IJPSR (2021), Volume 12, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 July 2020; received in revised form, 16 October 2020; accepted, 06 May 2021; published 01 July 2021

DEVELOPMENT OF TOPICAL NAIL LACQUER FORMULATION FOR THE MANAGEMENT OF ONYCHOMYCOSIS

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Keywords:

Nail lacquer, Permeation enhancers, Oxiconazole nitrate, Drug penetration, Antifungal activity

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ABSTRACT: Nail plate being a formidable barrier, drug permeation through this layer is limited. Taking this as a challenging task, the present study focuses on the formulation of an antifungal topical nail lacquer using Oxiconazole nitrate. The novelty of the studies can be stated as the use of a combination of permeation enhancers like salicylic acid, thioglycolic acid, and urea for effective therapeutic treatment of topical nail infection like Onychomycosis. **Results:** Optimized formulation F13 was subjected for incompatibility studies followed by post-formulation studies (drying time, water resistance, *etc.*). Clipping of nail part was examined for *in-vitro* drug release and anti-microbial activity followed by stability studies of the optimized formulation. The optimized F13 formulation was found to have a thickness (57±0.04µm), folding endurance (183±0.57 mm), and tensile strength (2.62±0.02 Kg/cm^2) values, respectively. The FTIR and XRD studies showed no interaction between drug-excipients. Permeation enhancer in the ratio of salicylic acid: thioglycolic acid: urea in hydrogen peroxide (1:1:1) with 5% concentration each showed *in-vitro* drug release rate of 96.03% at 48 hours both through cellophane membrane as well bovine hooves membrane methods. Antifungal activity exhibit a zone of inhibition of 11±0.03 mm. **Conclusion:** The present research with an attempt to use combinatorial permeation enhancer (salicylic acid: thioglycolic acid: urea in hydrogen peroxide) showed good permeability and antifungal activity. The F13 formulation possessed all the expected activities and thereof compared with marketed (Lakme) topical nail lacquer. From the above results, it can be concluded that *in-vivo* studies can be a promising approach in the future.

INTRODUCTION: Onychomycosis is a nail fungal disease caused by dermatophytes, non-dermatophytes, and yeast species afflicting both fingernails and toenails with a high recurrence rate [1, 2] that causes discoloration, thickening, and separation from the nail bed.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(7).3959-68

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(7).3959-68

Onychomycosis represents half of the nail abnormalities in the adult [3] with common risk factors includes reduced peripheral blood circulation, diabetes, slow-growing nails, family history of fungal infection, heavy perspiration, moist work environment, wearing artificial nail, wearing shoes and socks that prevent ventilation, walking barefoot in public places such as swimming pool, gyms, shower rooms, infected by AIDS [4] and those undergoing cancer therapy.

Onychomycosis Severity Index is one of the methods (scoring system) used to correlate the causes of infection to antifungal treatment; it also

helps to differentiate or classify the affected nail as mild, moderate, or severe [5]. The treatment regimen for nail infection involves oral therapy and topical application, which includes creams, ointment [6,] and nail lacquer with antifungal agents or antibiotics. Nail lacquers have been used as cosmetics for a long time for beautification and protection of nails, now recognized as a formulation that can treat many fungal nail infections commonly applied with a brush; there are also other types of application like spatulas and sponge tips. Colorless and non-glossy medicated nail lacquer is more acceptable by male patients [7].

Lacquer, when applied, forms an occlusive and adhesive film on the nail plate that acts as a drug depot for the sustained release of drug [6]. Thus, the duration of nail lacquer film on the nail plate is the most important property of nail lacquer formulation. Factors affecting diffusion of drugs through nails are physiochemical properties of the nail and physiochemical properties of active ingredient such as molecular solute size, hydrophilicity/hydrophobicity, ionization [8].

The human nail is a complex structure composed of a nail plate containing 80-90 layers of dead cells and mainly consists of the proximal nail fold (PNF), nail matrix, nail bed, and hyponychium [9]. The presence of many strands of keratin joined by disulfide bonds behaves as a concentrated hydrogel rather than a lipophilic membrane. Using this principle, a method to screen penetration enhancers is based on their "nail hydration capacity [8]".

Topical application bypasses first-pass metabolism, drug interaction and increases absorption of drug at the target site; furthermore, it provides controlled and prolonged release of drug through depot formulation and devoid from systemic effects [9]. Nail lacquers are used to treat severe diseases where oral therapy is contraindicated, for example, in children, in pregnant and breastfeeding women, in patients with hepatic and renal impairment [10].

Medicated nail lacquer formulation usually contains drug, film-forming polymer, solvent, suspending agent and for effective permeation, permeation enhancers are used [10]. In the present work, nail lacquer was formulated using different penetration enhancers like urea and thioglycolic acid and investigated the permeation of the antifungal drug, oxiconazole nitrate through the human nail plate. Oxiconazole citrate is a broad spectrum imidazole derivative that acts by impairing ergosterol growth in fungus, having a molecular weight of 429g/mol. Finally, the formulations were evaluated regarding their permeability enhancement factors by estimating the antimicrobial activity against the dermatophytes.

MATERIALS AND METHODS:

Materials: The following materials are used in this study: Oxiconazole nitrate (yarrow chemproducts), ethylcellulose (kemphasol), glycerin (ottokemi), propylene glycol (ottokemi), salicylic acid (nice chemicals), urea (medilise chemicals), hydrogen peroxide (nice chemicals), thioglycolic acid (nice chemicals), ethanol (changshuhongsheng, Chennai).

Methods of Preparation:

Preparation of Reagents: [9] Phosphate buffer pH 7.4 was prepared taking 50 ml of potassium dihydrogen phosphate solution and 0.2 M NaOH mix and make it up to 200ml.

Preparation of Stock Solution: [9] The standard stock solution 1mg/ml was prepared by dissolving Oxiconazole nitrate 100mg in 100ml of phosphate buffer. From the stock solution, the ultraviolet scan was taken between the wavelengths 200-400 nm, and λ_{max} was determined.

Formulation of Nail Lacquer: [9] Formulation of nail lacquer was prepared using a simple mixing method. All the formulations of nail lacquer contained drug (1%), ethyl cellulose (5%), glycerin (5%), propylene glycol (5%), salicylic acid, thioglycolic acid, and urea in hydrogen peroxide, non-volatile solvent. Set of formulation were prepared and coded as F1 to F13. The weight of Oxiconazole nitrate in all formulations was kept constant. In formulation F1, F2, F3 contained 1%, 3%, 5% of salicylic acid. F4, F5, F6 contained 1%, 3%, 5% of urea in H_2O_2 solution. Formulation F7, F8, F9 contained 1%, 3%, 5% of thioglycolic acid.

Formulation F10 having salicylic acid and thioglycolic acid in ratio 1:1. Formulation F11 having salicylic acid and urea in H_2O_2 ratio 1:1. Formulation F12 having thioglycolic acid and urea in ratio 1:1 and F13 having all 3 permeation enhancers in the ratio 1:1:1.

TABLE 1: FORMULATION DESIGN OF OXICONAZOLE NITRATE NAIL LACQUER

Ingredients	F0	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
Oxiconazole nitrate (g)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ethylcellulose (g)	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Glycerine (mL)	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Propylene glycol (mL)	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Salicylic acid (SA) (g)	-	1	3	5	-	-	-	-	-	-	3	3	-	3
Urea :H_2O_2 (mL)	-	-	-	-	1	3	5	-	-	-	-	3	3	3
Thioglycolic acid (mL)	-	-	-	-	-	-	-	1	3	5	3	-	3	3
Ethanol (mL)	100	100	100	100	100	100	100	100	100	100	100	100	100	100

*SA-salicylic acid, U- urea, TA-thioglycolic acid, HP-hydrogen peroxide

Evaluation Parameters:

Preformulation Studies of Oxiconazole Nitrate:

Construction of Calibration Curve: [10, 11] 10mg of drug was dissolved in phosphate buffer pH 7.4 and volume made up to 100ml to produce a stock solution-I of 100µg/ml. From this 10 ml of the solution was taken and further diluted to 100 ml with phosphate buffer pH 7.4 to produce a stock solution-II of 10 µg/ml solution. From these aliquots of 0, 2, 4, 6, 8, and 10 ml were taken and further diluted to 10ml to produce concentrations of 0, 2, 4, 6, 8, and 10µg/ml. The absorbance was measured by using a UV-visible spectrophotometer at 204 nm, and the results were tabulated.

Determination of Oxiconazole Nitrate Solubility: [12] Solubility of saturated Oxiconazole nitrate solution was determined in different solvents (water/methanol/ethanol). Then by using a mechanical shaker, the flasks were shaken for 48 h. The sample was withdrawn (1ml after filtration) was diluted with appropriate medium and analyzed by using UV spectrophotometer at 204 nm.

Drug Excipients Compatibility Studies: [12] The infrared (IR) spectra were recorded using an FTIR spectrophotometer by the KBr pellet method in the frequency range of 400-4000 cm^{-1}.

The spectra obtained for Oxiconazole and physical mixtures of Oxiconazole nitrate with other excipients were compared to check the compatibility of drug with excipients.

Optimization of Concentration of Ethyl Cellulose (EC): Six different concentration of ethyl cellulose 2%, 5%, 7.5%, 10%, 12.5%, 15% were prepared EF1, EF2, EF3, EF4, EF5, EF6 as various literature studies, optimum concentrations for film formation was determined by clarity, clear, gloss, smoothness of flow, film thickness, folding endurance, tensile strength, and water resistance

Evaluation of Oxiconazole Nitrate Loaded Nail Lacquer:

Non-volatile Content: [13] 10ml samples was taken in a petridish, and initial weight was recorded. The dish was placed in the oven at 105 °C for 1 h; the petridish was removed, cooled, and weighed. The difference in weight was recorded. The average of triplicate reading was noted, and the result was reported in **Table 5**.

Drying Time: [13] A film of the sample was applied on a petridish with the help of a brush. The time to form a dry to touch film was noted with the help of a stopwatch and given in **Table 6**.

Smoothness to Flow: [14] The sample was poured from a height of 1.5 inches into a glass plate and spread on a glass plate, and made to rise vertically.

Gloss: [14] Sample of nail lacquer was applied over the nail, and gloss was visually seen, compared with marketed cosmetic nail lacquer.

Drug Content Estimation: [15] Nail lacquer equivalent to 100mg was dissolved in 50 ml phosphate buffer solution of pH 7.4. Then the solution was ultrasonicated for 15 min. The resulting solution was filtered, made up to 100 ml with phosphate buffer solution of pH 7.4. From the above solution, take 10ml and made up to 100ml with PBS of pH 7.4. Then the diluted solution was estimated spectrophotometrically at wavelength of 223 nm and determined the drug content **Table 7**.

***In-vitro* Transungal Permeation Studies:** [15] Franz diffusion cell using an artificial membrane (cellophane) was used to carry out translingual permeation studies. Donor compartment is filled with test vehicle equivalent to 10 mg. The receptor compartment was filled with phosphate buffer pH 7.4. The whole assembly was maintained at 37 °C, and the speed of stirring was kept constant for 48 h.

An intermittent sample of 5ml was drawn from the receiver compartment at 2 h intervals for 48 h, and the amount of Oxiconazole nitrate transported was measured. An equal volume of fresh buffer was replaced in the receiver compartment followed by each sampling. The drug analysis was done using a double-beam UV Spectrophotometer (U.V.1700 Shimadzu Corporation) and reported in **Table 8**.

***In-vitro* Drug Release Study:** [15] Hooves obtained from freshly slaughtered cattle, free of adhering connective and Cartilaginous tissue, were soaked in distilled water for 24 h. From this hoof, approximately 1mm thickness membrane was removed by using a sharp knife. *In-vitro* permeation studies were carried out by Franz diffusion cell. The hoof membrane was placed carefully on the cell. The drug equivalent to 10 mg was applied evenly on the surface of the nail membrane.

The receptor compartment was filled with pH 7.4 phosphate buffers, and the whole assembly was maintained at 37 °C with constant stirring for 48 h. The 5 ml aliquot of drug sample was withdrawn after a fixed time interval and replaced by a fresh buffer. After 48 h of the study, the residual drug on the hoof was washed with fresh solvent.

The samples were analyzed using Shimadzu 1800 model UV-visible spectrophotometer at 204 nm; the absorbance data and consequently, the percent release of the drug at different times were obtained **Table 9**.

Anti Fungal Susceptibility Test: [15, 16] Each batch of lacquer formulation was subjected to antifungal activity, dermatophtye species, especially *Trichophyton rubrum*. Seeded agar plates containing *Trichophyton rubrum* are inoculated with the test solution and incubated at 130 ºC for 4 days. Zone of inhibition was measured and reported.

Stability Studies: [8] ICH guidelines were followed to carry out the stability studies of the optimized formulation. Samples were stored at a temperature of 40 ± 2°C/75 ± 5% RH for three months. The resultant samples were further analyzed for clarity, gloss, smoothness of flow, non-volatile content, drying time, drug content, antimicrobial study, and diffusion across hooves membrane **Table 10**.

RESULTS AND DISCUSSION:

Construction of Calibration Curve:

TABLE 1: STANDARD CURVE DATA FOR OXI SULPHATE IN PBS pH 7.4

Concentration (µg/ml)	Absorbance
0	0
2	0.098
4	0.174
6	0.254
8	0.339

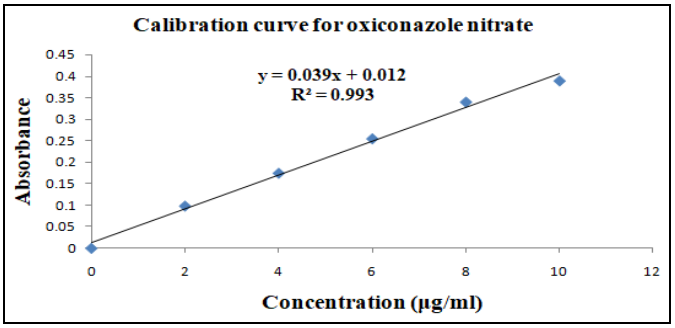

FIG. 1: CALIBRATION CURVE OF OXICONAZOLE NITRATE IN PBS OF pH 7.4

Determination of Oxiconazole Nitrate Solubility: Oxiconazole showed solubility in methanol of 20mg/ml and hence used has solvents for further studies.

TABLE 2: SOLUBILITY OF OXICONAZOLE NITRATE IN VARIOUS SOLVENTS

S. no.	Solvents	Solubility (mg/ml)	Inference
1	Methanol	20.01	Soluble
2	Ethanol	12.15	Sparingly soluble
3	Water	0.008	Very slightly soluble

Drug Excipients Compatibility Studies: FTIR spectrum of pure Oxiconazole nitrate showed characteristic peaks at 2970 cm^{-1} (C-H(methylene, CH_2)), 1507 cm^{-1} (C=N), 1241 cm^{-1} (N-O(of cis isomer)), 3246 cm^{-1} (C-H (aromatic)). All characteristic peaks of excipients were also observed. After spectral comparison, it was confirmed that all characteristic peaks of Oxiconazole mentioned above were observed in the physical mixture of the formulation. There were no new peaks or disappearance of characteristic peaks. The FTIR spectrum of standard drug Oxiconazole nitrate and its physical mixture with polymers correspond to similar wavenumbers. This indicates that there is no interaction between drug and polymers and that the polymers and drug are compatible with each other formulation.

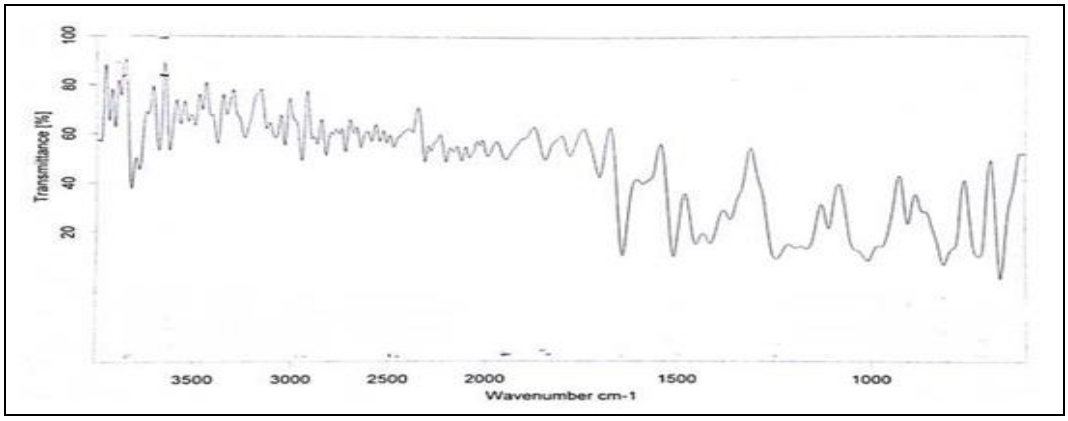

FIG. 2: FTIR OF DRUG OXICONAZOLE NITRATE

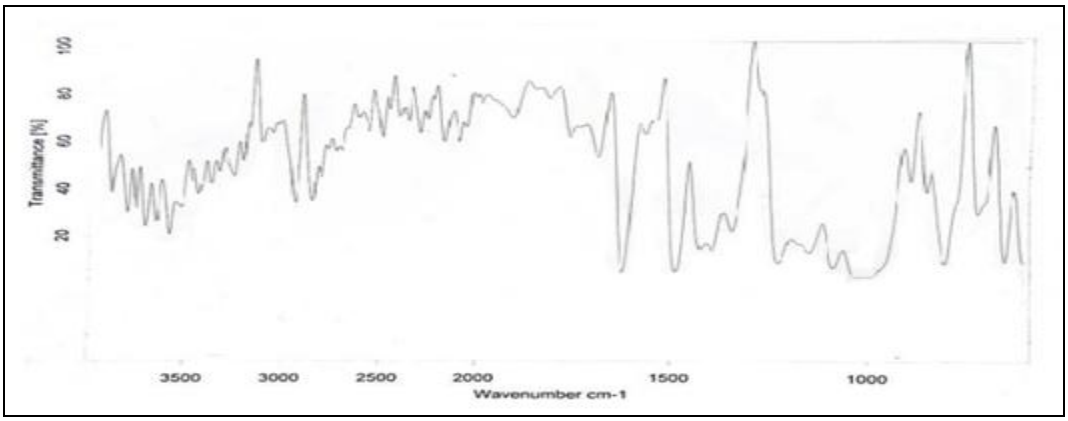

FIG. 3: FTIR OF OXICONAZOLE NITRATE PHYSICAL MIXTURES

Optimization of Concentration of Ethyl Cellulose (EC):

TABLE 3: OPTIMIZATION OF CONCENTRATION AND ETHYL CELLULOSE EVALUATION OF EC

Formulation code	EF2	EF3	EF4	EF5	Standard*
Thickness (µm)	57±0.04*	58±0.02	59±0.03	58±0.04	55±0.01
Folding endurance (mm)	183±0.57	176±0.57	125±0.57	175±0.57	185±0.57
Tensile strength (Kg/cm^2)	2.62±0.02	2.56±0.03	2.58±0.02	2.54±0.01	2.70±0.03

*Standard = lakme product, *Mean ± S.D

Film for nail lacquer was prepared using different concentration of ethyl cellulose and were subjected to thickness, folding endurance and tensile strength comparing with the standard marketed formulation. The marketed sample showed at thickness, folding endurance, and tensile strength of 55 µm, 185 and 2.70 kg/cm^2 respectively, EF2 with 0.5% concentration of ethyl cellulose had satisfactory folding endurance and tensile strength and was considered as an optimized formula to developed nail lacquer. To further sustain the drug release, which sustained the drug release to 48th h.

Evaluation of Oxiconazole Loaded Nail Lacquer:

Non-volatile Content: The desired amount of non-volatile matter was seen with complete evaporation of volatile matter leaving a thin film; it ranges from 21-22%. As the polymer concentration increases non-volatile content increases.

TABLE 4: NON-VOLATILE CONTENT OF NAIL LACQUER

Formulation code	Non-volatile content (%)
F1	22.31±0.57
F2	22.17±0.74
F3	22.34±0.17
F4	21.30±0.65
F5	21.92±0.34
F6	22.46±0.83
F5	21.91±0.25
F6	22.10±0.15
F9	22.90±0.62
F10	22.83±0.23
F11	21.96±0.06
F12	22.40±0.12
F13	21.22±0.54
Standard	21.33±0.05

Drying Time: The optimum time required for drying of medicated nail lacquer in literature is found to be less than 2 min. The drying time of formulated nail lacquer was found in the range of 58-71 sec. The result was found to be satisfactory.

TABLE 5: DRYING TIME OF DIFFERENT FORMULATION NAIL LACQUER

Formulation code	Drying time (sec)
F1	60±0.02
F2	63±0.98
F3	64±0.34
F4	63±0.66
F5	59±0.75
F6	62±0.45
F5	65±0.82
F6	68±0.93
F9	71±0.56
F10	64±0.05
F11	62±0.61
F12	61±0.35
F13	59±0.71
Standard	58±0.06

Smoothness to Flow: Smoothness of flow for formulations was found to be good on comparing with the standard and indicates that all the formulation can be easily applied to the nail plate with a brush.

Gloss: The gloss of nail lacquer was evaluated and compared with the standard. It was found to be satisfactory, and all the formulation possesses the acceptable range of gloss.

Drug Content Estimation: All the formulated contains 90% of the drug. Highest %age of drug content was found to be 97.89% (F13) indicates the stability of drug in the formulation and indicates the compatibility between drug and polymer.

TABLE 6: PERCENTAGE DRUG CONTENT OF DIFFERENT FORMULATION

Formulation code	Drug Content (%)
F1	92.03±0.70
F2	93.01±0.88
F3	93.02±0.32
F4	91.25±0.55
F5	94.38±0.15
F6	93.98±0.94
F5	90.54±0.08
F6	93.25±0.98
F9	93.01±0.18
F10	95.02±0.01
F11	96.54±0.25
F12	95.02±0.90
F13	97.89±0.05

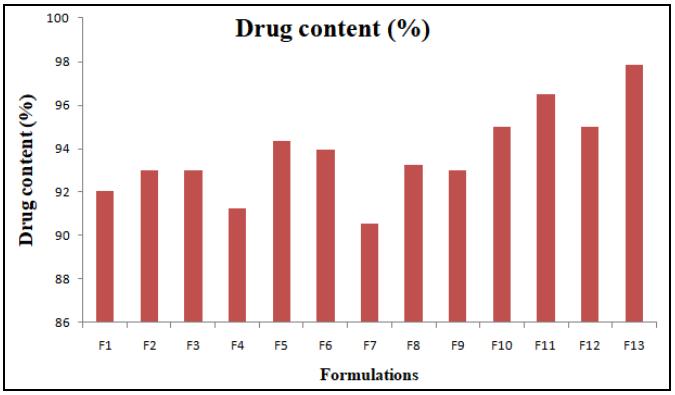

FIG. 4: DRUG CONTENT ESTIMATION

***In-vitro* Transungal Permeation Studies:** To carry out *in-vitro* drug release studies using cellophane membrane, 13 different formulation were prepared and code as F0-F13 with or without permeation enhancer according to Ashwani *et al.* [8] F0 formulation contained no permeation enhancer seem to have an *in-vitro* drug release of 21.98% till 48th h. Further studies were continued using salicylic acid as a keratolytic agent, incorporated to formulation F1, F2, and F3 (1%, 3%, and 5%) showed a drug release of 48.18%, 65.61%, and 65.01% respectively at 48th h.

From the above studies, salicylic acid was found to be a good permeation enhancer, but an increase in concentration showed no effect on further increase in drug release, and hence 3% permeation enhancer was found to be optimum. To further carry out the diffusion studies, urea and hydrogen peroxide in the proportion of 1:1 at 1%, 3%, and 5% (F4, F5, and F6) were attempted, these batches showed a drug release of 46.94%, 69.46%, and 68.95% respectively at 48th h. The trial was also carried out using another permeation enhancer, thioglycolic acid at a concentration of 1%, 3%, and 5% showed drug release of 41.45%, 60.09%, and 59.87% (F6, F7, and F8) respectively at 48th h.

Studies were extended using a combination of permeation enhancers, like salicylic acid, thioglycolic acid, and urea. The formulation F10 (3% of thioglycolic acid + 3% of salicylic acid) released 70.12% of the drug in 48th h across the artificial membrane. The formulation F11 (3% of salicylic acid +3% of urea in hydrogen peroxide) released 78.98% of the drug in 48 h across the artificial membrane. The formulation F12 (3% of thioglycolic acid + 3% of urea in hydrogen peroxide) shows the release of 76.67%. In batch

F13 the combination of three permeation enhancers (5% of thioglycolic acid + 5% of urea in hydrogen peroxide + 5% of salicylic acid) showed higher drug release when compared to F0 to F12, which sustained drug release to 48th h. Shireen *et al.*, in their studies found that 5% thioglycolic acid has shown a drug release rate of 96.37% within 36 h [10] which is in near correlation with the current results. The *in-vitro* diffusion studies showed a very good release of 96.03% in the 48th h in F13. Vipin *et al.*, in their studies found that a combination of salicylic acid and urea as permeation enhancer as shown a drug release rate of 96.03% was observed at 48th hrs for the formulation F10. This was due to the hydration and permeation property of propylene glycol, which improved penetration of the drug. The film characteristics, smoothness to flow, and drying time were also better for this formulation.

It has been found from the studies of Vipin *et al.*, that 0.5% of ethylcellulose has sustained the drug release to 48 h. Hence the same concentration of ethyl cellulose has been incorporated in all the formulations of the current study.

Release studies were also carried out using a bovine hoof membrane. Formulation F13, which exhibited ideal release characteristics with *in-vitro* drug release study, was selected for this further studies. A release of 95.31% was observed at 48th h. There was no significant difference in release pattern across artificial membrane and hooves membrane. The results showed a superimposable diffusion release profile. This further proved that artificial cellophane membrane mimicked the characteristic features of *ex-vivo* bovine hoof membrane.

TABLE 7: *IN-VITRO* DRUG RELEASE PROFILE OF F0, F1, F2, F3, F4, F5 AND F6 FORMULATION

	%Drug released						
	Formulation code						
	F0	F1	F2	F3	F4	F5	F6
	Concentration of permeation enhancer						
	0%	1%	3%	5%	1%	3%	5%
	Different Permeation enhancer						
Time		SA	SA	SA	U:HP 1:1	U:HP 1:1	U:HP 1:1
0	0	0	0	0	0	0	0
2	3.75±0.01	8.14±0.28	10.85±0.11	11.64±0.07	9.82±0.05	13.87±0.08	14.45±0.18
4	4.98±0.18	12.47±0.17	16.67±0.89	19.14±0.93	14.26±0.08	21.87±0.01	24.56±0.98
6	5.98±0.89	19.14±0.98	21.28±0.46	22.08±0.28	20.46±0.82	31.08±0.08	31.01±0.71
8	8.46±0.14	24.54±0.19	29.19±0.72	28.08±0.21	25.96±0.16	44.87±0.19	37.08±0.25
10	12.46±0.78	29.04±0.93	34.26±0.92	32.28±0.39	30.19±0.96	50.98±0.28	47.14±0.89
12	14.19±0.16	35.17±0.41	39.24±0.41	38.08±0.72	36.07±0.41	55.29±0.75	50.14±0.71
16	15.08±0.75	39.90±0.19	42.10±0.93	42.42±0.92	39.84±0.02	59.28±0.19	55.87±0.89
20	15.41±0.27	41.82±0.28	48.46±0..78	49.16±0.71	40.19±0.08	65.85±0.08	60.17±0.72
24	16.87±0.82	43.87±0.12	52.87±0.10	55.18±0.97	44.07±0.75	66.85±0.07	62.71±0.18
28	17.01±0.19	45.01±0.72	59.85±0.02	62.14±0.09	46.78±0.95	67.08±0.19	63.89±0.96
32	18.07±0.14	45.90±0.19	62.18±0.09	63.26±0.07	47.09±0.28	67.95±0.86	65.47±0.18
36	19.01±0.98	46.98±0.52	63.01±0.19	63.98±0.89	44.08±0.76	68.14±0.93	66.82±0.87
40	19.46±0.15	47.08±0.39	63.72±0.72	64.01±0.82	45.28±0.16	69.05±0.81	67.87±0.97
44	20.87±0.19	47.25±0.17	64.08±0.09	64.98±0.02	46.18±0.08	69.25±0.19	68.14±0.01
48	21.98±0.90	48.18±0.29	65.61±0.09	65.0±0.08	46.94±0.89	69.46±0.95	68.95±0.58

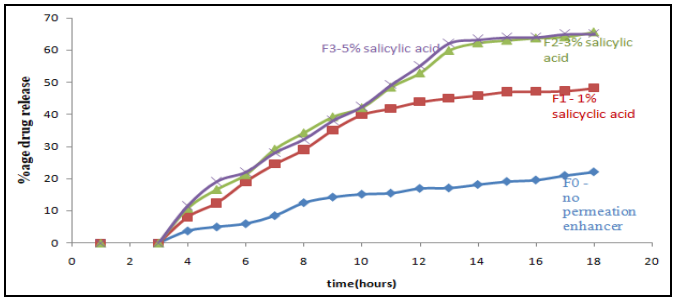

FIG. 5: *IN-VITRO* DRUG RELEASE PROFILES OF F0, F1, F2 AND F3 FORMULATION

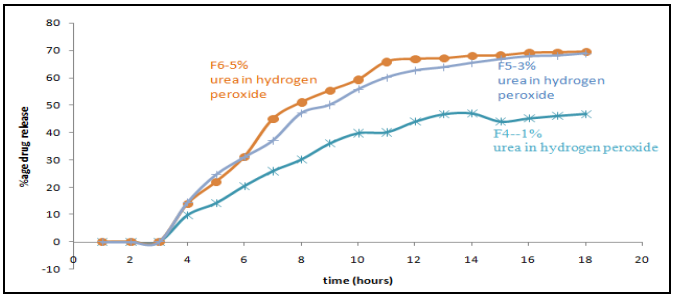

FIG. 6: *IN-VITRO* DRUG RELEASE PROFILES OF F4, F5 AND F6 FORMULATION

TABLE 8: *IN-VITRO* DRUG RELEASE PROFILE OF F7, F8, F9, F10, F11 F12 AND F13

	%Drug released						
	Formulation code						
	F7	F8	F9	F10	F11	F12	F13
	Concentration of permeation enhancer						
	1%	3%	5%	3%	3%	3%	5%
	Different Permeation enhancer						
Time	TA	TA	TA	TA:SA	SA : UinHP	TA : UinHP	SA: TA: UinHP
0	0	0	0	0	0	0	0
2	10.86±0.08	12.85±0.08	13.75±0.14	12.85±0.07	16.08±0.08	14.75±0.17	18.04±0.17
4	18.08±0.15	22.47±0.19	21.82±0.86	16.49±0.15	21.46±0.28	20.82±0.19	24.83±0.28
6	26.82±0.89	25.08±0.82	25.82±0.96	21.76±0.72	28.19±0.39	27.08±0.82	30.49±0.93
8	30.82±0.14	31.52±0.98	32.74±0.49	28.14±0.19	31.09±0.19	33.92±0.39	33.67±0.16
10	36.46±0.98	38.71±0.16	37.46±0.72	34.71±0.19	36.82±0.43	39.08±0.42	38.96±0.19
12	37.29±0.71	39.21±0.89	41.82±0.98	39.75±0.08	43.48±0.83	45.92±0.72	42.21±0.28
16	38.28±0.98	42.82±0.14	43.20±0.14	43.82±0.08	50.14±0.75	49.80±0.89	45.93±0.37
20	39.82±0.15	44.67±0.78	44.07±0.95	48.07±0.19	58.16±0.19	54.82±0.19	49.09±0.45
24	40.08±0.75	46.09±0.97	45.09±0.71	52.08±0.07	62.26±0.83	60.75±0.18	52.28±0.19
28	40.96±0.98	51.23±0.14	52.40±0.06	59.10±0.21	69.07±0.19	66.82±0.79	57.99±0.52
32	40.14±0.01	55.09±0.72	56.92±0.64	64.71±0.28	75.04±0.71	72.92±0.17	62.63±0.86
36	40.95±0.89	57.48±0.78	56.00±0.15	65.00±0.39	77.14±0.93	73.98±0.93	66.50±0.19
40	40.10±0.75	59.99±0.92	57.64±0.08	69.48±0.07	77.93±0.19	74.09±0.71	75.51±0.72
44	41.08±0.18	59.08±0.14	59.08±0.13	69.78±0.72	78.08±0.82	74.58±0.37	81.73±0.74
48	41.45±0.19	60.09±0.54	59.87±0.72	70.12±0.18	78.98±0.19	74.67±0.14	96.03±0.10

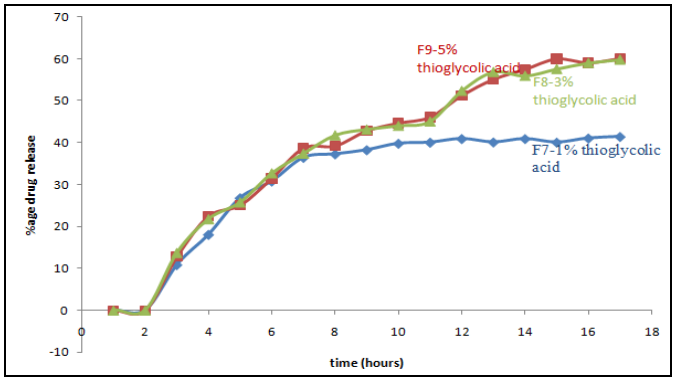

FIG. 7: *IN-VITRO* DRUG RELEASE PROFILES OF F7, F8 AND F9 FORMULATION

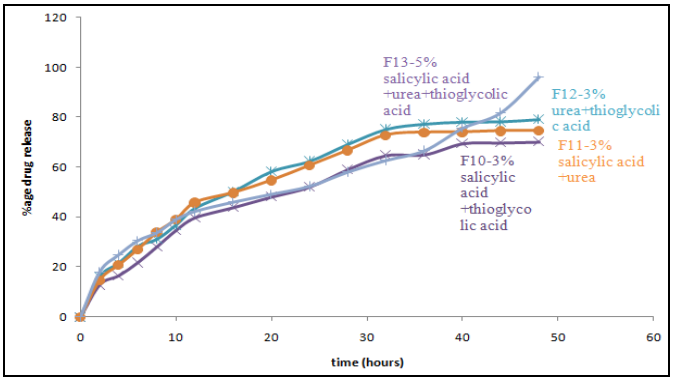

FIG. 8: *IN-VITRO* DRUG PROFILES OF F10, F11, F12 AND F13

TABLE 9: *IN-VITRO* TRANSUNGUAL PERMEATION STUDIES RELEASE DATA

Time (hr)	%Drug released through Hooves membrane	%Drug released through cellophane membrane
Formulation containing salicyclic acid: thioglycolic acid: urea in Hydrogen peroxide in ratio 1:1:1`(F13)		
0	0	0
2	17.54	18.04±0.17
4	24.83	24.83±0.28
6	29.49	30.49±0.93
8	32.67	33.67±0.16
10	39.69	38.96±0.19
12	43.21	42.21±0.28
16	47.91	45.93±0.37
20	49.89	49.09±0.45
24	53.28	52.28±0.19
28	57.99	57.99±0.52
32	64.13	62.63±0.86
36	68.50	66.50±0.19
40	76.51	75.51±0.72
44	82.91	81.73±0.74
48	95.31	96.03±0.10

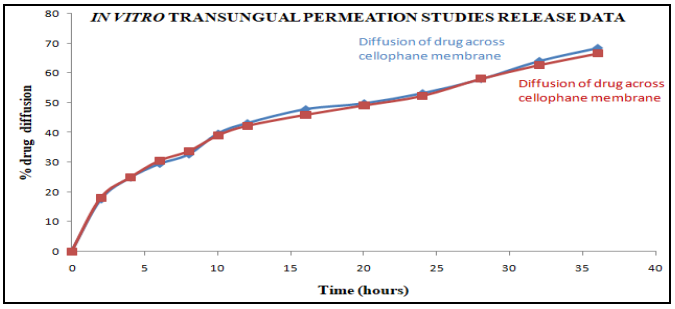

FIG. 9: DIFFUSION OF F13 ACROSS CELLOPHANE AND HOOVES MEMBRANE

Anti Fungal Susceptibility Test: The selected formulation was tested for antifungal activity. It was observed that the prepared medicated nail lacquer formulation has a zone of inhibition of 11±0.03 mm, which is comparable with the zone of inhibition of standard drug (12±0.05mm). This proves that the prepared formulation has sufficient antifungal property. The zone of inhibition is depicted in **Fig. N10**.

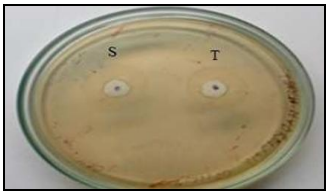

FIG. 10: ZONE OF INHIBITION OF OXICONAZOLE NITRATE

Stability Studies:

TABLE 10: STABILITY STUDIES DATA OF F13 FORMULATION

Formulation code	Clarity	
	Initial	**After stability**
	Clear	Clear
	Gloss	
	Initial	**After stability**
	+++	+++
	Smoothness of flow	
	Initial	**After stability**
	Smooth, evenly dispersed	Smooth, evenly dispersed
	Non volatile content	
	Initial	**After stability**
	21.22 ± 0.54	21.32±0.08
F13	**Drying time**	
	Initial	**After stability**
	59±0.71	58±0.07
	Drug content	
	Initial	**After stability**
	97.89±0.05	96.17±0.08
	Drug release	
	Initial	**After stability**
	96.03±0.15	94.81±0.14
	Anti-microbial activity	
	Initial	Final
	11mm	10mm

Optimized formulations (F13) were subjected for short-term stability studies *i.e.*, 90 days of storage at 40±2 ºC with 75±5% RH. Studies reported that there were no remarkable changes in the clarity, gloss, smoothness off flow but with slight variation in drying time, drug content and percentage drug release and anti microbial activity with drug release of 94.81% and zone of inhibition of 10mm, which is comparable with the zone of inhibition of standard drug (12mm) at the end of 3 months.

CONCLUSION: Efficient permeability across the nail plate is observed with medicated nail lacquer formulation. The evaluated combination of enhancers, Urea in H_2O_2, thioglycolic acid, and salicylic acid used in formulation F13 have a varying mechanism in enhancing oxiconazole nitrate's permeation and penetration into the nail plate resulting in its enhanced antifungal activity which can be correlated with the drug release rate of 96.03% at 48 hours both through cellophane membrane as well bovine hooves membrane. Since the formulated nail lacquer (F13) is having a rapid drying rate, the applied nail lacquer will not be wiped out from the nails like the conventional antifungal creams or lotions. Thus eliminating the repeated application of medicaments to the nail. As the developed nail lacquer is transparent, it can be prescribed for both genders. The oral route of administration of antifungal drugs like allylamines, azoles are associated with many side effects due to a large dose of administrations. These problems can be overcome by formulating these drugs as nail lacquer with much-reduced doses for treatment which in turn results in reduced side effects. Further clinical and pharmacokinetic studies are required to explore the potential of ungula drug delivery systems for use in humans.

ACKNOWLEDGEMENT: Authors would like to thank Pariyaram Medical College, Kannur, Kerala, and Yenepoya Pharmacy College and Research Centre, Mangalore, for providing the laboratory facilities to carry out the experimental works.

CONFLICTS OF INTEREST: All the authors herewith declare that they do not have any conflicts of interest

CONTRIBUTIONS OF AUTHORS: All the authors have equally contributed to conducting the research and writing the manuscript.

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How to cite this article:
Sindhu KK, Biju P, Shaik M, Augusthy AR and Revadigar V: Development of topical nail lacquer formulation for the management of onychomycosis. Int J Pharm Sci & Res 2021; 12(7): 3959-68. doi: 10.13040/IJPSR.0975-8232.12(7).3959-68.