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Phytochemical analysis and antimicrobial activity of *Hibiscus Rosa Sinensis*

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Abstract
Hibiscus rosa sinensis is a plant well known for its various medicinal properties. The aim of this research work was to find out the antibacterial activity and phytochemical analysis of whole plant extracts (root, flower, and leaves). In the present investigation phytochemicals analysis of various parts of the plants were done which shows presence of these phytochemicals alkaloids, terpenoids, tannins, flavonoids, saponins, phenolic compounds, carbohydrate, reducing sugar and triterpenoids. Further saponin content estimation revealed lowest amount of saponin in 2.5mg/g in HRlf followed by 3.1mg/g in HRfr and highest amount 4.3mg/g in HRrt. Moreover, antibacterial activities of different plant extracts were evaluated on gram negative bacteria *S.aureus* by Agar well-diffusion method in different concentration of 20, 50 and 100µg/ml and zone of inhibition were measured. The maximum zone of inhibition shown in concentration of 100µg/ml methanolic leaf extract of *H.rosa* sinensis (29.00±2.81mm), followed by methanolic flower extract (17.00±0.99mm), followed by aqueous flower extract(14.00±0.90mm) followed by methanolic root extract (13.00±0.75mm). MeOH extract of leafs exhibits highest antibacterial activity among tested ones. Current studies have shown that the *H.rosa sinensis* plant contains significant antimicrobial activities in the MeOH extracts. The antibacterial activities of *H.rosa* are may be due to presence of saponin, tannins, and flavonoids.

Keywords: antimicrobial activity, S.aureus, Bioactive compounds, methanolic extract, HRlf, Saponin content

Introduction
Hibiscus rosa sinensis commonly known as china rose, odhul in hindi and japakusum in sanskrit is a member of Malvaceae family is one of the important medicinal plants occurring throughout India and tropics and subtropical areas of world it is cultivated globally as an ornamental and medicinal plant [1]. This taxon propagates through cutting. Colorful and perennial blossom of the plant makes it the most ideal ornamental plant which does not require much care and attention of the plant grower *Hibiscus* species have long been known for their economic and therapeutic importance. Leaves and flowers are said to be emollient. Flowers are being employed as emmenagogue, sudorific and used to check excessive bleeding in menstruation. They are useful in epilepsy, leprosy, bronchial catarrh and diabetes [2]. The species has many vibrant colored flowers cultivar; however, red flower is considered more effective for medicinal use.

Previous studies have been suggested parts of the plant have been shown to possess different pharmacological actions [3] including antiproliferative and anticancerous. Roots possess hypolipidemic, antifertility and neuroprotective properties [4, 6] whereas leaves exhibited analgesic, antidiabetic, wound healing, and antimutagenic activities. [7, 11] Varied pharmacological and biological activities have been reported from the flower of this plant by different workers *viz*. antihyperlipidemic, wound healing, antimutagenic, anti-anxiety, anticonvulsant, cardioprotective, [12, 13] hypotensive, cholesterol lowering and hypoglycaemic. Flowers have also shown antibacterial, antioxidant activities as well as inhibitory effects on the alkaline phosphatase enzyme [14]. Contradictory actions like hair growth potential and hair growth retarding effect have also been reported [15].

The major constituents of *H. rosa sinensis* are anthocyanins, sterols, triterpenes, and phenolics, alkaloid, saponin and tannins.

Previous studies on other Hibiscus species such as Hibiscus sabdariffa and Hibiscus schizopetalus flowers reported activities against various pathogens found in humans and other mammals [16, 17]. Finding new antibacterial and medication has become very important nowadays in order to be able to face the escalating problem of bacterial resistance.

Natural agents, known for lower toxicity and higher effectiveness, are potential alternatives to more toxic and less effective treatments. Newly discovered natural medications that can be used for the treatment and prophylactic of bacterial and viral infections can be viable and safer alternatives to traditional medications. These biologically active metabolites identified from natural products may offer several advantages over synthetic ones including lower toxicity, more biodegradability, and lower costs. Plants are a rich source of bioactive compounds that can lead to the development of new, natural drugs that can have novel mechanisms. This is especially important given the spread of antimicrobial resistance.

The aim of the present study was phytochemical analysis and assessment of antibacterial activities of *H. rosa sinensis*.

Materials and Methods
Plant Material
The plant of *Hibiscus rosa sinensis* was collected from botanical garden of Ranchi university Ranchi. The plant was identified by comparing with the authentic samples at the Herbarium of taxonomy Department of Ranchi University. The whole plant material was washed carefully and shade

dried. After 4 weeks when leaves it dried completely, they were grounded into powder form. Then for the future use with proper labeling, the powder stored in air tight container.

Extraction of Plant Materials

The shade dried plant powder was soaked with methanol (1:10 ratio) and in other flask with distilled water covered with aluminium foil to prevent evaporation and then kept for 48 hr in shaker incubator. After 48 hr the solution was filtered with help of whattman No.1 filter paper and filtered was collected in beaker. Then the filtrate was kept in incubator at 37 ˚C to evaporate the solvent. The prepared extract was stored at 4˚C for further use.

Phytochemical Screening

Phytochemical screening was done to check the presence of of plant primary and secondary metabolites such as Phenol, saponin, tannins, reducing sugar, flavonoids, glycosides and alkaloids Qualitative analysis were carried out on the whole plant extract. The presence or absence of the bioactive compounds of material was analyzed using the following standard methods.

Test for Phenols

0.5 ml of aqueous plant extract was added in 5 ml of Folin Ciocalteu reagent than 4ml of aqueous sodium carbonate were added. Formation of blue color indicates the presence of phenols.

Test for Tannins

In 10 ml of distil water 0.5 g of the powdered plant material was mixed and boiled in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and appearance of blue-black or brownish green color indicates the presence of tannins [18].

Test for Flavonoids

Few drops of ferric chloride solution were added in plant extract aqueous solution formation of blackish red color indicates the presence of flavonoids.

Test for Carbohydrates Benedict's test

Few drops of Benedict's reagent was added in test solution (alkaline solution containing cupric citrate complex) and boiled in water bath, formation of reddish brown precipitate indicates the presence of carbohydrate.

Test for Alkaloids

1% of aqueous hydrochloric acid mixed with 1ml aqueous extract and then placed in hot water bath then, 1 ml of the filtrate was treated with Dragedorff's reagent. Presence of turbidity or precipitation shows the presence of alkaloids.

Test for Saponin

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

Test for Cardiac glycosides

5 ml of aqueous plant extracts was treated with 2 ml of glacial acetic acid containing one drop of Ferric chloride solution and then 1 ml of concentrated sulphuric acid were carefully added. Formation of brown ring at the interface indicates the presence of a deoxysugar which is characteristics of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just spreading gradually throughout the layer.

Test for Phlobatannins

Whole plant extracts were boiled with 1% aqueous Hydrochloric acid. Formation of red precipitate indicates the presence of phlobatanins.

Test for Terpenoids

5 ml of plant sample extract were mixed with 2 ml Chloroform than 3ml Concentrated sulphuric acid was carefully added to form a layer. A reddish brown color at the interface indicates the presence of terpenoids [19].

Determination of Total Saponin Content

Saponins were determined using the method of Obadoni and Ochuko [20]. The 2 g of air dried and ground plant sample were put into a conical flask and 20ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 20 ml 20% ethanol. The combined extracts were reduced to 4 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel than 10ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.6 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Microbiological Assay

The agar disc diffusion method was employed for the determination of antibacterial activities of the methanolic leaves and flowers *Hibiscus rosa sinensis* extract of (Mukherjee *et al*. [21]. The MIC of the extract was also determined using a two-fold dilution method. The bacteria were first grown in nutrient agar for 18 hour before use. The inoculum suspensions were standardized. It was performed using an 18 h culture at 37°C in 10ml of Mueller Hinton Broth. The cultures were adjusted to approximately 105CFU/ml with sterile saline solution. Five hundred micro liters of the suspensions were spread over the plates containing Mueller-Hinton agar using a sterile cotton swab in order to get a uniform microbial growth on test plates and then tested against the effect of the plant extracts at the concentration of 500mg/ml, 250 mg/ml, 125mg/ml, 62.5 mg/ml, and 31.25. Mg/ml. All petridishes were sealed with sterile laboratory para films to avoid eventual evaporation of the test samples. These plates were incubate for 24 hour at 37°C and measured the zone of inhibition in millimeter the plates later incubated at 37°C± 0.5°C for 24 hours after which they were observed for zones of inhibition. The effects were compared with that of the standard antibiotic Gentamicin at a concentration of 1mg/ml (Khan and Omotoso) [22]. This was used as positive control, while methanol was used as negative control. The inhibitory zone around test paper discs indicated as positive (growth inhibition observed) and absence of zone as negative [23].

Result and Discussion

The phytochemical screening of the plant part extracts of *H.rosa sinensis* showed that the plant are rich in secondary metabolites and certain biochemical compounds such as, phenols, alkaloids, tannins, flavonoids, carbohydrates/ reducing sugars, terpenoids, phlobatanin, cardiac glycoside and saponins.

Table 1: Phytochemical Analysis of the aqueous (HRA) and methanolic Extracts (HRME) of *Hibiscus rosa sinensis* plant parts.

`Sl No.	Phytochemicals	Name of the test	*Hibiscus rosa sinensis*		
			HRrt	HRlf	HRfr
1	Flavonoids	Ammonia test (modified)	++	+++	++
2	Carbohydrate/ Reducing sugar	Fehling's test	_	+	+
3	Alkaloids	Hager's test	+	+++	++
4	Polyphenols	Folin ciacalteu test	+++	+++	++
5	Cardiac glycosides	Killer-Killani's test	_	+	_
6	Phlobatannins	Ring test	+	_	+
7	Terpenoids	Salkowski test (modified)	_	++	+
8	Tannins	$FeCl_3$ test	+++	+++	+++
9	Saponin	Frothing test	+	+	++

Where +++ shows strong presence, ++ shows partially strong, + shows week and - shows absence of phytochemical activities.

Table 2: Saponin content estimated in *Hibiscus rosa sinensis*

Plant Extract	Concentration (in mg/g)
HRrt	4.3
HRlf	2.5
HRfr	3.1

Table 3: Zone of inhibition of aqueous extract of *Hibiscus rosa sinensis*

Plant Extracts	Zone of Inhibition in mm			Positive control
Concentration (in µg/ml)	20	50	100	
HRrt	Nil	Nil	9.00±0.65	18
HRlf	Nil	4.00±3.81	11.00±1.25	18
HRfr	Nil	Nil	14.00±0.90	18

Table 4: Zone of inhibition of methanolic extract of *Hibiscus rosa sinensis*

Plant Extracts	Zone of Inhibition in mm			Positive control
Concentration (in µg/ml)	20	50	100	
HRrt	Nil	Nil	13.00±0.75	25
HRlf	Nil	8.00±3.81	29.00±2.81	25
HRfr	Nil	6.75±0.76	17.00±0.99	25

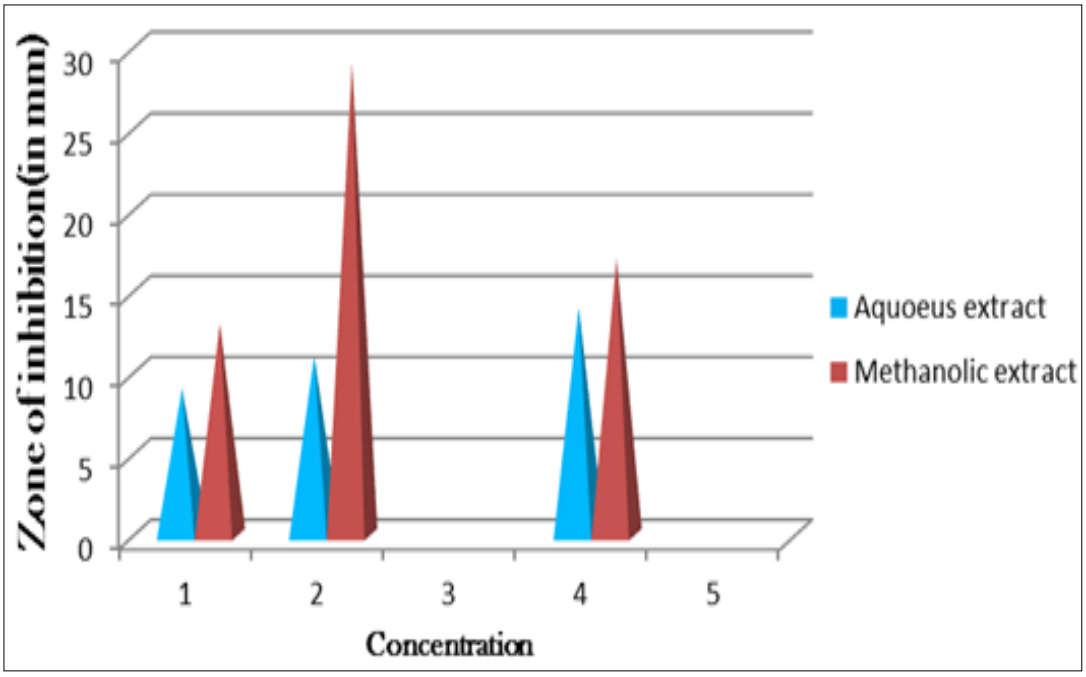

Fig 1: Inhibition zone of three solvent extracts from root, leaf, and flower extracts of *H.rosa* in 100 µg/ml concentration against *S.aureus*

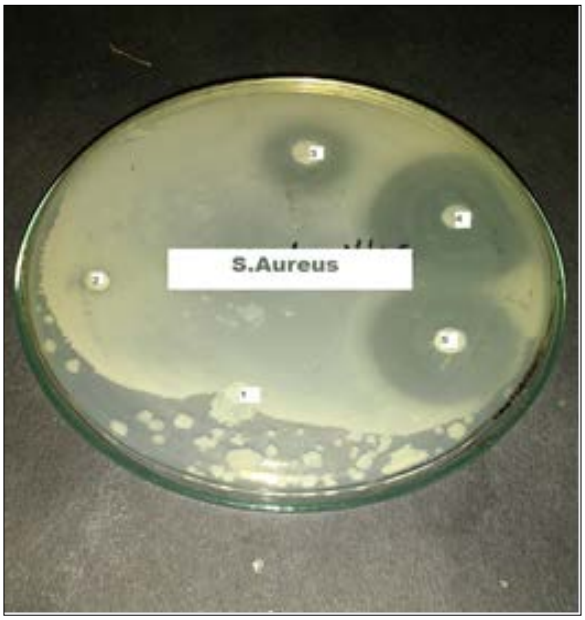

Fig 2: Activity of *H.rosa* methanolic leaf extract on *S.aureus*

The knowledge of medicinal properties of plants has been accumulated in the course of many centuries. The plant parts have been used by local indigenous people from ancient times and this knowledge passed from one to next generation by this tradition they have inherited rich traditional knowledge on the use of many plants or plant parts for treatment of common disease. Medicinal plants are accessible and culturally relevant sources of primary health care system. The remedies based on these plants often have minimal or no side effect [24]. The bioactive substances in plants are produced as secondary metabolites, which may not only be developmental stage specific but also organ and tissue specific. While plant leaf, stem and root extracts have been widely evaluated for phytochemicals, screening of plant flower has not been extensive. Secondary metabolites belonging to polyketide and nonribosomal peptide families constitute a major class of natural products with diverse biological functions and they have a variety of pharmaceutically important properties [25].

The phytochemical screening of root, leaf and flower extracts of *H. rosa-sinensis were car*ried out which shows presence of various important bioactive compounds such as tannins, alkaloids, flavonoid, phenol, terpenoid and saponins in aqueous and methanolic extracts are presented in Table 1. The presence of phytochemicals suggests both physiological and medicinal activities. From the general screening (Table 1) it was observed that methanol is a better solvent for extracting saponins.

In table 2 content of saponin were presented which was estimated in the plant sample which shows lowest amount of saponin were present in leaf extract (HRlf) 2.5 mg/gm followed by 3.1 in flower extract (HRfl) and highest amount in root extract (HRrt) 4.3mg/gm. Saponin is a nonnutritive compound found in many plant but also known for its medicinal properties. The nutritional significance of saponins stems largely because of their hypocholesterolaemic action, suggesting they may prove useful in the control of human cardiovascular diseases and also in case of Type 2 diabetes where blood cholesterol levels are raised along with elevated glucose levels. Saponins possess both beneficial and deleterious properties depending on its concentration in the sample [26]. In the study on the basis of finding low content of saponin in leaves of the plant seems a safer option for long term use in diabetes rather than root or flower.

The antibacterial activities of *H. rosa-sinensis* plant parts were carried out which showed in table 3 and 4. Most of the extract shows an antibacterial activity against the human pathogens *Staphylococcus aureus* in 100µg/ml concentration. *S.aureus* is a Gram-positive bacterium and causative agent of wide range of infectious diseases such as skin infections, bacteremia, endocarditis, pneumonia and food poisoning it causes wide range of infectious conditions both in nosocomial and community settings. The Gram-positive pathogen is armed with battery of virulence factors that facilitate to establish infections in the hosts. The organism is well known for its ability to acquire resistance to various antibiotic classes [27].

All the plant part extracts have shown the activity. Investigations were carried out of plant materials as alternative sources of antibacterial agents. It has become more common over the past few years, due to the increased rate of development of antibiotic resistance organism. The inhibition of bacterial growth *in-vitro* by the extracts of plants could be due to the presence of some active compounds in the extracts. These active compounds may act alone or in combination to inhibit bacterial growth. The crude extracts containing multiple organic components including flavonoids, tannins, alkaloids, triterpenoids, all of which are known to have antibacterial affects. HRrt, HRfr, and HRlf extract contain phenolics compounds like tannins and saponin that are very good antimicrobial agents [28]. Thus it may be summarized that the class of natural compounds must exhibit the antibacterial activity. The metabolites have been shown to be responsible for various therapeutic activities of medicinal plants [29]. Flavonoids especially are known to be effective antimicrobial agent against a wide array of microorganisms. The activity is attributed to their ability to complex with extra cellular and soluble proteins and with bacterial cell wall [30]. There are several reports published on antibacterial activity of different herbal extracts [31, 38]. Many antimicrobial screening studies use a relatively small number of microorganisms for testing.

In the present investigation root, leaf and flower extracts from *H. rosa-sinensis* were screened for antibacterial activity against human pathogenic bacterial strains *S. aureus* which is facultative anaerobe that grows by aerobic respiration or by fermentation which yields lactic acid. These are pathogenic to human beings. They cause a wild range of superlative infection as well as food poisoning and toxic shock syndrome. Thus the plant extracts can be used as an important antibiotic to cure above mentioned disorders caused by this strain of bacteria [39, 41]. The present studies conclude these extract could inhibit human pathogen *S. aureus* growth the maximum zone of inhibition were measured in methanolic leaf extract (Fig.1). The results are encouraging but precise assessment is utterly necessary before being situate in practice as well as the most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo secondary pharmacological evaluation.

Conclusion

The current study justified that antimicrobial activity was evaluated based on their use in ethnobotanical literature

using traditional herbal plants. From the results, it was concluded that methanolic leaf, root and flower extract of *Hibiscus rosa sinensis* showed potential antimicrobial activity against *S.aureus* among the plant parts best result were observed in methanolic leaf extract (HRlf). Hence, HRlf has effective bioactive compounds responsible for antibacterial activity. Therefore, further investigations on combined isolation, toxicology, and the effective compound require clinical trials and deserve extensive research.

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