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FORMULATION AND EVALUATION OF HERBAL ANTIFUNGAL NAIL LACQUER FOR THE TREATMENT OF ONYCHOMYCOSIS

Aneesha V.[1], Gopi S.[2], Jaison Rasel Jose[3], Karan S.[4], Snehadevi M.[5], Dr. S. Jeevanandham[6] and Akshara V. Anil[7]*

Sree Abirami College of Pharmacy, Coimbatore-21.

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***Corresponding Author**
Akshara V. Anil
Sree Abirami College of Pharmacy, Coimbatore-21.

ABSTRACT

Around 19% of the world population is suffering from onychomycosis among which 80% cases of Onychomycosis have affected toenails. There are varieties of topical and oral formulations used, like tablets, creams, solutions, gels, and nail patches. Traditional topical treatments for onychomycosis are not clinically effective because the nail barrier needs to be penetrated for formulations containing active agents to reach the target site at therapeutic concentrations. Nail is the less vascular area. Therefore, nail lacquer will be a more effective formulation than other formulations, mainly oral administration. Also, long-term use of antifungal drugs like amorolfine, ciclopirox, griseofulvin, and terbinafine has side effects. Here arises the importance of medicinal plants. Many of the herbs used today have been valued for their antimicrobial and antifungal effects. Mimosa pudica and Piper betel have excellent antifungal activity due to the presence of phenolic constituents. The objective of the present study is to formulate and evaluate an anti-fungal nail lacquer using Mimosa pudica and Piper betel. The herbs were collected, dried, extracted, and further subjected to phytochemical analysis, which included tests for phenols, tannins, steroids, glycosides, and flavonoids. The nail lacquer formulation was prepared by simple mixing using nitrocellulose, ethyl cellulose, ethyl acetate, salicylic acid, dibutyl phthalate, Mimosa pudica, and piper betel extract. The formulated herbal nail lacquer was subjected to a comparison study with a marketed formulation (ciclopirox nail lacquer) to prove the efficiency of herbal nail lacquer. It was analysed for non-volatile content, thickness of film, water resistance, antifungal activity, etc. All batches of formulation possess good film thickness, water resistance, drying time, folding

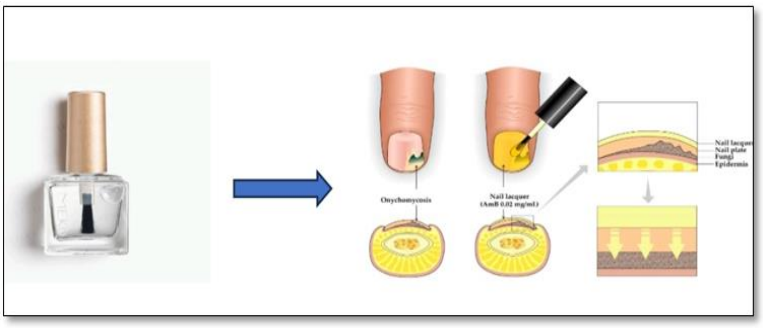
endurance, etc. The antifungal activity of the F3 and F4 formulations is higher than that of the standard fluconazole drug.

KEYWORDS: Antifungal agents, Mimosa pudica, Herbal nail lacquer, Onychomycosis.

1. INTRODUCTION

The Greek terms onyx, which means nail, and mykes, which means fungus, are where the word onychomycosis originates. Any part of the nail unit, including the nail plate, nail bed, and nail matrix, could be affected. Infecting 10–30% of people worldwide, onychomycosis is a widespread, persistent, and difficult to treat fungal illness of the toenails and fingernails. When onychomycosis is evident clinically, the skin becomes thicker, discoloured, and uneven. The most prevalent pathogens implicated in onychomycosis are dermatophyte like Trichophyton rubrum. Trichophyton interdigitale, Epidermophyton floccossum, Trichophyton violaceum, Mycosporum gypseum, Trichophyton, non dermatophyte, and candida albicans species. Historically, onychomycosis was treated with surgery to remove the infected nail, a very painful and traumatic procedure. Antifungal medications either topically (lotions, solutions, gels, and nail patches) or orally are now used to treat the illness. The medication diffuses into the nail plate after being absorbed into the systemic circulation after oral delivery.[1-3]

Significant obstacles to medication delivery to the nail (ungual drug delivery) include a lack of knowledge about the nail's barrier qualities and formulations for achieving enhanced horny structure delivery, which limits the effectiveness of topical therapies for nail disorders. And also have low patient compliance due to the lengthy treatment durations (up to 4–8 months) that are required.[2] Nail lacquer opens a new era for transungual drug delivey. Nail lacquer is a multicomponent composition that is used to paint or coat fingernails and toenails. Nail lacquer will function better than other formulations, especially when taken orally. They are harmless to nail and skin, convenient to use and stable during storage.



People have been using plants for sustenance and medicine for thousands of years; they are nature's remedies. Herbal medicine sometimes referred to as botanical medicine or phytomedicine, is the use of plant parts such as seeds, leaves, and roots. Because herbal medicines are low-cost, safe, and effective with little adverse effects, they are valuable in underdeveloped nations. Mimosa pudica and piper betel posses medicinal uses like antioxidant, antifungal, anticancer, antimicrobial etc. They contain phytoconstituents like tannins, phenols which make them good antifungal herbs. They are active against various pathogens like candida albicans, and dermatophytes.[4,5] Hence an attempt was made to formulate herbal antifungal nail lacquer for the successful treatment of onychomycosis.

1.1 Constituents of nail lacquer[7,8]

Film forming agents: One crucial step in creating a suitable nail lacquer type is choosing a film-forming agent. Includes nitrocellulose, cellulose acetate, cellulose acetate butylate, and ethyl cellulose.

Resinous substances: Resins give nail polish its glossy appearance and adhesive qualities. Eg: benzoin, shellac, damar, sandarac, and ester gums, synthetic resins, santolite MS 80% santolite MHP

Plasticizers: An essential component in the preparation of nail polish to increase the nail lacquer's pliability and reduce its propensity to shrink so that a homogeneous film forms. For example: dibutyl phthalate and castor oil.

Solvents: Solvents are volatile organic liquids that combine all the ingredients of lacquer formulation and make a homogeneous viscous preparation.

Low boiling point solvents: e.g. Ethyl ether, Acetone, Ethyl alcohol etc.

Medium boiling point solvents: e.g. N-Butyl acetate, n-Butyl alcohol etc.

High boiling point solvents: e.g. Ethyl lactate. Alcohols, particularly ethyl, isopropyl and butyl are very efficient diluents.

Plant extracts

Mimosa pudica: Leaves and roots provide more antifungal activity. Extracted by maceration for 24 hours using ethanol, water or methanol.

Piper betal: The betal leaves were extracted by using the maceration method with ethanol as a solvent.

2. MATERIALS

Fresh plant of Piper betel and mimosa pudica were collected in the month of October 2023 from Coimbatore district in tamilnadu. It was authenticated through botanist. Salicylic acid was purchased from labogens Ahmadabad and ethyl cellulose was purchased from central drug house (P) Ltd, New Delhi, India.

3. METHODS[9,10]

3.1 Phytochemical investigation

- To identify **phenols**, extract was treated with3-4 drops of ferric chloride solution to get in bluish black color.
- Ferric chloride test: Several drops of 10% w/v ferric chloride (FeCl3) solution were added to the sample solution. A brownish green color indicates the existence of **tannins**.
- 1ml of Glacial acetic acid and 1 ml of ferric chloride were transferred into 1 ml of sample solution and then 1 ml of concentrated sulfuric acid was added. The appearance of blue-green color represents the presence of **glycosides**.
- Ten drops of each solution A and B were added to a test tube containing sample solution. After heating for 15 min at 60 ± 0.5°C, orange red precipitate or green suspension was formed stipulating the existence of **reducing sugars**
- Dragendorff's test: The sample solution was acidified with diluted hydrochloric acid. The mixture was heated and filtered. Equal volumes of the resulting solution and Dragendorff's reagent were reacted. The formation of an orange red precipitate indicates the existence of **alkaloids**.
- Extract was mixed with chloroform and then add concentrated sulfuric acid to form a reddish-brown layer at the junction specifying the presence of **terpenoids**
- The **flavanoids** test was performed by treating extract with methanol and magnesium ribbon followed by few drops of concentrated hydrochloric acid to get orange or red color
- For the presence of **steroids**, the extract was treated with few drops o concentrated sulphuric acid to get red color.

3.2 Formulation of nail lacquer[10,12]

To obtain a clear solution, ethyl cellulose was dissolved in an adequate amount of ethyl acetate. The mixture above was mixed with dissolved salicylic acid and potassium hydrogen phthalate. Acetone and plant extracts were then added, and the mixture was continuously stirred at 100 rpm using a magnetic stirrer. Ultimately, enough ethyl acetate was added to the

nail lacquer to give it the right consistency. The formulation was coded as F1 to F3. F4 correspond to the marketed formulation - ciclopirox nail lacquer.

Table 1: Formulation of nail lacquer.

Ingredients	F1	F2	F3	F4(marketed formulation - ciclopirox nail lacquer)
Piper betel extract	0.1ml	0.2ml	0.3ml	-
Mimosa pudica extract	0.1ml	0.2ml	0.3ml	-
Nitrocellulose	2g	2g	2g	-
Ethyl cellulose	0.125g	0.25 g	0.50 g	-
Salicylic acid	0.70 g	0.70 g	0.70 g	-
Potassium dihydrogen phthalate	0.2 g	0.2 g	0.2 g	-
Acetone	0.1 ml	0.1 ml	0.1 ml	-
Ethyl acetate	q.s	q.s	q.s	-

3.3 Evaluation of nail lacquer[14.15]

Physical appearance

Visual observations were made of the nail lacquers' color, transparency, and application quality.

Smoothness of Flow and Gloss[14]

(F1 to F4) were spread out over a 1.5-inch area on a glass slide. It was spread out using a glass slide that was tilted. The flow's smoothness was assessed by contrasting it with nail lacquer that is sold. Glossiness was graded as good (++), very good (+++), and excellent (++++) based on visual inspection.

Lacquer film thickness

One ml of formulation was spread equally with an applicator brush in 8 cm diameter petri dish and was allowed to dry at room temperature. After drying nail lacquer film was isolated from the petri dish. The film thickness was measured at three different places using a micrometer screw guage and average was calculated

Non volatile content

The weight of the formulation that remained on the nail plate after application was calculated using the non-volatile content of F1 to F4. A glass Petri dish with a diameter of approximately 8 cm was filled with 5ml of nail lacquer. The sample was evenly distributed with a brush. After one hour at 105°C in the oven, the dish was cooled and weighed. The non-

volatile content of the nail lacquer explained the variation in sample weight between the pre- and post-drying times.

Drying time[14]

A glass petri dish measuring 4 x 4.5 cm^2 was marked, and a nail lacquer formulation and marketed product was applied to it using a brush. A stopwatch was used to record the amount of time needed for the film to dry. Three copies of the readings were taken.

Folding endurance

Folding endurance of the films was determined by repeatedly folding a small strip of the film (approximately 2x2 cm) at the same place till it broke. The number of times film could be folded at the same place, without breaking gives the value of folding endurance.

Water resistance test[14]

The petri dish was covered with a continuous film, which was then dried and submerged in water. The petri dish's weight was recorded both before and after immersion, and the weight increase was computed.

3.4 Determination of antifungal activity[15]

Trychophyton rubrum, Aspergillus niger and fusarium were employed for testing antifungal activity using Agar well diffusion. The fungal strain was maintained on nutrient agar. A loop full of culture from the slant was inoculated into the medium and incubated at 28°C for 48-72 hr and 0.1 ml of culture was evenly spread on the plates containing respective media. Wells were bored by using a sterile borer on the surface of media. Sample was added to each of the wells and incubated for 48hrs at 28°C. The zone of inhibition was recorded and compared with control. Fluconazole is used as standard.

4. RESULTS

Drug delivery must be targeted for onychomycosis. Treatment for onychomycosis is challenging due to the nail plate's barrier qualities. Under the nail plate, they can prevent the entry of antifungal medications in the necessary concentration to treat fungal infections. To get the most active ingredients through the nail plate and function as an antifungal, it was necessary to choose polyhebral constituents and a penetration enhancer.[15,16]

4.1 Identification test for phytoconstituents

By using the identification test, the presence of phenolic extract, tannins, terpenoids, alkaloids, and glycosides was verified.[16]

Table 2: Phytochemical test report.

Test	Piper betal	Mimosa pudica
Phenol	+++(confirmed)	+++(confirmed)
Tannins	+++(confirmed)	+++(confirmed)
Flavonoids	+++(confirmed)	+++(confirmed)
Volatile oil	+++(confirmed)	+++(confirmed)
Alkaloids	+++(confirmed)	+++(confirmed)
Reducing sugars	---not present	+++(confirmed)
Glycosides	+++(confirmed)	+++(confirmed)
Terpenoids	+++(confirmed)	+++(confirmed)

4.2 Physical appearance

Visual observations were made of the formulations' color, transparency, and application characteristics. (fig-1)

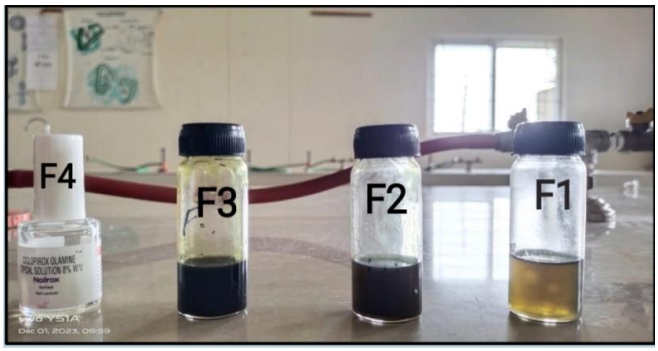


Fig. no: 1 Physical appearance.

Smoothness to Flow and Gloss

It was discovered that when nail lacquer was poured onto a glass plate, it spread and produced a smooth, uniform film. The applied lacquer's gloss was similar to that of marketed product (Table no:3).

Table 3: Appearance & smoothness to Flow and Gloss.

Formulation code	Clarity	Gloss	Smoothness to flow
F1	Clear	++	Smooth, evenly dispersed
F2	Clear	++	Smooth, evenly dispersed
F3	Clear	+++	Smooth, evenly dispersed
Marketed product	Clear	++++	Smooth, evenly dispersed

Film thickness

Uniform thickness indicates the uniformity of the formulations thereby suitability of the executed procedure. Thickness of all the films measured by using a micrometer screw gauge. Results showed that thickness of all formulations varied from to 63-71µm (Table:4).

Non-volatile content

Non-volatile content of F1-F4 was observed to be 34±0.4 to 38±0.2 (Table:4). Formulation F3 and F4 showed 37±0.1 and 38±0.2% of non-volatile content, respectively. Thus, it confirmed the uniformity in preparation of all batches.

Table 4: Non volatile Content & Film thickness.

Formulation code	Non-volatile content (%)	Film thickness (µm)
F1	34±0.4	63 ± 0.04
F2	36±0.3	67±0.02
F3	37±0.1	71±0.04
F4	38±0.2	70±0.02

Drying time

Mentioned in table no.6 below

Table 6: Drying Time and Folding endurance.

Formulation code	Drying time(sec)	Folding endurance
F1	75 sec	102
F2	73sec	155
F3	72 sec	176
F4	69sec	180

Folding endurance[18]

The folding strength is quoted as the number of double folds and the folding endurance is the log10 of the number of double folds. Higher the folding endurance values better will be the flexibility of the films. (F3 and F4) showed good folding endurance,(Table.6) thereby ensuring good flexibility.

F = log10 d,

Where, F= is the folding endurance

d =the number of double folds.

Water resistance

This is the measure of the resistance towards water permeability of the films. Higher the increase in weight lowers the water resistance.[17]

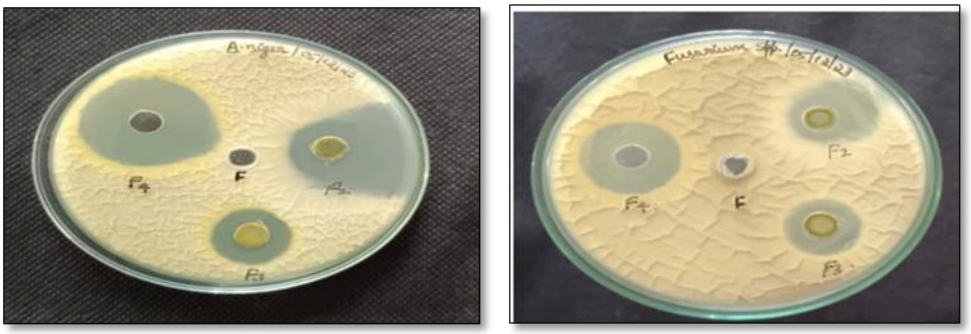
Table 5: Water resistance.

Formulation code	W1(g)	W2(g)	W1- W2(g)
F1	0.036	0.056	0.020
F2	0.036	0.052	0.016
F3	0.036	0.049	0.013
F4	0.036	0.044	0.008

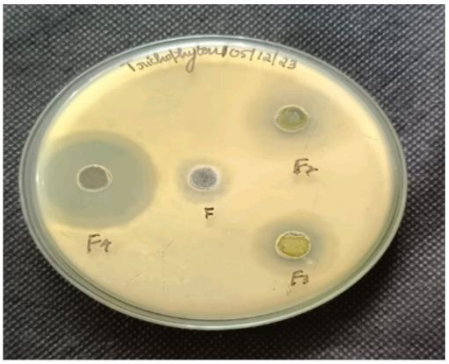
4.3 Antifungal activity[16-18]

Both potatoes dextrose and agar agar are weighted & mixed with distilled water. Then allow for sterilization for 15 minutes.

Prepare the media plates and allow for solidification, spread the fungus onto the plates and apply the herbal nail lacquer in different zone.



Aspergillus Niger **Fusarium**



Trichophyton rubrum

Fig. no. 2: Antifungal Activity and Zone of inhibition of fungal plates.

Antifungal study

The zone of inhibition for the F2 and F3 and F4 formulation was determined, which is comparable with that of standard (fluconazole). This indicates that F3 and F4 formulation Was sensitive to the microorganism trichophyton rubrum, Aspergillus, Fusarium species.(Table.7)

From these evaluation test, F3 And F4 formulation of nail lacquer were found very good against microorganism trychphyton rubrum, Aspergillus niger, Fusarium to treat onychomycosis. F3 batch of containing extract loaded nail lacquer showed good non-volatile content, drying time, and water resistance than other formulation of nail lacquer. Zone of inhibition also showed good result than other formulation. Therefore, F3 batch was considered as best batch of Herbal nail lacquer.

Table 7: Antifungal Activity and Zone of inhibition.

Name of the organism	Zone of inhibition(mm)			
	F (Fluconazole)	F2	F3	F4 (ciclopirox nail lacquer)
Trichophyton rubrum	2	13	15	31
Aspergillus niger	2.5	20	29	32
Fusarium spp	2	19	19	24

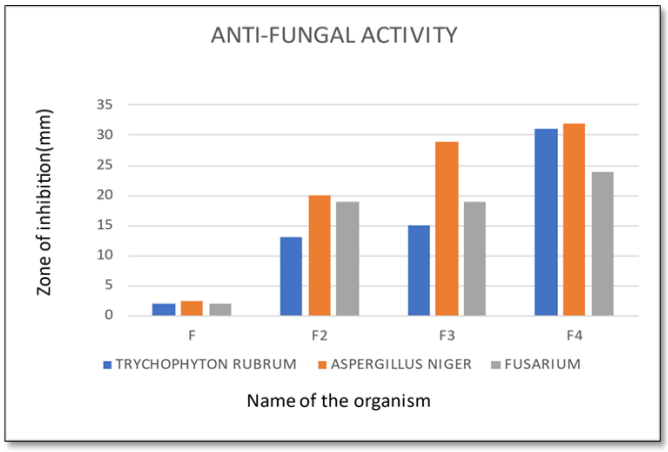


Fig. no. 3: Antifungal activities.

5. DISCUSSION

The nails are made beautiful and vibrant by nail lacquers. It can be tempting to use them, particularly if you're currently annoyed about a pretty nail issue, like fungal infections. It is the simplest method for treating nail fungal infections. Treating onychomycosis involves the use of specific nail lacquers. Through the nail plate, herbal extract-loaded nail lacquer was successfully applied. When comparing into the marketed ciclopirox formulation herbal nail lacquer also exhibited good non-volatile content, drying time, smoothness to flow, folding endurance, and antifungal activity. From this study the superior antifungal activity of F3 herbal nail lacquer than standard fluconazole is proved. However, the medications included in the marketed products have negative effects when used repeatedly. Because of the herbal extract, there is no harm to the skin or nails. Patients may accept herbal extract-loaded nail

lacquer because of its inherent appeal. Consequently, polyherbal extract is thought to be a good option for treating onychomycosis as an antifungal agent.[17,18]

6. CONCLUSION

The goal of this work was to create an herbal nail lacquer with antifungal properties that would help treat onychomycosis by combining extracts from mimosa pudica and piper betal. Traditional topical treatments for onychomycosis are not clinically effective because the nail barrier needs to be penetrated for formulations containing active agents to reach the target site at therapeutic concentrations. A range of research on physicochemical characteristics, such as the formation of films, non-volatile content, drying rate, gloss, flow smoothness, and antifungal studies, were assessed. Each of the aforementioned parameters was examined to determine compliance.

Conflict of interest

There is no conflict of interest in the study.

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