IN VITRO ANTIBACTERIAL ACTIVITY OF CRUDE METHANOLIC EXTRACTS FROM LEAVES OF JASMINUM SAMBAC

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Abstract: This study was carried out with an objective to investigate the antibacterial potentials of leaves of Jasminum sambac. In the present study, the antibacterial activity of methanolic extracts of leaves of Jasminum sambac was evaluated for potential antibacterial activity against medically important bacterial strains. The antibacterial activity was determined in the extracts using agar disc diffusion method. The antibacterial activity of extracts (25, 50, 100, 250, 500μg/ml) of Jasminum sambac were tested against four Gram-positive bacteria—Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Sarcina luteae and four Gram-negative bacteria—Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Shigella dysenteriae which are human pathogenic bacteria. Zone of inhibition of extracts were compared with standard Kanamycin disc at a dose of 25μg/disc for antibacterial activity. Significant antibacterial activity was found at a dose of 500μg/ml of methanolic extract. It was found 17 mm, 14 mm, 15 mm and 13 mm zone of inhibition against Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Sarcina luteae respectively and 14 mm, 15 mm, 15 mm and 16 mm zone of inhibition against Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Shigella dysenteriae respectively. The results showed that the methanolic extracts of Jasminum sambac have the significant inhibition of the bacterial growth against the tested organisms. The phytochemical analysis of the plant was carried out. The presence of alkaloids, tannins and resins are revealed in phytochemical analysis.

Key Words: Jasminum sambac, Antibacterial activity, Diffusion Method, Kanamycin, Zone of inhibition.

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INTRODUCTION:
Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, the main problem of using antibiotics is, many commonly used antibiotics have become less and less effective against certain illnesses not, only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Natural products from plant may offer new agents for antibacterial use. A special feature of plants is their capacity to produce a large number of organic chemicals of high structural diversity the so called secondary metabolites [1]. Plants are rich in a wide variety of secondary metabolites with antimicrobial properties, such as tannins, terpenoids, alkaloids and flavonoids [2-5].

Jasmines are an important group of flowering plants. They are widely cultivated and esteemed for their attractive fragrant flowers. This genus belongs to the family Oleaceae. Moreover, different parts of the plant such as the leaf, stem, bark, and roots are very useful and important in pharmaceutical industries. The fresh juice of leaves is applied to corns, and the leaves are chewed and used in the treatment of ulcersations of the mouth. The leaves contain resin, salicylic acid, and an alkaloid named jasmine. Jasminum sambac is used for the treatment of skin diseases, ulcers and fever. Recent study [6] on Jasminum sambac, a flowering plant abundant locally in Southeast Asia and traditionally used as remedies for skin problems such as acne, whitehead, and blackhead, has proven successful. This Arabian jasmine plant is distinctive for its flower’s white petals which emit a fragrance odour and bloom throughout the year. The leaves of Jasminum sambac contain the secoiridoid glycosides, jasminin, quercitrin, and rutin [7]. The preliminary phytochemical study of the antimicrobial activity of ethanolic callus extracts of jasmine focused on two species of Jasminum, Jasminum grandiflorum and Jasminum sambac, showed that the plants can be used as medicine for skin disorders [8]. As Jasminum sambac possesses antibacterial property, it can be used to treat acne and skin infections. Essential oil and extracts of Jasminum sambac have been shown to exhibit antibacterial and antifungal properties [9]. Jasmine oil has been proven to reduce skin inflammation, tones the skin by repairing skin cells by encouraging cell growth, and increases skin elasticity [10].

MATERIALS AND METHODS:
Collection of Plant Materials:
The fresh and healthy leaves of the plant Jasminum sambac were collected. The plant specimens were identified in Bangladesh national herbarium having accession no 42014.

Preparation of Plant Extract:
Extraction
The extraction of the Jasminum sambac leaves was carried out using known standard procedures [11]. The plant materials were dried and powdered in a mechanical grinder. The powder (200.0 g) of the plant materials were initially soaked in 500 ml methanol in amber colored reagent bottle. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stiring. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The extract yields a dark greenish solid residue. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use.

Preliminary Phytochemical Screening:
The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids, as described in literatures [12-14].

Test Microorganisms and Growth Media:
Bacteria tested:
The antibacterial activity of extracts (25, 50, 100, 250,500 μg/ml) of Jasminum sambac were tested against four Gram-positive bacteria—Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Sarcina lutea and four Gram-negative bacteria—Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Shigella dysenteriae which are human pathogenic bacteria. These were chosen based on their clinical and pharmacological importance [15].

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) those were incubated without agitation for 24 hrs at 37°C.

Antibacterial susceptibility test:
The disc diffusion method [16-18] was used to screen the antibacterial activity. In-vitro antibacterial
activity was screened by using Muller Hinton Agar (MHA). 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 methanolic extracts of concentration 25 µg/ml, 50µg/ml, 100µg/ml, 250µg/ml and 500µg/ml were loaded on 6 mm sterile disc. The loaded discs 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 Kanamycin disc of concentration 25 µg/disc was used as positive control.

**RESULTS:**
The results of the antibacterial determinations for the methanolic extracts of the leaf of *jasminum sambac* against the eight bacterial species are investigated in a disc-diffusion assay. The results are shown in the table 1.

<table>
<thead>
<tr>
<th>Name of Organism</th>
<th>Zone of inhibition (mm)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard (kanamycin)</td>
<td>Sample Concentration (µg/ml)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td><em>Sarcina luteae</em></td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em></td>
<td>22</td>
<td>5</td>
</tr>
</tbody>
</table>

The methanolic extracts of *jasminum sambac* when applied against four Gram (+ve) bacteria *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Sarcina luteae* it showed highest zone of inhibition against *Staphylococcus aureus* (17 mm) at a dose of 500 µg/ml.
Fig.1: Antibacterial activity against Gram (+ve) bacteria using methanolic extracts of *jasminum sambac*. (Standard: kanamycin, concentration 25 µg/disc)

The methanolic extracts of *jasminum sambac* when applied against four Gram (-ve) bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Salmonella paratyphi* it showed highest zone of inhibition against *Salmonella paratyphi* (16 mm) at a dose of 500 µg/ml.
DISCUSSIONS:
Plants belonging to family Oleaceae are rich sources of various biologically active substances with strong pharmacological activity. These species contains very important compounds like alkaloids, flavonoids, tannins and so on. The secondary plant metabolites steroids, alkaloids, flavonoids, tannins were reported to have cytotoxicity in different cell lines [19].

In our study, a wide range of human pathogenic microorganisms were examined, including Gram-positive and Gram-negative bacteria. This may partly indicate that the methanolic leaf extracts of *jasminum sambac* have broad inhibitory activities to pathogenic microorganisms and are promising to act as potential antibacterial agents from natural plant sources. Active compounds present in the crude extracts show the antibacterial activity with the dose dependant manner. If the dose is increased activity is also increased. If active principle is present in high quantities, there could be other constituents exerting antagonistic effects of the bioactive compounds. Polyphenolic, flavonoids and tannins present in the methanolic extract may be responsible for the antibacterial activity. Tannin is known to show the antibacterial activity by precipitation the microbial proteins. Flavonoids are produced by the plants for the defense against the infection. So, use of the crude
methylonic extract of this plant as an agent to control microbial pathogens needs further extensive research for their better economic and therapeutic utilization. The findings from this work may add to the overall value of the medicinal potential methylonic extract of leaf of Jasminum sambac. Further phytochemical studies are required to determine the purified fractions bioactive compounds responsible for the antibacterial activities of these species, which could serve as useful sources for new antibacterial agents.

CONCLUSION:

Jasminum sambac is very important medicinal plant with very diverse phytochemical constituents. The antibacterial studies show that the leaves of Jasminum sambac possess significant antibacterial activity. It may be useful for clinical evaluation and development of commercial drugs.

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COMPETING INTERESTS:

Author declares no conflict of interest.

REFERENCES: