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Research Article

Formulation and Evaluation of Topical Polyherbal Antifungal Spray

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ABSTRACT


Fungal infections in humans represent a growing concern in both clinical and public health settings, affecting millions worldwide. These infections can range from superficial skin condition to systemic illnesses with certain species exhibiting increase resistance to conventional antifungal treatments. The rise of antifungal resistance, combined with the side effects and limitation of synthetic antifungals, has led to a renewed interest in alternative therapies. This research explores the potential of developing and antifungal herbal spray formulation, harnessing the natural bioactive compounds of various plants with known antifungal activity. These plant extracts were carefully chosen for the bioactive compounds, which includes flavonoids, alkaloids, terpenoids and phenolic compounds known to exhibit antifungal, anti-inflammatory, and wound healing properties. This spray formulation consists of natural as well as synthetic ingredients such as Clove oil, Ajowan, Tulsi, Turmeric (*Curcuma Longa*), Neem (*Azadirachta indica*), Rosemary oil, Camphor, Sodium Citrate, Ethyl Alcohol, Salicylic acid, etc. which have antifungal and antibacterial properties. By assessing the efficacy, safety and stability of these herbal ingredients, this study aims to provide a novel, nontoxic and effective treatment for fungal infections. The proposed herbal spray formulation seeks to offer a safer and more accessible alternative to current antifungal therapies, contributing to the advancement of integrative medicine and improving patient outcome in management of fungal diseases. This is study contribute to the development of plant based antifungal therapies and supports the ongoing shift towards integrative approaches in managing dermatological infections.

INTRODUCTION

The most prevalent and bothersome illness in people is fungal infection. There are so many

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antifungal preparations available in the market in various forms (creams, lotions, ointments, powder, etc). Topical formulation of the natural herbal antifungal spray works well, and it may also be used to treat a variety of fungal infections. Antifungal and antibacterial properties are present in this formulation. The oral antifungal preparations are also used in therapeutic treatment, but it has many side effects which causes low patient compliance. By combining the antimicrobial property of selected plant extract with the convenience of a topical spray, these formulation aims to provide an effective, non invasive, and patient friendly alternative to current antifungal therapies. infections of the skin, such as dermatophytosis and candidiasis, are common and can cause significant discomfort and complications if left untreated. Conventional antifungal treatments, including synthetic drugs like azoles and allylamines, often come with drawbacks such as drug resistance, side effects, and high costs. As a result, there has been growing interest in the development of herbal-based antifungal formulations that offer safer and more sustainable alternatives. Poly-herbal formulations, which combine multiple plant extracts, have gained attention due to their synergistic effects, broad-spectrum activity, and reduced risk of resistance development medicinal plants such as *Azadirachta indica* (Neem), *Curcuma longa* (Turmeric), *Ocimum sanctum* (Tulsi), etc possess well-documented antifungal properties and have been traditionally used for skin infections. The Incorporation of these bioactive extracts into a topical spray formulation can enhance efficacy, ease of application, and patient compliance. The formulation process involves the careful selection of plant species, followed by the extraction of bioactive compounds through appropriate solvent

systems. The subsequent preparation of the polyherbal spray formulation requires consideration of factors such as stability, incompatibility and bioavailability of the active ingredients. In the research, the spray will undergo a series of evaluations to assess its antifungal efficacy, safety and potential side effects. The results of this study would have a substantial impact on dermatological therapy and provide a more accessible and safe substitute for traditional antifungal medications. Through careful scientific analysis and evaluation, this study aims to confirm the effectiveness of polyherbal formulations in the treatment of fungal infections and pave the way for further developments in herbal medicine-based therapies.

MATERIALS & METHODS:

- Plant Materials and Chemicals: Clove oil, Neem, Turmeric, Tulsi, Ajowan, Rosemary oil, Camphor, Salicylic Acid, Ethyl Alcohol, Glycerol, Sodium Citrate, etc.
- While some of these items are bought from the market, all of these materials are ready.
- Tulsi, neem, and other natural ingredients are gathered from Shivajirao S. Jondhale College of Pharmacy's institute garden in Asangaon, Thane.
- Pure chemical compound such as sodium citrate, ethyl alcohol, salicylic acid, glycerol are taken from the institutional laboratory of Shivajirao S. Jondhale College of Pharmacy, Asangaon, Thane.

Formulation ingredients and their properties: -

Sr. No.	Ingredients	Scientific Name or Formula	Properties	Drug Form
1.	Neem	<i>Azadirachta Indica</i>	Antimicrobial	Extract



2.	Tulsi	Ocimum sanctum Linn	Antifungal, Antibacterial, Anti inflammatory, Antiprotozoal	Extract
3.	Turmeric	Curcuma Longa	Anti inflammatory	Extract
4.	Ajowan	Trachyspermum ammi	Antimicrobial, Anti inflammatory	Extract
5.	Rosemary	Salvia rosmarinus	Antimicrobial, Anti inflammatory	Oil
6.	Clove	Syzygium aromaticum	Antimicrobial, Anti oxidant, Antimicrobial	Oil
7.	Camphor	C ₁₀ H ₁₆ O	Anti inflammatory, pain relief, Antifungal, Antispasmodic	Solvent
8.	Glycerol	C ₃ H ₈ O ₃	Moisturizing agent, solvent, emollient, permeation enhancer	Solvent
9.	Ethyl Alcohol	C ₂ H ₅ OH	Spray base, Preservative	Solvent
10.	Salicylic Acid	C ₇ H ₆ O ₃	API	Solvent
11.	Sodium Citrate	Na ₃ C ₆ H ₅ O ₇	pH Stabilizer	Solvent

Formula for Topical Antifungal Spray:

Ingredients	Quantity for 100ml	Percentage for 100ml Spray
Neem Extract	2 ml	2.0%
Tulsi Extract	2ml	2.0%
Turmeric Extract	2ml	2.0%
Ajowan Extract	2ml	2.0%
Rosemary Oil	2ml	2.0%
Clove Oil	2ml	2.0%
Camphor	2ml	2.0%
Glycerol	2ml	2.0%
Salicylic Acid	2ml	2.0%
Sodium Citrate	2gm	2.0%
Ethyl Alcohol	80ml	80.00%

Extraction Of Herbal Drugs:

1.Extraction Of Neem

- Fresh neem leaves (Azadirachta Indica) were collected.
- To get rid of dust and make the leaves clean, they are rinsed with distilled water.
- All of these leaves are cleaned and then allowed to dry in the shade. (not sun-dry)
- The aqueous leaf extract was prepared.



- After boiling 500 milliliters of distilled water with 50 grams of neem leaf powder for 30 minutes,
- the boiled solution was filtered through Whatman No. 1 filter paper to yield a clear aqueous leaf extract.

2. Extraction Of Tulsi:

- Fresh Tulsi (*Ocimum sanctum*) leaves collected, then washed with distilled water to remove the dust and for clean use of leaves.
- All of these leaves are cleaned and then allowed to dry in the shade.
- Dried leaves are converted into fine powder.
- Then the powder was boiled in distilled water.
- Then it was filtered to obtain extract.

3. Extraction Of Turmeric:

- The ground turmeric was extracted with distilled water.
 - Boil the turmeric powder in water.
 - Then the extract was filtered using Whatman No. 1 filter paper.
- ### 4. Extraction of Ajowan:
- The Ajowan powder was boiled in distilled water.
 - Then the extract was filtered by Whatman No.1 filter paper, so that obtain clear extract.

Procedure:

(1) Prepare the Herbal Extracts:

Combine the neem, tulsi, turmeric, ajowan, camphor, rosemary, and clove oils in a glass beaker or measuring cup. Stir well to combine.

(2) Prepare the Salicylic Acid and Sodium Citrate Solution:

Dissolve the salicylic acid and sodium Citrate in a small amount of glycerol. Stir until the acids are fully dissolved.

(3) Mix the Herbal Extracts with the Acid Solution:

The solutions of salicylic acid and sodium citrate were combined with the herbal extracts. Stir well to integrate.

(4) Add Ethyl Alcohol:

To avoid separation, gradually add the ethyl alcohol to the mixture while stirring continuously. Stirring continuously, so that ensure that the mixture is thoroughly mixed.

(5) Filter the Mixture:

Filter the mixture through filter paper or cheesecloth to remove any sediment or impurities. Discard the filter paper or cheesecloth.

(6) Fill the plastic container:

The filtered mixture fill into the plastic container, leaving 1-2 cm at the top. Make sure the bottle is securely sealed after attaching the spray nozzle.

(7) Label and Store:

Put the ingredients, date, and usage directions on the bottle's label. Keep the bottle somewhere dry and cool.



Mechanisms Of Antifungal Drug:**(1) Ergosterol Inhibition (Cell Membrane Disruption):**

Fungi, unlike humans, have ergosterol as a key component in their cell membranes. Antifungal agents that target ergosterol either bind to it or inhibit its synthesis. Ergosterol disruption compromises the fungal membrane's integrity. The fungal cell undergoes stress, becomes unstable, and eventually dies.

(2) Cell Wall Synthesis Inhibition:

Fungal cell walls are rich in β -glucans, which are absent in human cells.

Inhibiting β -glucan synthesis weakens the structural integrity of the fungal wall. This makes the fungi more prone to external stress and immune attack.

Due to weakened wall, it can't hold the internal contents, leading to cell lysis.

(3) DNA/RNA Synthesis Inhibition:

Some antifungals interfere with the nucleic acid synthesis in fungi, they may get incorporated into fungal DNA or RNA during replication. This halts replication and protein production. As a result, the fungus cannot grow, reproduce, or repair itself.

(4) Mitosis Inhibition:

Fungi, like other eukaryotic cells, reproduce through mitosis. Antifungal agents that disrupt microtubule formation inhibit mitosis. This prevents the fungus from multiplying. It successfully halts the infection's spread.

How It Reacts on The Skin?

- When applied, ethyl alcohol acts quickly to sanitize the skin.
- By penetrating the skin, salicylic and citric acids aid in pore unclogging and dead skin exfoliation.
- Essential oils and herbal extracts soothe inflammation, fight bacteria, and promote healing.
- Glycerol prevents excessive dryness from alcohol and acids.
- The combination of camphor and clove oil provides a cooling and slightly numbing effect, reducing irritation.

Evaluation & Result:**1. Characterization of topical antifungal spray**

Parameter	F1	F2
Spray pattern	High precipitation and even film quality	possess a high degree of homogeneity and film Spreadability.
Evaporation time	1 min. 20 sec.	1 min. 20 sec.
Leakage from container	No leakage	No leakage
PH	4 - 4.5	4 - 4.5

RESULT:

- F1 was found to produce a dense and even film with visible precipitation, indicating high

active concentration on the target area. F2 formed a more uniform mist and spread over a wider area, indicating better dispersion and spreadability.



- Both formulations exhibited quick drying on skin, which is ideal for patient compliance and comfort.
- the formulation suitable for commercial packaging and long-term storage.

2. Phytochemical Analysis:-

- The absence of leakage ensures product stability, safety, and ease of transport, making

Phytochemical Test	Neem	Turmeric	Clove	Tulsi	Ajowan	Rosemerry
Carbohydrate	++	--	++	++	++	++
Proteins	--	--	--	++	++	++
Glycosides	--	++	++	++	++	++
Steroids	++	--	++	++	++	++
Alkaloids	++	++	++	++	++	++
Flavonoids	++	++	--	++	++	++
Saponins	++	++	++	++	++	++
Anhraquinone	--	++	++	++	--	++
Tannins	++	--	++	++	++	--
Reducing Sugar	--	++	++	++	++	++
++ : Shows the presence of Phytochemicals -- : Shows absence of Phytochemicals						

3. Physical appearance of Antifungal spray :-

Formulations	Colour	Phase seperation	Grittiness	Homogeneity	Consistancy
F1	Yellow	None	Absent	Present	Present
F2	Yellow	None	Absent	Present	Present

Result:

- Both formulations (F1 and F2) exhibited a yellow colour, which may be attributed to the natural extracts such as turmeric, neem, and tulsi.
- No phase separation was observed in either formulation, indicating good miscibility and physical stability.
- The absence of grittiness confirmed that the sprays were smooth in texture and free from particulate matter that could block the nozzle or irritate the skin.

- Both formulations showed high levels of homogeneity and consistency, suggesting a uniform distribution of active and excipient components throughout the solution.

4. Pump Seal Efficiency Test :-

To ensure the reliability and hygiene of the antifungal spray product, a pump seal efficiency test was conducted. This test is critical for validating the integrity of the pump mechanism and its ability to prevent leakage, contamination, and product degradation during storage and use.

The test was performed on plastic containers equipped with standard pump Dispensers commonly used in pharmaceutical and cosmetic



packaging. The antifungal spray formulation was filled into the containers under controlled conditions. The pump assembly was then sealed according to manufacturing specifications.

Test Procedure:

1. Visual Inspection:

The sealed containers were first inspected for any visible defects in the pump mechanism, including misalignment or deformation.

2. Leakage Test:

Each container was inverted and subjected to mild pressure to simulate handling and transport conditions. Containers were left in both upright and inverted positions for 24 hours at room temperature and elevated temperature.

3. Actuation Consistency:

The spray mechanism was activated repeatedly (e.g., 20–30 actuations), and the pump's ability to return to a sealed state after use was evaluated.

Result: The plastic pump container demonstrated effective sealing properties, maintaining product containment and protecting against environmental exposure. These results show the pump's capacity to dispense the antifungal spray while maintaining product stability and hygiene.



Fig. No.1 Pump Seal Efficiency Test

5. Skin Irritation Test:

The skin irritation potential of the antifungal spray was evaluated to ensure its dermatological safety for topical application. The study was conducted in accordance with internationally accepted guidelines, specifically OECD Test Guideline 404 and ISO 10993-10, which outline procedures for assessing dermal irritation and corrosion.

Objective: To determine whether the antifungal spray formulation causes skin irritation when applied to human skin under controlled conditions

MATERIALS AND METHODS:

Test Subjects: The test was carried out on healthy adult volunteers ($n = X$), aged between 18 and 55 years, after obtaining informed consent.

Test Substance: Antifungal spray formulation, applied as-is (without dilution).

Application Site: A small area of the upper back or volar forearm or back side of hand (approximately $2\text{ cm} \times 2\text{ cm}$) was selected for application.

Procedure:

1. The application site was cleaned and marked.
2. The antifungal spray was applied onto a sterile gauze pad and secured to the skin using hypoallergenic tape (semi-occlusive dressing).
3. A control site (untreated or treated with vehicle/placebo) was also included for comparison.
4. After being applied to the skin for a whole day, the patch was taken off. The skin was then examined 24, 48, and 72 hours following application to look for any signs of irritation.

Evaluation Criteria: Skin reactions were assessed using the draize scoring system, which grades the severity of erythema (redness) and edema (swelling) on a scale from 0 (no reaction) to 4 (severe reaction). Any additional observations, such as dryness or rash, were also recorded.

Results: Most participants showed no visible skin reaction at any time point. Based on the test results and standardized scoring criteria, the antifungal spray was found to be non-irritating to human skin under the conditions tested. This confirms the product's suitability for safe topical application in its intended use.



Fig. No.3: Test of skin irritation

6. Anti-Fungal Test By Using Bread Mold

Method:

The bread mold inhibition test was chosen as a cost-effective, visual, and practical method to evaluate the antifungal activity of the developed herbal spray formulation. Bread, being rich in carbohydrates and moisture, offers an ideal medium for mold growth and allows observable comparison between treated and untreated samples.

Objective: To assess the antifungal effectiveness of the topical herbal spray by measuring the delay or inhibition of mold growth on bread slices treated with the formulation, individual ingredients, and control solutions.

Materials: Fresh white bread slices, Zip-lock bags or airtight containers, Control solutions, Test antifungal Spray, Labels and markers.

Procedure:

1. Label five bread slices as: Control (Water), Ethanol, Test Spray, Neem Extract Only, and Clove Oil Only.
2. Apply a consistent quantity (about 1 ml) of every treatment to the corresponding slices.
3. Each slice should be put in its own zip-lock bag.
4. Samples are kept in a warm, dark environment to encourage the formation of mold.
5. Keep track of the changes for ten days.

Parameters for Observation:

- Time of mold appearance (in days)
- Mold colour and type (visual)
- Area covered (surface %)
- Odour or degradation

Safety Tips:



Do not open the bags once mold appears to avoid inhaling spores. Dispose of moldy bread carefully in sealed bags.

Result:

Before Treatment shows dense mold colonies with sporulated hyphae covering the surface. After Treatment shows significant reduction in mold density, showing disrupted fungal structures. The herbal topical antifungal spray showed promising results in inhibiting mold growth, demonstrating its potential as an effective anti-fungal treatments.

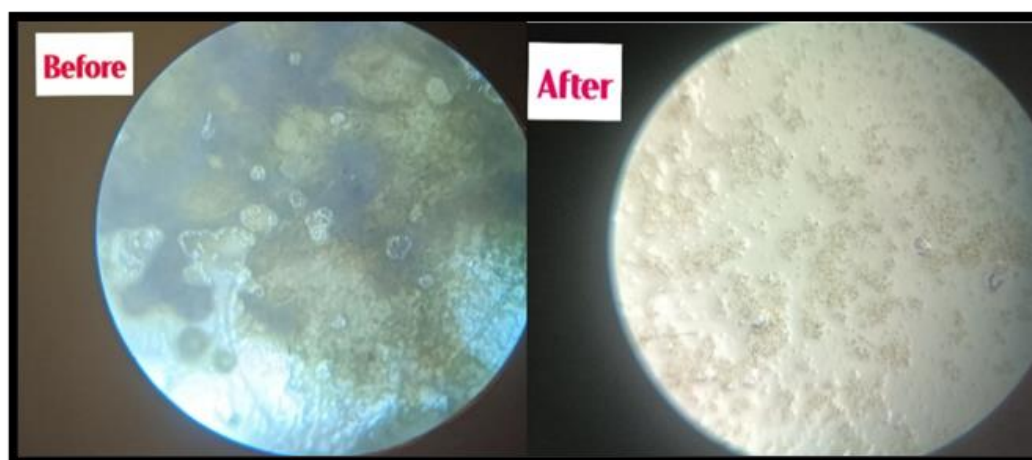


Fig. No.4: Before and after spraying antifungal spray.

7. UV Spectroscopy:

This antifungal spray formulation's medication content is investigated using spectrophotometry. Using a UV-Visible Spectrophotometer (Shimadzu, Japan) at λ max 380 nm, Equation 1 was used to determine the drug content using UVPC personal spectroscopy, software version 2. To put it briefly, 1 milliliter of spray solution is diluted with 25 milliliters of buffer solution. The concentration of the solution's active pharmaceutical ingredient was then measured using spectrophotometry at λ max 380 nm in comparison to the blank aqueous alcoholic spray. The size and form of the spray nozzle's aperture, which is also influenced by the pump capacity,

UV TEST	Wavelength	Absorbance
Formulation 1	380 nm	1.370
Formulation 2	400 nm	0.683

determines the spray displays. Every formulation study generated a consistent, round mess, indicating proper spray presentation.

Instrumentation:

Instrument: UV-Visible Spectrophotometer

Wavelength Range: 200 nm to 400 nm

Cuvette: Quartz cuvette, path length 1 cm

Blank Solution:

Distilled water (same solvent used for dilution).

Measurement:

Scan the sample from 200–400 nm. Note the whole absorbance spectrum and the highest absorbance wavelength, or λ max.

Result:

Overall, the test confirms that the formulation is stable and correctly prepared, with Formulation 1 showing better drug content performance.

8. Density & Viscosity:

Density: Density is a fundamental physical property that represents the mass per unit volume of a substance. It is usually measured in grams per cubic centimeter (g/cm³) or kilograms per cubic meter (kg/m³). For liquids, density also provides insights into their purity, composition, and suitability for specific applications.

Density(ρ) = Mass / volume

Viscosity: Viscosity measures a fluid's resistance to flow. It refers to the thickness or flowability of a liquid. The higher the viscosity, the thicker and more resistant to flow the liquid is conversely, the lower the viscosity, the thinner and more easily the liquid flow.

For Capillary Viscometer (Ostwald-type) Measurements:

When using a capillary viscometer (like an Ostwald viscometer), the dynamic viscosity is calculated by comparing the flow time of a liquid sample to that of a reference liquid (commonly water):

$$n_2 = \rho_2 t_2 / \rho_1 t_1 \times n_1$$

n_1 = Viscosity of Water at room temperature

n_2 = Viscosity of Sample

ρ_1 = Density of Water

ρ_2 = Density of Sample

t_1 = Flow time of Water

t_2 = Flow time of Sample

Apparatus:

Density bottle (25 ml), Analytical balance, Thermometer, Liquid sample, Distilled water (as a reference liquid), sample, Oswald viscometer, Stopwatch,

Procedure I : Determination of Density

1. Select a specific gravity container with a volume of 25 ml. Rinse the SG bottle with distilled water. Rinse the bottle with a small sample of liquid.
2. Weigh the empty specific gravity bottle. Let the weight be W_1 .
3. Fill the SG bottle with distilled water till it forms a meniscus. Place the lid and wipe the outer surface of the bottle filled with water. This is to ensure no droplets remain.
4. Weigh the SG bottle filled with water. Let the weight be W_2 .
5. Take out the bottle of distilled water. Use a small amount of acetone to rinse empty bottle.
6. Now transfer the sample liquid into the SG bottle up to the brim. Then keep the lid in place. Dry and clean the outer surface of the bottle using blotting paper.
7. Weigh the SG bottle filled with sample liquid. Let the weight be W_3 .

Procedure II: Determination of Time Flow of Liquid

1. Take an Ostwald viscometer. Clean it with chromic acid and then rinse with
2. acetone. Allow it to dry completely.
3. Transfer a known quantity of the liquid sample equivalent to the capacity of the



4. viscometer using a pipette through the side limb.
Density = $W_3 - W_1 / W_2 - W_1$
 $= 38.72 - 17.05 / 41.87 - 17.05$
5. Now, suck the liquid through the other limb up to a level higher than the upper mark.
 $= 21.67 / 24.82$
6. Allow the liquid to flow and start the stopwatch when the liquid passes the upper mark.
 $= 0.873 \text{ g/ml}$
Density of Formulation 0.873 g/ml
7. The upper mark and lower mark act as the start and end points. Use the stopwatch and note the reading.
8. Determine the average flow time by repeating the steps three or four times.
9. Similarly, find out the time required for an unknown liquid to pass from the upper to lower mark.

Calculation:**Part I:**Density of Sample = $W_3 - W_1 / W_2 - W_1$ Weight of empty density bottle (W_1) = 17.05 gWeight of bottle with water (W_2) = 41.87 gWeight of bottle with Sample (W_3) = 38.72 g**Part II:**Viscosity of water (n_1) = 0.719Density of water (ρ_1) = 0.9957Time of flow for water (t_1) = 20.72Density of test liquid (ρ_2) = 0.873Time of flow for test liquid (t_2) = 38.83Viscosity of test liquid (n_2) = ?

$$n_2 = \{ \rho_2 t_2 / \rho_1 t_1 \} \times n_1$$

$$n_2 = \{ 0.873 \times 38.83 / 0.9957 \times 20.72 \} \times 0.719$$

$$= 1.181 \text{ cp.}$$

Viscosity of Formulation is 1.181 cp.

Liquid	T1 (Sec.)	T2 (Sec.)	T3 (Sec.)	Mean time (Sec.)	Density (g/ml)	Viscosity (cp)
Water	21.06	20.03	21.08	20.72	0.9957	0.719
Sample	39.14	38.27	39.10	38.83	0.873	1.181

Result: The Density of formulation is 0.873 g/ml.**9. Stability Studies:**

The viscosity of formulation is 1.181 cp.

Parameters Evaluated	Freshly Prepared	After 1 Month	After 3 months
Appearance	Clear	Clear	Clear
PH (\pm SD)	4 - 4.5	4 - 4.5	4 - 4.5



Spray Pattern	Uniform	Uniform	Uniform
Temperature	34 ± 2	34 ± 2	34 ± 2

- Appearance test suggest that the formulation is physically stable over time.
- The Consistency in PH indicates chemical stability.
- Spray pattern is important for dose accuracy and patient compliance.
- The temperature remained stable at $34 \pm 2^\circ\text{C}$, showing that the formulation was stored under consistent conditions, and no temperature-dependent degradation occurred.

Evaluation Parameters:

Sr. No.	Parameters	Result
1.	Leakage Test	No leakage
2.	PH	4 – 4.5
3.	Density	0.873
4.	Viscosity	1.181
5.	Phase Separation	No Phase Separation
6.	Grittiness	Absent
7.	Homogeneity	Present
8.	Appearance Test	Clear
9.	Evaporation Time	1 Min.20 Sec
10.	Skin Irritation Test	No Skin Irritation Observed
11.	Stability	Stable
12.	Anti- Fungal Test	Inhibiting mold growth

Formulation and evaluation of polyherbal topical antifungal spray are done successfully and the antifungal spray gives action to kill the fungus.

CONCLUSIONS:

Based on the formulation experiments, it appears that this formulation's constituents work well to prevent fungal infections. This topical antifungal and antibacterial spray contain multiple

ingredients. It was successfully developed as a spray solution that can be used on additional subjects in subsequent research. The current research project is promising and offers a fresh approach to the transdermal antifungal treatment, according to the numerous investigations and findings. The antifungal polyherbal spray for topical application was formulated successfully with chosen medicinal plant extracts possessing antifungal activity. The efficiency of the polyherbal ingredients was confirmed by the antifungal activity on bread, which showed a noticeable suppression of fungal development, especially after the spray was used. Microscopic analysis also showed that after treatment, hyphal density and shape significantly decreased.

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