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FORMULATION AND EVALUATION OF ANTIDANDRUFF HAIR GEL FOR TREATMENT OF SEBORRHOEIC DERMATITIS

S.P. Dhamane*[1], N.V. Tayade[2], V.V. Potnis[1], A.S. Kulkarni[1] and A.S. Gadekar[1]

[1]JSPM'S Jayawantrao Sawant College of Pharmacy & Research, Hadapsar, Pune.

[2]Abbott Pharma Pvt. Ltd. Goa.

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***Correspondence for Author**
S.P. Dhamane
JSPM's Jayawantrao Sawant College of Pharmacy & Research, Hadapsar, Pune.

ABSTRACT

Seborrhoeic dermatitis is a chronic, non inflammatory condition of the scalp that is characterized by excessive scaling of scalp tissue. Dandruff may be a symptom of more serious problem, such as scalp become thick or yellowish colour seborrheic dermatitis. Various Antifungal agents are widely used in hair shampoos for the treatment of dandruff. These products show temporary effect for span of hours in a day on the scalp. Therefore, an attempt has been made for formulation of Fluconazole and Tea tree oil anti-dandruff gel which may give antidandruff action for number of hours. Placebo formulations were prepared by using different concentrations of carbopol 940, glycerine as a humectant and allantoin as a anti-irritant. All formulations were evaluated for physical appearance, pH, viscosity, extrudability, spradability. Based on viscosity, spreadability, extrudability formulation CGA8 containing 0.6% carbopol 940, glycerine and allantoin was selected as best formulation for addition of fluconazole and tea tree oil. The formulation FTCGA8 **(**containing 2% Fluconazole and 5% Tea tree oil) and TCGA8 (containing 5% Tea tree oil) shows good drug release than FCGA8 as it contains 2% fluconazole only. Antifungal activity shows that formulation FTCGA8 and TCGA8 shows higher efficacy.

KEYWORDS: Fluconazole, Tea tree oil, Seborrhoeic dermatitis hair gel, *Malassezia furfur* (MTCC-1374).

INTRODUCTION

Dandruff is a common embarrassing disorder which effects 5% of the global population.[1] *Malassezia furfur* is strongly suspected to play a role in the manifestation of the Seborrhoeic

Dermatitis**.** Currently available treatment options for the management of Seborrhoeic Dermatitis include therapeutic use of Selenium sulphide, zinc pyrithione, salicylic acid, imidazole derivatives, glycolic acid, steroids, and coal tar derivatives. However, these agents show certain limitations, either due to poor clinical efficacy or due to the compliance issues. Furthermore, these drugs are unable to prevent recurrence. Fluconazole and Tea tree oil are a broad spectrum synthetic antifungal agent having the chemical name 2-(2, 4-Difluorophenyl)-1,3-bis(1H,2,4-triazol-1-yl)propan2-ol and Empirical formula $C_{13}H_{12}F_2N_6O$, used in the treatment of variety of fungal infections. Various antifungal agents are widely used in hair shampoos for the treatment of dandruff/ Seborrhoeic Dermatitis These products show temporary effect for span of hours in a day on the scalp[2] Therefore, an attempt has been made for formulation of Fluconazole and Tea tree oil Antidandruff hair gels which may give antidandruff action for number of hours.

MATERIALS AND METHODS

Fluconazole was obtained as gift sample from Nulife Pharmaceuticals Pune. Tea tree oil was procure from Kelkar food and fragrance Pune, Carbopol 940, HPMCK4M, PEG 400, polyethylene glycol, methyl paraben, propyl paraben were procured from SD fine chemicals, Mumbai, India and all others chemicals and reagents were used of analytical grade. *Malassezia furfur* (MTCC 1374) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

METHODS

Formulation of Anti-dandruff hair gel

Measured quantity of methyl paraben, propyl paraben, glycerin, and weighed quantity of polyethylene glycol were dissolved in about 35 ml of water in beaker and were stirred at high speed using mechanical stirrer. Then carbopol 940 added slowly to the beaker containing above liquid while stirring allantoin was incorporated slowly in above dispersion (A). In one beaker, Fluconazole drug was dissolved in methanol and tea tree oil was dissolved in methanol in another beaker. Fluconazole solution was added slowly in dispersion A and stirred for 5min. then tea tree oil was added. Finally Triethanolamine (gelling agent) was added slowly while stirring till it attain neutralized gel structure. The details are shown in table no. 1.

Table 1: Formula of placebo hair gel.

S/N	Ingredients w/w	C1	C2	C3	CG4	CG5	CG6	CGA 7	CGA 8	CGA 9
1.	Carbopol 940	0.4	0.6	0.8	0.4	0.6	0.8	0.4	0.6	0.8
2.	Poly ethylene glycol	13	13	13	13	13	13	13	13	13
3.	Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
4.	Propyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
5.	Glycerin	-	-	-	6	6	6	6	6	6
6.	Allantoin	-	-	-	-	-	-	0.5	0.5	0.5
7.	Triethanolamine	q.s. to neutralize	q.s. to neutralize	q.s. to neutralize	q.s. to neutralize	q.s. to neutralize	q.s. to neutralize	q.s. to neutralize	q.s. to neutralize	q.s. to neutralize
8.	DistilledWater	qs	Qs	Qs	qs	qs	qs	qs	qs	qs

Table 2: Formula of antidandruff hair gel.

S/N	Ingredients w/w	FCGA8	TCGA8	FTCGA8
1.	Fluconazole	2	-	2
2.	Tea Tree oil	-	5	5
3.	Carbopol 940	0.6	0.6	0.6
4.	Poly ethylene glycol	13	13	13
5.	Methyl paraben	0.1	0.1	0.1
6.	Propyl paraben	0.1	0.1	0.1
7.	Glycerin	6	6	6
8.	Allantoin	0.5	0.5	0.5
9.	Triethanolamine	q.s. to neutralize	q.s. to neutralize	q.s. to neutralize
10.	DistilledWater	qs	qs	qs

Evaluation of hair gel

Physical examination

The prepared gel formulations were examined for their colour, clogging, homogeneity and presence of air bubbles.

pH

The pH of gel formulations was determined by using digital pH meter. About 1gm of non medicated gel was dissolved in 100 ml of distilled water. The measurement of pH of each formulation was made in triplicate and average values were calculated.

Viscosity: The gel formulations were assessed for viscosity (cp) using a Brookfield viscometer (Brookfield LVDV, spindle no. S64) at 25°C-28°C. The gels were rotated at 10, 5 and 2.5 rpm using spindle no.S64. At each speed, the corresponding viscosity (in cps) and torque (in %) values were noted.

Spreadability

An excess of gel (about 2 gm) was sandwiched between two glass slides A 1 Kg weight was placed on the top of the one slide for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off the edges. The top slide was then subjected to pull of 80 gms, with the help of string attached to the hook and then the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted.[4]

Spreading ability was calculated by using the formula

$$S = M. L /T$$

Where, S- Spreading Coefficient, M = weight tied to upper slide, L = length of glass slides

T = time taken to separate the slides.

Extrudability (Tube Test)

The method adopted for evaluating gel formulation for extrudability was based upon the quantity in percentage of gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds.[4]

Washability

Formulations were applied on the skin and then ease & extent of washing with water were checked manually.[5]

Drug Content Of Antidandruff Gel

Based on physical examination, viscosity, spreadability, Extrudability, pH formulation CGA8 was selected as a best formulation for preparation of antidandruff gel containing tea tree oil and fluconazole.

Fluconazole content

Drug content of the gels was determined by dissolving an accurately weighed quantity of gel (about 20 mg) in about 100 ml of ethanol.The solutions were then filtered.Concentration of drug in filtrate was estimated spectrophotometrically at 261 nm. Drug content was determined from the standard curve of Fluconazole.[6]

Drug Content = (Concentration × Dilution Factor × Volume taken) × Conversion Factor

Terpinen-4-ol content of formulations

The formulations (TCGA8, FTCGA8) were analyzed by the use of mixture of toluene and ethyl acetate (85:15) as a mobile phase used for HPTLC of tea tree oil. Formulations equivalent to 2 g were accurately weighed and dissolved in 100 ml of ethanol. To ensure complete extraction of the terpinen-4-ol it was sonicated for 15 min. The resulting solution was centrifuged at 3000 rpm for 5 min and the supernatant was analyzed for terpinen-4-ol content. One milliliter of the supernatant was dissolved in 10 ml of ethanol. Ten microliters of each of these solutions were spotted on plates and analyzed for terpinen-4-ol in the same way as described by the HPTLC method.[7]

***In vitro* diffusion study using synthetic membrane**

Diffusion study

Drug release (*in vitro*) from gel was estimated using Dialysis Membrane 50 which was placed between donor and receptor compartments of the diffusion cell. The quantity of gel equivalent to 20 mg of Fluconazole was spread uniformly on the dialysis membrane. Phosphate buffer solution (pH 7.4): methanol (9:1) was filled in the receptor compartment. This whole assembly was kept on a magnetic stirrer and the solution on the receptor side was stirred continuously using a magnetic bead, temperature of the medium was maintained at 37±0.5°C.Sample (2 ml) was withdrawn at suitable time intervals and replaced with equal amounts of fresh media. Samples were analyzed spectrophotometrically at 260 nm and the cumulative % drug release was calculated. The difference between the readings of drug release and control was used as the actual reading in each case.

***In vitro* anti-dandruff Activity**

The agar well diffusion method used for to perform antifungal efficacy of the prepared gel against *Malassezia furfur* (MTCC- 1374). Emmons modified of Sabourauds agar was used in the conventional manner for cultivation of fungi. The prepared Emmons modified of Sabourauds agar medium and corn oil was sterilized by autoclaving at 121^0 C, 15 lbs for 15 minutes then it was poured in the petri plates. By rotating the petri plates the medium was uniformly distributed and allow solidify. Fungi were taken from the previously subcultured *Malassezia furfur* culture by nichrome wire loop in the sterilized test tubes containing 10 ml of sterile saline (0.9%). A glass spreader was moistened in the above inoculum suspension and surfaces of Emmons modified sabourauds agar plates were streaked in 4 different

directions (at 90º angels), so as to cover the entire surfaces. Using sterile cork borer the medium was bored (10 mm) and the prepared test sample was taken and added in each bore. The plates were incubated at 28^0C for 4- 5 days. Later the values of zones of inhibition were recorded.[8]

Group I = gel base (vehicle control)
Group II = Optimized formulation of 2% w/w Fluconazole gel
Group III= optimized formulation of 5%w/w Tree tea oil.
Group IV = Marketed formulation (ketona shampoo 2% w/w)
Group V = Sterile distill water (Negative control)
Group VI = Formulation (FTCGA8)containing 2% fluconazole+ 5% tea tree oil
Significance of differences between zone of inhibition of each formulation was assessed by one way ANOVA followed by Tukey Kramer test.

Skin Irritation potential (*in vivo*)

In skin irritation study 12 (Adult,Wistar,Male) rats weighing about 150-200gm (approved by Institutional Animal Ethical Committee, JSPM's JSCOPR College, Pune, Maharashtra,) were used. Animals were divided in to 4 groups of 3 animals each. and were marked with identification codes using marker ink. The animal backs were carefully shaved using sterilized shaving blade to remove fur and area of 1 inch diameter was marked on both the sides. One side served as control while the other as test and animals were used after 24 hrs. About 1gm of the selected gels was applied on marked spots using previously sterilized absorbent cotton wool. The toxic manifestations if any on the skin were then assessed by observing skin areas at pre-selected time intervals after treatment e.g. 4 hrs, 24 hrs, 48 hrs and 72 hrs. The observations were recorded as numerical scores as follows for each animal.0 = no visible reaction,1 = mild erythema,2 = intense erythema,3 = intense erythema with edema,4 = intense erythema with edema and vesicular erosion.The scores for treatment group and control group animals were then compared.[9,10]

STABILITY STUDIES

The stability study was carried out for FTCGA8 and TCGA8 formulation containing 2% fluconazole and 5 % tea tree oil. Formulation was packed in a collapsible tube. Stability study of formulation was carried out at 25°c/60 % humidity and 40°c / 75% humidity for 45 days. Samples were withdrawn at time intervals of 15 days and evaluated for physical appearance, pH, rheological properties, drug content,drug release.

RESULT AND DISCUSSION

Evaluation of placebo gel

All placebo gels were semisolid, off white in color homogenous and free from gritty particles. The pH value of all gels ranged between 6.1 to 7.0 almost neutral. The pH is acceptable since it is closer to the normal pH of Scalp. Spreadability of all gel formulations was in the range of 14.74 to 17.05 g-cm/ sec. It was found that all the formulations were easily and uniformly extrudable from the collapsible tube.

Viscosities of gels (at 10 rpm)

Visosities of all formulations are as shown in table Formulation CGA8 was selected as best formulation for preparation of antidandruff gel. Viscosity of CG5 and CGA8 was closer to the marketed gel. CGA8 formulation contains allantoin which is used as anti-irritant hence, this formulation was selected as the best formulation.

Table no. 3: Viscosities of placebo gel at 10 RPM

Sr. No.	Formulation code	Viscosity in cps (at 10 RPM)	Sr. No.	Formulation code	Viscosity in cps (at 10 RPM)
1	C1	42675	6	CG6	48932
2	C2	47891	7	CGA7	41456
3	C3	49753	8	CGA8	43576
4	CG4	41989	9	CGA9	48553
5	CG5	45131	10	Marketed	44500

Viscosities of FCGA8, TCGA8 and FTCGA8 was found to be 44100, 43500 and 43200 cps respectively.

Drug Content Of Antidandruff Hair Gel

Percent content of fluconazole in FCGA8 and FTCGA8 was found to be 98.01% and 95.56% respectively.Terpinene-4-ol content of TCGA8 and FTCGA8 was found to be 80.76% and 73% respectively.

Diffusion (*in vitro*) of Fluconazole

Through synthetic membrane

All medicated gels indicated constant diffusion of Fluconazole through synthetic membrane over 2 hours. Drug release from formulation FTCGA8 is more as compared to marketed formulation and FCGA8. This may be due to addition of tea tree oil. At the end of 2 hrs the mean % drug release of each formulation is as shown in Figure 5.

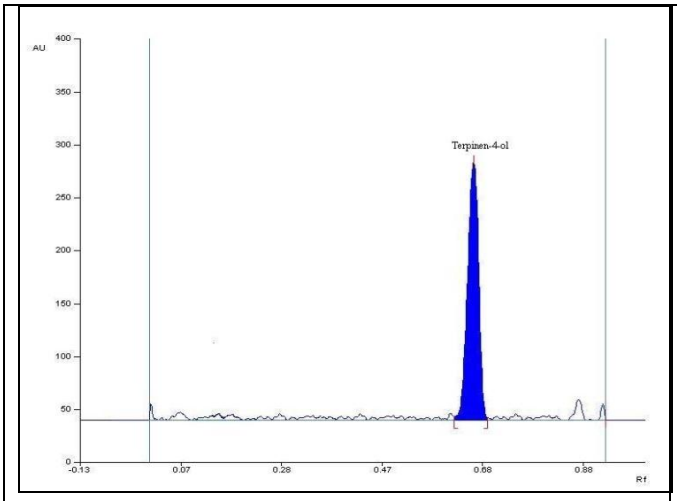

Fig.1: HPTLC densitogram of Terpinen-4- ol

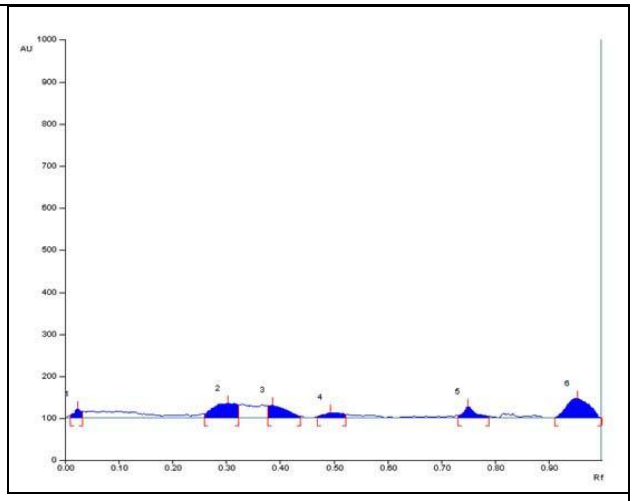

Fig.2: HPTLC densitogram of gel base

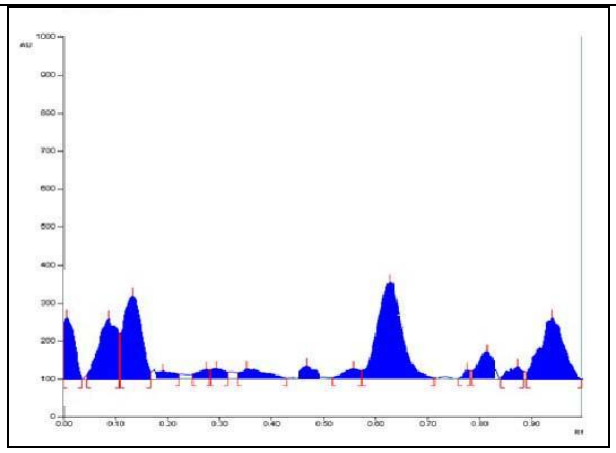

Fig.3: HPTLC densitogram of TCGA8

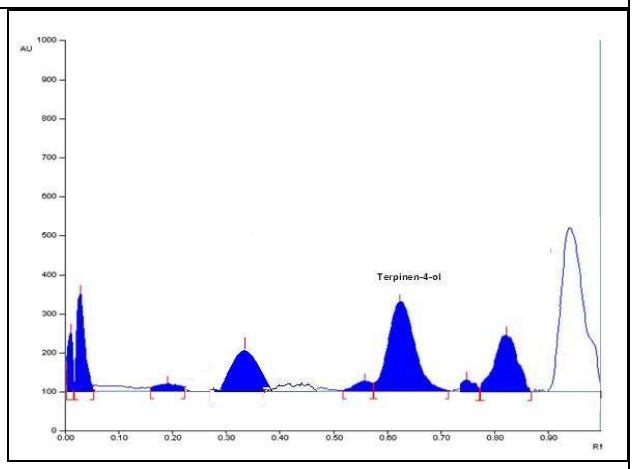

Fig.4: HPTLC densitogram of FTCGA8

Diffusion (*in vitro*) of Fluconazole

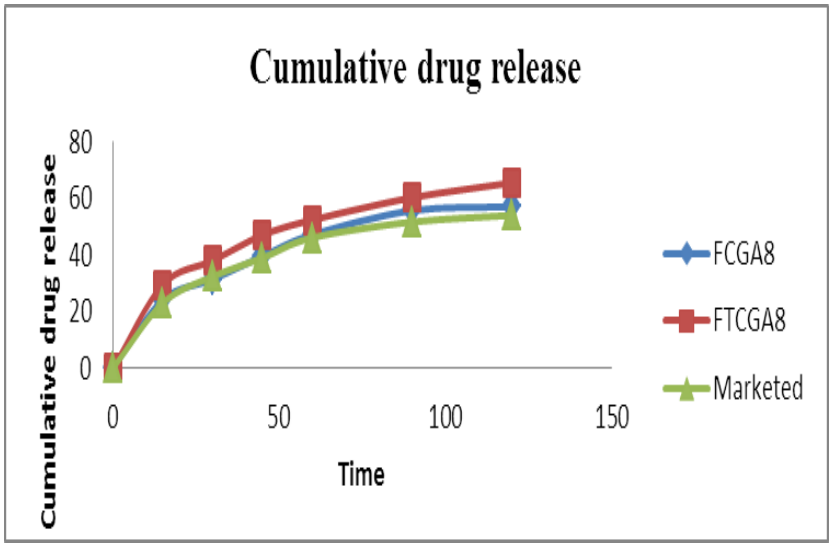

Fig.5: In *Vitro* drug release profile of gel

Glycerin reduces crosslinking of polymer. Release pattern of FCGA8,FTCGA8 formulations matches with the release profile of marketed formulation. In this formulation tea tree oil was added as herbal antifungal severel reports are there that tea tree oil has anti-fungal activity against *malassezia furfur*.From the physical observation it was observed that, formulation containing tea tree oil, as well as combination of TTO and fluconazole were off white and non gritty. Both formulations were homogeneous**.** All the gels were free from air bubbles. Both formulations were having pH in the acceptable range.Viscosity of both gels were not affected by addition of tea tree oil. Spreadability of TCGA8: 12.65 gm-cm/secs, FTCGA8: 12.23. Both formulations shown good wash ability.

Anti-fungal activities

The antifungal activity of fluconazole and tea tree oil from different gel formulae compared with marketed formulation(standard) as shown in table no.4 and fig. no. 6. The antifungal activity was determined by measuring the inhibition zone. Among all the formulations, formulation FTCGA8, TCGA8 and Marketed formulation shown maximum zone of inhibition than other formulation. Hence, Hair gel formulation (FTCGA8) was considered as best formulation.

Table no.4: Anti-fungal activities of gel

Sr. No.	Formulation	Zone of inhibition (mm)	Sr. No.	Formulation	Zone of inhibition (mm)
1	Gel base	Nil	4	TCGA8	20
2	Distill water (NC)	Nil	5	FTCGA8	22
3	FCGA8	Nil	6	Standard	25

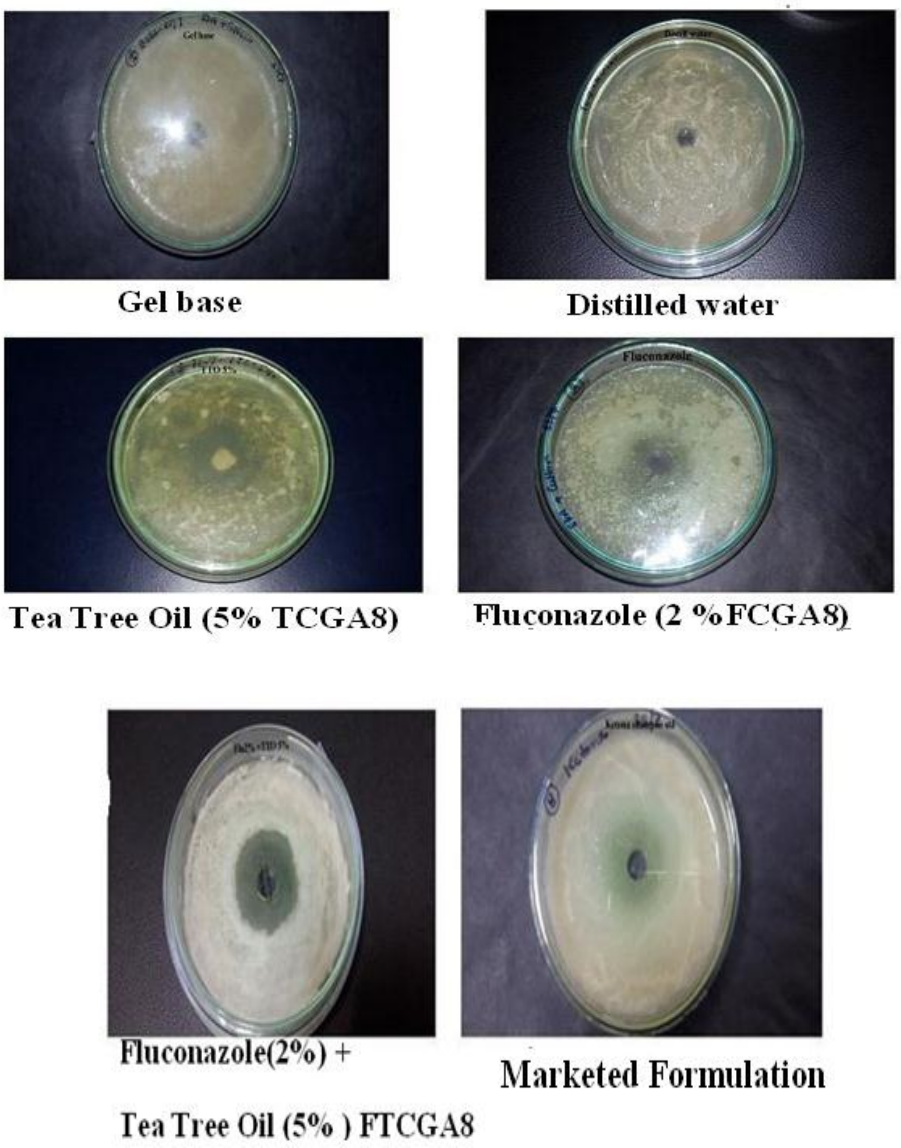

Fig.6: Antifungal activity of gel.

Significant difference was observed in zone of inhibition of gel base, TCGA8, FCGA8 and Marketed ($P<0.001$). Significant difference was observed in zone of inhibition of FCGA8, TCGA8, FTCGA8 and Marketed ($P<0.001$).Significant difference was not observed in zone of inhibition of gel base and FCGA8 ($P<0.05$) after one way ANOVA followed by Tukey Kramer test. Result of antifungal activity indicates Ketoconazole and Tea tree oil is active against *malassezia furfur* (MTCC 1374) while zone of inhibition of fluconazole was nil against the same strain.

Skin Irritation study

The fluconazole + Tea tree oil formulation did not show any irritation and erythema after 72hours. This indicates better skin acceptability of fluconazole and tea tree oil gel.

Stability Study of the Formulation

Assessment of stability of optimized formulation is important in drug product design and development. Stability can be observed from the physical property of drug content and drug release profile of formulation. Prepared formulation was found to be stable, where no significant change was observed in the parameters evaluated like physical appearance as color, syneresis, pH, rheological properties, drug release.

CONCLUSION

Hair gel formulation TCGA8 containing 0.6gm of Carbopol 940 was suitable for topical application as these formulations shows good results. Anti- fungal activity shows that formulation FTCGA8 and TCGA8 shows higher efficacy. Zones of Inhibitions for TCGA8 (containing 5 % Tea tree oil) and FTCGA8 (2% fluconazole and 5% Tea tree oil) were found to be significant while zone of inhibition for formulation FCGA8 (2% fluconazole) was found to be nil indicates that *Malassezia furfur* (MTCC 1374) may be resistant against fluconazole. Resistance study of *Malassezia fufur* against fluconazole needs to be carried. Zone of inhibition for FTCGA8 was may be due to presence of tea tree oil. Tea tree oil with active constituent 1, 4-terpeniol[11] may be responsible for the significant antifungal activity. Stability study of optimized gel formulation shows that Physical appearance, pH, Spreadability, drug release remains unchanged upon storage for 45 days.

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