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FORMULATION AND *IN VITRO* EVALUATION OF IN SITU GEL OF CALOTROPIS GIGANTEA IN TREATMENT OF FUNGAL INFECTION

Shirish B. Nagansurkar, Sanjay K. Bais, Onkar B. Pansare*
Fabtech College of Pharmacy, Sangola
Tal-Sangola, Dist.-Solapur
Maharashtra -413307

ABSTRACT

Tropical developing nations have higher rates of skin illnesses because of mycotic diseases. Herbal medicine has long been used in many regions of the world to treat skin-related issues, including mycotic infections. The medications used for the treatment of dermatophytosis have a number of adverse effects and possess a restricted level of effectiveness. Thus, the development of novel, safer, and more potent antifungal in situ gel is clearly needed. Since the relative toxicity of antifungal in situ gel and their increasing resistance to them are issues that traditional herbal medicine may be able to address with the use of herbal therapies. This study used dilution agar to assess the in-vitro antifungal efficacy of a leaf extract from Calotropis gigantea against three distinct genera of dermestids: Microscopium, Trichophyton, and Epidermophyton. In this study, an in-situ gel formulation containing Calotropis gigantea extract was developed and evaluated for its potential in treating fungal infections. The antifungal activity of the in-situ gel formulation was assessed using the agar diffusion assay against selected fungal strains. The formulation demonstrated significant antifungal activity, with zones of inhibition comparable to standard antifungal agents.

Keywords: *Calotropis gigantea Linn, antibacterial, antifungal, insecticidal activity.*

*Corresponding Author Email: - onkarbpansare9011@gmail.com
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INTRODUCTION

Fungal infections are exceedingly dangerous for human health when caused by certain pathogenic fungi, especially in those with compromised immune systems. The increasing resistance of fungi to conventional antifungal drugs has prompted the search for alternative remedies.[1] Plants have long been a source of bioactive compounds with potential therapeutic applications, such as antifungal properties. Calotropis gigantea, a member of the Apocynaceae family, is well-known for its antibacterial qualities among other medical benefits.[2]

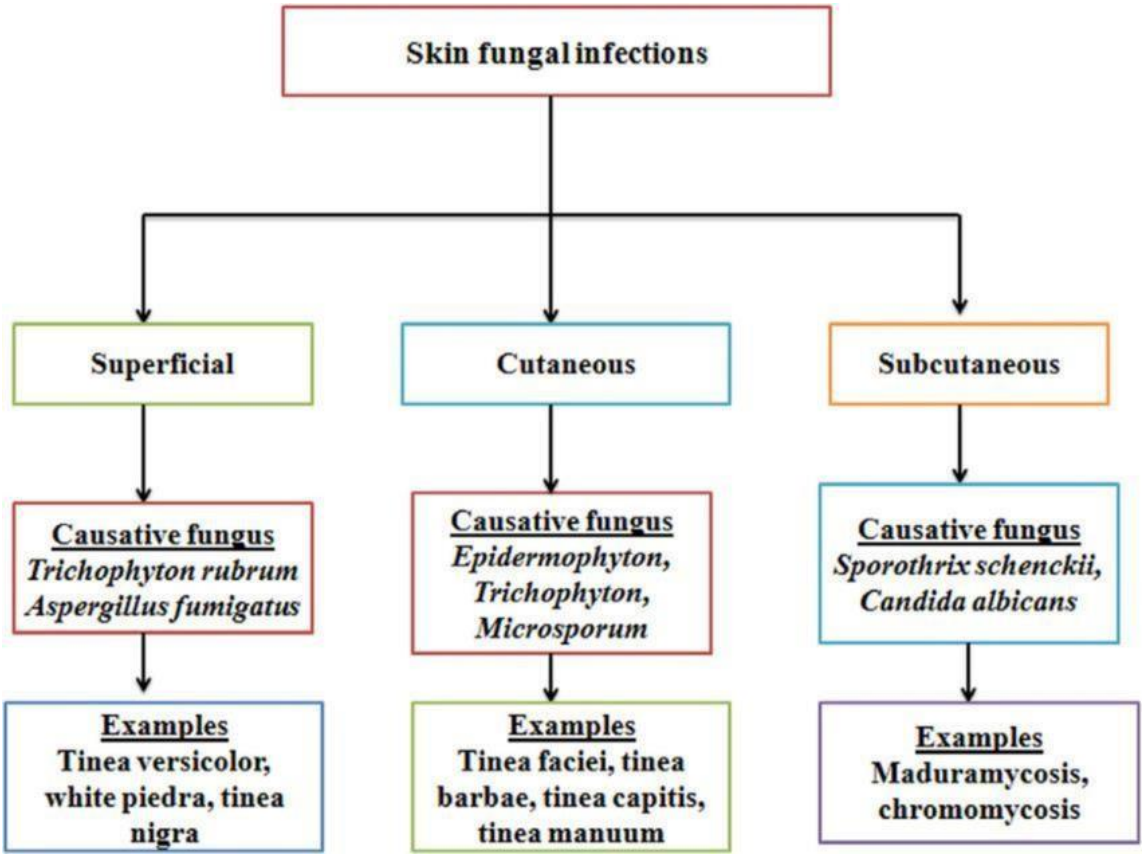
In recent years, there has been an increased focus on developing novel plant extract compositions for the treatment of fungal diseases. An illustration of such a formulation is an in-situ gel, which offers several advantages such enhanced simplicity of application, bioavailability and extended release of bioactive ingredients.[3]

Developing and evaluating an in-situ gel with Calotropis gigantea extract for the treatment of fungal infections is the aim of this study. The gel will be created using an appropriate polymer matrix, and its chemical and physical properties are to be evaluated. The antifungal activity of the in-situ gel will be assessed against clinically relevant fungal strains, and its efficacy will be compared to that of traditional antifungal drugs. Research on safety and stability will also be conducted to verify the safety of the in-situ gel when applied topically.[4]

An analysis of the antifungal activity of both individual and combination extracts in a single dose form was done to determine the possibility of synergistic antifungal action. Gel formulations are used to provide pharmaceuticals topically because they are simpler to apply, have a longer contact period, and have less adverse effects than different topical preparations and oral administration procedures.[5]

Figure No.1: Calotropis gigantea

Type of Fungal infection:



MATERIALS AND METHODS:

Plant Collection and Authentication: The leaves of Calotropis gigantea was collected in the month of February 2024 from Sangola, Solapur Dist. of Maharshtra, India. The plant was authenticated by Dr. Tembhurne R.R., Dept. of Botany from Sangola College, Sangola. The leaves were washed with tap water and dried under shade.

Preparation Of Plant Extracts of Calotropis gigantea: The fresh leaves were washed under running tap water, shed dried and coarsely powdered in a mechanical grinder.

Extraction Procedure:

Preparation Of Ethanolic Extracts:

A few changes were made to the extract preparation techniques from those outlined in. The leaf sample was washed with ordinary water, allowed to dry, and then placed in a blender to be ground into a powder. Ethanol is a solvent used in a range of ratios in the Soxhlet extraction method. Once the extract has been collected for a period of six to eight hours transfer it to 50 ml tubes, filter it using a muslin cloth, and centrifuge it for twenty minutes at twenty-five degrees Celsius and four thousand rpm. Once collected, the supernatant was set back for drying. [6,7]

Figure No. 2: Extraction Process

FORMULATION TABLE:

Sr. no.	Ingredient	Quantity
1.	Carbapol	1 w/v
2.	HPMC E15	1 w/v
2.	Distilled water	100 v/v
3.	Methyl paraben	0.5w/v
4.	Calotropis gigantea extract	2v/v
5.	Propylene glycol	0.5v/v
6.	Triethanolamine	1v/v

Table No.1: Formula for in situ gel

Preparation of in situ Gel formulation:

One w/v and fifty milliliters of v/v Carbopol were mixed together and continuously swirled. The required amount of methyl & propyl paraben was heated to dissolve them using a water bath after five v/v of distilled water was consumed. Propylene glycol was added once the solution was given time to cool. The volume was increased to one hundred v/v via the addition of distilled water.

Triethanolamine was progressively added to the formulation in order to balance the pH of the nail and provide the appropriate consistency for the in-situ gel, after all of the ingredients had been thoroughly combined into the Carbopol gel and frequently stirred by adding HPMC E15. This identical method was used to prepare extracts. [8,9]

EVALUTION TEST:

Physical evaluation: The color, texture, taste, and condition of the in-situ gel were visually assessed throughout this assessment test.[10]

Spreadability: He parallel plate technique was used to assess the spreadability of in situ gel. Two 20/20 cm glass slides were chosen. One of the slides was covered with around one gram of the gel formulation. A 125 grams weight was placed on the upper side of the other slide, that was placed on top of the in-situ gel such as to ensure that in situ gel had been placed between the slides and squeezed evenly to produce a thin layer. After releasing the weight, the smear that appeared was measured [11]

pH: The pH of the in-situ gel was determined by using the pH paper.

Viscosity: The in-situ gel's viscosity was assessed at regular intervals. The viscometer was used to gauge changes in viscosity. Both room temperature and 45 degrees Celsius are used to test stiffness.[12]

Homogeneity: After the in-situ gel was placed in a container, the uniformity of the gel formulation was examined aesthetically. They were examined for appearance and any presence of any aggregation in this homogeneity test.[13]

Washability test: The hand was treated with a little amount of in situ gel and then cleaned with tap water.[14]

Nutrient Media for Microorganism(fungi):

Sr. no.	Ingredient	Quantity
1	Beef Extract	0.3gm
2	Peptone	0.5gm
3	Sodium Chloride	0.05gm
4	Distilled	100ml

Table No.2: Formula for Nutrient Media

Firstly, sterilization all glassware in autoclave 121° C for 30 min.

Add Beef extract (0.3gm), peptone (0.5gm) , sodium chloride (0.05 gm) in 100 ml of water.

Boil the mixture for 30 min. and cool it.

Transfer the fungi in culture media and the procedure is carried out only in aseptic area to prevent microbial contamination.

Put the culture media in incubator for 48hrs at 37.8°C. [15,16]

Figure No.3.: Cultural Media

Antifungal activity:

Agar plate medium was made by heating and dissolving all of the elements in 15 g of nutritious agar powder in a hundred liters of distilled water. The dissolved mixture is autoclaved for 15 minutes at 121° C to allow it to cool but not harden. After that, the provided microbe (fungi) was injected into the nutrient agar medium and the mixture was placed onto plates to solidify. Then, using a borer, make holes in the same medium that are around 9 mm in diameter using the diffusion technique for agar wells. The extract of Calotropis gigantea was instantly inserted into the holes as an antifungal solution. The plates are incubated and reported in table no 2 and shown in figure 4. [17,18]

Figure No.4.: Agar plate media

RESULT:

The formulation no.3 (F3) of an anti-fungal in situ gel using Calotropis gigantea extracts shows promising results. Formulations were assessed in terms of certain physical parameters. The created in situ gels had a pH range of 6 to 6.7 and were transparent and having homogenous texture. Additionally, this formulation displayed appropriate spreadability qualities along with acceptable rheological behavior.

Figure No. 5:In situ Gel

Evaluation test of in situ gel:

Formulation	Physical appearance	pH	Spreadability	Homogeneity	Washability test
F1	Greenish in situ gel	6.7	5.47 ± 0.01	Good	Good washability
F2	Greenish in situ gel	6.5	5.66 ± 0.05	Good	Good washability
F3	Greenish in situ gel	6.9	6.41 ± 0.01	Good	Good washability

Table No.3: Evaluation test

In vitro study (Antifungal activity):

Concentration of gel	Diameter of the inhibition Zone(mm)
2 ml	6mm
4ml	8mm
6ml	10mm

Table No.4: Zone of Inhibition

DISCUSSION:

The initial step in the formulation involves extracting the bioactive compounds from Calotropis gigantea, known for its antifungal properties. The use of solvents like ethanol is crucial in obtaining a concentrated extract with maximum efficacy. The method ensures the preservation of phytochemicals responsible for antifungal activity. The formulation of an in situ gel is advantageous due to its ability to transition from a liquid to a gel state at the site of application, enhancing localized drug delivery. Using polymers like Carbopol 934 and HPMC E15 not only provides the necessary viscosity but also ensures controlled release of the active compound. An optimal viscosity is crucial for the application and retention of the gel at the site of infection. This parameter ensures the gel remains in place, allowing for prolonged drug release. An optimal viscosity is crucial for the application and retention of the gel at the site of infection. This parameter ensures the gel remains in place, allowing for prolonged drug release. The prepared formulation has shown promising results during the work, however further investigation for safety and efficacy in animal models is necessary**.**

CONCLUSION:

Even if some bacteria were less susceptible, the ethanolic extract of Calotropis gigantea leaves indicated a broad range of antifungal effectiveness in the n-hexane and ethyl-acetate fractions. as an alternative to common antibacterial and antifungal drugs, against them. Additionally, the two extracts showed insecticidal properties. This medicinal plant is used to cure infectious disorders brought on by harmful bacteria and

fungus as well as to manage insects. It is unknown which particular bioactive molecular structure or compounds were isolated from the leaves of Calotropis gigantea.

The study's findings suggest that there is great promise for the creation of accessible antifungal medications using an ethanol-based extract of Calotropis gigantea. To ascertain the effectiveness of this plant extract's active medicinal ingredient, more research is necessary.

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