

Phytochemical Screening and antibacterial evaluation of the aqueous extracts of *Hibiscus rosa-sinensis*' leaves against *Aeromonas Hydrophila*

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Abstract

Hibiscus rosa-sinensis is an important traditional plant with immense therapeutic value. To evaluate the scientific basis for the use of this plant, the phytochemical screening and its antibacterial activity was experimentally carried out. The aqueous crude extract of *Hibiscus rosa-sinensis* leaves were screened for its phytoconstituents and this extract revealed for the presence of saponins, phenols, alkaloids, proteins/amino acids, tannins, flavonoids, carbohydrates/reducing sugars and resins. These phytochemicals exhibits certain antibacterial activity which was further analyzed. The antibacterial activity of different concentrations of aqueous *H.rosa-sinensis*' leaves extract were investigated in-vitro against *Aeromonas hydrophila* by the agar well diffusion method. The results indicated that all concentrations showed an antibacterial activity against the tested bacteria. The highest activity was observed with an inhibition zone of 24 mm at 50% concentration.

Keywords: Hibiscus rosa-sinensis, phytochemical analysis, Aeromonas hydrophila, Antibacterial activity

1. Introduction

Plants synthesize a wide variety of chemical compounds, which can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines ^[1]. It is estimated that about 75% of the 120 biologically active plant derived compounds, presently in use worldwide, have been derived through follow up researches to verify the authenticity of the data from folk and ethno-medicinal uses. So, there is a great scope for new drug discoveries based on traditional plant uses ^[2].

The growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity ^[3, 4] has paved way for plant extracts as a potential and promising drug against microbial pathogens as an alternative tool for disease management. There has also been a revival of interest in herbal medicines due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases. Moreover, plant or herbal extract have advantages of minimal side effects, easy biodegradability, inexpensive and extracts that can be easily prepared.

Plants generally produce many secondary metabolites such as tannins, alkaloids and flavonoids which constitute an important source of inhibiting many microbial pathogens. Also, the successive isolation of such metabolites from the plant material largely depends on the type of solvent used in the extraction procedure. The traditional healers primarily use water as the solvent. Such identification and characterization of these metabolites along with their antimicrobial activity exhibited is significant.

A commonly found houseplant of interest in the tropical and subtropical regions of the world is *Hibiscus rosa-sinensis*, a

well-known member of the family Malvaceae. It grows as an evergreen shrub which is in leaf year round. This plant is extensively cultivated as an ornamental plant. Various parts of this plant, like leaves, flowers and roots, have been known to possess medicinal properties like antigraying ^[5], epilepsy, leprosy, bronchial catarrh and diabetes ^[6]. Bacterial infection plays a vital role leading to many of the pathological conditions where extracts of *H. Rosa-sinensis* extracts have been used as a traditional medication. One such study of Shivananda *et al.*, (2007) ^[7] confirmed this antibacterial activity *in vitro* as well as the wound-healing activity of the ethanol extracts of *H. rosa-sinensis* flowers *in vivo*.

Therefore, keeping in view the therapeutic potential of this plant, the present study is designed to evaluate the phytochemical properties and antibacterial activity of the aqueous extracts of *H. Rosa-sinensis* leaves against a pathogenic bacteria, *Aeromonas hydrophila*, which is an opportunistic pathogen of fish, amphibians and humans alike. This bacterium is heterotrophic, gram-negative, rod-shaped mainly found in areas with a warm climate and is resistant to most common antibiotics.

2. Materials and Methods

An investigation was carried out to evaluate the phytochemical constituency of the *Hibiscus rosa-sinensis*' leaves (aqueous extract) and to assess its antibacterial property against *Aeromonas hydrophila*.

2.1. Collection of plant leaves & preparation of aqueous extract

Fresh leaves of *Hibiscus rosa-sinensis* were collected from the localities of Coimbatore (India). It was thoroughly washed with tap water in order to remove the dust particles and debris from them and rinsed with distilled water. These

leaves were shade dried, ground into fine powder using an electric blender, passed through a sieve and stored for extract preparation. The powder was then used to obtain the crude extract following the cold aqueous extraction method [1].

2.2. Preliminary phytochemical analysis

The aqueous extracts of the selected plant's leaves were tested for saponins, phenols, alkaloids, protein/ amino acids, tannins, flavonoids, carbohydrates/ reducing sugars, phlobatannins, anthraquinone, terpenoids, glycosides and resin. This phytochemical screening of the extracts was carried out by standard methods.

2.2.1. Test for saponins

2ml of extract was shaken vigorously with 5 ml distilled water to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.

2.2.2. Test for phenols

To 1ml extract, add distilled water followed by few drops of 10% Ferric chloride. The formation of blue or black colour indicates the presence of phenolic groups.

2.2.3. Test for alkaloids

1ml of 1% HCL was added to the 2 ml of extract in a test tube and was treated with few drops of Mayer's reagent. A creamy white precipitate indicated the presence of alkaloids.

2.2.4. Test for protein / amino acids

To 1ml extract, add 2ml water followed by few drops of Conc. HNO₃. The formation of yellow colour indicates the presence of protein / amino acids.

2.2.5. Test for tannins

To 2 ml extract, 1ml of distilled water and 1-2 drops of Ferric chloride solution was added and observed for brownish green or a blue black coloration.

2.2.6. Test for flavonoids

A few drops of 1% NH₃ Solution were added to 2 ml of extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

2.2.7. Test for carbohydrates/ reducing sugars

5-8 drops Fehling's solution was added to 2ml extract. The mixture was heated in boiling water bath for 5 min. A red-brick precipitate shows the presence of reducing sugars.

2.2.8. Test for phlobatannins

1ml extract was boiled with 1% HCL and the disposition of red precipitate indicates the presence for phlobatannins.

2.2.9. Test for anthraquinone

2ml extract was mixed with chloroform and 1ml of 10% ammonia solution was added. The presence of a pink, red or violet colour indicates the anthraquinones.

2.2.10. Test for terpenoids

2ml extract was mixed with 2ml chloroform in a test tube. To this, 3ml Conc. H₂SO₄ was carefully added along the wall of the test tube to form a layer. An interface with a reddish brown coloration confirmed the presence of terpenoids.

2.2.11. Test for glycosides

2ml of 50% H₂SO₄ was added to the 2 ml of extract in a boiling tube. The mixture was heated in boiling water bath for 5 min. 10ml of Fehling's solution was added and boiled. A brick red precipitate indicated the presence of glycosides.

2.2.12. Test for resins

To 1ml extract, 5ml of boiling ethanol was added and filtered through what man no.1 filter paper and the filtrate was diluted with 4ml of 1% aqueous HCL. The formation of a resinous precipitate indicates the presence of resins.

2.3. In vitro antibacterial activity

2.3.1. Collection of test microorganism & culture media used

The bacterial pathogen *Aeromonas hydrophila* was used. *Aeromonas hydrophila* was collected from the Department of Microbiology, PSG Institute of Medical Sciences and Research, Coimbatore. This bacterium was cultured in nutrient broth (Himedia) for 24h at 37°C in an incubator.

2.3.2. In vitro antibacterial activity

The agar well diffusion assay [8] was adopted for the evaluation of the antibacterial activities of the selected plant extracts. The petriplates with nutrient agar medium was inoculated using sterile cotton swabs with bacterial suspension. The entire agar surface of each plate was inoculated with the swab first in horizontal direction then in vertical direction to get uniform lawn of bacteria. Using a flame cork-borer, wells of 0.5 cm in diameter was made in the media. These plates were labelled and 20µl extract of 10%, 20%, 30%, 40% and 50% concentrations were added and incubated at 37°C in an incubator for 24 hrs. The efficiency of extract was recorded by measuring the diameter of inhibition at the end of 24 hrs incubation. Positive (tetracycline) and negative (distilled water) wells were included as controls.

3. Results and Discussions

The phytochemical screening of the aqueous leaf extracts of *Hibiscus rosa-sinensis* showed that the leaves are rich in secondary metabolites and certain biochemical compounds such as saponins, phenols, alkaloids, proteins/amino acids, tannins, flavonoids, carbohydrates/reducing sugars and resins (Table – 1).

Table 1: Phytochemical Analysis of the aqueous Leaf Extracts of *Hibiscus Rosa-Sinensis*

S. No.	Phytochemicals	<i>Hibiscus rosa-sinensis</i>
1	Saponin	+ve
2	Phenol	+ve
3	Alkaloids	+ve
4	Proteins & amino acids	+ve
5	Tannin	+ve
6	Flavonoids	+ve
7	Carbohydrate/ Reducing sugar	+ve
8	Phlobatannin	-ve
9	Anthraquinone	-ve
10	Terpenoids	-ve
11	Glycosides	-ve
12	Resin	+ve

After preliminary phytochemical screening of the aqueous leaf extracts of *Hibiscus rosa-sinensis*, its antibacterial activity was analyzed. Its 50% concentration was found to be the most effective against *Aeromonas hydrophila* producing a maximum inhibitory zone of 24mm. Next to it, the control positive recorded a 22.25mm inhibition which was followed by 40% concentration of the aqueous leaf extract producing 22mm zone of inhibition. No inhibitory zone was found in the control negative indicating nil effects against the growth of *Aeromonas hydrophila* (Table – 2).

Table 2: Effect of Selected Leaf Extract against *Aeromonas Hydrophila*

Treatments	Zone of inhibition in mm
	<i>Hibiscus rosa-sinensis</i>
Control Positive	22.25
Control Negative	0.00
10%	20.00
20%	20.25
30%	20.75
40%	22.00
50%	24.00

Numerous studies have also been conducted in this regard with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds^[9,10]. The presence of antibacterial substances in higher plants is well established^[11]. This antimicrobial activity of plant extracts is mostly due to their essential oil fraction or sulphur containing compounds in aqueous phase^[12]. The present study was also supported by the work done by Uma maheshwari *et al.*, (2008)^[13] on the antimicrobial activity of different solvent extracts of leaf of *Bougainvillea spectabilis* against various bacterial pathogens i.e., *S.aureus*, *B.subtilis*, *Streptococcus faecalis*, *Micrococcus luteus*, *E. coli*, *P. aeruginosa*, *Salmonella typhi*, *K. pneumoniae*, *Proteus vulgaris*, *Serratia marcescens*, *Shigella flexneri* and *Vibrio cholerae*.

4. Conclusion

It is clear from the findings of this study, that *H. rosa-sinensis* leaves contain important constituents that confer its antibacterial activity and may be used in treating pathological conditions caused particularly by *Aeromonas hydrophila*. This notable antimicrobial activity against the tested microorganism was acceptable with respect to the standard antibiotic such as tetracycline and so can also be used as a natural remedy.

In the present study, only the susceptibilities of *Aeromonas hydrophila* to the extracts of *H. rosa-sinensis* leaves have been examined. Therefore, further microbiological studies would be carried out to determine the minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of the extracts prepared from *H. rosa-sinensis* leaves. Biochemical and pharmacological investigations would also be necessary to establish the exact antibacterial principles for use in complementary alternative medicine.

5. References

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