The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(1): 530-534, 2025 www.thebioscan.com

# Phytochemical analysis of *Hibiscus rosa-sinesis* and its biomedical application.

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**DOI:** https://doi.org/10.63001/tbs.2025.v20.i01.pp530-534

**KEYWORDS**
**Hibiscus rosa-sinesis, Phytochemical analysis, Antimicrobial, Biomedical applications.**
**Received on:**

**04-01-2025**

**Accepted on:**

**04-02-2025**

**Published on:**

**11-03-2025**

## ABSTRACT

*Hibiscus rosa-sinesis*, belonging to the family Malvaceae, is a tropical and subtropical plant widely recognized for its medicinal properties. The plant can grow up to 2–5 meters, and its leaves are dark green, glossy, and ovate with serrated edges. *Hibiscus rosa-sinesis* has been traditionally used in various treatments because of its rich phytochemical content. The bioactive compounds in *Hibiscus rosa-sinesis* leaves, including flavonoids, tannins, alkaloids, phenolic compounds, terpenes, and saponins, have been extracted using the Soxhlet extraction method, with ethanol as a solvent. The leaves exhibit potent anti-inflammatory, antioxidant, antimicrobial, antifungal, and Antidiabetic properties, making the plant a significant candidate for biomedical applications. These properties allow the plant to be effective in treating infections, managing inflammation, and controlling oxidative stress and diabetes. The total phenolic content and total flavonoid content in the leaves of *Hibiscus rosa-sinesis* to assess their contribution to the plant's medicinal potential. These phytochemicals are key contributors to the plant's therapeutic efficacy, particularly in oxidative stress reduction and inflammatory response modulation.

## INTRODUCTION

*Hibiscus rosa-sinensis* (family: Malvaceae) is a widely recognized medicinal plant, valued for its diverse therapeutic properties. The plant is a perennial hedge plant or small tree that generally attains a height of 2.5 to 5 meters (8 to 16 feet) and has a spread of 1.5 to 3 meters (5 to 10 feet). It features a taproot system with multiple branches and an aerial stem that is green, cylindrical, and branching in structure (Santhi et al., 2016). Different portions of this plant, such as the leaves, flowers, and roots, are known to produce a variety of bioactive compounds referred to as phytochemicals. These phytochemicals include glycosides, alkaloids, flavonoids, carotenoids, terpenoids, and tannins, which contribute to the plant's numerous medicinal properties (Kolhe, 2024).

The phytochemicals derived from *Hibiscus rosa-sinensis* have shown a broad spectrum of biological activities. These include antibacterial, antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Santhi et al., 2016). The plant's therapeutic potential is deeply rooted in its phytochemical composition, which enables it to act as a natural remedy for various health conditions. Historically, *Hibiscus rosa-sinensis* has been used in traditional medicine systems for treating ailments such as fever, and skin diseases, as well as for promoting hair growth and maintaining oral health. Its wide application in folk medicine highlights its significance as a valuable medicinal plant (Shukla, S. K.2019).

Phytochemicals, which are naturally occurring secondary metabolites, have extended considerable attention in recent years due to their effectiveness in preventing and managing diseases. These compounds are increasingly preferred over synthetic pharmaceuticals due to their minimal side effects and better compatibility with biological systems. For instance, flavonoids and carotenoids are well-known for their potent antioxidant properties, which play a crucial role in neutralizing free radicals and protecting cells from oxidative stress. Similarly, terpenoids and tannins have been extensively studied for their antimicrobial activities against a range of pathogenic microorganisms, making them potential candidates for the improvement of new antibacterial agents (Banu et al., 2015).

In the context of modern pharmacology, the exploration of secondary metabolites with previously unexplored pharmacological activities has opened new avenues for drug discovery. Researchers are particularly interested in phytochemicals with antibacterial efficacy, given the rising prevalence of antibiotic resistance among pathogenic bacteria. Secondary metabolites from plants like *Hibiscus rosa-sinensis* are increasingly being recognized as promising sources of novel therapeutic agents. Compounds with antibacterial and antimicrobial properties could play a significant role in addressing the global challenge of drug-resistant infections (Parekh et al., 2007).

**Materials and Methods:**

**Sample collection:**

Fresh leaves of *Hibiscus rosa-sinensis* were collected from Sangli, Maharashtra, India. The leaves were carefully rinsed with distilled water to remove impurities. They were then dried at 50°C using a hot air oven. After drying, the leaves were crushed with a mortar

and pestle to a fine powder. This powdered sample was subsequently utilized for phytochemical analysis.

**Extraction:**

The extraction process was performed using the Soxhlet extraction method. A measured quantity of 5 grams of powdered leaves was placed into a thimble, and 250 mL of ethanol was added to the Soxhlet column as the solvent. The apparatus was heated using a heating mantle, and six extraction cycles were carried out to ensure optimal extraction. The resultant extract was collected, stored in appropriate containers, and refrigerated for further use (Njoku et al., 2009).

**Phytochemical analysis**:

A phytochemical screening procedure was used to find out whether the plant contained primary and secondary metabolites. The bioactive components included in the extract were determined by both qualitative and quantitative analysis.

**1) Test for carbohydrates:**

**1.1. Benedict's test**: 2 mL of Benedict's reagent was mixed with the plant extract in a test tube and heated in a water bath for 10 minutes. The formation of a reddish-brown precipitate confirmed the presence of carbohydrates (Priya et al., 2021).

**1.2. Molisch's test**: The plant extract was combined with Molisch's reagent and mixed thoroughly. Two milliliters of sulfuric acid that had been concentrated was then added along the test tube's sides. The formation of a purple ring at the interface confirmed the occurrence of carbohydrates (Priya et al., 2021).

**2) Test for phenols:** A small amount of ethanolic plant extract was dissolved in 1 mL of water.

A few drops of ferric chloride were added, and the formation of a bluish-green color indicated the presence of phenols (Priya et al., 2021).

**3) Test for terpenoids:**

**3.1. Salkowaski test**: The plant extract was mixed with 2 mL of chloroform, followed by the careful addition of concentrated sulfuric acid. A red coloration in the lower layer confirmed the presence of terpenoids (Priya et al., 2021).

**4) Test for steroids:**

**4.1. Chloroform test**: After dissolving the crude extract in one milliliter of chloroform, a corresponding volume of strong sulfuric acid was cautiously applied along the test tube's walls. A red upper layer and a yellow sulfuric acid layer with green fluorescence indicated the presence of steroids (Dowara et al., 2024).

**5) Test for proteins:**

**5.1. Millon's test**: The plant extract was mixed with two milliliters of Millon's reagent. The appearance of a white precipitate confirmed the occurrence of proteins (Obidoa et al., 2009).

**5.2. Biuret test**: The plant extract was treated with 40% sodium hydroxide and a few drops of copper sulfate. The development of a violet color showed the occurrence of proteins (Obidoa et al., 2009).

**6) Test for tannins:**

**6.1. Gelatin test**: After dissolving the plant extract in 5 milliliters of distilled water, 10% sodium chloride and 1% gelatin solution were added. A white precipitate confirmed the presence of tannins.

**6.2. NaOH test**: 4 mL of 10% sodium hydroxide was added to 0.4 mL of the extract and shaken well. The formation of an emulsion indicated the presence of tannins.

**7) Test for starch:**

1 mL of the extract was added to 10 mL of sodium chloride solution and heated. The addition of starch reagent led to a blue or purplish coloration, confirming the presence of starch.

**Antidiabetic assay of *Hibiscus rosa-sinensis* by α-amylase method:**

The Antidiabetic properties of *Hibiscus rosa-sinensis* were assessed using the α-amylase inhibition method. Plant extract at varying concentrations was mixed with α-amylase enzyme, followed by the addition of a 1% starch solution (Latha et al., 2006). Acarbose was used as a standard inhibitor for comparison (Ghosh & Dey, 2011). The reaction mixture was treated with 3, 5-Dinitrosalicylic acid (DNSA), incubated, and its absorbance was measured. The inhibitory activity of the extract was calculated using a standard formula (Chung et al., 2007; Sasikumar et al., 2013).

$$\%\ of\ inhibition = \frac{Absorbace\ of\ untreated\ cells - Absorbance\ of\ treated\ cells}{Absorbace\ of\ untreated\ cells} \times 100$$

**Anti-inflammatory assay of *Hibiscus rosa-sinensis* by protein denaturation method:**

Egg albumin was combined with phosphate-buffered saline (PBS) at pH 6.9 as the protein source, with Diclofenac Sodium used as the standard reference (Gautam et al., 2010). The reaction mixture, which included the plant extract, was incubated at room temperature and then heated to 70°C to induce protein denaturation (Winter et al., 1962). The mixture was subsequently cooled, and absorbance was measured at 530 nm to determine the extent of protein denaturation inhibition (Gautam et al., 2010). The percentage of inhibition can be calculated using the following formula

$$\%\ of\ inhibition = \frac{Absorbace\ of\ untreated\ cells - Absorbance\ of\ treated\ cells}{Absorbace\ of\ untreated\ cells} \times 100$$

**Antioxidant assay for *Hibiscus rosa-sinensis* by DPPH method:**

The antioxidant activity of *Hibiscus rosa-sinensis* extracts was determined using the DPPH method, with azithromycin as a standard. The reaction mixture included DPPH solution and either the plant extract or standard, incubated at 37°C for 30 minutes. Absorbance was then measured at 510 nm (Sanchez-Moreno, 2002; Hatano et al., 1988). The percentage of inhibition can be calculated using the following formula

$$\%\ of\ inhibition = \frac{Absorbace\ of\ untreated\ cells - Absorbance\ of\ treated\ cells}{Absorbace\ of\ untreated\ cells} \times 100$$

**Antibacterial activity of *Hibiscus rosa-sinensis* by well diffusion method:**

Ethanol-extracted plant material was introduced into wells on agar plates previously inoculated with standardized bacterial suspensions (CLSI, 2015). The plates were incubated at 37°C for 24 hours, and antibacterial activity was assessed based on the zones of inhibition (Bauer et al., 1966; Eloff, 1998).

**Results:**

**1. Phytochemical test:**

The ethanolic extract of *H. Rosa-sinesis* subjected to phytochemical analysis revealed the presence of carbohydrates, phenol, steroid, protein, tannin, starch (table no. 1).

Table1: Phytochemical screening of *Hibiscus -rosa sinesis*

| Sr No. | Chemical components | Test | Results |
|---|---|---|---|
| 1. | Carbohydrates | 1) Benedicts test<br>2) Molisch test | Negative<br>Positive |
| 2. | Phenol | Ferric chloride test | Positive |
| 3. | Terpenoids | Salkowaki test | Negative |
| 4. | Steroid | 1)chloroform test | Positive |
| 5. | Protein | 1) Millon's test<br>2) Biuret test | Negative<br>Positive |
| 6. | Tannin | 1) Gelatin test | Positive |

| | | | |
|---|---|---|---|
| | | 2) NaOH test | Positive |
| 7. | Starch | Starch reagent test | Positive |

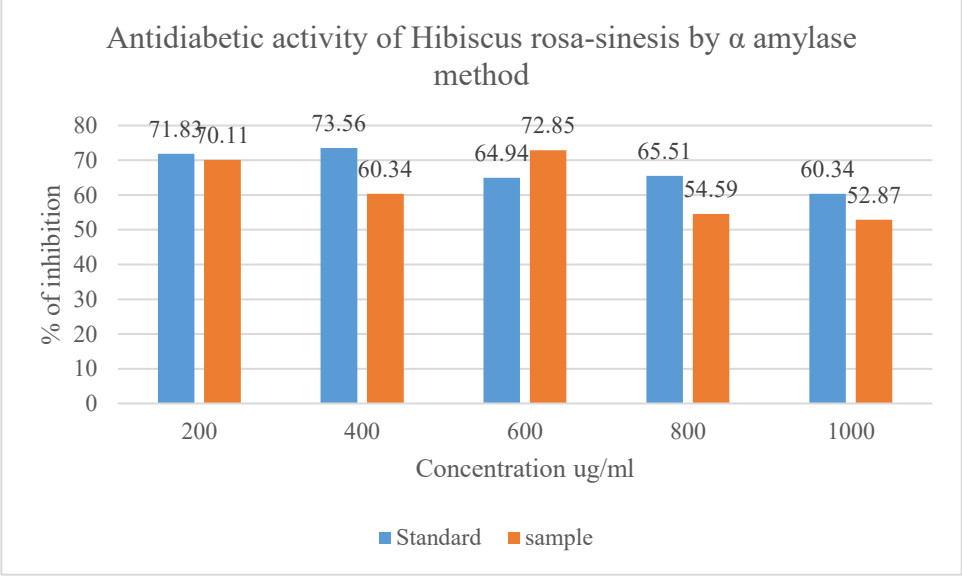
**2. Antidiabetic activity of Hibiscus rosa-sinesis by α amylase method:**

The *Hibiscus rosa-sinesis* extract showed significant α-amylase inhibition, indicating potential antidiabetic activity. The extract exhibited inhibition values comparable to the standard (table 2 & figure1).

Table 2: Antidiabetic activity of *Hibiscus rosa-sinesis* by α amylase method

| Concentration (ug/ml) | O.D at 540 nm | | Percentage of inhibition (%) | |
|---|---|---|---|---|
| | Standard | Sample | standard | Sample |
| 200 | 0.58 | 0.58 | 66.66 | 66.66 |
| 400 | 0.69 | 0.49 | 60.34 | 71.83 |
| 600 | 0.48 | 0.85 | 72.41 | 51.14 |
| 800 | 0.92 | 0.55 | 47.12 | 68.39 |
| 1000 | 0.38 | 0.82 | 78.16 | 52.87 |

| Concentration (ug/ml) | O.D at 660 nm | | Percentage of Inhibition (%) | |
|---|---|---|---|---|
| | Standard | Sample | Standard | sample |
| 200 | 0.49 | 0.52 | 71.83 | 70.11 |
| 400 | 0.46 | 0.69 | 73.56 | 60.34 |
| 600 | 0.61 | 0.72 | 64.94 | 72.85 |
| 800 | 0.60 | 0.79 | 65.51 | 54.59 |
| 1000 | 0.69 | 0.82 | 60.34 | 52.87 |




**Anti-inflammatory activity of *Hibiscus-rosa sinesis* by protein denaturation method:**

The *Hibiscus rosa-sinesis* extract exhibited significant anti-inflammatory activity, effectively reducing protein denaturation. Its inhibition was comparable to or greater than the standard in various concentrations (Table 3 & Figure 2).

Table 3: Anti-inflammatory activity of *Hibiscus rosa-sinesis* by protein denaturation method

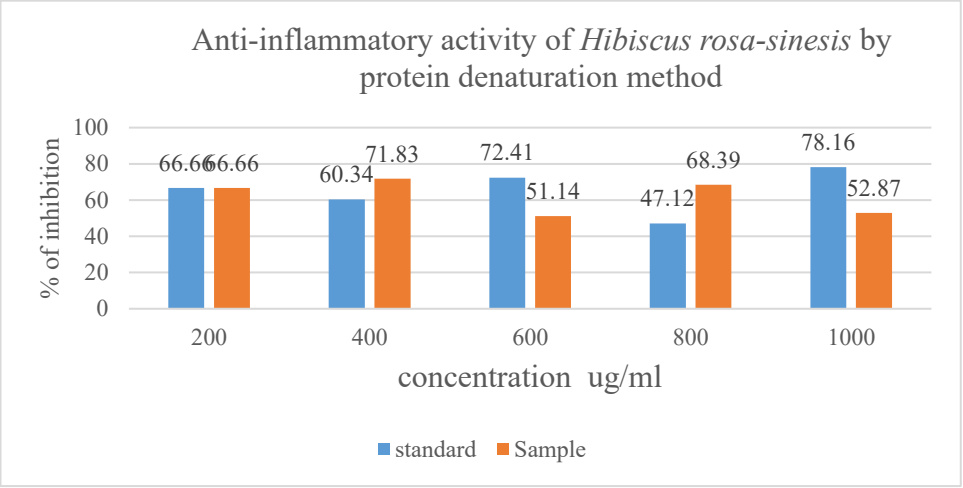


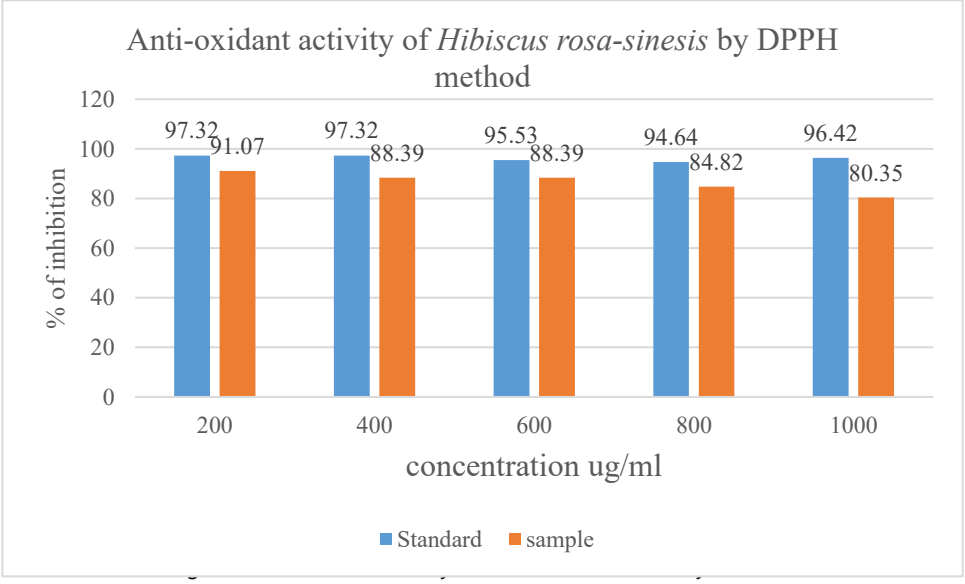

Figure 2: Anti-inflammatory activity of *Hibiscus rosa-sinesis* by protein denaturation method

**4. Anti-oxidant activity of *Hibiscus rosa-sinesis* by DPPH method:**

The *Hibiscus rosa-sinesis* extract exhibited significant inhibition, with the highest activity observed at the lower concentrations. The extract showed inhibition comparable to the standard across all tested concentrations (table 4 and figure 3).

Table 4. Anti-oxidant activity of *Hibiscus rosa-sinesis* by DPPH method

| Concentration (ug/ml) | O.D at 560 nm | | Percentage of Inhibition | |
|---|---|---|---|---|
| | Standard | Sample | Standard | sample |
| 200 | 0.03 | 0.10 | 97.32 | 91.07 |
| 400 | 0.03 | 0.13 | 97.32 | 88.39 |
| 600 | 0.05 | 0.13 | 95.53 | 88.39 |
| 800 | 0.06 | 0.17 | 94.64 | 84.82 |
| 1000 | 0.04 | 0.22 | 96.42 | 80.35 |




**5. Antimicrobial activity of *Hibiscus rosa-sinesis* by well diffusion method:**

An antimicrobial properties of *Hibiscus rosa-sinesis* against *Bacillus subtilis* and *Pseudomonas aeruginosa*. The outcome obtained from antimicrobial activity (table 5, figure 4).

Table 5: Antimicrobial activity of *Hibiscus rosa-sinesis* by well diffusion method

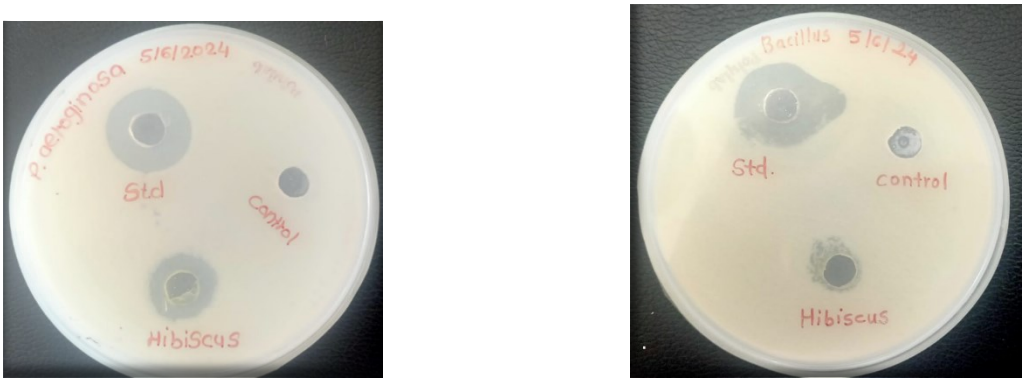


Figure 4: Antibacterial activity of plant *Hibiscus rosa-sinesis* using *Pseudomonas aeruginosa* and *Bacillus subtilis*

## CONCLUSION

*Hibiscus rosa-sinensis* is a versatile plant with significant biomedical applications. Its flowers and leaves possess antioxidant, anti-inflammatory, and antimicrobial properties, making it useful in treating wounds, skin disorders, and infections. The plant is known to lower blood pressure, regulate blood sugar levels, and support cardiovascular health. It is also widely used in cosmetics for promoting hair growth and skin rejuvenation, highlighting its therapeutic and commercial potential.

**Acknowledgement:**

The authors would like to thank Infinite Biotech Institute of Research and Analytics, Sangli, Maharashtra, India, and Yashavantrao Chavan Institute of Science, Satara (Autonomous), Maharashtra, India for the facilities and resources to conduct research.

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