Research Sutra

OPEN ACCESS
Journal of Research in Theoretical and Experimental Sciences
Vol 1 Issue 1 (2025)

DOI: 10.5281/zenodo.15774118

Antiseptic Cream Formulation Using *Tapinanthus globiferus* Leaf Extract as Bioactive Ingredient

Alang Michael Bong[1]*, Tsatsop Tsague Roli Karole[1], Fotio Defo Danielle[1], Nguemtchouin Mbouga Marie Goletti[1], Ngassoum Martin Benoit[1]
[1]Department of Applied Chemistry, ENSAI, The University of Ngaoundere, Cameroon.
balangmichael@yahoo.com

Received 18-04-2025; Revised 13-05-2025; Accepted 16-05-2025

Cite: Bong MA, Karole TTR, Danielle FD, Goletti M, Benoit NM. Antiseptic cream formulation using Tapinanthus globiferus leaf extract as bioactive ingredient. JRTES. 2025;1(1):2-19.

Abstract

Cosmetic substances like creams are used to beautify and enhance the appearance of the human body in a variety of ways. They might hydrate, moisturize and soften the human skin while some combat adverse microbial activities on the skin. Herbal products are usually included in cosmetic formulations to fortify the desirable properties of creams. In this study, the leaf extract of *Tapinanthus globiferus* rich in secondary metabolites with antioxidant properties and antimicrobial properties was used as a bioactive ingredient to formulate an antiseptic cream for body lotion aimed at combating resilient skin infections. Cream formulation was done here as a follow-up of a previous study in this series which had verified the presence of secondary metabolites, antioxidant and antimicrobial properties of *T. globiferus* and reported in a previous publication. A well-designed cream specification was used to formulate the body lotion cream. The plant extract was incorporated into the formulation base to obtain an antiseptic cream with optimal factors being bees wax 4.43251 g, acetyl alcohol 3.76749 g and xanthan gum 0.3g. Physico-chemical analysis of the cream gave an optimum viscosity of 23818.6 cP, a spread of 438.32 g.cm/s, a creaming index of 0% and a pH of 6.54. The results of antimicrobial tests of the base cream without the plant extract showed that the normal cream could inhibit 24.44% of E. coli, 22.22% S. aureus, 26.67% Streptococcus and 22.22% C. albicans while the cream with the optimal concentration of the plant extract (*T. globiferus*) inhibited 44.44% of E. coli, 47.78% of S. aureus, 46.67% of Streptococcus and 48.89% of C. albicans. Hence the results of antimicrobial tests showed that the cream containing *T. globiferus* as additive was twice more destructive to microbial pathogenic species compared to the blank. Hence the plant extract from *Tapinanthus globiferus* is a desirable product for quality upgrade when encompassed into a cream formulation.

Keywords: Cream formulation, Herbal products, bioactive ingredient, antioxidant properties, antimicrobial properties, antiseptic.

Introduction

Cosmetic creams are substances that are applied to the human body for purification, beautification, attractiveness and to change appearance without adverse effects to the body structure and function. Herbal cosmetics or creams with phyto-additives have the following main functions; to adjust the skin appearance, to enhance hair growth and maintenance and to treat or care for the skin amongst others [1]. The additives in creams usually differ as a function of the brand and desired functional properties of the final product [2]. Interest in medicinal and aromatic plants have experienced spectacular increase in recent times by both users and researchers. This may be due to the ease and efficiency of extraction of constituent bioactive compounds and the comparative low risks of exposure to phytochemicals relative to synthetic counterparts [3]. Medicinal plants are used in various folk medicine preparations to treat various infections such as bacterial and fungal infections which are increasingly resistant to traditional antibiotics and antifungals [4]. Medicinal plants are an important source of natural antimicrobial agents due to their inherent composition of secondary metabolites which constitute a preponderance of bioactive molecules [5]. Therefore, interesting molecules with potent therapeutic properties are being identified through research studies to produce phyto-medicines which are not only more effective against pathogenic infections but are also more cost effective [6]. Moreover, medicinal plants are a potent habitats of curative and preventive medicinal molecules for humans as well as a source of essential bioactive compounds for extractions targeting industrial applications [7]. Phytotherapy is therefore an essential component of public health amelioration procedures due to its rich supply phytochemicals with antioxidant and antimicrobial properties [8]. Hence, the antimicrobial and antioxidant potentials of some plant products represents a natural alternative to control many Gram-positive and Gram-negative bacteria in humans, livestock industry, and for food preservation. Recent investigations of natural products for the screening of active compounds has evolved into the discovery of natural occurring antioxidants applicable in foods to replace the carcinogenic synthetic antioxidants and antimicrobial agents [9-11]. As a matter of facts, many plants are a valuable reserve of new bioactive compounds useful in dietetic foods, pharmaceutical and cosmetic industries amongst others. This work is an engineering approach to use a previously investigated phyto-product (*T. globiferus*) as a bioactive component to formulate a skin-care product with antiseptic properties [12] targeting the mitigation of dermal ailments and related predicaments. *Tapinanthus globiferus* is a parasitic plant common in tropical and subtropical regions and is used to treat various conditions thanks to its richness in bioactive secondary metabolites. The leaf extract of this parasitic plant that is endowed with a plethora of bioactive ingredients was used as an additive ingredient to formulate an antiseptic cream to mitigate skin infections. Creams are usually semisolid and pasty types of dosage forms that are predominantly designed for topical application to the skin, location on the eye, nasal, rectal or vaginal usage for medicinal, protective, or cosmetic activity [13]. Herbal components in cosmetic products have recently increased due to an attendant increase in the demand for herbal cosmetics [14,15]. The goal here, was to obtain a formulation base to serve as a matrix for the incorporation of the ethanolic extract of *T. globiferus* to give a cream with desired antiseptic properties for skin care and related uses.

Materials and Methods

Materials

The following materials were chosen as components for the cream formulation; distilled water which was used as the dispersion solvent. The water was properly distilled to avoid possible contamination of the earmarked cream through the dispersion medium. The chemical reagents used in the formulation included the following; glycerin, xanthan gum, and EDTA for the aqueous phase. The chemical reagents were purchased from chemical shops (Sigma Aldrich). For the oily phase, the following materials were used; olive oil, beeswax and petroleum wax which were also bought from industrial chemicals suppliers. Other materials were stearic acid, acetyl alcohol, 5%, methylparaben, vitamin E, tocopherol, and triethanolamine (TEA), and lavender oil with each material fulfilling specific desired tasks. The leaf extract of *T. globiferus* was used as an additive to confer antiseptic properties to the cream formulation.

Methods

Cream Formulation Procedure

The different ingredients were weighed using a precision balance following the formulation specifications. The two oily and aqueous phases were heated using beakers in water baths at temperatures of 70°C for the oily phase and 74°C for the aqueous phase. When the temperature of the oily phase was 70°C and the temperature of the aqueous phase was 74°C, the oily phase was poured slowly with manual stirring into the aqueous phase maintained in the water bath. An electric stirrer was used to continue the homogenization with a speed of more 500 rpm. Then the stirring speed was reduced and gradually brought to zero. The emulsion was then cooled to a temperature of 50°C and triethanolamine was added and homogenized. Emulsification was done until a matrix for the active ingredient was obtained which is pasty in form and cooled to ambient temperature [16]. Then the additives and the bioactive ingredient were added to the cream to complete the formulation.

Optimization of the Cream Base Formulation

The objective here, was to determine the best proportion of each formulation ingredient: the thickener (xanthan gum); emollients (beeswax), emulsifier (cetyl alcohol). The choice of the domain of variation of each factor was made on the basis of literature review and preliminary tests done in the laboratory.

Table 1: Characteristic Factors of the Formulation Base

Factor Xi	Role	Associated Variable	Lower limit	Upper limit	Unit
Beeswax	Co-emulsifier	A	3.0	5.0	%
Cetyl alcohol	Co-emulsifier	B	3.5	5.0	%
Xanthane gum	Gelling agent	C	0.2	0.3	%

Formulation Experimental Matrix

The experimental matrix was obtained by the STATGRAPHICS Centurion XVII Software

Table 2: Experimental Matrix

	Block	Beeswax	Cetyl alcohol	Xanthane gum
1	1	4.80	3.50	0.20
2	1	3.30	5.00	0.20
3	1	4.70	3.50	0.30
4	1	3.20	5.00	0.30
5	1	4.40	3.88	0.23
6	1	3.65	4.63	0.23
7	1	4.35	3.88	0.28
8	1	3.60	4.63	0.28
9	1	4.05	4.25	0.20
10	1	4.75	3.50	0.25
11	1	3.25	5.00	0.25
12	1	3.95	4.25	0.30
13	1	4.00	4.25	0.25

Determination of Physicochemical Properties of the Formulation Base

The Creaming Index (CI)

The purpose of the CI is to evaluate the macroscopic stability of an emulsion. A sample cream (10 mL) was taken in a test tube and sealed. The emulsion stratified into two visible layers comprising of a white layer (the cream) and a translucent or transparent layer which is the aqueous phase. The height of the layer of the aqueous phase and that of the emulsion were measured. The creaming index was calculated using the formula [17].

$$Creaming\ Index = \frac{H_{aq}}{H_{em}} \times \frac{100}{1} \quad (1)$$

where,

H_{aq} Height of aqueous phase

H_{em} Height of emulsion phase

Water Washability Test

Water washability test was conducted to determine the ease of washing the cream off the skin of the human body. The water washability of the cream formulated in this study was done by placing small quantities of cream on the skin and spread. Then water was used to wash off the cream and ease of cream to be washed away was observed. The oil in water (O/W) emulsions are more easily rinsed off compared to water in oil emulsions.

Determination of Cream pH

The purpose of this test was to check whether the pH of the formulated cream falls within the range of the cream formulation specifications. The pH of the formulated cream was measured using a Hanna Instrument pH meter (model Hannah instruments). For this purpose, 1 g of cream was dissolved in 100 mL of distilled water. The mixture was left to stand for 2 hours and the pH of the suspension obtained was measured. The tests were carried out in duplicates.

Determination of Cream Viscosity

The main aim of determining the spreadability of the cream was to verify whether formulated samples fall in the range provided for in the cream formulation specifications. The spreading capacity is expressed as a function of the time (s) taken by two glass slides to separate from a cream (placed between the two blades) in the direction of a certain load. The lesser the time taken by the blades to separate, the better the spreading capacity. To measure the spreadability, 3g of cream was taken on a glass slide and another slide was placed over it, thus sandwiching the cream. A heavy mass (900 g) was placed on the upper plate to expel air for five minutes. The upper plate was pulled with a spring using a mass of 180.7g over a distance of 10 cm and time required for this displacement noted. The following formula was used to calculate the spreading capacity [19].

$$S = \frac{M \times L}{T} \tag{2}$$

where,

S	spreading capacity
M	mass of the load used (180.7 g)
L	distance traveled by the upper blade (10 cm)
T	time taken by upper blade to separate from fixed blade over a distance of 10 cm

Determination of Emulsion Particle Size

The particle size determination test was conducted to deduce droplet size of the cream formulation. The test was conducted using a device called Master Sizer and the master sizer was connected to software which allows someone to display the particle size results. To carry out this measurement, a solvent was used which could be water or alcohol. In this work, alcohol was used to disperse the cream particles. To accomplish this, a small quantity of the cream was dissolved in water. After rinsing the device, a quantity of alcohol was introduced until the pipes of the Master Sizer were filled with ethanol. Once filled, we calibrated so that the software takes into account the initial conditions (the white). Then a small quantity of dissolved cream in water was introduced into the alcohol contained in the device until a peak was observed which is on a red-green band. When this

peak arrived at the green band, we could read the grain size of the cream two or three times. After reading the particle size, we empty the tank of the device, rinse it again with water and resume the process.

Statistical Analysis of the Experimental Design and Validation of the Models

Statistical analyzes were carried out by StatGraphics software. Model validation was made by comparing the values of the regression coefficient (R^2) ($R^2 \geq 0.90$), the coefficient of adjusted regression (adjusted R^2) (adjusted $R^2 \geq 0.80$), Absolute Mean Deviation Analysis (AMDA) ($0 \leq$ AMDA ≤ 0.3).

$$AMDA = \sum_{i=1}^{n} \frac{\frac{Y_{obs} - Y_{cal}}{Y_{cal}}}{n} \tag{3}$$

where,

Y_{obs}	experimental response
Y_{cal}	theoretical response or calculated from the experimental model
i	number of trials
n	total number of experiences

Microbiological Analysis of the Cream Formulation

The microbiological analysis of the cream sample was carried out using culture media depending on the microorganism sought. The following culture media were used; The Potato Dextrose Agar (PDA) medium at concentrations 39g/L for the detection of molds. The medium Nutrient Agar (NA) at concentrations of 28 g/L for the detection of bacteria. 10g of the base cream sample was introduced into a test tube to which 90 mL of physiological water has been added. The mixture was stirred. Using a 1 mL pipette, decimal dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) were made for the sample starting from the sample stock solution. The test tubes were clogged with cotton and all operations were carried out under sterilized conditions beside a flame [20].

Preparation of Culture Media

The different culture media were prepared as recommended by the manufacturer. 11 g of the PDA and MHA culture each and 18 g of SDA medium were weighed and introduced into glass bottles each containing 270 mL of distilled water and boiled in autoclave at a temperature of 121°C for 1 hour. The culture media were then removed from the autoclave, cooled and poured into petri dishes. The cultures were prepared under sterile conditions near a flame. The culture media in the petri dishes were allowed to settle and solidify. Each culture medium was inoculated with 0.1 mL of different sample dilutions in the petri dishes using a 1mL pipette going from the least concentrated to the most concentrated medium. The culture media for the different petri dishes of the NA media were placed in an oven at 37°C for 24 hours and the medium of PDA was placed in the incubator at 37°C for 48 hours for microbial colonies to grow [21]. After the colonies were developed, they were identified by visual counting.

Incorporation of the Active Ingredient into the Cream Base

During this part of the work, *T. globiferus* extract was methodically introduced into the homogeneous cream formulation base. Two 100 g of base formulations were prepared and 5 mL of 500 mg/mL and 5mL of 250 mg/mL *T. globiferus* extract were respectively added to the formulations. The mixtures obtained were stirred at 700 rpm for 15 minutes. The validation criterion of the minimum antifungal and antibacterial concentration were verified by antimicrobial tests on the different products obtained.

Characterization of the Antiseptic Cream and Antimicrobial tests

The goal here was to verify whether the formulated cream has the characteristics predefined in the formulation specifications. Microorganisms were inoculated on petri dishes containing solidified NA medium and other media. Then 200 µL and 400 µL of antiseptic creams were introduced into the wells and the bacteria was incubate at 37°C and mold at 25°C for 48 hours. The ability of the antiseptic creams to destroy microscopic pathogens was noted. Other characterization procedures consisted of determining the pH, viscosity, emulsion quality and the creaming index through procedures mentioned earlier.

Stability Tests

This was a study of accelerated aging of the formulated cream in order to evaluate some specific parameters such as pH, coloring, physical appearance, spreading and the microbiological activity of the cream formulated at different temperatures (5°C, 25°C, 40°C and alternating 5°/40°) over time. Four batches of 50 g of cream were prepared and separated in 4 jars. For the macroscopic stability (aging test) the first jar was placed in an oven at 5°C, then the parameters were evaluated after 24 hours for 10 days. For the accelerated aging test, the second jar was placed in an oven at 25°C and 40°C, then the parameters were evaluated after 24 hours for 10 days. For stability at varying temperatures, the third jar was subjected to a freeze-thaw cycle at temperature (5°C-40°C), then specific parameters were evaluated after 24 hours for 10 days. Then for centrifugal stability, 1.5 mL of cream was taken in 4 Eppendorf tubes and centrifuging at speeds ranging from 1000 to 5000 rpm for 30 minutes [22].

Results and Discussion

Results of Experimental Matrix

The results of the screening analysis of the different formulation bases aimed at selecting the best proportion of each formulation ingredient are given in Table 3 below. The responses monitored were viscosity and spreading. The viscosity lies between 19050 and 28429 cP while the spreading values are in the range 117.26 and 880.5 g.cm/s respectively. Viscosity measures a fluid's resistance to flow. The variation of the cream viscosity is a function of the thickening agents. The higher the content, the higher the viscosity. The modeling of the cream formulation viscosity is given by the equation.

Table 3: Experimental Results of the Base Formulation

TESTS	VARIABLES			RESPONSES			
	A	B	C	Viscosity - Y1 (cP)		Spread - Y2 (g.cm/s)	
				Observed	Adjusted	Observed	Adjusted
1	4.80	3.50	0.20	22756.00	22516.10	390.90	423.94
2	3.30	5.00	0.20	25644.00	25835.10	199.53	257.14
3	4.70	3.50	0.30	28429.00	28504.80	117.26	110.33
4	3.20	5.00	0.30	21111.00	21758.10	309.65	309.50
5	4.40	3.88	0.23	20414.00	19222.40	606.09	576.62
6	3.65	4.63	0.23	19822.00	20815.70	580.88	538.97
7	4.35	3.88	0.28	21955.00	22150.60	403.22	465.57
8	3.60	4.63	0.28	20600.00	20035.40	398.36	519.40
9	4.05	4.25	0.28	19050.00	18232.90	880.50	839.54
10	4.75	3.50	0.25	26690.00	26387.20	129.90	101.35
11	3.25	5.00	0.25	25490.00	24673.40	200.55	117.53
12	3.95	4.25	0.30	19500.00	19188.70	779.53	708.92
13	4.00	4.25	0.25	19822.00	19587.50	580.88	608.44

$$Viscosity = 22516.2A + 27746.7B - 723351C - 27046.1AB + 897799AC + 698966BC \quad (4)$$

The experimental design was similar to those of literature reports [23]. The validation indicators such as R^2, adjusted R^2 and AMDA are all valid.

Table 4: Viscosity and Spread Validation Indicators

	Indicator	R^2	Adjusted R^2	AMDA
Viscosity	Value	96,04	93,20	0,02
	Observation	Valid	Valid	Valid
Spread	Value	94,28	90,20	0,15
	Observation	Valid	Valid	Valid

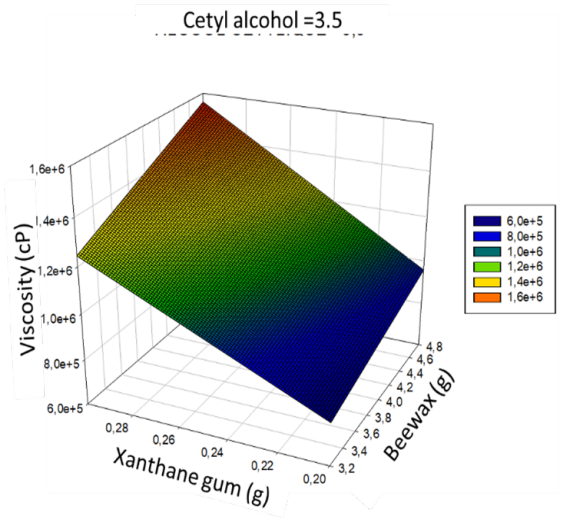

Figure 1: Surface Area Diagram: Viscosity Response

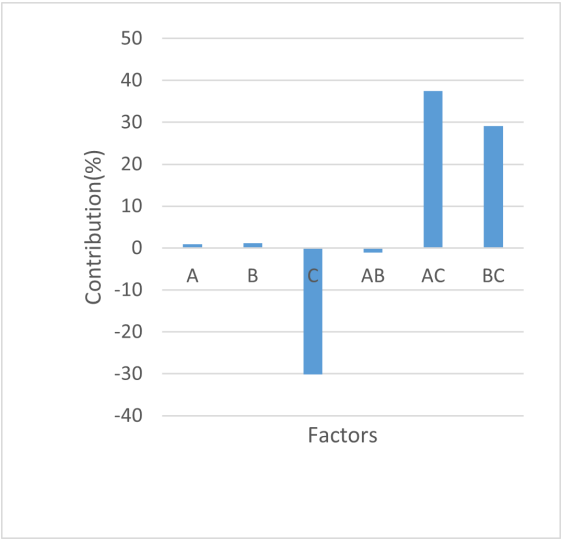

Figure 2: Factor Contribution Diagram: v, w, g

The goal of the optimization in this case was to maximize the viscosity. It was observed that the combined effect of beeswax and xanthan gum fortified the response (Figures 1, 2).

The Sprawl

Spreadability is the ability of a cream to spread over the skin. It plays an important role in administering a dose in a topical formulation. If the spreading of a cream increases, the cream is good because its application on the skin is easy and effective. The spread values are located between 117.26 and 880.5 g.cm/s. The cream formulation was modeled by the following mathematical equation for spread. The range of results obtained in this work are quite different from those of Avish and Swaroop [24] who obtained values ranging from 80 to 115 g.cm/s. The slight variation is due to differences in the formulation matrices.

$$Spread = 423.94A + 104.08B + 154561C + 2271.02AB - 169766AC - 161249BC \quad (5)$$

The validation indicators such as R^2, adjusted R^2, and AMDA are all valid.

Multi-response optimization of cream formulation

The experimental matrix brought out the proportions of the mixture constituting the optimal formula which served as a formulation matrix. The values are recorded in Table 5.

Table 5: Multi-Response Optimization Matrix

Hence, the optima base cream that was formulated and imparted with antiseptic properties by blending it with the ethanolic extract of *T.globiferus* , was formulated using the following optimum proportions of ingredients in percentages: 4.43% of beeswax, 3.77% of cetyl alcohol, and 0.30% of xanthan gum. The resultant base cream gave a viscosity of 23818.6 cP and a spread of 438.32 g.cm/s. The viscosity result was higher than that of Tsatsop et al [19] and Avish et al [24] which gave 15880 cP and 2851 cP, respectively. The difference is probably due to differences in experimental matrices.

Factor (%)	Lower limit	Upper limit	Optimum	Optimum Response
Cetyl alcohol	3.50	5.00	3.77	Viscosity = 23818.6 cP
Beeswax	3.20	4.80	4.43	Spread = 438.32 g.cm/s
Xanthan gum	0.20	0.30	0.30	

Result of the determination of pH, creaming index, and particle size

The pH results obtained on the experimental matrix were recorded in the following table:

Table 6: Physico-chemical parameters of the experimental matrix

Test	pH	Creaming Index (%)	Particle size (μm)
1	6.80 ± 0.21	0	26.81 ± 1.66
2	6.53 ± 0.07	0	76.23 ± 1.33
3	6.70 ± 0.24	0	33.40 ± 0.40
4	6.50 ± 0.14	0	41.08 ± 0.46
5	6.30 ± 0.21	0	24.72 ± 0.26
6	6.57 ± 0.02	0	39.60 ± 2.40
7	6.50 ± 0.15	0	14.95 ± 0.14
8	6.60 ± 0.22	0	48.34 ± 2.47
9	6.50 ± 0.25	0	36.66 ± 6.39
10	6.31 ± 0.01	0	45.03 ± 0.03
11	7.04 ± 0.08	0	27.8 ± 0.60
12	6.76 ± 0.04	0	38.9 ± 0.20
13	6.46 ± 0.02	0	66.07 ± 0.04

Table 6 gives the physicochemical properties of the different creams in the experimental matrix. It is observed that the pH varies from 6.3 to 7.04, the creaming index is 0% and the particle size varies from 14.95 to 76.23 µm. The pH values obtained are in the range recommended by specifications as well as the creaming index. This range is acceptable because the pH range of the skin is between 6.8 and 7.2 [25]. Out of this range, the cream may be irritating to the skin. The particle size tells

us about the size of the droplets of the dispersed phase; we can therefore say that we have a milky white macro-emulsion. This could be due to the type of stirring carried out which corresponds to a particular shear stress allowing poly-dispersion to occur.

Washability of the Emulsion

According to the washability tests carried out, the cream formulation base is easily washable with water, which mean that it is an oil-in-water (O/W) emulsion. The cream obtained here was easily removed by washing with water when applied on the skin, an observation similar to the report of Shubham et al [26].

Physico-chemical Characteristics of the Base Formulation

The results of the optimization process made it possible to obtain the basic formulation in which the active ingredients need to be incorporated. Viscosity, spreading (optimized parameters) and other physicochemical parameters of the base cream are reported in Table 7. It appears that these different parameters fall within the ranges provided for in the formulation specifications [27].

Table 7: Physico-Chemical Parameters of the Base Formulation

Parameters	Cream	Specification Range
Viscosity (cP)	23818.6	20000 – 30000
Spreading (g.cm/s)	438.32	300 – 600
pH	6.49	5 – 7
Creaming Index	0	0
Washability of cream	O/W	O/W

Microbiological Data

Cosmetics preparations may have antimicrobial properties or maybe susceptible to microbial attack if the products are not sterilized [28]. Some creams may harbor certain bacteria species like Pseudomonas aeruginosa, some gram-negative bacteria and Staphylococcus aureus. The quality of cosmetics products maybe dented by certain yeasts and molds. Hence it was expedient to carry out a thorough microbial screening of the formulated cream in order to validate its quality parameters. After obtaining the base cream, the microbiological analyses were done. These analyses made it possible to obtain the results in terms of the presence or absence of microbial colonies. Results given in Table 8 are microbial colonies per culture medium and each dilution. From Table 8, we see that the formulated cream was not potent enough to destroy all microorganisms that can affect the skin due to low concentration of bioactive ingredients. This microbial load did not satisfy the requirements of zero microbial load. However, cosmetic products like creams should be free from pathogens and total aerobic bacterial load should be within safe limits to eliminate the propensity to cause skin infections [29].

Table 8: Microbiological Results of Cream with 0.4% of *T. globiferus* ethanolic extract

Culture Medium	10^{-1}	10^{-2}	10^{-3}	10^{-3}
PDA	+	+	+	+
NA	+	+	+	+

The sign + means the effective presence of different colonies (bacteria and fungi).

To obtain a potent cream capable of reducing the microbial load to zero, a higher concentration of bioactive additive (0.8% w/v of *T. globiferus*) was used and the microbial load reduce to zero.

Antiseptic Cream and its Characteristics

This part aimed to know the minimum bactericidal and antifungal dose of the ethanolic extract of *T. globiferus* to be introduced into the base cream. The amounts of *T. globiferus* extract that was used to render the base cream antiseptic were the minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC) of the plant extract earlier determined during the evaluation of its antifungal and antibacterial activity [12]. Then when the MBC and MFC of the plant extract were used, the microbial load of the cream was brought down to zero in conformity with the antiseptic cream formulation specifications [30]. The characteristics of the antiseptic cream are reported in Table 9. It was noted that the pH value has slightly increased to 6.54 compared to that of the base cream which was 6.49 due to introduction of *T. globiferus* plant extract.

Effectiveness of Antiseptic Cream

The efficiency test was carried out by incorporating the MBC and MFC obtained above into the database formulation. It can be seen from the results that the cream without extract exhibited little activity towards the microbial strain studied. But the antimicrobial activity increases proportionately with the incremental addition of the ethanolic plant extract to the base formulation. That is as the concentration of the ethanolic extract incorporated into the base formulation increases. By comparing these results to those obtained during the antifungal and antibacterial activity evaluation of the extract, it is observed that the active principle incorporated in the formulation increases the inhibition percentage. This is in agreement with the work of Sanchita and Suchitra [31]. In some formulations, the same extract concentration gives higher antimicrobial effects. This may be due to the presence of some preservative additives like methylparaben in the base formulation which possesses some antimicrobial activity. Hence, it can be deduced that the extract of *T. globiferus* proffers antiseptic properties to the base cream at concentrations 500 mg/mL of extract and above.

From Table 10, it can be noticed that the normal base cream without the plant extract (negative control) has some limited antimicrobial activity probably due to the presence of the preservative additive (methylparabene). Then the extended antimicrobial activity of the base cream in which the plant extract is incorporate at a concentration of 250 mg/mL is higher than the negative control. The antimicrobial activity of the cream treated with an extract concentration of 500 mg/mL has greater lethal effects on microbes due to the increased concentration of the leaf extract of *T. globiferus*. The formulated cream stayed clear of microbes at lower and higher temperatures for a

period of 10 days of continuous monitoring.

Table 9: Physico-chemical Characteristics of the Antiseptic Cream

Parameters	Cream	Specification range
Viscosity (cP)	23050	20000–30000
Spreading (g.cm/s)	550.91	300–600
pH	6.54	5–7
Creaming index (%)	0	0
Washability	O/W	O/W

Table 10: Percentage inhibition of antiseptic cream

Batch	Treatment	Percentage Inhibition (%)			
		E. coli	*S. aureus*	*Streptococcus*	*C. albicans*
NC	Cream void of *T globiferus* Extract	24.44	22.22	26.67	22.22
PC	Cream with commercial antibiotic	86.67	86.67	83.33	77.78
Batch 1	Cream with 250 mg/mL of plant extract	40.00	40.00	38.89	44.44
Batch 2	Cream with 500 mg/mL of plant extract	44.44	47.78	46.67	48.89

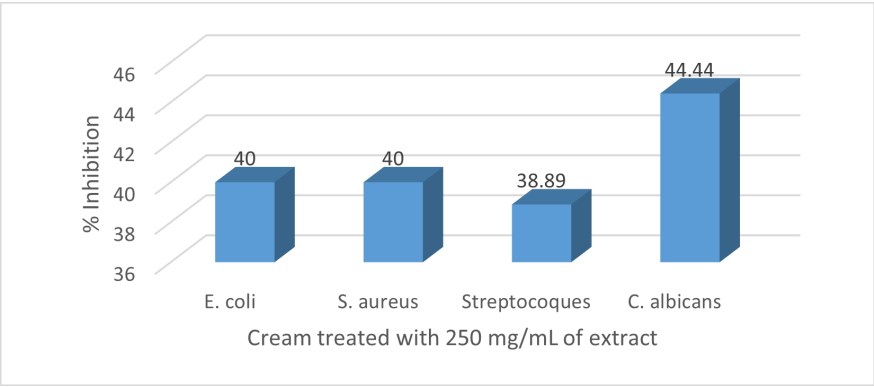

Figure 3: Percentage of inhibition of the cream with 250 mg/mL of *T. globiferus* extract

The 3D bar chart above (Figure 3) shows antiseptic properties of the cream treated with 250 mg/mL concentration of T globiferus leaf extract and Figure 4 is a 3D bar chart showing the inhibitory properties of the formulated cream treated 500mg/mL against pathogenic microbes. These 3D bar charts are more visible forms of Table 10.

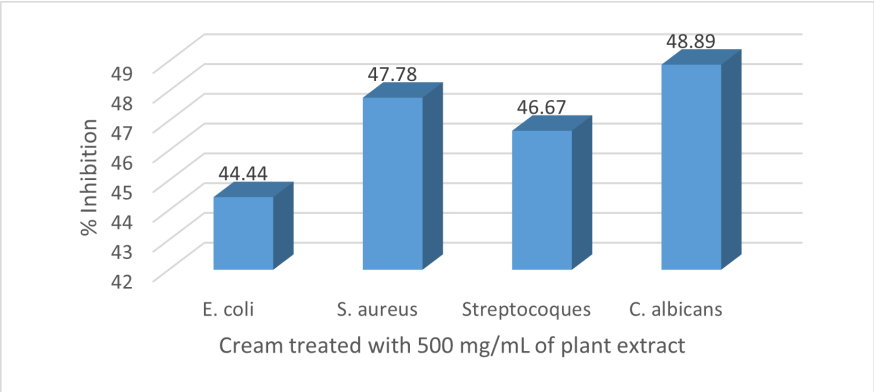

Figure 4: Percentage inhibition of cream with 500 mg/mL of *T. globiferus* extract

Cream Stability Study - Accelerated Aging

The stability of the product was investigated at different temperatures such 5°C, 25°C, 40°C, and alternating the temperatures such that products initially kept at 5°C, are then treated at 40°C and parameters such as pH, physical appearance, coloring, and microbiological parameters were recorded for 10 days. The measurement of these parameters was made before the introduction of these at different temperatures and after.

The evolution of the hydrogen potential is noted in Figure 5. This result shows that the pH varies very little at 5 and 25°C, increases at 40°C with time, and evolves in a sawtooth pattern with alternation between 5°C and 40°C. Cream formulated from a pH point of view is not stable at high temperatures. As for the cream undergoing temperature shocks (freeze-thaw cycle), we note increases when it is introduced at 40°C and tends not to change when it is at 5°C. The increase could be due to the fact that the triethanolamine (pH regulator) present in the medium has neutralized the acid molecules which are perhaps produced under the effect of the temperature, thus contributing to the increase in free molecules in the medium. The almost insignificant variation in pH at 5 and 25°C may be due to the fact that the molecules have not been inhibited, they are fixed in the matrix. Maintaining the cream at these temperatures will help keep the pH stable with time. For good skin tolerance, the cream must be stored at temperatures of 5°C and 25°C. The relative stability of the formulated cream is similar to reports documented by Apriani et al [25].

Physical Appearance (phase separation) - Centrifugation Test

To assess the phase separation, the centrifugation test was carried out and the results are recorded in Table 11. From the results, it is observed that each sample having been placed at different temperatures (5°C, 25°C, 40°C, and 5°C /40°C) is stable for 10 days at different centrifugation speeds, namely 1000 rpm; 3000 rpm and 5000 rpm; and only forms one phase. This stability could be explained by the use of two co-emulsifiers during the formulation, thus ensuring stability over a longer period. The fine particle size parameter is also a contributing factor as homogeneous droplets of small diameters contribute to the observed stability.

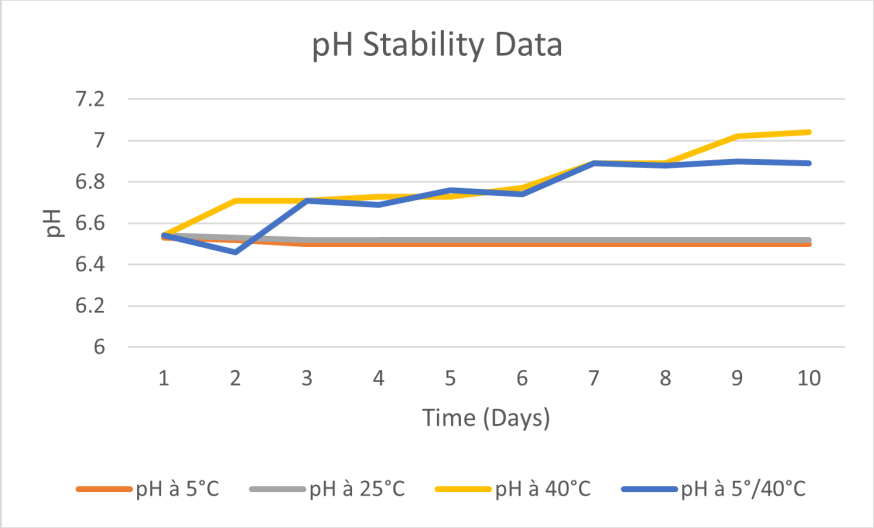

Figure 5: pH Stability Data Over Time

Table 11: Physical appearance of the cream during centrifugation

Temperature	Centrifugal Speed (rpm)		
	1000	3000	5000
5℃	Stable	Stable	Stable
25℃	Stable	Stable	Stable
40℃	Stable	Stable	Stable
5℃/40℃	Stable	Stable	Stable

Colour Stability Analysis

Table 12: Evolution of cream coloring as a function of time at specific temperatures

Table 12 shows the evolution of the coloring of the cream as a function of time at specified temperatures. We observe that at temperatures 5°C and 25°C there is no change in coloring over time but at temperatures 40°C and 5°C/40°C the color becomes more pronounced. These color changes may be due to oxidation which is a characteristic phenomenon of fatty acids going rancid. The increase in temperature over a long period contributes to the organoleptic degradation of the base matrix formulation. The effect of heat also promotes the release of free radicals which are chemically active. On the other hand, at 5 and 25°C, the organoleptic quality of the cream is stable because the free radicals trapped in the matrix by antioxidant compounds, thus shielding the free radicals from air, light, or heat. Hence, the oxidation phenomenon could not take place under such conditions. Therefore, the resultant cream from this work possesses desirable characteristics as it is unable to cause skin infections [32].

Temp.	1	2	3	4	5	6	7	8	9	10
5°C	pale yellow	pale yellow	pale yellow	pale yellow	pale yellow	pale yellow	pale yellow	pale yellow	pale yellow	pale yellow
25°C	pale yellow	pale yellow	pale yellow	pale yellow	pale yellow	pale yellow	pale yellow	pale yellow	pale yellow	pale yellow
40°C	pale yellow	pale yellow	pale yellow	yellow yellow	yellow yellow	yellow yellow	yellow yellow	yellow yellow	yellow yellow	yellow yellow
5°C/40°C	pale yellow	pale yellow	pale yellow	yellow yellow	yellow yellow	yellow yellow	yellow yellow	yellow yellow	yellow yellow	yellow yellow

Conclusion

In this study, the leaf extract of *T. globiferus* was used as a bioactive additive in the formulation of a base cream to impart antiseptic properties for body lotion. The plant extract used in this formulation had undergone thorough characterization in a previous study and found to contain almost all secondary metabolites, phenolic compounds, antioxidant properties, and antimicrobial activity on three pathogenic microbial species including E. coli, S. aureus, Streptococci, and a Candida albicans yeast. The dosage form chosen here was a cream. The search for the optimal formula for the base cream formulation gave the optimal result: 3.77%; 4.43%; 0.30% cetyl alcohol, beeswax, xanthan gum, respectively. Efficacy tests on the formulated cream showed on the one hand that 500 mg/mL has greater activity against microbes than that formulated at 250 mg/mL. Finally, stability studies showed that the antiseptic cream is more stable at 25°C and therefore at ambient temperatures. Hence, in this work, antiseptic cream has been formulated using the leaf ethanolic extract of *T. globiferus* as a bioactive additive.

References

1. Aravindhan V, Niranjanasree AC, Krishnan P, Thirumal V, Rishaanth M, Rohith S. Formulation and evaluation of all purpose cream using box behnken design. *IJRPR*. 2023;4(12):586-595.
2. Reddy PA, Saheb USK, Harika K, Lalitha S, Marina. Formulation, development and evaluation of multi-purpose skin cream. *World J Pharm Res*. 2018;7(9):641.
3. Mat Hussin Z, Irfan Zakry NA. Formulation and evaluation of cream from stem barks of *Pithecellobium jiringa*. *GJST*. 2020;3(1):91-9.
4. Budhiraja H, Gupta RK, Nand P. Formulation and characterization of *Cucumissativus* extract in the treatment of acne. *WJPPS*. 2014;3(12):1034-57.
5. Smaoui S, Hlima HB, Jarraya R, Kamoun NG, Ellouze R, Damak M. Cosmetic emulsion from virgin olive oil: Formulation and bio-physical evaluation. *AJB*. 2012;11(40):9664-71.
6. Rathore P, Patidar K, Soni M. Formulation and evaluation of polyherbal dermatological cream. *IJPBA*. 2014;5(5):153-7.

7. Kewlwni A. Phytochemical analysis and antimicrobial activity of selected medicinal plants. Dissertation report, Atmiya University; 2022.

8. Heredia-Castro PY, García-Baldenegro CV, Santos-Espinosa A, Tolano-Villaverde ID, Manzanarez-Quin CG, Valdez-Domínguez RD, et al. Phytochemical profile, antimicrobial and antioxidant activity of extracts of *Gnaphalium oxyphyllum* and *Euphorbia maculata* native to Sonora, Mexico. *Rev Mex Cienc Pecu*. 2022;13(4):928-942.

9. Daoud A, Malika D, Bakari S, Hfaiedh N, Mnafgui K, Kadri A, et al. Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of date palm pollen (DPP) from two Tunisian cultivars. *AJC*. 2019;12(8):3075-86.

10. Bakari S, Hajlaoui H, Daoud A, Mighri H, Ross-Garcia JM, Gharsallah N, Kadri A. Phytochemicals, antioxidant and antimicrobial potentials and LC-MS analysis of hydroalcoholic extracts of leaves and flowers of *Erodium glaucophyllum* collected from Tunisian Sahara. *Food Sci Technol*. 2018;38(2):310-17.

11. Kakade SS, Khedkar AN. Formulation and evaluation of herbal moisturizing cream. *IJNRD*. 2023;8(11):1-15.

12. Bong AM, Benoit NM, Sohanang N, Danielle FD, Akah NV. Phytochemical screening, antimicrobial properties and pharmaceutical applications of *Tapinanthus globiferus* leaf extract. *EJAS*. 2024;12(4):353-63.

13. Saptarini NM, Hadisoebroto G. Formulation and evaluation of lotion and cream of nanosized chitosan-mangosteen pericarp extract. *Rasayan J Chem*. 2020;13(2):789-95.

14. Hamishehkar H, Same S, Adibkia K, Zarza K, Shokri J, Taghaee M, et al. A comparative histological study on the skin occlusion performance of a cream made of solid lipid nanoparticles and vaseline. *Res Pharm Sci*. 2015;10:378-87.

15. Nakhli A, Mbouga MGN, Bergaoui M, Khafaoui M, Cretin M, Huguet T. Modeling of essential oils adsorption onto clays towards a better understanding of their interactions. *JML*. 2018;249:132-43.

16. Sharma PP. *Cosmetics-formulation, manufacturing and quality control*. Cap. 7. 5th ed. Delhi, India: Vandana Publications; 2014. p. 181-91.

17. Bhalekar M, Manish L, Krishna S. Formulation and evaluation of rice bran wax as ointment base. *Anc Sci Life*. 2004;24:52-9.

18. Akanksha D, Vikas G, Neetesh KJ, Shalendra S, Neelam B, Dinesh KJ. Formulation and evaluation of neomycin sulphate ointment containing natural wound healing agent *Curcuma longa*. *IJPSDR*. 2009;1:105-18.

19. Tsatsop RK, Djiobie G, Regonne K, Bama V, Mbawala A, Ngassoum M. Optimization of rheological properties in the formulation of an ointment base from natural ingredients. *IJSTR*. 2017;6(9):1-9.

20. Ibegbulam-Njoku PN, Chijioke-Osuji CC. Microbiological evaluation of cosmetics products sourced in Aba city, Nigeria. *IJSR*. 2015;2(4):74.

21. Ouryemchi I, Oubihi A, Taibi M, Elbouzidi A, Jaber H, Haida S, et al. Phytochemical components, antioxidant properties, antimicrobial effects, and bio-aphicidal prospects against the black bean aphid (*Aphis fabae* Scop) of *Olea europaea L.* leaves extracts from Morocco. *JEE*. 2024;25(3):64-74.

22. Tonapa MI, Pratiwi RD, Gunawan E. Formulation and stability test of lip cream preparation of kenop flower ethanol extract (*Gomphrena globosa L.*). *JPRD*. 3(2):57-64.

23. Claeys-Bruno M, Lamant J, Blasco L, Phan-Tan-Luu R, Sergent M. Development of a skin care formulation using experimental designs. *CILS*. 2009;96(2):101-7.

24. Maru AD, Lahoti SR. Formulation and evaluation of ointment containing sunflower wax. *Asian J Pharm Clin Res*. 2019;12(8):115-20.

25. Apriani EF, Nurleni N, Nugrahani N, Iskandarsyah I. Stability testing of azelaic acid cream based ethosome. *Asian J Pharm Clin Res*. 2018;11(4):1-4.

26. Pawar ST, Bhagat VP, Jadhav VA, Rode AR. Formulation and evaluation of moisturizing cream. *IRJMETS*. 2023;5(5):1-9.

27. Mali AS, Karekar P, Yadav AV. Formulation and evaluation of multipurpose herbal cream. *IJSR*. 2015;4(11):1495-98.

28. Jimenez L, Ignar R, Smalls S, Grech P, Hamilton J, Bosko Y, et al. Molecular detection of bacterial indicators in cosmetic/pharmaceuticals and raw materials. *JIMB*. 1999;22(2):93-5.

29. Siegert W. Microbiological quality management for the production of cosmetics and detergents. *Seifen Ole F Wachse*. 2012;138:30.

30. Eichie FE, Arhewoh MI, Isesele JE, Okoh EO. Antimicrobial activity of extract and topical cream formulation of *Mitracapus villosus* (Rubiaceae). *JPB*. 2011;8(2):76-9.

31. Choubey S, Godbole S. Methods for evaluation of microbiological safety, guidelines governing the quality and survey on microbial contamination of commercial cosmetic products: A review. *WJPMR*. 2017;3(8):85-94.

32. Aleem A, Khan M, Abid U, Ishfaq MF, Rouf A, Jamshaid T. Microbial analysis of selected brands of whitening creams. *Saudi J Med Pharm Sci*. 2020;6(2):178-182.