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Phytochemical Investigation of Hibiscus rosa-sinensis Flowers and Evaluation of Their Antimicrobial Activity

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Abstract: Medicinal plants are rich sources of bioactive compounds that play a significant role in the development of new therapeutic agents. Hibiscus rosa-sinensis, a widely used ornamental and medicinal plant, has been traditionally used for treating various ailments. The present study aims to investigate the phytochemical constituents of Hibiscus rosa-sinensis flower extracts and evaluate their antimicrobial activity against selected bacterial and fungal strains. Flower samples were subjected to successive extraction using solvents of increasing polarity. Preliminary phytochemical screening revealed the presence of flavonoids, tannins, phenolics, alkaloids, saponins, and glycosides carbohydrate. The antimicrobial activity was assessed using the agar well diffusion method against Escherichia coli. The results demonstrated significant antimicrobial activity, particularly in ethanol and methanol extracts, supporting the potential use of Hibiscus rosa-sinensis flowers as a natural antimicrobial agent.

Keywords: *Hibiscus rosa-sinensis, Phytochemical screening, Antimicrobial activity, Medicinal plants, Flower extract*.

I.INTRODUCTION:

Plants have long been recognized as vital sources of diverse natural compounds with significant medicinal value. In recent decades, the search for plant-based antimicrobial agents has intensified, largely due to the alarming rise of antibiotic-resistant bacterial strains that threaten global public health (Davis & Choisy, 2024),(Salmerón-Manzano et al., 2020). Conventional antibiotics, though effective in the past, are increasingly losing their efficacy as microorganisms rapidly develop resistance mechanisms, creating an urgent need for safer and more sustainable therapeutic alternatives (Afiune et al., 2017). Medicinal plants provide a biologically safe, economical, and environmentally sustainable alternative for drug development (Da-Costa-Rocha et al., 2014). Medicinal plants have emerged as promising candidates because they contain a wide array of bioactive secondary metabolites. These compounds—often produced as part of the plants' natural defense systems—exhibit potent antimicrobial, antioxidant, and anti-inflammatory properties. Importantly, plant-derived medicines are generally considered biologically safe, cost-effective, and environmentally sustainable, making them especially attractive for modern drug development (Da-Costa-Rocha et al., 2014; Ghadiya et al., 2024a).

Traditionally utilized in Ayurvedic and traditional medicine, Hibiscus rosa-sinensis (family: Malvaceae) is found across tropical and subtropical areas. There have been reports of antioxidant, anti-inflammatory, antidiabetic, wound-healing, and antibacterial qualities in various plant parts, including flowers, leaves, and roots (Mak et al., 2013). The blooms are especially high in anthocyanins, flavonoids, and polyphenols, all of which have antibacterial properties (Khadiga G. Abd Elaleem & Abd Elaleem, 2017). Hibiscus rosa-sinensis's wide range of bioactive components that support various pharmacological actions have drawn more and more interest from scientists.

Its promise as a natural antibacterial agent has been demonstrated by recent research, which show notable inhibitory effects against E. coli. These results imply that the plant's phytochemicals may disrupt metabolic pathways, protein synthesis, and the integrity of microbial cell walls, which would limit the growth and survival of dangerous-microbes.

Flowers and assesses the effectiveness of various antibiotics against particular harmful microbes (Zulkurnain et al., 2023),(V. Vastrad & A. Byadgi, 2018).

II.MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Hibiscus rosa-sinensis fresh flower were collected from CSEB Colony, Korba (C. G.). A botanist verified the plant's identity and taxonomy. For additional examination, collected flowers were cleaned, shade-dried, grind into a powder form (Figure 1).

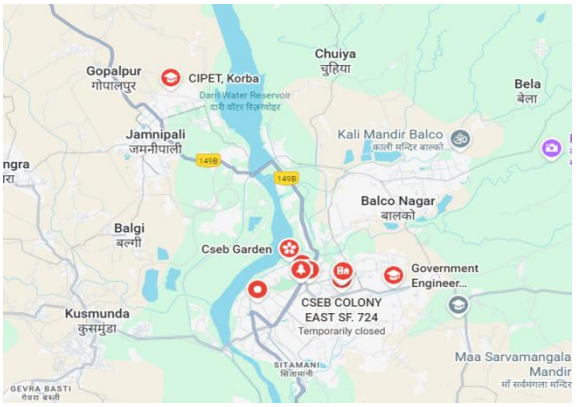

Figure 1- *Hibiscus rosa-sinensis*

2.2 Preparation of Flower Extracts

The dried flower powdered (50 gm) was subjected to Soxhlet extraction using a variety of solvents, including chloroform, ethanol and aqueous solvents. Before being used, the extracts were kept at 4 °C after being concentrated using a rotary evaporator (Vijayakumar et al., 2018), (Sharma et al., 2019),(Harini, 2024).

2.3 Preliminary Phytochemical Screening

The extracts were screened for the presence of various phytochemical constituents using standard qualitative methods (Vijayakumar et al., 2018).

Test for alkaloid -Mayers test - Few ml of plant extract add 1 -2 drop of mayer's reagent, cream coloured precipitate formed indicate the presence of alkaloids.

Test for Flavonoids -The extract is mixed with 2 ml of 2% NaOH solution followed by addition of dilute HCl, a yellow to colourless solution will be formed indicating presence of flavonoids.

Test for tannins -1 ml *Simarouba glauca* leaf extract with mixed 1% ferric chloride solution, a blue-green solution will be formed indicating presence of tannins.

Test for phenols - Ferric chloride test - 10 ml extract with add some drop of 1.0% ferric chloride solution, a green-black solution will be formed indicating presence of phenols.

Test for Saponin -1 ml extract with add 10 ml distilled water and boil for 5 min on 50^{O}C. The mixture was filtered and 2.5 ml of the filtered was added to 10 ml of distilled water in a test tube, test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Formation of honeycomb froth indicates the presence of saponin.

Test for Glycosides - Keller-Kiliani test -The extract is mixed with 4 ml acetic acid and 1.0 ml H_2SO_4 then add few drop 2% ferric chloride solution, a brown ring on surface will be formed indicating presence of glycosides.

Detection of Triterpenoids - Salkowski test - Filtrate and few drops of conc H_2SO_4 (Shake well and allowed to stand) golden yellow layer at the bottom indicate the presence of triterpenoids.

Test for phytosterols - few ml filtrate plant extract add few drops of conc. H_2SO_4 (shake;n well and allowed to stand) formed a red colour (lower layer) indicate the presence of phytosterol.

Test for Protein- Biuret test - 2 ml extract with add 1 ml of 1% sodium hydroxide solution and then add a few drops of 1% copper sulphate solution, a blue-green colour will be formed indicating presence of protein with weakly polypeptide bond in protein.

Test for Amino acid - Ninhydrin test - 0.5 ml extract with mixed two drop 2% ninhydrin reagent and heat this mixture for some time, a brownish or yellow brown colour indicating presence of amino acid with asparagine presence or a mix with proline-like compounds in leaf.

Test for Carbohydrate - Molisch's test - 0.5 ml extract was dissolved in 5 ml distilled water and filtered, then molish's reagent added to respective filtrates. The formation of a raddish violet ring at the junction of the filtrate and reagent indicated that the filtrate contains carbohydrates.

2.4 Antimicrobial test

2.4.1 Antibacterial activity

Antibacterial activity was assessed using the agar well diffusion method. Nutrient agar medium was prepared by dissolving 2.8 g NAM powder and 1.8 g agar-agar in 100 ml distilled water, sterilized by autoclaving at 121°C and 15 psi for 15 minutes, and poured into sterile Petri plates to solidify. A pure culture of Escherichia coli was evenly spread over the agar surface, and sterile wells were created using a well cutter. Test samples were introduced into the wells, and the plates were incubated at 37°C for 24 hours. Antibacterial activity was indicated by the clear inhibition zones around the wells (Ruban & Gajalakshmi, 2012)

III.RESULT AND DISCUSSION

3.1 Phytochemical Screening

The preliminary phytochemical screening of *Hibiscus rosa-sinensis* flower extracts revealed the presence of various bioactive constituents in different solvent systems. Ethanol extracts showed a rich presence of alkaloid, flavonoids, tannins, phenolic compound, saponins, glycosides, whereas chloroform extract contained such as alkaloids, tannins, phenolic compound, glycosides, triterpenoids, phytosterol. The aqueous extract showed a high concentration of tannins, phenolic compound, saponins, and carbohydrates. The variation in phytochemical composition among extracts may be attributed to the polarity of solvents used (Table 1).

Table 1: Preliminary Phytochemical Screening of *Hibiscus rosa-sinensis* Flower Extracts

Phytochemical constitution	Chloroform Extract	Ethanol Extract	Aqueous Extract
Alkaloid	+	++	+
Flavonoids	_	++	+
Tannins	+	++	++
Phenolic compound	+	++	++
Saponins	_	+	++
Glycosides	+	++	+
Triterpenoids	+	_	_
Phytosterols	+	_	_
Proteins & Amino acid	_	+	+
Carbohydrate	_	+	++

Note:

(++): Strongly present (+): Present (–): Absent

3.2 Antimicrobial Activity

3.2.1 Antibacterial activity

Escherichia coli and *Staphylococcus aureus*, antibacterial activity was evaluated using the agar well diffusion method. Three wells were prepared on each plate: one as the negative control containing only distilled water, and the other loaded with 50 µL ethanol extract and one 100 µL ethanol extract suspension. After incubation, zones of inhibition were observed in around e wells 1 mm. This result indicates that, under the tested conditions, show antibacterial effect against *E. coli (Figure 2, Table 2)* .

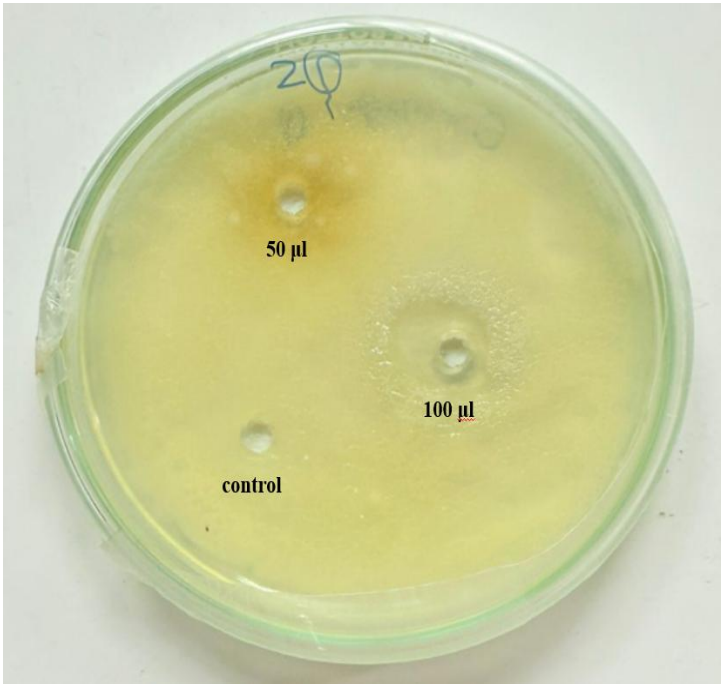

Figure 2 - Antibacterial of Hibiscus rosa against *E. Coli*

Table1: Zones of inhibition (ZOIs) of antibacterial activity (50, and 100µl) concentrations.

Bacteria	AuNPS	
	50 µl	100µl
E. coli	0mm	1mm

IV.DISCUSSUIN

The antimicrobial activity of *Hibiscus rosa-sinensis* flowers can be attributed to the presence of bioactive compounds such as alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, triterpenoids, phytosterol. These compounds are known to disrupt microbial cell membranes, inhibit enzyme activity, and interfere with nucleic acid synthesis. The higher activity observed in polar extracts suggests that the active constituents are predominantly polar in nature.

The findings of the present study are consistent with earlier reports that highlight the antimicrobial potential of *Hibiscus* species. The study supports the traditional use of hibiscus flowers in treating infectious diseases.

V.CONCLUSION

The present investigation confirms that *Hibiscus rosa-sinensis* flowers are rich in phytochemicals with significant antimicrobial activity. Ethanol, Aqueous and chloroform flower extracts showed promising inhibitory effects against pathogenic bacteria and fungi. These findings suggest that hibiscus flowers can serve as a potential natural source of antimicrobial agents and may be further explored for pharmaceutical applications.

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