

Formulation and Evaluation of Herbal Anti-Fungal Nail Lacquer

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ABSTRACT

Introduction:-Around 19% of the world population is suffering from onychomycosis among which 80% cases of Onychomycosis have affected toenails. The infection is associated with proximal subungal onychomycosis distal and primary total dystrophic onychomycosis. There are varieties of formulation for treatment of nails infection like creams, solution, gel, nail patches. Nail is the less vascular area. Therefore, nail lacquer will be more effective formulation than other formulation mainly oral administration. The objective of the present study is to formulate and evaluate the Anti-Fungal nail lacquer using various herbs. To screen Anti-Fungal activity of formulated nail lacquer. To find out effective concentration of penetration enhancer. **Method:-** Nail lacquer was prepared by using nitrocellulose, ethyl cellulose, ethyl acetate, salicylic acid (act as penetration enhancer), dibutylphthalate and various mixture of extract of *Calendula officinalis*, *Origanum vulgare*, *Allium sativum*, and oils of *Syzygium aromaticum*, *Melaleuca alternifolia*, *Cymbopogon*, *Ocimum tenuiflorum* and *Mentha piperita*. The mixture of extract and oils mixed with the continuous stirring. The nail lacquer of Fluconazole was prepared by same method and compare for Anti-Fungal activity with formulated herbal nail lacquer. Herbal nail lacquer were evaluated for appearance, non-volatile content, lacquer film thickness, drying time, gloss, smoothness, flow, water resistance test, peel adhesion test, Anti-fungal study. **Result:-**The evaluation results revealed the nonvolatile content helped for film making by evaporation of volatile content, the glossiness was visually seen required to accept medicated nail lacquer, it show good antifungal activity, the drying time increase as concentration of penetration enhancer increase, the flow was found to be smooth and the appearance was observed. Herbal nail lacquer showed improved antifungal activity as compare to Fluconazole. **Conclusion-** The extract loaded nail lacquer was successfully delivered through nail plate. The penetration enhancer proved to enhance transungual delivery. Herbal nail lacquer shows good antifungal activity. It could be a good choice of antifungal agent and accepted by patient due to natural appeal.

KEYWORDS: Polyhedral Nail Lacquer, Antifungal, Onychomycosis

INTRODUCTION

Nail anatomy and structure:

Nail is a horn-like envelope covering a dorsal part of the terminal phalanges of fingers and toes in people, most non-human primates, and a couple of different vertebrates. Nails which are similar to claws, are found on numerous other animals. Fingernails and toenails made up of a tough protein called keratin, similar to animals' hooves and horns. The nail consists of the nail plate, nails matrix and nail bed below it, and grooves surrounding it. Importance of nails in disease diagnosis: the color, appearance, shape and nature of the nails gives information about the general health and hygiene of a person. Nails are inspected as an everyday practice by all specialists to get a few pieces of information about fundamental infections. Just by taking a gander at nails we can recognize the cleanliness of an individual. The unusual nail might be congenital. The cause for changes in the nail extends from basic motivations to dangerous illnesses. Hence the study by a doctor is essential for diagnosis.(1)

Onychomycosis:

Onychomycosis is depicted as a contagious contamination influencing fundamentally finger and toe nails. It was accounted for to impact around 19% of the populace across the world and comprise around half of all the nail diseases..(2) Onychomycosis is characterized as a contagious disease influencing fingernails and toenails bringing about thickening, staining, and detachment from the nail bed. Onychomycosis influences around 10% of the normal populace yet, it is more pervasive in grown-ups. The gamble element of onychomycosis is 1.9-crease to 2.8-overly more in people experiencing diabetes correlation with the normal populace. Nonetheless, the over-simplification changes from 15 to 40% if there should arise an occurrence of patients contaminated with human immunodeficiency virus..(3) Onychomycosis is characterized into two kinds. The first is distal subungual onychomycosis influencing nail plate, nail bed, and hyponychium (contamination more conspicuously spreads from athlete's foot from the encompassing skin), diseased nails become dystrophic and thick and color of nails adjusts from yellowish-white to brown. The second is proximal subungual onychomycosis in which the parasite as a rule spreads through the proximal nail folds; the nail has a whitish appearance. The endonyx type of onychomycosis, influencing the nail plate without onycholysis or hyperkeratosis is likewise known. Shallow white kind of onychomycosis (nail becomes white with pasty texture) which is seen to be less frequent.(4)

Nail lacquer for transungual drug delivery:

Transungual drug delivery is defined as the system which is related to drug transport across the nail to gain targeted drug delivery in treatment of nail diseases. In the term transungual, "Trans" denote "through" and "unguis" denote "nails".(5) Transungual drug transport framework is thought to be viable to oversee nail issues on account of its better adherence confined activity, which gives least foundational side effects(6). Nail enamels have been utilized as restorative to give security to the nails, to design the nails.

Medicated nail enamels comprise an imaginative kind of definitions that have been utilized for transungual drug delivery.(7)After application to the nail plate, dissolvable dissipation happens. The film abandoned after dissolvable vanishing works like a medication warehouse. From this pharmacy, drug goes through delivery and entrance across the nail for an ideal time frame period. Highdissemination angle is produced for drug saturation into the nail plate.(8)Film development on nail plate causes decrease in water misfortune from the outer layer of nail surface into the environment. Hyper hydration of the upper nail plate layers happens, further aiding drug diffusion. The dynamic specialist entrance can additionally be libbed by means of purpose of infiltration enhancers like thiol compounds, hydrating agents, and keratolytics specialists.(9)

Nail lacquer:

Nail polish or nail varnish is applied to human fingernails or toenails to decorate and/or protect the nail plate. Conventional nail lacquers have been used as cosmetics since a long time for beautification and protection of nails. Topical nail preparations like lacquers, enamel and varnish are an integral part of today's beauty treatments. It protects the nail plate, but more importantly it enhances their beauty, imparting color and luster.(10)A model nail lacquer should have the following properties: It should be harmless to skin and nails, convenient and easy to apply, stable on storage, satisfactory film on nails. To achieve satisfactory film it should have the following characteristics:-It should have good wetting and flow properties so that the film formed is even, uniform color, good gloss, good adhesive properties, sufficient flexibility so that it does not crack or which is resistant to impact and scratch, reasonable drying time (1-2 minutes) without developing bloom, able to maintain the above-mentioned properties for a reasonable time (about 1 week).(11,12)

Constituents of nail lacquer:

The basic nail varnish consists of solvents, plasticizers which give flexibility and durability to the film, film forming polymers, resins which enable the film to adhere to nail plate and convey shining to the film, colouring agents and suspending agents.

- **Film formers:** A number of film forming substances have been suggested for nail enamels. Includes nitrocellulose, cellulose acetate, cellulose acetate butylate, ethyl cellulose, vinyl polymers and various polymers of methacrylate.
- **Resins:** Resins impart adhesion and improve gloss, commonly used resins are Santolite MHP and Santolite MS 80% which are claimed to increase moisture resistance, they are soluble in majority of solvents.
- **Plasticizers:** Plasticizers impart flexibility and adhesive properties to the film. The two types of plasticizers are solvent and non-solvent plasticizers. The amount of plasticizers which can be used in nail lacquers varies and may vary from 25% to 50% of film former. The amount depends on flexibility of film required. Dibutyl phthalate is most widely used plasticizer.
- **Solvents:** Although evaporation characteristics are of prime importance in nail lacquers, but rapid rate of evaporation causes a poor flow of enamel resulting in uneven and streaky application. According to their boiling points. 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: eg. Ethyl lactate. Alcohols, particularly ethyl, isopropyl and butyl are very efficient diluents.

Herbal importance:

Expensive prescription medications are still recommended by some doctors for the treatment of toenail fungus, although these remedies are not effective treatment for toenail fungus. In addition, each is associated with potentially adverse side effects. There are a number of natural anti-fungal supplements that can be used for the effective treatment of toenail fungus.

- *Origanum vulgare:* Oregano is a woody plant that is found mostly in the Mediterranean. Oregano oil has potent antiviral, antibacterial, and anti-fungal properties its primary component, carvacrol.(13)
- *Cymbopogon:* Lemongrass is an herb found in Southeast Asia, East and West India, South America, Africa, Australia, and the United States. It is widely used as insect repellent and for its anti-fungal and anti-bacterial properties.(13)
- *Allium sativum:* Treating toenail fungus with garlic is easy and painless. There are several methods that work well and depending on the severity of your fungal infection, you may wish to try one or more of them.(13) Garlic shown to possess fungistatic and fungicidal activity in vitro and in vivo.(14)
- *Calendula officinalis:* Constituents of the essential oil were the following: sesquiterpenols (27.0%), sesquiterpene hydrocarbons (68.0%), epi-a-murolol (12.87%), d-cadinene (22.53%), a-cadinol (20.40%).(15)
- *Syzygium aromaticum:* Eugenol is the main volatile compound of extracted oil from clove bud (*S. aromaticum* L.) is used as traditional medicine, as a fungicide, bactericide anaesthetic. However, as compare to bacteria antimicrobial activity is found to be higher in fungi. (9). Besides eugenol (49–87 %), it also contains eugenyl acetate (0.5– 21 %), β -caryophyllene (4–21 %), small amount of α -humulene is present.(16)
- *Melaleuca alternifolia:* Tea tree essential oil is particularly rich on terpinen-4-ol (40.44%), gamma terpinene (19.54%) and 1, 8-cineole (95.61%) and alpha-pinene (1.5%) for the *E. globulus* oil. *E. globulus* oil was more efficient and had the best antifungal effect on oral *Candida albicans*.(17)
- *Ocimum tenuiflorum:* Tulsi essential oil has emerged as the most inhibitory natural product against both the *Candida* species. The presence of 1, 8-cineole (eucalyptol), linalool, eugenol methyl and eugenol as the principle lead molecules. The antifungal activities of these major compounds, along with Tulsi Essential Oil, were explored in terms of the constituent effectiveness.(18)

- *Menthapiperita*: The *Mentha* species is the most widely used in terms of health and medicinal uses, because of Menthol and Mentone. The essential oil, comprising menthone (24.56%), menthol (36.02%), menthofuran (6.88%), menthyl acetate (8.95%); these are major components, and others are minor components. Significant antibacterial and antifungal activity than principle components. (19)

MATERIAL AND METHOD

Material

Fluconazole, nitrocellulose, ethyl cellulose, ethyl acetate, salicylic acid, dibutylphthalate and various mixture of extract of *Calendula officinalis*, *Origanum vulgare*, *Allium sativum*, *Curcuma longa* and oils of *Syzygium aromaticum*, *Melaleuca alternifolia*, *cymbopogon*, *Ocimum tenuiflorum* and *Menthapiperita*.

Method

Identification test for phytoconstituents

1. A thin section of drug and add alcoholic solution of Sudan red III turn red for presence of volatile oil.
2. A few drops of peppermint oil was mixed with 5ml of nitric acid solution (prepared by adding 1ml of nitric acid to 300ml of glacial acetic acid) heat on water bath but within 5 minutes liquid develops blue colour. (20)
3. Place a drop of chloroform concentrate of clove or clove oil or eugenol on slide and add to it a drop of 30% fluid arrangement of sodium hydroxide immersed with sodium bromide. Needle and pear molded precious stone of sodium euginate organized in rosette are seen very quickly. Place a drop of chloroform extract of clove or clove oil or eugenol on slide and add to it a drop of 30% aqueous solution of sodium hydroxide saturated with sodium bromide. Needle and pear shaped crystal of sodium euginate arranged in rosette are seen almost immediately. (21)
4. Dissolve 1gm of citral in 1gm of methanol, stationary phase- silicagel G Standard sample - Citral Detecting agent – 2,4-dinitrophenyl hydrazine reagent to produce Yellow to orange Colour spots Mobile phase – Pure Chloroform RF Value – 0.51. (22)
5. Dissolved 1mg of menthol in 1ml of methanol Stationary phase - Silica gel –G Standard sample - Menthol Detecting agent – 1% vanillin – sulphuric acid reagent and heat the plate 110°C for 10 minutes Mobile phase – Pure Chloroform RF Value – 0.48-0.62. (23)
6. To identify phenols, separate was treated with 3-4 drops of ferric chloride answers for get in pale blue dark.

Formulation of nail lacquer

Nitrocellulose and ethyl cellulose were dissolved in sufficient quantity of ethyl acetate to get clear solution. Salicylic acid was dissolved in above mixture and dibutyl phthalate was added. Then extract *Calendula officinalis*, *Origanum vulgare*, *Allium sativum*, and oils of *Syzygium aromaticum*, *Melaleuca alternifolia*, *cymbopogon*, *Ocimum tenuiflorum* and *Menthapiperita* And acetone were added with continuous stirring at 100 rpm on magnetic stirrer.

Ingredients	F1	F2	F3	F4	F5
Extract	0.05	0.06	0.07	-	-
Fluconazole	-	-	-	0.04	0.04
Nitrocellulose	2	2	2	2	2
Ethyl cellulose	1	1	1	1	1
Salicylic acid	0.30	0.45	0.60	0.45	0.60
Ethyl acetate	0.5	0.5	0.5	0.5	0.5
Dibutylphthalate	0.2	0.2	0.2	0.2	0.2
acetone	0.1	0.1	0.1	0.1	0.1

Evaluation of nail lacquer

Non-volatile content

Non-volatile content of F1 to F5 not set in stone to get the heaviness of definition that held on nail plate later application. Nail enamel (1 g) was taken in a glass petri dish of around 8 cm in distance across. Test was spread similarly utilizing brush. The dish was placed in a stove at 105°C for 1 hr, cooled and gauged. The distinction in weight of test when drying was the non-predictable substance present in the nail lacquer. (24)

Lacquer film thickness

One ml of detailing was spread similarly with a tool brush in 8 cm width petri dish and was permitted to dry at room temperature. In the wake of drying nail enamel film was disconnected from the petri dish. The film thickness was estimated at three better places utilizing a micrometre screw check and normal was calculated. (25)

Drying time and gloss

An area of 4 × 4.5 cm² was set apart on glass petri dish to which a film of nail finish definition (F1 to F5) 2nd promoted item was applied with the assistance of brush. The time taken for the film to dry was noted utilizing a stopwatch. The readings were gotten in three-fold. Shininess (F1 to F5) not entirely set in stone by visual examination and estimated as follows: great (++), generally excellent (+++) also, incredible (++++). It was contrasted and advertised restorative product. (26)

Smoothness of flow

Formulations (F1 to F5) were poured on a glass slide on an area of 1.5 inches. It was spread on a glass plate by making glass slide slant. Perfection of not set in stone on contrasting and advertised nail lacquer. (27)

Water resistance test

This test was performed to measure the opposition of nail veneer towards water penetrability of film. A constant film was applied on the petri dish, dried and afterward water was poured on it to inundate the film. The weight of petri dish was taken before and after immersion and increase in weight was calculated. (28)

Anti-Fungal Activity by In vitro transungual permeation study

The permeation study was performed using animal hooves as an alternative to human nail. The goat finger nail was collected from the slaughter house and stored in a -20°C freezer until further use. Before the use the nail softened at room temperature in phosphate buffer pH 7.4 solutions. The nail were rinsed with phosphate buffer solution and dried. The thin section of soft nail was cut and dried. The cut section act as nail membrane and was mounted among giver and receptor compartment with nail portion facing donor compartment of Franz diffusion cell. The nail lacquer was applied (1ml) on surface of nail section to cover the entire area of receptor compartment. The assembly was maintained at 32±0.5°C with constant stirring at 600 rpm for 36 hr. (29)(30)

Samples were withdrawn (1 ml) via sampling port at appropriate intervals (0, 6, 12, 24, 36, 48h) and analysed Antifungal study of formulations. These solution are used to find out the antifungal activity of the formulation.

RESULTS

Onychomycosis need targeted drug delivery. The treatment of onychomycosis is difficult because of barrier properties of nail plate. They can inhibit entrance of antifungal drugs in required concentration to treat fungal infection, beneath the nail plate. Therefore, selection of polyhebral constituents and penetration enhancer was required to pass maximum more amount of active constituents through nail plate and work against as an antifungal.

Identification test for phytoconstituents

The presence of volatile oil, peppermint oil, eugenol, citral, menthol, phenolic extract was confirmed by performing the test of identification.

Antifungal Activity

The antifungal activity of extract was performed against *C. albicans*

Time(hrs)	Zone of inhibition (in mm)
0	-
6	5±2
12	9±2
24	14±3
36	20±3
48	20±3

Formulation of Nail Lacquer

Herbal extract loaded Formulation of nail lacquer F1 – F3 and Fluconazole nail lacquer F4 – F5 were prepared using penetration enhancer.

Batches	Non-volatile content (%)	Lacquer Film Thickness(mm)	Drying Time(s)	Water Resistance (%)	Zone of inhibition(mm)
F1	34±0.3	0.16±0.3	58±2	92	18±2
F2	35±0.6	0.16±0.5	60±3	95	19±1
F3	36±0.9	0.17±0.9	58±3	98	23±1
F4	36±0.7	0.16±0.6	58±2	96	20±1
F5	37±0.1	0.18±0.2	61±2	97	21±1

Evaluation of nail lacquer**Non-volatile content**

Non-volatile content of F1-F5 was observed to be 34±0.3 to 37±0.1. Formulation F3 and F5 showed 36±0.9 and 37±0.1% of non-volatile content, respectively. Thus, it confirmed the uniformity in preparation of all batches.

Lacquer film thickness

After drying, the thickness of the film was found to range between 0.16±0.3mm to 0.18±0.2mm. The thickness was found to be uniform in all formulation.

Drying time and gloss

Drying time of F1 to F5 was found to be in range of 58±2 to 61±2. There was no significant difference in all batches. Drying time of F3 and F5 was observed to be, individually 58±3s and 61±2s. The glossiness of nail lacquer was evaluated by comparing with the marketed product. Nail lacquer was applied on the nail and gloss was visually seen and there was no significant difference in all batches. Glossiness of F3 and F5 was very good (+++) and good (++) . It was found to be satisfactory when compared to the marketed product.

Smoothness of flow

Smoothness of flow of all batches (F1 to F5) was compared with marketed product and found to be very good.

Water resistance test

Higher was the increase in weight, lower was the water resistance of nail lacquer. Formulation F1 and F4 showed very high permeation as compared to F3 and F5. F3 and F5 showed 98 and 97% water resistance.

Antifungal study

Extract exhibited very good antifungal action against *C. albicans*. Therefore, formulation F3 containing extract could be considered as a very good antifungal medicated nail lacquer.

From these evaluation test, F3 and F5 formulation of nail lacquer were found very good against *C. albicans* 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 medicated nail lacquer.

One way ANOVA test, using F distribution df

Source	DF	Sum of square	Mean square	F- Statistic	P value
Group(between groups)	1	1.6	1.6	0.4444	0.5237
Error(within groups)	8	28.8	3.6		
Total	9	30.4	3.3778		

H_0 hypothesis:

Since $p\text{-value} > \alpha$, H_0 is accepted.

The averages of all group assumed to be equal.

In other word, the difference between the averages of all groups is not big enough to be statistically significant.

DISCUSSION

The herbal extract loaded nail lacquer was transparent in colour. Phytoconstituents tests confirmed presence of volatile oil, citral menthol and phenolic extract. They have antifungal activity against *C. albicans* was due to linalool, α -cadinol, methyl eugenol, carvacrol, allicin, curcuminol, eugenol, terpinen-4-ol, citronellal, menthol. As the concentration of extract increased the antifungal activity also found to be increased.

In volatile content test the F3 and F5 showed good non-volatile content. The F3 formulation containing extract showed good non-volatile content than F5. The non-volatile content was helped for film making by evaporation of volatile content. The thickness of lacquer was found to be uniform in all formulation. The thickness of lacquer film was found increased with increases in concentration of salicylic acid.

F3 formulation showed faster drying time as compared to other formulation, which was convenient for patient to have the nail wet with nail lacquer for less time. Glossiness was visually seen required to accept medicated nail lacquer by patient as cosmetically aesthetic. There was no significant difference in all batches, thus confirmed uniformity in preparation of all batches. The smoothness of flow of F3 and F5 batches confirmed satisfactory compared to marketed product.

In the water resistance test, higher was the water permeation increase in weight of lacquer. Both batch F3 and F5 showed very less increase in weight after immersing in water. It concluded that nail lacquer has less permeability and thus high resistance towards water. The antifungal activity of extract was found to be effective in killing *C. albicans*. Petriplate with F3 formulation loaded with extract showed great antifungal activity movement compared within contrast with F5 formulation with Fluconazole.

Thus, extract was considered as good antifungal agent to be used in the medicated nail lacquer. F3 and F5 showed good non-volatile content and gloss, water resistance and less drying time, high drug retention in nail plate and better permeation. Nail F3 and F5 did not show any change in color thus confirmed the physical stability of formulations. No microbial growth was observed in F3.

CONCLUSION

Herbal extract loaded nail lacquer was successfully delivered through nail plate. The extract showed good antifungal activity than fluconazole. Because of the herbal extract it is safe for skin and nails, no harm. Herbal extract loaded nail lacquer could be accepted by patient due to its natural appeal. Thus, polyherbal extract considered as a good choice of as antifungal agent to cure onychomycosis.

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