

Antibacterial Potentiality of *Hibiscus rosa-sinensis* Solvent Extract and Aqueous Extracts Against Some Pathogenic Bacteria

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Abstract

Ayurvedic treatment exploits nature and its wealth are the medicinal plant, one such is *Hibiscus* which is used in discriminately so proper Antimicrobial assay was preformed to confirm its importance in traditional Holistic medicine. In this regard the solvent and the aqueous extract of the plants leaf and flower was obtained and its activity against gram positive and gram negative bacteria was tested by agar well diffusion and agar disk diffusion assay. They were highly positive suggesting the medicinal property of the plant.

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive 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 ailments of both microbial and non microbial origins (WHO 1978). 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 obsolete 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.

The use of plant extracts and phytochemicals, with known antibacterial properties may be of immense importance in therapeutic treatments. In the past few years, a number of studies have been conducted in different countries to prove such efficiency (Olukoya *et.al.*, 1993).

The plants *Hibiscus rosa-sinensis* belongs to the family malvaceae the roots are cylindrical 5-1 cm in length and 2 cm in diameter, off white and with light brown transverse tenticals. The roots taste sweet and are mucilaginous. The leaves are simple ovate or ovate lanceolate and are entire at the base and coarsely toothed at the apex. The corolla consists of 5 petals, red and about 8cm in diameter. Traditionally bark of the plants is used for ant fertility and is used for the control of dysfunctional citerine bleeding and as an oral contraceptive. The flowers are used as anti asthmatic agents (Stiffness *et.al.*, 1982). Many chemical compounds like Cyandin, Quercetin, Hentriacontane, Calcium oxalate, Thiamine, Riboflavin niacin and ascorbic acids have been isolated.

The present study has been designed to determine the role of flower and leaf extracts of *Hibiscus rosa-sinensis* was screened for potential antibacterial activity against two gram positive bacteria (*Staphylococcus*

aureus and *Bacillus subtilis*) and two Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*).

Materials and methods

The plant material used in this study was collected from Tamilnadu Agricultural University, during December 2008. The leaves and flowers were initially separated from the main plants body and rinsed with distilled water, Dried under shade paper towel in laboratory and then homogenized into fine powder and stored in air tight bottles and were used for all the extraction process.

Preparation of extracts

Extraction of aqueous component

Cold aqueous extraction

10g of each flower and leaves air dried powder was weighed and soaked separately in 50ml cold water in a conical flask stoppered with rubber cork and left undisturbed for 24 hrs and then filtered off using sterile filter paper (What Man No: 1) into a sterile conical flask and subjected to water bath evaporation, where the aqueous solvent was evaporated at its boiling temperature 100°C. The extract was got with the help of muslin cloth and was subjected to centrifugation at 5000Xg for 5 mts and the supernatant was obtained and stored at 4°C for further use (Farombi *et.al.*, 2003).

Hot aqueous extract

10g of each flower and leaves air dried powder was weighed and soaked separately in 50 ml of hot water which was then boiled for 30mts and kept in a conical flask for 24 hrs undisturbed and then filtered off using sterile filter paper in a sterile conical flask and subjected to water bath evaporation, where the aqueous solvent was evaporated at its boiling temperature 100°C. The extract was got with the help of a muslin cloth

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and was subjected to centrifugation 5000Xg for mts and the supernatant was stored at 4°C for further use (Ekpendu *et.al.*, 1994).

Solvent extraction

Methanol extract

10g of each leaf and flower air dried powder was weight and was placed in 100ml of organic solvent (methanol) in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 hrs after 24 hrs it was filtered with the help of muslin cloth and centrifuged at 5000Xg for 15 mts. The supernatant was collected and the solvent was evaporated to make the final volume of one-fourth of the original volume, giving a concentration of 40 mg/0.1ml. It was stored at 40°C in air tight bottles for further studies (Ikram *et.al.*, 1984).

Test Microorganism for Antibacterial Assay

The microbial strains are standard which were obtained from IMTECH, Chandigarh. The bacterial strains studied are *Staphylococcus aureus* MTCC 2940, *Bacillus subtilis* MTCC B441 the two Gram positive strains and *E.coli* MTCC739 and *P.aeruginosa* MTCC2453 the two Gram negative strains.

Culture Preparation for Antibacterial Assay

The cultures were grown on nutrient agar at 37°C for 18 hrs and the colonies were suspended in saline (0.85%Nacl) and its turbidity was adjusted to 0.5 Mac Far land standards (10⁸ CFU/ml). This saline culture preparation was used to inoculate the plates (Almagboulk *et.al.*, 1985).

Anti Bacterial Assay

Antibacterial assay was performed by two methods

Agar Disk Diffusion: In the agar disk diffusion method the test compound ie the flower and leaves aqueous and organic extract were introduced into a disk 0.7cm (hi-media) and then allowed to dry. Thus the disk was completely saturated with the test compound. Then these disks were placed directly on the surface of MHA plates swabbed with the test organism and the plates were incubated at 37°C for 24 hrs.

Agar Well Diffusion Method: Muller Hinton agar plates were prepared and wells of 6mm were cut and swabbed with different cultures and the cut wells were then filled with 50µl of both aqueous and solvent extracts of flowers and leaves separately and the plates were kept for incubation at 37°C for 24 hrs (Artizzu *et.al.*, 1995).

Result and Discussion

Antibacterial activity of the hot aqueous extract of Hibiscus rosa-sinensis leaf and flower by the two different methods towards the four different test organisms are shown in Table: 1.

The organism *Staphylococcus aureus* was found to be sensitive towards both the leaf and flowers hot aqueous extract by both the agar disk diffusion and Agar well diffusion method.

Bacillus subtilis was found to be resistant to both the hot aqueous extraction by both of the antibacterial assay method.

S No	Test organism	Hot Aqueous extract			
		Agar Disk Diffusion		Agar well Diffusion	
		Leaf	Flower	Leaf	Flower
1	<i>Staphylococcus aureus</i>	+	+	+	+
2	<i>Bacillus subtilis</i>	-	-	-	-
3	<i>Escherichia coli</i>	+	-	+	-
4	<i>Pseudomonas aeruginosa</i>	+	-	-	-

Table: 1 Antibacterial activity of hot aqueous extract of *Hibiscus rosa-sinensis* leaf and flowers.

Pseudomonas aeruginosa showed sensitivity towards the hot aqueous extract of leaf alone by the two methods. Where as it resistant to the flower extract.

E.coli was found to be sensitivity only to the hot aqueous extract of *Hibiscus rosa-sinensis* leaf that too by agar disk diffusion method and it was contrary to the result got in well diffusion method.

The antibacterial activity of the cold aqueous extract of *Hibiscus rosa-sinensis* leaf and flowers by the two different antibacterial assay methods are shown in Table: 2.

S No	Test organism	Cold Aqueous extract			
		Agar Disk Diffusion		Agar well Diffusion	
		Leaf	Flower	Leaf	Flower
1	<i>Staphylococcus aureus</i>	-	-	-	-
2	<i>Bacillus subtilis</i>	-	-	-	-
3	<i>Escherichia coli</i>	-	-	-	-
4	<i>Pseudomonas aeruginosa</i>	-	-	-	-

Table: 2 Antibacterial activity of cold aqueous extract of *Hibiscus rosa-sinensis* leaf and flowers.

The cold aqueous extract did not show any activity towards any of the 4 different test organisms by both of the two methods. This reveals that the active Antibacterial component of the flowers and leaves could not be extracted with the help of cold extraction which may be due to the nature of the chemical component present in plants leaves and flowers.

Antibacterial activity of the organic extract methanol of *Hibiscus rosa-sinensis* flower and leaves by both of the assay methods results are shown in Table: 3

S No	Test organism	Methanol Aqueous extract			
		Agar Disk Diffusion		Agar well Diffusion	
		Leaf	Flower	Leaf	Flower
1	<i>Staphylococcus aureus</i>	+	+	+	+
2	<i>Bacillus subtilis</i>	-	+	-	+
3	<i>Escherichia coli</i>	+	+	+	+
4	<i>Pseudomonas aeruginosa</i>	-	-	-	-

Table: 3 Antibacterial activity of Methanol extract of *Hibiscus rosa-sinensis* leaf and flowers.

The organism *Staphylococcus aureus* and *E.coli* were found to be sensitive to both the leaf and flower organic extract by both the assay method.

The test strain *Paeruginosa* was found to be resistant to the organic extract of both flower and leaf by two assay methods.

The results were interpreted from control plates which had only the organic solvent without the plant and leaf component.

The test strain *B.subtilis* was found to sensitive in both the methods to the flower extract. This property of Bacillus provokes eagerness to find the reason which may be a chemical component peculiar to the flower(Sousa *et.al.*, 1991).

Conclusion

From the preliminary screening, we have identified the methanol extract of Hibiscus has got phytochemical property it may be due to the nature of biologically active compounds present in hibiscus whose activity are enhanced in the presence on methanol and also methanol has a stronger extraction capacity which could have produced greater number of active constituents responsible for antibacterial activity(Barker *et.al.*, 1995).

The antimicrobial activities can be enhanced if the active components are purified and adequate dosage determined for proper administration. This may go a long way in preventing the administration of inappropriate concentration a common practice among many traditional medical practitioners.

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