Antimicrobial Assay of Zizyphus oenoplia (L.) Mill.

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INTRODUCTION

Microorganisms exist in every niche of our biosphere and occupy a peculiar place in the human view of life. The microbial population in the human body assists in its normal functioning like digestion and in enhancing resistance to infections. Without them, the pathogens can cause infections and abnormal digestion and other related problems in humans. Infectious diseases pervade human existence; they are the world’s leading causes of premature death killing almost 50,000 people every day (Mulligen et al. 1993). In recent years, drug resistance to human pathogenic microbes has been commonly reported from all over the world due to indiscriminate use of antibiotics (Ahmed and Beg 2001).

Human infection, particularly that involving microorganisms (bacteria, fungi, viruses, parasites, nematodes etc.), can cause serious problems in the tropical and subtropical regions of the world. In recent years, multiple drug resistance in human pathogenic microorganisms is a result of indiscriminate use of commercial antimicrobial drugs commonly administered in the treatment of such diseases. Over the last four decades, the intensive efforts have been made to discover clinically useful antimicrobial drugs (Perumal et al. 2004). This plant is also therapeutically used against cough, fever, dysentery, diarrohhea, ulcers, diabetes, fertility problems, snake-bite and skin disorders (Singh et al. 2002).

Hence, the present research investigates the antimicrobial activity of the leaf extracts (acetone and ethanol) of Z. oenoplia against the human pathogenic microbial populations (both bacteria and fungi).

MATERIALS AND METHODS

Plant material

The healthy leaves of Zizyphus oenoplia were collected from the foothills of Kolli hills (Namakkal District), one of the segments of Eastern Ghats of Tamil Nadu. The identification of the plant was confirmed with the assistance of the Book of Tamilnadu Carnatic (Matthew 1983) and the herbarium specimen was deposited in the Department of Botany, National College, Tiruchirappalli, Tamil Nadu. The plant materials were washed in tap water to remove the soil particles and shade dried for 10 days.

Microorganisms

In the present study, five bacterial strains (Pseudomonas putida, Vibrio cholerae, Shigella flexneri) and two Gram-positive bacteria (Staphylococcus aureus and Bacillus sp.) and two fungal strains (Candida albicans and Cryptococcus neoformans) by conducting a well-in-agar method. P. putida and V. cholerae exhibited better resistance against the plant extracts followed by Shigella flexneri and Bacillus sp. Other pathogens were ineffective against the plant extracts.

Keywords: antimicrobial activity, plant extracts, pathogenic microorganisms, Zizyphus oenoplia

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The greenish extracts obtained were passed through the Whatmann filter paper No.1 and the respective solvents were evaporated at 40°C with the help of heating mantle. The sticky black substances were obtained and stored in refrigerator and dissolved in DMSO (dimethyl sulfoxide) prior to use for the antimicrobial activity tests. Each extract was tested in triplicate and the standard deviations were calculated (Gupta 1977).

Preparation of inoculum

The test bacterial and fungal strains were inoculated onto nutrient broth/SD broth and incubated at 37°C (24 h) for bacteria and 25°C (24-72 h) for the fungal species. After the appropriate incubation period, the bacterial cultures were compared with the turbidity (opacity) standard.

Screening for antimicrobial properties

Antimicrobial properties of plant extracts were tested by well-inoculated agar method with some modifications. The culture plates were prepared by pouring twenty ml of Mueller Hinton Agar medium into sterile Petri dishes. The inoculum suspension was spread uniformly over the agar medium using sterile cotton swabs to get uniform distribution of bacteria and fungal spores. Using a cork borer, well of 5 mm diameter was made in the media at a distance of 1-2 cm from the periphery of the plates. These plates were labeled and 0.2 ml of each extract (at different concentration of extracts i.e. 100 μg, 200 μg, 300 μg and 400 μg) was added aseptically into the well. Then the plates were incubated for 24 h (at 37°C) for bacteria and 25°C (24-72 h) for the fungal species. After the appropriate incubation period, the extracts tested will help in formulating drugs against the pathogens.

The results of antimicrobial screening of the acetone and ethanol leaf extracts of Zizyphus oenoplia (Diameter of Growth Inhibition zone in mm).

<table>
<thead>
<tr>
<th>Solvents used</th>
<th>Extract conc. (μg)</th>
<th>Pseudomonas putida</th>
<th>Vibrio cholerae</th>
<th>Shigella flexneri</th>
<th>Staphylococcus aureus</th>
<th>Bacillus species</th>
<th>Candida albicans</th>
<th>Cryptococcus neofor mans</th>
<th>Tested microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>100</td>
<td>10.33 ± 0.94</td>
<td>10.00 ± 0.00</td>
<td>10.33 ± 0.47</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>200</td>
<td>11.00 ± 0.00</td>
<td>10.33 ± 0.47</td>
<td>10.00 ± 0.00</td>
<td>10.33 ± 0.47</td>
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<tr>
<td></td>
<td>300</td>
<td>11.66 ± 0.46</td>
<td>11.33 ± 0.47</td>
<td>11.00 ± 0.00</td>
<td>10.00 ± 0.00</td>
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<td>0</td>
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<tr>
<td></td>
<td>400</td>
<td>13.00 ± 0.82</td>
<td>12.33 ± 0.47</td>
<td>13.66 ± 0.46</td>
<td>11.00 ± 0.00</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Ethanol</td>
<td>100</td>
<td>10.33 ± 0.46</td>
<td>10.00 ± 0.00</td>
<td>0</td>
<td>0</td>
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<td></td>
<td>400</td>
<td>13.66 ± 0.94</td>
<td>14.00 ± 0.00</td>
<td>0</td>
<td>10.00 ± 0.00</td>
<td>0</td>
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<tr>
<td>Standard antibiotics</td>
<td>25.00 ± 0.00</td>
<td>20.33 ± 0.46</td>
<td>18.33 ± 0.46</td>
<td>15.00 ± 0.00</td>
<td>15.00 ± 0.00</td>
<td>15.00 ± 0.00</td>
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</tbody>
</table>

Antibiotics – Tetracycline (bacteria); penicillin (fungi)

RESULTS AND DISCUSSION

Based on our findings, most of the pathogens studied have considerable resistance against the various concentrations of leaf extracts, which is supported by Rajakaruna et al. (2002), who isolated phytochemicals from the fruit extracts of Zizyphus sativa. Similarly other plants belonging to Rhamnaceae have also been reported to have some antimicrobial properties e.g., Ventilago madaraspatana (Subhalakshmi et al. 2005), Cordialia buxifolia (Morel et al. 2002), Scutia buxifolia (Morel et al. 2005), etc.

REFERENCES


Charidy CM, Seaford CE, Phelps RH, Pollard GV, Khambay BP (1999) Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. Journal of Ethnopharmacology 64, 265-270

Gupta SP (1977) Statistical Methods, S. Chand &Co., New Delhi, total pp

Kinghorn AD (1987) Biologically active compounds from plants with reputed medicinal and sweetening properties. Journal of Natural products 50, 1009-1024

Matthew KM (1983) The flora of Tamil Nadu Carnatic, Diocesan press, Chennai, Tamil Nadu, India, total pp


Nayak Prokash, Calcutta, India, 317
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