



***In vitro* Anti-inflammatory Studies of Flavonoids from *Hibiscus rosa-sinensis* Linn**

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ABSTRACT

Flavonoids are a phytochemical substance found in a variety of plants, fruits, vegetables, and leaves that contain significant pharmacological activities such as anti-cancer, antioxidant, anti-inflammatory, anti-viral, and be beneficial in treating several disorders. The aim of the present study is the anti-inflammatory activity of the ethyl acetate fraction obtained from the fresh flowers of *Hibiscus rosa-sinensis* Linn. double yellow flowers with red color at the center was evaluated by *In vitro* hypotonicity induced RBC membrane stabilization of red blood cells. The effect of inflammation condition was studied on erythrocytes exposed to the hypotonic solution. The ethyl acetate fraction was found to show membrane stabilizing activity, optimum at 100 μ g.

Keywords: *Hibiscus rosa-sinensis* linn, *In vitro*, Anti-inflammatory, RBC membrane stabilization.

INTRODUCTION

Medicinal plants have a lot of biologically active chemicals with therapeutic capabilities that can be used to cure a variety of disorders, which induces prompting researchers to derive chemical substances from plants¹. More than 9000 phenolic compounds have been identified in plants as naturally occurring chemicals. Half of these phenolic molecules are flavonoids that include an aromatic ring with at least one hydroxyl group, appear as aglycones, glycosides, and methylated derivatives². Flavonoids have been reported on their effective antioxidants, anti-inflammation, anti-cancer, anti-bacterial, anti-malarial, immune system promoting, and skin protection from UV radiation³.

Inflammation occurs when infectious bacteria infiltrate the body, reside in tissues, and/or circulate in the bloodstream, it's either acute or chronic⁴⁻⁵. Lysosomal enzymes have a significant role in the development of acute and chronic inflammation, according to studies. Increased enzyme activity has been recorded in some instances of experimental inflammation, such as rat paws induced oedematous by phlogistic drugs⁶. Aspirin, sodium salicylate, phenylbutazone, and indomethacin have been widely used as remedial drugs for inflammation⁷⁻⁸. Anti-inflammatory medications limit the release of hemoglobin from Red blood cells when they are exposed to hypotonic stress; this is due to the drugs' membrane stabilization against hypotonicity-induced hemolysis. This is a very useful *In vitro* approach



for evaluating various drugs' anti-inflammatory effectiveness. Several researchers have employed RBC as a model system to examine medication interactions with membranes and are interested in the stability of RBC against hypotonic hemolysis⁹⁻¹². Flavonoids such as quercetin, rutin, hyperoside, naringenin, and naringin have been shown to prevent hypotonicity hemolysis¹³. Anti-inflammatory and anti-histaminic activities of *Daturastramonium* containing kaempferol and quercetin have been reported¹⁴. Leaf extracts of *Carica papaya* inhibited hemolysis significantly, suggesting that they could be used to treat diseases that cause cellular membrane instability¹⁵. The flowers of *Allamanda Cathartica Linn.* are also used as a laxative and the flavonoid extracted from it showed a substantial anti-inflammatory effect at a very low concentration¹⁶⁻¹⁷. For decades, Arab folk medicine has employed a concentrated, viscous aqueous extract of ripe carob to treat mouth inflammations and many studies have shown that furan natural derivatives derived from a variety of natural creatures, including plants, fruits, vegetables, and marine animals, have anti-inflammatory properties via various pathways¹⁸⁻¹⁹. *Hibiscus rosa-sinensis Linn.* of the Malvaceae family, is a magnificent evergreen woody shrub with glabrous leaves that is often known in Tamil as semparuthi (Double yellow bloom with red color at the center). It's a monster from China. It is used as a decorative plant in gardens throughout India. Numerous varieties of flowers in cherry, white, red, magenta, yellow, and striped were grown in sunny, semi-shaded, and shady settings and places. The blossoms are taken as food in fresh or in the form of pickles in the countries like China and the Philippines. Flowers have qualities such as demulcent, emollient, refrigerant, aphrodisiac, and emmenagogue. A paste made from these herbs is used to treat swellings and boils. An infusion of the blossoms is used to treat bronchial catarrh. The flowers are cooked in ghee and given to women suffering from menorrhagia²⁰. In Malaysia, a root- decoction is utilized to treat venereal infections and fevers. The juice of its root is used to cure menorrhagia, and powdered root is used to treat gonorrhoea. In Mysore, the root of *Hibiscus rosa-sinensis Linn.* is used to treat a variety of bovine ailments²¹. In this present investigation, the ethyl acetate concentrates of *Hibiscus rosa-sinensis Linn.* double yellow flowers with red color at the center have been tested for their anti-inflammatory activity and the results are exhibited hereunder.

MATERIALS AND METHODS

The mature flowers of *Hibiscus rosa-sinensis Linn.* (3000 g) were chosen, collected, and extracted with 90% methanol under reflux for 3 days. The alcoholic extract was concentrated in a vacuum, and the aqueous concentration was fractionated using approximately 2 L of solvents namely benzene, peroxide-free diethyl ether, and ethyl acetate. Mostly ethyl acetate fractions were used to obtain flavonoids, as they are polyphenolic compounds and they are active against inflammation. Therefore, ethyl acetate fraction (1 g) was concentrated in a vacuum and kept in an ice chest for a few days and it was studied for anti-inflammatory properties.

Anti-Inflammatory test (Hypotonicity induced RBC membrane stabilization)

Blood Collection

Sterilized hypodermic needles (22 gauzes) were used to draw blood from normal human subjects, which were then combined with an equal volume of sterilized Alsever's solution containing 0.55% citric acid, 0.8% sodium citrate, 2.05% dextrose, and 0.42% sodium chloride and kept at 4°C²².

Saline Solution Preparation

Different concentrations of saline were generated (Isosaline 0.85% and hyposaline 0.25%).

RBC Suspension Preparation

The collected blood sample was centrifuged at 3000 rpm and packed cells were rinsed with 0.85% iso- saline (pH7.2) thrice and 2% (V/V) suspensions were prepared with iso-saline.

Determination of RBC membrane stabilization

Assay mixture which contains the ethyl acetate fraction with different concentrations (10, 25, 50, 75, 100, 150, 200 µg/mL), 0.5 mL 2% RBC, 1 mL phosphate buffer (0.15M, pH 7.4), 2 mL hyposaline (0.25%) and the suspensions were taken in different tubes. Instead of the drug, distilled H₂O (2 mL) was utilized as a control. Both tubes were incubated for 30 min at 37°C. The samples were centrifuged after incubation; the hemoglobin level present in the supernatants was determined by utilizing a Digital photoelectric colorimeter (Type 115 Systronics) set to 560nm. The membrane stabilization or percentage inhibition of hemolysis were calculated²³ by applying the formula given below and has been mentioned

in Table 1. Ethyl acetate fractions with different concentrations were plotted against hemolysis inhibition percentage as shown in Figure 1.

$$\text{Percentage inhibition of hemolysis} = 100 \times \left\{ \frac{\text{OD1} + \text{OD2}}{\text{OD1}} \right\}$$

Where OD1 = optical density of the hypotonic + buffered saline solution alone

OD2 = optical density of the test sample in a hypotonic solution

Table 1: Effect of ethyl acetate fraction on hypotonicity-induced RBC membrane stabilization

S. No	Ethyl acetate fraction of <i>Hibiscus rosa-sinensis</i> ($\mu\text{g/mL}$)	Hemolysis Inhibition (%)
1	10	48
2	25	50
3	50	64
4	75	86
5	100	90
6	150	78
7	200	62

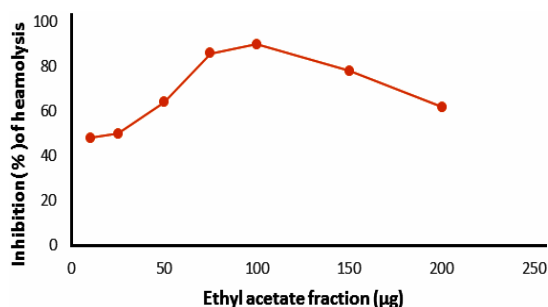


Fig. 1. HRBC membrane stabilization studies on *Hibiscus rosa-sinensis* Linn

RESULTS AND DISCUSSION

Inhibition of protein denaturation, lysosomal membrane stability, erythrocyte membrane stabilization, uncoupling of oxidative phosphorylation, fibrinolytic tests, and platelet aggregation are some of the techniques used to screen and study medicines' anti-inflammatory characteristics. The anti-inflammatory properties of ethyl acetate extracts may be due to binding to erythrocyte membranes and subsequent changes in cell surface charges. This could have facilitated dispersal by preventing physical contact with aggregating chemicals or by

the reciprocal repulsion of comparable charges involved in the hemolysis of red blood cells²⁴. *In vivo* and *In vitro* studies have revealed that some saponins and flavonoids have a strong stabilizing result on membranes of lysosomes, saponins, and tannins, and they can bind cations, stabilize erythrocytes and biological macromolecules²⁵. The ethyl acetate extract obtained from *Hibiscus rosa-sinensis* Linn. displayed membrane stabilizing properties after being exposed to hypotonicity. The percentage inhibition of hemolysis increased as the concentration of the isolated chemical was increased from 10 μg to 100 μg (up to 90%). At 100 μg , the hemolysis percentage inhibition drops. The percentage inhibition of hemolysis drops after 100 μg , indicating that the stabilizing effect is reduced by concentration variation. This is attributed to the biphasic property of the ethyl acetate fraction of *Hibiscus rosa-sinensis* Linn. showed successive anti-hemolytic activity which has been mentioned in previous studies. This biphasic property is a novel phenomenon in flavonoids.

CONCLUSION

The hypotonicity-induced RBC membrane stabilizing activity of ethyl acetate fraction obtained from *Hibiscus rosa-sinensis* Linn. was investigated. Even at a relatively low concentration of 100 μg of ethyl acetate fraction has been shown to have a substantial anti-inflammatory effect because, flavonoids are polyphenolic compounds and they are active against inflammation. As a result, the ethyl acetate fraction obtained from *Hibiscus rosa-sinensis* Linn. was discovered to be extremely efficient against the entire or any portion of acute or chronic inflammation.

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Conflict of interest

The authors declared no conflict of interest.

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