***In-vitro* ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY OF ETHANOLIC EXTRACT OF *Hibiscus rosa sinensis* LEAVES**

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ABSTRACT

Hibiscus is a genus of a gorgeous flowering potted plant. *Hibisceae* tribe is one of the ornamental plants that are most commonly available and it belonging to Malvaceae family is *Hibiscus rosa-sinensis*. The purpose of this work is to evaluate the in-vitro anti-arthritic effect of *Hibiscus rosa sinensis* at various levels of concentration. Here the percentage of membrane stabilization was carried out at various concentrations for ethanolic extract and Diclofenac sodium. Hibiscus extract was found to have maximum membrane stabilization of 94.97% at 500µg/ml. The highest percentage inhibition of denaturation of protein and membrane stabilization for these plant extracts were found to be 89.45% and 91.09% at a dose of 500µg/ml. Our research studies support the isolation and the use of active phytochemicals of hibiscus in the treatment of inflammation and arthritis.

Keywords: *Hibiscus rosa sinensis*, Anti-inflammatory, Anti-arthritic, Human Red Blood Cell (HRBC), Protein Denaturation.

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INTRODUCTION

Hibiscus rosa-sinensis is known as the rose of China, an evergreen herbaceous plant in certain markets. It is a tropical *Hibiscus* plant, known as, Shoeblack herb or Chinese Hibiscus and is a Chinese native that is now widely cultivated as an ornamental in the tropical and subtropical regions. It possesses dark-red, large, ornamental flower. Hibiscus leaves, roots and flowers are anodyne, emmenagogue and stimulate blood circulation and regulate menstruation.¹ Traditionally, flower extract is used for high blood pressure, liver disorder and as an aphrodisiac. Young flowers and leaves are used for headaches also. Hibiscus tea is rich in vitamin C. The leaves and flowers are used to extract the juice that is used for many years to treat some diseases, painful symptoms and herbal cosmetics. It has been reported that Hibiscus flowers are having antitumor, anti-inflammatory², anti-asthmatic, antipyretic, analgesic properties. Also, antimicrobial, anti-fungal, anti-oxidant properties are present in the flowers of *Hibiscus rosa-sinensis* that are proved by several studies. The photochemical components which are extracted from flowers, leaves, roots and stems from Hibiscus have shown beneficial effects for human health such as anti-oxidant property, which is the prevention of free radicals that can damage deoxyribonucleic acid. In several tropical countries, this plant is showing several important medicinal uses to treat gastric ulcers, hair loss, infections caused by bacteria and fungi, diabetes, fever, cough³, inflammation, and wounds. Studies on toxicity showed according to histological analysis, there were no signs of toxicity at higher doses in extracts from this plant. Photochemical analysis showed that alkaloids, saponins, terpenoids, tannins,

flavonoids which are the main bioactive phytochemicals are responsible for its therapeutic effects.⁴ Various studies have shown that Hibiscus flower and leaf extract has opted for nanoparticles synthesis due to the existence of phenolic hydroxyl groups that serve as natural reducing agents.

EXPERIMENTAL

Collection of Plant Material

The hibiscus leaves were collected from Visakhapatnam city, Andhra Pradesh, India. The leaves were dried under sunlight, powdered and sieved with 150 mesh size.⁵

Preparation of Extract

10gm of dried leaves powder was extracted with ethanol using a soxhlet extractor for 5hr. After this process, the ethanol solvent was distilled at a low-temperature level point of water bath⁶ then finally we get the crude extract.

Preparation of HRBC Suspension

HRBC is a Human Red Blood Cells. In this research, Alsever's sterilized solution is prepared with 0.8% sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), 0.42% sodium chloride (NaCl), 2% dextrose and 0.05% citric acid ($\text{C}_6\text{H}_8\text{O}_7$) in water. Now this sample was combined with an equal volume level of human blood. Then the mixed-blood sample was centrifuged up to 10-15 minutes at 3000 rpm.⁷ The cells were washed with isosaline up to two to three times. The mixed blood sample volume was measured and reformed as suspension (10% v/v) with isosaline.

Heat-Induced Hemolysis

Here hypo tonicity mediated to Human Red Blood cells membrane lysis is the concept involved here. The mixture of assay includes 2ml hyposaline (0.36%), 1ml of the buffer of phosphate (0.15M) at pH 7.4, 0.5ml of 10% v/v HRBC suspension with 0.5ml of crude extract and Diclofenac sodium standard drug of different levels of concentrations (25 to 500 $\mu\text{g/ml}$). Here control is distilled H_2O to produce hemolysis.⁸⁻⁹ Then incubated at 38°C for 30min and finally centrifuged. The suspension content was observed through UV spectrophotometer at 560nm. The Percentage of hemolysis of Human Red Blood cells membrane calculations:

$$\text{Percentage of Hemolysis} = (\text{OD of Test solution} / \text{OD of Control}) \times 100$$

The Percentage of Human Red Blood cells membrane stabilization calculations:

$$\text{Percentage of Protection} = 100 - [(\text{OD of Test solution} / \text{OD of Control}) \times 100]$$

Inhibition of Protein Denaturation

Here Hibiscus extract (test solution) and drug of diclofenac sodium (standard solution) were taken in different concentrations (25 to 500 $\mu\text{g/ml}$). These solutions were maintained at pH 6.3 with the aid of 1N hydrochloric solution.¹⁰ Now these solutions were incubated for 15-25 min at 38°C. After cooling these solutions, just phosphate buffer (2.5ml) is added. The OD was observed at 416nm by using UV-spectrophotometer. Here 100 percentage of protein denaturation is expressed by the control. These experimental results were observed with Standard drugs. The preparations of solutions were reported in Table-1.

Table1: Preparation of Solutions

S. No.	Preparation of Sample
1	Standard solution (0.5ml) = 5%W/V of BSA (0.45ml) + Diclofenac sodium (0.05 ml)
2	Test solution (0.5ml) = 5%W/V of BSA (0.45ml) + Test solution (0.05 ml)
3	Product control (0.5ml) = Distilled water (0.45ml) + Test solution (0.05 ml)
4	Test control (0.5ml) = 5%W/V of BSA (0.45ml) + Distilled water (0.05 ml)

The percentage inhibitions of denaturation of protein of various concentrations were reported in Table-3 and Fig.-3. The percentage inhibition of denaturation of protein calculations:

$$\text{Percentage of Inhibition} = [100 - (\text{OD of Test Solution} - \text{OD of Product Control}) \div (\text{OD of Test Control}) \times 100]$$

Effect on Membrane Stabilization / Inhibition of Membrane lysis

Here hypo tonicity mediated to Human Red Blood cells membrane lysis is the concept involved here.¹¹ The mixture of assay includes 2ml hyposaline (0.36%), 1ml of the buffer of phosphate (0.15M) at pH 7.4, 0.5ml of 10% v/v HRBC suspension with 0.5ml of crude extract and Diclofenac sodium standard drug of different levels of concentrations (25 to 500 µg/ml). Here control is distilled H₂O to produce hemolysis.¹² Then incubated at 38°C for 30 min and finally centrifuged. The suspension content was observed through UVspectrophotometer at 560nm. The percentage of membrane stabilization at various concentrations was reported in Table-2 and shown in Fig.-4. The percentage inhibition of membrane stabilization calculation:

$$\% \text{ Inhibition} = 100 - [(\text{OD of test solution}) \div (\text{OD of control}) \times 100]$$

RESULTS AND DISCUSSION

HRBC membrane lysis was induced by inhibition of hypotonicity i.e, the anti-inflammatory activity was assayed by HRBC membrane stabilization.¹³ The percentage of membrane stabilization for ethanolic extract (crude extract) and standard Diclofenac sodium drug were done at 25, 50, 100, 250, 500µg/ml concentrations. Ethanolic extract of Hibiscus are effective in inhibiting HRBC hemolysis¹⁴ induced by heat at various levels of concentrations (25-500µg/ml) and it showed in Table-2. The highest inhibition level was shown to be 90.75% at 500µg/ml. The hemolysis of membrane is reduced with increasing concentrations, as shown in Fig.-1 and membrane stabilization/support is increased as shown in Fig.-2.

Table-2: Effect of *Hibiscus* Plant Extract and Standard Drug on Membrane Stabilization and Hemolysis of HRBC Membrane

S.No.	Conc.(µg/ml)	% Hemolysis of <i>Hibiscus rosa sinensis</i>	% Stabilization of <i>Hibiscus rosa sinensis</i>	% Hemolysis of Diclofenac Sodium	% Stabilization of Diclofenac Sodium
1	25	31.26	68.52	48.17	54.72
2	50	20.70	78.47	24.56	80.03
3	100	17.09	84.70	18.80	89.57
4	250	11.67	88.64	15.78	94.60
5	500	8.40	90.75	7.91	97.78

Fig.-1: Effect of *Hibiscus* Plant Extract on Hemolysis of HRBC Membrane

Fig.-2: Effect of *Hibiscus* Plant Extract on HRBC Membrane Stabilization

At various concentrations, the ethanolic extract of Hibiscus showed substantial activity and its effect was contrasted with standard drug. 89.45% and 91.09% were found to be the highest percent inhibition of denaturation of protein¹⁵⁻¹⁶ and membrane stabilization at 500µg/ml as shown in Table-3. Auto antigen productions are formed in some arthritic disorders due to denaturation of protein and membrane lysis. From the results (Fig.-3 and 4) of this analysis, that ethanolic extract (Crude extract) can regulate the various autoantigen production¹⁷⁻¹⁸ and arrested to denaturation of protein and membrane lysis in the arthritic disorder.¹⁹⁻²⁰

Table-3: Effect of *Hibiscus* Plant Extract and Standard Drug on Inhibition of Membrane Stabilization and Denaturation of Protein

S. No.	Conc.(µg/ml)	% inhibition of <i>Hibiscus</i> Plant Extract	% inhibition of Diclofenac Sodium	% Stabilization of <i>Hibiscus</i> Plant Extract	% Stabilization of Diclofenac Sodium
1	25	61.64	65.72	66.97	58.08
2	50	69.30	73.68	78.54	76.00
3	100	77.25	88.98	83.50	87.40
4	250	84.90	93.06	89.47	92.67
5	500	89.45	96.81	91.09	97.88

Fig.-3: Effect of *Hibiscus* Plant Extract on Percentage Inhibition of Denaturation of Protein

Fig.-4: Effect of *Hibiscus* Plant Extract on Percentage Membrane Stabilization

CONCLUSION

Here studied to find out the anti-inflammatory activity and anti-arthritis activities of *Hibiscus* species. Inhibition of denaturation of protein and membrane stabilization was studied to the action of anti-arthritis activity of the *Hibiscus* plant. This research in-vitro experiment on hibiscus extracts shows the depression of inflammation. Due to the presence of phytochemicals like phenolic acids, phenolic compounds, alkaloids, tannins, saponins, bioflavonoids and polyphenolic compounds are responsible for these activities. Hence, Hibiscus can be used as an effective anti-inflammatory agent.

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