



Formulation And Evaluation Of Herbal Anti-Fungal Cream Using *Moringa Oleifera* Leaf Extract.

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Abstract :

In developing countries, traditional medicine derived from plants is increasingly relied upon. Thousands of pharmaceutical chemicals have been isolated from plant species, with many naturally occurring compounds used to treat various illnesses. *Moringa oleifera*, known as the “miracle tree,” offers numerous medicinal benefits beyond its culinary use. This study formulated and evaluated an anti-fungal herbal cream using hydro-alcoholic extract of *Moringa oleifera* leaves. The extract was obtained through microwave-assisted extraction and formulated into a cream. The cream's organoleptic properties, pH, viscosity, spreadability, homogeneity, and washability were assessed. Patch testing showed low skin irritation risk. The cream's anti-fungal activity against *Aspergillus niger* was evaluated using the well diffusion method, demonstrating an 18 mm zone of inhibition. The study confirms the retention of Moringa's anti-fungal properties in the cream formulation. The cream exhibited desirable properties, including a pH range suitable for skin (5- 6) and moderate viscosity. The findings suggest *Moringaoleifera* leaf extract's potential as an effective antifungal agent in herbal cream formulations. This research highlights traditional medicine's importance and natural compounds potential in developing innovative treatments. The formulated cream shows promise for treating fungal infections, warranting further research and development for potential therapeutic applications.

Index terms : Anti-fungal cream, Fungal strain, Hydro-alcoholic extract, *Moringa oleifera*

1. INTRODUCTION:

Approximately 90% of people in developing countries rely on traditional medicines, primarily derived from plants. Despite the potential of tropical forest plant species, fewer than 5% species have been examined for medicinal values. Currently, over 120 pharmaceutical chemicals are isolated from around 100 plant species, highlighting the importance of plant-based medicines, especially in Asian, African, and South American countries. Many traditional plant derived chemicals being used as drugs or medicines to treat various type of illness.¹

Moringa (Moringa oleifera) is a plant with many health benefits, known as the “miracle tree”. It's native to India, Himalayan region but now grows in many tropical countries like Africa, South America, Pakistan, Afghanistan, Nepal, etc. For centuries, the plant has been utilized in traditional medicine in the treatment of various conditions. It's rich in bioactive compounds and is considered a trustworthy and affordable alternative in the medicinal field. The other synonym used for moringa are horseradish tree, drumstick tree, marango, sajna, kelor, saijhan, Trishnagandha, saguna, Akshivaetc. There are 13 different known species of moringa belongs to the family moringaceae in which *Moringa oleifera* is most common species. The moringa plant is highly nutritious and has different medicinal values. Almost entire plant including leaves, roots, bark, stem, seeds, flower, gum can be utilized in treating various diseases.^{2,4,7}

Fig. No.1 *Moringa oleifera* leaves

The Taxonomical Classification of *Moringa oleifera* drug plant as follows:³

Kingdom : Plantae

Sub – kingdom : Tracheobionta

Super division : Spermatophyte

Division : Magnoliophyta

Class : Magnoliopsida

Sub-class : Dilleniidae

Order : Capparales

Family : Moringaceae

Genus : *Moringa*

Species : *oleifera*

Moringa has traditionally used for centuries to treat many illnesses, like asthma, bronchitis, and skin problems. It has many health benefits such as reducing fever, ulcers, and epilepsy, and also has cosmetic uses, like moisturizing skin and hair. *Moringa* oil has even been used in skin ointments since ancient Egyptian times. It's considered one of the most nutritious plants discovered, with many uses in healthcare industry and beauty products. *Moringa* shows various activities including anti-inflammatory, antibacterial, antioxidant, antifungal, antipyretic, anticancer, anti-aging, antimicrobial, antidiabetic, etc. So that *moringa* plant is called as the "Miracle tree".²

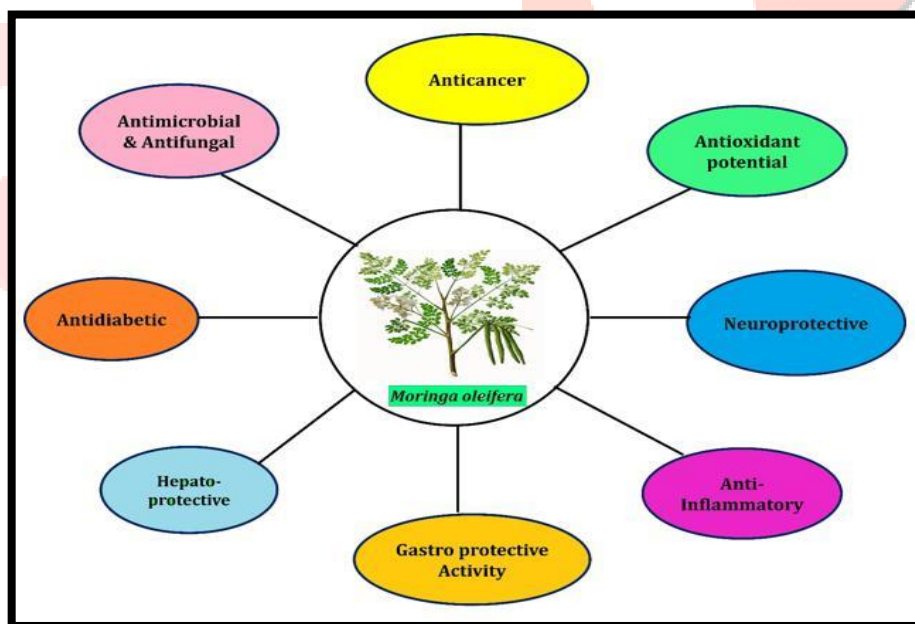


Fig. No.2 Pharmacological action of moringa

Moringa plant contains flavonoids, phenolic acids, phytosterols, fatty acids, vitamins, and minerals. The phytoconstituents present in *moringa* are rutin, quercetin, Kaempferol, Isorhamnetin, myricetin, ferulic acid, caffeic acid derivative (5-caffeoylquinic acid), zeatin, β -sitosteroletc. The *moringa oleifera* plant possess antifungal activity against various fungus. Flavonoids present in these plant shows mainly antifungal property.^{4,5}

Moringa oleifera has been found to have antifungal properties, effective against a wide range of fungi. Specifically:

- The root extract contains a compound (N-benzylethyl thioformateaglycone of deoxy niazimicin) that inhibits the growth of various fungi.

- Extracts from leaves, seeds, and stems have demonstrated to inhibit the growth of fungal species like *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus oryzae* etc.
- The fruit of *Moringa oleifera* contains compounds that inhibit the growth of *Candida albicans*, a common fungus.⁵

Overall, *Moringa oleifera* has been found to have significant antifungal properties, making it a potential natural remedy for various infections.⁵

Cream, a cosmetic formulation containing leaf extract of moringa naturally enhances hydration, making it ideal for moisturizing applications and management of dry skin. Cream are dosage form which are applied externally. They are usually semi-solid emulsion or viscous liquid. Cream are biphasic dosage form. There are two type of cream formulation o/w and w/o emulsion. They are opaque in nature and containing one or more medicinal ingredients. Cream are mainly applied topically on skin, sometimes applied to the vagina and rectum. Due to ease of spreading and removal cream are more frequently used compared to ointment by many patients.^{6,16}

The goal of this research was to assess the antifungal activity of *Moringa oleifera* leaf extract and its application in a cream formulation.⁶

2. MATERIAL AND METHODS:

Various materials and equipment's were used in this study of formulation and evaluation of herbal antifungal cream.

2.1 Chemicals: Bees wax, White Vaseline, Benzoic acid, Cetyl alcohol, Propylene glycol, Triethanolamine, Carboxy methyl Cellulose(CMC) and Moringa leaf extract(API), Ferric chloride reagent, Lead acetate solution, Ethanol, Distilled water

2.2 Equipment: Weighing Balance, Microwave oven, Hot plate, pH meter, Brookfield viscometer, Autoclave.

2.3 Collection of plant material:

Moringa oleifera leaf powder was collected from Bagwan Ayurvedic medical store which was made from dried leaves of *M. oleifera* so it is 100 % natural. This leaf powder was manufactured by Shree Narnarayan ayurvedic Pharmacy 1, Radhe Estate, Tajpur road, Ahmedabad-382 213(Gujarat). The leaf powder was must be **100 % pure**.



Fig. No.3 marketed leaf powder

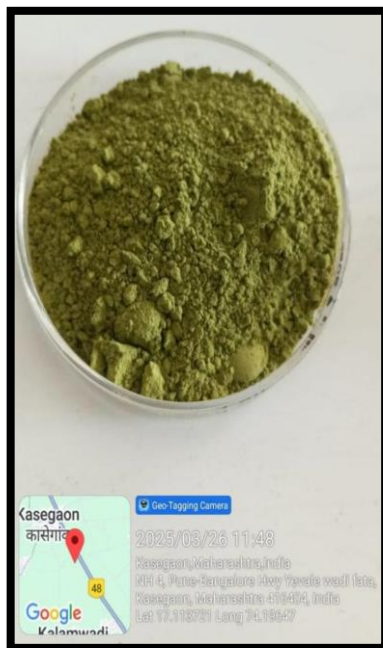


Fig. No.4 crude drug powder

2.4 Preparation of Hydro- alcoholic extract :

Microwave assisted extraction method was used for extraction due to its faster rate and effective extraction.¹¹

- 1) Extraction was performed on *Moringa oleifera* leaf powder in hydro alcoholic solvent (70% ethanol+30% water) by microwave assisted extraction method.
- 2) 25g of *M. oleifera* leaf powder was placed in vessel and 250 ml hydro alcoholic solvent was added into the vessel, then this vessel placed into the microwave oven for extraction.



Fig. No.5 Weighing of Powder



Fig. No.6 Microwave extraction

- 3) The extraction was performed at 400 W for 20 minutes.
- 4) Microwave radiation creates heat energy, leaf powder will absorb the energy and the bioactive constituent present in leaves will be dissolved into solvent.
- 5) After 20 min vessel removed from microwave and filtered by funnel and filter paper.
- 6) The filtrate collected into beaker and solvent was evaporated by hot plate at 60° temperature to obtained solid dry extract of moringa.
- 7) Collect the dry solid extract of leaf powder into tightly closed container and stored into dry place to prevent contamination.



Fig. No.7 Evaporation of Extract



Fig. No. 8 Weighing of Yield Extract

2.5 Phytochemical test :

Various chemical bioactive components found in the moringa leaf extract such as alkaloid, glycosides, tannins, phytosterols, fatty acids, vitamins, minerals, flavonoids, phenolic compounds. Among these phenolic compounds (Ex. Flavanoids, flavonols) shows antifungal activity against different fungi. To confirm presence of flavanoids into moringa leaf extract following phytochemical test were performed.

Table .1 Preliminary Phytochemical test for flavonoid

Sr. No.	Test	Procedure	Observation	Inference
1	Ferric chloride test	Mix 0.1 g of extract with 1 ml of ethanol then adding 1 ml of 10% ferric chloride. ¹²	Creation of brown colour solution and murky green precipitate.	Flavanoid present
2	Lead acetate test	When a small amount of extract is mixed with distilled water and then treated with lead acetate solution, a yellow-colored precipitate forms. ¹²	Yellow coloured precipitate	Flavanoid present



Fig. No.9 Phytochemical test of extract

2.6 Formulation Table :

Table . 2 Composition of cream formulation (for 20 gm)

Oil Phase (gm)		Water phase (gm/ml)			
Ingredient	Quantity	Role	Ingredient	Quantity	Role
White Bees wax	3 gm	Thickening agent	<i>Moringa oleifera</i> leaf powder extract	1.5 gm	Antifungal agent
White vaseline	4.5 gm	Moisturizer, soothing agent	Propylene glycol	4 ml	Humectant
Cetyl alcohol	3 gm	Emollient	Triethanolamine	1.5 ml	Stabilizer, pH adjuster
			Benzoic acid	0.5 gm	preservative
			Carboxy methyl cellulose	1.6 gm	Binding agent
			Perfume (lavender oil)	q.s	Fragrance
			Distilled water	q.s	solvent

2.7 Method of preparation of cream:

- 1) Weighing of all the ingredients accurately according to the formulation table.
- 2) Prepare the extract of *Moringa oleifera* leaves by dissolving it in small amount of water.
- 3) **Preparation of Oil phase:**
 - Combine the bees wax, white Vaseline into a beaker and heat the mixture to a temperature of around 70-80°C, stirring occasionally until its melted.
 - Then after melting of above mixture, add cetyl alcohol and melt it.
- 4) **Preparation of water phase:**
 - In separate beaker combine propylene glycol, triethanolamine, benzoic acid.
 - Heat the mixture to a temperature of around 60-70°C stirring occasionally until ingredients are fully dissolved.
- 5) **Development of cream formulation :**
 - Under the same temperature condition , slowly added the water based phase to the heated oil phase stirring constantly to ensure uniform blending.
 - Gradually add the CMC to the mixture, stirring constantly to ensure uniform dispersion. Add the moringa leaf extract solution to the mixture stir well.
- 6) Allow the mixture to cool to around 30°C or room temperature then add perfume to the mixture, stir well.
- 7) The o/w emulsion mixture was transferred into a thick cream formulation.
- 8) Pour the cream into suitable tightly packed container then perform the quality control test on the final cream product.^{3,6,7}

3. EVALUATION OF CREAM :

3.1 Physical appearance: Cream's physical characteristics can be evaluated based on its color, odor, texture, and consistency, which can be graded for quality assessment.³

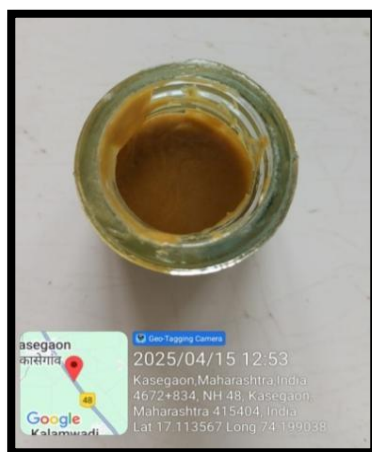


Fig. No.10 Appearance

3.2 Determination of pH: The small quantity of cream were transferred into a beaker and dissolved into distilled water then subjected to pH test using a pH paper.³

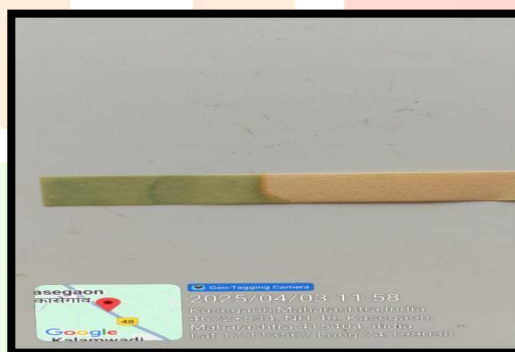


Fig .No.11 pH test

3.3 Determination of viscosity: The viscosity of the formulated cream was determined using a Brook-field viscometer with spindle number 3. The spindle was placed in a vessel containing the cream and rotated at 50 rotations per minute. The corresponding reading displayed on viscometer was recorded.^{3,6}



Fig.No.12 Viscosity

3.4 Spreadability: Small amount (1gm) of cream was placed between two glass slides and a weight is applied on the slides and measure the time taken for upper slide to move over lower slide (time taken to separate both the slides).^{3,16}

$$S = m \times l / t,$$

where ,

s- spreadability ,

m-weight applied on upper slide (200 gm)

l - length of the spreading area ,

t- time taken to separate both slides.



Fig.No.13 Spreadability

3.5 Homogeneity: The formulated cream were evaluated for homogeneity by visual inspection and touch. After feel The cream were evaluated for greasiness by applying a finger tip unit of the cream on the skin.^{3,7}

3.6 Washability : The cream's washability was tested by rinsing the applied area with running tap water to assess how easily it could be removed.³

3.7 Patch test : Applying a small amount of the product to a discrete area of skin (like wrist, dorsum of palm) and observing upto 24 hrs for any signs of irritation or allergic reaction such as redness, itching or swelling.¹⁴

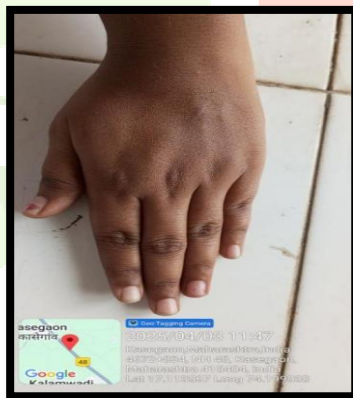


Fig. No.14 Patch test

3.8 Dilution test : A dilution test helps to determine if its an oil-in-water (o/w) or water-in-oil (w/o) emulsion by observing its miscibility with water or oil.

For o/w emulsions : Add water to the cream . If the cream and water mix well and form a stable solution, its an o/w emulsion.¹³



Fig. No.15 Dilution test

3.9 Determination of anti-fungal activity :

The sample of prepared cream was sent to **Infinite Biotech Institute of research and analytic, Sangli** to determine the anti-fungal activity of cream. In vitro anti-fungal assay of prepared cream was conducted by well diffusion method using Sabouraud agar media. The fungal strain *Aspergillus niger* was used in assay to assess potential of fungal inhibition of cream.^{8,9}

The experimental procedure of determination of anti-fungal activity of prepared cream as follows:

1. The inoculum of the microorganism was prepared from the bacterial cultures.
2. 15ml of Sabouraud agar (Hi media) medium was poured in clean sterilized Petri plates and allowed to cool and solidify.
3. 100 μ l of broth of fungal strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly.
4. 6mm in diameter wells were created using a sterile cork borer.
5. Solutions of the compounds (100 μ l/ml) were prepared in DMSO and 100 μ l of prepared test solutions and standard was added to the wells.
6. The petri plates incubated at 37°C for 24 hr.
7. Miconazole (1mg/ml) was prepared as a positive control and DMSO was taken as Negative control.
8. Antifungal activity was evaluated by measuring the diameters of the zone of inhibitions (ZI) all the determination were performed in triplicates.



Fig.No. 16 Antifungal assay (zone of inhibition)

4. RESULT AND DISCUSSION :

Table .3 Physical evalution of formulated cream

Sr. No.	Parameters	Result
1	Colour	Caramel brown
2	Odour	Aromatic
3	Texture	Smooth , Non greasy
4	pH	6 (compatible with skin)
5	Viscosity	2300 cp (Medium viscous)
6	Spreadability	11.66 g.cm/sec (Easily spreadable)
7	Homogenecity	Homogenous
8	Patch test	No skin irritation, rashes ,inflammation
9	Washability	Easily washable with water
10	Dilution test	Oil in water emulsion No phase separation

Table .4 Antifungal activity of test sample against *Aspergillus niger*

Sr. No	Samples	Zone of Inhibition in mm
1	Control	00
2	Standard (Miconazole)	28
3	MO cream (<i>M. oleifera</i> cream)	18

5. CONCLUSION :

This study demonstrated the hydro-alcoholic *Moringa oleifera* leaf extract possesses notable anti-fungal activity against *Aspergillus niger*. The developed *Moringa oleifera* anti-fungal cream showcased promising results in inhibiting fungal growth, underscoring its potential as a natural, effective, and sustainable treatment option for fungal infections. Rich in nutrients and anti-fungal compounds, the cream provides a holistic approach to skin health, offering a safer substitute to conventional treatments. The incorporation of herbal ingredients in the cream preparation supports skin health by providing essential nutrients and promoting biological functions. The cream's desirable characteristics, including good spreadability, consistency, smoothness, and skin-compatible pH, rendered the cream formulation suitable for use as topical application. Further investigation is necessary to fully harness the therapeutic potential of this innovative cream.

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