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SMART UNGUAL BIOPENETRANT FROM THE ROOTS OF BETA VULGARIS

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ABSTRACT

The main objective of the current research was to isolate biopenetrant from *Beta vulgaris* and formulate bioadhesive layers loaded with terbinafine using this biopenetrant for delivery through nail. Form the preparation of films, *Rosa centifolia* and *Vitis vinifera* were used as a biopolymer. *Beta vulgaris* was used as biopenetrant. The formulations prepared using Beta vulgaris biopenetrant were compared with formulations containing papain as standard penetrant. Four bio-adhesive films of different ratios were prepared by film casting method using each penetrant. The formulated films were evaluated for various parameters like weight, thickness, nail adhesivity, content uniformity, surface pH, folding endurance, and *in-vitro* drug permeation. The formulation LB4 (containing 1:4 Beta vulgaris biopenetrant) was found to be the best formulation having R2 value 0.990 with zero order as best fit model. The results obtained conclude that biopenetrants can be used as natural penetration enhancers.

INTRODUCTION

Onychomycosis, a nail fungal disease which constitutes about half of all nail disorders. It is one of the most common disorders in adults which can also affect children. It accounts for 2-3% of the population in U.S. up to 13% of Finnish population. It can affect any part of the nail. Although this disease does not lead to death but it causes severe pain and discomfort to the patient. Nail is highly keratinised and the chemical bonds present in nails are physically and chemically stable, which makes the nail hard and impermeable. The major problem while treating the nail diseases is to achieve therapeutically effective concentration of drugs in the deeper nail stratums during the course of treatment. The treatments available require large doses and frequent administration of drugs which may be associated with severe side effects. In order to obtain reduced side effects and better patient compliance, topical delivery is the most desired therapy. [1–4]

Ungual route is used for the delivery of drug across the nail plate by applying the formulation on the nail. This involves the transportation of drug directly to the site of infection with reduced cost of treatment. This therapy is preferred in case of geriatrics or the patients receiving multiple drugs to reduce drug-drug interaction. This lacks at one point i.e. poor trans-nail absorption of the drug. Unfavorable physicochemical properties of the drugs, lack of formulations that can overcome the barrier properties of the nail plate, short residence time of topical formulations and extensive binding of drug to the nail keratin are the major reasons for poor trans-nail absorption. Due to these reasons, it becomes quite necessary to design the formulation which has the ability to deliver the drug effectively across the nail plate.[1–4]. A variety of mechanical, chemical and physical methods have already been developed for effective drug permeation. Mechanical methods include nail avulsion and abrasion. These methods are invasive and painful. Chemical methods include the use of keratolytic agents (urea, salicylic acid, etc), compounds containing sulfhydryl groups (acetylcysteine, cysteine, and mercaptoethanol), keratinolytic enzymes, N-acetyl-l-cysteine and mercaptan compounds.

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Physical methods involved in this are iontophoresis, etching, hydration, occlusion, carbon dioxide laser. Phonophoresis and microsurgical laser apparatus (onycholaser) are the new emerging methods for enhancing nail penetration. However, discovery of most effective and safe enhancer is still a challenge [5]. Terbinafine [(2E)- 6,6- dimethylhept-2-en -4-yn-1-yl] (methyl) (naphthalen-1-ylmethyl) amine is a synthetic allylamine antifungal drug used for the fungal infections of toe or fingernails and skin due to its ability to accumulate in skin, nails, and fatty tissues. However, the oral dose required for the treatment is high and requires the continuation for a longer period of time. This also leads to several side effects and adverse drug reactions. By directly targeting the drug at the site of infection, the required dose for the treatment as well as the side effects can be reduced and the patient compliance can be improved. [6] In this study, biopenetrants are used as a means for nail penetration enhancer. Along with the biopenetrant, the drug can easily be permeated across the nail plate. Beetroot is the taproot of *Beta vulgaris* L., family Chenopodiaceae. It consists of saccharose and varying amounts of calcium, phosphorous, iron, vitamins A and C, niacin, thiamine, and riboflavin. The color is mainly derived from water-soluble nitrogenous pigments called betalains: betacyanins (red) or betaxanthins (yellow), including betanin, betanidin, betalmic acid, and vulgaxanthin. Traditionally it is used as an antitumor, carminative, emmenagogue, and hemostatic properties. It is used in food industry for its color. [7] The current objective of the research work is to isolate a biopenetrant from *Beta vulgaris* and to formulate bioadhesive layers for delivery of terbinafine through ungual route.

MATERIALS AND METHODS

Terbinafine was obtained as a gift from A.P.S. Biotech. Pvt. Ltd. *Beta vulgaris*, *Rosa centifolia* and *Vitis vinifera* were procured from local market. Dextrose, papain and ethanol used were of analytical grade.

BIOMATERIAL EXTRACTION

Extraction of biopolymer from rosa centifolia

500 gm *Rosa centifolia* petals were soaked in 1 litre of ethyl acetate and refrigerated for 24 hr to remove the color of petals. Ethyl acetate was decanted and decolorized. *Rosa centifolia* petals were taken. The petals were boiled in 1 litre distilled water in continuous stirring mode to avoid bumping. The petals were allowed to boil for 5 hr. After that it was filtered and the filtrate was allowed to cool at room temp. The petals were boiled again and again for complete extraction. 1000 ml distilled water obtained after filtration was added to equal amount of acetone (1000 ml) and refrigerated for 24 hrs. It was further subjected to centrifugation at 3000 rpm. The precipitate was collected which was allowed for air drying to obtain the polymer. Obtained dried polymer was passed through 120# sieve and stored in the container. [8]

Extraction of biopolymer from *Vitis vinifera*

1 kg *Vitis vinifera* seeds were washed properly and boiled in 1 litre distilled water in continuous stirring mode so as to separate out the chemical constituents of *Vitis vinifera* in distilled water. *Vitis vinifera* seeds were boiled again and again for complete extraction. The supernatant obtained was added to equal amount of methanol and refrigerated for 24 hrs. It was further subjected to centrifugation at 3000 rpm. The precipitate was collected which was air dried to obtain the polymer. Dried polymer obtained was stored in container for further use [8]

Extraction of biopenetrant from *Beta vulgaris*

500 gm beetroot was procured from market, washed properly and cut into small pieces. The pieces were mixed with 100ml of distilled water. The mixture was subjected to mechanical stirring for 1 hr. The mixture was filtered to obtain 100 ml beetroot juice. The cake of beetroot obtained was again mixed with 100ml of distilled water and subjected to mechanical stirring. This was repeated again and again till the liquid was decolorized. Beetroot juice was mixed with equal amount (i.e; 100 ml) of methanol and refrigerated for 24 hrs. The mixture was subjected to centrifugation at 3000 rpm to obtain reddish brown colored precipitate. The precipitate was collected which was allowed for air drying to obtain the biopenetrant. The dried biopenetrant obtained was passed through 120# sieve.

Formulation of bioadhesive layer for transungual delivery [9]

Bio-adhesive layers were prepared by solvent casting method. 200 mg *Rosa centifolia* bio-polymer and 10 mg *Vitis vinifera* bio-polymer were dissolved in distilled water at room temperature. 50 mg dextrose was added to this polymeric solution. 10 mg terbinafine and calculated amount of penetrant were dissolved in ethanol. The penetrants were used in four ratios (drug to bio-penetrant ratio) i.e; 1:0.1, 1:0.2, 1:2 and 1:4. The solution of terbinafine and penetrant in ethanol was

incorporated slowly to the polymeric solution and the volume was made to 10 ml with distilled water. This solution was poured in a petri-dish and allowed to dry in oven at 50ºC. The dried bioadhesive layers were obtained and packed in tightly closed container. Total 8 formulations with *Beta vulgaris* bio-penetrant and papain (as standard penetrant) were prepared.

TABLE I: Formula for Terbinafine Bioadhesive Layer Containing *Beta vulgaris* Biopenetrant

Formulations / Ingredients	LB1	LB2	LB3	LB4
Drug: Bio-Penetrant (ratio)	1:0.1	1:0.2	1:2	1:4
Terbinafine (mg)	10	10	10	10
Rosa centifolia Biopolymer (mg)	200	200	200	200
Vitis vinifera Biopolymer (mg)	10	10	10	10
Biopenetrant (*Beta vulgaris*) (mg)	1	2	20	40
Dextrose (mg)	50	50	50	50
Distilled Water (q.s.) (ml)	10	10	10	10

TABLE II: Formula for Terbinafine Bioadhesive Layer Containing Standard Penetrant Papain

Formulations / Ingredients	LP1	LP2	LP3	LP4
Drug: Bio-Penetrant (ratio)	1:0.1	1:0.2	1:2	1:4
Terbinafine (mg)	10	10	10	10
Rosa centifolia Biopolymer (mg)	200	200	200	200
Vitis vinifera Biopolymer (mg)	10	10	10	10
Penetrant (Papain) (mg)	1	2	20	40
Dextrose (mg)	50	50	50	50
Distilled Water (q.s.) (ml)	10	10	10	10

EVALUATION PARAMETERS

Physical Appearance

The formulations were visually inspected for various factors like color, clarity, and smoothness in order to ensure the uniformity in physical appearance of the films. [10,11]

Weight: A patch of area 2×2 cm^2 of each formulation was taken and weighed. [10,11]

Thickness

The thickness of the films for every formulation was measured using a micrometer at three different places and the mean value was calculated. [10,11]

Folding Endurance

Folding endurance was determined by repeatedly folding the film at the same place till it broke. The number of times the film could be folded at the same place without breaking was recorded which is known as the folding endurance. [10,11]

Surface pH

The individual film was placed in a petridish and moistened with 0.5 ml of distilled water and kept for 30 min. The surface pH was measured by using pH meter. [12]

Drug Content Uniformity

The film was dissolved in methanol and volume was made up to 100 ml. It was sonicated and kept for 24 hours. 0.1 mL was withdrawn from this and diluted to 10 ml. Absorbance was measured at λ-max of the drug i.e., 283 nm by using UV Spectroscopy. This was repeated for all the formulations. The drug content was measured against the reference sample without penetrant. From the drug content, % drug content was calculated. [10,11]

Nail Adhesivity

A patch of area 2×2 cm^2 of each formulation was taken. It was applied over the human nail until it got disadhered. The time of detachment of patch from the nail was noted down that showed nail adhesivity.

In-Vitro Drug Permeation Study

The *in-vitro* drug permeation study was carried out by using modified MS diffusion apparatus having two compartments - upper donor and lower receptor compartments. The assembly was made as follows:

Two pieces of thermocol was taken. The holes were made in thermocol pieces and plastic vials were fitted in the holes. The plastic vials in one piece were filled with buffer and acted as receptor compartment. Another thermocol piece with plastic vials was taken and nail clippings with the bioadhesive layer were attached to them on one side, this acted as donor compartment. The thermocol piece with plastic vials (containing nail clippings) was kept inverted on the receptor compartment. The nails were slightly dipped in buffer of receptor compartment. The whole assembly was adjusted properly. The formulated bioadhesive films were adhered onto a nail clipping of thickness 0.5 mm and it was fixed to a donor compartment at one end. This assembly was placed in the

receptor compartment containing 10 ml of pH 7.4 buffer solution. Samples were withdrawn completely at regular intervals for 6 days and replaced completely by fresh buffer each time**.** The samples were analyzed by UV spectroscopy at 279 nm to estimate the amount of the drug

Stability Studies

The formulated films were subjected to accelerated stability studies according to the ICH guidelines for six months. [13]

RESULTS AND DISCUSSION

Transungual drug delivery is one of the new emerging and promising route for drug delivery. This route by passes the first pass metabolism. It is a non-invasive technique thus improves patient compliance. The drug permeation can be increased by using biopenetrant and prolonged effect of the drug can be achieved. Terbinafine loaded bioadhesive films were prepared by solvent casting method using *Beta vulgaris* biopenetrant and papain as standard penetrant. The films prepared using *Beta vulgaris* biopenetrant were light pink opaque in color and the films prepared using papain as penetrant were white translucent in color. The films prepared were smooth in texture and flexible.

The bioadhesive films were evaluated for different parameters such as thickness**,** folding endurance, nail adhesivity, % drug content were found to comply with the ideal properties of the films. The drug interaction studies showed no interaction between the drug and bio-polymers.

Thickness of the bioadhesive layers LB1 to LB4 containing *Beta vulgaris* bio-penetrant ranged from 0.02 ± 0.001 to 0.03 ± 0.001 mm, LP1 to LP4 containing papain as penetrant ranged from 0.01± 0.001 to 0.03 ± 0.001 mm. The weight of the bioadhesive layers LB1 to LB4 containing Beta vulgaris bio-penetrant ranged from 21.23 ± 0.02 to 31.4 ± 0.02 mg, LP1 to LP4 containing papain as penetrant ranged from 17.41 ± 0.02 to 26.12 ± 0.02 mg. Little variation in thickness and weight of the bioadhesive layers ensuring uniformity was found to increase on increasing the penetrant weights from 1mg to 30 mg in formulations.

The micro environmental pH of the bioadhesive layers ranged from 5.08 to 5.62 which are nearer to the pH of the nail plate. It confirms that the formulations will not cause any irritation effect. Folding endurance of the bioadhesive layers ranged from 61 to 79 (times) which is indicative of reasonable flexibility of the bioadhesive layers so the formulation can be easily applied to the nail. The nail adhesivity of the bioadhesive layers ranged from 10.15 to 13.10 hrs which indicates that the formulation remain adhered at the site of action for the prolonged duration of time so the frequency of drug administration can be reduced.

Drug content uniformity of bioadhesive layers LB1 to LB4 containing *Beta vulgaris* biopenetrant ranged from 74.28 to 84.72 %. Drug content uniformity of bioadhesive layers LP1 to LP4 containing papain penetrant ranged from 67.27 to 74.66%. The percentage drug permeation data of formulations containing *Beta vulgaris* bio-penetrant was found to be in the order LB4> LB3> LB2> LB1. LB4 (1:4) was found to the best formulation. It shows that the drug release of the formulation increases on increasing the concentration of the biopenetrant. Depending on drug permeated the formulations containing papain were found to be in the order LP4> LP3> LP1> LP2. LP4 (1:4) was found to the best formulation.

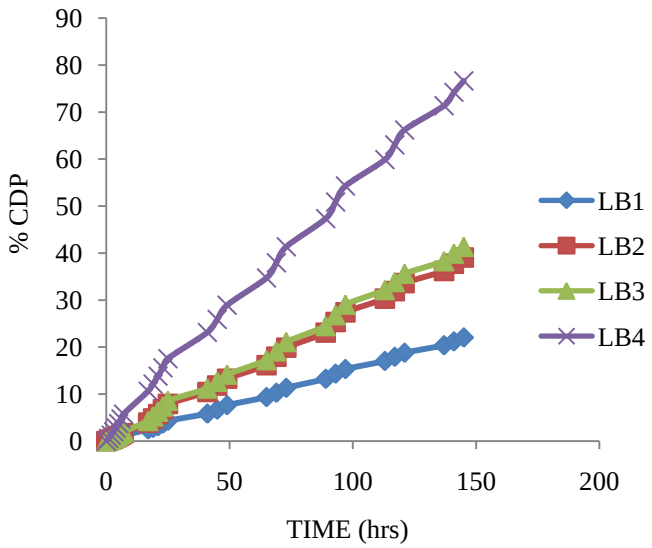

Fig 1. *In-vitro* drug permeation of formulations (*Beta vulgaris*)

The biopenetrant isolated from *Beta vulgaris* showed better permeation as compared to the standard penetrant, papain. It showed significant improvement in the penetration of the drug through nail. On comparing all the bioadhesive layers prepared, formulation LB4 (1:4) showed good nail adhesivity, retardibility, high folding endurance and R^2 value 0.990 with T_{50} 218.13 hrs and T_{80} 349.01 hrs showing zero order as the

best fit model. It showed better permeation characteristics among all for longer period of duration. Based on the above results, LB4 was found to be the best formulation. Stability studies indicated that all the formulations were stable as there was no change observed after six months. Above discussion conclude that the biopenetrant isolated from *Beta vulgaris* can be used to enhance the penetrability of the drug through the nail.

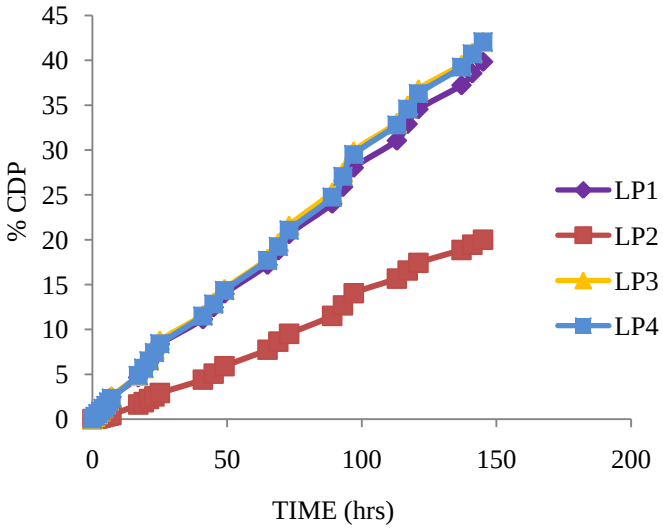

Fig 2. *In-vitro* drug permeation of formulations (Papain)

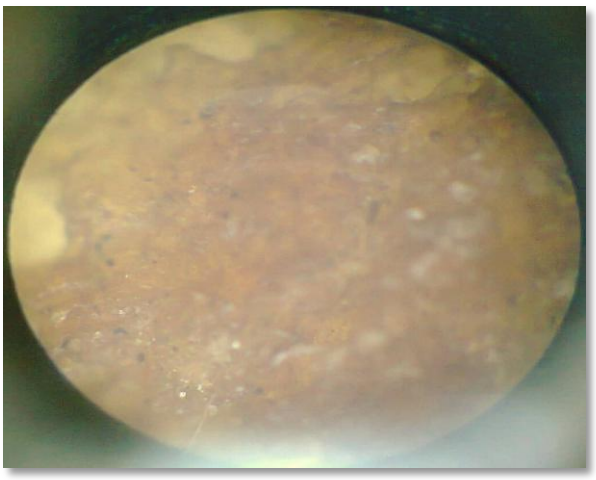

Fig 3. Drug permeation of formulation LB4 through nail

CONCLUSIONS

Ungual drug delivery system is considered as a beneficial strategy for the treatment of local and systemic diseases. Ungual dosage forms offer various future potential. A novelistic attempt has been made in the current research work by formulating bioadhesive layers loaded with terbinafine. The biopolymers and biopenetrant used were found to be biodegradable, non-toxic and can be effectively isolated in large quantity.

Table III: Evaluation of Terbinafine Bioadhesive Films

Formulation	Weight (mg)	Thickness (mm)	Surface pH	Folding endurance (times)	% Drug content (mg)	Nail Adhesivity (hr)
LB1	21.23 ± 0.02	0.02± 0.001	5.08	66	78.62	11.52
LB2	24.82 ± 0.02	0.02± 0.001	5.17	64	74.28	12.37
LB3	30.65 ± 0.02	0.03± 0.001	5.26	78	81.90	12.42
LB4	31.4 ± 0.02	0.03± 0.001	5.34	79	84.72	13.10
LP1	17.41 ± 0.02	0.01± 0.001	5.23	61	68.21	13.06
LP2	18.86 ± 0.02	0.01± 0.001	5.19	69	67.27	10.15
LP3	25.06 ± 0.02	0.02± 0.001	5.49	62	71.63	12.35
LP4	26.12 ± 0.02	0.03± 0.001	5.62	64	74.66	12.40

Terbinafine loaded bioadhesive layers were formulated which were further evaluated for various physico-chemical parameters.

The biopenetrant isolated from *Beta vulgaris* showed promising penetration of the drug through ungual route. It can serve as a biopenetrant as it is safe and the penetration mechanism may be due to the inbuilt chemical nature, low molecular weight and its compatibility with the nail structure that makes it possible to penetrate through the minor pores within the nail. This biochemical penetrant can be used for other API's by suitable formulations, without any etching of the nail structure. Moreover, since the bio-penetrant did not show any sign of acute toxicity, therefore the chronic toxicity study is not required. Since, the biopenetrant obtained from *Beta vulgaris* was economic, safe and showed good staining and penetration properties. Clinical trial studies are required before launching these terbinafine loaded bioadhesive layers in the market.

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