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Evaluating Antagonistic potential of leaves and flowers of Hibiscus rosa sinesis against Staphylococcus aureus MTCC-3160

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ABSTRACT

These days, natural plant products are widely used due to the rising number of illnesses. The plant known as Hibiscus rosa-sinensis L. (Family Malvaceae) is found all over the world. The Indian traditional medical system has used its leaves, barks, roots, and flowers as medicine to treat a variety of illnesses. Numerous studies have demonstrated the antioxidative, antimicrobial, antidiabetic, antiulcer, hepatoprotective, antifertility, antigenotoxic, and anti-inflammatory qualities of the Hibiscus rosa-sinensis plant, which aid in the treatment of a wide range of illnesses. Numerous herbal mixes and beverages have included hibiscus rosa-sinensis. The flowers and leaves of Hibiscus rosa-sinensis are evaluated in numerous animal-based research studies as antioxidant and antidiabetic substances. The goal of this review is to draw attention to the medicinal uses of Hibiscus rosa-sinensis.

Folk medicine has long employed crude preparations of Hibiscus rosa-sinensis leaves and flowers for a variety of purposes. In this work, we used the *Staphylococcus aureus* straightforward agar disc diffusion and method to assess the antibacterial activity of extracts from the leaves and flowers of Hibiscus rosa-sinensis against a few clinical isolates of bacteria. With the exception of *Klebsiella pneumoniae*, every bacterial isolate in the initial screening experiment displayed varied degrees of sensitivity to the flower extracts. The gram-positive bacterium Staphylococcus aureus proved to be the most responsive to flower extracts. However, with the exception of S. aureus, the majority of the tested bacterial isolates showed resistance to the leaf and flower extracts. Our research unequivocally shows that H. rosa-sinensis flower extracts had a greater antibacterial effect than leaf extracts.

Keywords:

Zone of inhibition, Hibiscus rosa-sinensis, Gentamycin, antibiotic resistance, and antibacterial activity, Staphylococcus aureus.

INTRODUCTION

The bushy evergreen shrub Hibiscus rosa-sinensis grows to a height of 2.5–5 m (8–16 ft) and a width of 1.5–3 m (5–10 ft). The taproot system of the plant is branched. It has an aerial, erect, green, cylindrical stem that is branching. Its petiolate, simple leaves have alternating phyllotaxy. The leaf has an ovate shape, a sharp tip, and a serrated margin. The leaves' veins are branched or divergent because they have unicostate reticulate veins. The RHS A-Z encyclopedia of garden plants describes their surfaces as glossy. There are free lateral stipules. It blooms in the summer and fall.

They are symmetrical and solitary (axillary). The RHS A-Z encyclopedia of garden plants states that they are usually red, have five petals that are 10 cm (4 in) in diameter, and have noticeable orange-tipped red anthers. For a variety of uses, medicinal plants are widely employed. The ability of the plants to synthesize chemical compounds that are important in preventing various diseases like diabetes, cancer, and other conditions has led to their identification as medicinal plants. Numerous other chemical compounds serve vital biological roles and protect living things from herbivorous mammals, fungi, insects, and other predators. Less than 10% of the total number of such compounds have been isolated thus far, with at least 12,000 having been found (Tapsell L.C. et al; 2006, Lai P.K., Roy J. et al; 2004).

They are used to treat a variety of conditions, including coughs, carbuncles, mumps, rashes, excessive and painful menstruation, cystitis, and feverish illnesses. Coughs have been treated with mucilage made from the root of H. rosa-sinensis (Duke and Ayensu et al., 1985, Chopra et al., 1986). According to ancient Indian medical literature, H. rosa-sinensis flowers have been shown to be beneficial for heart conditions (Nadkarni et al., 1976). The red hibiscus's historical origin and range are unknown. There are about 200 species in the genus Hibiscus, which are found in tropical and subtropical areas. The red hibiscus's historical origin and range are unknown. There are about 200 species in the genus Hibiscus, which are found in tropical and subtropical areas. placed in full sun on rich, well-drained soil that is rich in organic matter.

SOURCES AND METHODS

This study was carried out in the biotechnology research labs at R.B.S. College in Agra.

Making the crude extract: In September 2023, the botany department of R.B.S. Khandari Campus, Dr. Bhimrao Ambedkar University, Agra (U.P.), provided the leaves and flowers of Hibiscus rosa-sinensis. The plants' leaves and blossoms were meticulously cleaned with sterile water after being submerged in running tap water. After being air dried for two days at room temperature (300), they were ground into a fine powder with a sterile mortar and pestle and kept in airtight bottles. Four extracts in total were obtained by extraction using an aqueous and an ethanolic solvent. 150ml of aqueous was used as the solvent during the 12-hour mildly hot continuous percolation of 10g of ground-up leaves and flowers in a Soxhlet apparatus. The process for removing ethanol as a solvent from flowers and leaves was the same. A hot plate was then used to concentrate the extracts.

How to Make Mueller Agar Plates :utilizing a weighing balance to weigh the Mueller Hinton Agar. Put 7.5 grams of the medium in 250 milliliters of purified water. To fully dissolve the medium, boil for one minute while heating with frequent stirring. For 15 minutes, autoclave at 1210C. Let cool to room temperature.

The antimicrobial properties of H. rosa-sinensis: Disc diffusion method was used against various microorganisms, and disc diffusion method was used to investigate the antibacterial activity of plant extracts. Using this method, a stock culture is revived and the pure culture of microorganisms is subcultured in nutritional broth and incubated at 370C. Discs (6 mm) were prepared using Whatman's filter paper. These discs were allowed to dry in a laminar air flow while H. rosa-sinensis leaf extracts were serially diluted. These discs were exposed to varying concentrations of both aqueous and ethanol extract. Hibiscus rosa-sinensis leaves and flowers were serially diluted in aqueous and ethanol extracts. One way to prepare the dilution blanks is to fill tubes with the appropriate dilution liquid. 9ml of dilution blank tube 1 is filled with 1ml of the starting sample in order to perform serial dilution. The same process is then carried out, adding one milliliter from tube 1 to nine milliliters from tube 2, one milliliter from tube 2 to nine milliliters from tube 3, and so forth, until the required concentration is obtained. Therefore, the dilution in tubes 1, 1/10, and 1/100 became, correspondingly. A quarter of a microliter of the suspension was moved to the discs. The antimicrobial activity was assessed using gentamycin and vancoycin at a standard concentration of 0.1 mg/ml. The discs were prepared in the appropriate solvent for the negative control. Mueller Hinton Agar is the medium used to measure the antimicrobial activity. The Mueller hinton agar plate that had solidified was covered with the test organism, staphyllococcus aureus. After that, the discs that had been previously

prepared in various extracts were placed on MHA agar media. Following that, these plates were stored for 24 hours at 370C in a microbiological incubator. The clear area surrounding the disc, or the diameter of the zone of inhibition, served as a proxy for antimicrobial activity.

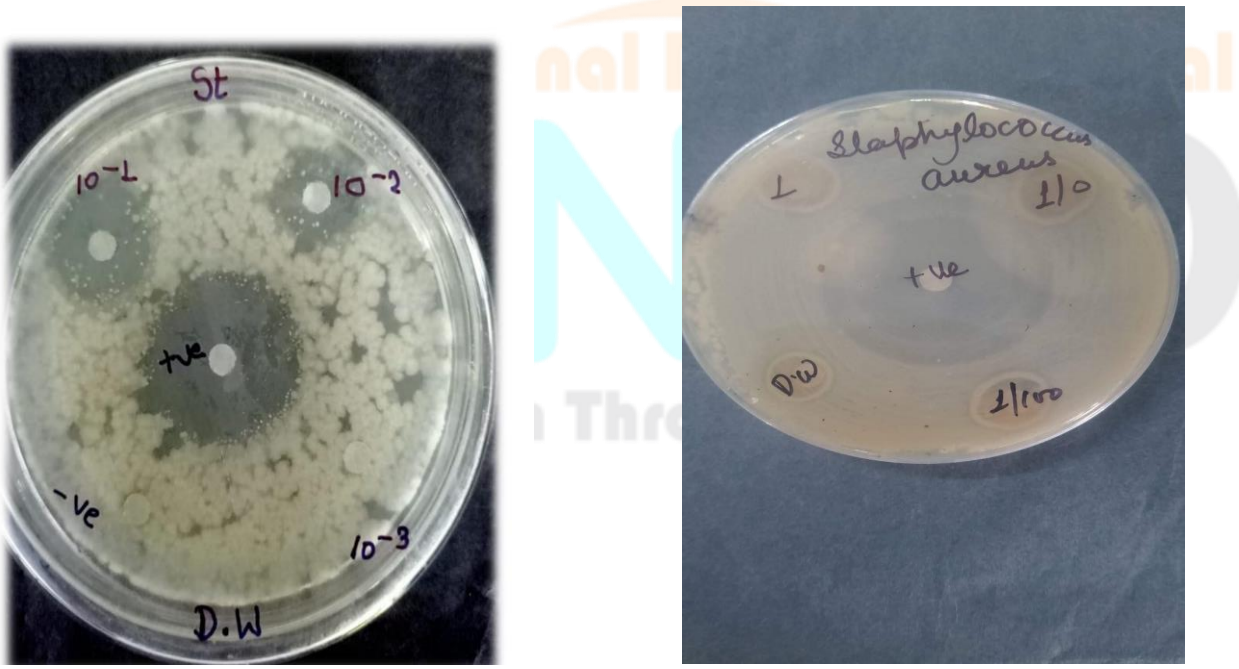
Result and Discussion

Various amounts of crude extracts were obtained when the plant parts, namely the leaves and flowers, were extracted. It was found that the yield of crude extract varied amongst all extracts in various solvents. The amount of plant part crude extract in various solvents.

Leaves

Using the disc diffusion method, the antimicrobial activity of leaf extracts in ethanol and chloroform was investigated. staphyllococcus aureus are test organisms used to measure antimicrobial activity (MTCC 3160). The diameter of the zone of inhibition is used to calculate the extract's activity against a specific microorganism. The extract is more effective against that microorganism if the zone of inhibition has a larger diameter. If there is no zone of inhibition, the test microorganism is not susceptible to that extract. Two antibiotic used to measure the antimicrobial activity of plant extracts are gentamycin and vancomycin.

. The figure illustrates the impact of various plant extract solvents on stapylococcus aureus.



(1) **(2)**

Fig. Effect of leaf extract on *staphyllococcus aureus* (1. Leaf chloroform , 2. Leaf ethanol)

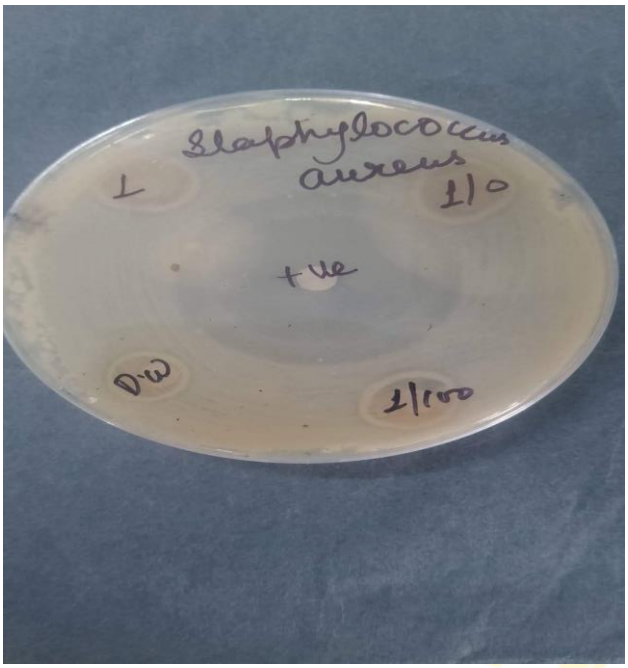
According to the research, the ethanol extract exhibited the maximum zone of inhibition, indicating that it is as effective as possible against staphyllococcus aureus. The antibacterial effect of leaf extract in ethanol was demonstrated. The zone of inhibition in gentamycin had a 27 mm diameter. The ethanol and chloroform extracts had 30% inhibition when compared to the standard. The same technique was used to assess antimicrobial activity against staphyllococcus aureus. The leaf ethanol extract was found to have the strongest anti-staphyllococcus aureus activity. In the case of concentrated chloroform and ethanol extract solution of leaves, the zone of inhibition's diameter measured 15/15 mm. The standard's leaf zone of inhibition measured 20/27 mm for both the ethanol and chloroform extracts.

Table 1: Effect of different solvents on *staphyllococcus aureus* bacteria.

S. No	Organism	Solvent	Zone of inhibition(mm) diameter				
1.	*Staphylococcus aureus*	Ethanol	+ve	-ve	1	1/10	1/100
		Antibiotic-(Gentamycin))	27	10	15	12	10
2	*Staphylococcus aureus*	Chloroform Antibiotic-Vancomycin	20	9.5	15	11	10

FLOWERS

Using the disc diffusion method, the antimicrobial activity of flower extracts in ethanol and water solutions was investigated. staphyllococcus aureus are test organisms used to measure antimicrobial activity (MTCC 3160). The diameter of the zone of inhibition is used to calculate the extract's activity against a specific microorganism. The extract is more effective against that microorganism if the zone of inhibition has a larger diameter. If there is no zone of inhibition, the test microorganism is not susceptible to that extract. Two antibiotic used to measure the antimicrobial activity of plant extracts gentamycin and vancomycin . The figure illustrates the impact of various solvent plant extracts on staphyllococcus aureus.



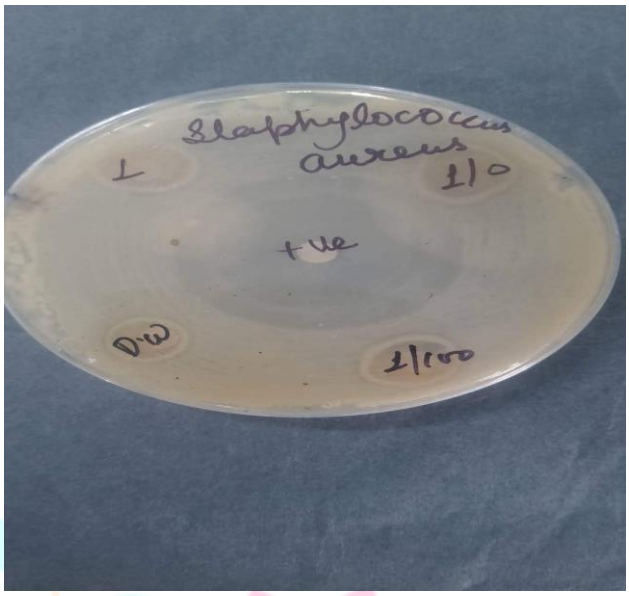


Fig Effect of flower extract on *staphyllococcus aureus* (1 flower chloroform , 2 flower ethanol)

According to the research, the ethanol extract exhibited the maximum zone of inhibition, meaning it was most effective against staphyllococcus aureus. The antibacterial effect of leaf extract in ethanol was demonstrated. The distilled water and ethanol extracts demonstrated 40% inhibition when compared to the standard, while the zone of inhibition in gentamycin had a diameter of 40 mm. The same technique was used to assess antimicrobial activity against staphyllococcus aureus. The leaf ethanol extract was found to have the strongest anti-staphyllococcus aureus activity. In the case of concentrated chloroform and ethanol extract

S. No	Organism	Solvent	Zone of inhibition(mm) diameter				
1.	*Staphylococcus aureus*	Ethanol	+ve	-ve	1	1/10	1/100
		Antibiotic-(Gentamycin))	40	15	21	16	17
2	*Staphylococcus aureus*	Chloroform	36	11	17	12	11
		Antibiotic-Vancomycin					

solution of leaves, the zone of inhibition had a diameter of 21-17 mm. The standard zone of inhibition's diameter for the ethanol extract of leaves was 40 mm.

FINAL VERDICT(conclusion)

Our results unequivocally show that H. rosa-sinensis flower extracts exhibited stronger antibacterial effects than leaf extracts.

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