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Formulation and Evaluation of Anti-Fungal Cream of *Bougainvillea Glabra* Flowers

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ABSTRACT:
The main aim of our research was to develop an Antifungal cream formulation consisting of B.glabra for the treatment of Fungal infections. Topical route is the most suitable route for skin infections.
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 B.glabra was prepared.
The formulation was subjected to in vitro diffusion studies. Microbiological studies were performed to find out the safety of materials used in the formulation.
The developed cream consisting of bougainvillea glabra was found to be safe and effective for the treatment of fungal infection.

Keywords: anti-fungal activity, topical Drug delivery, bougainvillea glabra.

1. INTRODUCTION

The genus,Bougainvillea is a widely dispersed group across the globe. It belongs to the Nyctaginaceae family and, according to the "Plant List," has roughly 18 species, four of which are commercially exploited: B. buttiana, B. glabra, B. spectabilis, and B. peruviana. However, there are more than 100 cultivars and three hybrids, one of which has yet to be recognized.
The active ingredients of hundreds of plant species that have been used traditionally as medicines have not been thoroughly studied. One such plant is B. glabra Choisy, a climbing plant native to Brazil that is a member of the Nyctaginaceae family and lives in warm climates. It is highly valued as an ornamental and horticultural plant because of its striking "inflorescences," which are formed by the involucre, which is composed of a set of colorful bracts and the true flower.
It is a very common genus of attractive flowering plants that Louis de Bougainville first studied in Brazil in the 18th century. He brought it to Europe, where it was widely welcomed and equally dominant. The most prevalent species in this genus, which is mostly grown as an ornamental plant, is Bougainvillea glabra, also referred to as paper flower or glory of the garden.
In traditional medicine, B. glabra is used to treat gastrointestinal issues including diarrhea and dysentery as well as respiratory conditions like the flu, cold, cough, bronchitis, and asthma. Because it contains active substances such as flavonoids, tannins, alkaloids, phenols, betacyanins, terpenoids, glycosides, and essential oils, it is also thought to have antibacterial properties.

2. METHOD AND MATERIAL

a) Plant material : [5][6]

In 2007, samples of Bougainvillea glabra were taken from beehives in Oaxaca State, Mexico, and plants were gathered in Morelos State, Mexico. Under the codes 26348 (B. glabra), 26347 (E. globulus), and 26346 (G. attenuatum), voucher specimens were verified by Juan Carlos Juárez and then placed at the HUMO Herbarium, CEAMISH (Centro de Educación Ambiental e Investigación Sierra de Huautla), UAEM.

In May 2021, B.spectabilis air pieces (flowers) were taken from Sari. I bought nettle seed from a nearby grocery store. Every chemical and reagent was bought from Sigma-Aldrich.

b) Preparation of Extract :[8]

The 500 g of coarsely powdered, shade-dried Bougainvillea glabra bracts was put through the Soxhlet extractor, first extracting with petroleum ether and then extracting again with ethanol. After being dried out under lowered pressure and regulated temperature, the extracts were refrigerated to ensure theirpreservation.

Figure no.2. Extraction of Bougainvillea Flowers

c) Antifungal Activity of Bougainvillea glabra :[11]

1. Using a sterile loop, inoculate the bread pieces with a B. glabra extract.
2. Slices of bread should be incubated at room temperature in a petri dish.
3. After a day, check the bread slices for colony formation and measure the colonies' diameter.
4. Treated extract with strong antifungal activity form a large zone of inhibition.

Control Test

Figure No.3. Antifungal Activity Of Bread Slice

d) Cream formulation :[8]

- A cream with an oil-in-water (O/W) emulsion base (semisolid formulation) was developed.
- In the oil phase (Part A), the emulsifier (stearic acid) and other oil-soluble ingredients (cetyl alcohol, almond oil) were separated and heated to 75 °C. The aqueous phase (Part B) was heated to 75 °C after the preservatives and other water-soluble ingredients (methyl paraban, propyl paraban , triethanolamine, and ethanol extract of Bougainvillea glabra) were dissolved in it.
- Following heating, the aqueous phase was gradually added to the oil phase while being constantly stirred.
- When the temperature dropped to (45±50) °C, perfume was applied . (Table no.1) provides the cream's formula.
- Place this cream on the slab, add a few drops of distilled water if needed, and mix the cream geometrically on the slab to ensure that all the elements are well combined and the cream has a smooth texture.
- This process is known as the extemporaneous method of cream preparation or slab technique.

Figure no.4. Formulation of Cream

Table 1. Composition of Bougainvillea glabra based on antifungal cream (g)

Ingredients	F_1	F_2	F_3	F_4	F_5
Bogainvillea Flowers Extract	200mg	400mg	600mg	800mg	1000mg
Stearic Acid	2.5gm	2.5gm	2.8gm	3gm	3.2gm
Triethanolamine	1.25gm	1.17gm	1.2gm	1.3gm	1.3gm
Cetyl Alcohol	2.43gm	2.3gm	2.32gm	2.5gm	2.6gm
Methyl Paraben	0.25gm	0.2gm	0.19gm	0.18gm	0.19gm
Propyl Paraben	0.22gm	0.23gm	0.18gm	0.2gm	0.21gm
Rose Water	1.84ml	1.85ml	1.8ml	2ml	1.8gm
Almond Oil	1ml	1ml	0.9ml	1ml	0.1ml
Total Weight	10gm	10gm	10gm	10gm	10gm

3. EVALUATION OF ANTIFUNGAL CREAM

a) Properties Of Cream:[8]

The following are some characteristics of the cream that were measured in the experiment:

- A common buffer solution was used to calibrate the pH meter. The pH of around 0.5 g of cream, weighed and diluted in 50 mL of distilled water, was determined.
- The formulation's viscosity was measured using spindle No. 7 on a Brookfield viscometer operating at 100 rpm.
- The color, fragrance of rose oil, and roughness of the cream were used to grade its appearance.
- By evaluating the compositions, visual appeal and tactile affinity, uniformity was verified.
- After applying a set quantity of cream, the amount of residue left behind, its emolliency, and its slipperiness were assessed.
- A film or smear was observed on the skin following the cream application.
- The spread ability and dampness were ruled out. An observer observed the qualities of a fixed quantity of cream applied to a human volunteer's dorsal skin surface.
- The kind of film or smear that developed on the skin after the cream was applied was examined.
- By using tap water to wash the area where the cream was applied, the cream's ease of removal was evaluated.

b) Irritancy Test:[8]

On the dorsal surface of the left hand, mark the area (1 cm^2). After applying the cream to that location, the time was recorded. After that, it is monitored for irritability, erythema, and any edema for up to 24 hours and reported. The results indicate that there are no symptoms of edema, erythema, or irritation with the cream.

c) Accelerated Stability Test:[8]

The formulation (10gm cream) tested for accelerated stability , stable samples are analyzed for Three month at room temperature and higher temp. (40 to 50°C) . Both room temperature and higher temperatures were used to observe the formulations.

d) Type Of Emulsion Under Dye Test:[8]

The cream and the scarlet crimson dye are combined. Before being viewed under a microscope, a drop of the cream was put on a microscopic slide and covered with a cover slip. If the ground is colorless and the dispersion globules look red, the cream is O/W type. W/O type cream exhibits the opposite phenomenon, where the dispersed globules appear colorless on the red ground.

e) Physical Evaluation:[10]

This test examined the color, odor, texture, and condition of formulations.

f) Washability Test: [10]

The washability test involved putting a tiny amount of cream on the hand and then washing it with tap water..

g) pH: [10]

The cream's pH was determined to be closer to the pH of skin, meaning that it is safe to use on skin.

h) Viscosity: [10]

Cream's viscosity was measured at 25 °C using a Brooke field viscometer and spindle number 63 spinning at 2.5 RPM. Based on the findings, the formulations exhibited sufficient viscosity.

i) Phase Separation: [10]

The prepared cream was stored away from light and at a temperature between 25 and 100 °C in a covered

container. Phase separation was then observed for 24 hours during a 30-day period. Any modifications to the phase separation were noted and verified..

j) Spredability: [10]

The spreadability was measured by measuring how long it took two slides to separate from the cream that was sandwiched between them under a specific load, measured in seconds. The spreadability improves when the two slides are separated in less time. We collected two sets of standard-sized glass slides. After that, a slide with the right dimensions was selected, and the cream formulation was put on it. On top of the formulation, another slide was then positioned. In order to create a thin layer of cream between the two slides, a weight or specific force was then applied to the upper slide.After removing the weight, extra formulation that had stuck to the slides was scraped off. The power of the weight attached to the upper slide allowed it to move off without any assistance. The amount of time it took for the upper slide to come off was recorded.

Spread ability= m × l/t

Where, m= Standard weight which is tied to or placed over the upper slide (30g)

l= length of a glass slide (5 cm)

t= time taken in seconds.

k) Greasiness: [10]

Here, the cream was smeared onto the skin's surface, and its oiliness or grease-likeness was assessed.

4. RESULT OF EVALUTIONS

Evaluation results of the cream is gives below**.**

a) Physical evaluation :

S. No.	Parameters	Result
1.	Colour	Pink
2.	Odour	Sweet
3.	Texture	Smooth
4.	Condition	Semisolid

b) Irritancy:

On the dorsal surface of the left hand, mark the area (1 cm^2). After applying the cream to that location, the time was recorded. After that, it is monitored for irritability, erythema, and any edema for up to 24 hours and reported. The results indicate that there are no symptoms of edema, erythema, or irritation with the cream.

Formulation	Irritability	Erythema	Edema
Cream	✓	✓	✓

c) Accelerated Stability Test : for 3 Month

Stability	F_1	F_2	F_3	F_4	F_5
Room Temperature	No change in weight	No change in weight	No change in weight	No change in weight	No change in weight
Higher Temperature	No change in weight	No change in weight	No change in weight	No change in weight	No change in weight

d) Wash ability :

The washability test involved putting a tiny amount of cream on the hand and then washing it with tap water.The cream was really easy to wash.

e) pH :

It is safe to use on skin.

S No.	Formulation	pH
1.	Cream	5

f) Viscosity

S. No.	Formulation	Viscosity(cps)
1.	Cream	15,640

g) Phase separation :

The prepared cream was stored away from light and at a temperature between 25 and 100 °C in a covered container. Phase separation was then observed for 24 hours during a 30-day period. Any modifications to the phase separation were noted and verified..

S. No.	Formulation	Phase separation
1.	Cream	No phase separation

h) Spreadability

The cream's spreadability was tested, and the evaluation test description states that the smaller the time it takes for the two slides to separate, the better the spreadability. In accordance with this assertion, the cream demonstrated better spreadability.

Spread ability = m × l/t

= 30×5/7

= 150/7

= 21.42 gm cm/sec

Uniform and easily spread.

i) Greasiness:

S. No.	Formulation	Greasiness
1.	Cream	Non Greasy

5. DISCUSSION AND CONCLUSION:

- **Antifungal Activity:**

Rhizopus stolonifer is commonly known as black bread mold. It is a member of Zygomycota and considered the most important species in the genus Rhizopus. It is one of the most common fungi in the world and has a global distribution although it is most commonly found in tropical and subtropical regions. In this study , Rhizopus stolonifer grow in wet bread piece and used for anti fungal activity of Bougainvillea glabra cream.

Formuations	Concentration(µg/ml)	Zone of inhibition(mm)
F_1	5	15
F_2	15	20
F_3	25	23
F_4	35	27
F_5	45	29
Nystatin	5	18
Griseofulvin	5	19

antifungal activities of the extracts increased linearly with increase in concentration of extracts (µg/ml). As compared with standard drugs, the results revealed that in the extracts for fungal activity, shows good result . The growth inhibition zone measured ranged from 15 to 29 mm for fungal strains.The purpose of this study was to develop an herbal cream. Furthermore, its effect remained even when it was included into the formulation of an antifungal cream. The results of different physicochemical tests showed a stable and good appearance of the cream. Cream has good anti- fungal activity Further detailed stability and safety studies are needed to improve the overall quality of the product. Furthermore, these parameters will be useful for the confirmation of the identity and authenticity of the *B. giabra* plant.

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