

Pharmacological studies of *Hibiscus rosa sinensis* leaf extract

Abstract-

We have undertaken to study the pharmacological activities of the hydro-methanolic leaves extract of *Hibiscus rosa sinensis* using standard screening methods such as disc diffusion and DPPH methods. In phytochemical screening, Secondary metabolites such as phenols, saponins, and tannins were present in *Hibiscus rosa sinensis* leaf extract. The dose dependent antibacterial and antioxidant activities was also observed. The present study is important because *Hibiscus Rosa sinensis* plant is important in traditional medicine to cure various ailments.

Key Words :Antibacterial , Phytochemical, *Hibiscus rosa sinensis*, Antioxidant

INTRODUCTION

Hibiscus rosa sinensis Linn. (Family Malvaceae) is a plant which is widely distributed throughout the world. It is used in the Indian traditional system as medicine to treat various diseases. *Hibiscus Rosa sinensis* extract is a source of many potentially active constituents such as quercetin, glycosides, riboflavin, niacin, carotene, malvalic acid gentisic acid, margaric acid and lauric acid". [1- 6]]. "*Hibiscus* flowers and leaves are used in India for the abortion, antifertility, contraceptive, Diuretic, Menorrhagia, Bronchitis, Emmenagogue, emulcent, and Cough" [7]. Methanol and ethanol extracts of the plant have been reported for antioxidant activity" [8 - 14]. "Ethanol extract of *Hibiscus rosa sinensis* at different doses was reported as hypoglycemic and hypolipidemic activity against streptozotocin induced diabetic rats [15 - 16], Hepatoprotective effect of flowers aqueous extract in male Wistar Albino rats. protective effect in tumour promotion stage of cancer development . "Cardioprotective effects of flowers extract and immunomodulatory activity of flowers extract in Wister albino rats were reported" [17 -23]. "Oral cancer cell lines KB (ATCC CCL-17) were reported with 75 µg and 125 µg of H. rosa sinensis oil extract for 24 hours. After subjecting the treated cells to DNA fragmentation assay and using agarose gel electrophoresis and was observed that the cell's DNA from both concentrations has been fragmented compared to control sample. This means that Hibiscus extract hindered the growth and proliferation of oral cancer cells" [24]. "It was also reported that 250 µg of 90% methanolic leaves extract inhibited HT-29 colorectal AGS cell lines by 100%. The cell viability percentage was measured using MTT assay and the calculated IC50 was found to be 90 µg/ml. Acetone extracts of H. Rosa sinensis flowers effect on HeLa cell lines viability was investigated. Using MTT assay, it was found that at a concentration of 1000 µg/ml resulted in only 12.96% cell viability. The presence of flavonoids, tannins and saponins were detected by FT-IR spectra and qualitative screening tests are suspected for

this activity” [26]. “Using albino rabbits as study models, flower ethanol extracts managed to lower blood glucose levels in a progressive manner in 72 hour period using all graded doses 50, 100, and 200 mg/kg. Glibenclamide (200 mg/kg p.o), which was used as positive control, caused a sharp decrease in blood sugar levels in 24 hour period, which is not desirable in clinical situations” [28].

MATERIALS AND METHODS

Extraction Process:-

Procedure:

The *Hibiscus rosa sinensis* leaves were collected from the local garden of Satna, Madhya Pradesh in the month of April 2023 and was identified by competent Botanist Dr. Manoj Tripathi of DRI, Chitrakoot. The leaves were cleaned and dried for few days in shade. Then powder was made with the help of grinder. 50 gms of leaves powder was taken in a separating funnel and added 50% methanol, then mixed it gently. After every 24 hours extract was collected in a beaker till the solvent appeared colorless. The final extract was pooled together and dried into powder at 40° C using water bath. The total weight of dried powder was weighed. On the day of experimentation, the desired amount of powder was suspended in double distilled water for the final administration. Phytochemical screening was done as per method reported by Agrawal, 2021 (27).

RESULTS and DISCUSSION

1.2 Antibacterial Activity:-

The test organisms *E. Coli* and *Bacillus subtilis* were obtained from the Department of Research, PBCRI Satna (M.P.). Antibacterial screening was done to find out the antibacterial properties of different concentration of 50% methanolic extract of *Hibiscus rosa sinensis* leaves under study. Kirby-Bauer Method (Disc diffusion method) was followed to test the antibacterial activity of different concentration of leaves extract. Nutrient agar broth media were used for the antibacterial activities. Nutrient Agar media was prepared and poured in Petri-plates after solidifying. Swab of the bacterial cultures sterile nutrient agar plates were inoculated with the test culture by surface spreading using sterile wire loops and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. 3 different concentrations of crude extract were prepared (100%, 50%, 25%) and were used for antibacterial analysis using

agar disk diffusion methods. Paper disks were made in each of the plate with a sterile 2.0 mm diameter .Each of the four disk was soaked in a given concentration of the extract mixed with plane sterile agar. The plates were then incubated at 37°C for 24 hours. The diameters of zones of inhibition were measured using a scale and the mean value for each organism was recorded.

Antioxidant assay. The radical scavenging activity of *Hibiscus rosa sinensis leaves* extracts against the DPPH radical was determined by the method of Brand Williams with slightly modified by Dudoné et al. (17). Determination procedures were as follow: 1 ml of 6×10^{-5} M DPPH radical solution (prepared daily) was mixed with 33.33 µL of methanolic solutions of *Hibiscus rosa sinensis* leaves extracts (maximum dissolved concentration). After 30 min incubation for at 37 °C and the absorbance was monitored at 515 nm. During reduction by the antioxidant, the solution colour changed from violet to yellow pale. DPPH radicals have an absorption maximum at 515 nm. Blank samples with 33.33 µL of methanol in the above DPPH radical solution were prepared and measured daily at same wavelength (*Ab*). The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula.

$$\text{Inhibition rate \%} = \frac{A_b - A_s}{A_b} \times 100$$

The 50% inhibitory concentration (IC₅₀) was expressed as the quantity of the extracts to react with a half of DPPH radicals.

1. Phytochemical screening

Table 1- Phytochemical present in *Hibiscus rosa sinensis* extract

S.No.	Phytochemical Test	Hydromethanolic Extract
I	Phenolic compound's	+
II	Tannins	+
III	Saponins	++
IV	Flavanoid	-

+ Present, - Absent

2. Antibacterial Activities

The different concentration i.e. 50%, 75%, 100% 50% of methanolic extract of leaves of *Hibiscus rosa sinensis* exhibited antibacterial activity in *Bacillus subtilis* (11-13 mm), and *E. coli* (12-14 mm)

TABLE 2. Antibacterial activity of *Hibiscus rosa sinensis* in different bacterial strains

Name of microorganisms	zone of inhibition (mm)]	% Concentration of Extract		
		50	75	100
<i>B.subtilis</i>	zone of inhibition (mm)]	11 ±1	12 ±2	13±2
<i>E. Coli</i>	zone of inhibition (mm)]	12 ±1	13 ±1	14 ±2

Antioxidant Activity

Table 3. In vitro antioxidant activity of *H. rosa sinensis* extracts Vs Ascorbic acid (standard)

Sr. No.	Concentration of ascorbic acid (µg)	% TBARS inhibition	Concentration of <i>H.rosa sinensis</i> (µg)	% TBARS inhibition
1	10	64.88	10	119.6
2	20	104.16	20	122.6
3	30	291.66	30	134.82
4	40	411.90	40	187.20

5	50	521.42	50	193.4
6	60	625	60	319.34
7	70	632.44	70	341.66
8	80	686.90	80	391.07
9	90	1421.42	90	429.76
10	100	1567.85	100	482.44

Indian plant possesses many therapeutic properties. Present study showed that the extract of *Hibiscus rosa sinensis* caused antimicrobial activity against gram positive and gram negative bacteria. Antioxidant activity was observed in DPPH assay activity which was also confirmed in other studies [14]. Its effects on antioxidant compounds may be correlated with the presence of Phenol compounds in *Hibiscus rosa sinensis* plant [8 - 13]. It is also suggested that this significant anticancer activity and antimicrobial was mostly due to flavonoids and terpenoids content in the leaves [25].

Conclusion

The study showed that the extract of *Hibiscus rosa sinensis* caused antimicrobial activity against gram positive and gram negative bacteria. The study is important for scientific community. Because this plant is used in traditional medicine to treat various diseases.

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