Research Article

Antimicrobial Activity of Cream Containing Polyherbal Extract: Development, Formulation and Evaluation

Gyati Shilakari *, Abhinav U., Anusha G., Siddhartha Reddy L.
Department of Pharmaceutics, Gokaraju Rangaraju College of Pharmacy, Hyderabad, Telangana, India.
***Corresponding author's E-mail:** gyatimmu@gmail.com

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ABSTRACT

Objective: To formulate and evaluate Antimicrobial herbal cream using *Aloe vera* gel, Turmeric, and dimethyl sulphoxide extracts of Neem (*Azadirachta indica*) and Tulsi extract (*Ocimum tenuiflorum).*

Methods: Various herbal cream was prepared using the cream base, Aloe vera gel, turmeric, and dimethyl sulphoxide extracts of Neem and Tulsi containing aqueous phase and oil phase. The first three herbal creams C1, C2 and C3 were prepared with aloe vera gel, turmeric, and extracts of neem and Tulsi. Whereas the next three formulations C4, C5, and C6 contain marketed Tulsi drops instead of Tulsi extract. All the developed creams were evaluated according to various parameters *viz.* appearance, pH, clarity, viscosity, dye test, dilution test, spreadability, and antimicrobial activity against Gram-positive, Gram-negative bacteria and fungi.

Results: Developed formulations C1, C2, C3, C4, C5, and C6 showed good appearance, adequate viscosity, pH, and were easily washable and no phase separation was observed. The formulations did not show redness, erythema, or irritation. All six formulations were found to be C1, C2, C3, C4, C5, and C6 and were stable at room temperature for one month. Developed polyherbal creams show good antibacterial activity against Gram-positive (*Staphylococcus aureus*), gram-negative (*E.coli*), and antifungal activity against *Candida albicans and Aspergillus niger.*

Conclusion: All creams contain herbal ingredients that show good antimicrobial activity and formulations are stable at room temperature and would be further evaluated for preclinical studies.

Keywords: *Aloe vera*, Turmeric, Neem, Tulsi and Antimicrobial Herbal cream.

INTRODUCTION

Cream is a term used to describe semisolid emulsions that are designed for external application. They can be either water in oil (w/o) emulsion or oil in water (o/w) type of emulsion. Its primary property is its ability to stay at the application site longer. It is applied to the outer or superficial part of the skin. The purpose of a skin cream is to provide the skin with soothing properties and protection from various environmental factors and weather. Numerous cream varieties exist, including cleansing, cooling, foundation, disappearing, night, massage, and hand and body creams.[1]

An herbal cosmetic has growing demand in the world market and is an invaluable gift of nature and herbal formulations always have attracted considerable attention because of their good activity and comparatively lesser or no side effects. Herbal formulation refers to the process by which herbal creams, or products, are created using a variety of approved pharmaceutical ingredients as a base, and then one or more herbal ingredients are added to provide specific benefits only.[2]

Herbal creams are semi-solid formulations that are used topically to treat infected skin. The active ingredients in the formulations are used to create antimicrobial creams that protect the skin from microbial infections.[3]

There are various common plants used in herbal cream preparation such as neem, tulsi aloe vera gel, turmeric, carrot, ashwagandha, papaya, and sandal etc. Some common plants are described in the below section.

Neem: Neem can be used as an anti-inflammatory, antibacterial, antiarthritic, antipyretic, hypoglycemic, antifungal, spermicidal, antimalarial, and diuretic properties and seeds of neem are used in home remedies and preparation of medicines.

Carrot: It is used as an Anti-oxidant and in wound healing.

Turmeric: It is an aromatic and used as an anti-inflammatory, and coloring agent.

Tulsi: Anti-inflammation, eliminates toxins, and protects against radiation and antioxidants.

Papaya: It helps in removing dead skin cells, prevents balding, is used to treat sore and cracked heels, and is used for skin whitening.

Sandalwood: Anti-cancer, insect repellent properties, antioxidant, anti-inflammatory, antipyretic, and antibacterial etc.

Neem: The *Azadirachta indica*, or neem tree, is an evergreen tree that grows in most tropical nations and is a member of the *Meliaceae* family. It is one of two species in the genus *Azadirachta* that are native to Burma and India. However, they have since naturalized in West Africa and

are commonly grown as both decorative and therapeutic plants in Nigeria. *Azadirachta indica* has yielded more than 135 chemicals that have been extracted from various portions of the plant, and its leaves have demonstrated significant efficacy against a range of bacterial and fungal species. Neem is used in different products, repelling insects, and mosquitoes (neem oil is used as natural insect repellent), Neem soaps, waxes and it is used in kinds of toothpaste and facewash.[4]

Table 1: Ingredients used in the formulation of Antimicrobial Herbal Cream [4-8]

S. No.	Ingredients	Role of Ingredients
1	*Aloe vera*	Anti-aging, anti-inflammatory, moisturizer, reduces acne and Pimples
2	Tulsi	aid in the healing of wounds and reduce redness, irritation, and dryness of the skin.
3	Neem	Antibacterial, adds glow to the face.
4	Beeswax	stabilizes the cream, emulsifies it, and adds thickness.
5	Liquid paraffin	Lubricating agent
6	Propylparaben	Preservative
7	Stearic acid	Emulsifier
8	Polyethylene glycol	Base
9	Borax	Alkaline agent reacts with emulsifying agent to form soap
10	Sodium lauryl sulphate	Surfactant

***Aloe vera*:** The entire plant, commonly referred to as Barbados or Curaçao Aloe (Syn. Aloe *barbadensis Mill*., Fam. *Xanthorrhoeaceae*), Using traditional remedies to treat and cure a range of illnesses for thousands of years. Despite being native to northern Africa, the plant has quickly spread over the world due to its ease of cultivation. Among the Aloe species, aloe vera is thought to have the highest level of biological activity. The plant has been shown to have more than 75 potentially active ingredients. Aloe vera is a natural ingredient that can do wonders for the skin, used in cleansers, face wash, face masks, gels, and shampoos, and used in antiperspirant deodorant.[5, 6]

Tulsi: The tree *Ocimum sanctum Linn*., also referred to as Krishna tulsi belongs to the Labiatae family. The antimicrobial, antifungal, antibacterial, and antioxidant properties of Krishna tulsi leaves were first discovered by Ayurveda. This naturally occurring, upright, herbaceous, multiflorous, softy hairy, biennial, or triennial plant, grows to a height of 30-75 cm. Pinnate green leaves are found in most species. Naturally, purple, or reddish flowers will bloom and bear little rust-coloured fruit. Tulsi is the best, and a new study is verifying its health benefits. There is growing evidence that tulsi's unique pharmacological activities can manage stress related to the body, mind, metabolism, and chemicals. It has been discovered that tulsi shields organs and tissues from physical stressors such as extended exercise, physical restraint, ischemia, exposure to cold, and loud noises, as well as chemical stress from industrial pollutants and heavy metals. Additionally, tulsi has been demonstrated to mitigate psychological stress by improving memory and cognitive function, regulating lipid levels and vital signs, and counteracting metabolic stress by normalizing blood glucose. It also possesses antidepressant and anxiolytic qualities. Due to its broad-spectrum antimicrobial activity, which includes activity against a range of human and animal pathogens, tulsi is frequently used as a mouthwash, hand sanitizer, and water purifier. It is also used in the preservation of food, animal husbandry, wound healing, as a raw herbal material, and for the health of travellers.[7]

Turmeric: It is preferable to formulate these extracts with antibacterial properties into semi-solid dosage forms with appropriate excipients to enhance the product's stability, drug release, and aesthetic acceptability for topical administration, among other factors. Diseases that are vulnerable to microbes can be treated with the herbal cream formulation. Turmeric has antiseptic properties that can help heal internal and external wounds. Turmeric is also used in baked goods, dairy products, ice cream, yogurt, orange juice, biscuits, popcorn, sweets, cake icings, cereals, sauces, and used in face creams.

Our primary aim is to create an herbal cream that serves as a moisturizer, decreases acne and skin irritation, treats skin conditions like psoriasis and eczema, and reduces dry skin, wrinkles, rashes, and more, all while improving the appearance of the face. Neem, Tulsi, Turmeric, and *Aloe vera* gel are the four natural substances we employed in our formulation. Aloe Vera gel is applied topically, and topically to heal burn wounds, to lessen acne, and minimize pimples. The antifungal and anti-inflammatory properties of neem help to lessen skin irritation, scarring, discoloration, and redness. Tulsi helps to heal wounds and gives the skin a radiant appearance. Turmeric contains antioxidants and anti-inflammatory components. These characteristics may provide glow and luster to the skin. Turmeric may also revive your skin by bringing out its natural glow.

The present study aims to formulate and evaluate the anti-microbial polyherbal creams comprising of extracts of dried fresh leaves of *Azardirachta indica*, Tulsi, and Aloe vera gel. turmeric against gram-positive and gram-negative bacteria and fungi.

MATERIALS AND METHODS

Material

Stearic acid, liquid paraffin, beeswax, polyethylene glycol, sodium lauryl sulfate, tri ethanol amine, propylparaben from SD Fine Chem, and Borax from Hi-Media Laboratories.

Test Microorganisms

The microorganisms used for the study were the following:

1. Bacteria: a) Gram-negative-*Escherichia coli* and b) Gram-positive- *Staphylococcus aureus.*

2. Fungi: *Candida albicans* and *Aspergillus niger*.

These microorganisms were obtained from the Microbiology Laboratory, Gokaraju Rangaraju College of Pharmacy, (GRCP).

Collection of herbs

The plants used in the study were taken from the botanical garden of Gokaraju Rangaraju College of Pharmacy, dried under shade, powdered coarsely & used for extraction (Fig 1).

Figure 1: Various herbs used in the preparation of herbal cream

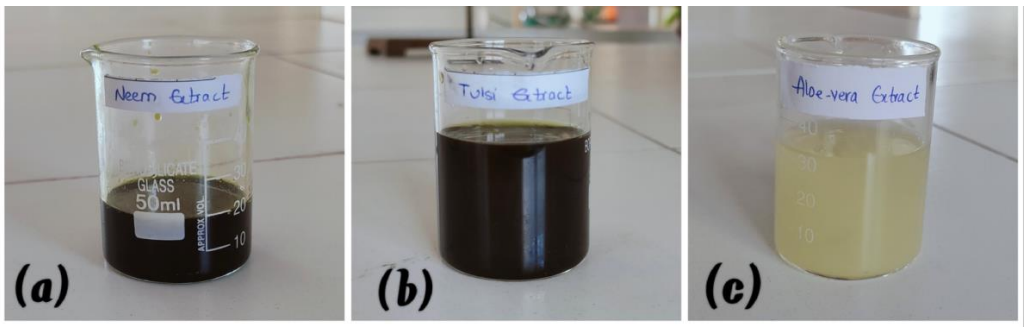

Figure 2: Extracts of (a) Neem (b) Tulsi (c) Aloe vera

METHODS

Extraction of Neem:

Collect fresh neem leaves and give them a thorough wash with distilled water. After drying it in a hot air oven, 5 grams of neem powder were taken and 20 ml of dimethyl sulfoxide was heated to 100 ^{o}C for five to ten minutes. After that, strain it through filter paper to obtain a clear solution (Fig 2a).[8]

Extraction of Tulsi:

The leaves of Tulsi were harvested and then rinsed with distilled water before being dried in a hot air oven. Once the leaves had sufficiently dried, they were ground into a fine powder using a pastel motor. 1g of Tulsi leaf powder was weighed and added into 10 ml of dimethyl sulfoxide in a conical flask and shaken the mixture for three days using a REMI RSB-12 mechanical shaker to make the mixture. After heating the solution for a few minutes at 80 to 100 ^{o}C over a water bath, it was concentrated to a volume of 5 ml and filtered through muslin cloth to remove impurities. After that, a clear solution or clear extract of Tulsi leaves was used to prepare the filtrate or filter product (Fig 2b).[9]

Extraction of *Aloe vera*:

Fresh, healthy, and mature leaves were selected, and then washed with distilled water. After the outer portion was cut longitudinally with a sterile knife. The colorless parenchymatous tissue that makes up aloe vera gel was then removed using the sterile knife. Then, muslin cloth was used to filter out the contaminants and fibres. Next, a transparent aloe vera gel known as the filtrate or filter product was employed in the procedure (Fig 2c).[8]

FORMULATION OF ANTIMICROBIAL HERBAL CREAM

Various antimicrobial polyherbal creams were prepared using different amounts of Tulsi, Neem extracts, Aloe Vera gel, and turmeric as shown in table no.2. Briefly oil phase was prepared by heating liquid paraffin, stearic acid, and beeswax in a borosilicate glass beaker to 75 °C and maintain the temperature followed by adding of tulsi, neem extracts, turmeric (oil phase). Borax, polyethylene glycol, sodium lauryl sulphate, triethanolamine, and methylparaben

(preservative) were dissolved in distilled water in a different beaker, and the mixture was heated to 75 °C to produce a clear solution (aqueous phase). Then aloe vera gel was added into the aqueous phase. Then aqueous phase was added to the heated oily phase gradually and mixed well with constant stirring using a glass rod until a smooth cream was obtained. Then, a few drops of rose oil were added for fragrance.

The first three herbal creams C1, C2 and C3 were prepared with aloe vera gel, turmeric, and extracts of neem and Tulsi. Whereas the next three formulations C4, C5, and C6 contain marketed Tulsi drops instead of Tulsi extract.

Table 2: Formulation Composition of Antimicrobial Herbal Cream

Oil Phase				Aqueous Phase			
Ingredients	C1	C2	C3	**Ingredients**	C1	C2	C3
Stearic acid	3%	3%	3%	**Polyethylene glycol**	3%	5%	7%
Liquid paraffin	10ml	12ml	15ml	**Sodium lauryl sulfate**	3%	3%	3%
Beeswax	3g	3.5g	4g	**Triethanolamine**	1%	2%	3%
Neem extract	3%	5%	7%	**Propylparaben**	0.2%	0.2%	0.2%
Tulsi extract	3%	5%	7%	**Borax**	0.2%	0.2%	0.2%
Turmeric	0.5g	0.5g	0.5g	***Aloe vera* extract**	2ml	1.5ml	1ml
-	-	-	-	**Water**	5ml	4ml	3ml

EVALUATION OF HERBAL CREAMS

1. Organoleptic properties

Physical Properties- The herbal cream was observed for color, odor and appearance by visual means and results were recorded.[10]

- **Homogeneity:** The formulations were tested for homogeneity by touch and visual appearance**.**[11]
- **Appearance**: The appearance of the cream was studied by its pearl sense, roughness and graded. After feeling emollience, slipperiness and amount of residue left after the application of the fixed amount of cream was checked.[12]
- **Type of smear:** After the application of cream, the type of film or smear formed on the skin was checked. The ease of removal of the cream applied was examined by washing the cream with tap water.
- **Irritancy:** Mark the area (1 cm^2) on the left-hand dorsal surface. Then the cream was applied to the area and the time was noted. After some time it is checked for irritant effect, erythema, and Oedema if any then reported.[10]

2. Determination of pH of the cream: The creams were transferred into a beaker and subjected to pH test using pH meter which was calibrated before each use with standard buffer solutions at pH 4, 7, 9. The electrode was inserted into the sample 10 min before taking the reading at room temperature and the readings obtained were recorded.[13]

4. Dye Test: A dye test was conducted using Amaranth dye and examined under a microscope. A drop of the cream was diluted with an amaranth dye solution that had been placed on a tiny slide and covered with a cover slip. If the continuous phase appears red, then it means that the emulsion is o/w type. If the scattered globules appear red and continuous phase colorless, then it is w/o type.[14]

5. Viscosity: The viscosity of the formulation was determined by using a Brookfield Viscometer at 20 RPM at a temperature of 25 °C, spindle number 64 was used for the study. The spindle was placed in a beaker containing the cream and rotated at 30 rotations per minute.[15]

6. Spreadability: The spreadability was measured by measuring the number of seconds it took for two slides separated by a cream layer under a specific load to separate from the cream. The ability to spread is improved by a shorter separation time between the two slides. Standard-sized glass slides were taken in two sets. Next, a slide with the appropriate dimensions was taken, and the cream formulation was put on it. After that, another slide was positioned above the formulation. Next, a weight was applied to the upper slide to evenly compress the cream between the two slides into a thin layer as shown in figure 3. After that, the weight was taken off, and any extra formulation that had stuck to the slides was scraped off. The force of the weight attached to the upper slide allowed it to slide off freely. It was noted how long it took for the upper slide to slip off.[10]

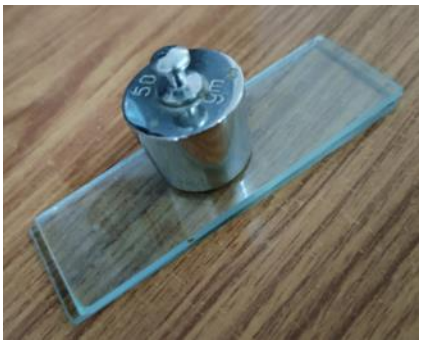

Figure 3: spreadability test

Spreadability is equal to m × L/T

Where,

m is the standard weight (50g) that is attached to or positioned over the upper slide.

L is the glass slide's length (5 cm)

T is time in seconds.

7. Antimicrobial activity

a. Antibacterial activity: Using the conventional agar well diffusion method, the antibacterial activity of various formulations against both gram-positive and gram-negative bacteria was assessed. Agar medium was prepared and sterilized using an autoclave at 121°C, 15lb pressure for 20 minutes. Sterilized molten agar media was inoculated with gram-positive bacteria *(Staphylococcus aureus)* and gram-negative bacteria *(E.coli)* separately by aseptic transfer under the laminar air flow bench. Immediately the inoculated agar media was poured into sterile petri plates and allowed for solidification. Using a sterile borer (6mm), wells were created on petri plates in the appropriate locations. The creams were diluted and applied into the wells and the petri plates were kept aside to allow the diffusion of sample through the agar medium and incubated at 37 °C for 24 hrs. After an incubation period, zones were measured in mm by a ruler for each well-exhibiting inhibition zones under and around cups or wells.[16]

b. Antifungal activity: Sabouraud agar media was used for determining the antifungal activity for the developed herbal formulations. Sabouraud agar media was prepared and sterilized using an autoclave at 121 °C, 15lb pressure for 20 minutes. Sterilized molten sabouraud agar media was inoculated with gram-positive fungi *(Candida albicans*) and gram-negative fungi *(Aspergillus niger)* separately by aseptic transfer under the laminar air flow bench. Immediately the inoculated Sabouraud agar media was poured into sterile petri plates and allowed for solidification. Using a sterile borer (6mm), wells were created on petri plates in the appropriate locations. The creams were diluted and applied into the wells and the petri plates were kept aside to allow the diffusion of the sample through the sabouraud agar medium and incubated at 25-28 °C for 24 hrs. Zones were measured in mm by a ruler for each well-exhibiting inhibition zone under and around cups or wells.[17]

RESULTS AND DISCUSSION

Physical evaluation: Physical evaluation was performed for all the developed creams by visual means and results are shown in table 3. All the creams were observed light green and have a strong odour might be due to the presence of Tulsi and Neem extracts with a smooth texture (fig 4.)

Figure 4: Herbal cream formulations

Irritation test: Irritancy test was performed by applying cream to the sensitive area of the skin and left for 24hrs. No irritant effects, erythema and edema were observed after application of any formulation at the end of the 24hrs as displayed in table 4.

Table 3: Physical evaluation of herbal cream

S. No.	Physical parameters	C1	C2	C3	C4	C5	C6
1	Colour	Light green	Light green	Dark green	Light green	Light green	Dark green
2	Oduor	pungent	pungent	pungent	pungent	pungent	Pungent
3	Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth

Table 4: Results of Irritant test

Formulation codes	Irritant effect	Erythema	Edema
C1	Nil	Nil	Nil
C2	Nil	Nil	Nil
C3	Nil	Nil	Nil
C4	Nil	Nil	Nil
C5	Nil	Nil	Nil
C6	Nil	Nil	Nil

pH Test: pH was observed using pH meter for all herbal formulations and results are shown in table 5. pH was found within the range of 5.90 – 7.87. It was in the range of skin pH, and all the formulations had good pH values.

Viscosity: The viscosity of the formulation was determined by using a Brookfield Viscometer at a temperature of 25 °C using spindle number 64 at 20 RPM and the results are shown in table 5. The viscosity of all herbal creams was found to be in the range of 150-450 cps.

Table 5: pH and viscosity of herbal cream

Formulation codes	pH	Viscosity (cps)
C1	6.30 ± 0.4	150
C2	5.90 ± 0.5	240
C3	6.70 ± 0.2	315
C4	7.12 ± 0.3	180
C5	7.64 ± 0.4	220
C6	7.87 ± 0.6	320

Type of Emulsion Test

Dye Test: Small amount of cream was taken and diluted with Amaranth was mixed and placed on the glass slide and it was observed under the microscope. It was found that the scattered globules appear red and continuous phase colorless and it was indicated that the cream is water in oil type of emulsion Fig 5.

Dilution Test: The creams are diluted with water and oil and it was observed that the creams are dissolving in oil. Hence, further confirmed that the creams were water in oil type of emulsion creams.

Phase separation: Prepared creams were kept in a closed container at room temperature away from light. Then phase separation was checked after 30 days. No phase separation was observed with the developed herbal creams.

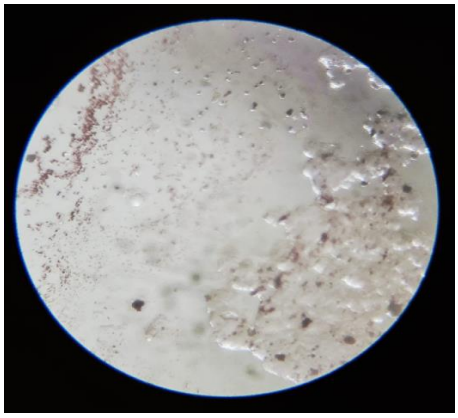

Figure 5: Dye test of creams under the microscope

Spreadability: The spreadability of the formulations was carried out and the results were shown in table no.6. Spreadability was found to be in the range of 9.0 – 31.02 g.cm/s. The time taken by the two slides to separate is less, then it has good spreadability.

Table 6: Spreadability of Herbal Cream

Formulation codes	Time (sec)	Spreadability (g.cm/sec)
C1	12	15.83
C2	15	12.86
C3	9	31.7
C4	10	22.2
C5	12	15.5
C6	14	17.5

Among all, C3 formulation was taken less time to separate two slides and showing good spreadability and considered as final formulation.

Antimicrobial activity: Antimicrobial activity was performed for developed herbal creams using gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*E.coli*) and by using fungal strains *Candida albicans* and *Aspergillus niger* using agar well diffusion method and zone of inhibition was measured and it was compared with the standard drug as shown in table 7. Antimicrobial activity was performed with standard drugs and results were shown in table no.8. It was found that C3 was showing a good zone of inhibition when compared with other formulations such as C1 and C2.

Among all the herbal creams, the C3 formulation displayed the highest zone of inhibition against bacteria and fungi as compared to C1 and C2 formulations. The zone of inhibition was higher i.e. 20.1 ± 00.1mm in the case of gram-positive bacteria (*S. aureus*) as compared to gram-negative ***(****E. coli****,*** 16.1 ± 0.02 mm) as shown in table 7 and fig 6 and 7**.**

Similarly, a higher zone of inhibition was recorded in the case of *Candida albicans (gram-positive)* than in *Aspergillus niger* (gram-negative) as shown in table 7 and Fig. 8 and 9.

The next three formulations that C4, C5 and C6 comprising marketed tulsi drops also displayed similar patterns in the zone of inhibition and close to the herbal creams containing fresh tulsi extract.

The zone of inhibition of the C3 formulation is very close to that of a standard drug in the case of bacteria as well as fungi as shown in table 8 and fig 10 and fig 11.

Table 7: Antibacterial and antifungal activity of Herbal creams (Zone of inhibition)

Microorganisms	Herbal Cream Formulations Zone of Inhibition (mm)					
	C1	C2	C3	C4	C5	C6
E. coli	15.0 ± 0.02	15.5 ± 00.3	16.1 ± 0.02	16.2 ± 0.01	18.5 ± 0.05	19.5 ± 0.04
Staphylococcus aureus	18.5 ± 0.04	19.0 ± 00.5	20.1 ± 00.1	19.5 ± 0.03	20.5 ± 0.02	20.5 ± 0.03
Candida albicans	15.51±0.03	15.7 ± 0.05	16.0 ± 0.04	12.5 ± 0.05	13.7 ± 0.01	16.0 ± 0.02
Aspergillus niger	13.2 ± 0.01	13.0 ± 0.04	13.5 ± 0.06	12.3 ± 0.06	13.3 ± 0.04	14.5 ± 0.03

Figure 6: Zone of inhibition against *Staphylococcus aureus*

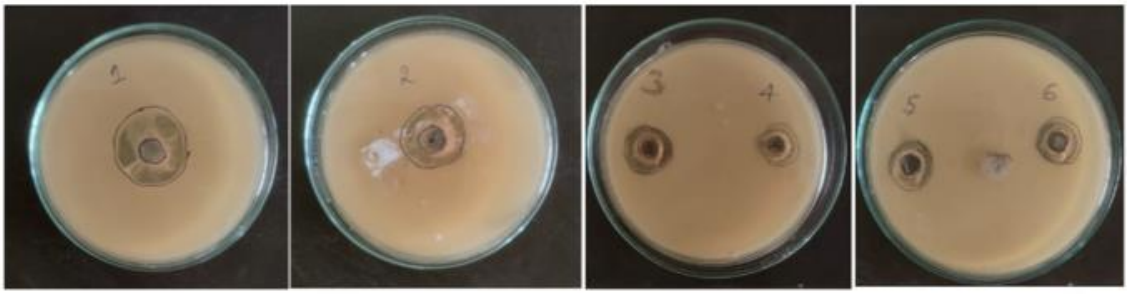

Figure 7: Zone of inhibition against of *E.coli*

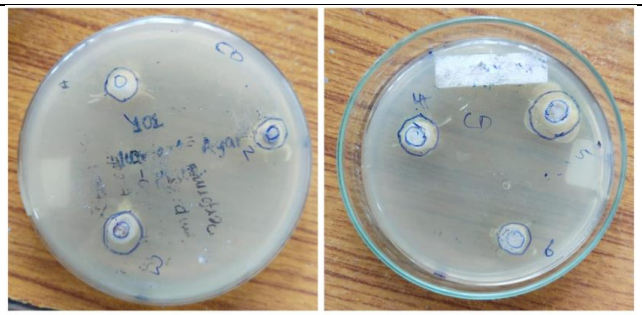

Figure 8: Zone of inhibition against *Candida albicans*

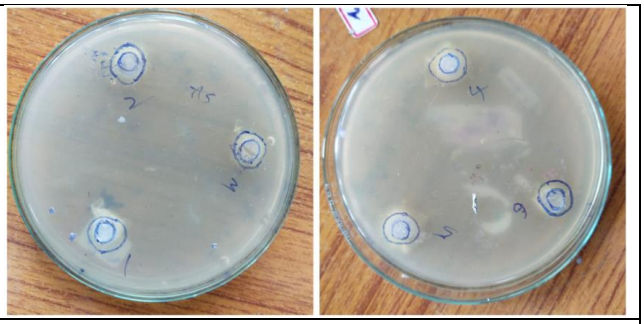

Figure 9: Zone of inhibition against *Aspergillus niger*

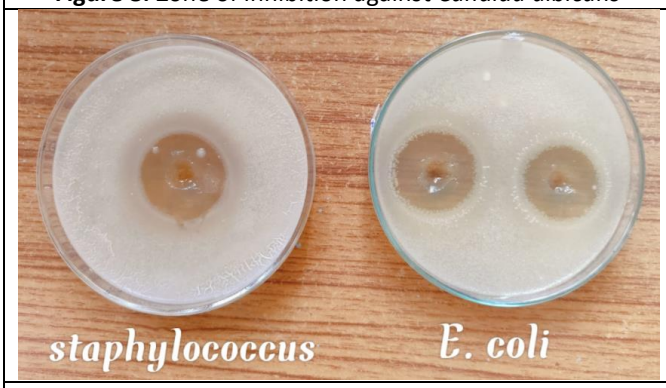

Figure 10: Zone of inhibition of standard antibiotic

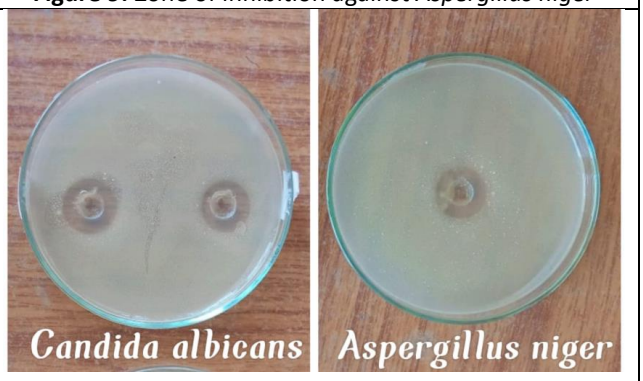

Figure 11: Zone of inhibition of standard drug (Ketoconazole)

Table 8: Zone of inhibition of standard drugs

Organisms	Standard drug	
	Antibacterial drug	Antifungal drug
E. coli	16.7 ± 0.02	-
Staphylococcus	23.0 ± 00.1	-
Candida albicans	-	17.7 ± 0.05
Aspergillus niger	-	14.54±0.02

Stability Studies

Stability studies were carried out for one month at room temperature and pH, appearance and phase separation were recorded after one month. All the herbal creams were found to be stable at the end of the storage period as shown in table 9.

Table 9: Stability studies at Room Temperature for one month

Formulation codes	pH	Appearance	Phase separation
C1	6.10	No change	No phase separation
C2	5.70	No change	No phase separation
C3	6.30	No change	No phase separation
C4	6.70	No change	No phase separation
C5	7.10	No change	No phase separation
C6	7.30	No change	No phase separation

CONCLUSION

The various herbal cream was prepared by using different concentration of Aloe vera gel, Turmeric, Neem and Tulsi extracts and characterized with various parameters such as pH, appearance phase separation viscosity, spreadability and antimicrobial activity. All the developed herbal cream showed a good antibacterial and antifungal activity. Based on results and discussion, the formulations C1, C2, C3, C4, C5, C6 were stable at room temperature and can be safely used on the skin. Among them C3 formulation was showing better results. It could be concluded that the herbal creams containing neem, tulsi extracts, aloe vera gel and turmeric powder displayed a promising formulation for future application.

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