Research

Formulation, Physicochemical Characterization, and In Vitro Antimicrobial Evaluation of a Polyherbal Face Cream Containing *Aloe vera*, *Azadirachta indica*, and *Ocimum sanctum*

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ABSTRACT

This study was conducted to formulate and characterize a face cream made up of herbs like Aloe barbadensis Miller (Aloe vera), Azadirachta indica A. Juss. (Neem), and Ocimum sanctum Linn. (Tulsi). Plant material that had been authenticated was processed separately and five formulations (F1-F5) were prepared by employing the technique of emulsification in different concentrations of herbal material. The formulations were characterized in terms of their physical properties, irritancy, washability, type of emulsion, after feel, viscosity, pH, spreadability, phase separation, compatibility by means of infrared spectroscopy, short term physical stability, and antimicrobial activity. All formulations possessed semisolid consistency with slight green colour and pleasant odour. Their pH ranged between 5.8 to 6.7, viscosity between 11,810 to 33,460 cP and spreadability between 5.5 to 8.0 g·cm/s. F1, F2 and F5 had oil in water nature while F3 and F4 had water in oil nature. Phase separation and poor physical stability were found in F3 while irritation and erythema was observed in F2. The polyherbal formulation showed concentration-based antimicrobial activity, with antibacterial inhibition zones ranging from 11 to 13 mm and antifungal inhibition zones ranging up to 10 mm for Candida albicans. On the basis of all physicochemical, sensory, stability and antimicrobial studies carried out, the formulation F5 was found to be the best formulation possessing all desirable characteristics including smooth consistency, washability, good spreadability and viscosity, lack of phase separation and physical stability at tested conditions.

Keywords: Aloe vera; Neem; Tulsi; polyherbal face cream; herbal cosmeceutical; topical formulation; antimicrobial activity; formulation evaluation

INTRODUCTION

Cosmetics are substances that can be applied to the human body for purposes of cleaning, beautifying, and making oneself attractive through alteration of one's physical appearance but not interfering with the structural or functional aspects of the human body. Herbal cosmetics are referred to as those beauty products that have desirable physiological activities including, but not limited to, the activities of healing, beautification, and conditioning because of the presence of herbs.[1] Creams extracted from natural sources are considered to be relatively safe compared to those that are synthetic. They contain natural ingredients that are characterized by fewer side effects and also give more skin glowing and whitening features.[2] There is an increased demand for herbal formulations worldwide. Literature has shown that plants have good anti-microbial, anti-oxidant and anti-inflammatory activities.[3,4] In our preparation, we have used three herbal ingredients including Aloe Vera gel, Neem and Tulsi. Aloe Vera gel acts as a moisturizer, for reducing pimples and acne, and treatment of burn wounds.

Uses of neem are as antifungal, anti-inflammatory, and it is also used for reducing scars, pigmentation, skin redness and itching. Uses of tulsi are to make skin glowing and for wound healing. [5]

Cream

According to the definition, creams are semi-solid emulsions that either are of o/w (oil in water) or w/o (water in oil) emulsions. These semi-solid emulsions have been formulated with the intention to be used externally. The classification of the skin cream is that of oil-in-water or water-in-oil emulsion.

The cream is applied to the outer layer of the skin and the key quality of the cream is that it can stay longer at the area where it has been applied. [6]

Classification of Creams

Cream types on the basis of purpose, characteristic features and type of emulsions:
1. Make-up Cream (o/w emulsion):
• Vanishing Creams
• Foundation creams
2. Cleansing cream, cleansing milk, cleansing lotion (w/o emulsion)
3. Winter Cream (w/o emulsion):
• Cold cream or moisturizing creams.
4. All-purpose cream and general creams
5. Night cream and massage creams.
6. Skin Protective cream
7. Hand and body creams.[7]
The main objective of our project is the preparation of herbal cream which will have multi functional effect, like moisturizer, anti-acne effect, skin irritation reduction and skin disease prevention.
Auxiliary components and herbs used along with their functions, like eczema, psoriasis, dry skin, wrinkles, rashes etc. and also making the face glow. [7]

Ideal Properties of Face Cream

• They are easy to apply.

• They are easy to spread on the skin.

• They are good looking.

• They irritate the skin less.

• They should melt easily.

These are also helpful in making the skin smooth, lubricated and protected other than their cleaning functions. [8]

MATERIALS & METHODS

Collection and Authentication of Plant Material

Fresh and healthy leaves of Aloe barbadensis Miller, Ocimum sanctum Linn., and Azadirachta indica A. Juss. were collected from the botanical garden of Shree Sainath College of Pharmacy, Nagpur, Maharashtra, India. Careful scrutiny of the collected plant parts was done to ensure that there was no presence of disease or any kind of contamination in these plants and then these plant parts were sent to the laboratory. Specimens of each of these plant parts were sent to the Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra, India, for their identification and authentication. These specimens were found to be authentic and were identified as Aloe barbadensis Miller (Asphodelaceae), Ocimum sanctum Linn. (Lamiaceae), and Azadirachta indica A. Juss. (Meliaceae). Herbarium specimens were prepared from these plants and preserved as reference materials. After authentication, these collected plant parts were washed using distilled water and further processing was done as given in the next section. [12]

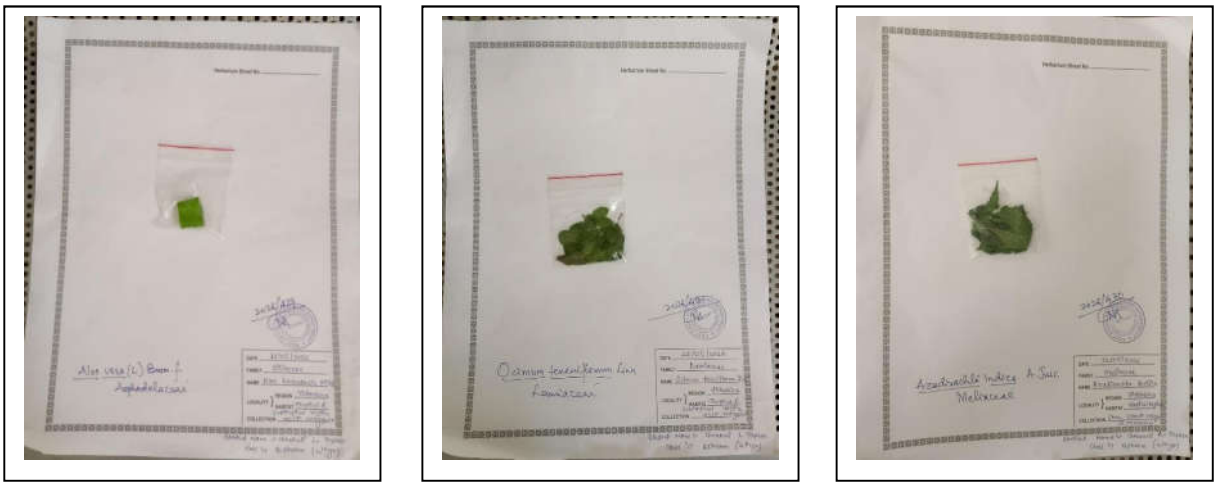


Figure 1: Botanical authentication of the plant materials used for the formulation. Representative herbarium specimens of **(A) *Aloe barbadensis* Miller (Aloe vera), (B) *Ocimum sanctum* Linn. (Tulsi), and (C) *Azadirachta indica* A. Juss. (Neem)**, authenticated by the Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra, India.

Preparation & Isolation of Herbal Extracts

The plant materials were then separately processed for the preparation of the herbal materials. The fresh Aloe vera leaves were washed using distilled water, dried and cut into strips along the midrib using a sterilized knife. The gel obtained from the parenchymatous tissue of the Aloe vera plant was filtered using muslin cloth to obtain a transparent gel which was then collected for formulating the herbal face cream. The dried and powdered Neem leaves (5 g) were separately extracted using methanol (50 mL) for three days using mechanical shaking with REMI RSB-12 mechanical shaker. The extracted solution was then subjected to heating in a water bath at 80-100°C till a concentration of about 20 mL was obtained after filtration through muslin cloth. Similarly, Tulsi leaves powder (1 g) was extracted using methanol (10 mL) by mechanical shaking in REMI RSB-12 shaker for three days. The extract was heated in a water bath at 80-100°C for some time, concentrated to about 5 mL and filtered through muslin cloth. [9,10]

Formulation of Herbal Face Cream

Ingredient and Role:

Table 1: Role of Ingredients

Sr.no.	Ingredient	Role
1	Aloe vera gel	Anti-ageing, anti-inflammatory, moisturizer.
2	Tulsi extract	Antibacterial, adds glow to the face.
3	Neem extract	Promote wound healing, relieves skin dryness.
4	Stearic acid	Emulsifier and thickener
5	Stearyl alcohol	Emollient and stabilizer
6	Liquid paraffin	Moisturizer (occlusive agent)
7	Carbopol 934	Gelling and thickening agent
8	Triethanolamine	Neutralizer and pH adjuster

9	Glycerin	Humectant (retains moisture)
10	Methylparaben	Preservative
12	Distilled water	Vehicle (base)

Preparation of Herbal Face Cream

The herbal face cream was prepared by an emulsion technique using Aloe vera gel, methanolic extracts of Neem and Tulsi, and cream base. Five formulations (F1-F5) were prepared by altering the concentration of the herbal ingredients and some other formulation constituents as detailed in Table 2 below. The preparation involved preparation of the oil and aqueous phases and then followed by controlled emulsification and addition of the herbal ingredients.

The preparation of the oil phase entailed weighing of stearic acid, stearyl alcohol, and liquid paraffin depending on the formula and placing them in a convenient vessel. The two components were heated to 70-75 °C until they formed a uniform oil phase.

For the preparation of the aqueous phase, methylparaben was first dissolved in warm distilled water followed by adding glycerin. Carbopol 934 was added gradually with constant stirring until uniform dispersion was achieved. The aqueous phase was kept at about 70-75 °C in order to promote emulsification.

The hot oil phase was slowly combined with the water phase while continuously stirring to make a uniform emulsion. Following the formation of the emulsion, triethanolamine was slowly added while continuously stirring to neutralize Carbopol 934 and enable formation of the required cream.[11]

The emulsion was cooled to below 40°C before incorporating the thermally sensitive herbs. Aloe vera gel, Neem extract, and Tulsi extract were then added to the mixture and well mixed to ensure even distribution in the cream base. Lastly, the perfume/rose oil was added, and the mixture stirred until the required herbal face cream was obtained. The prepared formulations were packed in suitable packs for evaluation. [12]

Table 2: Composition of the herbal face cream formulations (20 g batch size).

Sr. No.	Ingredient	F 1	F 2	F 3	F 4	F 5
1	Aloe Vera extract	2.0	2.5	3.0	2.0	1.6
2	Tulsi extract	0.5	0.5	0.7	0.3	0.4
3	Neem extract	0.5	0.7	0.5	0.3	0.4
4	Steric acid	2.0	2.5	3.0	2.5	2.4
5	Cetyl alcohol	1.0	1.2	1.0	0.8	0.6
6	Liquid paraffin	2.0	2.5	3.0	1.5	1.6
7	Carbopol 934	0.2	0.3	0.25	0.2	0.2
8	Triethanolamine	0.2	0.25	0.3	0.2	0.2
9	Glycerin	1.0	1.5	2.0	1.5	0.8

10	Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.02
11	Rose water	3.0	3.0	3.0	2.5	3.5	3.0
12	Distilled water	q.s.to 20	q.s.to 20	q.s.to 20	q.s.to 20	q.s.to 20	q.s.to 20

Evaluation Parameters of Herbal Face Cream

1. **Physical evaluation:** Colour, smell, physical form and consistency of the formulations were evaluated using visual and sensory observations. Consistency was measured through the manual application of a small amount of the cream.[13]
2. **Irritancy test:** A small patch of about 1 cm^2 from the dorsal region of the left hand was treated with the formulation. The treated patch was observed for a period not exceeding 24 hours for irritation, especially erythema and edema.[14]
3. **Washability test:** A small amount of the cream was applied to the hand and washed with tap water. The washability test of the formulation was performed visually.[15]
4. **Emulsion type:** The dilution technique was used to determine the type of emulsion. This involved testing whether the formulation can be diluted with aqueous and oily media to classify it as oil-in-water (O/W) or water-in-oil (W/O) emulsion.[16]
5. **After-feel test:** Evaluation was carried out after application of the formulations based on their emolliency, slipperiness and residual properties.[17]
6. **Viscosity:** The viscosity of the formulations was tested using the Brookfield viscometer set at 25 °C with spindle No. 63 at 2.5 rpm. Viscosity results were expressed in centipoise (cP).
7. **pH test:** In case of determining pH, 0.5 grams of cream was dissolved in 50 ml of distilled water, and its pH value was determined by digital pH meter.[18]
8. Phase Separation: Formulations were placed in proper wide-mouth bottles, and separation of oil and water phases was observed after 24 hours of storage.
9. **Spreadability:** Spreadability was determined using the glass-slide method. A fixed quantity of cream was placed between two glass slides, and the time required for the upper slide to separate under a specified load was recorded. Spreadability was calculated using:
10. $S = \frac{M \times L}{T}$

    where S = spreadability, M = applied weight (30 g), L = length of the slide (5 cm), and T = time taken for separation of slides in seconds.
11. **Compatibility study:** Compatibility between the herbal ingredients was determined using infrared spectroscopy. The reported wavelength range was 4000.12–525.03 cm^{-1}, and the characteristic absorption peaks of herbal ingredients in combination were compared with that of herbal ingredients separately.[19]
12. **Stability study:** The formulations were stored at 4 °C, room temperature, and 40 °C for a period of one month and observed for any physical change, especially color change and phase separation. [20]
13. **Antibacterial activity:** The antibacterial activity of the creams was tested against Staphylococcus aureus by the agar well diffusion technique using nutrient agar. Wells of about 8 mm diameter were made, the creams were poured in to the wells, and streptomycin served as the standard. The plates were incubated at 37°C for 18-24 hours, after which zones of inhibition in millimetres were measured.

14. **Antifungal activity:** The antifungal activity of the creams was tested against Candida albicans by the agar well diffusion technique using potato dextrose agar. Wells of about 6 mm diameter were made and were inoculated with different concentrations of the creams. The plates were incubated at 37°C for 24 hours, after which zones of inhibition in millimetres were measured. [21]

RESULT AND DISCUSSION

Face creams with herbal extracts of Aloe vera, Tulsi, and Neem in different amounts were formulated as F1 to F5. The formulated creams were analyzed in comparison to each other based on the physical properties, compatibility with the skin, emulsion behavior, rheological properties, pH, spreadability, physical stability, spectroscopic compatibility, and antimicrobial activity.

1. Physical characteristics

The physical properties of the formulations F1-F5 have been described in Table 3. All formulations displayed a faint green color and good smell, which is due to the presence of the herbal components. The formulations had semi-solid physical form. F2-F5 formulations had a smooth physical form while F1 was relatively non-smooth in nature. This disparity in the physical form of the formulations might be linked to differences in the concentration of herbal extract used in each formulation. It can be concluded that the mostly smooth semi-solid form of F2-F5 formulations is physically uniform in nature.

Table 3: Physical characteristics of the prepared herbal face cream formulations (F1–F5).

Sr. no.	Parameters	F1	F2	F3	F4	F5
1	Colour	Faint Green	Faint Green	Faint Green	Faint Green	Faint Green
2	Odour	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
3	Texture	Not smooth	Smooth	Smooth	Smooth	Smooth
4	State	Semisolid	Semisolid	Semisolid	Semisolid	Semisolid

2. Irritancy and washability

The irritancy test revealed that F1, F3, F4, and F5 do not cause any irritation effect, erythema, or edema within the conditions stated in the research. On the other hand, F2 causes irritation along with erythema, but there is no sign of edema. The results show that these formulations do not share the same irritancy behavior, and F2 needs special attention in future.

Table 4: Skin-irritancy assessment of the prepared herbal face cream formulations (F1–F5).

Sr. No.	Formulation	Irritant Effect	Erythema	Edema
1	F1	Nil	Nil	Nil
2	F2	Show	Show	Nil
3	F3	Nil	Nil	Nil
4	F4	Nil	Nil	Nil
5	F5	Nil	Nil	Nil

The washability property also differed from one formulation to another. F1, F4, and F5 showed easy washability property while F2 and F3 were difficult to wash. The difficulty in washing F2 and F3 could be due to different compositions of the oils used in their preparation. From the cosmetic acceptance point of view, easy washability property of F5 is better suited for face cream.

Table 5: Washability characteristics of the prepared herbal face cream formulations (F1–F5).

Sr. no.	Formulation	Washability
1	F1	Easily Washable
2	F2	Not Easily Washable
3	F3	Not Easily Washable
4	F4	Easily Washable
5	F5	Easily Washable

3. Emulsion type and after-feel

According to the dilution test results, F1, F2, and F5 samples were oil-in-water emulsions while F3 and F4 samples were water-in-oil emulsions. From the different emulsion types observed in the samples, it is clear that the relative concentrations of the components in the formulation affect the arrangement of the two phases.

Table 6: Emulsion type of the prepared herbal face cream formulations (F1–F5) determined by the dilution method.

Sr. no.	Formulation	Emulsion type
1	F1	o/w
2	F2	o/w
3	F3	w/o
4	F4	w/o
5	F5	o/w

A good after-feel was noted for all five formulations after application. All formulations were considered to have provided adequate emolliency and lubricity without having any unsatisfactory after-sensation. Hence, although varying in their types and physical properties, all formulations gave satisfactory results in sensory evaluation.

Table 7: After-feel characteristics of the prepared herbal face cream formulations (F1–F5).

Sr. no.	Formulation	After feel
1	F1	Good
2	F2	Good
3	F3	Good
4	F4	Good
5	F5	Good

4. Viscosity

Viscosity values of F1–F5 are 21,020, 11,810, 18,820, 33,460, and 29,000 cP, respectively. The formulation F4 has the highest value of viscosity, whereas formulations F5, F1, F3, and F2 have lower values. It can be seen that there is a three-fold difference between the viscosity values of F2 and F4; hence, there is a considerable variability in rheological properties of these formulations. This variability is important for topical formulations in relation to their use.

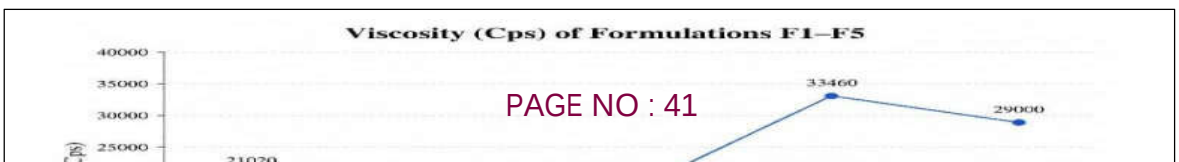


Figure 2: Comparative viscosity profile of the herbal face cream formulations (F1–F5).

It is noteworthy that the high viscosity values of F4 and F5 point to a higher structuring level in the cream system, while the low viscosity of F2 implies a less structured formulation. However, viscosity itself cannot serve as a basis for identifying the optimum formulation – it should be considered in connection with other factors.

Table 8: Apparent viscosity of the prepared herbal face cream formulations (F1–F5) measured at 25 °C.

Sr. no.	Formulation	Viscosity (Cps)
1	F1	21020
2	F2	11810
3	F3	18820
4	F4	33460
5	F5	29000

5. pH

F1-F5 had pH values of 6.7, 6.2, 6.6, 5.8, and 5.9 respectively. All the formulations thus exhibited pH values that were slightly acidic to neutral. F1 had the highest pH value while F4 had the lowest. The difference noted may be attributed to the different concentrations of the herbal components and the pH altering components in the different formulations.

Table 9: pH values of the prepared herbal face cream formulations (F1–F5).

Sr. no.	Formulation	pH
1	F1	6.7
2	F2	6.2
3	F3	6.6
4	F4	5.8
5	F5	5.9

This wide range of pH values suggests that the formulations tested are well suited for topical use; nevertheless, claims about skin compatibility must be considered in light of the irritancy results obtained. In fact, the irritation found with F2 proves that pH cannot be used as an index of topical tolerability.

6. Phase separation and Spreadability

No separation into phases was noted for F1, F2, F4, and F5, while F3 showed separation into phases. In terms of this finding, lower physical stability is evident for F3 in comparison with

other samples according to the conducted experiments. Phase separation is especially critical for the emulsion formulations used topically because it may affect the uniformity of dosage, appearance, and ease of application.

The values of spreadability of F1–F5 were 8.0, 5.5, 6.0, 5.8, and 6.6 g•cm/s, respectively. F1 had the highest spreadability, followed by F5, F3, F4, and F2. The high spreadability of F1 can be explained by the fact that comparatively low resistance was met during application, while F2 had the lowest value. It can be concluded that there is some relationship between the value of spreadability and viscosity, but not direct.

Table 10: Spreadability of the prepared herbal face cream formulations (F1–F5).

Sr. no.	Formulation	Spreadability (g.cm/sec)
1	F1	8 g.cm/s
2	F2	5.5 g.cm/s
3	F3	6 g.cm/s
4	F4	5.8 g.cm/s
5	F5	6.6g.cm/s

7. Compatibility of herbal ingredients

Infrared spectroscopic analysis was carried out to determine the compatibility of the herbal components. Characteristic infrared absorption bands for the herbal mixture were determined at 1026.79, 1368.24, 1438.73, 1604.78, 1728.45, and 3289.05 cm^{-1}, and all of the characteristic infrared absorption bands were also present in the spectra of the individual herbal components. The presence of the characteristic spectral bands implies that there was no significant change in the characteristic functional group peaks during the compatibility test.

Table 11: Infrared spectroscopic characteristics of the herbal ingredients used in the face cream formulation.

Functional group	Bond type	IR Ranges (cm^{-1})	Presence in herbs
Hydroxy	O-H streching	3200-3600	Neem, Tulsi, Aloe Vera
Alkane	C-H streching	2850-2960	Neem, Aloe Vera
Alkene	C=C stretching	1600-1680	Neem, Tulsi
Aromatic ring	C=C stretching	1450-1600	Neem, Tulsi
Carbonyl	C=O stretching	1700-1750	Neem, Aloe Vera
Ether/alcohol	C-O stretching	1000-1300	Neem, Tulsi, Aloe Vera
Amine	N-H stretching	3300-3500	Neem
Carboxylic acid	O-H stretching	2500-3300	Tulsi
Ester	C=O streching	1735-1750	Neem

Results of the functional groups showed the presence of –O-H, -C-H, =C-C, aromatic =C-C, C=O, C-O, -N-H, -O-H of carboxylic acid and -O-H of ester in the plant components. This observation from the spectra indicates a possible chemical compatibility between the herbal components, but chemical compatibility based on FTIR does not ensure total formulation stability.

8. Stability study

The physical stability study indicated that F1, F2, F4, and F5 were physically stable when stored under the stated storage conditions, while F3 was found to be physically unstable. The

physical instability of F3 is in line with the phase separation result obtained from the physical stability study. The concurrence between the two results further supports the claim that F3 was less physically stable than the rest.

However, the absence of phase separation results and the physical stability of F5 suggest comparatively greater physical stability of the drug formulation under the experimental conditions. However, it must be noted that the current study is only a short-term physical stability study and cannot be compared to a long-term stability study.

Table 12: Physical stability of the prepared herbal face cream formulations (F1–F5) under different storage conditions.

Sr. No.	Formulation	Stability
1	F1	Stable
2	F2	Stable
3	F3	Not stable
4	F4	Stable
5	F5	Stable

9. Antibacterial activity

The developed herbal ointment possesses antibacterial properties towards the bacterial test organism tested. At the mentioned concentrations of 25, 37.5, and 50 mg, the zones of inhibition measured were 11, 12, and 13 mm, respectively. The increment in the size of the inhibition zone with an increment in sample concentration denotes concentration-dependence of the antibacterial activity in the conditions of the experiment.

This activity can be related to the joint phytochemical contribution of Aloe vera, Neem, and Tulsi; nevertheless, at the current stage, it is impossible to determine the contribution of each plant separately based on the data obtained. As a result, the antibacterial activity can be characterized as polyherbal activity, and not the activity of a certain herb without further comparative experiments.

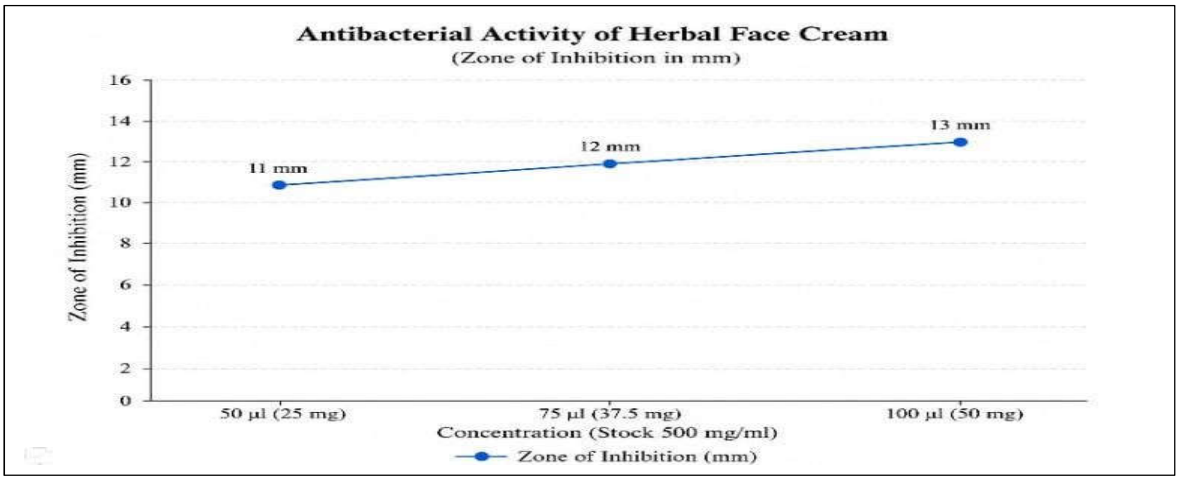


Figure 3: Antibacterial activity of the herbal face cream at different concentrations.

10. Antifungal activity

The preparation was found to exhibit antifungal effect against Candida albicans. The zones of inhibition observed were not observed at the lowest concentration used, but at 8 mm and 10 mm for the higher concentrations used. Therefore, the current observations reveal a concentration-dependent antifungal activity under the given conditions.

The concentration-dependent activities of the polyherbal preparation in both antifungal and antibacterial effects suggest its applicability as a topical antimicrobial cream formulation. Nevertheless, further studies using standard bacterial strains would help confirm this conclusion.

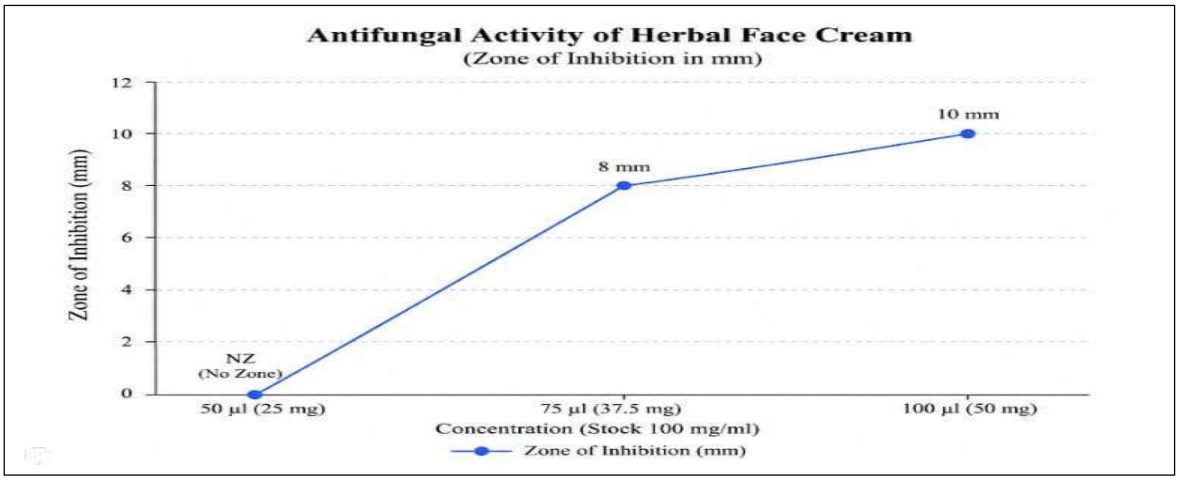


Figure 4: Antifungal activity of the herbal face cream at different concentrations.

11. Overall formulation performance

In view of the overall evaluation criteria, F1 had the best spreadability but did not show a smooth texture. F2 showed acceptable physical attributes, but was irritant, less washable, and had the lowest viscosity value. F3 showed smooth texture and acceptable pH and spreadability but exhibited poor washability, phase separation, and instability. F4 showed highest viscosity value and acceptable washability and stability but was found to be a W/O type emulsion. F5 showed a smooth semisolid texture, good odour, good after-feeling, easy washability, O/W type emulsion, relatively higher viscosity value, acceptable spreadability, no phase separation and stability during the reported stability test.

Taking into consideration the overall physicochemical and stability results, F5 can be termed as the most promising formulation out of all the formulations F1 to F5. This is based on its lack of irritancy and phase separation, easy washability, smooth texture, good after-feel, O/W type emulsion nature, and satisfactory viscosity and spreadability. Antimicrobial studies have further shown that this polyherbal formulation has significant antibacterial and antifungal activity.

CONCLUSION

The present research study has been performed for the development and evaluation of face creams based on neem, tulsi, and aloe vera to protect and hydrate the skin. Five different formulations were developed and evaluated based on tests like physical appearance, homogeneity, pH, viscosity, spreadability, washability, irritancy, type of emulsion, phase separation, stability study, after feel, and antimicrobial activity. It has been found from the evaluation results that formulations F1 has a non-smooth consistency whereas F2 and F3

show irritancy and poor washability. Formulations F2 and F4 have been found to be water-in-oil (W/O) emulsion type. Formulation F3 has phase separation and poor stability during storage showing lower stability of the formulation. However, all other formulations have shown satisfactory results in most of the evaluation tests.

It has been found that all the formulations have satisfactory pH suitable for application on skin and acceptable appearance, consistency, spreadability, and viscosity. Stable formulations have shown no growth of microorganisms and also good after-feel on application.

Based on the results of the overall assessment, formulations F5 were determined to be the most appropriate owing to their stability, good physical properties, non-irritant behavior, and cosmetic properties. Therefore, the prepared herbal face cream can be utilized as an effective topical application for skin care and moisturization.

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