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FORMULATION AND EVALUATION OF ANTIFUNGAL EMULGEL CONTAINING EXTRACTION OF CALOTROPIS GIGANTEA LATEX

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ABSTRACT
The present research has been undertaken with the aim to develop antifungal emulgel of calotropis gignatea latex extract. Emulgel comes to favour the hydrophobic drugs to give the advantage of gel. Emulgel have several advantages in the field of dermatology such as being thixotropic, greaseless, easily spreadable, easily removable, emollient non staining, bio friendly, transparent and pleasing appearance The ethanolic extract shows the presence of many biologically active molecules such as flavonoids, steroids, saponins, phenol and glycoside. The latex extract shows significant zone of inhibition and the result are compared with marketed formulation. from this study we conclude that latex extract possesses potent fungicidal activity which may be due to Presence of biologically active ingredient in the ethanolic extract of c. gignatea latex.

KEYWORDS: Calotropis gignatea latex, antifungal activity, zone of inhibition, disc diffusion method.

INTRODUCTION
Fungal infection of the skin is nowadays one of the common dermatological problems among the tropical formulation clear, transparent emulgel have widely accepted in both cosmetics and pharmaceutical.

Calotropis gignatea is a xerophytic erect scrub, growing widely throughout the tropical and subtropical regions of Asia and Africa. these plants are popularly known because it produces large quantity of latex. The plant is potent pharmacological properties. Large quality of latex can be easily collected from its green part of the plant. different parts of the plant show the various biological activities such as antipyretics, antifungal, anti-inflammatory and wound healing. The prevalence of invasive, fungal infection increases at an alarming rate specially in immune compromised individual. although it appears great array of antifungal drug.

MATERIAL AND METHOD
Plant material and latex collection
The fresh latex of C. gigantea was aseptically collected from the aerial parts of the healthy plants as described by Aworh et al., 16 in clean glass tubes containing distilled water to yield a dilution Rate of 1:1 (v/v). The latex mixture was gently handled to maintain homogeneity during transport to the laboratory where it was kept overnight at 4°C.

Preparation of C. gigantea latex extract
The supernatant was selectively decanted and centrifuged at 5000xg for 20 min at 25°C. The precipitated material showing rubber aspect **(**poly-isoprene) was pooled apart and the supernatant was decanted carefully and subjected to exhaustive dialysis using a membrane of 8000 MW cut off against distilled water at 25°C. Finally, the samples were centrifuged as previously described and the clear soluble supernatant was collected and lyophilized. The lyophilized fraction was subjected to ethanolic extraction (70% (v/v)) in Soxhlet extractor at room temperature and the extraction process was performed repeating 4 cycles 17.

The extract was filtered through Whatman No.1 filter paper and filtrate was concentrated with a rotary evaporator under reduced pressure at 60°C to afford crude. ethanolic extract. The dry-crude extracts were irradiated with ultraviolet light for 24 h for sterilization; sterility was confirmed by plating the sample suspension on Sabouraud Dextrose Agar and stored in labeled sterile brown glass containers at 4°C until used for the screening of antifungal activity. At that time, the extracts were freshly reconstituted to yield desired concentration.

Phytochemical screening of C. gigantea latex extract
The phytochemical screening of the ethanolic extract of C. gigantea latex was performed qualitatively for the presence of alkaloids, glycosides, flavonoids, phenols, tannins, saponins, sterols and triterpenes according to the method of Harborne 18.

Fungal strains and growth medium: Fungal cultures of Candida albicans (MTCC 227), Saccharomyces cerevisiae (MTCC 463).

Material and methods for emulgel preparation

Material

C.gigantea latex, Carbopol 940, liquid paraffin, tween 20, span 20, propylene glycol, ethanol, methyl paraben, ethyl paraben, clove oil, Mentha oil, water.

Method

The gel phase in the formulation was prepared by dispersing Carbopol 940 in purified water with constant stirring at a moderate speed using mechanical shaker. the ph. was adjusted to 6-6.5 using triethanolamine (TEA).

The oil phase of emulsion was prepared by dissolving span 20 in light liquid paraffin while the aqueous phase was separately heated to70-80 c then the oily phase was added to the aqueous phase with continuous stirring until it gets cooled to room temperature. The obtained emulsion was mixed with gel 1:1 ratio with gentle stirring to obtain the emulgel.

COMPOSITION OF DIFFERENT FORMULATION

Table 1: Composition of different formulation batches (% w/w).

Ingredients	F1	F2	F3	F4	F5
C.gigantea latex	1	1	1	1	1
Carbopol 934	-	-	-	0.5	1
Carbopol 940	O.5	1	1.5	-	-
Liquid paraffin	2.5	2.5	2.5	2.5	2.5
Span 20	0.3	0.3	0.3	0.3	0.3
Tween 20	0.4	0.4	0.4	0.4	0.4
Propylene glycol	2.5	2.5	2.5	2.5	2.5
Ethanol	1.25	1.25	1.25	1.25	1.25
Methyl paraben	0.01	0.01	0.01	0.01	0.01
Propyl paraben	0.05	0.05	0.05	0.05	0.05
Glutaraldehyde	0.05	0.05	0.05	0.05	0.05
Purified water	q. s	q. s	q. s	q. s	q. s

EVALUATION OF EMULGEL

All the prepared Emulgel formations were subjected for preliminary evaluations as follows:

1. Physical Appearance

The prepared emulgel formulation was inspected for their physical appearance including colour, consistency, homogeneity.

2. pH measurement

1gm of emulgel was dissolved in 100ml distilled water and kept aside for two hours. The pH developed formulation was determined using pH meter.

3. Viscosity

Brookfield viscometer was used to determine the viscosities of prepared emulgels. Prepared Emulgel formations was added to the beaker and settled it for 30min at 25-30°c. Adjust the spindal in that way that spindal does not touch the bottom of the jar and rotate at moderate speed 100RPM for 10min.the viscosity reading was noted.

4. Spreadability

Spreadabilty can be expressed as the extent of area to which the gel readily spreads on application to skin or affects part. Spreadability is calculated by using the formula: S = ml/ t, m = weight tied to upper slide, l = length moved on glass slide and t = time taken to separate the slides completely from each other. Spreadability of different formulation was recorded.

5. Drug content uniformity

Drug concentration in emulgel was measured by uv spectrophotometer. drug content was measured by dissolving specific quantity of emulgel in solvent with the help of sonication. Absorbance was measured after suitable dilution at 242nm in UV/VIS spectrophotometer.

6. Extrudability

Extrudability can be expressed as the force required to extrude material out of the tube determine the consistency of prepration and Extrudability was calculated using the following formulation.

Extrudability =Applied weight to extrude gel from tube (cm)2/Area (cm)2

7. In vitro diffusion study

Sample diffusion cell was used for the drug release studies. c. gigantea latex emulgel was applied onto the surface of egg membrane evenly. the egg membrane was clamped between the donor and receptar chamber of different cell. the receptor chamber of different cell was filled freshly prepared solution of phosphate buffer pH.5.5.the receptor chamber was stirred by magnetic stirrer. the sample were collected at suitable time interval. Sample were analyzed for drug content by uv visible spectrophotometer at 242nm after appropriate

dilution. the cumulative amount of drug release across the egg membrane was determined as function of time.

Antifungal activity of optimized formulation

Antifungal activity was checked by agar will diffusion method. certain volume of fungus suspension was poured into sterilization sabouraud's agar media (cooled at 40°c) and mixed systemically. About 20ml of this suspension was poured aseptically in Petri dish and kept till the solidification. The surface of agar plates was pierced by using a sterile cork borer. The prepared wells were filled with an equal volume of optimized batch of antifungal emulgel formulation after that it was incubated at 18-24°c for 24hrs.Fungal growth was found and the zone of inhibition was measured using antibiotic zone reader.

RESULT AND DISCUSSION

1. Physical Appearance

The physical observation of prepared Emulgel formulation is shown in Table 2.

2. PH measurement

The pH values of all prepared Formulation were range between 6 to 6.4 which are shown in table 2. The formulation is considered acceptable to avoid the risk of irritation upon application to the skin because adult skin PH is 5.5.

3. Viscosity

The test was performed at 100rpm for 10 min. result are shown in Table 2.

4. Spreadabilty

The values of spreadabilty indicate that the latex emulgel is easily spreadable by small amount of shear. The spredability of all prepared batches from F1 to F5 are shown in table 2.

5. Extrudability

The emulgel formulation were filled into collapsible metal tube or aluminium tube. the tube was passed to extrude the material and the Extrudability of formulation was observed. Result are shown in table 2.

6. Drug content

The drug content of prepared batches shown in table 2.

7. In- vitro diffusion study

The in- vitro diffusion study were carried out for all formulations using PBS (pH- 5.5) in vitro diffusion of all formulations is shown in table 3.

Table 2: Evaluation of Antifungal Emulgel formation.

Parameters	F1	F2	F3	F4	F5
Colour	White	White	White	White	White
Consistency	Excellent	Excellent	Excellent	Excellent	Excellent
Homogeneity	Good	Good	Good	Good	Good
pH	6.5	6.2	6.4	6.1	6
Viscosity	2231	2221	2236	2341	2346
Spreadabilty (gm.cm/sec)	21.12	24.63	27.52	24.52	27
Extrudability (g/cm2)	13	16	11	16	13
Drug content (%)	73	77	74	77	89

Table 3: In-vitro diffusion study.

Time (Hrs.)	% Drug Diffused				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	15.46	15.70	16.18	26.30	32.55
2	19.40	18.24	19.85	27.9	40.48
3	22.85	23.84	25.14	33.09	45.24
4	29.22	29.28	29.98	41.44	51.48
5	33.15	33.42	34.30	45.46	64.09
6	40.60	41.51	42.14	51.24	64.09
7	49.24	46.62	49.45	57.39	66.90

Antifungal activity of C. gigantea latex Emulgel

Table 4: Inhibition zone of optimized formulation (F5).

Optimized formulation	Inhibition zone (cm)
F5	0.4-0.6 cm
Blank	0.0
Fluconazole	0.6-0.8

CONCLUSION

In the present study, an attempt has been made to formulate the topical drug delivery systems of C. gigantea latex emulgel. C.gigantea latex is widely used Antifungal agent mostly used for fungal diseases. Emulgel was developed using the gelling agent Carbopol 940.span 20 an tween 20 used a emulsifiers. Propylene glycol used as a penetration enhancer. Methyl paraben and propyl paraben used as a preservative. all the formulation designed and evaluate for the post formulation studies like colour, pH, viscosity, spredability, Extrudability, drug content, Antifungal studies etc. Emulgel formation containing Carbopol 934 shown acceptable value as compared to Carbopol 940 so that F5 formulation selected as an optimized formulation. No phase separation was observed in F5 formulation. Drug content was found in range of 81%, spreadabilty in range of 27 g.cm/sec, Extrudability 13g/cm2, viscosity 2346 cos, In-vitro drug release 64.09%, Antifungal activity for Aspergillus Niger shown 0.5-0.7cm zone of inhibition found in range of 6 to 6.4 that suits the skin pH indicating skin compatibility. This is the primary requirements for a good topical formulation. from the above study we have concluded that the topical C. gigantea latex emulgel prepared from the Carbopol 934 having good spreadabilty, homogeneity and soothening effect. In these all aspects the Formulation F5 satisfied all the pharmaceutical parameters of emulgel and appears to be good topical agent.

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