Biocompatible Synergistic antifungal Essential Oil Cream for Topical Delivery of Fluconazole

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ABSTRACT: The incidence of antibiotic resistance and the widespread nature of fungus infections are recent issues in the field of public health. Utilizing the potential of natural bioactive compounds from plants is important to cure a fungal infection. Combining the effects of creams containing antibiotics with naturally occurring substances derived from plants is one method that might be used to develop a novel antifungal drug. Based on cinnamon, oregano, or clove essential oil (CIN, ORG, or CLV) as the oil phase and sucrose laurate (D1216) or sucrose palmitate (D1616) as surfactants, excipients with built-in antifungal action, we created topical biocompatible microemulsions. The intention was to improve fluconazole's absorption via the skin, tolerability, and therapeutic effectiveness. The research carried out revealed that antifungal cream combined with lemon grass oil had antifungal properties. This study intends to gain lemongrass oil's twin advantages as an antifungal and a penetration-enhancing agent. The addition of lemongrass to the fluconazole cream will have synergistic anti-fungal effects when fluconazole is combined with it and aid in penetrating the stratum corneum, the skin's protective barrier layer. The Fusion process was used to create several fluconazole creams that were oil-in-water emulsions. The cream formulation's pH, spreadability, viscosity, drug content, in vitro drug diffusion, and antifungal efficacy were all evaluated. Additionally, disc diffusion was employed. The formulation had a pH between 6 and 8, a spreadability between 3 and 3.5 cm, and a viscosity between 18000 and 19100 cp. The drug content varied from 92.5 to 98.1 percent, the in-vitro drug release varied from 91.8 to 97.0 percent, and the antifungal activity varied from 1.8 to 0.07 millimeters. Results from the F2 formulation were superior.

KEYWORDS: Fluconazole, Essential Oil, Lemon Grass Oil, Antifungal, Topical Delivery.

INTRODUCTION

Topical preparations with antifungal medicines, particularly azoles, are the first option for treating mild or localized types of superficial and cutaneous fungal infections because they allow you to focus treatment on the afflicted skin region while minimizing systemic side effects. The

topical antifungal preparations that are now on the market include traditional ointments, creams, gels, lotions, and sprays that contain the medication in free form. Poor drug penetration through the stratum corneum, the topmost layer of skin with strong barrier qualities, is the main disadvantage of traditional topical antifungal formulations. Due to the antifungal molecule's inability to effectively penetrate deeper skin layers like the living epidermis, their therapeutic efficacy and usefulness are constrained.

Modern pharmaceutical research is heavily reliant on topical medication administration since it provides a focused and effective means of treating a variety of mucosal and dermatological disorders. The use of essential oils as medication delivery vehicles is one such promising approach in topical drug delivery. This introduction addresses the novel and beneficial use of essential oils in combination with the antifungal drug fluconazole for localized treatment in this setting. 1-3

A major health problem across the world is fungus infections, which are mostly brought on by species of Candida and dermatophytes. Fluconazole stands out as a strong and often prescribed drug in the arsenal of antifungal medicines. Medical professionals can benefit from using it as a beneficial tool because of its well-established efficacy in treating a variety of systemic and superficial fungal diseases. Fluconazole (FZ), a fluorinated derivative of synthetic azole medications, is frequently used orally to treat systemic, cutaneous, and mucosal candidiasis despite its inherent adverse effects, mainly gastrointestinal problems.

It's been established that FZ has a far stronger affinity for skin keratin than other azoles, like itraconazole and ketoconazole, which are utilized as oral treatments for skin mycoses. Therefore, upon systemic injection, FZ quickly and considerably accumulates in the stratum corneum as an active, nonbinding form.

Its topical distribution must be optimised, nevertheless, to guarantee effective penetration, prolonged release, and improved therapeutic results while minimizing systemic exposure and negative effects. 4-6

Due to their intrinsic qualities, such as their antibacterial, anti-inflammatory, and permeation-enhancing qualities, essential oils made from aromatic plant sources have drawn interest. These oils not only have the potential to increase medication permeability through mucosal or skin barriers, but they also have additional advantages including calming and wound-healing qualities. A multidimensional strategy to improve the effectiveness of medications like fluconazole is the use of essential oils in topical preparations. Essential oils are complex blends of lipophilic volatile and non-volatile compounds that have shown antifungal activity and even synergistic effects with artificial antifungal medications like fluconazole. They are also efficient penetration enhancers and have received the GRAS (generally recognized as safe) certification from the FDA. These characteristics led to the combination of several essential oils as parts of the oil phase, sucrose esters as surfactants (stabilizers), isopropyl alcohol as a cosurfactant, and water to create stable ME systems for topical FZ administration.

In this investigation, we will dig into the benefits of employing essential oils as fluconazole delivery agents, emphasizing their modes of action, possible synergies, and prospective uses in the management of localized mycoses. Additionally, we will go through the difficulties and factors to be taken into account while developing such novel delivery methods, eventually

illuminating the changing environment of topical drug administration for better therapeutic results in dermatology and other fields.7, 8

There is evidence that essential oils and their terpene components improve the skin's ability to transport both hydrophilic and hydrophobic medicines. Among these oils are clove oil, turpentine oil, eucalyptus oil, peppermint oil, and lemongrass oil. The volatile compounds found in various plant components, such as flowers, fruits, leaves, and roots, are what make up essential oils, a sort of natural oil. These oils may penetrate the bottom layer of skin and are often regarded as safe and harmless for human use. A member of the Poaceae family of plants, Cymbopogon flexuosus Stapf. (syn. Andropogonnardus var. flexuous Hack.) produces lemongrass essential oil from its leaves. There are often terpenes and terpenoids, notably citral.

This oil has been employed as an effective permeation in several topical treatments. The main reason lemongrass can increase permeation is because the disturbance of the skin's lipid network alters skin diffusivity. in 2021) (Sorathia et al. Additionally used to treat fungus infections, essential oils are more biocompatible and less likely to cause negative effects. To cure cutaneous infections, plant-based essential oil is therefore seen as an alternative to manufactured antibiotics.One of the most popular essential oils for treating fungus infections is lemongrass. The physiologically active and predominant component oflemongrass oil is citral.

A synthetic antifungal medication of the imidazole family, fluconazole acts by inhibiting the development of infection-causing fungus. It is an azole derivative and a broad-spectrum antifungal medication. It is used daily in doses of 200 mg to 500 mg for the treatment of tinea versicolor and dermatophytosis. Fluconazole has poor solubility (1 mg/ml) and high permeability (log P=0.58) and is classified as a class 2 medication by the BCS. It is available in parenteral and oral dosage forms, which have a high likelihood of serious adverse responses, including nausea, vomiting, diarrhea, rash, and a decrease in red blood cell count. Patients using triazoles may also have hepatotoxicity. To avoid these adverse effects, it is thus strongly advised that efforts be made to manufacture topical medical dosage versions of FLZ.,[10].

It has been shown that topical antifungal medications are quite effective in treating fungal infections. Since fungus hyphae (mycelium) may penetrate deeply through the epidermal layers, it is ideal to improve the penetration of antifungal medications to the dermis layer of the skin. This study's objective was to determine the origins of fungus infections to create and assess FLZ cream, which contains lemongrass oil, as a topical treatment for a fungal infection. The newly

developed antifungal cream's efficacy will be contrasted with that of an antifungal topical cream based on fluconazole that is already on the market.[11].

MATERIAL AND METHOD

The fluconazole came from Sigma Aldrich in India. We purchased beeswax, cetostearyl alcohol, liquid paraffin, methyl paraben, propyl paraben, propylene glycol, triethanolamine, and carpool 940 from LOBA Chemie Pvt. Ltd. in Delhi. Borax, glycerin, and methanol were purchased from CDH Pvt. Ltd. in Delhi. Every reagent used was of the caliber of an analytical reagent.

METHODOLOGY:

FORMULATION OF FLUCONAZOLE CREAM CONTAINING ESSENTIAL OIL:

By fusing the oil and water phases, the formulation was created in the cream. When the aqueous phase and oil phase were heated to a temperature between 70 and 85 degrees Celsius and then combined while being well agitated, the medication was dissolved in the propylene glycol and an emulsion was created. A creamy texture was obtained (o/w) and the formulation of fluconazole cream incorporating essential oil as given in Table 1 after adding the drug solution and essential oil and triturating for 30 hours.[7].

Table 1: Formulation Table of Fluconazole Cream Containing Essential Oil.

FORMULATION CONTENT	**F1**	**F2**	**F3**	**F4**	**F5**
Fluconazole (mg)	500	500	500	500	500
Essential oil (lemongrass oil) (ml)	0.5	1	1.5	2	2.5
Beeswax (gm.)	1	1.5	1.5	1.5	1.5
Cetostearyl alcohol (gm.)	0.2	0.25	0.25	0.25	0.25

Carbopol 940 (gm.)	0.5	0.6	0.65	0.65	0.7
Liquid paraffin (ml)	3	4	4	4	5
Borax (gm.)	0.2	0.25	0.3	0.35	0.3
Glycerin (ml)	3	4	4.5	5	5.5
Methyl paraben (gm.)	0.04	0.04	0.04	0.4	0.04
Propyl paraben (gm.)	0.07	0.07	0.07	0.07	0.07
Triethanolamine (ml)	0.1	0.1	0.1	0.1	0.1
Propylene glycol (ml)	2	2	2	2	2
Water (ml)	60	70	75	80	85

EVALUATION:

Organoleptic properties: The viscosity, color, and homogeneity of the physical evaluation are taken into consideration.

pH:

A pH meter calibrated with standard buffer solutions at pH 4, 7, and 9 was used to measure the pH. The electrode was inserted into the sample ten minutes before the measurement was taken at room temperature.

Spreadability:

A sample of 0.5g of each formulation was taken after being crushed between two slides with 500g weights for about five minutes. As a baseline for spreadability (spread circle diameter - beginning diameter), spread circle diameters were measured in centimeters.

Viscosity:

Using a Brookfield viscometer, model-VL2 (Lemis Baltic), and spindle No. 6 4 at 30 rpm, the viscosity of fluconazole cream was evaluated.

Drug content

Determination of Fluconazole Content (Cream):

A predetermined amount of the cream, corresponding to 1 g of fluconazole, was transferred to a volumetric flask with a 10 ml alcohol content, sonicated, and then filtered to determine the amount of fluconazole in the cream. Next, the sample was appropriately diluted before being analyzed at a maximum of 260.

Then, using the provided formula, the drug content was determined:

$$\text{Drug content} = \frac{\text{Absorbance} \times \text{Dilution factor} \times 1/1000}{\text{Slope}}$$

In vitro diffusion study: The release of the medication from the skin and the egg membrane were the subjects of this investigation. the separation of the egg membrane from the egg shell, and the preparation of a 6.8 pH phosphate buffer. The egg membrane should be washed with distilled water after being separated, and then it should be kept in the phosphate buffer for 24 hours. Perform the in-vitro diffusion research after 24 hours.

In- vitro Antifungal activity

The Agar-based disc diffusion technique (ABDD) was used to test this formulation's antifungal efficacy against several commonly recognized dermatophyte species. Sample "A" Fluconazole Cream with Lemongrass Oil and Sample "B" Plain Cream (both equaling 2 g/disc) were made by dissolving the fluconazole cream, which includes lemongrass oil, in water. Then, 10 cc of each of these samples was placed onto a sterile disc. Sterile discs were also impregnated with 10 l of a water dilution of 1:100 as a control "B" experiment. The aforementioned 2 discs were placed on top of each infected and dried plate, which was subsequently incubated at 28 °C for up to 5 days. For each antifungal drug, the size of the zones of growth inhibition was measured.

Preparation of fungal culture

On Saubourd dextrose agar plates, stock cultures of Candida albicans were produced and stored in slants. Each culture was inoculated into a flask and then incubated at 280°C for 48 hours to establish the stock culture for the research. The stock culture was serially diluted 10 times with sterile peptone water, and 0.1 ml of each dilution was applied on seaboard dextrose agar plates. The plates were then incubated for 48 hours at 280 °C. The number of colony-forming units (CFU) in the stock culture was calculated after counting the CFU from the plates of each dilution.

RESULT AND DISCUSSION

Solubility: Table 2 displays the solubility of fluconazole in chloroform, methanol, propylene glycol, and water.

Drug	Soluble in	Sparingly soluble	Method of solubility
Fluconazole	Freely soluble in Chloroform, methanol(38mg/ml), propylene glycol,	Water (1mg/ml)	Shake flask method

Table 2: Solubility of fluconazole

UV-visible spectrophotometer scanning of the pure drug: The results of UV-visible scanning of the pure medication in methanol are shown in Figure 10 below. The drug's -max value is 260 nm, which is in line with the Indian Pharmacopoeia's standard value for fluconazole.

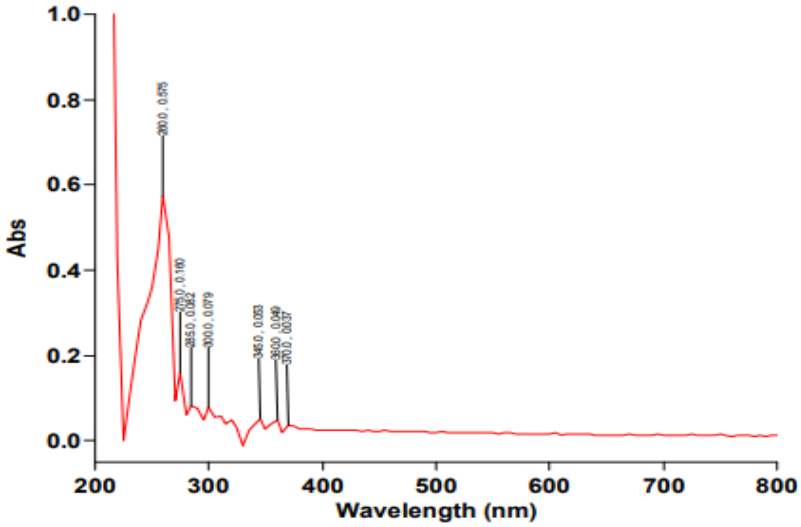

Fig 1: U.V Scanning of fluconazole pure drug in methanol

Calibration curve of Fluconazole:As illustrated in Table 3, the fluconazole standard calibration curve was prepared. Use the UV-visible spectrophotometer to calculate the absorbance at 260 nm. Fig. 4 below illustrates the graph that was created between the absorbance and concentration.

S. No.	Concentration in µg/ml	Absorbance
1.	2	0.0078
2.	4	0.0142
3.	6	0.0184
4.	8	0.0263
5.	10	0.0335

Table Standard Curve of Fluconazole

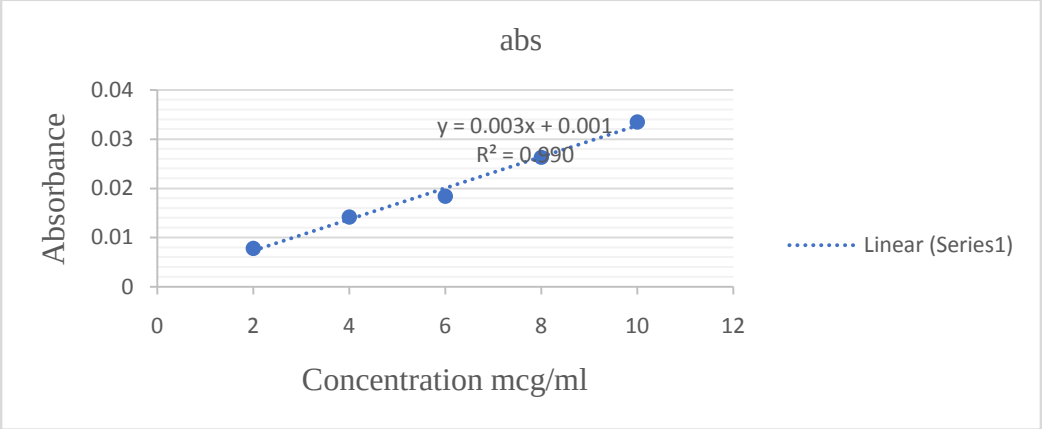

Fig 2: Standard calibration curve of fluconazole

Partition coefficient:Partition coefficient in Water and Octanol: Add the medication to the solution and rapidly mix water and octanol in a 1:1 ratio. Shake the mixture constantly for 30 minutes. After letting the solution remain for 24 hours to achieve layer separation, distinct layers were collected, and the absorbance of each layer was then measured at 260 nm using a UV-visible spectrophotometer. Afterward, note the outcomes. The phosphate buffer 6.8 and chloroform underwent the same process. Tables 4 and 5 provide the partition coefficient.

The absorbance of the octanol phase	**The absorbance of the water phase**	**Partition coefficient (K=Co/Cw)**
0.0490	0.2289	4.671

Table 4: Partition coefficient of octanol and water

The absorbance of the chloroform phase	The absorbance of phosphate buffer	Partition coefficient (K=Co/Cw)
0.1871	1.5911	0.11

Table 5: partition coefficient of chloroform and phosphate buffer

Fourier transformer infrared spectroscopy: Utilising Cary 360 Agilent technology in a 1:1 ratio of drug and polymer, FTIR of drug and excipient compatibility investigations were carried out. There is no significant difference between the peak of fluconazole alone and the combination of fluconazole and carbopol 940, and it was determined that there was no drug-polymerinteraction.

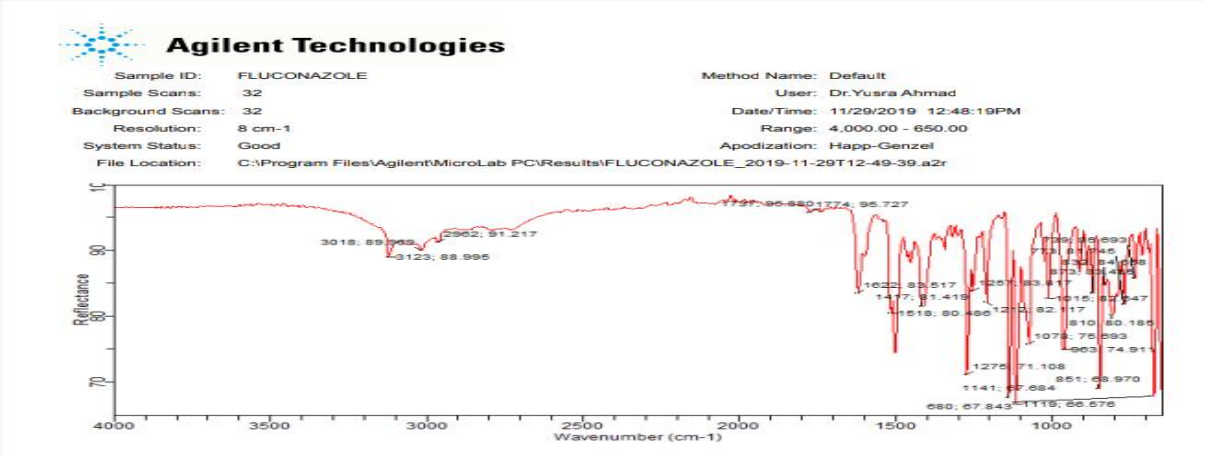

Fig 3: FTIR of Fluconazole API

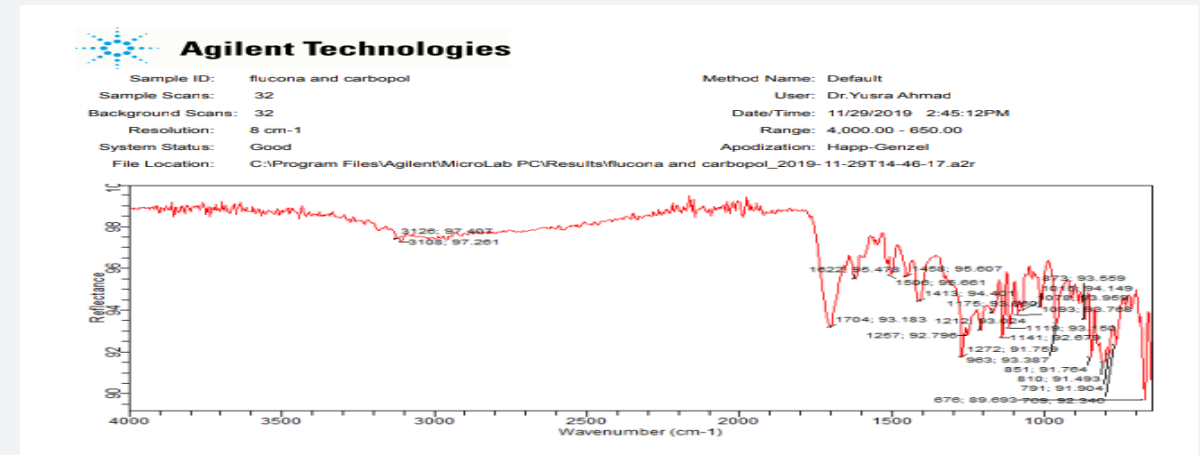

Fig 4: FTIR of Fluconazole and Carbopol 940

pH: Any topical preparation should have a pH of between 3 and 9 to effectively treat fungus infections. The pH was measured using a digital pH meter. pH as indicated in Table No. 6 below.

Formulation	**pH**
F1	6.8
F2	6.2
F3	6.3
F4	6.3
F5	6.0

Table: 6: pH of the cream formulation

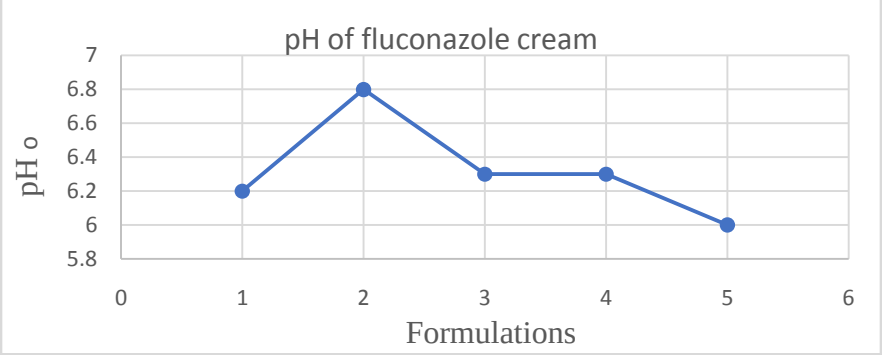

Fig 5: pH of cream formulation shown graphically

Spreadability: Spreadability was assessed using 3-3.3 cm samples of the various formulations depicted in Fig. 8.

Formulation	**Spreadability in cms**
F1	3.3
F2	3
F3	3.5
F4	3.3
F5	3.2

Table 7: Spreadability of cream

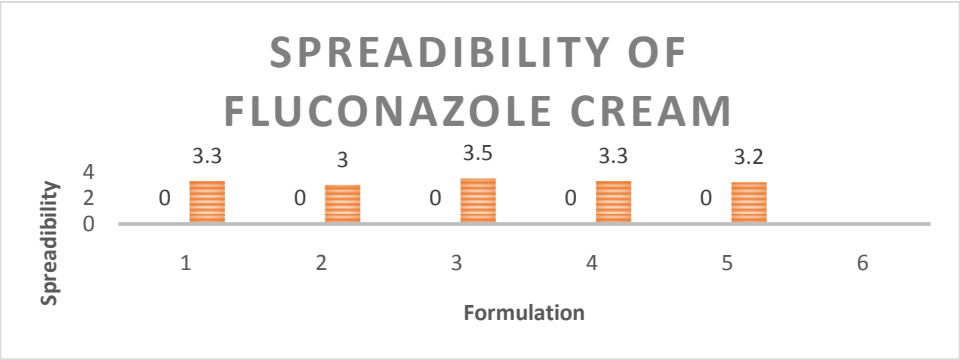

Fig 6: Fluconazole Cream Spreadability with Essential Oil

Viscosity: Oil in water cream viscosity ranges of 18000- 19100 were measured with spindle 64 at 30 rpm. presented in Table 9.

Formulation	**Viscosity**
F1	18360
F2	18000
F3	18540
F4	18700
F5	19100

Table 8: Viscosity of cream formulation

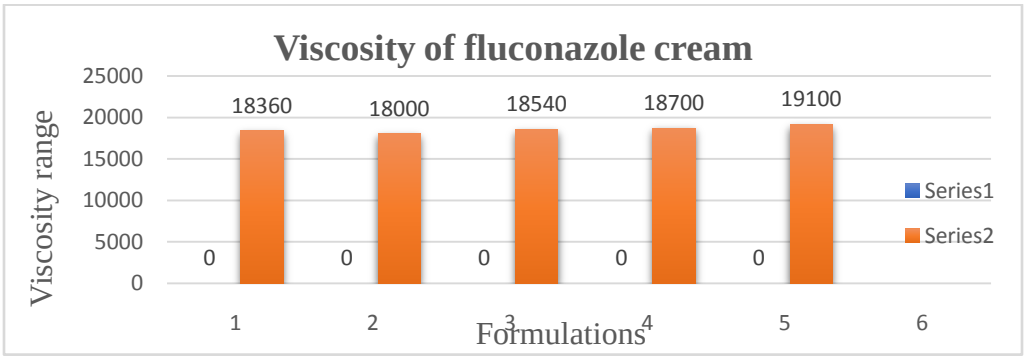

Fig 7: Graphical Representation of the Viscosity of the Cream

Drug content: Each formulation's drug concentration was calculated using U.V spectrophotometry at 260 nm, or between 92.50 and 98.10% of its wavelength. seen in Fig. 10.

Formulation	**Drug content**
F1	96.28%
F2	98.10%

F3	92.50%
F4	94.09%
F5	93.81%

Table 9: Drug content

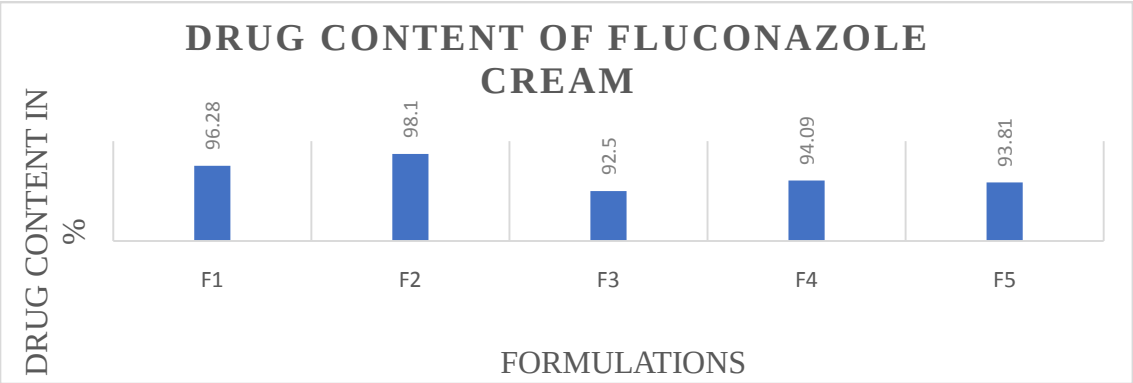

Fig 8: Fluconazole Drug Content in Fluconazole in Graphical Form

In-vitro drug diffusion study: Drug diffusion was assessed in vitro using the egg membrane. (%CDR) The cumulative drug release % was calculated and is shown in the table. 11.

Time (Hrs.)	**F1**	**F2**	**F3**	**F4**	**F5**
0	0 %	0 %	0 %	0 %	0 %
1	8 %	7.3 %	6.2 %	9.1 %	8.8 %
2	19.02 %	19.87 %	14.5 %	14.07 %	19.7 %
3	29.93 %	34.3 %	29.7 %	28.62 %	32.45 %
4	38.56 %	46.7 %	36.6 %	41.8 %	41.05 %
5	65.11 %	75.65 %	55.2 %	50.06 %	54.21 %
6	82.95 %	84.29 %	64.1 %	78.07 %	67.51 %
7	91.2 %	93.22 %	75.8 %	89.5 %	89.87 %
8	92.77 %	97.04 %	94.55 %	91.8 %	93.4 %

Table 10: Fluconazole In-Vitro Drug Diffusion and Cumulative Drug Release

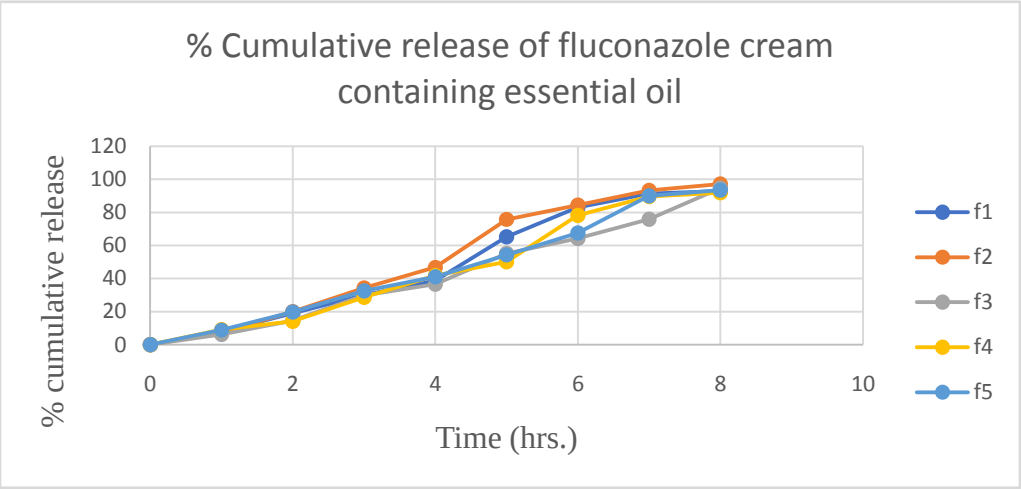

Fig 9: In-Vitro Drug Diffusion%Cumulative Drug Release in Graphical Form

In-vitro antifungal activity:

By using the disc diffusion method, it was determined that fluconazole cream has antifungal activity against Candida albicans, and the value of the zones of inhibition (ZOI) was found to be 1.8 0.07 mm.

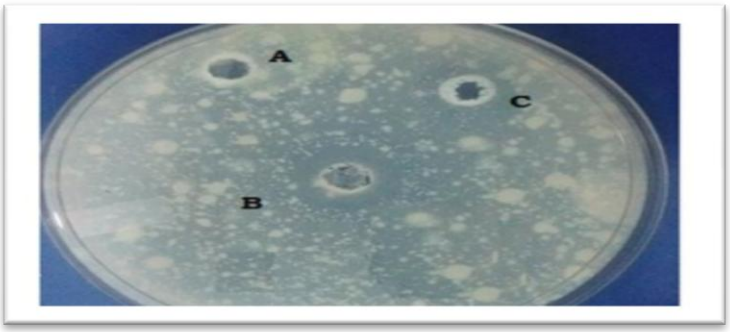

Fig: 12: Fluconazole's In Vitro Antifungal Activity Against Candida Albicans

CONCLUSION:

The trituration approach was effectively used to generate the fluconazole cream system, which reduced application frequency and provided a formulation with acceptable texture. Different drug-polymer ratios had a big influence on drug release, in vitro diffusion, and drug content. The fluconazole formulation was chosen for further investigation due to its superiority in terms of physiochemical characterization, in vitro diffusion, drug content, spreadability, and viscosity. Fluconazole cream had a considerably increased diffusion activity with lemon grass oil than ordinary creams, according to in vitro diffusion experiments. In the current study, the F2

formulation, which has a drug-to-polymer ratio of 1:2, exhibits improved outcomes with essential oil and also improves the drug's bioavailability and fluconazole's solubility.

In the future, fluconazole oil in aqueous cream infused with lemongrass oil will aid in the treatment of a fungus infection while enhancing the drug's solubility with essential oils. It has improved Candida Albican's zone of inhibition activity. According to this study, fluconazole cream infused with lemongrass oil can be used to treat fungus infections. **REFERENCES:**

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