



Formulation and Evaluation Antifungal Herbal Spray

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Abstract

In recent years, it has been found that fungal infections in humans have risen. Along with its growth rate, the kind of fungal species also increased considerably. An effective formulation is necessary to treat these fungal infections in a simple manner. The main aim of my research project was to develop a formulation of a topical antifungal spray. The formulation and development of tropical antifungal formulation have seen great results in the treatment of fungal infections. Over the conventional dosage forms, these topical drug delivery formulations have more benefits along with their extraordinary advantages. This spray formulation consists of natural as well as synthetic ingredients like Coconut oil (*Cocos nucifera*), Neem (*Azadirachta indica*), Turmeric (*Curcuma longa*), Tulsi (*Ocimum sanctum* Linn), Aloe vera (*Aloe barbadensis miller*), Salicylic acid, Curry leaves (*Murrayakoenigii*), Camphor, Sulphur, Apple cider vinegar (*Malus pumila* Mill), Clove oil (*Syzygiumaromaticum*), Sodium bicarbonate, Acetic acid, Mint (*Mentha piperita*), Garlic (*Allium sativum*) ingredients which have antifungal and antibacterial properties. The formulation and evaluation of topical antifungal spray were subjected to in vitro diffusion studies. The materials used in this formulation are studied microbiologically for analyzing their safety.

KEY-WORDS: Fungal infection, Topical Antifungal spray, Herbal Antifungal Spray, Coconut oil, Neem, Turmeric, Tulsi, Aloe Vera, Curry leaves, Apple cider vinegar, Clove oil, etc.

INTRODUCTION

Fungal infections are the most common, irritating disease in humans. There are so many antifungal preparations available in the market in various forms (creams, lotions, ointments, topical antifungal powders, etc. these are used for treatment purposes by dermatologists and skin therapists).

Natural ingredients like Coconut oil, Neem, Turmeric, Tulsi, Aloe vera, Curry leaves, Camphor, Apple cider vinegar, Clove oil, Mint, and Garlic has antifungal properties and are effectively used in topical preparation and can further be used in the treatment of various fungal infections. This preparation has both antifungal and antibacterial properties. It is applied topically using mechanical spray on various fungal infections. There are so many types of fungal infections some represent tissue invasions on the skin. This invasion of tissues on the skin is due to more than one fungi species. This cause local, superficial as well as deeper skin tissue infections. Such infections are also found in the blood known as septicemia or systemic disease. Pathogenic fungi cause infections directly to the immune system of the human body.

Topical antifungal treatments have a great impact on curing these skin infections. This includes the infection targeting, reducing possible side effects, more patient compliance, enhancing treatment efficacy, and much more. For the treatment of these dermatological fungal infections, various types of compounds are used. Nowadays, all these antifungal compounds are available in the market in a variety of conventional drug formulations like lotions, creams, oils, gels, sprays, etc. Systemic as well as topical antifungal and antibacterial agents are the most common therapeutic options in treating fungal infections. The oral antifungal preparations are also used in therapeutic treatment, but it has many side effects which cause low patient compliance.

MATERIALS AND METHODS:

- ❖ Plant Materials and chemicals Coconut oil, Neem, Turmeric, Tulsi, Aloe vera , Salicylic acid, Curry leaves extract, Camphor, Sulfur , Apple cider vinegar, Clove oil, Sodium bicarbonate, Acetic acid, Mint, Garlic extract.
- ❖ All these materials are prepared, and some materials are purchased from the market in Beed, Maharashtra.
- ❖ Dermal administration of the antifungal preparations should touch the high therapeutic level from the epidermis of the skin.
- ❖ The challenge in the administration of the dermal delivery of topical preparations is the stratum corneum.
- ❖ For the improvement of such part of the derma new drug delivery approaches are carried out.
- ❖ Natural ingredients like Tulsi, Aloe vera, Curry leaves, and Neem is collected from the institute garden of SHIVAJIRAO S JONDHALE COLLEGE OF PHARMACY, ASANGAON MAHARASHTRA.
- ❖ Pure chemical compounds such as salicylic acid, sulfur, sodium bicarbonate, and acetic acid are taken from the institutional laboratory of SHIVAJIRAO S JONDHALE COLLEGE OF PHARMACY, ASANGAON MAHARASHTRA.

❖ **Formulation ingredients and their properties**

Sr. No.	INFREDIENT	SCIENTIFIC NAMES	PROPERTIES	FORM
1.	Coconut	Cocos Nucifera	Anti Microbial	Oil
2.	Neem	Azadirachta Indica	Antifungal , Antibacterial Anti-Inflammatory, anticarcinogenic.	Extract
3.	Tulsi	Ocimum sanctum Linn	Antifungal, Antibacterial, Anti-Inflammatory , Anti Protozoal	Extract
4.	Turmeric	Curcuma Longa	Anti-Inflammatory	Extract
5.	Aloe Vera	Aloe Barbadensis	Anti -Inflammatory Anticarcinogenic	Extract
6.	Camphor	C ₁₀ H ₁₆ O	Antifungal, Antibacterial	Solvent
7.	Curry Leaves	Murraya Koenigii	Antiseptic	Extract
8	Mint	Mentha Piperita	Antifungal, Antibacterial, Anti- Inflammatory.	Extract

9	Garlic	Allium Sativum	Antioxidant, Antibacterial	Extract
10	Clove	Syzygium Aromaticum	Antifungal, Antibacterial, Anti-Inflammatory	Oil
11	Salicylic Acid	C7H6O3	Antimicrobial, Antibacterial	Solvent
12	Sulphur	Sulphur	Antibacterial	Solvent
13	Sodium Bicarbonate	NaHCO3	Anti-Inflammatory, Antiseptic	Solvent
14	Acetic Acid	Ethanoic Acid	Antifungal	Solvent
15	Apple Cider Vinegar	Malus Pumila Mill	Anti Bacterial	Solvent

Formulae for the preparation of topical antifungal spray

Ingredients	The percentage for 100ml spray	Quantity of ingredients for 100 ml	Drug Formulation
Coconut oil	0.6%	0.6ml	API
Neem extract	2%	2ml	API
Tulsi extract	2%	2ml	API
Turmeric extract	2%	2ml	API
Alovera extract	2%	2ml	API
Camphor	1.5%	1.5ml	API
Curry leaves extract	1.0%	1ml	API
Mint extract	1.0%	1ml	API
Garlic extract	0.9%	0.9ml	API
Clove oil	2%	2ml	API

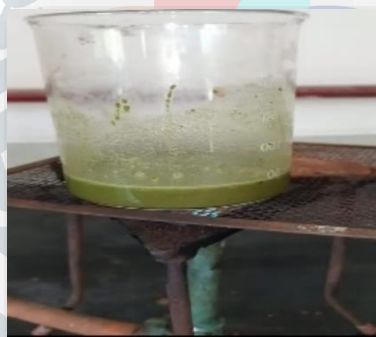
Salicylic acid	2%	2gm	API
Sulphur	2%	2gm	API
Sodium bicarbonate	1.0%	1gm	API
Acetic acid	1.0%	1ml	API
Apple cider Vinegar	1.0%	1ml	API
Ethyl alcohol	75%	75ml	Spray base / Preservative
Glycerol	3.0%	3ml	Permeation enhancer

Preparation of extracts (Neem):

- 1) Fresh neem leaves (*A. indica*) were collected from the SHIVAJIRAO S JONDHALE COLLEGE OF PHARMACY GARDEN , Asangaon, Maharashtra.
- 2) Taken leaves are washed with distilled water to remove the dust and for clean use of leaves.
- 3) After washing, all these leaves are dried in the shade.(no sundried)
- 4) The aqueous leaf extract was prepared as given,
 - i. Taking 50 gm of neem leaf powder.
 - ii. Mix about 500 ml of distilled water and boil it for 30 min.
 - iii. The boiled solution was filtered using Whatman No. 1 filter paper and clear aqueous leaf extract was obtained.

Preparation of extract (Tulsi):

- 1) Fresh Tulsi leaves were collected from the SHIVAJIRAO S JONDHALE COLLEGE OF PHARMACY GARDEN, Asangaon, Maharashtra.
- 2) Taken leaves are washed with distilled water to remove the dust and for clean use of leaves.
- 3) After washing, all these leaves are dried in the shade. (no sundried)
- 4) Dried leaves are converted into fine powder.
 - i. Then the powder was macerated with pure ethanol and filtered.
 - ii. By dissolving the 300gm of Tulsi powder in 1lit of ethanol, 18 gm of Tulsi extract is obtained.



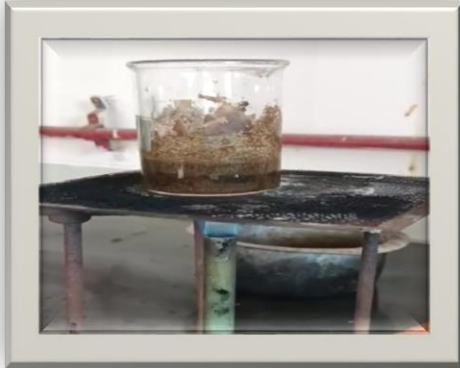
Preparation of extract (Aloe vera):

- 1) The thick leaves of the Aloe vera (*Aloe Barbadensis*) plant are obtained from the SHIVAJIRAO S JONDHALE COLLEGE OF PHARMACY Garden, ASANGAON, Maharashtra.
- 2) The leaves of aloe vera are collected and washed with distilled water.
- 3) The leaves are cut into pieces with a knife.
- 4) Knife or sharp cutter can be used to rub on the inner gel of the leaves to avoid the direct pieces.
- 5) The inner gel-like material is separated from the leaves.

Preparation of extract (Curry leaves):

- 1) Fresh Curry leaves are collected from the Shivajirao S Jondhale College of Pharmacy College Campus Garden.
- 2) Leaves are correctly washed with distilled water and dried in the shade. (no sundried)

- 3) The aqueous leaf extract was prepared as given,
- i. Taking 50 gm of Curry leaf powder.
 - ii. Mix about 500 ml of distilled water and boil it for 30 min.
 - iii. The boiled solution was filtered using Whatman No. 1 filter paper and clear aqueous leaf extract was obtained.



Selection of excipients:

- 1) The topical antifungal spray formula was prepared using Aqueous and non-aqueous solvents, cosolvents, diluent permeation enhancers, etc.
- 2) As a permeation enhancer Glycerol was used in the preparation.

Selection of spray bottle:

- 1) A polymer spray bottle that is nonreactive with the formulation is selected.
- 2) This Boston round spray bottle is selected and employed with the prepared antifungal transdermal formulation.
- 3) Such spray bottles are also used for mist spray, spray for cleaning purposes, sunscreen spray, insect repellents, etc.

Characterization of the topical antifungal spray :

Parameter	F3	F2	F1
Spray pattern	Have high uniformity and spread ability of films	High precipitate and low uniformity of films	High precipitate and low solubility of films
Mean particle size (nm) (\pm SD)	555.6 \pm 2.5	908.3 \pm 3.1	901.1 \pm 1.2
Evaporation Time	1-1.1 m	1.2-1.3 m	1.2-1.4 m
Leakage from container	No leakage	No leakage	No leakage
PH	4-4.5	4.2-4.6	4.4-4.7
Drug content per spray (%) (\pm SD)	100.05 (\pm 1.721) 0.46	99.65 (\pm 0.13) 1.45	98.89 (\pm 1.153) 1.09
Content uniformity	101.65 (\pm 1.569)	103.78 (\pm 0.462)	104.02 (\pm 1.432)

Formulation development of topical antifungal spray:**Preparation**

Antifungal Spray

Antibacterial Spray

PROCESS

75 % Ethyl Alcohol &
Remaining 25 % Coconut Oil,
Neem , Turmeric, Clove Oil.
Sodium Bicarbonate, Acetic Acid
Mint , Garlic Extract. In this formula.
Glycerol is used as the dermal Permeation enhancer.

Non-oil-based extracts are first mixed together, and remaining oil-based ingredients are added to it. For mixing the oils with extracts the emulsifier agent can be used (sodium phosphate).

After mixing all the ingredients stir this solution till the homogeneous mixture is obtained. After continuous stirring of the mixture the spray solution can be obtained, this mixture is then filled in a spray bottle and used as the antifungal agent.

Characterization of antifungal spray formulation:

Particle size distributions: The characterization of spray with the distribution of particle size is achieved by the Malvern zeta sizer nano 6.01 (Malvern Instruments Ltd, UK). The periodic time for sampling was automatically set. Three measurements are carried out in the alcoholic solutions.

The efficiency of the pump seal: The spray pump efficiency was estimated by the leak test. The spray bottle seal is tested by a pump seal test. The bottles of the same size and weight were tested for their weight variation. The bottles are balanced before and after the test stages and the leakages are tested.

PH and evaporation time of spray solution: For testing the evaporation time of the spray formulation, it is sprayed on ethanol-sensitive paper by doing this the evaporation time of the spray formulation is noted. The PH value of the spray formulation was estimated using the digital PH meter.

Drug content study: The drug content study is carried out with this antifungal spray formulation determined spectrophotometrically. At λ max 380 nm using UV-Visible Spectrophotometer (Shimadzu, Japan, UVPC personal spectroscopy, software version 2), the drug content was determined using equation 1. In brief, the 1ml of spray solution is diluted with the 25ml of buffer solution. Then the solution was analysed for its active pharmaceutical ingredients concentration spectrophotometrically at λ max 380 nm compared with the blank aqueous alcoholic spray. $\text{Drug content\%} = \frac{[\text{Concentration of entrapped drug}]}{[\text{Total drug concentration}]} \times 100$.

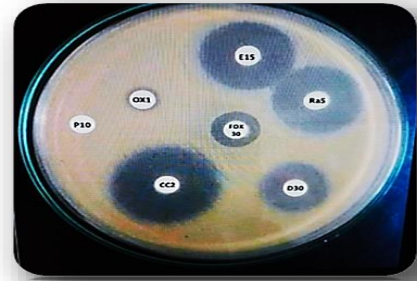
Antifungal spray stability study: This spray stability study is carried out by conventional methods of stability testing. The percentage valuation of the residual drug found estimated the formulation stability. The stability study is also carried out by using spray patterns, changes in PH, the drug release pattern, rate of leakage, etc.

Study regarding skin irritation: Five human volunteers aged 25-35 years group selected and ensured no past allergic history to them. The formulated topical antifungal spray preparation was sprayed on shaved hands of these volunteers. The test formulation was applied on the hand side. After waiting for some time the observations were spotted and analysed for Edema, skin irritation, erythema, etc. In this way, the skin irritation study was carried out.

❖ **TESTING:**

1. ANTIMICROBIAL TEST :

The study of the antifungal activity of the prepared antifungal spray was carried out by using the strains of bacteria as well as fungi. The agar well diffusion method helps us in this study. In this, the Mueller-Hinton agar is used at 4mm deep and poured in 125-150mm. The pH followed from 7.3 to 7.5 for this purpose aseptic technique was used. The sterile swab was placed in the broth culture of a particular organism, and by rotating the swab the excess fluid was removed. Uniform growth is obtained by streaking the plate in one direction the plate rotated at 90%. After waiting about 5 minutes these plates are allowed to dry. All these plates were incubated for a whole night at the temperature of 37°C (98.6°F). The inhibition zones were compared with the standard antifungal compounds.



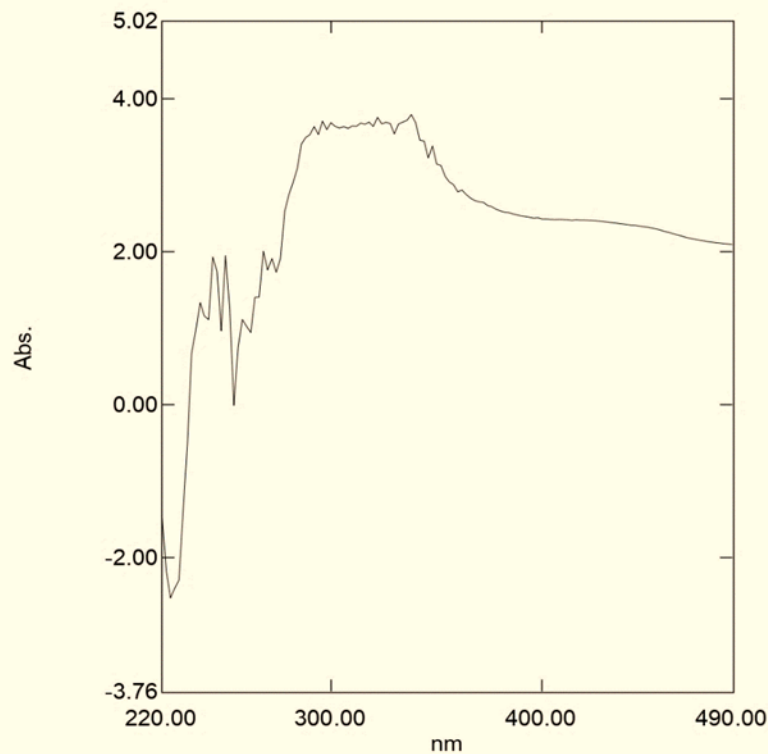
DURING INCUBATION

AFTREER INCUBATION

UV TEST:

The drug content study is carried out with this antifungal spray formulation determined spectrophotometrically. At λ max 380 nm using UV-Visible Spectrophotometer (Shimadzu, Japan, UVPC personal spectroscopy, software version 2), the drug content was determined using equation 1. In brief, the 1ml of spray solution is diluted with the 25ml of buffer solution. Then the solution was analyzed for its active pharmaceutical ingredient's concentration spectrophotometrically at λ max 380 nm compared with the blank aqueous alcoholic spray.

Spectrum Overlay Graph



III. RESULT AND DISCUSSION:

formulation	Flux(mg/cm ² .h)	*Q ₂₄ (mg/cm ²)
F1	0.0173	0.4369
F2	0.0199	0.4551
F3	F3.0276	0.6687

Table 1: permeation study of antifungal formulation

The spray displays affect by the shape and size of the orifice of the spray nozzle, this is also affected by the pump capacity. All formulation studies showed good spray displays by giving a uniform, spherical mess.

Spray formula:

This formula consists of 75% ethyl alcohol and the remaining 25% of the active natural ingredients. The optimized formula was selected based on the solution transparency, film thickness, rate of dehydration, and more. Formulation 1 F1 was just the drug solution which only consisted of ethyl alcohol this showed the high clarity with a high concentration of the ethyl alcohol. The film F3 was found to be transparent. The organic solvents used in the spray formulation have a high vaporization rate so they evaporate rapidly leaving behind a thin coating of APIs on the skin this last for 4 hours. By comparing the formulations F1, F2, and F3 found that these are better than other spray formulations.

* Amount of drug released per unit surface area after 24 h. Formulations F1 and F3 showed a zero-order kinetic model while formulation F2 showed root time kinetics with an R² value of 0.9608. [Formulations F2 and F3 (containing film formers and penetration enhancers) showed more flux and Q₂₄ value for the drug as compared to Formulation F1.

Stability studies: In terms of the physiochemical parameters the formulated drug preparation showed good stability. Under normal storage conditions for the formulations, its stability was found to be intact.

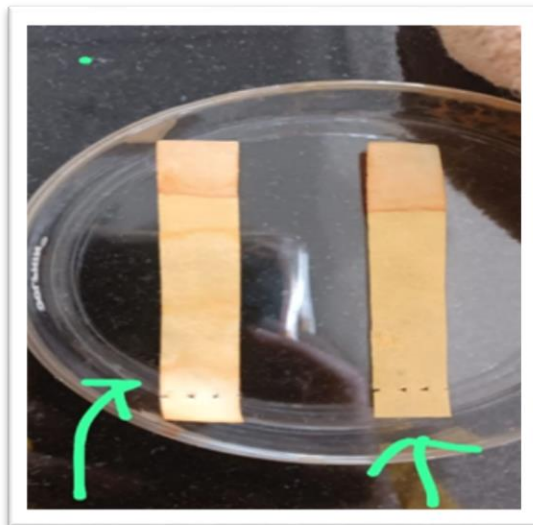
Stability Temperature at 37 ± 2° for F1

Parameters evaluated	Freshly prepared	After 1 month	After 3 months
Appearance	Clear	Clear	Clear
pH(+/- SD)	4.5(+/-)0.05	4.6(+/-)0.04	4.7(+/-)0.02
Spray pattern	Uniform	Uniform	Uniform
Percent drug remaining	100.05(+/-)0.46	99.65(+/-)1.45	98.89(+/-)1.09

Table: Stability study of the antifungal spray formulation within the time intervals

Skin irritation studies: Irritation was not found significantly on the human skin specifically the Edema and erythema. The formulation was found to be safe, and non-irritant for transdermal implementation. This formulation is effective till 24 hours, the results need to be rechecked by the pharmacokinetic studies.

Antifungal activities: The formulation study proves that the active pharmaceutical ingredients contain in the formulation are actively antifungal, antibacterial, and anti-inflammatory in nature.



PH TEST



APPERANCE TEST

CONCLUSIONS:

All the formulation studies suggest that the ingredients used in this formulation were found to be effective against fungal infections. This topical multi-ingredient antifungal and antibacterial spray was successfully formulated as the spray solution this can be used on the more volunteers for future trials. From the various studies and results, it is found that the present research work is promising as well as a novel approach to the transdermal antifungal treatment.

REFERENCES:

- [1]. Ginovyan M, Keryan A, Bazukyan I, Ghazaryan P, Trchounian A (2015) The large-scale antibacterial, antifungal and anti-phage efficiency of Petamcin-A: new multicomponent preparation for skin diseases treatment. (Translated from eng) *Ann Clin MicrobiolAntimicrob* 14: 28 (in eng).
- [2]. Pagano L (2013) Combined antifungal approach for the treatment of invasive mucormycosis in patients with hematologic diseases: a report from the SEIFEM and FUNGISCOPE registries. (Translated from eng) *Haematologica* 98(10): e127-130 (in eng).
- [3]. Mathpal D, Garg T, Rath G, Goyal AK (2015) Development and characterization of spray-dried microparticles for pulmonary delivery of the antifungal drug. *Curr Drug Deliv* 12: 464-471.
- [4]. Ribeiro RF (2016) Spray-dried powders improve the controlled release of antifungal tioconazole-loaded polymeric nanocapsules compared to lyophilized products. (Translated from eng) *Mater Sci Eng C Mater Biol Appl* 59: 875-884 (in eng).

- [5]. Carrillo-Munoz AJ, Tur-Tur C, Cardenes D, Rojas F, Giuliano G (2015) [In vitro antifungal susceptibility profile of *Scopulariopsis brevicaulis* isolated from onychomycosis]. (Translated from spa) *Rev Esp Quimioter* 28(4): 210-213 (in spa).
- [6]. Kumar L, Verma S, Bhardwaj A, Vaidya S, Vaidya B (2014) Eradication of superficial fungal infections by conventional and novel approaches: a comprehensive review. (Translated from eng) *Artif Cells Nanomed Biotechnol* 42(1): 32-46 (in eng).
- [7]. Subissi A, Monti D, Togni G, Mailland F (2010) Ciclopirox: recent nonclinical and clinical data relevant to its use as a topical antimycotic agent. (Translated from eng) *Drugs* 70(16): 2133-2152 (in eng).
- [8]. Xiao Y (2013) Antifungal screening of endophytic fungi from *Ginkgo biloba* for discovery of potent anti-phytopathogenic fungicides. (Translated from eng) *FEMS Microbiol Lett* 339(2): 130-136 (in eng).
- [9]. Cao LL, Zhang YY, Liu YJ, Yang TT, Zhang JL, et al. (2016) Anti-phytopathogenic activity of sporothriolide, a metabolite from endophyte *Nodulisporium* sp. A21 in *Ginkgo biloba*. *Pestic Biochem Physiol* 129: 7-13.
- [10]. Wei Y, Wang Y, Wang L, Hao D, Ma G (2011) Fabrication strategy for amphiphilic microcapsules with narrow size distribution by premix membrane emulsification. (Translated from eng) *Colloids Surf B Biointerfaces* 87(2): 399- 408 (in eng).
- [11]. Programmable Timing,” SAE Paper No. 910450.
- [12]. Schechter, M.; and Levin, M., 1998, “Camless Engine,” SAE Paper No. 960581
- [13]. INTERNATIONAL JOURNAL OF ROBUST AND NONLINEAR CONTROL, Int. J. Robust Nonlinear Control 2001; 11:1023}1042 (DOI: 10.1002/rnc.643)(