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Research Article

Formulation and Evaluation of Herbal Antifungal Ointment

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ABSTRACT

The rising resistance of harmful fungi to traditional antifungal medications has sparked a growing interest in natural remedies. This study aims to formulate and evaluate a topical herbal anti-fungal ointment using extracts from Melia Azedarach (chinaberry) and Hygrophila Auriculata (marsh barbel), Both known for their traditional medicinal properties. Ethanolic extracts of the plant materials was prepared and incorporated into an ointment base using standard methods. Then formulation was evaluated for physicochemical properties like pH, Spreadability, stability, and homogeneity. Antifungal activity was checked using the agar well diffusion method (MIC) against common fungus such as Candida albicans and Aspergillus niger. The formulated ointment showed promising antifungal effect, comparable to standard antifungal agents, and has favorable physical characteristics and stability when checked for 30 days' time period. These evaluations support the potential of Chinaberry and Marsh Barbel extracts as effective herbal ingredients in topical antifungal formulations, and can be used to treat various fungal infections.

INTRODUCTION

Fungal infections on or in your skin can look red, swollen or bumpy. They can look like a rash or you might be able to see a lump under your skin. Fungal infections in your nails can make them discoloured (yellow, brown or white), thick or cracked. Fungal infections in your mouth or throat can cause a white coating or patches. The most common fungal infections, like those on your skin or nails, aren't usually serious. If you have a weakened immune system, you're at higher risk of serious illness from certain fungal infections.

Types of Fungal Infections:

Superficial fungal Infections: Superficial fungal infections affect your nails, skin and mucous membranes (like your mouth, throat or vagina). Examples of superficial fungal infections include:

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Ringworm (dermatophytosis): A group of fungi that live off of skin, hair and nail cells (dermatophytes) cause ringworm. They can infect your feet (tinea pedis/athlete's foot), your groin and inner thighs (tinea cruris/jock itch), your scalp (tinea capitis), your hands (tinea manuum), your facial hair and skin around it (tinea barbae) and other parts of your body (tinea corporis).

Onychomycosis: Many types of fungi cause infections of your fingernails or toenails (onychomycosis). This can cause discoloured and cracked nails.

Candidiasis: Candida (usually Candida albicans) causes skin and mucous membrane (mucocutaneous) infections called candidiasis. These include oral thrush, some types of diaper rash, vaginal yeast infections (vulvovaginitis).

Tinea Versicolor/Pityriasis Versicolor: The fungus Malassezia causes skin discoloration called tinea versicolor or pityriasis versicolor.

Subcutaneous Fungal Infections: We can get a fungal infection under the surface of your skin (subcutaneous) if fungus gets into a cut or wound

Plant Profile:

Melia azedarach (China Berry)

Fig.1 Melia azedarach L.

Kingdom:	**Plantae**
Synonyms:	Melia azedarach var., japonica, melia toosendan
Common name:	Bakain, Pride-of-India, umbrella tree, persian lilac
Botanical name:	Melia azedarach L
Order:	Sapindales
Family:	Meliaceae
Genus:	*Melia*
Species:	*M. azedarach*

Description:

Melia azedarach is a deciduous tree growing to 50 ft. (15.2 m) in height and 2 ft. (0.6 m) in diameter.

Foliage: The leaves are alternate, bi-pinnately compound, 1-2 ft. (0.3-0.6 m) in length and turn golden-yellow in fall. the leaflets are dark green above and lighter green below, with serrate margins.

Flower: Flowering occurs in the spring, pale purple or lilac petals, when showy, lavender, 5-petaled flower develop in panicles.

Fruit: Fruit are hard, yellow, marble-sized, stalked berries that can be dangerous on sidewalks and other walkways. Seeds are spread by birds.

Uses

- Leaves have been used as a natural insecticide to keep with stored food.
- Chinaberry fruit was used to prevent insect larvae from growing in the fruit.
- leaves and trees has been used in the past to induce uterine relaxation

Fig.2 Hygrophila auriculata (Marsh Barbel)

Kingdom:	***Plantae***
Synonyms:	*Asteracantha auriculata Nees, Asteracantha longifolia Nees*
Common name:	*Marsh Barbel, Gokul Kanto, Ekhro, Kolshinda, Talimkhana*
Plant form:	*Herb*
Botanical name:	*Hygrophila auriculata*
Order:	*Lamiales*
Family:	*Acanthaceae*
Genus:	*Hygrophila*
Species:	*Articulata*
Native to:	*Angola, Assam, Bangladesh, Nepal, Burkina, Burundi,*

Description:

Hygrophila auriculata is a small herbaceous shrub or annual that grows up to 1.5 m tall. This plant has a straight, quadrangular, unbranched or sparsely branched, reddish-brown stem, covered in fine whitish hairs. The stem appears swollen and thickened at the nodes. The leaves are stalkless, lanceolate to linear lanceolate, with pointed tips, growing between 50-100 mm long and 15-25 mm wide. They grow in whorls, 4 pairs of leaves emerge from each axil, with outer pairs appearing longer and larger, they grow facing upwards. They are covered with bristly hairs on both surfaces, and the margins are finely toothed**.**

Uses:

- It is hailed for its anti-diabetic, anti-tumor and antibacterial properties, and dubbed safe as it does not cause side effects.
- The roots secrete an aqueous substance that is used in different treatments for liver ailments, liver damage and jaundice.
- *H. auriculata* is also used in medication to treat cancer and has antifungal properties.

MATERIAL AND METHOD:

Collection, Drying of Plant Material: The leaves were collected, coarsely powdered and well-stoppered container. The dried material of two plants was then used further work.

Material: - Mature plants of chinaberry were used for this preparation and were collected from Gondia city. The analytical balance was used for weighed chinaberry powder. Sieve was used for separate fine particle from chinaberry powder. Heating Mantle was used for heating the solution

during Soxhlet extraction time. Soxhlet chamber was used for the extraction process. Round bottom flask and measuring cylinder were used for measuring solvent.

Extraction Method of Chinaberry Leaves:

Soxhlet extraction: -25 gm of shade dried, powder of plant materials was filled separately in the thimble and extracted successively with 150 ml each of methanol using a Soxhlet extractor for 48 hrs. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extracts was weighed and preserved at 40 °C. Celsius in airtight bottles until further.

Extraction Method of Marsh Barbel:

Maceration Extraction: -

The dried plants were subjected to pulverization using a mixer, to get coarse powder. To 20 gm of the coarse powder, 100 ml of absolute alcohol (Analytical Reagent) was added. The mixture was kept soaking for 24 hrs. Then the mixture was filtered using Whatman no. 41 filter paper. The filtrate of the methanol extract was stored in air tight container in a refrigerator

Phytochemical Screening Experimental Procedure of Chinaberry and Marsh Barbel Extract (Test for both Plant Extract)

a. Test for Alkaloids (Mayer's Test)

- Add 2 mL of extract + few drops of Mayer's reagent.
- **Result**: Creamy white precipitate indicates alkaloids.

b. Test for Flavonoids (Shinoda Test)

- Add magnesium turnings + concentrated HCl to extract.
- **Result**: Red/pink color indicates flavonoids.

c. Test for Tannins

- Add 1% ferric chloride to the extract.
- **Result**: Greenish-black color indicates tannins.

d. Test for Saponins

- Shake extract with water vigorously.
- **Result**: Persistent froth indicates saponins.

e. Test for Terpenoids (Salkowski Test)

- Add chloroform + conc. sulfuric acid to extract.
- **Result**: Reddish-brown layer indicates terpenoids.

f. Test for Steroids

- Mix extract with acetic anhydride + sulfuric acid.
- **Result**: Green-blue color confirms steroids.

g. Test for Glycosides (Keller-Kiliani Test)

- Add glacial acetic acid, ferric chloride & sulfuric acid to extract
- **Result**: Reddish-brown ring at the junction.

Developing herbal ointment: -

Table:1 Formulation table of Ointment

Ingredients	Role	Quantity
Chinaberry extract	API	1.5gm
Marsh barbel extract	API	1.5gm

Stearic acid	Thickening	1.5gm
Potassium hydroxide	Alkaline properties	1gm
Sodium carbonate	Alkaline properties	1.5gm
White soft paraffine	Emoollient: softening skin	1.5gm
Methanol	Solvent in plant extract	1.5gm
Glycerine	Moisturizer	2ml
Methyl paraben	Preservative	1.5gm
Rose oil	Cooling effect, antiseptic	0.5ml
Distilled water	Dissolves ingredient	q.s

Steps involve in formulation of ointment:

1. Weighing Ingredients: Accurately measure all the required ingredients according to the formula.

2. Melting the Base: Melt the oil-soluble components (soft paraffin) in a water bath at around 70°C.

3. Adding Aqueous Components: Heat the water-soluble components to the same temperature (around 70°C).

4. Mixing Phases: Gradually add the aqueous phase to the melted base with continuous stirring to form an emulsion.

5. Cooling and Stirring: Allow the mixture to cool while stirring to ensure a homogenous product.

6. Adding Heat-Sensitive Ingredients: Incorporate any heat-sensitive ingredients (e.g., certain APIs or volatile oils) once the mixture has cooled down sufficiently.

7. Packaging: Transfer the ointment into suitable containers and label appropriately.

Evaluation of herbal ointment: A visual examination was used to check physical characteristics, including colour and smell.

a. Consistency: Smooth consistency and no indications of greed are present. Solubility Ether, alcohol, and chloroform are all soluble in and miscible with boiling water.

b. Washability: After the combination had been applied to the skin, the ease of water washing was evaluated.

c. pH: A digital pH metre was used to determine the herbal ointment's pH. One hundred millilitres of distilled water was used to make the ointment solution, which was then left to settle for two hours. The solution's pH was measured three times, and the average value was determined was 6.93 pH.

d. Spreadability: To test the spread ability, we sandwiched extra sample between two slides that had been uniformly crushed using a predetermined weight for a specific amount of time. To determine spread ability, the time needed to separate the two slides was utilised. Improved spread ability is the end consequence of speedier slide separation. Spread ability was determined using the formula below.

$S=M\times L/T$ Where, S = Spread ability M denotes the weight of the upper slide. L = Glass slide length T is the duration needed to separate the slides.

e. Extrudability: A tube-shaped container was used to store the mixture. Extrudability was calculated as the weight of cream needed to extrude 0.5 cm of cream ribbon in 10 seconds.

f. Diffusion analysis: The diffusion research was carried out using the agar nutritional medium. A board with a hole in the middle was filled with

ointment. It was apparent how long the ointment took to diffuse. (After 60 seconds)

g. Stability study: A four-week physical stability test on the herbal cream was conducted at different temperatures, including 2°C, 25°C, and 37°C. It was found that the herbal ointment was physical steadiness across a range of temperatures, including 2°C, 25°C, and 37°C, four weeks soon. For this test we used well diffusion method on agar plate, prepared agar plate bored 6mm hole by borer and inoculated with microorganism (Aspergillus niger fungus) and the prepared ointment filled in the cavity, incubated for 24hr at 37 degrees Celsius and studied the zone of inhibition. From the above antimicrobial activity, the antimicrobial chewing gum showed inhibition of microorganism in the range of 4.8 mm to 6.0 mm and it can show significance application in treating halitosis.

RESULT & DISCUSSION:

Table:2 Standard Phytochemical Screening Test Observation

Sr. No.	Phytochemical	Test Name	Melia Azedarach L	Hygrophila auriculata
1	Alkaloids	Mayer's test	+	+
2	Flavonoids	Shinoda test	+	+
3	Tannins	Ferric chloride test	+	+
4	Saponins	Froth test	+	+
5	Terpenoids	Salkowski test	+	+
6	Phenols	Ferric chloride test	+	+
7	Glycosides	Keller-Kiliani test	+	-

Table:3 Antifungal activity of melia azedarach L extract

Sr.no.	Concentration (ml)	Fungus: Aspergillus niger (Radius)
1.	0.1 ml	-
2.	0.2 ml	2.0 mm
3.	0.3 ml	2.3 mm
4.	0.4 ml	3.0 mm
5.	0.5 ml	4.1 mm
6.	0.6 ml	5.6 mm
7.	0.7 ml	7.0 mm
8.	0.8 ml	7.0 mm
9.	0.9 ml	7.2 mm
10.	1.0 ml	8.0 mm

Table:4 Antifungal Activity of Hygrophila Auriculata Extract

Sr. No.	Concentration (ml)	Fungus: Aspergillus niger (Radius)
1.	0.1 ml	-
2.	0.2 /ml	2.0 mm
3.	0.3 /ml	2.0 mm
4.	0.4 /ml	3.0 mm
5.	0.5 /ml	4.0 mm
6.	0.6 /ml	6.0 mm
7.	0.7 /ml	6.5 mm
8.	0.8 /ml	6.7 mm
9.	0.9 /ml	7.0 mm
10.	1.0 /ml	7.0 mm

Maximum zone of inhibition of *Melia Azedarach L* extractwas found to be 8.0 mm at 1.0 ml *andHygrophila Auriculata* extract was found to be 7.0 mm, at 0.9ml dilution respectively. That's why I had chosen 1.0ml and 0.9ml concentration of drug to use as in combinations. (12 plates for each combination).

Table :5 Antifungal activity of combinations of both drug combinations

Sr. No.	Concentration (ml) of Both Drug	Zone of Inhibition (Radius)(mm)
1.	1.0 ml + 0.9 ml	6.5 mm
2.	1.0 ml + 0.9 ml	6.2 mm
3.	1.0 ml + 0.9 ml	7.0 mm
4.	1.0 ml + 0.9 ml	6.0 mm
5.	1.0 ml + 0.9 ml	6.2 mm
6.	1.0 ml + 0.9 ml	8.0 mm

7.	1.0 ml + 0.9 ml	6.0 mm
8.	1.0 ml + 0.9 ml	7.5 mm
9.	1.0 ml + 0.9 ml	8.0 mm
10.	1.0 ml + 0.9 ml	7.3 mm
11.	1.0 ml + 0.9 ml	6.0 mm
12.	1.0 ml + 0.9 ml	8.0 mm

For The Combination the MIC was found between 6.0 mm to 8.0mm for 0.9ml and1.0ml dilution.

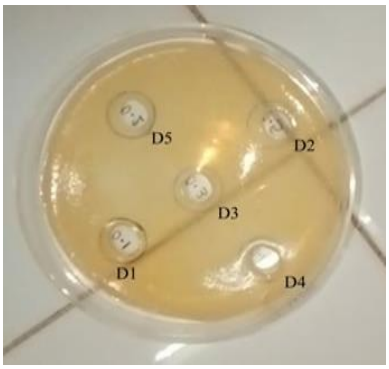

Fig.3 Antifungal Activity of Drug Combination

Table 6: Stability Studies Data of Formulated Ointment

No. Of days	Physical appearance	pH evaluation
Initial	No change in colour	6.8
30	++	6.8
60	Slight change in colour	6.5
90	+	6.4

Table 7: Analysing a herbal ointment physical and chemical composition

Sr. No.	Physicochemical Parameters	Observation
1	Intensity of Colour	Greenish Black
2	Odour	Characteristic
3	Consistency	Smooth
4	pH	5.4
5	Spreadability (seconds)	7
6	Extrudability	0.4 gm
7	Diffusion Study (after 60 min)	0.7 cm
8	Loss on Drying	30%
9	Solubility	In boiling water, alcohol, ether etc
10	Washability	Good
11	Non-irritancy	Non-irritant
12	Stability Study	Stable

Table 8: Antifungal Activity of Formulated Ointment

Sr. No.	Formulations	Zone of Inhibition (Radius)
1.	F1	6.0 mm
2.	F2	5.5 mm
3.	F3	4.8 mm

CONCLUSION:

The present study focused on the formulation and evaluation of a herbal antifungal ointment incorporating extracts of *Melia azedarach* L. and *Hygrophila auriculata*, two medicinal plants traditionally used for their antimicrobial properties. The ointment was successfully prepared using a suitable base, and various concentrations of the plant extracts were incorporated to assess their antifungal efficacy. Phytochemical screening of the extracts confirmed the presence of bioactive compounds such as alkaloids, flavonoids, tannins, and saponins, which are known for their antimicrobial activity. In vitro antifungal evaluation demonstrated significant inhibitory effects against common fungal pathogens, indicating that the combination of these plant extracts possesses potent antifungal properties. The formulated ointment was subjected to standard evaluation parameters including physical appearance, pH, Spreadability, stability, and microbial activity. The results showed that the formulation was stable, aesthetically acceptable, and effective in inhibiting fungal growth, making it a promising candidate for topical antifungal therapy.

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