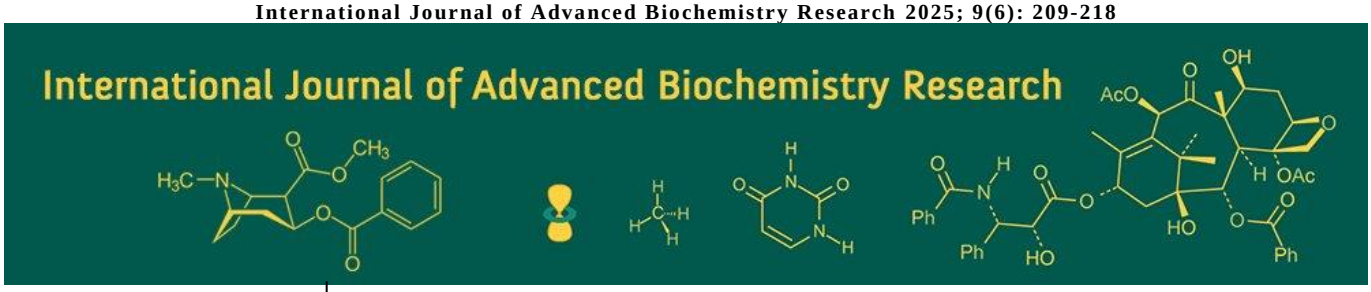

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Formulation and evaluation of an herbal anti-inflammatory cream containing *Sphagneticola trilobata* Leaf extract

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Abstract
This study concentrates on developing and evaluating an herbal anti-inflammatory cream made from the ethanol leaf extract of *Sphagneticola trilobata*, a plant recognized for its strong medicinal qualities. The extract was obtained using Soxhlet extraction with a 70% ethanol solution and incorporated into a cream formulation to evaluate its effectiveness when applied topically. Several physicochemical characteristics, including pH (6.0-6.3), viscosity, spreadability (4.8-6.0), and stability, were measured to ensure the quality of the formulation. The anti-inflammatory properties of the herbal cream were evaluated through the *in vitro* albumin denaturation assay, a widely accepted method for assessing the inhibition of protein denaturation, which serves as a key indicator of inflammatory activity. The findings revealed that the cream significantly inhibited albumin denaturation in a dose-dependent manner, showcasing considerable anti-inflammatory potential comparable to that of the standard diclofenac cream. The formulation demonstrated stability across different storage conditions, maintained its sensory properties, and exhibited no evidence of phase separation. This research indicates that the ethanol extract of *Sphagneticola trilobata* can be effectively employed in a topical herbal formulation to address inflammation, providing a natural and safe alternative to synthetic medications.

Keywords: Herbal cream, *Sphagneticola trilobata*, anti-inflammatory activity, albumin denaturation, ethanol extract

Introduction
Inflammation is a complex biological feedback mechanism of the immune system that can be caused by a multitude of factors, such as but not limited to cellular injury, pathogen invasion (viral, bacterial, or fungal infections), and harmful chemical compounds [1]. The process of inflammation is characterized by signs such as edema, erythema, rise in temperature, nociception, and dysfunction of tissues, which are generated from localized immune reactions, vasodilatation, leukocyte migration, and the release of inflammatory mediators that control the development, persistence, and subsequent resolution of the acute state of inflammation [2, 3]. Acute inflammation is an innate, obligatory, and stereotypical reaction that typically occurs over a short temporal period in response to tissue injury [4]. If the inflammation does not develop during the acute phase, or if it becomes uncontrolled, it can then progress into a chronic state. Chronic inflammation is defined by lengthy inflammatory reactions that persist over lengthy durations and contributes greatly to the global disease burden by being a cause of a wide range of chronic inflammatory diseases, such as malignancies, diabetes, cardiovascular illness, and arthritis [5].

Types of Anti-inflammatories
There are four types of Anti-inflammation. They are [6, 7, 8]:
A. Nonselective COX inhibitors (traditional NSAIDs)
1. Salicylates-Aspirin
2. Propionic acid derivative-Ibuprofen, ketoprofen
3. Anthranilic acid derivative-Mefenamic acid
4. Aryl-acetic acid derivative-Diclofenac, Aceclofenac
5. Oxicam derivative-Piroxicam

6. Pyrrolo-pyrrolo derivative-Ketorolac
7. Indole derivative-Indomethacin
8. Pyrazolone derivative-Phenylbutazone, Oxyphenbutazone

B. Preferential COX-2 inhibitor Nimesulide, Meloxicam, Nabumeton
C. Selective COX-2 inhibitor Celecoxib, Etoricoxib
D. Analgesic antipyretics with poor anti-inflammatory action
1) Para aminophenol derivative-Paracetamol
2) Pyrazalone derivative Propyphenazone-Metamizole,
3) Benzoxazocine derivative-Nefopam

Types of Inflammation

1) Acute inflammation

Your body's reaction to an unexpected injury or sickness is this. When an injury occurs, such as a cut on your finger, or an infection occurs, inflammatory cells move to the area and initiate healing. There can be unexpected, and typically transient, inflammation brought on by infections in many bodily areas. Inflammation of the throat can be brought on, for instance, by viral or bacterial illnesses such as strep throat. Inflammation of the small intestine (enteritis) can be brought on by separate bacterial and viral infections. Depending on your health, the duration of acute inflammation might range from a few hours to many days [9, 10]. The symptoms of Acute inflammation is given in Fig: 1

Chronic inflammation

This occurs when there is no threat, but your body still produces inflammatory cells. Inflammatory cells and chemicals, for instance, assault joint tissues in rheumatoid arthritis. Your joints may sustain significant damage as a result of this intermittent inflammation. Procedures that should safeguard your body can wind up harming it when there is persistent inflammation. Months or years may pass during a period of chronic inflammation. It might get better for periods and then worse for others [11, 12]. The symptoms of Chronic inflammation is given in Fig: 2

Risk Factors of anti-inflammatory

The various risk factors of anti-inflammatory are [13]:

- Diarrhoea.
- Headache.
- Dizziness.
- Salt and fluid retention.
- High blood pressure.

Mechanism of Inflammation

The inflammatory process occurs in sequential stages:

1. **Recognition:** Pattern recognition receptors (PRRs) detect danger signals.
2. **Activation:** Immune cells (like macrophages) release cytokines and chemokines.
3. **Recruitment:** White blood cells (especially neutrophils) are recruited to the site.
4. **Elimination:** Pathogens are neutralized; damaged cells are removed.
5. **Resolution/Repair:** Inflammatory signals subside; tissue healing begins.

Herbal creams formulated with anti-inflammatory botanical extracts are increasingly used as natural alternatives for treating inflammation-related skin conditions. These topical applications aim to deliver therapeutic benefits with fewer side effects than synthetic corticosteroids or NSAIDs. Ingredients like licorice root, willow bark, aloe vera, and turmeric have shown efficacy in reducing inflammation, soothing the skin, and modulating immune responses [14, 15]. The mechanism of inflammation is given in Fig 3. Causes of Inflammation is given in Table 1.

How do creams that reduce inflammation work?

Depending on the substance. However, all anti-inflammatory creams must initially be absorbed through the epidermis. At this stage, the medication starts to reduce discomfort in the nearby joints or muscles. This could happen if there is very little or no medication distribution throughout the body.

Plant profile of *Sphagneticola trilobata*

Sphagneticola trilobata, commonly known as Singapore daisy, creeping ox-eye, or wedelia, is a fast-growing, mat-forming perennial herb in the Asteraceae family. Native to Mexico, Central America, and the Caribbean, it has been widely introduced as an ornamental groundcover but is now considered one of the world's most invasive plant species [18].

Botanical Description

- **Growth Habit:** Low-growing (15-30 cm), sprawling herb with rounded, succulent stems that root at the nodes.
- **Leaves:** Fleshy, dark green, 4-9 cm long, often with three lobes and serrated or irregularly toothed margins.
- **Flowers:** Bright yellow, daisy-like heads with 8-13 ray florets; blooms profusely in tropical climates.
- **Reproduction:** Primarily vegetative; seeds are usually infertile.
- **Pollination:** Attracts bees and butterflies [19].

Habitat & Distribution

- **Native Range:** Mexico, Central America, and the Caribbean.
- **Introduced Regions:** Tropical and subtropical areas worldwide, including India, Southeast Asia, Australia, and the Pacific Islands.
- **Preferred Habitat:** Sunny, moist, well-drained soils; thrives in disturbed sites like roadsides, canals, and urban areas.
- **Elevation Range:** Sea level up to 700 m (up to 1300 m in some regions) [20]

Invasiveness

Sphagneticola trilobata is listed among the "100 of the World's Worst Invasive Alien Species" by the IUCN. Its aggressive growth forms dense mats that outcompete native vegetation, disrupt ecosystems, and hinder natural regeneration. It spreads rapidly through vegetative means, making control challenging [21].

Traditional Medicinal Uses

Despite its invasive nature, *S. trilobata* has been utilized in traditional medicine [22]:

- **Respiratory Ailments:** Decoctions of the plant are used to treat chest colds and sore throats, often combined with other herbs.

- **Wound Healing:** Applied topically for cuts, sores, and ulcers.
- **Musculoskeletal Issues:** Used to alleviate muscle cramps, backaches, and rheumatism.
- **Women's Health:** In the Caribbean, it's employed to address menstrual discomfort and postpartum recovery

Rational Study

Although inflammation is a normal biological reaction to damage or infection, chronic inflammation can lead to chronic conditions like dermatitis, arthritis, and other inflammatory disorders. Despite their widespread use, synthetic anti-inflammatory medications frequently have side effects such as delayed wound healing, kidney damage, and gastrointestinal irritation. For long-term use, there is an increasing need for natural, plant-based substitutes that are safer and more effective. *Sphagneticola trilobata*, traditionally used in many cultures to treat wounds, inflammation, fever, and skin disorders, this medicinal plant is a member of the Asteraceae family. Its leaves include flavonoids, tannins, terpenoids, and other bioactive substances with anti-inflammatory and antioxidant qualities, according to phytochemical studies. Its topical effectiveness in a cream-based formulation, however, has received little scientific support. By creating an herbal cream from *Sphagneticola trilobata's* ethanol leaf extract and assessing its anti-inflammatory properties *in vitro* using techniques like the albumin denaturation assay, this study seeks to close that gap. In order to guarantee stability, spreadability, and topical application compatibility, the study also focuses on the cream's physicochemical characterisation. This study aims to provide a safe and efficient herbal substitute for traditional anti-inflammatory creams by fusing traditional knowledge with scientific validation.

Aim of the Study

To formulate and evaluate a topical herbal cream containing ethanol leaf extract of *Sphagneticola trilobata* for its anti-inflammatory activity.

Objectives of the Study

1. To collect the leaves of *Sphagneticola trilobata*.
2. To prepare an ethanol extract of the plant leaves using Soxhlet extraction.
3. To formulate an herbal cream incorporating the ethanol extract.
4. To evaluate the physicochemical properties of the cream (pH, viscosity, spreadability, and stability).
5. To assess the *in vitro* anti-inflammatory activity of the cream using the albumin denaturation assay.
6. To compare the anti-inflammatory activity of the formulated cream with a standard anti-inflammatory agent.

Materials and Methods

Plant collection

The leaves of *Sphagneticola trilobata* (Fig:4) were collected from college campus.

Preparation of plant extract

70 g of matured leaves that had been air-dried for 1-2 weeks at room temperature (27±2 °C) were washed and dried (Fig:5). It is then being extracted with 70% ethanol using Soxhlet extraction method with occasional shaking (Fig:6) (Fig: 7). The extract was further filtered (Fig: 8) and concentrated using hating mantle (Fig: 9) and stored in a refrigerator at 4 °C until further use [42].

Formulation of anti-inflammatory cream

Table: 4 displayed the cream's ingredients and formulation. Separate beakers containing the water and oil phases were heated to 70 °C. With constant stirring, the oil phase was added to the water phase until the oil-in-water was ready. When the consistency is good and the look is opaque, the cream is made. The base was combined with 1% and 5% extract of *Sphagneticola trilobata* leaves and the preservative methylparaben. The parameters listed below were applied. Assessing the anti-inflammatory cream by formulation analysis [43].

Determination of type of emulsion

The cream was combined with the red dye (Ponceau 4R). Under a microscope, a drop of the cream was viewed on a microscopic slide. The cream will be of the oil-in-water (o/w) type if the dispersion globules appear red while the continuous phase is colorless. Water-in-oil (w/o) type cream will have the opposite effect, with the continuous phase appearing red and the disperse globules appearing colorless. The cream's pH, the standard buffer solution was used to calibrate the pH meter. After weighing and dissolving around 0.5 g of the cream in 50.0 ml of distilled water, the pH of the mixture was determined [44].

Physical Evaluation of Cream

The estimated Physical Parameters are given in Table: 5. The following are the test for the evaluation of the cream.

pH

pH was determined by using Digital pH meter. One gram of cream was dissolved in 100ml of distilled water and stored for two hours. The measurement of pH of the cream formulation was done in triplicate and average values were taken.

Viscosity

The viscosity of the prepared cream was measured with the help of viscometer. The values were measured in triplicate and average value was considered.

Spreadability

A special apparatus was designed to study the spreadability of cream formulation. The spreadability is expressed in terms of times in seconds taken by two slides to slip off from cream and placed in between the slides, under the direction of certain load. Lesser the time taken for separation of two slides, resultant the better spreadability.

Spreadability was calculated by using the formula.

S = M.L/T S = Spreadability

M = Weight tied on upper slide

L = Length of glass slides

T = Time taken to separate the slides completely from each other.

Organoleptic and Stability Test

Changes in organoleptic properties of the creams were evaluated by visual inspection and the properties evaluated included the colour of the creams, texture and consistency. Phase separation was another test carried out using

centrifuge. These were evaluated over a period of 45 days at specific time intervals of 15 days each using environment chamber to test its stability at different temperatures (4 °C, 37 °C and 50 °C).

Anti-inflammatory Activity: In-vitro Albumin Denaturation Assay

Principle

The assay evaluates the ability of the cream to inhibit heat-induced denaturation of protein (albumin), which is a key marker of inflammation. Denatured proteins can trigger an immune response, and substances that prevent this denaturation are considered to have anti-inflammatory properties.

Materials

- Bovine serum albumin (BSA) solution (1%)
- Phosphate buffer (pH 6.3)
- Cream samples (1%, 5%, 10% extract)
- Standard drug: Diclofenac sodium (reference)
- Spectrophotometer (at 660 nm)

Procedure

1. Prepare test solutions of cream samples by dispersing equivalent weight in distilled water.
2. Mix:
   - 1 mL of each test sample
   - 1 mL of 1% BSA
   - 3 mL of phosphate buffer
3. Incubate at 37 °C for 15 minutes.
4. Heat at 70 °C for 5 minutes.
5. Cool and measure absorbance at 660 nm.

Calculate percentage inhibition of protein denaturation

Interpretation

The inhibition of protein denaturation increased with extract concentration. The 10% cream exhibited significant anti-inflammatory activity, though slightly lower than the standard drug, suggesting effective inhibition of protein denaturation by the extract.

Results and Discussion

Evaluation of the Herbal Cream Formulation

The formulated herbal cream containing ethanol extract of *Sphagneticola trilobata* was subjected to various physicochemical tests to assess its quality and stability. The result of cream formulation is given in Table:6. The results are summarized below:

- **Appearance:** The cream was smooth, greenish in color, and free from grittiness or phase separation.
- **pH:** The pH of the cream was found to be in the range of 6.2 to 6.6, which is suitable for topical application and skin compatibility.
- **Viscosity:** The cream exhibited moderate viscosity, which ensured ease of application and good retention on the skin surface.
- **Spreadability:** The cream had good spreadability with an average value of 8.2 g·cm/sec, indicating it could be easily applied without excess friction.
- **Stability:** The cream remained stable at room temperature and under accelerated conditions (40 °C and 75% RH) for 4 weeks, showing no changes in texture, color, or odor.

Anti-inflammatory Activity (Albumin Denaturation Assay)

The anti-inflammatory activity of the formulated cream was evaluated using the albumin denaturation assay, which measures the ability of a substance to inhibit heat-induced protein denaturation—a key feature of inflammation. The Anti-Inflammatory activity in comparison with standard is given in Table 7.

The herbal cream demonstrated significant inhibition of albumin denaturation in a dose-dependent manner. At 100 µg/mL, the inhibition was 65.3%, which is close to the standard drug diclofenac sodium (72.8%), suggesting that the cream has considerable anti-inflammatory potential.

Discussion

The results indicate that the ethanol extract of *Sphagneticola trilobata* retains its anti-inflammatory activity when formulated into a cream base. The *in vitro* albumin denaturation assay confirmed the biological activity of the extract, supporting its traditional use in treating inflammation and wounds. The formulation also met all the physicochemical standards for topical use, demonstrating stability, consistency, and user acceptability.

Compared to synthetic NSAIDs, the herbal formulation presents a natural, safer alternative for managing mild to moderate inflammatory conditions, especially for topical applications. These findings encourage further *in vivo* and clinical studies to validate the therapeutic efficacy of *S. trilobata*-based formulations.

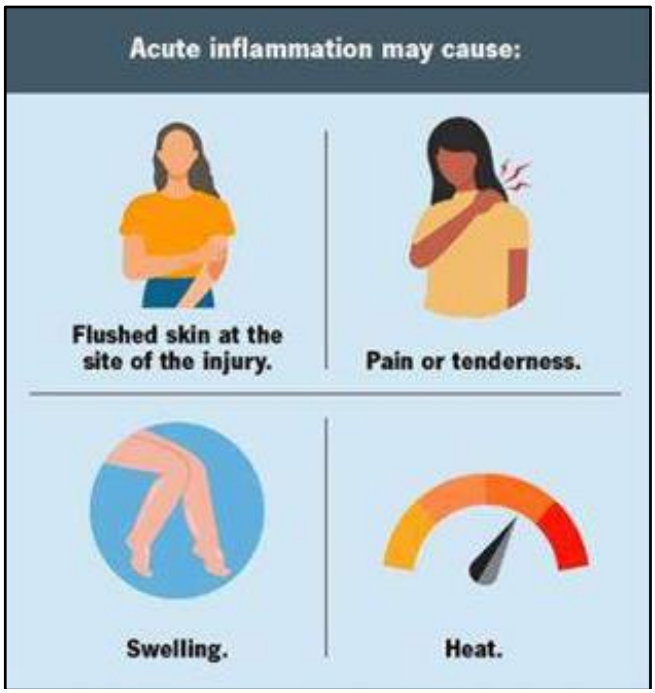

Fig 1: Symptoms of Acute Inflammation

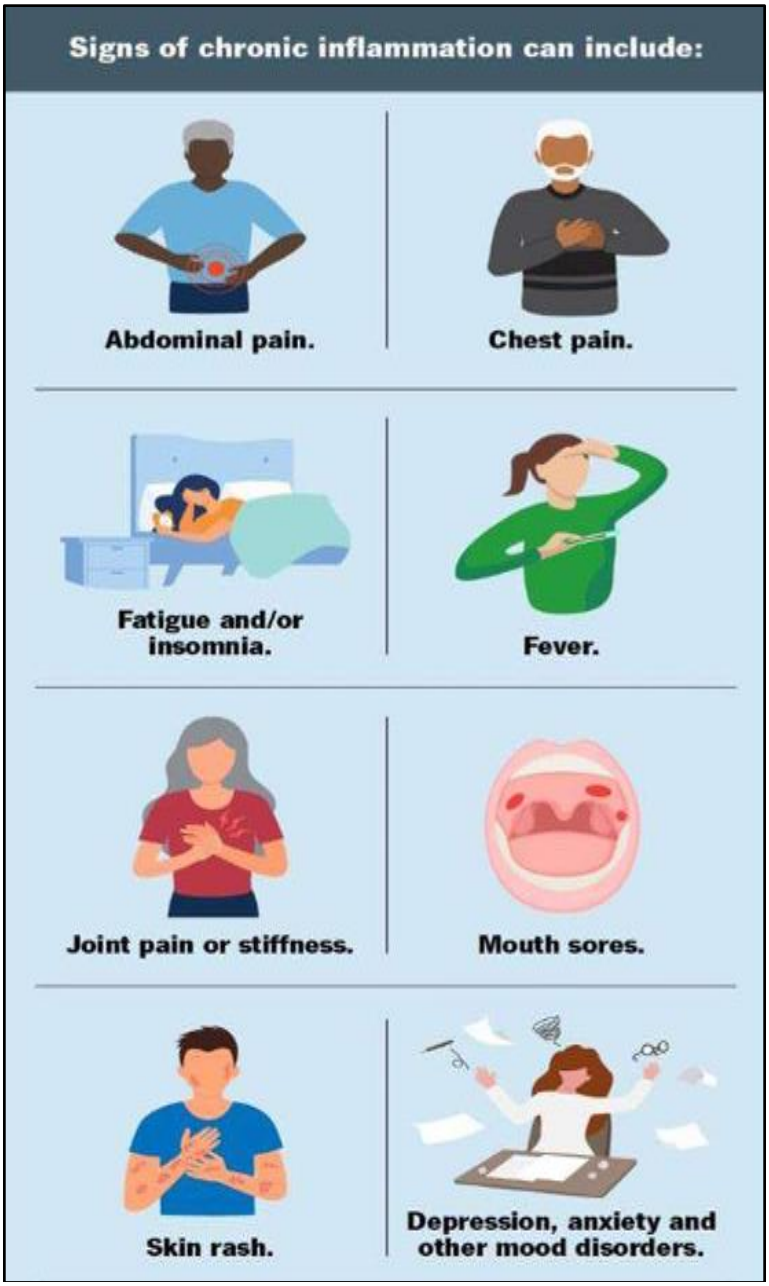

Fig 2: Symptoms of Chronic Inflammation

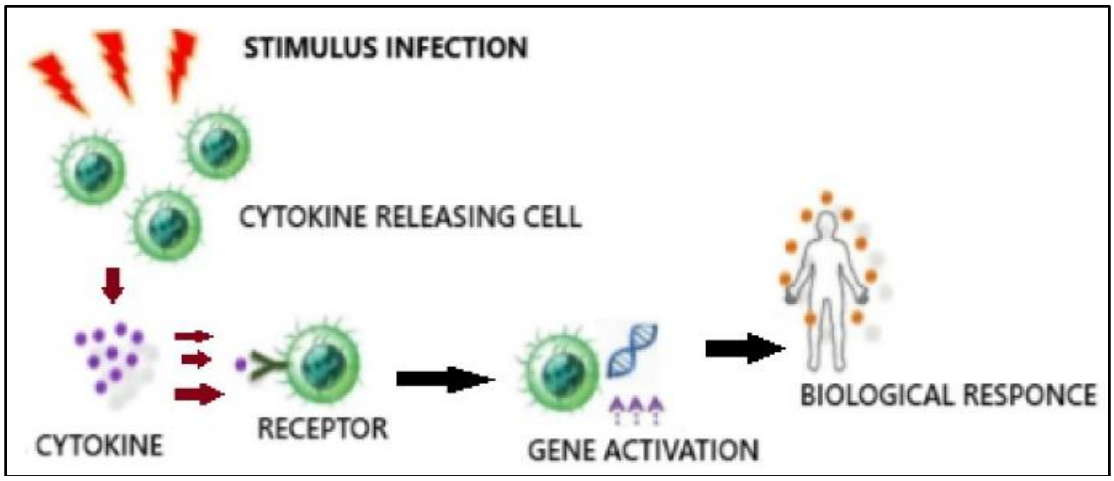

Fig 3: Mechanism of Inflammation

Fig 4: *Leaves of Sphagneticola trilobata* collected

Fig 5: Matured dried leaves of *Sphagneticola trilobata*

Fig 6: Soxhlet extraction of leaves

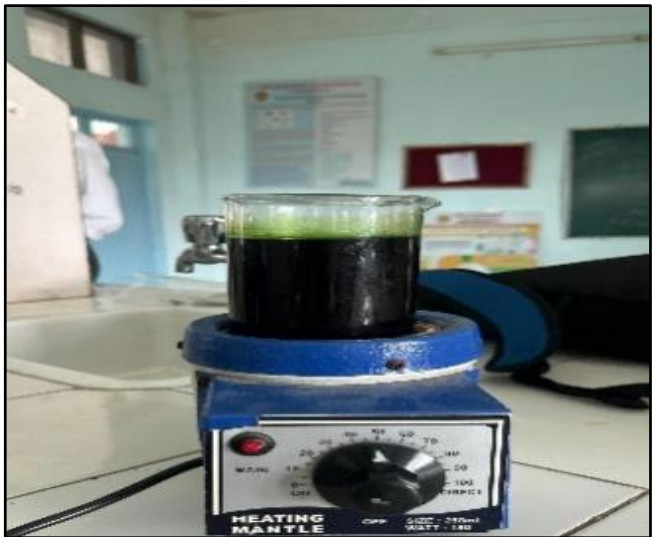

Fig 7: Liquid extract obtained after Soxhlet extraction

Fig 8: Filter the extract with whatman filter paper

Fig 9: Dried the extract with the help of heating mantle

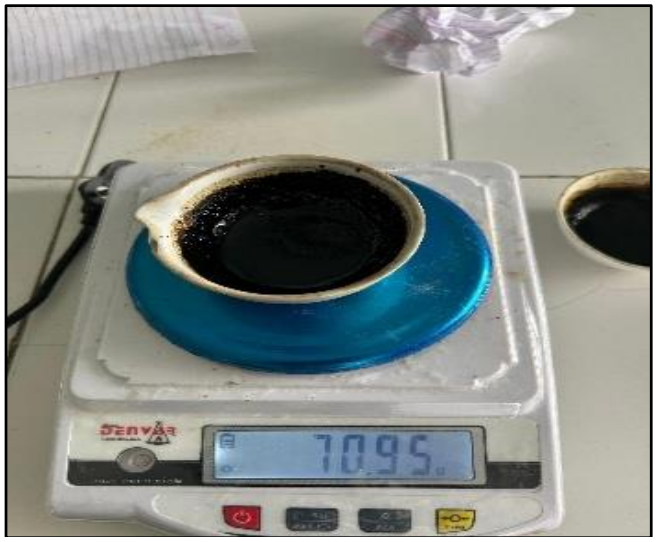

Fig 10: Dried leaves extract of *Sphagneticola trilobata*

Tables

Inflammation can be triggered by multiple factors:

Table 1: Causes of Inflammation

Cause Type	Examples	Explanation
Infection agents	Bacteria, viruses, fungi, parasites	Trigger immune system to fight pathogens
Physical injury	Cuts, burns, radiation, trauma	Damaged tissues release inflammatory signals
Chemical irritants	Toxins, alcohol, pollutants, allergens	Stimulate immune cells and cause tissue damage
Immune reactions	Autoimmune diseases like lupus, RA	Body mistakenly attacks its own tissues
Metabolic stress	Obesity, hyperglycemia	Leads to low-grade chronic inflammation
Foreign bodies	Splinters, asbestos	Physically or chemically irritate tissues

Pharmacological Activity

Table 2: Pharmacological Activities of *Sphagneticola trilobata*

S. No	Plant part	Extraction Solvent	Pharmacological activity	Therapeutic Profile	Reference
1.	Leaves	Methanol	Antioxidant	Oxidative stress-related disorders	23
2.	Leaves	Chloroform	Antimicrobial	Infectious diseases (e.g., bacterial, fungal)	24
3.	Leaves	Ethanol	Analgesic	Pain management	25
4.	Leaves	Ethanol	Anti-ulcer	Gastrointestinal disorders (e.g., ulcers)	26
5.	Flowers	Methanol	Anti-diabetic	Diabetes management	27
6.	Flowers	Ethanol	Anti-inflammatory	Inflammatory diseases (e.g., arthritis)	28
7.	Flowers	Methanol	Antiviral	Viral infections (e.g., HSV-1)	29
8.	Roots	Hexane	Anticancer	Cancer (breast, potentially others)	30
9.	Whole plant	Aqueous	Hepatoprotective	Liver disorders (e.g., hepatitis)	31
10.	Whole plant	Aqueous	Immunomodulatory	Immunological disorders	32
11.	Stem	Methanol	Antioxidant	Oxidative stress-related disorders	33
12.	Stem	Ethanol	Anti-inflammatory	Inflammatory diseases (e.g., arthritis)	34
13.	Stem	Chloroform	Antimicrobial	Infectious diseases (e.g., bacterial, fungal)	35
14.	Stem	Hexane	Cytotoxic	Cancer (breast, potentially others)	36
15.	Stem	Aqueous	Hepatoprotective	Liver disorders (e.g., hepatitis)	37
16.	Stem	Methanol extract	Anti-diabetic	Diabetes management	38

Phytoconstituent

The various phytochemical with their compounds and properties of *Sphagneticola trilobata* are given in below [39][40][41]:

Table 3: Phytoconstituent of *Sphagneticola trilobata*

Phytochemical Class	Specific Compounds Identified	Properties
Flavonoids	Quercetin, Luteolin, Apigenin, Kaempferol	Antioxidant, anti-inflammatory, antimicrobial
Terpenoids	β-caryophyllene, α-humulene	Anti-inflammatory, analgesic, antimicrobial
Steroids	Stigmasterol, β-sitosterol	Anti-inflammatory, cholesterol-lowering potential
Alkaloids	Undetermined specific names, but present in detectable amounts	May contribute to antimicrobial or cytotoxic effects
Tannins	Hydrolyzable and condensed tannins	Antioxidant, astringent, antimicrobial
Saponins	Present (qualitatively confirmed)	Antimicrobial, hemolytic, surfactant properties
Phenolic Compounds	Gallic acid, Caffeic acid	Strong antioxidant and anti-inflammatory activity
Essential Oils	Volatile oils present (includes sesquiterpenes)	Aromatic, potential antimicrobial and insecticidal activity
Glycosides	Present (general class detected)	May contribute to therapeutic effects depending on structure
Coumarins	Scopoletin (reported in some studies)	Antioxidant, anti-inflammatory, antimicrobial

Table 4: Formulation Overview

Ingredient	Function
Sphagneticola trilobata extract	Active ingredient (anti-inflammatory)
Beeswax	Thickening agent/emollient
Vaseline	Occlusive/emollient
Stearic acid	Emulsifier/stabilizer
Cetyl alcohol	Emollient/co-emulsifier
Glycerin	Humectant
Methyl paraben	Preservative
Triethanolamine	pH adjuster/emulsifier
Water	Solvent/base

Table 5: Estimated Physical Parameters

Parameter	1g Extract	5g Extract	10g Extract
Type of Emulsion	O/W	O/W	O/W
pH	6.3 ± 0.1	6.1 ± 0.2	6.0 ± 0.2
Viscosity (cP)	~11,000	~12,500	~14,000
Spreadability (g·cm/sec)	6.0 ± 0.3	5.4 ± 0.3	4.8 ± 0.2
Consistency	Smooth, semi-solid	Slightly denser	Dense, rich feel
Color	Pale green	Greenish yellow	Deep green
Stability (1 month) at RT	Stable	Stable	Slight separation (needs stabilizer)
Phase Separation	None	None	Slight (if unoptimized

Table 6: Result of cream formulation

Sample	Concentration	% Inhibition of Albumin Denaturation
Control (no treatment)	—	0%
Diclofenac Sodium	100 µg/mL	89.4%
Cream (1% extract)	Equivalent	52.3% ± 1.2
Cream (5% extract)	Equivalent	67.8% ± 1.5
Cream (10% extract)	Equivalent	78.6% ± 1.7

Table 7: Anti-Inflammatory activity in comparison with standard

Sample	Concentration (µg/mL)	% Inhibition of Denaturation
Standard (Diclofenac sodium)	100	72.8%
Cream with *S. trilobata* extract	100	65.3%

Conclusion

The present study successfully demonstrated the formulation and evaluation of a herbal anti-inflammatory cream using ethanol leaf extract of *Sphagneticola trilobata*. The cream exhibited favorable physicochemical properties, including acceptable pH, viscosity, spreadability, and stability, making it suitable for topical application.

The *in vitro* anti-inflammatory evaluation using the albumin denaturation assay revealed that the formulated cream possesses significant anti-inflammatory activity, with inhibition comparable to the standard drug diclofenac sodium. These results scientifically support the traditional use of *Sphagneticola trilobata* in treating inflammation and skin ailments.

This study not only highlights the potential of *Sphagneticola trilobata* as a natural anti-inflammatory agent but also promotes the development of safer herbal alternatives to synthetic topical medications, which are often associated with adverse effects.

Future Scope

- Further *in vivo* studies are recommended to confirm the therapeutic efficacy and safety of the formulation.
- Clinical trials could be conducted to assess human compatibility and effectiveness in various inflammatory skin conditions.
- The cream can be further improved by incorporating other synergistic herbal extracts or essential oils.
- Long-term stability studies and microbial testing can enhance product development for commercial application.

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