Exploring the potential of *Aerva Lanata* extract in a herbal ointment for fungal infection treatment

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Abstract

Background: Fungal infections are a major health concern, and the search for effective and safe antifungal agents is ongoing. The use of natural products, including herbal extracts, has gained popularity due to their potential therapeutic benefits and minimal side effects. In this study, we aimed to formulate and develop an herbal ointment containing extract of *Aerva Lanata* for the treatment of fungal infections.

Methods: The ointment formulations were prepared using various concentrations of wool fat, hard paraffin, and cetosteryl alcohol. The physical properties of the ointment formulations, including extrudability, spreadability, pH, viscosity, and in vitro drug release, were evaluated. The in vitro antifungal activity of the optimized herbal ointment and *Aerva Lanata* extract were evaluated against *Candida albicans* and *Aspergillus niger* using the agar well diffusion method.

Results: The FTIR and DSC analysis showed that there was no significant drug-excipient interaction in the herbal ointment formulation containing *Aerva Lanata* extract. The viscosity of the ointment formulations ranged from 11,632±132cps to 16,423±172cps, indicating that the ointment formulations have good consistency and are suitable for topical application. In vitro drug release studies suggest that the ointment formulations containing *Aerva Lanata* extract exhibit sustained release of the drug over a period of 12 hours. The optimized herbal ointment (BF7) exhibited a zone of inhibition of 20.5±0.12 mm against *Candida albicans* and 17.8±1.15 mm against *Aspergillus niger*.

Conclusion: The study successfully developed and evaluated an herbal ointment containing *Aerva Lanata* extract with good physical properties and sustained drug release. The *Aerva Lanata* extract and the optimized herbal ointment showed promising antifungal activity, suggesting their potential as an alternative to existing antifungal agents.

Keywords: *Aerva Lanata*, herbal ointment, FTIR, DSC, in vitro drug release, antifungal activity.

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INTRODUCTION

Fungal infections are a significant health concern worldwide, affecting millions of people each year. They can be particularly challenging to treat, as fungi can develop resistance to conventional antifungal medications. Therefore, there is a growing interest in the development of alternative, natural therapies for the treatment of fungal infections. One such alternative is the use of herbal medicines.[1]Conventional antifungal agents such as amphotericin B, fluconazole, itraconazole, and terbinafine are effective, but they have several limitations, including adverse effects, drug interactions, and the emergence of resistant strains. Moreover, these drugs are expensive and may not be affordable to all patients. Therefore, there is a growing interest in alternative therapies for fungal infections, including herbal medicines.[2]

Herbal medicines have been used for centuries to treat various ailments, including fungal infections. They offer several advantages over synthetic drugs, including affordability, availability, and fewer side effects. *Aerva lanata*, commonly known as mountain knotgrass or kapok bush, is a medicinal plant that has been traditionally used to treat a variety of health problems, including fungal infections. *Aerva lanata* belongs to the *Amaranthaceae* family and is native to India and other tropical regions. It has been shown to possess various pharmacological activities, including antifungal, antibacterial, anti-inflammatory, and antioxidant properties.[3-6]

Topical ointments are a common form of medication for the treatment of fungal skin infections. They provide a localized effect, allowing for high concentrations of the active ingredient to be delivered directly to the affected area. Therefore, the development of a herbal ointment containing extract of *Aerva lanata* could offer a natural, effective and safe alternative to conventional antifungal ointments.[7-9]

In this research article, we present the formulation and development of a herbal ointment containing extract of *Aerva lanata* for the treatment of fungal infections. We evaluate the physicochemical properties of the ointment and determine its in vitro antifungal activity. The results of this study could provide valuable insights into the potential use of *Aerva lanata* as a natural remedy for the treatment of fungal skin infections.

MATERIAL AND METHODS

Materials:

Aerva Lanata plant materials were collected from a local region and authenticated by a qualified botanist. The voucher specimen (Ref. No. RMI lab/Auth/2023/0024) was deposited in the institutional herbarium. Excipients such as wool fat, hard paraffin, cetosteryl alcohol, and soft paraffin were purchased from Research Lab fine chem industries, Mumbai.

Methods

Preparation of plant extract:

The collected *Aerva Lanata* plant materials were shade-dried and ground into a fine powder. The extraction process was carried out using 95% ethanol as the solvent in a Soxhlet apparatus. The extraction process continued for 48 hours at a temperature of 60-70°C. After the extraction, the solvent was evaporated using a rotary evaporator at 40°C to yield the crude extract.[10]

Table 1: The Composition of various ointment formulations (BF1 to BF9).

Formulation Code	*Aerva Lanata* (mg)	Wool fat (gm)	Hard Paraffin (gm)	Cetosteryl alcohol (gm)	Soft paraffin (gm)
BF1	100	2.5	2	3.5	q.s
BF2	100	5	2	3.5	q.s
BF3	100	7.5	2	3.5	q.s
BF4	100	2.5	3.5	3.5	q.s
BF5	100	5	3.5	3.5	q.s
BF6	100	7.5	3.5	3.5	q.s
BF7	100	2.5	5	3.5	q.s
BF8	100	5	5	3.5	q.s
BF9	100	7.5	5	3.5	q.s

Formulation of ointment:
Nine formulations (BF1-BF9) of the herbal ointment were prepared by varying the concentrations of wool fat, hard paraffin, cetosteryl alcohol, and soft paraffin. The *Aerva Lanata* extract (100 mg) was incorporated into each formulation, as shown in Table 2. The ointments wereprepared using the fusion method, where the lipid components (wool fat, hard paraffin, cetosteryl alcohol, and soft paraffin) were heated separately until completely melted. The *Aerva Lanata* extract was then dispersed into the molten lipid mixture, and the resulting blend was cooled to room temperature to form the final ointment.[11]

Evaluation of Ointment Formulations:
Colour and Odour
Physical parameters like colour and odour were examined by visualexamination.[12]
Consistency
The consistency and color of the ointment formulations were visually inspected.[13]

Extrudability:
Extrudability is a measure of the ease with which the ointment can be extruded from a tube or applicator. The extrudability of each ointment formulation was determined using a modified texture analyzer (Stable Micro Systems, India). A cylindrical sample of the ointment weighing 2 g and measuring 2 cm in height and 2 cm in diameter was placed in the apparatus. The probe of the texture analyzer was lowered onto the surface of the ointment sample with a force of 5 N, and the distance that the probe traveled into the sample was measured over a period of 30 seconds. The extrudability was calculated as the distance traveled by the probe in centimeters during the 30-second period. The experiment was performed in triplicate, and the mean extrudability value was calculated for each ointment formulation.[14]

pH
An electronic PH metre was used to measure the PH of the produced herbal ointment. 100ml of distilled water was used to make the ointment solution, which was then left to sit for two hours. The solution's PH was measured three times, with the average value being computed.[15]

Spread ability:
Spreadability was measured using a modified slide method. A glass slide was coated with a thin layer of the ointment, and a second glass slide was placed on top of the first slide. A weight of 100 g was placed on the top slide for 5 minutes to allow the ointment to spread. The diameter of the spread circle was measured using a Vernier caliper. The spreadability was calculated using the following formula:

$$Spreadability = \frac{(M \times L)}{T} \quad (1)$$

Where, M is the weight in grams placed on the top slide, L is the length of the spread circle, and T is the time in seconds taken for the top slide to move a distance of 7.5 cm. Three measurements were taken for each formulation, and the mean value was calculated.[15]

Viscosity:
The viscosity of the ointment formulations was measured using a Brookfield viscometer (DV-II+ Pro). The spindle used was S-64, and the speed was set at 10 rpm. Approximately 10 g of the ointment was placed in the sample chamber of the viscometer, and the spindle was lowered into the sample. The viscosity reading was taken after 30 seconds of immersion. Three readings were taken for each formulation, and the mean value was calculated. The viscosity measurements were reported in centipoise (cps).[16]

Differential Scanning Calorimetry (DSC):
DSC analysis was conducted to study the thermal behavior of the herbal ointment formulations containing extract of *Aerva Lanata*. The DSC measurements were performed using a DSC instrument (Model: Micro Cal PEAQ, India) equipped with a platinum pan. About 5-10 mg of the ointment sample was placed in the platinum pan and sealed with a lid. The instrument was calibrated using an indium standard. The temperature was ramped from 25°C to 200°C at a rate of 10°C/min under a nitrogen atmosphere. The

melting point and enthalpy of the ointment formulations were determined using the DSC curves.[17]

Fourier Transform Infrared Spectroscopy (FTIR):

FTIR analysis was conducted to identify the functional groups present in the herbal ointment formulations containing extract of *Aerva Lanata*. The FTIR measurements were performed using a FTIR spectrometer (Model: 1650s) equipped with a diamond ATR accessory. About 2-3 mg of the ointment sample was placed on the diamond ATR crystal and pressed with a pressure arm. The FTIR spectra were recorded in the range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1}. The spectra were processed using the software provided by the instrument manufacturer. The FTIR spectra were analyzed for the presence of characteristic functional groups of the ointment formulations.[18]

***In-vitro* drug release Study**

The in vitro drug release study was carried out using the Franz diffusion cell apparatus. The apparatus consisted of two compartments separated by a cellulose membrane (pore size 0.45 µm) mounted on the diffusion cell. The upper compartment of the diffusion cell contained 1 g of the ointment formulation, and the lower compartment contained 10 ml of phosphate-buffered saline (pH 7.4) as the release medium. The temperature was maintained at 37 ± 0.5 °C, and the apparatus was stirred at 100 rpm.At predetermined time intervals, 1 ml of the release medium was withdrawn from the lower compartment and replaced with fresh medium. The amount of drug released was determined spectrophotometrically at 346nm using a UV-Visible spectrophotometer. A calibration curve was prepared using standard solutions of *Aerva Lanata* extract in phosphate-buffered saline (pH 7.4) in the concentration range of 5-50 µg/ml.[19,20]

Antifungal Activity:

The optimized ointment formulation was evaluated for its antifungal activity against *Candida albicans* and *Aspergillus niger* using the agar well diffusion method. Sabouraud dextrose agar plates were prepared, and 100 µl of the fungal suspension containing 10^5 CFU/ml was spread on the agar plates using a sterile cotton swab.After the plates were allowed to dry, wells of 6 mm diameter were made in the agar using a sterile cork borer. The ointment was dissolved in ethanol and added to the wells in various concentrations (150µg/ml). The plates were incubated at 37°C for 24 hours for *Candida albicans* and 48 hours for *Aspergillus niger*. The zones of inhibition around the wells were measured using a Vernier caliper. Fungiwin ointment (Marketed standard) (150µg/ml) was used as a positive control.[20,21]

RESULTS AND DISCUSSIONS

Results of Drug-excipients compatibility study.

Results of FTIR spectral analysis

The FTIR spectra of *Aerva Lanata* pure extract and the herbal ointment containing extract of *Aerva Lanata* with excipients were analyzed to determine the compatibility of the drug with the excipients.

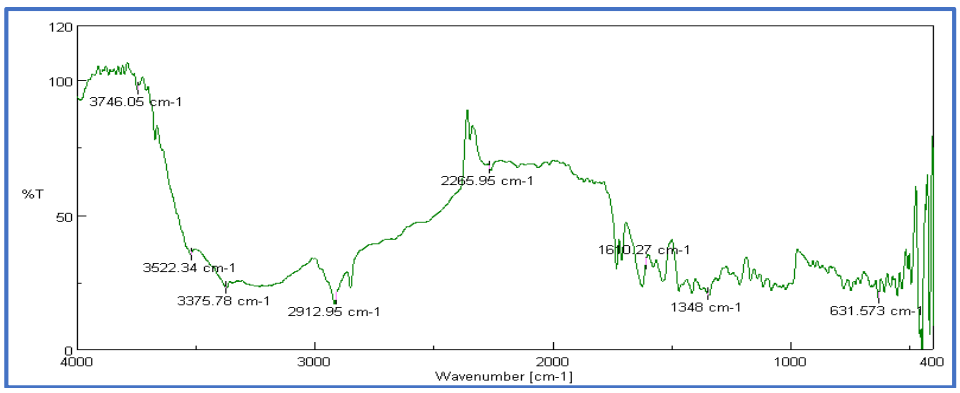

Figure 1: FT-IR spectra of Pure extract (*Aerva Lanata*)

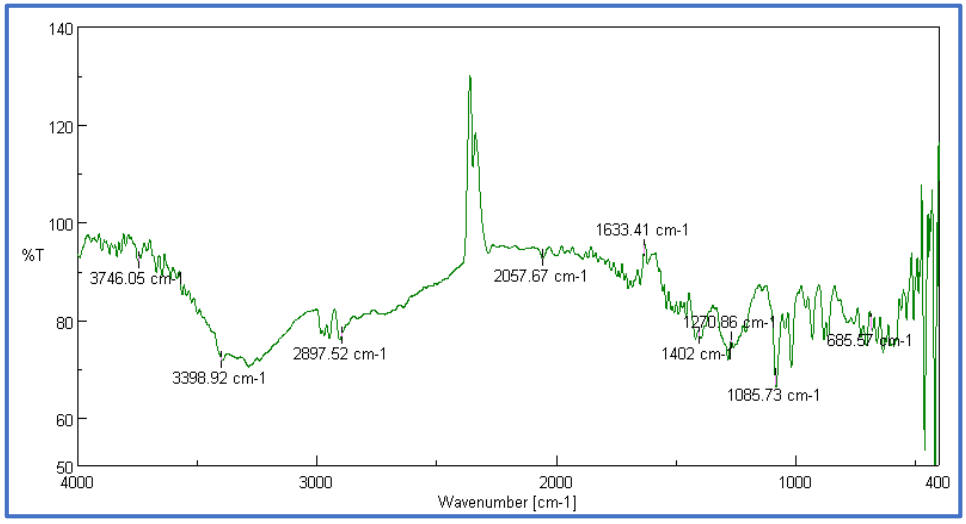

Figure 2: FT-IR spectra of Ointment (Pure extract + Excipients)

Results of Differential scanning calorimetry (DSC) studies

DSC thermogram of*Aerva Lanata* and physical mixture (ointment) of formulation is shown is Figure 4 and 5.

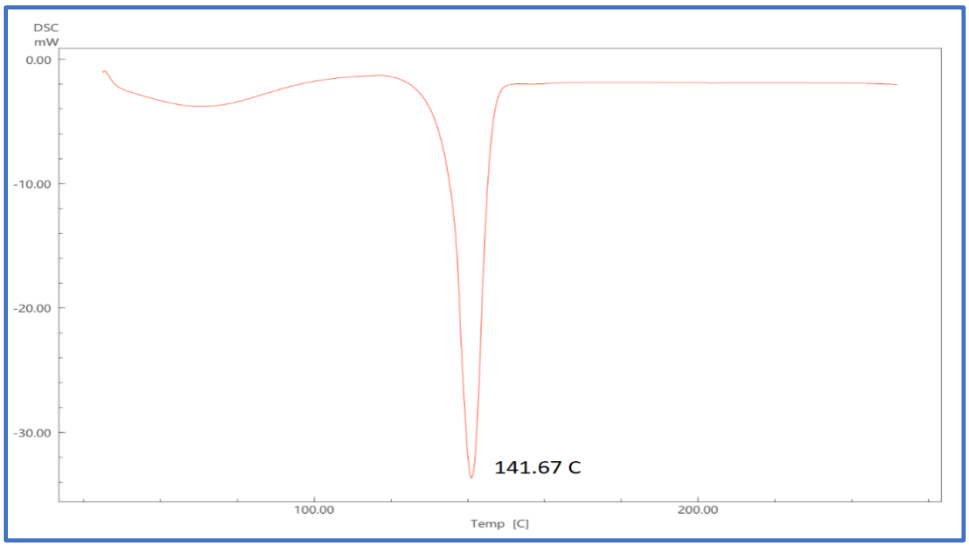

Figure 3: DSC of extract (*Aerva Lanata*)

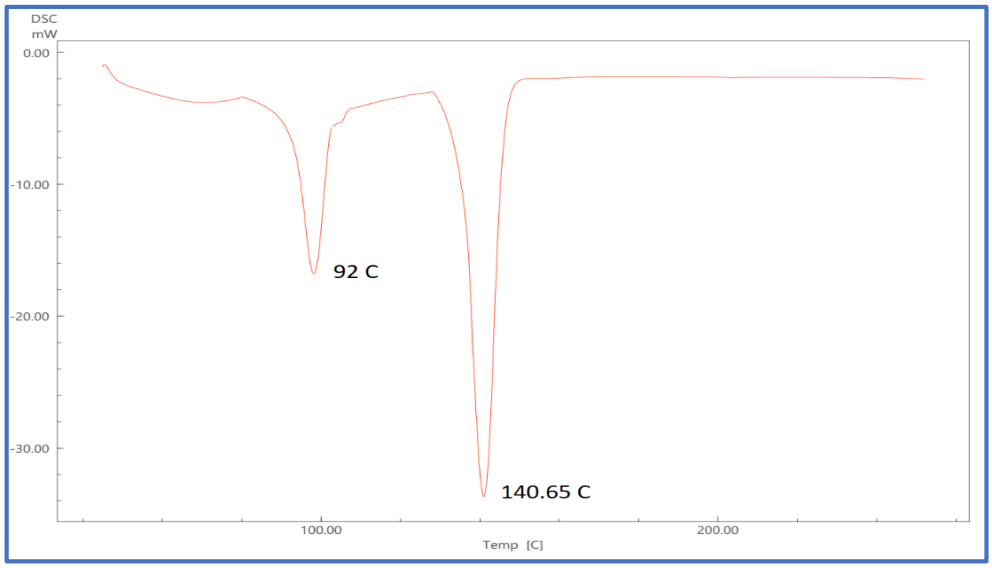

Figure 4: DSC of extract + Excipients (ointment)

Results of evaluation of ointment formulations

Table 2: Consistency and colour of Ointment Formulations

Formulation Code	Consistency (mm)	Colour
BF1	3.48±0.02	Pale yellow
BF2	4.11±0.03	Pale yellow
BF3	4.89±0.02	Pale yellow
BF4	3.56±0.01	Pale yellow
BF5	4.52±0.04	Pale yellow
BF6	5.05±0.05	Pale yellow
BF7	3.62±0.02	Pale yellow
BF8	4.60±0.03	Pale yellow
BF9	5.06±0.04	Pale yellow

Values are expressed in mean±SD, (n=3)

Table 3: Results of Extrudability and Spreadability of Ointment Formulations

Formulation Code	Extrudability (g/cm^2)	Spread ability (cm)	pH	Viscosity (cps)
BF1	12.5±0.8	3.52±0.05	5.78±0.03	11,632±132
BF2	19.4±1.1	4.07±0.03	5.67±0.02	12,923±145
BF3	26.9±1.2	4.69±0.02	5.61±0.03	14,576±156
BF4	13.4±0.9	3.57±0.03	5.80±0.02	11,942±125
BF5	23.7±1.3	4.48±0.04	5.69±0.03	13,875±138
BF6	27.8±1.4	5.02±0.05	5.56±0.02	15,324±165
BF7	14.9±0.7	3.64±0.02	5.82±0.01	12,498±136
BF8	22.1±1.2	4.55±0.03	5.71±0.02	14,364±142
BF9	28.6±1.3	4.98±0.04	5.65±0.01	16,423±172

Values are expressed in mean±SD, (n=3)

Results of *In-vitro* drug release Study

Table 4: In-vitro drug release profile of the ointment formulation

Time (hr)	BF1	BF2	BF3	BF4	BF5	BF6	BF7	BF8	BF9
0	0	0	0	0	0	0	0	0	0
1	2.75	3.38	4.19	2.83	3.64	5.6	2.78	3.87	4.38
2	8.02	9.79	12.04	8.24	10.52	15.89	14.1	11.17	12.57
4	17.73	21.38	25.88	18.18	22.85	33.21	26.89	24.15	26.91
6	38	33.56	41.03	32.76	35.98	54.43	46.76	40.54	47.54
8	42.73	49.7	57.5	43.64	52.34	68.44	67.05	54.6	59.17
10	56.66	64.32	72.29	57.68	67.1	72.27	78.03	69.41	73.91
12	67.2	74.7	81.93	68.23	77.29	81.04	91.57	79.39	83.33

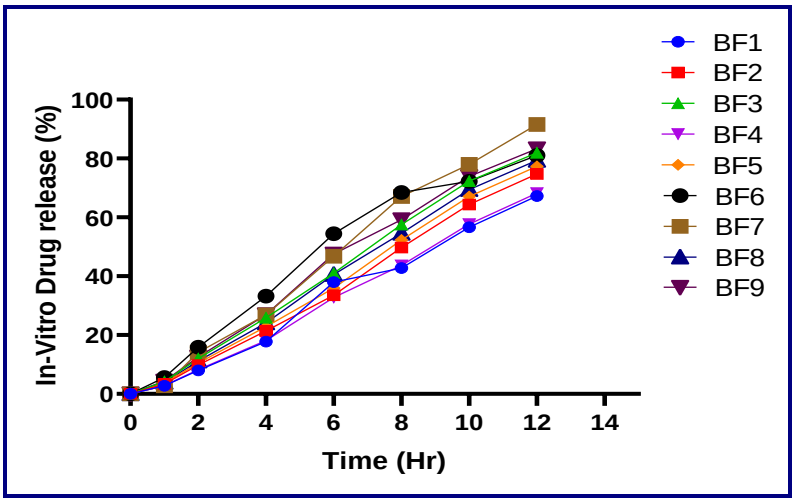

Figure 5: Graphical representation of *In-vitro* drug release profile of the ointment formulation

Results of *In-vitro* Antifungal activity of extracts and ointment formulation

Table 5: Anti-microbial activity of *Aerva Lanata* extract, Herbal ointment (BF7) and Fungiwin ointment.

Sr. No.	Name of Sample	Zone of Inhibition (mm)*	
		Candida albicans	***Aspergillus niger***
1.	*Aerva Lanata extract*	21.2±0.52	19.7±0.65
2.	Herbal Ointment (Optimized batch BF7)	20.5±0.12	17.8±1.15
3.	Marketed standard (Fungiwin ointment)	26.5±0.76	28.4±0.86

Values are expressed in mean±SD, (n=3).

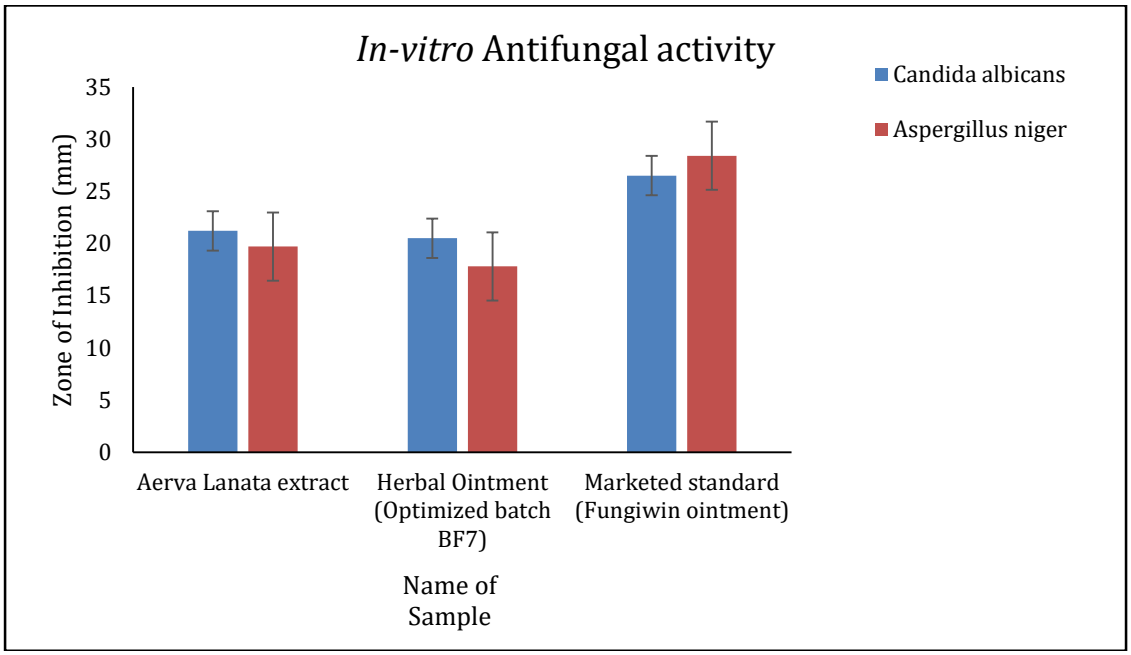

Figure 6: Graphical Representation of *In-vitro* Antifungal activity

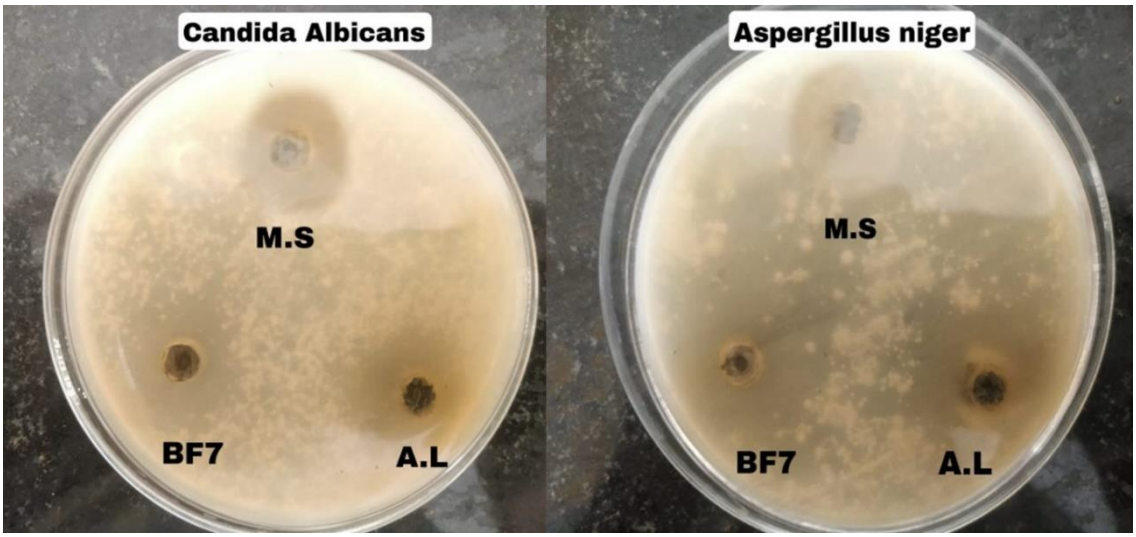

Figure 7: *In-vitro* antifungal activity of the *Aerva Lanata* extract (A.L), the optimized herbal ointment (BF7), and the marketed standard (M.S) for *Candida albicans* and *Aspergillus niger*

DISCUSSION

The results of DSC are showed in Figure 1 and 2, The OH stretch at 3746.05 cm^-1 and NH stretch at 3375.78 cm^{-1} are characteristic peaks for *Aerva Lanata* pure extract and were also present in the ointment formulation. This suggests that there was no significant interaction between the drug and the excipients in terms of hydrogen bonding.The C-H bend at 2912.95 cm^{-1} and the stretch at 2265.95 cm^{-1} are characteristic peaks for the excipients used in the ointment formulation, and were present

in both the pure excipients and the ointment formulation. This indicates that there was no significant interaction between the excipients and the drug in terms of hydrophobic interactions.

The C-Cl stretch at 631.57 cm^{-1} is a characteristic peak for one of the excipients used in the ointment formulation, and was present in both the pure excipient and the ointment formulation. This suggests that there was no significant interaction between the excipient and the drug in terms of electrostatic interactions.The C=C aromatic stretch at 1610.27 cm^{-1} is a characteristic peak for *Aerva Lanata* pure extract and was not present in the ointment formulation. However, this peak was not observed in the FTIR spectra of the excipients used in the ointment formulation either. This indicates that the absence of this peak in the ointment formulation may be due to dilution of the drug extract rather than any significant interaction with the excipients.Overall, the FTIR results suggest that there was no significant drug-excipient interaction in the herbal ointment formulation containing extract of *Aerva Lanata*.

DSC thermogram of*Aerva Lanata* and physical mixture of formulation is shown is Figure 3 and 4.DSC analysis was performed to study the thermal behaviour of *Aerva Lanata* pure extract and the physical mixture of the herbal ointment formulation containing extract of *Aerva Lanata* with excipients. The DSC thermogram of *Aerva Lanata* pure extract showed an endothermic peak at 141.67°C, corresponding to its melting point.

The DSC thermogram of the physical mixture of the ointment formulation containing extract of *Aerva Lanata* with excipients showed peaks around 92°C and 140.65°C. These peaks indicate the melting points of the excipients used in the formulation. The absence of any additional peaks or shifts in the melting point of the drug peak in the DSC thermogram of the physical mixture suggests that there is no significant interaction between the drug and the excipients.Overall, the DSC results suggest that there is no significant drug-excipient interaction in the herbal ointment formulation containing extract of *Aerva Lanata*. The absence of any additional peaks or shifts in the melting point of the drug peak in the DSC thermogram of the physical mixture indicates that the drug and excipients are physically compatible and can be used together in the formulation.

The consistency and colour of the nine ointment formulations prepared using the optimized concentration of wool fat and hard paraffin are summarized in **Table 8**.The consistency of the ointment formulations was determined using the penetrometer method. The results show that the consistency of the ointment formulations ranged from 3.48±0.02 mm to 5.06±0.04 mm, indicating that the ointment formulations have a soft to semi-solid consistency.

The colour of the ointment formulations was determined visually, and all the formulations were found to be pale yellow in colour. This suggests that the addition of extract of *Aerva Lanata* did not significantly affect the colour of the ointment formulations.The results of the extrudability, spreadability, pH, and viscosity measurements for the nine ointment formulations are summarized in Table 3.The extrudability of the ointment formulations was measured using a texture analyzer. The results show that the extrudability of the ointment formulations ranged from 12.5±0.8 g/cm^2 to 28.6±1.3 g/cm^2, indicating that the ointment formulations have good extrudability.The spreadability of the ointment formulations was measured using a spreadability apparatus. The results show that the spreadability of the ointment formulations ranged from 3.52±0.05 cm to 4.98±0.04 cm, indicating that the ointment formulations can be easily spread on the skin.The pH of the ointment formulations was measured using a pH meter. The results show that the pH of the ointment formulations ranged from 5.56±0.02 to 5.82±0.03, indicating that the formulations are slightly acidic and compatible with the skin.The viscosity of the ointment formulations was measured using a Brookfield viscometer. The results show that the viscosity of the ointment formulations ranged from 11,632±132cps to 16,423±172cps, indicating that the ointment

formulations have good consistency and are suitable for topical application.Overall, the results of extrudability, spreadability, pH, and viscosity measurements suggest that the ointment formulations containing extract of *Aerva Lanata* have good physical properties and are suitable for topical application.[22-24]

Table 9 and Figure 5 shows the in vitro drug release profile of the ointment formulations at different time intervals (0, 1, 2, 4, 6, 8, 10, and 12 hours). The results indicate that the release of the drug from the ointment formulations was time-dependent and followed a sustained release pattern.At 1 hour, the percentage of drug released from the ointment formulations ranged from 2.75% (BF1) to 4.38% (BF9). At 2 hours, the percentage of drug released increased to a range of 8.02% (BF1) to 15.89% (BF6). After 4 hours, the percentage of drug released increased to a range of 17.73% (BF1) to 33.21% (BF6). At 6 hours, the percentage of drug released ranged from 32.76% (BF4) to 54.43% (BF6). After 8 hours, the percentage of drug released increased to a range of 43.64% (BF4) to 68.44% (BF6). At 10 hours, the percentage of drug released ranged from 56.66% (BF1) to 73.91% (BF9). After 12 hours, the percentage of drug released increased to a range of 67.20% (BF1) to 91.57% (BF7).Overall, the in vitro drug release studies suggest that the ointment formulations containing extract of *Aerva Lanata* exhibit sustained release of the drug over a period of 12 hours. The sustained release pattern observed in this study could be attributed to the use of natural polymers in the ointment formulations, which are known to provide sustained release of drugs.

In-vitro antifungal activity of the *Aerva Lanata* extract, the optimized herbal ointment (BF7), and the marketed standard (Fungiwin ointment) was evaluated against *Candida albicans* and *Aspergillus niger* using the agar well diffusion method. **Table 10, Figure 6 and 7** shows the zone of inhibition (in mm) exhibited by the samples against the test organisms.

The results indicate that the *Aerva Lanata* extract exhibited a zone of inhibition of 21.2±0.52 mm against *Candida albicans* and 19.7±0.65 mm against *Aspergillus niger*. The optimized herbal ointment (BF7) exhibited a zone of inhibition of 20.5±0.12 mm against *Candida albicans* and 17.8±1.15 mm against *Aspergillus niger*. The marketed standard (Fungiwin ointment) exhibited a zone of inhibition of 26±0.76 mm against *Candida albicans* and 28±0.86 mm against *Aspergillus niger*. The results suggest that both the *Aerva Lanata* extract and the optimized herbal ointment exhibit antifungal activity against *Candida albicans* and *Aspergillus niger*, although the activity is lower than that of the marketed standard (Fungiwin ointment). The observed activity could be attributed to the presence of bioactive compounds in the *Aerva Lanata* extract and the use of natural antifungal agents in the formulation of the herbal ointment. However, further studies are required to evaluate the in vivo efficacy and safety of the optimized herbal ointment.

CONCLUSION

The present study reports the formulation and development of a herbal ointment containing extract of *Aerva Lanata* for the treatment of fungal infections. The FTIR and DSC results suggest that there is no significant interaction between the drug and excipients, indicating their compatibility. The ointment formulations prepared using the optimized concentration of wool fat and hard paraffin exhibited good physical properties, including extrudability, spreadability, pH, and viscosity, making them suitable for topical application. In vitro drug release studies indicated that the ointment formulations exhibit sustained release of the drug over a period of 12 hours, possibly due to the use of natural polymers in the formulations. The optimized herbal ointment (BF7) exhibited antifungal activity against Candida albicans and Aspergillus niger, although its activity was lower than that of the marketed standard (Fungiwin ointment). The observed activity of the optimized herbal ointment could be attributed to the presence of bioactive compounds in the *Aerva Lanata* extract and the use of natural antifungal agents in the formulation. Further studies are required to evaluate the in vivo efficacy and safety of the optimized herbal ointment. In conclusion, the

herbal ointment containing extract of *Aerva Lanata* has potential as a natural alternative for the treatment of fungal infections.

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