

Formulation and Evaluation of Topical Spray from the Extract of *Datura Stramonium L* for the Treatment of Fungal Infection.

Shreyash V. Kirgat¹, Pragati S. Karande², Karan M. Pandirkar³, Rutuja S. Kawale⁴, Umesh Kamble⁵

UG Students, Department of Pharmaceutics^{1,2,3,4}

Assistant Professor, Mahadevrao Wandre Institute of Technology, Turkewadi⁵

Corresponding Author Mail Id- shreyashvirkat@gmail.com

Email Id- karandepragati4321@gmail.com, karanpandirkar@gmail.com, rutujakawale2001@gmail.com

Abstract: *It has been found that the incidence of fungal infections in humans has recently increased. Numerous antifungal medications were used to treat different fungal infections. They come in a variety of formulations, including sprays, creams, ointments, shampoos, vaginal pills, oral medications, and injections. My research project's primary goal was to create a topical antifungal spray formulation. The treatment of fungal infections has greatly benefited from the formulation and development of tropical antifungal formulations. Both natural and synthetic chemicals with antifungal and antibacterial qualities, such as Datura stramonium, turmeric, peppermint oil, and comphor oil, are used in this spray composition*

Keywords: Antifungal spray, Datura Stramonium L., Turmeric, Comphor oil, Peppermint oil

I. INTRODUCTION

1.1 Fungal Infection

Fungal diseases accounted for a significant portion of all animal and human diseases, which are ranked third behind bacterial and viral diseases.¹

Dermatophytes are among the most frequent causes of tinea, along with barbae, pedis, capitis, corporis, and onychomycosis. Pityriasis versicolor and candidal infections are two other common superficial cutaneous fungal infections. Clinical treatments for these fungal infections consist of four primary classes of synthetic antifungal medications: azoles, allylamines, echinocandins, and polyenes.

Despite its advancements, modern treatment has not been without issues and difficulties, most notably medication resistance. But azole resistance is well recognized.²

Fungal infections of the skin are the most prevalent type of fungal disease and so play an important role in dermatology. Dermatophytosis refers to superficial infections of keratinized tissues, hair follicles, and nails produced by dermatophytic fungus. Dermatophytoses are among the most common human skin diseases. Approximately 10-15% of the global population may be infected. The most prevalent yeast infection is cutaneous candidosis, caused by *Candida spp*, which infects the skin and mucous membranes (e.g., oral or vaginal mucosa). *albicans* is the most common and significant causative agent.

Adults' natural skin flora includes lipophilic *Malassezia* yeasts. They can induce superficial skin infections like pityriasis (tinea) versicolor and contribute to *Malassezia* folliculitis. The significance of their participation in seborrheic dermatitis and certain types of atopic dermatitis remains unclear. Antimycotic drugs, including griseofulvin, allylamines, morpholine derivatives, and azoles, can treat fungal infections on the skin.³

Due to a lack of general knowledge on the prevalence and implications of fungal infections, the development of antifungal drugs for the treatment of systemic fungal infections lags far behind that of antibacterial treatments. It has only been recently recognized that systemic fungal infections are a significant cause of morbidity and mortality. While medical mycology has emerged as an infection disease specialization in recent years, dermatological mycoses are well-known clinical disorders that have long been a concern in the field of dermatology. The prevalence of systemic illness has increased with advancement in medical practice, particularly in the treatment of critically ill patients receiving

corticosteroids, dialysis, chemotherapy, radiation therapy, parenteral nutrition, IV catheters, broad spectrum antibiotics, organ transplantation, and other major surgeries.⁴

1.2 Topical spray

Topical drug delivery routes have a wide accessible surface area, prevent first-pass metabolism, and mitigate the effects of low pH and gastrointestinal tract enzymes. They also aim for systemic or local effects. 1-6 Drugs applied topically are typically manufactured in a dosing method, such as a patch, gel, lotion, cream, ointment, or spray, to enhance therapeutic efficiency or pharmacokinetic characteristics.⁵

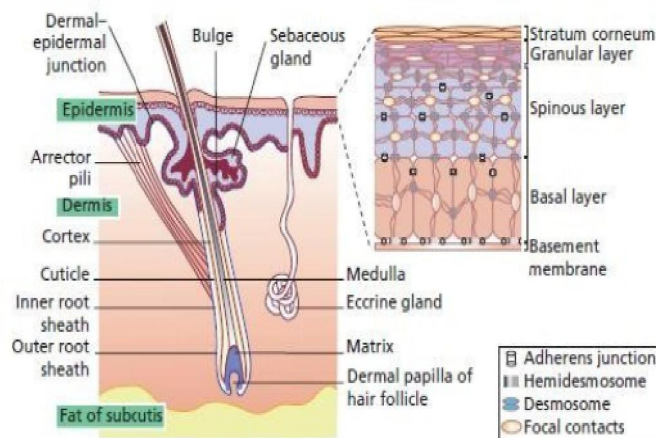
Sprays are applied topically and to the nasal pharyngeal tract. These suspensions and solutions are placed into spraying bottles, and the sprays themselves can take the form of coarse droplets or eventually split solids of aqueous or oleaginous solution.^{6,7}

Advantages of Topical Drug Delivery Systems

- Preventing first-pass metabolism.
- Convenient and simple to use.
- Preventing gastrointestinal compatibility issues.
- Improve patient compliance.⁸

1.3 Skin

The skin is the body's largest organ, making up over 10% of its total mass, and the organ that allows the body to have the closest possible relationship with its surroundings. The stratum corneum (nonviable epidermis), the remaining layers of the epidermis (viable epidermis), the dermis, and the subcutaneous tissues make up the skin's four basic layers. Nails, sweat ducts, apocrine glands, and hair follicles are some of the additional appendages that are connected. The majority of the physiological functions of mammals and humans in a generally hostile environment can be categorized as vital to their survival. This includes several functions of the skin. These roles can be broadly categorized as sensing, protecting, or preserving homeostasis. The skin's barrier quality serves as one example of the significance of the skin's protective and homeostatic roles.⁹



1.4 Candida albicans

Candida albicans is a common opportunistic pathogenic yeast found in the human intestine. It can also survive in the absence of a human body. In 40–60% of healthy individuals, it is observed in the oral and gastrointestinal tract. Although it is usually a commensal organism, it can become pathogenic in immunocompromised individuals for a variety of reasons. This species is one of the uncommon ones in the genus *Candida* that causes candidiasis in humans. An overabundance of the fungus is what causes the sickness. For instance, candidiasis is frequently seen in people who are HIV-positive. The most often isolated fungus from biofilms on human tissue or (permanently) implanted medical devices is *Candida albicans*. *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida glabrata* together

account for 50–90% of human cases of candidiasis. The mortality rate for patients with systemic candidiasis associated to *Candida albicansis* 40% .¹⁰

I. Preformulation Study

The first step in the methodical development of a pharmacological substance's dosage forms is preformulation testing. Preformulation research is the process of maximizing medication delivery by identifying the novel compound's phytochemical characteristics that may have an impact on the medicine's performance and creating a dosage form that is safe, effective, and stable. It provides the details required to characterize the substance's nature and offer a structure for the drug's combination with pharmaceutical excipients in the dosage form. Therefore A preformulation research was conducted to identify and determine the compatibility of the herbal extracts

II. DRUG PROFILE

2.1.1 *Datura (Datura Stramonium L.)* :



Fig No. 2.1 *Datura stramonium L.*

Synonyms : *Datura bernhardii*, *Stramonium vulgare*.

Biological source:

Datura Stramonium is the term by which it is known in botany. It goes by the names jimsonweed and devil's snare as well. It is a member of the family Solanaceae. The dried leaves and flowering tops constitute the biological source.

Scientific Name:

Thornapple, moon flower, hell's bells, devil's trumpet, devil's weed, tolguaicha, Jamestown weed, stinkweed, locoweed, pricklyburr, false castor oil plant, and devil cucumber.

Collected of *Datura stramonium L.*

The *Datura (Datura stramonium L.)* were collected from the Turkewadi, Kolhapur and authenticated (accession no: RMRC-1832) by Dr. Harsha Hegde, Scientist-E, Indian Council of Medical Research (ICMR) Belagavi, Karnataka, India.

Preparation of extract:

We gathered fresh *Datura* leaves (*Datura stramonium*) from Turkewadi in Kolhapur, Maharashtra. Clean distilled water is used to wash these leaves until all of the dust has been removed. All of these leaves are dried in the shade after being cleaned. The extraction was done using the Soxhlet extraction method. In a different Soxhlet extractor, the 25 gm of finely crushed *Datura stramonium L.* leaves were securely packed. The extraction solvent used was 250 cc of methanol. With this procedure, the liquid was evaporated to a dryness at 60 degrees Celsius under reduced pressure using an evaporator.

Uses:

- It have Parasympatholytic and Anticholinergic properties.
- It has CNS depressant effects.
- It is used to treat Asthma.
- It is also used in cerebral excitement.
- It is Aphrodisiac.
- It is used as wound and burn healer.
- It is used to treat Colds.¹¹

2.1.2 Turmeric (*Curcuma Longa L.*)

Synonyms : Saffron Indian; Haldi (Hindi); Curcuma; Rhizoma cur-cumae

Biological Source :

Turmeric is the dried rhizome of *Curcuma Longa Linn.* belonging (syn. *C.domestica* Valetton) family: *Zingiberaceae*.



Collection of Turmeric:

The turmeric (*Curcuma Longa*) was collected from local field Turkewadi, Kolhapur, Maharashtra.

Preparation of extract:

After the turmeric was properly dried in the shade and ground into a fine powder, it was used to make the herbal extract. The turmeric was then cleaned under running water to get rid of any foreign materials. Microwave aided extraction was used to complete the extraction. The microwave oven's center was filled with a mixture of 100ml (95% ethanol) and turmeric powder with a maximum power of 900 W. In order to prevent solvent boiling, the extraction process was conducted in cycles of 30 seconds of radiation and 10 minutes of cooling. The extract was gathered and kept in storage.

Uses :

- Improve skin condition.
- It helps to prevent cancer .
- Reduce side effect of chemotherapy.
- Turmeric is a powerful anti-inflammatory and antiseptic, useful for bronchial asthma, chronic cough.
- Turmeric has an anti-coagulant action, keeping the blood thin.¹¹

Table No. 2.1 List Of Ingredients And Its Uses

Sr.No	Ingredients	Scientific names	Uses	Form
1	Datura	Datura stramonium Family: <i>Solanaceae</i>	Antifungal, Antibacteria.	Extract
2	Turmeric	Curcumin longa Family: <i>Zingiberaceae</i> .	Anti-inflammatory, antifungal activity, Antibacterial.	Extract

3	Glycerol	Glycerin	Balancing pH, Permeation enhancer.	Solvent
4	Comphor oil	Cinnamomum camphora Family: Lauraceae	Antifungal.	Oil
5	Peppermint oil	Mentha Piperita Family: Lamiaceae	Anti-itching	Oil
6	Ethyl alcohol	Ethanol	Penetrating, Antifungal.	Solvent
7	Rose water	Rosa damascena Family: Rosaceae	Fragrance, Perfume	Solvent

Table No. 2.2 Formulation Table For Transdermal Spray

Ingredients	F1	F2
Datura stramonium	1.5gm	2.5gm
Turmeric	0.35gm	0.75gm
Glycerol	5ml	5ml
Comphor oil	0.45ml	0.45ml
Peppermint oil	0.25ml	0.25ml
Ethyl alcohol	QS	QS
Rose water	1ml	2ml
Total volume	30ml	30ml

Method Of Preparation :

- 10g of turmeric should be weighed and then dissolved in 100ml of 95% ethanol. Microwave aided extraction was the method used to carry out the extraction. Take another 25 grams of Datura Stramonium powder and dissolve it in 250 ml of methanol.
- Use a Soxhlet equipment to perform the extraction. Following the evaporation of the solvent from both extractions in a rotary evaporator.
- The solid extracts of datura stramonium and turmeric are dissolved in 95% ethyl alcohol and glycerol, comphor oil, peppermint oil, rose water with ethyl alcohol making up the volume.
- After that, the mixture was put into a container and labelled

Method Of Preparation of topical spray:



Fig No. 4.8 Ingredient

Fig No. 4.9 Soxhlet extraction



Fig No. 4.11 Extraction



Fig No. 4.10 Microwave Extraction



Fig No. 4.12 Other excipients



Fig No. 4.13 Formulation F1, F2

2.7. Evaluation Parameter of antifungal spray

2.7.1 Evaporation Time

Spraying the formulation on white paper allowed for the estimation of the evaporation time, which is the amount of time needed for spray film to dry. The drying time was then recorded for each formulation.¹²

2.7.2 Spray pattern

Applying the spray through the TS onto white paper allowed for the evaluation of the spray pattern. The formulation was sprayed onto the paper, which was clipped to a board, 2.5 to 3.0 cm from the plat.¹²

2.7.3 Spray angle

The spray was directed horizontally onto a piece of white paper that was positioned 15 cm away from the nozzle. Using various angles, the radius of the circle that was drawn on the paper was measured. After three attempts at the test, the average was calculated. The spray angle was computed using the subsequent formula.

Spray angle (θ) = $\tan^{-1}(h/r)$

where r is the circle's average radius and h is the paper's distance from the nozzle.¹³

2.7.4 Average weight per dose

The containers' starting weight was noted. The TS sprayed the five subsequent deliveries, and the containers' weight increased once more. To calculate the average weight per dose, the difference between the container's beginning and final weights was divided by the number of deliveries.¹⁴

$$\text{Average weight per dose}(W) = (\text{initial weight}(W_i) - \text{final weight}(W_f)) / \text{number of deliveries} (N)$$

2.7.5 pH

Below 5, the skin becomes highly sensitive.

The spray's pH has a significant impact on skin quality and reduces inflammation.

The majority of antifungal formulations have a mildly acidic quality that prevents microbial development.

Using a pH meter, the formulation spray's pH was ascertained.

2.7.6 Leak test

Delayed leak test: Aerosol containers with precise weights were kept at room temperature for two months. Two months later, the containers are weighed once more. The difference in a container's weight is the indicator of container leakage.¹⁵

III. RESULTS & DISCUSSION

3.1 Formulation of transdermal spray

The substances listed in table number were employed to produce the transdermal spray, and the plant material used in the formulation is abundant in a variety of phytochemicals. Alkaloids, flavonoids, phenolic compounds, phenols, Glycosides Lignins, Saponins, Sterols & Tannins which exhibits antifungal activity are the components of these phytochemicals. For the formulation to flow out of the container more easily, a good transdermal spray formulation needs to have an optimal spray pattern. An extensive battery of physiological and chemical tests is used to assess transdermal spray. Numerous formulation parameters were revealed by this test, and the findings were summarized.

3.2 Evaluation tests for the antifungal spray:

The antifungal spray is evaluated by means of several physiological and chemical tests. These tests yield information on several formulation parameters. The results of the testing were recorded.

3.2.1 Phytochemical screening:

Chemical compounds known as phytochemicals are found in plants naturally. They give the plant its color and organoleptic properties. Phytochemicals with physiological and therapeutic effects include alkaloids, phenols, tannins, flavonoids, saponins, carbohydrates, and phytosterols.

Numerous secondary metabolites were examined, such as proteins, steroids, alkaloids, glycosides, flavonoids, saponins, lignins, tannins, and phenolic substances. The same's findings are given in Table No.5.1

Table No. 3.1: Phytochemical Screening Of Methanolic Extracts Of Plant

Test	<i>DATURA STRAMONIUM L.</i>
Alkaloids	+
Sterols	+
Tannins	+
Phenols	+
Lignins	+
Saponins	+
Flavonoids	-
Glycosides	-

(+) indicates presence whereas (-) indicates absence of the phytochemical

3.2.2 Physical appearance and content analysis:

1. Evaporation Time:

Monitoring the formulation's evaporation time was crucial to the product's quality control process. The definition of a product's aesthetics, including its clarity and spreadability, is significantly influenced by its evaporation duration. The created formulation's evaporation time was discovered to be 111 seconds.

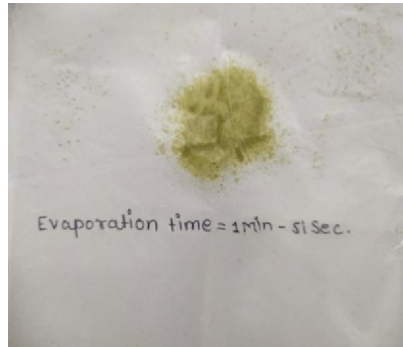


Fig No. 4.15 Evaporation Time

2. Spray pattern:

When there are uniform, spherical spots, the spray pattern formulation shows good spray patterns. It was discovered that the spray pattern's average mean was 2cm.

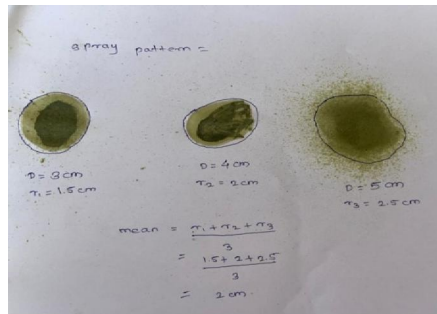


Fig No. 4.16 Spray pattern

3. Spray angle:

In order to maximize surface area and facilitate the activation of the medication solution from the container, the spray angle must to be less than 85°. It was discovered that the average spray angle was 77. 38°.

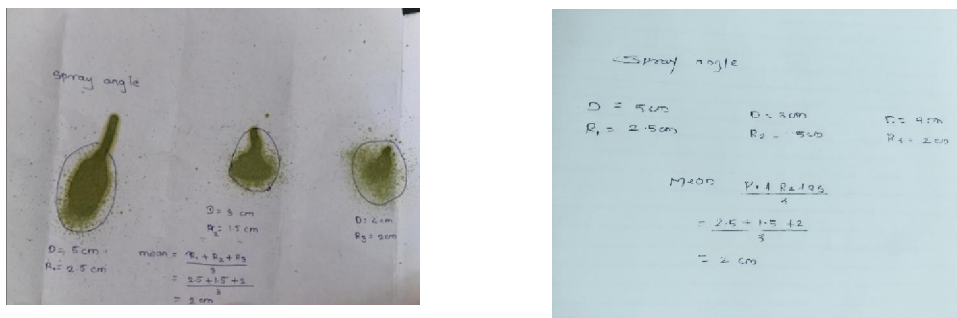


Fig No. 4.17 Spray angle

4. Average weight per dose:

The following delivery formulation is determined using the average weight per dose and the weight of each dose on average was found to be 0.08gm/ml.

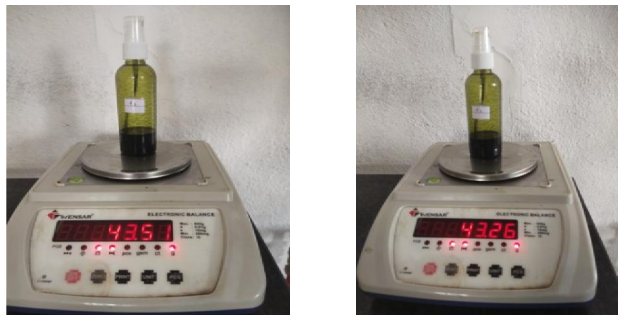


Fig No. 4.18 Average weight per dose

5. pH:

pH is calculated by using a pH meter, The observed pH of formulation is 6.86.



Fig No. 4.19 pH

6. Leak test:

This test assessed the spray pump's seal's effectiveness as well as its capacity to hold product contents. After three days of being upright and at a temperature of thirty degrees, the filled containers were weighed both before and after to ensure that no formulation was leaking out of the container. As a result, there was no discernible weight variation.

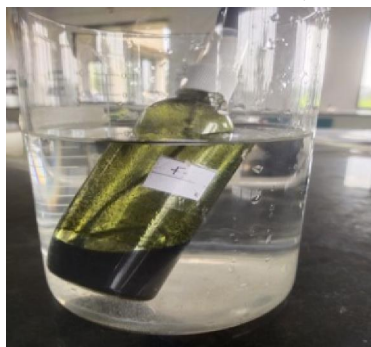


Fig No. 4.20 Leak test

7. Anti-Bacterial Activity:

The methanol extracts of leaves of *Datura stramonium* L. Showed the bactericidal activity against Gram-positive bacteria in a dose-dependent manner. Methanol extract exhibited the highest inhibitory activity against lactobacillus bacteria. *D.stramonium*L.was very effective against various strains



Fig No. 5.1 Antibacterial Activity

8. MIC test

The given sample Sample – F1, Sample – F2 used to carried out for this assay to determine the minimum inhibition concentration. The different concentration 100 mg/ ml to 6.25mg/ml used. The sample F1 and sample F3 showed 25 µl /ml ,50µl /ml which is low concentration for the inhibition of 50- 60 % growth of fungal strain against *candida albicans*.

Sample Description :

The MIC of the combinations of Sample on *Candida albicans* was carried out using broth dilution method. Culturing of microorganism, inoculum development, and MIC determination were carried out in laminar air flow. Samples were prepared mg/ml and then appropriately diluted at different serial dilutions ranging from 100 µl to 6.5 µl The inoculum of cultures (single cultures) was developed in broth medium. The cultures were then incubated and subsequently, serially diluted to reach the density of 2×10^4 cells per ml. Cell counting was done using hemocytometer. Two milliliters of sabroud’s dextrose both was dispensed in tubes, and 100 µL of cell culture was inoculated in it. Then, 100 µL of different concentration of extract was added to each tube. Each experiment was carried out in a triplicate set. Growth control was run in parallel with every experiment. All the experimental tubes were incubated in incubator for 48 h. After completion of incubation period, the optical density was measured at 600 nm using spectrophotometer.

MIC was defined as the minimum concentration of extract that caused 20% inhibition in growth of test microorganism.
 $MIC = \frac{\text{control} - \text{test}}{\text{control}} * 100$

Observation table:

Table No. 3.2 Determination of MIC by using different conc. For *Candida albicans*

Sr.No	Sample	Concentration(µl/ml)	<i>Candida albicans</i>
1.	Sample – F 1	100	-
		50	-
		25	-
		12.5	+
		6.25	+
2.	Sample – F 2	100	-
		50	-
		25	+
		12.5	+
		6.25	+

(“+” Turbidity/growth, “-” No turbidity/no growth, “++” Extreme growth)

Sr.no	Sample	Strain	Concentration μl/ ml	O.D	% inhibition	MIC
1.	Control		-	0.82	-	
2.	Sample F1	<i>Candida albicans</i>	100	0.24	70.73	25 μl/ml
			50	0.29	64.63	
			25	0.33	59.75	
			12.5	0.48	41.46	
			6.25	0.69	0.15	
3.	Sample F2	<i>Candida albicans</i>	100	0.26	68.29	50 μl ml
			50	0.30	63.41	
			25	0.46	43.90	
			12.5	0.48	41.46	
			6.25	0.74	0.09	

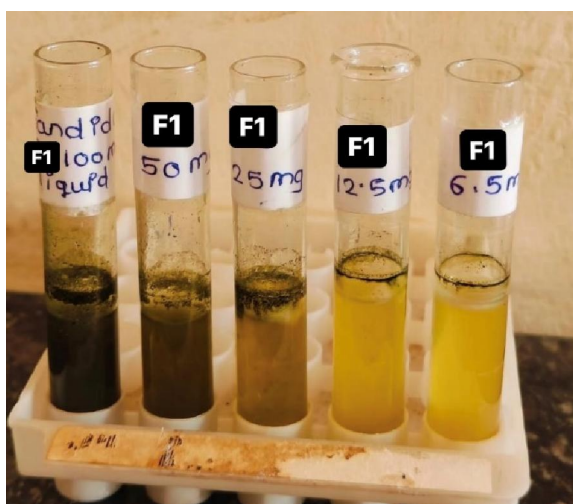


Fig No. 5.2 Sample F1

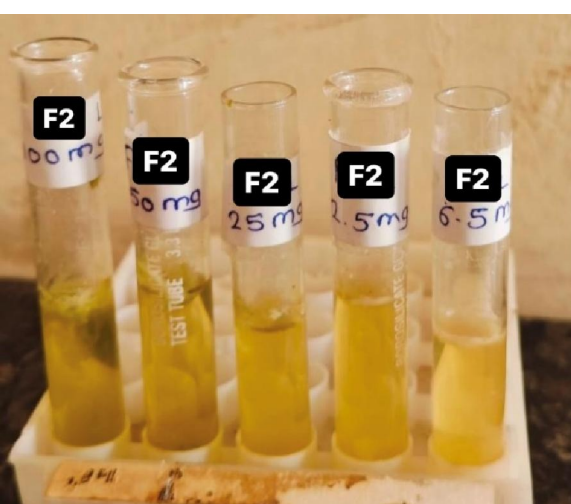


Fig No. 5.3 Sample F2

IV. CONCLUSION

Due to the growing negative impacts of the synthetic chemicals employed in product development, it has been noted that there is a greater demand in recent years for plant-based healthcare and cosmetic preparations.

This study's primary goal was to create a transdermal spray that was both stable and functionally useful without the need for artificial ingredients. Natural materials have been employed in place of the chemicals that are typically included in the marketed formulations. The transdermal spray was made from turmeric, or *Datura stramonium L.*

Datura stramonium L. and the rhizomes of turmeric have antibacterial, antifungal, antioxidant, and anti-inflammatory qualities.

The given sample Sample - F1, Sample - Fused to carried out for this assay to determine the minimum inhibition concentration. The different concentration 100 mg/ ml to 6.25mg/ml used. The sample F1 and sample F2 showed 25 ul/ml, 50ul/ml which is low concentration for the inhibition of 50- 60 % growth of fungal strain against *candida albicans*.

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