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EXPLORATION AND ELUCIDATION OF THE PHYSICAL PROPERTIES OF HPMC BASED HERBAL TRANSDERMAL PATCHES, DEVELOPED FOR THE TREATMENT OF FUNGAL SKIN INFECTIONS

B. N. Ramakrishna [*1], D. M. Surendra [2] and Santhosh C. Siddhashrama [3]

Department of Physics [1], Government College for women, Mandya - 571401, Karnataka, India.
Department of Chemistry [2], Bharathi College PG and Research Centre, Mandya - 571422, Karnataka, India.
Department of Studies in Chemistry [3], University of Mysore-Manasa Gangotri, Mysuru - 570005, Karnataka, India.

Keywords:

Dermal patch, HPMC, *Rubia cordifolia*, Fungal infection, *Candida albicans*

Correspondence to Author:
B. N. Ramakrishna

Assistant Professor,
Department of Physics,
Government College for women,
Mandya - 571401, Karnataka, India.

E-mail: ramakrishnabnphy@gmail.com

ABSTRACT: Fungal infections represent a significant global challenge within the realm of dermatology. The prevalence of fungal infections is notably high, affecting approximately 40 million individuals in developing and underdeveloped countries. Considering this, our study proposes a novel approach: transdermal drug delivery utilizing herbal formulations to enhance efficacy &minimize side effects in the treatment of fungal infections. Seven Ayurveda plants extracts were selected & formulated in a 1:1 ratio. These herbal formulations were incorporated into dermal patches composed of HPMC& PEG. Subsequently, the herbal transdermal patch was subjected to characterization like UV spectrometry, folding endurance, thickness measurement, & surface pH determination. The antifungal property of these patches was evaluated against *Candida albicans* by determining MIC and Agar Well method. The prepared patches exhibited distinctive UV-visible spectra within the 200-800 nm range for the HPMC-based, displayed a thickness of 0.063 ± 0.01, a weight of 206 ± 5.1 mg, and a surface pH of 5.7. The transdermal drug delivery system demonstrated potent fungal inhibition at a concentration of 2.5 mg/ml, as evidenced by MIC, while the Agar Well method exhibited significant inhibition of *Candida* growth. Transdermal drug patches hold promising rapid &potent treatment for skin fungal infections.

INTRODUCTION: Transdermal drug delivery system (TDDS) has been an increased interest in the drug administration *via* the skin for both local therapeutic effects on diseased skin (topical delivery) as well as for systemic delivery of drugs [1].

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The skin as a site of drug delivery has a number of significant advantages over many other routes of drug administration, including the ability to avoid problems of gastric irritation, pH and emptying rate effects, avoid hepatic first-pass metabolism thereby increasing the bioavailability of drug, reduce the risk of systemic side effects by minimizing plasma concentrations [2] compared to oral therapy, provide a sustained release of drug at the site of application; rapid termination of therapy by removal of the device or formulation, the reduction of fluctuations in plasma levels of drugs, and avoid pain associated with injections.

Transdermal delivery can also eliminate pulsed entry into the systemic circulation, which might often cause undesirable side effects [3]. Fungal infection is one of the major burdens of skin disease worldwide. The reported prevalence of fungal infection is about 40 million people in developing & underdeveloped countries. Fungi usually attack the skin surface during the initial phase and later invade into the deeper layer by desquamation. Candida species is one of the fungi which has the most superficial cutaneous infection [4]. Fungal infection expressed in deeper layers of skin called cutaneous mycoses". Cutaneous fungal infections are commonly known as "Dermatophytes. Fungi is commonly involved in different dermatomycoses including Tinea corporis, Tinea pedis and Tinea cruris [5].

Once, fungal infection further penetrates deeper skin tissue is known as "Subcutaneous mycosis. Skin fungal infections are a major cause of visits by patients to dermatology clinics. Although a large number of antifungal agents are available for treatment of skin fungal infections, their toxic profile and physicochemical characteristics reduce therapeutic outcome. When these antifungal agents are delivered topically using conventional formulations like creams and gels, they may cause various side effects like redness, burning, and swelling at the site of application. Therefore, various vesicular formulations (phospholipid based or non-phospholipid based) have been explored by pharmaceutical scientists to treat skin fungal infections topically [6, 7]. But till now there is no proper accurate diagnosis and treatment for skin fungal infection. The disadvantages of the topical creams were longer durations, steroidal treatments, and low efficacy. Also, currently available anti-fungal creams were having side effects in one or the other way. So, in the current study, herbal formulation was developed, depending on the Ayurveda backroad and incorporated in to the epidermal patch to deliver the drug directly in to the infection site with high efficacy.

Holostemmaada-kodien (jeevanti) is an ayurvedic herb belongs to the family Apocynaceae, root extract of the plant is using in India Ayurveda and Siddha system to treat skin diseases [8], also it reported to have the antimicrobial and anti-inflammatory property [9]. *Rubia cordifolia* (Manjista) root is one of the important plants in the Ayurveda which is reported to have the anti-inflammatory, antimicrobial and antioxidant activity [10, 11]. In Ayurveda, Root extract of Manjista was used treat acne, scars, and skin infections. Haridra, Turmeric is one of the known, ancient herbal treatments for anykind of skin infections due to its anti-inflammatory effect [12, 13]. *Wrightia tinctoria* also called swethakuthajais the most prescribed Siddha herbal medication for skin diseases, in specific psoriasis. The "777 oil" made from the fresh leaves of the plant exhibits various analgesic, anti-inflammatory, and antipyretic activities and it is a highly cited medication for the treatment of psoriasis [12]. *Hydnocarpus wightianus* (Chaulmoogra oil) use to sooth the skin inflammation in psoriasis condition [13, 14]. Coconut endosperm, also called malai, is one of the famous ingredients currently using in skin care industry as a moisturizer and antioxidant [17, 18]. Sandalwood powder was used in the formulation to sooth the skin infection. In the present study, HPMC was used as polymer to develop the epidermal patch by inclusion of herbal formulation in it. The polymer patch was evaluated for anti-fungal activity against *Candida albicans*.

MATERIALS AND METHODS:

Materials: *Holostemmaada-kodien* (jeevanti) root, *Rubia cordifolia* (Manjista) root, *Curcuma longa* (Haridra), *Wrightia tinctoria* (streekutaja), *Cocoa nucifera L*,(coconut endosperm), *Hydnocarpus wightianus* (Chaulmoogra oil), sandal wood powder, fish card liver oil, HPMC, PVA *etc.*

Collection of Plants: *Holostemmaada-kodien* (jeevanti) root, *Rubia cordifolia* (Manjista) root, *Curcuma longa* (Haridra), *Wrightia tinctoria* (streekutaja), and *Hydnocarpus wightianus* (Chaulmoogra oil) were purchased from the NKCA Ayurveda pharmacy, Mysuru, Karnataka, India. Fish card liver oil capsules, Coconut endosperm and sandalwood powder were purchased from the local venders.

Extraction and Formulation of Plant Extracts: *Holostemmaada-kodien* (jeevanti) root powder, *Rubia cordifolia* (Manjista) root powder, Haridra and *Wrightia tinctoria* (streekutaja) were subjected for the hot water extraction separately. 10g of each powder was taken in 150 ml beaker, to that 100 ml

distilled water was added and kept on magnetic stirrer at 60^{o}C for 1hr, after, extract was filtered through Whatman paper and condensed using rota evaporator and stored in airtight container until use. Fish cord liver oil capsules were directly used for the formulation. Coconut endosperm was collected and homogenized for 10min using homogenizer and stored at 4^{o}C until use.

Preparation of Herbal Formulation:

TABLE 1: NAME OF THE AYURVEDA HERBS USED FOR THE FORMULATION

Plant extract	Ratio w/w
Holostemmaada-kodien (jeevanti) root	15mg/100mg
Rubia cordifolia (Manjista) root	25mg/100mg
Haridra	10mg/100mg
Wrightia tinctoria (streekutaja)	10mg/100mg
Chaulmoogra oil	1ml
Fish card liver oil capsules,	500ul
Coconut endosperm	2ml
sandalwood powder	15mg/100mg

Formulation was prepared for 100mgw/e. All the plant extracts were taken as described in above **Table 1**. All the plant extracts and oils were mixed by adding 1ml of distilled water and 500ul of tween 80 as a surfactant. Keep the reaction mixer in magnetic stirrer until both water and oil face get mixed and form emulsifier. At the end of the reaction, a think semisolid emulsion. The emulsion was air dried and stored in an airtight container.

Fabrication of Transdermal Patch [19]: Transdermal films containing herbal formulation were cast on glass slide by solvent evaporation method using HPMC (100 M) in presence of a plasticizer. PEG 400 (5%) was used as a plasticizer in all cases. **Table 2** shows the formulae and composition for the different types of formulated patches. 25 and 50 mg of Herbal formulation was dissolved in water-methanol solvent mixture (6:4). The drug matrix was prepared by dissolving varying concentrations (1% and 1.5% w/v) of HPMC in the same solvent system. The solution was kept undisturbed for 24 h. Then, with the help of syringe, the solution was poured into a glass plate of 5.0-cm diameter placed on the glass surface. The solvent was allowed to evaporate for overnight in a thermostatically controlled oven at 60 °C. The patches were stored in an airtight container under ambient conditions for 7 days prior to use.

TABLE 2: FABRICATION OF TRANSDERMAL PATCHES AND THEIR CODES

% Of HPMC	Drug conc, in mg	% of PEG400	Code
1.5	25	5	A
1.5	50	5	A1
1	25	5	B
1	50	5	B1

Evaluation and Characterization of Transdermal Patch:

Physical Appearance [20]: All the prepared patches were visually inspected for color, clarity, flexibility, and smoothness.

Thickness of the Patch: The thickness of the drug loaded patches was measured by using a screw gage micrometer at three different points on the patches. Average values and standard deviation values of the three readings were calculated for each drug loaded patch.

Uniformity of Weight: The patches were subjected to weight variation test by weighing all the patches on a digital weighing machine. The determinations were carried out in triplicate for each formulation. Average weight and standard deviation values were then calculated.

Folding Endurance: This test was carried out to check the efficiency of the plasticizer and the strength of the patch prepared using different polymers. Folding endurance is defined as the number of folds required to break any polymeric patch. The folding endurance was measured manually by repeatedly folding a small strip of the film (2 × 2 cm) at the same place until it broke. The number of times the patch could be folded at the same place without breaking/cracking gave the value of folding endurance. Three patches of each type were taken for the test.

Surface pH: Patches were kept in contact with 0.5 ml of double distilled water for 1 h in glass tubes and were allowed to swell. A combined glass electrode was brought near the surface of patch and pH readings were taken after allowing an equilibration period of 1 min.

UV-visible Spectroscopic Analysis of Polymer: Fabricated polymers were soluble in PBS and scanned in UV visible spectrometer at the range of 200 to 800nm. PBS was used as a blank.

Percentage Moisture Absorption/Water Vapor Absorption: The percent moisture absorption test was carried out to check the physical stability and integrity of the films in high humid conditions. The prepared films (3.14 cm2) were individually weighed accurately and exposed to 85 ± 5% relative humidity in a desiccator containing 100 ml of saturated solution of potassium chloride at room temperature.

During this period, the films were weighed at regular time intervals of 24, 48 and 72 h. The percent moisture absorption was determined from the following formula:

% moisture uptake ¼ ð Þ Final weight – Initial weight =Initial weight 100

Percentage Moisture Content: This test was also carried out to check the integrity of films under dry conditions. The individual transdermal films (of specified area) were kept in a desiccator containing fused anhydrous calcium chloride at room temperature. During this period, the films were weighed at regular time intervals of 24, 48, and 72 h. The percentage moisture content was determined by using the following formula:

% Moisture content ¼ ð Þ Initial weight – Final weight =Initial weight 100

Water Vapor Transmission: Water vapor transmission rate (WVTR) is defined as the quantity of moisture transmitted through unit area of film in unit time. Glass vials of equal volume and diameter were used as transmission cells. The cells were washed properly and dried in oven.

Then, about 1 g of anhydrous fused calcium chloride was placed in each vial, and the patch was fixed over the brim of the vial with the help of an adhesive tape. These vials were then weighed and placed in desiccators containing saturated solution of potassium chloride to maintain 84% relative humidity. These cells were removed from the desiccators and weighed after 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th day. The water vapor transmission rate was determined as follows:

W: V: T: ¼ W L=S

Where W is the weight of water vapors transmitted, L is the thickness of patch and S is the surface area exposed in square centimeters.

Minimum Inhibitory Concentration Analysis of the Fabricated Transdermal Patch by Resazurin Method:

Minimum Inhibitory Concentration Assay by Resazurin Method: The MIC was determined based on the broth dilution method using 96 well plates according to NCCLS M27-P (1990). For the determination of MIC, inoculum suspension was prepared from 24 h broth cultures. Bacterial culture was adjusted to 0.5 McFarland turbidity standards (1.5×10^8 CFU/mL) and 10 µL of diluted suspensions of bacterial culture was added to 50 µL of various concentrations of given extract in to the well.

The 100mg/ml stock of the polymer were serially diluted concentrations of extract ranged from 50mg/ml, 25, 125, 62.5, 31.2, 15.6, 7.8, 3.9 mg/ml. 50 µL Streptomycin concentration 50, 25, 125, 6.25, 3.125 µg/ml were used as a Standard. PBS was used as vehicle control. Sterile LB broth served as a media blank and only bacterial culture without treatment (10 µl inoculum, 40 µl sterile Double strength LB media and 50 µl of water) was considered as control. Plates were incubated at 37 °C for 24 h. After incubation OD was taken at 600nm to analysed the bacterial inhibition.

The MIC was defined as the lowest concentration that restricted the bacterial growth to an absorbance lower than 0.05 at 600 nm (invisible growth). After taking OD reading 30 µl of 0.015 % resazurin was added to all wells for visual observation of MIC and incubate at 37 °C for 3 hrs. The experiment was conducted in triplicate with 3 independent experiments. The colour change was then assessed visually. Any color changes from purple to pink were recorded as positive (pink color indicates growth and blue means inhibition of growth). The lowest concentration at which color change occurred was taken as the MIC value. The average of three values was calculated and that was the MIC for the test material and bacterial strain [21].

Antimicrobial Activity by well Diffusion Method [22]: Antimicrobial activity of transdermal patches

was investigated using well diffusion method. Test plates (diameter 10 cm) were prepared with 20 mL of LB agar (LBA). After media get solidified, 100 µl of 48 h fungal (*C. albicans*) culture (1.5 × 10^8 CFU/mL) was added and uniformly spread over plates using L shaped loop. Then make well (about 6mm diameter) and add 50 µL different concentration of the polymer 10, 50 and 100mg/ml respectively. The wells loaded with sterile media are considered Blank. Clotrimazole 1mg/ml was used as a standard. After loading, plates were kept in sterile condition until complete absorption of the test compounds. Plates were incubated at 37°C in an appropriate gaseous condition for 24 hrs. Zones of inhibition of microbial growth around the well were measured and recorded after the incubation time. The inhibitory zone was considered the shortest distance (mm) from the outside margin of the samples to the initial point of the microbial growth. All measurements were performed twice by the same blind operator. Three replicates were done.

RESULT & DISCUSSION:

UV-visible Spectrometer:

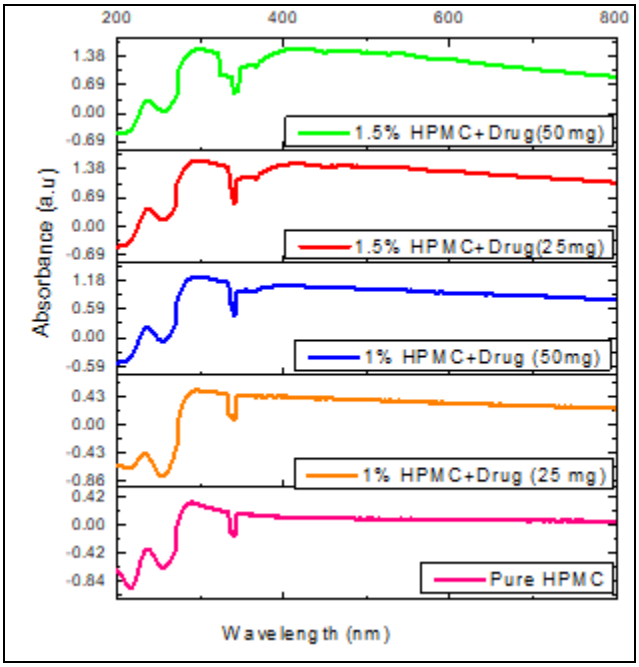

FIG. 1: UV-VISIBLE SPECTROMETER OF THE TRANSDERMAL PATCHES WITH DIFFERENT DRUG RATIO

Physical Characterization data for HPMC Based Transdermal Patch: The prepared transdermal patches were evaluated to study the effect of different concentration of herbal formulation with HPMC polymer (with varied concentration), concentration of HPMC, and the presence of PEG 400 as plasticizer on the release kinetics of drug and on the physical characteristics of the film. These were evaluated for their physical appearance, weight uniformity, thickness uniformity, surface pH, flatness test, water vapor absorption, drug content uniformity.

TABLE 3: PHYSICAL CHARACTERIZATION EVALUATION OF TRANSDERMAL PATCHES

Formulation code	Weight (mg) ± SD	Thickness (mm) ± SD	Folding endurance ± SD	Surface pH
A	241±2.18	0.061±0.01	261±3.71	5.7
A1	296±7.2	0.086±0.05	245±1.87	5.4
B	206±5.1	0.063±0.01	300±3.33	5.7
B1	203±3.7	0.063±0.001	304±2.76	5.7

All the films were evaluated for their physical parameters (weight, thickness, folding endurance, diameter, area, flatness, and surface pH), and they were found to be flexible, uniform, smooth, and transparent **Table 3**. All the formulations were uniform in their weight, thickness, folding

endurance, and with low SD values. The weight of the prepared transdermal patches for different type of formulations ranged between 241 ± 2.18 mg and 203 ± 3.7 mg, but within a formulation, all the patches showed low standard deviation values. Low standard deviation values in the film thickness measurements ensured uniformity of the patches which further indicated the reproducibility of the procedure followed for the preparation of the patches. Folding endurance values varied between 261 ± 3.71 and 304 ± 2.76. The flatness study showed that all the formulations had the same strip length before and after their cuts, indicating 100% flatness. Thus, no amount of constriction was observed which indicated that all patches had smooth flat surface which would be maintained when the patches are applied to the skin.

Moisture Content: Water vapor transmission studies Water vapor transmission studies were carried out to determine the permeability characteristics of the transdermal patches.

The water vapor transmission rate for the prepared patches ranged were 0.41,0.35, 0.51 and 0.71% for 24h **Table 4** indicating that all the formulations were permeable to water vapor. The low water vapor transmission (WVT) rates again emphasize the stability aspects on long-term storage. There is a statistically significant difference was seen with the change in type and concentration of the polymer, however, there is no significant changes in the change in the herbal drug concentration in the polymer.

TABLE 4: MOISTURE CONTENT EVALUATION OF TRANSDERMAL PATCHES AT DIFFERENT DAYS

	Weight of the patch					
Formulation code	**Average initial weight in mg**	**Day 1**	**Day 2**	**Day 3**	**Total Moisture gain**	**% of moisture gain in mg**
A	182.33±1.72	182.77±2.33	183.01±1.55	183.08±1.12	0.75±0.38	0.41±0.38
A1	196±2.17	196.88±1.24	196.91±1.35	196.95±2.23	0.68±0.34	0.35±0.34
B	220.22±3.1	221.03±2.2	221.11±1.19	221.34±1.35	1.12±0.56	0.51±0.56
B1	200.12±1.72	200.81±1.33	201.11±0.99	201.55±1.33	1.43±0.72	0.71±0.72

Minimum Inhibitory Concentration assay of Herbal Transdermal Patch against *Candida albicans*: Minimum inhibitory concentration of the developed transdermal patch was evaluated against *C. albicans* by resazurin dye quenching method. *C. albicans* are the main fungi which causes usual skin infection which may persist for longer time, if left untreated further may leads to the secondary complications. Developed transdermal HPMC based polymer patch was screened of % of inhibition of candida growth **Fig. 3**. A1 (1.5% HPMC+50mg herbal formulation) showed MIC at the concentration of 2.5mg/ml, whereas, A and B showed at 10mg/ml. B1 (1% HPMC and 50mg drug) showed MIC 0.625mg/ml concentration, % of inhibition was 18.69±0.02. Similarly, A1 showed 87.32±0.01 at 10mg/ml concentration. A and B at 10mg/ml showed 62.84±0.13 and 57.16±0.11 respectively.

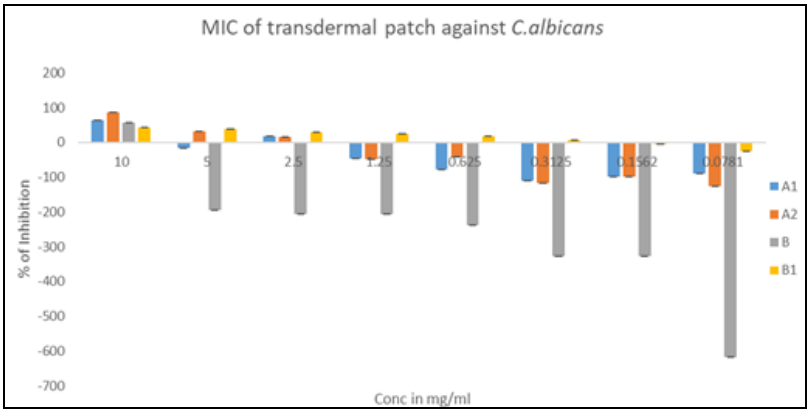

FIG. 2: SHOWING % OF INHIBITION OF HERBAL TRANSDERMAL PATCH AGAINST *C. ALBICANS*

Anti-fungal Activity of the Herbal Transdermal Patch against *C. albicans* by well Diffusion Method: Anti-fungal activity of the developed herbal transdermal patch was evaluated against *C.*

*albicans*by well diffusion method in the concentration range of 50, 100 and 200mg/ml. **Fig. 3.** Transdermal patch with 50mg drug infusion (A1 and B1) showed promising result at the highest concentration and formed the cleared zone by inhibiting the fungal growth. There was no zone found in the HPMC alone treatment, therefore, herbal formulation synergistically playing the role of antifungal effect against *C. albicans*.

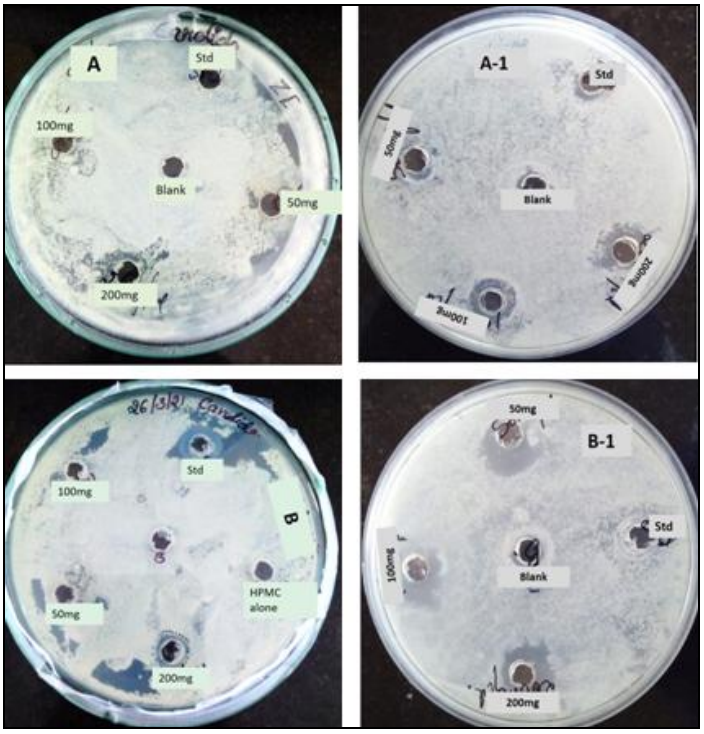

FIG. 3: SHOWING ANTI-FUNGAL ACTIVITY OF THE HERBAL TRANSDERMAL PATCH AGAINST *C. ALBICANS* BY WELL DIFFUSION METHOD

TABLE 5: ZONE OF INHIBITION IN CM OF TRANSDERMAL PATCHES AGAINST *C. ALBICANS*

Zone of inhibition in cm					
Organism: *Candida Albicans*					
Test Compounds	**Std 1**	**Blank**	**50mg/ml**	**100mg/ml**	**200mg/ml**
A	---	---	----	----	0.8
A1	0.2	--	----	1.0	1.1
B	0.5	---	-----	----	0.7
B1	----	---	1.1	1.3	1.7

CONCLUSION: Transdermal films of Ayurveda herbal formulation have been successfully formulated by solvent evaporation technique. Evaluation of the prepared films in terms of physical appearance, weight uniformity, thickness uniformity, surface pH, suggest that the method employed for formulation of the transdermal patches was reproducible and ensured excellent quality and uniformity in patch characteristics with minimum variability. Further, anti-fungal activity of the transdermal patch showed promising results in inhibiting the candida growth and showed dose dependent % of inhibition against *C. albicans*. These results show that transdermal delivery of developed herbal formulation can have good potential applications in therapeutic area offering advantages in terms of reduced dosing frequency, improved patient compliance, non-invasive characteristics, improved bioavailability, and easy termination of therapy. Further drug kinetics and skin irritation studies are needed to explain the half-life and toxicity.

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