IN VITRO ANTIBACTERIAL ACTIVITY OF OPUNTIA FICUS-INDICA L. (PRICKLY PEAR) AGAINST MULTIPLE DRUG RESISTANT (MDR) BACTERIA ISOLATED FROM CLINICAL SAMPLES

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ABSTRACT

The present study was done for evaluation of antibacterial activity of Opuntia ficus-indica L. against multiple drug resistant (MDR) bacteria isolated from clinical specimen. The antibacterial activity of Opuntia ficus-indica L. were evaluated on MDR strains such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Salmonella spp., Enterococcus faecalis, Citrobacter freundii, Acinetobacte baumannii, Streptococcus pneumoniae, Enterococcus faecium and Enterobacter cloacae. Antibacterial activity of five different solvent extracts (Methanol, acetone, ethanol, petroleum ether and n-Hexane) were prepared by using Soxhlet extractor. In-vitro antibacterial activity was performed by agar well diffusion method. The ethanolic and methanolic extract of Opuntia ficus-indica L. showed the presence of Saponins, Glycosides, Alkaloids, Flavonoids, Phenolic substance, Di & Tri-terpenes and Proteins. The methanolic extract of leaf of Opuntia ficus-indica L. found highest activity against C. freundii and S. pneumoniae (18 mm). S. aureus showed 16 mm whereas E. coli, K. pneumoniae, E. faecalis and E. cloacae showed 14 mm zone of inhibition. The lowest MIC value found against S. pneumoniae and highest MIC value found in the range of 25-50mg/ml in both ethanolic and methanolic extract. Acetone extract also found to be effective against MDR strains. Petroleum ether and n-Hexane showed least antibacterial activity against all MDR strains.

KEYWORDS: MDR, Antibacterial Activity, Opuntia ficus-indica L., Soxhlet extractor
INTRODUCTION

Opuntia, also known as nopales or paddle cactus, is a genus in the cactus family, Cactaceae. Currently, only prickly pears are included in this genus of about 200 species distributed throughout most of the Americas. The most common culinary species is the Indian fig opuntia (O. ficus-indica). **Opuntia ficus-indica** is a species of cactus that has long been a domesticated crop plant important in agricultural economies throughout arid and semiarid parts of the world. It is thought to possibly be native to Mexico. Some of the common English names for the plant and its fruit are Indian fig opuntia, barbary fig, cactus pear, spineless cactus, and prickly pear, although this last name has also been applied to other less common **Opuntia** species.

Most culinary uses of the term "prickly pear" refer to this species. Prickly pears are also known as "tuna", "nopal" or **nopales**, from the Nahuatl word nōpalli for the pads, or nostle, from the Nahuatl word nōchtli for the fruit; or paddle cactus. The genus is named for the Ancient Greek city of Opus where, according to Theophrastus, an edible plant grew which could be propagated by rooting its leaves.\(^1\) Prickly pears typically grow with flat, rounded cladodes (also called platyclades) that are armed with two kinds of spines; large, smooth, fixed spines and small, hairlike prickles called glochids, that easily penetrate skin and detach from the plant. Many types of prickly pears grow into dense, tangled structures. **Opuntia** spreads into large clonal colonies, which contributes to its being considered a noxious weed in some places.\(^2\)

Prickly pears (mostly **Opuntia stricta**) were originally imported into Australia in the 18th century for gardens, and were later used as a natural agricultural fencing and in an attempt to establish a cochineal dye industry. They quickly became a widespread invasive weed. The fruit of prickly pears, commonly called cactus fruit, cactus fig, Indian fig or **tuna** in Spanish\(^3\) is edible, although it has to be peeled carefully to remove the small spines on the outer skin before consumption. If the outer layer is not properly removed, glochids can be ingested, causing discomfort of the throat, lips, and tongue, as the small spines are easily lodged in the skin. Cactus figs are often used to make candies, jelly, or drinks such as vodka or lemonade. The prickly pear fruit is also used as the main ingredient of a popular Christmas beverage in the British Virgin Islands, called "Miss Blyden".

**In folk medicine and research:** Most species of **Opuntia** contain a range of alkaloids in variable quantities, such as substituted phenethylamines. Identified compounds that may have
biological activity include 3-methoxytyramine, candicine and hordenine, as examples. The Sicilian prickly pear contains betalain, betanin and indicaxanthin, with highest levels in their fruits.\[4\] Some species of Opuntia have been investigated in preliminary research. One study on O. megacantha raised concern about toxic effects on the kidney and extracts of O. streptacantha may inhibit alpha-glucosidase activity.\[5\]

The gel-like sap of prickly pears might be useful as a hair conditioner. In Mexican folk medicine, its pulp and juice have been used to treat numerous maladies, such as wounds and inflammations of the digestive and urinary tracts.\[6\] Opuntia stricta has been used in traditional folk medicine because of its role in treating a number of diseases and conditions, including anti-inflammatory effects\[7\], hypoglycemic properties, inhibition of peptic ulceration\[8\], neuroprotective effects\[9\], antioxidant actions and also used for treating burns and asthma.\[10\]

MATERIALS AND METHODS

1) Plant Materials
Medicinal plants and their parts were collected from different areas of Nagpur city. This plant then authenticated from P.G. Department of Botany, R.T.M. Nagpur University, Nagpur. Leaves were collected washed with sterile distilled water and air dried at room temperature. Dried leaves were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers for further use.

2) Herbal preparations
The dried plant materials (20 gm) were extracted with 200 ml of each solvent separately by using Soxhlet extractor for 2 to 5 hr at a temperature not exceeding the boiling point of the Solvent. The solvents used for the study were methanol, ethanol, petroleum ether, acetone and n-hexane. The extracts were filtered and then concentrated to dryness. The extract were transferred to glass vials and kept at 4°C before use. The extracts were dissolved in 20% aqueous dimethyl sulfoxide (DMSO) to produce a stock solution of 100 mg/ml. The stock solutions were stored in a refrigerator until needed.\[11\]

3) Phytochemical analysis
The phytochemical screening of all the extracts was carried out to determine the presence of the following compounds; alkaloid, flavonoids, polyuronides, reducing sugars, cyanogenic
glycoside, saponins, terpenes, anthracenosides, phytosterols and phenols as described below.[12]

3.1: Saponins (the Froth test)
2 ml of the extract was added to distilled water and shaken vigorously. A froth (foam) that persisted for more than 10 minutes indicated the presence of saponins.

3.2: Glycosides
To the solution of extract in glacial acetic acid few drops of ferric chloride and conc. H$_2$SO$_4$ are added and observed for reddish brown coloration at the junction of 2 layers and bluish green color in upper layer.

3.3: Polyuronides / Polyamides
Ten milliliters of acetone was added to 2ml of the extract in a test tube. The appearance of a Precipitate indicated the presence of polyuronides.

3.4: Reducing sugars
Two milliliters 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.

3.5: Alkaloids
Twenty milliliters of the alcohol extract was evaporated to dryness on a water bath. Five to ten milliliters of 10% hydrochloric acid (HCl) and CHCl$_3$ were added to the extract. Concentrated ammonia was added to the aqueous layer to obtain a pH of between 8 and 9. The solution was then extracted in a separating tube with chloroform or ether. The a polar solvent was evaporated to dryness in an evaporated dish in a water bath and the residue was dissolved with 5ml of HCl (2N) and the solution was divided into three separate test tubes. Two to three drops of Mayer’s reagent was added to one and the same amount of Bertrand’s reagent to the other, while the third test tube served as a reference. The appearance of an opalescent or yellow-white precipitate with the reagents indicated the presence of alkaloids.

3.6: Anthracenosides
Four milliliters of the extract was concentrated to 2ml with 2ml of 25% of ammonia solution added and shaken. A cherry red colour of the alkaline layer indicated the presence of emodols (aglycones of anthracenosides) in an oxidized form–Borntrager’s reaction.
3.7: Flavonosides
Five milliliters of the extract was evaporated to dryness. The residue was dissolved in 2ml of 50% methanol by heating and 4 grams of metal magnesium and 6 drops of concentrated HCl added. A red solution indicated the presence of flavonoids, while an orange solution indicated the presence of flavones.

3.8: Phenolic substances
Two to three drops of 10% Ferric chloride solution was added to 5ml of extract in a test tube and observed. Dark Green color was develops indicated positive results.

3.9: Sterols and Triterpenes
Ten milliliters of the extract was evaporated to dryness. The residue was dissolved in 0.5ml of acetic aldehyde and 0.5ml of CHCl₃ added and transferred into a dry test tube. About two milliliters of concentrated sulphuric acid (H₂SO₄) was added to the bottom of the tube using a pipette. A brownish red or violet ring at the contact zone of the two liquids indicated the presence of sterols and triterpenes. The greenish and brownish red (wine) nature of the supernatant indicated the presence of sterols and triterpenes respectively.

3.10: Test for Tannins
To 0.5 ml of extract solution 1 ml water and 1-2 drops of ferric chloride solution was added. Blue color was observed for garlic tannins and greenish black for catecholic tannin.

3.11: Test for amino acids
1 ml of plant extract add 2 ml of Ninhydrins. For positive results indicates forming purple color.

3.12: Test for proteins
1 ml of dilute extract add 1 ml of 5% CuSO₄ add 1% of 1ml of NaOH. Deep blue color indicates positive results.

3.13: Test for Saponin
To 50 mg powder and add 20 ml distilled water shake for 15 minutes. Forming 2 cm foam was produced in measuring cylinder indicated positive results.

4. Bacterial Isolates: Multiple drug resistant bacteria were isolated from different clinical specimen such as urine, blood, wound swabs/pus, cerebrospinal fluid and sputum. The MDR
strains were identified on the basis of their morphology, cultural, biochemical characteristics as well as antibiotic susceptibility test. These all MDR bacteria were resistant to more than 10 antibiotics. The MDR strains used for the antibacterial activity were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Salmonella spp., Enterococcus faecalis, Citrobacter freundii, Acinetobacter baumannii, Streptococcus pneumoniae, Enterococcus faecium and Enterobacter cloacae.*

5: Determination of the potency of the herbal preparation

The agar diffusion method was used to investigate the antibacterial activity of the crude extracts. Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterilized swab was aseptically dipped into the suspension. The dried surface of a Mueller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface with bacteria. A sterilized cock borer of an internal diameter of about 6 mm was used to punch holes in the medium and plant extracts were dispensed into the respective labeled holes. 20 % v/v DMSO was used as negative controls. Triplicates of each plate were made and the procedure was repeated for the other microorganisms. The plates were kept in the refrigerator for about 4 hours for complete diffusion of the extract and incubated at 37ºC for 24 hours. After the incubation period, the diameter of each zone of inhibition was measured in millimeters (mm) with zone measuring scale. \[^{[11]}\]

6: Determination of minimum inhibitory concentration (MIC) of the crude extracts

MIC for each test organism was determined by following the modified agar well diffusion method. A twofold serial dilution of each extract was prepared by first reconstituting the dried extract (100 mg/ml) in 20% DMSO followed by dilution in sterile distilled water (1:1) to achieve a decreasing concentration range of 50mg/ml to 0.195 mg/ml. A 100 μl volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100μl of standardized inoculum (10⁶cfu/ml) of the test microbial strain. All test plates were incubated aerobically at 37⁰C for 24 hrs and observed for the inhibition zones. MIC, taken as the lowest concentration of the test extract that completely inhibited the growth of the microbe, showed by a clear zone of inhibition (>8mm), was recorded for each test organism.\[^{[17]}\]

RESULTS AND DISCUSSION

Literature studies showed that there is not enough information in the field of antimicrobial activity of cactus plant. In the present study, the phytochemical analysis was carried out for
the presence of secondary metabolites. The ethanolic and methanolic extract Cactus leaf pad showed the presence of Saponins, Glycosides, Alkaloids, Flavonoids, Phenolic substance, Di & Tri-terpenes and Proteins shown in Table No. 1. After phytochemical analysis these plant extract performed for the antibacterial activity against MDR strains. The antibacterial results showed that the leaf pad of *Opuntia ficus-indica* L. showed the least (minimum) inhibitory activity against the MDR strains isolated from clinical specimen. The ethanolic extract showed zone of inhibition against *S. aureus* and *S. pneumoniae* having 14 mm zone of inhibition. 12 mm zone of inhibition were found against *E. coli, K. pneumoniae, S. typhi* and *C. freundii*. On the other hand, methanolic extract also exhibited better antibacterial activity against tested bacteria. The methanolic extract of leaf pad of *Opuntia ficus-indica* L. found highest activity against *C. freundii* and *S. pneumoniae* (18 mm). *S. aureus* showed 16 mm whereas *E. coli, K. pneumoniae, E. faecalis* and *E. cloacae* showed 14 mm zone of inhibition [Table No. 2]. The lowest MIC value found against *S. pneumoniae* and highest MIC value found in the range of 25-50mg/ml in both ethanolic and methanolic extract. Acetone extract also found to be effective against MDR strains [Table No. 3]. The antibacterial activity of acetone extract against *C. freundii* was observed that 14 mm zone of inhibition. Acetone extract showed 12 mm zone of inhibition against *E. coli, E. faecalis* and *A. baumannii*. Petroleum ether and n-Hexane showed least antibacterial activity against all MDR strains. The study conducted by Somaie Shafiei et. al (2013) was found that antimicrobial activity of cactus *Opuntia stricta* extracts against both gram-positive and gram-negative bacteria was investigated.[14] It was clarified that *Opuntia stricta* extract is an effective extract with antibacterial and anti-fungal activity. The most sensitive microorganism was *Staphylococcus aureus* (PTCC 1764).The findings of present study may form the basis for further investigation to isolate active compounds, elucidate the structure and evaluate it against wide range of antibiotic resistant bacteria with the subject to find new therapeutic principles. Many reports have been showed the effectiveness of traditional herbs against microorganisms.[15][16] Shahidi et al (2004), antibacterial and antifungal activity of methanol plant-extracts of 221 species from 98 families which had documented uses in Iranian herbal-medicine were screened for against 11 standard bacterial strains and 3 fungal species at 20 mg/ ml.[17] Eighty one samples in 39 families showed antibacterial and/or antifungal activity against at least on one of the tested microorganism.[17] Adomi et al the antibiotic activity of aqueous and ethanol extracts of the root bark of two plants Alstonia boonei De wild and Morin dalucida against seven bacteria and showed different bioactive components are present in each species.[18]
Table No. 1: Phytochemical analysis of *Opuntia ficus-indica* L.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Phytochemical analysis</th>
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<td>Polyamides</td>
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<td>4</td>
<td>Reducing Sugars</td>
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<td>9</td>
<td>Di &amp; Tri-terpenes</td>
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<td>Proteins</td>
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Table No. 2: Antibacterial activity of *Opuntia ficus-indica* L. (Cactus) against multiple drug resistant (MDR) bacteria

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<th>Plant Part used</th>
<th>Solvent</th>
<th>Zone of inhibition in mm</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>K. pneumoniae</th>
<th>P. mirabilis</th>
<th>S. typhi</th>
<th>E. faecalis</th>
<th>C. freundii</th>
<th>A. baumannii</th>
<th>S. pneumoniae</th>
<th>E. faecium</th>
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NZ – No Zone

Table No. 3: MIC of *Opuntia ficus-indica* L. (Cactus) against Multiple drug resistant (MDR) bacteria

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<th>Plant Part used</th>
<th>Solvent</th>
<th>Minimum Inhibitory Concentration in mg/ml</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>K. pneumoniae</th>
<th>P. mirabilis</th>
<th>S. typhi</th>
<th>E. faecalis</th>
<th>C. freundii</th>
<th>A. baumannii</th>
<th>S. pneumoniae</th>
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REFERENCES


