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Formulation and Evaluation of Antifungal Herbal Cream

Alok Bhargava[1], Dr. Rajshree Mishra[1] and Ravishankar Rao[2]

[1]Department of Pharmacognosy, Sunrise Pharmacy College, Sunrise University, Alwar, Rajasthan.

[2]Department of Pharmaceutics, Sunrise Pharmacy College, Sunrise University, Alwar, Rajasthan

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Corresponding author: Alok Bhargava

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Abstract:

Fungal infections caused by Candida albicans are among the most common microbial infections affecting the skin and mucosal surfaces. The present study was undertaken to formulate and evaluate an antifungal herbal cream containing 5% w/w extract of Phyllanthus acidus and to assess its antifungal efficacy. The antifungal activity of the plant extract was evaluated through in vivo studies using albino mice infected with C. albicans. The treated group receiving 50 mg/kg plant extract exhibited a mean survival time of 19.84 ± 2.36 days compared to 15.52 ± 2.81 days in the infected untreated group, indicating significant antifungal activity. An oil-in-water cream formulation was prepared using stearic acid, cetyl alcohol, liquid paraffin, glycerin, triethanolamine, methylparaben, and the herbal extract. The prepared cream was evaluated for physical appearance, pH, viscosity, spreadability, and stability. The cream exhibited a smooth appearance, pleasant odor, pH of 6.8, viscosity of 221022 cps, and spreadability of 32.4 g·cm/sec. Stability studies conducted for one month under accelerated conditions showed no significant changes in the evaluated parameters. The results suggest that the formulated herbal cream possesses satisfactory physicochemical properties, good stability, and promising antifungal activity, making it a potential alternative to conventional antifungal formulations.

Keywords: Antifungal Herbal Cream, *Phyllanthus acidus, Candida albicans*, Topical Drug Delivery, Herbal Formulation

Introduction:

Fungal infections represent a significant global health concern, particularly those caused by Candida albicans, which is responsible for various superficial and systemic infections. The increasing incidence of fungal infections and the emergence of resistance to conventional antifungal drugs have prompted the search for safer and more effective therapeutic alternatives. Herbal medicines have gained considerable attention due to their therapeutic efficacy, reduced adverse effects, affordability, and widespread availability.

Phyllanthus acidus is a medicinal plant known for its diverse pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, and antifungal properties. The bioactive phytoconstituents present in the plant

contribute significantly to its therapeutic potential. Topical drug delivery systems such as creams are widely used for the treatment of fungal infections because they provide localized action, enhance patient compliance, and minimize systemic side effects.

The present study focuses on the formulation of an oil-in-water herbal cream containing Phyllanthus acidus extract and the evaluation of its physicochemical characteristics and antifungal efficacy. The developed formulation aims to provide an effective and stable topical treatment option for fungal infections while utilizing the therapeutic benefits of herbal medicine.

Materials and method

Phyllanthus acidus leaves extract was selected as the active ingredient for the preparation of antifungal herbal cream based on its reported antifungal activity. The formulation ingredients included stearic acid, cetyl alcohol, liquid paraffin, triethanolamine, glycerin, methylparaben, and purified water. Stearic acid served as an emulsifying agent, cetyl alcohol as a stiffening and emollient agent, liquid paraffin as an emollient, glycerin as a humectant, triethanolamine as a neutralizing and emulsifying agent, and methylparaben as a preservative. Purified water was used as the vehicle for the preparation of the oil-in-water cream. All materials employed in the study were of analytical grade and used as received without further purification.

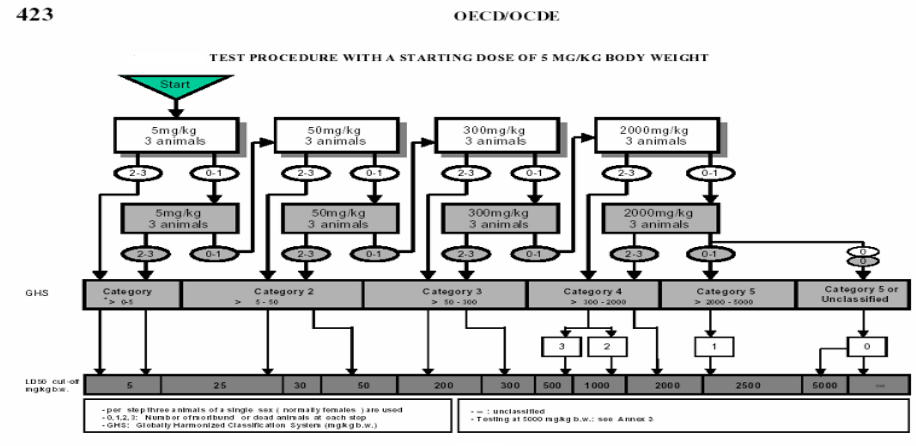
Extraction of dried leaves

50gms of dried shaded leaves powder will be exhaustively extracted with chloroform, ethanol, water and hydroalcohol (1:1) using soxhlet extraction apparatus. The extracts were evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

***In vivo* antifungal activity**

Animals

Animals were procured from Local market area, Rajasthan. Female Albino mice (20-25gm) of almost the similar age were used for the activity. Polypropylene cages were used for housing of animals, standard rodent nutrition along with water ad libitum and an alternate cycle of twelve hours of darkness and light were provided for their nourishment. Drug given orally by means of orogastric cannula. Animals were subjected to fasting for minimum 12hours, before any of the experiment perform; measures for test were presented for the assessment of the Institutional Animals Ethical Committee and were passed by the correspondent. Giving to CPCSEA rules for care of research creatures and the moral rule for examinations of exploratory torment in cognizant creatures, tests were acted in morning.



Acute toxicity studies (Determination of LD_{50})

For LD_{50} determination, an oral acute toxicity study is to perform for which guidelines were regulated by OECD. OECD is an international organization named as Organization for Economic co-operation and Development, working for animal welfare. The objective is to reduce number of animals and the extent of pain accompanying with acute toxicity study (www. oecd. org).

Guidelines followed by OECD:

- Guideline 420, fixed dose procedure. (5 animals used)
- Guideline 423, acute toxic class. (3 animals used)
- Guideline 425, up and. down method. (1 animal used)

Guideline 423

a) Principle

Acute oral toxicity is a process that includes the identification of a dose level that causes mortality. This test generally includes female animals with three animals in each dose group. Test samples were used at a pre-specified fixed dose of 5mg/kg, 50mg/kg, 300mg/kg, 2000mg/kg which is given orally to fasted healthy young adults and detect their mortality by the effect of this doses. After giving dose to animals, they were keep under observation for upto 15 days and determine body weight and necropsy.

Procedure

OECD Guidelines

- For the acute oral toxicity studies overnight fasted mice was selected and weighed.
- All three plants extract were dosed in a stepwise procedure separately. The selection of initial dose was aimed to cause some indications of toxicity which required a two weeks' observation.
- According to Guideline 423, toxic dose was selected.

***In-vivo* activity**

Survival study

C. albicans was used for infection. This isolate was maintained between studies at 4°C on Sabouraud dextrose agar. Before these studies, the isolate was transferred to brain heart infusion broth and grown at 37°C overnight. The inoculum was washed three times in saline, and an aliquot was used for the hemacytometer counting. The inoculum was adjusted to 0.2 ml/mouse, and the count of viable organisms was determined by colony count dilutions. Male ICR mice (30 g, 10 mice/group) were infected through tail vein (intravenously [i.v.]) inoculation of 5 × 106 CFU/mouse (0.2 ml of 2.5 × 107 CFU/ml). At 24 h postinfection, the animals were treated with Plant extract at concentration of 50mg/kg (p.o), 0.2 ml/mouse as a once-daily dose for 5 days. Comparison was achieved with groups of mice receiving Fluconazole 3mg/kg p.o. The survival of animals was monitored and compared to controls for 28 days postinfection

Tissue burden study

For determination of tissue burden, mice were infected and treated as described above and the tissue burden was assessed on days 2, 4, 7, 10, 14 and 28 post-infection. The kidneys were aseptically removed and homogenized in 1 ml of sterile normal saline and cultured on SDA plates and assessed by determination of fungal colonization.

Histopathological analyses

Tissues were fixed in 10% neutral buffered formalin (Merck, Germany) for at least 2 days and blocked by paraffin wax using a Shandon Automated Tissue Processor (ThermoShandon, PA, USA). The blocked tissues were cut using a Leica microtome (Leica, model RM2025, Germany) in 4-µm thickness. The sections were stained by hematoxylin and eosin

(H&E) and periodic acid shiff's (PAS) and viewed under light microscopy

Formulation of herbal cream using leaves extract

An oil-in-water (O/W)-based cream (20 g) will be formulated. The emulsifier (stearic acid) and other oil-soluble components, thickening agent (cetyl alcohol) and emollient or lubricant (liquid paraffin) were dissolved in the oil phase and heated to 75 °C (Part A). The aqueous phase will be prepared by dissolving the required amount of extract in propylene glycol solvent and then adding it to water. The preservatives (methylparaben, triethanolamine) and the other water-soluble component glycerin, which will be used as humectant, were dissolved in the aqueous phase and heated to 75 °C. After heating, the aqueous phase (Part B) will be added in portions to the oil phase (Part A) with continuous stirring until cooling of emulsifier occurred. The creation of the galangal extract cream will be done according to the method mentioned in reference. A 5% w/w cream will be prepared using the formula given below

Table 1 Formulation of Herbal Cream

Components	Ingredients	Amount in Grams (g) for 5% *w*/*w* Cream
Oil phase (Part A)	Extract of *P. acidus*	1
	Stearic acid	2.2
	Cetyl alcohol	0.8
	Liquid paraffin	0.8
Aqueous phase (Part B)	Water	14.7
	Triethanolamine	0.3
	Glycerin	1
	Methylparaben	0.2

Evaluation of herbal cream

Physical Properties: The Cream will be observe for colour, odour and appearance

Determination of pH: 0. 5 ± 0.01g of the Cream will be weighed accurately in a 10ml test tube. 4.5ml of water will be added & dispersed the Cream in it. The pH of the suspension will be determined at 270 C using the pH meter.

Viscosity: Viscosity will be measured by Brookfield Viscometer. The determination will be carried out in triplicate and the average of three readings will be recorded.

Spreadability: An important criteria for semisolids is that it possess good spreadability. "Spreadability is a term expressed to denote the extent of area to which the cream readily spreads on application to the skin". The therapeutic efficacy of a formulation also depends on its spreading value. A special apparatus has been designed to study the spreadability of the formulations. Spreadability is Expressed in terms of "time in seconds" taken by two slides to slip off from the formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of the two, better the spreadability. Two glass slides of standard dimensions will be selected. The

formulation whose spreadability had to be determined will be placed over one of the slides. The other slide will be placed on top of the formulations will be sandwiched between the two slides across the length of 5 cm along the slide. 10 g weight will be placed up on the upper slide so that the formulation between the two slides will be pressed uniformly to form a thin layer. The weight will be removed and the excess of formulation adhering to the slides will be scrapped off. One of the slides will be fixed on which the formulation will be placed. The second movable slide will be placed over it, with one end tied to a string to which load could be applied by the help of a simple pulley and a pan. A 3g weight will be put on the pan and the time taken for the upper slide to travel the distance of 5.0cm and separate away from the lower slide under the direction of the weight will be noted. The spreadability will be then calculated from the following formula: [79]

Spreadability= m ×l /t Where, m = weight tied to the upper slide (3g) l =length of glass slide (5cm) t =time taken in seconds

Stability studies- Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, stability studies will be done according to ICH guidelines. The stability studies will be carried out as per ICH guidelines. The cream filled in bottle and kept in humidity chamber maintained at 30 ± 2°C/ 65 ± 5 % RH and 40 ± 2°C / 75 ± 5 % RH for a month. At the end of studies, samples will be analyzed for the physical properties and viscosity.

Results and discussion

***In-vivo* Anti-fungal Activity**

Survival study

Table 2: Mean survival time

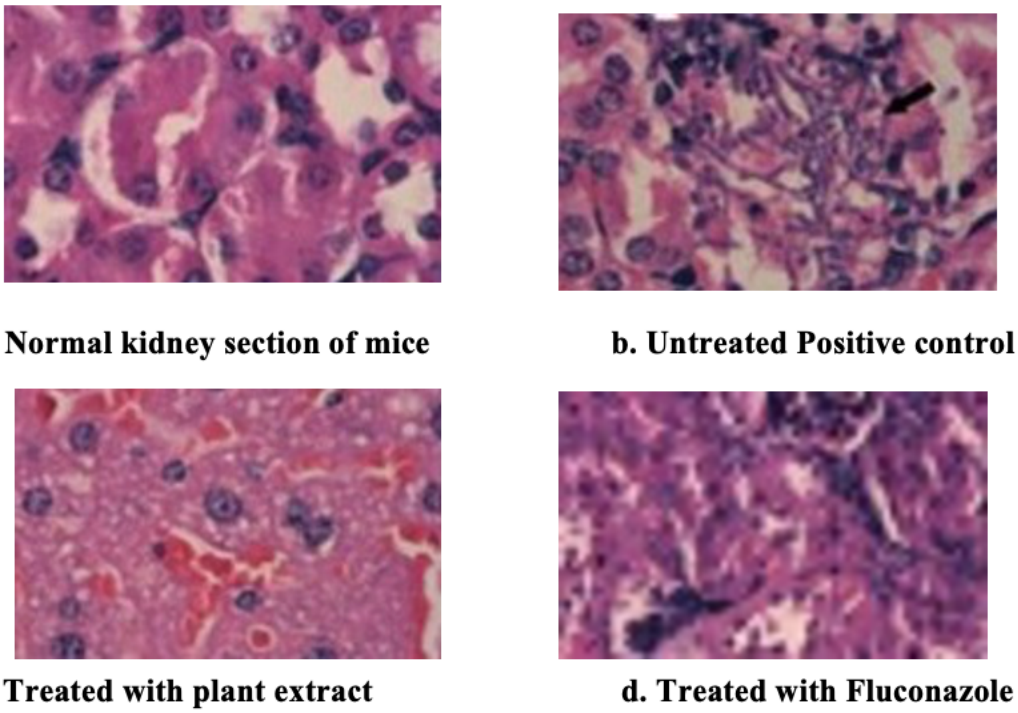
S No.	Group	Dose mg/kg	Mean survival time (In days)
1	Group I	Control Treated with saline	-
2	Group II	Injected with C. Albicans	15.52±2.81
3	Group III	Injected with C. Albicans, treated with 50mg/kg Plant extract	19.84±2.36
4	Group IV	Injected with C. Albicans, treated with 3mg/kg Fluconazole	24.24±2.67

The survival study demonstrated the antifungal potential of Phyllanthus acidus extract against Candida albicans infection in mice. The infected untreated group (Group II) showed a mean survival time of 15.52 ± 2.81 days, indicating severe fungal infection and disease progression. Treatment with P. acidus extract at 50 mg/kg (Group III) significantly increased the mean survival time to 19.84 ± 2.36 days, suggesting effective inhibition of fungal growth and improvement in the health status of infected animals. The standard drug fluconazole (3 mg/kg) exhibited the highest protection with a mean survival time of 24.24 ± 2.67 days, confirming its established antifungal efficacy. The results indicate that the plant

extract possesses considerable antifungal activity, although slightly lower than the standard antifungal drug.

Tissue fungal load

Plant extract was efficient at inhibiting the growth of *C. albicans* , *in vivo*. The results of fungal burden determination in the kidney at different time points, indicated a significant reduction in CFU/g in the tissue ($p<0.05$) starting from day 2 post-infection. Cell viability was significantly higher in mice kidney tissues treated with *plant extract* 28 days post-infection. Histopathological analysis showed the presence of *C. albicans* between the tubules, as well as a few scattered lymphocytes and moderate congestion in the infected kidney 10 days post-infection. While in kidney section from mice treated with *plant extract*, mild congestion within the interstitial tissue was observed. On the other hand, kidney section from mice treated with fluconazole, showed a normal appearance of closely-packed tubules without any congestion.



a. Normal kidney section of mice **b. Untreated Positive control**

c. Treated with plant extract **d. Treated with Fluconazole**

Fig. No. 1 Histological structure of normal, treated and untreated mice kidney sections

Formulation and development of cream:

Table no. 3 Composition of herbal cream

Components	Ingredients	Amount in Grams (g) for 5% *w/w* Cream
Oil phase (Part A)	Extract of *P. acidus*	1
	Stearic acid	2.2
	Cetyl alcohol	0.8
	Liquid paraffin	0.8
Aqueous phase (Part B)	Water	14.7
	Triethanolamine	0.3
	Glycerin	1
	Methylparaben	0.2

Evaluation of cream

Cream was evaluated using various parameters like color, odor, appearance, pH, viscosity and spreadability.

Table no. 4 : Results of evaluation of cream

S. No.	Parameter	Results
1	**Color**	Brown
2	**Odor**	Pleasant
3	**Appearance**	Smooth
4	**pH**	6.8
5	**Viscosity**	221022Cps
6	**Spreadability**	32.4 (g X m X sec)

The prepared cream showed a brown color, which may be attributed to the presence of plant constituents in the extract. The formulation possessed a pleasant odor and smooth appearance, indicating good aesthetic acceptability. The pH value of 6.8 was within the acceptable range for topical formulations and compatible with skin pH, minimizing the risk of irritation. The viscosity (221022 cps) indicated good consistency and ease of application. The spreadability value (32.4 g·cm/sec) suggested that the cream could be uniformly spread over the skin surface, ensuring effective drug distribution. Overall, the evaluation results confirmed the suitability of the cream for topical antifungal therapy.

Stability studies

Parameters of evaluation of cream show satisfied results and the cream undergoes for stability studies.

Table no. 5 : Results of evaluation of stability studies of cream for one month

S. No.	Parameter	30 ± 2°C/ 65 ± 5 % RH	40 ± 2°C / 75 ± 5 % RH
1	**Color**	Brown	Brown
2	**Odor**	Pleasant	Pleasant
3	**Appearance**	Smooth	Smooth
4	**pH**	6.8	6.7
5	**Viscosity**	221022Cps	220088Cps
6	**Spreadability**	32.4 (g X m X sec)	32.6 (g X m X sec)

The herbal cream remained stable under both 30 ± 2°C/65 ± 5% RH and 40 ± 2°C/75 ± 5% RH storage conditions for one month. No noticeable changes were observed in color, odor, or appearance during the study period. Only minimal variations in pH, viscosity, and spreadability were recorded, indicating good physical stability of the formulation. The results suggest that the developed herbal cream possesses satisfactory stability and can maintain its quality during storage. Therefore, the formulation can be considered stable and suitable for further pharmaceutical development and therapeutic application.

Conclusion

The present study successfully formulated and evaluated a 5% w/w antifungal herbal cream containing Phyllanthus acidus extract. The in vivo antifungal studies demonstrated significant activity against Candida albicans, as evidenced by increased survival time and reduced fungal burden in treated animals. The formulated cream exhibited desirable physicochemical properties, including smooth appearance, acceptable pH, suitable viscosity, and good spreadability. Stability studies conducted under different storage conditions confirmed the stability of the formulation without significant alterations in its characteristics. Overall, the developed herbal cream demonstrated promising antifungal potential and could serve as a safe, effective, and stable topical formulation for the management of fungal infections. Further clinical studies are recommended to establish its therapeutic efficacy in humans.

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