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Original Article

The Efficacy of a Topical Antifungal Preparation from *Hibiscus sabdariffa*: A Formulation and *in-vitro* Analysis

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ABSTRACT

Background: Fungal infections are a growing global health concern, necessitating the development of safer and more effective antifungal therapies. *Hibiscus sabdariffa* (roselle) is a medicinal plant traditionally used for its antimicrobial properties, attributed to bioactive constituents such as flavonoids, tannins, alkaloids, and saponins. This study aims to formulate and evaluate a herbal antifungal cream incorporating *H. sabdariffa* ethanolic extract as a natural alternative to synthetic antifungal agents. **Materials and Methods:** The ethanolic extract of *H. sabdariffa* calyces was prepared using Soxhlet extraction and subjected to preliminary phytochemical screening. Cream formulations were prepared using Beeswax, Cetyl alcohol, Glycerine, and Essential oils as the base, incorporating varying concentrations of the extract. Physicochemical parameters, including pH, spreadability, stability, and viscosity, were evaluated. Antifungal activity against *Candida albicans* was determined by the agar well diffusion method, and inhibition zones were measured. Stability studies were conducted over four weeks at controlled conditions to assess colour, odour, and phase separation. **Results:** Phytochemical analysis confirmed the presence of flavonoids, tannins, alkaloids, and saponins. The prepared creams exhibited pH values within the ideal topical range (4.5-6.5), good spreadability, and no phase separation during the stability study. Antifungal testing revealed concentration-dependent inhibition of *C. albicans*, with higher extract concentrations producing larger inhibition zones, comparable to standard antifungal agents. No significant changes in physicochemical properties were observed during the four-week stability study. **Conclusion:** The *H. sabdariffa*-based herbal cream demonstrated promising antifungal activity and stability, suggesting its potential as a safe and effective natural alternative to conventional antifungal formulations. Further *in vivo* investigations are warranted to confirm its therapeutic efficacy and safety for clinical use in managing fungal skin infections.

Keywords: Antifungal cream, *Candida albicans*, Flavonoids, Herbal formulations, *Hibiscus sabdariffa*.

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INTRODUCTION

The skin, being the largest organ of the human body, acts as a protective barrier against environmental factors such as dust, harmful UV rays, chemicals, and disease-causing microbes. It also reflects an individual's internal health and aging. Due to its constant contact with the environment, the skin is vulnerable to various infections, particularly fungal infections. For centuries, traditional systems of medicine have relied on plant-based treatments to manage such conditions. Known as herbal medicine, this approach utilizes different parts of plants—like leaves, stems, roots, flowers, and seeds to prepare formulations including creams, tinctures, and oils. Many modern drugs are developed from these traditional practices because they are effective, affordable, and usually have fewer side effects. With the rising resistance to conventional antifungal drugs, there is an increasing interest in exploring plant-based options that offer safe and reliable alternatives. Studying plant-derived compounds helps confirm their medicinal potential and identifies active ingredients with therapeutic benefits (Vikhe *et al.*, 2024; Jadhav *et al.*, 2020; Thirumurugan *et al.*, 2010; Cowan *et al.*, 1999; Koley *et al.*, 2024; Almajid *et al.*, 2023).

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The present research is aimed at developing and analyzing an herbal antifungal cream formulated with *Hibiscus sabdariffa*, a plant recognized for its skin-protective and antimicrobial qualities. Its extract is rich in beneficial compounds like flavonoids, tannins, and organic acids, which may contribute to antifungal activity. *Hibiscus sabdariffa* L., from the *Malvaceae* family, is a widely grown shrub in tropical regions, native to areas from Malaysia to India. presents its taxonomical classification. The cream will be evaluated for key parameters such as pH, viscosity, spreadability, and antifungal activity to assess its potential as a natural treatment for fungal infections (Ali *et al.*, 2005; Da-Costa-Rocha *et al.*, 2014; Herrera-Arellano *et al.*, 2004; Ibrahim *et al.*, 2020; Pawar *et al.*, 2020; Sindi *et al.*, 2023).

MATERIALS AND METHODS

Collection and Authentication of the Plant

The calyces of plant were collected from Loni Bk, Ahmednagar Dist. Maharashtra, India during the month of October 2024. The plant was identified and authenticated by Dr. S. P. Giri, Department of Botany and Research Centre P.V.P. College Loni, Ref. No./PVPC/Bot/2024-25/311.

Extraction

Soxhlet extraction is a reliable technique for isolating bioactive compounds from *Hibiscus sabdariffa* calyces. The dried and powdered calyces are placed in a Soxhlet apparatus with a solvent like ethanol. The system is heated between 50-80°C, allowing the solvent to repeatedly pass through the sample, extracting key phytochemicals such as anthocyanins and flavonoids. The process continues until no more compounds dissolve, after which the solvent is evaporated to obtain a concentrated extract. The extract is then stored at 4°C for further study and formulation. This method is widely used for obtaining plant-based compounds for medicinal and cosmetic purposes. The extractive value of the *Hibiscus sabdariffa* is mentioned in Table 1. The extraction of *Hibiscus sabdariffa* shown in the (Hapsari *et al.*, 2021; Egesie *et al.*, 2019; Ahmad *et al.*, 2017).

Preliminary Phytochemical Screening

A series of chemical tests were conducted to analyse the phytochemical composition of *Hibiscus sabdariffa* extracts. The study focused on detecting the presence of flavonoids, phenolic acids, saponins, ascorbic acid, citric acid, glycosides, and terpenes using standard qualitative methods.

Detection of Flavonoids

Lead Acetate Test

A few drops of lead acetate solution were added to the extract. The development of a yellow precipitate indicated the presence of flavonoids.

Sulfuric Acid Test

Concentrated Sulfuric Acid (H_2SO_2) was added to the extract. The formation of an orange colour confirmed the presence of flavonoids.

Shinoda's Test

The extract was mixed with small pieces of magnesium turnings, and concentrated Hydrochloric Acid (HCl) was added dropwise. A change in colour to pink, red, or occasionally green or blue indicated the presence of flavonoids.

Tests for Phenolic Compounds

Ferric Chloride Test

- **Procedure:** Add 2 mL of extract to a test tube and mix with a few drops of 1% ferric chloride solution.
- **Observation:** Formation of a blue, green, or black coloration confirms the presence of phenolic compounds.

Bromine Water Test

- **Procedure:** Add bromine water dropwise to 2 mL of extract.
- **Observation:** Decolorization of bromine water confirms the presence of phenolics.

Liebermann's Test

Two to three drops of extract were combined with acetic anhydride, followed by the addition of concentrated Sulfuric Acid (H_2SO_4). The development of a blue, green, or red colour indicated the presence of phenolic compounds.

Alkaloid Detection

Mayer's Test

The extract was treated with Mayer's reagent. The formation of a yellowish-white precipitate confirmed the presence of alkaloids.

Wagner's Test

The extract was combined with Wagner's reagent. The appearance of a brown or reddish-brown precipitate indicated the presence of alkaloids.

Dragendorff's Test

A few drops of Dragendorff's reagent (potassium bismuth iodide solution) were added to the extract. The formation of a reddish-brown coloration confirmed the presence of alkaloids.

Glycoside Identification

Borntrager's Test

2 drops of extract were mixed with dilute sulphuric acid, boiled for five minutes, and filtered. The filtrate was combined with an equal amount of chloroform, and the organic layer was separated. The addition of ammonia to this layer resulted in a pinkish-red colour, indicating the presence of glycosides (Akinjogunla *et al.*, 2011; Dineshkumar *et al.*, 2010; Harborne *et al.*, 1998; Wang *et al.*, 2015; Zhang *et al.*, 2021).

Thin Layer Chromatography

Thin Layer Chromatography (TLC) was performed to identify bioactive compounds such as flavonoids, anthocyanins, and polyphenols in the ethanolic extract of *Hibiscus sabdariffa*. The extract was prepared by drying and grinding the calyces, followed by ethanol extraction and concentration. A small amount of the extract was applied to a silica gel TLC plate and developed using a mobile phase of chloroform-methanol (4:1). After the solvent front reached an optimal height, the plate was dried and observed under UV light at 254 nm and 366 nm. The Retention factor (R_f) value was calculated using the formula: R_f = Distance travelled by solute/Distance travelled by solvent. The observed R_f value (0.89) was compared with the standard R_f value (0.92), confirming the presence of flavonoids. TLC operates on the principle of adsorption and partition chromatography, utilizing silica gel G as the stationary phase and ammonia as a spraying reagent. This analysis verified that *Hibiscus sabdariffa* contains flavonoids, supporting its potential pharmacological benefits. TLC results are depicted (Dewi *et al.*, 2023; Kartini *et al.*, 2023; Okoye *et al.*, 1998; Sirirat *et al.*, 2010; Maryani *et al.*, 2015).

RF VALUE = = = 0.89

- **Std R.F value:** 0.92.
- **Observation:** TLC confirms the presence of flavonoids.

Formulation of Herbal Cream

Selection of excipients

The formulation of the herbal cream began with the selection of excipients, where *Hibiscus sabdariffa* calyces were collected from Loni BK, Ahmednagar, Maharashtra, India, in October 2024. The plant was identified and authenticated by Dr. S. P. Giri from the Department of Botany and Research Centre, P.V.P. College, Loni (Ref. No./PVPC/Bot/2024-25/311). Raw materials and chemicals were sourced from Pravara Rural College of Pharmacy, Loni BK.

Method of preparation

The ethanolic extract of *Hibiscus sabdariffa* calyces was prepared, and the formulation process involved heating the oil phase (beeswax and cetyl alcohol) in a water bath at 70-75°C. Simultaneously, the aqueous phase, containing glycerine, distilled water, and preservatives (methyl and propyl paraben), was heated to the same temperature. The ethanolic extract was then added to the aqueous phase with constant stirring, ensuring uniform mixing. The oil phase was gradually incorporated into the aqueous phase with constant agitation to achieve proper emulsification. The mixture was stirred continuously until it cooled to room temperature and reached the desired consistency. Finally, rose oil was added for fragrance, and the cream was transferred into sterilized containers for storage and further evaluation. A detailed composition of ingredients and their respective quantities is provided in Table 2 shows the formulation of cream (Maryani *et al.*, 2015; Ningsih *et al.*, 2023; Susilawati *et al.*, 2022; Patel *et al.*, 2009; Sharma *et al.*, 2012).

Evaluation of Cream

Physical Evaluation

Physical parameters similar as colour and appearance were estimated.

Homogeneity

Formulated cream passed unity testing through visual examination post-container settling, assessing their appearance and the absence of any summations.

pH

The pH of different cream phrasings was assessed using a digital pH cadence. Specifically, 2.5 g of cream sample was precisely counted and dispersed in 25 mL of distilled water, allowing it to sit for 2 hr. pH measures for each expression were conducted and the value was reported.

Spreadability

The Spreadability of creams was assessed using a rustic block outfit equipped with a pulley at one end. This system measured the slip and drag characteristics of creams. Roughly 2 g of the cream under study were placed on a fixed ground slide. Another glass slide of the same confines as the ground slide, equipped with a hook, was placed on top to sandwich the cream. A weight of 1 kg was applied to the top slide for 5 twinkles to remove air and insure a livery cream film between the slides. Redundant cream was removed from the edges. Latterly, a 50 g weight was pulled with a string attached to the hook, and time is taken for the top slide to travel a distance of 6.5 cm was recorded. A shorter time interval indicated better.

Spreadability

Spreadability was calculated using the following formula:

$S = M \times L / T$

M = Weight in the visage (tied to the upper slide).

L = Length moved by the glass slide and.

T = Time taken to separate the slide fully each other.

Irritancy test

This test was conducted on healthy adult levy. 1 cm^2 area on the rearward face of the left hand was marked, a pea-sized quantum of cream was applied. The operation time was recorded at regular interval over a 24-hr period for the signs of erythema, edema or other adverse responses.

Wash ability

A test was conducted where a portion of the cream was applied to the skin on the reverse of the hand. After operation, the area was exposed to flowing water for a duration of 10 twinkles. The time taken for the cream to be fully removed under these conditions was recorded.

In vitro Screening for Assessment of Antifungal Activity

The agar well prolixity system begins with the medication of nutrient agar and nutrient broth, where the agar is poured into sterile Petri dishes for fungal growth, and the broth is used to culture the test fungus. A loopful of *Candida albicans* or the test fungus is invested into 2-3 mL of Mueller-Hinton Broth (MHB) and incubated at 37°C. The dressed fungi are also spread unevenly on nutrient agar plates using a sterile cotton tar, rotating the plate at a 60-degree angle for invariant distribution. Once dried, wells are created in the agar using a sterile cork borer, and these wells are filled with a positive control (Clotrimazole), a negative control (Distilled water), and the test excerpt. The plates are incubated at 27°C for 48 hr, after which the zones of inhibition are observed and measured using an antibiotic zone anthology to determine the antifungal effectiveness. To sure delicacy, the agar must be fully dissolved before sterilization at 121°C for 15 twinkles, cooled to 40 to 50°C before pouring, and allowed to solidify duly to avoid redundant humidity, which could lead to impurity and affect results. The zone of inhibition (Figure 1 and Table 1) the compliances are given (Gupta *et al.*, 2023; Gupta *et al.*, 2009; Nweze *et al.*, 2004; Parekh *et al.*, 2007).

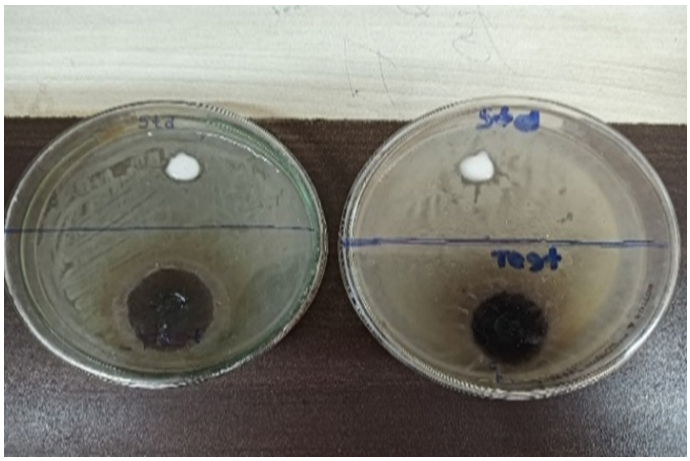

Figure 1: *In vitro Antifungal activity.*

RESULTS

Extraction of *Hibiscus sabdariffa*

The calyces of *Hibiscus sabdariffa* were shade dried, powdered, and extracted using ethanol by the Soxhlet extraction method. After concentration, a semisolid extract was obtained. The percentage yield of the extract was found to be 25%.

Physicochemical evaluation of cream

Physical Appearance

Formulation was found to be homogeneous Brown cream preparation.

Homogeneity

Developed cream was tested for homogeneity by visual inspection after the creams have been set in the container.

Measurement of pH

The pH values of prepared formulation ranged from 6-7 which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5.

Spreadability

Formulated cream has quite good spreadability.

Irritancy test

The formulated cream is non-irritant.

Washability

The cream is readily removed with water, leaving no noticeable residue.

The results of the below physical evaluation, homogeneity, pH determination, spreadability, irritancy test, and washability tests are presented in Table 3.

In vitro antifungal activity

The extract of *Hibiscus sabdariffa* exhibited greater antifungal activity than fluconazole, as indicated by its larger zone of

Table 1: ***In vitro*** **antifungal activity of the extract by agar well diffusion method.**

Sl. No.	Drug	Concentration	Zone of inhibition
1.	Fluconazole (Positive control)	100	23 mm
2.	Test sample (Extract)	100	25 mm
3.	Distilled water (Negative control)	-	No zone

Table 2: Formulation of Herbal Antifungal Cream.

Sl. No.	Ingredients	Quantity	Role of ingredients
1.	Ethanolic extract of *H. sabdariffa*	0.5	Active ingredient
2.	Beeswax	5 g	Emolient
3.	Cetyl alcohol	1.6 g	Co-emulsifier
4.	Potassium hydroxide	0.5 g	Alkali reagent
5.	Glycerine	15 mL	Moistening agent
6.	Propyl paraben	0.2 g	Preservative
7.	Methyl paraben	0.2 g	Preservative
8.	Distilled water	Q.S.	Vehicle
9.	Rose oil	Q.S.	Perfume

Table 3: Evaluation of Herbal Antifungal Cream.

Sl. No.	Parameter	Observation
1.	Colour	Light brown
2.	Appearance	Smooth, uniform
3.	Homogeneity	Homogenous
4.	pH	6.5
5.	Spreadability	10.50 g.cm/s
6.	Irritancy	Non-irritant
7.	Washability	Easily washable

inhibition. This finding highlights the extract's strong potential as an effective antifungal agent.

DISCUSSION

This study demonstrates the antifungal potential of *Hibiscus sabdariffa*, with the extract yielding 25% and containing key bioactive compounds like flavonoids and tannins. TLC analysis identified active constituents, while antifungal testing against Candida albicans showed a dose-dependent inhibitory effect, confirming its effectiveness. A herbal antifungal cream was successfully formulated, exhibiting smooth texture, good spreadability, stable pH, and long-term stability, making it suitable for topical application. The results suggest that *Hibiscus sabdariffa* could be a promising natural alternative to synthetic antifungal treatments, with potential applications in pharmaceuticals and skincare products. Further research, including *in vivo* studies and advanced compound analysis, is recommended to validate its clinical safety and therapeutic efficacy.

CONCLUSION

The formulated herbal antifungal cream containing *Hibiscus sabdariffa* demonstrated significant antifungal activity, supporting its potential as a natural remedy for fungal infections. Its effectiveness, safety, and consistency make it a promising option for further development. The study highlights the value of plant-based ingredients in dermatology, with the cream's antifungal properties attributed to bioactive compounds such as flavonoids, anthocyanins, and organic acids. Its effectiveness, stability, and safety make it a promising alternative to conventional treatments. The formulation maintains its stability over time while preserving its antifungal efficacy, making it a viable alternative to conventional antifungal treatments. Additionally, it offers benefits such as suitability for individuals seeking plant-based solutions, reduced side effects, and a lower risk of resistance development. Further studies, including safety evaluations and clinical trials, are necessary to confirm its effectiveness and explore its potential for commercial dermatological applications.

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ABBREVIATIONS

C: Degree Celsius; **CM:** Centimetre; **g:** Gram; **h:** Hour; **HCl:** Hydrochloric acid; **H_2SO_4:** Sulfuric acid; **kg:** Kilogram; **L:** Length (in cm); **M:** Mass (in g); **MHB:** Mueller Hinton broth; **min:** Minute; **mL:** Millilitre; **mm:** Millimetre.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL STATEMENT

Informed consent was obtained from healthy volunteers for the skin irritancy tests conducted during the study.

AUTHOR CONTRIBUTIONS

Dattaprasad Vikhe Ganesh Shinde contributed to Planning, conceptualization, data Collection, and paper writing Rutuja Shashikant Kamble, Vaibhav Vitthal Bhone, Sagar Dattatraya Magar contributed to data collection, literature review, and data interpretation. All authors contributed to the completion of the manuscript.

SUMMARY

Fungal skin infections remain a significant global health issue, prompting the search for safe and effective herbal alternatives to synthetic antifungal agents. This study developed and evaluated a herbal antifungal cream containing ethanolic extract of *Hibiscus sabdariffa* (roselle), known for its bioactive compounds such as flavonoids, tannins, alkaloids, and saponins. The extract was prepared via Soxhlet extraction and confirmed through phytochemical screening. Cream formulations incorporating varying extract concentrations were prepared using beeswax, cetyl alcohol, glycerine, and essential oils. Physicochemical properties, including pH, spreadability, stability, and viscosity, were assessed, alongside antifungal activity against Candida albicans using the agar well diffusion method. Results demonstrated pH within the ideal topical range, good spreadability, and stability without phase separation over four weeks. Higher extract concentrations produced inhibition zones.

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