www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(1): 27-30

www.thepharmajournal.com
Received: 08-11-2022
Accepted: 11-12-2022

Sushma Ghadigaonkar
Assistant Professor, Mumbai Veterinary College, Maharashtra Animal and Fishery Science University, Nagpur, Maharashtra, India

Corresponding Author:
Sushma Ghadigaonkar
Assistant Professor, Mumbai Veterinary College, Maharashtra Animal and Fishery Science University, Nagpur, Maharashtra, India

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

Sushma Ghadigaonkar

DOI: https://doi.org/10.22271/tpi.2023.v12.i1a.18010

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 has several forms of flowers with varying colours. *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. Which were purchased from Omkar traders was used for Antibacterial sensitivity test.

Penicillin susceptibility test discs 10 units/disc (Penicillin antibiotic disk) batch No. 0000182528 manufactured by HI Media Laboratories Pvt. Ltd. purchased from Omkar Traders was used for Antibacterial sensitivity test.

Preparation of inocula

The bacterial strains were maintained in nutrient agar (HI media) at 35 °C anaerobic conditions. All the organisms were sub cultured every 2 weeks before testing. The bacterial inoculums were prepared & cultivated on Nutrient broth for 12 h at incubator temperature of 37 °C. The microbial cultures were serially diluted (10 fold increment) in sterile broth to obtain the cell suspension of 1.0 x 10^5 CFU/ml. To standardize the inoculums density for test, $BaSO_4$ turbidity standard equivalent to 0.5 McFarland standards was used. A 0.5 McFarland standard was prepared as described in NCCLS of Sulphuric acid was prepared by adding 1 ml concentration Sulphuric acid to 99 ml of water & mixed well. A 1.175% w/v solution of barium chloride was prepared by dissolving 2.35 gm of dehydrated barium chloride ($Bacl_2.H_20$) in 200 ml of distilled water. To make the turbidity 0.5 ml of the barium chloride solution was added to 1% 99.5 ml Sulphuric acid & mixed well. A small volume of those turbid solutions was transferred to a screw capped tube of the same type as used for preparing control inoculate & stored in the dark at room temperature. Inocula were obtained from an overnight agar culture of the test organism. Inoculum prepared by taking top of each colony was touched with a sterile loop and growth was transferred into a tube containing 4 to 5 ml normal saline. The broth culture achieved the turbidity of the 0.5 McFarland standards. This result suspension containing approximately 1-2 x10^8CFU/ml. The turbidity of actively growing broth culture was adjusted with sterile broth to turbidity comparable to that of the 0.5 McFarland standards.

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

Sr. No.	Antibacterial activity	Antibacterial activity of different dose		
		50 µg/ml	100 µg/ml	200 µg/ml
	Gentamicin + E. coli	Alcoholic leaf extract of *H. rosa-sinensis + E. coli*	Alcoholic leaf extract of *H. rosa-sinensis + E. coli*	Alcoholic leaf extract of *H. rosa-sinensis + E. coli*
	Gentamicin + E. coli	Aqueous leaf extract of *H. rosa-sinensis + E. coli*	Aqueous leaf extract of *H. rosa-sinensis + E. coli*	Aqueous leaf extract of *H. rosa-sinensis + E. coli*

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* $1x10^9$ CFU/ml (group II) and 60% of the infected antibiotic treated group (group VI) manifested the symptom of watery diarrhea 2nd day after inoculation with *E. coli.* Group II positive control infected with suspension of *E. coli* $1x10^9$CFU/ml showed diarrhea from 3rd day of inoculation and remain up to 10 days. Group IV treatment group infected with *E.coli* $1x10^9$CFU/ml and received 2.5 mg/kg of ethanolic leaf extract of *Hibiscus rosa-sinensis* showed diarrhea on 3rd day and diarrhea subsides on 5th day of treatment. Group IX infected with *E.coli* $1x10^9$ CFU/ml and treated with standard antibiotics Gentamicin @ 4 mg/kg body weight showed diarrhea on 3rd day and stopped on 5th day. Group III positive control infected with $1x10^9$ CFU/ml of *Staphylococcus aureus* showed diarrhea on 2nd day. Group VI treatment group infected with suspension of *Staphylococcus aureus* $1x10^9$ CFU/ml and received 2.5 mg/kg of ethanolic leaf extract of *Hibiscus rosa-sinensis* showed diarrhea on 3rd day and stopped diarrhea on 5th day. Group IX infected with *Staphylococcus aureus* $1x10^9$CFU/ml and treated with Penicillin (Ampicillin) @ 10,000IU/kg body weight showed diarrhea on 2nd day and subsides on 6th 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, 6th 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 $x10^3$-$8x10^3$ CFU/g, 1 $x10^3$-5 $x10^3$ CFU/g, 2 $x10^3$-4 $x10^3$ CFU/g,$1x10^3$-$4x10^3$ CFU/g, $2x10^3$ -$4x10^3$ CFU/g, 3 $x10^3$-5 $x10^3$ CFU/g, $2x10^3$ -$4x10^3$ CFU/g and $2x10^3$ -$5x10^3$ 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) [2]. 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. Khuntia 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 Mistra 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 against *S. aureus* 10.64 and 8.09 mm zone of diameter respectively, MIC of leaf extract *Hibiscus rosa-sinensis* against *E. coli* and *S. aureus* were 2.5 mg/ml and 0.312 mg/ml respectively.

There were no *in-vivo* studies references available for 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) [16] who reported that most antibiotics often eradicate intestinal commensal bacterial more than the pathogenic bacteria.

References

1. Das K, Tiwari SKR, Shrivastav KD. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research. 2010;4(2):104- 111.
2. Namita P, Mukesh R. Medicinal plants used as antimicrobial agents: A review. International Research Journal of Pharmacy. 2012;3(1):31-40.
3. Khan AV, Ahmed QU, Khan AA, Shulka I. *In vitro* antibacterial efficacy of some important traditional medicinal plants in India against *Escherichia coli* and *Staphylococcus aureus* strains. Journal of Medicinal Plants Research. 2013;7(7):329-338.
4. Somchit MN, Reezal I, Nur IE, Mutalib AR. *In-vitro* antimicrobial activity of ethanol and water extracts of *Cassia alata.* Journal of Ethnopharmacology. 2003;84(1):1-4.
5. Sumana D, Das K, Dubey V. Inhibitory activity ad phytochemical assessment of ethno medicinal plants against some human pathogenic bacteria. Journal of medicinal plants Research. 2011;5(29):6536-6543.
6. Rosenthaler L. The Chemical Investigation of Plants, 1931.
7. Argal Kuma MS, Chourdhari H, Thakker R, Verma S, Seniya S. The efficacy of *Murraya koenigii* leaf extract on some bacterial and a fungal strain by disc diffusion method. Journal of Chemical and Pharmaceutical Research. 2011;3(5):697-704.
8. Kuntia TK, Panda DS. Evaluation of antibacterial activity of *Murraya koenigii* Spreng. Pharma Science. 2012;2(2):105-110.
9. Miles, Misra SS, Irwin JO. The estimation of the bactericidal power of the blood. The Journal of Hygiene. 1930;38(6):732-49.
10. Saini Mahak, Pradeep Kumar, Mahesh Kumar, Vasudevan Mani, Rakesh Kumar Mishra. In-vitro antimicrobial, anticancer evaluation and QSAR studies of benzohydrazides. Arabian Journal of Chemistry. 2014;7(4):448-460.
11. Gonzalez-Lamothe R, Mitchell G, Gattuso M, Diarra SM, Malouin F, Bouarab K. Plant antimicrobial agents and their effects on plant and human pathogens. International Journal of Molecular Sciences. 2009;10(8):3400-3419.
12. Haraguchi H. Mode of action of antimicrobial substances in plants. Journal of Recent Research Developments in Agricultural and Food Chemistry. 1998;2(1):258-268.
13. Olila D, Olwa O, Opuda-Asibo J. Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, Ugandan medicinal plants. Journal of African Health Science. 2001;1(2):66-72.
14. Somchit MN, Reezal I, Nur IE, Mutalib AR. In vitro Antimicrobial Activity of Ethanol and Water Extracts of Cassia alata. Journal of Ethnopharmacology. 2003;84(1):1-4.
15. Lewis K, Ausubel FM. Prospects of plant derived antibacterials. Journal of Natural Biotechnology. 2006;24:1504-1507.
16. Jin-Hyung L, Sushil CR, Jung-Ae K, Moo HC, Hyungdon Y, Chang-Soo L. Apple flavonoid phloretin inhibits Escherichia coli O157:H7. Biofilm and ameliorates colon inflammation in rats. Infection and Immunity. 2011;79(12):4819-4827.