

RESEARCH PAPER

Formulation and Evaluation Of Polyherbal Topical Formulation For Diabetic Wound Management

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Abstract

Diabetic wounds are known for delayed tissue repair, oxidative stress, impaired cellular responses, and increased susceptibility to microbial infection, creating a need for effective topical therapeutic approaches. The present study aimed to formulate and evaluate a polyherbal topical formulation containing *Aloe vera*, *Curcuma longa*, and *Centella asiatica* for potential application in diabetic wound management. The formulation was developed using emulsifying wax, beeswax, liquid paraffin, polyethylene glycol 400, and propylene glycol and optimized using a Design of Experiments approach based on response-surface methodology. The prepared formulations were evaluated for physicochemical properties, drug-content uniformity, in vitro release, antibacterial activity, and DPPH free-radical scavenging activity. The optimized formulation (F2) exhibited a pH of 5.91, moisture content of 6.65%, viscosity of 26 cP, and spreadability of 6.93 g·cm/s. In vitro studies demonstrated time-dependent release of all three herbal components. The formulation exhibited concentration dependent antibacterial activity against *Escherichia coli*, producing zones of inhibition of 17 and 25 mm at 5 and 10 mg/mL, respectively, and demonstrated 97.66% DPPH radical-scavenging activity at 100 µg/mL. These findings demonstrate favorable pharmaceutical characteristics together with measurable antioxidant and antibacterial potential, supporting further investigation of the developed polyherbal formulation for topical diabetic wound management. However, additional stability, safety, and validated wound-healing studies are required to establish its therapeutic efficacy.

Keywords: *Aloe vera*; *Curcuma longa*; *Centella asiatica*; polyherbal formulation; diabetic wound; topical formulation; antioxidant activity; antibacterial activity.

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INTRODUCTION

Among the most difficult and dangerous consequences of diabetes mellitus is diabetic wounds, which occur when tissue repair is impaired, peripheral neuropathy is present, vascular insufficiency is present, oxidative stress is increased, and microbiome infection is more likely to occur as a result of prolonged hyperglycemia. A well-coordinated process including haemostasis, inflammation, proliferation, angiogenesis, collagen deposition, re-epithelialization, and tissue remodelling is involved in wound healing under normal physiological settings ^[1,3]. But in diabetes, these cascades are thrown off by chronic hyperglycemia, which increases reactive oxygen species production, decreases angiogenesis, changes collagen synthesis, inhibits fibroblast and keratinocyte activity, and impairs inflammatory responses.^[4] As a result, diabetic wounds are more likely to experience issues such as infection, ulceration, delayed closure, and poor granulation tissue production. Traditional methods for managing diabetic wounds involve washing the wound, removing dead tissue, controlling infections, using

the right dressings, managing blood sugar levels, and administering topical or systemic therapeutic agents. Nevertheless, there is a need for supplementary therapeutic approaches due to issues such as long treatment duration, high treatment costs, microbial resistance, side effects, and insufficient healing in certain chronic wounds. Flavonoids, phenolics, tannins, terpenoids, alkaloids, and saponins are just a few of the bioactive phytoconstituents found in medicinal plants that have garnered a lot of attention for their potential use in wound management. These compounds may have antioxidant, antimicrobial, anti-inflammatory, and tissue-repair promoting effects. The therapeutic effects of a polyherbal approach may be more extensive than those of a single herbal component because it uses a combination of medicinal plants with different but complimentary action mechanisms.^[7] The inclusion of these herbal extracts to an appropriate topical formulation might further improve the wound environment by allowing for localised application, extended contact with the afflicted area, easy administration, and the delivery of phytoconstituents. Consequently, the purpose of this research was to create and assess a topical polyherbal mixture for the

treatment of diabetic wounds. A stable, effective, and patient-convenient polyherbal topical formulation is the overarching goal of this study, which employs experimental methods to systematically prepare and incorporate selected herbal extracts into an appropriate topical base, optimise the formulation, assess relevant physicochemical and pharmaceutical characteristics, and evaluate its wound-management potential.^[7,8,9]

MATERIALS AND METHODS

Materials

Aloe vera leaf and *Curcuma longa* dried rhizome from local area near Mohol, Maharashtra, India, while *Centella asiatica* extract was procured from YUCCA Enterprises, Mumbai, Maharashtra, India. The study utilised a variety of analytical-grade chemicals and reagents, including emulsifying wax, beeswax, liquid paraffin, propylene glycol, ethanol, methanol, and other necessary components for extraction, phytochemical screening, formulation, and evaluation. The total phenolic content was estimated using the Folin-Ciocalteu reagent, sodium carbonate, and gallic acid. The antioxidant assessment was carried out using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ascorbic acid/Trolox. For antimicrobial assessment, *Escherichia coli* (ATCC 8739) and Antibiotic Assay Medium No. 19 were utilised. Electronic analytical balance, mechanical stirrer, heating mantle, water bath, Soxhlet extraction apparatus, rotary evaporator, digital pH meter, Brookfield viscometer, UV-Visible spectrophotometer, Franz diffusion cell, and other conventional laboratory glassware and equipment were utilised as major instruments during the study. Whenever necessary, we used purified or distilled water, and all of the chemicals and solvents were of analytical quality.

Methods

a. Collection and authentication of plant

Aloe vera leaf and *Curcuma longa* dried rhizome from local area near Mohol, Maharashtra while *Centella asiatica* extract was procured from YUCCA Enterprises, Mumbai, Maharashtra, India. The identification of aloe vera, turmeric was carried by the department of Botany K. B. P. Mahavidhyalaya, Pandharpur.

b. Extraction of plant material

Prior to extraction, the powdered *Curcuma longa* and *Aloe vera* materials were passed through sieve No. 16. Hydroalcoholic extraction was carried out using an ethanol-water solvent solution on around 50 g of powdered plant material for each compound. The material was ground into a fine powder and then extracted using about 200 mL of hydroalcoholic solvent in a cellulose thimble for about six to seven hours. This process is known as

Soxhlet extraction. Filtering and reduced-pressure evaporation with a rotary evaporator yielded the crude extract after extraction; this was then transferred to an appropriate sterile glass container and kept in the fridge until needed.^[10, 11, 12]

c. Preliminary phytochemical screening

Standard chemical assays for important types of secondary metabolites, such as steroids, glycosides, saponins, flavonoids, alkaloids, tannins, phenolic compounds, and anthraquinones, were used to conduct qualitative phytochemical screening.^[13,14,15]

d. Total phenolic content determination

The Folin-Ciocalteu technique, using gallic acid as the reference standard, was used to determine the total phenolic content. To summarise, 2.5 millilitres of diluted Folin-Ciocalteu reagent was combined with 0.5 millilitres of adequately diluted gallic acid standard or extract. The mixture was then left to stand for 5 minutes. After that, 2.0 millilitres of 7.5% w/v sodium carbonate solution was added. After a half an hour of dark incubation at room temperature, the absorbance at 760 nm was recorded using a UV-Visible spectrophotometer. Mg GAE/g extract is the unit of measurement for total phenolic content, which was determined using the gallic acid calibration curve.^[11]

Preparation of ointment using DoE

The polyherbal topical formulation was developed using emulsifying wax, beeswax, liquid paraffin, PEG 400 and propylene glycol as principal formulation components, together with *Aloe vera*, *Curcuma longa* and *Centella asiatica*. The oily and watery components were made independently and heated at around 70-75 degrees Celsius. To create a uniform emulsion, the water phase was added to the oil phase little by little while stirring constantly. After letting the mixture cool to around 40–45°C, the herbal components were added slowly while stirring constantly to ensure even distribution. After the formulations were cooled to room temperature, they were transferred to clean, properly sealed containers and kept under the right conditions until further examination. Stirring was maintained during this process.^[16] An experimental design based on response-surface methods, utilising a quadratic model and a Central Composite Design, was used for the creation and optimisation of the formulation. We looked at beeswax, PEG 400, emulsifying wax, liquid paraffin, and moisture content as formulation factors, and viscosity and moisture content as formulation responses. In order to optimise the formulation, we generated experimental batches with varying amounts of the ingredients per the experimental design and tested how changing the variables affected the most important properties.^[17]

Table no. 01 Experimental range of independent variables for a four factors CCD

Independent variables	Coded levels		
X1(Liquid Paraffin)	-1	0	+1
X2(Emulsifying Wax)	-1	0	+1
X3(Beeswax)	-1	0	+1
X4(PEG 400)	-1	0	+1

Table no. 02 Experimental runs for a four factor CCD

Formulation	X1 Liquid Paraffin (%)	X2 Emulsifying wax (%)	X3 Beeswax (%)	X4 PEG 400 (%)
1	10	10	5	10
2	30	10	5	10
3	10	25	5	10
4	30	25	5	10
5	10	10	20	10
6	30	10	20	10
7	10	25	20	10
8	30	25	20	10
9	10	10	5	50
10	30	10	5	50
11	10	25	5	50
12	30	25	5	50
13	10	10	20	50
14	30	10	20	50
15	10	25	20	50
16	30	25	20	50
17	10	17.5	12.5	30
18	30	17.5	12.5	30
19	20	10	12.5	30
20	20	25	12.5	30
21	20	17.5	5	30
22	20	17.5	20	30
23	20	17.5	12.5	10
24	20	17.5	12.5	50
25	20	17.5	12.5	30
26	20	17.5	12.5	30
27	20	17.5	12.5	30

Evaluation of ointment parameters

- Organoleptic Properties:** Organoleptic properties such as look, colour, smell, consistency, and homogeneity were assessed in the made-up polyherbal concoctions.^[18]
- pH:** A calibrated digital pH meter was used to determine the pH following the formulation's proper dispersion or dilution in distilled water.^[18]
- Viscosity:** Viscosity was determined using a Brookfield viscometer under controlled experimental conditions with an appropriate spindle and rotational speed.^[17]
- Spreadability:** Spreadability was assessed using the glass-slide method and calculated as

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

Where,

S represents spreadability

M is the applied weight

L is the distance travelled by the slide and

T is the time required for separation^[18]

- Moisture content:** Using a precisely measured amount of formulation, the moisture content was assessed using a loss-on-drying method.^[18]

$$\% \text{ Moisture Content} = \frac{W1-W2}{W1-W0} \times 100$$

Where,

W0 weight of empty porcelain dish

W1 wt. porcelain dish+ sample Wt. before drying

W2 wt. porcelain dish+ sample Wt. after drying

- Drug Content Uniformity:** A suitable solvent was used to extract representative samples from different parts of the formulation. Then, the spectrophotometric determination of selected marker constituents, such as aloin, asiaticoside, and curcumin, was performed at their analytical wavelengths to determine the

herbal constituents' uniformity. ^[19]

- g. In-Vitro Release Test:** A Franz diffusion cell that had a cellulose membrane attached to it was used to measure in-vitro diffusion. The donor compartment was evenly coated with around 1 g of formulation, while the receptor medium, which consisted of phosphate buffer pH 7.4, was kept at $37 \pm 0.5^\circ\text{C}$ with constant stirring. Using the right analytical approach, samples were taken at regular intervals and analysed. ^[20]
- h. Anti-microbial Activity:** Antimicrobial activity was evaluated by the agar-well diffusion method using *Escherichia coli* (ATCC 8739) as the test organism. From a live culture, a standardised microbiological suspension was made and then added to sterile Antibiotic Assay Medium No. 19 in an aseptic environment. Following the solidification of the seeded material in sterile Petri plates, a sterile cork borer was used to aseptically produce wells measuring around 6 mm in diameter. Each of the specified wells was then filled with an equal volume of test formulation/sample solution, reference standard, and negative control. After the proper pre-diffusion time, the plates were placed in an incubator that was conducive to bacterial growth. The antimicrobial activity was evaluated by measuring the millimetre width of the inhibitory zones that formed. ^[21, 22, 23]
- i. Antioxidant Activity:** A DPPH free-radical scavenging experiment was used to determine the antioxidant activity. For 30 minutes at

room temperature and in the dark, a mixture of the test sample at the correct quantities was incubated with a freshly made 0.1 mM DPPH solution in methanol. A DPPH solution devoid of the test sample and an appropriate reference antioxidant, like ascorbic acid or Trolox, were comprised of the control. We used a UV-Visible spectrophotometer to detect absorbance at 517 nm. We then computed the radical-scavenging activity relative to the control. All experiments were conducted in a controlled laboratory setting with tools that were properly calibrated and analysed according to established protocols. ^[24]

j. Statistical analysis:

An aloe vera, curcuma longa, and Centella asiatica polyherbal ointment was fine-tuned using response surface methodology (RSM). It assessed how the chosen answer played out. The optimal formulation with the desired physicochemical and medication release properties for effective diabetic wound treatment was identified with the use of response surface plots, which showed the interaction between responses and independent variables. ^[17]

- k. Stability study:** For three months, the prepared herbal ointment was placed in aluminum collapsible tubes (5g) and tested for stability at 5°C , $25^\circ\text{C}/60\% \text{ RH}$, $30^\circ\text{C}/65\% \text{ RH}$, and $40^\circ\text{C}/75\% \text{ RH}$. The samples were taken out and assessed for rheological characteristics, pH, and physical appearance. ^[18]

Table no. 03 Batch formulation

Ingredient	F1	F2	F3	F4	F5	F6
Emulsifying wax (g)	6.25	4.375	4.375	4.375	4.375	4.375
Beeswax (g)	5.00	3.13	5.00	3.13	3.13	3.13
Liquid paraffin (mL)	2.90	8.80	5.90	5.90	5.90	5.90
PEG 400 (mL)	11.00	6.60	6.60	2.20	11.00	6.60
Propylene glycol (mL)	3.60	3.60	3.60	3.60	3.60	3.60
<i>Aloe vera</i> (g)	0.050	0.050	0.050	0.050	0.050	0.050
<i>Curcuma longa</i> (g)	0.125	0.125	0.125	0.125	0.125	0.125
<i>Centella asiatica</i> (g)	0.050	0.050	0.050	0.050	0.050	0.050

RESULTS

Preliminary Phytochemical Screening

Proteins were found in *Centella asiatica* and *Aloe vera*, but not in *Curcuma longa*. All three materials contained carbohydrates, glycosides, alkaloids, and flavonoids. The presence of many phytochemical

classes lends credence to the polyherbal system's phytochemical complexity and lays the groundwork for future investigations into its antioxidant and antibacterial capabilities.

Total Phenolic Content of *Curcuma longa* Extract

The *Curcuma* extract exhibited a concentration-dependent increase in total phenolic content when

evaluated against the gallic acid standard. The phenolic content increased from **10.17 mg GAE/g extract at 10 µg/mL** to **76.03 mg GAE/g extract at 100 µg/mL**, indicating a steady accumulation of phenolic constituents with increasing concentration. The results

suggest that Curcuma extract possesses significant phenolic content capable of contributing to free radical scavenging and therapeutic efficacy in herbal preparations.

Physicochemical evaluation of formulations F1–F6

Table no. 04 Physicochemical examination study

Parameters	F1	F2	F3	F4	F5	F6
Appearance	Yellowish-brown	Yellowish-brown	Yellowish-brown	Yellowish-brown	Yellowish-brown	Yellowish-brown
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Phase separation	No	No	No	No	No	No
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Washability	Good	Good	Good	Good	Good	Good
Solubility	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble
pH	5.9	5.91	6.15	6.23	6.37	6.49
Moisture content (%)	5.99	6.65	8.3	8	5.43	5.99
Viscosity (cP)	30	26	26	25	29	28
Spreadability (g.cm/s)	-	6.93	-	-	-	-

Drug-Content Uniformity of the Optimized Formulation(F2)

The drug-content values were 95.55%, 99.22%, and 98.92% for *Aloe vera*, *Centella asiatica*, and *Curcuma longa*, respectively. The corresponding %RSD values were below 1%, indicating low variability among replicate measurements and satisfactory analytical repeatability under the experimental conditions employed.

In Vitro Release Study

The in vitro drug release study was carried out using

a France diffusion cell with phosphate buffer (pH 7.4) at $37 \pm 0.5^\circ\text{C}$ under continuous stirring. The in vitro release profiles of *Aloe vera*, *Centella asiatica*, and *Curcuma longa* from the optimized polyherbal formulation were evaluated up to the final sampling point of 240 min. The ointment showed slow and sustained drug release. The Korsmeyer-Peppas model showed good linearity for aloe vera extract ($R^2=0.9908$) and curcuma longa ($R^2=0.9973$). *Centella asiatica* showed best fit of Zero order model ($R^2=0.9985$).

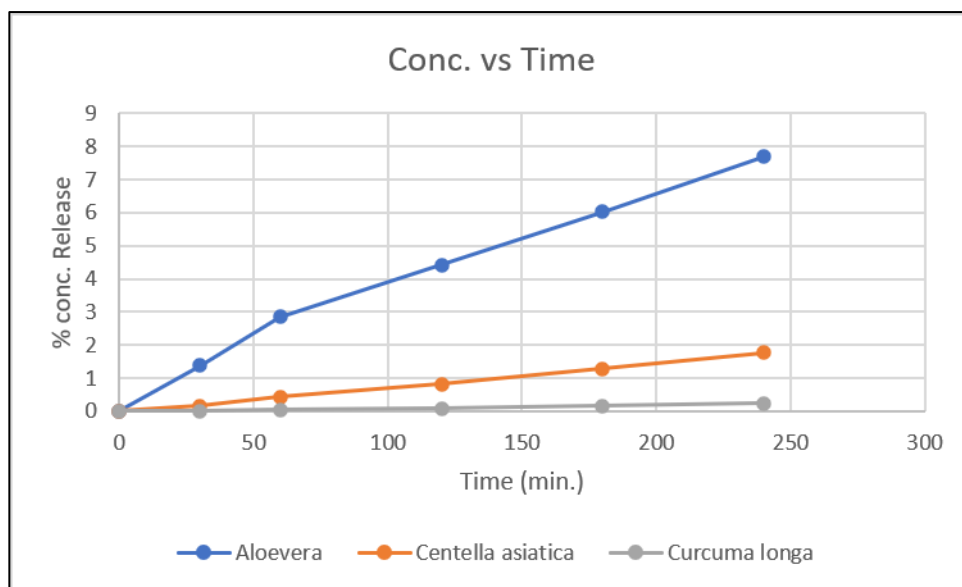


Fig.no.01 Conc. Vs Time plot extracts release

Table no. 05 Kinetic data of Formulation F2

Formulation parameter	Aloe vera extract	Centella asiatica extract	Curcuma longa extract
Zero order model (R^2)	0.9854	0.9985	0.9754
First order model (R^2)	0.9878	0.9984	0.981
Higuchi model (R^2)	0.9625	0.8979	0.8228
Korsemeyer-Pappas model (R^2)	0.9908	0.9958	0.9973

Antimicrobial Activity

The antibacterial activity of the optimized polyherbal ointment was investigated against *Escherichia coli*

using the agar-well diffusion method, with streptomycin serving as the reference antibacterial agent.

Table no. 06 Antibacterial activity of the optimized polyherbal ointment

Sr. No	Sample	Concentration	Zone of Inhibition (mm) <i>E. coli</i>
1.	Control	-	-
2.	Standard Streptomycin	1mg/ml	32
3.	Sample-Polyherbal ointment	5 mg/mL	17
		10mg/mL	25

The polyherbal ointment produced zones of inhibition of 17 and 25 mm at concentrations of 5 and 10 mg/mL, respectively, demonstrating a concentration-dependent antibacterial response against *E. coli*. Streptomycin at 1 mg/mL produced a 32 mm zone of inhibition. Although direct potency comparison is limited because the standard and test formulation were evaluated at different concentrations, the results demonstrate measurable antibacterial activity of the optimized polyherbal

formulation.

Antioxidant Activity

The polyherbal ointment's ability to scavenge DPPH radicals increased clearly when the concentration was raised. The formulation's scavenging activity was just 0.77 percent at 10 µg/mL, in contrast to ascorbic acid's 27.81 percent. By raising the concentration of the formulation to 20 and 40 µg/mL, respectively, the inhibition was enhanced to 35.34

and 67.77%. The formulation demonstrated high scavenging efficacy at concentrations of 60 µg/mL (96.10%), 80 µg/mL (96.75%), and 100 µg/mL (97.66%).

The polyherbal ointment's radical-scavenging reaction was comparable to the ascorbic acid reference at the higher concentrations that were studied. The formulation had a reported value of 97.66% while ascorbic acid had a value of 96.81% at 100 µg/mL. To conclude that one treatment had substantially higher antioxidant activity than the other, however, statistical comparison is necessary.

Statistical analysis

A three-dimensional surface plot illustrating the

effect of beeswax (X3) and PEG 400 (X4) on the polyherbal ointment's moisture content was created. At lower concentrations of beeswax and PEG 400, the moisture content began to decline, and it continued to do so until it reached an intermediate value. The moisture content, however, rose once more at elevated concentrations of the two variables. The curving response surface shows that the formulation's moisture retention is affected by the interaction between the Beeswax and PEG 400.

(A= Liquid paraffin, B= Emulsifying wax, C= Beeswax & D= PEG 400)

$$Y_1 (\text{Moisture Content}) = 2.51363 + 0.0475045 * B + -0.106626 * C + -0.103986 * D + -0.0749793 * CD + -0.174534 * B^2 + 0.226839 * C^2$$

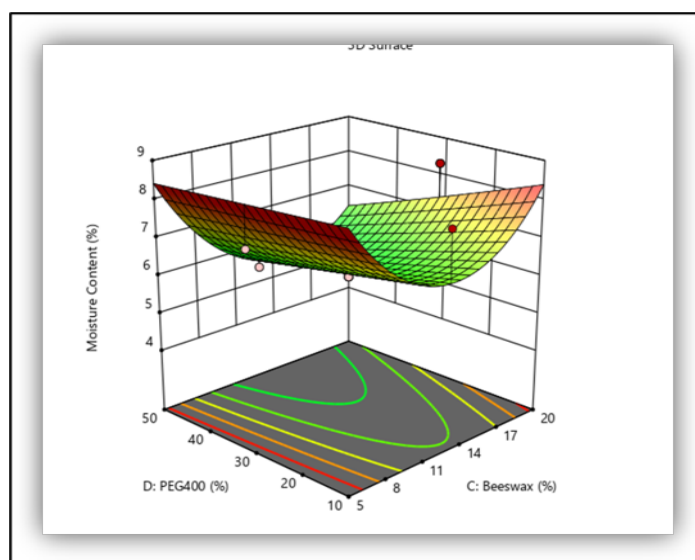


Fig.no.02 Response surface 3D graph of Moisture content

The 3D surface plot shows the effect of Beeswax (X3) and PEG 400 (X4) on the viscosity of the prepared polyherbal ointment. The response surface shows a gradual upward trend, indicating that increasing the amount of Beeswax contributes considerably to the thickening of the formulation, while PEG 400 also

influences the viscosity. The curved surface suggests an interaction between the two factors in determining the rheological properties of the ointment.

$$Y_2 (\text{Viscosity}) = 5.24114 + 0.0483069 * A + 0.261773 * B + 0.293508 * C + 0.134123 * D + 0.105231 * BD + -0.258184 * A^2 + -0.412976 * C^2$$

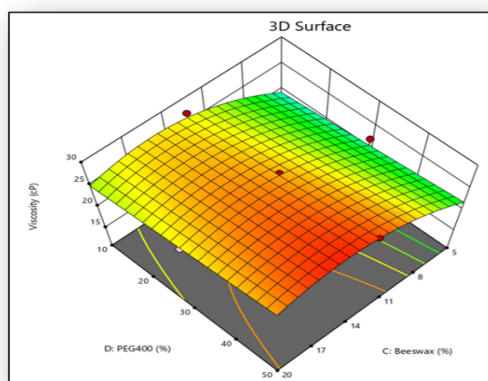


Fig.no.03 Response surface 3D graph of Viscosity

To evaluate how different formulation factors affected attractiveness, moisture content, and viscosity, response-surface and contour plots were employed. The selected design region yielded a desirability rating close to 1, suggesting that all of the established optimisation criteria were satisfied at the same time. Predicted viscosity was at 25.92 cP and moisture content was around 6.80% at about 16% emulsifying wax and 22% PEG 400. While emulsifying wax caused very little changes in the depicted region, the moisture-content response tended to decrease as the PEG 400 concentration increased within the analysed design space.

The correlation between moisture content and the

three-dimensional response surface that depicted the combined influence of beeswax and PEG 400 was nonlinear. The expected moisture response changed when the relative concentrations of both components were changed, showing that both formulation variables had to be considered at the same time for the semisolid base to be optimised. An upward trend was observed on the viscosity response surface as the quantities of emulsifying wax and PEG 400 were increased. Both components likely played a role in the formulation's rheological behaviour, as the higher expected viscosity shown in the top part of the experimental design indicated.

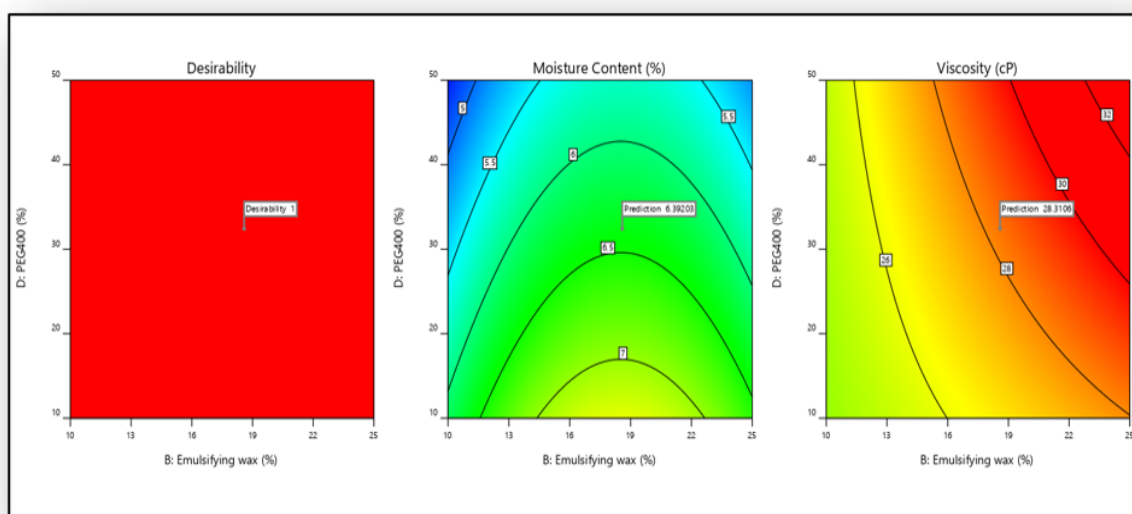


Fig.no.04 Contour plots showing the effects of formulation variables on desirability, moisture content, and viscosity.

Stability Data

The optimized film remained unchanged throughout

the study time period. No significant change in colour, appearance, texture and other parameters.

Table no. 07 Stability data of 3 months

Parameter	Initial	1 month	2 month	3 month
Appearance	Yellowish-brown	Yellowish-brown	Yellowish-brown	Yellowish-brown
Texture	Smooth	Smooth	Smooth	Smooth
Phase separation	No	No	No	No
pH	5.91	5.93	5.94	5.97
Moisture content (%)	6.65	6.70	6.73	6.78

DISCUSSION

The optimized polyherbal topical formulation containing *Aloe vera*, *Curcuma longa*, and *Centella asiatica* demonstrated acceptable gross physical characteristics, a pH of 5.91, moisture content of 6.65%, viscosity of 26 cP, and spreadability of 6.93 g·cm/s. A relatively even distribution of the measured herbal components was shown by drug-content values ranging

from 95.55% to 99.22% and %RSD values below 1%. The formulation also showed DPPH radical-scavenging action that was concentration-dependent and detectable antibacterial activity against *E. coli*. Results from physicochemical, phytochemical, antibacterial, and antioxidant analyses corroborate the formulation's promise as a polyherbal topical system. Appropriate wound-healing investigations and

biological validation are necessary to confirm effectiveness in diabetic wound therapy, as the published experiments mainly aim at establishing formulation properties and in vitro biological activity.

CONCLUSION

In this study, a polyherbal topical formulation was created and optimised using a systematic Design of Experiments approach. The formulation includes *Aloe vera*, *Curcuma longa*, and *Centella asiatica*. Appropriate physicochemical properties, such as an appropriate pH, adequate moisture content, viscosity, spreadability, homogeneity, and drug-content uniformity, were displayed by the chosen formulation. The phytochemical screening of the *Curcuma longa* extract revealed the presence of many bioactive component classes, and the total phenolic content provided evidence of phenolic compounds that may have antioxidant properties. At higher tested concentrations, the optimised formulation demonstrated substantial DPPH free-radical scavenging activity and demonstrated measurable, concentration-dependent antibacterial activity against *Escherichia coli*. Depending on the specific herbal component, in vitro diffusion tests revealed a time-dependent release of the integrated components; the data from this release fit well to either the zero-order or Korsmeyer-Peppas models. Taken together, these results suggest that the created polyherbal formulation has promising pharmaceutical properties, antioxidant capabilities, and antibacterial potential; hence, it appears to be a good candidate for additional research into its use as a topical formulation for the management of diabetic wounds. To confirm its therapeutic effectiveness and clinical relevance, additional research is needed that incorporates validated wound-healing models, safety evaluations, stability testing, and appropriately designed biological investigations. The current findings, however, are mainly derived from physicochemical and in vitro assessments.

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