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ORIGINAL ARTICLE

Formulation and Evaluation of an Anti-Aging Cream Incorporating *Sauropus androgynus* Leaf Extract: Harnessing Natural Antioxidants for Skin Health

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ABSTRACT
This study aimed to develop an effective anti-aging cream by incorporating Sauropus androgynus leaf extract, renowned for its antioxidant properties. Various formulations were prepared and evaluated for physicochemical properties, antioxidant activity, and antibacterial efficacy. Sauropus androgynus leaf extract was obtained through Soxhlet extraction and cold maceration. Formulations of anti-aging cream were prepared and assessed for appearance, pH, spreadability, and viscosity. The DPPH assay was used to measure antioxidant activity, and antibacterial efficacy was evaluated using the agar well diffusion method. Accelerated stability testing was conducted to assess formulation stability.Physicochemical evaluation revealed desirable properties in formulations with higher concentrations of Sauropus androgynus leaf extract. The optimized formulation demonstrated the highest antioxidant activity and potent antibacterial efficacy against tested strains. Stability testing confirmed the formulation's stability under accelerated storage conditions.The study successfully developed an anti-aging cream incorporating Sauropus androgynus leaf extract with potent antioxidant and antibacterial properties. These findings highlight the potential of natural ingredients in skincare formulations and contribute to the development of novel anti-aging solutions. Further research is warranted to optimize formulation parameters and explore additional benefits of Sauropus androgynus in skincare.
***Keywords**: Skin aging, Sauropus androgynus, antiaging cream, gallic acid, antioxidant, DPPH assay.*

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INTRODUCTION

Skin aging is a complex process marked by changes in texture, elasticity, and appearance. It is influenced by intrinsic factors like genetics and hormones, and extrinsic factors such as sun damage and pollution. Together, these factors lead to wrinkles, dryness, and loss of firmness. [1].

Central to the aging process is the role of free radicals, highly reactive molecules that induce oxidative stress and cellular damage. Reactive oxygen species (ROS), a subset of free radicals generated primarily by oxygen metabolism, instigate cellular deterioration, leading to various skin manifestations including age spots, sagging, and wrinkles. Consequently, the imbalance between the production of free radicals and the body's antioxidant defense mechanisms becomes a pivotal determinant in the progression of skin aging [2].

Antioxidants emerge as critical agents in mitigating the effects of oxidative stress on skin health. Their ability to scavenge free radicals and neutralize their harmful effects underscores their significance in

skincare formulations. Natural antioxidants, such as vitamins C and E, alongside botanical extracts rich in polyphenols and flavonoids, offer promising avenues for combating oxidative damage and preserving skin vitality [3].

In response to the demand for anti-aging solutions, the cosmetic industry has developed topical formulations with active ingredients, antioxidants, and moisturizers for skin rejuvenation. This study aims to incorporate *Sauropus androgynus* extract, known for its antioxidant properties, into an anti-aging cream. The goal is to assess the cream's effectiveness in addressing age-related skin concerns, promoting skin fairness, and providing antimicrobial benefits, blending traditional knowledge with modern science [4].

MATERIAL AND METHODS

Materials

Stearic acid, cetostearyl alcohol, potassium hydroxide were purchased from S D Fine-Chem, Mumbai. 2,2-Diphenyl-1-picryl hydrazyl (DPPH) was obtained from Sigma Aldrich. All other chemicals used were of analytical grade.

Plant Extraction

The methanolic leaf extract of *Sauropus androgynus* plant was prepared following a modified method described by Sujitha Kuttinath et al. The collected leaves were washed thrice with water and then shade dried for 14 days. After drying, the leaves were ground to a coarse powder (140 gm) and stored in an air-tight container. The extract was obtained through Soxhlet extraction and cold maceration using 70% methanol. The resulting mixture was filtered using Whatman filter paper, and excess methanol was evaporated at 70°C. The obtained semisolid extract was then stored in an air-tight container [5].

Phytochemical screening of leaf extract of *Sauropus androgynus*

The freshly prepared crude extract of *Sauropus androgynus* underwent phytochemical screening to identify its chemical constituents. Carbohydrates were detected through a reaction with Molish reagent and concentrated H2SO4, resulting in the formation of a brown ring. Glycosides were identified by producing a pink color when treated with pyridine, sodium nitroprusside, and NaOH. Flavonoids were confirmed by observing a color change (purple, orange, or red to purple) upon treatment with ethanol, magnesium chips, and concentrated HCl. Tannins were also detected through a similar color change. Finally, alkaloids were indicated by the formation of a reddish-brown precipitate upon mixing with Dragendorff's reagent [6].

Total phenolic content: Standard Calibration Curve of Gallic Acid: Gallic acid standard solutions ranging from 2 to 28 µg/mL were prepared in methanol. Each solution was mixed with 10% Folin-Ciocalteu reagent and 20% Na_2CO_3, adjusted to 10 mL, and incubated for 30 minutes. Absorbance was measured at 658 nm [7].

Sample Preparation: A plant extract solution (100 µg/mL) was prepared in methanol. This solution was mixed with 10% FC reagent, 20% Na_2CO_3, adjusted to 10 mL, and treated similarly to the standard solutions. TPC was determined using the calibration curve, and results were expressed as mg GAE/g. All experiments were triplicated.

Total Flavonoid Content Determination:

Standard Calibration Curve of Quercetin: A Quercetin standard solution (100 µg/mL) was prepared by dissolving 10 mg of pure Quercetin in 100 mL methanol. Concentrations ranging from 2 to 24 µg/mL were prepared, mixed with distilled water, and then treated with NaNO2, AlCl3, and NaOH. Absorbance was measured at 510 nm, and triplicate readings were taken to construct the calibration curve [8].

Sample Preparation: A 10 mg plant extract was dissolved in 100 mL methanol to achieve a concentration of 100 µg/mL. This solution was mixed with water and treated similarly to the standard solutions. Absorbance was measured at 510 nm, and triplicate readings were taken to determine the Total Flavonoid Content (TFC) using the calibration curve. Results were reported as mg quercetin equivalents per gram of dry weight (mg QE/g).

Quantification of plant extract by HPLC

HPLC was employed to quantify gallic acid in *Sauropus androgynus* leaf extract, following the method by Rency Elizabeth Thomas et al. A standard solution of gallic acid (1000 mg) was prepared in 70% methanol and injected into the HPLC system. The separation occurred in a column, where the components were separated based on their chemical properties. Detection was performed using a suitable detector, with quantification based on detector response. The methanolic extract of *Sauropus androgynus* was similarly processed. Retention times and peak properties from the obtained chromatograms were used to determine the concentration of gallic acid in the extract [9].

***In vitro* antioxidant study plant extract**

The antioxidant activity of *Sauropus androgynus* leaf extract was evaluated via the DPPH free radical scavenging assay. DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL methanol, covered for 30 minutes. Standard ascorbic acid solution was prepared by dissolving 10 mg of ascorbic acid in 100 mL methanol, diluted to concentrations of 20, 40, 60, 80, and 100 µg/mL. Similarly, the extract solution was prepared by dissolving 10 mg of leaf extract in 100 mL methanol, diluted to the same concentrations. Each concentration (1 mL) was mixed with 3 mL DPPH solution and adjusted to 10 mL with methanol, followed by absorbance measurement at 517 nm. A control solution of 6 mL methanol and 3 mL DPPH solution was also prepared for comparison [10].

% Scavenging Activity =(Absorbance of Control – Absorbance of sample)/(absorbance of control)× 100

Drug-excipient compatibility study by FTIR

The cream formulation's stability by storing it in amber-colored bottles in a stability chamber set to 40°C ± 2°C and 75% ± 5% RH for one month. [11] Following this, samples underwent FTIR spectroscopy (FTIR-8400S, Shimadzu, Japan) to evaluate API-exicipient compatibility over a scanning range of 400-4400 cm^{-1}.

PREPARATION OF ANTIAGING CREAM O/W CREAM

The required quantities (Table.1) of the oily phase ingredients (stearic acid, cetostearyl alcohol, lanolin, and sunflower oil) were taken in a china dish and heated to 75°C. In another china dish, propylene glycol and KOH were added to distilled water and also heated to 75°C. The aqueous phase was then added to the oily phase with vigorous agitation. The extract was dissolved in methanol and sonicated for 10 minutes. Preservatives and a flavoring agent were added to the extract solution. Finally, the medication combination was slowly added to the mixture with gentle agitation to form the anti-aging cream [12, 13].

EVALUATION OF ANTIAGING CREAM

pH determination

The pH of the formulations was measured using a digital pH meter. The electrode was calibrated with a phosphate buffer (pH 7.4). A 10% solution of the formulation was prepared, and the electrode was immersed in the solution. Readings were taken in triplicate [13].

Spreadability

The spreadability of the cream was evaluated using the parallel plate method. Two glass slides, each 20 cm in length, were utilized for the assessment. One gram of cream was placed on one glass slide, and another slide was placed on top. A weight of 125 g was applied to the upper slide for 1 minute. After removing the weight, the diameter of the spread circle was measured in centimeters [14].

Viscosity determination

The viscosity of the prepared anti-aging cream was measured using the Brookfield Viscometer DV-2P device with spindle no. TL4 at a speed of 10 rpm [15].

Dye test

Cream evaluation incorporates the use of a water-soluble dye, like scarlet red. A drop of the cream is positioned on a microscope slide, covered with a slip, and observed under a microscope. In an O/W type cream, dispersed globules would be red, with the continuous phase colorless. Conversely, in a W/O type cream, dispersed globules would be colorless [16].

***In vitro* drug diffusion study**

The permeation study of the anti-aging cream utilized a Franz diffusion cell with a 150 µm dialysis membrane pre-soaked in pH 7.4 phosphate buffer for 24 hours. One gram of the cream was evenly spread on the membrane between donor and receptor chambers, with the latter containing 12.5 mL of pH 7.4 phosphate buffer as the dissolution media. The setup was maintained at 37±0.5°C, stirring the receptor compartment with a magnetic bead. Samples were withdrawn at specified intervals over 5 hours, diluted with buffer solution, and analyzed for absorbance at 270 nm using a UV spectrophotometer to assess the cream's permeation characteristics over time [17].

***In vitro* antibacterial study**

The antibacterial activity of antiaging cream F6 was assessed and compared with a ciprofloxacin standard disc using Whatman filter paper. Staphylococcus epidermidis and Pseudomonas aeruginosa were employed as test organisms. Mueller Hinton agar (MHA) was prepared by autoclaving a specified quantity of powder in distilled water, which was then poured into Petri plates. These plates were inoculated with the test organisms and subsequently incubated at 37°C for 24 hours. A concentration of 100 µg/mL of the antiaging cream was utilized for the assay. Clear zones of inhibition observed around the wells containing the cream or ciprofloxacin disc after incubation indicated antibacterial activity against the tested organisms [18].

Accelerated Stability study

The chosen cream formulation was securely sealed and subjected to storage at 40°C ± 2°C / 75% RH ± 5% RH for 3 months. Subsequent assessments included evaluating physical appearance, pH, viscosity, phase separation, as well as In vitro antioxidant and drug release studies [17].

RESULTS

Yield from Extraction Methods

The yield from Soxhlet extraction was found to be 14.23%, whereas cold maceration yielded 7.02%.

Phytochemical screening of plant extract:

Table 2 summarizes the results of phytochemical tests conducted on the *Sauropus androgynus* leaf extract, revealing the presence of carbohydrates, glycosides, flavonoids, tannins, phenols, and alkaloids.

Total phenolic content:

The quantitative assessment of total phenols was conducted using a standard curve derived from gallic acid. The calibration curve demonstrated linearity over the concentration range of 2 to 28 µg/mL for gallic acid, exhibiting a strong correlation with an R-squared value of 0.991. The total phenolic content in the plant extract was determined to be 9.83 mg GAE/gm of dry extract.

Total flavonoid content:

Total flavonoids were quantitatively analyzed using a standard curve with quercetin, showing excellent linearity (2-24 µg/mL) and a high correlation (R-squared = 0.9904). The plant extract contained 7.41 mg quercetin equivalents per gram of dry extract.

Quantification of plant extract by HPLC:

Quantitative analysis of plant extract was quantified by HPLC. Gallic acid, the marker present *Sauropus androgynus* plant was estimated. The gallic acid in the plant extract was found to be 24.06 % shown in Table 3.

***In vitro* antioxidant activity of plant extract:**

The I*n vitro* antioxidant study was carried out by DPPH Scavenging Assay. Plant extract showed the highest % of inhibition to DPPH when compared to standard drug ascorbic acid. Plant extract showed 80 % of inhibition while ascorbic acid 73 % of inhibition and the antioxidant activity of ascorbic acid and plant extract was shown in Table 4 and Figure 1.

Compatibility study using FTIR Spectroscopy:

The peaks can be regarded as characteristic peaks of pure gallic acid, plant extract, and the cream formulation. The wave numbers associated with different functional groups obtained from the literature match the reported values. These results indicate that the excipients used in the cream formulation are compatible with the drug and do not cause any significant alteration in the pharmacological activity of the drug. FTIR spectra was shown in Figure. 2, 3 & 4

Evaluation parameter:

Appearance

The formulations exhibited appearances ranging from pale green to pale green-white.

pH

pH values ranged from 6.3 to 6.8, with slight variations observed among the formulations.

Spreadability

Spreadability, measured in centimeters, ranged from 5.4 to 6.0 cm.

Viscosity

Viscosity, measured in centipoises (cps), ranged from 26544 to 27550 cps.

***In vitro* antioxidant activity of cream:**

Among all the prepared formulations, F3 and F6 showed the highest % of inhibition to DPPH. As the quantity of plant extract increases the inhibition also increased. F3 and F6 contains 4.5 % of plant extract which scavenges 49.97 % & 53.27 % of DPPH respectively and In vitro anti-oxidant activity of prepared formulations are shown in Table 5.

Dye test:

When cream was mixed with water soluble dye scarlet red and observed under the microscope, the dispersed globules appeared red and continuous phase appeared colorless. It is concluded that the prepared antiaging cream are O/W cream.

***In vitro* drug release study:**

The % cumulative drug permeation values of all formulations after 5 hrs. ranged between 73 % to 88 %. F6 showed the highest drug permeation at the end of 5 hrs i.e.87.89 %. The comparative diffusion profile of all formulations shown in figure.5

***In vitro* antibacterial study:**

The assessment of antibacterial activity involves measuring the diameter of the zone of inhibition in millimeters, representing the area where bacterial growth is absent. The optimized formulation F6 exhibited a zone of inhibition measuring 13 mm against Pseudomonas aeruginosa and 10 mm against Staphylococcus epidermis, as illustrated in Figure 6

Accelerated stability study: Stability study on optimized formulation F6 was carried out at 40 °C±2 °C/75 %RH ± 5 %RH. The formulation was found to be stable throughout the Accelerated storage conditions 40 °C±2 °C/75 %RH±5 %RH. There was no phase separation found in cream formulation.

Table 1: Formulation table of antiaging cream

Ingredients %	Category	F1	F2	F3	F4	F5	F6
S.A. leaf extract (mg)	API	1.5	3	4.5	1.5	3	4.5
Stearic acid (mg)	Emulsifier	13	13	13	13	13	13
Cetostearyl alcohol (ml)	Emulsifier	2	2	2	2	2	2
Sunflower oil (ml)	Emollient	4	4	4	4	4	4
Lanolin (ml)	Emollient	0.5	0.5	0.5	0.5	0.5	0.5
KOH (mg)	Stabilizer	0.36	0.36	0.36	0.36	0.36	0.36
Tween 80 (mg)	O/W surfactant	-	-	-	2	2	2
Propylene glycol (ml)	Humectants	3.7	3.7	3.7	6	6	6
Tea tree oil (ml)	Preservative	0.2	0.2	0.2	0.2	0.2	0.2
Orange oil (ml)	Flavouring agent	0.5	0.5	0.5	0.5	0.5	0.5
Distilled water (ml)	Vehicle	qs	qs	qs	qs	qs	qs

Table 2: Phytochemical tests of plant extract

Sr.no.	Phytochemicals	*Sauropus androgynus* leaf extract
1	Carbohydrates	+
2	Glycosides	+
3	Flavonoids	+
4	Tannins	+
5	Phenols	+
6	Alkaloids	+

Table 3: Data obtained from the HPLC chromatograms

Title	Retention time	Area	Drug content (%)
Gallic acid	3.43	7455719	100
Plant extract	3.39	1522169	24.06

Table 4: Antioxidant activity of ascorbic acid and plant extract

Sr. no.	Concentration (mg/ml)	Ascorbic acid (%inhibition)	Plant extract (%inhibition)
1	20	34.3	39.44
2	40	41.67	47.39
3	60	49.5	56.99
4	80	59.37	68.12
5	100	73.45	80.27

Table 5: pH, Spreadability, viscosity and *In-vitro* antioxidant activity of prepared formulations

Formulation	pH	Spreadability (cm)	Viscosity (cps)	% inhibition
F1	6.3 ± 0.05	5.4 ± 0.1	27125	28.11
F2	6.4 ± 0.11	5.5 ± 0.05	26544	40.52
F3	6.4 ± 0.17	5.6 ± 0.05	27230	49.57
F4	6.6 ± 0.05	5.6 ± 0.15	27237	30.64
F5	6.6 ± 0.05	5.7 ± 0.05	27383	41.34
F6	6.8 ± 0.05	6.0 ± 0.15	27550	53.27

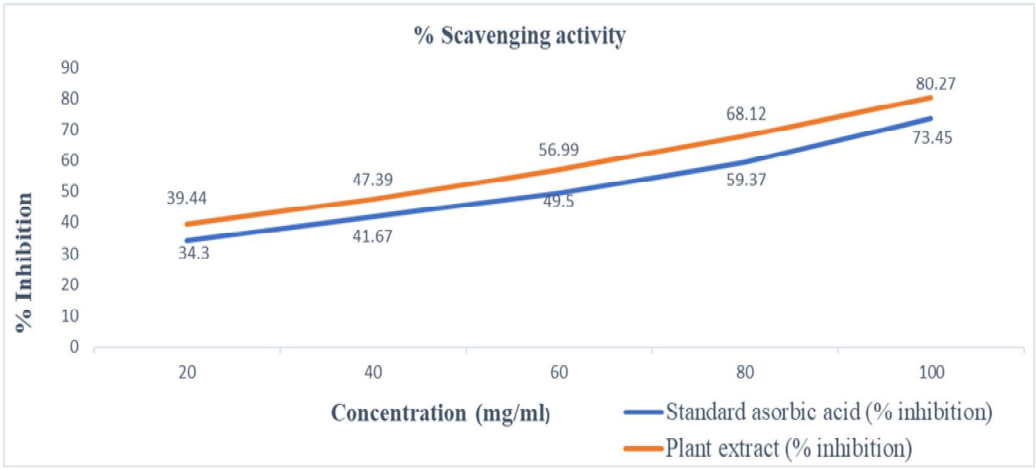


Figure. 1: Antioxidant activity of ascorbic acid and plant extract

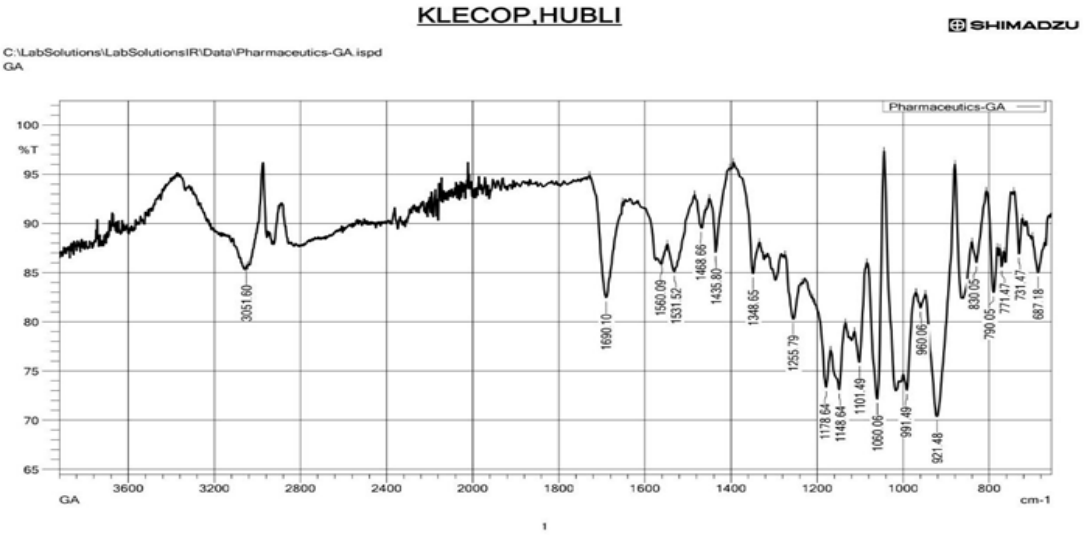


Figure 2: FTIR Spectrum of Gallic acid

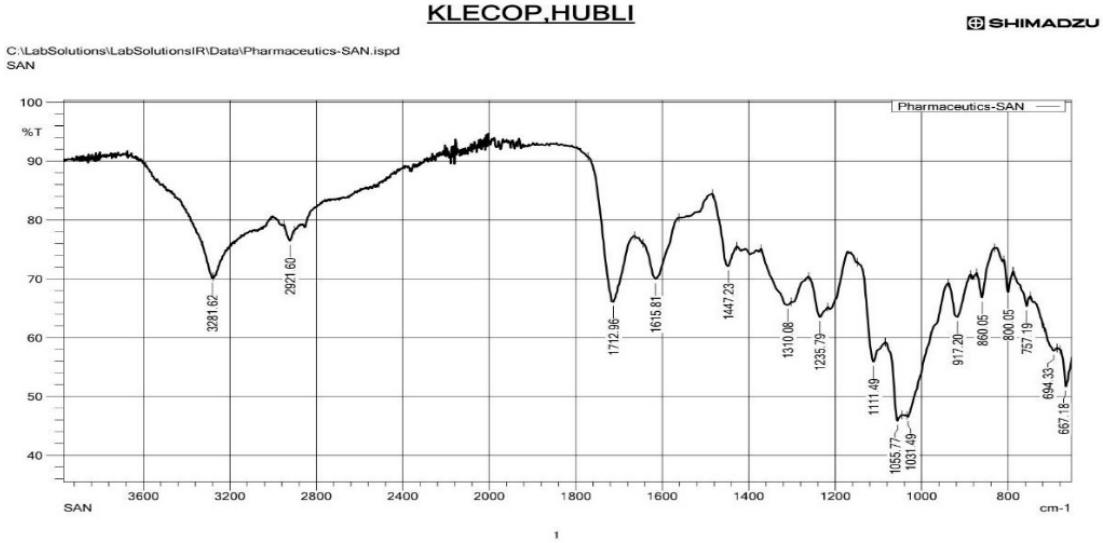


Figure 3: FTIR spectrum of *Sauropus androgynous* extract

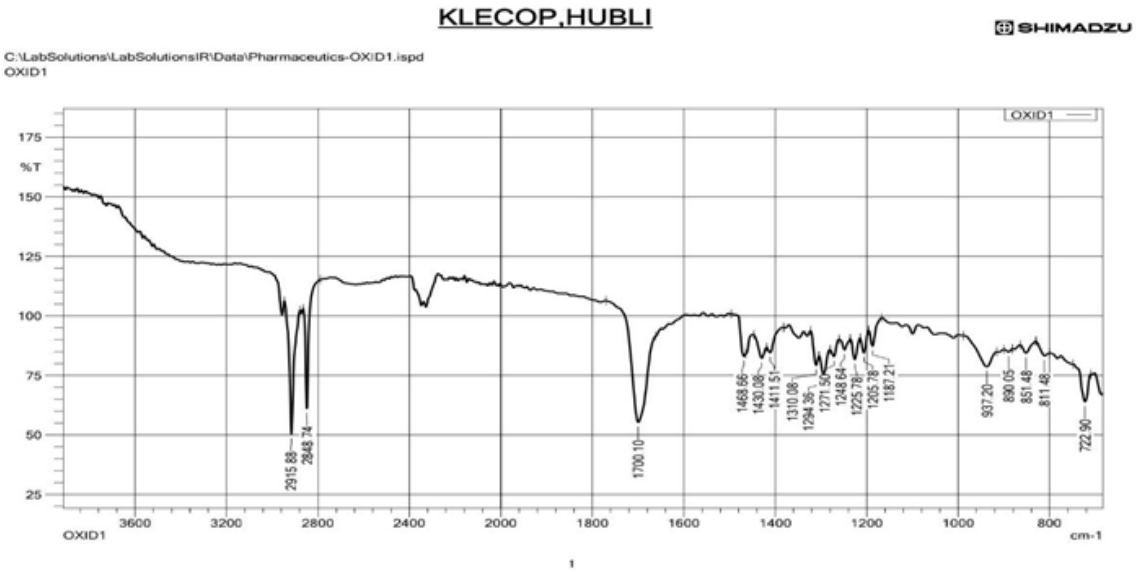


Figure 4: FTIR spectrum of antiaging cream formulation

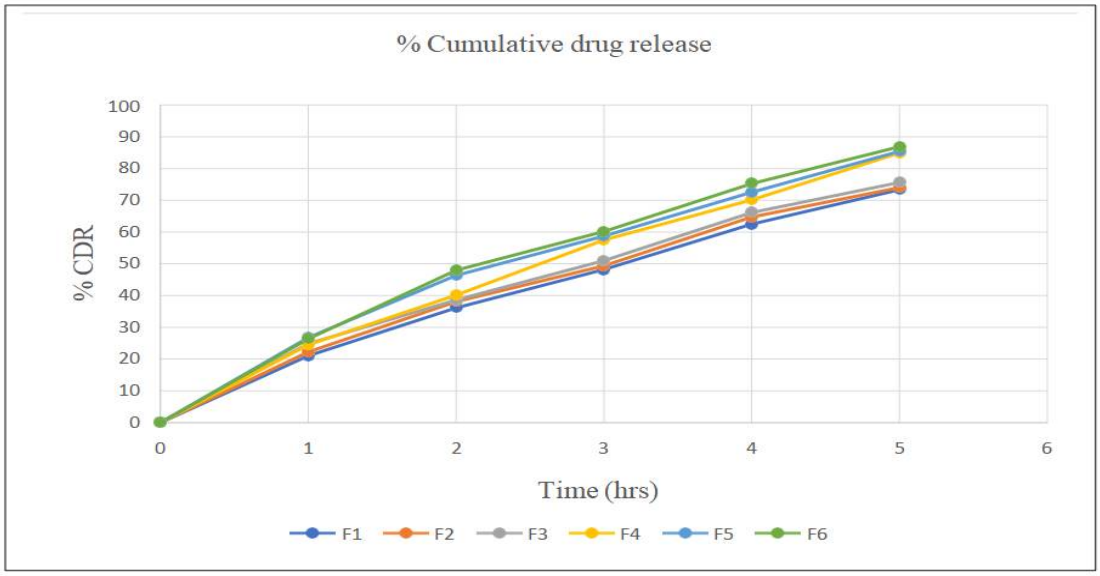
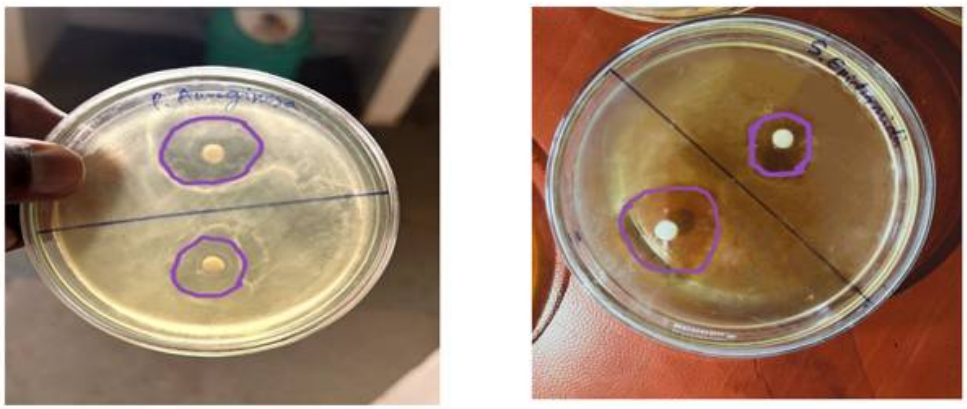


Figure 5: *In vitro* drug diffusion study of formulations.



a) b)

Figure 6 : zone of inhibition a) *Pseudomonas aeruginosa* b) *Staphylococcus epidermis*

DISCUSSION

The Soxhlet extraction method resulted in a higher percentage yield compared to cold maceration. The phytochemical screening of the extract revealed the presence of carbohydrates, glycosides, flavonoids, tannins, and alkaloids. The quantification of the extract was done using UV spectroscopy and HPLC. FTIR analysis indicated good compatibility between the plant extract and the excipients used in the formulation, with no reported interactions.

A successful antiaging cream was formulated using *Sauropus androgynus* leaf extract. The color of the formulations ranged from pale green-white to pale green, and their consistency was rated as good to excellent. The pH of all formulations fell within the range of 6.3 to 6.7, indicating compatibility with the skin and minimizing the risk of skin irritation upon application. The spreadability of the formulations ranged from 5.4 cm to 5.7 cm, indicating easy application. The viscosity of the formulations was within the ideal range for topical preparations.

The formulations were also tested for their antioxidant activity, and F3 and F6 showed the highest percentage of antioxidant activity. However, F3 faced stability issues, leading to F6 being selected as the best formulation. The drug release from F6 was found to be 87.89 %.

Based on its antioxidant activity, physical appearance, and drug release profile, F6 was identified as the best formulation. The results confirmed that F6 possessed antibacterial activity, and it remained stable throughout the accelerated storage conditions.

CONCLUSION

The issue of aging has become a significant concern for individuals, leading them to turn to anti-aging cosmetics. Unfortunately, these products have resulted in harmful consequences, prompting the shift towards cosmetics that incorporate herbal ingredients with fewer adverse effects. The primary components of anti-aging cosmetics are antioxidants.

Antiaging cream using *Sauropus androgynus* leaf extract was prepared successfully. Six formulations of cream were prepared using stearic acid, sunflower oil, lanolin, Tween 80, propylene glycol and different concentration of plant extract. All the formulations exhibited antioxidant activity. The prepared antiaging cream also possessed antibacterial capacity. Among them F6 formulation had the best physiochemical properties, highest antioxidant capacity (44.27 %), *In vitro* drug release (87.89 %) and stable throughout the stability studies.However, further research is needed to explore the full potential of *Sauropus androgynus* in anti-aging cosmetics. It is important to identify the optimal concentration and combination of herbal ingredients to maximize their anti-aging effects.

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