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Formulation, Optimization, and Evaluation of Herbal Antifungal Cream using *Achyranthes aspera* and *Cassia tora* Extract

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Abstract: Fungal infections have become a major concern and are caused by various fungi. Treatment of these infections is a real challenge to health professionals. The field of herbal medicine has experienced tremendous expansion during the past decades. The current study made an attempt to formulate, optimize, and evaluate the herbal antifungal cream containing *Achyranthes aspera* leaves and *Cassia tora* seed extracts for the treatment of fungal disease. An ethanolic extract was prepared using the Soxhlet apparatus. The cream was formulated by using a three-level two factorial design. The prepared cream was evaluated using a variety of criteria, including pH, homogeneity, spreadability, viscosity, phase separation, washability, drug content, *in vitro* diffusion, antifungal as well as stability study. The antifungal activity of the cream was carried out by the Agar diffusion cup plate method against *Candida albicans*. The ATR-FTIR results proved that the API and the excipients were physically and chemically compatible. The TLC results show the accurate results of R_f values for both plants. The best cream formulation among all possible combinations was batch F5 (Stearic acid 2 per cent and Cetyl alcohol 1.25 per cent), according to the experimental design analysis. For the optimized formulation, an *in-vitro* drug release analysis confirmed approximately 98-99 % release after 12 h. The optimized batch F5 showed potent antifungal activity with a zone of inhibition of 22 nm for *Candida albicans*. The developed cream was found to be safe and effective for the treatment of fungal infections.

Keywords: *Achyranthes aspera*, *Cassia tora*, Herbal cream, Antifungal activity, Fungal infections

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Introduction

Around the world, fungus infections have been a big problem. That area of the primary target is

the skin for fungal infections. The majority of fungi are responsible for skin conditions such as

dermatitis, candidiasis, and eczema. A fungal infection can cause symptoms such as itching, redness, swelling, irritation, etc. Topical or systemic antifungal treatment is frequently used to treat fungal infections (Jasim and Alkhuzai, 2023). Topical fungal therapy is typically favoured because it offers tailored treatment and has fewer adverse effects. Many formulations have been created to treat fungal infections, including ointments, creams, gels, emulsions, etc. Additionally, topical administration prevents pre-systemic metabolism and results in lessened systemic toxicity. Site-specific drug administration decreased systemic toxicity, increased patient compliance, increased treatment efficacy, and improved bioavailability are further benefits of topical delivery (Hoenigl *et al.*, 2022; Rukari *et al.*, 2023).

The use of medicinal plants in treatment is an ancient practice. Infectious and non-infectious skin conditions have all been treated using medicinal herbs over the years due to their extensive biological activities, efficacy and superior therapeutic results, ease of availability, and higher safety margin than synthetic drugs. Due to their more affordable pricing compared to synthetic or allopathic drugs, which have a number of therapeutic complications, herbal medicines have experienced a significant increase in demand in recent years in both developed and developing countries (Pathakumari *et al.*, 2020). As a result, by conducting this experiment using only natural medicines, we can develop a newer, safer, and more effective pharmacological therapy for one of the most fungal infections. The present study intends to develop, improve, and assess an herbal cream made from extracts of *Achyranthes aspera* and *Cassia tora* for the treatment of fungal disease (Bao *et al.*, 2023).

Herbal medication or other alternative formulations can also be made as creams. These creams refer to a viscous semisolid preparation that is administered topically to bodily surfaces like skin, vaginal, nasal, and ocular mucous membranes. These creams have unique

therapeutic benefits. A medicinal ingredient is combined, suspended, or emulsified in the emulsifying base of the medicated creams. Externally used herbal creams include those with antipruritic, keratolytic, protestant, antiseptic, emollient, and astringent properties (Han *et al.*, 2020).

Achyranthes aspera L. Belongs to Amaranthaceae family. It is also referred to as latjira, chirchira, and apamarga. It is a weed that grows as a native of South Andaman Island, Australia, and India's roadsides, field boundaries, and waste areas. *Achyranthes aspera* L. has several medicinal applications in folkloric research. Stomach tonic, diuretic, laxative, anthelmintic, anti-hyperlipidemic, expectorant, anti-inflammatory, anti-bacterial, anti-fungal, hypoglycaemic, anti-asthmatic, and anti-allergic are all conditions in which *Achyranthes aspera* L. is used to treat. Additionally, it has hepatoprotective effects. When formulated as a cream for topical application, *Achyranthes aspera* L. has antibacterial action and great potential as an antifungal agent (Pakhale *et al.*, 2023).

Cassia tora L. (family Leguminosae) is a major weed that is primarily found in South-East Asia and the South-West Pacific. It is an annual herbaceous foetid plant. It is one of the acknowledged plants that contains anthraquinone (organic chemicals). The seeds and leaves effectively treat skin conditions, particularly ringworm and itching. The seeds are recognized in the Japanese Pharmacopoeia and are effective against ringworm and other skin conditions as a tonic and stomachic. Additionally, seeds are useful for earaches, liver issues, and eye conditions. The ringworm fungus *Microsporon nanum*, *Candida albicans*, was resistant to the seed extract's antifungal effects. Both plants have historically been used to treat fungus-related skin illnesses for many generations, and they may be sources of antifungal agent development (Shadab *et al.*, 2019).

Materials and Methods

Collection of plant materials:

Fresh leaves of *Achyranthes aspera* and seeds of *Cassia tora* were purchased from a local herbal vendor. Authentication of *Achyranthes aspera* leaves and *Cassia tora* seeds powder authenticated and identified by Prof. Vijayalaxmi S. Shelke, Department of Botany, R. C. Patel College of Arts, Commerce, and Science, Shirpur, Dist. Dhule, India. Then, for additional study, freshly dried leaves and seeds were processed into a coarse powder and kept in a dark, cool, and dry place. Excipients were purchased from Modern Industries in Nashik for the study.

Pharmacognostic study:

We examined the morphological and microscopical characteristics of fresh *Achyranthes aspera* leaves and *Cassia tora* seeds (Kumar *et al.*, 2023).

Physicochemical evaluation of leaves and seeds:

The physicochemical assessment was performed utilizing a variety of measures, including ash value, foreign matter, extractive value, swelling index, foaming index, LOD, etc (Maurya *et al.*, 2021).

Preparation of extracts:

A sample of the finely ground powder used in this extraction was placed in a porous muslin-fabric thimble. The finely ground material was extracted in a total amount of 200 g, first with petroleum ether at 40°C and then with ethanol at 60 °C to 70 °C. Using Whatman filter paper, the filtrate was created after the marc had been extracted. The filtrate was dried to remove the solvent at 40 °C to 70 °C temperatures. The refrigerator's temperature was set at 4 °C for the dried sample (Pingale and Ravindra, 2019).

Physicochemical evaluation of extracts:

Both plants' ethanol extracts were tested for alkaloids, glycosides, flavonoids, tannins, and other physicochemical components, as well as colour, odour, nature, yield etc. (Singh and Samanta, 2022).

*Determination of λ max and Calibration curve of *Achyranthes aspera* and *Cassia tora*:*

The maximum wavelength of *Achyranthes aspera*

was found to be 272 nm and that of *Cassia tora* to be 279 nm using ultraviolet (UV)-visible spectroscopy. The standard stock solution of *Achyranthes aspera* and *Cassia tora* was produced by precisely weighing 10 mg of the drug and transferring it to a volumetric flask with a 10 ml capacity. The drug was dissolved in saline phosphate buffer 7.4 pH and the final volume was made with saline phosphate buffer to achieve a concentration of 1000 g/ml. Aliquots of various sizes were obtained from working dilution into 100 ml volumetric flasks and were made up of Saline phosphate buffer (pH 7.4) to achieve standard drug concentrations between 2 and 10 g/ml. These *Achyranthes aspera* and *Cassia tora* dilutions were estimated to have absorbances of 272 nm and 279 nm, respectively (Pradhan *et al.*, 2023).

Drug and Excipients compatibility study:

To verify the results of the medication and excipient compatibility tests, the Bruker Alpha- II ATR- FTIR was used. To determine whether the API and excipients employed in the formulation were compatible, the *Achyranthes aspera*, *Cassia tora*, and excipients were each studied and evaluated separately at room temperature. The physical mixture of *Achyranthes aspera*, *Cassia tora*, and excipients was also evaluated. The compatibility of the medicine and excipients was also examined in the IR of the optimized batch (Wani *et al.*, 2023).

Thin layer Chromatography:

Aluminium TLC sheets with a silica gel coating, 60 F₂₅₄ 8.5 cm x 6.5 cm glass capillaries were used to spot the samples (*Achyranthes aspera* and *Cassia tora*) and reference solution (oleanolic acid and emodin) onto TLC plates. Multiple solvent systems were used for elution, and ascending development was performed in a 20 x 10 cm twin trough chamber that had already been solvent-saturated for 30 min at RT. The plate was then dried, placed in a UV cell for visualization, and sprayed with 10% methanolic H₂SO₄ and 10% ethanolic KOH

when the elution was complete (Patel and Pingale, 2014).

Formulation of trial batches:

Different medication concentrations were used in the experimental batches, which were taken into consideration. The creams are created in trial batches utilizing various medication concentrations. Following that, the doses of the two medications were chosen based on evaluation criteria like drug diffusion and antifungal research. *Achyranthes aspera* and *Cassia tora* dosages in the T1 batch were 1 and 4 g, respectively. The dosage for *Achyranthes aspera* and *Cassia tora* in the T2 batch was 2 and 5 g, respectively. The dosage for *Achyranthes aspera* and *Cassia tora* in the most recent batch of T3 was 3 and 6 g, respectively. From the aforementioned trial batches, the third trial batch was deemed to be the ideal batch and was chosen for examination of additional manufacture. The third trial batch, which was chosen for consideration of additional manufacture, was considered to be the ideal batch from the aforementioned testing batches (Patil *et al.*, 2022).

Optimization data analysis:

Using Design Expert 13 version, each variable's impact on the chosen response was examined. Surface plots were made to show the statistical significance of each response coefficient. The 3^2 full factorial design was utilized. In the current study, the effects of these variables on viscosity, the %CDR of *Achyranthes aspera*, and the %CDR of *Cassia tora* were investigated using design expert 13 software. Nine formulations were created using a 3^2 factorial design. The independent variables stearic acid (X1) and cetyl alcohol (X2) were found to be two different factors. Three responses (Dependent variables) were identified: Y1 for viscosity, Y2 for the percentage of CDR for *Achyranthes aspera*, and Y3 for the percentage of CDR for *Cassia tora* (Akram and Garud, 2021).

Preparation of herbal antifungal cream by Experimental design:

Antifungal herbal creams were made using the

beaker method based on information obtained from the preliminary investigation. Response surface methodology was applied by Design Expert 13 software to optimize and statistically validate the developed herbal antifungal cream. It was determined how independent parameters affected their response using a three-level, two-factor factorial design (quadratic model). Stearic acid (1, 2, 3% w/w) and cetyl alcohol (1, 1.25, 1.5% w/w) were employed in varying amounts to make the herbal antifungal cream. A semisolid cream based on an oil-in-water (O/W) emulsion was developed. The oil phase (Part A) was warmed up to 75° C while the oil-soluble ingredients (stearic acid, cetyl alcohol, tulsi oil, karanj oil, isopropyl myristate, and phenoxyethanol) were dissolved in there. Preservatives and other water-soluble ingredients (Triethanolamine, *Azadirachta indica* ethanol extract, Methylparaben, Propylparaben, and Propylene Glycol) were mixed in the water-base phase (Part B) and warmed to 75 °C. After being heated, the aqueous phase was continually incorporated into the oily phase while the emulsifier was constantly stirred till it chilled. Table 1 lists all formulations' ingredients based on a 3^2 -factorial design (Refat *et al.*, 2022; Reka *et al.*, 2023).

Evaluation of herbal cream:

Physical evaluation: The following physical parameters were used to further assess the formulation of herbal creams i.e. colour, smell, consistency, and formulation state (Türedi and Acarali, 2022).

pH: Using a digital pH meter, the pH of herbal creams that had been made was measured. 100 ml of distilled water were used to produce the cream solution, which was then left to aside for 2 h. The pH of the solution was determined three times, then the mean result was estimated.

Spreadability: By enclosing the sample between two slides and compressing it to a consistent thickness with a specific weight for a specific amount of time, the spreadability of cream

Table 1: Composition of formulations as per 3² full factorial design

Formulation %	F1	F2	F3	F4	F5	F6	F7	F8	F9
<i>Achyranthes aspera</i> leaves extract	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
<i>Cassia tora</i> seeds extract	3	3	3	3	3	3	3	3	3
Tulsi oil	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Karaj oil	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Aloe vera gel	5	5	5	5	5	5	5	5	5
Stearic acid	1	2	3	1	2	3	1	2	3
Cetyl alcohol	1	1	1	1.25	1.25	1.25	1.5	1.5	1.5
Glycerine	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Isopropyl myristate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Aluminium hydroxide	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phenoxy ethanol	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Potassium hydroxide	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Methyl paraben	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Propyl paraben	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lemon oil	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Water (q.s.)	100	100	100	100	100	100	100	100	100

formulations was assessed. Spreadability was calculated as the required time to separate the two slides. Better Spreadability was demonstrated with a shorter gap period between the two slides. Spreadability was calculated using the formula below:

$$S = M \times L / T$$

Where S= Spreadability, M= the higher slide's weight tide, L= Length of a glass slide, and T= the time required to separate the slides.

Washability: All creams were applied to the skin after which the easiness of washing with water was assessed.

Non-irritancy test: The non-irritancy test formulation of herbal creams was assessed. The areas were observed for 24 h.

Viscosity: Cream viscosity was measured using a Brooke field viscometer at a spindle speed of 5 rpm at an ambient temperature of 25 °C.

Phase separation: The creams were placed in a suitable wide-mouth container after being

produced. After 24 hours of storage, the separation of the oily phase and water-base phase could be seen.

Extrudability study: Extrudability was evaluated based on how much cream was extruded when finger pressure was applied. Extrudability improved with more quantity extruded.

Greasiness: The greasiness of the formulations was observed by applying the cream to the skin.

Homogeneity: After the creams had been placed in the container, a visual inspection was used to check for homogeneity in all of the formulations. They were examined for the presence of any aggregation and how it appeared.

After feel: Emolliency, slipperiness, and the amount of residue left after applying the prescribed amount of cream were all examined.

Type of smear: Examined was the kind of film or smear that appeared on the skin following cream application.

Drug content:

A 100 ml volumetric flask containing 1 g of each formulation's cream was filled to capacity with pH 7.4 phosphate buffer and continuously agitated to mix the active ingredients in a solvent. The solution was sonicated for a short while, and then Whatman filter paper was used to filter it. The filtrate was then pipetted out in 0.1 ml portions and diluted with pH 7.4 buffer to a final volume of 10 ml. The content of active constituents was estimated spectrophotometrically by using 272 nm and 279 nm λ_{max} of herbal cream. The linear regression analysis of the calibration curve was employed for estimating the drug content of the formulation (Ashok Babu *et al.*, 2022).

In vitro Drug Release Study:

The release of medication from the cream was examined using an egg membrane attached between the donor and receptor compartment in a Franz diffusion cell while keeping the temperatures of both compartments at 37 °C. 1 g of cream was put into the donor compartment, and 25 ml of newly made phosphate buffer (pH 7.4) was added to the receptor compartment. A magnetic stirrer was used to mix the receiver compartment's contents. For the duration of the investigation, the egg membrane was in contact with a phosphate buffer (pH 7.4). At 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h, aliquots of 1 ml from the receiver compartment were taken and replaced with fresh buffer until the experiment was finished. A UV-visible spectrophotometer was utilised to assess the absorbance at 272 nm and 279 nm after the samples had been suitably diluted. Higuchi, first order, zero order, and Korsmeyer-Peppas release models, which indicated the goodness of fit in terms of R^2 values, were used to investigate the release mechanism and kinetic profile of the optimized cream batch (Saracino *et al.*, 2022).

Determination of in vitro antifungal activity:

The Agar cup plate diffusion method was employed to evaluate the antifungal effectiveness of the superior batch. The antifungal study utilized the *Candida albicans*. The necessary amount of

cream was put into the Petri plate cavities, and the plates were refrigerated for an hour to facilitate pre-incubation diffusion. After refrigeration, the plates were normalized at RT and then incubated at 37 ± 10 °C for 3 days. The common medication Nystatin was used in the same trial (Chandrasekar, 2022).

Stability studies as per ICH guidelines:

The stability studies were performed individually for the respective topical cream by keeping it at 40 ± 2 °C with $75 \pm 5\%$ RH for a period of three months. The various parameters such as physiological parameters, content uniformity, pH, viscosity, and *in vitro* drug release of preparation were recorded (Pingale *et al.*, 2020).

Results and Discussion

Raw material standardization:

The *Achyranthes aspera* leaves powder was green in colour with a bitter taste. The *Cassia tora* seeds powder has been found to be a pale brown colour as well as possess a bitter taste. The foreign matter determination by visualization method showed the absence of any admixture or any other obnoxious materials. The particle size of the sample was characterized to 120 # by sieve analysis.

Physical constant values for parameters like ash, water-soluble ash, foreign matter, loss on drying, and extractive value were calculated. We investigated the extractive value and colour of the extract. For *Achyranthes aspera* the total ash value was found to be - 10.5 %, water soluble ash value - 7.2%, acid insoluble ash value - 0.6%, foreign matter - Nil, loss on drying - 12.9%, swelling index - 2.2 cm and the alcohol soluble extractive value was found to be 9.23%. For *Cassia tora*, the total ash value was found to be - 4.25 %, water soluble ash value - 3.7 %, acid insoluble ash value - 1.05 %, foreign matter - Nil, loss on drying - 8.15 %, swelling index - 1.8 cm and the alcohol soluble extractive value was found to be 12.08 %.

Alkaloids, glycosides, tannins, steroids, flavonoids, and saponins were present during a

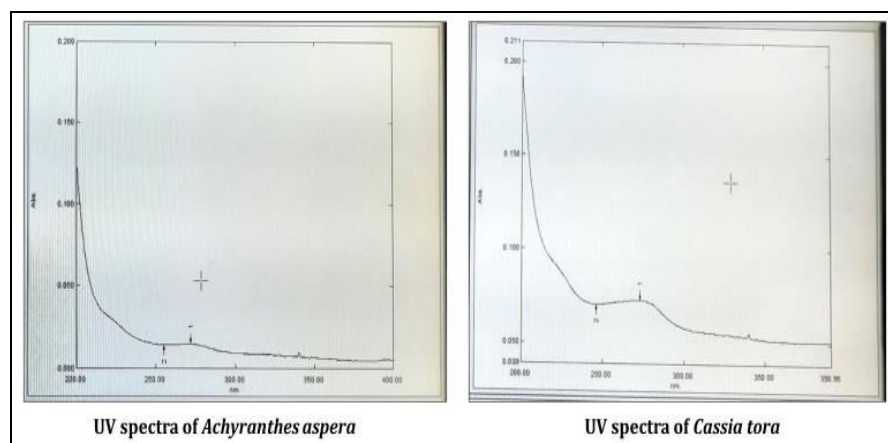


Fig. 1: UV spectra of *Achyranthes aspera* and *Cassia tora*.

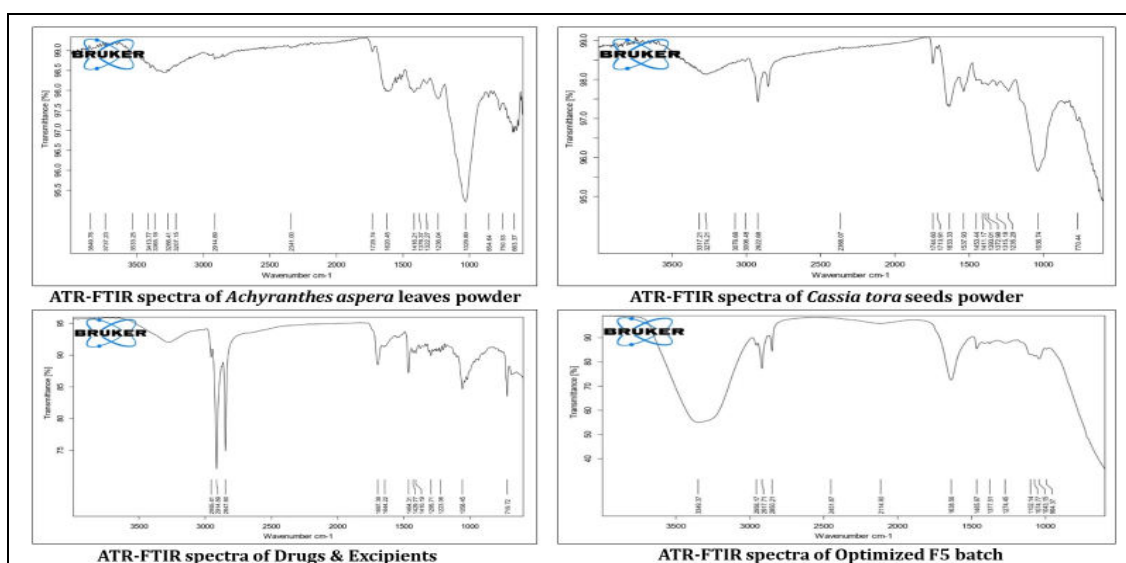


Fig. 2: ATR-FTIR spectra of *Achyranthes aspera*, *Cassia tora*, drug and excipients, and optimized F5 batch.

preliminary qualitative phytochemical screening. Results revealed that Alkaloids, glycosides, cardiac glycosides, flavonoids, and saponins show positive results in *Achyranthes aspera* while terpenoids, steroids, and tannins are absent. In the *Cassia tora*, the alkaloids, glycosides, cardiac glycosides, terpenoids, flavonoids, and saponins are present and tannins and steroids are absent. The values of the quantitative standards test are found to be complying with the standard given in Ayurvedic pharmacopeia.

λ max Estimation:

A double-beam UV visible spectrophotometer (Shimadzu) was utilized to determine the UV

spectrum for wavelengths between 200 and 400 nm. Figure 1 illustrates the computed absorbance of the two medication dilutions in phosphate buffer pH 7.4 for *Achyranthes aspera* and *Cassia tora*, respectively.

ATR-FTIR Studies:

A physical combination of *Achyranthes aspera* and *Cassia tora* and excipients (Fig. 2) and optimized formulation of cream suggests that the drug is compatible with stearic acid, and the presence of cetyl alcohol confirms the excipients compatibility. *Achyranthes aspera*'s FTIR peak positions are 1029, 1620, and 663 cm^{-1} , while *Cassia tora*'s peak positions are 1036, 1537, and 2925 cm^{-1} .

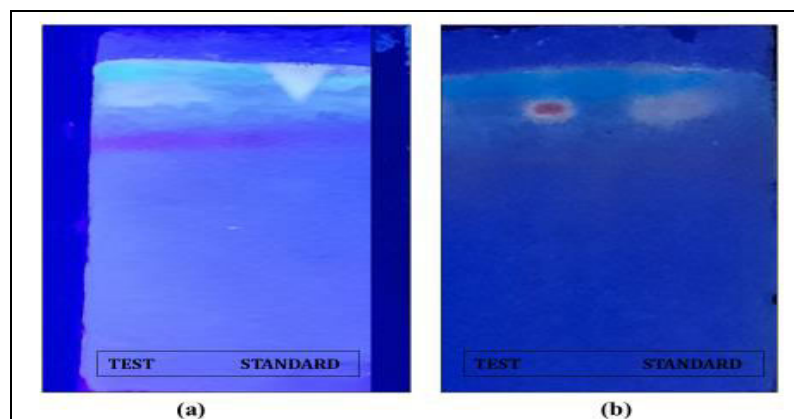


Fig. 3: (a) TLC for *Achyranthes aspera* as a test sample against oleanolic acid as a standard solution; (b) TLC for *Cassia tora* as a test sample against emodin as a standard sample.

Table 2: Physical evaluation of batches F1-F9

Batch	Colour	Odour	pH	Viscosity (cps)	Consistency	Spreadability g.cm/s	Phase separation	Type of smear
F1	Yellowish brown	Pleasant	6.7	12805	Creamy	18.29	No	Non-greasy
F2	Yellowish brown	Pleasant	6.8	13236	Creamy	15.00	No	Non-greasy
F3	Yellowish brown	Pleasant	6.7	13996	Slightly hard	10.13	No	Greasy
F4	Yellowish brown	Pleasant	6.5	15423	Poor	10.41	No	Non-greasy
F5	Yellowish brown	Pleasant	6.9	16593	Creamy	12.50	No	Non-greasy
F6	Yellowish brown	Pleasant	6.7	17989	Creamy	8.33	No	Non-greasy
F7	Yellowish brown	Pleasant	6.3	16879	Creamy	9.375	No	Greasy
F8	Yellowish brown	Pleasant	6.7	17390	Slightly Hard	8.52	No	Greasy
F9	Yellowish brown	Pleasant	6.4	18637	Slightly Hard	8.06	No	Greasy

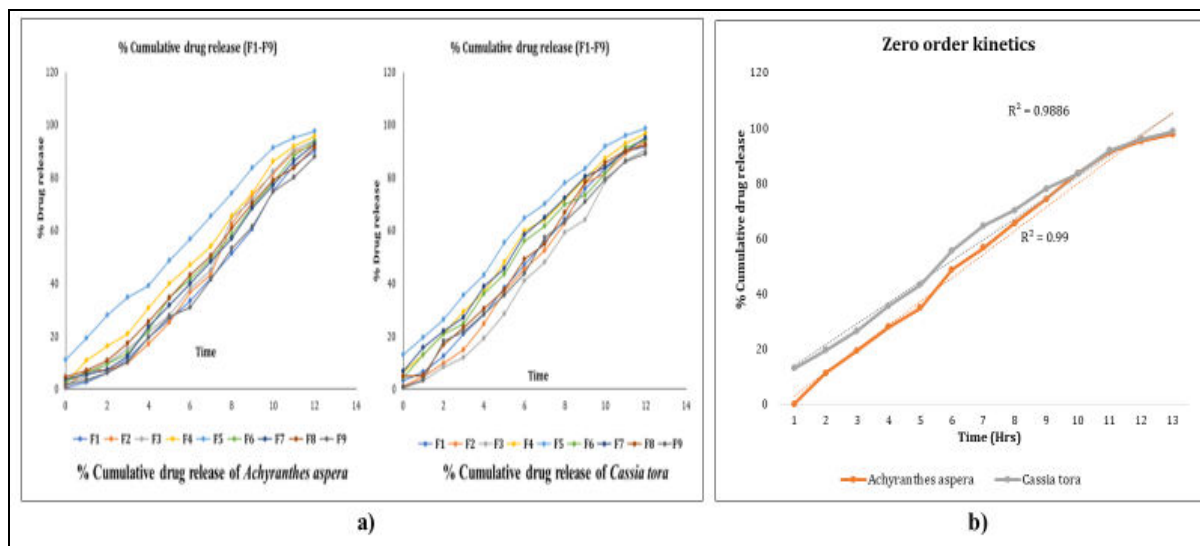
The physical evaluation of the extracts of the *Achyranthes aspera* leaves and *Cassia tora* seeds are carried out. The colour for *Achyranthes aspera* is found to be dark green and for *Cassia tora* it is dark yellowish-red brown. The odour is unpleasant for *Achyranthes aspera* and agreeable for *Cassia tora*. Nature is semisolid for both extracts. % yield is 8.09% (w/w) for *Achyranthes aspera* and 10.45% (w/w) for *Cassia tora*. Both extracts are soluble in water and ethanol. pH for

Achyranthes aspera extract is 6.2 and 5.7 for *Cassia tora* ethanolic extract.

The TLC method was performed with standard saponin (oleanolic acid) and anthraquinones (emodin) components such as plant extract samples, in which total saponins and anthraquinones content were presented. The chromatographic result of standard oleanolic acid, emodin, and plant sample extract is shown in Figure 3, respectively. TLC plates were observed

Table 3: Comparative study of kinetic models for F5 batch

Name of Model	<i>Achyranthes aspera</i>		<i>Cassia tora</i>	
	Linearity Equation	R ² Value	Linearity Equation	R ² Value
Zero order	$y = 8.5537x - 5.5192$	0.99	$y = 7.6181x + 6.4308$	0.9886
First order	$0.1262x + 2.3457$	0.8872	$y = -0.1337x + 2.3269$	0.8502
Higuchi	$y = 8.5537x - 5.5192$	0.99	$y = 7.6181x + 6.4308$	0.9886
Korsmeyer Peppas	$y = 0.1181x + 0.7354$	0.6953	$y = 0.0694x + 1.2202$	0.9019

Fig. 4: (a) % Cumulative drug release of F1 to F9 batches of *Achyranthes aspera* and *Cassia tora*; (b) Zero order kinetic study data of F5 batch.

under Normal light, Short UV, and Long UV. A similar R_f value (0.83) of oleanolic acid, and R_f value (0.78) of emodin, as well as a plant extract sample was recognized. Chromatogram analysis of saponins and extracts sample encompasses Ethyl acetate: Toluene: Formic acid (4.5: 3.5: 2 v/v) as mobile phase. Chromatogram analysis of anthraquinones and extracts sample encompasses Toluene: Ethyl acetate (9:1, v/v) as mobile phase.

Evaluation of herbal antifungal cream:

All cream formulations were found to be translucent and homogenous and yellowish-brown in colour. The odour for all batches was found to be pleasant. The state of the cream was semisolid. They were easily spreadable, viscous with a smooth texture, and non-greasy on application. The outcome is displayed in Table 2.

The pH values of formulated cream range from 6.3 to 6.9 which lies in the normal range. The measurement of viscosity shows that formulated cream was of low viscosity which satisfies the ease of application on skin. The viscosity of the cream was adjusted by the addition of a small quantity of Stearic acid and cetyl alcohol. All cream formulations were found to have viscosities that ranged from 12805 cps to 18637 cps. In the glass plate method, the spreadability ranges from 8.06 to 18.29 g.cm/s. All prepared cream formulations exhibited excellent consistency and without any lumps. The prepared batches of cream were easily washed except for batches F6, F8, and F8. None of the nine formulations caused irritancy, erythema, or edoema. The prepared cream of all batches is homogeneous and the cream was smooth. The phase separation was examined and evaluated for

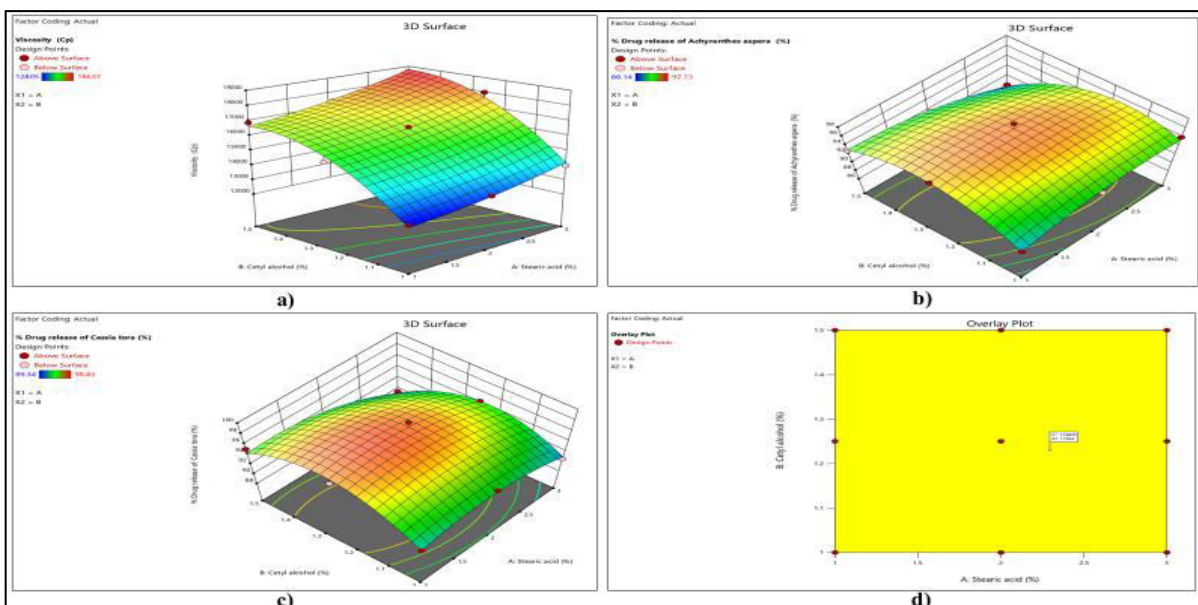


Fig. 5: (a) 3D surface of Response 1 (viscosity); (b) 3D surface of Response 2 (% CDR of *Achyranthes aspera*); (c) 3D surface of Response 3 (% CDR of *Cassia tora*); (d) Overlay plot.



Fig. 6: Zone of inhibition of optimization batch F5.

any changes. According to the findings, none of the nine formulations exhibit phase separation. The nine formulations tested did not leave any residue on the skin. All batches show the non-greasy type smear except F3, F7, F8, and F9 batches as shown in Table 2.

Drug Content:

A per cent of *Achyranthes aspera* and *Cassia tora* extract content in all cream formulations F1-F9 was found to be in the range of 95.91 % to 99.73% for *Achyranthes aspera* and 92.29% to 98.79% for *Cassia tora*. Batch F5 has good drug content with a

range of 99.73 for *Achyranthes aspera* and 98.79% for *Cassia tora*, respectively.

Drug release study:

Batch F5 has a good cumulative per cent drug release of 97.73% for *Achyranthes aspera* and 98.83% for *Cassia tora* as depicted in Figure 4a.

Kinetic study:

The optimum formulation was discovered to follow the zero-order kinetics model (Fickian diffusion) as shown in Table 3. Table 3 and Figure 4b explain that the formulation follows a zero-order kinetic model.

Table 4: Stability test data for optimized batch F5

S. No.	Parameters		0 Day	30 Day	60 Day	90 Day
1	Colour		Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown
2	Phase separation		No	No	No	No
3	pH		6.9	6.8	6.7	6.9
4	Viscosity (cps)		16593	18324	18983	17014
5	Spreadability g.cm/s		12.50	11.73	11.06	12.36
6	Drug Content (%)	<i>Achyranthes aspera</i>	99.73	99.02	98.75	99.58
		<i>Cassia tora</i>	99.58	99.14	98.82	98.43
7	Drug release (%)	<i>Achyranthes aspera</i>	97.73	96.64	97.20	97.81
		<i>Cassia tora</i>	98.83	99.16	98.77	98.62

Data analysis:

The 3² complete factorial design was employed, and the 2 independent factors were analysed at 3 levels, each are Stearic acid (X1) and Cetyl alcohol (X2), with the viscosity, per cent drug release of *Achyranthes aspera*, and per cent drug release of *Cassia tora* as the dependent variables. The data was processed using the Design Expert version 13 software, which was then statistically analysed using analysis of variance (ANOVA). To investigate the interaction of Stearic acid (X1) and Cetyl alcohol (X2) on the dependent variable. Additionally, the data was provided to 3-D response surface methodology and contour plot. The values of X1 and X2 were determined to be significant, suggesting that both factors had a significant effect on the responses studied. Based on optimization analysis data, batch F5 was selected as the best formulation, with Stearic acid 2% (w/w) and Cetyl alcohol 1.25% (w/w). Figure 5 shows a 3-D response surface plots for each of the response variables studied.

ANOVA for Quadratic model:

Overlay plot b) indicates the optimized quantity of X1 and X2. The confirmation analysis of design expert software confirmed that the F5 batch was found to be the best batch among the 9 formulation batches.

In vitro antifungal study:

The antifungal activity of the superior composition

F5 was examined while the zone of inhibition was calculated. Figure 6 displays the antifungal activity results obtained *in vitro*. For the optimized cream, a microbiological examination for *Candida albicans* had been performed and matched to a control and pure medication solution of Nystatin. The diameter of the inhibitory zone obtained using an optimized cream is shown in (Fig. 6). The zone of inhibition for the Nystatin drug solution was 22 mm, while the zone of inhibition of optimized batch F5 was 26 mm. This demonstrated that the improved formulation had a strong antifungal activity.

These findings reveal that when both drugs are used together, they have a synergistic impact against fungal strains. In combination, they effectively inhibit fungal infection compared to the pure synthetic drug solution of Nystatin. From the above results it was concluded that *Achyranthes aspera* leaves and *Cassia tora* seeds, have antifungal activity for *Candida albicans* infection. The efficacy of the optimized herbal cream is higher than the standard drug solution.

Stability studies:

A stability investigation of the optimized F5 batch was run at 40 ± 2°C with 75 ± 5% RH for 3 months. Accelerated stability at room temperature: 40 ± 2°C with 75 ± 5% RH. The outcomes obtained concluded that the formulation was found to be

stable with minimum changes in parameters (Table 4).

Conclusion

Topical medication delivery is a viable alternative to oral drug administration. According to the literature, *Achyranthes aspera* and *Cassia tora* are particularly important plants due to their wide range of therapeutic characteristics. *Achyranthes aspera* and *Cassia tora* when used in the combination form, they exhibit strong synergistic activity for antifungal activity. That is why they are used in the combined form to show effective action against fungal infection. The physiological, pharmacognostic, spectrophotometric analysis, ATR-FTIR estimation, preliminary qualitative, quantitative test, and thin layer chromatography was performed and they show results within the range. *Achyranthes aspera* and *Cassia tora* both possess strong antifungal activity and some excipients used in the cream also have antifungal activity so the formulated preparation has better antifungal activity. The developed herbal antifungal cream for topical administration for an antifungal drug *Achyranthes aspera* and *Cassia tora* by using Stearic acid and Cetyl alcohol in different concentrations. The pH, viscosity, phase separation, spreadability, homogeneity, washability, drug content, *in vitro* drug release, antifungal activity, and stability study of the cream were considered. The results of various physicochemical testing revealed that the cream was stable and had a good appearance. Formulation F5 was found to be optimized due to its desirable drug release approximately 98-99%. A 3²-factorial design revealed that the amounts of Stearic acid and Cetyl alcohol have a substantial effect on the dependent variables such as viscosity, and % cumulative drug release for both drugs. Thus, it was concluded that a promising non-invasive drug delivery system with increased patient compliance had been developed. It is clear from the research that applying herbal antifungal cream is a feasible alternative. The developed

formulation may be a promising and valuable alternative for the treatment of fungal disease.

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