

## **DEVELOPMENT AND EVALUATION OF A TOPICAL FILM-FORMING SPRAY CONTAINING SENNA ALATA EXTRACT FOR FUNGAL INFECTIONS**

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### **ABSTRACT**

**Background:** Topical drug delivery systems, including sprays, are gaining popularity for the treatment of skin disorders such as fungal infections. These systems allow direct application of the active drug to the affected area, offering localized treatment without the side effects associated with oral administration. In this study, a novel film-forming topical spray containing *Senna alata* (L.) Roxb. extract was developed for the treatment of fungal skin infections. **Objective:** To formulate and evaluate a film-forming spray using ethanolic extracts of *Senna alata* for its antifungal activity and assess its physical properties and performance in treating common dermatophyte infections. **Methods:** The ethanolic extract of *Senna alata* was incorporated into three different formulations of a film-forming spray. The spray's components included Isopropyl Alcohol (IPA), Isopropyl Myristate (IPM), Propylene Glycol (PG), and various polymers like Carbopol 940, HPMC, and CMC. The physical properties of the formulations, such as pH, viscosity, drying time, and stickiness, were evaluated. The antifungal efficacy was tested against three fungal species: *Aspergillus flavus*, *Penicillium spp.*, and *Candida albicans*, using the zone of inhibition method. **Results:** The film-forming sprays demonstrated favorable physical characteristics, including non-sticky films, optimal pH, and appropriate viscosity. Formulations F1 and F2 showed significant antifungal activity against *Aspergillus flavus* and *Penicillium spp.*, with zones of inhibition up to 18 mm. However, all formulations exhibited reduced efficacy against *Candida albicans* compared to the standard ketoconazole. **Conclusion:** The developed *Senna alata* extract-based topical spray was successful in enhancing the antifungal effects on selected dermatophytes, demonstrating its potential as an alternative treatment for fungal skin infections. Further optimization and clinical trials are needed to confirm its efficacy and safety in broader populations.

**KEYWORDS:** *Senna alata*, Topical spray, Fungal infection, Dermatophytes, Skin treatment, Zone of inhibition.

## 1. INTRODUCTION

A topical medication is a medication that is applied to a particular place on the body. Most often topical administration means application to body surfaces such as the skin or mucous membranes to treat ailments via a large range of classes including creams, foams, gels, lotions, and ointments. Topical products do not cross the blood brain barrier, and it is not meant to be ingested by humans or animals. Most commonly, a topical drug delivery system is applied to the skin, where the medicine either treats only the area of application or absorbed into the bloodstream through the dermis.

Film forming spray generally consist of active substances, enhancers, and polymers that are dissolved in organic solvents' thin, non-sticky film forms that can increase the contact time & permeability of the drug, resulting in continuous drug release, and can prevent crystallization so that more drug is available to provide therapeutic effect compared to other conventional topical preparation. The type of nozzle, the size of the aperture, the pressure of spray applied, and the nature of the liquid strongly influence the spray ability of film forming spray.

Topical spray offer many advantages compared to conventional topical preparations because they can provide uniform drug distribution and dose, increased bioavailability, lower incidence of irritation, continuous drug release, and accelerated wound healing through moisture control. The spray film formed can either be a residual liquid film that is absorbed instantly via the stratum corneum or a solid polymeric substance that behaves like a matrix for the prolonged drug release.



**Fig. 1: Topical Spray.**

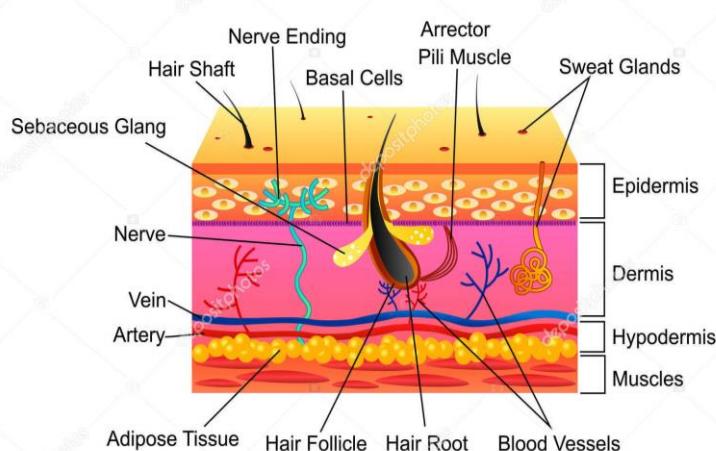
### 1.1 Anatomy and Physiology of Skin

The human body has two systems that protect it from the harmful organisms existing in the environment. The internal defense system destroys microorganisms and bacteria that have already attacked the body. The external defense system prevents microbial microorganisms to enter the body. Skin is biggest external defense system. Skin covers the outside of the body but has other functions beside the defense mechanism. It serves as a mechanical barrier between the inner part of the body and the external world. Temperature of skin varies in a range of 30 to 40°C degree depending on the environmental conditions. Anatomy of skin is the largest organ in the body. It consists of three layers. The outer layer is called epidermis, the middle layer is dermis and the inner most layer is hypodermis.

Epidermis are consists of epithelial cells. Among these cells, both living cells and dead cells can be found. These new cells at the bottom of epidermis divide fast and push the older cells upward. The epidermis does not have any direct source of blood veins to provide nutrition. It takes its nutrients from the diffusion of necessary molecules from a rich vascular network in the underlying dermis. Epidermal cells are connected very strongly by desmosomes. Desmosomes are in contact with the intracellular keratin filaments. Keratin filaments produce keratin. Keratin cells accumulate and

crosslink with the other keratin cells in the cytosol during their maturation. Afterward when the older cells die, this network of keratin fibres remains and provides a tough and hard protective layer in epidermis, called protective keratinized layer.

This layer is water proof and airtight. It prevents most substances to enter the body or leave from the body. In diseased skin, particularly burns, epidermis is destroyed causing potential loss of body fluid and an increase in susceptibility to microbial infections, leading to fatal consequences untreated.



**Fig. 2: Human Skin.**

### 1.2 Fungal Infection

A fungal infection, also called mycosis, is a skin disease caused by a fungus. There are millions of species of fungi. They live in dirt, on plants, on household surfaces, and on your skin. Sometimes, they can lead to skin problems like rashes or bumps. Different types of fungi can cause fungal infections. In some cases, fungi typically found on or inside your body can multiply out of control and cause an infection. Fungal infections can be contagious. They can spread from one person to another.

Currently, fungal skin infection is one of the most serious dermatological concerns in the world. It has been found that in developing and underdeveloped countries, about 40 million people have suffered from fungal infections. One of the most common causes of tinea and onychomycosis is dermatophytes. Also, among the most common surface skin fungal infections. Topical distribution is an effective route for local and systemic treatment of the infections. Drug delivery to the skin is a well-known method of treatment for local dermatological diseases. It can result in deeper penetration into the skin and thus improve the absorption of the drug.

A fungus that invades the tissue can cause a disease that's confined to the skin, spreads into tissue, bones and organs or affects the whole body. Approximately 40 different species of fungi can cause ringworm; the scientific names for the types of fungi that cause ringworm are *Trichophyton*, *Microsporum* and *Epidermophyton*. Anti-fungal drugs are used to prevent fungal growth; active against fungi. Anti-fungal liquids or sprays can be used to treat skin fungal infections, scalp and nails. These antifungal drugs can cause various types of adverse effects such as the site of application. There are over 100,000 fungal species, about 150 species are pathogenic to humans.



**Fig. 3: Fungal Infection.**

## 2. PLANT PROFILE

*Senna alata* (L.) Roxb. Is a flowering shrub of the fabaceae family. It has the name “candle bush” owing to the framework of its inflorescences. It is an annual and occasionally biannual herb, with an average height of 1 to 4m, burgeoning in sunlit and humid zones. The leaves are oblong, with 5 to 14 leaflet sets, robust petioles (2 to 3 mm), caduceus bracts (2=3/1=2 cm), and dense flower (20=50 /3=4 cm).zygomorphic flowers have bright yellow color with seven stamens and a pubertal ovary. The fruit exist as a 10 to 16=1.5 cm tetragonal pod, thick, flattened wings, brown when ripe with many diamond –shaped brown seeds. It is propagated by seeds and dispersed to about 1500 meter above the sea level.



**Fig. 4: *Senna alata* (L.).**

### 2.1 Scientific Classification

- **Kingdom:** Plantae
- **Order:** Fabales
- **Family:** Fabaceae
- **Subfamily:** Caesalpinoideae

### 2.2 Vernacular Name

- **Scientific name:** *Senna Alexandrina*.
- **Tamil Name:** Seemai Agathi, Vandu raali.
- **English Name:** *Cassia alata*, craw- craw plant acapula / ringworm plant.
- **Sanskrit Name:** Chakra Maida/ Dadrughna.
- **Local Name:** Eth thora, Rata tora.
- **Kanada Name:** Seema Tangadi.
- **Telugu Name:** Sema Tangedu.

### 2.3 Morphological Characters

- **Weed Type:** Broad leaf
- **Global Description:** Shrub to 3 meter or more. Branchlets olive-brown, longitudinally ridged, usually minutely puberulous, glabrescent.
- **Stem:** The plants are branched, woody, and upright.
- **Leaves:** par pinnate; stipules asymmetrically triangular, persistent; rachis generally 25 to 75cm long; leaflets in (5)7-12 pairs, 6.5 to 8 into 3-3.5cm (much larger in south American material), oblong, asymmetrically rounded at base, rounded and apiculate at apex, sparsely pubescent mainly on the veins beneath glabrescent.
- **Inflorescence:** Inflorescence racemose, axillary, to 25cm long; bracts orange, 2.1-1.3cm, broadly obviate, falling before anthesis but prominent earlier. Petals golden yellow with dark veins, particularly when dry, 13-15 = 10-12mm, broadly elliptic. Stamens 10, two with anthers 10mm long, five with anthers 4-5mm long; three with anthers 1-2 mm long. Ovary 10-12mm long, densely pubescent, longitudinally ridged; style 1-2mm long; style 3-4mm long, almost glabrous.

### 2.4 Pharmacological Activities

Medicinal plants belonging to the Fabaceae family have extensively been investigated for their pharmacological activities. Plants synthesised array of secondary metabolites which contribute to its therapeutic activities. Therapeutic appraisal of *S. alata* authenticates the ethno biological claims and establishes the pharmacological activities. There are many published articles connected to diverse therapeutic activities of *S. alata* which are mainly related to antibacterial, antidiabetic, antilipogenic, antifungal, antioxidant, dermatophytic, antihyperlipidemic, and anthelmintic activities among others. Few studies have also reported its antimalarial activities.

## 3. MATERIALS AND METHODS

### 3.1 Preparation of Ethanolic Extracts

Collect fresh whole plant of *senna alata* were cleaned with water & shade dried until a constant weight was obtained & subsequently powdered & sieved mesh no 40. Powdered material 5kg was defatted with petroleum ether & marc was extracted with of 90% of ethanol v/v at 50 degree in soxhlet apparatus 1L for 72hr. dark brown semi – solid residues. 525g was obtained by evaporating the ethanol extract under reduced pressure.

### 3.2 Formulation of Spray

**Table 1: Formulation Table.**

Ingredients	F1	F2	F3
Plant Extract	5 ml	5 ml	5 ml
IPA	2 ml	2 ml	2 ml
IPM	1.5 ml	1.5 ml	1.5 ml
PG	1.5 ml	1.5 ml	1.5 ml
Carbopol 940	0.50 gm	-	-
HPMC	-	0.75 gm	-
CMC	-	-	1.0 gm
Methyl Paraben	0.5 gm	0.5 gm	0.5 gm
Menthol	1 gm	1 gm	1 gm
Citric Acid	0.8 gm	0.8 gm	0.8 gm
Acetone	25 ml	25 ml	25 ml
Alcohol	65 ml	65 ml	65 ml

## Procedure

The spray developed as topical solution made up of non aqueous vehicle, cooling agent. Plant extract was dissolved in ethanol in a separate vessel until clear solution was obtained. The polymeric system was prepared by incorporating Isopropyl alcohol and acetone into solvent system. With continuous stirring propylene glycol, Menthol and isopropyl myristate was added to the mixture. The pH was adjusted by adding citric acid into spray system. Volume of final solution was made up by ethanol in such a way that desired amount of drug could be obtained after each actuation, solution was stirred and pH was observed.



**Fig. 5: Anti – Fungal Topical Spray F1 – F3.**

### 3.3 Evaluation Parameters

#### 3.3.1 pH

Using the digital pH meter, the pH of the optimized spray solution was calculated. The pH meter was adjusted using phosphate buffer of different pH values (4.0, 7.0 and 9.0) before calculating the pH of the optimized formulation. The pH was determined for the spray solution. Each formulation was measured in triplicate and then the mean values were calculated.

#### 3.3.2 Viscosity

Viscosity was calculated at  $25 \pm 1^\circ\text{C}$  using a Ostwald viscometer.

#### 3.3.3 Drying Time

Evaporation time is the time needed to dry the spray film. It was measured by spraying the formulation on a glass slide and noting down the drying time.

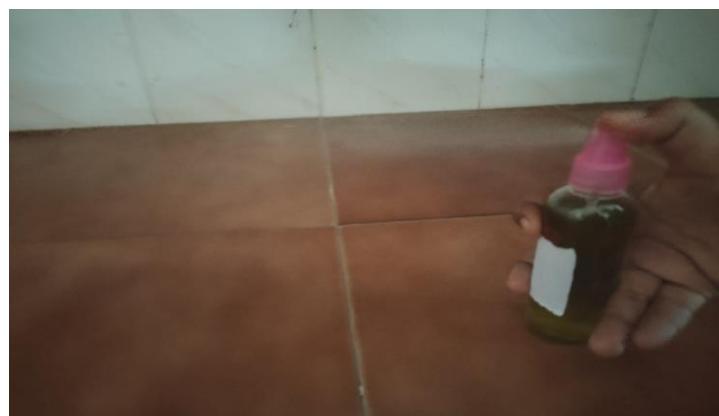
#### 3.3.4 Stickiness of the Film after Evaporating the Solvent

Low pressure cotton wool is used to press the dry film in order to determine the stickiness of it. The stickiness is rated depending on how much of the cotton fibers is retained by the film. The stickiness is rated high if there is a thick accumulation of fibers on the film, medium if there is a thin fiber layer on the film and poor if fiber adherence occurs

rarely or never. This parameter of assessment is important, as the developed formulation is supposed to be non-sticky to prevent sticking to the clothing of the patients.

### 3.3.5 Flammability & Flame Extension

To evaluate flame projection, flammability testing was done by filling the container with LPG gas in the pressurized gas system. Flame projection (cm) was maintained as a medium with a flame gas and a spray distance of 50 cm which was applied to paper to evaluate flashback of LPG gas flame (cm).



**Fig. 6: Flammability & Flame Extension.**

### 3.3.6 Leak Test

As mentioned below, two types of leak tests were performed.

- **Immediate leak test:** After filling in warm water (around 50°C), the aerosol containers were allowed to sink for about 10 seconds. In the jar, if the bubbling takes place, it indicates the leakage.
- **Delayed leak test:** Exactly weighed aerosol containers were stored for 2 months at room temperature. The containers are weighed again after two months. The leakage of container is identified as difference in the weight of a container.

### 3.3.7 Spray Angle

First, the distance from nozzle between papers was fixed. After that, one actuation was sprayed onto paper and the circle size was measured. Spray angle is calculated as:

$$\text{Spray angle } (\theta) = \tan^{-1} 2 \times \left( \frac{1}{R} \right)$$

Where, 1 and R are the paper's distance from the nozzle and average circle radius, respectively.



**Fig. 7: Spray Angle.**

### 3.4 Anti – Fungal Activity

#### 3.4.1 Preparation of Agar Medium

Prepare MHA from the dehydrated medium according to the manufacturer's instructions. media should be prepared using distilled water or deionizer water. Heat with frequent agitation and boil to dissolve the medium completely. Sterilized by autoclaving at 121°C for 15 minutes.

Check the PH of each preparation after it is sterilized, which should be between 7.2 and 7.4 at room temperature. This is done by macerating a small amount of medium in a little distilled water or by allowing a little amount of medium to gel around a PH meter electrode. Cool the agar medium to 40 to 50°C. Pour the agar into sterile glass or plastic petri dish on a flat surface to a uniform depth of 4mm.

Allow to solidified. Prior to use, dry plates at 30-37°C in an incubator, with lids partly agar, for not more than 30minutes or until excess surface moisture have evaporated. media must be moist but free of water droplets on the surface. presence of water droplets may result to swarming bacterial growth, which could give in accurate results. They are also easily contaminated.

#### 3.4.2 Inoculums Preparation

From a fungal culture (not more than 48 hours, old except for slow growing organism) take 4 or 5 colonies with a wire loop. Transfer colonies to 5ml of trypticase soy broth or 0.9% saline. Incubate the broth at 30°C at an optimum growth temperature until it achieves or exceeds the turbidity of 0.5 macfarland standard (prepared by adding 0.5ml of 0.048m BaCl<sub>2</sub> to 99.5ml of 0.36 NH<sub>2</sub>SO<sub>4</sub>; commercially available). Compare the turbidity of the test bacterial suspension with that of 0.5 macfarland (vigorously shaken before use) against a white background with contrasting black line under adequate light. Arrow points to tube with correct turbidity. Reduce turbidity by adding sterile saline or broth.

#### 3.4.2 Inoculation of Plates

- Dip a sterile cotton swab into the standardized fungal suspension.
- Remove excess inoculums by lightly pressing the swab against the tube wall at a level above that of the liquid.
- Inoculated the agar by streaking with the swab containing the inoculums.
- Rotate the plate by 60 °C and repeat the rubbing procedure. Repeat two times. This will ensure an even distribution of the inoculums.
- Allow the surface of the medium to dry for 3-5 minutes but not longer than 15 minutes to allow for absorption of excess moisture.



**Fig. 8: Inoculums Preparation.**

#### 4. RESULTS AND DISCUSSION

##### 4.1 Physical Evaluation

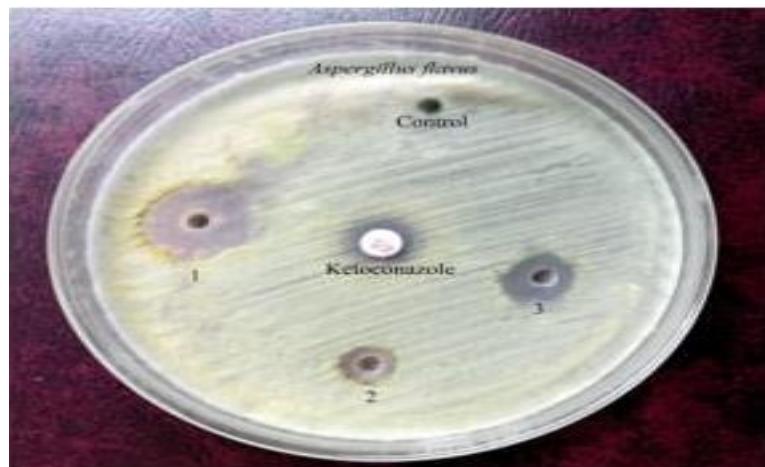
Table 2: Physical Evaluation.

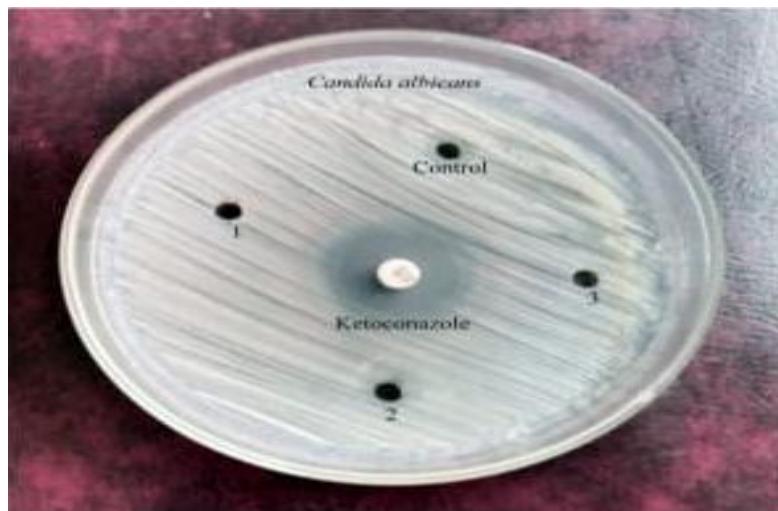
S. No	Parameters	F1	F2	F3
1	pH	$7.09 \pm 0.05$	$7.20 \pm 0.04$	$7.19 \pm 0.01$
2	Viscosity	$3.48 \pm 0.37$	$3.77 \pm 0.25$	$4.02 \pm 0.55$
3	Density	$0.688 \pm 0.01$	$0.679 \pm 0.02$	$0.689 \pm 0.01$
4	Flame Extension	69 cm	71 cm	70 cm
5	Flash Back	10 cm	12 cm	11 cm
6	Spray Angle	220	240	220
7	Leakage Test	No Leakage	No Leakage	No Leakage
8	Dry Time	30 Sec	33 Sec	31 Sec

##### 4.2 Anti – Fungal Activity

Table 3: Anti – Fungal Activity.

S. No	Microorganism	Control	F1	F2	F3	Ketaconazole (Standard)
		Zone of Inhibition in mm				
1	<i>Aspergillus flavus</i>	-	18	08	12	10
2	<i>Penicillium sps</i>	-	12	10	15	17
3	<i>Candida albicans</i>	-	2	1	1	18





**Fig. 9: Anti – Fungal Activity.**

#### 4.3 DISCUSSION

The study highlights the potential of plant-based formulations, particularly *Senna alata* extract, in the treatment of skin fungal infections. The use of topical sprays offers several advantages over conventional topical forms, such as creams and ointments. The ability of the spray to form a thin, non-sticky, and continuous film on the skin enhances the contact time of the active ingredient and supports prolonged drug release. This feature is particularly beneficial for treating skin conditions like fungal infections, where continuous drug action is necessary.

The physical evaluations showed that the formulations exhibited optimal pH, which is essential for maintaining skin integrity and avoiding irritation. The viscosity values were within the acceptable range, allowing easy application without excessive dripping. Drying time, another critical factor in topical sprays, was found to be acceptable across all formulations, further ensuring user convenience.

Despite the promising results, the antifungal activity of the spray formulations was less potent against *Candida albicans*, a common causative agent of fungal infections. This reduced efficacy suggests that further formulation optimization is required, potentially by adjusting the concentration of *Senna alata* extract or incorporating additional antifungal agents. The results also suggest that the formulation containing *Senna alata* can serve as a viable adjunct to existing antifungal therapies, offering a more natural alternative for the treatment of superficial skin infections.

#### 5. CONCLUSION

This research successfully developed a topical film-forming spray containing *Senna alata* extract with potential antifungal activity. The spray demonstrated favorable physical characteristics and provided a foundation for further optimization and clinical trials. Given the growing concern regarding fungal skin infections, such a formulation could offer a new, effective, and natural treatment option.

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