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Invitro Anti-Inflammatory Activity Of *Abutilon Crispum*

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Abstract: The present study investigates the *in vitro* anti-inflammatory potential of the ethanolic extract of *Abutilon crispum* leaves, employing the protein denaturation assay using egg albumin as the model system. Protein denaturation is a widely accepted mechanism implicated in inflammation, and its inhibition serves as an indicator of anti-inflammatory activity. The leaf extract was prepared through Soxhlet extraction with ethanol as the solvent and evaluated at concentrations ranging from 100 to 500 µg/mL. Distilled water was used as the diluent for preparing test solutions, while acetylsalicylic acid (aspirin) was employed as the standard reference drug for comparison. The results demonstrated that the *Abutilon crispum* extract significantly inhibited protein denaturation in a concentration-dependent manner, achieving a maximum inhibition of 72.1% at 500 µg/mL, whereas aspirin exhibited an inhibition of 80.4% at the same concentration. This notable inhibitory effect indicates that the extract possesses considerable *in vitro* anti-inflammatory activity, which may be attributed to its bioactive phytoconstituents. These findings substantiate the traditional medicinal use of *Abutilon crispum* for inflammatory conditions and provide a scientific basis for its potential therapeutic application. However, further *in vivo* studies and detailed phytochemical investigations are recommended to isolate and characterize the active constituents and to elucidate the precise mechanisms underlying its anti-inflammatory effects.

Keywords: *Abutilon crispum*, Anti-inflammatory activity, Phytochemical screening, Soxhlet evaporation, Aspirin.

I. INTRODUCTION:

Inflammation is a nonspecific, defensive response of the body to tissue damage. Among the conditions that may produce inflammation are pathogens, chemical irritations, distortions or disturbances of cells, and extreme temperatures. The four characteristic signs and symptoms of inflammation are redness, pain, heat and swelling. The inflammatory response has three basic stages:

1. Vasodilation and increased permeability of blood vessels
2. Emigration (movement) of phagocytes from the blood into interstitial fluid.
3. Tissue repair.[1]

Histamine, kinins, prostaglandins (PGs), leukotrienes (LT) and various complement system components are

the compounds that contribute to vasodilation, increased permeability and other characteristics of the inflammatory response. There are two primary forms of inflammation: acute inflammation, which is linked to leukocyte emigration, capillary infiltrationand increased vascular permeability. Prolonged inflammation is linked to fibroblast activation, proliferation, fibrosis, neutrophils, monocytes, macrophages and mononuclear immune cell infiltration.[2] *Abutilon crispum*, a member of the Malvaceae family, is traditionally used for various ailments including inflammation. However, scientific validation of its anti-inflammatory potential is limited. This study aims to assess the in vitro anti-inflammatory activity of its ethanolic leaf extract using the egg albumin denaturation method, with aspirin as the standard drug. [3]

1.1.ETHANOMEDICAL STUDIES

Abutilon crispum, a member of the Malvaceae family, has long been recognized in traditional medicinal systems for its therapeutic benefits. In various regions of India, especially in Tamil Nadu and Andhra Pradesh, local communities have employed different parts of this plant to treat a range of ailments. [4] The leaves are commonly used to prepare poultices for treating swellings, wounds, and localized pain, indicating its potential anti-inflammatory and analgesic properties. The root decoction is traditionally consumed for fever management and urinary disorders, while the flowers are used in folk remedies to address respiratory issues like cough and bronchitis [5].

Traditional healers in rural parts of Sri Lanka and southern India report the use of *Abutilon crispum* in managing joint pain and skin infections.[6] These practices support the ethnopharmacological relevance of the plant and suggest that its therapeutic efficacy may be due to the presence of bioactive compounds such as flavonoids, tannins, glycosides, and triterpenoids, many of which are known to exhibit anti-inflammatory action [7].

The alignment of these traditional uses with modern pharmacological findings underscores the importance of integrating ethnomedicinal knowledge into scientific research. These observations not only validate the plant's traditional applications but also provide a rationale for further investigation into its bioactive constituents and mechanisms of action [8].

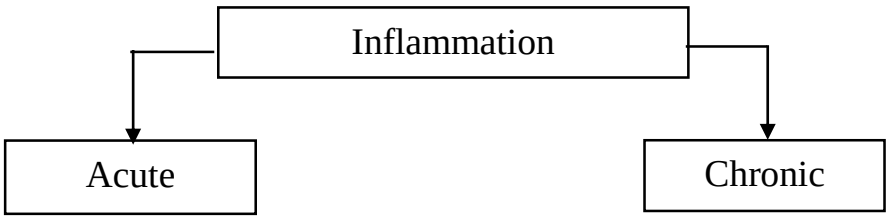
1.2 CARDINAL SIGNS OF INFLAMMATION

Rubor: Hyperaemia
Warm: Hyperaemia
Pain: Chemical mediators
Swelling: Exudation
Loss of Function: Dysfunction [9]

1.3 MECHANISM OF ACTION OF ANTI-INFLAMMATORY AGENTS

The main mechanism of action of NSAIDs is the inhibition of the enzyme cyclooxygenase (COX). Cyclooxygenase is required to convert arachidonic acid into thromboxanes, prostaglandins, and prostacyclins. The therapeutic effects of NSAIDs are attributed to the lack of these eicosanoids. Specifically, thromboxanes play a role in platelet adhesion, prostaglandins cause vasodilation, increase the temperature set point in the hypothalamus, and play a role in antinociception.[10] There are two cyclooxygenase isoenzymes, COX-1 and COX-2. COX-1 is constitutively expressed in the body, and it plays a role in maintaining the gastrointestinal mucosa lining, kidney function, and platelet aggregation. COX-2 is not constitutively expressed in the body; instead, it is inducibly expressed during an inflammatory response. [11] Most of the NSAIDs are nonselective and inhibit both COX-1 and COX-2. However, COX-2 selective NSAIDs (e.g., Celecoxib) only target COX-2 and therefore have a different side effect profile. Importantly, because COX-1 is the prime mediator for ensuring gastric mucosal integrity and COX-2 is mainly involved in inflammation, COX-2 selective NSAIDs should provide anti-inflammatory relief without compromising the gastric mucosa. [12]

1.4 TYPES OF INFLAMMATION FLOW CHART



- Quick and short-term (e.g., a cut, infection)
- Signs: Redness, swelling, pain, heat and loss of function
- Helps with healing

- Long-lasting, can persist for months or years
- Often silent and hidden
- Linked to diseases like arthritis, heart disease, diabetes and even cancer

Figure - 1: Types of inflammation

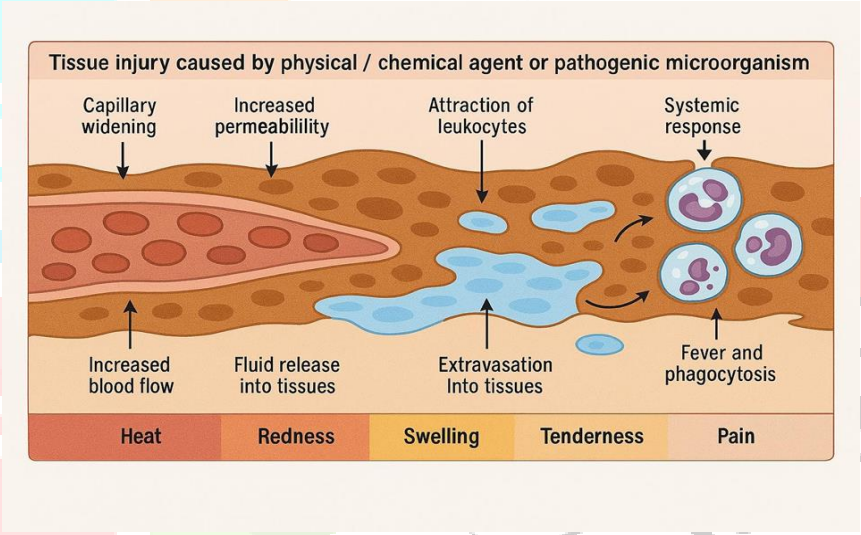


Figure - 2: Site of the inflammation process

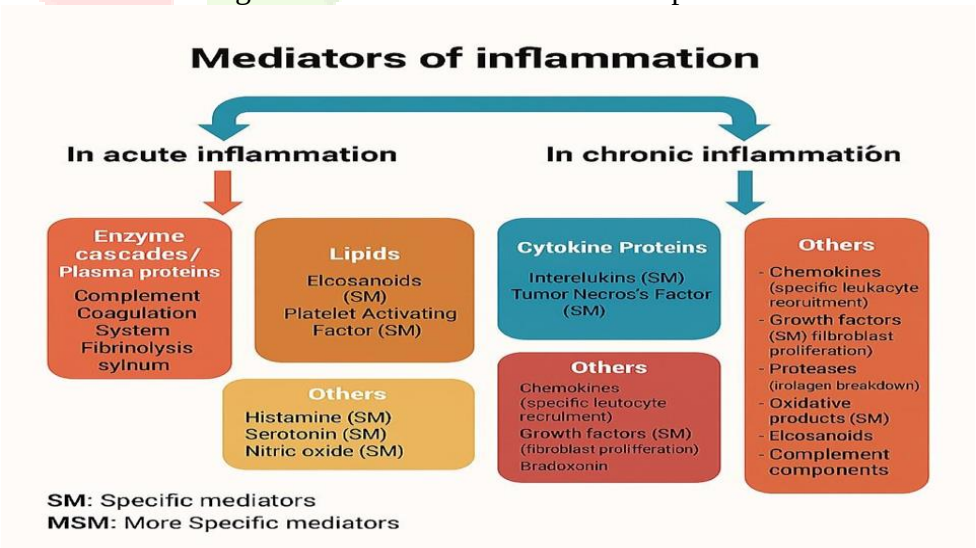


Figure -3: Mediators of inflammation

II. MATERIALS AND METHODS

2.1 Plant Collection:

Abutilon crispum (L.) Medik (Malvaceae) whole plant was collected from Chittoor district, Andhra Pradesh, India, in July. The selected material was identified by Dr. K. Madhava Chetty, Botany Department, Rtd. Assistant Professor, Sri Venkateshwara University, Tirupati, Chittoor district. Later, a voucher sample of the same was placed in the herbarium for reference (0477). [13]

2.2 Extraction by Soxhlet evaporation:

Abutilon crispum whole plant material was dried out in the shade and crushed to obtain a coarse powder. The material was passed over sieve no. 40 and kept in a plate. Material of *Abutilon crispum* was extracted using a Soxhlet apparatus with 95% ethanol for 6-8 hours. The extract was concentrated using a rotary evaporator and stored at 4°C. [14]

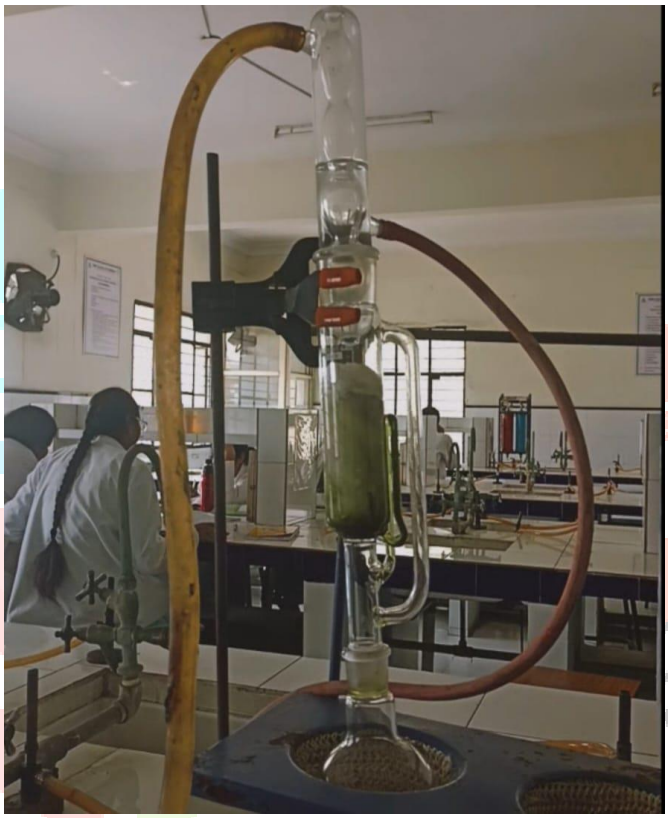


Figure-5: Soxhlet extraction

2.3 Chemicals and Reagents

Egg albumin (fresh hen egg), distilled water, acetylsalicylic acid (standard), and ethanol.

2.4 Methods:

2.5 Phytochemical Screening: Plant bioactive compounds play a crucial role in human health and well-being. Plants exhibit a wide range of primary and secondary metabolites, including flavonoids, terpenoids, phenols, quinones, and coumarins, among others, which are useful for diagnosing various diseases. [15]

Test for Phenols:

Lead Acetate Test: A 10% lead acetate solution is added to 1 ml of plant extract supernatant after it has been diluted with 5 ml of distilled water. When white precipitate is seen, it means that phenol is present.

Saponin Test:

***Test of Foam*: 3 ml** of distilled water is added to 1 ml of plant extract to dilute it. Saponins are present when a 1 cm layer of foam forms.

Test for Tannins:

***Braymer's Test*: 2 ml** of distilled water is mixed with 2 ml of filtrate. Add a couple of drops of 10% $FeCl_3$ solution to this. The presence of tannins is shown by the color change to green

Test for Flavonoids:

***Zinc-Hydrochloride Reduction Test*:** Add a few drops of petroleum ether and a few pieces of zinc dust to 1 ml of the plant sample. Afterward, dropwise add 2 ml of strong hydrochloric acid. Flavonoids are present, as shown by the emergence of the magenta color.

Test for Quinones:

***Sulfuric Acid Test*:** Extract diluted in isopropyl alcohol to 1 ml. 1 ml of concentrated solution to this Drop by drop, H_2SO_4 is added. Quinones are present when the color is red.

Test for Alkaloids:

***Bertrand's Test*:** Add 2 drops of potassium mercuric iodide to 1 milliliter of supernatant. Alkaloids are present when a pale cream color is present. [16]

Test for Terpenoids:

***Salkowski's Test*: 5 ml** of ethanol was added to 2 ml of plant sample. Add 2 cc of mildly warmed and then cooled chloroform to this. Next, pour 1 cc of concentrated H_2SO_4 along the test tube sides. The formation of a grey-colored solution is indicative of terpenoids being present.

Test for Glycosides:

***Legal Test*:** Add 1 ml of the 0.3% sodium nitroprusside reagent and 2 drops of 10% sodium hydroxide to 5 ml of the extract. Glycosides are indicated by a pink to red color.

Test for Coumarins:

***NaOH Test*:** Add a few drops of chloroform and 1 ml of 10% NaOH to 1 ml of sample. The presence of coumarins is indicated by yellow color.

Test for Anthocyanins: HCl Test: Add 2 ml of 2N HCl and 2 ml of ammonia to 2 ml of sample extract. Anthocyanins are indicated by a pinkish-red color that turns bluish-violet when ammonia is added.[17]

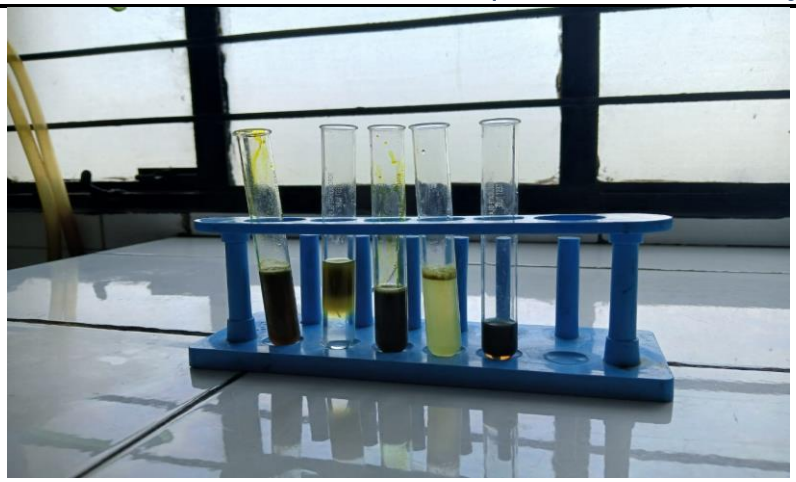


Figure- 6: Phytochemical Tests

Preparation of Test Solutions

The extract was dissolved in distilled water to obtain concentrations of 100, 200, 300, 400, and 500 µg/mL. Aspirin standard was prepared at the same concentrations for comparison.

2.6 In Vitro Anti-Inflammatory Assay

1. Reaction Mixture:
1 mL of egg albumin solution (5% in distilled water), 1 mL of extract or aspirin (test) - 8 mL distilled water [18]
2. Procedure:
The mixturewas incubated at 37°C for 15 minutes.
- Heated at 70°C for 5 minutes, then cooled to room temperature.
- Absorbance measured at 660 nm using a UV-Visible spectrophotometer.

Calculation

% Inhibition = ((Ac - As) / Ac) × 100

Where:
- Ac = absorbance of control (distilled water + egg albumin)
- As = absorbance of sample or standard. [19]

The results of the screening of the ethanolic extract of *Abutilon crispum* (L.) are shown in the table.

Table 3: *Abutilon crispum* phytochemical constituents

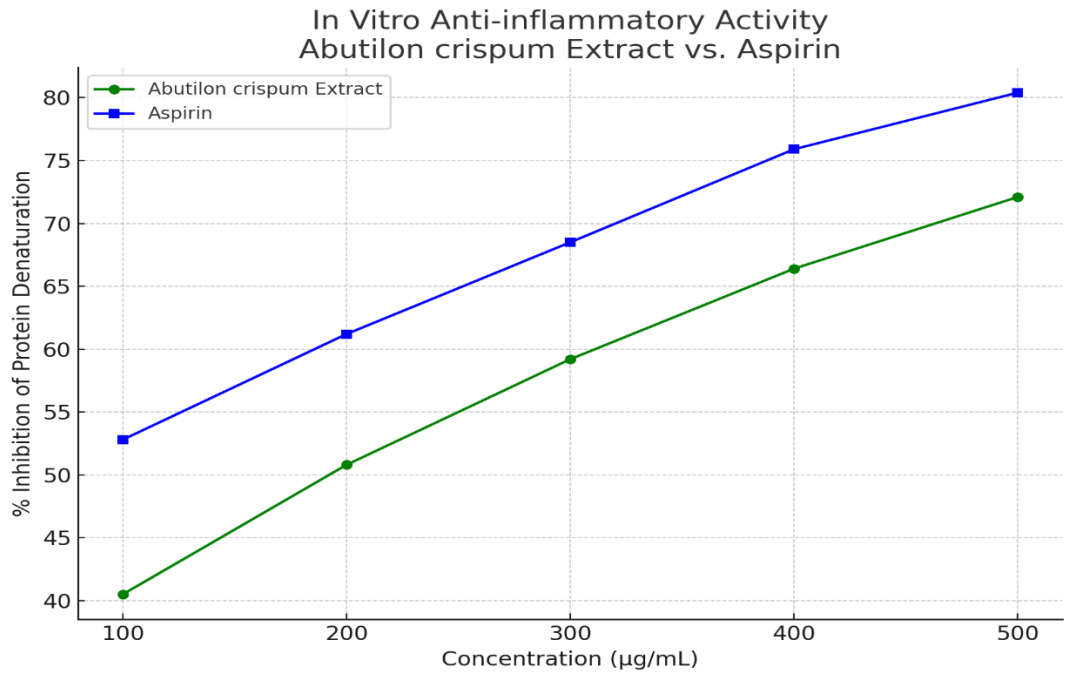
S. No	Chemical test	Observation	Inference		
A	**Alkaloids**		**EtoAc**	**MeOH**	**$CHCl_3$**
	Dragendorff's	Reddish brown precipitate	--	++	++
	Mayer's	White precipitate			
B	**Proteins**	Violet/Pink color	--	--	--
	Million's	Orange color			
	Biuret	Red color precipitate			
C	**Amino acids**		--	--	--

	Ninhydrin	Bluish/ Purple color			
D	**Steroids** Salkowski reaction Lieberman-Burchard reaction	 Yellow Fluorescence Green color	++	++	++
E	**Glycosides** Cardiac glycosides (Keller-Killiani) Test	 ---	++	++	+ +
F	**Flavonoids** Shinoda test	 Pink color	++	++	++
G	**Tannins** Test	 White Precipitate	--	++	--

III. RESULTS

To further confirm anti-inflammatory activity, the extract was analyzed using a UV-Vis spectrophotometer. The scan spectrum curve showed characteristic absorbance peaks at specific Wavelengths, indicating the presence of bioactive compounds. These absorbance patterns suggest the presence of polyphenolic and flavonoid compounds that may contribute to the overall impact of anti-inflammatory activity.

The UV spectrum graph is attached below as instrumental evidence of the extract's photochemical profile.



IV. DISCUSSION

The ethanolic extract of *Abutilon crispum* demonstrated noteworthy anti-inflammatory activity in vitro assays, specifically through its capacity to inhibit heat-induced protein denaturation. [20] Protein denaturation is a well-established model for evaluating anti-inflammatory agents, as the process mimics the alterations that occur during inflammation in biological systems. The ability of the extract to suppress protein denaturation suggests that it may effectively interfere with inflammatory pathways. [21] This bioactivity is likely due to the presence of various phytochemical constituents, notably flavonoids, alkaloids, and tannins, which are well-documented for their anti-inflammatory and antioxidant properties. Flavonoids, for example, are known to inhibit enzymes involved in the inflammatory response such as cyclooxygenase and lipoxygenase, while tannins may stabilize proteins against denaturation and alkaloids may modulate immune responses. [22] Although the standard reference drug, aspirin, exhibited slightly higher inhibition in the assay, the ethanolic extract of *A. crispum* produced comparable results, indicating promising therapeutic potential. Unlike synthetic drugs, which often carry adverse side effects with long-term use, plant-derived compounds may offer a safer alternative for managing chronic inflammatory conditions. The results obtained in this study provide pharmacological evidence that supports the ethnomedicinal use of *Abutilon crispum* in the treatment of inflammation-related disorders. Further studies, including *invivo* evaluations and isolation of individual active constituents, are warranted to fully explore the therapeutic potential and mechanisms of action of this plant. [23]

V.CONCLUSION

The ethanolic extract of *Abutilon crispum* demonstrates notable *in vitro* anti-inflammatory activity, as evidenced by its capacity to inhibit protein denaturation effectively. When compared to the standard drug aspirin, the extract exhibits a comparable and concentration-dependent percentage of inhibition, indicating its promising potential as an anti-inflammatory agent. These findings suggest that *Abutilon crispum* may contain bioactive phytoconstituents responsible for its anti-inflammatory effects. However, to validate its therapeutic efficacy and safety, further *in vivo* studies and comprehensive phytochemical investigations are essential. Such studies could help isolate and characterize the active principles, understand their mechanisms of action, and evaluate their pharmacological profiles. Ultimately, this could lead to the development of a natural and effective alternative or complementary anti-inflammatory treatment derived from *Abutilon crispum*.

V. ACKNOWLEDGEMENT

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