Research Article

Phytochemical Screening and *in vitro* Antibacterial Activity of *Hibiscus rosa-sinensis* L. Leaf Extracts

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ABSTRACT

Aim: People have used plants as medicine from the beginning of time to treat a variety of diseases. Different bioactive chemicals found in plants make them viable substitutes for traditional antibacterial treatments. *Hibiscus rosa-sinensis* L. is a popular ornamental plant employed in traditional medicine to facilitate childbirth, soothe menstrual cramps, induce abortion, and treat headache, fever, and inflammation. The current study aims to analyze the phytochemical components and antibacterial properties of aqueous and solvent extracts of *Hibiscus rosa-sinensis* L. leaf. **Materials and Methods:** Phytochemical analysis was performed to evaluate the phytoconstituents of leaves using standard tests. Agar well diffusion method was used to screened the antibacterial activity of leaf extracts and results were interpreted in terms of diameter of zone of inhibition. **Results:** The preliminary phytochemical analysis revealed the presence of flavonoids, tannins, steroids, phenolic compounds, saponins in methanolic extract of leaf. In *in vitro* antibacterial activity screening indicated that all the extracts exhibited inhibitory activity against tested bacteria. The highest potential was observed in acetone extract against *Escherichia coli*, *Salmonella typhimurium*, and *Bacillus subtilis* with zones of inhibition of 9.60±0.10, 7.93±0.05 and 6.10±0.10 respectively. **Conclusion:** The current findings confirm the use of *Hibiscus rosa-sinensis* L. leaves in traditional medicine to treat conditions like coughs, intestinal tract infections, urinary trat infection, foodborne illnesses and pneumonia caused by these pathogenic bacteria and support the potentiality to serve as a foundation for isolating antibacterial compounds from *Hibiscus rosa-sinensis* L.

Keywords: Antimicrobial, Phytoconstituents, Traditional medicine, Aqueous extract

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INTRODUCTION

Nature has provided a plethora of medicinal plants and a staggering number of recent pharmaceuticals have been derived from those plants. Since the dawn of time, plants have remained a key component of managing many economic and health challenges. Over the last few decades, a growing belief in traditional medicine, has led to a resurgence of interest in medicinal plants for fewer side effects than allopathic medicine.[1] According to the WHO (World Health Organization) research, more than 80% of people throughout the world, especially those who belong to rural regions, utilize plant derived medicines as their major source of medication.[2] Herbs have been identified in several studies as prospective sources of modern pharmaceuticals as well as a possibility for the identification of novel therapeutic agents for a variety of ailments in the future.

Plants contain different bioactive compounds and secondary metabolites, viz., alkaloids, glycosides, terpenoids, saponin, flavonoids, tannins, quinones, and coumarins[3] that make them efficient to treat various health problems.[4] Plant derived secondary metabolites are important for plant's adaptation to their environment and major sources of active pharmaceutical compounds.[5] Most of drug used in recent dates are

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derivatives of bioactive compounds identified from plant crude extracts.

Hibiscus rosa-sinensis L. (family Malvaceae) is a tropical evergreen shrub, traditionally used in wound treatment, inflammation, fever and coughs, diabetes, various bacterial and fungal infections, dysentery, diarrhea, menstruation problems and to stimulate blood circulation.[6] Various extracts of *H. rosa-sinensis* L. have been shown in recent studies to have a wide spectrum of antipyretic, hypotensive, anti-cancer, antioxidant, antibacterial, anti-inflammatory, anti-bacterial, anti-diabetic, wound-healing, and abortifacient effects.[7] Despite the wide folkloric use of leaves and flowers of the plant, little research has been done to explore them. Thus, in order to give a scientific rationale for these folk treatments, it was proposed for the current study to evaluate its antibacterial activity using aqueous and organic plant extracts against some clinically significant bacteria. The presence of major physiologically active phytoconstituents were evaluated through phytochemical screening.

MATERIALS AND METHODS

Collection and processing of the plant materials

The fresh leaves of *Hibiscus rosa-sinensis* L. were collected from the localities of Dhemaji, Assam. After being rinsed with distilled water the leaves were shade dried and thoroughly homogenized into a fine powder, and finally put into airtight containers.

Preparation of plant extract

Plant extracts were prepared in three different organic solvents- acetone, methanol and chloroform by soaking 5 g of plant powder in 50 mL of the solvents (1:10 w/v) and kept undisturbed for 72 hr. The 50 mL of aqueous extract was also prepared. The extracts were filtered using Whatman No. 1 paper, allowed to dry to sticky mass in dry bath at 65°C and preserved in air tight glass containers at 4°C for further experiments.[8] Before subjected to qualitative phytochemical analysis and antimicrobial activity studies, the crude extracts were dissolved in Dimethyl Sulfoxide (DMSO) to make 1 mg/mL of concentration.[9,10]

Qualitative phytochemical estimation of the plant extract

For qualitative phytochemical estimation of *H. rosa-sinensis* L. leaf extracts were performed using standard protocols for detection of tannins, flavonoids, amino acids, alkaloids, steroids, terpenoids, phenols, and saponin.[11-13]

Test for phenols and tannins

2-3 mL of crude extract was mixed with 2 mL of 2% $FeCl_3$ solution. Here, phenols and tannins could be detected by their blue-green or black colouration.

Test for flavonoids

Alkaline reagent test

1 mL of crude extract was treated with 2 mL of 2% NaOH solution. The presence of flavonoids was revealed by the formation of a bright yellow colour that became colourless when a few drops of diluted HCl acid were added.

Test for amino acids

Ninhydrin test

2 mL ethyl extract was taken and 0.5 mL ninhydrin was added to it. It was mixed well and boiled for few min. Appearance of violet colour indicated the presence of amino acids.

Millon's test

Crude extract was mixed with 2 mL of Millon's reagent. Amino acids were detected by the appearance of a white precipitate that turned reddish upon mild heating.

Test for alkaloids

To the crude extract, 2 mL of 1% HCl was added and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbid precipitation was taken as confirmation for the presence of alkaloids.

Test for steroids

Concentrated H_2SO_4 was added sideways to the extract after 2 mL of chloroform had been added. The presence of steroids was detected by the emergence of a red colour in the lower chloroform layer.

Test for terpenoids

First the crude extract was dissolved in 2 mL of chloroform and evaporated to dryness. Then 1-2 mL of concentrated H_2SO_4 was added to it and heated for 2 min. A grayish colour confirmed the presence of terpenoids.

Test for saponin

In a test tube, crude extract was mixed with 3-4 mL of distilled water and shaken vigorously. The formation of foam was taken as evidence for the presence of saponins.

Screening of antimicrobial activities of plant extracts

Collection and culture of the test pathogens

The reference bacterial strains *Escherichia coli* (MTCC 443), *Bacillus subtilis* (MTCC 441), *Salmonella typhimurium* (NCIM 2501), and *Staphylococcus aureus* (NCIM 2079) used in this study were obtained from MTCC (Microbial Type Culture Collection), Institute of Microbial Technology (IMTECH), Chandigarh and NCIM (National Collection of Industrial Microorganisms), Pune. These were stored on Nutrient Agar (NA) slants, sub cultured regularly and stored at 4ºC.

Preparation of inoculums

Gram staining technique was carried out to check the cultured bacterial strains. A loopful of isolated colonies was inoculated 5 mL of nutrient broth medium (0.013g/mL; pH 7.0) and incubated without agitation for 24 hr at 37ºC. The turbidity of growing bacterial suspension was compared visually with 0.5 McFarland standard, which is equivalent to approx. $2x10^8$ Colony Forming Units per mL (CFU/mL).[14]

Culture media preparation and sterility test

Nutrient agar media was prepared (0.028 g/mL) and poured in sterile petri plates. The plates were incubated for 24 hr at 37ºC and checked for contamination.

Screening of antibacterial activity of plant extracts

The activity of different extracts against bacterial isolates was measured in terms of zone of inhibition using agar well diffusion method.[15] The bacterial suspension was swabbed evenly over the MHA (Mueller Hinton Agar) (HiMedia) plates and 6 mm wells were prepared using a sterile cork borer. In each well, 50 µL of plant extract was loaded. DMSO was used as the negative control. The plates were incubated at 37ºC for overnight and the sizes of Zone of Inhibition (ZOI) was measured.[16] The results were expressed in terms of the average diameter of the ZOI in mm±SD (standard deviation).

Statistical analysis

Statistical software-16.0 version was used to analyze the data. Multiple Analysis of Variance (MANOVA) was done with Duncans Multiple Range Test (DMRT) @ $p \leq 0.05$ for well diffusion method. Antibacterial activity of different solvent extracts was expressed as the mean±SD values of triplicates.

RESULTS

Phytochemical screening of plants extracts

The phytochemical analysis conducted on aqueous extract of *H. rosa-sinensis* L. revealed the presence of tannin, flavonoids, amino acids, steroids, alkaloids, phenols, terpenoids and saponin whereas in methanol extracts amino acid is absent. The acetone and chloroform extracts showed the presence of total five phytochemicals except amino acids, steroids and terpenoids (Table 1).

Table 1: The phytochemical components of *H. rosa-sinensis* L. based on preliminary screening of crude leaf extracts

Phytochemicals	Aqueous	Acetone	Methanol	Chloroform
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Amino acids	+	-	-	-
Steroids	+	-	+	-
Phenolics	+	+	+	+
Alkaloids	+	+	+	+
Terpenoids	+	-	-	-
Saponin	+	+	+	+

'+'represents the presence of phytoconstituents, '-'represent the absence of phytoconstituents

Antibacterial activity of leaf extracts

The result demonstrated that the aqueous extract of leaf exhibits different inhibitory activity against at different solvent concentration. At the concentration of 200 µg/mL, the aqueous leaf extract had the highest inhibition effect against *S. aureus* (16.00±1.00) and the lowest inhibition effects against *B. subtilis* (11.50±1.50) (Table 2). The acetone extract showed the highest inhibitory activity among all the solvent extracts. The zone of inhibition was recorded against *E. coli* (9.60±0.10), *S. typhimurium* (7.93±0.05) and *B. subtilis* (6.10±0.10) for the acetone extract. Whereas methanol extract showed inhibition against *E. coli* (7.83±0.47) and *S. aureus* only (13.00±00). The chloroform extract showed the maximum ZOI against *S. typhimurium* with 11.00±0.47.

Table 2: Antibacterial activity of *H. rosa-sinensis* L. aqueous and solvent extracts at concentration 200 µg/mL

Test Pathogens	Aqueous extract	Methanol extract	Acetone extract	Chloroform extract
E. coli MTCC 443	14.50±0.50	7.83±0.47	9.60±0.10	-
S. typhimurium NCIM 2501	14.00±1.00	13.00±0.18	7.93±0.05	11.00±0.47
B. subtilis MTCC 441	11.50±1.50	-	6.10±0.10	-
S. aureus NCIM 2079	16.00±1.00	-	0±0.00	-

'-' indicates no zone of inhibition.

DISCUSSION

For a very long time, medicinal plants served as a source of healing, and plant-based products were fundamental to the practice of traditional medicine. Higher plants have been essential to maintaining human health as a source of therapeutic chemicals from the dawn of mankind. In this study, both aqueous and solvent extracts reveal the presence of same type of phytochemicals like alkaloids, saponin, flavonoids, tannins, phenolic compounds and these are well defined as antimicrobial agents exhibit in plants. It can be inferred that plant secondary metabolites were more soluble in methanol than acetone and chloroform based on the increased yield of the methanol extracts when compared to both the solvent extracts. In addition, all the phytochemicals detected from the methanolic leaf extract of H. rosa-sinensis in the current study are the same as those revealed from ethanolic extract of flowers.[17]

Numerous reports have stated that *H. rosa-sinensis* contains anthocyanins and flavonoids such as cyanidin-3-sophoroside-5-glucoside, quercetin-3,7-diglucoside, quercetin-3-diglucoside, cyanidin-3,5-diglucoside, cyclopeptide alkaloids, hentriacontane and vitamins like riboflavin, ascorbic acid and thiamine. A GC-MS analysis of the phytocompounds in the methanolic extract of H. rosa sinensis flowers also revealed the presence of 3,3'-dithiobis(2,2-dimethyl-SS), 2,3-Hexanediol, 2-Hydroxy-2-Methylbutyric Acid, n-Hexadecanoic Acid, Heptanoic Acid, 2-Ethyl-Trans-(2-Ethylcyclopentlyl) Methanol, 3-N-Hexylthiolane, SS-dioxide Hexanedioic Acid.[18] Flower, leaf and stem extract of *Hibiscus* exhibited strong antioxidant capacity also that lowers the risk of many diseases in *in vitro* investigations.[19]

Using the agar well diffusion method, the antibacterial properties of *H. rosa-sinensis* leaf extracts were examined against certain pathogens in the current study. Of them, aqueous extract showed maximum inhibition activity against Gram-positive bacteria. Whereas, among solvent extracts methanolic extracts showed the highest activity against *S. typhimurium*. The ZOI against *S. typhimurium* was 13.00±0.18 mm in methanol extract, 11.00±0.47 mm in chloroform extract, 7.93±0.05 mm in acetone extract and 14.00±1.00 mm in aqueous extract of 200 µg/mL concentration. Whereas for *E. coli*, 7.83±0.47 mm, 9.60±0.10 mm, 14.50±0.50 mm of inhibition zones were observed in methanol, acetone and aqueous extract respectively. On the other hand, chloroform extract showed less inhibitory activity compared to other extracts. Ruban *et al*., (2012) confirmed the antibacterial activity of *H. rosa-sinensis* flower petals against important human pathogens such as *E. coli, B. subtilis, Pseudomonas aeruginosa, Salmonella* sp., and *S. aureus*.[20] However, in a report Uddin *et al*., (2010) reported that leaves of *H. rosa-sinensis* exhibit significantly higher antibacterial activity than flower. Interestingly, the resemblance of the findings can be observed in the study where they demonstrated the antibacterial potential of *H. rosa-sinensis* leaf against *S. typhimurium* and *B. subtilis*[21] Similar to this, Udo *et al*., (2016) found that the human pathogens *E. coli, B. subtilis*, and *S. aureus* were considerably inhibited by aqueous and solvent extracts of the *H. rosa-sinensis* L. leaf which support the current investigation also[22] In another study, the aqueous extract of *H. sabdariffa* showed higher inhibition activity against *E. coli* and *S. aureus* (40 mm) than *S. mutans* (28 mm) and *P. aeruginosa* (27 mm).[23] Additionally, the majority of people who utilize *H. rosa-sinensis* leaves as a conventional method of treating numerous illnesses, employ a water-based extract of the leaf. However, very less studies have been reported on the antibacterial activity of aqueous extract of this particular plant. Thus, the current study has accomplished to validate the indigenous use of *H. rosa-sinensis* leaves in various health issues.

The purpose of the study was to ascertain whether there was any scientific support for the traditional applications of herbal remedies made from the plant *H. rosa-sinensis* in treating some disorders brought on by bacterial infection. The results obtained from this study demonstrated that *H. rosa-sinensis* leaves contain significant phytocomponents that give its antibacterial action and may be employed in treating pathological disorders, notably those caused by *S. typhimurium* and *E. coli*. In this study, the antibacterial activity was examined only for susceptible bacterial isolates. Further, more *in vitro* analysis could be done against drug-resistant bacterial isolates to establish their potent inhibitory activities.

CONCLUSION

The ethnomedicinal plants are abundant in bioactive compounds that have potential therapeutic uses and can be utilized as an alternative to traditional treatment. For a very long time, numerous researchers and experts have been working to find fresh, pharmacologically potent phytoconstituents from plants to combat a variety of pathophysiological illnesses like cancer, cardiovascular disease, metabolic disorders and microbial infections. Due to its antibacterial properties, leaf extracts of H. rosa-sinensis have the potential to offer additional health advantages and can be utilized as a natural alternative to synthetic antimicrobials. Future research is necessary,

nevertheless, to pinpoint the active substances present in the leaves and other parts of this plant.

CONFLICT OF INTEREST

The author declares no competing interests that are relevant to the content of this article.

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AUTHORS' CONTRIBUTIONS

The experimental design and experimental studies were done by Mregangka Dowara. The data analysis, manuscript preparation, editing and manuscript reviewing were done by Indrani Gogoi and Shyamalima Saikia.

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None

ABBREVIATIONS

WHO: World Health Organization; **ZOI:** Zone of Inhibition; **NA:** Nutrient Agar; **MHA:** Mueller Hinton Agar; **MIC:** Minimum inhibitory concentration; **CFU:** Colony Forming Unit; **MTCC:** Microbial Type Culture Collection; **NCIM:** National Collection of Industrial Microorganisms.

SUMMARY

The objective of this original work is to analyze the phytochemical components and antibacterial properties of aqueous and solvent extracts of *Hibiscus rosa-sinensis* L. leaf. The preliminary phytochemical analysis showed the presence of tannins, flavonoids, steroids, phenolic compounds, saponins in methanolic extract of leaf. In *in vitro* antibacterial activity screening revealed that all the extracts exhibited inhibitory activity against the tested bacteria confirming the potential role of *Hibiscus rosa-sinensis* L. leaves as traditional medicine to treat different clinical conditions by these pathogenic bacteria.

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