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In vitro antibacterial activity of *Hibiscus rosa-sinensis* flower extract against human pathogens

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ABSTRACT

Objective: To access the *in vitro* antibacterial activity of *Hibiscus rosa-sinensis* (*H. rosa- sinensis*) flower extract against human pathogens. **Methods:** Antibacterial activity was evaluated by using disc and agar diffusion methods. The protein was run through poly acrylmide gel electrophoresis to view their protein profile. **Results:** The results showed that the cold extraction illustrates a maximum zone of inhibition against *Bacillus subtillis* (*B. subtillis*), *Escherichia coli* (*E. coli*) *viz.*, (17.00 ± 2.91), (14.50 ± 1.71) mm, followed by hot extraction against, *E. coli*, *Salmonella* sp. as (11.66 ± 3.14), (10.60 ± 3.09) mm. In methanol extraction showed a highest zone of inhibition recorded against *B. subtillis*, *E. coli* as (18.86 ± 0.18), (18.00 ± 1.63) mm pursued by ethanol extraction showed utmost zone of inhibition recorded against *Salmonella* sp. at (20.40 ± 1.54) mm. The crude protein from flower showed a maximum inhibitory zone observed against *Salmonella* sp., *E. coli* *viz.*, (16.55 ± 1.16), (14.30 ± 2.86) mm. The flower material can be taken as an alternative source of antibacterial agent against the human pathogens. **Conclusions:** The extracts of the *H. rosa-sinensis* are proved to have potential antibacterial activity, further studies are highly need for the drug development.

1. Introduction

Nature has been a source of medicinal agents for thousands of years and a striking number of modern drugs have been isolated from natural source, many based on their use in traditional medicines or phytomedicines. Over the years, World Health Organization (WHO) advocated traditional medicines as safe remedies for aliments of both microbial and non microbial origins[1]. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry. Some antibiotics have become almost archaic because of drug resistant and consequently new drugs must be sought, for which herbal treatment is one possible way to treat diseases caused by multi drug resistant bacteria.

It is well known that plants, through lacking the typical immune response, have an in-built system for production against biotic and abiotic, stress conditions. Since plants have coevolved with pathogens, they understandably have also developed the chemical protection pathways against the parasitic organisms. Therefore, it is reasonable to expect a verity of plant compounds with specific as well as general antimicrobial activity and antibacterial potential. The plants *Hibiscus rosa-sinensis* (*H. rosa- sinensis*) belongs to the family Malvaceae. Traditionally the flowers can be used as anti asthmatic agents[2,3]. Many chemical constituents such as cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acids have been isolated from this plant. Resistance towards reveling antibiotics having become widespread among bacteria and fungi, new class of antimicrobial substances are urgently required. There are several studies which reveal the presence of such compounds with antimicrobial properties in various plant parts[4,5]. The petals have some protective mechanism against microbial attack in most of the plants. The *H. rosa-sinensis* flower petals of a large number of plant species growing in the vicinity of our environment were

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screened for their antibacterial activity.

The present study has been designed to determine the role of flower in *H. rosa-sinensis* extract in the *in-vitro* antibacterial activity against human pathogens *viz*., Gram positive bacteria [*Staphylococcus aureus* (*S. aureus*), *Streptococcus*, *Bacillus subtillis* (*B. subtillis*)] and Gram negative bacteria [*Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Salmonella* sp.] and view their protein profile.

2. Materials and methods

2.1. Collection of samples

The flowers were initially separated from the main plants body and rinsed with distilled water and shade dried and then homogenized into fine powder and stored in air tight bottles.

2.2. Extraction of aqueous component

2.2.1. Cold extraction

A total of 10 g of dried flower was soaked in 50 mL of cold water in a conical flask for 24 h and then filtered off using sterile Whatman No. 1 filter paper into a sterile conical flask and evaporated by using solvent distillation apparatus. The extract was got with the help of muslin cloth and centrifuged at 10 000 rpm for 5 min. The supernatant was obtained and stored at 4 ℃ for further use[6].

2.2.2. Hot extraction

A total of 10 g of dried flower was soaked in 50 mL of hot water which was then boiled for 30 min and kept for 24 h undisturbed and then filtered through sterile filter paper, evaporated by using solvent distillation apparatus. The extract was got with the help of a muslin cloth, centrifuged at 10 000 rpm for 5 min and the supernatant was stored at 4 ℃ for further use[7].

2.3. Methanol and ethanol extractions

A total of 10 g of flower air dried powder was weighed and was placed in 100 mL of organic solvents (methanol and ethanol) in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 h. And then it was filtered with the help of muslin cloth and centrifuged at 10 000 rpm for 5 min. The supernatant was collected and the solvent was evaporated by solvent distillation apparatus to make the final volume of one-fourth of the original volume, giving a concentration of 40 mg/mL. It was stored at 40 ℃ in air tight bottles for further studies[8,9].

2.4. Extraction of protein

One gram of dry weight of the flower was dissolved into 2 mL of phosphate buffer and mixed nicely. Centrifuged at 10 000 rpm for 15 min at 4 ℃, supernatant was taken. Further, the equal volume of acetone was added and centrifuged at 10 000 rpm for 15 min at 4 ℃. The pellet was taken and dissolved in phosphate buffer. This was taken as pure sample. The process was preceded in the same way as above for the extraction of crude sample which was dividing of acetone[10].

2.5. Protein profile

The estimated protein was resolved in poly acrylmide gel electrophoresis (PAGE) in which the sample was diluted with sample buffer in the ratio of 1:1 in 2× sample buffer at 100 volt.

2.6. Test microorganism for antibacterial assay

For the *in vitro* antibacterial assay the following human bacterial pathogens were studied such as *S. aureus*, *Streptococcus*, *B. subtillis*, *E. coli*, *P. aeruginosa* and *Salmonella* sp.

2.7. Culture preparation for antibacterial assay

The cultures were grown on nutrient agar at 37 ℃ for 18 h and the colonies were suspended in saline (0.85% Nacl) and its turbidity was adjusted to 0.5 Mac Farland standards (108 CFU/mL). This saline culture preparation was used to inoculate the plates[11].

2.8. Anti bacterial assay

2.8.1. Disc diffusion

In the agar disc diffusion method the test compounds, *i.e.* the flower aqueous and organic extract were introduced into a disc 0.5 mm (hi-media) and then allowed to dry. Thus the disc was completely saturated with the test compound at concentration of 40 mg/mL. Then these discs were placed directly on the surface of Muller Hinton agar plates, swabbed with the test organism and the plates were incubated at 37 ℃ for 24 h.

2.8.2. Agar well diffusion method

Muller Hinton agar plates were prepared and wells of 5 mm were cut and swabbed with different cultures. The cut wells were then filled with 50 μL of both aqueous and solvent extracts of flowers and leaves separately and the plates were kept for incubation at 37 ℃ for 24 h[12].

2.9. Statistical analysis

The results were analyzed by using standard deviation (SD) statistical method[8].

3. Results

3.1. Aqueous extraction

The results clearly showed that cold extractions of flower inhibited *B. subtillis*, *E. coli* with (17.00 ± 2.91), (14.50 ± 1.71) mm, respectively. Hot extraction showed an antibacteria activity against *E. coli*, *Salmonella* sp. at (11.66 ± 3.14), (10.60 ± 3.09) mm. The hot and cold extractions had very low inhibition effects against *B. subtillis*, *P. aeruginosa* at (1.00 ± 0.81), (0.00 ± 0.00) mm and *Staphylococcus* sp., *Salmonella* sp. (8.00 ± 1.63), (8.76 ± 2.71) mm (Table 1).

3.2. Solvent extraction

Methanol extraction showed a highest zone of inhibition recorded against *B. subtillis*, *E. coli* as (18.86 ± 0.18), (18.00 ± 1.63) mm followed by ethanol extraction showed maximum zone of inhibition recorded against *Salmonella* sp. with (20.4 ± 1.54) mm (Table 2).

3.3. Protein assay

The crude protein from flower shows a maximum inhibitory zone observed against *Salmonella* sp, *E. coli viz.*, (16.55 ± 1.16) and (14.30 ± 2.86) mm. Pure protein showed zone of inhibition against *Staphylococcus* sp, *E. coli* such as (11.4 ± 1.74), (12.25 ± 0.97) mm (Table 3).

3.4. Protein profile

The crude and pure *H. rosa-sinensis* flower proteins were run in PAGE. The different band patterns are showed in Figure 1. The gel shows a different band separation, the crude protein shows a thick band and more bands were observed.

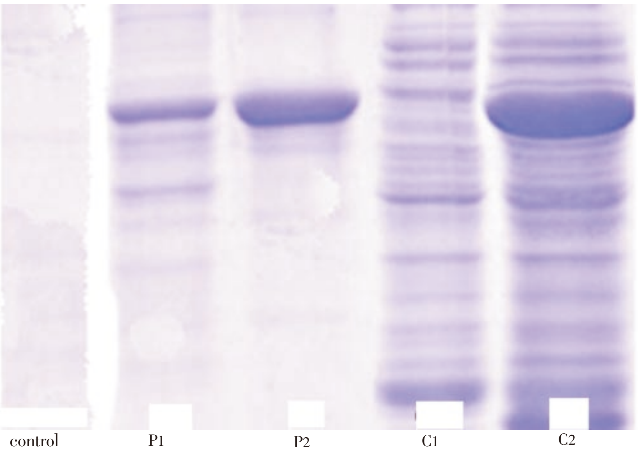

Figure 1. Protein profile from *H. rosa-sinensis* flower.
P-1 and P-2: pure protein; C-1 and C-2: Crude protein

Table 1
Antibacterial activity of cold and hot aqueous extract of *H. rosa-sinensis* in agar and disc diffusion method (Mean ± SD) (mm).

Test organisms	Agar diffusion method		Disc diffusion method	
	Cold aqueous extract	Hot aqueous extract	Cold aqueous extract (mm)	Hot aqueous extract
S. aureus	11.43 ± 2.85	0.00 ± 0.00	8.00 ± 1.63	1.71 ± 1.47
Streptococcus sp.	13.80 ± 3.23	5.44 ± 2.74	11.77 ± 4.22	3.50 ± 3.45
B. subtillis	17.00 ± 2.94	1.66 ± 2.35	9.00 ± 2.16	1.00 ± 0.81
E. coli	14.50 ± 1.71	11.60 ± 3.14	13.66 ± 3.85	9.00 ± 2.44
Salmonella sp.	13.15 ± 1.71	10.66 ± 3.09	8.76 ± 2.71	10.00 ± 2.44
P. aeruginosa	14.00 ± 3.26	0.00 ± 0.00	13.56 ± 2.36	2.88 ± 1.50

Table 2
Antibacterial activity of solvent extraction in *H. rosa-sinensis* in agar and disc diffusion method (Mean ± SD) (mm).

Test organisms	Agar diffusion method		Disc diffusion method	
	Methanol extract	Ethanol extract	Methanol extract	Ethanol extract
S. aureus	0.00 ± 0.00	10.00 ± 1.84	0.66 ± 0.94	13.27 ± 0.75
Streptococcus sp.	14.18 ± 2.80	10.16 ± 1.64	16.26 ± 1.67	12.66 ± 1.24
B. subtillis	18.86 ± 0.18	12.80 ± 2.75	16.50 ± 1.22	12.41 ± 1.62
E. coli	17.16 ± 1.64	0.33 ± 0.47	18.00 ± 1.63	0.00 ± 0.00
Salmonella sp.	0.00 ± 0.00	15.30 ± 2.35	0.00 ± 0.00	20.40 ± 1.54
P. aeruginosa	0.00 ± 0.00	16.30 ± 0.94	0.00 ± 0.00	15.58 ± 0.54

Table 3
Antibacterial activity of protein in *H. rosa-sinensis* in agar and disc diffusion method (Mean ± SD) (mm).

Test organisms	Agar diffusion method		Disc diffusion method	
	Crude protein	Pure protein	Crude protein	Pure protein
S. aureus	0.00 ± 0.00	11.00 ± 2.94	1.00 ± 0.81	11.40 ± 1.74
Streptococcus sp.	12.04 ± 0.86	1.76 ± 0.88	13.06 ± 0.89	2.80 ± 0.57
B. subtillis	0.00 ± 0.00	1.00 ± 1.41	1.06 ± 0.09	4.16 ± 0.60
E. coli	13.70 ± 0.87	12.25 ± 0.97	14.30 ± 2.86	11.00 ± 1.63
Salmonella sp.	15.21 ± 2.28	1.86 ± 0.65	16.55 ± 1.16	4.26 ± 0.73
P. aeruginosa	11.50 ± 2.28	1.86 ± 0.65	13.01 ± 1.63	2.44 ± 1.02

4. Discussion

The knowledge of medicinal property of plants has been accumulated in the course of many centuries. The local inhabitants have inherited rich traditional knowledge on the use of many plants or plant parts for treatment of common disease. Medicinal plants provide accessible and culturally relevant sources of primary health care. The remedies based on these plants often have minimal side effect[13]. The bioactive substances in plants are produced as secondary metabolites, which may not only be developmental stage specific but also organ and tissue specific. While plant leaf, stem and root extracts have been widely evaluated for bioactive compounds, screening of plant flower has not been extensive. Secondary metabolites belonging to polyketide and nonribosomal peptide families constitute a major class of natural products with diverse biological functions and they have a variety of pharmaceutically important properties. Experimental studies have shown that the biosynthetic machanism for polyketide and nonribosomal peptides involves multi-functional megasynthases[14].

The antibacterial activities of *H. rosa-sinensis* flower petals were carried out. Most of the extract shows an antibacterial activity against the human pathogens such as *E. coli*, *B. subtillis*, *P. aeruginosa*, *S. aureus*, *Streptococcus* sp. *Salmonella* sp. All the extracts of flower have shown the activity. Investigations were carried out of plant materials as alternative sources of antibacterial agents. It has become more common over the past few years, due to the increased rate of development of antibiotic resistance organism. The inhibition of bacterial growth *in-vitro* by the extracts of flower could be due to the presence of some active compounds in the extracts. These active compounds may act alone or in combination to inhibit bacterial growth. The crude extracts containing multiple organic components including flavonoids, tannins, alkaloids, triterpenoids, all of which are known to have antibacterial affects. Flower extract contain phenolics compounds like tannins that are very good antimicrobial agent[15]. Thus it may be summarized that the class of natural compounds must exhibit the antibacterial activity. The metabolites have been shown to be responsible for various therapeutic activities of medicinal plants[16]. Flavonoids especially are known to be effective antimicrobial agent against a wide array of microorganisms. The activity is attributed to their ability to complex with extra cellular and soluble proteins and with bacterial cell wall[17]. There are several reports published on antibacterial activity of different herbal extracts[18-25]. It supports the earlier investigation that the tannins isolated from the flower possess remarkable toxic activity against bacteria and may assume pharmacological importance[26-38]. Many antimicrobial screening studies use a relatively small number of microorganisms for testing. It is possible that these plant materials contain antibacterial compounds against pathogenic bacteria other than those tested in this study. In addition, the lack of activity may be because of degradation of active chemicals during the drying process, the extraction process.

In the present investigation flower extracts from *H. rosa-sinensis* were screened for antibacterial activity against human pathogenic bacterial strains. Most of the extracts have shown antibacterial activity against these pathogens. *E. coli* are common member of the normal flora of large intestine. It is predominant facultative organism in the gastrointestinal tract and colonizes the tract within hours or few days. It is responsible for causing diarrhea which is characterized by rapid onset of watery non bloody fluid. *Pseudomonas* sp. is the epitome of an opportunistic pathogen to human. It causes urinary tract infection, respiratory system infection, dermatitis soft tissue infection, gastrointestinal infection and a variety of systemic infection. *S. aureus* is a facultative anaerobe that grows by aerobic respiration or by fermentation which yields lactic acid. These are pathogenic to human beings. They cause a wild range of superlative infection as well as food poisoning and toxic shock syndrome. *Salmonella* sp. includes a large number of pathogens of human beings as well as mammals. These are pathogenic when acquired by oral route. Broadly they may cause enteric fever, septicemia and enteritis. The enteric fever and septicemia are caused by thousand of *Salmonella*. Thus the plant extracts can be used as an important antibiotic to cure above mentioned disorders caused by the different strains of bacteria. The present studies conclude these extract could inhibit human pathogens growth[39-41]. The results are encouraging but precise assessment is utterly necessary before being situate in practice as well as the most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo secondary pharmacological evaluation.

Conflict of interest statement

We declare that we have no conflict of interest.

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