**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 100µg.

**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 plants. 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 derivatives. Flavonoids have been reported on their effective antioxidants, anti-inflammation, anti-cancer, anti-bacterial, anti-malarial, immune system promoting, and skin protection from UV radiation.

Inflammation occurs when infectious bacteria infiltrate the body, reside in tissues, and/or circulate in the bloodstream, it's either acute or chronic. 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 drugs. Aspirin, sodium salicylate, phenylbutazone, and indomethacin have been widely used as remedial drugs for inflammation. 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 hemolysis. Flavonoids such as quercetin, rutin, hyperoside, naringenin, and naringin have been shown to prevent hypotonicity hemolysis. Anti-inflammatory and anti-histaminic activities of Daturastramonium containing kaempferol and quercetin have been reported. Leaf extracts of Carica papaya inhibited hemolysis significantly, suggesting that they could be used to treat diseases that cause cellular membrane instability. 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 concentration. 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 pathways. Hibiscus rosa-sinensis Linn. 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 menorrhagia. In Malaysia, a root- a 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 gonorrhea. In Mysoore, the root of Hibiscus rosa-sinensis Linn. is used to treat a variety of bovine ailments. 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°C.

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 calculated 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.

Percentage inhibition of hemolysis = 100 X (OD1+OD2/OD1)

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

<table>
<thead>
<tr>
<th>S. No</th>
<th>Ethyl acetate fraction of Hibiscus rosa-sinensis (µg/mL)</th>
<th>Hemolysis Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>64</td>
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<td>90</td>
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<tr>
<td>6</td>
<td>150</td>
<td>78</td>
</tr>
<tr>
<td>7</td>
<td>200</td>
<td>62</td>
</tr>
</tbody>
</table>

The percentage inhibition of hemolysis drops after 100µg, 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.

### 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 cells.[24] 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 macromolecules.[25] The ethyl acetate extract obtained from *Hibiscus rosa-sinensis Linn.* displayed membrane stabilizing properties after being exposed to hypotonicity. 

### 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 100µg 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.

### REFERENCES


