ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS OF PLUMERIA RUBRA L.

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ABSTRACT

Petroleum ether, carbon tetrachloride, chloroform and ethyl acetate extracts of Plumeria rubra leaves were studied for their antimicrobial activities against eleven human pathogenic bacteria, viz., Shigella dysenteriae, S. sonnei, Salmonella typhi, S. paratyphi, Bacillus subtilis, B. megaterium, B. cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Vibrio cholerae and four human pathogenic fungi, viz., Aspergillus niger, A. ochraceus, A. ustus and Candida albicans using disc diffusion and poisoned food method, respectively. Chloroform and ethyl acetate extract exhibited moderate to good antibacterial and antifungal activity against all the pathogens tested. The ethyl acetate extract exhibited the largest zone of inhibition (25 mm in diameter with 2000 μg/disc extract) against E. coli. The highest inhibition of fungal radial mycelial growth (62.00% with 100 μg extract/ml medium) was recorded against A. ustus with ethyl acetate extract. The MICs were determined by broth macrodilution technique. The ethyl acetate extract exhibited the lowest MIC (750 μg/ml) against E. coli. However, for fungi the lowest MIC was 500 μg/ml against A. ustus with the same extract.

Key words: Antimicrobial activity, crude extract, leaf, Plumeria rubra.

INTRODUCTION

Various plant species have been serving as the natural source of drugs and medicines from the beginning of civilization. The use of, and search for drugs and dietary supplements derived from plants have accelerated in recent years. Traditional healers have long used plants to prevent or cure infectious conditions and western medicine is trying to duplicate their successes. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, saponins, glycosides, quinolines, essential oils and flavonoids, which have been found in vitro to have antimicrobial properties (Ahmed et al. 2002, Aureli et al. 1992, Rahman et al. 1999). They are capable of mitigating and curing human
sufferings, healing wounds, cuts, burns and other antimicrobial source. Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases (Davis 1994). This situation forced scientists to search for new antimicrobial substances from various sources. Now-a-days, the natural products have been accepted as important sources of biologically active antimicrobial substances, and the major sources of which are still left undiscovered. But a few works have been done in this field in Bangladesh (Rahman et al. 1999, Ahmed et al. 1999).

*Plumeria rubra* L. is a small tree with crooked trunk, rough bark and pink fragrant flowers, which belongs to the family Apocynaceae. It grows everywhere in Bangladesh as an ornamental plant. Plant principally contains triterpenes, plumeric acid, glycosides, plumieride and methyl ester. Juice of leaves is used as poultice to swelling and stem bark is used in diarrhoea and piles (Ghani 1998). Considering above mentioned facts, the present work has been undertaken to observe antimicrobial activity of extracts of *P. rubra*.

**MATERIALS AND METHODS**

*Collection and extraction of plant material*

Leaves of *Plumeria rubra* were collected in fresh condition from the Chittagong University campus, Chittagong, Bangladesh. The collected and cleaned samples were cut into small pieces (1-2 cm), dried in air to make it suitable for grinding. The samples were ground to fine powder mechanically and then 50 g of the dried powder was kept steeped for 72 hours in petroleum ether, chloroform, ethyl acetate and carbon tetrachloride. The extracts thus obtained separately were filtered, centrifuged at 5000 rpm for 20 minutes and concentrated to a gummy material under reduced pressure at 50°C by rotary vacuum evaporator. The gummy materials were then collected in small vials and dried to obtain the crude extract.

*Test organisms*

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The test organisms were collected from Department of Microbiology, University of Chittagong, Bangladesh.

Determination of antimicrobial activity

The in vitro antibacterial and antifungal activities of the crude extract of the plant were determined by disc diffusion method (Bauer et al. 1966) and poisoned food technique (Miah et al. 1990), respectively. Mueller-Hinton (agar and broth) medium was used for culture of bacteria and Sabouraud (agar and broth) medium was used for fungi. A 10% solution of the crude extract was used as the test material. All the results were compared with the standard antibacterial antibiotic ampicillin [20µg/disc, BEXIMCO Pharma Bangladesh Ltd.] and antifungal antibiotic nystatin [100µg/ml medium, BEXIMCO Pharma Bangladesh Ltd.]. MIC of the crude extract was determined by macro-dilution broth technique (Jones et al. 1985).

RESULTS AND DISCUSSION

The crude extracts (petroleum ether, chloroform, ethyl extract and carbon tetrachloride extracts) obtained from Plumeria rubra were screened for their antibacterial activity against eleven human pathogenic bacteria and compared to that of standard antibacterial antibiotic ampicillin. The results of the sensitivity test are presented in Table 1. Among the four solvent extracts, only chloroform and ethyl acetate extracts showed antibacterial activity. The chloroform and ethyl acetate extracts exhibited good antibacterial activity against the test organisms tested. But crude extracts of petroleum ether and carbon tetrachloride did not show antibacterial activity at a concentration of 2000 µg/disc extract. The ethyl acetate extract exhibited the largest zone of inhibition (25 mm in diameter with 2000 µg/disc extract) against E. coli. The standard antibiotic ampicillin (20µg/disc) was also found to be active against all the bacteria tested. Similar antibacterial activity of other plant extracts has been reported previously (Sarker et al. 1991, Rojas et al. 1992, Brantner and Grein 1994, Rahman et al. 1998, Ahmed et al. 1999).

The antifungal activity of the crude extract (100 µg/ml medium) against four human pathogenic fungi was studied and compared with that of standard antifungal antibiotic nystatin. The results of the inhibition of radial mycelial growth of fungi are summarized in Table 2.
TABLE 1: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS FROM *PLUMERIA RUBRA*.

<table>
<thead>
<tr>
<th>Name of bacteria</th>
<th>Diameter of inhibition zone in mm. (Crude extract 2000µg/disc)</th>
<th>Ampicillin* 20µg/disc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Petroleum ether</td>
<td>Carbon tetrachloride</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. megaterium</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. cereus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. sonnei</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. paratyphi</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Standard antibacterial antibiotic

TABLE 2: ANTIFUNGAL ACTIVITY OF THE CRUDE EXTRACTS FROM *PLUMERIA RUBRA*.

<table>
<thead>
<tr>
<th>Name of fungi</th>
<th>Percentage inhibition of fungal mycelial growth(^a) (100 µg/ml medium)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Petroleum ether</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>-</td>
</tr>
<tr>
<td>A. ochraceus</td>
<td>-</td>
</tr>
<tr>
<td>A. ustus</td>
<td>-</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>-</td>
</tr>
</tbody>
</table>

* Standard antifungal antibiotic ; Minus(-) mean no growth;
\(^a\)Growth measured- radial growth in mm.
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From Table 2, it appeared that the petroleum ether and carbon tetrachloride extracts have no antifungal activity at a concentration of 100 $\mu$g/ml medium. On the other hand, chloroform and ethyl acetate extract of P. rubra inhibited the radial mycelial growth of all the test fungi at a concentration of 100 $\mu$g/ml medium. The highest inhibition (62.0%) of fungal radial mycelial growth was recorded against A. ustus with ethyl acetate extract at a concentration of 100 $\mu$g/ml medium. Antifungal antibiotic nystatin (100$\mu$g/ml medium) exhibited inhibitions of radial mycelial growth of all the four fungi. Similar antifungal activities on plant extracts of other plants have also been previously reported (Naidu and John 1981, Shetty and Shetty 1987, Miah et al. 1990, Stange et al. 1993, Anwar et al. 1994).

The MIC values of the crude extracts obtained from P. rubra leaf are summarized in Table 3. It appeared that the chloroform and ethyl acetate extract exhibited the MIC values from 750 $\mu$g/ml to 2000 $\mu$g/ml against the bacterial pathogens. But petroleum ether and carbon tetrachloride extract did not show MIC up to the extract concentration of 2500 $\mu$g/ml. The lowest MIC (750 $\mu$g/ml) was recorded against E. coli with ethyl acetate extract. In case of fungi, chloroform and ethyl acetate extracts exhibited MICs from 500 $\mu$g/ml to 2000 $\mu$g/ml against the fungal pathogens. The lowest MIC (500 $\mu$g/ml) was recorded against A. ustus with ethyl acetate extract.
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TABLE 3: MICS OF CRUDE EXTRACTS FROM PLUMERIA RUBRA.

<table>
<thead>
<tr>
<th>Bacteria / fungi</th>
<th>MIC (Crude extract µg/ml medium)</th>
<th>Pet. ether</th>
<th>C. tetrachloride</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Bacteria:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>2000</td>
<td>1500</td>
</tr>
<tr>
<td>B. megaterium</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>1500</td>
<td>2000</td>
</tr>
<tr>
<td>B. cereus</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>1500</td>
<td>1000</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>2000</td>
<td>1000</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>2000</td>
<td>750</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>1500</td>
<td>1000</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>2000</td>
<td>1500</td>
</tr>
<tr>
<td>S. sonnei</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>1500</td>
<td>1000</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>1500</td>
<td>1500</td>
</tr>
<tr>
<td>S. paratyphi</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>1500</td>
<td>2000</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>1500</td>
<td>1000</td>
</tr>
<tr>
<td><strong>B. Fungi:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>2000</td>
<td>1000</td>
</tr>
<tr>
<td>A. ochraceus</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>1500</td>
<td>750</td>
</tr>
<tr>
<td>A. ustus</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>Candida albicans</td>
<td></td>
<td>NF</td>
<td>NF</td>
<td>750</td>
<td>750</td>
</tr>
</tbody>
</table>

NF – not found up to 2500 µg/ml

The present investigation confirms that there are antibacterial and antifungal properties in the crude extract of *Plumeria rubra* leaf. However, it is important to point out that crude extract such as this needs to be further processed to obtain pure compound(s) which can then be tested for antimicrobial activity.
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