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Formulation Development, Evaluation and Anti-*Candida* Activity of Conventional and Novel Herbal Cream for the treatment of Vaginal candidiasis

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Abstract

Vaginal candidiasis, the second most prevalent vaginal infection, is so common that women frequently turn to herbal therapy after trying allopathic medicines. It is responsible for approximately 25% of cases, while *Candida albicans* is responsible for 85-95% of those cases. According to observations and reports, around 75% of females acquire vaginal candidiasis once throughout their lives, 50% twice, and 5% annually. Several plants have been found as viable remedies after reviewing the literature on herbal medicine. In the present investigation the hydroalcoholic extract of leaves of *Trianthema portulacastrum Linn*. were taken to formulate herbal cream. The conventional cream was formulated using extract and novel herbal cream was formulated using ethosomal suspension. The formulations were evaluated and anti-candidal activity was screened.

Key-words: Vaginal candidiasis, Herbal Cream, *Trianthema portulacastrum*

Introduction

Women are significantly more susceptible to gynaecological disorders than men because of a variety of issues, such as compromised immunity, hormonal fluctuations, elevated sugar consumption in meals, prevalent or daily consumption of antimicrobial agents, inadequate cleanliness, emotional and physical strain, as well as certain infections caused by microbes such as bacteria and yeast which trigger catastrophic fungal or additional infections of the sensitive female sexual body part. Vaginitis is a term used to describe conditions that include vaginal swelling or infection. It is an infected or non-infectious inflammation of the vulva and, to a lesser extent, the vaginal mucosa. Testing vaginal secretions allows for the diagnosis of vaginitis. Yeast being a widespread form of fungi, although infections caused by yeast, which include Candida, tend to be more frequent among women. Depending on the underlying cause, vaginitis can be treated with anti-fungal medications, antibacterial medications that can be applied topically, taken orally, or both in the event of severe infections. [1]

Many herbal medicines are routinely used to treat various forms of gynaecological issues. The majority of gynaecological disorders affecting women in low-income countries are vaginitis, irregular periods, menorrhagia, leucorrhoea, and so on. Furthermore, some women may develop infertility or the inability to conceive. The underlying problem is not addressed by modern synthetic medications. Certain gynaecological issues, on the other hand, can be effectively treated with herbal medication. Several herbs are often used to treat vaginal infections, including *Chlorophytum tuberosum* Linn., *Aloe vera* L., *Syzygium cumini* (L.) Skeels., *Carica papaya* L., *Ipomoea paniculata*(L.) R.Br., *Ficus racemosa* L., *Asparagus racemosus* Willd., *Saraca indica* Auct., *Hibiscus rosa-sinensis* L., *Michelia champaca* L., *Terminalia arjuna* Roxb., *Emblica officinalis* Gaertn., *Vitex negundo* L. [2]

Trianthema portulacastrum Linn. also known as the Patharchataa belongs to family: Lamiaceae is traditionally used as analgesic, stomachic, laxative, treatment of blood disease, anemia, inflammation, and night blindness. Laboratory investigations on extracts of the plant have demonstrated significant pharmacological activities, such as antioxidant, diuretic, analgesic, hepatoprotective, and anticarcinogenic. Due to wide therapeutic efficacy of the plant selected in the treatment of fungal infection and an attempt was made for formulate and evaluate herbal cream from leaves extract of *Trianthema portulacastrum Linn*. [3-4]

Material and Methods

Selection Collection and Authentification of Plant Material

Trianthema portulacastrum Linn. (Leaves) TPL was chosen for use in treating vaginal infections based on folklore literature. *Trianthema portulacastrum Linn*. (Leaves) TPL was collected from Malwa region of Madhya Pradesh in the month of Oct-Dec 2023. The plant specimen was authenticated by Dr. S.N. Dwivedi, Retd. Professor and Head of the Department of Botany at Janata PG College, A.P.S. University, Rewa (M.P.); Voucher number J/Bot/TPL-013.

Extraction of Plant Material

250 gm of coarsely powdered, air-dried *Trianthema portulacastrum Linn* leaves was put in a soxhlet apparatus and extracted with ethanol and water until the extraction was finished. The filtrate was evaporated after extraction to produce the extract. [5]

Formulation of Herbal Cream [6-8]

Formulation of Conventional Cream

Bees wax is first melted at 60 to 70 degrees Celsius, followed by the addition of paraffin wax, soya powder, olive oil, and tocopherol. This creates a water-in-oil emulsion cream. Aqueous phase and extract were added, and they were heated to 50°C. Glycerine and rose water were then

added, and the mixture was cooled to 40°C. For homogeneous dispersion, the two phases were continually combined. After being slowly cooled, peppermint and sandalwood oils were added for scent.

Formulation of Novel Cream

Bees wax was first melted at 60–70°C, followed by the addition of paraffin wax, soya powder, olive oil, and tocopherol (Phase I), creating a water-in-oil emulsion cream. Taking ethosome suspension and warming it slightly at 35 °C (Phase II) Phase III involved the separate warming of glycerine and rose water. Phases I and II were first continuously combined for homogeneous dispersion and then gently chilled. Phase III was then slowly added and homogeneously mixed, and peppermint and sandalwood oils were added for scent.

The following is a description of the numerous steps that go into making a herbal cream: [10-12]

Preparation of Oil Phase

In accordance with tables 4.2 and 4.3, the appropriate amounts of beeswax, paraffin wax, and olive oil were taken and melted at 70°C in a porcelain dish.

Preparation of Aqueous Phase

PFL, glycerine, soy powder, water, and the extract of the dried plant material from Trianthema portulacastrum Linn. were added to another clean dish and heated to 70°C.

Addition of Aqueous Phase to Oil Phase

At room temperature, the aqueous phase was continuously stirred into the oil phase. The last ingredients were rose water, peppermint oil, and sandalwood oil. The mixture was then transferred into an appropriate container.

Table 1: Composition of Conventional and Novel Cream

Ingredients	CCI	CCII	CCIII	NCI	NCII	NCIII
Bees Wax (gm)	6	6	6	6	6	6
Paraffin wax (gm)	4	4	4	4	4	4
Olive Oil (ml)	10	10	10	10	10	10
Soya Powder (gm)	1.5	1.5	1.5	1.5	1.5	1.5
Tocopherol (ml)	0.5	0.5	0.5	0.5	0.5	0.5
HAETPL (%)	2.5	5	10	-	-	-
Ethosome Suspension with HAETPL (%)	-	-	-	2.5	5	10
Glycerine (ml)	2.5	2.5	2.5	2.5	2.5	2.5
Rose water(ml)	1	1	1	1	1	1
Peppermint oil	1	1	1	1	1	1
Sandalwood oil	1	1	1	1	1	1
Aqueous Phase (ml)	qs	qs	qs	qs	qs	qs

Evaluation Parameters of Herbal Cream [6-8]

Physical Evaluation

Clarity and transparency of the herbal cream were assessed visually as part of its physical test. The samples were examined against a white background in the light.

Determination of pH

The pH metre was calibrated initially, and a reading of zero was noted. The samples were placed in a beaker after being diluted with water to produce a suspension, and measurements were obtained using a calibrated electrode. Three average readings were taken after repeating the technique.

Determination of Viscosity

Utilising spindle no. 01 at 20 rpm and temperatures between 4 and 37 degrees Celsius, the viscosity of the herbal cream was measured using a Brookfield viscometer. A beaker was filled with 15 ml of samples from each batch, and the spindle was then submerged in the formulation. The reading was taken at different temperatures both before and after rotation. The reading was three times recorded.

Determination of Homogeneity

By visually inspecting each batch of manufactured herbal cream, homogeneity was verified and the existence of any aggregates in the formulation was assessed.

Determination of Spreadibility

The glass slide with the recipes on it was topped with another glass slide that was empty and contained no gel. The formula was set up so that it was sandwiched between two slides. The slides were found to be occupied at a distance of 7.5 cm. The herbal cream was pressed into a thin, even coating and placed between two slides. The herbal cream was freed from its weight. It was removed the extra herbal cream seen on the slides. The two slides were fastened together, and 20 0.5 g of weight were connected to the upper glass slide. The time it took to cover the 7.5 cm separation distance was recorded as both slides being separated due to weight. The mean time was calculated after the three readings were obtained. Spreadability was determined as follows:

$$S = m \times \frac{l}{t}$$

Where, l: length of the slide (7.5 cm), m: the weight which is tied to slides, and t: time taken in second.

Determination of Wetness

Applying the prepared herbal cream to the skin's surface helped evaluate how moist it was. The quantity of cream extracted from each batch was enough to cover a 10 mm2 area of skin surface.

Determination of type of Smear

The produced herbal cream was applied to the skin's surface, and the type of film or smear generated on the skin was noted. The amount of cream collected from each batch was enough to cover a 10 mm2 area of skin surface.

Determination of Emolliency

We evaluated the herbal cream's emolliency, slipperiness, and amount of residue that remained after application. The quantity of cream taken from each batch was enough to cover a surface area of the skin 10 mm2 in size.

Determination of type of Emulsion

Dilution Test

Depending on the type of emulsion, either o/w or w/o, the created herbal cream was diluted with oil or water, and the results were recorded.

Dye Solubility Test

The resulting herbal cream was combined with an amaranth-based water soluble dye and examined under a microscope. The collected results were interpreted.

Drug Content

The drug content was determined as per method earlier described. The herbal cream's composition was calculated using a UV-Visible spectrophotometer. The dosage was roughly 1g of the formulation in 50 ml of volumetric flask. The right amount of ethanol was added to the solution. Whatman filter paper Grade 1 was used to filter the mixture after it had been shaken. The filterate was further diluted to a volume of 10 ml with solvent before being estimated at the

appropriate wavelength. The standard curve created at the maximum wavelength, or 340 nm, was used to assess the amount of medication present in the extract.

Anti-*Candida* Activity of Optimized Formulation

By using disc diffusion methods, the antifungal activity (anti-candida activity) of standard antifungal cream formulations containing Clotrimazole and optimised herbal cream formulations was assessed, and the results obtained for Zone of inhibition was compared. To compare all the outcomes, marketed Clotrimazole cream (MCC) 1% w/w IP was utilized. [9-10]

Results and Discussion

The traditional and innovative herbal creams both comprise hydroalcoholic extracts of *Trianthema portulacastrum Linn* dried plant material. Prepared and analysed were leaves and excipients in various proportions. No defects were noted in any of the batches. According to the findings, innovative cream with ethosome suspension offers more promising outcomes than traditional cream (Tables 3).

It was determined that CCIII and NCIII have the highest drug content 86.30% and 95.24% . Further CCIII and NCIII were screened for anti-Candida activity. The NCIII has the best and most substantial anti-candida activity when compared to the marketed conventional cram formulation (MCC) and CCIII, and as a result, it may be used to treat vaginal infection, according to the results (table 3).

Table 3: Evaluation parameters of Convention cream containing EEPFL

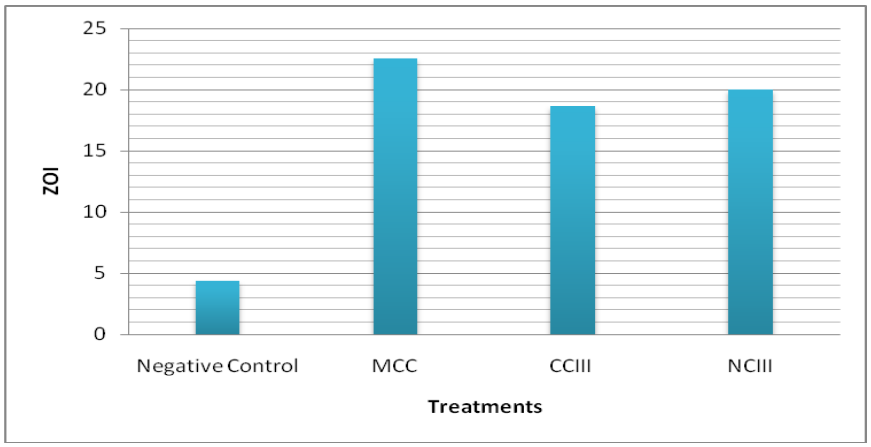
S/No.	Parameters	CCI	CCII	CCIII	NCI	NCII	NCIII
1.	Appearance	Clear	Clear	Clear	Clear	Clear	Clear
2.	pH	6.8	6.9	7.1	7.0	7.0	6.9
3.	Viscosity(cp)	3814	4216	4432	4612	4838	4844
4.	Homogeneity	H	H	H	H	H	H
5.	Spreadibility (gcm/sec)	50.38	50.12	52.72	54.38	52.80	54.14
6.	Wetness	++	++	+++	+++	+++	+++
7.	Type of Smear	NG	NG	NG	NG	NG	NG
8.	Emolliency	NRL	NRL	NRL	NRL	NRL	NRL
9.	Type of Emulsion	o/w	o/w	o/w	o/w	o/w	o/w
10.	Drug Content	70.21	74.86	86.30	84.39	90.46	95.24

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

Table 8: Anti-*Candida* Activity of CCIII and NCIII

S/No.	Test	Zone of Inhibition (mm)
1.	Negative Control	4.39±0.21
2.	MCC	22.58±0.19**
3.	CCIII	18.67±0.20**
4.	NCIII	20.04±0.29**

Note: Every value is presented as Mean (X) ± SEM, n = 3. Values are statistically significant *P<0.01, **P<0.001 when compared to control and standard, following a one-way ANOVA and student test.



Graph 1: Anti-*Candida* Activity of CCIII and NCIII

Conclusion

The anti-Candida activity of novel herbal cream formulation (NCIII) is best than conventional herbal cream (CCIII) CCE3 and NCE3. As a result, formulation NCIII was determined to be the best formulation for the treatment of candidal infections and vaginal infections.

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