International Journal of Pharmacognosy and Clinical Research

ISSN Print: 2664-763X
ISSN Online: 2664-7648
Impact Factor: RJIF 8.00
IJPCR 2023; 5(2): 12-19
www.pharmacognosyjournal.in
Received: 13-04-2023
Accepted: 20-05-2023

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Formulation, Development and Evaluation of Antibacterial and Antifungal Skin Ointment by Pongamia Pinnata (Karanja)

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DOI: https://doi.org/10.33545/2664763X.2023.v5.i2a.35

Abstract
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.
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.
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.
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.

Keywords: *Pongamia pinnata*, antibacterial, antifungal, skin ointment, Karanja seed

Introduction
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. The purpose of topical dosage forms is to conveniently deliver drugs to a localized area of the skin.
Psoriasis is a common, noncontagious, chronic, inflammatory, multisystem, and genetic disease of the immune system which affects predominantly skin and joint manifestations affecting approximately 2% of the population. Psoriasis is driven by the immune system, especially involving a type of white blood cell called a T cell. Normally, T cells help protect the body against infection and disease. In the case of psoriasis, T cells are put into action by mistake and become so active that they trigger other immune responses, which lead to inflammation and to rapid turnover of skin cells.
These cells pile up on the surface of the skin, forming itchy patches or plaques. The first outbreak of psoriasis is often triggered by emotional or mental stress or physical skin injury, but heredity is a major factor as well. The lesions vary in appearance with the type of psoriasis. There are various forms of psoriasis i.e., Plaque psoriasis or psoriasis vulgaris, Guttate or Eruptive Psoriasis, Inverse Psoriasis, Seborrheic Psoriasis, Nail Psoriasis, Generalized Erythrodermic Psoriasis, Pustular Psoriasis, Psoriatic arthritis.

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, co-surfactant, and oil.

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.

Karanja oil, one of the natural oils, is a non-edible semi drying fixed oil obtained from seeds of *Pongamia pinnata* belogs to the family Fabaceae. The literature survey revealed that karanj oil is used as an antipsoriasis 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 anti-psoriatic drug widely used for the treatment of psoriasis and Alopecia areata.

Anthralin has abilities to induce lipid peroxidation and reduce levels of endothelial adhesion molecules which are markedly elevated in psoriatic patients. It is very effective in arthritis by exerting anti-inflammatory and anti-proliferative effects. High onset and long duration of action makes anthralin a good candidate for treating psoriasis. But its poor water solubility makes it effective in psoriasis for topical drug delivery system.

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 [1, 6].

Active Ingredients Used in Ointment

1) *Pongamia pinnata* (Karanja oil)

Fig 1: Karanja Seed

Synonyms: Karanja oil is another name for *Pongamia pinnata* seed oil. Some synonyms for karanja oil include Indian beech oil and pongamia oil.

Biological sources: Karanja oil is derived from the seeds of the *Pongamia pinnata* tree, which is native to India and is now found in many tropical regions around the world.

Chemical constituents

Karanja oil contains several bioactive compounds, including fatty acids, flavonoids, and terpenoids. The most well-known and studied of these compounds are pongamol and karanjin, which have been shown to have antifungal, antibacterial, and insecticidal properties.

Uses

Skin diseases: Karanja oil has been used traditionally in Ayurvedic medicine to treat various skin conditions, including eczema, psoriasis, and dermatitis. It is believed to have anti-inflammatory, antifungal, and antibacterial properties that may help alleviate symptoms.

Wound healing: Karanja oil has been traditionally used as a natural remedy for wound healing due to its antibacterial and antifungal properties. It may help promote the healing process and prevent infection.

Hair care: Karanja oil is believed to have conditioning properties that may help improve the overall health and appearance of hair. It is used in some hair care products to help promote healthy hair growth and reduce dandruff.

Pain relief: Karanja oil has been traditionally used as a natural remedy for pain relief due to its anti-inflammatory and analgesic properties. It may be applied topically to alleviate pain associated with arthritis, muscle soreness, and other inflammatory conditions.

2) Neem seed oil

Fig 2: Neem Seeds

Synonyms: Neem seed oil is derived from the seeds of the neem tree, *Azadirachta indica*. Some synonyms for neem seed oil include margosa oil and neem oil.

Biological sources: Neem seed oil is extracted from the seeds of the neem tree, *Azadirachta indica*. The neem tree is

native to India and is also found in other parts of Asia, Africa, and South America.

Chemical constituents: Neem seed oil is composed of a variety of bioactive compounds including triglycerides, sterols, terpenoids, and fatty acids such as oleic acid, stearic acid, and linoleic acid. It also contains azadirachtin, nimbin, nimbidin, and nimbinin, which are known for their insecticidal and antifungal properties.

Uses:
Acne: Neem seed oil can help treat acne due to its antibacterial properties. It can be applied topically to the affected area, or used as an ingredient in face washes and creams.

Eczema: Neem seed oil can be used to relieve the symptoms of eczema such as itching and inflammation. It can be applied topically to the affected area.

Psoriasis: Neem seed oil can help reduce the scaling and itching associated with psoriasis. It can be applied topically to the affected area.

Ringworm: Neem seed oil has antifungal properties that make it effective in treating ringworm. It can be applied topically to the affected area [3, 5, 10].

3) Coconut oil

Fig 3: Coconut oil

Synonyms: Coconut oil is also known as copra oil or coconut palm oil.

Biological sources
Coconut oil is derived from the kernel or meat of mature coconuts harvested from the coconut palm (Cocos nucifera). This plant is widely grown in tropical regions around the world, and is an important source of food, oil, and other products.

Chemical constituents
Coconut oil is primarily composed of medium-chain fatty acids, including lauric acid, myristic acid, caprylic acid, and capric acid. It also contains small amounts of polyphenols, phytosterols, and vitamins E and K.

Uses: Coconut oil uses in skin disease

Coconut oil has been used for its skin benefits for centuries. It is rich in medium-chain fatty acids, which have antimicrobial and anti-inflammatory properties. Here are some ways in which coconut oil can be used for skin diseases:

Eczema: Coconut oil can help relieve the symptoms of eczema such as itching and inflammation. It can be applied topically to the affected area.

Psoriasis: Coconut oil can help soothe the skin and reduce the redness and scaling associated with psoriasis. It can be applied topically to the affected area.

Acne: Coconut oil can help treat acne due to its antibacterial and anti-inflammatory properties. It can be applied topically to the affected area, but it should be used with caution on acne-prone skin as it can also clog pores.

Dry skin: Coconut oil can help moisturize and nourish dry skin. It can be applied topically to the affected area as a natural moisturizer.

Sunburn: Coconut oil can help soothe sunburned skin and promote healing. It can be applied topically to the affected area.
It's important to note that coconut oil may not be suitable for everyone and can cause allergic reactions in some people. It may also clog pores in some individuals, leading to breakouts. Therefore, it's best to patch test coconut oil on a small area of skin before using it regularly [3, 5].

4) Turmeric powder

Fig 4: Turmeric Powder

Synonyms: Turmeric powder is also known as Indian saffron or haldi powder.

Biological sources: Turmeric powder is derived from the root of the turmeric plant (*Curcuma longa*), which is a member of the ginger family. The turmeric plant is native to India and other Southeast Asian countries, and is now cultivated in many tropical regions around the world.

Chemical constituents
The active ingredient in turmeric powder is curcumin, which is a polyphenolic compound that gives turmeric its bright yellow color. Turmeric powder also contains other bioactive compounds, including turmerone, atlantone, and zingiberene.

Uses: Turmeric powder is widely used as a spice in cooking, especially in Indian and Middle Eastern cuisine. It is also used as a natural food coloring and flavoring agent. In addition, turmeric powder has been used for centuries in traditional medicine to treat a variety of ailments, including digestive disorders, respiratory infections, and skin diseases. More recently, curcumin has been studied for its potential health benefits, including its anti-inflammatory and antioxidant properties, which may help to reduce the risk of chronic diseases such as cancer, Alzheimer's disease, and heart disease. Turmeric powder is also used in cosmetics and personal care products due to its antibacterial and anti-inflammatory properties, and it is used as a natural dye for textiles [3, 5].

5) Bees Wax

Fig 5: Bees Wax

Synonyms: Cera alba, White wax, yellow wax, Honeycomb wax

Biological sources: Beeswax is produced by honeybees, primarily of the genus Apis, which collect it from glands on their abdomens and use it to build honeycomb cells to store honey and to protect larvae.

Chemical constituents

Beeswax is primarily composed of esters of fatty acids and alcohols, with the major components being palmitate, palmitoleate, and oleate esters of long-chain alcohols, along with hydrocarbons and free fatty acids.

Uses

Beeswax is commonly used in cosmetics and personal care products as an emulsifier, thickener, and moisturizer. It is also used in the manufacture of candles, furniture polish, and other products. Beeswax has a long history of use in medicine, and is used in some traditional remedies for conditions such as burns, wounds, and skin infections [3, 5]

Formulation Table

Table 1: Formulation of Ointment Base (According to 30gm)

Sr. No.	Ingredients	Quantity (gm)
1	Wool fat	1.5gm
2	Cetostearyl alcohol	1.5gm
3	Bees Wax	1.5gm
4	White soft paraffin	25.5gm

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Fig 6: Ingredients used for Ointment base

Table 2: Formulation of Herbal ointment

Sr. No.	Ingredients	Quantity Taken			Uses
		F1	F2	F3	
1	Karanja seed oil	1.3%	1.5%	1.65%	Antifungal, Anti-inflammatory
2	Neem seed oil	1.2%	1.3%	1.5%	Antiseptic
3	Turmeric	0.003%	0.005%.	0.0062%	Coloring Agent, Antiseptic
4	Coconut oil	0.3%	0.5%.	0.63%	Rubefacient and antiseptic
5	Ointment Base q.s.	Up to 30gm	Up to 30gm.	. Up to 30gm.	Ointment Base

Extraction Procedure

Soxhlet Extraction

Principle: Soxhlet extraction is a laboratory technique used for the extraction of compounds from solid or semi-solid samples, such as plant material, soils, and food products. The principle of Soxhlet extraction is based on the repeated extraction of a sample with a small volume of solvent over a prolonged period, resulting in the complete extraction of the desired compounds from the sample.

The procedure involves placing the sample to be extracted in a porous thimble made of filter paper, which is then placed in a Soxhlet extraction apparatus. The sample is repeatedly extracted with a small volume of solvent, typically a non-polar organic solvent such as hexane, until all the desired compounds are extracted from the sample.

Fig 7: Soxhlet Extraction

Soxhlet extraction Procedure

Soxhlet extraction has been performed by using different solvents system and selected based on polarity of the solvent system after defatting of the crude drug by petroleum ether. Solvents used were petroleum ether, ethyl acetate, ethanol and distilled water. The moderately coarse powder of the drugs e.g. Momordica charantia (MC), Pongamia glabra (PG) and Piper nigrum (PN) were subjected to Soxhlet extraction with different solvents in increasing order of polarity from non-polar to polar. The 80 gm of dried coarsely powdered drug was packed in Soxhlet apparatus and defatted with 1000 ml of petroleum ether at 40-600C temperature, until the complete defatted. Complete defatting ensured by placing a drop by thimble on the filter paper which did not exhibit any oily spot. The defatted material was removed from the Soxhlet apparatus and air dried to remove the last traces of petroleum ether. The defatted material was subjected to extraction by ethyl acetate and then with ethanol as solvent by Soxhlet apparatus and finally with distilled water by maceration process. The completion of extract was confirmed by evaporating a few drops of the extract on the watch glass and ensuring that no residue remained after evaporating the solvent. The marc was air dried before extracted with the next solvent. Dried marc was macerated with water for 24 h. The extracts were evaporated under reduced pressure at low temperature (30oC) to dryness to yield different extracts, stored in an airtight container in refrigerator for further experimental studies. They were weighed to a constant weight and percentage w/w basis was calculated.

The extraction process involves the following steps:

The solvent is heated to its boiling point, and the vapors rise to the top of the extraction chamber. The condensed solvent drips back into the extraction flask, which contains the sample to be extracted. The solvent dissolves the target compound(s) in the sample and becomes saturated with the compound (s). The solvent then re-enters the extraction chamber, where it is heated and evaporated, leaving behind the extracted compound (s) in the extraction flask. The extraction process is repeated several times to ensure complete extraction of the target compounds. Soxhlet extraction is a time-consuming process but is considered a reliable and effective method for extracting a wide range of compounds from solid and semi-solid samples. It is commonly used in analytical chemistry, food science, and environmental testing [7, 8].

Method of Preparation

Preparation of Ointments by Fusion Method

The fusion method is one of the common techniques used in the preparation of ointments. This method involves heating the ingredients together until they melt and form a homogeneous mixture. The following are the steps involved in preparing ointments by the fusion method:

- **Weigh and measure the ingredients:** The first step is to weigh and measure the ingredients needed to make the ointment. The ingredients may include the active ingredient, the base, and any other additives.
- **Melt the base:** Heat the base in a water bath or a melting pot until it melts completely. The base should be melted over a low heat to avoid burning or overheating.
- **Add the active ingredient:** Once the base has melted, add the active ingredient to the melted base and stir it until it dissolves completely.
- **Incorporate any additives:** If any other additives are needed, such as preservatives, fragrances, or colorants, add them to the mixture and stir until they are well incorporated.
- **Pour the mixture into a container:** Once all the ingredients are mixed, pour the mixture into a clean, dry container while it is still warm and in a liquid state.
- **Allow to cool and solidify:** The ointment should be allowed to cool and solidify at room temperature or in a refrigerator. Once it solidifies, it can be labeled and stored for use.

Overall, the fusion method is a simple and effective technique for preparing ointments. However, it is important to ensure that the ingredients are mixed thoroughly to achieve a homogeneous mixture and that the ointment is cooled properly to avoid any separation or crystallization.

Collection Plant Material

Oil of Neem and Karnaj seed was collected from local market of bodhegaon. The part used for extraction of oil is seeds. The method which is used for extraction process is cold pressed method. The quality of extracted oil is 100% pure, authentic, and natural. This data is obtained from technical data sheet provided by the industry. The extracted oil is stored in air tight container amber colored bottle with the dropper

Herbal ointment was made by levigating correctly weighed Neem and Turmeric extract into the ointment base to make a smooth paste with 2 or 3 times the weight of base, progressively incorporating more base until homogeneous ointment was formed, and then transferred to a suitable container.

Evaluation of Herbal Ointment

1. Colour
2. Odour
3. Consistency
4. pH
5. Spreadability
6. Extrudability
7. Diffusion study
8. Loss On drying (LOD)
9. Washability
10. Non irritancy Test
11. Viscosity
12. Stability study

Colour: Physical parameters colour examined by visual examination and it is found to be Yellow colour.
Odour: Physical parameters odour is found to be Characteristics.
Consistency: Smooth and no grittiness were observed.
pH: pH of prepared herbal ointment was measured by using digital pH meter. The solution of ointment was prepared by using 100 ml of distilled water and set aside for 2 h. pH was determined in triplicate and average value was calculated and it is found to be 5.79

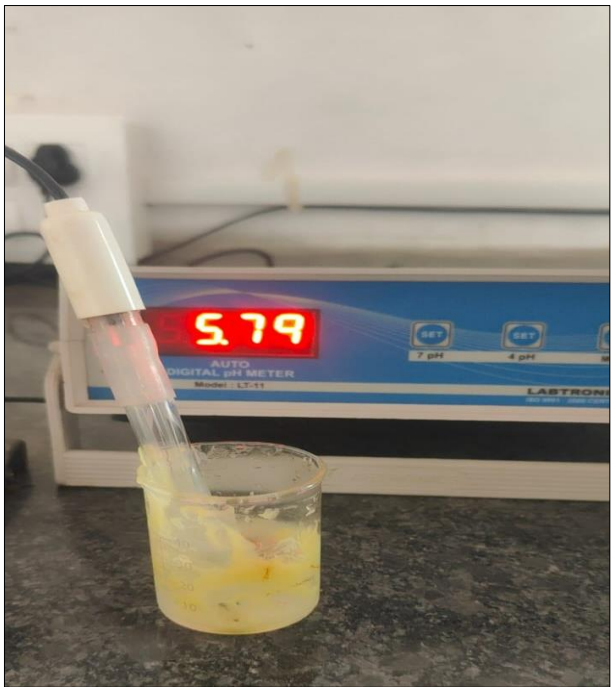

Fig 8: Determination of PH

Spread ability
Spread ability is determined by clamping the excess sample between two compressed blades to a uniform thickness by applying a fixed weight for a fixed period of time. The spread is determined by the time it takes to separate the two slides. The shorter the time it takes to separate two slides, the better the spread.
Spread ability was calculated by following formula.
Spread ability was calculated by following formula:

$$S=M\times L/T$$

Were,
S= spread ability
M= weight tide to the upper slide
L= length of glass slide
T= time taken to separate the slides.

Extrudability: The formulation was filled in collapsible tube container. The extrudability was determined in terms of weight of ointment required to extrude 0.5 cm of ribbon of ointment in 10 seconds.

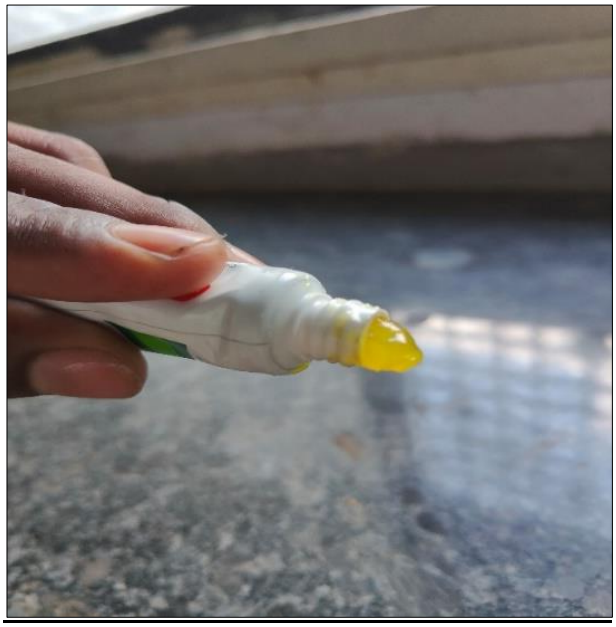

Fig 9: Determination of Extrudability

Diffusion study: The diffusion study was carried out by preparing agar nutrient medium. A hole has made on a board at the center and poly-herbal ointment was by placed in it. The time taken by ointment to get diffused through was noted after 1h.

Fig 10: Determination of Diffusion

Loss on drying (LOD): LOD was determined by placing the poly-herbal ointment formulation in Petri dish on water bath and dried at 105^{o}C temperature.

Solubility
Solubility is main criteria for the ointment formulation to check the cleansing properties solubility has determined in boiling water, distilled water and other solvent including alcohol, ether and chloroform.

Fig 11: Determination of Solubility

Wash ability

Formulation was applied on the skin and then ease extend of washing with water was checked and it is found to be easily washable.

Before After

Before **After**

Fig 12: Determination of wash ability

Non irritancy Test

Herbal ointment prepared was applied to the skin of human and observed that the prepared ointment was not showing any irritancy to the skin.

Fig 13: Determination of irritancy test

Stability study

Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 20 °C, 25 °C and 37 °C. The herbal ointment was found to be physically stable at different temperature i.e., 20 °C, 25 °C and 37 °C within four weeks [11].

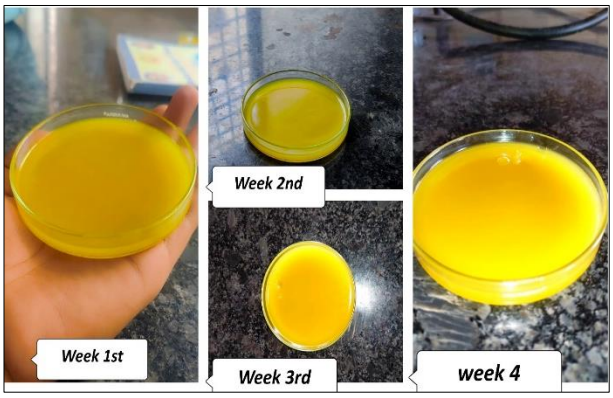

Fig 14: Stability Study

Results

Physicochemical evaluation of formulated ointment

Table 3: Physicochemical evaluation of formulated ointment

Physiochemical parameters	Observations
Colour	Yellow
Odour	Characteristic
Consistency	Smooth
pH	5.79
Spreadability (seconds)	8 Sec
Extrudability	0.45 gm
Diffusion study	0.5cm
Loss on drying	12%
Solubility	Miscible with alcohol, ether, chloroform
Washability	Good
Non irritancy	Non- irritant
Stability study (25 °C and 37 °C)	Stable

Discussion

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 oil-in-water emulsion method. The aqueous phase was prepared by dissolving *Pongamia pinnata* extract in distilled water, while the oil phase was prepared by heating a mixture of mineral oil, beeswax, and cetyl alcohol. The two phases were then mixed while continuously stirring, resulting in the formation of an emulsion. The emulsion was cooled to room temperature while being stirred, and then methylparaben and propylparaben were added as preservatives. 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, viscosity, and spread ability.

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 spread ability of the ointment was found to be 8 sec, indicating that it can cover a larger area of skin.

The biological activity of the ointment was evaluated by testing its antibacterial, antifungal, and antipsoriatic activity. 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. Additionally, the ointment showed significant anti-inflammatory activity in the mouse ear swelling test, which suggests its potential for the treatment of psoriasis.

Conclusion

Pongamia pinnata, also known as the Indian Beech tree, is a plant with a variety of medicinal properties. Its seeds contain compounds such as pongamol, karanjin, and flavonoids that possess antibacterial, antifungal, anti-inflammatory, and anti-psoriatic properties.

To formulate an ointment using *Pongamia pinnata*, the seeds are first extracted using a suitable solvent such as ethanol or methanol. The extract is then incorporated into a suitable ointment base, such as white petrolatum or beeswax.

The ointment is then evaluated for its antibacterial, antifungal, and anti-psoriatic properties using various *in vitro* and *in vivo* tests. *In vitro* tests can include agar diffusion and broth microdilution assays to determine the minimum inhibitory concentration (MIC) of the ointment against different strains of bacteria and fungi. *In vivo* tests can include animal models of psoriasis to determine the efficacy of the ointment in treating the skin condition.

In conclusion, the ointment containing *Pongamia pinnata* extract was successfully formulated using a simple oil-in-water emulsion method. The ointment exhibited good physicochemical properties and significant biological activity against bacterial and fungal infections, as well as anti-inflammatory activity. Therefore, *Pongamia pinnata* can be considered a promising natural source for the development of new and effective topical formulations for the treatment of skin infections and psoriasis. However, further studies are needed to confirm its safety and efficacy in humans.

Overall, the formulation and evaluation of an ointment using *Pongamia pinnata* can provide a natural and effective alternative to conventional treatments for bacterial, fungal, and psoriatic skin infections.

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