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Development Evaluation and Estimation of Phytobioactive Antifungal Compounds for the Treatment of Atopic Dermatitis

Shweta Shukla[1*], Shreetama Sarkar[2], Jyoti Thakur[3], Tamalika Chakraborty[4], Ravi Shankar Kumar[5] and Faheemah Hossain[6]

[1]Bhabha university, Bhopal, (M.P.) - India
[2]SSKM College of Pharmacy , 24pgs, (W.B.) - India
[3]NRITP College, Bhopal (M.P.) - India
[4]Guru Nanak Institute of Pharmaceutical Science and Technology, Kolkata(W.B.) - India
[5]Dasmesh College of Pharmacy, Faridkot, (Punjab) - India
[6]School of Health and Medical Sciences, Adamas University, (W.B.) - India
Corresponding Email: *shuklasweta1624@gmail.com

Abstract: One of the most prevalent chronic inflammatory skin illnesses, atopic dermatitis (AD) is brought on by a variety of reasons, such as infections, host immunological responses, genetics, skin barrier abnormalities, allergen sensitivity, and environmental influences. Patients with AD frequently have bacterial and viral infections in their eczematous lesions, which obviously exacerbates the symptoms. Despite reports indicating that some dermatophytes, Candida, and Malassezia can impact AD symptoms, research on fungal infections in AD is very scarce. The current study created and assessed an antifungal cream that contained hydro-alcoholic extracts of *Tecoma stans* leaves. Chlorogenic acid, a phytobioactive antifungal component, was also evaluated in the produced formulation and extract.

Key-words: *Tecoma stans,* Anti-fungal cream, Phytobioactive

1. Introduction

An inflammation of the skin is generally referred to as dermatitis. Dermatitis is a frequent ailment with a wide range of causes and manifestations. Usually, there is a rash on puffy, reddish skin or itchy, dry skin. Or it may cause the skin to boil, leak, and crust or flake off. Dandruff, contact dermatitis, and atopic dermatitis (eczema) are a few examples of this disorder.
Eczema, also known as atopic dermatitis, is a chronic (long-lasting) skin condition that causes skin irritation, redness, and inflammation. It's a prevalent ailment that typically manifests in childhood, however it can strike anyone at any age.[1-2]
People are turning back to nature in the hopes of safety and security as herbal preparations have grown in popularity as consumer goods and the name "herbal" has come to represent safety in contrast to synthetic preparations, which have a negative impact on human health. The medicinal, flavouring, and aromatic qualities of the herbs have made them highly valued, and they are now used in many different ways within civilization.[3]
Tecoma stans family Bignoniaceae is one of the most used plant traditionally for the cure of the treatment of various diseases. Leaves, barks and roots have been used for a variety of purposes in the field of herbal medicine. Applications include the experimental treatment of diabetes, digestive problems, control of yeast infections and other medicinal applications.[4] The aim of the present work is to develop and evaluated the anti-fungal cream and estimates the phytobioactive compounds using spectroscopic techniques.

2. Material and Methods

Selection, Collection and authentication of Plant Material

The plant parts viz., TSB: *Tecoma stans* (L.) Juss. Ex Kunth (Leaves), was selected based on literature review, collected from local area of Bhopal region Madhya Pradesh and identified & authenticated Botanist and was deposited in our Laboratory. Voucher specimen was allotted.

Preparation of Extract
The powdered leaves were extracted using ethanol-water (90:10) in Soxhlet apparatus. After extraction the extract was dried and stored for further use.[5]

Development of Anti-fungal Cream
Stearic acid, cetyl alcohol, almond oil in desired quantity were taken in porclean dish and was melted at 70^0C. Hydro-alcoholic extracts of leaves *Tecoma stans* (HAETSL), glycerol, methyl paraben, triethanolamine and water were taken in another porclean dish and were heated at 70^0C. The aqueous phase was added to the oil phase with continuous stirring at room temperature. Perfume was added at last and the formulation was transferred in a suitable container. [6]

Table 1: Development of Anti-fungal Herbal Cream

Ingredients	**Formulation Code**		
	AFC-I	**AFC-II**	**AFC-III**
HAETSL	0.5	0.75	1.0
Stearic acid	5	5	5
Cetyl alcohol	10	10	10
Almond oil	5	5	5
Glycerol	3	3	3
Methyl paraben	0.02	0.02	0.02
Triethanolamine	qs	qs	qs
Water (100 ml)	qs	qs	qs
Total weight	100	100	100

Note: All values are taken in gm

Evaluation of Anti-fungal Cream
The developed formulations were evaluated for physical parameters, pH, viscosity, homogeneity, spreadibility, type of smear, emolliency, type of emulsion and drug content as per standard procedure prescribed.[7-8]

Estimation of active phytobioactive antifungal compounds
UV-Visible Spectrophotometer (Shimadzu, UV-1800) was used for estimation of active phyto-constituents content i.e, Chlorogenic acid against standard in final optimized formulation. Accurately weighed 10 gm of active phyto-constituents (Chlorogenic acid) was transferred in 100 ml volumetric flask and dissolved in and diluted to 100 ml with methanol. The final solution contained 100 µg of the active phyto-constituents (Chlorogenic acid) per ml of the solution. Standard solutions of active phyto-constituents (Chlorogenic acid) was pipetted into concentration range 5-30 µg/ml in a series of five 25 ml volumetric flask. The absorbance of the active phyto-constituents was measured at 335 nm wavelength against methanol as solvent.[9-10]

3. Results and Discussion

Hydro-alcoholic extracts of leaves *Tecoma stans* (HAETSL) with excipients were mixed according to the formula mentioned in table 1 and various evaluation parameters were carried out to validate the efficacy of the prepared formulation. The formulated anti-fungal herbal cream containing HAETSL was evaluated as per standard protocols. The results are mentioned in table 2. The drug content was found maximum in FC3 i.e., 98.12 %

Table 2: Evaluation parameters of anti-fungal herbal

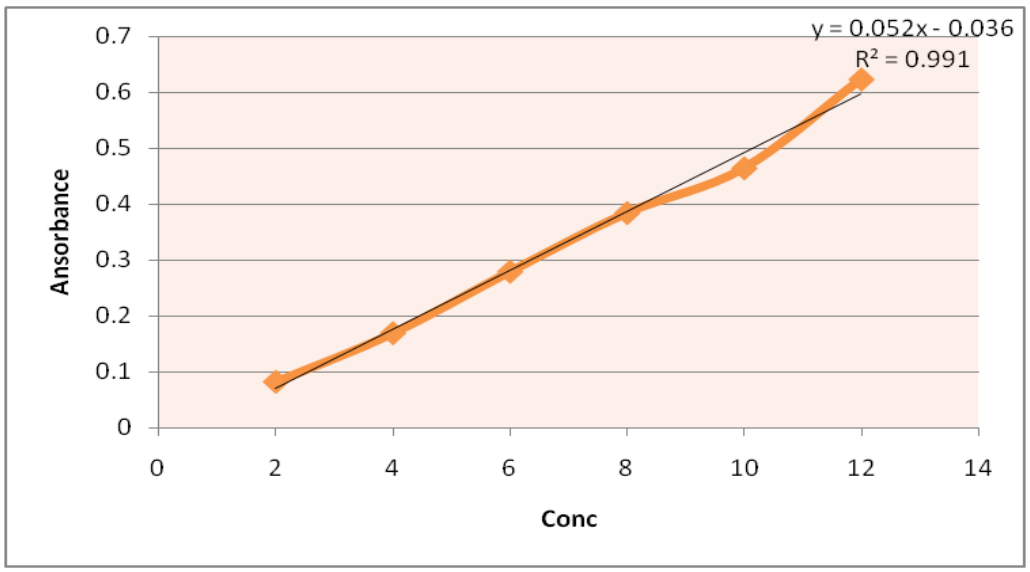
FC	Appearance	pH	Viscosity	Homogeneity	Spreadibility	Smear	Emollience y	Emulsion	Drug Content
AFC-I	Light green	6.8	27452	H	57.43	NG	NRL	o/w	97.32
AFC-II	Light green	6.8	28310	H	58.89	NG	NRL	o/w	97.46
AFC-III	Light green	6.9	28774	H	60.44	NG	NRL	o/w	98.12

Note: H=Homogeneous, NH=Non homogeneous,, G=Greasy, NG= Non-greasy, NRL=No residue left, LR=Residue left

Standard solutions of Chlorogenic acid were pipette into concentration range 5-30 µg/ml in a series of five 25 ml volumetric flask. The absorbance of the Chlorogenic acid acid was measured at 335 nm against methanol. The absorption maxima and Beer's law limit were recorded and data that prove the linearity and obey Beer's law limit were noted (Table 3). The linear correlation between these concentrations (X-axis) and absorbance (Y-axis) were graphically presented and the slope (b), intercept (a), and correlation coefficient (r^2) were calculated out for linear equation (Y= bx+a) by regression analysis using the method of the least square (Graph 1).

Table 3: Calibration curve data for Chlorogenic acid

Concentration (µg/ml)	**Absorbance**
2	0.082
4	0.169
6	0.279
8	0.384
10	0.464
12	0.623



Graph 1: Calibration curve of Chlorogenic acid

The limit of detection (LOD) is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value. The limit of quantitation (LOQ) is the lowest amount of analyte which can be quantitatively determined with suitable precision. The LOD and LOQ of the developed method were determined by injecting progressively low concentration of the standard solution and the lowest concentrations assayed (Table 4).

Table 4: Validation parameter of Chlorogenic acid

Parameter	Observations
Absorption Maxima	335 nm
Beer's Law limit	2-12µg/ml
Regression equation (y= bx+a)	y = 0.052x - 0.036
Intercept (a)	-0.036
Slope (b)	0.052
Correlation coefficients (r^2)	R^2 = 0.991
Precision (n=6, % RSD)	0.389
Accuracy (%)	99.24
LOQ	0.005 µg/ml
LOD	0.015 µg/ml

The appropriate aliquots from Chlorogenic acid extract of formulated anti-fungal cream FC and raw material HAETSL separately were withdrawn in 10 ml volumetric flask. Absorbance for aliquots of each was noted at 335 nm. The corresponding concentration of Chlorogenic acid against respective absorbance value was determined using the Chlorogenic acid calibration curve. The statistical analysis for checking uniformity in batches is also performed (Table 5).

Table 5: Estimation of Chlorogenic acid in extract and optimized anti-fungal crea

S. No.	Sample	Chlorogenic acid content (% w/w)
1.	HAETSL	4.12±0.036
2.	AFC-III	0.412±0.012

Note: Reading are expressed as Mean ± SD; n=6

4. Conclusion

The antifungal herbal cream containing hydroalcoholic extract of T. stans was evaluated and it was noted that the drug content was found maximum in AFC-III. Also, the active phyto-constituents Chlorogenic acid was estimated in optimized cream and it was found to be 0.412±0.012.

5. References

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