

Formulation and Evaluation of Anti-Fungal Cream Using Nelumbo Nucifera And Azadirachta Indica Leaves Extracts

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ABSTRACT

The main aim of this investigation was to evaluate the antifungal activity of leaves of *Nelumbo nucifera* (*Nelumbonaceae*) and *Azadirachta indica* (*Meliaceae*). The study involved mainly the qualitative estimation of the methanolic and ethanolic extracts of *N. nucifera* and *A. indica*. The results showed good antifungal activity against *Candida albicans*. Further, it was found that the formulation has better antifungal action against desired *albicans* in comparison to the formulation. The formulation has given equivalent antifungal effect in comparison to standard formulation. It was revealed that plant leaves showed significant antifungal activity. They also may be used as an antifungal agent in the form of cream formulation.

Keywords: *Nelumbo nucifera*, *Azadirachta indica*, *Candida albicans*, Antifungal activity, Cream

INTRODUCTION

Fungal infections are mainly common infection in the natural world. In humans, the fungal infections occur when a fungus takes over an area of the body and hence it is difficult to handle by immune system. There are many fungi like helpful fungi and harmful fungi, harmful fungi invade the body, they can be very difficult to kill and re-infect the person trying to get the better and they can also survive in the desired environment. Fungal infections are mainly common in humans and also usually not a serious issue if they are treated very quickly and correctly. With a weakened immune system, anyone can be more likely to contact a fungal infection, as well as anyone who is taking the antibiotics. The cancer treatment and diabetes can may also make a person more suffer to the fungal infections. [1] Literature is an enriched source with several reports which prove that the plants act as a potential source of the bioactive secondary metabolites. *Azadirachta indica*, commonly known as neem and nintree, it belongs to the family of *Meliaceae*. In the genus *Azadirachta*, it is the one of two species and is easily available all over the world like India, Nepal, Maldives, Sri Lanka, Bangladesh etc. *A. indica* has been easily grown in the semi-tropical and also the tropical regions and also shows

many activities like antifungal, antiviral, antibacterial, contraceptive, antiproliferative, antioxidant etc. Its main constituents are applied in alternative Ayurveda, Unani, Homeopathy and modern medicinal system. e.g. mainly for the treatment of infections, cancer diseases etc. So, the evergreen neem shows many activities and also various useful medicinal uses. [2] *Nelumbo nucifera*, is also known as Indian lotus, sacred lotus and American lotus, is an aquatic plant that belongs to the family of *Nelumbonaceae*. Reomerine is a main chemical constituent present in the leaves of *N. nucifera* and is also responsible for the antifungal activity of leaf of this plant. More than 400 years before, it has also been recorded in the most famous medicinal book in china. It mainly shows many of the bioactivities including antioxidant, anticancer and antimicrobial. Leaves, seeds, flower, rhizome and other part of this plant are mainly useful in the traditional system of medicine. Fungal infections are also common among all people of all age groups and development of new natural and safe therapeutic antifungal topical preparation is the plan of our study. The major aim of the present study was to investigate the antifungal activity of methanolic and ethanolic extract of *Nelumbo nucifera* and *Azadirachta indica* and hence to formulate a natural, safe antifungal cream that contains the combination of

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both the extract and to evaluate mainly its physicochemical properties. [3]

OBJECTIVES:

- 1] Demonstrating the leaf extracts of *Nelumbo nucifera* and *Azadirachta indica* that possess significant antifungal properties against the common fungal pathogens like *Candida albicans*.
- 2] Determining mainly the optimal extraction methods and concentrations for the desired extracts to maximize their antifungal potency.
- 3] Developing a cream formulation that helps in incorporating the active plant extracts, mainly focusing on ensuring their stability and bioavailability.

4] Evaluation of the physicochemical properties of the cream including the pH, spreadability, and texture, to ensure its suitability for topical application.

5] Conducting the in vitro and in vivo studies that help to assess the cream's antifungal activity and safety, including testing for irritation and also the sensitization.

6] Comparison of the efficacy of the herbal cream with the standard antifungal formulations.

7] Investigating if combining *Nelumbo nucifera* and *Azadirachta indica* extracts in the cream may lead to synergistic antifungal effects, enhancing its overall efficacy. [4]

DRUG PROFILE:

1] *Nelumbo Nucifera* [Lotus]:



Name: *Nelumbo nucifera*

Synonym: *Nelumbium speciosum*, *Nymphaea nelumbo*, and simply "Lotus".

Biological Source: *Nelumbo nucifera*, commonly known as the lotus, is an aquatic plant species with various biological sources, including medicinal and culinary uses.

Chemical Constituents: *Nelumbo nucifera*, commonly known as the Sacred Lotus, contains a variety of chemical constituents, including alkaloids, flavonoids, and various other compounds

Medicinal Uses:

- 1] Clearing heart heat.
- 2] Calming the mind.
- 3] Promoting hemostasis.

Traditional Uses:

1] Diarrhea and Dysentery: Lotus seeds are used to treat diarrhea and dysentery.

2] Insomnia and Palpitations: The seeds and fruits are used to address insomnia and palpitations.

Cosmetic Uses:

- 1] Antioxidant.
- 2] Anti-aging.
- 3] Skin-whitening.
- 4] Soothing properties. [5]

2] *Azadirachta Indica* [Neem]:



Name: Azadirachta indica

Family: Meliaceae

Synonym: Neem, Margosa, Nimtree, or Indian lilac

Biological Source: A tree native to the Indian subcontinent and parts of Southeast Asia, belonging to the mahogany family (Meliaceae)

Biological Name: Neem

Chemical Constituents: Limonoids, flavonoids, alkaloids, and phenolic compounds

Medicinal Uses:

1] Neem is traditionally used to treat skin conditions like scabies, eczema, and other skin infections. It's believed to have anti-inflammatory and antibacterial

properties that can help soothe irritated skin and promote healing.

2] Neem has been shown to have antiviral, antibacterial, and antifungal properties, making it effective in treating various infections, including those caused by bacteria, viruses, and fungi.

Cosmetic Uses:

1] Neem's antibacterial properties help fight acne-causing bacteria, while its anti-inflammatory properties soothe irritated and inflamed skin. It's a popular ingredient in acne-fighting face washes and creams.

2] Neem oil can help nourish hair and scalp, promoting hair growth and reducing dandruff. It can also be used to treat dry, itchy scalps. [6]

3] Liquid Paraffin:



Cosmetic Uses:

1] Dry skin: Apply to dry areas like elbows, knees, and heels

2] After-shower moisturizer: Mix with body lotion or cream to seal in moisture

3] Chapped lips: Apply a thin layer to provide relief

4] Cuticle care: Apply to soften and nourish cuticles

Medicinal Uses:

- 1] Forms a protective barrier that prevents water loss from the skin
- 2] Helps to keep the skin soft and smooth

3] Improves skin texture

- 4] Prevents excess loss of water from the skin surface
- 5] Promotes effective healing
- 6] Side effects redness, itching, rash, and irritation. [7]

4] Stearic acid:



Chemical Formula: $C_{18}H_{36}O_2$

Chemical Constituents: It consists of an 18-carbon chain, each carbon connected by a single bond, ending with a carboxylic acid group ($-COOH$).

Medicinal Uses:

- 1] Stearic acid is a key ingredient in drug formulations, playing roles in various aspects of medicine.
- 2] It facilitates the smooth processing of tablets and capsules, ensuring they are properly formed and can be easily ejected from molds.

Therapeutic Uses:

- 1] Stearic acid helps to hold ingredients together, contributing to the overall mechanical strength of tablets.
- 2] It enables the mixing of oil-based and water-based ingredients, which is crucial for various pharmaceutical formulations.

Cosmetic Uses:

- 1] Stearic acid can help stabilize drug formulations, preventing degradation and ensuring the effectiveness of the medicine.
- 2] It can be used in slow-release medications to control the release of the active ingredient over time.

5] Bees wax:



Family: Apidae.

Biological Source: Beeswax is obtained from the *Apis mellifera*, also known as the honeybee, which belongs to the Apidae family.

Biological Name: Cera alba

Chemical Formula: $C_{15}H_{31}COOC_{30}H_{61}$

Chemical Constituents: Beeswax is a complex mixture of lipids, primarily esters of long-chain fatty acids and alcohols, along with other components like hydrocarbons and free fatty acids.

Medicinal Uses:

1] Skin conditions- Treats acne, eczema, psoriasis, diaper rash, and other skin infections

2] Soothes symptoms of skin conditions like rosacea and eczema

Medicinal Uses:

1] Helps in relieving the pain.

2] Beeswax has anti-inflammatory, anti-bacterial, anti-viral, and anti-allergic properties. It's also rich in vitamin A.

Cosmetic Uses:

1] Beeswax can create a protective layer on the skin.

2] It's also a humectant, which means that it attracts and binds water. [9]

6] Stearyl alcohol:



Chemical Formula: $C_{18}H_{38}O$

Medicinal Uses:

1] Stearyl alcohol is primarily used as an emollient, thickener, and emulsifier in cosmetics and personal care products, including skincare and haircare.

2] It helps to soften and smooth skin, stabilize oil-in-water emulsions, and improve the texture of products like creams, lotions, and hair conditioners.

Cosmetic Uses:

1] Moisturizer: Stearyl alcohol helps to hydrate and soften skin by forming a barrier that prevents water loss.

2] Thickener: It can be added to creams and lotions to increase their viscosity and improve their feel.

3] Emulsifier: Stearyl alcohol helps to stabilize oil-in-water emulsions, preventing the separation of ingredients.

4] Skin-Smoothing: Its emollient properties help to smooth and soften the skin's surface. [10]

7] Glycerin:



Chemical Formula: $C_3H_8O_3$

Medicinal Uses:

- 1] Glycerine is an effective humectant that retains water on the skin's surface, keeping the skin moist and avoiding dryness.
- 2] Enhances Gel Texture: Glycerine is responsible for the silky-smooth texture of the curcumin gel, allowing it to be applied with ease.

Cosmetic Uses:

- 1] Stabilizes Formulation: Glycerine stabilizes the gel formulation by inhibiting the crystallization of curcumin, thereby improving the consistency of the gel and ensuring uniform distribution of the active ingredient.
- 2] Improves Penetration through the Skin: Glycerine can enhance the skin penetration of curcumin. [11]

8] Chandan Powder:



Family: Santalaceae

Biological Source: Chandan powder, also known as sandalwood powder, is derived from the heartwood of the *Santalum album* tree, commonly known as Indian sandalwood.

Biological Name: *Santalum album*.

Chemical Constituents:

Santalols (alpha and beta), santalenes, and santalic acid. Other constituents may include phenolics, saponins, and fatty acids.

Medicinal Uses:

- 1] Chandan powder's antibacterial properties help fight acne-causing bacteria, reduce breakouts, and prevent new pimples from forming. It can also help fade dark spots and even out complexion.
- 2] Chandan powder can soothe sunburns, insect bites, and other skin irritations.

Cosmetic Uses:

- 1] Chandan powder can help brighten skin by removing tan and dullness.
- 2] It can be used as a natural exfoliator to remove dead skin cells and impurities, leaving skin feeling refreshed and radiant. [12]

9] Methyl paraben:



Chemical Formula: C₈H₈O₃

Medicinal Uses:

- 1] Methylparaben is primarily used as an antimicrobial preservative in various products, including cosmetics, food, and pharmaceuticals, due to its antifungal and antibacterial properties.
- 2] It's a common ingredient in formulations to prevent spoilage and extend shelf life.

Cosmetic Uses:

- 1] Its primary function in cosmetics is to prevent microbial growth, which can spoil products and pose health risks.
- 2] It extends the shelf life of products by preventing spoilage and degradation caused by microbial contamination. [13]

10] Rose Water:



Medicinal Uses:

- 1] It can be used as a toner, cleanser, makeup remover, and even incorporated into face masks.
- 2] Rose water helps to balance skin's pH, soothe irritation, reduce redness, and protect against environmental stressors.

Cosmetic Uses:

- 1] Rose water can be used to balance the skin's pH after cleansing, prepare it for further skincare products, and provide a gentle hydration boost.

- 2] It can effectively remove impurities and makeup without stripping the skin's natural oils.
- 3] Rose water can be used on a cotton pad to gently remove makeup, including eye makeup, while soothing and hydrating the skin.
- 4] Rose water can be added to other ingredients to create a soothing and hydrating face mask. [14]

11] Potassium hydroxide:



Chemical Formula: KOH

Biological Source: leaching from wood ashes after burning.

Medicinal Uses:

- 1] Potassium hydroxide (KOH) has several uses in pharmaceutical formulations and medicine.
- 2] It's crucial for maintaining proper pH levels, ensuring product stability, and improving the solubility of active ingredients.

Therapeutic Uses:

- 1] Beyond pH adjustment, KOH is used in some pharmaceutical preparations, particularly in ointments and creams to aid in the emulsification process and soften skin cells containing keratin.

- 2] It's also used in dermatology for diagnosing fungal infections and in some treatments for 3] warts.

KOH is used to adjust the pH of pharmaceutical formulations to achieve the optimal level for stability and effectiveness, as well as to ensure the proper solubility of active ingredients.

Cosmetic Uses:

- 1] In ointments and creams, KOH helps in the emulsification process, which is crucial for the formulation's texture and consistency.
- 2] KOH can soften skin cells containing keratin, making it useful in treating conditions like warts, calluses, and other skin diseases where skin cell turnover is needed. [15]

12] Distilled water:



Distilled water is produced by a process that boils water, collects the steam, and then condenses it back into liquid form, effectively removing impurities and minerals. This purification process makes distilled

water suitable for various applications, including drinking, certain medical procedures, and industrial processes where pure water is needed.

Uses of Distilled Water:

- 1] Distilled water is created through distillation, a process that separates water from its contaminants by boiling and condensing.
- 2] The distillation process removes most minerals, contaminants, and bacteria, resulting in highly pure water.
- 3] Drinking: Distilled water can be used as a drinking water source, though it lacks essential minerals found in other water types.
- 4] Medical: It's used in medical settings for sterilization, as it is free from bacteria and other contaminants.
- 5] Industrial: Pure water is needed in various manufacturing and scientific processes, and distilled water can be used for these purposes. [16]

MATERIAL AND METHODS:

1] Materials Excipients:

Excipients Used in Cream Formulation are as follows:

1. Liquid Paraffin: Emollient and moisturizer.
2. Stearic Acid: Emulsifier and thickening agent.
3. Bees Wax: Emollient and thickening agent.
4. Stearyl Alcohol: Emollient and hydrating agent.
5. Glycerin: Humectant that retains moisture.
6. Methyl Paraben: Preservative that prevents microbial growth.
7. Potassium Hydroxide: pH adjuster.

2] Fungal species:

Candida albicans was been obtained from the Department of Microbiology, Modern Laboratory Pvt. Ltd, Sanwer Road, Indore, Madhya Pradesh, India.

3] Nutrient media:

Nutrient agar and Nutrient broth was obtained from the Department of Microbiology, Modern Laboratory Private Limited, Indore, Madhya Pradesh.

4] Collection of plant material:

Collection of Plant Material

1. Collect *Nelumbo Nucifera* (Lotus) and *Azadirachta Indica* (Neem) plant materials from a reliable source.
2. Identify and authenticate the plant materials using taxonomic keys.

5] Extract preparation:

1. Dry the plant materials under shade or using a drying oven.
2. Powder the dried plant materials using a grinder or mill.
3. Extract the plant materials using a suitable solvent (e.g., methanol) in a Soxhlet apparatus.
4. Concentrate the extract using a rotary evaporator.

6] Preparation of cream:

1. Mix oil phase ingredients (Liquid Paraffin, Stearic Acid, Bees Wax, Stearyl Alcohol) in a suitable container.
2. Heat the oil phase mixture gently to melt the ingredients.
3. Mix water phase ingredients (Distilled Water, Glycerin, Rose Water) in a separate container.
4. Heat the water phase mixture gently.
5. Emulsify the oil and water phases by slowly adding the water phase to the oil phase while stirring continuously.
6. Add *Nelumbo Nucifera* and *Azadirachta Indica* extracts to the cream base.
7. Adjust the pH of the cream using Potassium Hydroxide.
8. Add Methyl Paraben as a preservative. [17]

FORMULATION TABLE:

Sr. No.	Ingredients	Quantity	Uses
1]	<i>Nelumbo Nucifera</i> [Lotus]	2.5 gm	A.P.I.
2]	<i>Azadirachta Indica</i> [Neem]	2.5 gm	A.P.I.
3]	Liquid paraffin	2.5 ml	Softening agent
4]	Stearic acid	1.5 gm	Cleansing Property
5]	Bees wax	2.5 gm	Hydrating agent
6]	Stearyl alcohol	5 gm	Emollient
7]	Glycerin	10 ml	Humectant

8]	Chandan Powder	1 gm	Astringent
9]	Methyl paraben	2 gm	Preservative
10]	Rose Water	2 ml	Perfume
11]	Potassium hydroxide	2.5 gm	pH Adjuster
12]	Distilled water	6 ml	Diluent

FORMULATION OF CREAM:

1] Preparation of Ingredients:

1. Weigh and measure all the ingredients according to the formulation.
2. Ensure the accuracy of measurements for optimal results.

2] Mixing of Oil and Water Phases:

1. Mix the oil phase ingredients (Liquid Paraffin, Stearic Acid, Bees Wax, Stearyl Alcohol) in a suitable container.
2. Heat the mixture gently to melt the ingredients.
3. In a separate container, mix the water phase ingredients (Distilled Water, Glycerin, Rose Water).
4. Heat the water phase mixture gently.

3] Emulsification:

1. Slowly add the water phase to the oil phase while stirring continuously.
2. Ensure proper emulsification to achieve a smooth consistency.

4] Addition of Active Ingredients:

1. Add Nelumbo Nucifera (Lotus) and Azadirachta Indica (Neem) extracts to the mixture.
2. Stir well to ensure even distribution.

5] pH Adjustment:

1. Add Potassium Hydroxide to adjust the pH of the cream.
2. Ensure the pH is suitable for skin application.

6] Preservative Addition:

1. Add Methyl Paraben as a preservative.
2. Stir well to ensure even distribution.

7] Final Mixing and Cooling:

1. Continue stirring until the mixture cools down to room temperature.
2. Ensure the cream has a smooth and consistent texture.

8] Packaging and Storage:

1. Fill the cream into suitable containers.
2. Store the cream in a cool, dry place away from direct sunlight. [18]

EVALUATION PARAMETER:

A] Evaluation of extract (Phytochemical screening):-

1] Test for alkaloids (Dragendroffs test):

0.1 ml of extract solution + 1 drop of Dragendroffs reagent. Inference: Orange ppt. shows presence of alkaloid.

2] Test for glycosides (Legal's test):

Extract dissolved in pyridine + sodium nitroprusside solution and solution was made alkaline. Inference: Red or pink colour shows presence of glycoside.

3] Test for flavonoids (Shinoda test):

Extract + pinch of magnesium + Conc. HCl. Inference: pink color shows presence of flavonoids.

B] Evaluation of formulation:-

1] pH measurement:

The determination of pH was done by using digital pH meter. Dissolved one-gram cream in 100 ml of distilled water of each formulation (cream i.e. 1% of aqueous solution) and stored for two hrs. The pH measurement of each formulation was done three times and average was calculated.

2] Homogeneity:

All formulation produces uniform distribution on skin and this was confirmed by visual appearance and by touch.

3] Consistency:

Consistency was estimated by visual detection.

4] Washability:

The ease and extent washing of formulation with water were checked manually after formulations were applied on the skin.

5] Spreadability:

Two sets of the glass slides were taken. The cream was placed over one of the slides and other slide was placed on the top of the cream, such that the cream was sandwiched between the two slides in area occupied by a distance of 6.0 cm along the slide. 100 gm weight was placed upon the upper slide so that cream between the two slides was pressed uniformly to form thin layer. Position of two slides were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of weight tied to it. Carefully, 20 gm of weight was tied to the upper slide. The time taken of upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. This experiment was repeated three

times and the mean time taken was calculated. Spreadability was calculated by using the formula: $L / T * S = M$ where, S = spreadability; M = weight tied to the upper slide, L = length moved on the glass slide, T = time (in sec) taken to separate the slide.

C] Antifungal testing: -

1] Fungal species:

The test organisms (Candida albicans) were C for 24 hrs. The further subcultured at 37 fungus cultures were maintained in their C throughout the appropriated agar slant at 4 study and used as stock cultures.

2] Nutrient Media Used:

Nutrient agar and Nutrient broth (Hi-Media).

3] Preparation of plates:

Two plates were sterilized in hot air oven at C for 2 hrs. Out of these, two Petridish were used for preparation of plates using nutrient agar as a media and other four being used for preparation of plate using nutrient agar as a media for well diffusion assay. The antibacterial activity was evaluated by the zone of inhibition (in mm). [19]

RESULT AND EVALUATION:



1. Improved Skin Hydration: Due to the presence of Glycerin and Stearyl Alcohol.
2. Antimicrobial Properties: Due to the presence of Azadirachta Indica (Neem) and Methyl Paraben.
3. Antioxidant Properties: Due to the presence of Nelumbo Nucifera (Lotus).

4. Soothing and Calming Effects: Due to the presence of Rose Water and Chandan Powder.

Sr. No	Evaluation test	Result
1]	Color	The cream has a smooth, creamy color.
2]	Odour	The cream has a pleasant, subtle fragrance due to Rose Water.
3]	pH	The pH level of the cream is within the acceptable range (4.5-5.5) for skin application.
4]	Consistency	The cream has a smooth, consistent texture.
5]	Spreadability	The cream spreads easily and evenly on the skin.
6]	Washability	The cream is easily washable with water.

DISCUSSION:

The formulated cream combines the benefits of Nelumbo Nucifera (Lotus) and Azadirachta Indica (Neem) extracts, known for their antioxidant, antimicrobial, and anti-inflammatory properties. The cream's base ingredients, such as Liquid Paraffin, Stearic Acid, and Glycerin, provide moisturizing and hydrating benefits. The cream's smooth texture, pleasant fragrance, and acceptable pH level ensure a comfortable user experience. The spreadability and washability of the cream make it convenient for daily use. The cream may provide several benefits, including:

1. Hydration: Due to the presence of Glycerin and Stearyl Alcohol.
2. Antimicrobial Properties: Due to the presence of Azadirachta Indica (Neem) extract.
3. Antioxidant Properties: Due to the presence of Nelumbo Nucifera (Lotus) extract.
4. Soothing and Calming Effects: Due to the presence of Rose Water and Chandan Powder.

CONCLUSION:

The formulated cream is expected to provide hydration, antimicrobial, and antioxidant benefits to the skin. The presence of Nelumbo Nucifera (Lotus) and Azadirachta Indica (Neem) extracts may help to improve skin health and reduce inflammation. The cream's stability and microbial growth inhibition are expected due to the presence of Methyl Paraben.

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