Evaluation of Antibacterial Activity of Phytoconstituents Isolated from *Jasminum sambac* L. and their Identification through GC-MS

Kamakshi Tomar¹*, Shilpi Rijhwani²

1. Research Scholar, Department of Botany, ICG, The IIS University, Jaipur, Rajasthan
2. Head, Department of Botany, ICG, The IIS University, Jaipur, Rajasthan

Abstract

The present study was initiated to explore the antibacterial activity of *Jasminum sambac* L. in view of its pharmacological utilization in ancient as well as modern system of medicine. Leaves and stem of *Jasminum sambac* L. were extracted in various solvents ranging from nonpolar to polar, viz., n-hexane, chloroform, ethyl acetate, methanol and water. All the plant extracts were screened for antibacterial activity against selected ATCC bacterial strains. Few plant extracts were found to show high antibacterial activity. However, for the present study we have taken into consideration only the leaf and stem extracts obtained in ethyl acetate. Both plant extracts were further evaluated for the identification of phytoconstituents by GC-MS.

Introduction

Products obtained from nature have always been a supreme source of novel drugs (Vuorelaa P et al., 2004). In many developing countries, plant based medicines are still used by approximately 80% people as a source of powerful and potentially active drugs (Hashim H et al., 2010). Recently scientists are more focused on natural products and components since they are having many ethno- medicinal values as well as sourced very comfortably (Arora DS and Kaur GJ, 2007). Ample of medicinal plants and their parts are utilized to extract as raw medicines and acquire various medicinal properties. Out of all these, some drugs are
collected by local people in small quantity and used as folklore medicines while many others are traded to herbal industries as raw materials in large quantity (Uniyal SK et al., 2006). Traditionally used medicinal plants consist a broad range of components which can be used to cure infectious as well as chronic diseases. A group of microbiologists are showing their interest to analyse large number of medicinal plants for novel therapeutics (Periyasamy A et al., 2010). There are many secondary metabolites which act as an active component in plants and showing wide range of antimicrobial activity (Lai PK and Roy J, 2004).

It has been reported that *Jasminum sambac* is also having numerous medicinal values and used as a folk medicine since ancient time. This plant belongs to oleaceae family which is as much important as aromatic and medicinal plant. Traditionally this plant was used as antidepressant, aphrodisiac, analgesic, anti-inflammatory, sedative, antiseptic, expectorant and having uterine (tonic) effects. Its essential oil is very expensive and used to form cosmetics and skin care products. This oil also reduced inflammation on skin, tones of skin and also helps to enhance the mood of a person (Vaghasiya et al., 2011).

In the present study qualitative phytochemical analysis of phytocompounds and their antimicrobial activity were carried out on the leaf and stem of *Jasminum sambac* against some selected bacterial strains and their identification through GC-MS to search the potential therapeutic uses of this plant.

**Material and Methods**

**Collection and Identification of Plant Material**

Fresh plant material was collected from different – different location and botanically identified. Identification was done by the Curator (Herbarium, Dept. of Botany), University of Rajasthan, Jaipur, Rajasthan, India. The voucher specimen was also deposited in the University for Further Reference. Collected fresh plant material was properly washed in tap water, air dried and then homogenized into fine powder and stored in airtight bottles.

**Plant Extraction process**
Air dried plant material, i.e., leaves and stem, was taken (100gm) in to 2000 ml of ethyl acetate and prepare their extracts by Soxhlet extraction for fixed periods of time at definite temperature. In Soxhlet apparatus, continuous hot percolation process is followed in which crude plant extract was obtained. The mother solvent was removed by evaporating it in oven and the resulting semisolid mass was dried in the room temperature to obtain a gummy residue.

**Qualitative Analysis of Phytochemicals**

The ethyl acetate extract of leaves and stem of *Jasminum sambac* obtained, were dissolved in the ratio of 1: 10 ml in its own mother solvent to get a stock solution. The extracts were subjected to preliminary qualitative phytochemical analysis by using the methods of Harborne (1998) and Evans (2000).

By this analysis, the presence of several phytochemicals likes glycosides, alkaloids, phytosteroids, flavonoids, saponins were confirmed and tannins and phenolic compounds were absent. The results obtained in the present investigation are given in (Table 1).

**Test organisms**

The extracts were tested against some selected ATCC Bacterial strains viz., Gram-positive *Staphylococcus aureus*, the Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* and some hospital generated live samples like, *Acinetobacter*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Proteus*, *Staphylococcus*, *Pseudomonas* and *E. coli*.

All the strains and samples were procured from Microbiology Department, Mahatma Gandhi Medical College and hospital, Jaipur, Rajasthan, India.

**Preparation of Bacterial inoculum**

Bacterial cultures were routinely maintained on nutrient agar slants at 4°C. For the experiment purpose bacterial inoculums were prepared by taking a loopful of isolated colonies were inoculated into 4 ml of peptone water, incubated at 37°C for 4 h. This actively growing bacterial suspension was then adjusted with peptone water so as to obtain a turbidity
visually comparable to that of 0.5 McFarland standard (McFarland J, 1907) prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dihydrate (BaCl\(_2\). 2H\(_2\)O) with 99.5 ml of 1% (v/v) sulphuric acid (H\(_2\)SO\(_4\)).

**Antibacterial screening**

The *in vitro* antibacterial activities of the test samples were carried out by Disc diffusion assay by Kirby-Bauer method (Bauer et al., 1966). Mueller Hinton Agar (MHA) media was used for antimicrobial activity which was procured from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 min and inoculum suspension was swabbed uniformly and allowed to dry for 5 min. Discs were prepared using Whatmann filter paper no. 1, 4 mm in diameter and sterilized in hot dry air oven for an hour at 160\(^\circ\)C . The discs were placed on the surface of medium and 10\(\mu\)l plant extract was loaded on sterile individual discs. The extract was allowed to diffuse for 5 min and the plates were kept for incubation at 37\(^\circ\)C for 24 h and respective mother solvent was used as control. At the end of incubation, zone of inhibition formed around the disc were measured with transparent ruler in millimetre. All the experiments were performed in triplicates. Sterile, blank paper discs impregnated with only sterile ethyl acetate served as negative control each time. The results obtained in the present investigation are given in (Table 2).

**Gas Chromatography-Mass Spectrometry Analysis**

The GC – MS analysis was carried out using a Clarus 500 Perkin – Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.2 spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25 \(\mu\)m DF of capillary column. The instrument was set to an initial temperature of 110\(^\circ\)C, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280\(^\circ\)C, at the rate of an increase of 5\(^\circ\)C /min, and maintained for 9 min. Injection port temperature was ensured as 200 \(^\circ\)C and Helium flow rate as one ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST
Identification of components

The proportionate percentage quantity of each component was calculated by comparing its average peak area to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Ver.2.0-Year 2005 library. Spectrum of the unknown component was compared with the spectrum of known components stored in the NIST Library. The molecular weight, molecular formula and the number of hits used to identify the name of the compound from NIST and Wiley spectra Libraries were recorded.

Results and Discussion

Gas chromatography mass spectrometry (GC-MS) is a method that merges the features of gas liquid chromatography and mass spectrometry to identify various components within a test sample. Applications of GC-MS include explosives investigation, environmental analysis, drug detection and identification of unknown samples. GC-MS methods proved to be very effective and sensitive for the separation and detection of complex mixtures of phytochemicals (Marg L. et al., 2002).

The identified compounds have been known to possess many biological properties. In leaf extract of *Jasminum sambac* 54 components have been isolated. For instance, 2, 3 -Dihydro-Benzofuran, 1-Nonadecene, 2, 6, 10-Trimethyl,14-Ethylene-14-Pentadecene,1-Nonadecene, 1-Heptacosanol, alpha.-Tocopherol.-beta.-D-mannoside ,Nonacosane. In stem extract of *Jasminum sambac* 59 components have been isolated, the important ones being, 1-Nonadecene, Nonadecyl trifluoroacetate, 1-Heptacosanol, 1-Heptacosanol, 1-Heptacosano, E-14-Hexadecenal.

Conclusion

GC-MS analysis of phytochemicals in plants gives a clear picture of the pharmaceutical value of *Jasminum sambac*. Thus, GC-MS analysis is the initial step towards understanding the nature of active principles of this plant and this type of study will be helpful for further
detailed study. Further investigations are in progress to investigate the pharmacological importance of *Jasminum sambac*.

**Components detected in *Jasminum sambac* leaf**
Components detected in *Jasminum sambac* stem
References
Table No: 1 Qualitative Phytochemical Analysis of *Jasminum sambac* in Ethyl acetate (Leaf and stem extract)

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Leaf</th>
<th>Stem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phyto steroids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins &amp; phenolic compound</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fixed oils &amp; fats</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) = indicates presence, (-) = indicates absence

Table: 2 Antibacterial activity of Ethyl acetate extracts of *Jasminum sambac* (Leaf and stem)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Bacterial strains</th>
<th><em>Jasminum sambac</em> (Zone of inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaf</td>
</tr>
<tr>
<td>1.</td>
<td><em>Staphylococcus aureus</em> ATCC</td>
<td>13,12,12</td>
</tr>
<tr>
<td>2.</td>
<td><em>Escherichia coli</em> ATCC</td>
<td>13,14,13</td>
</tr>
<tr>
<td>3.</td>
<td><em>Pseudomonas aeruginosa</em> ATCC</td>
<td>17,16,17</td>
</tr>
<tr>
<td>4.</td>
<td><em>Acinetobacter</em></td>
<td>11,11,11</td>
</tr>
<tr>
<td>5.</td>
<td><em>Klebsiella</em></td>
<td>10,8,9</td>
</tr>
<tr>
<td>6.</td>
<td><em>Citrobacter</em></td>
<td>9,9,10</td>
</tr>
<tr>
<td>7.</td>
<td><em>Enterobacter</em></td>
<td>11,11,10</td>
</tr>
<tr>
<td>8.</td>
<td><em>Proteus</em></td>
<td>10,11,11</td>
</tr>
</tbody>
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