



Antimicrobial and Antioxidant Potential of *Hibiscus Rosa-sinensis* L. in Western Himalaya

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ABSTRACT: *Hibiscus rosa-sinensis* L. is a medicinal plant found in western Himalaya. Methanol and ethanol flower extracts of *Hibiscus rosa-sinensis* were investigated in present study for its antibacterial and antioxidant activities from western Himalaya. Disc diffusion method was used for determining antimicrobial properties of flower extracts of the plant against standard and clinical isolates of pathogenic bacteria. Methanolic and ethanolic extract showed maximum antimicrobial activity against standard isolate of *Pseudomonas aeruginosa* which was observed as 11.4 ± 1.7 mm and 13.60 ± 2.1 mm respectively. The ethanolic extract showed more antimicrobial activity than methanolic extract. The antioxidant property was evaluated by free radical scavenging activity by DPPH assay with IC_{50} value of 19.54. Thus, the medicinal potential of plant by virtue of its antioxidant and antimicrobial activity could be harnessed for drug formulation.

Keywords: Medicinal plants; Antimicrobial activity; Antioxidant activity; DPPH assay; Disc diffusion.

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INTRODUCTION

Medicinal plants are used traditionally to prevent and cure diseases all over the world (Nair *et al.*, 2005; Verma *et al.*, 2016a). Herbal medicines play an important role in rural areas and various locally produced drugs are still used as household remedies for different ailments (Qureshi and Ghufraan, 2005; Kumar *et al.*, 2016; Abdalla and Abdallah, 2016; Verma *et al.*, 2016b). Plant derived medicines are widely used because they are relatively safer than the synthetic alternatives, as they are easily available and cheaper (Iwu *et al.*, 1999). Approximately 500 species of medicinal plants and 150 species of aromatic plants have been reported from the Himachal Pradesh, which represents quite a high percentage out of 3500 recorded plant species (Chauhan, 1999). The plant-based, traditional medicine systems continue to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al.*, 2007).

Plants of the genus *Hibiscus* belongs to family Malvaceae is widely cultivated in the tropical, subtropical and sub-temperate regions as an ornamental plant. Chinese *Hibiscus* is the english name of *Hibiscus rosa-sinensis*. It is an evergreen woody glorious showy shrub of 1.5-2.4m in height. Flowers are axillary,

solitary, campanulate, red coloured, capsules are rotund and many seeded (Upadhyay *et al.*, 2011).

Studies have shown that the plants of the *Hibiscus* genus have the potential to provide biologically active compounds which act as anti-oxidants and cardio protective agent. Hence, *Hibiscus* genus may be a great natural source for the development of new drugs and may provide a cost-effective mean for the treatment of cancer and other diseases in the developing world (Maganha *et al.*, 2009). Sachdewa *et al.* (2001) reported anti-diabetic activity of plant in rural populations and in hyperglycaemic rats. The wound-healing activity of the ethanolic flower extract of *H. rosa-sinensis* was determined in rats, using excision, incision and dead space wound models (Shivananda *et al.*, 2007). Plant extract exerts a protective effect against tumour promotion stage of cancer development (Sharma and Sultana, 2004) and hypoglycemic activity (Sachdewa and Khemani, 1999). *Hibiscus* anthocyanins have shown antioxidant activity in protecting against hepatotoxicity in rats which still needs to be investigated in humans. Presence of various biochemicals have been reported in the *Hibiscus rosa-sinensis* plant like flavonoids, flavonoid glycosides, hibiscetin, cyanidine, cyanin glycoside, campesterol, cyclopropenoids, taraxeryl acetate, sitosterol, stigmasterol, ergosterol, citric acid, tartaric and oxalic acids and anthocyanin pigments (Gilani, 2005).

MATERIALS AND METHODS

In the present research work antimicrobial effect of the ethanolic and methanolic flower extract of *Hibiscus rosa-sinensis* were investigated against pathogenic bacterial strains viz. *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella* sp. The flowers of the plant were also assessed for its antioxidant potential for ensuring its medicinal potential. *Hibiscus rosa-sinensis* flowers used in the present study were collected from Solan (Arki), Himachal Pradesh, India. Solvents used were of analytical grade. Microbial cultures were procured from School of Bioengineering and Food Technology, Shoolini University, Solan Himachal Pradesh, India. DPPH and Mueller Hinton Agar was purchased from International Scientific and Surgicals, Solan, India.

A. Preparation of plant extracts

The flowers of plants were collected for extract preparation which were then washed with water to remove soil and dust particles. The plant sample was identified in the School of Biological and Environmental Sciences in Shoolini University. Then they were dried thoroughly in shaded place and grounded to fine powder form and stored in airtight containers at room temperature. Methanol and ethanol extracts were prepared by soaking 10 g of the dry powdered petals in 50ml of methanol and ethanol respectively. The flasks in which extracts were made were kept on orbital shaker at 40°C for 48 hours. The extracts were filtered through Whatmann filter paper and concentrated using a water bath set at 40°C. Plant extracts once obtained were preserved at 4°C till further use.

B. Antimicrobial activity

The antimicrobial activity was determined by disc diffusion method (Bauer *et al.*, 1966). Sterilized Mueller Hinton agar was poured into sterilized Petriplates. After solidification, 100µl bacterial inoculums adjusted to an OD of 0.8 were swabbed on the respective plates. Whatman No.1 filter paper was punched into 5mm disc and sterilized. Each sterile disc was incorporated individually with different concentration of plant extracts using micropipette. Various concentrations (80µg/disc, 120µg/disc, 160µg/disc and 200µg/disc) of plant extracts were added to the sterile discs along with positive (ampicillin) and negative control separately. The plates were incubated for overnight at 37°C. After incubation the diameter of inhibitory zones formed around each disc were measured in mm and recorded.

C. Antioxidant activity

The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical (Brand-Williams *et al.*, 1995).

DPPH (0.002%) was freshly prepared in methanol and kept in the dark for 30 minutes. One ml of four different concentrations of the plant extracts (25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml) were taken and added with one ml of DPPH prepared in methanol. Absorbance was measured spectrophotometrically at 517 nm. Methanol was used to set the absorbance zero and DPPH solution was used as reference. All determinations were performed in triplicate. The radical scavenging activities of the samples, expressed as percentage of inhibition were calculated according to the following equation.

$$\text{Percent (\%)} \text{ inhibition of DPPH} = [(A - B) / A] \times 100$$

Where A is the absorption of the blank sample and B is the absorption of the plant extract. Further IC₅₀ value was calculated for effective inhibitory concentration of DPPH scavenging effect by interpolating graph and analysis.

RESULTS AND DISCUSSION

Antimicrobial activities of ethanolic and methanolic extract of plant were observed against various clinical and standard isolates of bacterial sp. *Hibiscus* showed inhibitory effect against most of tested pathogenic bacteria. The results of antimicrobial activity by methanolic plant extract are mentioned in Fig 1 which shows maximum activity against standard strain of *Pseudomonas aeruginosa* (11.4±1.7) at concentration of 200µg/disc. Minimum inhibition zone was observed against clinical strain of *Staphylococcus aureus* (9.3±2.9) at 200µg/disc. Based on the available reports, ethanol extracts have been shown to result in high extraction yields with strong antibacterial activities. Ethanolic extract results of antimicrobial activity of *Hibiscus rosa-sinensis* showed maximum activity against standard isolate of *Pseudomonas aeruginosa* (13.6±2.1) at 200µg/disc and minimum activity observed against standard isolate of *Salmonella* sp. (11.3±0.6) at same concentration. Available reports have shown that crude plant extracts exhibit higher antibacterial activities against Gram-positive bacteria than Gram-negative bacteria (Kabuki *et al.*, 2000; Tian *et al.*, 2009). According to Uddin *et al.* (2010) *Staphylococcus aureus*, a Gram-positive bacterium is most sensitive to the extracts of flowers at applied doses of 50ml and 100ml in well diffusion method. Present study revealed that ethanolic extract showed greater antimicrobial activity than methanolic extract. The lack of activity may be due to degradation of active chemicals during drying and extraction process (Ezeani *et al.*, 2011). Biologically active compounds from plants are assumed to be more acceptable and non-hazardous than synthetic compounds and represent a rich source of potential disease-control agents (Bhattarai and Jha, 2016).

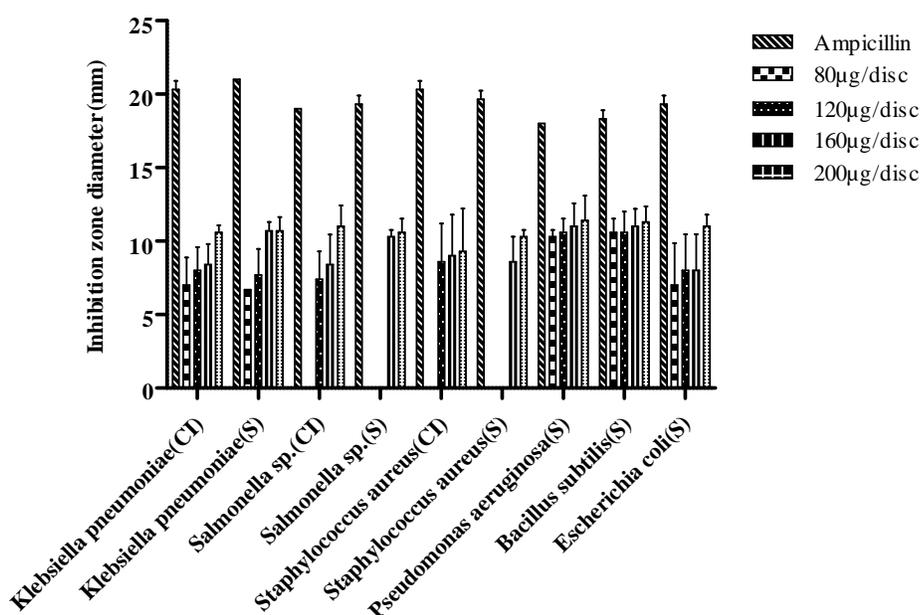


Fig. 1. Antimicrobial inhibition by methanolic flower extract of *Hibiscus rosa-sinensis* against bacterial pathogens. Where values are calculated as mean±standard deviation, (n=3).

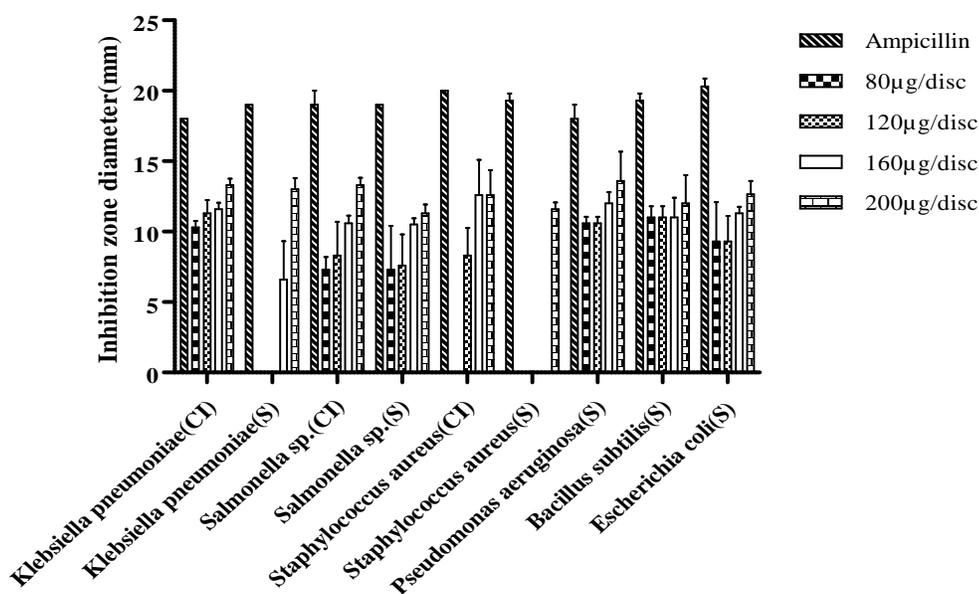


Fig. 2. Inhibition zone diameter of ethanolic extract of *Hibiscus rosa-sinensis* flowers against bacterial pathogen. Where values are calculated as mean±standard deviation, (n=3).

Antioxidant values are shown in Fig. 3 with 19.54 as its IC₅₀ value. The DPPH radical scavenging activity of *Hibiscus rosa-sinensis* flowers was observed using different concentrations of the methanolic extract. It is clear from the table that the % inhibition of the methanol extract was highest at 100µg/ml concentration, followed by 75µg/ml and 50µg/ml

concentration and lowest with 25µg/ml concentration. The % inhibition of the methanol extract increased as the concentration increased. In the present study, radical scavenging activity of methanolic extract of *Hibiscus rosa-sinensis* varied from 51.05% to 57.12% with the increasing plant extract concentration.

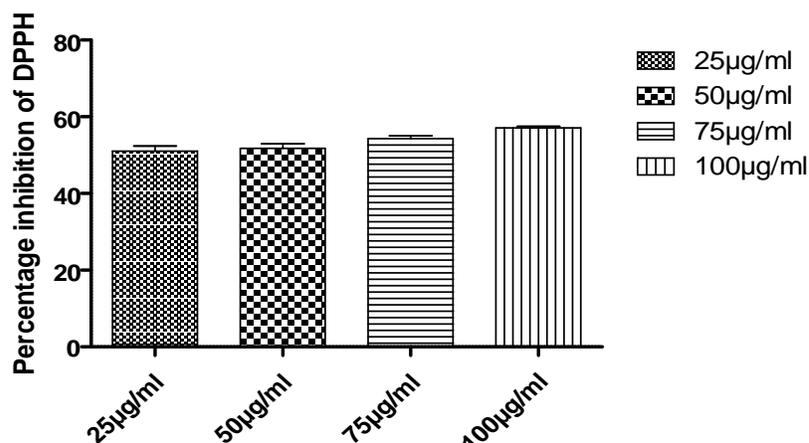


Fig. 3. DPPH radical scavenging activity of *Hibiscus rosa-sinensis* L. flower extract. Where values are calculated as mean±standard deviation (n=3).

According to Wong *et al.* (2009) antioxidant properties of leaves of highland populations of *H. rosa-sinensis* were greater than those of lowland. Considerable scavenging potential of methanol extract showed promising antioxidant behaviour in flower extract of *Hibiscus rosa-sinensis*. The IC_{50} value of methanolic extract of *Hibiscus rosa-sinensis* 19.54. High IC_{50} value indicates less antioxidant capacity in DPPH assay and vice versa.

CONCLUSION

Present study on profiling of antimicrobial and antioxidant properties of *Hibiscus rosa-sinensis* from Solan, Himachal Pradesh has confirmed medicinal potential of plant. The secondary metabolites of the plant can help in virtue of modern medicines. The flower extract of *Hibiscus rosa-sinensis* has wide range of biological activities such as antibacterial, anti-cancerous, antifungal, antiviral and anti-inflammatory. Results of antimicrobial activity against bacterial strains showed maximum activity against standard strain of *Pseudomonas aeruginosa* (13.6 ± 2.07 mm) in ethanolic extract at concentration of $200 \mu\text{g}/\text{disc}$.

In present study comparison of both methanol and ethanol extract shows wide range of antimicrobial activity against different bacterial strains and suggests that ethanolic extract shows more inhibition than methanolic extract at $200 \mu\text{g}/\text{disc}$. The results of the DPPH assay suggest that *Hibiscus rosa-sinensis* have antioxidant potential or free radical scavenging activity. The antioxidant potential harnessed by the consumption of food made by medicinal plant parts are of immense benefit as it provides health benefit and protect from

diseases by virtue of their medicinal potential. Further, most of the biological damage may be due to lipid peroxidation process which triggers certain chain reactions via free radical mechanism. Inhibition of lipid peroxidation is involved in protecting the body from lethal diseases by plants (Khan *et al.*, 2014) due to their antioxidant property. Thus, the greater antioxidant and antimicrobial activity in plant, the more will be its therapeutic benefit to individuals.

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