

Formulation, Physical Evaluation and Pharmacological Screening of Herbal Spray Using PAA Leaves for Anti-Inflammatory Activity

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ABSTRACT:

The present study was carried out to formulate, physically evaluate, and screen the anti-inflammatory activity of an herbal preparation prepared from the leaves of Pongamia pinnata, Azadirachta indica, and Aloe barbadensis. Pharmacological screening was performed using Wistar rats to assess the anti-inflammatory potential of the prepared formulation. The experimental animals were divided into four groups, namely healthy, control, standard, and test groups. Inflammation was induced using a standard experimental method. The control group received only the vehicle, while the standard group was treated with a known anti-inflammatory drug. The test group received the formulated herbal preparation. The study was conducted for a period of 21 days, and observations were recorded on days 1, 3, 7, 14, and 21. The degree of inflammation and wound healing was evaluated through visual scoring and photographic documentation. The results showed a significant reduction in inflammation in the test group when compared with the control group. The activity of the test formulation was found to be comparable to that of the standard drug. These findings indicate that the herbal formulation possesses promising anti-inflammatory activity and supports its potential use in the development of safe and effective herbal therapeutic agent.

Keywords: Pongamia Pinnata, Azadirachita Indica, Aloe Barbadensis, Herbal spray, Anti – inflammatory.

I. INTRODUCTION

Inflammation is the body's natural immune response to injury, infection, or irritation, acting as a defense mechanism to remove harmful stimuli and initiate healing. It typically presents with redness, heat, swelling, pain, and loss of

function. While acute inflammation helps heal injuries, chronic inflammation can damage healthy tissues.

Purpose: It acts as an alarm system to fight bacteria, viruses, or toxins and repair damaged cells.

1.1 Types:

Acute: Develops quickly, lasts a few days, and is necessary for recovery (e.g., a scraped knee or sore throat).

Chronic: A long-lasting, low-grade response that can last months or years, often damaging tissues and contributing to diseases like arthritis, cancer, and heart disease.

Signs & Symptoms: The five cardinal signs are pain, heat, redness, swelling, and loss of function.

Causes: Infections (bacteria/viruses), physical trauma, toxins, or chronic stress.

Significance of the study: This study is important as inflammation is associated with many acute and chronic diseases. Although synthetic anti-inflammatory drugs are effective, prolonged use may lead to adverse effects. Herbal formulations are considered safer and more economical alternatives. The selected medicinal plants are known for their traditional therapeutic properties. This study provides scientific evidence for their anti-inflammatory activity and supports the development of safe and effective herbal formulations.

II. PLANT PROFILE(PAALEAVES)

2.1PONGAMIA PINNATA:

- **Botanical Name:** Pongamia pinnata
- **Family:** Fabaceae
- **Common Name:** Indian Beech
- **Vernacular Name:** Karanj (Hindi), Pungai (Tamil)

- **Geographical Distribution:** *Pongamia pinnata* is widely found in India, Sri Lanka, and Southeast Asia. It grows in tropical and subtropical regions.
- **Description:** It is a medium-sized evergreen tree with compound leaves and purple flowers. The seeds are flat and contain oil.
- **Chemical Constituents:** It contains flavonoids, karanjin, pongamol, and fixed oils.
- **Medicinal Uses:** Traditionally, it is used in skin diseases, wounds, inflammation, and rheumatism. It also shows antibacterial and anti-inflammatory activity.



Fig: 1 *Pongamia Pinnata*

2.2 AZADIRACHTA INDICA:



Fig: 2 *Azadirachta Indica*

- **Botanical Name:** *Azadirachta indica*
- **Family:** Meliaceae
- **Common Name:** Neem
- **Vernacular Name:** Neem (Hindi), Vembu (Tamil), Bevu (Kannada)
- **Geographical Distribution:** Neem is widely found in India, Bangladesh, Sri Lanka, and tropical regions. It grows well in dry and semi-dry areas.
- **Description:** Neem is a fast-growing evergreen tree with compound leaves and small white

flowers. The fruits are oval-shaped and yellow in color.

- **Chemical Constituents:** Neem contains Azadirachtin, nimbin, nimbidin, flavonoids, and tannins.
- **Medicinal Uses:** Neem is used in treating skin diseases, wounds, inflammation, infections, and fever. It has antibacterial, antifungal, and anti-inflammatory properties.

2.3 ALOE BARBADENSIS:



Fig: 3 *Aloe Barbadensis*

- **Botanical Name:** *Aloe barbadensis*
- **Family:** Liliaceae
- **Common Name:** Aloe vera
- **Vernacular Name:** Kathalai (Tamil), Gwarpatha (Hindi), Lolesara (Kannada)
- **Geographical Distribution:** Aloe vera is found in tropical and subtropical regions. It is commonly cultivated in India, Africa, and Asia.
- **Description:** Aloe vera is a short-stemmed succulent plant with thick, fleshy, green leaves. The leaves contain clear gel inside.
- **Chemical Constituents:** It contains aloin, aloe-emodin, polysaccharides, vitamins, enzymes, and amino acids.
- **Medicinal Uses:** Aloe vera is used in wound healing, burns, skin care, inflammation, and digestive problems. It has soothing, moisturizing, and anti-inflammatory effects.

III. MATERIALS AND METHODS

3.1 Collection Of Crude Material:

Freshly collected *Pongamia pinnata* and *Azadirachta indica* from surrounding area. The freshly leaves are thoroughly washed with fresh water. The leaves were subjected to shade drying for 30 days. The shade dried leaves were finely coarse powdered separately. The finely leaves

powered were passed sieve no.85 separately from impurities.

Lately collect the aloe vera freshly and clean thoroughly and add in after maceration process

3.2 Collection Of Solvent:

90% ethanol was collected from our college

3.3 Maceration Process:

The 500ml conical flask were taken for this process, weigh accurately 30g of Pongamia pinnata and 30g of Azadirachta indica was taken and they transferred into mortar and pestle for mix uniformly. 250ml of 90% absolute ethanol in to 500ml conical flask and the uniform mixer of Pongamia pinnata and Azadirachta indica into it. The mouth of conical flask was covered with cotton plug and aluminium foil. The conical flask lasted 7 days with intermittent shaken. After filter with filter paper using funnel. The filtered solvent is transferred into 500ml freshly conical flask and covered with cotton plug.

3.4 Formulation Of Anti Inflammation Spray:

A 500ml conical flask was washed with water and then it was rinsed thoroughly with 90% ethanol. The required chemical was measured accurately using 100ml measuring Cylinder. Isopropyl alcohol (40ml); acetone (25ml); were first accurately measured and transferred into a 500 ml conical flask. Mix uniformly using a glass rod /stirrer. Then add herbal extract 5ml [Pongamia pinnata, Azadirachta indica - 3ml; aloe barbadensis - 2ml] to the conical flask and mix then. Then incorporated water (25ml) to the 500ml conical flask and finally added glycerine (5ml) into the 500 ml conical flask. Mix the contents in the conical flask thoroughly using a stirrer until we get a uniform mixture. Transfer the prepared solution into the sterile spray apparatus.

S.NO	INGREDIENTS	QUANTITY
1.	Herbal extract	5ml
2.	Iso propyl alcohol	40ml
3.	Acetone	25ml
4.	Glycerine	5ml
5.	Purified water	25ml

Table: 1 Formulation Of Spray

3.5 Preliminary Phytochemical Screening Of Herbal Spray:

3.5.1 Test for Flavonoids: Lead acetate test: Add a few drops of 10% lead acetate solution to the plant aqueous extract. A positive result for flavonoid is indicated by the formation of a yellow precipitate.

3.5.2 Test For Tannins: Ferric chloride test: Add a few drops of a 1% ferric chloride solution to a plant extract; a positive result for tannin is a colour change to a greenish black precipitate.

3.5.3 Test for Phenol: Add a few drops of 1% of 2ml ferric chloride solution to a plant extracts; a positive result for phenol is a colour change to greenish black precipitate.

3.5.4 Test for Glycosides: Keller Killani test: Add 2ml of extract and add 1ml of glacial acetic acid and add a few drops of ferric chloride. Carefully add 1ml of conc. H₂SO₄ inside the test tube. A positive result for glycoside is a reddish-brown ring at the junction.

3.5.5 Test for Terpenoids: Salkowski test: Add 5ml of extract and add 2ml of chloroform and then carefully add 3ml of conc. H₂SO₄. A positive result for terpenoids is reddish brown colouration at the interface.

3.5.6 Test for Carbohydrate: Molish test: 2 drops of molish's reagent to the test solution. Carefully add conc. H₂SO₄ down the side of the test tube. A positive result for carbohydrates is formation of a violet at the interface.

3.5.7 Test for Protein: Biuret test: Add 2ml of sample and 2ml of 10% NaOH and mix and add 2-4 drops of 1% CuSO₄ drop wise. The test stands for 1-2 mins. A negative result for protein is the appearance of a reddish orange colour.

3.5.8 Test for Saponin: Foam test: Take a sample and add 10ml of distilled water. Vigorously shake for 30 seconds and stand for 15 minutes. A negative result for saponin is no persistent foam is observed.

3.6 Evaluation Of Herbal Spray:

3.6.1 Physical Parameters:

Colour: Appearance: Examine the Herbal Spray colour, which can range from clear to tinted. The colour should be uniform and stable over time.

pH Level: Acidity/Alkalinity: Measure the serum's pH to ensure it's within a safe range for skin, typically between 4.5 and 6.5. This helps prevent irritation and ensures compatibility with the natural nail environment.

Density: Weight: Determine the density of the Herbal Spray, which influences how much product is needed per application and how it feels on the nails.

3.6.2 Flame Test: Take a small lighter and ignite a steady flame. Hold the spray can about 15cm away from the flame. Press the nozzle so that the spray passes directly through the lighter flame. Observe whether the flame gets extended or if the flame returns back to the nozzle.

3.6.3 Spray Pattern: Take a clean filter paper and place it on a flat surface. Hold the spray bottle 10cm above the centre of the paper. Press once to spray. Allow the liquid to spread for 1 min. Measure the diameter of the stain formed on the filter paper.

3.6.4 Primary Skin Irritation Test: The prepared formulations were assessed for primary skin irritation test. Healthy human volunteer was selected for the study choose a small area of skin, cleanse the area with mild soap and water, let it dry completely. Apply a small amount of the serum to the test area allow to spread on skin. Leave the serum on your skin for 24 to 48 hours. After 24 to 48 hours observe the area for any sign of irritation, redness, swelling, itching or any other reactions.

3.7 Pharmacological Screening:

Experimental animals:

Healthy adult Wistar albino male sex rats, weighing between 150g –200g, were used for the study. The animal was obtained from an authorized animal house. They were kept under standard laboratory condition with controlled temperature, humidity, and 12hrs light and dark cycle. Standard pellet diet and water provided. The study was conducted according to ethical guidelines.

Grouping of animals

The animal was divided into four groups, there are,

GROUP NO.	GROUP NAME	TRESTMENT
1.	Healthy group	Normal diet only
2.	Control group	Inflammation induced only
3.	Standard group	Inflammation induced and standard drug
4.	Test group	Inflammation induced and test drug

Table: 2 Grouping Of Animals

IV. RESULT AND DISCUSSION

4.1 Extraction Appearance And Percentage Yield:

Drug	Pongamia Pinnata, Azadirachita Indica, Aloe Barbadensis
Solvent	Ethanol
Colour	Dark Green
Consistency	Liquid
Percentage yield	69.5 % w/w

Table: 3 Extraction Appearance And Percentage Yield

4.2 Evaluation Of Herbal Spray:

S.NO	PARAMETERS	INFERENCE
1	State	Liquid
2	Color	Light green
3	Odour	Aromatic Odour
4	PH	7.37
5	Specific gravity	1.015
6	Sensitivity test	No irritation
7	Irritation test	No irritation

Table: 4 Evaluation Of Herbal Spray

4.3 Preliminary Phytochemical Test Results Of Herbal Spray:

S.NO	PHYTOCHEMICAL TEST	PRESENCE OR ABSENCE
1.	Flavonoids	+
2.	Tannins	+
3.	Phenol	+
4.	Glycosides	+
5.	Terpenoids	+
6.	Carbohydrate	+
7.	Protein	-
8.	Saponin	-

Table: 5 Preliminary Phytochemical Test Results Of Herbal Spray



Fig: 4 Preliminary Phytochemical Test Results Of Herbal Spray

4.4 Flame Test:

Take a small lighter and ignite a steady flame. Hold the spray can about 15cm away from the flame. Press the nozzle so that the spray passes directly through the lighter flame. Observe whether the flame gets extended or if the flame returns back to the nozzle.



Fig: 5 Flame Test

4.5 Spray Pattern:

Take a clean filter paper and place it on a flat surface. Hold the spray bottle 10cm above the centre of the paper. Press once to spray. Allow the liquid to spread for 1 mins. Measure the diameter of the stain formed on the filter paper.



Fig: 6 Spray Pattern

4.6 Graph:

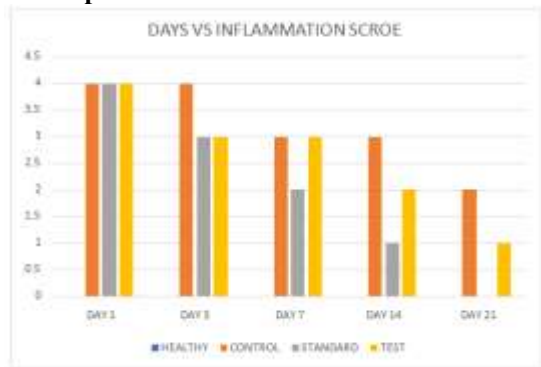


Fig: 7 Values Are Expressed As Mean Inflammation Score. The Test And Standard Group Showed A Gradual Reduction In Inflammation Compared To Control

4.7 Observation Table Scale:

GROUP	DAY1	DAY3	DAY7	DAY14	DAY21
HEALTHY	0	0	0	0	0
CONTROL	4	4	3	3	2
STANDARD	4	3	2	1	0
TEST	4	3	3	2	1

Table: 6 Observation Table Scale

4.8 Percentage Improvement Table:

GROUP	% IMPROVEMENT (21 DAYS)
HEALTHY	-
CONTROL	5
STANDARD	100
TEST	75

Table: 7 Percentage Improvement Table

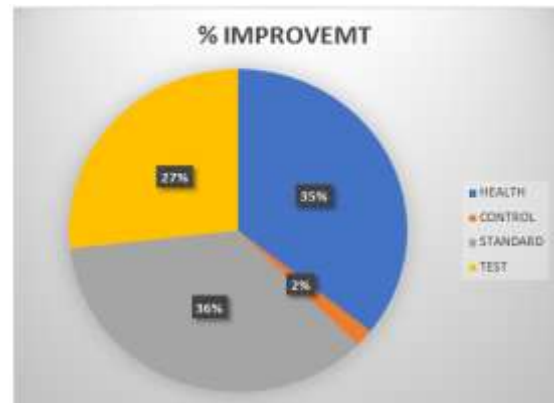


Fig: 8 Percentage Improvement Table

4.9 Pie Chart:

GROUP	% IMPROVEMENT (21 DAYS)
HEALTHY	-
CONTROL	5
STANDARD	100
TEST	75

Table: 8 The Pie Chart Represents The Percentage Improvement In Inflammation On 21 Days, Showing Comparative Efficacy Of Control, Standard And Test.

4.10 Inflammation Score Scale:

SCORE	OBSERVATION
0	NORMAL SKIN
1	MILD REDNESS
2	MODERATE SWELLING
3	SEVERE INFLAMMATION
4	VERY SEVER INFLAMMATION

Table: 9 Inflammation Score Scale

4.11 Observation Days:





















GROUP/ DAYS	DAY 1	DAY 3	DAY 7	DAY 14	DAY 21
HEALTHY GROUP					
CONTROL GROUP					
STANDAR D GROUP					
TEST GROUP					

Table: 10 Observation Days

V. CONCLUSION

An herbal spray was formulated using Pongamia pinnata (fixed oils, Furano flavonoids, sterols, glycoside, tannins, saponins, alkaloids, flavonoids), Azadirachta indica (limonoids, flavonoids, sterols, tannins, fatty acids,

polysaccharides, amino acids) and Aloe barbendensis (polysaccharides, anthraquinones, chromones, enzyme, vitamins, minerals, amino acid and other constituents). These mainly contains three compounds namely like karanjin, nimbidin, and anthraquinones are good anti-inflammatory

property. These phytoconstituents are possess for their anti-inflammatory, anti-oxidants, and wound-healing properties. Preliminary evaluation showed that the spray was stable and effective in anti-inflammatory activity. The combination of *Pongamia pinnata*, *azadirachta indica* and *Aloe barbadensis* showed better activity than individual components, suggesting a synergistic effect. The herbal spray was found to be safe in the flame test, showing no sustained ignition. The spreadability test confirmed uniform spreading, ensuring easy application and effective skin coverage. the combination showed synergistic anti-inflammation within 21 days in an albino rat. Though silver sulphadiazine acted faster, the spray efficacy positions it as a viable natural alternative for skin burn wound. The pharmacological screening confirm that the formulated herbal spray is safe, stable, and effective wound healing properties, using as topical herbal formulation.

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