Evaluation of antibacterial activity of ethanolic and aqueous leaf extract of *Hibiscus rosa-sinensis* in mice

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**Abstract**

Infectious diseases are the second leading cause of death worldwide, killing almost 50,000 people every day. The worldwide emergence of multidrug resistant *Escherichia coli* and many other β-lactamase producers has become a major therapeutic problem. The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. In rural and backward areas of India, several plants are commonly used as herbal medicine for the treatment of infectious diseases. *Hibiscus rosa-sinensis* commonly found plants were screened for potential antibacterial activity. The present study was conducted to evaluate the *In-vitro* and *In-vivo* evaluation of the antibacterial activity of ethanolic and aqueous leaf extract of *Hibiscus rosa-sinensis* in mice. *In-vitro* activity of ethanolic and aqueous leaf extract of *Hibiscus rosa-sinensis* at concentrations of 50, 100 and 200 mg/ml was tested against *E. coli* and *Staphylococcus aureus* using the agar well diffusion method and was compared with penicillin and gentamicin. Ethanolic leaf extracts showed higher inhibitory activity compared to aqueous extract at 50 and 100 mg/ml concentrations. MIC value of *Hibiscus rosa-sinensis* was found to be 2.5 and 5 mg respectively.

**Keywords**: Antibacterial, *Hibiscus rosa-sinensis*, *E. coli*, *Staphylococcus aureus*, mice

**Introduction**

Infectious diseases are the leading cause of death worldwide, killing almost 50,000 people every day. Infections due to a variety of bacterial etiologic agents such as pathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, *staphylococcus aureus* spp, *salmonella* spp, *vibrio cholerae*, *Shigella* spp, *Klebsiella* spp, *Campylobacter* spp are most common. (Parmar and Rawat 2012) [2]. Antibacterial agents provide the main basis for the therapy of microbial (bacterial and fungal) infections. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens and has further complicated the treatment of infectious diseases. The worldwide emergence of multidrug resistant *Escherichia coli* and many other β-lactamase producers has become a major therapeutic problem (Khan et al., 2004) [3].

Plants form an integral part of life in many indigenous communities as a readily and cheaply available alternative to allopathic medicines. Such plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections (Somchit et al., 2003) [4], caused by bacteria often known to resist various classes of conventional antibiotics. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Traditional medicine is in practice for many centuries by a substantial proportion of the population.

*Hibiscus rosa-sinensis*, a member of the Malvaceae family, is widely cultivated in the tropics as an ornamental plant. It is often planted as a fence or hedge plant and was commonly found plants were screened for potential antibacterial activity. *Hibiscus rosa-sinensis* is a glabrous shrub widely cultivated in the tropics as an ornamental plant. It is also used in traditional medicine to induce abortion, ease menstrual cramps, assist in childbirth and relieve headache, fever and inflammation. Previous studies have showed that *Hibiscus rosa-sinensis* possesses many biological activities, such as anticomplementary, antidiuretic and antiphlogistic activity. It has also been reported that the flower possesses antispermatogenic, androgenic, antitumor and anticonvulsant properties; in addition, the leaves and flowers have been found to be hair growth promoters and aid in the healing of ulcers.
Materials and Methods

Collection of plant material and Preparation of extract
The leaves of *H. rosa-sinensis* were collected from their natural habitat in Shirval village, Satara, Maharashtra. The plant materials were identified by Botanist from Pune University. Each plant samples were carefully dried in the laboratory at room temperature. All the plant samples were separately pulverized to a fine powder with a mixer grinder and kept in an airtight container at room temperature. The powdered leaves of *Hibiscus rosa-sinensis* were extracted with ethanol using the Soxhlet extraction apparatus and the extractability percentage was determined as per the method suggested by Rosenthaler (1931) [6].

Bacterial strains
Standard strain of Gram-positive bacteria (*Staphylococcus aureus*) and Standard strain of Gram-negative (*Escherichia coli*) were used for antibacterial studies which were obtained from MTCC, Chandigarh (No.2940 & 739) & Department of Veterinary Microbiology, KNPVC, Shirval.

Drugs: Gentamicin susceptibility test discs 10 mcg/disc (Gentamicin Antibiotic disk) Batch No.0000183307 manufactured by HI Media Laboratories Pvt. Ltd. was used for Antibacterial sensitivity test. Penicillin susceptibility test discs 10 units/disc (Penicillin antibiotic disk) batch No. 000182528 manufactured by HI Media Laboratories Pvt. Ltd. purchased from Omkar Traders was used for Antibacterial sensitivity test.

### Table 1: In-vitro antibacterial activity of *Hibiscus rosa-sinensis*

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Antibacterial activity</th>
<th>Antibacterial activity of different dose</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 μg/ml</td>
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<tr>
<td>Gentamicin + <em>E. coli</em></td>
<td>Alcoholic leaf extract of <em>H. rosa-sinensis</em></td>
<td>Alcoholic leaf extract of <em>H. rosa-sinensis</em> + <em>E. coli</em></td>
</tr>
<tr>
<td>Gentamicin + <em>E. coli</em></td>
<td>Aqueous leaf extract of <em>H. rosa-sinensis</em> + <em>E. coli</em></td>
<td>Aqueous leaf extract of <em>H. rosa-sinensis</em> + <em>E. coli</em></td>
</tr>
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</table>

The antibacterial activity of ethanolic & aqueous extract of *Hibiscus rosa-sinensis* leaves was tested against *E. coli* and *S. aureus* by agar-well diffusion method as described by Perez et al., 1990 with minor modifications.

Experimental Design
The mice of either sex with an average weight of 15-20 gm procured from the Institute of Bioscience, Pune, were used in the present investigation. The experiment protocol was approved by (IAEC) the Institutional Animal Ethics Committee of the college. The experimental protocol met the guidance as per the recommendation of the committee for the purpose of Control & Supervision on Experiments on Animals (CPCSEA), Ministry of social justice & Empowerment, Govt. of India, New Delhi. Test animals were housed in a polypropylene cage covered with a stainless steel wire mesh and a paddy husk bed, with adequate provision for feed and water. Test animals were maintained on commercial feed manufactured by Amrut Laboratory Animal feed, Pranav Agro Ltd., Sangli. The mice were housed in clean polypropylene cages, under controlled environments conditions temperature (18-25 °C), relative humidity (50-70%), 12:12 light: dark cycle and other micro and macro environment conditions as suggested by the Committee for the purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Results
The ethanolic extract of *Hibiscus rosa-sinensis* at the concentration of 50 μl, 100 μl and 200 μl (mg/ml) showed inhibitory activity against *Staphylococcus aureus*, zone of diameter 15.66±0.88, 17.33±0.66 and 17.33±0.66 mm respectively. While the antibacterial effect of ethanolic extracts of *Hibiscus rosa-sinensis* against the standard strains of *Staphylococcus aureus* at dose rate of 50 mg, 100 mg and 200 mg showed inhibitory activity with zone of diameter 15.66±0.33, 16.66±0.66 and 21.33±0.66 mm respectively. Penicillin was used as standard drug for *in-vitro* antibacterial activity against *Staphylococcus aureus*, and showed 23.33±0.66 mm zone of diameter. Ethanolic extract of *Hibiscus rosa-sinensis* at 50 mg showed less inhibition 15.66±0.88 mm zone of diameter as compared to dose rate of 100 and 200 mg showed 17.33±0.66 and 17.33±0.66 mm zone of diameter respectively against *Staphylococcus aureus*.

The inhibitory effect of ethanolic extract leaf of *Hibiscus rosa-sinensis* against *E. coli* at the concentration of 50, 100, 200 mg/ml showing 17.00±0.57, 18.00±1.15 and 18.66±1.33 mm zone of diameter respectively. The ethanolic leaf extract
at all concentrations showed an intermediate degree of inhibitory effect. While the antibacterial effect of ethanolic extracts of *Hibiscus rosa-sinensis* at different concentration (50, 100, 200 mg) against the standard strains of *E. coli* showed 17.33±0.66, 18.66±1.15 and 21.33±0.66 mm respectively. The ethanolic leaf extract at 200 mg showed highest degree of inhibition with increase dose increased inhibition. The ethanolic extract at 50 and 100 mg showed intermediate zone of inhibition. Thus ethanolic extract is found to be good bioactive compound to inhibit growth of *E. coli* at higher concentration than lower concentration. Gentamicin was used as reference antibiotic for comparative study, gentamicin showed inhibitory effect with 30.66±0.66 mm zone of diameter.

The MIC values of Ethanolic leaf extract of *Hibiscus rosa-sinensis* against the standard strains of *Staphylococcus aureus* and *E. coli* were 2.5 mg and 2.5 mg respectively. The in-vivo antimicrobial efficacy of HREE in Mice was determined. All rats were found negative for *E. coli* and *S. aureus* in faeces before inoculation and treatment with ethanolic leaf extract of *Hibiscus rosa-sinensis* Linn and antibiotic (Penicillin and gentamicin).

The presence of diarrhea in mice after inoculation and treatment with extract and antimicrobial agents were presented in Table 2. Group I disease control does not receive any bacterial suspension and treatment, was administered on normal saline. However 100% of the infected non treated group *E. coli* 1x10^7 CFU/ml (group II) and 60% of the infected antibiotic treated group (group VI) manifested the symptom of watery diarrhea 2^nd^ day after inoculation with *E. coli*. Group II positive control infected with suspension of *E. coli* 1x10^7 CFU/ml showed diarrhea from 3^rd^ day of inoculation and remain up to 10 days. Group IV treatment group infected with *E.coli* 1x10^5 CFU/ml and received 2.5 mg/kg of ethanolic leaf extract of *Hibiscus rosa-sinensis* showed diarrhea on 3^rd^ day and diarrhea subsides on 5^th^ day of treatment. Group IX infected with *E.coli* 1x10^5 CFU/ml and treated with standard antibiotics Gentamicin @ 4 mg/kg body weight showed diarrhea on 3^rd^ day and stopped on 5^th^ day. Group III positive control infected with 1x10^5 CFU/ml of *Staphylococcus aureus* showed diarrhea on 2^nd^ day. Group VI treatment group infected with suspension of *Staphylococcus aureus* 1x10^5 CFU/ml and received 2.5 mg/kg of ethanolic leaf extract of *Hibiscus rosa-sinensis* showed diarrhea on 3^rd^ day and stopped diarrhea on 5^th^ day. Group IX infected with *Staphylococcus aureus* 1x10^5 CFU/ml and treated with Penicillin (Ampicillin) @ 10,000 IU/kg body weight showed diarrhea on 2^nd^ day and subsides on 6^th^ day. The result revealed that there was a significant interaction between treatment and time (p>0.05) over the course of the study. However when comparing treatment groups at specific observation days, the proportion of albino mice showing diarrhea in infected antibiotic treated group was significantly higher than infected non treated groups on 5, 6^th^ days. None of the mice group suffered from bloody diarrhea. It was observed that there was more reduction in the number of mice defecating watery stool over time among the infected non treated group of mice than the infected antibiotic treated group. Thus, the defecation of watery diarrhea by the rats lasted between 7 to 8 days in group II and group III and 5 to 6 days in group IV, V. The groups of the infected animals treated with *Hibiscus rosa-sinensis*, penicillin and gentamicin stopped shedding *E. coli* and *S. aureus* at quantifiable concentration levels at days 5-5, 6-6, 5 and 6 days respectively Variation was also apparent in the amount of *E. coli* and *S. aureus* shed in feces among the various mice groups. Thus, during the course of experiments the concentration of the organisms (*E. coli* and *S. aureus*) in feces of the positive animals in groups II to IX ranged between 3 x10^3-8x10^5 CFU/g, 1 x10^5-5 x10^7 CFU/g, 2 x10^7-4 x10^9 CFU/g, 1x10^9-4x10^7 CFU/g, 2x10^7-4x10^9 CFU/g, 3 x10^5-5 x10^7 CFU/g, 2x10^7-4x10^9 CFU/g and 2x10^7-5x10^6 CFU/g respectively Statistical analysis of the results showed that there was a significant time effect, but no significant treatment effect among some of the infected mice groups treated with the *M. Koengii* and *H. Rosa-sinensis* plant extract. However, a significant difference (p<0.05) was observed in treatment effects among the *E. coli* infected non treated group of animals and those treated with the plant extracts. While, no significant difference was in treatment effects among *S. aureus* infected non-treated group of animals and those treated with the plant extracts. Among the mice that suffered from diarrhea and abnormalities such as general weakness with slow movement, loss of appetite and loss of weight were observed in them. No pathological changes were observed in other mice groups all through the course of the experiment.

**Discussion**

Infectious diseases are the leading cause of death world-wide, killing almost 50,000 people every day. Infections due to a variety of bacterial etiologic agents such as pathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* spp, *Salmonella* spp, *Vibrio cholerae*, *Shigella* spp, *Klebsiella* spp, *Campylobacter* spp are most common. (Parmar and Rawat 2012) [7]. Previous studies described the antimicrobial activity of ethanolic and Methanolic leaf extract of *Hibiscus rosa-sinensis* against *E. coli* and *Staphylococcus aureus* with zone of diameter 16 mm, 11 mm and 14 mm respectively (Argal et al., 2011) [7]. Methanolic and ethanolic extract of *Hibiscus rosa-sinensis* showed higher zone of inhibition i.e. 16 mm and 16 mm against *E. coli* compared to 11 mm and 14 mm against *Staphylococcus aureus*. Hence solvent extract has been found more antibacterial activity compared to aqueous extract for tested microorganism. Khunita and Panda (2011), also reported similar observation, petroleum ether extract of *Hibiscus rosa-sinensis* at 5 mg/ml and 10 mg/ml against *E. coli* and *Staphylococcus aureus* showed 20 mm & 19 mm zone of diameter and 20 mm & 21 mm zone of inhibition respectively. The MIC values of *Hibiscus rosa-sinensis* were determined for microorganisms (*Staphylococcus aureus* and *E. coli*) that were found to be sensitive during the agar well diffusion assay. The MIC was determined by broth dilution method described by Miles and Misra method (1938). Nutrient broth was used to determine MIC. The MIC values of Ethanolic leaf extract of *Hibiscus rosa-sinensis* against the standard strains of *Staphylococcus aureus* and *E. coli* were 2.5 mg and 2.5 mg respectively. Similar observations were reported by Saini et al. (2013) who observed that methanolic and aqueous leaf extract *Hibiscus rosa-sinensis* showed antimicrobial activity against *E. coli* 7.05 and 3.22 mm zone of diameter and those treated with the plant extracts. Among the mice that suffered from diarrhea and abnormalities such as general weakness with slow movement, loss of appetite and loss of weight were observed in them. No pathological changes were observed in other mice groups all through the course of the experiment.
The ethanolic extract of *Hibiscus rosa-sinensis* against *E. coli* and *S. aureus*. MEPB inhibited bacterial growth in a dose dependent manner. The extract at the dose of 125, 250 and 500 mg/kg effectively reduced shigella density from the seventh day of therapy and beyond; the percentage of reduction of *Shigella flexneri* density was respectively 69.03%, 75.54% and 80.37% compared to the value administered. Thus, the dose 500 mg/kg of the extract appeared to be more active than ciprofloxacin (70.63%) compared to the negative control.

**Conclusion**

The present study has concluded, that the inhibitory effect of the antibiotic drug against the organism *in-vivo* seemed to be less effective than the effect of the plant extract; hence, 60% of the rat had the symptoms of diarrhoea. The reason for the less effectiveness of the antibiotic as compared to the plant extracts could be attributed to the fact that the antibiotic drug inhibited the competitive microorganism in the gut more than *E. coli* O157:H7 strain. This condition enables the proliferation of *E. coli* O157:H7 in the gut and also enhances the development of disease conditions such as watery diarrhoea. This suggestion was supported by Jin-Hyung et al. (2011) [10] who reported that most antibiotics often eradicate intestinal commensal bacteria more than the pathogenic bacteria.

**References**


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