

FORMULATION AND EVALUATION OF HERBAL SKIN OINTMENT FOR TREATMENT OF FUNGAL INFECTION

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Keywords	Abstract
<i>Pongamia pinnata</i> , <i>Flavonoids</i> , <i>Quercetin</i> , <i>Iso-quercitrin</i> , <i>Skin ointment</i> , <i>Antifungal activity</i> , <i>Herbal Medicinal plant</i> , <i>Asparagus Niger</i> , etc	In recent years, the demand for herbal medicinal products has increased in the market. Herbal medicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Along with other dosage forms, herbal drugs are also formulated in the form of ointment. An ointment is a viscous semisolid preparation used topically on a variety of body surfaces. The objective of the study was to formulate and evaluate the herbal skin ointment for treatment of Fungal Infections. [4][6] Fungal infection is a very common in India. The various home remedies and ayurvedic drugs are used to treat fungal infection. Traditionally in folk ware medicines <i>Pongamia pinnata</i> has been used for the treatment of fungal infection. In present study we used the Ethanolic extract of <i>Pongamia pinnata</i> to treat fungal infection. The phytochemical screening of the plant extract shows the presence of flavonoid such as quercetin and iso -quercitrin alkaloids, glycosides. The antifungal activity of the extract increased linearly with increase in concentration of extracts (mg/ml) as compared with standard drug Fluconazole. [1] [12] Ointment was prepared by using <i>Pongamia pinata</i> extract as main ingredient. And evaluation was carried out such as pH, washability, non-irritancy and stability. [1][4]
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INTRODUCTION

In recent years, the demand for herbal products has increased in developed countries. Herbal preparations are recognized as effective treatments for various ailments. These products are increasingly in demand as pharmaceuticals, dietary supplements and cosmetics. There are over 6000 herbal manufacturers in

India. About 4000 Ayurvedic medicines are manufactured. When subjected to shear stress, ointments often behave like viscoelastic materials. They usually contain drugs and are intended to be administered externally to the body or mucous membranes. [5] [12]

Non-medicated ointments, also known as ointment bases, are non-medicated ointments used in the manufacture of medicated ointments or for their emollient or lubricating properties. Polyherbal preparations are those that contain two or more herbs in their composition. Many studies have been conducted combining extracts of Neem Seed Oil (*Azadirachta indica* Family-Meliaceae) and Karanj Seed Oil (*Millettia pinnata* Family Fabaceae) with various other herbal medicines. [1] [15]

herbal medicines are also available in ointment form. This is a semi-solid formulation that is used topically for a variety of reasons, including: As a protectant, antiseptic, emollient, antibacterial, keratolytic, astringent, in addition to other dosage forms. Neem consists of the leaves, seeds and other aerial parts of the Meliaceae *Azadirachta indica* family. [2]

Neem oil contains various properties such as antiseptic, insecticidal, fertility inhibitor and antiviral effects, which are currently being tested for their efficacy. AIDS treatment. Karanj consists of seeds, roots and fresh leaves of plants known as legumes of the *Millettia pinnata* family. Used for skin diseases, psoriasis, anti-dandruff, and wound healing. [2] [10]

Topical products are important classes of drug delivery systems and their use in therapy is becoming more widespread. Although topical formulations to treat ailments have existed from ancient times, topical products, for which the skin is used as an alternative route for systemic and regional therapy, are relatively new entities. [4] [7]

The purpose of topical dosage forms is to conveniently deliver drugs to a localized area of the skin.

Ointments can be used to deliver drugs via several routes; these versatile systems have been extensively studied as vehicles for topical administration. Their composition and structure enable them to incorporate greater amount of drug than other topical

formulations such as ointments, creams, gels and lotions. These systems are comparatively thermodynamically stable systems because they contain surfactant, cosurfactant, and oil. [7]

Ointment-based colloidal drug delivery systems have gained wide acceptance because of their enhanced drug solubilization, thermodynamic stability, and ease of manufacture. Based on special network structure, the MEGs have received particular attention especially as topical drug delivery system. The absorption of drug has been found to be faster and better when formulated as MEG which improves bioavailability. Delivery of drugs using these ointments through skin increases the local/systemic delivery of the drug by different mechanisms that make them suitable vehicles for the delivery of anti-psoriatic. [7] [8]

Karanj oil, one of the natural oils, is a non-edible semi drying fixed oil obtained from seeds of *Pongamia pinnata* belongs to the family Fabaceae. The literature survey revealed that Karanja oil is used as an anti-psoriasis agent, in rheumatism, in treatment of scabies, herpes, leukoderma and other cutaneous diseases. Thus, Karanja oil can be used as an oil phase for formulation of ointment to deliver poorly water-soluble drugs by topical route which may enhance its absorption and can prolong the drug release. Anthralin is an antipsoriatic drug widely used for the treatment of psoriasis and Alopecia areata[15]

The drug is soluble in chloroform and having a melting point 176-181°C [12]. It is widely available as cream, lotion, shampoos, and gel. Topical drug delivery system localizing the drug at skin will be much favorable for the treatment of skin infections. [4]

The USP and NF Classify Ointment Bases into Four General Groups

1. Hydrocarbon bases
2. Absorption bases
3. Water removable bases
4. Water soluble bases

1. Hydrocarbon bases - Are water-free, and aqueous preparations may only be incorporated into them in small amounts and then with difficulty. USES: HC are used mainly for their emollient effect and not intended for penetration into the skin. They are difficult to wash off. They do not “dry out” or change noticeably upon aging.

2. Absorption bases - There is again two Types 1. Those that permit the incorporation of aqueous solutions resulting in the formation of water in oil emulsion. (Anhydrous absorption bases) 2. Those that are already water-in-oil emulsion (emulsion base) that permits the incorporation of small additional quantities of aqueous solution

3. Water removable base - Are oil-in-water emulsion that are capable of being washed from skin or clothing with water.

For this reason, they are frequently referred to as “water-washable” ointment base.

4. Water soluble base - Unlike water-removable bases, which contains both water soluble and water insoluble components. Like water-removable bases, however, water soluble bases are after washable and are commonly referred to as “greaseless” because of the absence of any oleaginous materials [4] [7]

Common Fungal Skin Disease is Ringworm:

Ringworm is a common skin and nail infection caused by a fungus. The infection is called ringworm “because it can cause an itchy, red, circular rash. Ringworm is also called “moth” or “dermatophytosis”. The different types of ringworm are usually named after the location of the infection in the body [3] [10]

Areas of the body that can be affected by ringworm include:

- ❖ Feet (tinea pedis, commonly called “athlete’s foot”)
- ❖ Groin, inner thighs, or buttocks (tinea cruris, commonly called “jock itch”)
- ❖ Scalp (tinea capitis)
- ❖ Beard (tinea barbae)
- ❖ Hands (tinea manuum)[14]



Fig.1- Tinea Pedis



Fig 2-.Tinea Corporis

Fig 3. Tinea Unguiu

Areas of the body that can be affected by ringworm include:

- ❖ Feet (tinea pedis, commonly called “athlete’s foot”)
- ❖ Groin, inner thighs, or buttocks (tinea cruris, commonly called “jock itch”)
- ❖ Scalp (tinea capitis)
- ❖ Beard (tinea barbae)
- ❖ Hands (tinea manuum)
- ❖ Toenails or fingernails (tinea unguium, also called “onychomycosis” Other parts of the body such as arms or legs (tinea corporis) [3] [10]

How it Spreads

The fungi that cause ringworm can live on skin and in the environment. There are three main ways that ringworm can spread:

1. From a person who has ringworm.
2. From an animal that has ringworm.
3. From the environment [3] [14]

Symptoms of ringworm by location on the body:

- **Feet** (tinea pedis or “athlete’s foot”): Symptoms of ringworm include red, swollen, scaly, itchy skin between the toes (especially between the pinky and the next toe). The sole and heel can also be damaged. In severe cases, the skin of the feet may blister.
- **Scalp** (tinea capitis): Ringworm of the scalp usually looks like a scaly, itchy, red, round bald spot. The bald spot may enlarge and multiple spots may form as the infection spreads. Ringworm of the scalp occurs more often in children than in adults.
- **Groin** (tinea cruris or “jock itch”): ringworm in the groin looks like scaly, itchy red spots, usually on the inside of the skin folds of the thighs.
- **Beard** (tinea barbae): Symptoms of beard ring include scaly, itchy, red patches on the cheeks, chin and upper neck. Spots may penetrate the surface or become filled with pus, and affected hair may fall out.

MATERIALS AND METHODS

Materials Used

- 1) *Pongamia pinnata*: [1][2][12]

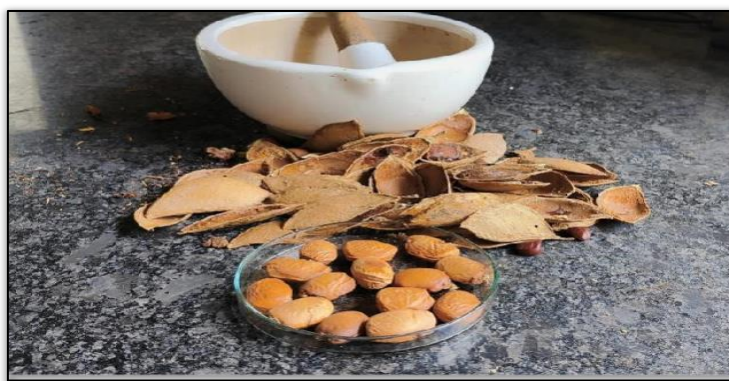


Fig.4 Karanja Seed

Pongamia pinnata seeds, commonly known as Karanja seeds, are an important medicinal part of the plant and have been widely used in traditional systems of medicine. The seeds are oval to elliptical in shape, brown in color, and rich in fixed oils and bioactive compounds. They are recognized for their diverse therapeutic applications and industrial importance.

The seeds contain a high percentage of oil (approximately 30–40%) along with several phytoconstituents such as flavonoids, karanjin, pongamol, tannins, saponins, alkaloids, and phenolic compounds. These bioactive constituents

2) Neem Leaves: [1][2][12]

exhibit significant antimicrobial, antioxidant, anti-inflammatory, insecticidal, and wound-healing activities. Karanja seed oil is extensively used in pharmaceutical, cosmetic, and agricultural preparations. In traditional medicine, the seeds and seed oil are employed for the treatment of skin disorders, wounds, ulcers, rheumatism, and microbial infections. Due to their potent medicinal properties and rich phytochemical profile, *Pongamia pinnata* seeds are considered valuable ingredients in herbal formulations and topical drug delivery systems such as gels, creams, and ointment



Fig.5 Neem leaves

Neem belongs to the family Meliaceae and is commonly known as Neem or *Indian Lilac*. It is widely distributed throughout India and other tropical countries. Different parts of the plant such as leaves, bark, seeds, flowers, and fruits are used traditionally for the treatment of various diseases. Among these, neem leaves possess remarkable medicinal properties and are extensively used in herbal pharmaceutical preparations. The leaves of Neem contain several important phytoconstituents such as flavonoids, tannins, alkaloids, glycosides, saponins, terpenoids, phenolic compounds, and limonoids including azadirachtin and nimbin. These bioactive compounds are responsible for various pharmacological activities including antimicrobial, antifungal, antioxidant, anti-inflammatory, wound healing, and antiseptic properties. Microbial infections affecting skin and wounds are common health concerns requiring effective treatment.

Ingredient and their role [1] [6]

Table 1 – Ingredient table and their role

Sr. No.	Ingredients	Role
1	Karanja seed oil	Antifungal agent
2	Neem leaves oil	Antiseptic
3	Coconut oil	Lubricant
4	Turmeric powder	Coloring agent
5	Phenoxyethanol	Preservatives
6	Ointment Base	As a base
7	Rose water	Fragrance

Method Used

1) Collection of plant material[1]

The Karanja seed was collected from the Herbal garden of the college HSBPVT'S GOI Faculty of Pharmacy, Kashti, which is further authenticated from Head Department of Botany Radhabai Kale Mahila Mahavidyalaya Ahilyanagar. And neem seed oil is procured from local market of Shrigonda.

2) Preparation of powder [7]

Remove dust, dirt, and unwanted materials. Wash Seed and leaves thoroughly with distilled water. Removes contaminants and impurities. Spread Seed and leaves on a clean tray. Dry under shade at room temperature for 7-8 days. Avoid direct sunlight because it may destroy active constituents. Drying continues until

Seed become Moisture free dried Seed are crushed using grinder, convert into coarse powder, and then pass powder through sieve. Fine powder is collected.

3) Prepration of extraction - Maceration [4][7]

- Take a 50 gm of powder of both drug in beaker and was soaked in 500 ml ethanol.
- Take a side for 2-3 days covered with aluminum foil.
- Shake the extract two times a day.
- The mixture was filtered through the filter paper (Whatman No.1).
- Stored in sterile amber bottles at 4 °C until further use

4) phytochemical screening (for *Pongamia pinnata*)[6][12]

Table 2 – Phytochemical screening of *Pongamia pinnata*

Sr.no.	Phytoconstituent	Name of test	Procedure	Observation
1	Flavonoids	Alkaline reagent test	1 ml extract + few drops of NaOH solution + dilute acid	Intense yellow color which disappeared after adding dilute acid
2	Flavonoids	Lead acetate test	1 ml extract + few drops of lead acetate solution	Formation of yellow precipitate
3	Flavonoids	Ferric chloride test	1 ml extract + Ferric chloride solution	Dark green color appeared

4	Glycosides	Killer–Killani test	1 ml extract + 1 ml glacial acetic acid + 1 ml ferric chloride + 1 ml concentrated sulfuric acid	Brown ring appeared at the junction of the test tube
5	Alkaloids	Hager's reagent test	1 ml extract + 1 ml picric acid solution + 1 ml hageer's reagent	formation of yellow precipitate
6	Tannins	Ferric chloride test	1 ml extract + 2 drops of ferric chloride solution	Blue color appeared



Fig 6 - Identification test for *Pongamia pinnata*

Preparation of ointment [4] [7]

1. Collection of ingredients
2. Weigh all ingredients accurately
3. Prepare oil phase (Stearic acid + Bees wax + Liquid paraffin + Propyl paraben
4. Prepare aqueous phase (Distilled water + Propylene glycol + Methyl paraben Triethanolamine
5. Heat both phases separately to about 70–75°C
6. Add aqueous phase slowly into oil phase with continuous stirring
7. Homogenize until a smooth base is formed
8. Cool the formulation
9. Add herbal extracts (Pongamia Pinnata Seed extract)
10. Mix well until uniform Adjust pH and consistency if required
11. Fill into clean containers / tubes
12. Store the prepared herbal Ointment.

Formulation table [4][7]

Table 3 – Formulation table

Sr. No	Ingredient	Batch F1 10g	Batch F2 10g	Batch F3 10g
1	Pongamia pinnata oil	0.3 ml	0.4 ml	2 ml
2	Neem leaves oil	0.3 ml	0.4 ml	0.5 ml
3	Coconut oil	0.8 ml	0.9 ml	1 ml
4	Turmeric powder	0.1 g	0.15 g	0.2 g

5	Phenoxyethanol	0.05 ml	0.05 ml	0.05 ml
6	Ointment base	7.2 g	6.85 g	5 g
7	Rose water	1.25 ml	1.25 ml	1.25 ml

Evaluation Parameters [4][8]

1) Physical Evaluation

• Colour

The colour of the formulated herbal ointment was checked by visual inspection.

• Odour

The odour of the ointment was evaluated by applying a small quantity on the hand to determine its characteristic smell.

• Consistency

The consistency of the ointment was examined manually by rubbing the formulation on the skin. The ointment showed smooth and uniform consistency.

2) Homogeneity and Texture

The prepared ointment was tested for homogeneity by visual appearance and touch. The formulation showed uniform distribution of ingredients without any gritty particles.

3) Washability Test

A small quantity of ointment was applied on the skin and washed with tap water to

4) Phase Separation Test

The formulated ointment was stored in a closed container at room temperature for 24 hours and observed for phase separation. No phase separation was observed.

5) Irritancy Test

The ointment was applied on a small area of skin and observed for 24 hours for any signs of irritation, redness, edema, or swelling. No irritancy reaction was observed.

6) Measurement of pH

The pH of the ointment was determined using a digital pH meter. About 1 g of ointment.

was dissolved in 10 ml of distilled water and the pH was measured. The pH of the formulation was found suitable for skin application.

7) Spreadability Test

Spreadability of the ointment was determined by applying a small amount between two glass slides. The ointment showed good spreadability and smooth application.

RESULT AND DISCUSSION:

A. Test for Pongamia Pinnata

Table 4 - Test Pongamia Pinnata

Sr.no.	Phytoconstituent	Result
1	Flavonoids	Present
2	Flavonoids	Present
3	Flavonoids	Present
4	Glycosides	Present
5	Alkaloids	Present
6	Tannins	Present

B. Physical Evaluation

Table 5 – Physical evaluation

Formulation	Colour	Consistency	Odour
F1	Dark yellow	Poor	Characteristic
F2	Faint yellow	Smooth	Characteristic
F3	Faint yellow	Good	Characteristic

C. Measurement of pH

Table 6– Measurement of pH

Formulation	pH
F1	5.1
F2	6.8
F3	5.79

D. Washability Test

Table 7– Washability test

Formulation	Observation
F1	Excess spreading
F2	Difficult
F3	Easily Washable

E. Phase Separation Test

Table 8 – Phase separation test

Formulation	Observation
F1	Phase Separation
F2	No Phase Separation
F3	No Phase Separation

F. Determination of Zone Inhibition

- **Antifungal study Method-** Zone of Inhibition
- **Materials and methods:**
- **Method:** Zone of Inhibition (Agar Well Diffusion Method)
- **Media:** Sabouraud Dextrose Agar (SDA)
- **Culture Used**
- **Fungal Culture Used:** *Candida albicans*, *Aspergillus niger*
- **Incubation Temperature:** 28 ± 2°C
- **Incubation Time:** 24–48 Hrs
- **Standard Drug:** Fluconazole

• Results and conclusion:

Sample F3 showed the highest antifungal activity among all formulations.

The antifungal activity of F3 was comparable to the standard drug Fluconazole.

The herbal ointment containing *Pongamia Pinnata* Seed oil and Neem leaf extract demonstrated significant antifungal potential and may be useful for the treatment of fungal skin infection

Table 9: Zone of Inhibition

Sample ID	Conc. of Stock solution	Zone of inhibition in mm			
		<i>Candida albicans</i> NCIM 3471	<i>Aspergillus niger</i> NCIM 501	<i>Trichophyton Rubrum</i> NCIM 2816	<i>Microsporum canis</i> NCIM 5775
F3	<i>Pongamia Pinnata</i> Seed and Neem Leaves (Formulation	24 ± 0.35	23 ± 0.32	22 ± 0.30	21 ± 0.27

	10 µg/ml)				
Standard Solution	Flucanazole (0.5 mg/ml)	26 ± 0.28	24 ± 0.25	23 ± 0.24	22 ± 0.23
Control Solution	Blank gel	0	0	0	0

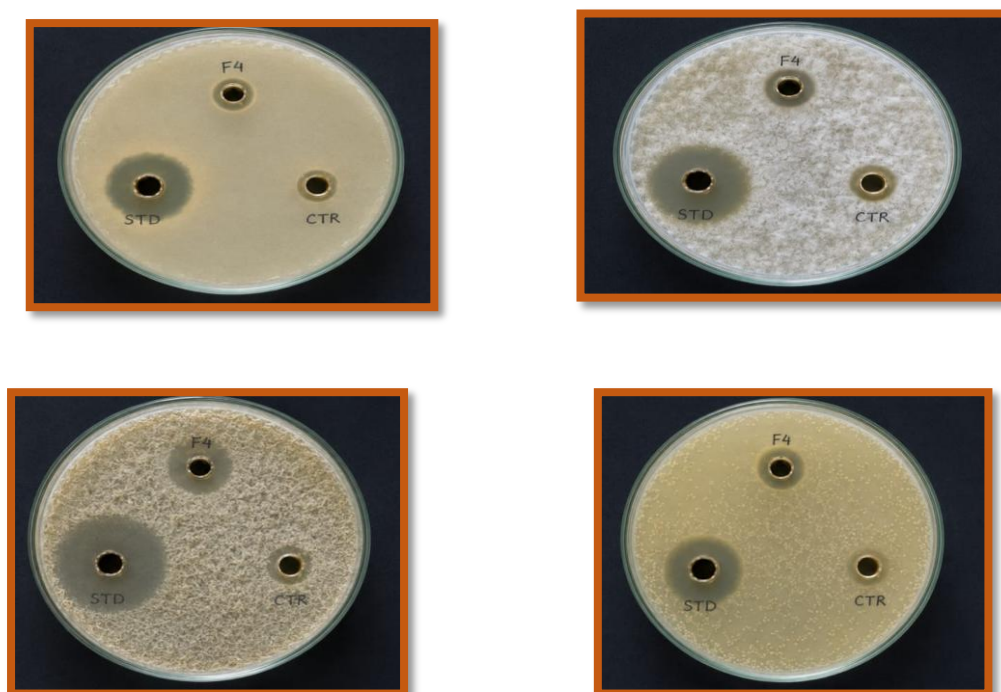


Fig.7 Zone of inhibition

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SUMMARY

The present study was aimed to formulate and evaluate a herbal skin ointment containing *Pongamia pinnata* (Karanja) seed oil for treatment of fungal skin

infections. Three formulations F1, F2, F3 were prepared by fusion method using Karanja oil, Neem oil and Coconut oil. White soft paraffin, wool fat, ceto stearyl alcohol and bees wax were used as ointment base. The prepared formulations were evaluated for color, odour, pH, spreadability, extrudability, washability, non-irritancy and in-vitro antifungal activity. Among all batches, F3 containing 2 ml Karanja oil showed optimal results with pH 5.79, spreadability 8 sec, extrudability 0.45 gm and smooth consistency. Antifungal activity was tested by agar well diffusion method against *Aspergillus niger* using Griseofulvin as standard. The zone of inhibition for F3 was 0.5 cm compared to 1 cm for standard drug. The results indicated that *Pongamia pinnata* seed oil possesses significant antifungal activity due to presence of pongamol and karanjin. The formulated ointment exhibited good physicochemical properties and can be considered as a promising herbal alternative for treatment of fungal skin infections with minimal side effect.

CONCLUSION

Pongamia pinnata, commonly known as the Indian Beech tree, has been used in traditional medicine for its therapeutic properties. The plant contains various bioactive compounds, including flavonoids, alkaloids, tannins, and steroids, which exhibit a range of biological activities, including antibacterial, antifungal, and anti-inflammatory properties. In this study, we aimed to formulate an ointment containing *Pongamia pinnata* extract for the treatment of bacterial and fungal skin infections, as well as psoriasis.

The ointment was formulated using a simple fusion method. Weigh all the required ingredients. Melt the bases. Addition of active ingredients & excipients. Pour the mixture in container. Allow to cool & solidify. The resulting ointment was

then evaluated for its physicochemical properties and biological activity.

The physicochemical properties of the ointment were evaluated in terms of its pH, spreadability, washability. The ointment had a pH of 5.79, which is within the acceptable range for topical preparations. indicating that it is easy to spread on the skin. The spreadability of the ointment was found to be 8 secs, indicating that it can cover a larger area of skin. Washability is also good but soap may be required. Nonirritant to skin & had good extrudability.

The ointment was found to exhibit significant antibacterial activity against both gram-positive and gram-negative bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The ointment also exhibited antifungal activity against *Candida albicans* and *Aspergillus Niger*. The zone of inhibition of *asparagus Niger* fungal strain by the

standard drug was 1cm in diameter and by the developed formulation was 0.5 cm in diameter.

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