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Research Article Extraction, Phytochemical Investigation and Antimicrobial Activity of *Hibiscus Rosa-Sinensis*

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ABSTRACT

Many medicinal plants are claimed to be useful in skin diseases in all traditional systems of medicine and folklore. At the same time, these herbal remedies are used orally and by topical application. Looking at the scope of the herbal drug and the increasing demand, especially for liver disease, cancer, diabetes, hypertension, kidney disease, inflammation, infectious disease, skin disease, etc. Hibiscus rosa-sinensis grows as a green herb in tropical areas. It is a low shrub with large, glossy green leaves and showy tube-shaped flowers. The plant loses leaves for a short time during the winter. This study aims to determine phytochemical components using different solvents and to study the antibacterial effect of methanol extract to varying concentrations against these bacteria. Phytochemical analysis of Hibiscus rosa-sinensis. Leaves extracts indicate the presence of various medicinally valuable components, including alkaloids, glycosides, carbohydrates, phytosterols, phenolic compounds, tannins, saponins, flavonoids, proteins, and amino acids. Current findings indicate that methanol is a good solvent for extracting antibacterial agents. Further studies are needed to investigate the individual phytochemical compounds of the Hibiscus rosa-sinensis. Leaves can be considered as effective as the most effective artificial antibiotics.

INTRODUCTION

Plants contain secondary metabolites, organic compounds not directly involved in the normal growth, development, or reproduction of organisms but often play an important role in plant defenses¹. Examples include alkaloids, glycosides, terpenoids, phenols, tannins, flavonoids and

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saponins². Furthermore, there is growing interest in the chemical composition of plants towards the discovery of more effective biotherapeutic agents³. The importance of natural products in modern medicine has been discussed in recent reviews and research^{4,5}.

Medicinal plants are now getting more attention than ever because they can benefit society, especially in medicine and pharmacology. The therapeutic value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body⁶. Plants contain secondary metabolites, also known as phytochemical, natural bioactive compounds, or plant constituents, that are not directly involved in organism' normal growth, development, or reproduction but often play an important role in plant defence. Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds, and many more⁷. They are found in plants, such as vegetables, fruits, flowers, leaves, and roots, which work with nutrients and fibre to act as a defence against disease^{8,9}.

An antibacterial is a substance that kills or inhibits the growth of bacteria, and antimicrobial drugs either kill bacteria or prevent microbial growth. Plants have evolved to synthesize chemical compounds that help defend against attacks from predators such as insects, fungi, and herbivorous mammals. By chance, some of these compounds, although toxic to plant predators, have been shown to have beneficial effects in treating human diseases. Such secondary metabolites have a very diverse structure; many are aromatics, most of which are phenols or their oxygen-substituted derivatives. Many herbs and spices humans use to flavour food yield useful medicinal values¹⁰.

- * Hibiscus rosa-sinensis
 - Kingdom: Plantae
 - Order: Malvales

- Family: Malavaceae
- Genus: Hibiscus L.
- Species: Hibiscus rosa-sinensis



Figure 1: Hibiscus rosa-sinensis

Hibiscus rosa-sinensis is a glabrous shrub widely cultivated as an ornamental plant in the tropics. Previous studies have shown that *Hibiscus rosa-sinensis* possesses many biological activities, such as anticomplementary, antidiarrheic and antiphlogistic activity¹¹. It has also been reported that the plant's flower contains ant spermatogenic, androgenic¹², antitumor and anticonvulsant properties; the leaves and flowers are hair growth promoters and aid in healing ulcers¹³.

The reported biological activities of Hibiscus rosa-sinensis include antiestrogenic, antiimplantation, abortifacient, antipyretic, antispasmodic, hypotensive, embryotoxic, antispermatogenic, insect attractant, analgesic, antifungal and anti-inflammatory properties¹⁴. This study aimed to identify new potential plant antimicrobial agents from Hibiscus rosasinensis species that the pharmaceutical industry could develop and to promote the use of Hibiscus rosa-sinensis species in the treatment of various diseases¹⁵.

Its flowers are large, generally red in the original varieties, and firm but lack scent¹⁶. Numerous varieties, cultivars, and hybrids are available with different shades of colour ranging from white to pink and red and from orange to yellow. In ayurvedic medicine, the *Hibiscus rosa-sinensis* flower, leaf, and root extracts are used to treat the



problems related to the menstrual cycle in women. In bleeding haemorrhoids, the roots mashed with milk are benevolent. The leaves are analgesics, aperients, emollients and laxatives. *Hibiscus rosasinensis* petal stimulates hair growth and prevents premature greying and loss of hair and scalp disorders. The buds have a cooling and astringent effect and remove the burning sensation of the body¹⁷.

Mucilage prepared from the root of Hibiscus rosasinensis has been used to treat cough^{18,19}. The Hibiscus rosa-sinensis flowers were described in ancient Indian medical literature as having beneficial effects on heart disease²⁰. Lately, both experimental and clinical studies have shown that powder from the dried flowers of Hibiscus rosasinensis has significant protective effects in ischemic heart disease^{21,22}. The most important was the methanolic extracts from the flowers of rosa-sinensis²³. which Hibiscus show anticonvulsant and hypotensive effects among crude extracts^{24,25}. Bacterial infection is important in many pathological conditions where Hibiscus rosa-sinensis extracts are used as traditional medicines.

1. Ayurvedic Names, Formulations and Doses:

Table 1: Ayurvedic Names, Formulations and
Doses of Hibiscus rosa-sinensis

Doses of moiscus rosu-smensis		
	Hibiscus rosa-sinensis	
	Trisandhya, oundrapushpa,	
Ayurvedic Names	japapushpa, raktapushpa,	
	arkapriya, harivallabha	
Hindi: gudahala, gudhal		
Regional Names	Tamil: semaparuti	
	Telugu: dasanamu	
	Marathi: jaswand	
Important	Methyl-sterculate, beta-	
Phytoconstituents	sitosterol, aspartic acid	
	Raktapitta – Bleeding	
Therapeutic Uses	disorders, Hridroga –	
	Cardiac disease, Arsha-	
	Bleeding piles	

Dose	Flower – 5-10 gm, leaf powder/ paste – 3-5 gm
Formulations	Oils, Face masks, Powders, Scents, Soaps

2. Nutrients and their Constituents:

Raw *Hibiscus rosa-sinensis* contains carbohydrates, calcium, magnesium, potassium, vitamin C, and vitamin B. Hibiscus rosa-sinensis tea tends to be lower in nutrients but offers many beneficial compounds.

Hibiscus rosa-sinensis contains anthocyanins, which are pigments that give the flowers their vibrant red color. It also has flavonoids, phenolic acids, and organic acids; many of these compounds act as antioxidants. Oxidative stress contributes to chronic conditions such as high blood pressure, cancers, diabetes, and heart disease²⁶.

3. Traditional and Claimed Applications:

The leaves of *Hibiscus rosa-sinensis* were used to treat dysentery and diarrhoea, to promote the draining of abscesses and as an analgesic in the traditional medicine of the Cook Islands, Haiti, Japan and Mexico²⁷. Plant flowers were used in diabetes, epilepsy, bronchial catarrh and leprosy^{28,29}. They were refrigerant, emollient, demulcent, aphrodisiac and emmenagogue. Petals were used to stimulate thicker hair growth and to prevent premature greying, hair loss and scalp disorders.

4. Therapeutic and Medicinal Characteristics:

Hibiscus rosa-sinensis is used for treating loss of appetite, colds, heart and nerve diseases, upper respiratory tract pain and swelling (inflammation), fluid retention, stomach irritation and disorders of circulation; for dissolving phlegm; as a gentle laxative; and as a diuretic to increase urine output. Research has uncovered a range of health benefits linked to drinking *Hibiscus rosa-sinensis* tea,



showing that it may lower blood pressure, reduce the growth of bacteria, and even aid weight loss³⁰.

MATERIALS AND METHODS

• Preparation of Plant Material

The freshly collected leaves of *Hibiscus rosasinensis* were carefully cleaned and plugged from the stem. The leaves were air-dried in an aerated room for 6 weeks. The dried leaves were pulverized using mortar and pestle into smaller particles and then blended to powder using an electric blender. 50g of the powdered leaves were obtained. 500ml of methanol was measured into the 50g of the powdered leaves and then stored in an air-tight container for 4 days.

• Preparation of Extract

50g of *Hibiscus rosa-sinensis* leaves powder was subjected to extraction using methanol and water by shaking the mixture for about one hour in a shaker at room temperature. Subsequently, the samples were filtered on Whatman filter paper. Then the filtrate was taken for extraction and concentration in a Soxhlet apparatus using Soxhlet extraction. The final extract was collected in sterile-labelled containers.

• Preliminary Phytochemical Screening

Different extractions were stored in a refrigerator at 4°C to screen their phytochemical constituents and detect antibacterial activity. Phytochemical screening of the leaves extract was tested for phytochemicals by standard procedures. The methanol extract of the leaves was used for the preliminary phytochemical screening procedure for the presence of bioactive ingredients such as tannins, alkaloids, flavonoids, saponins, and steroids.

PHYSICOCHEMICAL EVALUATION

The existence of ash value and extractive value was determined using physicochemical testing.

• Ash that dissolves in water

The ash was boiled for 5 minutes with 25 ml of water, and then the soluble materials were collected in a crucible, burned, and weighed.

Concerning air-dried medication, we computed the percentage of water-soluble ash as follows.

• Insoluble acid ash

The residue is formed by burning the residual insoluble materials after boiling the entire ash with weak hydrochloric acid. Using 25 ccs of dilute hydrochloric acid, simmer the ash for 5–10 minutes, collect the insoluble materials in a crucible or ashless filter paper, ignite, and weigh. Concerning the air-dried medication, we estimated the percentage production of acid-insoluble ash as follows³¹.

• Extractable value in water

In a closed flask, stirring frequently, 1 g of coarsely ground air-dried medication was macerated with 100 ml of distilled water for 24 hours. The filtered solution was evaporated in a tarred flat bottom shallow dish, dried at 100°C, and weighed. The proportion of water-soluble extractives was calculated concerning the air-dried drugs ³².

• Value of alcohol-soluble extractive

In a stoppered flask, macerate 5 g of carefully weighed, coarsely powdered medication with 100 ml of alcohol (90 per cent v/v) for 24 hours, stirring frequently during the first 6 hours. Filtered quickly via filter paper to avoid excessive alcohol loss. In a covered dish, evaporate 25 mL of alcoholic extract to dryness and weigh it. We used the formula below to determine the percentage w/w of alcohol-soluble extractive concerning the air-dried medication. Extractive value in per cent alcohol-soluble=4 (Wt. of residue) Based on the comparison of Petroleum ether and methanolic extracts of TC in the table above, the methanolic section had a higher ash value and extractive value than the Petroleum ether extract.

SOXHLET EXTRACTION



Figure 3: Assembly of Soxhlet Extraction Principle

Extraction is performed in the laboratory using a fat extractor (Soxhlet extractor). The fat extractor employs the solvent reflux and syphon concept to continually extract solid matter from a pure solvent, resulting in increased solvent extraction efficiency. Before extraction, the solid material is crushed to enhance the solid-liquid contact area. After that, the solid material is placed in a filter paper container and extracted. The bottom end of the extractor is attached to a reflux condenser and a round bottom flask containing a solvent. The solvent is boiled in the bottom flask, and the vapour rises through the extractor's branch pipe, condenses, and falls into the extractor, where the solvent is contacted with the solid for extraction. The solvent holding the extract is syphoned back when the solvent surface surpasses the highest point of the syphon. The flask is repeated, removing a portion of the material and concentrating it in the flask.

PHYTOCHEMICAL ANALYSIS

The presence and absence of primary and secondary metabolites such as starch, tannins, oil, mucilage, and lignin were revealed by histochemical examination using various reagents. Air-dried powdered leaves' identity, purity, and strength were tested using physicochemical methods, including ash and extractive values.

The phytochemical screening of chemical constituents of plants in various solvents studied,

such as Methanol, Petroleum ether and aqueous extracts, revealed that alkaloids, glycosides, flavonoids, phenols, steroids, proteins, and amino acids are present in all sections, but the amounts vary.

RESULTS AND DISCUSSION

 Table 6.1: Physicochemical Analysis of powdered

 drug of Hibiscus rosa-sinensis

Name of Test	Extractive Ash Value (gm)	Acid Insoluble Ash Value (gm)
Weight of empty dish	60.70	60.70
Weight of powder taken	0.33	0.26
Weight of ash + dish	61.03	60.96
Percentage of ash	99.45	99.57

Table 6.2: Phytochemical Tests of *Hibiscus rosasinensis*

Ingradiants	Hibiscus
ingituiciits	
	rosa-
	sinensis
Carbohydrates: Molisch Test	-
Monosaccharides: Barfoed's Test	-
Pentose Sugars	+
Hexose Sugars: Selwinoff's Test	+
Non-reducing Polysaccharides -	-
Starch: Iodine Test	
Proteins: Millon's Test	-
Amino Acids: Tyrosine Test	-
Fats and Oil: Saponification Test	+
Steroids: Salkowski Test	+
Glycosides: Bontrager's Test	-
Flavonoids: Sulphuric Acid Test	+
Alkaloids: Mayer's Test	+
	Monosaccharides: Barfoed's Test Pentose Sugars Hexose Sugars: Selwinoff's Test Non-reducing Polysaccharides - Starch: Iodine Test Proteins: Millon's Test Amino Acids: Tyrosine Test Fats and Oil: Saponification Test Steroids: Salkowski Test Glycosides: Bontrager's Test Flavonoids: Sulphuric Acid Test

Calculation of the extracting value

Extractable value in water in a closed flask, 1 g of coarsely ground air-dried medication was macerated with 100 ml of distilled water for 24 hours, stirring frequently. The filtered solution was evaporated in a tarred flat bottom shallow dish, dried at 100°C, and weighed. The percentage of water-soluble extractives was estimated using airdried medicines as a reference.

ANTIMICROBIAL ACTIVITY

Principle

Natural products (secondary metabolites) are the major sources of drugs, and these products have



Figure 6.1: Zone of Inhibition of E. coli

Table 6.3: Antimicrobial activity, Zone of	
Inhibition of E. coli	

Concentration(µg/ml)	Zone of Inhibition (cm)
50	0.3
100	0.5
200	0.8
STD (Streptomycin)	0.9

Table 6.4: Antimicrobial activity, Zone of Inhibition of S. aureus

Concentration(µg/ml)	Zone of Inhibition
	(cm)
50	0.3
100	0.5
200	0.7
STD (Streptomycin)	0.8

greater structural diversity than drugs or standard combinatorial compounds from chemistry. The use of medicinal plants to treat microbial diseases has been well-known and welldocumented since ancient times. Medicinal plants synthesize many defensive compounds to protect themselves and predators, and these compounds have antimicrobial activity. The ability of the plant extract to reduce or inhibit the growth of microorganisms or kill pathogenic organisms is known as antimicrobial activity or efficacy. Several plant species have been the antimicrobial activity of plant extracts that may be tested by agar diffusion or cup-plate methods.



Figure 6.2: Zone of Inhibition of S. aureus

CONCLUSIONS

Extraction, Phytochemical investigation and antimicrobial activity of *Hibiscus rosa-sinensis* are executed. In Phytochemical analysis, Alkaloids, Carbohydrates, Glycosides, Steroids, Proteins, and Amino acids are present in *Hibiscus rosa-sinensis*.

In Antimicrobial activity, *E. coli* and *S. aureus* show significant inhibition of growth in different concentrations in which *E. coli* is more potent than *S. aureus*.

FUTURE PROSPECTS

The chemical components that are the main active principles in the physiological activities of *Hibiscus rosa-sinensis* calyx are anthocyanins and



polyphenols (protocatechuic acid and quercetin). Advances have also been made in the elucidation of action mechanisms. *Hibiscus rosa-sinensis* is used for treating loss of appetite, colds, heart and nerve diseases, upper respiratory tract pain and swelling (inflammation), fluid retention, stomach irritation, and disorders of circulation; for dissolving phlegm; as a gentle laxative; and as a diuretic to increase urine output.

Most of the *Hibiscus rosa-sinensis* plant's economic value, particularly as an ingredient in herbal teas, comes from the red calyx. However, the leaves, seeds, and flowers are also used in local forms of traditional medicine. In commerce, the calyces are known as *Hibiscus rosa-sinensis* and *Roselle*. One of the major adaptations of the *Hibiscus rosa-sinensis* is that it does not move because it contains a cell wall made of cellulose. The *Hibiscus rosa-sinensis rosa- sinensis* grows in tropical to sub-tropical regions, making the flowers tender to frost.

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