Antibacterial ,antioxidant and Phytochemical studies of *Hibiscus rosa sinesis leaf*extract

Abstract-

The present study was undertaken to explore the phytochemical screening,antioxidant and anti-bacterial activities of the hydro-methanolic leaves extract of *Hibiscus rosa sinesis*using standard screening methods such as disc diffusion and DPPH methods. In phytochemical screening, *Hibiscus rosa sinesis leafextract* showed presence of secondary metabolites such as Carbohydrate,phenols, saponins, and tannins. It also showed dose dependent antibacterialand antioxidant activities.

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

INTRODUCTION

The natural plant products are widely used nowadays because of increasing the burden of diseases. Hibiscus rosa sinensis Linn. (Family Malvaceae) is a plant which is widely distributed throughout the world. Its leaves, barks, roots and flowers have been used in the Indian traditional system as medicine to treat various diseases. Hibiscus rosa sinensis has been used in many herbal mix and drinks. *Hibiscus rosa sinesis*extract is a source of many potentially active antioxidants and anticancer 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, Emmengogue, Demulcent, and Cough[7]. Methanol and ethanol extracts of Hibiscus have been reported for antioxidant activity [8 - 13]. Anticancer and antioxidant activities of 90% methanolic leaves extract was reported in MTT reduction assay, DPPH assay, FRAP assay and SOD activity [14] Ethanolic extract of *Hibiscus rosa sinesis at different doses* was reported as Antioxidant, hypoglycemic and hypolipidemic activity against streptozotocin induced diabetic rats [15 - 16] Medicinal properties of flower aqueous extract of 500 mg/kg was reported for Reversible suppression of spermatogenesis, cholesterol level and glucose level in adult Swiss albino mice ,antioxidant and antigenotoxic effectsof flowerextract in

Swiss albino mice , 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].

MATERIALS AND METHODS

Extraction Process:-

Procedure:

The *Hibiscus rosa sinensis* leaves were collected from the local area of Satna,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 60^{o} 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,RC[24]

1.2 Antibacterial Activity:-

The test organisms *E.Coli*.and Bacillus subtiliswere 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 sinesis*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 prepared and poured in Petri- plates after solidifying swab of the bacterial cultures on the plates and allowed for incubation at 37^{0}C for 24 hrs.Measurement of Zone of Inhibition (In mm).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 inoculums.3 different concentrations of crudeextract 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 meter rule and the mean value for each organism was recorded.

DPPH radicalscavenging assay. The radicalscavenging activity of *Hibiscus rosa sinesisleaves*extracts against the DPPH radical was determined by the method of BrandWilliams with slightly modified byDudonńe et al. (17).. Determination procedures were as follow: 1 ml of6 × 10−5 M DPPH radical solution (prepared daily) was mixed with 33.33 µL of methanolic solutions of *Hibiscus rosa sinesis*leavesextracts (maximum dissolved concentration). After 30 min incubation for at 37 °C, absorbance decrease of the mixture 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 radicalsolution were prepared and measured daily at same wavelength (*Ab*). The experiment was carried out in triplicate. Radical scavenging activity was calculated using the followingformula.

Inhibition rate %= Ab -As ×100 Ab

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

RESULTS

1. **Phytochemical screening**

Table 1- Phytochemical present in the hydromethanolic extract of *Hibiscus rosa sinensis* extract

S.No.	Phytochemical Test	Hydromethanolic Extract
I.	Test for Carbohydrates and reducing sugars	
A	Fehling's Test	+

II	Test for Phenolic compound's	
A	Ferric Chloride test	+
III	Test for Tannins	+
V	Test for Proteins	-
VI	Test for Saponins	++
VII	Test for Flavanoid	-

+ Present, - Absent

2. Antibacterial Activitys

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

TABLES 2. Antibacterial activity of*Hibiscus rosa sinensis* against bacterial strains

Name of microorganisms	**% Concentration of Extract [zone of inhibition(mm)]**		
	50	**75**	**100**
B.subtilis	11	12	13
E. Coli	12	13	14

Antioxidant Activity

Table 3. In vitro antioxidant activity of 50% methanolic*H. Rosa sinesis*extracts Vs Ascorbic acid (standard)

Sr. No.	Concentration of ascorbic	% TBARS inhibition	Concentration of *h.Rosasinesis*(µg)	% TBARS inhibition

	acid (µg)			
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

DISCUSSION

Indianplant possesses manytherapeutic properties. Recent scientific research has established the presence of many active compounds in this plant that are known to possess specific pharmacological properties. Present study showed that hydromethanolic extract of Hibiscus rosa sinesisleaves caused antimicrobial activity against gram positive and gram negative bacteria . Methanol and ethanol extracts have been reported for antioxidant activity [8 - 13]. Anticancer and antioxidant activities 0f 90% methanolic leaves extract was reported in MTT reduction assay, DPPH assay, FRAP assay and SOD activity [14] Phenolic compounds which naturally present in Hibiscus rosa sinesisplant can reduce the risk of many diseases and its effects which correlated with the antioxidant compounds. Methanol and ethanol extracts have been reported for antioxidant activity [8 - 13]. Therefore, it is evident that the plant contains active ingredients flavanoid and terpenoid are also known to possess astringent and antimicrobial property.

CONCLUSION

Hibiscus rosa sinensis is widely employed by users of natural health for contraception, controlling uterine bleeding, menorrhagia, venereal diseases, cough, fever, refrigerant and

vitaliser in palpitation. The pharmacological studies reported in the present studies confirm the therapeutic value of *Hibiscus rosa sinensis* is important

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