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Antioxidant And Antimicrobial Activities Of *Hibiscus Tiliaceus*

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ABSTRACT

The antioxidant activity of aqueous flower extract of *Hibiscus tiliaceus* (Malvaceae) was accessed on the basis of the radical scavenging effect of the stable DPPH free radical inhibition assay. The extract showed significant activity at a concentration of 250 μg/ml. Antibacterial potency of water extract of flower and ethanolic leaves extract of the plant was studied by agar disc diffusion method. Both the extracts showed good amount of antimicrobial activity.

INTRODUCTION

Hibiscus tiliaceus (Malvaceae) is also known as traditionally, it is used as febrifuge, laxative, resolvent, emollient. The fruit juice is rubbed on skin to cure weakness and flowers, boiled in milk, are used in the treatment of earache[1-3].

The plant of *Hibiscus tiliaceus* (Malvaceae) was collected locally from the University campus and identified by Dr. B.D.Vashisht, Botanist, Department of Botany, Kurukshetra University, Kurukshetra, Haryana.

Antioxidant assay

The free-radical scavenging activity of *Hibiscus tiliaceus* flower extracts was measured by decrease in the absorbance of methanol solution of DPPH. It is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. It involves reaction of specific antioxidant with a stable free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH*). As a result, there is reduction of DPPH concentration by antioxidant, which decreases the optical absorbance of DPPH. A stock solution of DPPH (33 mg in 1L) was prepared in methanol, which gave initial absorbance of 0.576, and 5ml of this stock solution was added to 1ml of *Hibiscus tiliaceus* flower extract solution at different concentrations (250-2500 μg/ml), shaken well and mixture was incubated at 37 ^{0}C for 30 min. Then absorbance

was measured at 517 nm. Antiradical activity was calculated as % inhibition from the given formula[8]:

$$\%\ \text{Anti}-\text{radical activity} = \frac{\text{Control Abs} - \text{Sample Abs}}{\text{Control Abs}} \times 100$$

Preparation of extracts

The flowers were macerated with water and leaves were successively extracted with ethanol (95%) in a soxhlet extractor. The extracts were concentrated to dryness *in vacuo*.

Screening of antioxidant activity

5 mg/ml stock solution was prepared by dissolving the extract in distilled water and 250, 500, 1000, 1500, 2000, 2500 µg/ml solutions were prepared, the filtrates were used for the experiment. Ascorbic acid was taken as standard and a stock solution (100 µg/ml) was prepared by dissolving it in distilled water. From this 5, 10, 15, 20, 25, & 30 µg/ml ascorbic acid solutions were prepared.

ANTIBACTERIAL ACTIVITY

The antimicrobial activity of the extracts was evaluated by agar disc diffusion method[4-7]. Both the extracts were screened for their antimicrobial using *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 121), *Pseudomonas aeruginosa* (MTCC 741), *Escherichia coli* (MTCC 51). The cultures of microorganisms were procured from MTCC, Chandigarh.

The bacterial cultures were maintained on nutrient agar (Peptone 5.0 g, Beef extract 3.0 g, Sodium Chloride 5.0 g, Agar 15.0 g and Distilled water 1000 ml) by subculturing them on fresh slants after every four weeks and incubating them at respective mentioned temperature for 24 hrs. All stock cultures were stored at refrigeration at 4^0C. For the evaluation of antimicrobial activity, 24 hours fresh culture of bacteria were suspended in sterile distilled water to obtain a uniform suspension of microorganism (about 10^6 Cfu /ml). The working concentration of sample used for both extracts was 1 mg/100 µl.

Nutrient agar plates were swabbed with the broth culture of the microorganisms (diluted to 0.5 McFarland Standard) and kept for 15 min for absorption to take place. Wells of 8 mm diameter were punched into the agar medium and filled with 100 µl each of the extracts. DMSO was taken as solvent blank. The inoculated plates were incubated at 37 ^{0}C for 24 hrs. All the tests were made in triplicate and mean of the diameter of inhibition zone was calculated. The antimicrobial activity of the extracts was compared with chloramphenicol (30 µg/100 µl) and ampicillin (10 µg/100 µl).

RESULTS AND DISCUSSION

The percentage of DPPH scavenging activity of flower extract was found to be 86.28 % at concentration 250 µg/ml with reference to 94.62 % at 30 µg/ml of L-ascorbic acid.

The study also reveals that aqueous extract of flowers exhibited significant activity against all the tested bacterial organisms at concentration of 1 mg/100 µl. Ethanolic extract of leaves showed comparatively low activity against *S.aureus*, *B.subtilis* and *E.coli*.

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