



Preparation and optimization of medicated cold cream using *Caralluma adscendens* var. *attenuata* for the treatment of *Candida* skin infection

MADASAMY SUNDAR^{1,2}, SUNDAN SURESH², KRISHNASAMY LINGAKUMAR^{1*}

¹Centre for Research and Postgraduate Studies in Botany, Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India

²Department of Botany, Madurai Kamaraj University, Madurai, Tamil Nadu, India

Abstract

Fungal skin infection is a major skin health issue worldwide. For the treatment of fungal infections, systematic antifungal therapies are frequently prescribed. The aim of this study is to prepare an antifungal cold cream from *Caralluma adscendens* var. *attenuata* to treat deep dermal fungal infection in the skin layer. To achieve this, different concentrations of plant extract-based cold cream were prepared, and their *in vitro* characteristic features such as color, texture, pH, viscosity, spreadability, stability, permeation, were analyzed together with *ex vivo* evaluation to identify their applicability in the treatment of acute rat skin irritation. After 72 h of induction of *Candida albicans* infection in rats (7 days, two times/day), *C. adscendens* var. *attenuata* cold cream was applied topically. In rats with *C. albicans* induction without any treatment, adverse skin damages were visible in the form of red rashes, whereas in those with the formulated cold cream application, significantly less skin damage and inflammation were observed on a dose-dependent basis. Moreover, the reduced microbial colonization and histopathology of the rat skin without any treatment indicated the successful invasion of *C. albicans* and showed the morphological changes caused by candidal infection. However, treatment with the *C. adscendens* var. *attenuata* cream significantly inhibited candida colonization and reversed the morphological changes. In addition, the formulated *C. adscendens* var. *attenuata* cold cream showed good spreadability, permeation, and viscosity. Hence, it can act as a potent antifungal topical agent for the treatment of *C. albicans* skin infection without any irritation, thus safeguarding the skin tissue.

Key words: *C. adscendens* var. *attenuata*, cold cream formulation, *C. albicans*, antifungal treatment, histopathology

Introduction

Fungal infection is the most predominant superficial infectious disease that affects the skin surface, nails, hair, and subcutaneous tissues under the skin. Appearing on the surface of the skin as a commensal, *Candida albicans* is the major infectious agent that affects human skin (Garber, 2001; Guégan et al., 2016). Candidal infection results in skin rashes, red itching, and inflammation that can spread all over the body and is rapidly transmitted from infected persons to healthy ones. It mainly affects wet skin and damp and furrowed areas, such as subarms and intergluteal areas (Kuh-

bacher et al., 2017). Upon infection, *C. albicans* damages the skin surface initially, and later, it invades deep dermal layers through desquamation, causing subcutaneous mycosis. If the host immune system is weak, it can also invade deep tissues and cause a wide range of life-threatening neutropenia conditions (Ameen, 2010; Havlickova et al., 2008; Queiroz-Telles et al., 2003; Zhang et al., 2007).

Reports published in 2020 indicate that about 40 million individuals in underdeveloped and developing countries were affected by fungal infection (Coates et al., 2020). Researchers around the world are actively investi-

* Corresponding author: Centre for Research and Postgraduate Studies in Botany, Ayya Nadar Janaki Ammal College, Sivakasi – 626124, Tamil Nadu, India; e-mail: kris.lingakumar@gmail.com

gating the application of antifungal chemotherapies to treat surface and deep fungal infections (Ansari et al., 2019; Dave et al., 2017; Garg et al., 2020; Gupta and Cooper, 2008; Wingard and Leather, 2004). Resistance to antifungal drugs has increased dramatically in *C. albicans*, resulting in reduced effectiveness of treatment for *Candida* infections (Herederro-Bermejo et al., 2020; Zamani et al., 2020). Topical therapy is a conventional systematic treatment for fungal infections (Alqahtani et al., 2020; Zhang et al., 2007). When topical treatment is performed directly on the infected site, a significant reduction in disease symptoms is observed (Deyno et al., 2019; Kowshihan et al., 2020). However, the stratum corneum, the outer layer of the skin, is the critical barrier to drug permeation. Therefore, for an antifungal drug to be effective, it should have a drug delivery system that can pass the barrier of high mechanical strength to the subcutaneous tissue (Khalid et al., 2021; Ré et al., 2021).

However, topical application of antifungal drugs can trigger adverse reactions in the skin such as allergic reactions and itching (Dismukes, 2000; Naik et al., 2000; Silva et al., 2014). Although more synthetic antifungal formulations are commercially available nowadays, such as creams, gels, and lotions, they are not suitable for pharmaceutical applications due to poor absorption and other adverse effects. New drug delivery mechanisms are being developed to overcome these challenges and to develop a topical cold cream formulation with reduced local side effects and increased therapeutic effectiveness (Rapalli et al., 2020; Sonia et al., 2017; Ugazio et al., 2020). Cold cream is an excellent face cleanser, and it protects the skin against environmental stress; sometimes, it is used as a topical drug carrier for pharmaceutical applications (Sonawane et al., 2021; Sonia et al., 2017).

Caralluma adscendens var. *attenuata* grows in dry deciduous forests and scrub jungles (Kumar et al., 2014) and is a good source of steroids and glycosides (Kiranmayee et al., 2016). *Caralluma* exhibits effective antioxidant, anti-inflammatory, antidiabetic, and antimicrobial activities and so can be used in pharmaceutical applications (El-Shiekh et al., 2021; Madasamy et al., 2020; Vajha et al., 2010). Therefore, a cold cream based on the extract of *C. adscendens* var. *attenuata* was formulated in this study to treat *C. albicans* infection and tested on the rat skin under laboratory conditions.

Methods

Extraction and preparation of Caralluma cold creams

Cladodes of *C. adscendens* var. *attenuata* were collected in June around Sivakasi (latitude: 9.463898°N 77.760829°E), Tamil Nadu, India. The collected plants were verified, and their identification was confirmed by comparing with an authentic specimen by a taxonomist at the Centre for Research and Postgraduate Studies in Botany, Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India. The collected plant cladodes were dried in a shadow for a week and then finely powdered. Then, 100 g of the powdered plant material was subjected to 12 h extraction in 600 ml of 95% methanol (AR grade) using the Soxhlet apparatus. The supernatants thus obtained were evaporated and dried in a vacuum under reduced pressure at 55–60°C. Then, the residues were collected and stored at 4°C for further use.

Cold creams (water/oil emulsion) were prepared according to the procedure of Carter (2008) with slight modifications. The basic composition of the cold creams is presented in Table 1. The oil-phase components such as beeswax, paraffin wax, cetyl alcohol, and mineral oil were taken in a beaker, mixed, and heated in a water bath at 70°C for 10 min. Simultaneously, borax, methanolic extract of *C. adscendens* var. *attenuata*, and sterile distilled water were mixed in another beaker and heated up to 70°C for 10 min, which formed the aqueous phase. After heating for 10 min, the aqueous phase was added slowly to the oil phase with continuous stirring until a uniform smooth paste was formed. The prepared cream was packed in a sterile plastic container and stored in a refrigerator at 4–8°C for further use.

Evaluation of physical appearance and visual examination of the cold creams

The following characteristics were evaluated in the formulated creams: color, texture, homogeneity, stability, pH, spreadability, viscosity, and *in vitro* permeation.

Determination of pH

pH was measured in triplicate for each cold cream using a digital pH meter (Hanna-98107, India).

Homogeneity

The homogeneity parameter determined the consistency of the cream. It was tested manually by pressing

Table 1. Basic composition of cold cream formulation using methanolic extract of *Caralluma adscendens* var. *attenuata*

Basic composition	Concentration in grams			
	FCA-50	FCA-100	FCA-200	Plain cream
Bees wax	1.12	1.12	1.12	1.12
Mineral oil	6.86	6.86	6.86	6.86
Paraffin wax	0.98	0.98	0.98	0.98
Cetyl alcohol	0.14	0.14	0.14	0.14
Borax	0.05	0.05	0.05	0.05
Purified water	5.8	5.75	5.65	5.85
<i>Caralluma</i> extract	50 mg	100 mg	200 mg	Nil
Total volume	15 g	15 g	15 g	15 g

FCA – formulated *C. adscendens* var. *attenuata* cream

a small quantity of the formulated cream between the thumb and index finger. Immediate skin feel (such as stiffness, grittiness, and greasiness) was evaluated.

Spreadability test

Spreadability of the formulations was evaluated by measuring the spreading diameter of 300 mg of the sample after placing it for 1 min between two horizontal glass plates (glass plate size 7.5 × 2.5 cm). The weight of the top plate was 20 g. Each formulation was tested in triplicate (Haneefa et al., 2010). The results were recorded, and spreadability was calculated by using the following formula:

$$S = \frac{m \times l}{t} \quad (1)$$

where S is spreadability, m is the weight tied to the top slide, l is length (7.5 cm), and t is time (s).

Stability studies

Stability tests were performed in compliance with the International Council on Harmonisation criteria to determine drug and formulation stability (Bajaj et al., 2012). The cream sample was filled in a glass bottle and kept under accelerated conditions of $40 \pm 2^\circ\text{C}/75 \pm 5\%$ relative humidity (RH) in a programmable environmental test chamber (Remi CHM-16S) for 90 days. Samples were taken at the end of the experiment, and physical properties and viscosity were evaluated.

Viscosity measurement

Viscosity of the formulated creams under different shear rates was calculated using the Brookfield visco-

meter (Spindle type, S-24; LVDV-E model) in the range of 10–100 rpm (revolutions per minute) at 25°C . A sample of 20 g of cream was taken in a beaker, the spindle was dipped into the beaker for around 5 min, and then, the reading was taken.

In vitro skin permeation studies

In vitro skin permeation tests were performed using a dialysis membrane (AB-253a, Abron Exports, India) of pore size 10–100 kDa. To create a uniform surface to place the transdermal patch, the membrane was connected with one side and its other side was an open-ended cylinder. The side where the transdermal patch resided was considered the donor compartment. The membrane's whole surface is in contact with the receptor compartment containing 150 ml of phosphate buffer (pH 6.8) and maintained at a temperature of $37 \pm 2^\circ\text{C}$. A magnetic stirrer was used to stir the contents of the receiver compartment at a constant speed of 100 rpm. Two milliliters of the sample was collected from the receiver compartment every hour up to 8 h, and an equal quantity of fresh receptor medium was added every time to maintain the volume of the content in the receiver compartment constant. Then, the antioxidant activity of the contents in the receptor compartments was tested using DPPH. This experiment was performed in triplicate, and the results were presented as the mean \pm standard deviation (Mahdi et al., 2011).

$$\text{Percentage release} = \frac{\text{Activity of plant extract [\%]}}{\text{Activity of formulated cream [\%]}} \times 100 \quad (2)$$

Animals

Thirty albino Wister female rats (170 ± 10 g) were obtained from an animal house, and the experimental procedure used in this study was approved by the Institutional Ethics Committee of K. M. College of Pharmacy, Madurai, Tamil Nadu, India. The Committee approved the study protocol (clearance no.: 661/PO/Re/S/02/CPCSEA) based on the control and supervision of experimental animal guidelines in India. The animals were then divided into six groups ($n = 5/\text{group}$), housed in polypropylene cages under aseptic conditions, and fed with standard pellet and water *ad libitum*.

Skin irritation test

The skin irritation test was carried out on albino rats as follows: the animal fur was shaved from the backside in an area of 3 cm^2 . The first rat group served as control, in which 5% sodium lauryl sulphate in distilled water was applied; in the second group, standard plain cream was applied; and in the remaining three groups, FCA 50 mg, FCA 100 mg, and FCA 200 mg (concentrations of the prepared cold cream) were applied (Hiremath et al., 2008). A pinch of cream was rubbed on the rats' skin twice a day for 3 days. The experiments were performed in triplicate to evaluate the morphological changes on the skin surface.

Preparation of immunosuppressed animals

Immunosuppressed albino Wister rats were used for testing the effect of the prepared formulations in preventing and treating deep fungal skin infections in the case of extreme skin infections. The rats were housed in individual cages and allowed to receive food and water *ad libitum*. To suppress rat immunity, cyclophosphamide ($150 \text{ mg/kg} \times 3$ days) was intraperitoneally injected (Aldawsari et al., 2015; Gupta and Vyas, 2012).

Fungal strain preparation

Candida albicans MTCC7315 (obtained from MTCC – Microbial Type Culture Collection and Gene Bank, Chandigarh, India) was inoculated in potato dextrose broth (Hi-Media, Bangalore, India) and allowed to grow at 30°C for 48 h. The fungal cell pellets were collected via centrifugation after incubation, washed twice with PBS and 70% ethanol, and resuspended in PBS solution.

Infusion of candidal infections and cold cream treatment

To study the effects of the prepared topical creams on fungal infections, the animals were divided into six groups ($n = 5/\text{group}$): group 1, left untreated and considered control; groups 2–6 were subjected to skin candidiasis by means of *C. albicans*. Each rat from groups 2–6 was injected intraperitoneally with $100 \mu\text{l}$ of 10^7 CFU/ml *C. albicans* cell suspension in the middle of the shaved region, following the procedure of Qushawy et al. (2018). The injected site was rubbed until the tiny edema had vanished and were observed for 72 h. After 72 h, 300 mg of the appropriate cream formulation was applied on the infected rat skin twice a day and rubbed, following the protocol of Qushawy et al. (2018). Group 2 served as a control for *C. albicans* infection without treatment, group 3 was treated with plain cream, group 4 was treated with FCA-50 cream, group 5 was treated with FCA-100 cream, and group 6 was treated with FCA-200 cream. After 7 days of treatment, the skin condition was observed and photographed.

Microbial count

After completion of the treatment, the rats were anesthetized, and the infected skin samples were collected, washed, spread-plated onto potato dextrose agar medium (Hi-Media, Bangalore, India), and incubated for 48 h at $37 \pm 1^\circ\text{C}$. The viable values of colony-forming units (CFUs) were then recorded, and all the results were presented as mean \pm standard deviation (Aldawsari et al., 2015).

Histopathology analysis

At the end of the experiments, the rats were anesthetized, and the infected skin was collected, sliced, fixed with 10% formaldehyde, and then paraffin-blocked. Then, slides were made from paraffin blocks and dyed with hematoxylin-eosin, all according to the procedure of Abdellatif et al. (2020). The slides were visualized using an optical microscope ($40\times$; Olympus microscope, CH20iBIMP with micro view $\times 86$ Software), which showed signs of inflammation and epidermal and dermal changes.

Statistical analysis

One-way analysis of variance with post hoc comparison was used to investigate the significant difference be-

tween results. $P < 0.05$ was considered statistically significant. All statistical analyses were carried out using SPSS package, version 21.0 (IBM corporation, Armonk, New York).

Results and discussion

The efficacy of the *C. adscendens* var. *attenuata* extract cold cream was evaluated ex vivo in albino Wister female rats, after inducing skin infection with *C. albicans*. All the prepared formulations were evaluated for physical appearance, homogeneity, stability, pH, spreadability, viscosity, *in vitro* permeability, and skin irritation.

Physical appearance and stability

All the formulated creams were semisolid, with a pale green to dark green color depending on the concentration of the extract, and the plain cream was white (Table 2). All these creams showed consistent texture with good homogeneity and without lumps. Therefore, the formulated creams showed good topical applicability (Chandrasekar et al., 2018).

Stability studies

A herbal formulation's quality, safety, and efficacy for commercialization are dependent upon its stability. Thus, stability studies are essential for the development and improvement of formulations by establishing their validity and monitoring their physical and chemical properties (Burki et al., 2020). For stability analyses, all the prepared formulations (FCA-50, FCA-100, FCA-200, and plain cream) were kept at a temperature of $40 \pm 2^\circ\text{C}$ and RH of $75 \pm 5\%$ for 90 days, and the time required for phase separation and changes in color, pH, homogeneity, and spreadability were recorded. Before stability studies, all the formulated creams showed a smooth texture, and phase separation (oil/water) was not observed (Table 2). After 90 days of stability studies, all the formulated creams were still stable under $40^\circ\text{C} + 75\%$ RH, and no phase separation or color change was observed. These results demonstrated that the formulations were relatively stable under accelerated temperature and humidity conditions.

pH value

The pH of all the prepared formulations was tested. The plain cream showed a pH value of 6.1; for FCA-50,

FCA-100, and FCA-200, the pH values were 6.1, 6.22, and 6.3, respectively, which were acceptable for skin formulations (pH ranging from 4 to 6, according to Lukić et al., 2021). The pH of all the formulations was analyzed at 0, 30th, 60th, and 90th days. Interestingly, slight variations in pH were observed during 90 days of storage, but they were within the normal skin pH range (Table 2). Throughout the experiment, the pH of the formulated products was within the acceptable range for dermal preparation.

Spreadability

Spreadability analyses of the formulated creams indicated that all the prepared formulations were evenly and easily spread on the skin surface (Khan et al., 2020). As summarized in Table 2, FCA-200 exhibited the highest spreadability calculated by means of the time required by the top slide to slip over the bottom slide (12.62 ± 0.5 s; the lower, the better). In comparison, the plain cream showed a spreadability of 18.6 ± 0.5 s. Interestingly, FCA-50 and FCA-100 showed a spreadability of 13.32 ± 0.5 and 12.74 ± 0.5 s, respectively. Consistent with this study, Ijaz et al. (2021) reported that the spreadability of the formulations containing herbal cosmetic cream of *Aloe vera* gel and tomato powder was in the range of 9–13 s, which was in the acceptable range. As mentioned earlier, spreadability plays an essential role in administering a medicated formulation to the skin and also in the efficacy of the formulation (Dhyani et al., 2019).

Viscosity

Viscosity refers to the stickiness or thickness of a semisolid cream and is the most important quality control parameter of the formulated creams. All the tested creams showed gradual changes in viscosity with an increase in rpm. FCA-50 and FCA-100 showed a change in viscosity from 19 153 to 4895 and from 19 850 to 4139 cP, respectively. Based on these values, both FCA-50 and FCA-100 creams had a similar viscosity curve. FCA-200 showed a higher viscosity, ranging from 22 345 to 3857 cP, and the plain cream showed the lowest viscosity, ranging from 18 738 to 4365 cP (Fig. 1). Viscosity is considered crucial as it defines the shelf-life stability, product esthetics such as clarity and ease of flow of the product package, and the spreadability of the cream on the skin (Alquadeib et al., 2018; Khan et al., 2020).

Table 2. Evaluation of physical parameter studies of prepared *C. adscendens* var. *attenuata* cold cream

Test	Accelerated stability condition		Plain cream	FCA-50 mg cold cream	FCA-100 mg cold cream	FCA-200 mg cold cream
	$40 \pm 2^\circ\text{C}/75 \pm 5\% \text{RH}$	initial period after three month				
pH	$40 \pm 2^\circ\text{C}/75 \pm 5\% \text{RH}$	initial period after three month	6.1 ± 0.1 within range	6.1 ± 0.1 within range	6.22 ± 0.1 within range	6.3 ± 0.1 within range
Nature	$40 \pm 2^\circ\text{C}/75 \pm 5\% \text{RH}$	initial period after three month	white colour transparent NCC	pale green transparent NCC	pale green transparent NCC	dark green colour transparent NCC
Physical appearance	$40 \pm 2^\circ\text{C}/75 \pm 5\% \text{RH}$	initial period after three month	semi solid NCC	semi solid NCC	semi solid NCC	semi solid NCC
Odor	$40 \pm 2^\circ\text{C}/75 \pm 5\% \text{RH}$	initial period after three month	characteristic NCC	characteristic NCC	characteristic NCC	characteristic NCC
Texture	$40 \pm 2^\circ\text{C}/75 \pm 5\% \text{RH}$	initial period after three month	smooth NCC	smooth NCC	smooth NCC	smooth NCC
Spreadability [g/cm/sec]	$40 \pm 2^\circ\text{C}/75 \pm 5\% \text{RH}$	initial period after three month	18.6 ± 0.5 within range	13.32 ± 0.5 within range	12.74 ± 0.5 within range	12.62 ± 0.5 within range

NCC – non characteristic changes

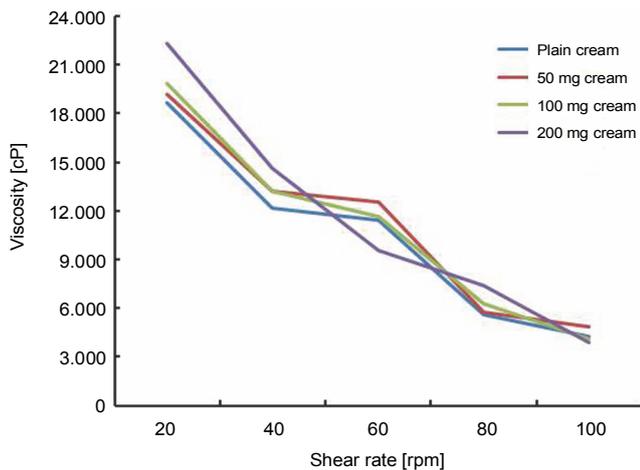


Fig. 1. Graph representing the viscosity-versus-shear rate plot of different concentrations of FCA cold cream formulations, 50 mg, 100 mg, and 200 mg

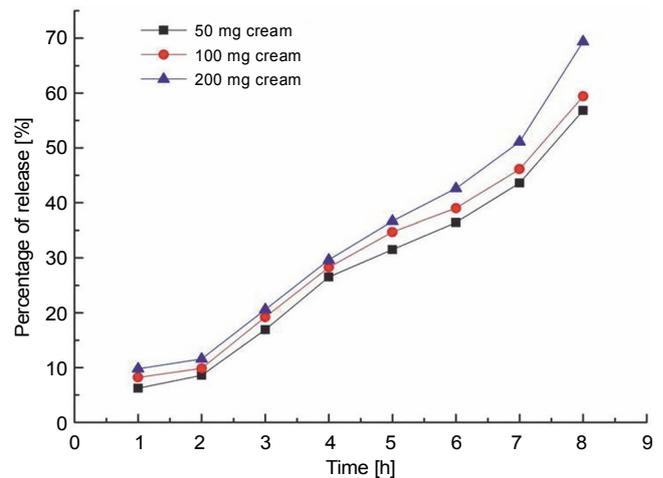


Fig. 2. *In vitro* permeation of 50 mg, 100 mg, and 200 mg FCA cold cream profiles through Franz diffusion dialysis membrane at 8 h of permeation assay ($n = 3$, mean \pm SD)

In vitro permeation test

The most essential step in designing a successful topical therapy is maintaining sufficient permeability of drugs through the skin (Pithayanukul et al., 2002; Sequeira, 1990). Permeation analysis was used to evaluate the time taken for the sustained release of the drug across the dialysis membrane. The data obtained for permeation analysis of the prepared samples are shown in Figure 2. The time necessary for the release of the formulated creams across the membrane was calculated as a percentage of the same value obtained for the control sample. When compared with the control group, FCA-50 showed a gradual increase from 6.2 to 56.8%, FCA-100 from 8.2 to 59.3%, and FCA-200 from 9.7 to 69.3% after the first and eighth hours of suspension in the apparatus. According to a prior study, a sertaconazole nitrate-loaded nanovesicular gel with high drug penetration offers significant antifungal activity (Abdellatif et al., 2017). Similar to the creams formulated in the present study, the sertaconazole nitrate-loaded nanovesicular gel exhibited a high degree of permeability. Thus, the *in vitro* skin permeation test accurately predicts the result of an *in vivo* experiment and offers a credible estimate of bioequivalence.

Skin irritation study and treatment with cold cream formulations

The formulated cold creams were non-irritant and did not cause adverse effects on the applied area after 72 h of the study, indicating that they were safe for the skin.

The antifungal properties of the prepared cold creams were tested by inhibiting *C. albicans* infection of the epidermal skin of the studied rats. *Candida albicans* mycoses induced on the skin surface appeared reddish with lesions on the extensor surfaces and coarse blisters (Fig. 3). The formulated cold creams, when applied on the infected skin region, reduced the *C. albicans* infection. A high reduction in skin fungal infection was observed after 7 consecutive days of topical application of 200 mg FCA (i.e., in rats from group 6). Topical application of 50 and 100 mg FCA showed moderate effects in inhibiting the fungal infection (i.e., in rats from groups 4 and 5, respectively). No inhibition of skin infection was reported in groups 2 and 3 (the control group for *C. albicans* infection and the group that received the plain cream, respectively, Fig. 3). *Candida albicans* initially invades the epidermal skin layer and suppresses or alters the immune response to stimulate lower cytokine response, thus causing severe damage (Mukaremera et al., 2017; Netea et al., 2015). We demonstrated that *C. albicans* caused morphological changes in rat skin similar to those in human skin (Hube et al., 2015; Schaller et al., 2000). We showed in our previous report that terpenes and essential oils were present in the methanolic extract of *C. adscendens* var. *attenuata* triterpenes (Sundar et al., 2021). A previous study has also shown that these plant metabolites are active antimicrobial compounds (Espino et al., 2019). The mechanism of action of this class of compounds is

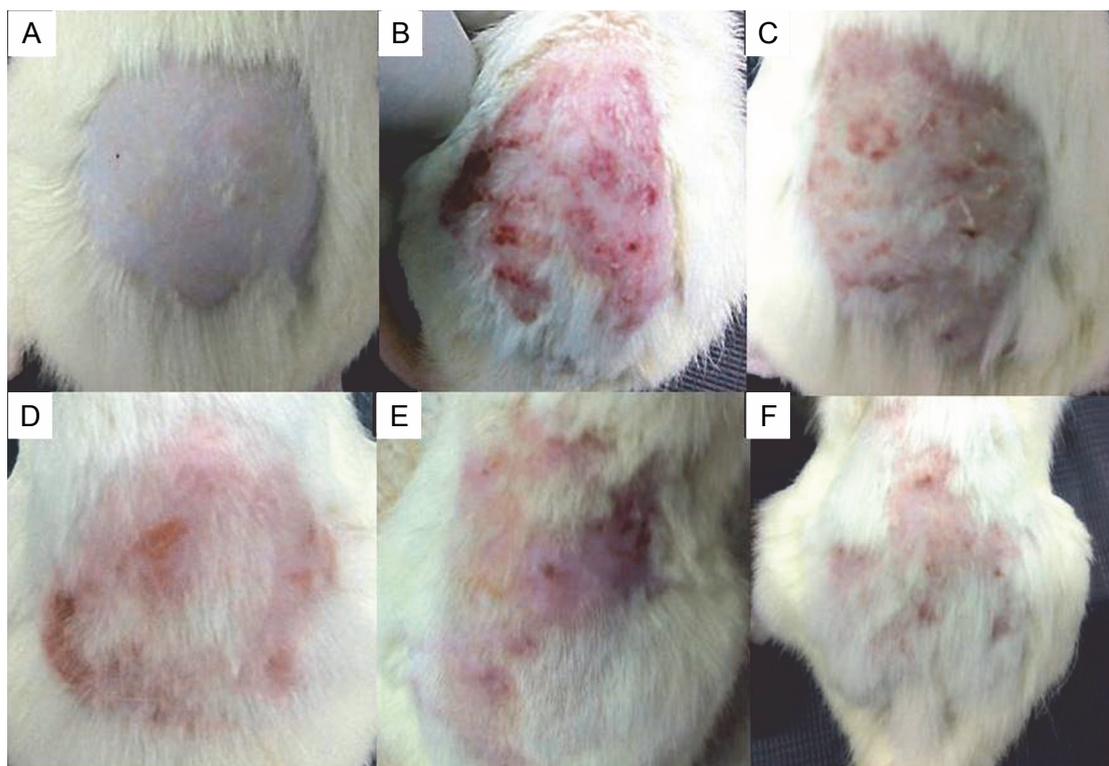


Fig. 3. Images showing experimental animals initially infused with $100\ \mu\text{l}$ of 10^7 CFU/ml of *Candida albicans*; after 72 h, 300 mg of *Caralluma adscendens* var. *attenuata* cold cream was applied on specific skin surfaces; A) group 1, B) group 2, C) group 3, D) group 4, E) group 5, and F) group 6; at the end of the treatment, on the dorsal skin of each rat, the occurrence of patches, spots, and hair growth was noted, and the skins were photographed

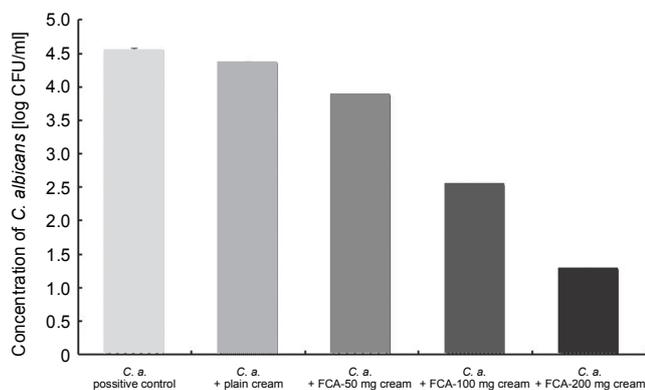


Fig. 4. Mean CFU of each rat group after the treatment period at $P < 0.05$

speculated to involve fungal membrane disruption and fungal mitochondrial destruction (Lagrouh et al., 2017; Tian et al., 2012). In a previous study in which a promising natural compound in different topical cream formulations of *C. albicans* was tested, a reduction in the total fungal load was observed 6 days after the treatment was started (Zinsou et al., 2020). Moreover, Campos et al.

(2018) found that the herbal formulation of the *Mitracarpus frigidus* extract showed acceptable physicochemical and potent antifungal properties against *C. albicans* skin infections.

The microbial count is essential in the determination of infection ranges in the skin samples (Gravning et al., 2021). CFU was calculated in the skin of all the rat groups; groups 2 and 3 showed well-defined infections, with the microbial count of 4.55 and $4.36 \log^{10}$ CFU/ml, respectively. The post hoc comparison revealed that group 6 (i.e., treated with 200 mg FCA) showed a significant decrease in the CFU value of the infected site to $1.28 \log^{10}$ CFU/ml, compared with control samples ($P < 0.05$) (Fig. 4). Groups 4 and 5 (i.e., FCA-50 and FCA-100 groups), showed CFU values of $3.88 \log^{10}$ and $2.53 \log^{10}$ CFU/ml, respectively, indicating a slight decrease in the infection severity, compared with plain cream treatment (group 3). Based on these results, FCA-200 cream showed a high therapeutic efficacy as it resulted in significant fungal colony eradication in the skin epidermal layer, which is consistent with the pre-

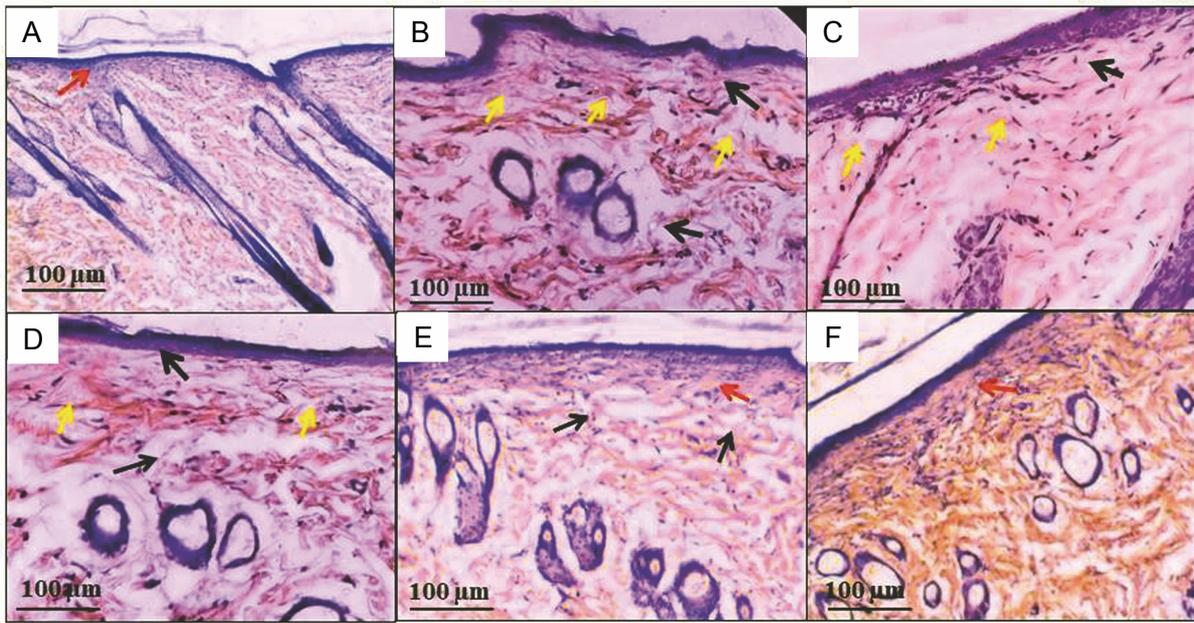


Fig. 5. Histopathological observations of the epidermal skin layer; *Candida albicans* induced epidermal damage, and the treatment with formulated *Caralluma adscendens* var. *attenuata* cold cream inhibited the epidermal damage; hematoxylin and eosin staining of rat dorsal skin under (40 \times) view; scale bar: 100 μ m; A) group 1, B) group 2, C) group 3, D) group 4, E) group 5, and F) group 6

vious report (Bonifácio et al., 2019), where the antifungal activity of *Astronium urundeuva* was observed in suppression, germination, and inhibition of mycelial growth in female Wistar rats. The high antifungal activity of FCA-200 could be due to the presence of numerous active compounds in the cold cream, such as triterpenes, terpenes, and essential oils, as reported in a previous study (Sundar et al., 2021).

Histological observation of skin

Histopathological analysis is a quick and inexpensive way to diagnose invasive fungal infections. The uninfected skin tissue is arranged into uniform dermis and epidermis layers, with the basal cells lining the base and spinous keratinocytes with notable intracellular structures overlaid (red arrow, Fig. 5A). Extensive skin damage and abnormalities were observed after the induction of *C. albicans* infection. At the same time, fungal hyphae were noted in the superficial epidermal cells (yellow arrow, Fig. 5B), as well as the focal interface dermatitis (black arrow) and dense and chronic hyperkeratosis of the dermis layer (black arrow, Fig. 5C), which is in agreement with a previous report indicating that *Candida* spp. affected the epidermal and epithelial tissues (Gácsér et al., 2007). By contrast, in group 6 (rats treated

with 200 mg FCA cream), effective regeneration of the epidermal cell structure with a mildly compact hyperkeratosis layer was observed, compared with groups 4 and 5 (rats treated with 50 and 100 mg FCA). Groups 4 and 5 showed a marked reduction in cell inflammatory infiltration, which remained mostly in deep layers of the lamina propria (Fig. 5D–F). A previous study demonstrated that plant-based (*M. frigidus*) formulations have mitigated the pathological leukocyte infiltration and high tissue reproducible capacity in candidal infections (Campos et al., 2018).

Conclusions

The results of this study indicate that the cold cream formulations developed based on the methanolic extract of *C. adscendens* var. *attenuata* showed antifungal activities against *C. albicans* in rat skin. These findings suggest that the prepared cold cream could be effectively used to treat fungal infections without damaging the skin surface and also that the topical formulation *C. adscendens* var. *attenuata* cold cream showed good stability and permeability, with no signs of irritation. The prepared cold cream formulations were tested against *C. albicans* infection and found to be helpful in reconstituting the

original structure of the dermal layer in the subcutaneous layer of the skin.

Funding

This research work was duly done by the self-financial scheme, and the authors did not receive financial support from the university or any other funding agencies.

Author contributions

K.L. and M.S. designed and conceptualized the work. M.S. was responsible for the entire experimental portion of the work and the preparation of the manuscript draft. K.L. aided in the completion of the critical revision. S.S. and K.L. provided technical assistance. All authors have read and approved the final manuscript.

Conflict of interest

All authors state that there are no conflicts of interest.

Acknowledgments

The authors thank the Department of Pharmaceutics, Sankaralingam Bhuvaneshwari College Pharmacy, Sivakasi, and K.M. College of Pharmacy, Madurai, for providing facilities to carry out the work.

References

- Abdellatif M.M., Khalil I.A., Elakkad Y.E., Eliwa H.A., Samir T.M., Al-Mokaddem A.K. (2020) *Formulation and characterization of sertaconazole nitrate mucoadhesive liposomes for vaginal candidiasis*. Int. J. Nanomed. 11(15): 4079–4090. <https://doi.org/10.2147/IJN.S250960>
- Aldawsari H.M., Badr-Eldin S.M., Labib G.S., El-Kamel A.H. (2015) *Design and formulation of a topical hydrogel integrating lemongrass-loaded nanosponges with an enhanced antifungal effect: in vitro/in vivo evaluation*. Int. J. Nanomed. 29(10): 893–902. <https://doi.org/10.2147/IJN.S74771>
- Abdellatif M.M., Khalil I.A., Khalil M.A. (2017) *Sertaconazole nitrate loaded nanovesicular systems for targeting skin fungal infection: in-vitro, ex-vivo and in-vivo evaluation*. Int. J. Pharm. 527(1–2): 1–11. <https://doi.org/10.1016/j.ijpharm.2017.05.029>
- Alqahtani S.S., Makeen H.A., Menachery S.J., Moni S.S. (2020) *Documentation of bioactive principles of the flower from Caralluma retrospiciens (Ehrenb) and in vitro antibacterial activity-Part B*. Arab. J. Chem. 13(10): 7370–7377. <https://doi.org/10.1016/j.arabjc.2020.07.023>
- AlQuadeib B.T., Eltahir E.K.D., Banafa R.A., Al-Hadhairi L.A. (2018) *Pharmaceutical evaluation of different shampoo brands in local Saudi market*. Saudi Pharma. J. 26(1): 98–106. <https://doi.org/10.1016/j.jsps.2017.10.006>
- Ameen M. (2010) *Epidemiology of superficial fungal infections*. Clin. Dermatol. 28(2): 197–201. <https://doi.org/10.1016/j.clindermatol.2009.12.005>
- Ansari M.T., Sami F., Majeed S., Hasnain M.S., Badgujar V.B. (2019) *Design and evaluation of topical herbal antifungal stick containing extracts of Rhinacanthus nasutus*. J. Herb. Med. 17: 100290. <https://doi.org/10.1016/j.hermed.2019.100290>
- Bajaj S., Singla D., Sakhuja N. (2012) *Stability testing of pharmaceutical products*. J. App. Pharma. Sci. 2(3): 129–138. <https://doi.org/10.7324/JAPS.2012.2322>
- Baskararaj S., Panneerselvam T., Govindaraj S., Arunachalam S., Parasuraman P., Pandian S.R.K., Sankaranarayanan M., Mohan U.P., Palanisamy P., Ravishankar V. (2020) *Formulation and characterization of folate receptor-targeted PEGylated liposome encapsulating bioactive compounds from Kappaphycus alvarezii for cancer therapy*. 3 Biotech. 10(3): 1–18. <https://doi.org/10.1007/s13205-020-2132-7>
- Burki I.K., Khan M.K., Khan B.A., Uzair B., Braga V.A., Jamil Q.A. (2020) *Formulation development, characterization, and evaluation of a novel dexibuprofen-capsaicin skin emulgel with improved in vivo anti-inflammatory and analgesic effects*. AAPS PharmSciTech. 21(6): 1–14. <https://doi.org/10.1208/s12249-020-01760-7>
- Bonifacio B.V., Vila T.V.M., Masiero I.F., Da Silva P.B., Da Silva I.C., de Oliveira Lopes É., Bauab T.M. (2019) *Antifungal activity of a hydroethanolic extract from Astronium urundeuva leaves against Candida albicans and Candida glabrata*. Front. Microbiol. 2642(10): 1–12. <https://doi.org/10.3389/fmicb.2019.02642>
- Campos L.M., de Melo L., Lemos A.S.O., Guedes M.C.M.R., Silva T.P., Figueiredo G.F., Junior J.L.R., Rocha V.N., Melo R.C.N., Araujo M.G.F. (2018) *Mitracarpus frigidus: a promising antifungal in the treatment of vulvovaginal candidiasis*. Ind. Crops Prod. 123: 731–739. <https://doi.org/10.1016/j.promfg.2018.07.038>
- Carter S.J. (2008) *Cooper and gunns dispensing for pharmaceutical students* (SJ Carter (ed.); 12th ed.). CBS Publishing and Distributors.
- Chandrasekar R., Priyanka K., Sakhira K., Sreeprada K., Harshitha K., Haripriya B., Babu M.N. (2018) *Formulation and stability evaluation of natural preservatives in poly-herbal skin care cream*. Int. J. Res. Deve. Pharm. Life Sci. 7(3): 2999–3005. [https://doi.org/10.21276/IJRDPL.2278-0238.2018.7\(3\).2999-3005](https://doi.org/10.21276/IJRDPL.2278-0238.2018.7(3).2999-3005)
- Coates S.J., Enbiale W., Davis M.D., Andersen L.K. (2020) *The effects of climate change on human health in Africa, a dermatologic perspective: a report from the International Society of Dermatology Climate Change Committee*. Int. J. Dermatol. 59(3): 265–278. <https://doi.org/10.1111/ijd.14759>
- Dave V., Sharma S., Yadav R.B., Agarwal U. (2017) *Herbal liposome for the topical delivery of ketoconazole for the effective treatment of seborrheic dermatitis*. App. Nanosci. 7(8): 973–987. <https://doi.org/10.1007/s13205-020-2083-z>
- Deyno S., Mtewa A.G., Abebe A., Hymete A., Makonnen E., Bazira J., Alele P.E. (2019) *Essential oils as topical anti-infective agents: a systematic review and meta-analysis*. Compl. Ther. Med. 47: 102224. <https://doi.org/10.1016/j.ctim.2019.102224>
- Dhyani A., Chander V., Singh N. (2019) *Formulation and evaluation of multipurpose herbal cream*. J. Drug Deliv. Ther. 9(2): 341–343. <https://doi.org/10.22270/jddt.v9i2.2540>

- Dismukes W.E. (2000) *Introduction to antifungal drugs*. Clin. Infect. Dis. 4: 653–657. <https://www.jstor.org/stable/4461123>
- El-Shiekh R.A., Salama A., Al-Mokaddem A.K., Bader A., Abdel-Sattar E.A. (2021) *Russelioside B; A pregnane glycoside for treatment of gastric ulcer via modulation of heat shock protein-70 and vascular endothelial growth factor*. Steroids 165: 108759. <https://doi.org/10.1016/j.steroids.2020.108759>
- Espino M., Solari M., de los Angeles Fernández M., Boiteux J., Gómez M.R., Silva M.F. (2019) *NADES-mediated folk plant extracts as novel antifungal agents against Candida albicans*. J. Pharm. Biomed. 167: 15–20. <https://doi.org/10.1016/j.jpba.2019.01.026>
- Gácsér A., Schäfer W., Nosanchuk J.S., Salomon S., Nosanchuk J.D. (2007) *Virulence of Candida parapsilosis, Candida orthopsilosis, and Candida metapsilosis in reconstituted human tissue models*. Fungal Genet. Biol. 44(12): 1336–1341. <https://doi.org/10.1016/j.fgb.2007.02.002>
- Garber G. (2001) *An overview of fungal infections*. Drugs 61(1): 1–12. <https://doi.org/10.2165/00003495-200161001-00001>
- Garg A., Sharma G.S., Goyal A.K., Ghosh G., Si S.C., Rath G. (2020) *Recent advances in topical carriers of anti-fungal agents*. Heliyon 6(8): 04663. <https://doi.org/10.1016/j.heliyon.2020.e04663>
- Gravning G.E.N., Rørtterud O.J., Björkrøy S., Forseth M., Skjerve E., Llerena A.K., Lian A., Johannessen G.S., Hauge S.J. (2021) *Comparison of four sampling methods for microbiological quantification on broiler carcasses*. Food Control. 121: 107589. <https://doi.org/10.1016/j.foodcont.2020.107589>
- Guégan S., Lanternier F., Rouzaud C., Dupin N., Lortholary O. (2016) *Fungal skin and soft tissue infections*. Curr. Opin. Infect. Dis. 29(2): 124–130. <https://doi.org/10.1097/qco.0000000000000252>
- Gupta A.K., Cooper E.A. (2008) *Update in antifungal therapy of dermatophytosis*. Mycopathologia 166(5): 353–367. <https://doi.org/10.1007/s11046-008-9109-0>
- Gupta M., Vyas S.P. (2012) *Development, characterization and in vivo assessment of effective lipidic nanoparticles for dermal delivery of fluconazole against cutaneous candidiasis*. Chem. Phys. Lipids 165(4): 454–461. <https://doi.org/10.1016/j.chemphyslip.2012.01.006>
- Havlickova B., Czaika V.A., Friedrich M. (2008) *Epidemiological trends in skin mycoses worldwide*. Mycoses 51: 2–15. <https://doi.org/10.1111/j.1439-0507.2008.01606.x>
- Heredero-Bermejo I., Gómez-Casanova N., Quintana S., Soliveri J., de la Mata F.J., Pérez-Serrano J., Sánchez-Nieves J., Copa-Patiño J.L. (2020) *In vitro activity of carbosilane cationic dendritic molecules on prevention and treatment of Candida albicans biofilms*. Pharmaceutics 12(10): 918. <https://doi.org/10.3390/pharmaceutics12100918>
- Hiremath S.S.P., Dasankoppa F.S., Nadaf A., Jamakandi V.G., Mulla J.S., Sholapur H.N. (2008) *Formulation and evaluation of a novel in situ gum based ophthalmic drug delivery system of linezolid*. Sci. Pharm. 76(3): 515–532. <https://doi.org/10.3797/scipharm.0803-17>
- Hube B., Hay R., Brasch J., Veraldi S., Schaller M. (2015) *Dermatomycoses and inflammation: The adaptive balance between growth, damage, and survival*. J. Mycol. Med., 25(1): e44–e58. <https://doi.org/10.1016/j.mycmed.2014.11.002>
- Ijaz N., Durrani A. I., Rubab S., Bahadur S. (2021) *Formulation and characterization of Aloe vera gel and tomato powder containing cream*. Acta Ecol. Sinica 1–9. <https://doi.org/10.1016/j.chnaes.2021.01.005>
- Khalid A., Ahmed N., Qindeel M., Asad M.I., Khan G.M., Rehman A. (2021) *Development of novel biopolymer-based nanoparticles loaded cream for potential treatment of topical fungal infections*. Drug Dev. Ind. Pharm. 1–10. <https://doi.org/10.1080/03639045.2021.1957914>
- Khan B.A., Ullah S., Khan M.K., Alshahrani S.M., Braga V.A. (2020) *Formulation and evaluation of Ocimum basilicum-based emulgel for wound healing using animal model*. Saudi Pharma. J. 28(12): 1842–1850. <https://doi.org/10.1016/j.jsps.2020.11.011>
- Kiranmayee P, Anitha K, Usha R. (2016) *Isolation and identification of steroid triterpenoids from the polar and non-polar fractions of Caralluma attenuate (Wight) roots*. Int. J. Pharm. Phytochem. Res. 7(5): 1120–1124.
- Kowshihan P., Roy A., Somasundaram J., Lakshmi T. (2020) *Therapeutic approach on oral mucositis*. Indian J. Forensic. Med. Toxicol. 14(4): 5328–5335. <https://doi.org/10.37506/ijfimt.v14i4.12458>
- Kuhbacher A., Burger-Kentischer A., Rupp S. (2017) *Interaction of Candida species with the skin*. Microorganisms 5(2): 32. <https://doi.org/10.3390/microorganisms5020032>
- Haneefa K.P.M., Saraswathi R., Mohanta G., Nayar C. (2010) *Formulation and evaluation of herbal gel of Pothos scandens Linn*. Asian Pac. J. Trop. Med. 3(12): 988–992. [https://doi.org/10.1016/S1995-7645\(11\)60015-1](https://doi.org/10.1016/S1995-7645(11)60015-1)
- Kumar K.M.P., Murshida U.C., Thomas B., George S., Balachandran I., Karuppusamy S. (2014) *Notes on Caralluma adscendens (Roxb.) Haw. var. attenuata (Wight) Grav. and Mayur. (Apocynaceae: Asclepiadoideae)*. J. Threat. Taxa. 6(9): 6282–6286. <https://doi.org/10.11609/JoTT.o3523.6282-6>
- Lagrouh F., Dakka N., Bakri, Y. (2017) *The antifungal activity of Moroccan plants and the mechanism of action of secondary metabolites from plants*. J. Mycol. Med. 27(3): 303–311. <https://doi.org/10.1016/j.mycmed.2017.04.008>
- Lukić M., Pantelić I., Savić S.D. (2021) *Towards optimal pH of the skin and topical formulations: From the current state of the art to tailored products*. Cosmetics 8(3): 69. <https://doi.org/10.3390/cosmetics8030069>
- Madasamy S., Sundan S., Krishnasamy L. (2020) *Preparation of cold cream against clinical pathogen using Caralluma adscendens var. attenuata*. Asian J. Pharm. Clinic. Res. 13(9): 120–123. <https://doi.org/10.22159/ajpcr.2020.v13i9.37976>
- Mahdi E.S., Noor A.M., Sakeena M.H., Abdullah G.Z., Abdulkarim M.F., Sattar M.A. (2011) *Formulation and in vitro release evaluation of newly synthesized palm kernel oil esters-based nanoemulsion delivery system for 30% ethanolic dried extract derived from local Phyllanthus urinaria*

- for skin antiaging. *Int. J. Nanomed.* 6: 2499. <https://doi.org/10.2147/IJN.S22337>
- Mukaremera L., Lee K.K., Mora-Montes H.M., Gow N.A.R. (2017) *Candida albicans* yeast, pseudohyphal, and hyphal morphogenesis differentially affects immune recognition. *Front. Immunol.* 8: 629. <https://doi.org/10.3389/fimmu.2017.00629>
- Naik A., Kalia Y.N., Guy R.H. (2000) *Transdermal drug delivery: overcoming the skin's barrier function*. *Pharma. Sci. Technol. Today* 3(9): 318–326. [https://doi.org/10.1016/S1461-5347\(00\)00295-9](https://doi.org/10.1016/S1461-5347(00)00295-9)
- Netea M.G., Joosten L.A.B., Van Der Meer J.W.M., Kullberg B.J., Van De Veerdonk F.L. (2015) *Immune defence against Candida fungal infections*. *Nat. Rev. Immunol.* 15(10): 630–642. <https://doi.org/10.1038/nri3897>
- Ouassou H., Bouhrim M., Kharchoufa L., Imtara H., Elhouda Daoudi N., Benoutman A., Bencheikh N., Ouahhoud S., Elbouzidi A., Bnouham M. (2021) *Caralluma europaea* (Guss) NE Br.: a review on ethnomedicinal uses, phytochemistry, pharmacological activities, and toxicology. *J. Ethnopharmacol.* 273(12): 113769. <https://doi.org/10.1016/j.jep.2020.113769>
- Pithayanukul P., Chansri N., Sugibayashi K. (2002) *The enhancing effects of common pharmaceutical solvents on the in vitro skin permeation of estradiol*. *Thai. J. Pharm. Sci.* 26(3): 109–119.
- Queiroz-Telles F., McGinnis M.R., Salkin I., Graybill J.R. (2003) *Subcutaneous mycoses*. *Infect. Dis. Clin. North Am.* 17(1): 59–85. [https://doi.org/10.1016/S0891-5520\(02\)00066-1](https://doi.org/10.1016/S0891-5520(02)00066-1)
- Qushawy M., Nasr A., Abd-Alhaseeb M., Swidan S. (2018) *Design, optimization and characterization of a transferosomal gel using miconazole nitrate for the treatment of candida skin infections*. *Pharmaceutics* 10(1): 1–26. <https://doi.org/10.3390/pharmaceutics10010026>
- Rapalli V.K., Waghule T., Gorantla S., Dubey S.K., Saha R.N., Singhvi G. (2020) *Psoriasis: pathological mechanisms, current pharmacological therapies, and emerging drug delivery systems*. *Drug Discov. Today.* 25(12): 2212–2226 <https://doi.org/10.1016/j.drudis.2020.09.023>
- Ré A.C.S., Martins J.F., Cunha Filho M., Gelfuso G.M., Aires C.P., Gratieri T. (2021) *New perspectives on the topical management of recurrent candidiasis*. *Drug. Deliv. Translational. Res.* 11: 1568–1585. <https://doi.org/10.1007/s13346-021-00901-0>
- Schaller M., Schackert C., Korting H.C., Januschke E., Hube B. (2000) *Invasion of Candida albicans correlates with expression of secreted aspartic proteinases during experimental infection of human epidermis*. *J. Invest. Dermatol.* 114(4): 712–717. <https://doi.org/10.1046/j.1523-1747.2000.00935.x>
- Sequeira J.A. (1990) Optimization of the skin availability of topical products. *Cosmet. Toiletries* 105(12): 114–121.
- Silva H., Luz G.M., Satake C.Y., Correa B.C., Sarmiento V.H., Oliveira G.H.D., Carvalho F.C., Chorilli M., Gremião M.P.D. (2014) *Surfactant-based transdermal system for fluconazole skin delivery*. *J. Nanomed. Nanotechnol.* 5(5): 1–10. <http://dx.doi.org/10.4172/2157-7439.1000231>
- Smaoui S., Hlima H., Ben Jarraya R., Kamoun N.G., Ellouze R., Damak M. (2012) *Cosmetic emulsion from virgin olive oil: Formulation and bio-physical evaluation*. *Afr. J. Biotech.* 11(40): 9664–9671. <http://dx.doi.org/10.5897/AJB12.163>
- Sonawane T.N., Chaudhari D.D., Mali S.D., Shaikh A.Z. (2021) *A short review on skin cares cream*. *Res. J. Topical Cosmetic Sci.* 12(1): 1–3. <http://dx.doi.org/10.52711/2321-5844.2021.00001>
- Sonia S., Ruckmani K., Sivakumar M. (2017) *Antimicrobial and antioxidant potentials of biosynthesized colloidal zinc oxide nanoparticles for a fortified cold cream formulation: a potent nanocosmeceutical application*. *Mater. Sci. Eng.* 1(79): 581–589. <https://doi.org/10.1016/j.msec.2017.05.059>
- Sundar M., Suresh S., Lingakumar K. (2021) *Influence of Caralluma adscendens Var. attenuata cold cream on UV-B damaged skin epidermal cells: a novel approach*. *3 Biotech.* 11(4): 1–16. <https://doi.org/10.1007/s13205-021-02694-y>
- Tian J., Ban X., Zeng H., He J., Chen Y., Wang Y. (2012) *The mechanism of antifungal action of essential oil from dill (Anethum graveolens L.) on Aspergillus flavus*. *PLoS One* 7(1): e30147. <https://doi.org/10.1371/journal.pone.0030147>
- Tsutsumi-Arai C., Terada-Ito C., Tatehara S., Imamura T., Takebe Y., Ide S., Satomura K. (2021) *Fungicidal activity of grapefruit seed extract against the pathogenic Candida species causing oral candidiasis*. *J. Oral. Maxillofac. Surg. Med. Pathol.* 33(6): 626–632. <https://doi.org/10.1016/j.ajoms.2021.03.001>
- Ugazio E., Tullio V., Binello A., Tagliapietra S., Dosio F. (2020) *Ozonated oils as antimicrobial systems in topical applications. Their characterization, current applications, and advances in improved delivery techniques*. *Molecules* 25(2): 334. <https://doi.org/10.3390/molecules25020334>
- Vajha M., Amrutha V., Audipudi M.K. (2010) *Evaluation of immunostimulating activities of Caralluma spp.* *Int. J. Pharmacogn. Phytochem. Res.* 2(4): 1–4. <https://doi.org/10.13140/2.1.4134.4641>
- Wingard J.R., Leather H. (2004) *A new era of antifungal therapy*. *Biol. Blood Marrow Transplant.* 10(2): 73–90. <https://doi.org/10.1016/j.bbmt.2003.09.014>
- Zamani N., Fazilati M., Salavati H., Izadi M., Koochi-Dehkordi M. (2020) *The topical cream produced from phycocyanin of Spirulina platensis accelerates wound healing in mice infected with Candida albicans*. *Appl. Biochem. Microbiol.* 56(5): 583–589. <https://doi.org/10.1134/S0003683820050166>
- Zhang A.Y., Camp W.L., Elewski B.E. (2007) *Advances in topical and systemic antifungals*. *Dermatol. Clin.* 25(2): 165–183. <https://doi.org/10.1016/j.det.2007.01.002>
- Zinsou A., Assanhou A.G., Ganfon H., Sounouvou H., Kassehin U.C., Lawson R.F., Medegan S., Evrard B., Quetin-Leclercq J., Gbaguidi F.A. (2020) *Development of new dermatological formulations for the treatment of cutaneous candidiasis*. *Sci. African.* 8: e00342. <https://doi.org/10.1016/j.sciaf.2020.e00342>