ANTIMICROBIAL ACTIVITY OF HIBISCUS SABDARIFFA (FLOWERS)

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disc diffusion method.

Chloramphenicol.

The aim of the present study was to investigate the antimicrobial effect of the sample isolated from the ethyl acetate fraction of flowers of \textit{Hibiscus sabdariffa}. This compound was shown to possess antimicrobial activity against bacteria and fungi, viz. Six bacterial strains were \textit{Salmonella typhi}, \textit{Escherichia coli}, \textit{Enterococcus faecalis}, \textit{Bacillus cereus}, \textit{Bacillus subtilis}, \textit{Lacto bacillus} and two fungal strains \textit{Curvularia lunata} and \textit{Candida albicans} by using disc diffusion method. The anti bacterial activity of the compound isolated from ethyl acetate fraction is almost comparable with standard solvent control \textit{Chloramphenicol}. The anti fungal activity is almost comparable with standard solvent control \textit{Fluconazole}. From this study, it can be concluded that \textit{Hibiscus sabdariffa} (flowers) reveal antimicrobial activity against various human pathogenic bacteria.

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INTRODUCTION

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value [1]. Plants are used in modern medicine where they occupy a very significant place as raw material for important drugs [2]. Plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. Plants are rich sources of ecologically developed secondary metabolites, which are potential remedies for different ailments. Extreme interest in plants with microbial activity has revived as result of current problems such as resistance associated with the use of antibiotics obtained from micro organisms [3].

_Hibiscus sabdariffa_ L., (Malvaceae), commonly known as “roselle”, is an important medicinal plant native to India and Malaysia, although it grows widely in the tropics and subtropics of both hemispheres and has become naturalized in many areas in Central America [4]. The plant is an annual, erect, herbaceous sub-shrub with a deep root system. The plant has fibrous stems, small branches, as well as bright red and acidic-tasting calyces. In folk medicine, an infusion from the calyces is used as a diuretic and to treat gastrointestinal disorders, liver diseases, fever, hypercholesterolemia, and hypertension [5]. Extracts from the calyces are reported to have a variety of therapeutic effects _in vivo_ and _in vitro_, including anticancer and antioxidant properties [6-9]. Considering these facts, it is expected that the screening and scientific evaluation of the flowers of _H.sabadariffa_ may provide novel antimicrobial compounds.

MATERIALS AND METHODS

Collection of Flowers

Fresh flowers of _Hibiscus sabdariffa_ 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. DP003 dated: 22/01/2016). St.Joseph’s College (Campus),Trichy, Tamil Nadu, India.

Extraction and fractionation

Fresh flower (1kg) of _Hibiscus sabdariffa_ 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 [10].
Table No. I: Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of Hibiscus sabdariffa.

<table>
<thead>
<tr>
<th>S.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>Salmonella typhi</td>
<td></td>
<td>20</td>
<td>8 10 12 14</td>
</tr>
<tr>
<td>2</td>
<td>Escherichia coli</td>
<td></td>
<td>26</td>
<td>6 8 11 14</td>
</tr>
<tr>
<td>3</td>
<td>Enterococcus faecalis</td>
<td></td>
<td>21</td>
<td>7 9 12 14</td>
</tr>
<tr>
<td>4</td>
<td>Bacillus cereus</td>
<td></td>
<td>25</td>
<td>0 8 14 16</td>
</tr>
<tr>
<td>5</td>
<td>Bacillus subtilis</td>
<td></td>
<td>19</td>
<td>7 8 12 14</td>
</tr>
<tr>
<td>6</td>
<td>Lactobacillus</td>
<td></td>
<td>24</td>
<td>8 10 11 14</td>
</tr>
</tbody>
</table>

Fig. I: Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of Hibiscus sabdariffa.

Graph No. 1: Graphical representation of antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of Hibiscus sabdariffa. (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 Sabouraud’s 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 Hibiscus sabdariffa.

<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>28</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Candida albicans</td>
<td>18</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

Fig. II: Antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of Hibiscus sabdariffa.

Graph No.2: Graphical representation of antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of Hibiscus sabdariffa. (Standard: Fluconazole, concentration 1 mg/ml).

RESULTS AND DISCUSSION
In the present study, Hibiscus sabdariffa flowers were screened for antimicrobial activity and compared with standard drug. It is evident from the data presented in Table I (Fig I) that the compound isolated from the ethyl acetate fraction of Hibiscus sabdariffa flowers possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 8 mm, 6 mm, 7 mm, 0 mm, 7 mm and 8 mm for 30 mg/ml as 10 mm, 8 mm, 9 mm, 8 mm, 8 mm and 10 mm for 40 mg/ml showing 12 mm, 11 mm, 12 mm, 14 mm, 12 mm and 11 mm for 50 mg/ml as 14 mm, 14 mm, 14 mm, 16 mm, 14 mm and 14 mm for the test sample against Salmonella typhi, Escherichia coli, Enterococcus faecalis, Bacillus cereus, Bacillus subtilis and Lacto bacillus respectively when compared with standard drug Chloramphenicol showing 20 mm, 26 mm, 21 mm, 25 mm, 19 mm and 24 mm zone of inhibition respectively.

Then it is evident from the data presented in Table II (Fig II) that the test sample possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 7 mm and 0 mm, for 30 mg/ml as 10 mm and 8 mm, for 40 mg/ml as 12 mm and 11 mm, for 50 mg/ml as 14 mm and 15 mm for the test solution against Curvularia lunata and Candida albicans respectively when compared with standard drug Fluconazole showing 28 mm and 18 mm of inhibition respectively.

CONCLUSION
Hibiscus sabdariffa is very important medicinal plant with very diverse phytochemical constituents the antimicrobial studies support its traditional uses and may prove to be useful for clinical evaluation and development of commercial drugs.
REFERENCES


