Phytochemical Profiling, Antioxidant and Antibacterial Activity of *Hibiscus rosa-sinensis* against Pathogenic bacteria

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Abstract- Plants have been a valuable resource of natural products for human health. *Hibiscus rosa-sinensis* (Gurhal) is cultivated ornamental and medicinal plant widely used in traditional healthcare systems. The present study focuses on the phytochemical composition and antioxidant and antibacterial activity from the flowers of Gurhal. Phytochemical screening revealed the presence of important bioactive constituents including flavonoids, saponins, glycosides, phenolic compounds, alkaloids, tannins and terpenoids. These secondary metabolites are responsible for the therapeutic and pharmacological properties of the plant. Antioxidant activity of the extracts was evaluated using standard free radical scavenging assays, which demonstrated significant antioxidant potential due to the high content of phenolic and flavonoid compounds. In addition, antibacterial studies showed effective inhibitory activity against *staphylococcus aureus*. Among the tested extracts, the acetone and methanol extracts exhibited comparatively higher biological activity than the other extracts. The finding suggests that *Hibiscus rosa-sinensis* can serve as a valuable source of natural antioxidants and antibacterial agents for pharmaceutical and medicinal and therapeutic applications.

Key words: *Hibiscus rosa-sinensis*, Phytochemical screening, Antioxidant & Antibacterial Activity.

Introduction

Hibiscus rosa-sinensis, commonly known as China rose and gurhal, belongs to the family Malvaceae and is widely cultivated. A highly potential functional and valuable medicinal plant has been reported in the ancient medicinal literature with beneficial effects in various disorders of humans[(1)].

Medicinal plants are considered an important source of natural therapeutic agents because of their phytochemical constituents. *Hibiscus rosa-sinensis* is rich in secondary metabolites such as flavonoids, phenolic compounds, alkaloids, tannins, saponins, glycosides and terpenoids. These phytochemicals play a vital role in the plant's biological and pharmacological activities. The flower petals, in particular, contain anthocyanins and flavonoids that contribute to their antioxidant properties. They can function as sources for nutritional supplements or serve as natural antioxidants, which help maintain food quality and prolong shelf-life[(2-3)].

The natural antioxidants may have free-radical scavengers, reducing agents,

potential complexes of pro-oxidant metals, quenches of singlet oxygen [(4)]. Recently, interest has increased considerably in finding natural occurring antioxidants for use in medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity [(5)]. Crude alcoholic extracts of *Hibiscus rosa-sinensis (H. rosa-sinensis)* flower petals and leaves are used *in vivo* for their antioxidant properties in some studies [(6)].

Rising antimicrobial resistance is forcing a search for new, natural medicine sources. *Staphylococcus aureus* is a Gram-positive coccus, and is the common cause of staph infection. About 20% of human population if carries *Staphylococcus aureus* can cause gor long term illness of skin infection, such as pimples, impetigo, and cellulitis folli-cutis. Extracts from *Hibiscus rosa-sinensis* flowers successfully inhibit *staphylococcus aureus* making them ideal candidates for herbal remedies. This therapeutic power relies on the synergistic effects of its natural plant compounds. While *H. rosa-sinensis* and *H. acetosella* are highly valuable, *H. sabdariffa* stands out globally for its culinary appeal and proven antioxidant, anti-inflammatory, and antimicrobial health benefits[(7)]. Antioxidant prevent occurrence of these diseases by inhibiting the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress[(8)].

Therefore, the present study aims to investigate the phytochemical profile, antioxidant potential, and antibacterial activity of *Hibiscus rosa-sinensis*. These findings highlight its importance as a valuable medicinal plant with promising pharmaceutical applications.

Material and Methods

Fresh flowers of *Hibiscus rosa-sinensis* were collected from healthy plants growing in the local area. The plant material was washed thoroughly with distilled water to remove dust and other impurities and then shade dried at room temperature for one week. The dried sample was powdered using mechanical grinder and stored in airtight containers for further analysis.

Preparation of plant extract- The powdered plant material was extracted using different solvents such as methanol, ethanol, acetone, hydro-alcoholic, hexane and distilled water by maceration method. 20 g of powdered sample was mixed with 200 ml of solvent and kept for 24- 48 hours with occasional shaking. The extracts were filtered with whatman no.1 filter paper and concentrated using water bath.

Phytochemical Screening- Phytochemical analysis of the extracts was carried out using standard qualitative methods to identify the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, saponins, phenolic compounds, and terpeniods.[(9)]

1) **Flavonoids** (Shinoda Tests) - Took 2-3 ml of the extract solution in a test tube. Added a few magnesium turnings, few drops of conc. HCl was added drop-wise. Pink to red coloration develops.

2) **Alkaloids** (Mayer's test)- Extracts were treated with Mayer’s reagent (potassium mercuric chloride). Formation of a yellow colored precipitate indicates the presence of alkaloids.

3. Tannins- To the extracts, a few drops of 10% ferric chloride solution were added. Appearance of a green or blue colour indicates the presence of tannins.

4) Phenolic compound (Ferric chloride test)- Two ml of diluted extracts were treated with dil. $FeCl_3$ solution. Appearance of a violet color indicated the presence of phenol like compounds.

5) Saponins (Foam test)- The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicated the presence of saponins.

6) Terpenoids (Salkowski Test)- Five ml of the extract were mixed with 2ml of chloroform and 3ml of conc. H_2SO_4 solution. A reddish-brown colour at the interphase indicated the presence of terpenoids.

Antibacterial Activity- The antibacterial activity of the extracts was evaluated against selected bacterial strain (*Staphylococcus aureus - ATCC 6538*) using the agar well diffusion method. Sterile nutrient agar plates were inculcated with microbial cultures, and wells were made using a sterile cook borer. The known concentration of plant extracts were added into the well, while standard antibiotics were used as positive controls. The plates were incubated at 37^0C for 24 hours for bacterial cultures. The antibacterial activity was determined by measuring the diameter of the zone of inhibition around each well[(10)].

Antioxidant Activity- The radical scavenging activity (antioxidant activity) was determined according to a previously reported method[(11)] with slight modifycations. A stock solution of the sample (50mg/ml) was diluted for different concentrations. The following concentrations of extracts were prepared 20µg/mL, 40µg/mL, 60µg/mL, 80µg/mL, 100µg/mL and 120µg/mL. Each concentration was tested in duplicate. The 3.0ml of sample solution was mixed with 1.0ml of 0.1mM 1, 1-Diphenyl-2-2picrylhydrazyl (DPPH, in methanol) and allowed to stand at room temperature for 30 minute under light protection. The absorbance was measured at 517nm. The scavenging activity of the samples at corresponded intensity of quenching DPPH. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The difference in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as (%) scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

Inhibition % = Ac-As/Ac×100
Where, Ac is the absorbance of the control
As is the absorbance of the sample

Result and Discussion

The present study was undertaken to evaluate the phytochemical constituent, antibacterial activity, and antioxidant activity of flower extract of *Hibiscus rosa-sinensis*. The extracts were subjected to phytochemical screening, antibacterial assessment against *staphylococcus aureus*, and antioxidant evaluation using the DPPH radical scavenging assay.

The preliminary phytochemical screening of different extracts of *Hibiscus rosa-sinensis* flowers revealed the presence of several bioactive constituents shown in Table -1. Alkaloids, tannins, saponins, terpenoids, flavonoids, and phenolic compounds were detected in most of the extracts. The aqueous extracts showed a comparatively higher abundance of alkaloids, indicating that water was more

effective in extracting polar phytoconstituents. The presence of phenols tannins, flavonoids and terpenoids is significant because these compounds are known to possess antioxidant and antibacterial properties.

Table-1 Phytochemical Screening of *Hibiscus rosa-sinensis.*

Sr.No.	Chemical Test	Ethanol	Aqueous	Methanol	*Hexane*	*Acetone*	*Hydro-Alcoholic*
1	*Alkaloids*	+	++	+	+	+	+
2	*Flavonoid*	-	+	-	-	-	+
3	*Tannins*	+	+	+	-	+	+
4	*Saponins*	+	+	+	-	+	+
5	*Terpenoids*	+	+	+	-	+	+
6	*Phenol*	+	+	+	-	+	+

The antibacterial activity of different flower extracts of *Hibiscus rosa-sinensis* against *staphylococcus aureus* is presented in table-2. Among all extracts, the acetone extract exhibited the highest antibacterial activity 20mm, followed by methanol extract 17mm. the antibacterial effect observed in acetone and methanol extract may be attributed to the presence of phenolic compounds, tannins, and terpenoids, which are known to disrupt bacterial cell membranes and inhibit microbial growth. Hydro-alcoholic, aqueous, and ethanol extracts showed moderate antibacterial activity with inhibition zones of 14mm, 13mm and 13mm respectively. No antibacterial activity was observed in the hexane extract. The positive control (ofloxacin) produced a zone of inhibition of 36mm, which was significantly higher than all plant extracts.

Table-2 Antibacterial activity of *Hibiscus rosa-sinensis* **against** (*Staphylococcus aureus* **-** ***ATCC 6538*** **)**

Name of the plant extract (Flower)	**Zone of inhibition against *S.aureus (mm)***
Methanol	**17**
Hexane	**ND**
Acetone	**20**
Hydro-Alcoholic	**14**
Aqueous	**13**
Ethanol	**13**
Positive Control (ofloxacin)	**36**
Negative Control	**ND**

Note: ND= Not Detected

The DPPH free radical scavenging activity of the *Hibiscus rosa-sinensis* flower extract is presented in table-3. The extract showed 43.83% inhibition at 20µg/ml, which gradually increased to 91.23% at 120µg/ml. The concentration required to inhibit 50% of DPPH radicals (IC_{50}), was calculated to be approximately 29.18µg/ml for *Hibiscus rosa-sinensis* and 5.19 µg/ml for Ascorbic acid, indicating potent antioxidant activity.

The increase in antioxidant activity with concentration suggests that the methanol extract contains significant amounts of phenolic and flavonoid compounds capable of donating hydrogen atoms or electrons to neutralize free radicals. The strong antioxidant activity observed in the methanol extract correlates with the phytochemical screening results, which confirmed the presence of phenols, tannins, terpenoids, and other secondary metabolites. These compounds are well able to scavenge reactive oxygen species.

Table-3 Antioxidant activity of methanol extract of *Hibiscus rosa-sinensis*

Concentration (µg/ml)	**Plant extract (%) inhibition**
20	43.83%
40	57.26%
60	61.09%
80	70.41%
100	90.68%
120	91.23%

Conclusion

The present study demonstrated that *Hibiscus rosa-sinensis* flower extracts possess considerable phytochemical, antibacterial and anti-oxidant properties. The phytochemical analysis confirmed the presence of several bioactive metabolites that may contribute to the observed biological activities. Among the tested extracts, the acetone extract showed the strongest antibacterial activity against *Staphylococcus aureus.* The methanol extract exhibited remarkable antioxidant potential with an IC_{50} value 29.18 µg/ml. These findings suggest that *Hibiscus rosa-sinensis* is a promising natural source of bioactive compounds with potential pharmaceutical and nutraceutical applications.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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