

**Research on Formulation and evaluation of in vitro polyherbal
Antifungal gel****Shirish B. Nagansurkar, Sanjay K. Bais, Akshata S. Zapke****Fabtech College of Pharmacy, Sangola**Tal-Sangola, Dist.-Solapur**Maharashtra -413307***ABSTRACT**

In the Indian medical system, Ayurveda, piper betle and Ocimum tenuiflorum, has been described as a therapy for several infectious infections and maladies. The overall function of several active ingredients is what determines the effectiveness of herbal medicines since these ingredients work in concert to increase the therapeutic value of the product. The present study objective was to create and assess a multiherbal gel combining liquid piper betle extract as well as Ocimum tenuiflorum based on the folklore use. Gel formulations (A and B) were created using ethanolic extracts of Ocimum tenuiflorum and Piper betle at concentrations in a base. It was found that the polyherbal gel compositions have antibacterial properties. The formulation's plant ingredients' synergistic action may be responsible for the effective activity. Numerous actions, including antioxidant, antidiabetic, chemo preventive, anti-ulcer, and immunological response regulation, have been reported for Ocimum tenuiflorum. It is well known that piper betles contain a number of medicinal qualities, including antibacterial, hypoglycemic, antifungal, and antioxidant effects.

Keywords: *Ayurveda, polyherbal gel, Piper beetle, Ocimum tenuiflorum*

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Received on 02 July, 2024, Accepted 10 July, 2024

Please cite this article as: Zapke Akshata et al. Research on Formulation and Evaluation of in Vitro Polyherbal Antifungal Gel
International Journal of Pharmacy And Herbal Technology 2024.

INTRODUCTION

Ecologically and biologically friendly plant-based products have received a lot of attention lately as a means of treating and preventing various human illnesses. It is known that traditional medicine, especially plant-based medicines, has been used for primary healthcare by the majority of people on Earth.^[1] Since all of the active ingredients work in concert to increase the therapeutic value of the product, the total function of these components determines the activity of herbal use.^[2] When compared to synthetic pharmaceuticals, herbal medications are often regarded as secure and few adverse consequences which has led to a steady a recent resurgence of interest of using herbs of medicine in poor nations in recent years.^[3] Comparing topical gel administration to cream and ointment at pathological locations, there are significant benefits for a quicker release of a medication to the area of action.^[4]

Numerous actions, including antioxidant, antidiabetic, chemopreventive, anti-ulcer, anticarcinogenic, anti-stress, and immune response regulation, have been reported for *Ocimum tenuiflorum*.^[5] It is well known that piper betles contain a number of medicinal qualities, including antibacterial, hypoglycemic, antifungal, and antioxidant effects. This knowledge led to the decision to create a gel of herbs using extracts of herbs that have greater action and work better against germs.^[6] Thus, by evaluating the inhibitory zones using the agar well diffusion method against *Candida albicans*, the current study was designed as the first step toward thoroughly describing the properties opposition to a candidacy of *ocimum tenuiflorum* and piper betle.^[7] These plants have strong antibacterial properties, however applying them in their raw form to the skin's surface might be challenging. Following preparation, the formulations were assessed for physical appearance, medication content, spreadability, pH, viscosity, washability, and antibacterial effectiveness.^[8]

The gel can be advantageous in many factors such as they can help to reduce redness, swelling, and discomfort associated with fungal infections.^[9] Capacity to focus on the problem region for quick alleviation and therapy. Following application sites with diligence. Avoiding undesirable side effects by avoiding the digestive tract. Some side effects also be there such as the gels' ingredients could irritate skin. Temperature, humidity, and other environmental conditions can have an effect on effectiveness.^[10]



Fig. No. 1.: Herbal Antifungal gel

MATERIALS AND PROCEDURES:

Gathering as well as Authentication of Plant Material:

Gather the leaves of *Ocimum tenuiflorum* and Piper beetle fresh.

Thoroughly wash the plant material with water to get rid of any dirt or contaminants.

To eliminate extra moisture, either let the plant material air dry or use a drying oven set at a low temperature (such as 40–45°C).

The Department of Botanical Sciences at Sangola Science College in Sangola, Maharashtra, handles the sample's verification. The sample was taken in the summer from the college's Botanical Garden.

Authentication: - In the Sangola, Solapur district of Maharashtra, leaves of the Piper beetle and *Ocimum tenuiflorum* were harvested in February 2024. Dr. Tembharne certified the plant's authenticity. R.R. Botany Department, Sangola College, Sangola.



Fig. No.2: Leaves of Piper beetle



Fig. No. 3: Leaves of *Ocimum tenuiflorum*

Extraction Method:

Soxhlet Extraction: In the Soxhlet extractor's thimble, add the powdered botanical material. In the Soxhlet apparatus, pour the solvent into the flask with a circular bottom. The solvent will cycle through the thimble for several hours while the flask is heated to reflux.^[11]



Fig. No. 4.: Extraction of piper beetle *Ocimum tenuiflorum*

Filtration and Concentration:

Use a filter paper or cloth to remove any solid particles from the extract after the extraction time.

To get rid of the solvent and create a concentrated extract, concentrate the filtered extract using a rotary evaporator or another appropriate technique.^[12]

Sr. No.	Ingredient	Quantity
1.	Carbopol	1w/v
2.	Extract of Piper beetle	6w/v
3.	Extract of <i>Ocimum tenuiflorum</i>	6w/v
4.	Propylene glycol 400	4v/v
5.	Methyl paraben	0.5w/v
6.	Propyl paraben	0.5w/v
7.	Triethanolamine	2v/v
8.	Distilled water	100v/v

Table no. 1. Formulation

PREPARATION OF GEL FORMULATION:

Initial Plant Extract Preparation:

1. Piper beetle juice: Pick up fresh Piper beetle leaves, give them a thorough wash, and let them air dry. Using a mill and pestle, finely grind the dried leaves to powder.

Use a Soxhlet device and an appropriate solvent (ethanol) to extract the active chemicals for six hours.

To achieve a concentrated extract, filter the mixture and evaporate the solvent under low pressure.^[13]

The process for making Ocimum tenuiflorum extract involves gathering fresh leaves, washing them, and letting them air dry. 2. Using a grinder, finely ground the dried leaves. 3. Using a Soxhlet device and a solvent approach akin to that indicated for Piper beetle, extract the active components. 4. To get the concentrated extract, filter the mixture and then evaporate the solvent.^[14]

Mixing continuously, 50ml of distilled water and 1g of Carbopol were blended. A water bath was used to dissolve the required amount of methyl and propyl paraben after five millilitres of distilled water were added. Propylene glycol was added after allowing the solution to cool. To get the volume up to 100 millilitres, distilled water was added. To get the required consistency for the gel and to balance the pH, triethanolamine was gradually added to the formulation after all of the ingredients had been thoroughly combined and the mixture had been stirred constantly.^[15]



Fig. No.5 Preparation of antifungal polyherbal gel

Assessment of Antifungal Study:

Using the Agar cup bioassay method, the antifungal effectiveness was assessed.

Preparation of nutrient agar media:

First, sterilize all glassware using an autoclave it at 121 degrees Celsius and 30 minutes. Place 0.5g of sodium chloride, 5gram of peptone, and 3gram of beef extract in 100ml of distilled water. After 30 minutes of boiling, allow the mixture to cool.^[17] If possible, keep the process contained to aseptic spaces to prevent microbiological contamination when transferring the bacteria to culture media. Give the culture medium a 48-hour incubation period at 37C.^[16]

Determination of Zone of inhibition:

15 g of nutritious agar powder were added to 100 liters of distilled water, heated, and all ingredients were dissolved to create the agar plate medium. The dissolved liquid is allowed to cool but not harden for 15 minutes at 121° C in the autoclave. After that, add the specified fungi to the nutritional agar medium and pour the mixture into plates, allowing it to solidify. Next, holes with a borer of roughly 9 mm in diameter were made in the same medium using the agar well diffusion method. Each hole was filled with the test and control materials, and after the medium was cultured for 24 hours at 37°C, their diameters were determined to be the diameter of the inhibition.^[18]



Fig. No.6.: Preparation of nutrient agar media

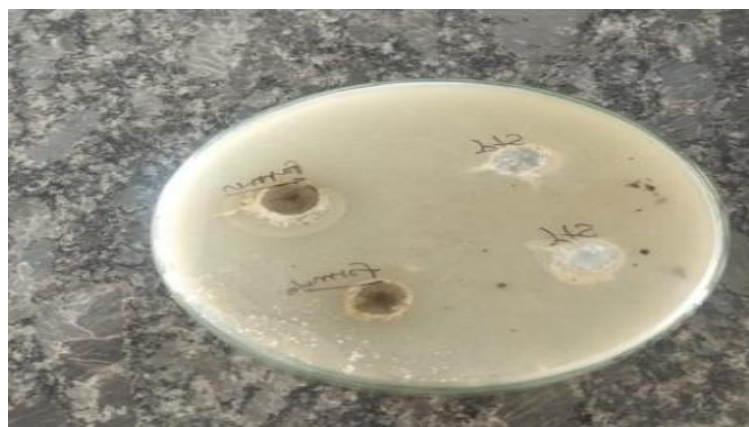


Fig. No.7.: Determination of zone of inhibition

Composition	Amount (gm)
Beef extract	3gm
Peptone	5gm
Sodium Chloride	0.5gm
Distilled water	1lit.

Table no. 2.: Composition of Agar medium

EVALUATION TEST:**A. Organoleptic character:**

The color, smell, and condition of the gel were evidently assessed throughout this test.^[19]

Spreadability:

The spread ability of gel was determined by the parallel plate technique. There were two 20/20 cm glass slides were chosen. Over one of the slides, approximately 1 gram of the gel formulation was applied. The gel was sandwiched between the second slide and the gel itself when a 125-gram weight was placed on the upper side of the slide, pressing the gel between the weight was removed was measured.^[20,21]

pH:

Using the digital pH meter, the gel's pH was ascertained.^[22]

Viscosity:

Viscosity of the gel was determined after a regular interval of time. Changes in the viscosity were determined by using the viscometer. Viscosity is measured at room temperature and at elevated temperature, 45° C.^[23]

Homogeneity:

After the gel was set in container, the uniformity of the gel formulation was examined visually. They were examined for appearance and the existence of any aggregates in this homogeneity test.^[24]

Washability test:

The hand was treated with a modest amount of gel and then cleaned with the water in the tap.^[25]

RESULTS:

Formulations	Physical Appearance	Spreadability	pH	Viscosity	Homogeneity	Washability test
F1	Semisolid, blackish green gel.	5.47+_0.01	6.35	1834.65	Good	Good washability, non-greasy Properties
F2	Semisolid, blackish green gel.	5.69+_0.01	6.19	2165.035	Good	Good washability, non-greasy properties.
F3	Semisolid, blackish green gel.	6.14+_0.01	5.65	2639.5	Good	Good washability, non-greasy properties.

Table No. 3.: Results of Evaluations

Antifungal Activity:

Concentration of gel	Diameter of zone of inhibition (mm)
1%	7mm
2%	6mm
3%	6mm

Table No. 4.: Zone of Inhibition



Fig.No.8: Diameter of Zone of Inhibition

DISCUSSION

The gel formulations showed good appearance and homogeneity. The pH of gel was in the range of normal pH range. Viscosity of polyherbal gel were determined by using Brookfield viscometer. The values of spreadability the gel was easily spreadable by small amount of shear. Washability of gel is easily washable.

Antifungal Activity:

Zone of inhibition presence and diameter around the disks serve as indicators of antifungal action. Results of antifungal activity using candida albicans are displayed below. This aids in selecting a formulation that works better.

CONCLUSION:

According to the findings, the gel compositions. Antifungal activity of the polyherbal gel formulations was noted. The produced gels underwent assessments for their viscosity, homogeneity, grittiness, drug content, swelling index, pH, spreadability, and diffusion studies. It was concluded from the data that the gel formulations had good look and uniformity. Study findings demonstrated that, as compared to the control, every formulation exhibited a greater zone of inhibition. As a result of formulation's higher concentration of herbal extracts than that of formulation, formulation demonstrated the greatest efficacy against the chosen strains. The polyherbal gel compositions to have antifungal efficacy.

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