

Pharmacognostical Standardization and Modernization of An Official Siddha Formulation-Mayilarakaathi Chooranam

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ABSTRACT:

Traditional siddha medicine offers a vast repository of therapeutic agents with proven clinical efficacy but limited modern dosage form standardization. The present study was undertaken to develop a topical herbal formulation containing “**MayilirakaathiChooranam**”, a traditional siddha medicine known for its Anti-inflammatory properties and also used for Wound healing & Antimicrobial. The “**Herbal Formulation**” was formulated into a ointment and it is evaluated for physicochemical parameters including appearance, pH, spreadability, viscosity, and stability. The “**MayilarakaathiChooranam**” can definitely be a potential, natural, alternative medicine for skin care and management of infection. The primary objectives of this study was to formulate a stabilized, topical herbal formulation which containing “**MayilirakaathiChooranam**” to enhance patient compliance and provide a localized delivery system for treating infections and promoting tissue regeneration.

KEYWORDS:

Mayilarakaathichooranam, Anti-inflammatory, Therapeutic effect, Tropical drug delivery, Nigella sativa seeds, Bees wax, glycerin, Bovine Serum Albumin Assay, Siddha vaithya Thirattu, Enhanced local delivery, Wound Healing, Tissue regeneration, Phytochemical analysis, Evaluation.

I. INTRODUCTION:

Traditional Siddha and Ayurvedic systems of medicine have been widely practiced in India for centuries for the effective management of various skin disorders. **MayilarakaathiChooranam** is a classical polyherbal formulation described in Siddha literature, traditionally valued for its anti-inflammatory, antimicrobial, antiseptic and wound-healing properties. It has been commonly used in the treatment of skin infections, wounds, and inflammatory skin condition.

Conventionally, MayilarakaathiChooranam is administered in powdered form for internal use.

However, the direct topical application of powder may be inconvenient, less adherent to the skin surface. This limits its prolonged contact time with the affected area and may reduce therapeutic effectiveness in certain dermatological conditions.

To overcome these limitations, the formulation can be converted into a semi-solid dosage form such as an ointment. Ointments are highly suitable for topical drug delivery as they provide better occlusion, enhanced skin hydration and prolonged retention time at the site of application. The oleaginous base of an ointment facilitates deeper penetration of herbal active constituents, thereby improving therapeutic efficacy. In addition, ointments protect the wound surface, reduce moisture loss, and create a favorable environment for healing.

An ointment formulation of MayilarakaathiChooranam offers improved patient compliance, ease of application, and enhanced local drug delivery. It can be effectively utilized in the management of skin infections, & wounds.

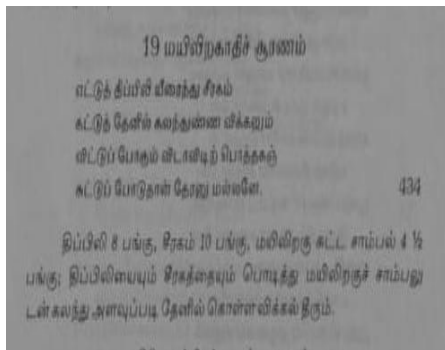
SIDDHA SYSTEM OF MEDICINE:

The “**SIDDHA SYSTEM**” of medicine is one of the oldest traditional systems in India, which is originating in Tamil Nadu and deeply rooted in ancient Tamil culture. It is believed to have been developed by enlightened sages known as “**SIDDHARS**”, with “**AGASTYA**”. The term “**SIDDHA**” means achievement and “**SIDDHARS**” were saintly personalities, who attained proficiency in medicine through practice of “**BHAKTI and YOGA**”. This is the system of preVedic period identified with Dravidian culture, and it is largely therapeutic in nature. Like Ayurveda, this system believes that all objects in universe are made up of **FIVE** basic elements, namely *Earth, Water, Sky, Fire and Air*. The identification of causative factors of diseases is done through pulse reading, colour of body, study of voice, urine examination, status of digestive system, and examination of tongue. Three humors

(Mukkuttram) are: Vatham (Air), Pitham (Fire), Kabam (Water/Earth).Siddha uses a wide range of natural substances:

- Herbs and plants.
- Minerals and metals.
- Animal products^[1].

A number of other dosage forms like: Churna, Avaleha, Ghrita, Sandhana kalpa, Bhasma, are prepared from them. Mostly, all of them are polyherbal formulations. Some of the important herbs in ayurveda are Piper longum, Cuminum cyminum, cassia angustifolia, sesamum indicum, etc.^[1]



AIM:

The aim of the project is to formulate and evaluate a semi-solid dosage form containing a traditional siddha medicine to improve its patient compliance, acceptability, and therapeutic efficacy compared to conventional chooranam.

PLAN OF WORK:

- ♣ Literature Review
- ♣ Collection and preparation of raw materials
- ♣ Preformulation studies
- ♣ Formulation of ointment
- ♣ Evaluation of ointment
- ♣ Documentation and Results
- ♣ Conclusion

METHODOLOGY:

- Procurement of ingredient.
- ↓
- Preparation of ointment base.
- ↓
- Incorporation of Extraction.
- ↓
- Attaining consistency.
- ↓
- Evaluation of final ointment.

NIGELLA SATIVA:

Nigella sativa (N. sativa) (Family Ranunculaceae) is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine, like Siddha,

Ayurveda, and Unani. N. sativa is commonly known as black seed. N. sativa is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia [4]. N. sativa has been extensively studied for its biological activities and therapeutic potential and shown to possess wide spectrum of activities viz. as diuretic, antihypertensive, antidiabetic, anticancer and immunomodulatory, analgesic, antimicrobial, anthelmintics, analgesics and anti-inflammatory, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, renal protective and antioxidant properties. The seeds of N. sativa are widely used in the treatment of various diseases like bronchitis, asthma, diarrhea, rheumatism, and skin disorders. It is also used as liver tonic, digestive, anti-diarrheal, appetite stimulant, emmenagogue, to increase milk production in nursing mothers to fight parasitic infections, and to support immune system^{[5]-[10]}.

MORPHOLOGY OF THE PLANT:

N. sativa is an annual flowering plant which grows to 20-90 cm tall, with finely divided leaves, the leaf segments narrowly linear to threadlike. The flowers are delicate, and usually coloured white, yellow, pink, pale blue or pale purple, with 5-10 petals. The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds^[10-12].



CHEMICAL COMPOSITION OF BLACK SEEDS:

The most important active compounds are thymoquinone(30%-48%),thymohydroqui-none, dithymoquinone, p-cymene (7%-15%), carvacrol (6% 12%), 4-terpineol (2%-7%), t-anethol (1%-4%), sesquiterpene longifolene (1% 8%) α -pinene and thymol, etc.

Black seeds also contain some other compounds in trace amounts. Seeds contain two different types of alkaloids; i.e. isoquinoline alkaloids, e.g. nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine. Moreover, N. sativa seeds also contain alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anticancer agent.^[13-14]

The seeds of N. sativa contain protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fibre (8.4%) and total ash (4.8 %). The seeds are also containing good number of various vitamins and minerals like Cu, P, Zn and Fe etc. The seeds contain carotene which is converted by the liver to

vitamin A. Root and shoot are reported to contain vanillic acid.^[14-15]

PREPARATION OF NIGELLA SATIVA SEED AND MAYILIRIKAATHI CHOORANAM EXTRACT:

- ✓ Nigella sativa seeds were powdered and 15g of the powder was taken.
- ✓ Similarly, 15g of Mayilirikaathichooranam was taken.
- ✓ Both powders were mixed together to obtain a total of 30g of samples.
- ✓ The mixed sample was subjected to extraction using a Soxhlet apparatus.
- ✓ The mixed sample [30g] was packed in tissue paper and placed in the Soxhlet extractor.
- ✓ Water was used as a solvent, and about 300ml of water was taken in a round bottom flask. } The Soxhlet apparatus was assembled and extraction was carried out. } The process continued for approximately 8 hours and about 3 extraction cycles were completed.



PHYTOCHEMICAL ANALYSIS:

TEST FOR ALKALOIDS:

After adding 3 drops of Wagner's reagent to 4ml of extract, it was alone for 5 minutes. Reddish-brown precipitate indicates the presence of alkaloids.

TEST FOR FLAVONOIDS:

A diluted solution of hydrogen chloride and sodium hydroxide was used to dissolve 0.2g of extract. The solution was stored at a low temperature. The absence of yellow indicates the presence of flavonoids.

TEST FOR TERPENES:

Add 12 drops of $\text{Cu}[\text{OAc}]_2$ solutions to 5ml of extract and allow it to settle for a while. The formation of a beryl green color indicates the presence of terpenes.

TEST FOR SAPONINS:

The presence of saponin is shown by a stable form, which forms after 2ml of water is added to 3ml of extract and aggressively agitated for approximately 10 minutes.

TEST FOR PHENOLS:

A solution of 0.5ml, plant extract and 5ml. H_2O was heated for 10 minutes. Three drops of 10% ferric chloride solution were added to 2 millilitres of recovered filtrate. The presence of phenolic compounds is indicated by the appearance of greenish blue or violet color.

TEST FOR TANNINS:

A thorough mixing was done between 4ml of extract and 4ml of $\text{Pb}[\text{C}_2\text{H}_3\text{O}_2]_2$ solutions. An indication of the presence of plants and tannins is the presence of white precipitate.

TEST FOR COUMARIN:

Two milliliters of extract were combined with a 1N sodium hydroxide solution's presence of coumarin glycosides is indicated by bluish green fluorescence.

TEST FOR CARDIAC GLYCOSIDES:

A few milliliters of the extract were dissolved in water using a solution of ferric chloride, strong acid [such as sulfuric acid], and glacial acetic acid. A brown ring that forms at the junction suggests the presence of cardiac glycosides.



FORMULATION

OINTMENT:

1. Mayilirakaathichooranam extract [7.5ml]-Anti inflammatory
2. Nigella sativa seed extract [7.5ml]-Anti inflammatory
3. Bees wax [15g] Thickening agent
4. Glycerin 15ml[1:1ratio] Vehicle

PREPARATION OF OINTMENT BASE:

- ◆ First, take the required quantity of beeswax in a clean beaker.
- ◆ Heat the beaker using a water bath until the beeswax melts completely.
- ◆ After the beeswax melts, add the plant to extract slowly and mix well. ◆ Then add the required amount of glycerin to the mixture.
- ◆ Stir the mixture continuously to obtain a uniform consistency.
- ◆ Allow, the mixture to cool at room temperature.
- ◆ After cooling, the ointment is formed.
- ◆ Transfer the prepared ointment into a clean and dry container and store it properly.



S.NO	INGREDIENTS	B1	B2
1.	Mayilirakaathichooranam extract	4ml	7.5ml
2.	Nigella sativa seed extract	4ml	7.5ml
3.	Bees wax	5g	15g
4.	Glycerin	15ml	15ml

EVALUATION OF OINTMENT:

PH OF THE CREAM:

The pH of the ointment was found to be in the range of 7.3, which is good for skin ph.

Procedure: The pH meter was calibrated using a standard buffer solution. About 0.5g of the ointment was weighed and dissolved in 50ml of distilled water, and its pH was measured.

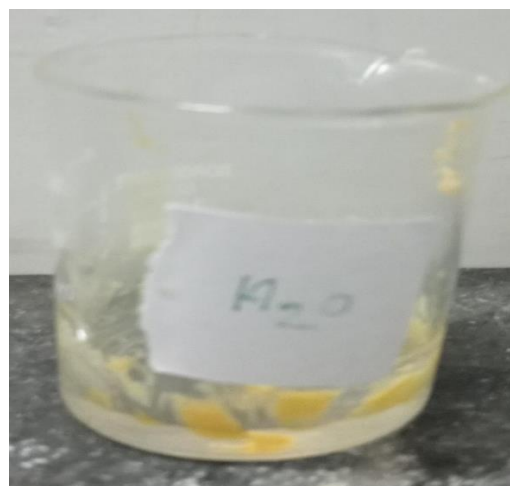


APPEARANCE:

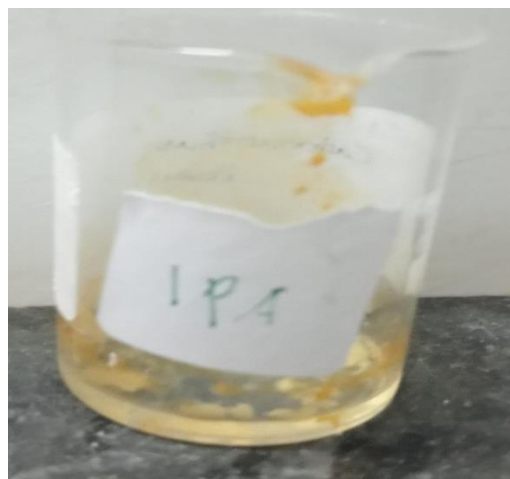
The appearance of the ointment was judged by its color, consistency, and texture. Color- Yellowish
 Consistency- Creamy Texture-Smooth. 25

SOLUBILITY:

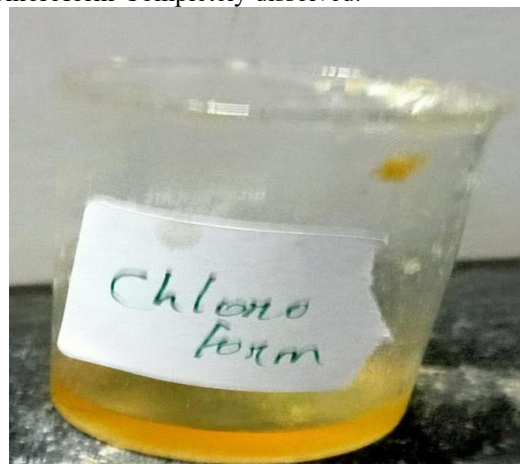
Water-Partially dissolved



Isopropyl Alcohol-Partially dissolved



Chloroform-Completely dissolved.



SPREADABILITY:

Spreadability is an important characteristic of these formulations and is responsible for correct dosage transfer to the target site, ease of application on the substrate, extrudability from the package, and most important, consumer preference.

Procedure:

Spreadability test is done by using the parallel plate method. The sample is placed between two glass plates. A 50g of samples were placed on top for 1min. The spreadability is determined by measuring the increased diameter. The spreadability value +8.33 has been found in our test.



IRRITANCY TEST:

The formulation shows no redness, inflammation, or irritation during irritancy study. These formulations are safe to use for skin.

REMOVAL:

The ointment applied on skin was easily removed by washing with tap water.

AFTER FEEL:

Smooth non-irritating and easily spreadable on the skin.

LITERATURE OF BSA:

⊞ Bovine serum albumin (BSA or "Fraction V") is a serum albumin protein derived from cows. It is often

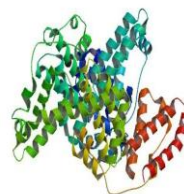
used as a protein concentration standard in lab experiments

⊞ The nickname "Fraction V" refers to albumin being the fifth fraction of the original Edwin Cohn purification methodology that made use of differential solubility characteristics of plasma proteins. By manipulating solvent concentrations, pH, salt levels, and temperature, Cohn was able to pull out successive "fractions" of blood plasma. The process was first commercialized with human albumin for medical use and later adopted for production of BSA.

PROPERTIES:

1. The full-length BSA precursor polypeptide is 607 amino acids (AAs) in length. An N-terminal 18-residue signal peptide is cut off from the precursor protein upon secretion; hence, the initial protein product contains 589 amino acid residues. An additional six amino acids are cleaved to yield the mature.

2. BSA protein that contains 583 amino acids. BSA has three homologous but structurally different domains. The domains, named I, II, and III, are broken down into two sub-domains, A and B.



Identifiers	
Organism	Bos taurus (domestic cow) ⓘ
Symbol	ALB
Entrez	280717 ⓘ
HomoloGene	105925 ⓘ
RefSeq (mRNA)	NM_180992 ⓘ
RefSeq (Prot)	NP_851335 ⓘ
UniProt	P02769 ⓘ
Other data	
Chromosome	6: 91.54 - 91.57 Mb ⓘ
Search for [show]	



IN-VITRO OF ANTI-INFLAMMATORY ACTIVITY:


www.tanbio.in
tbrds.res@gmail.com

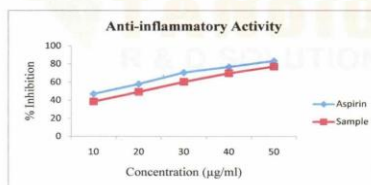
ANTI-INFLAMMATORY ACTIVITY WITH BSA ASSAY

Sample Details:	V. Sangamithra ,	Standard	• Aspirin
Client's Name :	B. Yamunasri, S. Srija pillay N. Kaviyasri		
University [Institute]	S. Vanitha, • EGS Pillay College of Pharmacy, Nagapattinam	Sample Name	: Unknown
Test Name	: Anti-inflammatory activity	Sample Nature : Semi-Solid dosageform	
Test Method	• BSA Assay	Reference Date	: 14/03/26

Result:

Table 1: Determination of Anti-inflammatory Activity with BSA Assay

S.No	Concentration (µg/ml)	% Inhibition	
		Standard	Sample
1.	10	47.11±0.06	38.66±0.13
2.	20	58.02±0.39	49.25±0.72
3.	30	70.56±0.51	60.34±1.09
4.	40	76.82±0.98	69.81±0.08
5.	50	82.39±0.43	77.15±0.54



Interpretation:

From the given result, we concluded that the sample has an anti-inflammatory activity when compared with the standard (Aspirin) with the different concentrations from 10, 20, 30, 40 and 50 respectively. As the concentration increases, the absorbance also increases in both the sample and the standard. It shows that the sample has an anti-inflammatory activity.

Analyst Signature

Test Method — BSA (Bovine Serum Albumin) Assay:
Procedure:

The anti-inflammatory activity of the extract was determined by using the BSA (Bovine Serum Albumin) assay. BSA solution (0.4%, w/v) was prepared in Tris Buffered Saline (one tablet is dissolved in 15 mL of deionized water to yield 0.05M Tris and 0.15M sodium chloride, pH 7.6 at 25 °C). The pH was adjusted to 6.4 with glacial acetic acid. Stock solutions of extract were prepared in methanol at a concentration of 50 µg/mL or 0.005%, w/v. Respective aliquots of 5.0 µl, 10 µl and 20 µl representing concentrations of 0.25 µg/mL, 0.50 µg/mL and 1.00 µg/mL of the stock solutions were added to test tubes containing 1 mL of 0.4%, w/v BSA buffer solution. Both negative (methanol) and positive (aspirin) controls were assayed in a similar manner. The solutions were then heated in a water bath at 72 °C for 10 minutes and cooled for 20 minutes under laboratory conditions. The turbidity of the solutions (level of protein precipitation) was measured at 660 nm in a Hach Spectrophotometer using an air blank. The experiments were conducted in duplicate and the mean absorbance values were recorded [19]. The percentage inhibition of precipitation (protein denaturation) was determined on a percentage basis, relative to the negative control using the following equation:

$$\% \text{ Anti-Denaturation Activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

$$\% \text{ Anti-Denaturation Activity} = \% \text{ Inhibition of Protein Denaturation} = \% \text{ Anti-inflammatory Activity}$$

II. RESULT AND DISCUSSION:

The evaluation of the formulated ointment yielded several findings regarding its physicochemical properties and therapeutic potential they are,

Traditional Foundation:

The study validates that MayilarakaathiChooranam, sourced from the classical text Siddha Vaithya Thirattu, retains its traditional anti-inflammatory and antimicrobial properties when standardized into a modern semi- solid dosage form.

Physicochemical Standardization:

The formulation was successfully evaluated for parameters such as appearance, pH, spreadability, viscosity, and stability to ensure it meets standard pharmaceutical requirements.

Therapeutic Protection:

The ointment creates a favorable environment for healing by protecting the wound surface and reducing moisture loss, which promotes tissue regeneration.

Enhanced Local Delivery:

The traditional powder (chooranam) into an ointment provides better occlusion and enhanced skin hydration. This allows active ingredients to remain in contact with the affected area longer, improving therapeutic efficacy for skin infections and wounds.

III. CONCLUSION:

During the conversion of Mayilirakaathichooranam into ointment form, the anti-inflammatory activity was found to be altered.

To enhance the anti-inflammatory property of the formulation, *Nigella sativa* seed extract, which possesses known anti-inflammatory activity, was incorporated into an ointment.

♣ The extract was prepared and incorporated into an ointment base containing beeswax and glycerin.

♣ The prepared ointment was evaluated for its anti-inflammatory activity by using the BSA [Bovine Serum Albumin] assay method.

♣ The results obtained from the BSA assay indicated that the formulation showed anti-inflammatory activity. Further evaluation tests such as physical appearance, consistency and spreadability of the ointment confirmed that the formulation was suitable for topical application.

♣ Thus, the study concludes that the ointment prepared from *Mayilirakaathichooranam* and *Nigella Sativa* seed extract may possess potential anti-inflammatory properties and can be considered as a natural herbal formulation for reducing inflammation.

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