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FORMULATION AND EVALUATION OF POLYHERBAL SPRAY CONTAINING *VITEX NEGUNDO* EXTRACT, *AZADIRACHTA INDICA* OIL FOR IN-VITRO ANTI-INFLAMMATORY ACTIVITY

Dr. M. Senthil Raja, Mrs. K. Sojarna*, S. Bharath, V. Logeshwaran, T. Poovarasu, S. Sridhar

P.S.V College of Pharmaceutical Science and Research, Orappam(Village), Krishnagiri (Dt) Tamilnadu-635108 Bachelor of Pharmacy (B Pharm).

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***Corresponding Author**
Mrs. K. Sojarna
P.S.V College of Pharmaceutical Science and Research, Orappam(Village), Krishnagiri (Dt) Tamilnadu-635108 Bachelor of Pharmacy (B Pharm).

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1. ABSTRACT

Vitex negundo extract and *Azadirachta indica* oil was used in the study to create a herbal anti-inflammatory spray. The Maceration method was used to prepare ethanolic (F1) and (F2) extracts, respectively. Comparing their physicochemical characteristics and anti-inflammatory effectiveness was the goal. Appearance (colour, odour, clarity), pH measurement, viscosity, stability analysis, and leak test were all included in the evaluation. The protein denaturation assay was used to evaluate the anti-inflammatory efficacy. Proteins become denatured (lost their normal structure) during inflammation. Compounds that stop proteins from becoming desaturated are thought to have anti-inflammatory properties. The compositions dried quickly, were non-sticky, and stable (pH: F1, F2). Good physicochemical characteristics and a significant inhibition of protein denaturation demonstrated the formulation's promising anti-inflammatory capability, pointing to potential alleviation from inflammatory pain. The results showed that the herbal topical formulation has good formulation qualities. By preventing, the study recommends aqueous extraction. Denaturation of proteins and cell membrane stabilization. Strong anti-inflammatory properties were shown by the *Vitex negundo* herbal spray, which may help with pain relief, swelling reduction, and muscular spasms. Additional research on in vitro efficacy and long-term stability is necessary.

KEYWORDS: *Vitex negundo*, Phytochemicals, Maceration, Protein denaturation assay, Evaluations, Anti-inflammatory activity.

2. INTRODUCTION

The body's natural defines mechanism against damage, infection, irritation, and other harmful stimuli is inflammation. It is a crucial biological function for the body's healing and defense systems. On the other hand, excessive or persistent inflammation can cause tissue damage, pain, swelling, and discomfort. Therefore, creating potent anti-inflammatory drugs is crucial for treating inflammatory diseases. Although many people utilize conventional anti-inflammatory medications, long-term usage of some synthetic medications may have negative effects. This has sparked interest in herbal remedies and medicinal plants as sources of possible naturally occurring therapeutic ingredients. Nirgundi, or Vitex negundo Linn., is a significant medicinal plant belonging to the Lamiaceae family. Its analgesic and anti-inflammatory properties have led to its traditional use. Flavonoids, alkaloids, glucosides, saponins, and volatile oils are among the phytoconstituents found in the leaves that give them their therapeutic properties. Using the proper spray apparatus, a liquid preparation is dispersed into tiny droplets to create a medicinal dose form known as spray. It provides a useful method of applying a composition and allows the active elements to be distributed across the required area. A perfect herbal spray must have good stability, a suitable pH, a consistent spray pattern, decent spread ability, fast drying, a bearable odour, and non-irritating qualities. In this work, a herbal spray is made by combining *Vitex negundo* leaf extract with camphor and neem oil. Two formulations, F1 and F2, are prepared with different concentrations of leaf extract for comparison. The prepared formulations are evaluated for several physicochemical parameters, including organoleptic properties, homogeneity, consistency, clarity, evaporation time, stickiness, pH, solubility, and leak test, to determine the physical quality, stability, and suitability of the herbal spray formulation. *Vitex negundo* extract's anti-inflammatory qualities are evaluated using the protein denaturation assay. This method investigates the extract's ability to stop protein denaturation at different concentrations. The project results demonstrate a concentration-dependent increase in inhibition from 18% at 100 µg/mL to 72% at 500 µg/mL, suggesting promising in-vitro anti-inflammatory efficacy.

3. AIM

• The aim of the present research is to formulate and evaluate an herbal spray using ethanolic methods of selected medicinal plants and to compare their physicochemical properties, stability

and anti-inflammatory activity.

4. OBJECTIVES

- To achieve the aim, the following objectives were set:
- To select suitable medicinal plants based on their known anti-inflammation properties

➢ Ethanolic extract

- To formulate polyherbal spray formulation using extracts and appropriate excipients.
- To carry out organoleptic evaluation for both formulations.
- To determine physicochemical parameters, including:

➢ pH

➢ Spray angle

➢ Leak test

➢ Stickiness test

- To evaluate anti-inflammatory activity of both formulations against protein denaturation assay.
- To compare the results of F1and F2 formulations and determine which extraction method is more effective for herbal spray preparation.
- To provide a scientific rationale for the use of herbal combinations in topical spray formulations.

5. DRUG PROFILE

5.1 VITEX NEGUNDO

Nirgundi (Vitex negundo Linn.)

Synonym: Sindhuvara, swetapushpa, sinduka, sinduvaraka, nilapushpi, suvaha.

Biological source: Vitex negundo Linn.

Family: Lamiaceae.

Geographical Source: This species is native to the tropical regions of Eastern and Southern Africa, as well as parts of Asia.

USES: Nirgundi (Vitex negundo) has traditionally been utilized for its anti-inflammatory and analgesic properties. It is used as Rheumatoid Arthritis (RA) and joint pain.

5.2 AZADIRACHTA INDICA

SYNONYMS: Melia azadirachta L., Antelaea azadirachta (L.) Adelb.

Biological Source: Azadirachta indica A.

Geographical source: Neem is mainly distributed throughout the Indian subcontinent and is native to India. It is widely cultivated in tropical and subtropical regions, particularly in dry and semi-arid areas.

Uses: Neem (Azadirachta indica) is traditionally used for its antimicrobial, anti-inflammatory, antipyretic and wound-healing properties. It is used in the management of skin infections, acne, eczema and minor wounds.

6. METHODOLOGY: MATERIAL AND METHODS

Collection of plant material	Fresh, healthy Vitex negundo leaves and authentication, were collected, cleaned, and washed thoroughly to remove foreign matter and impurities.
Shade drying	After washing, the leaves were shade-dried for seven to ten days at room temperature (25 to 30°C), rotating them occasionally, until they were totally dry and crisp.
Powder preparation	Vitex negundo leaves that had been dried were ground into a fine powder, sieved through 40 mesh, and kept in an airtight container.
Preparation of extract	25 g of powdered Vitex negundo leaves were extracted by maceration using 70% ethanol.
Maceration method	250 mL of ethanol was used to soak 25 g of dried Vitex negundo leaf powder for 72 hours at room temperature with sporadic shaking. The mixture was filtered, and a water bath was used to concentrate the filtrate at 40–50°C. The ethanolic extract that was produced was gathered, weighed, and kept for later research in an airtight container.

Fig. 1: ***Vitex negundo.***

7. INGREDIENT USED IN FORUMULATIONS

SI.NO	INGEDIENTS	FUNCTIONS	F1(50)	F2(50ml)
1.	***Vitex negundo* leaf extract**	Main herbal (Active anti-inflammatory agent)	1.0g	2.0g
2.	**Neem oil**	Active anti-inflammatory agent	1ml	1ml
3.	**Camphor**	Cooling and pain-relieving agent	1g	1g
4.	**Ethanol 95%**	solvent	40.00ml	40.00ml
5.	**Propylene glycol**	Co-solvent and humectant	5ml	5ml
6.	**Glycerine**	Moisturizer	5.00ml	5.00ml
7.	**Tween 80**	Solubilizing agent(emulsifier)	2.00ml	2.00ml
8.	**Sodium benzoate**	preservative	0.10g	0.10g
9.	**Citric acid**	PH adjustments	0.05g	0.05g
10.	**Distilled water**	Vehicle (Base)	q.s for 50ml	q.s for 50ml

7.1 PREPARATION PROCEDURE

Preparation of oil phase: Fill a clean beaker with 15 ml of 95% ethanol. Stir in 1 g of camphor until it dissolves all. Mix thoroughly after adding 1 mL of neem oil. To achieve a clear, homogeneous oil phase, add 1 mL of Tween 80 and mix for 5 to 10 minutes.

Preparation of aqueous phase: Fill a separate beaker with 20–25 mL of filtered water. 0.05 g of sodium benzoate should be well dissolved. Stir with 0.05 g of citric acid until it dissolves. Stir constantly while you add 2.5 mL of glycerine and 5 mL of propylene glycol. Slowly add 2.5 g of Vitex negundo leaf extract and stir until the mixture is uniform.

Mixing: Use a glass rod to continually agitate the aqueous phase or place it on a magnetic stirrer. Stir constantly as you gradually add the oil phase dropwise into the aqueous phase. To achieve a consistent and stable composition, keep stirring for 15 to 20 minutes.

Make Up the Volume: Add purified water (q.s.) to make the final volume 50 ml.

Filteration: To get rid of any particles that haven't dissolved, filter the mixture using Whatman No. 1 filter paper.

Filling: Fill a sterile 50 mL spray container with the filtered mixture. Put a label on it and keep it out of direct sunlight in a cool, dry location.

8. FORUMULATION PROCEDURE F1 & F2

1. **Preparation of Herbal extract:** To get a clear extract, prepare the Vitex negundo leaf extract and filter it.
2. **Preparation of solvent phase:** Take 15 mL of ethanol (95%) in a clean beaker. Add 1 g of camphor and stir until it dissolves completely.
3. **Addition of cosolvent:** Add 5 ml of propylene glycol. 2.5 mL of glycerine should be added. To get a homogenous solution, thoroughly mix.
4. **Addition of Emulsifier:** Add 1 mL of Tween 80 and mix thoroughly.
5. **Addition of Active ingredients:** Add F1[1.0 g] and F2[2.0 g] of *Vitex negundo* leaf extract while stirring constantly. Mix in 1 mL of neem oil until the mixture is uniform.
6. **Addition of preservative:** Add 0.05 g of sodium benzoate to the formulation after dissolving it in a little amount of pure water.
7. **PH adjustments:** Gradually add 0.05 g of citric acid until the pH is between 5.5 and 6.5.
8. **Make up the final volume:** Make the final volume 50 mL by adding purified water (q.s.). For ten to fifteen minutes, stir constantly.
9. **Filteration:** To get a clear spray solution, filter the mixture using muslin cloth & Whatman No. 1 filter paper.
10. **Filling and storage:** Fill a sterile 50 mL spray container with the filtered mixture. Put a label on it and keep it out of direct sunlight in a cool, dry location.

9. PHYTOCHEMICAL TEST

PHYTOCHEMICAL CONSTITUENT	TEST USED	PROCEDURE	POSTIVE OBSERVATION
Flavonoids	Shinoda test	Magnesium ribbon And concentrated HCL added to extract	Red/pink coloration formed
Flavonoids	Lead acetate test	Extract added to lead acetate solution.	Yellow-precipitate formed
Alkaloids	Mayers test	Extract treated with Mayers reagents.	White precipitate formed
lkaloids	Wagners test	Extract treated with wagers reagents.	Reddish-brown precipitate formed
Phenolic compound	Ferric chloride test	Fecl3 solution added to extract.	Blue, green or violet colour formed
Carbohydrates	Molish test	Molish reagent and Concentrated H2SO4 added to extract.	Violet or purple ring layer formed
Triterpenoids	Libermann-burchard test	Add acetic anhydride and concentrated H2SO4 to extract and mix gently.	Green/blue-green colour formed
Saponins	Foam test	Extract shaken vigorously with water.	Formation of persistent froth/foam layer.

10. PHYSICOCHEMICAL EVALUATION TEST

ORGANOLEPTIC EVALUATION

1. Colour

Light green to dark green.

2. Odour

Characteristic herbal odour.

3. Homogeneity

The prepared Vitex negundo anti-inflammatory spray was found to be homogeneous, showing uniform distribution of the extract without phase separation, sedimentation, or visible particles.

4. Consistency

Free-flowing liquid with low viscosity, suitable for spraying.

5. Clarity

The formulation was clear and free from visible particulate matter, indicating proper filtration and uniformity.

6. Evaporation time

The formulation showed rapid evaporation which is desirable for topical spray in f1 100 second and f2 110second.

7. Stickiness test

The formulation was found to be non-sticky with after drying, indicate better patient acceptability.

8. pH measurement

The PH of the formulations was the acceptable range for the topical application, indicating skin compatibility.

9. Solubility test

- Take two beakers, in one beaker take 5ml water and another beaker take 5ml ethanol.
- Add 1ml spray in both beakers and check the solubility.

10. Leak test

The leak test was performed to evaluate the integrity of the spray containers were tightly closed and cleaned externally to remove any adhered formulation. Each container was then kept in the inverted position at room temperature for 24hrs. The containers were visually examined at regular intervals for of no leakage, wetness around the closure or loss of contents. The absence of leakage, wetness or loss of formulation indicated that the spray container successfully passed the leak test.

11. RESULT AND DISCUSSION

1. Phytochemical screening

Phytochemical test	Test method	Observation	Inferences
Flavonoids	Shinoda test	Red/pink coloration formed	Flavonoids present
Flavonoids	Lead acetate test	Yellow-precipitate formed	Flavonoids present
Alkaloids	Mayers test	White precipitate formed	Alkaloids present
Alkaloids	Wagners test	Reddish-brown precipitate formed	Alkaloids present
Phenolic compound	Ferric chloride test	Blue, green or violet colour formed	Phenolic present
Carbohydrates	Molish test	Violet or purple ring layer formed	Carbohydrates present
Triterpenoids	Libermann-burchard test	Green/blue-green colour formed	Triterpenoids present
Saponins	Foam test	Formation of persistent froth/foam layer.	Saponins present

2. EVALUATION OF FORUMULATION

1. Organoleptic Evaluation

The spray formulation was found to be aesthetically acceptable with a uniform and clear appearance. No particulate matter or phase separation was observed, indicating good formulation stability.

Parameter	F1	F2
Colour	Light green	Dark green
Odour	Characteristic herbal odour	Characteristic herbal odour
Homogeneity	Homogeneous,uniformdistribution,no sedimentation or visible particles	Homogeneous; uniform distribution, nosedimentation or visible particles
Consistency	Free-flowing liquid with low viscosity, suitable for spraying	Free-flowing liquid with low viscosity, suitable for spraying
Clarity	Clear	Clear

2. Evaporation Time

The formulation showed rapid evaporation, which is desirable for topical sprays.

SI.NO	Formulations	Time
1.	F1	100 Sec
2.	F2	110Sec.

3. Stickiness test

The formulation was found to be non-sticky with after drying, indicate better patient acceptability.

SI.NO	Formulations	Result
1.	F1	Non-sticky
2.	F2	Non-sticky

4. pH measurement

The PH of the formulation was the acceptable range for the topical application, indicating skin compatibility.

SI.NO	Formulations	PH
1.	F1	4.8
2.	F2	5.8

5. Solubility test

Take two beakers, in one beaker take 5ml water and another beaker take 5ml ethanol. Add 1ml spray in both beakers and check the solubility.

SI.NO	Formulations	Water(5ml)	Ethanol(5ml)
1.	F1	Soluble	Soluble
2.	F2	Soluble	Soluble

6. Leak test

No leakage was observed during the 24-hour test period, confirming the integrity of the container.

SI.NO	Formulation	Observations time
1.	F1	24hours
2.	F2	24hours

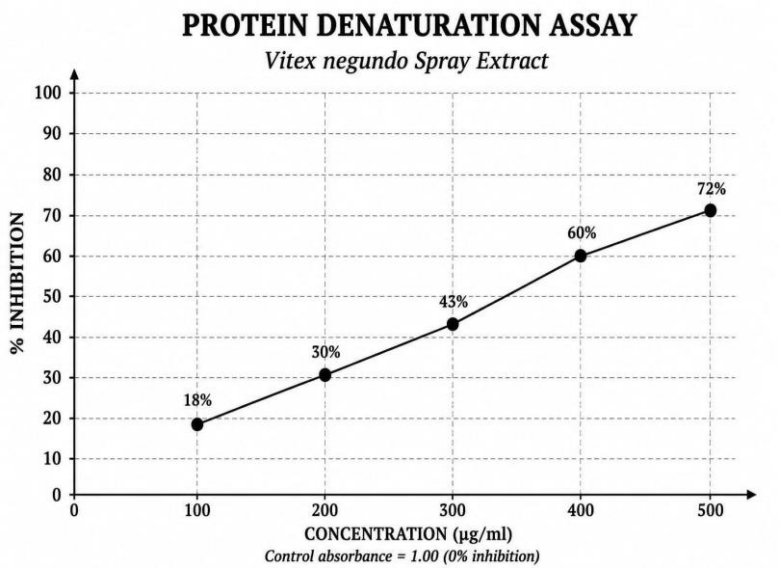
3. Anti-inflammatory activity

Protein Denaturation Assay

Fresh egg white was used as the protein source for the investigation. Various concentrations of

Vitex negundo extract (100, 200, 300, 400 and 500 µg/ml) were prepared using distilled water. In each test tube, 0.2 ml of egg white, 2.8 ml of phosphate buffer (pH 6.4), and 2 ml of Vitex negundo extract were added. For the control tube, 0.2 ml egg albumin, 2.8 ml phosphate buffer and 2 ml distilled water were mixed, while for the standard tube, 0.2 ml egg albumin, 2.8 ml phosphate buffer and 2 ml Diclofenac sodium solution were mixed. All the test tubes were incubated at 37°C for 15 minutes. After incubation, the mixtures were heated at 70°C for 5 minutes in a water bath to promote protein denaturation and then allowed to cool to room temperature. The absorbance of each sample was measured at 660 nm using a UV-Visible spectrophotometer. The percentage inhibition of protein denaturation was calculated to evaluate the anti-inflammatory activity of Vitex negundo extract.

Concentration	Absorbance	% Inhibition
100 µg/ml	0.82	18%
200 µg/ml	0.70	30%
300 µg/mL	0.57	43%
400 µg/ml	0.40	60%
500 µg/ml	0.28	72%



The spray extract of Vitex negundo showed concentration dependent increase in inhibition of protein denaturation. The percentage inhibition was increased from 18 % at 100 µg/ml to 72 % at 500 µg/ml which indicated a promising in-vitro anti-inflammatory activity.

12. CONCLUSION

The current study was successfully completed in order to formulate and assess a herbal anti-inflammatory spray that contains leaf extract from Vitex negundo. The goal of the study was

to create an appropriate herbal formulation by using Vitex negundo's medicinal qualities. Important phytoconstituents that may contribute to its medicinal effect, including flavonoids, alkaloids, glycosides, saponins, phenolic compounds, and volatile oils, were found by the phytochemical screening. Acceptable physical and physicochemical properties, such as appearance, homogeneity, consistency, pH, clarity, evaporation duration, and stability, were demonstrated by the developed formulations F1 and F2. The protein denaturation method was used to assess the in-vitro anti-inflammatory efficacy. The findings demonstrated the promising anti-inflammatory potential of Vitex negundo leaf extract by showing a rise in percentage inhibition with an increase in extract concentration. The study comes to the conclusion that Vitex negundo leaf extract has promising anti-inflammatory properties and can be successfully added to a herbal spray composition. For more research and the creation of anti-inflammatory medications, the proposed formulation might offer a helpful herbal approach.

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