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Study on Phytochemical Screening and Antibacterial Potential of Methanolic Flower and Leaf Extracts of *Hibiscus rosa-sinensis*

Tej Prakash

P.G. Department of Botany, Dr. Ashok Kumar Smarak P.G. College, Akbarpur, Ambedkar Nagar (U.P.), India

Abstract

Hibiscus rosa sinensis is member of the family Malvaceae and grows as an evergreen herbaceous plant in tropical regions. Hibiscus species are used in the treatment of various diseases. They have many pharmacological properties including antipyretic, antispasmodic, hypotensive, antifungal, anti-inflammatory and many more. Therefore, the present study was aimed at evaluating the antibacterial activity of methanolic extract from the leaves and flowers of *H. rosa sinesis* and identification of secondary metabolites in these extracts. The phytochemical analyses showed the presence of alkaloids, glycosides, flavonoids, tannin and phenols in hibiscus leaf extract, while hibiscus flower extract contained alkaloids, protein, steroid and carbohydrate.

Antibacterial activity has been checked in terms of zone of inhibition by disc diffusion method against microorganisms *E.coli* and *S. aureus* for different concentrations of methanolic leaf and flower extracts ranging from 31.25 to 500 mg/disc which were compared with positive control gentamicin (1mg/disc). Both extracts showed increasing antibacterial property with increase in the extract concentration. Maximum zone of inhibition observed for both methanolic leaf and flower extracts of *H. rosa sinesis* at concentration 500 mg for *E. coli* 23±1.01mm and 13.75±0.99 mm, respectively. However, for *S.aureus* methanolic leaf and flower extracts of *H. rosa sinesis* at concentration 500 mg showed maximum zone of inhibition 19.33±0.29mm and 9.75±0.76 mm. These results suggest that methanolic extracts of both leaf and flower of *H. rosa sinensis* contained varied types of pharmacologically active compounds that have potential as antibacterial agent for treating infection caused by these microorganisms.

Keywords

Hibiscus rosa sinensis, antibacterial activity, zone of inhibition, phytochemicals.

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Introduction

Since ancient time higher plants, are sources of medicinal compounds and play a dominant role in the maintenance of human health (Farombi, 2003). Over 50% of all modern clinical drugs are of natural product origin (Sumathi and Krishnaveni, 2012). Microorganisms are the most important pathogens causing severe morbidity and fatal infections in humans. The range of pathogenic bacteria is wide and

so is the variety of diseases caused by them (Barbour et al., 2004; Machado et al., 2003). Hence, many efforts have been exploited to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nostro et al. 2000, Arullappan et al. 2009, Baker et al, 1995). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Erdogrul, 2002).

Hibiscus rosa sinensis a well known member of the family Malvaceae, grows as an evergreen herbaceous plant. Leaves and stems of hibiscus contain β-sitosterol, stigmasterol, taraxeryl acetate and three cyclopropane compounds and their derivatives. Flowers contain cyaniding diglucoside, flavonoids and vitamins, thiamine, riboflavin, niacin and ascorbic acid (Ghani, 2003). *H. rosa sinensis* has many pharmacological properties including antioestrogenic, anti-implantation, abortifacient, antipyretic, antispasmodic, hypotensive, embryotoxic, antispermatogenic, insect attractant, analgesic, antifungal and anti-inflammatory activities (Herbal Medicine Research Centre 2002). *Hibiscus rosa-sinensis* flowers contains anti-mitotic constituents that can stop the mitosis in anywhere of the cell cycle and therefore, probably affect the cytoskeleton by tubulin polymerization or degradation (Ali, 2010).

The objective of this study was to investigate antibacterial potential of methanolic leaf and flower.

Materials and Methods

Collection of plant materials

H. rosa sinensis leaves and flower were procured from plant pathology lab garden of Department of Botany, Dr.Ashok Kumar Smarak P.G.College, Akbarpur, Ambedkar Nagar and the identification of the plant was confirmed by the taxonomist of the same department.

Preparation of *Hibiscus rosa sinensis* extract

Crude leaf and flower extracts were prepared by soxhlet extraction method described by Okeke et al (2001). About 45 gm of leaves and flower powder material were uniformly packed into a thimble and run in soxhlet extractor separately. It was exhaustible extracted with 250 ml methanol for the period of about 48 hours and 22 cycles or till the solvent in the siphon tube of an extractor become colourless. After that extracts were filtered with the help of filter paper and solvent evaporate from extract in rotary evaporator to get the syrupy consistency. The residue was dried over anhydrous sodium sulphate to remove trace of alcohol. Then extract was kept in refrigerator at 4°C for detect antibacterial activity and analyzed their physical and chemical property.

Phytochemical screening of crude extract

Extracts were tested for the presence of active principle such as steroid, tannins, phenols, flavonoid, alkaloids, glycoside, triterpinoids, carbohydrates and proteins by standard procedures followed by Debela (2002).

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., 1995). 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 10 ml of Mueller Hinton Broth. The cultures were adjusted to approximately 10^5CFU/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 parafilms 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, 2003). 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.

Statistical analysis

All measurements of were carried out in triplicates. The results are expressed as mean values ± standard deviation (SD).

Results and Discussion

In the present investigation table 1 shows the presence of phytochemicals in methanolic extracts of hibiscus flower and leaves. The phytochemical analyses showed the presence of alkaloids, glycosides, flavonoids, saponins, tannin and phenols in hibiscus leaf extract, while hibiscus flower extract contained alkaloids, saponins, protein, phytosterols and carbohydrate. Table-2 shows antibacterial patterns for methanolic leaf and flower extracts of *Hibiscus rosa sinensis* for different concentrations ranging from 31.25mg–500mg/disc. Results showed maximum inhibition zone as 23±1.01mm and 13.75±0.99 in leaves and flower, respectively in case of *E. coli*, while in case of *S. aureus* maximum zone of inhibition was observed 19.33±0.29 mm and 9.75±0.76 mm in leaves and flowers, respectively as compared with the standard positive standard gentamicin (1 mg/disc).

Table 1: Table showing phytochemicals present in *Hibiscus rosa-sinensis* (leaves and flowers)

Phytochemicals	Name of phytochemical test	Methanolic leaf extract of *Hibiscus rosa sinensis*	Methanolic flower extract of *Hibiscus ros. sinensis*
Alkaloids	Mayers& Dragendorff's	+	+
Glycosides	Keller Kilani	+	-
Flavonoids	Shinoda's & Zn-HCl	+	-
Proteins and amino acids	Ninhydrin, Biuret	-	+
Phytosterols	Salkowski & Liebermann Burchad	-	+
Carbohydrates	Benedicts and Fehling	-	+
Tannin& phenol	Ferric chloride	+	-
Saponins	Frothing test	+	+

Table 2:Antibacterial activity of methanolic extracts *Hibiscus rosa sinensis* (leaves and flowers) against the Gram-positive (*S. aureus*) and Gram-negative bacteria (*E.coli*) based on disc diffusion assay

Samples	Concentration (mg/disc)	Microorganisms (Inhibition zone diameter, mm)	
		Gram positive bacteria (*S. aureus*)	Gram negative bacteria (*E. coli*)
Hibiscus rosa sinensis (leaves)	0.5	19.33±0.29	23±1.01
	0.25	15.87±0.58	20.34±0.98
	0.125	10.56±0.47	16.78±0.75
	0.062	8.85±0.25	11.97±.82
	0.031	-	8.72±0.32
Hibiscus rosa sinensis (flowers)	0.5	9.75±0.76	13.75±0.99
	0.25	8.96±0.37	11.23±0.86
	0.125	7.25±0.26	9.68±0.46
	0.062	-	7.86±0.38
	0.031	-	-
Gentamicin	1	35±1.57	36±1.63
Methanol	-	-	-

The antibacterial activity of plant extracts depends on the available bioactive secondary metabolites in the plant part. It has been reported that *H. rosa-sinensis* possess alkaloid, glycosides, flavonoids, tannin and phenols (Krishnaiah, et al., 2009, Anyasor et al., 2010). From the preliminary screening, it has been identified that methanol extract of hibiscus exhibits phytomedical property which may be due to the presence of biologically active compounds in hibiscus whose activity are enhanced in the presence on methanol. Furthermore, methanol has stronger extraction capacity which could be helpful in extracting greater number of active constituents responsible for antibacterial activity (Baker et.al., 1995). 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 (Ionela and Ion, 2007; Kenneth and Golomeke, 2015). The antimicrobial activity of phenolics have been reported from various plant resources. Their mode of actions are well explained by many workers (Cowan 1999; Nohynek et al. 2006; Veldhuizen et al. 2006; Tiwari et al. 2014). Their basic mode of action is as follows. 1) The position and number of hydroxyl groups is related to the level of toxicity in case of some phenols e.g carvacrol. 2) In case of simple phenols such as catechol and epicatechin, inhibition is mainly due to substrate deprivation and membrane disruption. 3) Other mechanisms of action by phenolic acids, flavonoids and tannins involve enzyme inhibition, enzyme inactivation, formation of complexes with cell walls and metal. 4) Disruption of cell homeostasis leading to growth inhibition and cell death,and also involved in many physiological activities such as stimulation of phagocytic cells, host-mediated tumor activity. They are soluble in water, alcohol and acetone and gives precipitates with proteins (Basri and Fan, 2005). There are evidences that phenolics were traditionally used for protection of inflammed surfaces of the mouth and treatment of wounds, hemorrhoids and diarrhoea (Ogunleye and Ibitoye, 2003). They are also found to stimulate macrophages, which are antiinfective (Dewick, 2002; Spatafora and Tringali, 2012). Saponins have been found to be potentially useful for the treatment of hypercholesterolemia which suggests that saponins might be acting by interfering with intestinal absorption of cholesterol. (Olaleye, 2007; Patel et al 2012; Nayak et al., 2007). *H. rosa-sinensis* also aids in wound healing and this can be explained due to the existence of tannins and terpenoids which plays important role in promoting wound healing. Several plants which are rich in tannins have been shown to possess antimicrobial activities against a number of microorganisms (Nayak, et al 2007, Okwu et,al 2006). In the present study *E. coli* is more susceptible to antibacterial activity of both extracts of hibiscus. The varying degrees of sensitivity of the bacterial test organisms may be due to the intrinsic tolerance of microorganisms. However, Banso and Adeyemo (2007) investigated gram positive bacterial strains were more susceptible to the extracts when compared to gram negative bacteria, which may be due to the two groups differ by its cell wall component and its thickness (Yao et al., 1995) and also the ability of tannin compounds to cause the bacterial colonies to disintegrate probably results from their interference with the bacterial cell wall; thereby inhibiting the microbial growth (Erasto et al., 2004; Viljoen et al.,2009).

Based on these results, we may conclude methanolic extracts of leaves and flowers of *Hibiscus rosa sinensis* contains phytochemical compounds with antibacterial activities against the sensitive study bacteria. Pharmacology and toxicology of *H. rosa sinensis* the should be further studied to determine how it can be utilized to treat bacterial infections.

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