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(RESEARCH ARTICLE)

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Formulation and evaluation of antifungal herbal gel using Aloe Vera and Betel leaves extract for the treatment of candidiasis

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Abstract

The primary objective of the current study was to develop and evaluate an antifungal herbal gel for the treatment of candidiasis using an extract from aloe vera and betel leaves. Herbal remedies are now widely used for both medicinal and commercial reasons. The antibacterial gel made from herbal plants works better than synthetic medications and has fewer side effects. Aloe vera belongs to the Asphodelaceae family, which also includes Aloe barbadensis. One species in the *Piper betle* L. family of Piperaceae is the betel leaf. Aloe vera and betel leaf products, such as plant branches, roots, and leaves, have also been shown to be abundant in phytochemicals. The most popular herbs for treating fungus infections are aloe vera and betel leaves, which also contain potent anti-inflammatory, antibacterial, and antifungal properties. Methods: Hydroalcoholic extract was prepared by the maceration method. Carbopol 940 was used as a gelling agent to create the gel formulation. Candida albicans has been used to test its antifungal properties. Evaluation:The pH, viscosity, homogeneity, grittiness, extrudability, spreadability, antifungal activity and in vitro drug release research were assessed for the optimally designed herbal gel. Results:In comparison to marketed 1% clotrimazole gel 25 mm and marketed herbal gel 20 mm, the aloe vera and betelleaf extract herbal gel (S4 Batch) was found to be brown in color, homogenous, had good extrudability, no grittiness, pH 6.8, viscosity of 3424±0.45, spreadability of 4.3±0.3, and zone of inhibition of 23 mm. After being stored for 60 days, the herbal gel mixture, including extracts of aloe vera, tulsi, and peppermint leaves, did not alter in appearance. The pH has not changed in two months.

Keywords: Aloe vera; Betel leaves (*Piper betle* L.); Candidiasis; Herbal gel; Carbopol 940; Antifungal study

1. Introduction

In developing nations, herbal medications are currently much sought-after for basic health care due to their low cost, higher cultural acceptability, improved bodily compatibility, and fewer side effects. These days, fungus-induced skin infections rank among the most prevalent dermatological issues [1]. There are several options available for therapy, including liquid dosage formulation, semisolid dosage form, and solid dose form. For primary healthcare, the majority of people on the planet have used traditional medicine, especially plant-based medications. Herbal medications are essential to the medical system [2]. One prevalent opportunistic pathogenic yeast found in the human gut flora is Candida albicans. It is also able to endure outside of the body. Forty to sixty percent of healthy people have it in their mouths and gastrointestinal tracts [3]. Any kind of yeast (Candida) can cause the fungal illness known as candidiasis. Thrush is the term for a fungal infection of the mouth. White spots on the tongue or other parts of the throat and mouth are indications and symptoms of thrush. Other signs and symptoms include soreness and difficulty swallowing [5].

Yeast infection is the term for the fungal infection that affects the vaginal region. Yeast infection symptoms include burning, itching, and thick, white discharge coming from the vagina. Penis were also affected by yeast infections, albeit

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itchy rashes are less prevalent in these cases. Rarely, a yeast infection can also spread to other areas of the body. Fever is a typical sign of a yeast infection, while other symptoms may vary based on the affected body area [6].

One species within the Asphodelaceae family is Aloe vera, also known as Aloe barbadensis miller. *Piper betle* L., family Piperaceae, has a species of leaves known as betel. Plant leaves, stems, and roots have also been shown to be abundant sources of phytochemicals in aloe vera and betel products. Because of its potent anti-inflammatory, antibacterial, and antimicrobial properties, aloe vera and betel leaves are most frequently used to treat fungus infections.

Aloe vera contains a variety of phytoconstituents, including aloe-emodin, aloin, aloesin, and emodin. Maceration can be used to extract many different compounds from betel leaves, including eugenol, methyl eugenol, hydroxycatechol, caryophyllene, 1,8-cineol, α-pinene, and β-pipine. Aloe vera and betel leaf extract have a variety of phytoconstituents that give them their anti-fungal properties. Because we are concentrating on herbal formulations, aloe vera and betel leaf extract are appropriate for use in formulations [7].

2. Drug profile

2.1. Name : Aloevera

Figure 1 Aloe Vera

- Botanical name : *Aloe barbadensis* miller
- Plant part used : leaves
- Solubility : soluble in water, methanol and ethanol
- Scientific classification:
  - Kingdom- Plantae
  - Family- Asphodelaceae.
  - Genus- *Aloe L*.
  - Species- *Aloe vera*(L.) Burm.
- Category:Wound healing, anti-microbial, anti-inflammatory, anti-oxidant, anti- fungal.
- Chemical Constituents: Proteins, carbohydrates, amino acids, emodin, anthraquinone glycosides, cardiac glycosides, tannins, and phenolic substances are all present in aloe vera extract.
- Use: Skin illnesses, fungal infection, acne, wound healing, diuretic, antibacterial, antiulcer, and anti-inflammatory properties [9,12].

2.2. Name: Betel Leaf

Figure 2 Betel leaf

- Botanical Name : *Piper betle* L.
- Plant part used : Leaves
- Solubility : slightly soluble in water and dissolve fairly well in ethanol, mix well organic solvent chloroform and ether
- Scientific classification:
  - Kingdom –Plantae
  - Family– Piperaceae
  - Genus - piper L.
  - Species- piper betel L.
- Category :anti-microbial, anti-inflammatory, anti-oxidant, anti- fungal, anti cancer , anti diabetic, gastro protrctive.
- Chemical Constituents: Betel leaf extract contains the following compounds: campene, f-pinene, u-limonene, 1-,9-cineol, methyl eugenol, phenol, chavicol, chavibetol, hydroxychavic, and caryophyllene.
- Use : boosting immunity, preventing infections, healing wounds, reducing inflammation, fighting fungal infections, and treating skin conditions [9,10,11].

3. Material and methods

3.1. Sample Collection

The Aloe vera and Betel leaves was collected from Nagarjuna Medicinal plant garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India and authenticated by Dr. D.K. Koche Head of Botany Department by Shri Shivaji Education Sociaty Amravati's Shri Shivaji College of Arts, Commerce and Science, Akola, Maharashtra and notated as identification voucher No. SSC / B-9/ 2024.

3.2. Plant Extract

After adding 33 g of aloe vera powder to 66 ml of hydroalcoholic solvent, the mixture got to maceration for 24 to 42 hours at room temperature. The filtrate was then collected and filtered, and the solvent evaporated on a hot plate at 50 to 60 °C to produce a fine brown powder. The identical protocol was used for the extraction of betel leaves [17].

3.3. Preparation of Inoculum

The current study includes Candida albicans ATCC 10321 as the fungal pathogen. To obtain an inoculum for testing, the fungus was grown on Saboured dextrose medium and incubated for 24 hours at 37 °C [16].

3.4. Preparation of Saboured Dextrose Agar Media

Dextrose (10 g), peptone (2.5 g), agar (3.75 g), and water (250 ml) were combined to create the medium, which was then adjusted to pH 5.8. After that, the medium was autoclaved for 15 minutes at 121 °C and 15 pounds of weight.

3.5. Determination of Zone of Inhibition.

Usinga 250 ml conical flask, dissolve the Sabouraud's dextrose agar medium in 100 ml of distilled water. The pH was adjusted to 5.6±0.2. After 15 minutes of autoclaving at 15 pounds and 121 °C, the medium was allowed to cool to room temperature before being transferred onto sanitized petri dishes that were placed under a laminar air flow device. A loop of diluted suspension culture (Candida albicans) was then applied to the surface of the solidified agar and evenly distributed with the aid of a spreader. Medium-filled petri dishes were then placed in a laminar air flow unit to solidify.

3.6. Determination of minimum inhibitory concentration

3.6.1. Brothdilution method

Nutrient broth test tubes were filled and labeled. The first tube A (UT), which was not infected, served as a negative control. Tube B (CT) received control inoculum but no extract. Extract was applied to tubes C, D, E, and F at concentrations of 50, 100, 150, and 200 ug/ml. To determine the ultimate microbe concentration, 3-4 drops of inoculum were given to each tube. Extract was placed in all test tubes except the uninoculated (negative control) and control (positive) tubes. The positive control tube (CT) was used to determine the medium's appropriateness for Candida albicans growth. All test tubes were shaken and incubated for 48 hours at 37 °C. All experiments were conducted three times, with the findings represented as averages.

Table 1 Determination of Minimum Inhibitory Concentration by brothdilution method

Tube Number	Volume of Double Strength Medium (ml)	Conc. Of Extract (ug/ml)
(UT) A	5	0.0
(CT) B	5	0.0
C	5	50
D	5	100
E	5	150
F	5	200

3.7. Formulation of Gel

While another beaker contained the weighed and necessary amount of extracted drug powder, which was dissolved in polyethylene glycol and sonicated for 15 minutes, different ratios of Sodium CMC and Carbopol 940 were distributed in distilled water with constant stirring with the aid of a mechanical stirrer. Following 15 minutes of sterilized, this mixture was added to the first solution, which included a combination of carbopol 934 and sodium CMC, while stirring continuously. The necessary amount of sodium benzoate, on the other hand, was dissolved by boiling 5 ml of distilled water in a water bath. Polyethylene glycol was added and mixed with the previously mentioned solution once the solution had cooled. In order to get the desired consistency for the gel, all components were finally thoroughly combined with the carbopol 940 while being stirred continuously. Using this technique, four herbal gel formulations were produced, each containing 0.2%, 0.4%, 0.6%, and 0.8% of aloe vera and betel leaf extract, respectively.

Table 2 Formulation Table

Sr.No	Ingredients	S	S1	S2	S3	S4
1	Extract (aloe vera and betel leaves)	0	200	400	600	800
2	Carbopol 940 (g)	6	3	3	3	3
3	Sodium carboxy methylcellulose (g)	1	0.5	0.5	0.5	0.5
4	Polyethylene glycol (ml)	2	1	1	1	1
5	Sodium benzoate (g)	0.25	0.25	0.25	0.25	0.25
6	Distilled water	q.s	q.s	q.s	q.s	q.s

3.8. Evaluation and characterization of prepared gel

3.8.1. Physical appearance

Visual inspection was done to assess the produced gel formulation's color and clarity.

3.8.2. pH

he produced gel formulation's pH is measured using a digital pH meter. After dissolving 1 g of gel in 100 ml of distilled water, it was kept for 2 hours. In order to prevent any kind of skin irritation, the pH of the topical gel formulations was determined in the range of 6.8–7.1, which is close to the natural pH of the skin.

3.8.3. Grittiness

A microscopical analysis was performed to determine if the generated gel formulation included fine particles.

3.8.4. Homogeneity

Visual examination was used to assess the homogeneity of produced gel formulations once the gel had solidified in the container. It was recognized by the way the aggregates in the gel formulations looked and felt.

3.8.5. Viscosity

The Brookfield viscometer (LVDVE model, Brookfield Engineering Ltd.) was used to measure the viscosity of the gel. Spindle number 64 was used to rotate the gels at 10 rpm, and the dial reading was recorded.

3.8.6. Extrudability

10 grams of gel compositions were placed into either a metal or aluminum collapsible tube. The gel was extruded from the collapsible aluminum tube by pressing the tube with the finger. a higher extrusion volume of gel guarantees improved extrudability. By quantifying the quantity of gel extruded from an aluminum collapsible tube, the extrudability of the formulations was examined.

3.8.7. Spreadability

Using a CT3 Texture Analyzer (Brookfield) in compression mode and the spread ability attachment (TA-BT-KIT) (Brookfield Engineering Corporation, USA), the spreadability of the gel was ascertained. In order to prevent air pockets from entering the samples, the female probe was filled with an optimized gel formulation. A conical analytical male probe with a diameter of 45 mm and a specified pace of 1 mm/s was driven down into each sample to a predetermined depth of 10 mm. The samples were analyzed at least three times.

3.8.8. Invitro Antifungal study

In a 250 ml conical flask, dissolve the Sabouraud's dextrose agar medium with 100 ml of distilled water. After adjusting the pH to 5.6 to 0.2, the medium was autoclaved for 15 minutes at 121 °C. After cooling to ambient temperature, it was transferred onto sterile petri plate plates that were placed under a laminar air flow unit. Once the medium-filled petri dishes were placed in a laminar airflow unit to solidify, a diluted suspension culture loop (Candida albicans) was added to the surface of the solidified agar and evenly distributed with the aid of a spreader culture. The culture was then sterilized, and cups were punched using sterile corkborer, aloe vera, and betel leaf extract gel. Mixture: 0.5 ml of 1% Clotrimazole gel, as sold in the market, were added to each cup individually. After that, the zone of inhibition was evaluated in petri dishes that had been cultured for 24 hours at 37 °C.

4. Results and Discussion

4.1. Determination of minimum inhibitory concentration of aloe vera & betel leaves extract

The lowest concentration of aloe vera and betel leaf extract that prevents candida albicans from growing visibly is known as the minimum inhibitory concentration. The test tubes were designated as CT-control and UT-uninoculated, meaning that candida albicans was not injected into them. Candida albicans was fully grown in sample C (50 µg/ml). Candida albicans in test tubes D (100µg/ml) did not appear to be much eliminated.The aloe vera (150 µg/ml) exhibited a wonderful clear medium that demonstrated its ability to prevent the development of Candida albicans in test tubes.

Likewise, betel leaf extract (200 µg/ml) displayed amazing transparent media, suggesting that the betel leaf extract impeded Candida albicans development. As a result, the MIC values for the aloe vera and betel leaf extracts' antifungal activities were 150 and 200 µg/ml, respectively.

Table 3 Determination of MIC by broth dilution method

Tube No.	Volume of Double Strength Medium (ml)	Concentration of Extract (ug/ml)	Visual Result for Aloe Vera	Visual Result for Betel Leaves
(UT)A	5	0.0	Clear	Clear
(CT)B	5	0.0	Turbid	Turbid
C	5	50	Turbid	Turbid
D	5	100	Slightly turbid	Turbid
E	5	150	Clear	Slightly turbid
F	5	200	Clear	Clear

4.2. Percentage yield of hydroalcoholic extract of aloe vera and betel leaves

Table 4 Percentage yield of hydroalcoholic extract of aloe vera & betel leaves

Sr.No.	Extract	Solvent Used	Extraction Process	%Yield
1.	Aloe Vera	Hydroalcoholic	Maceration	10.27%
2.	Betel Leaves	Hydroalcoholic	Maceration	12.39%

4.3. Formulation and Development

Four trial batches were formulated and evaluated for pH, viscosity, appearance. Batch S4 selected as optimized batch because they have very high spreading coefficient.

Table 5 Formulation table of gel

Sr.No.	Ingredients	S4
1.	Extract (Aloe vera, and betel leaves) (mg)	800
2.	Carbopol 940 (g)	3
3.	Sodium carboxy methyl cellulose (g)	0.5
4.	Polyethylene glycol (ml)	1
5.	Sodium benzoate (g)	0.25
6.	Distilled water	q.s

Based on the preceding data, we may conclude that the S4 batch has a satisfactory pH. As a result, it was chosen as the optimal batch for further development of antifungal gel for the treatment of candidiasis.

4.4. Evaluation of antifungal gel

4.4.1. Homogeneity and grittiness

There was no grittiness and good homogeneity in any of the developed gel compositions.

4.4.2. Appearance

The developed gel compositions had a smooth look, an aromatic scent, a brown color, and a slightly thick consistency.

4.4.3. pH

Gel formulations were found to have pH values between 5.9 and 6.9, which is within the pH range of normal skin. (Table 6).

4.4.4. Extrudability

All gel formulations were determined to have acceptable extradability.

Table 6 Colour pH, homogeneity of gel formulations

Batches	Colour	pH	Homogeneity	Grittiness
S1	Brown	5.9 ± 0.038	Homogeneous	No
S2	Brown	6.4 ± 0.005	Homogeneous	No
S3	Brown	6.6 ± 0.013	Homogeneous	No
S4	Brown	6.8 ± 0.034	Homogeneous	No

4.4.5. Viscosity

Table 7 displays the viscosities of several aloe vera and betel leaf extract gel formulations. There is a range of viscosity from 2685 to 3424 cps. The findings indicate a greater viscosity in the S4 formulation. The S1 formulation was found to have a low viscosity.

Table 7 Viscosity, spread ability, extrudability of gel formulations

Batches	Viscosity	Spread ability	Extrudability
S1	2685±0.33	2.5±0.2	Good
S2	2865±0.41	3.2±0.1	Average
S3	3167±0.23	3.7±0.4	Excellent
S4	3424±0.45	4.3±0.3	Excellent

4.4.6. Spreadability

The spreadability of a gel refers to how easily it covers a certain area after being applied. Spreadability is dependent on the gel's viscosity; as viscosity rises, spreadability falls. Good adhesiveness, adhesive power, and hardness (firmness) were demonstrated using S4 fomulation. Gel hardness and compressed gel structural strength are correlated. Gel has a thicker consistency the higher its hardness value. The polymer's concentration and viscosity determine how spreadable it is. Good spreadability (4.3±0.3) was seen in the S4 gel.

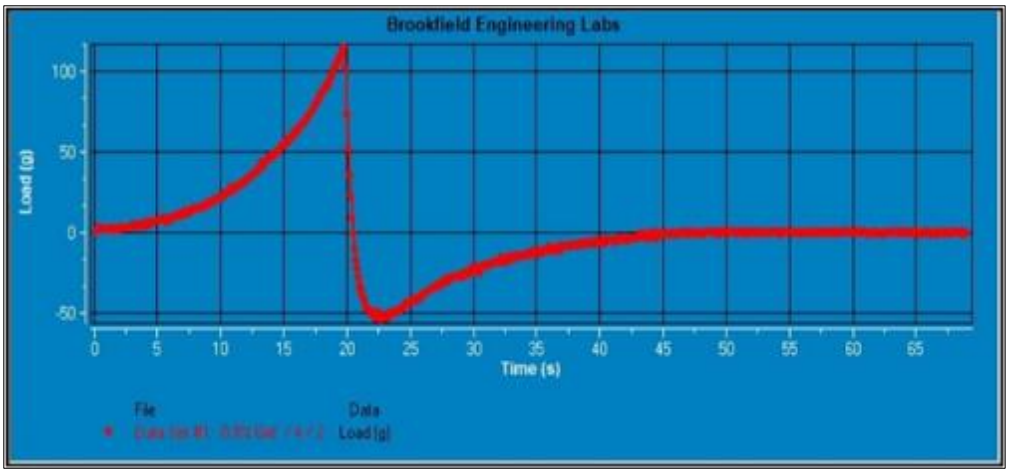

Figure 3 Texture analyser for formulation of gel

4.4.7. In vitro antifungal study

The antifungal properties of commercialized 1% clotrimazole gel and an extract gel made from aloe vera and betel leaves were tested using the cup plate technique, as illustrated. As indicated the zones of inhibition for the marketed 1% clotrimazole gel, 0.8% aloe vera and betel leaf extract gel, and marketed herbal gel were determined to be 23 mm, 25 mm, and 20 mm, respectively. In comparison to commercialized herbal gel, the zone of inhibition of the 0.8% extract gel of betel leaves and aloe vera (optimized S4 batch) demonstrated 23 mm of efficient inhibition against *C. albicans.* Thus, it has been demonstrated that 0.8% aloe vera and betel leaf extract gel is efficient against candida albicans. The zones of inhibition for the 0.2%, 0.4%, and 0.6% aloe vera and betel leaf extract gel were 19 mm, 20 mm, and 22 mm, respectively. So, S4 batch has been optimized.

Table 8 Zone of inhibition shown by aloe vera and betel leaves extract, 0.8% aloe vera and betel leaves extractgel (S4), marketed herbal gel, marketed 1% clotrimazole gel for *C.albicans.*

Sr. No.	Sample Name	Zone Of Inhibition In Mm, Mean ±SD (N=3)
1.	Aloe vera and betel leaves extract.	23mm
2.	0.8% aloe vera and betel leaves extract gel.	23mm
3.	Marketed herbal gel.	20mm
4.	Marketed 1%clotrimazole gel.	25mm

5. Conclusion

Aloe vera and betel leaves are therapeutic plants that contain numerous phytoconstituents. Herbal formulations are becoming increasingly popular in the global market. A topical gel containing aloe vera and betel leaf extract, with Carbopol 940 as a gelling agent (3 gm), was effectively made and tested for candidiasis therapy. In the antifungal investigation, regular 1% clotrimazole gel (25 mm) and marketed herbal gel (20 mm) outperformed the ethanolic extract (23 mm) of the herbal gel formulation against Candida albicans.The antifungal action of aloe vera and betel leaf extract is due to phytoconstituents and phytochemicals. As a result, it can be stated that a topical gel comprising 0.8% aloe vera and betel leaf extract might be utilized as an alternative to commercially available herbal gel.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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