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APPLICATION OF LEMONGRASS OIL AS AN ANTIFUNGAL AND PENETRATION ENHANCER AGENT IN THE FORMULATION AND EVALUATION OF FLUCONAZOLE CREAM

Gulafshan Praveen [1]*, Mohit [2], Neha Joshi [3], Arun Kumar Maurya [4], Vineet Singh [5], Kaustuv Sahoo [6] and Lubhan Singh [7]

[1] Assistant Professor, Faculty of Pharmacy,
Veer Madho Singh Bhandari Uttarakhand Technical University, Suddhowala,
Dehradun, Uttarakhand, India. *Corresponding Author E-mail: gparveen.pharma@gmail.com
[2] Principal and Professor, Guru Nanak College of Pharmaceutical Sciences,
Jhajra, Dehradun, Uttarakhand, India.
[3] Assistant Professor, College of Pharmacy, Graphic Era Hill University,
Bhimtal Campus, Bhimtal, Nainital, Uttarakhand, India.
[4] Principal and Professor, JBIT College of Pharmacy, Dehradun, Uttarakhand, India.
[5] Associate Professor, Shri G.S Institute of Technology and Science, 23-Park Road, Indore, India.
[6] Adjunct Faculty, Shir G.S Institute of Technology and Science, 23-Park Road, Indore, India.
[7] Professor and Head, Department of Pharmacology, Kharvel Subharti College of Pharmacy,
Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India.

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Abstract

Aim: Essential oils have been widely reported to have therapeutic activity, and have been incorporated into a wide range of pharmaceutical formulations. Topical delivery is the most important and convenient route for drug delivery through the skin in various diseases including fungal infections. Skin is the largest organ of the body that is widely used to provide local and systemic effects. Fluconazole is a broad-spectrum antifungal drug commonly used for dermatophytosis. It is a poorly soluble drug of BCS class II. Essential oils are commonly used to treat fungal infections and also act as penetration enhancers. Lemongrass is one of the most common essential oils used in the pharmaceutical industry as it has broad-spectrum antibacterial, antifungal and insect repellant activities on topical application. It also acts as a penetration enhancer. The aim of this research is to get the dual benefits of lemongrass oil as an antifungal as well as a penetration enhancer agent. The incorporation of lemongrass in the cream of fluconazole will provide synergistic anti-fungal effects in the combination of fluconazole and help in penetrating the barrier layer of skin i.e., stratum corneum. **Methodology:** Different oil-in-water emulsion-type cream of fluconazole was prepared by using Trituration method. The cream formulation was evaluated for pH, spreadability, viscosity, drug content and in vitro drug diffusion and antifungal activity. **Result:** The fluconazole-turpentine oil cream was successfully prepared and evaluated. The pH, spreadability and viscosity of formulation were found in between 6-8, 3-3.5cm and 18000-19100cp respectively. The drug content range was found 92.5-98.10%, and the In-vitro release of drug diffusion was found 91.8-97.04%. **Conclusion:** Fluconazole cream containing lemongrass essential oil is safe and effective for topical application to the skin in fungal infections. The addition of lemongrass oil gave a synergistic effect with the drug for anti-fungal activity. The F2 formulation showed a better pH, and spreadability. It is avoiding.

Keywords: Topical, Turpentine Oil, Fungal, Skin, Oil Phase, Aqueous Phase.

INTRODUCTION

Fungal infection of the skin is one of the major dermatological problems. Various antifungal agents like fluconazole, ketoconazole, clotrimazole, miconazole, itraconazole and griseofulvin are used to treat fungal infection. Skin is one of the most effective means for local therapy of fungal infection. Topical drug delivery is a localized mean of administrating drugs through the skin, rectal, vaginal, and ophthalmic routes. Skin is the most affordable and accessible part of the topical route of drug administration. It covered almost $2m^2$ in an adult body. Essential oils and their terpenes

components have been reported to act as an effective penetration enhancer through the skin for the delivery of both hydrophilic and hydrophobic drugs. Among these oils are clove oil, turpentine oil, eucalyptus oil, peppermint oil and lemongrass oil. Essential oils are natural oil containing a mixture of volatile compounds found in different parts of plants, such as flowers, fruits, leaves, and roots. These oils are generally considered safe and nontoxic to human application and have the ability to permeate into the lower layer of skin(Alhasso et al., 2022). Lemongrass is an essential oil obtained from leaves of the plant *Cymbopogon flexuosusStapf*. (syn. *Andropogonnardus var. flexuosus* Hack.), belonging to the family *Poaceae*. It usually contains terpenes and terpenoids mainly citral [1].

This oil has acted as an effective permeation in various topical formulations. The permeation enhancement effect of lemongrass is primarily due to altered. Lemongrass is one of the most common essential oils for the treatment of fungal infections. Citral is the biologically active and most abundant constituent of lemongrass oil. Fluconazole is a synthetic antifungal agent of the imidazole class; it works by slowing the growth of fungi that cause infection. It is a broad-spectrum antifungal drug which belongs to azole derivatives. It is used for dermatophytosis and tinea versicolor in a dose of200mg-500mg per day. Fluconazole belongs to the BCS classification class 2 drug and has low solubility(1mg/ml) and high permeability (log P=0.58).It is presented as oral and parenteral dosage forms which can be associated with serious side effects as stomach upset, diarrhea, feeling sick, vomiting, rash, and reduction in red blood cells. In addition, there is incidence of hepatotoxicity in patients receiving triazoles. Therefore, trying to develop topical pharmaceutical dosage forms of FLZ is highly indicated to avoid such adverse events. [12]

The topical antifungal agents are found to be quite satisfactory in the treatment of fungal infections. Improving the permeation of the antifungal agents to the dermis layer of skin is desired as fungi hyphae (mycelium) can enter deeply through the epidermal layers. Therefore, the aim of the current study was to develop and to evaluate FLZ cream containing lemongrass oil for topical treatment of fungal infection. In addition, to antifungal effect, lemongrass also enhances the penetration of drug into the skin. The efficacy of prepared antifungal cream will be compared to the antifungal topical cream of fluconazole currently available in the market.

Fig 1: Lemongrass

MATERIAL AND METHOD

Fluconazole was obtained from Sigma Aldrich, India. Bees wax, Cetostearyl alcohol, Liquid Paraffin, Methyl Paraben, Propyl Paraben, Propylene Glycol, Triethanolamine, and Carbopol 940 was obtained from LOBAChemie Pvt. Ltd. Delhi. Borax, glycerin, and Methanol were obtained from CDH Pvt. Ltd. Delhi. Other reagents used were of analytical reagent grade.

Method:

Preformulatıon Studıes:

Solubility study:

The solubility of fluconazole in many lipophilic environments was determined by conventional equilibration method. The concentration of drug was analyzed using double beam UV–Visible spectrophotometer (UV–Vis Systronics-2201) after appropriate dilution with carbinol at 276 nm.

Partition coefficient: Partition coefficient (oil/water) estimation of the drug's lipophilicity.

Po/w = (Coil / C water).

Partition coefficient in octanol and water/ Chloroform and water: The partition coefficient was determined by the shake flask method the drug dissolves in water and octanol phases. Take 62.5 ml of octanol and 62.5 ml of water in 1:1 ratio in a separating funnel and weigh the 50 mg of drug were added into the solution than shake separating funnel vigorously for 30 min. and mixed well for dissolving the drug properly. Then stand for 24 hours the separated layers of organic and aqueous phases were collected from the funnel into the beaker and then filtered out both solution with Watt man filter paper and see the absorbance using UV spectrophotometer at a wavelength 260nm.

Fourier transformer infrared spectroscopy:

Fourier transformer infrared spectroscopy was done by the drug and the mixture of drug and polymers analyzed. Drug and polymer were taken in a 1:1 ratio and the data was obtained and recorded for the characteristic group peaks present in the drug.

Procedure:

Formulatıon Of Fluconazole Cream Contaınıng Essentıal Oıl:

Formulation was developed in the cream by the fusion method of oil and water phase. Oil phase was melted and drug was dissolved in the propylene glycol and it into oil phase and aqueous phase was heated then mixed both phases temperature maintained at 70-85^{o}C and stirred well emulsion was formed the drug solution and essential oil was added now triturate it for 30 hours, then keep aside for 2 hours the creamy texture was obtained (o/w)[10].

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
Bees wax (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 physical evaluation is considered a series of physical characteristics (viscosity, color, and uniformity).

pH: The pH was measured in every formulation, using a pH meter, which was calibrated before every use with standard buffer solutions at pH 4, 7, 9. The electrode was inserted in to the sample 10 min priors to taking the reading at room temperature.

Spreadibility: A sample of 0.5g of each formulation was pressed between 2 slides with 500g weights and left for about 5 min where no more spreading was expected. Diameters of spread circles were measured in cm and were taken as comparative values for spreadability (diameter of the spread circle –initial diameter.

Viscosity: The viscosity of Fluconazole cream was measured in Brookfield viscometer, model-VL2 (Lemis Baltic) with spindle No 6 4 at the 30 rpm.

Drug content: Determination of Fluconazole content (Cream): Fluconazole content in the cream was determined by taking required quantity of the cream which is equivalent to 1 gm of fluconazole transferred to 10 ml volumetric flask containing alcohol and it allowed to sonicate and filtered. Then, suitably diluted and analyzed at λ max 260.

Then the drug content was calculated by the given formula:

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

Slope

In vitro diffusion study: This study was done by the egg membrane and release of drug from the skin. **Preparation of 6.8 pH phosphate buffer, Separation of egg membrane from the egg shell.** After the separation of egg membrane wash it with distilled water and store it in the phosphate buffer for 24 hours. After 24 hours perform the in-vitro diffusion study.

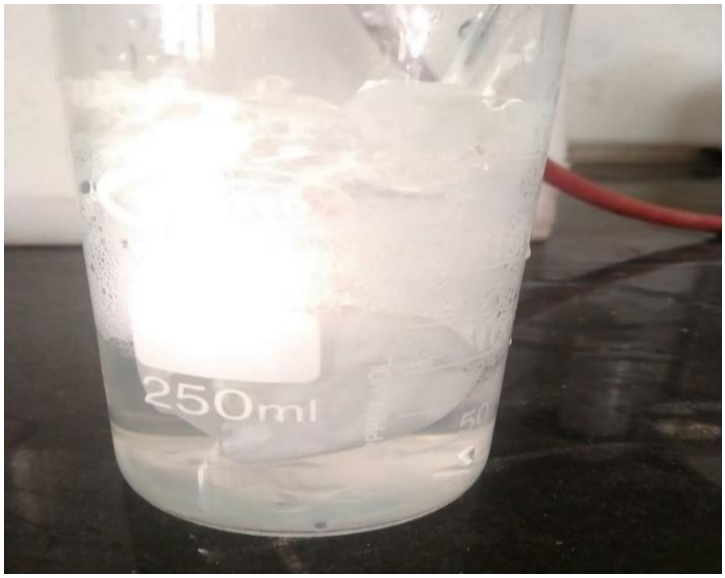

Fig 2: Separation of egg membrane from the egg shell

RESULT & DISCUSSION

Solubility:

Table 2: Solubility of fluconazole

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

Scanning of pure drug by UV-visible spectrophotometer: UV- visible scanning of pure drug in methanol has been summarized below figure 10.Theλmax value of the drug 260nm which is related to the standard value of the fluconazole (Indian pharmacopoeia).

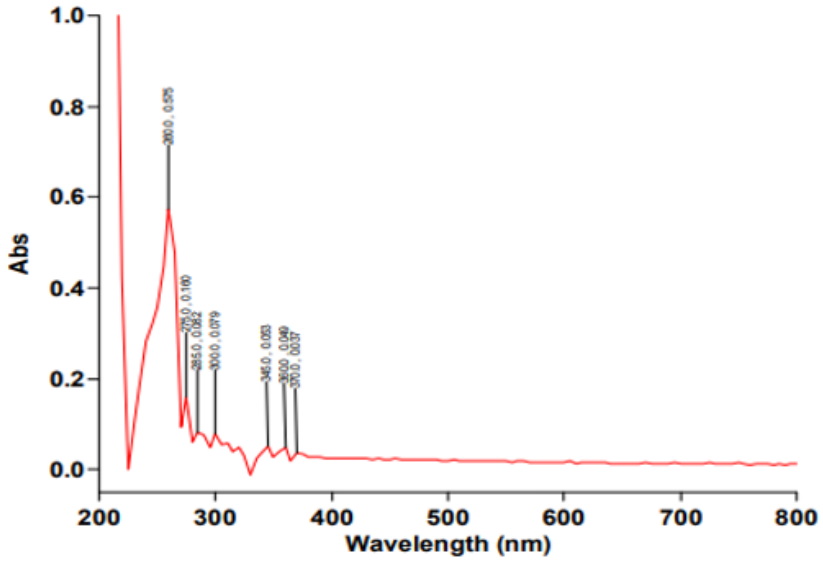

Fig 3: U.V Scanning of fluconazole pure drug in methanol

Calibration curve of fluconazole:

Preparation of standard calibration curve of fluconazole in methanol: Measure the absorbance at 260nm by using the UV-visible spectrophotometer. Plotted the graph in between the absorbance and concentration which is shown below in fig:4.

Table: 3 Standard Curve of Fluconazole:

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

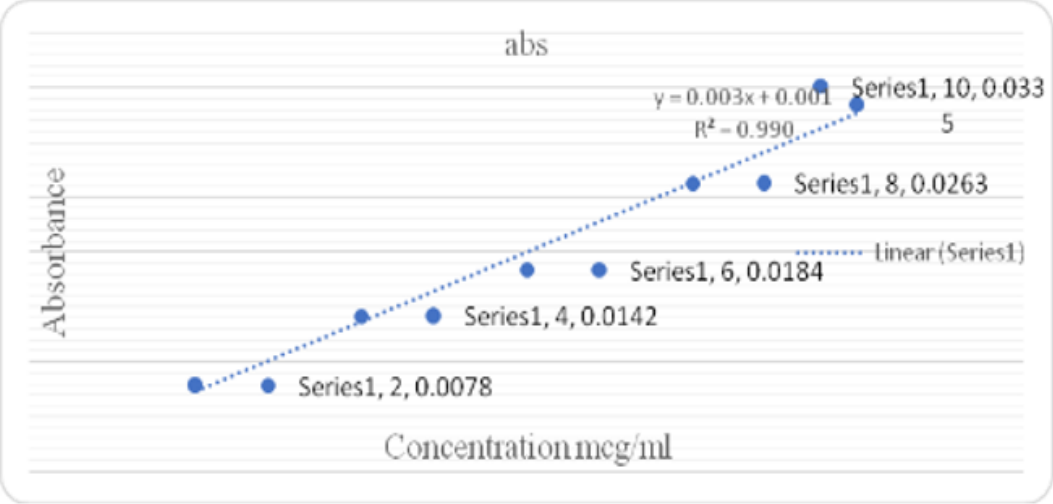

Fig 4: Standard calibration curve of fluconazole

Partition coefficient: Partition coefficient in Water and Octanol Take water and octanol in 1:1 ratio vigorously mixed and added the drug into the solution and shake continuously for 30 min. Then stand the solution for 24 hours until the separation of layers obtained and collected the layers separately than measured the absorbance in the U.V visible spectrophotometer at the 260nm. Than record the results. Same procedure followed for the chloroform and phosphate buffer 6.8. Partition coefficient shown in below table 5 & 6.

K=Co /Cw

Table 5: Partition coefficient of octanol and water.

Absorbance of octanol phase	Absorbance of water phase	Partition coefficient (K=Co/Cw)
0.0490	0.2289	4.671

Table 6: partition coefficient of chloroform and phosphate buffer.

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

Fourier transformer infrared spectroscopy: FTIR of drug and excipients compatibility studies was performed using cary 360 Agilent technology in 1:1 ratio of drug and polymer.There is no major change in the peak of fluconazole alone and the mixture of fluconazole and carbopol 940 and it was destinguished no interaction between drug and polymer.

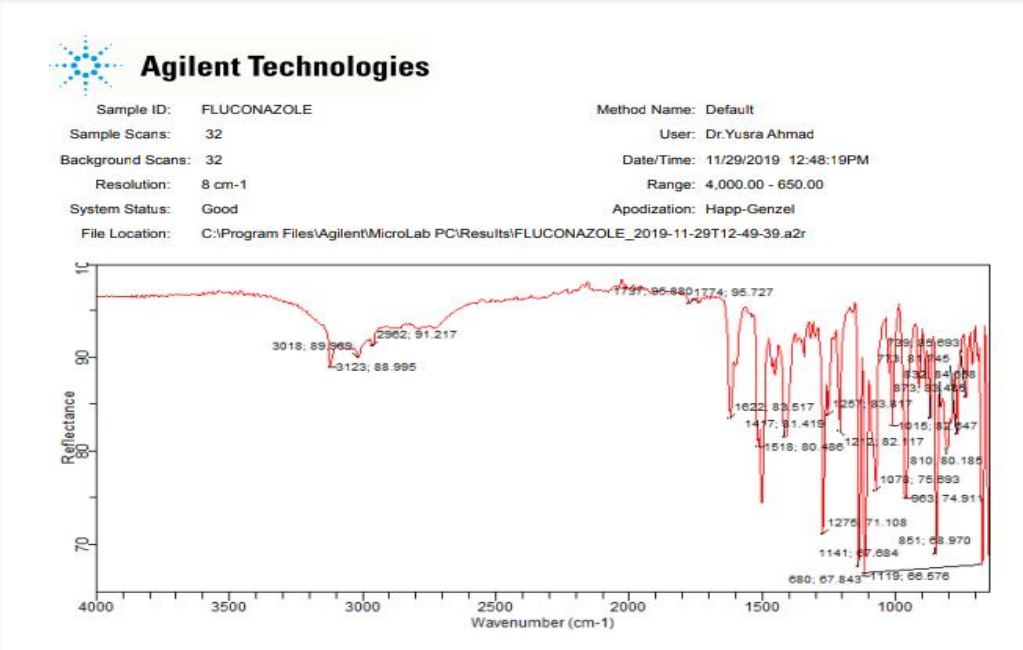

Fig 5: FTIR of Fluconazole pure drug

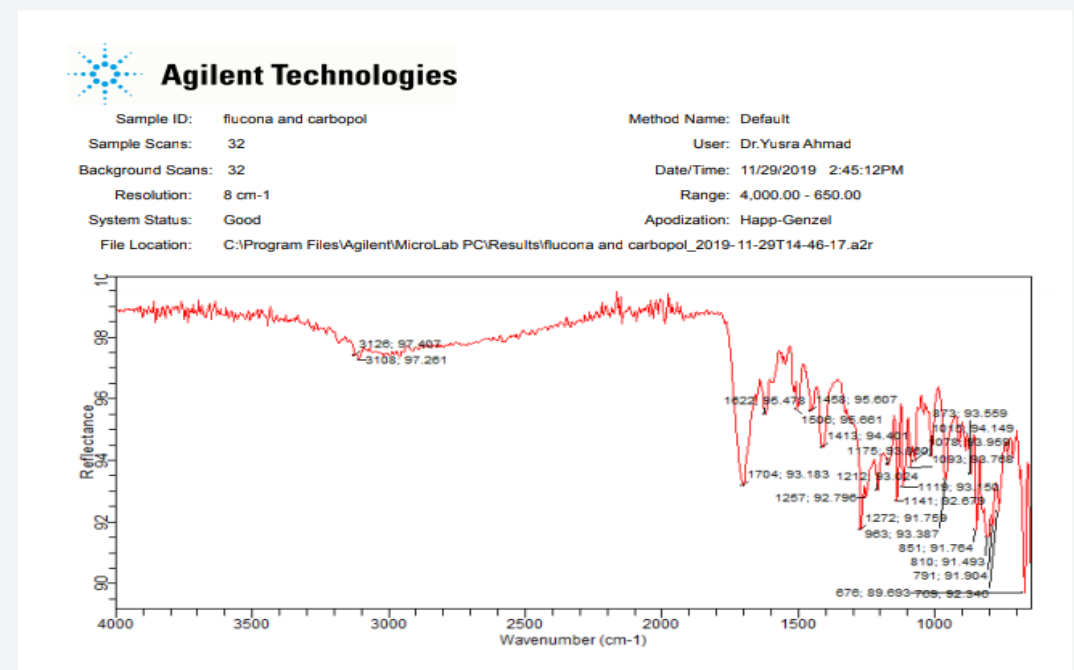

Fig 6: FTIR of Fluconazole and Carbopol 940

pH: pH of the any topical formulation should be between in 3-9 for treat the fungal infections. pH was determined by the digital pH meter. pH shown below the table no. 7.

Table 7: pH of the cream formulation

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

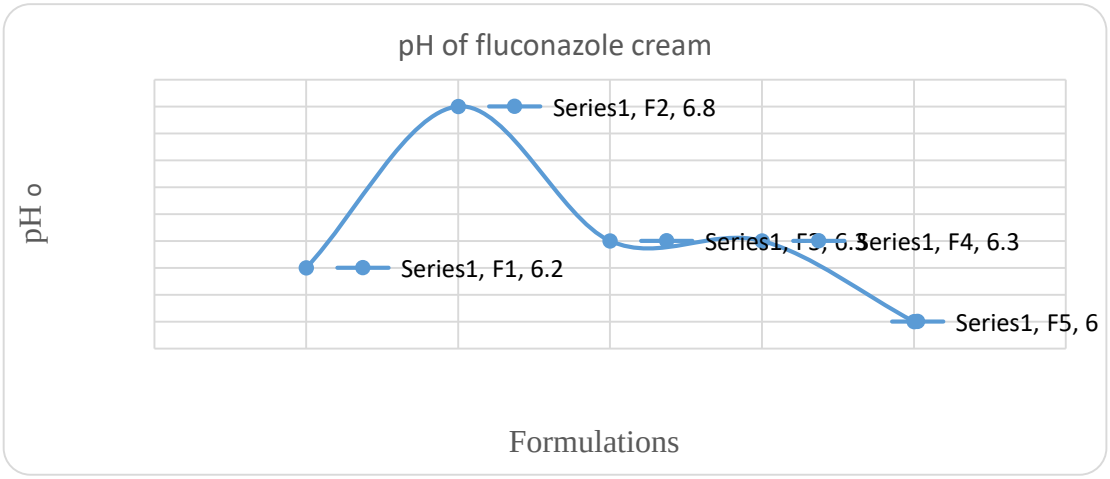

Fig 7: Graphical representation pH of cream formulation

Spreadability: Spreadability was determined 3-3.3 cm of different formulation shown in the fig. 8.

Table 8: Spreadability of cream

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

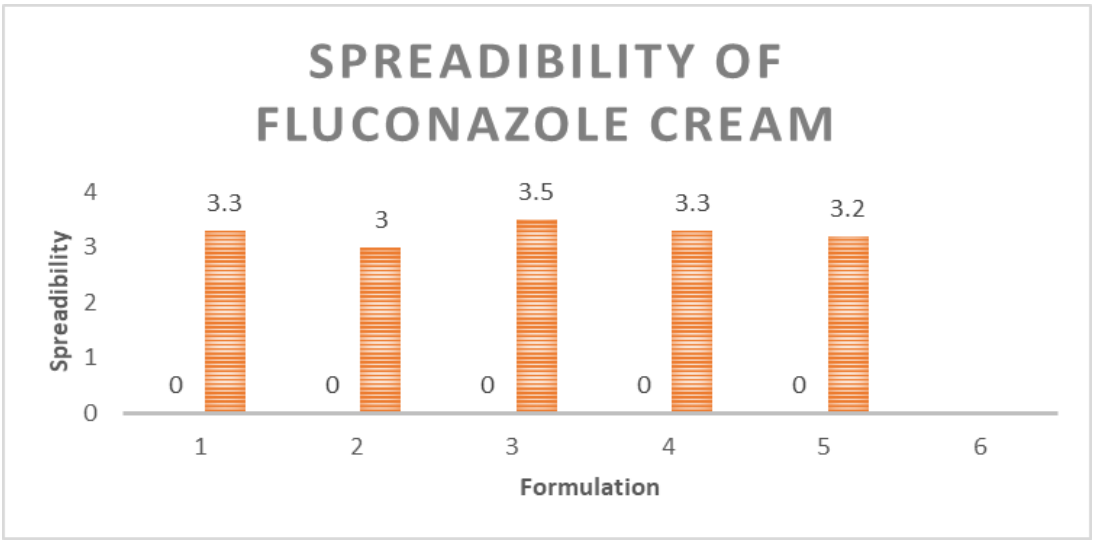

Fig 8: Spreadibilityof Fluconazole Cream Containing Essential Oil

Viscosity: Oil in water cream viscosity ranges was determined 18000- 19100 with spindle 64 at 30 rpm. Shown in the table 9.

Table 9: Viscosity of cream formulation

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

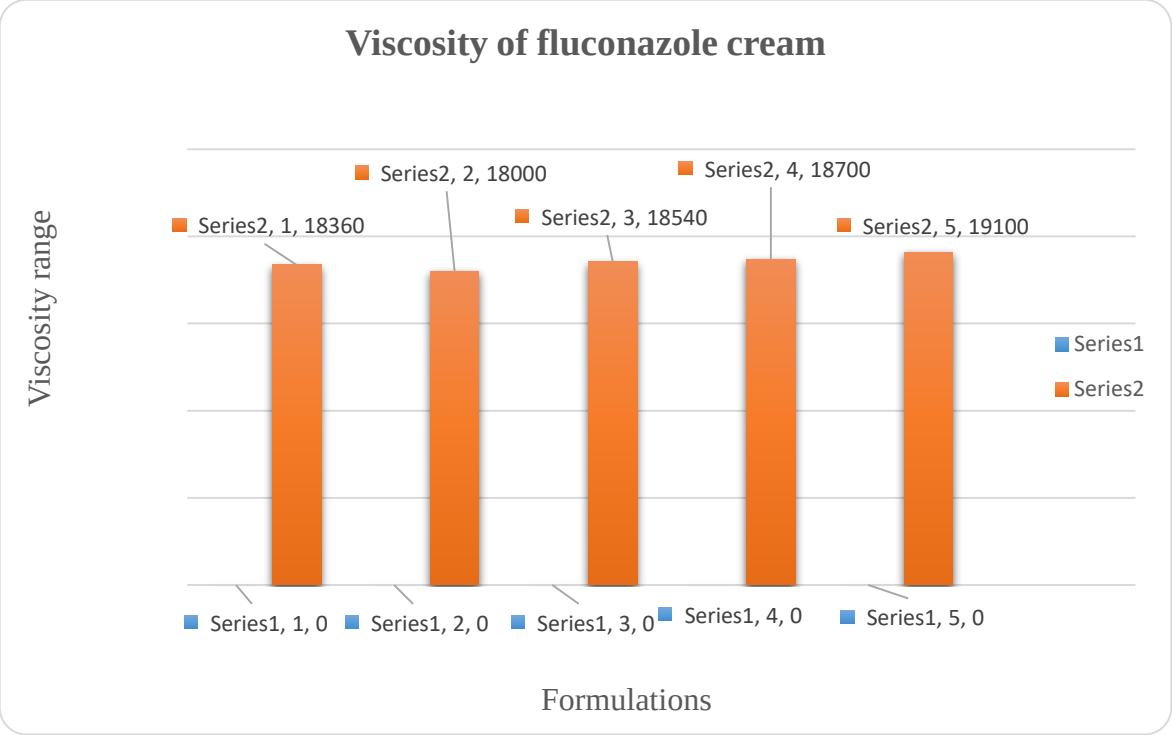

Fig 9: Graphical Representation of the Viscosity of the Cream

Drug content: Drug content was determined each formulation by the U.V spectrophotometry at the wavelength 260 nm it is between 92.50-98.10%. Shown in the fig. 10.

Table 10: Drug content

Formulation	Drug content
F1	96.28%
F2	98.10%
F3	92.50%
F4	94.09%
F5	93.81%

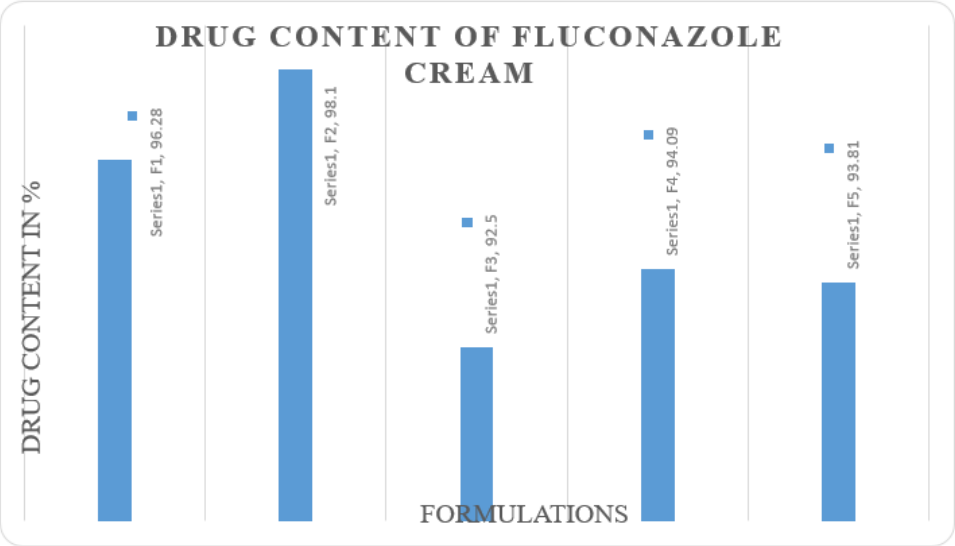

Fig 10: Graphical Representation of Percentage Drug Content of Fluconazole

In-vitro drug diffusion study: In- vitro drug diffusion was determined from the egg membrane. (%CDR) Percentage Cumulative drug release was determined and it given in the table. 11. [12]

Table 11: % In-vitro Drug Diffusion and Cumulative Drug Release of Fluconazole

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 %

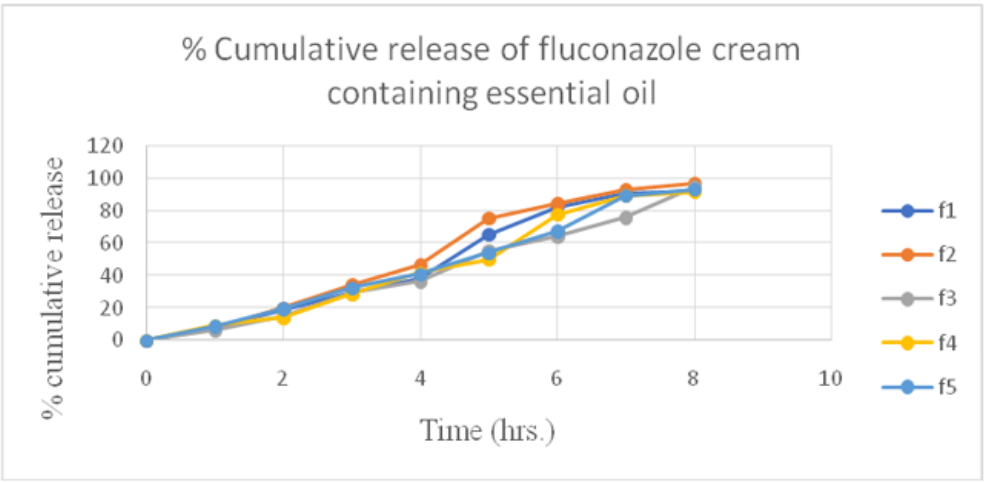

Fig 11: Graphical Representation In-Vitro Drug Diffusion %Cumulative Drug Release

In-vitro antifungal activity:

The zones of inhibition (ZOI) were seen the fungal strains. The zone of inhibition of Fluconazole microsponge formulation was 1.9 mm and no zone of inhibition was seen with disk containing DMSO and plain gels against Candida Albicans. The results of zone of inhibition are summarized in Table:12.[11]

Table: 12: In-vitro antifungal activity of Fluconazole Cream

Parameter	Fluconazole Cream
Zone of Inhibition	18±0.07

Zone of inhibition

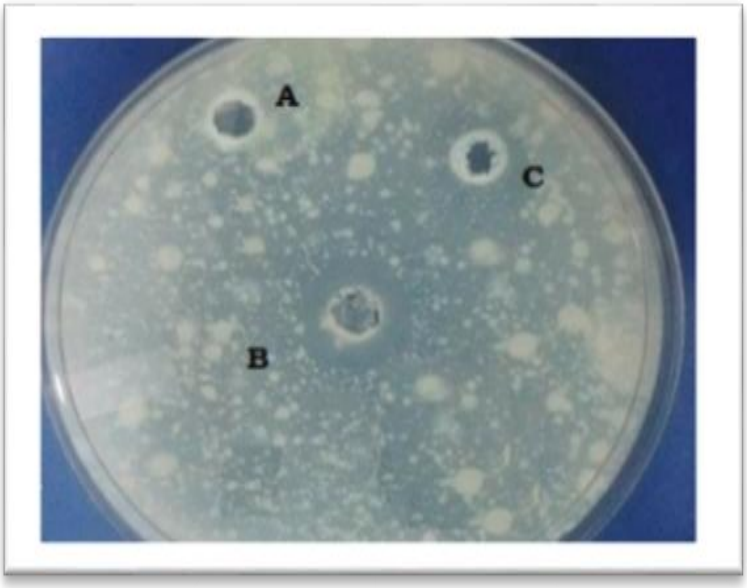

Fig: 12: Invitro Antifungal Activity of Fluconazole against *Candida Albicans*

DISCUSSION

The physical appearance of fluconazole drug was found to be a white fine powder with its odorless property and it was sparingly soluble in the water (1mg/ml) and freely soluble in methanol (l38mg/ml), freely soluble in warm propylene glycol and also freely soluble in the chloroform.

Melting point was found to be 138^{o}C and calibration curve was also prepared for the estimation of fluconazole in methanol at 260nm. Partition coefficient was found in octanol and water 4.671 and in chloroform and phosphate buffer is 0.11. FTIR was determined carry 360Agilent. There is no change in the peaks of fluconazole with Carbopol 940. Resolution is 8cm^{-1} at the 400.00-600.00 range. After the formulation it was evaluated by the evaluation parameters that is pH is found in between 6-8, spreadability was found at 3-3.5cm. Viscosity was found 18000-19100cp, Drug content range was found 92.5-98.10%, and the In-vitro release of drug diffusion was found 91.8-97.04%.

Invitro Antifungal Activity was found to 1.8±0.07, it showing a better zone of inhibition against *Candida Albicans.*

CONCLUSION

Fluconazole cream system was developed successfully using Trituration method for topical delivery up to prolonged period so as to reduce application frequency, formulation with good texture. Varied drug-polymer ratio reflected remarkable effect on drug content, in vitro diffusion,and drug release. The Fluconazole formulation was chosen for further study on the basis of its superiority in terms of physiochemical characterization, In vitro diffusion, drug content, spreadibility, viscosity. *In vitro* diffusion studies revealed that, the cream of Fluconazole had shown a significantly higher diffusion activity with lemon grass oil than plain creams. Present research F2 formulation which have the ratio of drug and polymer 1:2 Shows better results with essential oil and it is also increases the bioavailability of the drug and solubility of the fluconazole. In future the fluconazole oil in water cream with lemongrass oil will be helpful for treating the fungal infection and essential oil helps the increase the solubility of the fluconazole drug. In future the fluconazole oil in water cream with lemongrass oil will be helpful for treating the fungal infection and essential oil helps to increase the solubility of the fluconazole drug. It shows the better zone of inhibition activity against the *Candida Albicans.* In this research it is concluded that, fluconazole cream with lemongrass oil can treat the fungal infections.

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