Antibacterial activity of Methanolic extract and Semi Alkaloidal Fraction of flower of *Sphaeranthus indicus* Linn.

Mahajan Namrata G., Chopda, Manojkumar Z.* and Mahajan Raghunath T.
Department of Zoology, Moolji Jaitha College, Jalgaon, North Maharashtra University, Jalgaon 425002 M.S. India
mzczoo@yahoo.co.in

Available online at: www.isca.in, www.isca.me
Received 16th November 2015, revised 12th January 2016, accepted 22nd January 2016

Abstract

*Many herbal remedies have been used in various medical systems for treating and managing various diseases. The weed *Sphaeranthus indicus* Linn been used in different systems of traditional medicine for the treatment of disorders and diseases in human beings. The antibacterial potential of flower of *S.indicus* against seven bacterial pathogens cultures was specifically studied with respect to methanolic extract and semi alkaloidal fraction. Preliminary screening of in vitro antibacterial activity of methanolic extract and semi alkaloidal fraction was carried out by the agar well diffusion method. The highest zone of inhibition was measured against *K. pneumoniae* (18.60 mm), whereas, least in *E. coli* (13.83 mm) at 1000μg/ml of MeOHx. *K. pneumoniae* was sensitive at the highest MIC (8 mg/ml) while lowest in *E. coli* MIC (4 mg/ml) to the SAF.*

**Keywords:** *Sphaeranthus indicus*, Antibacterial activity.

Introduction

*Many infectious diseases have been recognized to be treated with herbal remedies throughout the history of mankind. Infectious diseases are the chief reason of death world-wide. Resistance to antibiotics has become a global problem*1. The clinical effectiveness of many existing antibiotics threaten the emergence of multidrug-resistant pathogens2. Natural products, either as pure compounds or in the form of standardized herbal extracts, provides unlimited opportunities for new drug leads of unmatched availability of chemical diversity.

There is a continuous and pressing need to find out new antimicrobial compounds with different chemical structures and new mechanisms of action for emerging and re-emerging infectious diseases3.

Thus, researchers are increasingly turning their attention to ethnobotanical medicine, looking for new leads to the development of more superior drugs against microbial infections4.

Growing inability chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity5. In recent years, plants secondary metabolites, previously unknown to the pharmacological activity, have been well studied as a source of medicines6. Thus, it is assumed that the phytochemicals with adequate antimicrobial efficiency will be used to treat bacterial infections. Since times immemorial, parts of plants has used in the treatment and prevention of various ailments7. In the present study, the *in vitro* effect of the MeOHx and SAF of *S. indicus* on certain pathogenic microorganisms is evaluated.

Materials and Methods

The plant was collected (January 2008, 2009 and 2010) from North Maharashtra Region, Maharashtra State, India. The flowers were separated and shade dried. After complete drying, the material was pulverized to form coarse powder. Then, dried flower powder thoroughly extracted in a Soxhlet apparatus with methanol. Obtained solvent extract was then filtered to eliminate any suspended impurities under vacuum.

The extract was separately concentrated under reduced pressure and at 55°C to 60°C temperature. This methanolic extract (MeOHx) of flower was conserved in dry, cool condition in a desiccator. Thus, it was screened for its biological activity and on the basis of promising results, it proceed for fractionation to isolate the active ingredient especially alkaloid.

Fractionation and Characterization of the MeOHx of flower of *S. indicus*: A number of methods are described in the literature to isolate the natural plant product present in a crude extract. These include adsorption column chromatography, thin layer chromatography, gel filtration etc. depending upon the nature of the plant products present in crude extract. The various adsorbents used are silica gel, silicic acid, neutral aluminium oxide, charcoal and fuller’s earth etc. Similarly, depending upon the nature of the active constituents different solvent systems are employed.

---

**International Science Community Association**
Fractionation of MeOHx of flower of *S. indicus* was carried out and evaluated for its biological activities.

Naqvi, was unable to deduce structure of sphaeranthine – S1, S2, S3, S4, S5, S6, S7 and S8any alkaloid. In relation to this we followed repeatedly the method for isolation of sphaeranthine, an alkaloid fraction given by Naqvi, we failed to purify any kind of sphaeranthine – S1, S2, S3, S4, S5, S6, S7 and S8. We find it was very complicated. Therefore, some modifications are attempted to isolate alkaid fraction.

**Test pathogens:** Seven bacterial pathogens like, five Gram negative (*Pseudomonas aeruginosa* (Schroeter) Migula: 2200, *Escherichia coli* (Migula) Castellani and Chalmers: 2065, *Proteus vulgaris* Hauser: 2027, *Proteus mirabilis* Hauser: 2241 and *Klebsiella pneumoniae* (Schroeter Trevisan: 2706) and two Gram positive (*Staphylococcus aureus* Rosenbach: 2079 and *Bacillus subtilis* (Ehrenberg) Cohn: 2010) were obtained from the culture collection of Microbiology Department, Moolji Jaitha College, Jalgaon, Maharashtra state, India. Pathogens subcultured and maintained on standard nutrient agar (Hi-media). Fresh bacterial culture for 24 h at 25°C in Nutrient Broth (NB) (Hi-media) was used in experimentation and particular density was monitored. A standardized concentration of 10⁸ cells ml⁻¹ was used in experimentation.

**In vitro antibacterial assay:** By the cup plate method the antibacterial activity of MeOHx and SAF was determined against seven bacterial pathogens. This technique was used to determine the pathogenicity against bacterial pathogens, which multiply sufficiently to detect growth or inhibition within 24-48 h of incubation. Aliquots of 100 μl were spread evenly onto individual NA plates. On each plate, four equidistant wells were made in the agar with a sterilized cork borer of 6 mm diameter and 12 mm away from the edge of the plate. Fifty μl of MeOHx (500 and 1000μg/ml) and SAF (50 and 100μg/ml) were transferred to a respective well and plates were incubated at 37°C for 24-48 h. The same volume of antibiotic Gentamycin (10μg/ml) was used as a positive control. Extraction solvent [methanol] was considered as a negative control. Experiments were performed in triplicate. The appearance of visible inhibition zones around the wells considered positive results and measured in mm, initial diameter of the well was subtracted from the diameter of resultant zone of inhibition.

**Determination of MIC:** The minimum inhibitory concentration (MIC) of the MeOHx and SAF was estimated for each bacterial pathogen in triplicates. Two ml of nutrient broth was added in 0.5ml of varying concentrations of the MeOHx (20.0, 40.0, 60.0, 80.0 and 100.0 mg/ml) and SAF (2.0, 4.0, 6.0, 8.0 and 10.0 mg/ml) and then a loop full of the test organism diluted to 0.5 McFarland turbidity standard was put to the tubes. The procedure was followed for the standard antibiotic (Gentamycin). The tube containing only the nutrient broth seeded with test organisms, as described above, to serve as a control. However, the tubes containing nutrient broth and test material at various concentrations were considered as a negative control. Then tubes containing bacterial cultures were incubated at 37°C for 24 h. The growth of organisms was observed and subjected for optical density evaluation at 620 nm to determine the MIC.

**Results and Discussion**

**Results:** In the *in vitro* semi-qualitative experiment, MeOHx and SAF of *S. indicus* showed broad spectrum antibacterial activity to the microbial pathogens challenged. Results obtained in the present study reveal that, the MeOHx and SAF possess potential antibacterial activity in opposition to *E. coli*, *P. vulgaris*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and *K. pneumoniae* (Table - 1). When tested by Cup plate method, the MeOHx (1000μg/ml) showed significant activity (p<0.01 and p<0.001) against *E. coli*, *P. vulgaris*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and *K. pneumoniae* around 13.83 ± 0.47 to 18.60 ± 0.2 mm zone of inhibition (Figure - 1). The highest zone of inhibition was measured against *K. pneumoniae* (18.60 mm), whereas, least in *E. coli* (13.83mm) at 1000μg/ml of MeOHx. *P. mirabilis* showed resistance to both tested samples. SAF at 100 μg/ml concentration exhibited highest antibacterial activity (97.14 ± 3.45%) against *S. aureus* and lowest activity was demonstrated against *P. aeruginosa* (78.78 ± 4.02%) when compared to the standard drug Gentamycin. The observations of the MIC study is given in Table - 2 and it is noted that *E. coli*, *P. vulgaris* and *B. subtilis* (MIC-80 mg/ml) are the most and equally sensitive organisms to the MeOHx, whereas, *P. aeruginosa*, *S. aureus* and *K. pneumoniae* were inhibited at 100 mg/ml of MeOHx. *K. pneumoniae* was sensitive at the highest MIC (8 mg/ml) while lowest in *E. coli* MIC (4 mg/ml) to the SAF (Figure - 1). SAF exhibited same MIC 6 mg/ml to *P. vulgaris* and *B. subtilis*, while, *P. aeruginosa* and *S. aureus* showed MIC 5 mg/ml.

**Discussion:** Demonstration of antibacterial activity against Gram-positive and Gram-negative bacteria may indicate the presence of broad spectrum antibiotic compounds. It will be a huge advantage in the fight against the threat of antibiotic agents of refraction, which are so common in recent years. Zachariah et al., evaluated antibacterial activity of the hexane, chloroform, ethyl acetate, ethanol, methanol and aqueous extracts of whole plant including flower heads of *S. indicus* among them, the methanolic extract at 4000 μg/ml exhibited strong antibacterial activity. They have not tried activity on either fraction or pure compound. Naqví reported that ethanolic extract and alkaloidal fraction of flowers of *S. indicus* had effective antibacterial activity against Gram-negative and Gram-positive bacteria. Khare mentioned that fruits of *S. indicus* contain alkaloids, tannins and volatile oil and antibacterial properties of it against *Vibrio cholera* and *Micrococcus* present results are also in accordance with Naqví and Khare that the SAF is more effective than MeOHx, this may be due to the alkaloids present in it.
Table-1

*In vitro* antibacterial activity of the MeOHx and SAF of the *S. indicus*

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Dose (μg/ml)</th>
<th>Std. (10)</th>
<th>MeOHx 1 (500)</th>
<th>MeOHx 2 (1000)</th>
<th>SAF 1 (50)</th>
<th>SAF 2 (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>11.33 ± 0.33</td>
<td>9.66 ± 0.76 (85.98 ± 7.64)</td>
<td>13.83 ± 0.47*** (122.4 ± 4.68)</td>
<td>1.5 ± 0.25 (13.09 ± 2.01)</td>
<td>10.33 ± 0.33 (91.69 ± 4.52)</td>
<td></td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>11.5 ± 0.34</td>
<td>10.50 ± 0.5 (91.41 ± 3.84)</td>
<td>18.00 ± 0.51*** (157.7 ± 8.52)</td>
<td>2.41 ± 0.35 (20.73 ± 2.73)</td>
<td>9.66 ± 0.21 (84.19 ± 1.18)</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>11.08 ± 0.32</td>
<td>9.91 ± 0.71 (89.07 ± 4.7)</td>
<td>16.6 ± 0.25*** (150.5 ± 5.7)</td>
<td>1.5 ± 0.34 (13.48 ± 3.02)</td>
<td>8.66 ± 0.21 (78.78 ± 4.02)</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11.6 ± 0.25</td>
<td>9.76 ± 0.85 (84.22 ± 7.22)</td>
<td>17.47 ± 0.33*** (150.9 ± 4.35)</td>
<td>1.91 ± 0.23 (16.5 ± 1.93)</td>
<td>11.23 ± 0.25 (97.14 ± 3.45)</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>12.00 ± 0.36</td>
<td>10.83 ± 0.54 (90.49 ± 4.46)</td>
<td>14.42 ± 0.32*** (120.5 ± 3.28)</td>
<td>2.16 ± 0.27 (18.21 ± 2.55)</td>
<td>10.43 ± 0.55 (86.86 ± 3.30)</td>
<td></td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>4.93 ± 0.06</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>11.60 ± 0.25</td>
<td>10.6 ± 0.41 (91.67 ± 4.30)</td>
<td>18.60 ± 0.20*** (160.8 ± 4.48)</td>
<td>2.16 ± 0.42 (18.82 ± 3.88)</td>
<td>10.00 ± 0.93 (86.5 ± 8.29)</td>
<td></td>
</tr>
</tbody>
</table>

Std. = Gentamicin, Nil = no activity, Values are expressed as mean ± S.E., n= 6 replicas. **P<0.01 and ***P<0.001 Vs standard and percentages in parenthesis

Figure-1

*In vitro* antibacterial activity of the MeOHx and SAF

Table-2

*In vitro* MIC of the MeOHx and SAF against some bacteria

<table>
<thead>
<tr>
<th>Organisms</th>
<th>MeOHx (μg / ml)</th>
<th>SAF (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>80</td>
<td>4</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>100</td>
<td>8</td>
</tr>
</tbody>
</table>

NIL = No Activity
Conclusion

The present study indicates that, the plant possess a significant antibacterial activity and may be of use for development of phytomedicine for the therapy of infectious disease. Excellent wound healing property of this plant may be recognized due to occurrence of potent antibacterial natural product.

References


