RESEARCH ARTICLE

Formulation Development, Evaluation and Anticandidal Screening of Herbal Cream of *Trianthema portulacastrum* Linn.

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ABSTRACT

Trianthema portulacastrum, commonly known as black pigweed (E) and patharchata (H) belongs to the family Lamiaceae is a flowering medicinal pants. The plant contains various bioactive compounds such as trianthenol, leptormol, cinnamic acid, beta cyanin and ecdysterone. Traditionally, almost every part of the plant is used for the treatment of inflammation, fungal infection, pain, liver disorders, etc. In the present study, hydroalcoholic extract of leaves of *Trianthema portulacastrum* was taken to formulate herbal cream in three different batches, i.e., C-I, C-II and C-III, using different concentrations of the extract and prepared cream. Also, the anti-candidal activity of the herbal cream was determined in the fungus *Candida albicans.* The results obtained indicated that the herbal cream containing hydroalcoholic extract of the selected plant possesses significant anti-candidal activity and hence it may used in the treatment of fungal infection.

Keywords: Leaves, Extract, Fungus, Formulation, Herbal.

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INTRODUCTION

In emerging nations like India, fungal illnesses are becoming more common. People are turning to nature in their quest for a prominent and efficient antifungal treatment despite the fact that there are several antifungal drugs on the market. A number of fungal infection diseases may be effectively treated using a variety of herbal medications. There is no underlying treatment for modern synthetic medications. Herbal treatment, on the other hand, effectively addresses a number of fungal issues. Because of their unique climate and socioeconomic circumstances, Indians are disproportionately prone to fungal infections, which a wide variety of fungi may cause. About 85 to 95% of the population will have a fungal infection at some point in their lives, and fungal infections make up a quarter of all illnesses.[1] A large number of plants have been found for the treatment of herbal medications, according to the literature. When it comes to treating fungal diseases, these plants are often used: *Aloe vera* L., *Asparagus racemosus* Willd., *Chlorophytum tuberosum* Linn., *Emblica officinalis* Gaertn., *Ficus racemosa* L., *Saraca indica* Auct., *Syzygium cumini* (L.) Skeels., *Terminalia arjuna* Roxb., *Carica papaya* L., *Vitex negundo* L., *Ipomoea paniculata* (L.) R.Br., *Hibiscus rosa-sinensis* L., *Michelia champaca* L.[2]

The lack of clarity on the active components is a big problem with herbal formulations. In order to standardize the product, it is crucial to identify the active ingredient(s). Although there are now a number of herbal formulations available to patients who take them as prescribed by their doctors, an herbal formulation is necessary to ensure their efficacy and safety.[3] In the present investigation, *Trianthema portulacastrum* hydroalcoholic extract obtained from the leaves was taken to formulate, evaluate and screen the anti-candial activity.

MATERIAL AND METHODS

Preparation of Hydroalcoholic Extract

Extract preparation

About 500 g of coarsely powdered plant material, i.e., leaves of *T. portulacastrum* were taken and loaded into a soxhlet apparatus using ethanol:water (90:10) as solvent for 72 hours.

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Table 1: Composition of herbal cream

Ingredients	*HAETPL*		
	C-I	*C-II*	*C-III*
Paraffin wax in gm	3	3	3
Tocopherol in mL	0.5	0.5	0.5
HAETPL [extract (%)]	2.5	5	10
Glycerine in mL	2	2	2
Sandalwood oil in mL	0.5	0.5	0.5
Aqueous phase in mL	Qs	Qs	qs

Formulation of herbal cream

First, a water-in-oil emulsion cream is made by melting beeswax at 60 to 70°C. Then, soya powder, olive oil, tocopherol, and paraffin wax are added (Phase I). Phase II: Ethosome suspension, gently warmed at 35°C. In phase III, the glycerine and rose water were heated independently. Before adding the third phase-fragranced with peppermint and sandalwood oil phases I and II were combined constantly to ensure homogeneous dispersion; the mixture was then cooled gently. Here are the several stages that go into making ethosomal polyherbal cream.[4,5]

Preparation of oil phase

The beeswax, paraffin wax, and olive oil were melted in a porcelain dish at 700C according to the amounts specified in Table 1.

Making the water-based substrate

Guizotia abyssinica (L.f.) Cass. and *Sarcostemma acidum* (Roxb.) flower extract in an ethosomal suspension. In a separate, clean dish, the ingredients listed in Tables 2 and 3, including water, glycerine, and soya powder, were heated to 70°C.

The process of adding an aqueous to an oil phase

While swirling continuously at room temperature, the water phase was introduced to the oil phase. The next step was to add sandalwood oil, peppermint oil, and rose water to the mixture before transferring it to an appropriate container.

Evaluation of creams

The developed formulations were assessed for the following parameters.[6-7]

Visual examination

A visual assessment of the herbal cream's transparency and clarity served as the basis for its physical examination. The samples were examined on a white backdrop with illumination.

pH

pH was determined using a calibrated pH meter.

Viscosity

A Brookfield viscometer was used to measure the herbal cream's viscosity. Spindle number 01 was adjusted to rotate at a speed of 20 revolutions per minute (rpm), while maintaining temperatures of 4 and 37°C. were used. A beaker was used to collect about 15 ml of each batch's sample, and the spindle was then submerged in the mixture. The measurement was taken both before and after the rotation at various temperatures. The results were documented three times.

Ascertaining uniformity

We visually inspected each batch of herbal cream to ensure it was homogeneous and checked for aggregates in the final product.

Calculating the spreadability

All of the herbal creams that were created had their spreadability tested. Once the gel-containing slide was positioned on top of the formulation-containing slide, the empty slide was then placed on top of both. It was positioned so that the formulation was sandwiched between two slides. We measured a 7.5 cm occupied distance for the slides. A thin, homogeneous coating of the herbal cream was formed by pressing it between the slides. We took off the weight that had been holding the herbal cream. We removed the extra herbal cream that was visible on the slides. The weight of the two slides caused them to split, and the time it took to accomplish this separation, which measured 7.5 cm, was recorded. The mean time was calculated from the three recorded measurements.

Determining humidity

After applying the herbal lotion to the skin, its hydration level was measured. We were able to apply enough cream from each batch to cover a 10 mm^2 region of skin.

Smear types

It was recorded using the application of cream from each batch to cover a 10 mm^2 region of skin.

Emolliency determination

We conducted measurements on the emolliency, slipperiness, and residual amount of the herbal cream that we manufactured. We were able to apply enough cream from each batch to cover a 10 mm^2 region of skin.

Emulsion Type Determination

A test of dilution

The results were documented after the process of diluting the herbal cream formulation with either o or w (oil-in-water or water-in-oil).

Color dissolvability assay

Before being examined under a microscope, the herbal cream that had been made was combined with amaranth, a pigment that dissolves in water. We analyzed the data and made sense of it.

Drug content

We used a UV-visible spectrophotometer to determine the drug content of the herbal cream. In a 50 mL volumetric flask, about 1 g of the formulation was taken. Using methanol, the solution was brought to the mark. After shaking, the mixture was filtered using Whatman Grade 1 filter paper. The filtrate, which was initially 0.1 mL in volume, was diluted to 10 mL using solvent and then measured at an appropriate wavelength.

From the standard curve that was constructed at the optimal maximum wavelength, 286 nm, the drug concentration in the extract was determined.

Anti-candidal Activity[8]

Strain

The selected fungal strain is *Candida albicans. T*his strain was obtained from Pathology Lab, Indore

Screening of Antifungal activity (Disc diffusion method)

Disc training

A quarter-inch-diameter disc of Whatsmann filter paper was made and autoclaved for sterilization.

Sample preparation with an entrapped disk:

Different concentrations of stock solution were prepared and the Whatman filter paper disc was loaded with all of the produced dilutions using a micropipette. After that, they dried and sterilized the discs.

Setting up the culture plate

Sabouraud's agar and Mueller-Hinton agar media were used in the present investigation.

Assessment of inhibition zone

The antifungal efficacy of the re-cultured fungal strains was determined by noting the zone of inhibition.

- *Data analysis by statistic*

The data was evaluated using ANOVA, using student t-test. The values have shown a noteworthy statistical influence (*p <0.00, ** p <0.01). The findings are shown as the average value plus or minus the standard deviation of the average (SEM).

RESULTS AND DISCUSSION

Three herbal formulations coding C-I, C-II and C-III were formulated using different concentrations of HAETPL and excipients. The results of evaluation parameters are given in Table 2. The reading for drug content is illustrated in Figure 1. Results from disc diffusion techniques were used to test the antifungal activity of herbal cream and results were compared with standard antifungal cream containing clotrimazole. The findings are given in Table 3.

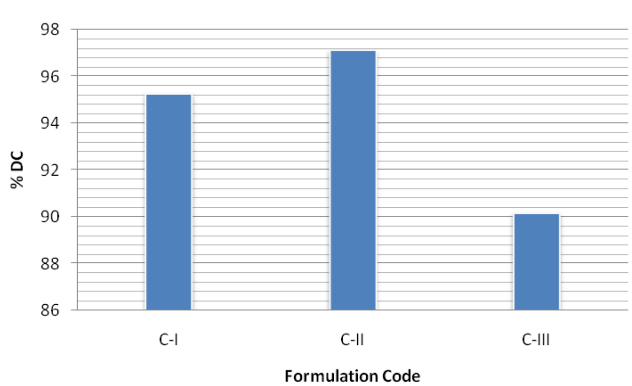

Figure 1: %Drug content of herbal cream

Table 2: Evaluation parameters of herbal cream

Parameters	*Formulation code*		
	C-I	*C-II*	*C-III*
Appearance	Light green and clear	Light green and clear	Light green and clear
pH	6.9	6.8	6.9
Viscosity (cp)	4652	4678	4812
Homogeneity	H	H	H
Spreadability (gcm/Sec)	50.29	52.32	51.28
Wetness	+++	+++	++
Type of smear	NG	NG	NG
Emolliency	NRL	NRL	NRL
Type of emulsion	o/w	o/w	o/w
Drug content (%)	95.26	97.11	90.16

Abbr: H=Homogeneous, +=Good, ++=Better, +++=Best, G=Greasy, NG= Non-greasy, NRL=No residue left, o/w=oil in water

Table 3: Anti-candidal activity of herbal cream

Test	*ZOI*
Negative Control	3.86 ± 0.18
STD. (Clotrimazole)	24.28 ± 0.11**
C-I	20.13 ± 0.25**
C-II	22.37 ± 0.18**
C-III	20.86 ± 0.19*

Note: The data are shown as the mean (X) with the standard error of the mean (SEM), based on a sample size of 3 (n=3). The statistical analysis included doing a one-way ANOVA followed by a Student's t-test. The obtained results were found to be statistically significant, with a significance level of *P<0.001 and **P<0.01, when compared to both the control and standard groups.

CONCLUSION

The results of the present investigation conclude that the herbal cream containing 5% hydroalcoholic extract of *T. portulacastrum* was found to have significant anti-candidal activity and maximum drug content.

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