

Development and Evaluation of Psidium guajava Loaded Herbal Nail Lacquer for the Treatment of Onychomycosis Fungal Infection

¹Ritika Chauhan, ²Navneet Mehan, ³Manit Singh, ⁴Himanshu Sharma, ⁵Suman Anjali, ⁶Lalit Sharma

^{1,2,3,4,5,6}Ganpati Institute of Pharmacy, Bilaspur, Yamunanagar, Haryana, India, 135102.

²M.M. College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala, Haryana, India

Abstract- A well-known nail fungus known by the names Dermatophytes onychomycosis and Tineaunguim is onychomycosis. The pathogens that cause onychomycosis are dermatophytes, Candida, and non-dermatophytic molds. This study has produced a natural nail lacquer that is antifungal. The objective of this study was to reduce the course of treatment and deliver a consistent release of Psidium guajava over an extended period of time—up to 24 hours. It was anticipated that this would increase patient cooperation as well as potency. The nail lacquer preparation was created through straightforward mixing and its non-volatile content was examined. Functional group identification, drug content estimation, gloss, viscosity, drying time, drug diffusion investigations, and anti-fungal studies are among the applications for FTIR analysis. The nail lacquer with the best non-volatile content, drug release, drug content estimation, and zone of inhibition was made up of 4% Psidium guajava, 8% Eudragit RL-100, 2% ethyl cellulose, 2% ethyl acetate, 0.8% Dibutylphthalate, 10% salicylic acid, and 0.4% acetone out of all the formulations. It is possible that the medication release continued for up to 24 hours, and 72.25% of the drug was delivered overall. Phenols and flavonoids were detected in the acetone extract of the Psidium guajava-loaded nail lacquer, according to FTIR analysis. An expedited stability assessment of the specially developed formulation F4 was carried out for one month at 42 ± 2 °C in accordance with ICH rules, revealing that no significant alteration in the original features was observed. Thus, it follows that antifungal nail lacquer is among the ground-breaking drugs that could revolutionize the pharmaceutical and medical fields.

Keywords: Psidium guajava, Nail lacquer, Onychomycosis, fungus, Tineaunguim.

INTRODUCTION

People with weakened immune systems and the elderly are more susceptible to these nail problems. Candida albicans is the most common cause of yeast infections. persons with diabetes are more likely than non-diabetic persons to develop onychomycosis. The big, pointed edges of the patient's infected nail disintegrate the nail bed under pressure and infect the surrounding skin tissue [5]. The most common treatment for onychomycosis is still oral antifungal therapy, however it has a number of disadvantages.

Prolonged, methodical surgery can cause severe side effects and damage to the liver; also, because of restricted blood flow, a small amount of medication can never reach its objective. Topical therapy is therefore usually thought to as a good substitute [6]. Despite the fact that topical administration techniques offer many benefits. Formulations that are gel, cream, or liquid are insufficient for transngual distribution; in the interim, they can be removed by rubbing or flushing. Their inefficiency is explained by this event at the application site. Trans unguial delivery systems, or drug-containing nail lacquers, are relatively new formulations for treating nail infections [7, 8]. In addition to offering a long-lasting exterior coating to make thin, friable, susceptible, and uneven nails appear better, a coat of nail lacquer can protect them. stronger and more appealing [9]. The materials employed in this investigation were carefully selected to guarantee the nail lacquer's quality and safety. Solvent evaporation occurs after lacquer application, forming a lacquer layer. For medicine to be administered effectively, nail lacquers' films need to stick to the nail surface. The quantity of volatile ingredients in nail lacquer affects how much the medication concentrates. This method is believed to be useful for treating onychomycosis because a film develops a reservoir of the medicinal substance in the affected nail plate. As a result, the drug substance continues to permeate, which is necessary for effective treatment [10, 11]. Natural substances are generally more palatable, easily obtained, and thought to be non-toxic. It has been found that a wide range of naturally occurring chemicals produced from plants, including as flavonoids, terpenoids, alkaloids, tannins, lignans, phenolic acids, quinones, and coumarins, have strong antioxidant properties and are important in the treatment of fungal illnesses. The purpose of this research was to use Psidium guajava plant extract to make non-toxic lacquers. The family Myrtaceae, which includes Psidium guajava Linn., is known by several names, including guave, goyave, or goyavier in French; guave, Guavenbaum, Guayave in German; banjiro in Japanese; goiaba, goiabeiro in Portugal; araçá-

goiaba, araçá-guaçu, guaiaba in Brazil; guayaba, guayabo in Español and guava in English [12]. The tree is a little one, standing only 10 metres tall, with peeling, thin, smooth bark. The opposite, shortpetiolate leaves. The flowers are rather spectacular, with many stamens and pale petals that can reach a length of 2 cm [13]. Fruits are 5 cm in diameter, juicy yellow globose to ovoid berries with an appetising pink mesocarp that contains many little, hard white seeds. α -pinene, β -pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β -bisabolene, cineol, caryophyllene oxide, β -copanene, farnesene, humulene, selinene, cardinene, and curcumene are the primary constituents of leaves [14]. The leaves have been used to separate flavonoids, saponins, and oleanolic acid [15]. Moreover, ursolic, crategolic, guayavolic, β -sitosterol, and nerolidol have been found. *Psidium guajava* has been shown to have antigenotoxic, antimutagenic, anti-allergic, anti-diarrheal, anti-microbial, anti-malarial, anti-tussive, hepatoprotective, antioxidant, free radical scavenger, and radioprotective properties [16].

Because it dissolves different active components in herbal extracts and is less hazardous than other organic solvents used in nail lacquers, acetone could be a good choice for a solvent in nail lacquers. Salicylic acid was successfully used as a permeation enhancer, and its effectiveness in boosting impregnation over the human nail and bovine hoof was established.

METHODOLOGY

Medicinal plant selected for the study: *Psidium guajava* leaves were selected for this investigation due to their purported therapeutic effectiveness in the past for treating fungal illnesses.

Collection and authentication of *Psidium guajava* leaves: We harvested fresh leaves of *Psidium guajava* (Figure 1 and Figure 2), a member of the Myrtaceae family, from the campus garden at GIP, the Ganpati Institute of Pharmacy, in Bilaspur. The director of the college department verified the authenticity of the plant portion. Fungal strains that were clinical isolates were gathered from the GIP microbiology laboratory located in BILASPUR.



Figure 1: Plant of *Psidium guajava*.



Figure 2: A) Fresh *Psidium Guajava* leaves; B) Dry *Psidium guajava* leaves; C) Super dry *Psidium guajava* leaves; D) Powder of *Psidium guajava* leaves

Extraction of plant material: After giving the leaves a thorough cleaning under running tap water, they were washed with distilled water. The leaves were placed in a shaded area and allowed to dry for two weeks before being processed into a powder using an electric grinder. A conical flask with 250ml of acetone and 50g of powder was macerated for two days with sporadic shaking. Using Whatman no. 1 filter paper, the extract was refined to produce *Psidium guajava* acetone extract. The filtrates were then collected for further solvent evaporation. The extract was dried entirely by

evaporating it. Up to its subsequent use, the yield of the recovered residue was reported. The weight of the medication divided by the initial weight of the plant powder was used to calculate the extract percentage (Figure 3) [17].

It was calculated by using formulae:

$$\text{Percentage yield} = (\text{Weight of drug} / \text{Weight of the plant powder}) \times 100$$



Figure 3: Extraction of the Psidium guajava leaves.

Preparation of Nail Lacquer: Eudragit RL-100 and cellulose were dissolved in an appropriate volume of ethyl acetate to yield a transparent solution. Salicylic acid was dissolved in the aforementioned mixture, then dibutyl phthalate was added. Then, acetone and Psidium guajava extract were added, and a magnetic stirrer was used to continuously agitate the mixture at 100 rpm. Formulation codes were F1 through F4 (Table 1).

Table1: Formulation of nail lacquer.

Ingredients	F1	F2	F3	F4
Psidiumguajava(ml)	4	4	4	4
Eudragit RL-100(g)	3	6	7	8
Ethyl cellulose(g)	0.50	1	1.5	2
Ethyl acetate(ml)	4	4	4	2
Dibutyl phthalate(ml)	0.8	0.8	0.8	0.8
Salicylic acid(ml)	2	4	7	10

Evaluation of Herbal Nail Lacquer

Organoleptic Assessment: The organoleptic attributes of several physical characteristics, including colour, odour, and appearance, were investigated.

Non-volatile content

Each formulation's first millilitre of sample was put in a Petri plate, and the initial weights were recorded. The plates were removed from the oven after an hour at 105 °C, allowed to cool, and then weighed. The difference in weight was observed [19].

Non -volatile content = $(W3 - W1 / W2) \times 100$ W1=weight of the empty dish.

W2= weight of the sample before the test.

W3= weight of dish with dried sample It was calculated by using formulae.

Fourier-transform infrared spectroscopy (FTIR) analysis

An IR affinity spectrophotometer was used to enrol waves with an amplitude range of 450 to 4000 cm⁻¹ in order to resolve the IR for nail lacquer with the goal of separating functional groups [20].

Drug content estimation

A 50 ml phosphate buffer solution with a pH of 7.4 was used to dissolve 2 millilitres of nail lacquer. After that, the solution received a 15-minute ultrasonic treatment. The mixture was refined and then diluted with a pH 7.4 phosphate buffer solution to reach a final volume of 100 millilitres. After taking 10 millilitres of the previous solution, mix 100 millilitres of pH 7.4 PBS into it. After that, spectrophotometric analysis was performed at a wavelength of 223 nm to determine the drug concentration of the diluted solution [20&21].

$$\% \text{age drug content} = [\text{absorbance (test)} / \text{absorbance (standard)}] \times 100$$

Smoothness to flow

The nail lacquer for each formulation was transferred from a 1.5-inch height into a different glass plate, spread out, and allowed to rise vertically before being closely inspected for film smoothness.

Gloss

After the nail lacquer formulation was put over the nail, the shine was visible.

Viscosity

The viscosity of nail lacquer can be measured with a viscometer. The Brookfield Viscometer was used to measure viscosity [22].

Drying time

A thin layer of lacquer that is spread out or flows out for inspection covers a clean, translucent surface. The drying time is estimated using a timer and confirmed by placing a fingertip against the film when there is no visible mark. The films should be examined with your fingertips. The films should also feel dry to the touch at that moment. The term "dry-to-touch" describes a film's capacity to be touched with the tip of a finger without leaving any residue behind.

Lacquer film thickness

One millilitre of each mixture was placed into an 8 cm diameter petri dish, spread equally with an applicator brush, and allowed to dry at room temperature. After curing, the nail polish film was taken off of the Petri plates. The film thickness was tested three times with a micrometre screw gauge, and the average was found.

$$\text{Determination of pitch} = \text{Distance moved by pitch} / \text{number of full rotations given to screw}$$

$$\text{Least count} = \text{Pitch} / \text{Number of divisions on the circular scale}$$

Folding endurance

Folding endurance describes the film's elasticity. To test the films' adaptability, folding endurance tests were the only exercises available. A layer's folding endurance is determined by the total number of folds it can sustain without being disrupted [24].

Water resistance test

The purpose of the experiment was to assess the resistance to water permeability. A continuous film was put to the petri dish, allowed to dry, and then the film was immersed in water. The weight of the petri dish was measured before and after immersion, and the weight gain was calculated [23].

$$\text{Water resistance} = (\text{Loss of the weight of lacquer} / \text{Actual weight}) \times 100$$

In vitro translingual permeation studies

Following a cattle slaughter, the hooves were submerged in distilled water for a whole day without the presence of any adhesive connective tissue. Roughly 1 mm thick hoof membranes were extracted. For the in vitro permeation studies, the hoof membrane was carefully placed on a Franz diffusion cell. Next, 2ml of nail lacquer was applied equally on the surface of the nail membrane. The receptor compartment was sealed with a pH 7.4 phosphate buffer solution, and the assembly was kept at 37°C and shaken constantly for a whole day. The 5ml aliquot of the drug sample was mixed with the new solvent after 2, 4, 6, 8, 10, 12, 16, and 24 hours had passed. To examine the data, a double-beam UV spectrophotometer tuned to 223 nm was used.

$$\text{Drug release} = (\text{Absorbance} / \text{Standard absorbance}) \times \text{Standard concentration} \times 100 / \text{Amount taken} \times \text{Dilution factor}$$

Determination of antifungal activity

The cup-plate method was used to measure the antifungal activity in *Candida albicans*. With Sabouraud's agar slants, the culture was maintained. The Petri plates were filled with 20 millilitres of ground-up Sabouraud's agar medium and 0.2 millilitres of a suspension of *Candida albicans*. The plates were then left for fifteen minutes. After punching the Petri plate cups (10 mm in diameter), 0.08 cc of the sample solution was added to each one. Moreover, promote nail lacquers and formulations devoid of salicylic acid. The zone of inhibition was measured and contrasted with formulations without salicylic acid and the control. Before being incubated at 30°C for 48 hours, the plates were held at 40°C for one hour to allow for diffusion. After the incubation time was over, the zone of inhibition was measured in millimetres [25].

Stability study

Studies on nail lacquers' stability were carried out in compliance with ICH regulations. The samples were stored at 40 ± 20 °C and 75 % relative humidity for a month. Research was done to verify alterations in physical attributes such as hue and scent, gloss and smoothness, one month of drying at three distinct temperatures (40°C).

III. Results and Discussion

Percentage yield

By dividing the initial amount of plant material used for extraction by the amount obtained after extraction, the percentage yield of the extract was calculated, and the yields were reported to be 8.2% W/W.

Organoleptic evaluation

It was notable for a number of sensory attributes, including colour, smell, look, etc. All four recipes yield consistent outcomes. These characteristics—color, scent, and appearance—give nails a refined look that boosts customer approval.

Non-volatile content

The entire volatile stuff vanished and a thin film parted, revealing the non-volatile component. The results, which vary from 26-39%, are shown in (Table 2). The evanescence of volatile content in the F4 formulation provided good non-volatile content aid for filmmaking among all formulations. It was observed that the non-volatile content rose along with the polymer concentration. The non-volatile content is contingent on the polymer concentration employed.

Table 2: Evaluation of nail lacquer

S. NO	Non- volatile Content (%)	Drug content Estimation(%)	Viscostiy	Drying Time (s)	Thickness (mm)	Water resistance
F1	28.11	89.12	97	78.6	0.064	0.26
F2	28.23	92.8	112	73	0.079	0.32
F3	36.06	92.7	139	66	0.13	0.11
F4	39.22	92.8	149.7	64	0.16	0.06

Fourier-transform infrared spectroscopy (FTIR) analysis

Major bands were seen in the nail lacquer's FTIR spectrum, as indicated in Table 3. Strong and broad absorption maxima in the range $449-4024\text{cm}^{-1}$ were observed in the FT-IR spectrum of guava leaf extract (Figure 4), which aligned with the stretching ambiances of hydroxyl groups (O-H). This indicates that phenolic and alcoholic groups are present, along with a significant shift in hydrogen bonding. Peaks at 2973.01 on the FTIR spectrum of the herbal nail lacquer could be explained by the existence of O-H, which stretches to suggest the presence of phenols. The presence of flavonoids and phenols is shown by the FTIR data.

Figure 4: Fourier-transform infrared spectroscopy (FTIR) analysis of the Psidium Guajava leaves.

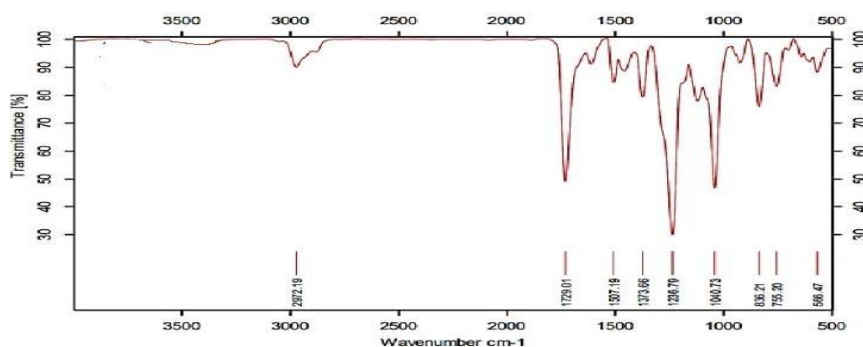


Table 3: FT-IR spectra of Nail lacquer

Wave Number (cm-1)	Functional group
2971.79	O-H Stretching
1728.06	C=O Aromatic
1506.68	N-O Stretch
1373.01	O-H Bending
1237.02	C-N Stretch
1041.05	CO Stretch
835.67	C=C Bending
754.01	C-H bending(aromatic)
565.98	Aromatic CH stretch

Drug content estimation

The drug content percentages of all formulations, which ranged from 89.01 to 93.5%, were judged to be appropriate (Table 04). The results indicated that 89.01% appeared to have the least amount of drug, while the greatest drug content was 93.5% (F4). Because flavonoids are present, the formulations F1, F3, and F4 have a drug content of above 90%, which indicates a significant amount of drug contained in the formulation. Additionally, a high medication content assures that positive therapeutic outcomes are possible.

Smoothness to flow

On the glass plate, it was discovered that the nail lacquer spread and formed a homogeneous, smooth film. The reduced concentration of polymer allowed for good smoothness in all formulations.

Gloss

The F4 formulation was found to have the best glossiness out of all of the formulations. It was determined that glossiness was necessary to give the patient a cosmetically pleasing nail lacquer.

Viscosity

Table 4 lists all of the formulas' viscosities. Formulations F2, F3, and F4 exhibit favourable outcomes as a result of a higher concentration of polymer. As the concentration of polymer grew, so did the formulation's viscosity. The formulation's viscosity varies from 105 to 215 centipoises, although it was found that 98 to 145 centipoises produced clear, glossy results. Viscosity above this threshold causes clouding and reduces lustre, which is unsuitable from a decorative standpoint.

Drying time

The drying rate increases from 70 to 80 seconds as the polymer concentration rises from F1 to F4 (Table 4). Because Formulation F4 contains a higher concentration of polymer and a higher viscosity, it dries faster than other formulations. This is suitable for people who want to keep their nails wet with nail lacquer for a shorter period of time. The characteristics of the solvent's volatility, and hence the drying period, have a significant impact on the application and cohesiveness of nail covering. The drying time increases as the polymer content rises.

Thickness (mm)

After drying, there were differences in all preparations' thicknesses, ranging from 0.067 to 0.13 mm. The results that were observed are shown in Table 4. It was discovered that the lacquer's thickness was consistent across all formulations. Due to a higher concentration of polymer and plasticizer, the thickness of the F4 formulation exhibits exceptional strength, increased flexibility, and resistance to water. The layer thickness observations agreed with the benefits of thickness as described by a review of the film literature.

Folding endurance

The growth of the film's flexibility depends on its folding endurance. The folding endurance results are presented in Table 4. F4 outperformed the other formulations in folding endurance, indicating that the polymer and plasticizer gave the film good flexibility. The films are more flexible the higher the folding endurance values.

Water resistance

In this case, the F4 formulation is more water resistant and weighs less overall. The information was referenced in Table 4. The F4 preparation exhibited no cloudiness, burning, or mass variation throughout the water's presence. It was discovered that the preparation's resistivity to water increased as the concentration of plasticizer and polymer increased.

Table 4: Characterization of Anti-Fungal Nail Lacquer.

F. Code	Viscosity	Drying time	Thickness	Folding endurance	Water resistance
FG1	562	76 Sec	0.067	Good	Good
FG2	143	70 Sec	0.013	Good	Good
FG3	206	80 Sec	0.053	Good	Good
FG4	105	70 Sec	0.041	Good	Good

In vitro transungual permeation studies

Transungual diffusion investigation was performed in vitro to determine the drug's capacity to penetrate salicylic acid. The information was referenced in Table 5. F3 to F4: The rate of diffusion increased along with the formulae's increased salicylic acid composition. Because salicylic acid releases its medication quickly, it is a great permeation booster. The keratolytic substance salicylic acid is what causes the nail plate to demulcent. The F4 formulation demonstrated a higher bioavailability within 24 hours compared to the F3 and F2 preparations.

Table 5: In vitro transungual permeation studies

S.No	Drug Diffusion
F1	45.2 ± 0.124
F2	44.97± 0.234
F3	55.12± 0.123
F4	72.25± 0.143

Anti-fungal study

Zones of inhibition for all formulations were assessed and found to range from 12 to 16 mm, which is comparable to those of nail lacques that are sold and formulations that do not contain salicylic acid. A zone of inhibition of around 18 mm is shown by the optimised formulation F4 and commercial nail lacquer, compared to a zone of inhibition of about 16 mm in the formulation without salicylic acid. A Table 6 presents the results. This suggests that nail lacquer's zone of inhibition is unaffected by formulations containing or lacking salicylic acid. The antifungal activity of the extract was shown to be efficient. The antifungal activity of the extract was tested against *C. albicans* to see how effective it was. phenols and flavonoids because of the presence of albicans. The marketed Amrolifine nail lacquer formulation and a Petri plate with optimised F4 preparation that contains extract showed the same outcome (zone of inhibition). Herbal nail lacquer's FTIR spectra, which shows peaks at 2972.19, may explain the presence of O-H, stretching to infer the presence of flavonoids and phenols. The presence of phenols and flavonoids was revealed by FTIR, which is in line with multiple previous studies that demonstrated the phenol molecules in the sample had strong antifungal activity.

Table 6: Zone of inhibition.

Formulation Code	Zone of inhibition (mm)		
	Prepared herbal Nail lacquer	Marketed Amrolifine nail lacquer	Formulation without salicylic acid
F1	13	16	15
F2	15	17	17
F3	16	18	14
F4	17	18	16

Stability studies

The shelf life and storage conditions of a product were ascertained through stability studies. In order to confirm the changes in physical properties such colour, odour, gloss, and smoothness, as well as the drying time at $(42 \pm 2^\circ\text{C})$ for one month, it was found that the F4 formulation was stable for one month. According to Table 7 stability study input, the numbers didn't alter all that much. It was discovered that the F4 formulation met the requirements for stability and compliance with ICH protocols. improved F4 nail lacquer composition.

Table 7: Stability study of nail lacquer formulations.

Parameters	After Stability studies			
	F1	F2	F3	F4
Color	Transparent pale green	Light Pale green	Light green	Transparent Light green
Odour	Sweet aromatic odour	Fruity odour	Fruity odour	Sweet fruity odour
Gloss and Smoothness	No gloss and good smoothness	Less gloss and smoothness	Gloss and smoothness	Gloss and smooth
Drying time	More drying time	Slow drying time	Less drying time	Less time drying

Conclusion

A leaf extract from *Psidium guajava* has been developed to treat onychomycosis. The phenol and flavonoid contents of the *Psidium guajava*-loaded nail lacquer were detected in the acetone extract, according to the FTIR data. Eudragit RL100 and the proper plasticizers were used to develop the formulation for the translingual delivery system (nail lacquer), which enabled the formation of uniform, even, translucent, and flexible films. The F4 formulation outperformed the others in terms of zone of inhibition, drug release, thickness, water resistance, and drying time. Salicylic acid nail lacquer formulation F4 shows increased translingual delivery of 75.72%. It was discovered that the nail lacquer F4 formulation

was highly effective at preventing the growth of fungi. All formulations underwent an accelerated stability testing for one month at $42 \pm 2^\circ\text{C}$, following ICH guidelines. The results showed that the F4 formulation showed no appreciable variation in the early features. Because flavonoids and phenols are present, *Candida albicans* produced the intended zones of inhibition. Thus, it is possible that the nail lacquer will be the first novel dosage form that completely transforms the pharmaceutical and healthcare industries.

Consent for Publication

Not Applicable

Funding

None

Conflict of Interest

Not Applicable

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