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In-vitro anti-inflammatory activity of methanolic extract of *Mirablis jalapa* and *Costus igneus*

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Abstract

A protective immunological reaction to irritations, infections, or tissue damage is inflammation. It includes vascular permeability, elevated blood flow, production of signalling chemicals like cytokines and histamine. Enzymes that cause different illnesses can be released when lysosomal membranes lyse during inflammation. By blocking these enzymes or maintaining membrane stability, NSAIDs assist medications like methylprednisolone, diclofenac, aspirin, indomethacin, Diclofenac, Ibuprofen, and Hydrocortisone. Traditional medicine has utilised plants like *Costus igneus* and *Mirabilis jalapa* to treat a variety of illnesses. β-sitosterol, β-amyrin are found in *Mirabilis jalapa* and *Costus igneus* give it anti-inflammatory qualities respectively.

Method: The leaves of *Mirabilis jalapa* and *Costus igneus* were extracted using methanol, and a phytochemical screening was performed. The human red blood cell (HRBC) membrane stabilization method and heat-induced haemolysis were employed to evaluate the anti-inflammatory properties of *Costus igneus* and *Mirabilis jalapa.* The evaluation was conducted at varying concentrations (40, 80, and 100 ug/ml), and the activity was noted in a dose-dependent way.

Result and discussion: With the use of HRBC membrane stabilization method, the percentage protection was discovered to be 70%, 78%, and 81%, respectively. Additionally, the heat-induced haemolysis approach yielded 72%, 76%, and 79%, respectively. The HRBC membrane stabilization approach provides 86% protection for the standard aspirin (100 ug/ml), while heat-induced haemolysis provides 82% protection.

Conclusion: The bioactive components are associated with their traditional and pharmaceutical application in the management of inflammation, it can be concluded. Both plants have strong anti-inflammatory properties.

Keywords: Heat-Induced Hemolysis; HRBC Membrane Stabilization; *Costus igneus*; *Mirabilis jalapa.*

1. Introduction

The complicated process of inflammation, which is often linked to pain, includes changes in membranes, increased vascular permeability, and increased protein denaturation. Stress is a type of injury that occurs when the cells are injured by bacteria, Chemical substances or physical force.

When the body reacts to stress, tissue inflammation occurs.[5]. The body uses inflammation as a defense mechanism in reaction to chemical irritants, allergies, microbial infections, and tissue damage. It is an essential immune response that attempts to get rid of harmful stimuli and initiates the repair process for damaged tissue. [6]

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Increased blood flow and vascular permeability, as well as the activation of humoral and cellular defense systems, are some of the intricate pathophysiological processes that lead to inflammation. [2] These processes are mediated by a variety of signaling molecules produced by mast cells, brain terminals, endothelial cells, granulocytes, platelets, lymphocytes, and macrophages. Lipids (leukotrienes, prostanoids, and platelet activating factor) are among the mediators.

Proteins and peptides (cytokines, growth factors, antibodies, kinines), physiologically active amines (histamine, serotonin), and superoxide anion, hydroperoxide, and hydroxyl radicals are examples of activated oxygen species. Numerous human illnesses are caused, maintained, exacerbated, and influenced by these mediators. The development of endogenous antiphlogistic and immunomodulatory mechanisms adds to the complexity of these processes, in addition to the vast number of proinflammatory actors. [7]

Inflammation symptoms is defensive reaction include Loss of function and redness, discomfort, and edema in the injured area. [5] Histamine, prostroglandins, and kinins are released by wounded tissue cells. Together, these lead to increased capillary permeability and vasodilation, or the widening of blood vessels. Increased blood flow to the damaged area results from this. Through a process called chemotaxis, these compounds also act as molecular messengers that attract certain of the body's defensive cells.[7] By either stabilizing the lysosomal membrane or preventing the

Lysosomal membrane stabilization is essential for lowering the inflammatory response because it prevents the release of lysosomal components of active neutrophils, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release. [12] Acute and chronic inflammation are two different categories. The body's first reaction to damaging stimuli is acute inflammation, which is brought on by an increase in the flow of plasma and leukocytes from blood into the wounded tissues. Chronic inflammation is characterized by the simultaneous destruction and healing of the tissue caused by the inflammatory process, and it results in a progressive change in the kind of cells present at the site of inflammation. [5] Inflammation can cause lysosomal membrane lysis, which releases the enzyme components that lead to a variety of diseases. [7] Non-steroidal anti-inflammatory drugs (NSAIDs) function by either blocking the release of lysosomal enzymes or stabilizing the lysosomal membranes. Hemolysis, or the lysis of red blood cell membranes, and hemoglobin oxidation can result from exposure to toxic chemicals. The following compounds are harmful: heat, methyl salicylate, phenyl hydrazine, and hypotonic medium.[8]

A hypotonic solution causes the RBC membrane to rupture due to an excessive build up of fluid inside the cells. RBC haemolysis occurs at the end. The cell is more susceptible to secondary damage from lipid peroxidation caused by free radicals when the red cell membrane is damaged. Bacterial enzymes and proteases are found in the lysosomes of active neutrophils. Leakage of lysosomal components upon extracellular release causes further tissue damage and inflammation. The integrity of the lysosome membrane is therefore essential for controlling the inflammatory response. This is going to stop its contents from spilling.[8] Human erythrocytes can be utilized to test the extract capacity to stabilize the membrane through heat-induced hemolysis and hypotonic solution-induced hemolysis. [9] The lysis of human red blood cell membranes (HRBCs) caused by hypotonicity can be used as an in-vitro test to determine whether a drug or plant extract has anti-inflammatory properties. [10] [11]

One important source of compounds with pharmacological activity is natural products, particularly medicinal plants. Herbs are frequently chosen over synthetic substitutes because of their natural sources, possibility for fewer adverse effects, and more comprehensive therapeutic approach. Instead of only treating symptoms, they are said to be kinder to the body and have the ability to promote recovery.

Mirabilis jalapa in Ayurveda also called “gulambasa.” It has been claimed that leaves contain a variety of substances, including β-sitosterol, stigmastrol, ursolic acid, leaves and stems are used as tonics and diuretics. To lessen itching in ailments like urticarial, leaf juice is traditionally administered to wounds and bruises. [2] Constituents isolated from the root and aerial parts of the plant include some rotenoids, an isoquinoline derivative, terpenoids, steroids, phenolic compounds, d-glucoside, ursolic acid, mirabalisoic acid, trigonelline, an antiviral protein, alanine, alpha-amyrins, arabinose, beta-amyrin, campesterol, daucosterol, and dopamine. [4]

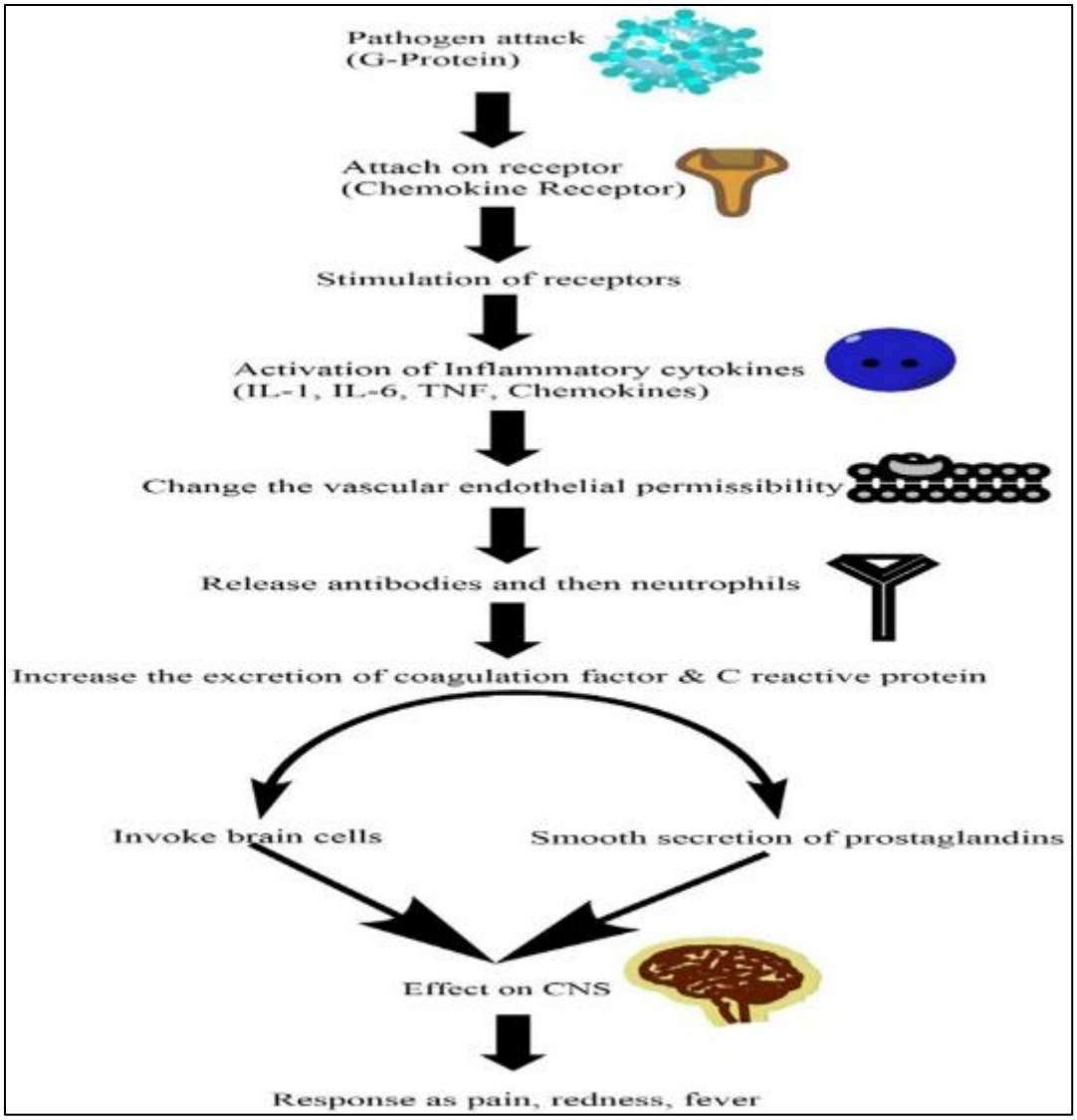

Figure 1 Mechanism of Inflammation

Proteous substances have been reported to have anti-tumor and plant virus inhibitory effect, while leaves have been utilized to treat and reduce inflammation. These studies have resulted in the isolation of soluble starch, pigments of centrospermae, sterols and fatty acids, sugars, amino acids, and triterpenes. [13] The plant's aerial components, which include four steroids, B-sitosterol acetate, ursolic acid 4, and one diterpene, trans-phytol triterpenes, are discussed in this communication. [2]

Costus igneus called "Fiery Costus" and "Spiral Flag" are other names for the medicinal plant *Costus igneus.* [2] Native to South and Central America. Commonly referred to as the "insulin plant". This plant is relatively new to India, having originated in South Central America.[13] In Southern India, the entire *Costus igneus* plant is utilized for its anti-diabetic properties, which also shield the body from illness, preserve mental health, and increase life expectancy. Preliminary phytochemical screening revealed that this plant extract included proteins, carbohydrates, steroids, alkaloids, tannins, glycosides, saponins, fixed oils, and flavonoids. [13]

Based on previous studies, there is a strong belief that eating the leaves of this plant can help diabetics control their blood glucose levels. It was discovered that people who consumed the plant's leaves had lower blood glucose levels. It has been demonstrated that the administration of *Costus igneus* stem extract in both aqueous and ethanolic form inhibits the production of urinary stones in rats with experimentally induced urolithiasis by ethylene glycol. The components of the *Costus igneus* plant preparation indicate that it may reduce oxidative stress. [14] The goal of this work was to identify the mechanism by which β-amyrin, which was extracted from *Costus igneus* leaves,[2] β-sitosterol which extracted from *Mirabalis jalapa* had an anti-inflammatory effect [1]. Phenol, cardiac glycosides, triterpene, tannin, carbohydrates,

alkaloids, terpenoid, and protein presence techniques were among the main chemicals that were examined. Major chemicals found in *Costus igneus*, along with their pharmacological and therapeutic properties. Pharmacological property include anti-diabetic, anti-proliferative, anti-microbial, anti-urolithiatic, anti-inflammatory, anti-oxidant, neuroprotective, hypolipidemic, and other effects. [4]

2. Experimental work

Collection and authentication of plant: Both plants were gathered during the July-August monsoon season from local area (garden) of Ichalkaranji, Kolhapur, Maharashtra. To preserve the phytochemical content, the plant materials were shade-dried at room temperature (around 25 °C) after being cleaned with distilled water, and authenticated by Dattajirao Kadam Arts, Science and Commerce college Ichalkaranji, Kolhapur, Maharashtra, India. Voucher specimens were prepared and archived for reference.

- ***Mirabilis jalapa*:** Known as Gulambasa in Ayurveda is known for its diverse bioactive compounds like beta-sitosterol, ursolic acid, and anthraquinone. Traditionally, its leaf juice is used for wounds, itching, and bruises. [3][2]
- ***Costus igneus*:** Referred to as the "insulin plant," is prized for its anti-diabetic**,** anti-inflammatory, and antioxidant properties. Rich in phytochemicals like flavonoids, alkaloids, and glycosides, its leaves help lower blood glucose and cholesterol levels. [4][13]

Figure 2 Leaves of *Mirabilis jalapa*

Figure 3 Leaves *Costus igneus*

Table 1 Taxonomy of *Mirabilis jalapa*

Botanical Name	***Mirabilis jalapa***
Kingdom	Plantae
Family	Nyctaginaceae
Genus	*Mirabilis*
Species	*jalapa*

Table 2 Taxonomy of *Costus igneus*

Botanical Name	***Costus igneus***
Kingdom	Plantae
Family	Costaceae
Genus	*Costus*
Species	*igneus*

- **Biological and pharmacological properties *Mirabalis jalapa:*** Diuretic, carminative, conjunctivitis, abdominal colic, purgative, anti-bacterial, anti-fungal, anti-inflammatory, anti-spasmodic, anti-viral, and anti-gonorrheal
- **Biological and pharmacological of properties Of *Costus igneus*:** anti-inflammatory, anti-diabetic, anti-microbial, anti-oxidant, anti-Urolithiatic, neuroprotective, hypolipidemic, and anti-proliferative [4] [13]

3. Material and methods

3.1. Extraction of *Mirabilis jalapa* and *Costus igneus*

The coarsely powerdered leaves of *Costus igneus* were extract with methanol by using maceration process of extraction. About 50 gram powdered leaves extracted with 250 ml of methanol through a week of cold maceration at room temperature.[15] The coarsely powerdered leaves of *Mirabilis jalapa* were extracted with methanol by using maceration process of extraction. 200 mL of methanol were used to extract roughly fifty grams of powdered leaves over the course of 48 hours at room temperature. [16] Extract of both supernatant evaporated after they had been filtered through Whatman No. 1 filter paper. The extract amounts for *Costus igneus* and *Mirabilis jalapa* were 1.96 g and 1.26 g, respectively.

3.2. Procedure for analysis of anti-inflammatory activity:

- Preparation of Red Blood Cells (RBCs) (10% v/v)
- Method for analysis anti-inflammatory activity
  - Human red blood cell Stabilization method
  - Heat induced hemolysis [20]

Preparation of Red Blood Cells (RBCs) (10% v/v): Human donors who were willing and in good health provided the red blood cells. (Never took NSAIDS before to expressing). The EDTA tube was filled with three mL of the red blood cells, which were then centrifuged at 3000 rpm for fifteen minutes at room temperature. After removing the supernatant and placing the residue in the centrifugation tube, sufficient isosaline solution was added, and centrifugation was repeated. Three iterations of the procedure were carried out until the isosaline solution's colour became evident. To make a 10% red blood cell suspension, one drop of red blood cells was combined with nine drops of isosaline. [19]

Method for analysis anti-inflammatory activity: HRBC membrane Stabilization method: Different concentration of test solution (40,80,100ug/ml)consisted of 1 mL sample solution; 2 mL hyposaline;1 mL of sodium phosphate buffer and 0.5 mL (10% v/v) suspension of RBCs in isosaline. The test control solution consisted of 2 mL hyposaline; 1 mL of sodium phosphate buffer, 1 mL of isosaline and 0.5 mL (10% v/ v) of RBCs suspension in isosaline. The standard solution consisted of 2 mL hyposaline; 1 mL of sodium phosphate buffer, 1 mL aspirin (100µg/mL) and 0.5 mL (10% v/v) suspension of RBCs in isosaline. After 30 minutes of incubation at 37°C, each assay mixture was centrifuged at 3000 rpm. Liquid supernatant was decanted, a colorimeter set at 570 nm was used to measure the absorbance. The formula is used to determine the percentage of protection and hemolysis. [7] [18] [20]

$$\% \text{ Hemolysis} = (\text{Abs of Test} / \text{Abs of Control}) \times 100$$

$$\% \text{ inhibition} = (\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$$

Abs control =Absorbance of control

Abs sample = Absorbance of sample

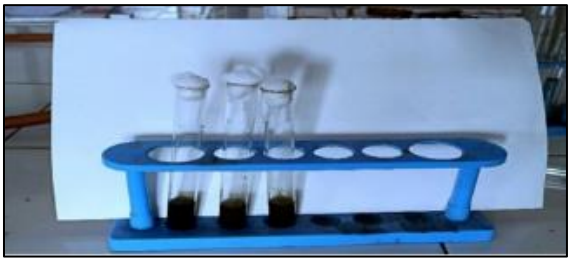

Figure 4 Effect of extracts different concentration for HRBC membrane stabilization method

Heat induced hemolysis: Two mL of hyposaline, one mL of sodium phosphate buffer, half a mL of 10% v/v RBC suspension, and one mL of sample were used to make the test solution. 1 mL standard solution, 2 mL hyposaline, 1 mL sodium phosphate buffer, and 0.5 mL (10% v/v) suspension of RBCs in isosaline. 0.5 mL (10% v/v) RBC suspension in isosaline, 1 mL of sodium phosphate buffer, 1 mL of isosaline, and 2 mL of hyposaline made up the control solution. After that, the solution was incubated in a water bath at 56°C for 30 minutes. After that, the fluid was centrifuged at 3000 rpm for 15 minutes. The absorbance of the solution was measured using a colorimeter set at 570 nm. A formula was used to calculate the protection and hemolysis percentages.[7][18][20]

% Hemolysis = (Abs of Test / Abs of Control) x 100

% inhibition = (Abs control – Abs sample) x 100 /Abs control

Abs control =Absorbance of control

Abs sample = Absorbance of sample

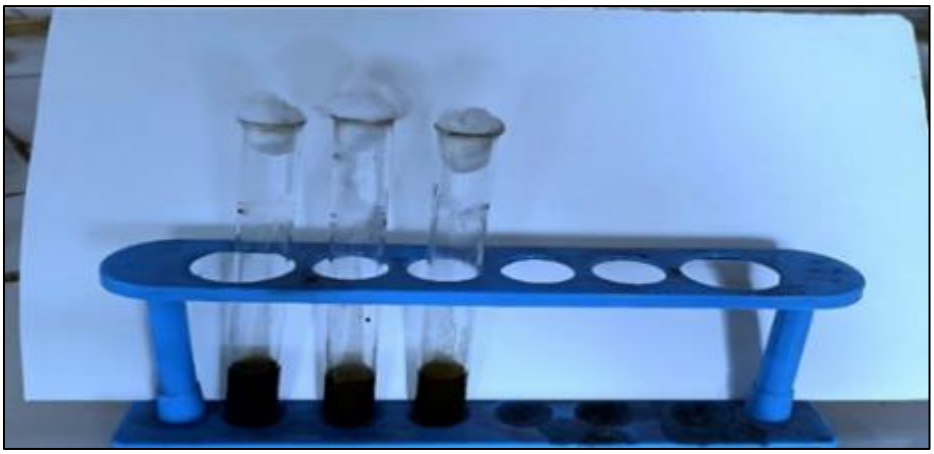

Figure 5 Effect of extracts different concentration for heat induced haemolysis method

3.3. Statical analysis

The statistical analysis utilizing one-way ANOVA for both the heat-induced hemolysis and HRBC membrane stabilization procedures revealed no significant differences between the groups. The HRBC method had an F-value of 11.781 and a p-value of 0.0280, whereas the heat-induced hemolysis method had an F-value of 11.3649 and a p-value of 0.0265. These results showed that the methanol extracts of *Mirabilis jalapa* and *Costus igneus* exhibited anti-inflammatory activity similar to the reference drug ($p<0.5$), supporting the extracts' consistency and effectiveness in both in vitro models.

4. Result and discussion

Table 3 Phytochemical test for *Mirabalis jalapa*

Sr. No.	Test	Procedure	Inference
01	Salkowaski test	Filtrate + few drops of Conc. H_2SO_4.	+
02	Copper acetate test	Extract + distilled water+ 3-4 drops of copper acetate solution	+
03	Libermann burchard test	Extract+ 2ml acetic acid solution+ 1-2 drops of Conc. H_2SO_4 along the side of test tube.	+

Table 4 Phytochemical test for *Costus igneus*

Sr. No.	Test	Procedure	Inference
01	Salkowaski test	Filtrate + few drops of Conc. H_2SO_4.	+
02	Copper acetate test	Extract + distilled water+ 3-4 drops of copper acetate solution	+
03	Libermann burchard test	Extract+ 2ml acetic acid solution+ 1-2 drops of Conc.H_2SO_4 along the side of test tube.	+

Table 5 Human red blood cells stabilization method

Sr. No.	Treatment	Concentration (µg/mL)	% Haemolysis	% Protection
01	Control	-	100	00
02	Test	40	30	70
03	Test	80	21	78
04	Test	100	18	81
05	Standard	100	14	86

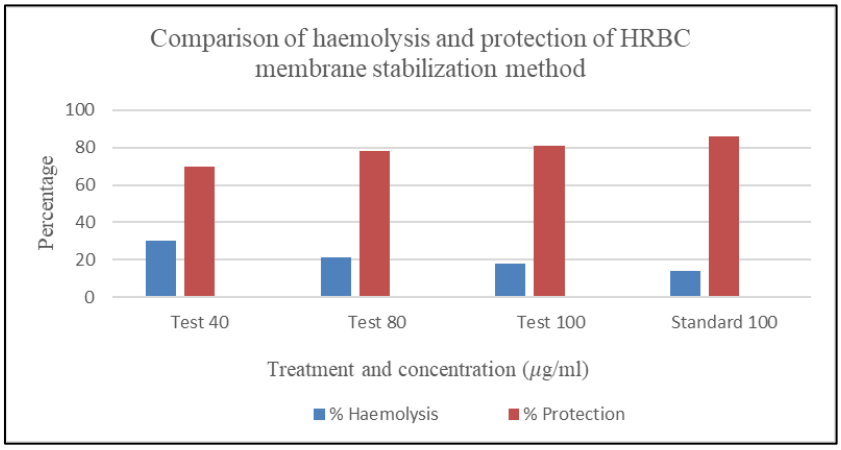

Figure 6 Comparison of % haemolysis and protection of HRBC membrane stabilization method

Human red blood cells stabilization method: The methanolic extract of the leaves of *Mirabilis jalapa* and *Costus igneus* was tested for its in vitro anti-inflammatory qualities using the HRBC membrane stabilization technique. The strongest anti-inflammatory activity and 81% HRBC protection in hypotonic solution were demonstrated by 100 µg/ml out of all doses. The results showed 86% protection display the benchmark.

Table 6 Heat induced hemolysis method

Sr. No.	Treatment	Concentration (µg/mL)	% Haemolysis	% Protection
01	Control	-	100	00
02	Test	40	27	72
03	Test	80	23	76
04	Test	100	20	79
05	Standard	100	18	82

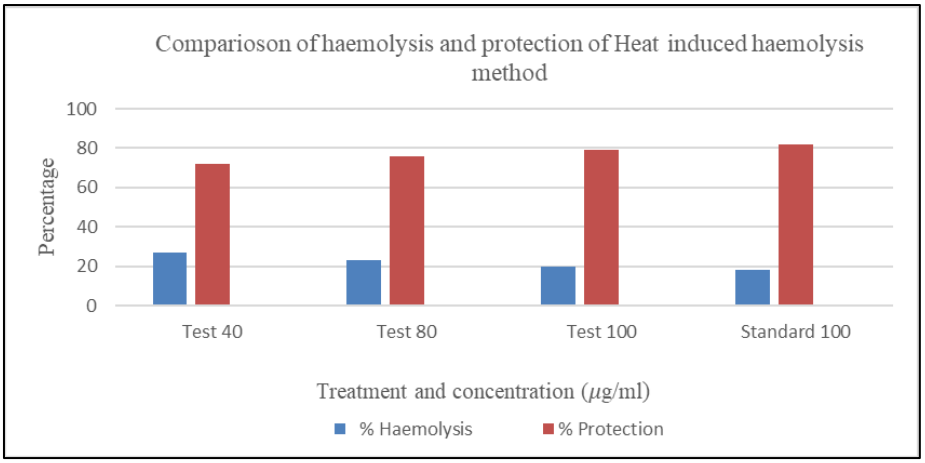

Figure 7 Comparison of % haemolysis and protection of Heat induced haemolysis method

Heat induced hemolysis method: The mechanism of the anti-inflammatory action of the methanolic extract of *Costus igneus* and *Mirabilis jalapa* at varying doses was investigated using heat-induced hemolysis. It proves that all concentrations effectively stopped heat-induced hemolysis. The greatest percentage protection was 79% at concentrations of 100 µg/ml, while the lowest percentage was 72% at values of 40 µg/ml.

The present study confirm that phytoconstuituents derived from *Mirabilis jalapa* and *Costus igneus* has anti-inflammatory activity. In the HRBC technique, the extract at 100 µg/ml showed 81% protection, close to the standard aspirin (86%), suggesting strong membrane stabilization, which may prevent lysosomal enzyme release and reduce inflammation. In the heat-induced hemolysis method, the extracts also showed effective protection (72–79%) across tested concentrations. One-way ANOVA analysis confirms the statistical analysis for the HRBC membrane stabilization and heat-induced haemolysis method. Both shows that There are no any notable variations between the groups ($p<0.5$) and confirm that *Mirabalis jalapa* and *Costus igneus* have anti-inflammatory property.

5. Conclusion

Both *Costus igneus* and Mirabilis jalapa are excellent medicinal herbs that have been utilised for millennia in Ayurvedic medicine. They found ß-sitosterol, ß-amyrin are among the bioactive substances found in *Mirabalis jalapa* leaves and *Costus igneus* respectively. Significant anti-inflammatory action was shown by the current investigation for both plant extracts, their active ingredients show anti-inflamentory property.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Nath LR, Manjunath KP, Savadi RV, Akki KS. Anti-inflammatory activity of Mirabilis jalapa Linn. Leaves. Journal of basic and clinical pharmacy. 2010 May 15;1(2):93.

[2] Krishnan K, Mathew LE, Vijayalakshmi NR, Helen A. Anti-inflammatory potential of β-amyrin, a triterpenoid isolated from Costus igneus. Inflammopharmacology. 2014 Dec;22:373-85.

[3] Liya FI, Yasmin MF, Chowdhury NS, Charu TK, Fatema IB. Mirabilis jalapa: A review of ethno and pharmacological activities. Advancement in Medicinal Plant Research. 2021;9(1):1-0.

[4] Mathew F, Varghese B. A review on medicinal exploration of Costus igneus: the insulin plant. Int J Pharm Sci Rev Res. 2019 Jan;54(2):51-7.

[5] Leelaprakash G, Dass SM. Invitro anti-inflammatory activity of methanol extract of Enicostemma axillare. International Journal of Drug Development and Research. 2011 Jul;3(3):189-96.

[6] Yadav A, Vaidya M. In vitro Cyclooxygenase inhibitory activity and GC-MS profiling of bioactive compounds in Bauhinia racemosa Lam. Journal of Drug Delivery & Therapeutics. 2024 Oct 1;14(10).

[7] Safayhi H, Sailer ER. Anti-inflammatory actions of pentacyclic triterpenes. Planta medica. 1997 Dec;63(06):487-93.

[8] Sarveswaran RJ, Jayasuriya WJ, Suresh TS. In vitro assays to investigate the anti-inflammatory activity of herbal extracts a review.

[9] Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. Int J Pharm Sci. 2010 Jan;2(1):146-55.

[10] Fadly D. Antibacterial and Anti-inflammatory Activities of Ethanol Extract Obtained from The Hooks of Uncaria Tomentosa (Wild. Ex Schult) DC Originated Kalimantan, Indonesia. Systematic Reviews in Pharmacy. 2020 Jul 1;11(7).

[11] Moualek I, Aiche GI, Guechaoui NM, Lahcene S, Houali K. Antioxidant and anti-inflammatory activities of Arbutus unedo aqueous extract. Asian pacific journal of tropical biomedicine. 2016 Nov 1;6(11):937-44.

[12] Kumari CS, Yasmin N, Hussain MR, Babuselvam M. Invitro anti-inflammatory and anti-artheritic property of Rhizopora mucronata leaves. Int. J. Pharm. Sci. Res. 2015;6:482-5.

[13] Rani K, Priya S, Singh N, Bansal R. Antidiabetic potential of the insulin plant (Costus pictus). In Antidiabetic Plants for Drug Discovery 2022 Dec 29 (pp. 105-146). Apple Academic Press.

[14] Arivu I, Muthulingam M, Selvakumar G. Detailed study on Costus igneus plant for its medicinal importance-A review. International Journal of Zoology and Applied Biosciences. 2023;8(1):34-9.

[15] Okoye NN, Ajaghaku DL, Okeke HN, Ilodigwe EE, Nworu CS, Okoye FB. Beta-Amyrin and alpha-amyrin acetate isolated from the stem bark of Alstonia boonei display profound anti-inflammatory activity. Pharmaceutical biology. 2014 Nov 1;52(11):1478-86.

[16] Shehu S, Bello ZU, Danmalam UH, Ilyas N, Danjuma NM. Topical anti-inflammatory effect and HPLC fingerprint of ethanol extract of the rhizome of Costus spectabilis (Fenzl) K. Schum. Igbinedion University Okada Journal of Pharmaceutical Sciences. 2023 Nov 27;2(1):27-35.

[17] Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. International journal of chemical studies. 2020 Mar 1;8(2):603-8.

[18] Fadly D. Antibacterial and Anti-inflammatory Activities of Ethanol Extract Obtained from The Hooks of Uncaria Tomentosa (Wild. Ex Schult) DC Originated Kalimantan, Indonesia. Systematic Reviews in Pharmacy. 2020 Jul 1;11(7).

[19] https://www.researchgate.net/post/How_to_prepare_2_RBC_suspension/58f5f001ed99e1a6b66bef1d/citation/download

[20] Parthiban S, Kumar KG, Boopathi T, Sangeetha G, Kokila T, Devan VS. In vitro anti inflammatory activity of stem of Zanthoxylum Rhetsa (Roxb.) DC. World Journal of Pharmaceutical Research. 2017;6(3):591-600.