DEVELOPMENT AND CHARACTERIZATION OF TOPICAL PHYTO-FORMULATION FOR ANTIFUNGAL ACTIVITY
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The present study was aimed to develop a potent antifungal topical gel using Curcumin and fluconazole in suitable combination. In these studies we have prepared different gel formulations using polymer like carbopol, Hydroxy propylmethyl cellulose, as well as excipients like tri-ethanolamine, methanol, glycerin and purified water. The formulated gel were evaluated in context of different parameters like drug content, pH, spreadability viscosity, in-vitro drug release, anti-microbial effect etc. Candida albicans was used as a model fungus to evaluate the antifungal activity of the prepared formulations.

Key words: Pharmaceutical gel, Curcumin, Fluconazole, Carbapol, HPMC, Evaluation of topical gel

INTRODUCTION
Curcumin is a natural compound isolated from the turmeric plant known as Curcuma longa. It has a variety of biological activities and pharmacological actions, such as Anti inflammatory, antioxidant, anti-carcinogenic, anti-virus properties, anti diabetic, and anti depressant as well as shows promising clinical applications due to its low toxicity. It is reported that in some cases curcumin used in combination with other synthetic drug to increase their activity and reduce the toxicity. In present study, the antimicrobial activity of curcumin in combination with fluconazole was studied. The potent combination was introduced in a gel base. The objective of this study was to develop a potent antimicrobial topical gel formulation using carbopol, HPMC, and mixtures of carbopol – HPMC at different ratios. All the prepared formulation was subjected to evaluation of different essential parameters. The hypothesis of this research is that the use of HPMC to carbopol may give a gel of suitable physical properties, high viscosity, high drug release and good bioavailability.

METHOD
Fluconazole was supplied as gift sample from Synergene Active Ingredients (P) Ltd., curcumin was procured from Molychem Pvt Ltd., ethanol from Rankem Lab, Mumbai, HPMC & carbopol, Peptone purchased from Sd Fine Chem. Limited, Agar from Lobachem, Sodium chloride, Glycerine, yeast extract & Beef extract were purchased from Molychem Pvt Ltd.

Preparation of topical gel
Carbopol 940, Hydroxy propylmethyl cellulose and purified water were taken in a beaker and allowed to soak for 24 hours. Required amount of drug (Curcumin and fluconazole) was added in the gel base and it was than neutralized with sufficient quantity of tri-ethanolamine (1-2 drop). Glycerin as moistening agent and benzyl alcohol as a preservative was added to the gel mass with slow and continuous stirring. Five gel formulations were prepared by using different ratios of Carbapol-P934 and Hydroxypropylmethyl cellulose respectively (1:1), (1:2), (1:3), (1:4), (3:1).

Evaluation of topical gel
All the prepared formulations were evaluated for parameters like determination of drug content, determination of PH, Viscosity and Rheological studies, In-Vitro Diffusion Study, Spreadability, and Antimicrobial estimation.

Determination of drug content
Weighed 10 gm of each gel formulation were transferred in 250 ml of volumetric flask containing 20 ml of methanol and stirred for 30 min. The volume was than made up to 100 ml and filtered. 1 ml of above solution was further diluted to 10 ml with methanol. The absorbance of the solution was measured spectrophotometrically at 261 & 422 nm respectively.

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**Determination of pH:**
The pH of gels was checked by using a digital Elico pH meter at room temperature. Initially, the pH meter was calibrated using standard buffers solution of pH 4, 7 and 9. After calibration all the prepared formulation were subjected to evaluate the pH.

**Viscosity and Rheological studies**
The viscosity of gels was determined by using Brookfield viscometer. The gel was placed in the sample holder and the suitable spindle selected was lowered perpendicularly into the sample. The spindle was attached to viscometer and then it was allowed to rotate at a constant optimum speed at room temperature. The readings were noted after 2 minutes.

**In-Vitro Diffusion Study**
In vitro evaluation studies of topical gel were performed using a Franz diffusion cell. A well treated semi permeable dialysis membrane was placed between the donor and reservoir compartment. 10 mg of formulated gel were placed on the surface of the membrane and the cell was immersed into diffusion cell containing 100 ml of phosphate buffer of pH 4.5, and maintains temperature at 37±1ºC. The sink conditions were maintained throughout the period of experiment. After suitable dilution, the sample was analyzed by using UV visible spectrophotometer at 261 & 422 nm respectively.

**Spreadability:**
The parallel plate method is the most widely used method for determining and quantifying the spreadability of gel preparations. The spreadability can be determine by using formula

$$S = \frac{M \cdot L}{T}$$

M = weight tied to upper slide, L = Length of glass slide, T = Time taken to separate the slide.

**Antimicrobial estimation:**
Firstly media was prepared and sterilized for 15 min at 121ºC at 15 lb pressure in autoclave. Then it was cooled at room temperature and the fungal strain (*Candida albicans*) was dispersed in the medium and then the medium was poured it in to the three petridish and allowed it cool it for sometime at room temperature until it forms solidifies at room temperature and then the three cups are bored in each petridish with the help of sterile steel bore of 6 mm and calculated concentration of the standard drug (Curcumin and fluconazole), gel formulations and placebo gel were placed in the bores and incubated the petridish plates for 72 h at 37ºC in incubators. Then the zone of inhibition was calculated.

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Drug Content (%)</th>
<th>pH</th>
<th>Spreadability (cm²)</th>
<th>Viscosity (Cp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>97</td>
<td>5.66</td>
<td>6.2</td>
<td>42500</td>
</tr>
<tr>
<td>F2</td>
<td>98.5</td>
<td>5.55</td>
<td>9.3</td>
<td>42000</td>
</tr>
<tr>
<td>F3</td>
<td>96</td>
<td>6.62</td>
<td>12.5</td>
<td>66000</td>
</tr>
<tr>
<td>F4</td>
<td>98</td>
<td>6.6</td>
<td>15</td>
<td>54000</td>
</tr>
<tr>
<td>F5</td>
<td>97.5</td>
<td>5.12</td>
<td>8.3</td>
<td>7000</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**
In the present study, Curcumin and Fluconazole gel was prepared by using carbopol 934P, HPMC, Triethanolamine, glycerin and distilled water. Five formulations different formulations were prepared using combination of both the drug. The drug content of the formulated gel was estimated by spectrophotometer at λ max 261 & 422 nm respectively using methanol as solvent. It was found that the combination of carbopol and HPMC modified the characteristics of the gel structure and enhanced the drug diffusion from the gel matrix. The Drug content of all the formulations were ranged from 96 to 98 (Table 1). The pH values all the gel formulations were found in the range of 5 to 6. The lowest pH value was 5.12 and was obtained from the gel containing high amount of carbapol, while the highest pH value of 6.62 was obtained from gel containing high amount of HPMC. The gel formulations containing equal amount of carbopol and HPMC, had 5.6 pH. As the ratio of HPMC is increased, the pH of the gel is
slightly increases. The study was performed there time to get more accurate results.

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Concentration (µg/ml)</th>
<th>Zone of inhibition (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>5000</td>
<td>1.1304</td>
</tr>
<tr>
<td>F2</td>
<td>5400</td>
<td>1.1304</td>
</tr>
<tr>
<td>F3</td>
<td>6.664</td>
<td>1.1304</td>
</tr>
<tr>
<td>F4</td>
<td>8000</td>
<td>2.268</td>
</tr>
<tr>
<td>F5</td>
<td>10000</td>
<td>3.454</td>
</tr>
</tbody>
</table>

**Table 2:** Zone of inhibition of Curcumin and Fluconazole gel

The spreadability is very much important as it shows the behavior of gel comes out from the tube as well as the ability to spread out to the applied area. It was found that all the formulation showing satisfactory results. Spreadability of the gel formulation decreases with the increase in the concentration of the polymer. Spreadability of the formulation F4 shows highest value 15 cm². The values of spreadability indicated that the gel were easily spreadable. The rheological behaviors of all gel formulations were investigated. Gel formulations containing higher amount of carbopol showed an approximate viscosity 7000 cp, while formulations containing combination of carbopol : HPMC in the ratio 1:1 showed an approximately viscosity 42500 cp and formulation containing carbopol : HPMC at 1 : 2 ratio showed approximate viscosity 42000 cp. The formulation containing Carbapol: HPMC at ratio 1:3 showed approximate viscosity 86000 cp. These data indicated that the incorporation of HPMC to carbopol increases the viscosity of the gel base.

The release of topical gel was varied according to concentration of polymer. The progressive increase in the amount of drug diffusion through a skin from formulation F1 attributed to gradual decrease in the concentration of polymer. It has been concluded that, if we have increase the concentration of polymer, the diffusion of drug through the skin also decreases. The amount of drug diffused from formulation F1 was around 100 % within 6 hrs which was higher among all the gel formulation.

In this anti fungal study Candida albicans was used as fungal stain. The studies were carried for the different formulations and highest zone of inhibition observed for F5 (3.454cm²).

![Fig 1 Drug diffusion of F1, F2, F3, F4, F5 formulation](image)

**CONCLUSION**

In consideration to the above results and discussion it can be concluded that formulations prepared using suitable combination of synthetic and natural antifungal agent showing effective antifungal activity. Again the formulations were found shows satisfactory physicochemical stability as well as effective drug release pattern.

**ACKNOWLEDGEMENT**

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