Antimicrobial Activity of *Datura innoxia*(Flowers)

**ABSTRACT**

A large number of medicinal plants are claimed to be useful in treating skin diseases in all traditional systems of medicine. The present study was carried out to investigate the antimicrobial effect of the compound isolated from the ethyl acetate fraction of flowers of *Datura innoxia*. This compound was shown to possess antimicrobial activity against bacteria and fungi. Four bacterial strains *Salmonella typhi*, *Escherichia coli*, *Enterococcus faecalis*, *Bacillus cereus* and two fungal strains *Curvularia lunata* and *Candida albicans* were tested by using disc diffusion method. The antibacterial activity of the compound isolated from ethyl acetate fraction is almost comparable with standard solvent control *Chloramphenicol*. The antifungal activity is almost comparable with standard solvent control *Fluconazole*.

**Keywords:** *Datura innoxia*; antibacterial activity; antifungal activity; diffusion method; chloramphenicol; fluconazole.
INTRODUCTION

Infectious diseases are the leading foundation of death worldwide. Antibiotic resistance has become a global concern [1]. The medical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens [2]. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [3]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [4]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have led to the screening of several medicinal plants for their potential antimicrobial activity [5,6].

*Datura* is known (vellum mattai) as thorn apple, prickly burr, jimson weed, moon flower, devil’s weed, devil’s cucumber and devil’s trumpet. All *Datura* plants found to contain tropane alkaloids such as scopolamine, hyoscyamine, and atropine, primarily in their seeds and flowers [7]. *Datura innoxia* (Family: Solanaceae) is used for many medicinal purposes. *Datura* precise and natural distribution is uncertain, owing to its extensive cultivation and naturalization throughout the temperate and tropical regions of the globe. In Sudan, *Datura innoxia* is widely spread in AL Jazeera State and other States. It is native to Central and South America, and introduced in Africa, Asia, Australia and Europe [8]. It contains tropane alkaloids such as scopolamine, hyoscyamine, hyoscine, norscopolamine, meteloidine, [9, 10] flavonoids, cardacis glycosides, essential oils, saponins and phenols [11,12]. Traditional medicine uses flowers, leaves and seed of *Datura innoxia* to treat skin eruptions, colds, and nervous disorders [13]. It has been used in the earlier period as antispasmodic, hallucinogenic, hypnotic and narcotic and also in the treatment of insanity, impotence, asthma, diarrhea, as an analgesic, to control fever, kill parasites, and skin diseases [14].
MATERIALS AND METHODS

Collection of Flowers

Fresh flowers of *Datura innoxia* were collected from S. Pudur, Sivagangai (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. AR001 dated: 08/01/2016). St. Joseph’s College (Campus), Tiruchirappalli, Tamil Nadu, India.

Extraction and fractionation

Fresh flower (3 kg) of *Datura innoxia* 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 of 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 the 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 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 petri plates. 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 compounds of concentration 20 mg/ml, 30 mg/ml, 40 mg/ml, 50mg/ml were loaded on 6 mm sterile disc. The loaded discs 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 [15].

<p>| Table No. I Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of <em>Datura innoxia</em> |</p>
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Organisms</th>
<th>Zone of inhibition(mm)</th>
<th>Standard (Chloramphenicol)</th>
<th>Sample Concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Salmonella typhi</em></td>
<td>22</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td><em>Escherichia coli</em></td>
<td>20</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td><em>Enterococcus faecalis</em></td>
<td>21</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td><em>Bacillus cereus</em></td>
<td>19</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

Fig. I: Images showing the antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Datura innoxia*
Graph No. I: Graphical representation of antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Datura innoxia* (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 HiMedia Pvt Ltd, 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) 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 (Fluconazole, 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 \textit{Datura innoxia}

<table>
<thead>
<tr>
<th>Sr. 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>\textit{Curvularia lunata}</td>
<td>20</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>\textit{Candida albicans}</td>
<td>18</td>
<td>12</td>
<td>18</td>
</tr>
</tbody>
</table>

![Curvularia lunata and Candida albicans](image)

Fig. II: Images showing the antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of \textit{Datura innoxia}

![Graphs](image)

Graph No. II: Graphical representation of antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of \textit{Datura innoxia} (Standard: Fluconazole, concentration 1 mg/ml)
RESULTS AND DISCUSSION

In the present study, Datura innoxia 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 Datura innoxia flowers possesses antibacterial activity. The result of disc diffusion method showed the zone of inhibition for 20 mg/ml as 0 mm, 7 mm , 9mm and 10 mm, for 30 mg/ml as16 mm, 17 mm, 17mm and 20 mm, for 40 mg/ml showed as 21 mm, 24 mm, 23mm and 22 mm, and for 50 mg/ml as27 mm, 27 mm, 28mm and 30 mm, for the test sample against Salmonella typhi, Escherichia coli, Enterococcus faecalis and Bacillus cereus respectively when compared with standard drug chloramphenicol showing 22 mm, 20 mm , 21 mm and 19 mm zone of inhibition respectively.

It is evident from the data presented in Table II that the test sample possesses antifungal activity. The result of disc diffusion method showed the zone of inhibition for 20 mg/ml as 11 mm and 12 mm, for 30 mg/ml as16 mm and 18 mm, for 40 mg/ml as 21 mm and 21 mm, and for 50 mg/ml as 29 mm and 28 mm for the test solution against Curvularia lunata, and Candida albicans respectively when compared with standard drug Fluconazole showing 20 mm and 18mm zone of inhibition respectively.

CONCLUSION

Our investigation showed that the antimicrobial activity of the compound isolated from the ethyl acetate fraction of flowers from Datura innoxia might be due to the presence of flavonoids. The flowers studied here can be a source of high pharmacological importance and potential source of new drugs. Further studies on such bioactive compound will unravel the potentiality of these traditional medicines.

REFERENCES