allowed to dry for one hour. The bacterial/yeast suspension equal 1.5×10^8 cells /ml in sterile normal saline was prepared following the method [20] and inoculated on nutrient agar media by sterile cotton swabs. 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 μ l of the different concentration 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. All tests were done in triplicate. DMSO solvent is considered as negative control. Streptomycin was used as a standard antibacterial drug.

Antifungal activity

Yeast extract peptone dextrose agar media (YPDA) were prepared and 25ml of each was poured in to sterile Petri dishes with different species of *candida* fungus. Using a sterile cork borer (6mm diameter) four holes per plate was made and a total of 20 µl of plant extracts was poured in to the wells. The plates were incubated at 28°C for 36 to 48hrs and the zone diameter was then recorded. Ketoconazole (10µg/ml) was used as a standard antifungal drug.

Results

The antimicrobial potential of both the plant part extracts was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standards, viz., streptomycin, ketacanazole (10 mg/ml). For bacteria and *candida* respectively the results revealed that all the extracts are potent antimicrobials against all the microorganisms studied. Among the different solvent extracts studied methanol showed a high degree of inhibition compared to chloroform. Methanol showed maximum antibacterial activity in fruit extract maximum inhibition zone diameter was obtained is (23mm) against *B. Subtilis*. (21mm) in *E.coli*. Similarly, Methanol cladode 40mg/ml extract showed maximum inhibition zone with diameter of (20.3)mm against *B. Subtilis*. More specifically, chloroform and methanol extract represented higher susceptibility to all bacterial strains with a concentration of (10mg/ml) For the antifungal activity, *Candida albicans* (21.4 mm) showed efficient antifungal activity for methanol fruit and methanol lowest concentration 10mg/ml extract showed lowest inhibition zone with diameter ranging between (5.2-5.4) mm compared to the chloroform extract (8.2-8.9) against all pathogenic fungal strains, respectively (Table.1) all the chloroform and methanol fruit and cladode antibacterial and antifungal activity shown in (Fig 2,9)

Table 1: Zone of inhibition in diameter mm, no inhibition, (--) DMSO – dimethyl sulfoxide, –ve control +ve control for Bacteria – streptomycin For fungal *Candida* sps – Ketoconazole C,- Cladode, F- Fruit

Test organisms	Plant part	CHOLOROFORM				METHENOL				-ve controle (DMSO)	+ve contrloe
		40mg/ml	30mg/ml	20mg/ml	10mg/ml	40mg/ml	30mg/ml	20mg/ml	10mg/ml		
<i>E. coli</i>	C	20	18.2	16	10.6	19.9	21	14.2	10	---	35
	F	18.2	16.2	16.2	8.2	20.2	20	15	11	--	35
<i>B.subtilis</i>	C	20	18.2	16	10.6	20.3	20.4	20	10	--	33.8
	F	18	14	18	12	19.6	23-	18.2	11.4	--	33.8
<i>S.aureus</i>	C	20.1	14.3	18.6	12.4	19.2	19.4	20.2	0.8	--	30.5
	F	18.6	16.6	16	10	20	16.7	17	0.7	--	30.5
<i>P. aurginosa</i>	C	18.7	16.8	16.6	13	18.2	18.4	16.1	0.8	--	30
	F	18.4	17	14	10	18.2	17.4	16.5	0.6	--	30
<i>Candida albican</i>	C	20.1	18.6	15	12	21.3	18.4	15	10	--	36
	F	20.2	17.2	14.2	10.3	21.4	16.2	12.4	8.2	--	36
<i>C. glabrata</i>	C	18.3	15	12.6	10.2	18.4	17.4	11.4	7.3	--	35
	F	19.1	17.2	16.2	12.1	17.2	15.2	14.3	6.2	--	35
<i>C.haemulinii</i>	C	17.5	15	13	10	17.5	18.4	14.5	5.4	--	32
	F	17.2	16.1	12.2	8.2	16	16.2	13.6	5.3	--	32
<i>C tropicalis</i>	C	18.5	15.2	13.3	8	16.8	16.8	13.8	5.2	--	30
	F	17.5	16.2	14.5	8.5	16.7	16.6	13.4	6	--	30

Fig. 2 Antibacterial activity of chloroform extract of cladode

Fig. 3 Antibacterial activity of chloroform extract of fruit

Fig. 4 Antibacterial activity of Methanol extract of cladode

Fig. 5 Antibacterial activity of Methanol extract of fruit

Fig. 6 Antifungal activity of chloroform extract of cladode

Fig. 7 Antifungal activity of chloroform extract of fruit

Fig. 8 Antifungal activity of Methanol extract of cladode

Fig. 9 Antifungal activity of Methanol extract of fruit

Discussion

Recently, much attention has been directed toward plant extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal agents with significant activity against infective microorganisms [21, 22]. On the basis of the result obtained in this present investigation, we conclude that the methanol cladode and fruit extract had significant *in vitro* antimicrobial activity. The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium [23]. The present observation suggests that the organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they supported by many investigators [24, 27]. Candidiasis of the oesophagus and mouth by *Candida albicans*, is the most common infection observed in the immunosuppressed individual [28]. Because there are limited effective antifungal drugs currently available for the treatment of candidiasis, it is critical to discover and evaluate new sources of antifungal agents. In previous studies [29] it has been shown that the only cladodes have possessed the antimicrobial activity but in this investigation we have taken fruit as well as cladode and obtained results which indicate that both parts of plant have significant antimicrobial agent.

Conclusion

There are no reports till now regarding *Opuntia cochenillifera* plant applications in health care system. The present results can be useful in further investigations on antimicrobial studies of this plant and other aspects. The cladode and fruits can be used in the treatment of diseases caused by bacteria *Candida*.

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