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Antibacterial activity and qualitative phytochemical study of Hibiscus rosa sinensis

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Abstract
Phytochemical screening showed the presence of biologically active phytochemical Flavonoids, Tannin, Glycosides, Triterpinoids, Steroids. Antibacterial activity of powder of *Hibiscus rosa-sinensis* was evaluated using disc diffusion and method. The zone of inhibition of hibiscus rosasinensis show better antibacterial activity against four pathogen like positive pathogen *Staphylococcus aureus, Bacillus Subtilis* and negative pathogen *Pseudomonas* Aeruginose and Escherichia coli.

Keywords: qualitative phytochemical, Hibiscus Rosa sinensis

Introduction
The Malvacea family includes Hibiscus rosa sinensis [1]. It growns in evergreen plant. It is used to cure many diseases. Alkaloids, protein, steroid, and carbohydrates were found in this phytochemical analysis [2]. It is an ornamental plant that grows and has antibacterial action as evidenced by disc diffusion zones of inhibition. It's methods against *Escherichia coli, Staphylococcus aureus* microorganism [3]. Discs containing methanolic floral extracts ranging from 31.25 to 500mg. It is compared to gentamicin, a positive control (1mg\disc). It flower extract observed at concentration 500mg for *Escherichia coli* 23+ 1.01mm and 13.75+ 0.99mm by maximum zone of inhibition [4]. Flow extract of Hibiscus rosa sinensis is effective against human infections. It is also highly need for drug development. Antibacterial activity are proved by extract of Hibiscus rosa-sinensis [5]. Hibiscus flower and leaf extracts are used to treat a variety of ailments. This flower shades are white to pink and red and from orange to yellow [6]. Petals are used to promote hair development, hair loss, and scalp problems. Medicinal plants have potential of myriad benefits. *Hibiscus rosa-sinensis* were tested against positive bacteria antimicrobial is a substance to kill or inhibits the growth of microbes [7]. Microbes such as bacteria, fungi or viruses. Usually plants have ability to synthesis chemical compounds [8]. That chemical compounds are used to defend against attack from a wide variety of predators such as insects, fungi. It is descripted asjasum, Chinese hibiscus, jasuba, japa, mondaro, dasanamu, semparuti in many states. And also various countries to say angharachindi, kaungyan, hong can, rosa de china [9]. Calcium, phosphorus, magnesium, sulphur, potassium, chloride and sodium are all found in this south-eastern Asian native. Zinc, iron, silicon, manganese, copper, fluoride and other trace elements are essential [10]. Trace minerals are necessary in small amounts, less than 100mg per day, or less than 0.01 percent of body weight, and include critical trace elements including zinc, iron, silicon, manganese, copper, fluoride, iodine and chromium [11]. Furthermore, these ingredients help to lower a variety of individual risk factors. These are associated with both human and animal cardio vascular illness [12].

Material and Method
The fresh flower were collected from Villupuram, Tamilnadu, India. These fresh flower was washed under running up water, and then dried out it under shadow. Then the dried flower was grinded to fine powder using electrical grinder and stored the powder for tests.

Fig 1: Hibiscus Rosa Sinensis

Qualitative phytochemical analysis

Test for Alkaloids: Dragendroff's reagent was used to test this, and wagner's reagent was used to confirm it. 2ml strong HCL acid was carefully added to 4ml crude ethanolic extract in a test tube. The mixture was warmed for 15 minutes, then cooled before being filtered through whatman No.1 filter paper (125mm diameter). After that, the filterate was put through the dragendroff and wagner tests. Wagner's reagent was added to 1ml of the filterate. The presence of alkaloids was revealed by the formation of a brownish precipitate.

Test for Phenol

Ferric Chloride Test: Three drops of alcoholic FeCL3 solution were added to the test extract. The creation of a bluish black colour indicates the presence of phenol.

Test for Steroid

In a test tube, 2ml of the extract was mixed with 20ml of chloroform. Concentrated H2SO4 acid was slowly added into the liquid through the tube walls without agitation. The presence of a red interface and yellow-greenish fluorescence in the H2SO4 acid layer indicates the presence of steroid in the plant extract.

Test for Tannins

Lead Test: In a test tube, 10mg of hibiscus Rosa sinensis was dissolved in 3ml of distilled water, and a few drops of ferric chloride were added to the solution, which was then examined for blue or green colour.

Test for Saponins

Foam Test: 50mg of Hibiscus Rosa sinensis was dissolved in 5ml distilled water and forcefully agitated until a stable, persistent froth formed. The foam was then blended with 3 drops of olive oil and violently agitated before being examined for emulsion**.**

Test for Flavonoids

Ferric Chloride Test: In 3-4ml of distilled water, 40mg of Hibiscus Rosa sinensis were dissolved. A 0.5ml solution of weak ammonia was added to it. Later on, Concentrated Sulphuric acid was added. The presence of flavonoids was indicated by a yellow colour. After letting the solution to sit for a while, the yellow colour fades.

Test for Terpenoids

Salkowaski's Test

25mg of Hibiscus rosasinensis were dissolved in 2ml of chloroform, then 3ml concentrated sulphuric acid were added. The presence of terpenoids was revealed by the appearance of a reddish brown discoloration at the contact.

Test for Carbohydrates

Fehling's Solution Test: 1ml of fehling's solution –A is mixed and 1ml of fehling's solution –B are combined. A small amount of the item dissolved in water is added to the mixture, agitated thoroughly, and heated in a boiling water bath. Red brown precipitate is formed indicates the presences of carbohydrates.

Test for Proteins

Biuret's Test: 2ml biuret reagent + 2ml extract = 2ml biuret reagent + 2ml extract = 2ml biuret reagent + 2ml shaken it well before warming it in a water bath. The presence of proteins is indicated by the appearance of red or violet colour.

Fixed Oils and Fatty Acid

Spot Test: The presence of fixed oil and fats was indicated by a prepared area on the filter paper with the test solution and oil staining on the filter paper. It shows that carbohydrates are present.

Proteins

Xanthoproteic Test: A few drops of concentrated HNO3 were added to the extract. The presence of proteins is shown by the formation of a yellow colour.

Amino Acids

Ninhydrin Test: 2 ml on ninhydrin reagent was added to the 2ml extract and cooked for a few minutes; the production of blue colour indicates the presence of amino acid.

***In vitro* antibacterial activity**

Among the most common infectious diseases are bacterial infections. As a result, almost 50 years of intensive study has gone into developing novel antibacterial drugs derived from various sources. Despite advancements in the creation of antibacterial medicines, the rise of multidrug-resistant bacteria has necessitated the development of new antibacterial drugs. The MIC of aqueous extract of C.longa rhizome was determined in an antibacterial research (minimum inhibitory concentration).

Result and Discussion

Table 1: Qualitative Analysis of Hibiscus Rosa Sinensis

Phytochemical	Water	Methanol
Alkaloids Wager's		
	+	+
Saponin	+	+
Steroid	_	+
Tannin	+	+
Protein	_	_
Amino acid	_	_
Carbohydrade Fehling test	+	+
Flavonoids		
	+	_
10%NaOH	+	+
10%NH4OH Mg test	+	+

Fig 2: Qualitative Analysis of Hibiscus Roso Sinensis

In vitro Antibacterial Activity

Diffusion method is done using *Escherichia coli, Pseudomonas aeruginosa*. For the experiment, plant powder solutions are prepared. For antibacterial activity testing, the dried plant powder was weighed (10mg/ml) and dissolved in sterile and distilled water.The antibacterial activity was performed by disc prepared appropriate dilution to get required concentrations of about 50µl (50µg), 100µl (100µg) and 150µl (150µg). Unless they were utilized in the experiment, they were kept in the refrigerator. To compare the test solution, a standard solution containing gentamicin for bacteria and amphotericin for fungus was utilized. For experimental purposes, they were stored in a refrigerator.

Preparation of Dried Filter Paper Disc

Whatmann filter paper (No: 1) was used to make discs that are roughly 6mm in diameter and sterilized with hot air. After sterilization, the discs were loaded with various amounts of hibiscus rosa-sinensis plant extract solution and refrigerated for futher 24 hours.

Microorganism

In this investigation, bacteria that cause infectious diseases in both animals and humans were employed. Gram-positive bacteria such as Pseudomonas and Escherichia coli were used in the study.

Application of Disc to Inculated Agar Plate

On the surface of the inoculated agar plate, previously prepared paper discs were dispersed. Each was firmly pressed against the agar surface to ensure complete contact. The discs were evenly spaced on the medium, and the plates were incubated at 5 degree c for 1 hour to allow for proper diffusion before being transferred to a 37 degree c incubator for 24 hours. After the plates had been inverted and placed in an incubator set to the appropriate temperature for 24 hours, they were inverted and placed in an incubator set to the appropriate temperature for 24 hours.

Antibacterial Activity of Hibiscus Rosa Sinensis

The powder of Hibiscus rosa-sinensis were evaluated for antibacterial activity by disc diffusion method different concentrations like (25, 50, 75,100 µg/ml) were prepared by reconstituting with water. The test microorganisms such as *Pseudomonas* and *Escherichia coli* were seeded into respective medium by spread plate method 10µl (106 cells/ml) with the 24 hours cultures of bacteria grown in nutrient broth. After solidification, sterile filter paper discs (6mm in diameter) impregnated with the powder were placed on test organism plates. Gentamycin (20µg/ml) was used as standard anti-biotic. The anti assay plates were incubated at 37º c for 24 hours. The diameters of the inhibition zones were measured in mm.

Measurement of Zone of Inhibition

The antimicrobial potential of test compounds was measured in millimeters by the mean diameter of the inhibitory zone around the disc. A millimeter scale was used to assess the zones of inhibition of the tested microorganisms by the powder.

Table 2: Zone of inhibition of the extract *Hibiscus rosa-sinensis*

S.No	Positive and negative pathogen	Zone of inhibition(diameter in mm) for *Hibiscus rosa-sinensis*				Standard (Gentamicin) 50µg/mL
		25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	
1	Staphylococcus aureus	9	12	15	17	17
2	Bacillus Subtilis	9	11	12	17	18
3	Pseudomonas aeruginosa	8	10	13	16	19
4	Escherichia coil	10	13	15	18	20
5	Control(DMSO)	NIL	NIL	NIL	NIL	NIL

NI: No inhibition

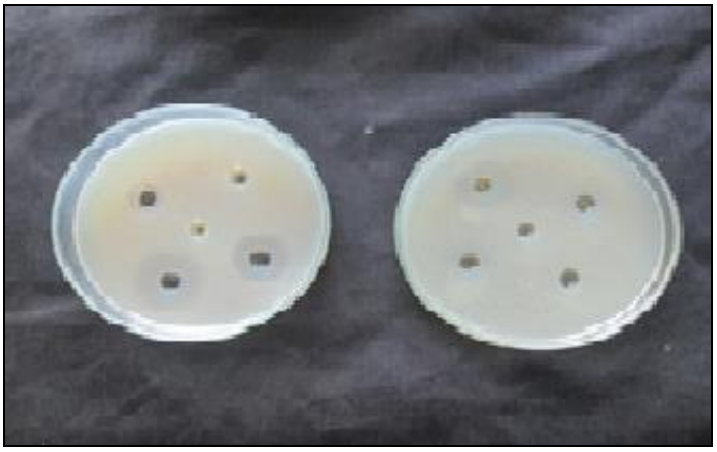

Fig 3: Antibacterial activity against S. aureus and B. Subtilis

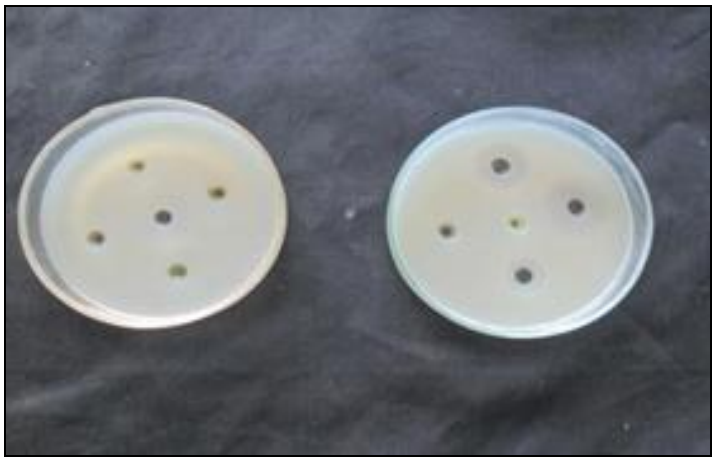

Fig 4: Antibacterial activity against P.aeruginosa and E. Coli

Among the four pathogen *Escherichia Coli* show the better antibacterial activity with inhibition of 18mm at 100µg/mL compared with other pathogen. In positive pathogen *Bacillus subtilis* show the better antibacterial activity with inhibition of 17mm at 100µg/mL compared with Standard. Based on the Table 2 it shows when concentration increases anbacterial activity also increases for*Hibiscus rosa-sinensis.,*

Conclusion

*Hibiscus rosa-sinensis*were tested for phytochemical analysis its indicate the Flavonoids, Tannin, Glycosides, Triterpinoids, Steroids. The Zone of inhibition of *Hibiscus rosa-sinensis* show better antibacterial activity against four pathogen like positive pathogen Staphylococcus aureus, Bacillus subtilis, and negative pathogen Pseudomonas aeruginosa and Escherichia coli when concentration increases antibacterial activity also increases.

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