ANTIBACTERIAL ACTIVITY OF METHANOLIC FRUIT EXTRACT OF RANDIA DUMETORUM LAMK

Movalia Dharmishtha¹* and Gajera Falguni¹

¹Dept. of pharmacognosy, S. J. Thakkar Pharmacy College, Rajkot-360005, India.

*Email : dvdharmi@gmail.com, dharmishtha24@yahoo.com Tel.: +91-820-2922482

ABSTRACT : The goal of this study was to determine the preliminary antibacterial activity of Methanolic extract of Randia dumetorum Lamk. (Xeromphis spinosa Thumb.) belonging to family Rubiaceae toward some phytopathogenic bacteria. The antibacterial activity of the extract was done on some standard and wild pathogenic bacterial strains such as Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis, Escherichia coli and Salmonella typhi. The testing was done by the agar cup plate method using sterile top agar. Zone of inhibition of extract (50, 100 and 150 mg/ml) was compared with that of standard Amoxicillin (0.5 and 1 mg/ml) prepared in DMSO. The extract shows potential antibacterial properties comparable with that of standard amoxicillin against the organisms tested. The methanolic extract of R. dumetorum displayed a concentration related antibacterial activity. The results show that the inhibition of the bacterial growth was more pronounced on Escherichia coli as compared to the other tested organisms.

Keywords: Antibacterial activity, Randia dumetorum, cup plate method, Xeromphis spinosa Thumb.

INTRODUCTION
The most critical problem related with health and causes of mortality rates in society is infectious disease¹. Since last decade, there has been an increasing evidence of bacterial and fungal infections due to population explosion, pollution, changed environmental conditions, wastes from different sources, which may effect food with perfect nutrition value. This factor causes less immunogenecity in human beings and animals. This fact coupled with the resistance developed in microorganisms to allopathic agents, antibiotics with increased toxicity in human being and animal during prolonged treatment with several antimicrobial drugs². These facts motivate us to find out a new herbal moiety, which can act as an antimicrobial biomolecules to treat opportunistic microbial infections³. This paper reports the in-vitro antimicrobial screening of methanolic extracts of R. dumetorum against clinical isolates of six bacterial species.

In Indian system of medicine Randia dumetorum (Xeromphis spinosa Thumb.) belonging to family Rubiaceae is an important medicinal plant and popularly known as metic nut. It is found in waste places & jungles all over India, extending northwest to the Bias river & ascending to outer Himalaya to 4000 ft⁴. Literature survey reveals that the fruit is bitter, sweet; heating, aphrodisiac, emetic, purgative, carminative, antipyretic; cures abscess, ulcers, inflammations, wounds, tumors, skin diseases and have antibacterial activity. The pulp of fruit is believed by many practitioners to also have anthelmintic properties, and also used as an abortifacient as folklore remedy⁵⁻⁶. But the data of systemic and scientific antimicrobial study is not available so far. Therefore it was our intention to study the antibacterial profile of R. dumetorum by using different microbial species.

MATERIALS AND METHODS
Plant collection and authentication
Fruits of Randia dumetorum were collected during November from Botanical garden of M.S.U. Baroda and were identified by Head of Botany department, M. S. University, Baroda. A voucher specimen has been deposited in the museum of department of Pharmacognosy, M.S.U. Baroda. Voucher specimen (PH-805) was deposited in the herbarium of Pharmacy Department of M.S.U. Baroda.

Plant preparation and extraction
The fruits were dried in sunlight and reduced to a coarse powder. Then the powder was subjected to soxhlet extraction with methanol for 72 hours at a temperature of 50-60°C. The extract was concentrated and the solvent was completely removed. They were freeze dried and stored in the vacuum dessicator until further use.

Preliminary phytochemical screening⁷
Preliminary phytochemical screening revealed the presence of phytosterol, polyphenols, saponins, flavonoids and carbohydrates.

**Microorganisms**

Standard cultures of following microorganisms were obtained from Food and Drug Laboratory, Baroda. The microorganisms were identified by staining techniques. The organisms were maintained by sub culturing at regular intervals in nutrient agar medium.

**Gram +ve bacteria:** Staphylococcus aureus
Staphylococcus epidermidis
Bacillus cereus
Bacillus subtilis

**Gram -ve bacteria:** Escherichia coli
Salmonella typhi

**Preparation of inoculums**

The suspension of all organisms were prepared by inoculating one colony of the strain in 20 ml of nutrient broth in conical flask and incubated at 37°C for 24 hours to activate the strain. The suspension is adjusted such that it contained approximately 1 x 10^8 colony forming units/ml. It was obtained by calculating the cells by Neubers chamber. Nutrient agar (HiMedia) was prepared for the study.

**Culture medium**

The medium was prepared by dissolving 13 gm of nutrient broth in 1000ml of distilled water pH to (7.3 ± 0.2), and subjecting it to sterilization in an autoclave at 121°C for 15 min.

**Antimicrobial Agent**

The reference standard amoxicillin was procured as gift sample from Hindustan Antibiotics Ltd., Pune.

**Determination of minimum inhibitory concentration**

The molten nutrient agar media was prepared and distributed in Mc cartney bottles, 20 ml each, prior to sterilization. A measured amount of the methanol extract was added to each bottle in such a manner that the final concentration per ml of the agar medium was 0 (control), 5, 15, 25, 50 and 100 mg. the final mixture was poured individually into 100 mm sterile petriplates. For uniform diffusion of the drug throughout the medium, the nutrient agar plates containing different concentrations of the drug were refrigerated overnight at 4°C and then dried for 24h at 37°C before inoculation. One loopful (loop diameter – 2mm) of an over night grown bacteriological culture of the test organism at concentration – 10^6 colony forming units (cfu/ml) was placed in all the petriplates marked by checkerboard technique. The spot inoculated plates were incubated at 37°C for 24h and then observed for any growth of microorganisms. The minimum concentration of extract which prevent bacterial growth was taken as MIC (Table 1). The antibacterial growth was observed by formation of bacterial colony or turbidity around the inoculum’s spot.

**RESULT AND DISCUSSION**

The observations of the MIC study has been tabulated in table 1 and it was found that the minimum inhibitory concentration for methanolic fruit extract against E. coli is 15 mg/ml, where as for Salmonella typhi, Staphylococcus aureus and Staphylococcus epidermidis it was 25 mg/ml and for, Bacillus cereus and Bacillus subtilis were inhibited at 50 mg/ml. From the data it is evident that the methanolic extracts is active against both Gram positive and bacteria but more active against Gram negative at low concentration. The results of zone of inhibition of the methanolic fruit extract and comparison with standard antibiotic amoxicillin were recorded in Table 2. The result shows that the methanolic fruit extract of R. dumetorum displayed concentration dependent antibacterial activities. It indicates that R. dumetorum shows antibacterial activity towards all six investigated phytopathogenic bacteria. The highest antibacterial activity was found towards E. coli, while it was less active against S. aureus. The compounds responsible for this antimicrobial property were not investigated. However preliminary phytochemical analysis of the methanolic extract revealed the presence of phytosterol, polyphenols, saponins, flavonoids and carbohydrates. The antimicrobial potency of the plant may be attributed to the single or combined effect of the above mentioned chemical groups. The methanolic fruit extract of R. dumetorum had impressive antibacterial and could lead to the discovery of new antibiotics. This becomes more relevant as the current antibiotics in use are fast loosing effectiveness due to emergence of resistant microorganisms. The isolation of components of fruits of R. dumetorum methanol extract is in progress as very potent antimicrobial agents.
Table 1. Determination of MIC of methanolic fruit extract of R. dumetorum against different bacteria

<table>
<thead>
<tr>
<th>Name of bacteria</th>
<th>Growth in nutrient agar containing different concentration of extract in mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>S. aureus</td>
<td>+</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>+</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>+</td>
</tr>
<tr>
<td>B. cereus</td>
<td>+</td>
</tr>
<tr>
<td>E. coli</td>
<td>+</td>
</tr>
<tr>
<td>S. typhi</td>
<td>+</td>
</tr>
</tbody>
</table>

‘0’ – Control (without extract); ‘+’ – Growth; ‘−’ – No growth

Table 2. Antibacterial activity of Amoxicillin and fruits methanolic extract

<table>
<thead>
<tr>
<th>Micro organism</th>
<th>Zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extract Conc. mg/ml</td>
</tr>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td>S. aureus</td>
<td>12 ± 0.42</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>14 ± 0.51</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>7.5 ± 0.22</td>
</tr>
<tr>
<td>B. cereus</td>
<td>6 ± 0.31</td>
</tr>
<tr>
<td>E. coli</td>
<td>20 ± 0.73</td>
</tr>
<tr>
<td>S. typhi</td>
<td>14.5 ± 0.48</td>
</tr>
</tbody>
</table>

REFERENCES
9. Pelczar M.J. and Reid J.D., Microbiology, Tata Mcgraw Hill, New Delhi, 1974, 473.