Available online on 15.08.2023 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

Open Access **Research Article**

Formulation & Development of Transdermal Spray of Turmeric Lemongrass as Antifungal

Shirke Bhushan, Shirke Divya*

Gourishankar Institute of Pharmaceutical Education and Research, Limb, Satara

A B S T R A C T

The study aims to find out the effectiveness of the combination of citral and curcumin as antifungal compounds. *Lemongrass* (Cymbopogon citratus) is a valuable family of grass known due to its flavoring, medicinal, and fragrance application. Haldi (Turmeric) scientifically known as *Curcuma longa* belongs to familthey *Zingiberaceae.* Its polyphenolic compound curcumin has been showing a variety of *antifungal investigations* due to extensive traditional uses and very low side effects.Turmeric has beenutilized in traditional medicine for various diseases counting diabetes, hepatitis, hemorrhoids, hysteria, indigestion, skin disease, inflammation, anorexia, hepatic disorders, cough, and sinusitis. In this formulation substudy combination of two bioactive oils is considered to form an effective *antifungal* spray preparation. The spray preparation is helpful to achieve fast absorption of the drugs through the *transdermal* way of drug administration. The effectiveness and activity rate of spray preparation is more beneficial. *Lemongrass* oil and curcumin are dissolved in ethanol to form a stable, safe, and effective spray formulation.

Keywords: *Lemongrass, Zingiberaceae,* Transdermal, Antifungal

A R T I C L E I N F O: Received 23 April 2023; Review Complete 27 May 2023; Accepted 12 Jul 2023; Available online 15 August 2023

Cite this article as:

Bhushan S, Divya, S, Formulation & Development of Transdermal Spray of Turmeric Lemongrass as Antifungal, Asian Journal of Pharmaceutical Research and Development. 2023; 11(4):12-23. DOI: http://dx.doi.org/10.22270/ajprd.v11i4.1279

*Address for Correspondence:
Shirke Divya, Gourishankar Institute of Pharmaceutical Education and Research, Limb, Satara

INTRODUCTION:

The versatile nature of the skin makes it the most useful site for drug administration. It has been used preferably for the management of several skin diseases such as inflammation, microbial infections, psoriasis, dermatitis, and many more. The transdermal route of drug administration has been employed constantly in the management of various systemic disorders such as hypertension, arthritis, diabetes, cancer, etc. It helps overcome drawbacks associated with oral and intravenous routes [1].

Dermal and transdermal routes offer a larger surface area available for drug absorption, ease in accessibility and termination of therapy whenever required Drug delivery through the skin helps in the management of both topical as well as systemic disorders [2].

It is a pain-free method of administration, facilitates self-medication in patients, is preferred in long-term term management of the ailments like chronic pain, and avoid hepatic first-passss metabolism [3].

Skin acts as a protector of the internal organs by shielding against external agents, and sunburn, and by regulating body temperature; however, sometimes pathogens invade the body and disturb the skin'sprotective properties, leading to skin diseases or infections [4] Bacteria, viruses, parasites, and fungi can cause skin diseases. Fungal infections are more severe because they occur on the third layer of the skin [5]. Fungi act on keratin tissue such as skin, nails, and hair [2].

Fungal diseases are difficult to manage because they tend to be chronic, hard to diagnose, and difficult to eradicate with antifungal drugs[6].In the skin, fungi lead to subcutaneous infections, and over the past years, the

cases of fungal skin infection shave been increasing rapidly, especially in immune-compromised individuals [7].

Fungal infections are typically recognized by symptoms such as itchy red color patches, hair loss, and crusted patches [8]. Some common conditions leading to fungal infection are wearing tight-fitting clothes or sharing a locker room, clothes, or furniture with an infected person [9]. Antifungal drugs, primarily topical, oral, and intravenous, are used to treat various types of fungal infections; however, oral antifungal drugs are more toxic to the human body as compared to topical antifungal drugs. Additionally, commonly used antifungal drugs contain different types of broad categories of components such as azole, echinocandin, and polyenes [10]. Azoles inhibit the oxidative enzymes present in the fungal cell membrane, which prevents the cell wall of the fungus from forming sterol (ergosterol), and due to incomplete synthesis, cells become permeable. On the other hand, echinocandins inhibit the synthesis of important polysaccharides (1,3-β-glucan) responsible for developing the cell wall, whereas polyenes directly bind to the ergosterol and move inside the cell through the cell membrane by creating pores, and through these pores, cellular organelles come out that cause the death of the cell [11]

Due to the immediate release of the drug, treatments for an extended period are sometimes needed due to low penetration. Additionally, these drugs may not reach the target location, which could lead to incomplete clearance of the infection. To overcome this problem, the use of natural plant extracts and oils as antifungal agents could be a practical approach [12].

Fungal infections:

Fungal infections are widespread in the population, generally associated with skin and mucous membranes. A disquietening trend after the 1950s is the rising prevalence of fungal infections due to the increasing use of broad-spectrum antibiotics, corticosteroids, anticancer/immunosuppressants, the emergence of AIindwellingling catheters, implants,nts, and dentures. They lead to the breakdown of the host defense mechanism, so saprophytic fungi easily invade living tissue.

Fungal infections can be classified as

- Superficial
- Systemic

The superficial fungal infection may be further classified to

1. Dermatophytosis which includes infection of the skin, hair, and nails
2. Candidiasis which includes infection of mucous membranes of mouth or vagina Dermatophytes are located in the stratum corneum within the keratinocytes. The signs and symptoms that pearinfected inviduals to cute and chronic inflammatory changes that appear in the dermis. For these reasons, antifungal agents should have the ability to penetrate the stratum corneum cells to be efficient when applied topically.

Dermatophytes may be classified according to the genera, ecology and, patterns of infection. The clinical picture forms distinct entities grouped according to the infected site, namely tineacapitis, tineabarbae, tineafavosa, tineacorporis, tineaimbricata, tineacruris, tineapedis, tineamanuum and tinea unguium.

Fungal Infections-Dermatophytosis

The management of dermatophytosis begins with topical agents. These agents should penetrate the skin and remain there to suppress the fungus. In the last 50 years, numerous drugs have been introduced for the treatment of superficial infections. The choice of treatment is determined by the site and extent of the infection, the species involved as well as by the efficacy and safety profile, and the kinetics of the drugs available. For localized non-extensive lesions caused by dermatophytes topical therapies with imidazole, allylamines, tolnaftate, morpholinederivates, etc is generally used.[13-14]

The developed formulation will facilitate rapid evaporation of solvents providing a cooling effect and reducing the tendency of rubbing off by forming a uniform thin layer onto the skin and infection site. The formulation will give rapid action as it will penetrate through the skin faster. Since it is self-applicable and easy to use, patient compliance/patient acceptability will improve, and a minimum quantity of dose will be able to maximize the compliance, ensuring a high margin of safety. This study will result in the development of an efficient, easy-to-apply, spray formulation containing a citral and curcumin for treating superficial fungal infections against candida species in nails and peripheries of the skin [15]

EXPERIMENTAL WORK

Materials and methods:

Ingredients used:

- Lemongrass
- Turmeric
- Glycerol
- Propylene glycol
- Ethyl alcohol
- Peppermint

Instruments used:

- Electronic balance
- Microwave-assisted
- Soxhlet apparatus
- Rotary evaporator
- Sonicator
- Magnetic stirrer
- Incubator
- Autoclave

Evaluation of spray

1. **Organoleptic characteristics –**

- Color
- Odour

2. Evaluations related to formulation

- pH
- Viscosity
- Drying Time
- Stickiness of the spray after evaporating the solvent
- Spray angle
- Solution volume delivered at each actuation
- Spray patterns
- Leakage test
- Invitro study

Plant Profile : -

Figure 1: Curcuma longa

1]. Turmeric (curcuma longa) -

Part used=roots

Scientific name and family:-Cucuma longa from Zingiberaceae

Uses=Curcumin also displayed various pharmacological Activities including antioxidant, antineoplastic, antiviral, antiinflammatory, antibacterial, antifungal, antidiabetic,anticoagulant, antifertility, cardiovascular protective, hepatoprotective, and immunostimulant activities in living organisms.

2]. Lemongrass (Cymbopogon flexuosus) -

Part used: Stalk, leaves, roots etc.

Figure 2: Cymbopogon flexuosus

Scientific name and family: Cymbopogon flexuosus with poaceae.

Uses: Cymbopogon flexuous activities such as anti-amoebic, antibacterial, antidiarrheal, antifilarial, antifungal and antiinflammatory properties. Cymbopogon citratus essential oil is used in aromatherapy. Lemongrass is antifungal and antibacterial in nature owing to citral, an organic compound that is found in its leaf, stalk and roots.

Pre-formulation of the drug:

For the most part, the pre-formulation studygenerates useful data to develop stable dosing forms. Regarding this project, studies about the application and effectiveness of lemongrass extract along with curcumin extract have been performed by going through the references available. The bioactivity of lemongrass has been extensively studied, including especially its antibacterial, and antifungal.

Haldi (Turmeric) scientifically known as Curcuma longa belongs to the family Zingiberaceae. Its polyphenolic compound curcumin has been varied as of antifungal investigations due to extensive traditional uses and very low side effects. The promising results for the antifungal activity of Curcuma longa made it a good candidate to enhance the inhibitory effect of existing antifungal agents. The basic aim of the study is to prove that turmeric can be used as a natural antifungal agent.

Studies of the combined effect of these two elements were performed

Organoleptic characteristics:

The physical inspection was conducted to test organoleptic curcumin and lemongrass characteristics, including color and odor.

Determination of the curcumin melting point

The melting point of curcumin was determined using the capillary rising method. Melting point of curcumin 183 °C.

Determination of solubility of curcumin and lemongrass in various solvents.

Ethanol is the most preferred solvent for extracting curcumin 100% ethanol (at about 1mg/ml) or DMSO (25mg/ml).

Materials and methods.

1. Sample collection

The C. citratus plant leaves were used for this study. The lemongrass plant leaves were collected from G.l.P.E.R, Limb, Satara.

Figure 3: Steam Distillation

2. Method. Steam Distillation Methods: Put 150 grams of fresh lemongrass sample into 1 lighted round bottom flask with 250 ml of distilled water. The flask is equipped with a rubber stopper to Connect to the condenser and heat. 0°C water Condensation through the condenser in the countercurrent to ensure steam. When water it reaches 100 °C, it starts to boil Essential oil from lemongrass. When the lemongrassis heated and the essential oil is extracted from

Leaves mixed with water vapor. Through the

Condenser and steam Condensed into liquid. With the use of ice cubes, Make cooling possible and volatilize Avoid using essential oils. Condensate uses a 500ml beaker to collect directly, then pour into the separatory funnel. This forms two Oil layers and water layer. Separated faucet Open the funnel to release water, and the oil Collect immediately 100ml stoppered bottle. The bottle is tightly closed to prevent the evaporation of essential oils. Oil is collected Weigh the volume of oil obtained [16-17]

Extraction method Turmeric

Materials and Methods

Raw material

Dried turmeric from Premium Food Co., Ltd.Satara was milled by hammer mill machine and sieved. Particle size of turmeric powder was at 1.2 mm. and stored in aluminum foil bags under vacuum. Turmeric powder was analyzed for total phenolic content, antioxidant capacity (EC50) and curcuminoid content.

Microwave-assisted extraction of turmeric

Tumeric powder 5 g was mixed with 95% ethanol (50 ml) for the extraction procedure. The mixture was placed in the center of microwave oven using different power; 400 and 800 watts and different duration time 1, 2, 3, 4 and 5 mins. The extracts were filtered and concentrated by rotary evaporator.[18]

Figure 4: Microwave-assisted

Curcumin extraction:

Conventional extraction using Soxhlet:

1. Fresh rhizomes were cleaned, washed with denoised water, sliced and dried in the sun for one week and dried again at 105ºC in a hot air oven for three hours.
2. Dried rhizomes were triturated using mortar and screened through a sieve with mesh 80 to obtain uniform powder with particle size of 0.18 mm.
3. The turmeric powder was stored in refrigerator to prevent moisture uptake.
4. The Soxhlet extraction was per-formed as follows: 15 g ground turmeric powder was weighed and embedded in a thimble and put in the Soxhlet apparatus which was gradually filled with acetone as the extraction solvent.
5. The extraction experiment was carried out at 60 °C within 8 h.
6. Upon completion of the extraction, the acetone was separated from the extract using rotary evaporator under vacuum at 35 °C. The residue was dried and weighed.

Figure 5: Soxhlet Appratus

Figure 6: Rotary Evaporator

Preparation of formulation

Lemon grass oil dissolve in Propylene glycol by using magnetic stirrer.Curcumin dissolved by using ethyl alcohol by using ultrasonicator. Curcumin solution is added step by step into lemon grass oil solution.The mixture is stirred by magnetic stirrer for 5 min.Ethyl alcohol is added into mixture in qs. and stirred for 2 min and at the end few drops of peppermint oil is added.The prepared formulation is filled into suitable container.

Formulation Ingredients Table

Table.1: Spray formulation

Composition of Ingredients				
Sr.no.	**Ingredient**	**F1**	**F2**	**F3**
1	Lemongrass oil	0.750ml	1.03ml	1.5ml
2	Curcumin	0.375gm	0.75gm	0.75gm
3	Glycerol	5ml	5ml	5ml
4	Proplyene glycol	4ml	5ml	6ml
5	Peppermint	0.25ml	0.25ml	0.25ml
6	Ethyl alcohol	Q.S	Q.S	Q.S
Total volume		30ml	30ml	30 ml

Excipients and their role

Table 2: Excipient and their role

Sr. No	Ingredient	Role
1	Glycerol	➢ Permeation enhancer ➢ Balancing of PH of media
2	Propylene glycol	➢ Solvent
3	Peppermint	➢ Anti-itching agent ➢ Perfume
4	Ethyl alcohol	➢ Analytical Solvent ➢ Penetrating agent ➢ Antifungal agent

Figure 8: Formulation 1

Figure 9: Formulation 2

Figure 10: Formulation3

EVALUATIONS

Evaluations of antifungal spray:

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.[24]

2. Viscosity:

Viscosity was calculated at 25±1°C using a Brookfield viscometer (digital viscometer model). The rotation of the ULA spindle was kept as 1 rpm.

The solution equivalent of 10ml was taken into a volumetric flask (100ml) and diluted using methanol. [24]

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 [24].

4. Stickiness of the spray after evaporating the solvent

Low pressure cotton wool is used to press the dry film to determine the stickiness of it. The stickiness is rated depending on how much of the cotton fibresis retained by the film. The stickiness is rated high if there is a thick accumulation of fibres on the film, medium if there is a thin fibre layer on the film and poor if fibre adherence occurs rarely or never. This parameter of assessment is important.[24]

Container related evaluations

5. 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:Spray angle (Θ) $=\tan^{-1}$ (h/r)

Where, l and r are the paper's distance from the nozzle and average circle radius, resp. [24].

6. Solution volume delivered at each actuation

The following equation was used to measure how much solution is delivered at each actuation.

AL=WO-Wt/D

Where, VL — Solution volume supplied at each

Actuation, Wt — Formulation weight after

Actuation, Wo — Formulation initial weight before

Actuation, and D — Formulation density (Measured using a pycnometer)[24].

7. Spray patterns:

A pH-sensitive paper was prepared by dipping the Whatman filter paper in a methyl red solution. The formulation (one actuation) was sprayed onto this paper.

The distance between the container and the destination was kept constant at 5 cm. Then, the pattern of spray was assessed by spraying the concentrates vertically and horizontally.

8. Short-term stability study :

The engineered batch's short-term stability reached 25 ± 2°C and RH 60 ± 50, for one month. The stability testing aimed to provide proof of how the quality of a formulation changes over time due to environmental factors such as viscosity, pH, solution volume on actuation, spray angle, and optimized batch ex-vivo physical characteristics remained unchanged during the analysis.[24]

9. Leakage test:

Leakage of canisters was verified by passing the canisters at 55°C and variability in weight in the water bath. Testing was done on selected samples. This examination was passed in batches.[24]

10.In-vitro antifungal activity:

Materials and Methods

2.1. Phytoconstituent, Antifungal Standard, and Substances

The following substances used in this work were obtained commercially: citral (purity 95%)

Culture Media

To test the biological activity of the product, Sabouraud dextrose agar (SDA) was purchased from agar-cornmeal from HiMédia Laboratories (Mumbai, India) culture media were used. They were prepared and used according to the manufacturer's instructions.

Fungal Strains

The experiment was performed with strains ofstandard C. albicans strains. Strains belong to the collection of the Microbiology Department of Pharmaceutical Sciences, G.I.P.E.R Limb Satara. These strains were maintained in SDA at 35°C and 4°C until used in tests.

Inoculum Preparation

The suspensions were prepared from recent C. albicans cultures, plated on SDA, and incubated at 35°C for 24–48 h. After incubation, we transferred roughly c. albicans colonies (with a sterile loop) to test tubes containing 5 mL of saline solution 0.9% [19-21].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The MIC was defined as the lowest citral concentration that produced visible inhibition of fungal growth. The antimicrobial activity of the products was interpreted (considered active or not), according to the criteria proposed by Morales et al. [22]. Strong/good activity (MIC: <100 µg/mL); moderate activity (MIC: 100–500 µg/mL); weak activity (MIC: 500–1000 µg/mL); and inactive product/no antimicrobial effect (MIC: >1000 µg/mL).5% lemongrass oil significant elimination of fungal infection.

Determination ofMinimum inhibitory concentration and minimum fungicidal concentration of Curcuma longa

The culture plate that did not demonstrate visible growth corresponds with the MIC of the antimicrobial agent. The MIC endpoint is the lowest concentration of the C. longa extract at which there was no visible growth in the tubes. The culture plate demonstrating no visible growth was subculture to Sabouraud agar plates, and MFC was determined by comparing the growth with the positive control. The MFC endpoint is defined as the lowest concentration of antimicrobial agent that kills >99.9% of the initial fungal population where no visible growth of the fungi was observed on the SDA plates[23].

RESULTS AND DISCUSSION:

1. pH Test:

pH of the formulation was determined using a pH meter.

pH of formulation was found to be 6.21as shown in Fig. 11. This is compactable with skin. Hence there is no irritation.

Figure 11: pH meter

2. Viscosity:

The viscosity of all the formulations was found to be in the range of 9 cps as shown in fig. 12.

Figure 12: Brookfield Viscometer

3. Evaporation Time:-

Evaporation time is the time required for spray film to dry and it was estimated by spraying the formulation on white paper and then the drying time was noted for each formulation is in between 1.53-3 min as shown in fig. 13.Indicate better penetration into skin.

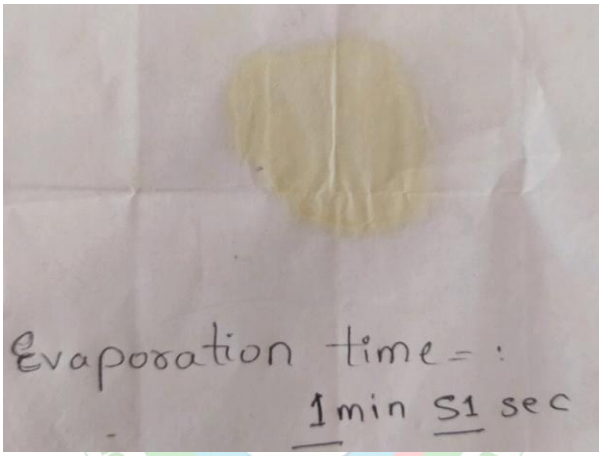

Figure 13: Evaporation Time of formulation

4. Stickiness of Spray:

No stickiness found in formulated spray which mentioned in fig. 14.

Figure 14: Stickiness of Spray

5. Spray angle:

Spray angle(ø)= $\tan^{-1}{}_{(h/r)}$

Where, h is the distance of paper from the nozzle &r is the average radius of the circle.

Therefore spray angle was found to be 80^0

6. Spray pattern:

-Spray pattern was assessed by delivering the spray on a paper. The good spray pattern result in uniformand spherical spot after actuation .Therefore spray pattern was found to be 1.6 cm. as shown in below fig. 15.

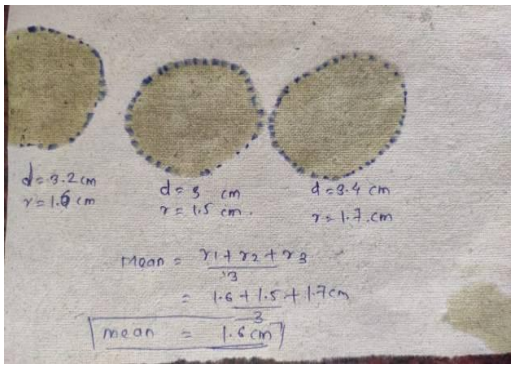

Figure 15: Spray pattern

1. Average weight per dose:

Average weight per dose(W)= (initial weight(W.)-final weight(W))/number ofdeliveries (N)

= 3.509 – 3.445

= 0.064 ml

Figure 16: Before spray

Figure 17: After spray

2. Short term stability study

The quality of a formulation changes over time due to environmental factors such as viscosity, pH, solution volume on actuation, spray angle, and optimized batch ex-vivo physical characteristics remained unchanged during the analysis**.**

2. Leak test:

Effectiveness of pump seal of a spray and its ability to store the contents of the products. Hence there is no leakage in container.

Figure 18: Leak test

Evaluation of spray

Table 3: Evaluation of spray

Sr. No	Parameter	Result	
		F1	F2
1.	pH	6.1	6.21
2.	Viscosity	9 cps	12 cps
3.	Drying time	2-3 min	1.53-3 min
4.	Stickiness of spray	No stickiness	No stickiness
5.	Spray angle	76.5^0	80^0
6.	Average weight for dose	0.068 ml	0.064 ml
7.	Spray patterns	1.6cm	1.6cm
8.	Short term stability	stable	stable
9.	Leak test	No leakage	No leakage

In-vitro antifungal activity

Result of MIC (Antifungal activity of Turmeric, Lemongrass oil and formulation against candida alibicans) as shown in below fig. 19,fig. 20 and fig. 21 respectively.

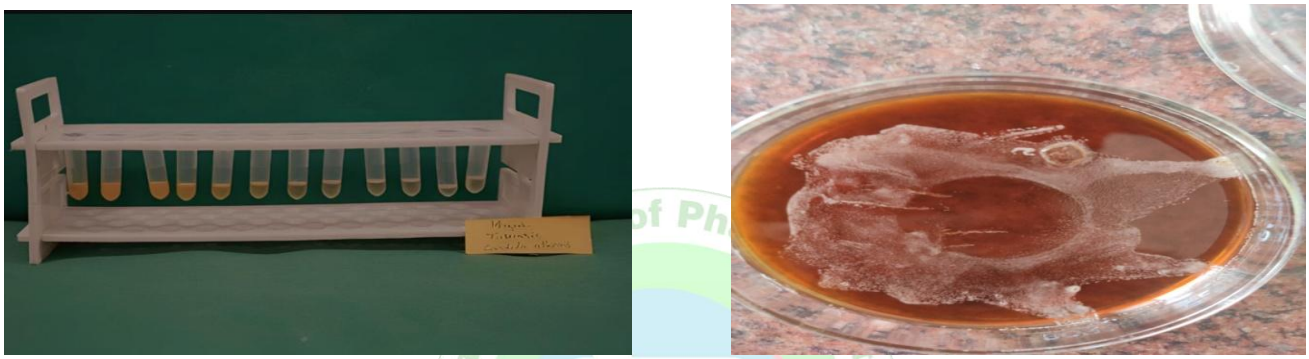

Figure 19: Result of MIC: curcumin

Figure 20: Result of MIC: lemongrass oil

Fig.21. Results ofMIC Test of Formulation

Table 4: Result of Minimum Inhibitory Concentration (MIC) turmeric extract and lemongrass oil against *Candida albicans*

Sr. no	samples	100 µl/ml	50 µl/ml	25 µl/ml	12.5 µl/ml	6.25 µl/ml	3.15 µl/ml	1.6 µl/ml	0.8 µl/ml	0.4 µl/ml
01	Turmeric extract	S	S	S	S	S	S	S	S	R
02	Lemongrass oil	S	S	S	S	S	S	S	R	R

NOTE **S=** Sensitive **R=** Resistant Standard value [fluconazole]=16µ/ml

RESULTS OF ANTIFUNGAL ACTIVITY.

Test Compound: 01 Compound

Method used for testing antifungal activity: Minimum Inhibitory Concentration (MIC)

Control: For MIC: Growth Control and Broth Control

Organism Tested: Standard strain of Candida albicans Results of MIC for F2 formulation

Table.5: Result of Minimum Inhibitory Concentration (MIC) of final formulation against *Candida albicans*

Sr.no	Samples	100 µl/ml	50 µl/ml	25 µl/ml	12.5 µl/ml	6.25 µl/ml	3.15 µl/ml	1.6 µl/ml	0.8 µl/ml	0.4 µl/ml
01	Extract	S	S	S	S	S	S	S	S	R

NOTE **S=** Sensitive **R=** Resistant Standard value [fluconazole]=16µ/m

CONCLUSION:-

This complete study and project work has confirmed of synthesis of antifungal spray preparation prepared by combining Cymbopogonflexuosus extract with the Curcuma longa extract and making a stable spray preparation that is effective in terms of antifungal activities. This spray preparation is based on the usage of the most basic and sufficiently available elements;its high bioavailability makes it more therapeutic effective and easy to produce formulation.After considering the elements used, it shows that it has no human harm.

The base solvent used in the formulation is ethanol which makes it not only easier for the application but also easierto absorption of the pharmaceutical component by penetrating it intothe skin easily and rapidly.The stability of the formulation was confirmed using proper solubility tests.The stability of solution is much more stable by using standard ingredients. The spray preparation has effectively worked on the fungal colonies prepared and has shown good results with its antifungal properties. The preservatives added might affect the quality and stability of the formulated product.This study effectively shows itsup-to-mark results which might be used in upcoming times to enhance and improve the formulation

The optimized yield of a formulated product has been confirmed by performing test activities on fungal colonies which were prepared in vitro. It has been performed in three different trials as F1, F2, and F3. Considering these trials F2 was the most effective trial ofall. In the F1 trial, the concentration of citral was 0.750 ml which was found to be less effective. In the F3 trial as the citral oil concentration increased solubility get decreased. The F2 trial concentration was 1.03 ml citral which was found to be very effective than the F3 trial.(F2>F1>F3)

ACKNOWLEDGEMENT:

The authors are thankful to the Gourishankar Institute of Pharmaceutical Education and Research, Limb Satara, and Yashwantrao Chavan Institute of Science, Satara for providing the necessary facilities.

REFERENCES:

1. Khurana S, Jain NK, Bedi PM. Nanoemulsion based gel for transdermal delivery of meloxicam: physicochemical, mechanistic investigation. Life sciences. 2013 Mar 14;92(6-7):383-92.
2. Rai, M.; Ingle, A.P.; Pandit, R.; Paralikar, P.; Gupta, I.; Anasane, N.; Dolenc-Volj˘c, M. Nanotechnology for the Treatment of Fungal Infections on Human Skin. Clinical Microbiology Diagnosis, Treatment and Prophylaxis of Infections the Microbiology of Skin, Soft Tissue, Bone and Joint Infections; Kon, K., Rai, M., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; Volume 2, pp. 169–184
3. Schmook FP, Meingassner JG, Billich A. Comparison of human skin or epidermis models with human and animal skin in in-vitro percutaneous absorption. International journal of pharmaceutics. 2001 Mar 14;215(1-2):51-6.
4. AbdElaziz, D.; Abd El-Ghany, M.; Meshaal, S.; El Hawary, R.; Lotfy, S.; Galal, N.; Ouf, S.A.; Elmarsafy, A. Fungal infections in primary immunodeficiency diseases. J. Clin. Immunol. 2020, 219, 108553.
5. Shields, B.E.; Rosenbach, M.; Brown-Joel, Z.; Berger, A.P.; Ford, B.A.; Wanat, K.A. Angioinvasive fungal infections impacting the skin: Background, epidemiology, and clinical presentation. JAAD 2019, 80, 869–880.
6. Tribhuvan G.S*, VelhalA. B, Jadhav. P.D, Redasani V.K., Review on Collagen as a wound healer., Asian Journal of Pharmaceutical Research and Development. 2022; 10(4):98-101.
7. Bongomin, F.; Gago, S.; Oladele, R.O.; Denning, D.W. Global and multi-national prevalence of fungal diseases—Estimate precision. J. Fungi 2017, 3, 57.
8. AbdElaziz, D.; Abd El-Ghany, M.; Meshaal, S.; El Hawary, R.; Lotfy, S.; Galal, N.; Ouf, S.A.; Elmarsafy, A. Fungal infections in primary immunodeficiency diseases. J. Clin. Immunol. 2020, 219, 108553.

9. 14. Jain, A.; Jain, S.; Rawat, S. Emerging fungal infections among children: A review on its clinical manifestations, diagnosis, and prevention. J. Pharm. Bioallied. Sci. 2010, 2, 314–320.
10. Nami, S.; Aghebati-Maleki, A.; Morovati, H.; Aghebati-Maleki, L. Current antifungal drugs, and immunotherapeutic approaches as promising strategies to treatment of fungal diseases. Biomed. Pharmacother. 2019; 110, 857–868.
11. Marek, C.L.; Timmons, S.R. Antimicrobials in Pediatric Dentistry. Pediatr. Dent. 2019; 128–141.e1.
12. Tabassum, N.; Hamdani, M. Plants used to treat skin diseases. Pharmacogn. Rev. 2014, 8, 52.
13. Rang HR,DaleMM,Ritter JM, Flower RE,Rang& Dales Pharmacology, 'Fungi and Fungal Infections,6th edition pg no:692-693.
14. Tripathi K.D,"Antifungal drugs" Essentials of Medical Pharmacology, Jaypee Brothers publication 2004; 715-24.
15. Mrunal Desai, Ajeet M Godbole*, Sandesh Somnache, Pankaj Gajare, Arti Pednekar, Siddhi Manerikar, KrutikaKudaskar, Vivek Naik. Design, Development, and Characterization of Film Forming Spray as Novel Antifungal Topical Formulation for Superficial Fungal Infections. Indian Journal of Pharmaceutical Education and Research |Vol 56 | Issue 4 (Suppl) | Oct-Dec, 2022DOI: 10.5530/ijper.56.4s.211
16. Suryawanshi MA, Mane VB, Kumbhar GB. Methodology to extract essential oils from lemongrass leaves: solvent extraction approach. International Research Journal of Engineering and Technology 2016, 03(08).
17. Akash M Wagh, Swapnil G Jaiswal and Deepak T Bornare. Extraction of essential oil from lemon grass as a preservative for animal products.Journal of Pharmacognosy and Phytochemistry 2021Sp, 10(3):26-31.
18. Kanjana Singh, PiyawanSimapaisanandNiramonUtama-ang. Effect of microwave-assisted extraction on curcuminoid from turmeric and application in germinate-coated rice. Food and Applied Bioscience Journal, 2017; 5(1):11-22.
19. Cleeland R, Squires E. Evalution of new antimicrobials in vitro and experimental animal infections. In: Lorian V, editor. Antibiotics in Laboratory Medicine. 3rd edition. Baltimore, Md, USA: Lippincott Williams & Wilkins; 1991. pp. 739–786.
20. Hadacek F, Greger H. Testing of antifungal natural products: methodologies, comparability of results and assay choice. Phytochemical Analysis. 2000; 11:137–147.
21. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. 2nd edition. CLSI Document M27-A2. Philadelphia, Pa, USA: CLSI; 2002.
22. Belletti N, Lanciotti R, Patrignani F, Gardini F. Antimicrobial efficacy of citron essential oil on spoilage and pathogenic microorganisms in fruit-based salads. Journal of Food Science. 2008; 73(7):M331–M338.
23. Jeevitha Murugesh,1Rajeshwari G Annigeri,2 G K Mangala,3 P Hema Mythily,4 and J Chandrakala5.Evaluation of the antifungal efficacy of different concentrations of Curcuma longa on Candida albicans: An in vitro study National Library of Medicine
24. Harshil M Patel*, AasmaGangat, Urvashi B Patel, BhaveshAkbari. Fabrication and Characterization of Luliconazole Film-Forming Topical Spray for the Treatment of Fungal InfectionsManipal Journal of Pharmaceutical Sciences | September 2020 | Volume 6 | Issue 231 Jul 2020.