IN VITRO EVALUATION OF ANTIMICROBIAL PROPERTIES OF TWO SPECIES OF GENUS GOMPHOSTEMMA

DEKA, H.†, GOGOI, D. K., GOGOI, H. K. AND HANDIQUE, P. J.†

Defence Research Laboratory, Post Bag No: 2, Tezpur-784001, †Department of Biotechnology, Gauhati University, Guwahati-784 014, India. E. mail: hemashree_deka@redifmail.com

Received: February 25, 2006; Revised: April 26, 2006; Accepted: May 26, 2006

Abstract: Two rare species of the genus Gomphostemma (G. crinitum and G. niveum), belonging to family Lamiaceae, available only in few pockets of North East India, were evaluated for their antibacterial and antifungal properties. Leaf extracts, prepared in different organic solvents, were screened against five human pathogenic microorganisms viz., two Gram positive (Bacillus subtilis and Staphylococcus aureus), two Gram negative (Escherichia coli and Klebsiella sp.) and a fungal pathogen (Candida albicans). Out of five microbial test organisms, S. aureus was found to be the most susceptible. On the other hand, the extract of both the species of Gomphostemma showed weak inhibitory effect against B. subtilis and C. albicans. The MIC of the crude extracts ranged from 180µg/ml to 600µg/ml. Comparative study of the activity of the standard antibiotics and chloroform extracts of both the plants revealed that these extracts have broad spectrum activity than some commercially available antibiotics, therefore, can be tested further for raising important antimicrobial agent against important pathogen.

Key words: Antimicrobial activity, Gomphostemma crinitum, Gomphostemma niveum

INTRODUCTION

Medicinal and aromatic plants have been the subject of man’s curiosity and usages, ever since man began settled life some 8000-10000 years ago. The revival of interest in natural drugs started mainly because of the widespread belief that “green medicine” is healthier than synthetic products [1]. The effect of herbal compounds and phytochemicals on human pathogenic microorganisms has been well documented [2-7].

The North East India being one of the mega-biodiversity hot spots [8] has enormous store of germplasm. Due to wide variation in soil and climate, a large of medicinal and aromatic plants grow in these area, which can be screened and used as raw materials for pharmaceuticals, perfumery, cosmetics, flavour, food and agrochemical industry. Two species of the genus Gomphostemma belonging to family Lamiaceae are available only in few pockets of North East India. Locally the people have been using the plants as antipyretic medicine since the time immemorial, but till today there are no reports on antimicrobial property of this genus. Therefore, in present study antimicrobial activity of both the species of Gomphostemma against five human pathogenic microorganisms has been screened.

MATERIALS AND METHODS

Plant material: Fresh leaves of the two species of Gomphostemma viz., G. crinitum and G. niveum were collected from the foot hills area of Arunachal Pradesh and Assam during the month of March, 2004. The plants were identified at BSI, Kolkata.

Preparation of extracts: The leaves were shade dried for two weeks and crushed to coarse powder. The powders were extracted in Soxhlet apparatus with petroleum ether, acetone, chloroform and methanol by standard method [9]. The extracts concentrated under vacuum and stored at 4.4 °C for further use.
Microorganisms and media: Five human pathogenic microorganisms were used as test organisms. Out of these two were Gram positive {Bacillus subtilis (MTCC-736) and Staphylococcus aureus, (MTCC-96)} two were Gram negative {Escherichia coli (MTCC-739) and Klebsiella sp. (MTCC-304) and one fungal pathogen Candida albicans (MTCC-227). All the test organisms were obtained from MTCC, Chandigarh. The media used were NA (nutrient agar) and NB (nutrient broth). They were procured from Himedia Ltd, Mumbai, and DMSO (Merck Ltd. Mumbai) and used for preparation of crude extracts.

Screening for antimicrobial activity: The efficacy of extracts prepared in various solvents (petroleum ether, acetone, chloroform, methanol) were screened against five test organisms. Screening was carried by Agar well diffusion method [10] and Disc diffusion method.

(a) Agar well diffusion method: A single bacterial colony was suspended in 1 ml sterile saline and added into 20 ml media at 45 °C, then mixed thoroughly and poured into sterile petriplates. After solidification of media, wells of about 6 mm in diameter were cut in agar and 5 mg/ml of the crude extracts of the plants G1 and G2 were loaded in the wells. The plates were incubated at 37 °C and observations were made after 24 hours. The experiments were repeated thrice and the results were expressed as the average of three.

(b) Disc diffusion method: Discs (4 mm diameter) were prepared from Whatman No.4 filter paper. Different doses of extracts were added on it and kept in the oven at 60 °C for one hour for complete drying. Nutrient agar plates were prepared and swabbed with sterilized cotton buds with inoculums. Then the discs were treated with the crude extracts and placed aseptically onto the plates.

Comparative study with commercially available antibiotic discs: Some of the human pathogenic microbes are multidrug resistant strains, showing resistant to most of the commercially available antibiotics. Therefore, a comparative screening was carried out against six commercially available antibiotics and one antifungals by disc diffusion method. The reference antibiotics used were Tetracyclin, Ciprofloxacin, Ofloxacin, Chloramphenicol, Ampicillin, Penicillin G and one antifungal Fluconazole. Antibiotic discs were obtained from Himedia Ltd, Mumbai. Fluconazole was procured from Torrent Pharmaceutical Ltd, India.

Determination of minimum inhibitory concentration: Different concentrations of the extracts 1000 µg/ml, 950 µg/ml, 900 µg/ml, 850 µg/ml upto 50µg /ml were prepared in nutrient broth (NB) tube. Then the tubes containing 10 ml NB media were inoculated with 100 µl of the standardized test organisms containing 10^8 cfu/ml and incubated at 37 °C overnight. The results were taken by visual observation for turbidity in the tubes for growth of microorganism. A tube containing NB media and without inoculums were taken as control. MIC was determined as the least concentration of extracts inhibiting the growth of the test organism.

**RESULTS**

In vitro antimicrobial bioassay of the extracts of both Gomphostemma crinitum and Gomphostemma niveum in five organic solvents showed that the chloroform and petroleum ether extracts have broad spectrum activity against almost all the test pathogenic microbes (Figs.1,2) Both Gram positive (B. subtilis, S. aureus), Gram negative pathogenic microbes (E.coli, Klebsiella sp.) and fungal pathogen C. albicans, clearly showed reduction in their growth. Considering the best activity of chloroform extract among all the solvents, the crude chloroform extract of Gomphostemma crinitum and G. niveum were selected for further experiments.

<table>
<thead>
<tr>
<th>Antibiotic discs / Extracts</th>
<th>Concentration</th>
<th>Test organisms (Inhibition zone diameter in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B. subtilis</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>25 µg/disc</td>
<td>0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30 µg/disc</td>
<td>15</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>10 µg/disc</td>
<td>19</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>10 unit/disc</td>
<td>0</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>05 µg/ml</td>
<td>18</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30 µg/ml</td>
<td>17</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>04 mg/ml</td>
<td>0</td>
</tr>
<tr>
<td>Gomphostemma sp.1</td>
<td>04 mg/ml</td>
<td>20</td>
</tr>
<tr>
<td>Gomphostemma sp.2</td>
<td>04 mg/ml</td>
<td>22</td>
</tr>
</tbody>
</table>
Table: 2 The Minimum inhibitory concentration (MIC) of crude chloroform extract of *Gomphostemma* species

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Minimum inhibitory concentration (µg/ml)</th>
<th>Gomphostemma sp.1</th>
<th>Gomphostemma sp.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>600</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>200</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>400</td>
<td>350</td>
<td></td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>200</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>500</td>
<td>500</td>
<td></td>
</tr>
</tbody>
</table>

Chloroform extract of *Gcrinitum* against *S.aureus*

Chloroform extract of *Gcrinitum and G niveum* against *E.coli*

Acetone extract of *Gcrinitum and G niveum* against *B.subtilis*

Chloroform extract of *Gcrinitum and G niveum* against *C.albicans*

**Plate:**
Antimicrobial activity of crude solvent extracts of *Gomphostemma* species
Agar cup diffusion method was followed to screen the antimicrobial activity and in this, the concentration of crude chloroform extract (5mg/ml) G. niveum was strongly active against the microorganisms with maximum activity against S. aureus showing inhibition zone diameter (IZD) of 28 mm. It was followed by activity against Klebsiella sp and Candida albicans with IZD of 25 mm each and least activity was found against B. subtilis and C. albicans with IZD 22 mm each. In the other hand G. crinitum showed highest activity against S. aureus with IZD 26 mm, followed by E. coli with IZD 24 mm and Klebsiella sp showing IZD 23 mm. Least activity was found against B. subtilis (20 mm) and C. albicans (19 mm).

The minimum inhibitory concentration of crude chloroform extracts of both the species were carried out (Table 2). This revealed that the chloroform extract of G. crinitum was highly active against S. aureus (200µg/ml) followed by Klebsiella sp. (400µg/ml), E. coli (400µg/ml), C. albicans (500µg/ml) and B. subtilis (600µg/ml). In case of G. niveum the MIC was least against S. aureus (180µg/ml) followed by E. coli (350µg/ml), Klebsiella sp. (400µg/ml), C. albicans (500µg/ml) and B. subtilis (600µg/ml).

The crude chloroform extracts were compared with six commercially available standard antibiotic discs and one antifungal. In this screening all the test organisms were found to be resistant against Penicillin G and Ampicillin, except E. coli. (Table 1). Tetracyclin, chloramphenicol, ciprofloxacin and ofloxacin were found to be active for almost all the test organisms. None of the above antibiotics showed any activity against C. albicans, therefore, Fluconazole with concentration 4 mg/ml was used as a reference antifungal. It was observed from the above experiment that the chloroform extract (4 mg/ml) of the two species of Gomphostemma have both antifungal and antibacterial actions, which were even not found in any of the above mentioned standard antibiotics.

**DISCUSSION**

A positive approach for scientific research on existing natural sources has been actively encouraged by WHO [11]. An analysis of antimicrobial activity of leaves extracts of G. crinitum and G niveum in different solvent indicated that the chloroform extracts had greater inhibition against tested microbes than methanol, ethanol, petroleum ether and acetone extracts. Further, the chloroform extract showed strong inhibition in the growth of all 5 human pathogenic microorganisms included in the study {viz., two Gram positive bacteria (S. aureus and B. subtilis) two Gram negative bacteria (E. coli, Klebsiella sp.) and a fungal pathogen (Candida albicans)}. Medicinal plant having similar activity against both Gram positive and Gram negative bacteria were reported by several workers [12-15]. The study clearly indicates that both the species of genus Gomphostemma have a broad spectrum antimicrobial activity.

The minimum inhibitory concentration of the chloroform extracts of both the species of Gomphostemma revealed that S. aureus was highly sensitive to the plant extract with MIC of 200µg/ml, 180µg/ml respectively, while B. subtilis was found to be most resistant against both G. crinitum and G. niveum as it has got the MIC recorded were 600µg/ml against each.

A comparison of minimum inhibitory concentration of chloroform leaves extracts of both the plant species showed almost similar effect in all the pathogens as evident in table 2, except Klebsiella sp. in G. niveum extract treatment, where double the concentration was required. The comparative study of both the species of genus Gomphostemma against some commercially available standard reference antibiotics infer that, both of the species have broad spectrum antimicrobial activity which is not found in any of the above mentioned commercial antibiotics. Now-a-days while on one hand, a number of pathogenic strains are gaining resistance against already existing drugs [16], on the other hand side effect and narrow specificity of synthetic and inhibitory concentration, BSI-Botanical Survey of India, IZD-Inhibition Zone Diameter semi synthetic drugs are always a matter of limitation for many human, as well animal diseases. Therefore, search for newer drug with broad spectrum activity is always welcomed by pharmaceuticals. The present discovery of broad spectrum antimicrobial property of both the species of genus Gomphostemma is highly promising for further phytochemical evaluation is being carried on by the authors.

**ACKNOWLEDGEMENTS**

The authors are highly thankful to Director DRL, Tezpur, for constant inspiration and providing all facilities to carry out the experiment. Thanks are also due to DRDO, for
providing funds in terms of Senior Research Fellowships.

REFERENCES
