ANTIMICROBIAL ACTIVITY OF OPUNTIA STRICTA (FLOWERS)

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ABSTRACT
A large number of medicinal plants are claimed to be useful in treating skin diseases in all traditional system of medicine. The purpose of the present study was to examine the antimicrobial effect of the sample from the ethylacetate fraction of flowers of Opuntia stricta. This sample was shown to possess antimicrobial activity against bacteria and fungi, viz. Six bacterial strains were Salmonella typhi, Escherichia coli, Enterococcus faecalis, Bacillus cereus, Bacillus subtilis, Lactobacillus and two fungal strains Curvularia lunata and Candida albicans by using disc diffusion method. The anti bacterial activity of the sample from ethyl acetate fraction is almost comparable with standard solvent control Chloramphenicol. The anti fungal activity is almost comparable with standard solvent control Fluconazol. From this study, it can be concluded that Opuntia stricta (flowers) reveal antimicrobial activity against various human pathogenic bacteria.

KEYWORDS: Opuntia stricta; antibacterial activity; antifungal activity; diffusion method; Chloramphenicol;

INTRODUCTION
Nature is and will still serve as man’s primary source for the cure of his ailments. However, the potential of higher plants as a source for new drugs is still largely unexplored.¹ The traditional system of using medicinal plants for curing many diseases dates back to the age of Rig Veda. Many microbial diseases can be cured by medicinal plants without any side effects and economical issues.² Multidrug resistance towards antibiotics and their related effects has
an added effect to pursue the use of natural drugs.\[^3\] Infection with various microorganisms is one of the leading causes for a number of diseases.\[^4\]

The Opuntia genus belongs to the Cactaceae family and comprises from 200 to 300 species. These plants grow in arid and semiarid regions because of their low water requirements, and are considered by the FAO to be a valuable alternative for agriculture development in dry regions, where water supply is scarce and expensive.\[^5\] Several species of the Opuntia genus are grown for fresh consumption and forage. Fruits are incorporated in confectionery specialities, such as jam, candy, liquor, juice, etc. Some Opuntia species seem to contain agents that have shown analgesic, anti-inflammatory, antioxidant, hypoglycemic and neuroprotective effects, and might be useful in cancer chemoprevention.\[^6–9\]

Opuntia stricta occurs naturally in Texas, Alabama, Georgia, Mississippi, Florida and South Carolina in the United States as well as the Bahamas, Bermuda, the Caribbean, eastern Mexico, Central America, northern Venezuela, and Ecuador. Opuntia stricta has been introduced to many parts of the world, including Africa, Southern Europe, Australia and Southern Asia.

Opuntia stricta can grow up to 2 meters in height and produce lemon yellow flowers followed by purplish-red fruits. The term ‘prickly pear’ also relates to the fruits which are often spiny and pear-shaped. Common names of Opuntia stricta are Erect Prickly Pear, prickly cactus pear, Haw and Nopal Estricto (Spanish), Bengali: nagphana, phenimama; Gujarati: chorhathalo, zhorhatheylo; Hindi: hathhathoria, samar; Oriya: nagophenia, nagopheni, poturiyasiju, Punjabi: chhittarthohar; Sanskrit: kanthari, vidara; Tamil: manjarnagadali, mullukkallli, nagadali, nagathali, sappathikkalli; Telegu: nagadali, nagajanadu, nagajemudu, nagamullu, Urdu: nagaphani, thuar.\[^10–12\] Considering these facts, it is expected that the screening and scientific evaluation of the flowers of O. stricta may provide novel antimicrobial compounds.

**MATERIALS AND METHODS**

**Collection of Flowers**

Fresh flowers of Opuntia stricta were collected from Z. Suthamalli, Ariyalur (Dt), Tamil Nadu, India, during the month of January and identified by Dr. S. John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. DP005 dated: 22/01/2016). St. Joseph’s College (Campus), Trichy, Tamil Nadu, India.
Extraction and fractionation
Fresh flower (1kg) of Opuntia stricta collected at Z. Suthamalli, Ariyalur (Dt), Tamil Nadu, India were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80°C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Petroleum ether fraction and diethyl ether fraction did not yield any isolable material. Ethyl acetate fraction on concentration yielded a dry powder which was dissolved in DMSO to get various concentrations and were used for further study.

Antimicrobial procedure
Screening of antibacterial activity
Bacteria tested
Four bacterial strains were used throughout investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

Preparation of inoculums
Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton Broth (MHB) that were incubated without agitation for 24 hrs at 37°C. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0x10^6 colony forming units (CFU/ml).

Antibacterial susceptibility test
The disc diffusion method (Bauer et al., 1966) was used to screen the antibacterial activity. In-vitro antibacterial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The compound of concentration 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml were loaded on 6 mm sterile disc. The loaded disc were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with
transparent ruler in millimeter. Standard antibiotic Chloramphenicol of concentration 1mg/ml was used as positive control.[13]

Table No. I: Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of Opuntia stricta

<table>
<thead>
<tr>
<th>S.No</th>
<th>Organisms</th>
<th>Zone of inhibition(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Standard (Chloramphenicol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>Salmonella typhi</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Escherichia coli</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>Enterococcus faecalis</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>Bacillus cereus</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>Bacillus substilis</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>Lactobacillus</td>
<td>24</td>
</tr>
</tbody>
</table>

Fig. I: Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of Opuntia stricta
Graph No.1: Graphical representation of anti bacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Opuntia stricta* (Standard: Chloramphenicol, concentration 1 mg/ml)

**Screening of antifungal activity**

**Culture Media**
The media used for antifungal test was Sabouraud’s dextrose agar/broth of Hi media Pvt. Bombay, India.

**Inoculum**
The fungal strains were inoculated separately in Sabouraud’s dextrose broth for 6 h and the suspensions were checked to provide approximately 105 CFU/ml.

**Determination of antifungal activity**
The agar well diffusion method (Perez, 1993) was modified. Sabouraud’s dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabourauds dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with sample solution and solvent blanks (hydro alcohol, and hexane). Standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37°C for 72 hrs. The diameters of zone of inhibition observed were measured.

**Table No. II: Antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of Opuntia stricta**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Organisms</th>
<th>Zone of inhibition (mm)</th>
<th>Standard (Fluconazole)</th>
<th>Sample Concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>Curvularia lunata</td>
<td>27</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Candida albicans</td>
<td>19</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

In the present study, Opuntia stricta flowers were screened for antimicrobial activity and compared with standard drug. It is evident from the data presented in Table I that the compound isolated from the ethyl acetate fraction of Opuntia stricta flowers possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 6 mm, 0 mm, 6 mm, 0 mm, 0 mm and 6 mm for 30 mg/ml as 9 mm, 9 mm, 9 mm, 0 mm, 8 mm and 9 mm for 40 mg/ml showing 14 mm, 14 mm, 11 mm, 10 mm, 11 mm and 12 mm for 50 mg/ml as 16 mm, 17 mm, 16 mm, 14 mm, 13 mm and 15 mm for the test sample against Salmonella typhi, Escherichia coli, Enterococcus faecalis, Bacillus cereus, Bacillus substilis and Lacto bacillus respectively when compared with standard drug Chloramphenicol showing 20 mm, 23 mm, 25 mm, 24 mm, 18 mm and 24 mm zone of inhibition respectively.

Then it is evident from the data presented in Table II that the test sample possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 0
mm and 6 mm, for 30 mg/ml as 8 mm and 9 mm, for 40 mg/ml as 11 mm and 12 mm, for 50 mg/ml as 15 mm and 15 mm for the test solution against Curvularia lunata and Candida albicans respectively when compared with standard drug Fluconazole showing 27 mm and 19 mm of inhibition respectively.

CONCLUSION
In recent years molecules from natural origin had gained more popularity due to less side effects and better therapeutic action, particularly in antimicrobial field because of rapidly developing resistance to synthetic molecules. Present study indicates that the sample from the ethyl acetate fraction of flowers of Opuntia stricta shows good pharmacological action. That means Opuntia species has wide scope to isolate various phytochemical constituent and evaluate their pharmacological screening to get better therapeutic value. However, further works are needed to evaluate the actual clinically effective antibacterial compounds inherent in the plant material.

REFERENCES


