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Research Paper

Development of Herbal Film- Forming Spray for the Management of Ringworm

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ABSTRACT

Topical fungal infections such as dermatophytosis (ringworm) remain a common dermatological concern, particularly in tropical regions, and require effective, patient-friendly treatment options. The present study aimed to develop and evaluate an herbal film-forming spray (FFS) containing Cassia alata extract as a potential alternative to conventional topical formulations. Film-forming sprays are designed to form a thin, flexible film on the skin after application, thereby enhancing drug residence time, improving adherence, and enabling sustained drug release. The Cassia alata extract was prepared and subjected to phytochemical screening, Further standardization using thin layer chromatography (TLC). The prepared formulations were evaluated for physicochemical parameters including pH, viscosity, drying time, etc. Formulations demonstrated acceptable properties, forming clear, uniform, and non-sticky films with suitable pH for skin application. Overall, the developed herbal film-forming spray showed promising antifungal efficacy along with desirable formulation characteristics, suggesting its potential as a convenient and effective alternative for the management of superficial fungal infections. Further studies focusing on clinical evaluation and long-term stability are warranted to support its practical application.

INTRODUCTION

The skin is the largest and most accessible organ of the human body, serving as a primary protective barrier against environmental agents, including microorganisms and various chemical substances.

Its low permeability plays a crucial role in preventing the entry of both micro- and macromolecules. In an average adult, the skin covers an approximate surface area of 2 m² and receives nearly one-third of the body's total blood circulation, highlighting its physiological

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significance. In recent years, the incidence of fungal infections has increased considerably, emerging as a major concern for healthcare systems worldwide and posing significant challenges for clinicians in terms of effective management and treatment. Fungal infections involving the skin, nails, hair, and mucous membranes are all examples of topical fungal infections. ⁽¹⁾

Ringworm, also known as dermatophytosis or tinea, is a common superficial fungal infection affecting keratinized tissues such as the skin, hair, and nails. It is caused by a group of fungi known as dermatophytes, primarily belonging to the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*. These organisms have the unique ability to utilize keratin as a nutrient source, enabling them to colonize and infect the

outer layers of the body. Despite its name, ringworm is not caused by a worm but by fungi that produce characteristic ring-shaped, erythematous, and scaly lesions on the skin. The infection is typically limited to the non-living, cornified layer of the epidermis and rarely invades deeper tissues in immunocompetent individuals. ⁽²⁾ Dermatophytosis is one of the most prevalent fungal infections worldwide and represents a significant public health concern, particularly in tropical and developing regions. It spreads through direct contact with infected individuals or animals, as well as indirectly via contaminated objects or environments. Clinically, ringworm manifests in different forms depending on the site of infection, such as tinea capitis (scalp), tinea corporis (body), tinea pedis (feet), and tinea cruris (groin). ⁽³⁾



Figure 1. Ringworm infection showing round, reddish, scaly patches on the skin.

Natural plant extracts are increasingly being recognized for their potential in treating infections because they are rich in bioactive compounds that can target specific pathogens while gradually breaking down into harmless substances. Two well-known tropical plants that have been traditionally used for medicinal purposes are *Cassia alata* L. (ringworm cassia) and *Curcuma longa* L. (turmeric). These plants are native to regions across Southeast Asia, Africa, South America, and Northern Australia and have long been valued by local communities for their healing properties.

In Thailand, *C. alata* is commonly grown both as an ornamental plant and as a medicinal herb, especially for treating ringworm and other skin conditions, while *C. longa* is a familiar household plant used in daily cooking as well as a traditional remedy for various ailments. The leaves of *C. alata*, in particular, are known to have multiple health benefits: they act as a strong laxative, reduce inflammation, relieve pain, stimulate urination and perspiration, aid digestion, repel insects, and combat bacteria, fungi, and other parasites. Research has shown that these effects are largely due to the presence of active

compounds such as alkaloids and flavonoids, which directly contribute to the plant's antimicrobial activity against harmful bacteria and fungi. ⁽⁴⁾



Figure 2. *Cassia alata* Plant

Topical drug delivery systems are designed to achieve either localized or systemic therapeutic effects through application on the skin. This route offers several advantages, including the avoidance of first-pass hepatic metabolism, protection of drugs from degradation by gastric pH and enzymes, and utilization of the large surface area of the skin for drug absorption. To enhance therapeutic efficacy and optimize pharmacokinetic profiles, drugs intended for topical administration are commonly formulated into various dosage forms such as patches, gels, lotions, creams, ointments, and sprays. ⁽⁵⁾

Despite these benefits, conventional topical formulations present certain limitations. Transdermal patches, for instance, may retain residual drug after use, raising concerns regarding drug wastage and potential misuse. Additionally, they are often associated with adverse skin reactions such as hypersensitivity, irritation, and blister formation. Manufacturing challenges, particularly during scale-up, can also arise due to issues related to drug stability and the risk of crystallization during storage. Similarly, semisolid formulations like creams and ointments can be inconvenient in daily use, as they may adhere to

clothing and require manual application, increasing the risk of contamination and cross-infection, especially in wound management. ⁽⁶⁾

In comparison, topical spray formulations offer several notable advantages. They provide ease of application, reduce the likelihood of direct contact and contamination, and ensure better patient compliance. Sprays also allow uniform drug distribution over the affected area, offer adjustable dosing, and are generally associated with a lower incidence of skin irritation. Furthermore, their ability to deliver sterile formulations makes them particularly suitable for sensitive or infected skin conditions. In recent decades, significant advancements have been made to develop more efficient and effective spray-based drug delivery systems. Among these, film-forming sprays (FFS) have emerged as a promising and versatile innovation, finding applications across diverse fields such as the food industry, cosmetics, pharmaceuticals, and agriculture. In pharmaceutical applications, FFS are particularly valued for their ability to form a thin, uniform film upon application, which enhances drug retention and controlled release at the site of action. Typically, film-forming sprays are composed of active pharmaceutical ingredients, film-forming polymers, and permeation enhancers, all dissolved or dispersed in suitable volatile organic solvents. Upon application, the solvent rapidly evaporates, leaving behind a coherent film on the skin that facilitates sustained drug delivery and improves therapeutic effectiveness. A thin, non-sticky film forms that can increase the contact time and permeability of the drug, resulting in continuous drug release, and can prevent crystallization so that more drug is available to provide therapeutic effects compared to other conventional topical preparations. ⁽⁷⁾

Topical film-forming systems (FFSs) are a promising approach for antifungal therapy. After application, the solvent evaporates, leaving a thin

polymeric film on the skin. This film acts as a drug reservoir, increasing residence time, improving patient comfort, and offering greater convenience compared with conventional creams and gels. FFSs show superior benefits in treating superficial fungal infections by enhancing substantivity, providing sustained drug delivery, and improving adherence. ⁽⁸⁾

MATERIALS AND METHOD:

MATERIAL:

Cassia alata extract acts as the active ingredient, providing antifungal and skin-protective effects, especially useful for treating infections like ringworm. Distilled water and Ethanol serves as the primary solvent and vehicle, ensuring safe, uniform dissolution and application of all components. Hydroxypropyl methylcellulose E15 (HPMC E15) functions as the film-forming polymer, creating a thin, protective layer on the skin after spraying, which helps in sustained drug release and protection of the affected area.

Polyethylene glycol 400 (PEG 400) acts as a plasticizer and co-solvent, improving the flexibility, spread ability, and uniformity of the formed film. Rosemary extract is included as a natural preservative due to its antioxidant and antimicrobial properties, helping to prevent microbial growth and enhance the stability of the formulation. Together, these ingredients form an effective and stable topical film-forming spray system.

METHOD OF EXTRACTION

Extraction was carried out by the Cold Maceration Method.

Powdered leaves of *C. alata* (10 g) were subjected to maceration using 80% ethanol (100 mL) as the extraction solvent. The process was continued and repeated until complete extraction was achieved, as confirmed by a negative Borntrager's reaction. The resulting macerates were pooled, filtered to remove particulate matter, and concentrated by evaporation to obtain the ethanolic extract in liquid form. ⁽⁹⁾

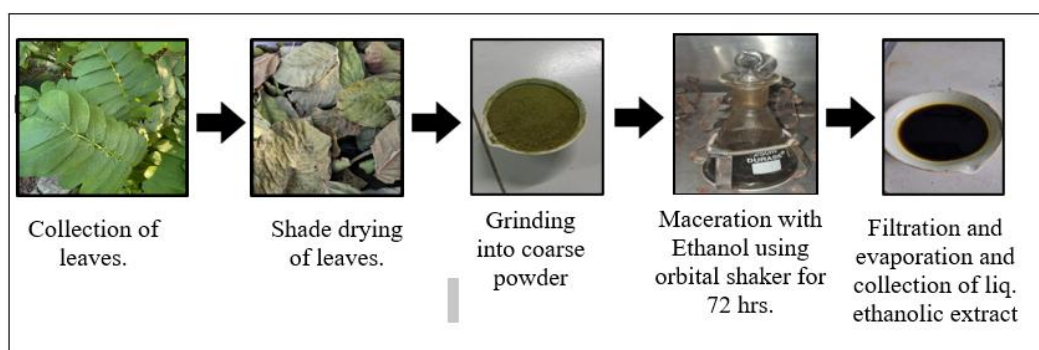


Figure 3. Extraction method

STANDARDIZATION OF EXTRACT

The different qualitative chemical tests were carried out on the extract using standard procedures to identify the constituents

Phytochemical screening

Preliminary qualitative phytochemical screening of the aqueous extract was carried out using standard procedures to identify the major classes of bioactive constituents.

a) Test for Alkaloids : To 1 mL of the extract, 2 mL of Dragendorff's reagent was added. The formation of a turbid orange precipitate indicated the presence of alkaloids.

b) Test for Tannins: A mixture of 1 mL of the extract and 2 mL of ferric chloride solution produced a dark green coloration, confirming the presence of tannins.

c) Test for Saponins (Foam Test): The extract (1 mL) was diluted with 2 mL of distilled water and

shaken vigorously. The formation of stable, persistent foam lasting for about 10 minutes indicated the presence of saponins.

d) Tests for Proteins and Free Amino Acids: A small quantity of the extract was mixed with water and subjected to the following tests:

Millon's Test: Addition of a few drops of Millon's reagent (mercuric nitrate solution) resulted in the development of a pink colour, indicating the presence of proteins.

Ninhydrin Test: Treatment with 0.1% w/v ninhydrin solution in n-butanol produced a purple coloration, confirming the presence of free amino acids.

e) Test for Anthraquinone Glycosides (Borntrager's Test): The hydrolysed extract was shaken with 1 mL of chloroform. The chloroform layer was separated and treated with an equal volume of dilute ammonia solution. The appearance of a pink colour in the ammoniacal layer confirmed the presence of anthraquinone glycosides.

f) Test for Flavonoids (Alkaline Reagent Test): The extract was treated with a few drops of sodium hydroxide solution, producing an intense yellow colour. This colour disappeared upon the addition of dilute hydrochloric acid, indicating the presence of flavonoids.

g) Test for Carbohydrates (Benedict's Test): The extract was treated with Benedict's reagent and heated. The formation of a green, yellow, or brick-red precipitate confirmed the presence of reducing sugars. ⁽¹⁰⁾

THIN LAYER CHROMATOGRAPHY:

The present study involves the standardization of *Cassia alata* Leaf extract using Thin Layer Chromatography (TLC). TLC analysis was carried out on silica gel using toluene, ethyl acetate, and formic acid (5:4:1) as the mobile phase. The extract was applied on the plate and developed in a saturated chamber. After development, the plate was dried and observed under UV light at 254nm. Further detection was done by spraying with 10% KOH solution, which produced pink to red spots indicating the presence of anthraquinones. The R_f values of the spots were calculated and used to characterize the phytoconstituents present in the extract. The TLC profile showed multiple spots indicating different compounds and provided a characteristic fingerprint for the identification and standardization of *Cassia alata* extract. ⁽¹¹⁾

PREPARATION OF FILM- FORMING SPRAY:

All the ingredients were measured according to quantity mentioned in table no:1 and three batches were prepared. Then first, Mix Ethanol and distilled water in a beaker (8:2), then, Dissolve Film-forming agents (HPMC E15) completely, and then add the drug, and mix it on a magnetic stirrer till it becomes homogeneous for 20min. Lastly, add PEG400 to the formulation and stir it again for 15 minutes, then Transfer this FFS (film-forming solution) into a container. ⁽¹²⁾

Table 1. Formulation Composition

Sr.no	Ingredients	Purpose	Formula1 (F1)	Formula2 (F2)	Formula3 (F3)
1.	<i>Cassia alata</i> extract	Antifungal agent	5%	7.5%	10%
2.	HPMC E15	Polymer	4%	4%	4%
3.	PEG 400	Plasticizer	2%	2%	2%
4.	Rosemary extract	Preservative	0.5%	0.5%	0.5%
5.	Ethanol : Water (8:2)	Solvent	q.s	q.s	q.s



EVALUATION PARAMETERS:

The prepared FFS was visually inspected for color, homogeneity, and clarity.

Physical appearance:

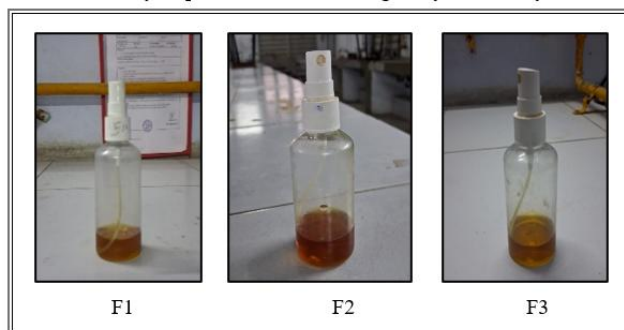


Figure 4. Pictures of prepared formulation

pH:

The pH of the optimized formulation was determined using a calibrated digital pH meter. The electrode was immersed directly into the film-forming solution, and the pH value was recorded once a stable reading was obtained. Adjusting the pH of the formulation is essential to minimize the risk of skin irritation and to maintain the physiological conditions required for effective wound healing. Furthermore, the pH of the dosage form plays a crucial role in drug permeation through the skin, as it influences the degree of ionization of the drug, thereby affecting its absorption and overall therapeutic efficacy. ⁽¹³⁾

Viscosity:

The viscosity of film-forming solutions is significantly influenced by the type and concentration of the polymer used. Variations in these parameters can lead to noticeable differences in the flow behavior of the formulation. Viscosity is a critical factor affecting the spray ability of the system, as it determines the ease with which the formulation can be atomized and uniformly applied. An increase in polymer concentration generally leads to higher viscosity, which may reduce the spray coverage area and hinder uniform distribution of the formulation over the skin surface. Therefore, optimizing viscosity is essential to ensure efficient application and

consistent performance of the film-forming spray. ⁽¹⁴⁾

Water Washability:

The ease of film removal was evaluated on the dried film to determine its washability. The formed film was exposed to water and gently rinsed, after which its removal was assessed using an ordinal scale categorized as easily washable, moderately washable, or poorly washable. This parameter is particularly important for ensuring user safety and convenience, especially in cases where the formulation may accidentally come into contact with sensitive areas such as the eyes or oral cavity. An easily washable film is desirable to allow quick and effective removal without causing irritation or discomfort. ⁽¹⁵⁾

Occlusion Potential or Water Vapor Permeability of the Film:

The permeability of the film to wound fluid is also vital to determine because it affects the moisture of the wound. Excessive humidity will trigger the growth of microorganisms and lead to infection. This test is done by covering the mouth of a glass beaker containing 50 mL of water with filter paper. One of the papers is sprayed with a film-forming solution and allowed to form a film. The beaker is then stored at room temperature and humidity. The permeability of the film to water is determined based on the reduced water weight in the beaker.

The assessment is determined using the following formula.

$$F = \frac{A - B}{A} \times 100$$

Where F is the occlusivity factor and A is the reduction in water weight in the glass beaker covered with the filter paper without a film. In contrast, B value is the reduction in water weight in the glass beaker covered with the filter paper coated by the film. The smaller the occlusivity factor value, the better the film's permeability. ⁽¹⁶⁾

Stickiness:

The stickiness of the dried film was evaluated using a cotton wool adhesion test. In this method, the dried film surface was gently pressed with a piece of cotton wool, and the extent of fibre attachment was observed. The degree of adhesiveness was categorized based on the amount of cotton fibres adhering to the film: a high level of stickiness was indicated by the attachment of many fibres, moderate stickiness by a smaller amount of fibre attachment, and low stickiness by minimal or no fibre adherence. This parameter is important in determining the practical usability of the formulation, as excessive stickiness may lead to unintended adhesion to clothing or other surfaces during daily activities. Therefore, an optimal level of adhesiveness is desirable to ensure both effective film formation and patient convenience. ⁽¹⁷⁾

Drying Time:

The drying time of the formulation was determined by applying the film-forming solution onto the inner surface of the forearm or alternatively onto a Petri dish. At predetermined time intervals, a clean glass slide was gently placed in contact with the film surface and then removed. The film was considered completely dry when no visible liquid residue adhered to the glass slide upon removal. Drying time is a critical parameter in evaluating the performance of film-forming sprays, as a shorter drying duration

enhances patient convenience and compliance. An efficient formulation should exhibit minimal drying time to ensure rapid film formation without causing discomfort or delay during application. ⁽¹⁸⁾

Volume Actuated Upon Each Spray:

After each actuation, the weight difference was recorded and the volume delivered was estimated using the formula,

$$WT = WO - AL/Dn,$$

Where, AL = amount of solution delivered with each actuation, WT = formulation's load following an actuation, WO = formulation's initial weight before an actuation, Dn = density. ⁽¹⁹⁾

Thin layer Chromatography:

Prepared formulation was evaluated by TLC to identify the presence of active constituents like anthraquinone in the formulation. TLC analysis was carried out on silica gel using toluene, ethyl acetate, and formic acid (5:4:1) as the mobile phase. After development, the plate was dried and detection was done by spraying with 10% KOH solution, which produced pink to red spots indicating the presence of anthraquinones. Thin layer chromatography (TLC) is commonly employed for identification, purity assessment, and compatibility studies during formulation development. ⁽²⁰⁾

Microbial Assay:

The antifungal activity of *Cassia alata* extracts was evaluated using agar well diffusion method, the antifungal activity of *Cassia alata* extracts was assessed and contrasted with ketoconazole, a common antifungal drug. After 48 hours of incubation at 28 – 30°C, the zone of inhibition against the tested fungal strain was determined using extracts made at concentrations of 5%, 7.5%, and 10% extracts, respectively. The results showed with zones of inhibition of 25.5mm, 28mm and 30.5mm for 5%, 7.5%, and 10% extracts, respectively, the result showed a concentration-dependent antifungal action of *Cassia alata*. By contrast, at the equivalent concentrations,



ketoconazole showed zones of inhibition of 25mm, 25mm at the corresponding concentrations. These findings indicate that *Cassia alata* extract has strong antifungal activity, which at higher concentration increases that of standard drug. Hence *Cassia alata* has antifungal activity which can be used for topical applications. ⁽²¹⁾

RESULT & DISCUSSION

Standardization of extract:

a. Phytochemical screening

Phytochemical screening showed presence of flavonoid, anthraquinone, alkaloids, tannins, carbohydrate, and amino acids. some components like saponins and proteins were absent. This confirms antifungal potential of the extract.

Table 2. Phytochemical test

Sr No.	Test	Constituents	Inference
1.	Alkaline reagent test	Flavonoid	(+) Positive

2.	Bontrager's test	Anthraquinone	(+) Positive
3.	Dragendorff's test	Alkaloids	(+) Positive
4.	Ferric chloride test	Tannin	(+) Positive
5.	Benedict's test	Carbohydrate	(+) Positive
6.	Foam test	Saponins	(-) Negative
7.	Ninhydrin Test	Amino acid	(+) Positive
8.	Millon's Test	Protein	(-) Negative

b. Thin Layer Chromatography

TLC was performed to standardize the ethanolic extract of *Cassia alata* and to confirm the presence of active constituents, mainly anthraquinones. A distinct fluorescent/ yellow spot was observed under UV light and pink spot was observed after spraying 10% KOH alcoholic solution which confirm the presence of anthraquinones. The R_f value obtained was 0.58, which lies within the standard range (0.45- 0.65).

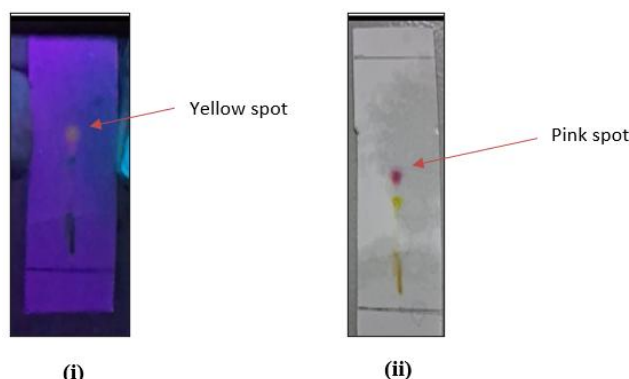


Figure 5. Pictures of TLC Plates (i) under UV observation, (ii) detection by reagent

➤ EVALUATION PARAMETERS RESULTS

Table 3. Results

Sr.no.	Parameters	F1	F2	F3
1.	Color	Yellowish- brown	Yellowish- brown	Yellowish- brown
2.	pH	5.5 ± 0.12	5.5 ± 0.08	6.5 ± 0.15
3.	Viscosity	$80 \pm 2.5\text{cP}$	$90.6 \pm 3.1\text{cP}$	$64 \pm 1.8\text{cP}$
4.	Water Washability	$37 \pm 2\text{sec}$ (Moderately wash)	$30 \pm 1.5\text{sec}$ (Easily wash)	$23 \pm 1.2\text{sec}$ (Easily wash)
5.	Occlusion Factor	$22\% \pm 1.1$	$24\% \pm 0.9$	$33.33\% \pm 1.4$

6.	Stickiness	No	No	No
7.	Drying Time	3min 20sec $\pm$ 5sec	3min 25sec $\pm$ 4sec	3 min 35sec $\pm$ 6sec
8.	Volume/spray	0.093 $\pm$ 0.004ml	0.069 $\pm$ 0.003ml	0.082 $\pm$ 0.005ml

➤ Microbial Assay:

The antifungal activity of the developed formulations was evaluated against *Candida albicans*. All formulations exhibited clear zone of

inhibitions with F3 demonstrating the highest activity(30.5 $\pm$ 0.9 mm), significantly out performing F1(25.5 $\pm$ 0.8 mm) and F2(28 $\pm$ 1.2 mm).

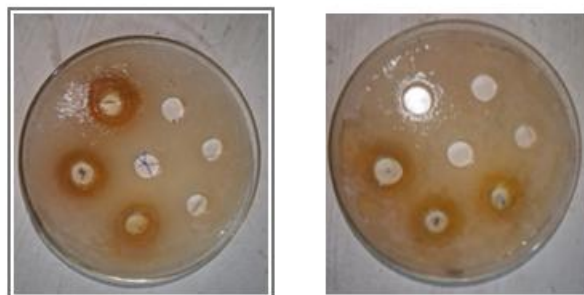


Figure 6. Zone of Inhibition

Table 4. Result of Zone of Inhibition

FORMULATION	ZONE OF INHIBITION (mm)
F1	25.5 $\pm$ 0.8
F2	28.0 $\pm$ 1.2
F3	30.5 $\pm$ 0.9
Standard (Control)	32.0 $\pm$ 0.5

➤ TLC of Formulation:

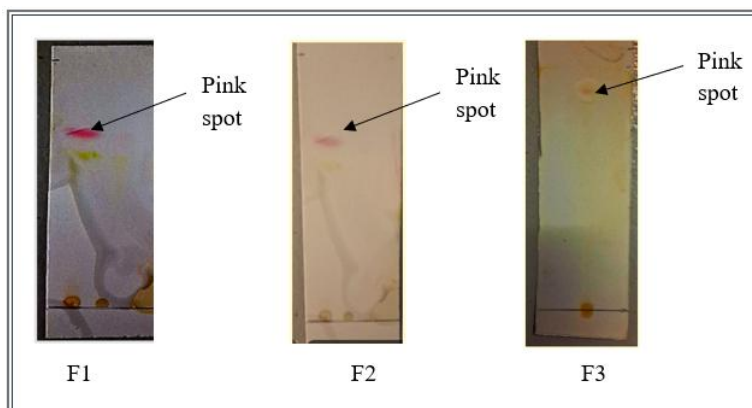


Figure 7. TLC Plate of Formulation

CONCLUSION

In conclusion, the development of an herbal antifungal film-forming spray incorporating *Cassia alata* extract has demonstrated promising

potential as an effective alternative treatment for topical fungal infections. The formulation exhibited notable antifungal activity, as *Cassia alata* contains bioactive compounds known to be effective against dermatophytes and other

pathogenic fungi. The prepared spray showed desirable physicochemical properties, including appropriate viscosity, rapid drying time, uniform film formation, and clarity, indicating its suitability for easy application and improved patient compliance.

Among the different formulation variants developed, the F3 formulation showed superior film-forming ability, good adhesion, resulting in a smooth, non-sticky protective layer on the skin. The absence of phase separation and the stability of the formulation further support its reliability as a shelf-stable product. However, further studies are recommended to evaluate its clinical efficacy, long-term stability, and wider applications in dermatological therapy.

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