Phytology

Antioxidant and antibacterial activity of leaves of *Hibiscus rosa-sinensis*

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Abstract

Hibiscus rosa-sinensis has been bestowed with multifaceted chemical constituents which would be used for variety of human ailments, the plant has long history of uses to cure and treat various condition the leaves of plant are deep green in color would trove important antioxidant and antibacterial compounds. The ethanolic extract of leaves has been proven to be good antioxidant potential with IC_{50} of 18.70 compared to standard IC_{50} of 6.35. The antibacterial potential has been tested with gram positive *S. aureus* and gram-negative *E. coli.* The ethanolic extract of leaf showed higher potential of antibacterial action at 2% concentration as compared to 1%, the study also revealed that the extract of leaf had greater antibacterial against gram negative *E. coli.*

Keywords: hibiscus, antioxidant, antibacterial, antiaging

Introduction

Hibiscus rosa sinensis vernacularly known as China rose or red Hibiscus is an average to large sized shrub. The plant supposed to native of Southeast Asia, belonging to mallow family Malvaceae. The plant is cultivated since long for their prominent beautiful flowers and medicinal attributes. Hibiscus flowers and leaves had been used in variety of condition like menorrhagia, emmenagogue, abortion, antifertility, contraceptive, demulcent, Cough. The leaves of the *Hibiscus* are ovate and alternatively arranged on the branches. The leaves would be intense green with serrate or toothed margins. The red flowered species would be preferred over others for therapeutic purpose.[40] the major constituents in the leaves are alkaloids, tannins, flavonoids, steroids, saponins, phenols, proanthocyanidin. The plant extract would be used in various skin disorders and protect the cells from damaging effect of oxidative stress and UV radiation. Due to presence of potential active metabolites, antioxidant and antibacterial activity of hydroalcoholic extract of leaves of *Hibiscus rosa sinensis* has been chosen for the study. Antioxidants are compounds which primarily neutralize the free radical in our body and protect the cell and tissue from invidious effects of oxidative stress. [1,2]

Materials and methods

Analytical grade chemicals and reagents had been used for the purpose of study all the chemicals were procured from Central Drug House (P) LTD. New Delhi, the glassware used in the study was borosilicate and ASGI mark. Pharmaspec Shimadzu UV-VIS Spectrophotometer model UV-1700, Japan has been used.

Collection and processing of plant material

The leaves of *Hibiscus rosa sinensis* has been collected from the herbal garden at BCP, Bhopal M.P. India, in the month of august. Plant sample were authenticated by Dr. S. Mishra, Scientist, MFP-PARK Laboratory, Bhopal, MP, India. The collected plant materials washed with tap water to remove debris and dirt. The plant was kept in shade until complete dried, the shade dried plant sample then grounded using electric grinder to get coarse powder, and the coarse powder was subjected to extraction with hydroalcoholic solvent.

Extraction of plant material

The hydro alcoholic extract has been prepared by soaking the powdered sample in 80% ethanol. 250g of coarsely powdered plant sample was macerated with 80% ethanol for seven consecutive days in closed flask with periodically stirring. The extract was collected and filtered using Whatman filter paper, the filtrate was evaporated and concentrated to remove excess solvent under reduced pressure at 35°C in rotary evaporator. The concentrated extract was then placed in the desiccators to eradicate residual solvent.

In-vitro anti-oxidant Activity

Antioxidant potential of *Hibiscus rosa sinensis* leaves extract had been evaluated by DPPH radical scavenging method. The comparison of sample data was made with ascorbic acid as standard antioxidant compound. 0.1mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was prepared in methanol [3].

Preparation of sample/standard

One mg of ascorbic acid and *Hibiscus rosa sinensis* dried powdered extract were dissolved in 1ml of methanol to get 1mg/ml standard and sample stock solution. Various dilution was made to get the desirable concentration of 20,40,60,80,100

µl/ml for both standard and sample in methanol. 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly to each test tubes of sample and standard. The mixture is then incubated for 30 minutes in dark condition away from light then absorbance of standard and sample were recorded at the wavelength 517 nm [4, 5].

Preparation of control

Three milliliters of 0.1mM DPPH solution was prepared. The solution was incubated for 30 minutes at room temperature in dark condition. Absorbance of the control solution has been recorded against methanol as blank at 517 nm. The antioxidant activity of sample/ standard was reckoned by using formula [6, 7].

Percentage Inhibition = [(Abs of control- Abs of sample/ Abs of control x 100]

Antibacterial activity

Antimicrobial efficiency of the sample extract has been tested through well diffusion assay against gram negative bacteria *E. coli* MTCC 42 and gram-positive bacteria *S. aureus* MTCC 10787.The nutrient culture media was prepared by incorporation of twenty eight gram nutrient agar in one liter of distilled water. pH of media was checked after formulation the same has been recorded for future reference. The media was autoclaved at 121ºC at 15 lbs pressure for 15 minutes, further sterilized media was allowed to cooled and poured into plates before it gets solidified all operation had been done in laminar flow [8-10].

Well diffusion assay

The sample for the test has been prepared by mixing 1% and 2% of test extract distinctly with distilled water. The culture of bacterial strain was spread on prepared media. Standard solution for comparison with test has been prepared by dissolving one milligram of ofloxacin and gentamycin in 1ml of distilled water to get 1mg/1ml of standard solution. The inoculum of *E. coli* MTCC42 and *S. aureus* MTCC 10787 were prepared; primarily test organisms were inoculated in 10 mL of nutrient broth. The bacterial suspension was optimized to get 10^8 CFU/ml. 100 µl of the inoculum was taken and transferred in to pristine and sterile solidified agar media. Three wells of 6 mm had been made by sterile cork-borer. The initial two wells were filled with test sample with concentration of 1% and 2% further, third well were filled with 50µl of standard drug. The sample and standard were kept in sterile condition and allowed to diffuse for 30 minutes at room temperature further all samples were incubated for 24 hours at 37ºC. The incubated plates were investigated for effect of test sample and standard. The clearing zone observed around the well was the harbinger of antimicrobial efficiency of tested compounds. The zone of inhibition was observed and measured in mm using ruler on the back of the inverted petri plate [11-14].

Table 1: DPPH radical scavenging activity of ascorbic acid

Concentration (µg/ml)	Percentage inhibition
20	57.928
40	66.067
60	74.735
80	82.241
100	88.583
Control	0
IC50	6.35

Table 2: DPPH radical scavenging activity of *Hibiscus rosa sinensis*

Concentration (µg/ml)	Percentage inhibition
20	51.0570
40	62.790
60	68.498
80	77.589
100	79.809
control	0
IC50	18.70

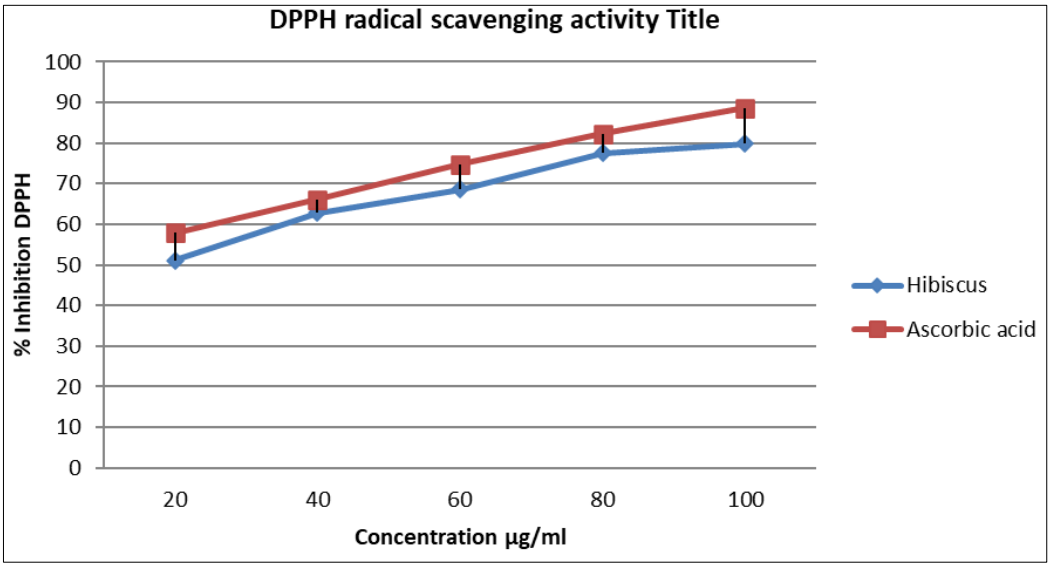

Fig 1: Graph represents the percentage inhibition *vs* concentration of sample extracts

Table 3: Antimicrobial activity of extract against *S. aureus*

Extract	Plate 1	Plate 2	Plate 3	Mean ± SD
1%	5 mm	6 mm	6mm	5.66±0.533
2%	9 mm	10 mm	10 mm	9.66±0.577
Control	0mm	0mm	0mm	0±00
Ofloxacin (1mg/ml)	22 mm	20 mm	25 mm	22.33±3.056

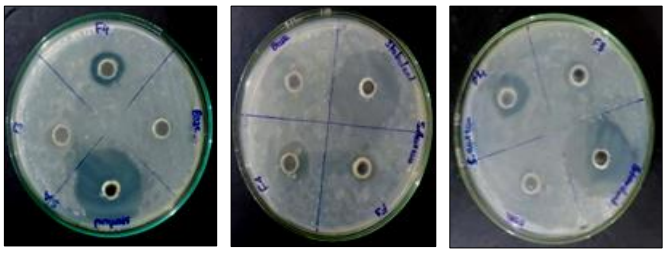

Fig 2: Antimicrobial activity of extract against *S. aureus*

Table 4: Antimicrobial activity of extract against *E. coli*

Extract	Plate 1	Plate 2	Plate 3	Mean±SD
1%	11 mm	10 mm	8 mm	9.66±1.231
2%	14 mm	13 mm	14 mm	13.33±0.577
Control	0mm	0mm	0mm	0±00
Gentamycin (1mg/ml)	25 mm	26 mm	24 mm	25±1.487

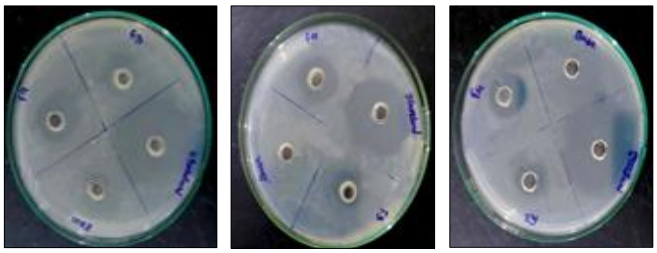

Fig 3: Antimicrobial activity of extract against *E. coli*

Result and discussion

The DPPH radical scavenging potential of standard and extract has been compared to access the antioxidant potential of ethanolic extract the result indicated that the ethanolic extract of leaves had good antioxidant potential with IC_{50} of 18.70 compared to standard IC_{50} of 6.35. The antibacterial efficacy has been tested with gram positive *S. aureus* and gram-negative *E. coli.* bacteria. The ethanolic extract of leaf showed higher potential of antibacterial against *S. aureus* at 2% concentration with the inhibition of 9.66±0.577 as compared to 1% with the inhibition of 5.66±0.533. the antibacterial potency of leaves extract against *E. coli.* has been found higher for 2% concentration with the zone of inhibition of 13.33±0.577 as compared to 1% with inhibition of 9.66±1.231, while compared both strain it was found that the extract had greater antibacterial against gram negative *E. coli.* The results supported that the plant had good antioxidant and antibacterial action that could be used for similar action in formulation.

Conclusion

Increasing demand of herbal products inclined to explore the new potential substance from natural origin that could give better and safer alternative and efficiently used in various formulation. In this study we have tested the efficiency and potential of newer effects of *Hibiscus rosa sinensis* leaves extracts that might be useful to explore the plant in the newer area of herbal formulation. The results indicated that the *Hibiscus rosa sinensis* extracts had positive result on the parameter tested and could be used as anti-ageing, antibacterial and antioxidant potential. Further study needed to refine the extract by using isolated components and some more pharmacological evaluation needed that could broader the effect of drug.

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