Evaluating antagonistic potential of leaves and flowers extract of *Hibiscus rosa-sinensis* against *Klebsiella pneumoniae* strains

1Rajni, 2Tushi Singh, 3Jagriti Sharma

Department of Biotechnology
R.B.S. College Khandari, Agra.

Abstract- The natural plant products are widely used nowadays because of increasing the burden of diseases. *Hibiscus rosa-sinensis* L. (Family Malvaceae) is a plant which is widely distributed throughout the world. Its leaves, barks, roots and flowers have been used in the Indian traditional system as medicine to treat various diseases. Various research studies proved that the different parts of *Hibiscus rosa-sinensis* plant possesses Antioxidant, Antimicrobial, Antidiabetic, Antiulcer, Hepatoprotective, Antifertility, Antigenotoxic, and Antiinflammatory properties, which helps in treatment of many diseases. *Hibiscus rosa-sinensis* has been used in many herbal mix and drinks. Many research studies conducted in animal modal evaluate *Hibiscus rosa-sinensis* flowers and leaves as antidiabetic and antioxidant compounds. This review attempt to highlight the therapeutic application of *Hibiscus rosa-sinensis*.

Crude preparations of the leaves and flowers of *Hibiscus rosa-sinensis* have been traditionally used in folk medicine for various purposes. In the present study, we have evaluated the antibacterial activity of the extracts of *Hibiscus rosa-sinensis* leaves and flowers against some clinical isolates of bacteria by simple agar disc diffusion and method. In the preliminary screening experiment, all of the bacterial isolates showed varying degrees of sensitivity to the flower extracts excluding *Klebsiella pneumoniae*. We found *Staphylococcus aureus*, a gram positive bacterium as the most sensitive to the extracts of flowers. On the other hand, most of the tested bacterial isolates were resistant to the extracts of leaves and flowers excluding *S.aureus*. Our findings clearly demonstrates that the flower extracts *H.rosa-sinensis* had stronger antibacterial effect than that of leaves.

Keywords: Antibacterial activity, Antibiotic resistance, Gentamycin, *Hibiscus rosa-sinensis*, *Klebsiella pneumoniae*, Zone of inhibition.

INTRODUCTION

*Hibiscus rosa-sinensis* is a bushy evergreen shrub or small tree growing 2.5-5m (8-16ft) tall and 1.5-3m (5-10ft) wide. The plant has a branched taproot system. Its stem is aerial erect green cylindrical and branched. Its leaves are simple and petiolate, with alternate phyllotaxy. The leaf shape is ovate, the tip is acute, and the margin is serrated. Venation is unicostate reticulate, meaning the leaves veins are branched or divergent. Their surfaces are glossy (*RHS A-Z encyclopedia of garden plants*). Free lateral stipules are present. Its flowers bloom in summer and autumn. They are solitary (axillary) and symmetrical. They are typically red with five petals 10cm (4in) diameter, with prominent orange tipped red anthers (*RHS A-Z encyclopedia of garden plants*). The medicinal plants are widely used for the various purposes. The plants have identified as medicinal plants because of the ability to synthesize chemical compounds which play major role to prevent different diseases like cancer, diabetes, etc. A wide variety of chemical compounds also have important biological functions, and to defend against attack from predators such as fungi, insects and herbivorous mammals. At least 12000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (*Tapsell L.C. et al; 2006, Lai P.K., Roy J. et al; 2004*). They are used in the treatment of excessive and painful menstruation, cystitis, venereal diseases, feverish illnesses, carbuncles, mumps, sores, coughs. Mucilage prepared from the *H. rosa-sinensis* root has been used in the treatment of coughs (*Duke and Ayensu etal; 1985, Chopra etal; 1986*). The flowers of *H. rosa-sinensis* have been repoted in the ancient Indian medicinal literature with beneficial effects in heart diseases (*Nadkarni etal; 1976*). The origin and historical distribution of red Hibiscus as unknown. The genus Hibiscus contains approximately 200 species distributed throughout tropical and subtropical regions. Hibiscus is commonly cultivated in garden ornamental from sea level to 500m altitude. It is frost sensitive and will freeze in mild winters but will resprout from the base in spring. It does best in full sun in well drained fertile soil high in organic matter.

MATERIAL AND METHOD

This study was conducted in research laboratories department of biotechnology R.B.S. College Agra.
**Preparation of crude extract:** The leaves and flowers of *H. rosa-sinensis* were collected from the botany, R.B.S. Khandari Campus, Dr. Bhimrao Ambedkar University, Agra(U.P.) during September 2023. The leaves and flowers of the plants were carefully washed under running tap water followed by sterile water. They were then air dried at room temperature (30°C) for two days, pulsed to a fine powder using a sterilised mortar and pestle and stored in air tight bottles. Aqueous solvent and Ethanol solvent were used for extraction to obtain a total 4 extracts. The 10g of pulsed leaves and flowers were packed in a Soxhlet apparatus and subjected to mild hot continuous percolation for 12 hours at 100°C in 150ml of aqueous as solvent. The same procedure was followed for extraction of ethanol as solvent from leaves and flowers. The extracts were then concentrated using hot plate.

**Preparation of Mueller Agar Plates** Weighing the Mueller Hinton Agar on the weighing balance. Suspend 7.5gm of the medium in 250ml of distilled water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121°C for 15min. Cool to room temperature.

**Antibacterial activity of *H. rosa-sinensis*** Disc diffusion method used to study the antibacterial activity of plant extracts, agar diffusion method was used against different microorganisms. In this method the pure culture of microorganism is sub cultured nutrient broth and is kept for incubation at 37°C and the the stock culture is revived. For preparation of discs (6mm) Whatman’s filter paper was used. These discs were left to dry under laminar air flow in the serial dilution of leaves extracts of *H. rosa-sinensis*. These discs were left in different concentration of ethanol extract and aqueous extract. Serial dilution of the leaves and the flowers of aqueous extract and ethanol extract of Hibiscus rosa-sinensis. The dilution blanks are prepared by having tubes filled with an appropriate dilution liquid. To perform serial dilution, 1ml of the starting sample is added to 9ml of dilution blank tube 1. This is when followed by the same procedure, where 1ml from tube 1 is added to 9ml of tube 2, 1ml from tube 2 is added to 9ml from tube 3, and so on until the desired concentration is reached. So the dilution in these tube 1, 1/10, 1/100 became respectively. 25 micro litre of the suspension were transferred to the discs. Gentamycin in concentration 0.1mg/ml was used as standard to check antimicrobial activity. For negative control the discs were prepared in respective solvent. To check the antimicrobial activity the media used is Mueller Hinton agar. The test organism that is *Klebsiella pneumoniae* was spread over the solidified Mueller hinton agar plate. Then the discs already prepared in different extracts were positioned on MHA agar media. Then these plates were kept in microbiological incubator at 37°C for 24 hours. Antimicrobial activity was interpreted by the size of diameter of zone of inhibition i.e., the clear area around the disc.

**RESULT AND DISCUSSION**
In extraction of plant part i.e., leaves and flowers different amount of crude extracts were obtained. It was observed that all the extracts in different solvents are having different yield of crude extract. The amount crude extract of plant part in different solvents.

1. **LEAVES**
Antimicrobial activity of aqueous and ethanol solvent extract of leaves was studied by disc diffusion method. Test organisms for antimicrobial activity are *Klebsiella pneumoniae* (MTCC 109). The activity of extract against a particular microorganism is calculated by the diameter of zone of inhibition. Higher diameter of zone of inhibition means the extract is more effective against that microorganism. No zone of inhibition means the microorganism to be tested is resistant against that extract. Gentamycin is an antibiotic which is used as standard to check the antimicrobial activity of plant extract. Effect of different solvent of plant extract on *Klebsiella pneumoniae* is shown in figure.

(1)

**Fig. Effect of leaf extract on *Klebsiella pneumoniae*** (1. Leaf distilled water, 2. Leaf ethanol)
From the studies the ethanol extract showed maximum zone of inhibition which indicates that ethanol extracts having maximum efficiency against *Klebsiella pneumoniae*. Leaf extract in ethanol had shown the antimicrobial effect. In Gentamycin the zone of inhibition was having diameter of 23mm. When compared with the standard, the distilled water and ethanol extracts had shown 30% inhibition. Antimicrobial activity against *Klebsiella pneumoniae* was checked using same method. It was found that ethanol extract of leaves had shown maximum activity against *Klebsiella pneumoniae*. The diameter of zone of inhibition in case of concentrated d. water and ethanol extract solution of leaves was 15/18mm. The ethanol extract and distilled water extract of leaves diameter of zone of inhibition in standard were 23/21mm.

Table 1: Effect of different solvents on *Klebsiella pneumoniae* bacteria.

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>ORGANISM</th>
<th>SOLVENT</th>
<th>ZONE OF INHIBITION (MM) IN DIAMETER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>Distilled water Antibiotic-(Gentamycin)</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanol Antibiotic-(Gentamycin)</td>
<td>23mm</td>
</tr>
</tbody>
</table>

**FLOWERS**

Antimicrobial activity of aqueous and ethanol solvent extract of flowers was studied by disc diffusion method. Test organisms for antimicrobial activity are *Klebsiella pneumoniae* (MTCC 109). The activity of extract against a particular microorganism is calculated by the diameter of zone of inhibition. Higher diameter of zone of inhibition means the extract is more effective against that microorganism. No zone of inhibition means the microorganism to be tested is resistant against that extract. Gentamycin is an antibiotic which is used as standard to check the antimicrobial activity of plant extract. Effect of different solvent plant extract on *Klebsiella pneumoniae* is shown in figure.
From the studies the ethanol extract showed maximum zone of inhibition which indicates that ethanol extract having maximum efficiency against *Klebsiella pneumoniae*. Leaf extract in ethanol had shown the antimicrobial effect. In Gentamycin the zone of inhibition was having diameter of 38mm. when compared with the standard, the distilled water and ethanol extracts had shown 40% inhibition. Antimicrobial activity against *Klebsiella pneumoniae* was checked using same method. It was found that ethanol extract of leaves had shown maximum activity against *Klebsiella pneumoniae*. The diameter of zone of inhibition in case of concentrated d. water and ethanol extract solution of leaves were 13/18mm. The ethanol extract of leaves diameter of zone of inhibition in standard was 40mm.

**Table 2: Effect of different solvents on *Klebsiella pneumoniae* bacteria**

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>ORGANISM</th>
<th>SOLVENT</th>
<th>ZONE OF INHIBITION (MM) IN DIAMETER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>Distilled water</td>
<td>+ve  -ve  1  1/10  1/100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antibiotic- (Gentamycin)</td>
<td>38mm  -----  13mm  10mm  5mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanol</td>
<td>Antibiotic- (Gentamycin)</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Our findings clearly demonstrate that the flower extracts of *H. rosa-sinensis* had stronger antibacterial effects than that of leaves.

**REFERENCES:**

1. RHS A-Z encyclopedia of garden plant.