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## Research Article

# A Comparative Study on The Anti-inflammatory Activity of *Boerhavia Diffusa* and *Scoparia Dulcis*

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### ABSTRACT

Inflammation is a reaction of living tissues towards injury, and it comprises systemic and local response which is an effective function of immune system. Herbal plants play a major role in the treatment of various types of inflammation. This study evaluates the anti-inflammatory potential of Scoparia dulcis and Boerhavia diffusa, two medicinal plants traditionally used for their therapeutic properties. Ethanolic extracts of both plants were assessed using in vitro methods, including human red blood cell (HRBC) membrane stabilization and protein denaturation assays. Both plants exhibited significant, concentration-dependent anti-inflammatory activity, with Boerhavia diffusa demonstrating superior efficacy. The HRBC stabilization assay showed effective inhibition of haemolysis, while the protein denaturation assay highlighted the ability of the extracts to prevent heat-induced protein denaturation. These effects are attributed to the phytochemical composition of the plants, which includes flavonoids, alkaloids, and other bioactive compounds. Thus, investigational findings suggest that Scoparia dulcis and Boerhavia diffusa possess potential anti-inflammatory activity, so these plants are promising candidates for developing safer, plant-based alternatives to conventional anti-inflammatory drugs. The characterization & isolation of the active principle may help to disclose the mechanism by which they elicit anti-inflammatory activity.

## INTRODUCTION


### 1.1 Inflammation

Inflammation is a complex biological reaction of vascular tissues to adverse stimuli such as infections, damaged cells, and irritants. It occurs

when body tissues are exposed to infections and physiological tissue damage<sup>[1]</sup>. Inflammation is a vital physiological response that are important in deciding whether tissues survive or are destroyed following a variety of traumas. As a defence mechanism, inflammation has developed in higher species in reaction to harmful stimuli such tissue

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damage, microbial infection, and other unpleasant circumstances. It's a vital host immunological response that permits the elimination of dangerous stimuli and the repair of injured tissue. This defence response involves immune cells, changes to blood vessels, and molecular mediators. Inflammation is represented by the development of granulomas, leukocyte infiltration, and edema. Endogenous stress signals, also known as structural pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs), are the cause of inflammation, according to the receptors for pattern recognition (PRRs) encoded in the germline and this PRRs are expressed by various kinds of cells, but it is mainly expressed on myeloid cells, including monocytes, macrophages, neutrophils, and dendritic cells. It is crucial to note that during inflammation, each tissue displays unique inflammatory features due to both local and general molecular, immunological, and physiological factors. It is now abundantly evident that inflammation has both beneficial and detrimental effects, acting as two sides of a single sword. Understanding the regulating mechanisms and their boundaries will aid in the future comprehension and treatment of a variety of illnesses. Developing conceptual and mechanistic knowledge of inflammation leads to novel therapeutic methods and insights into human diseases. [2]

### 1.1.1 Types of inflammation

Inflammation is mainly classified into two types, acute inflammation and chronic inflammation. Acute inflammation is a physiological process that occurs swiftly in response to injury or infection, typically manifesting within minutes to hours and lasting for a short duration, often resolving within days. This rapid onset is marked by the exudation of plasma proteins and fluids from the bloodstream into the surrounding tissue, leading to localized

swelling and redness. A hallmark of acute inflammation is the migration of leukocytes, especially neutrophils, to the site of injury. In addition to combating infections, the acute inflammatory response also serves to facilitate tissue repair. The damaged body part and personal characteristics determine the cause and length of the condition. By recruiting additional immune cells and releasing signalling molecules, this process helps to clear out debris and promote healing in the affected area [3]. When inflammatory processes continue after they are needed or happen without a genuine stimulation, it is known as chronic inflammation. Chronic inflammation frequently results from Infections, abnormal immune reactions, obesity, exposure to environmental triggers, unhealthy diet, smoking, sleep problems, high stress levels and that can lead to the consequences like DNA damage, cancer, atherosclerosis, heart disease, Alzheimer's disease, asthma, rheumatoid arthritis, diabetes, and other conditions [4].

### 1.1.2 Causes of Inflammation

Various physical and chemical agents can cause inflammation. The physical agents are mechanical injuries, extreme temperatures, radiation, triggering inflammatory pathways and the chemical agents that leads to the inflammation are a growing array of drugs and environmental toxins. Biological agents like bacteria, viruses, fungi, and parasites play a significant role in the development of inflammation [5]. Additionally, immunologic disorders, characterized by hypersensitivity reactions, autoimmunity, and immunodeficiency states, can lead to inappropriate or insufficient inflammatory responses. Genetic and metabolic disorders, such as gout and diabetes mellitus, often involve inflammatory mechanisms as part of their pathophysiology, highlighting the multifaceted nature of inflammation as both a



protective and a pathological process in the body [6].

### 1.1.3 Pathophysiology

Inflammation is a complex immune response triggered by injury, infection, or harmful stimuli, involving vascular changes, immune cell activation, and the release of signalling molecules. The development of inflammation involves a series of coordinated steps that aim to eliminate harmful stimuli, repair tissue damage, and restore homeostasis [7]. The process begins with the release of pro-inflammatory mediators like cytokines, histamines, and prostaglandins. A key component of the inflammatory response is the COX (cyclooxygenase) pathway, where the enzyme COX-1 and COX-2 convert arachidonic acid into prostaglandins and thromboxane. COX-1 is constitutively expressed and maintains normal physiological functions, such as protecting the stomach lining, while COX-2 is induced during inflammation and produces prostaglandins that promote pain, fever, and vasodilation. These prostaglandins increase vascular permeability, allowing immune cells and proteins to reach the site of injury or infection. Chronic inflammation can lead to tissue damage and fibrosis, often associated with dysregulated COX activity, contributing to various inflammatory diseases, such as arthritis [8].

### 1.1.4 Current Treatment

Three main categories of medications used to treat inflammatory reactions. Corticosteroids, DMARDs, or disease-modifying anti-rheumatoid medications NSAIDs, or non-steroidal anti-inflammatory drugs, include naproxen, ibuprofen, and aspirin. Many inflammatory illnesses can be effectively treated with these commonly used medications. For the treatment of inflammation, NSAIDs are most

frequently recommended [9]. In order to minimize temperature and pain during inflammation, NSAIDs work by inhibiting the activity of COX which is implicated in inhibiting PG production. The NSAIDs are known for their three primary effects: analgesia (pain reduction), anti-inflammatory, and antipyretic (fever reduction). They provide an anti-inflammatory effect that helps treat a number of ailments, such as rheumatoid arthritis, osteoarthritis, musculoskeletal diseases, and pericarditis. Analgesia is used to relieve mild to severe pain. They don't lead to dependence, but their maximum therapeutic efficacy is far less than that of opioids. By inhibiting the COX enzymes and lowering prostaglandins throughout the body, nonsteroidal anti-inflammatory medications (NSAIDs) lower fever, discomfort, and inflammation. NSAIDs are frequently used to treat both acute and chronic disease symptoms. Asthma, psoriasis, and arthritis are examples of chronic inflammatory conditions that have been treated with NSAIDs [10].

### 1.2 Herbal Remedies & its significance

India is one of the largest storehouses of medicinal plants in the world, and herbal remedies are very common in India. Herbal remedies are used for various ailments due to their immediate availability, accessibility, and affordability. About 50% of modern drugs are derived from plants, and plant products have pharmacological properties including inflammatory, antipyretic, and analgesic activities [11]. Plants have the ability to synthesize a wide variety of phytochemical compounds, and consumption of plant products reduces the risk of developing pathological conditions such as cancer, nervous system disorders, cardiovascular, genetic, and inflammatory diseases. One major drawback of the powerful anti-inflammatory synthetic medications currently on the market is their toxicity and the recurrence of symptoms after



stopping use. Long-term NSAID use damages the cardiovascular and renal systems and causes gastrointestinal ulcers can cause a number of serious adverse effects, including osteoporosis, diabetes mellitus, insulin resistance, hyperglycaemia, and anxiety. Non-selective NSAIDs can cause serious injury to the upper gastrointestinal tract, including perforations, ulcers, and bleeding. COX-2 selective inhibitors are associated with less upper gastrointestinal tract toxicity<sup>[12]</sup>. These adverse effects can be reduced by the use of herbal drugs having anti-inflammatory activity. Many researchers have isolated a greater number of phytochemicals from various medicinal plants and reported as anti-inflammatory agents. These phytochemicals inhibit the anti-inflammatory mediators. The main

target was Cyclooxygenase (COX) enzyme. Large number of herbal species has been used traditionally or as folk medicines against inflammatory disorders. Many of them have been studied scientifically and proved to be beneficial anti-inflammatory agents. Despite the divergent bioactivities of the plant medicines against various diseases, active components of most plant extracts have not been elucidated thoroughly, due their complex mixtures<sup>[13]</sup>. However, the core chemical classes of anti-inflammatory agents from natural sources have been reported to engage a vast range of compounds such as polyphenols, flavonoids, terpenoids, alkaloids, anthraquinones, lignans, polysaccharides, saponins and peptides. Some plants with anti-inflammatory activity are listed below<sup>[14]</sup>.

**Table.1. List of herbal plants with anti-inflammatory activity**

Sl. No	Botanical Name	Plant/Family	Parts Used	Constituent Compounds
1	<i>Acacia catechu</i>	Mimosaceae	Bark, wood, flowering tops, gum.	Tannin, gum, catechuic acid
2	<i>Azadiracta indica</i>	Meliaceae	Leaf, root, oil, seed, gum, fruit, flower.	Margosine, bitter oil, azadirachtin.
3	<i>Cassia angustifolia</i>	Caesal Pinaceae	Pods, dried leaves.	Emodin, eatharitin, mucilage, senna-picrin
4	<i>Foeniculum vulgare</i>	Apiaceae	Fruit, root, seeds, leaves	Ascorbic acid, estragole, coumaric acid, caeic acid, -terpinene, scoparone, scopoletin, cynarin, D-limonene, -phellandrene.
5	<i>Aquilaria agallocha Roxb.</i>	Thymelaeaceae	Barks	Agarospinol, jinkoh-eremol and hinesol
6	<i>Curcuma longa</i>	Zingiberaceae	Rhizomes	curcumin
7	<i>Mangifera indica Wall.</i>	Anacardiaceae	Fruits	Mangiferin
8	<i>Coriandrum sativum</i>	Umbelliferaeapiaceae	Leaf, bark, flower	Tannin, cathartin, Malic acid,
9	<i>Cuscuta reflexa</i>	Convolvulaceae	Plant, seed, fruit, stem.	Cuscutine, flavonoid, glucoside,
10	<i>Euphorbia hirta</i>	Euphorbiaceae	Plant, roots, leaves	Ascorbic acid, amyirin, choline

11	<i>Ficus carica</i>	Moraceae	Fruit, root.	Alkaloids, ascorbic acid, caffeic acid, niacin,
12	<i>Foeniculum vulgare</i>	Apiaceae	Fruit, root, seeds, leaves.	Ascorbic acid, estragole, coumaric acid, caffeic acid, terpinene, scoparone, scopoletin, cynarin, D-limonene, phellandrene.
13	<i>Momordica charantia</i>	Cucurbitaceae	Whole plant	cholesterol, lutein, diosgenin, lanosterol, lycopene
14	<i>Nicotiana tobacum</i>	Solanaceae	Leaves.	1,8-Cineole, 4-vinylguaiacol, acetaldehyde, acetophenone, alkaloids, anabasine, nicotinic acid, nicotine, scopoletin, quercitrin, sorbitol, tocopherol, stigmasterol, trigonelline.
15	<i>Ocimum basilicum</i>	Lamiaceae	Whole plant	Acetic acid, ascorbic acid, aspartic acid, Apigenin, arginine.

### 1.3 *Boerhavia diffusa* & Its significance

#### Taxonomy

**Botanical name:** *Boerhavia diffusa*

**Family:** Nyctaginaceae

**Order:** Caryophyllales

**Genus:** Boerhavia

**Phylum:** Tracheophyta

**Kingdom:** Plantae

**Class:** Magnoliopsida

**Species:** *Boerhavia diffusa* L.



**Figure 1. Boerhavia Diffusa**

*Boerhavia diffusa*, a creeping perennial weed commonly found in tropical and subtropical regions, is a well-known ethno-medicinal plant.

Various parts of the plant, including the leaves, roots, and stems, as well as its extracts, have been extensively used in traditional and folk medicine to treat a wide range of ailments. The plant contains a diverse array of phytochemicals, such as flavonoids (e.g., C-methyl flavone, 5,7-dihydroxy-3',4'-dimethoxy-6,8-dimethylflavone, boerhavone), alkaloids (like punarnavine), glycosides (such as punarnavoside and eupalitin), rotenoids (boeravinones A-H), as well as steroids, triterpenoids, lipids, lignans, carbohydrates, proteins, and glycoproteins [15]. Numerous studies have confirmed the biological, pharmacological, and clinical activities of the plant and its constituents. Notable therapeutic effects include diuretic, hepatoprotective, anti-inflammatory, anti-cancer, anti-diabetic, and immunomodulatory properties, as well as anti-fibrinolytic, anti-lymphoproliferative, and analgesic effects. It is also used in the treatment of pulmonary tuberculosis. In addition, the plant has shown some less prominent activities, including non-teratogenic, antioxidant, anti-viral effects against plant viruses, anti-bacterial, anti-fungal, adaptogenic, anti-amoebic, lipotropic, and anticonvulsant activities. Because of these proved pharmacological actions this plant has a major role in the medical world [16].

## 1.4 *Scoparia Dulcis* & its Significance

### Taxonomy

**Botanical name:** *Scoparia dulcis* Linn.

**Family:** Scrophulariaceae

**Kingdom:** Plantae

**Subkingdom:** Trachcobionta

**Division:** Magnoliophyta

**Genus:** Scoparia

**Class:** Magnoliopsida

**Subclass:** Asteridae

**Order:** Lamiales

**Phylum:** Tracheophyta

**Species:** Dulcis



Figure 2. *Scoparia dulcis*

*Scoparia dulcis* commonly known as ‘sweet broom weed’ is distributed throughout the tropical and subtropical region of the world. The plant is used traditionally as used as a remedy for treating various disease. Its ethno-medicinal uses amongst various indigenous tribes in the rain-forest zone are well-documented. In fresh or dried form *S. dulcis* plants have been traditionally used as remedies for Diabetes mellitus in India and hypertension in Taiwan. It is used in curing ailments such as fever, diarrhoea, ulcer, cancer, wounds, skin rash, cough and tuberculosis. The fresh or dried plant has been used for treating stomach aches, inflammation, bronchitis,

hemorrhoids and hepatitis. In the western part of Orissa its root is traditionally is used as an effective remedy for Jaundice and diarrhoea. It is also used as an analgesic and antipyretic, in stomach troubles, bronchitis, as well as inhibition of herpes simplex virus replication, gastric H<sup>+</sup>,K<sup>+</sup>-ATPase activation and antitumor activity. It is deemed to be a panacea for all ills. In Gambia, a lotion prepared from the plant is used in curing fever. A hot water infusion or decoction of the leaves or whole plant is used medicinally by indigenous tribes of Nicaragua to treat malaria, stomach disorders, menstrual disorders, insect bites, fevers, heart problems, liver disorders and venereal diseases. It has been used for blood cleansing, in childbirth and as a general tonic<sup>[16 c]</sup>. *Scoparia dulcis* is a rich source of various bioactive compounds, including flavones, terpenes, steroids, phenols, tannins, saponins, amino acids, coumarins, and carbohydrates. Key chemical constituents of the plant are scopadulcic acids A and B, scopadiol, scopadulciol, scopadulin, scoparic acids A–C, and betulinic acid. Additionally, other notable compounds present are acacetin, amyirin, apigenin, benzoxazin, benzoxazolin, benzoxazolinone, cirsimarin, cirsitakaoside, coixol, coumaric acid, cynaroside, daucosterol, dulcinol, dulcioic acid, gentisic acid, glutinol, hymenoxin, linarin, luteolin, mannitol, scoparinol, scutellarein, scutellarin, sitosterol, stigmasterol, taraxerol, vicenin, and vitexin which are responsible for the wide range of pharmacological actions<sup>[17]</sup>. Traditionally the fresh or dried plant has been used as a remedy for treating diseases such as, stomach ailments, kidney stones, hypertension, diabetes, inflammation, bronchitis, haemorrhoids, analgesic, antipyretic and urinary disorders. Plant is also used for upper respiratory bacterial and viral infections, to relieve from all types of pain, to tone balance, strengthen heart function, for veneral diseases and urinary tract infections. The leaf of

*Scoparia dulcis* is used for diabetes in India. Plant is also reported to possess cytotoxic, anti-cancerous, antimicrobial, anti-malarial, anti-ulcer, antacid, ant cholesterol and antioxidant actions<sup>[18]</sup>.

### 1.5 Problem in Hand & Solution

Existing anti-inflammatory drugs, such as NSAIDs and corticosteroids, have several notable deficiencies. They often come with significant side effects, including gastrointestinal issues, cardiovascular risks, and potential kidney damage. Many patients experience limited efficacy, requiring higher doses that can exacerbate side effects. Additionally, these medications may have a short duration of action, necessitating frequent dosing, and some, like opioids, pose a risk of dependence. Corticosteroids can suppress the immune system, increasing infection risk, while newer biologics may be prohibitively expensive. The complexity of their use, potential for drug interactions, and delayed onset of action further complicate their effectiveness, underscoring the need for better therapeutic options. Herbal remedies often have fewer side effects, making them suitable for long-term use without the risk of dependency or severe adverse reactions<sup>[19]</sup>. The use of plants as therapeutic agents serves multiple objectives, including the isolation and concentration of bioactive compounds for direct use as drugs, the production of novel or known bioactive substances for semi-synthesis into more potent, patentable entities with improved efficacy or reduced toxicity, and the use of plant compounds as pharmacological tools for research. Additionally, whole plants or their parts may be

utilized as herbal remedies in traditional medicine. A key distinction of plant-derived drugs is their molecular diversity, which far exceeds that of synthetic compounds, despite advancements in chemical synthesis. This diversity provides a broader range of biological functions and potential therapeutic applications, making plant-derived compounds promising candidates for developing treatments for various diseases. The complexity and variability of these natural products provides opportunities for discovering novel drugs with unique mechanisms of action that may offer advantages over conventional synthetic medications.<sup>[20]</sup>

## 2. Aim & Objectives

### 2.1. Aim

To conduct a study on the anti-inflammatory activity of *Scoparia dulcis* and *Boerhavia diffusa* and compare it.

### 2.2 Objectives

1. Identify and collect the plants *Scoparia dulcis* and *Boerhavia diffusa*.
2. Prepare the alcoholic extracts of the plants *Scoparia dulcis* and *Boerhavia diffusa*.
3. Evaluate the anti-inflammatory activity of *Scoparia dulcis* and *Boerhavia diffusa*.
4. Compare the anti-inflammatory activity of *Scoparia dulcis* and *Boerhavia diffusa*

## 3. MATERIALS AND METHODS

### 3.1. Materials Used

**Table.2. List of Materials Used**

Sl. No	Name	Manufacturer
1	Mixer Grinder	Sujatha
2	Beaker	Borosil



3	Heating mandle	Mild laboratory heating mantle for heaters
4	90% Ethanol	Cochin Petromins Pvt. Ltd
5	Vacuum rotary evaporator	Buchi
6	Diclofenac sodium	AdvaCare Pharma
7	Aspirin	Kureasia pharma pvt.ltd
8	Phosphate buffered saline	Otto chemie pvt ltd
9	UV/Vis spectrophotometer	Shimadzu
10	Sterile saline	Fresenius Kabi India Pvt.Ltd
11	Distilled water	Caritas college of pharmacy
12	Centrifuger	Apollo Machinery
13	Funnel	Borosil
14	Iodine flask	Borosil
15	100 ml standard flask	Borosil

### 3.2. Collection of plants

#### 3.2.1 Collection of *Scoparia dulcis*

For the present study, the investigated plant *Scoparia dulcis* were collected from Ettumanoor, Kottayam district on October 24<sup>th</sup> 2024. The collected plant parts (complete herb) were sorted to remove any undesirable materials or plant sections. They were dried in shade for two week and powdered to a coarse form.



**Figure 3. *Scoparia Dulcis* plant**



**Figure 4. Dried powder of *scoparia Dulcis***

#### 3.2.2 Collection of *Boerhavia Diffusa*

The whole plant of *Boerhavia diffusa* were collected from Pala, Kottayam district on 1/11/2024. They were dried in shade for three weeks and ground to a coarse form.



**Figure 5: *Boerhavia Diffusa* Plant**



**Figure 7. Extraction of *Scoparia Dulcis***



**Figure 6. Dried powder of *Boerhavia diffusa***



**Figure 8. Extract of *Scoparia Dulcis***

### 3.3 Preparation of Extract

#### 3.3.1 Extraction of *Scoparia Dulcis*

Macerate the dry powder of *Scoparia dulcis* in ethanol at a ratio of 1:4. Kept the mixture at room temperature with occasional stirring for 5 days. Then, concentrated and dried the filtrate using a rotary vacuum evaporator [46].

#### 3.3.2 Extraction of *Boerhavia Diffusa*

100 grams of the dried powder of *Boerhavia Diffusa* is taken into a sterile iodine flask, 500 millilitres of ethanol is added and shaken vigorously. Covered the bottle tightly and let it sit for 5 days. Filtered the mixture with Whatman filter paper at room temperature. Concentrated the extract, dried it at room temperature, and stored it in a refrigerator [47].



Figure 9. Extraction of *Boerhavia Diffusa*



Figure 10. Extract of *Boerhavia Diffusa*

### 3.3 Calculation of Percentage Yield

The percentage yield was calculated for each plant extract and major compounds with reference to the crude material taken using the formula. Percentage yield with reference to crude plant material = (Weight in grams of extract obtained / Weight in grams of plant material taken) x 100

### 3.4 In vitro anti-inflammatory screening methods

#### 3.4.1 Protein Denaturation Using Egg Albumin

##### Preparation of 1% of egg albumin solution:

For making egg-albumin solution using a fresh hen's egg properly involves carefully cracking an egg, transferring 1 mL of the translucent portion to 100 mL of w/V distilled water, and stirring thoroughly. The clear component of the egg is called egg albumin. The water should be cold when making the solution. Water will coagulate if it is heated to a boil.

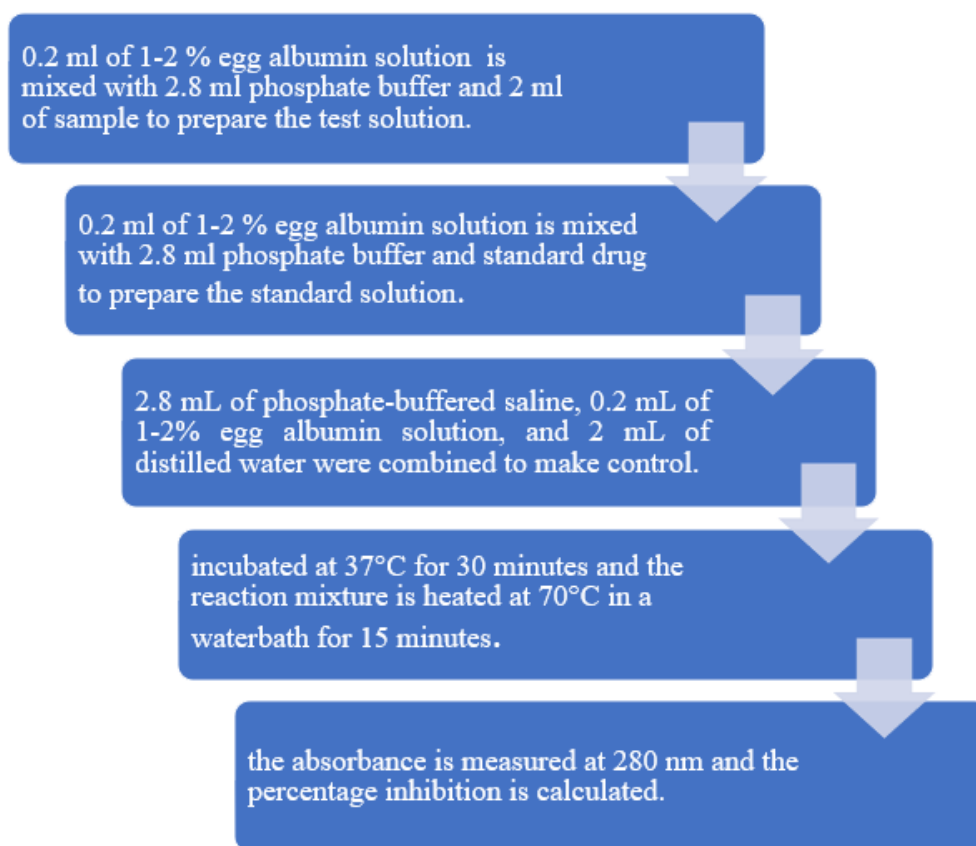
##### Procedure:

Unknown crude extracts' anti-inflammatory properties can be assessed in vitro by preventing the denaturation of the protein egg albumin. To create a reaction mixture with a total volume of 5 mL, 0.2 mL of 1-2% egg albumin solution (from fresh hen's eggs or commercially available egg albumin powder), 2 mL of sample extract or standard (Diclofenac sodium), and 2.8 mL of phosphate buffered saline (pH 7.4) were combined. 2.8 mL of phosphate-buffered saline, 0.2 mL of 1-2% egg albumin solution, and 2 mL of distilled water were combined to make a total volume of 5 mL of the control. After 30 minutes of incubation at  $37 \pm 2^\circ\text{C}$ , the reaction mixtures will be heated for 15 minutes in a water bath at  $70 \pm 2^\circ\text{C}$ . After cooling, distilled water was used as the blank and the absorbance was measured at 280 nm using an appropriate UV/Vis spectrophotometer<sup>[48]</sup>.

absorbance of control - absorbance of test sample

Percentage inhibition = ----- x 100

absorbance of control



### 3.4.2 Human red blood cell membrane stabilization method

The in vitro HRBC (Human Red Blood Cell) membrane stabilization method is used for evaluating anti-inflammatory activity of plants. Fresh blood is collected and mixed with an equal volume of sterile saline to prepare a suspension of red blood cells (RBCs). 1 ml of phosphate buffer,

2 ml hyposaline and 0.5 ml of HRBC suspension were added to the prepared plant extracts. It was incubated at 37 °C for 30 min and centrifuged at 3000 rpm for 20 min. Absorbance of the released haemoglobin is measured using a spectrophotometer at 560nm. Diclofenac sodium was used as reference standard and the percentage inhibition of haemolysis is calculated.<sup>[49,50]</sup>

$$\text{Percentage inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test sample}}{\text{absorbance of control}} \times 100$$

RBC suspension is prepared by mixing of equal volume of fresh blood and sterile saline.

1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added to the prepared plant extracts

Incubated at 37°C and centrifuged to separate the supernatant.

The absorbance is measured at 560nm and the percentage inhibition is calculated.

### 3.8 Statistical Analysis

Values are expressed as mean  $\pm$  Standard error mean, where n = 6. The results were analysed by one-way analysis of variance [ANOVA] followed by post Dunnett's post hoc multiple comparison test. The difference between groups were considered significant at P<0.05\*, P<0.01\*\*, P,0.001\*\*\*, P<0.0001\*\*\*\*. The statistical analysis was carried out using Graph Pad Prism version 10.4.1 for windows was used to analyze the data

## 4. RESULTS AND DISCUSSION

### 4.1. RESULTS

#### 4.1.1 Practical Yield of the Plant Extracts

The plant extracts were collected and the practical yield was calculated for each plant.

Sample	Extraction Technique	Practical yield
<i>Scoparia Dulcis</i>	Maceration	20%
<i>Boerhavia Diffusa</i>	Maceration	28%

#### 4.1.2. Anti-inflammatory activity by HRBC membrane stabilization

Anti-inflammatory activity of *Scoparia dulcis* and *Boerhavia diffusa* in HRBC membrane stabilization was evaluated by measuring percentage inhibition. (Table.3)

**Table 3: percentage inhibition exhibited by *Scoparia dulcis* and *Boerhavia diffusa* in HRBC membrane stabilization**

Sl. No	Sample	Concentration ( $\mu\text{g/ml}$ )	Percentage inhibition (%)
1	Standard Drug (Aspirin)	100 $\mu\text{g/ml}$	89.67 $\pm$ 2.459%****
2	<i>Scoparia dulcis</i>	100 $\mu\text{g/ml}$	9 $\pm$ 1.065%*** ##
		200 $\mu\text{g/ml}$	38.00 $\pm$ 1.414%**** ###
		400 $\mu\text{g/ml}$	47.67 $\pm$ 1.282%**** ###
		100 $\mu\text{g/ml}$	11.50 $\pm$ 0.7638%*** ##
		200 $\mu\text{g/ml}$	44.67 $\pm$ 2.290%**** ###



3	<i>Boerhavia diffusa</i>	400 µg/ml	57.50 ±1.118%**** ###
4	Control	--	--

All the values are in Mean±SEM. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001 when compared to control. #P<0.05, ##P<0.01, ###P<0.001 when compared to standard.

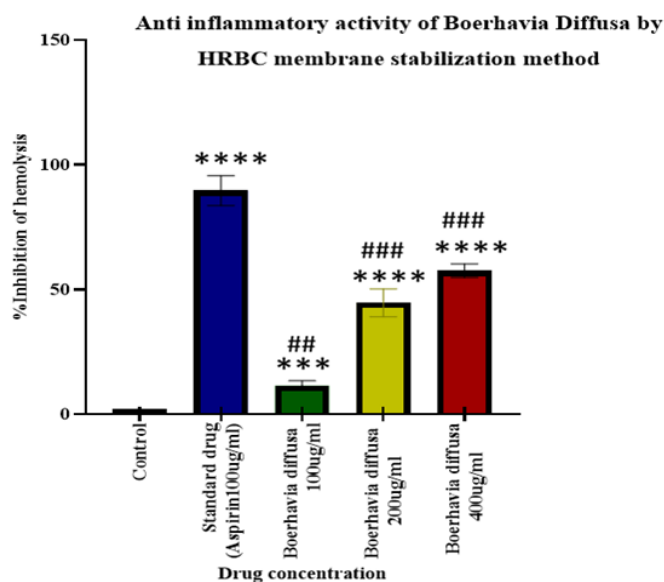


Figure 11. Anti-inflammatory activity of *Boerhavia diffusa* by HRBC Method

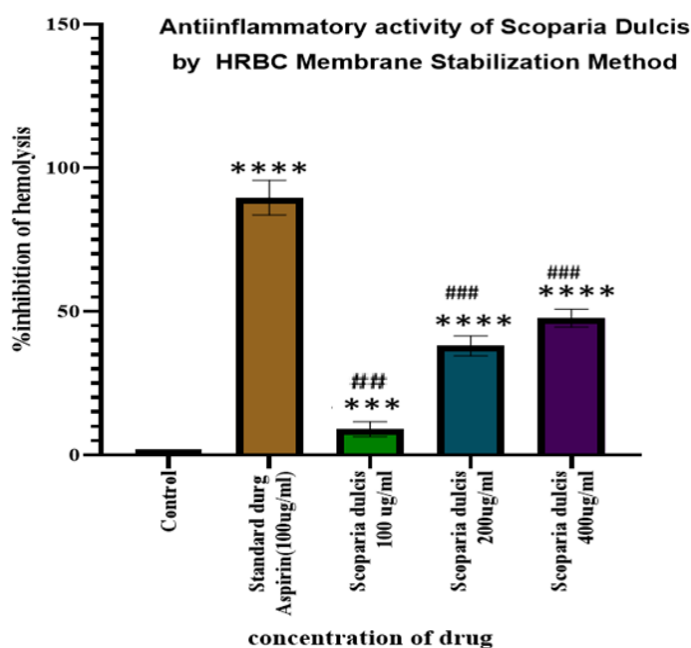


Figure 12. Anti-inflammatory activity of *Scoparia dulcis* by HRBC Method

In HRBC membrane stabilization method 100 µg/ml Aspirin is used as standard drug and 100,200,400 µg/ml of both the plant extracts are used as test doses. The standard drug Aspirin at the

concentration of 100 µg/ml shows significant (P<0.01) anti-inflammatory activity when compared to the control with 89.67±2.459 % inhibition of haemolysis. 100 µg/ml of *Scoparia*

*dulcis* and *Boerhavia diffusa* shows effective anti-inflammatory activity with  $9 \pm 1.065\%$  and  $11.50 \pm 0.7638\%$  haemolysis inhibition respectively ( $P < 0.01$ ) when compared to the control. *Scoparia Dulcis* and *Boerhavia Diffusa* in the concentration of  $200 \mu\text{g/ml}$  exhibit an effective anti-inflammatory activity with  $38.00 \pm 1.414\%$  and  $44.67 \pm 2.290\%$  inhibition of haemolysis respectively ( $P < 0.001$ ) when compared with the control. In comparison with the control,  $400 \mu\text{g/ml}$  of *Scoparia Dulcis* and *Boerhavia Diffusa* gives an effective anti-inflammatory activity with  $47.67 \pm 1.282\%$  and  $57.50 \pm 1.118\%$  inhibition of hemolysis ( $p < 0.001$ ). The anti-inflammatory activity of both the plant extracts, was compared with the anti-inflammatory activity of the standard drug Aspirin with 89% of haemolysis inhibition. When comparing the effect of  $100 \mu\text{g/ml}$  *Scoparia Dulcis* with standard drug ( $100 \mu\text{g/ml}$ , the test group shows moderate anti-inflammatory activity ( $P < 0.01$ ) with 9% of haemolysis inhibition. At the concentration of  $200 \mu\text{g/ml}$  *Scoparia Dulcis* showed a significant ( $P < 0.01$ ) anti-inflammatory activity when compared to the standard drug Aspirin with 38% of haemolysis inhibition. The higher concentration of drug extract ( $400 \mu\text{g/ml}$ ) *Scoparia Dulcis* shown a significant ( $P < 0.01$ ) anti-inflammatory activity when compared to the

standard drug Aspirin with 47% of haemolysis inhibition. When comparing the effect of  $100 \mu\text{g/ml}$  *Boerhavia Diffusa* with standard drug ( $100 \mu\text{g/ml}$ , the test group shows moderate anti-inflammatory activity ( $P < 0.01$ ) with 11% of haemolysis inhibition. The concentration of  $200 \mu\text{g/ml}$  *Boerhavia diffusa* showed a significant ( $P < 0.01$ ) anti-inflammatory activity when compared to the standard drug Aspirin with 44% of haemolysis inhibition. The higher concentration of drug extract ( $400 \mu\text{g/ml}$ ) *Boerhavia diffusa* shown a significant ( $P < 0.01$ ) anti-inflammatory activity when compared to the standard drug Aspirin with 57% of haemolysis inhibition. From the observed results it is clear that both *Scoparia dulcis* and *Boerhavia diffusa* have significant anti-inflammatory activity when compare to both standard and control.

#### 4.1.4 Anti-inflammatory activity by protein denaturation method

Protein denaturation using egg albumin is an effective in vitro anti-inflammatory screening technique to evaluate the effect of test drugs by measuring percentage inhibition of protein denaturation. (Table.4)

**Table.4 Percentage inhibition of protein denaturation by Scoparia dulcis and Boerhavia diffusa in protein denaturation method**

Sl. No	Sample	Concentration (100 $\mu\text{g/ml}$ )	Percentage inhibition (%)
1	Standard Drug (Diclofenac Sodium)	100 $\mu\text{g/ml}$	$88.33 \pm 2.261\%$ ****
2	<i>Scoparia dulcis</i>	100 $\mu\text{g/ml}$	$10.50 \pm 0.7638\%$ **** #
		200 $\mu\text{g/ml}$	$29.00 \pm 1.461\%$ **** ##
		400 $\mu\text{g/ml}$	$44.83 \pm 1.014\%$ **** ###
3	<i>Boerhavia diffusa</i>	100 $\mu\text{g/ml}$	$11.67 \pm 0.8819\%$ **** #
		200 $\mu\text{g/ml}$	$35.17 \pm 2.257\%$ **** ###
		400 $\mu\text{g/ml}$	$53.00 \pm 0.9661\%$ **** ###
4	Control	--	---

All the values are in Mean  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$  when compared to control.

# $P < 0.05$ , ## $P < 0.01$ , ### $P < 0.001$  when compared to standard.

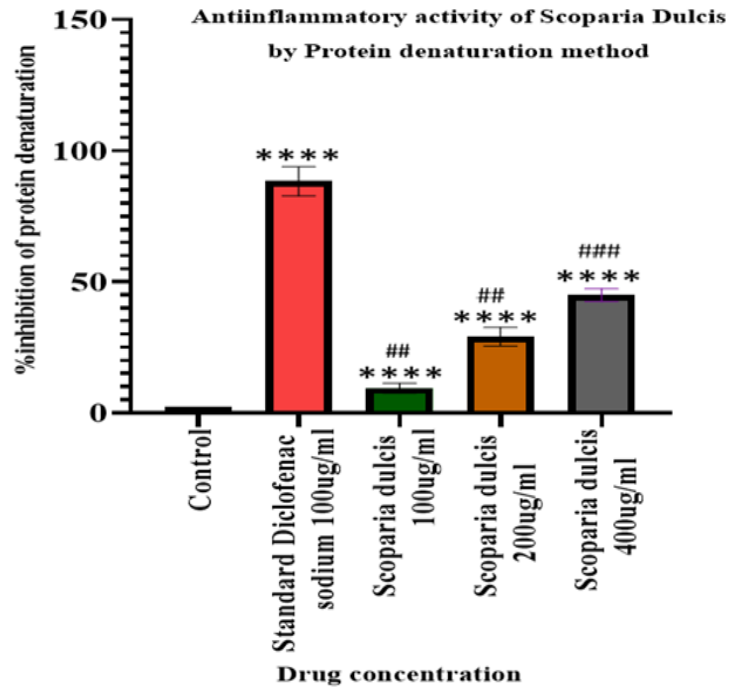


Figure 13. Anti-inflammatory activity of *Boerhavia diffusa* by Protein denaturation

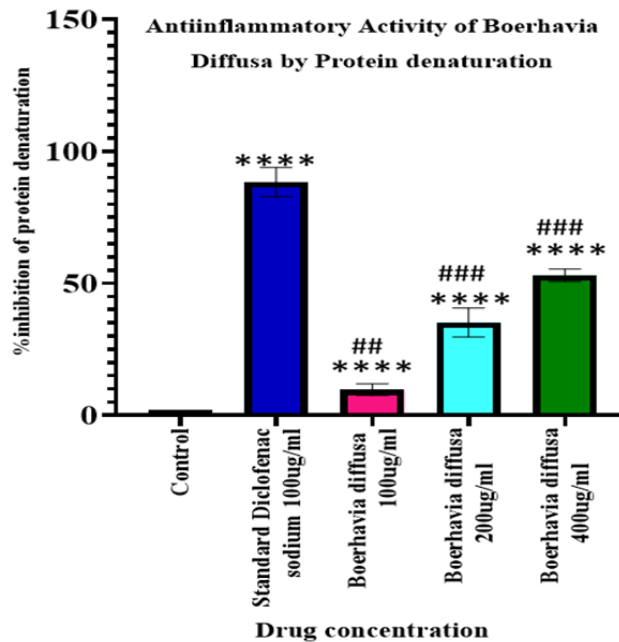


Figure 14. Anti-inflammatory activity of *Scoparia dulcis* by Protein denaturation

The test samples used in this method is 100, 200, and 400 µg/ml of the ethanolic extracts of Scoparia dulcis and Boerhavia diffusa and diclofenac sodium ( 400 µg/ml ) is used as standard drug. The standard drug Diclofenac sodium in the

concentration of 100 µg/ml shows significant anti-inflammatory activity when compared to the control with  $88.33 \pm 2.261\%$  inhibition of protein denaturation ( $P < 0.001$ ). 100 µg/ml of Scoparia dulcis and Boerhavia diffusa shows effective anti-

inflammatory activity with  $10.50 \pm 0.7638\%$  and  $11.67 \pm 0.8819\%$  inhibition of protein denaturation respectively ( $P < 0.001$ ) when compared to the control. *Scoparia dulcis* and *Boerhavia diffusa* in the concentration of  $200 \mu\text{g/ml}$  exhibit an effective anti-inflammatory activity with  $29.00 \pm 1.461\%$  and  $35.17 \pm 2.257\%$  inhibition of protein denaturation respectively ( $P < 0.0001$ ) when compared with the control. In comparison with the control,  $400 \mu\text{g/ml}$  of *Scoparia dulcis* and *Boerhavia diffusa* gives an effective anti-inflammatory activity with  $44.83 \pm 1.014\%$  and  $53.00 \pm 0.9661\%$  inhibition of protein denaturation ( $p < 0.001$ ). The anti-inflammatory activity of both the plants by measuring their percentage inhibition of protein denaturation is further compared with the anti-inflammatory activity of the standard drug Diclofenac sodium by measuring its percentage inhibition of protein denaturation. When comparing the effect of  $100 \mu\text{g/ml}$  *Scoparia Dulcis* with standard drug ( $100 \mu\text{g/ml}$ , the test group shows moderate anti-inflammatory activity ( $P < 0.01$ ) with 10% of haemolysis inhibition. At the concentration of  $200 \mu\text{g/ml}$  *Scoparia Dulcis* showed a significant ( $P < 0.01$ ) anti-inflammatory activity when compared to the standard drug Diclofenac sodium with 29% of haemolysis inhibition. The higher concentration of drug extract ( $400 \mu\text{g/ml}$ ) *Scoparia Dulcis* shown a significant ( $P < 0.01$ ) anti-inflammatory activity when compared to the standard drug Diclofenac sodium with 44% of haemolysis inhibition. When comparing the effect of  $100 \mu\text{g/ml}$  *Boerhavia Diffusa* with standard drug ( $100 \mu\text{g/ml}$ , the test group shows moderate anti-inflammatory activity ( $P < 0.01$ ) with 11% of haemolysis inhibition. The concentration of  $200 \mu\text{g/ml}$  *Boerhavia diffusa* showed a significant ( $P < 0.01$ ) anti-inflammatory activity when compared to the standard drug Aspirin with 35% of haemolysis inhibition. The higher concentration of drug extract ( $400 \mu\text{g/ml}$ ) *Boerhavia diffusa* shown a

significant ( $P < 0.01$ ) anti-inflammatory activity when compared to the standard drug Aspirin with 53% of haemolysis inhibition. When comparing the anti-inflammatory effect of both the plants by protein denaturation assay it is evident that, the *Boerhavia diffusa* shows potent anti-inflammatory activity than *Scoparia dulcis* in all the test concentrations.

## 4.2 DISCUSSION

The main objective of the present study is to compare the anti-inflammatory activity of both the plants *Scoparia dulcis* and *Boerhavia diffusa* by using two important invitro methods such as HRBC membrane stabilization and protein denaturation bioassay. In HRBC membrane stabilization technique the ability of the extract to stabilize the erythrocyte membrane was evaluated. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extracts may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. Disrupting the red blood cell (RBC) membrane can be achieved through several approaches, with one of the most commonly employed methods being hypotonicity-induced haemolysis. Hypotonicity-induced haemolysis involves exposing red blood cells to a hypotonic solution, which has a lower concentration of solutes than the cells' interior. This causes an influx of water into the cells, leading to swelling and eventual rupture of the RBC membrane. This method is frequently used in laboratory settings to study RBC membrane properties, cellular contents, and various biochemical processes. The successive extracts of *Scoparia dulcis* and



Boerhavia diffusa (100,200,400µg/ml) exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane in a dose dependent manner. The protein denaturation assay is a critical experimental approach to study the structural integrity and stability of proteins under various conditions. Egg albumin, a readily available and rich source of protein, serves as an ideal model for investigating denaturation processes. The experiment utilized egg albumin as a substrate to monitor denaturation under different conditions, including heat. Spectrophotometric analysis was employed to measure turbidity as an indicator of protein precipitation and denaturation. The protein denaturation is a major event in the process of inflammation. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The increments in absorbances of test samples with respect to control indicates stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation. Diclofenac sodium at a concentration of 100 µg/ml was used as the standard drug. Various studies has been reported that non-steroidal anti-inflammatory drugs are able to stabilize (prevent denaturation) heat treated albumin at the physiological pH (pH: 6.2-6.5). In this study the ethanolic extracts of the test drugs Scoparia dulcis and Boerhavia diffusa in various concentration such as 100µg/ml, 200 µg/ml, 400 µg/ml were evaluated for anti-inflammatory activity by protein denaturation assay using egg albumin. From the observed test result it is found that ethanolic extract of Scoparia dulcis and Boerhavia diffusa possessed marked in vitro anti-inflammatory effect against the denaturation of protein when compared to standard dug (Diclofenac 100µg/ml) and control in a dose dependent pattern. On the basis of in vitro evaluated results Scoparia dulcis and Boerhavia diffusa showed significant anti-inflammatory

activity as compared to control and standard in both the HRBC membrane stabilization technique and in protein denaturation assay using egg albumin. When analysing the anti-inflammatory activity produced by the two plants, it is clear that they have a concentration dependent anti-inflammatory activity, that is as the concentration of the drug increases the anti-inflammatory activity also increases. When comparing the above obtained values of percentage inhibition of haemolysis and percentage inhibition of protein denaturation of Scoparia dulcis and Boerhavia diffusa. it is clear that both the plants have significant anti-inflammatory activity and the Boerhavia diffusa has more anti-inflammatory activity than Scoparia dulcis. Further definitive studies are necessary to ascertain the mechanisms and constituents behind its anti-inflammatory actions. The characterisation, quantification of lead phytoconstituent and the study about it will give a potential drug candidate for the treatment of inflammation.

## 5.CONCLUSION

The study titled "A Comparative Study on Anti-Inflammatory Activity of Boerhavia diffusa and Scoparia dulcis" highlights the significant therapeutic potential of two widely used medicinal plants in combating inflammation. The research utilized in vitro assays HRBC membrane stabilization and protein denaturation to evaluate their anti-inflammatory activities. Both Boerhavia diffusa and Scoparia dulcis exhibited promising anti-inflammatory effects in a concentration-dependent manner, demonstrating their ability to mitigate the inflammatory process effectively. Boerhavia diffusa, however, consistently outperformed Scoparia dulcis in both assays, showcasing greater inhibition of haemolysis and protein denaturation. These results indicate a higher efficacy in preventing cellular and protein



damage, key contributors to inflammation. The superior performance of *Boerhavia diffusa* can be attributed to its rich phytochemical profile, which includes flavonoids, alkaloids, and other bioactive compounds known for their anti-inflammatory properties. The HRBC membrane stabilization assay revealed that both plant extracts help to protect red blood cell membranes from hypotonicity-induced lysis, a mechanism analogous to stabilizing lysosomal membranes *in vivo*. This property is critical for limiting the release of harmful enzymes and mediators that exacerbate tissue damage during inflammation. Similarly, the protein denaturation assay demonstrated the extracts' capacity to inhibit heat-induced protein denaturation, a process often implicated in inflammatory and arthritic diseases. By offering comparable efficacy with a potentially safer profile, *Boerhavia diffusa* and *Scoparia dulcis* emerge as valuable candidates for developing plant-derived therapeutic agents. In conclusion, this research underscores the significant anti-inflammatory potential of *Boerhavia diffusa* and *Scoparia dulcis*, with *Boerhavia diffusa* demonstrating superior efficacy. The results reflect the anti-inflammatory potential of *Boerhavia diffusa* and *Scoparia dulcis* characterized by the dose dependent increase in percentage inhibition of haemolysis and protein denaturation. Future investigations should focus on isolating and characterizing the specific active compounds responsible for these effects, as well as conducting *in vivo* studies and clinical trials to validate their therapeutic applications. This approach could pave the way for safer, cost-effective, and sustainable anti-inflammatory treatments derived from natural sources.

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