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Original Article

FORMULATION AND EVALUATION OF ANTIFUNGAL CREAM OF CHLORPHENESIN

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ABSTRACT

Objective: The main aim of our research was to develop an Antifungal cream formulation consisting of Chlorphenesin for the treatment of Fungal infections. Topical route is the most suitable route for skin infections.

Methods: The development of topical drug delivery systems designed to have systemic effects appears to be beneficial for a number of drugs on account of several advantages over conventional dosage forms(or) routes of drug administration. An Antifungal cream formulation consisting of Chlorphenesin was prepared.

Results: The formulation was subjected to *in vitro* diffusion studies. Microbiological studies were performed to find out the safety of materials used in the formulation.

Conclusion: The developed cream consisting of Cholrphnesin was found to be safe and effective for the treatment of fungal infection.

Keywords: Fungal infection, Chlorphenesin, Antifungal cream

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INTRODUCTION

Several antifungal agents are available on the market in different topical preparations (e. g., creams, ointments, and powders for the purpose of local dermatological therapy). One of these antifungal agents is chlorphenesin (CHL), which has both anti-fungal and antibacterial properties. It is applied locally in mild uncomplicated dermatophyte and other cutaneous infections [1, 2].

Fungal infections (also called mycoses) represent the invasion of tissues by one or more species of fungi which may cause superficial, localized, deeper tissue infections to serious lung, blood (septicemia) or systemic diseases. Some fungi are pathogenic, causing disease whether the immune system is healthy or not [3].

Topical treatment of fungal infections has several superiorities including, targeting the site of infection, reduction of the risk of systemic side effects, enhancement of the efficacy of treatment and, high patient compliance. Different type of topical effective antifungal compounds has been used in the treatment of a variety of dermatological skin infections. Currently, these antifungal drugs are commercially available in conventional dosage forms such as creams, gels, lotions and sprays [4].

The most common therapeutic options are systemic and topical antifungal agents; however, oral antifungals are associated with adverse effects that can cause patients to discontinue treatment, which may be complicated by the presence of comorbid conditions [5].

Antifungal drugs should reach effective therapeutic levels in viable epidermis after dermal administration. The greatest challenge for dermal delivery is stratum corneum, in order to improve its permeability, new formulation approaches have been investigated [6, 7].

MATERIALS AND METHODS

Materials

Propylene glycol, bees wax, stearyl alcohol, cetyl alcohol, Triethanolamine, propylparaben, methyl paraben, liquid paraffin. Stearic acid And Cholrphenesin were purchased from NS Chemicals, New Mumbai.

Table 1: Formulae for cream base

	S. No.	Ingredients	Quantity in gm (20 gm)							
			F1	F2	F3	F4	F5	F6	F7	F8
Oil phase (A)	1	Stearic acid	3	3.5	4	4.5	4	4.5	5	5
	2	Cetyl alcohol	0.1	0.2	0.1	0.2	-	-	-	-
	3	Potassium Hydroxide	-	0.1	0.2	0.3	-	-	-	-
	4	Sodium Hydroxide	0.03	0.02	0.01	-	-	-	-	-
	5	Coconut oil	-	-	-	-	1	2	3	4
	6	Liquid Paraffin	-	0.5	1	1.5	3	3	3	3
Aqueous	7	Glycerine	1	1.5	2	2.5	3	3	3	3
phase (B)	8	Triethanolamine	-	-	-	-	1.5	1.5	1.5	1.5
	9	Methyl Paraben	0.04	0.03	0.04	0.02	0.02	0.02	0.02	0.02
	10	Propyl Paraben	0.04	0.03	-	0.02	0.02	0.02	0.02	0.02
	11	Aloe vera gel	-	-	-	-	2	4	6	8
	12	Perfume	Qs	qs	Qs	qs	qs	qs	Qs	qs
	13	Water	qs to 20 ml	qs to 20 ml	qs to 20 ml	qs to 20 ml	qs to 20 ml	qs to 20 ml	qs to 20 ml	qs to 20 ml

Preparation of o/w cream formulation

These o/w emulsion-based preparations contain the aqueous phase and oil phase. The ingredients of oil phase (A) were mixed together by melting in a china dish at 70 °C on a water bath with constant stirring. The components of the aqueous phase (B) were mixed together separately in a beaker and heated about the same temperature as of the oil phase on a water bath. The aqueous phase was added to the oil phase drop by drop with constant stirring using an emulsifier. The therapeutically active Chlorphenesin is dissolved in distilled water and add to the above mixture and stir continuously until formation of cream. The preservatives propylparaben and methylparaben were added after cooling to 40 °C.

RESULTS

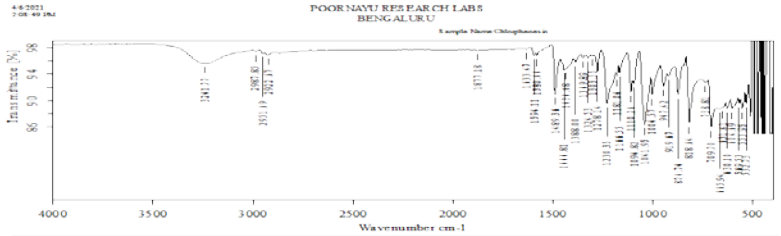
Evaluation parameters

Take about 1 gram of cream in a clean petri dish and observe visually.

Infrared spectral analysis

IR spectral analysis is one of the most powerful analytical techniques which offer possible chemical identification. In the present work, IR spectrum of Chlorphenesin pure drug and Chlorphenesin along with other excipients in the formulation was studied for their interactions [8].

The results are shown in table 2 to 7, and fig. 1 to 3.



Item	Value
Sample name	Chlorphenesin
Sample ID	Solid
Date of Measurement	4/6/2021
Intensity mode	Transmittance
Apodization	APF
No. of Scans	16
Resolution	4

Analysed by: Page 1 of 2 Checked by:

Fig. 1: FTIR-graph of pure chlorphenesin API

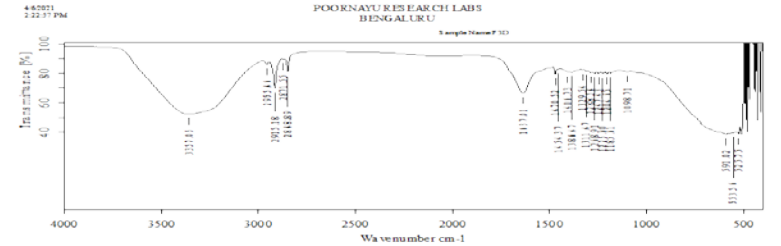
IR Spectrum of pure drug Chlorphenesin exhibited its characteristic absorption bonds in the following IR region in, and showed in table 4 and 5

Table 2: R Spectrum of pure drug chlorphenesin exhibited its characteristic absorption bonds

Frequency	Bonds	Inference
3241.77 cm^{-1} (3190-3390)	Broad peak	Broad peak due to hydrogen bonded O-H stretching

Table 3: IR Interpretation of chlorphenesin

Wavelength	Group
2987.85 cm-1	Aromatic C-H Stretch
2951.69 and 2922.67 cm-1	C–H Stretching of CH_2 Group
1633.47, 1594.11, 1580.44, 1489.36 cm-1	C=C Ring Stretching
1444.81 and 1388.00 cm-1	C–H Bonding of CH_2 Group
1278.14 cm^{-1}	O–H Bonding
1094.82 cm^{-1}	C–O–C
818.64 cm^{-1}	Para Substitutional Benzene
665.94 cm^{-1}	C–Cl



Item	Value
Sample name	FID
Sample ID	Semisolid
Date of Measurement	4/6/2021
Intensity mode	Transmittance
Apodization	APF
No. of Scans	16
Resolution	4

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Fig. 2: FTIR-graph of pure chlorphenesin with other excipients

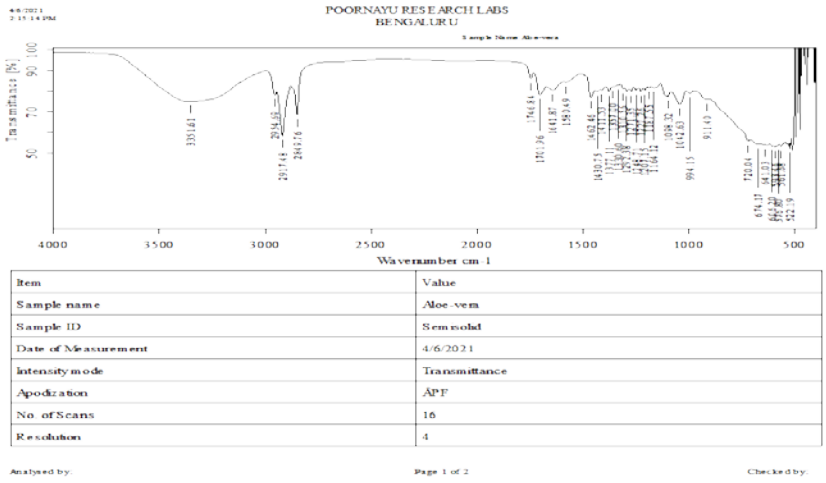
IR Spectrum of Chlorphenesin with other excipients exhibited its characteristic absorption bonds in the following IR region in

Table 4: IR Spectrum of chlorphenesin with other excipients exhibited its characteristic absorption bonds

Frequency	Bonds	Inference
3351 cm^{-1} (3190-3390)	Broad peak	Some OH peaks of excipients must have merged with OH peaks of the drug. Hence very broad peak of hydrogen-bonded O-H stretching.

Table 5: IR Interpretation of chlorphenesin with other excipients

Wavelength	Group
2954.69 and 2917.48 cm-1	C-H Stretching of CH_2 groups
1641.87, 1580.49, 1462.46 cm-1	C=C Ring Stretching
1430.76 and 1377.11 cm-1	C-H bonding of CH_2 Group
1271 cm-1	O–H Bonding
1098.32 cm^{-1}	C–O–C
674.17 cm^{-1}	C–Cl



Item	Value
Sample name	Aloe-vera
Sample ID	Semsolid
Date of Measurement	4/6/2021
Intensity mode	Transmittance
Apodization	APF
No. of Scans	16
Resolution	4

Analysed by: Page 1 of 2 Checked by:

Fig. 3: FTIR-graph of pure chlorphenesin with other excipients and Aloe vera gel

IR Spectrum of Chlorphenesin with other excipients and Aloe vera gel exhibited its characteristic absorption bonds in the following IR region in

Table 6: IR Spectrum of chlorphenesin with other excipients and aloe vera gel exhibited its characteristic absorption bonds

Frequency	Bonds	Inference
3357 cm^{-1} (3190–3390)	Broad peak	Hydrogen bonded O-H Stretching of OH groups

Table 7: IR Interpretation of chlorphenesin with other excipients and aloe vera gel

Wavelength	Group
2955.46 and 2915.18 cm-1	C-H Stretching of CH_2 groups
1637, 1470.52 cm-1	C=C Ring Stretching
1454.36 and 1386.47 cm-1	C-H bonding of CH_2 Group
1268 cm-1	O–H Bonding
1098.71 cm^{-1}	C–O–C
650 and 591 cm^{-1}	C–Cl

Physical examination

The prepared topical creams were inspected visually for their color, homogeneity, consistency, spreadability and phase separation. The results are shown in table 8 and 9.

Determination of pH

Weigh about 5 gm of the cream and dispersed in 45 ml of water in a 100 ml beaker. The pH was determined at 27 °C using the pH meter. The results are shown in table 8 and 9 [9].

Viscosity

The viscosity of formulated creams was measured by Brook field Viscometer LVD using spindle S 94 at varying speed and shear rates. The measurements were done over the range of speed setting from 0.10, 0.20, 0.30, 0.40 and 0.50 rpm in 60 s between two successive speeds as equilibration with the shear rate ranging from 0.20 s-1 to 1.0 s-1. Viscosity determinations were performed at room temperature [10]. The results are shown in the table 10 and fig. 4

Table 8: Physico-chemical evaluation of formulation

S. No.	Formulation code	Appearance	pH	Consistency
1	F1	White	7	Poor(liquid)
2	F2	White	7.08	Creamy
3	F3	White	7.06	Hard (solid)
4	F4	White	7.1	Creamy
5	F5	White	6.8	Creamy
6	F6	White	6.86	Creamy
7	F7	White	6.81	Creamy
8	F8	White	6.8	Creamy (smooth)

All the above formulations contain 0.3% of Chlorphenesin, From the above results, F8 formulation is considered as the finally optimized formulae, and that formulation was evaluated with the process such as homogenizer speed (RPM), time of homogenization, water bath temperature, sampling points. Further studies is carried out and are evaluated for parameters such as pH, spread ability, drug content, diffusion and stability studies.

Table 9: Physico-chemical evaluation of F8 formulation and marketed product

	Trial code	Appearance	pH	Drug content	Tube extrudability	Spreadability
1	Marketed product	White	6.8	98.42	98.7%	18.3
2	F8	White	6.7	97.60	98.6%	18.5

Table 10: Viscosity data of prepared cream formulation F8 containing 0.3% of chlorphenesin

S. No.	RPM	Centripois	Torq
1	10	287	2.3
2	20	192	3.2
3	30	148	3.7
4	50	120	5
5	60	104	5.4
6	100	82	6.9

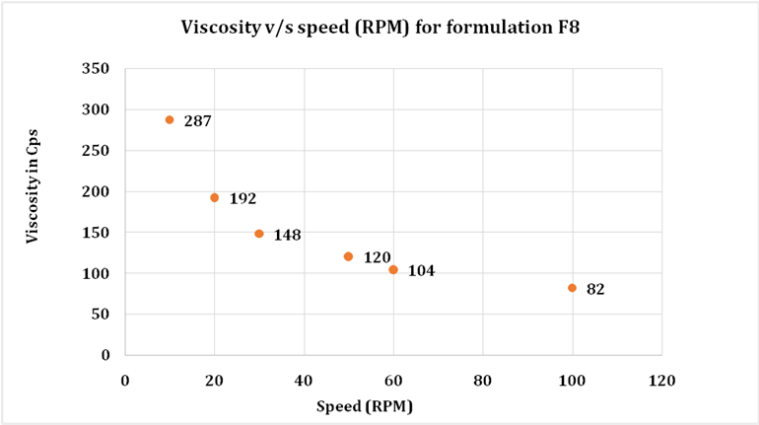


Fig. 4: Viscosity v/s speed (RPM) for formulation F8

Tube extrucibility

In the present study, the method adopted for evaluating cream formulation for extrudability was based upon the quantity in percentage cream extruded from tube on the application of finger pressure. More quantity extruded better the extrudability.

The formulations under study were filled in a clean, lacquered aluminum collapsible 5 grams tube with a nasal tip of 5 mm opening. The pressure was applied on the tube by holding it in between the thumb and index finger for 1 sec. Tube extrudability was then determined by measuring the amount of cream extruded through the tip when the pressure was applied [8]. The results are shown in table 8.

***In vitro* drug diffusion**

A glass cylinder with both ends open, 10 cm height, 3.7 cm outer diameter and 3.1 cm inner diameter was used as permeation cell. A cellophane membrane prehydrated in pH 7.4 buffer (24 h. before use) was fixed to one end of the cylinder with the aid of an adhesive to result in permeation. One gram of semisolid formulation was taken in the cell (donor compartment) and the cell was attached to a beaker containing 140 ml of drug-free pH 7.4 phosphate buffer as receptor compartment. The medium in the receptor compartment was agitated using a magnetic stirrer and a temperature of 37 °C±1 °C was maintained. Samples of 1 ml from the receptor compartment were taken at various intervals over a period of 3 h with replacement of an equal amount of drug-free buffer (7.4 Phosphate). The samples were estimated by measuring the absorbance at 279 nm in a UV-1700 Shimadzu spectrophotometer [11]. The results are shown in table 12.

Table 11: *In vitro* drug diffusion studies

S. No.	Time interval (min)	%CDR F8
1	30	30.01
2	60	40.25
3	90	50.58
4	120	65.74
5	150	80.95
6	180	96.19

In vitro antimicrobial studies

Topical formulation with broad, non-resistance promoting activity against Aspergillus Niger can be of great use in dermatology preparation were infections are often mixed. Since formulation containing antifungal agent as active moiety, it is likely to protect from fungal growth. To determine the activity of formulation is subject to study the prepared formulation with standard method called Disk diffusion method and the inhibition zone diameters were measured with the help of zone reader. The results are shown in the table 12 and fig. 5.

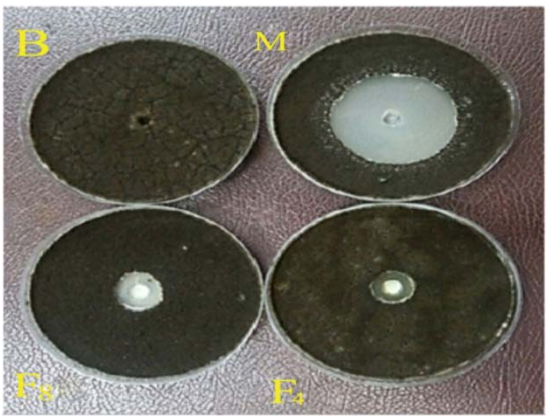


Fig. 5: *In vitro* antifungal studies showing zone of inhibition, (B: Blank, M: marketed product, F8: Formulation 8, F4: Formulation 4)

Table 12: Zone of inhibition of chlorphenesin and marketed cream (Clotrimazole)

Drugs	Zone of inhibition (mm)
BLANK (B) (without drug)	00
Marketed product (M)	42
F8	19
F4	18

DISCUSSION

From the above-compiled data, the study clearly shows that the formulation is showing good *in vitro* antifungal activity against Aspergillus Niger.

As a part my research work, Infra-red spectra of pure drug Chlorphenesin and its formulation are taken interpretation of above IR spectra reveals that the characteristic absorption bonds of different function groups and bonds present in the drug are present in both formulations, The positions of characteristics bonds of pure drug also presence in the spectra of its formulations. Even if slight variation in the position of absorption bonds is observed it is negligible and it is within the permissible range, this clearly suggests that there is no interaction of the drug with excipients used in the preparation of formulations. Hence it may be concluded that the drug has no interaction with the excipients used and thus, there is drug-excipients compatibility [12-22].

CONCLUSION

The formulation of the antifungal agent Chlorphenesin exhibited an enhanced rate of diffusion and anti-activity. The results of different chemical and physical tests of cream showed that it could use topically in order to protect against skin infections caused by fungus.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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