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Formulation and Evaluation of Herbal Moisturizing Cream using Polyherbal Extract

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ABSTRACT

Herbal cosmetic formulations are increasingly preferred for their safety and multifunctional skin benefits. This study aimed to formulate and evaluate a herbal moisturizing cream containing *Laurus nobilis* leaf extract, *Cucumis sativus* extract, and *Aloe vera* gel, and to assess its antimicrobial activity, anti-inflammatory activity, and cytotoxicity by MTT assay. Hydroethanolic polyherbal extract was incorporated into oil-in-water creams, alongside a placebo base. The formulations were evaluated for organoleptic properties, pH, viscosity, spreadability, homogeneity, washability, irritancy, and stability. Antimicrobial activity was determined by agar well diffusion against *Staphylococcus aureus*, and *Escherichia coli*. Anti-inflammatory activity was assessed *in vitro* by protein denaturation inhibition using bovin serum albumin as standard. Cytotoxicity was evaluated by MTT assay on a suitable cell line, with cell viability. The creams were smooth, homogeneous, and spreadable, with a skin-compatible pH (5.5-6.5) and no phase separation during stability testing. The herbal cream showed concentration-dependent antimicrobial activity and significant anti-inflammatory activity comparable to the standard drug. The MTT assay indicated high cell viability and low cytotoxicity at tested concentrations. The polyherbal cream was stable, safe, and effective, offering a promising natural alternative for skin hydration and management of mild skin inflammation and infections.

***Keywords*:** Herbal cream, Polyherbal extract, antimicrobial, anti-inflammatory, MTT assay

1. INTRODUCTION

Skin, an important external organ, protects the body from various infections and injuries and is also highly susceptible to infection due to microbial infection, stress, weather condition etc. In India, the common skin diseases reported amongst the populations are acne, psoriasis, scabies, tinea, impetigo, inflammation leucoderma, and eczema **(Sardana *et al.*, 2009).** Many medico-ethnobotanical surveys have conducted, and data are published on the medicinal plants used in skin disorders sought that, still most of the inhabitants, both from rural/urban and indigenous are, rely on the herbal formulation in the treatment of skin diseases **(Nagariya*et al.*, 2010)**

The skin serves three primary functions: it acts as the first line of defense against external threats such as pathogens, chemicals, and physical interactions; it prevents water loss; and it regulates body temperature (**Kabashima*et al.*, 2019).** This protective mechanism involves a complex series of tissue alterations designed to eliminate initial sources of cellular damage, which can stem from infectious agents, metabolic by products (including microorganisms and toxins), physical factors (such as radiation, burns, and trauma), or chemical agents (like corrosive substances). Common signs of inflammation include localized redness, swelling, pain, elevated temperature, and reduced functionality **(Clara dos Reis Nunes *et al.*, 2020)**

The necessity for exploring alternative options that may prove to be more advantageous has become evident. A review of existing literature indicates that numerous plant species across the botanical spectrum possess anti-inflammatory properties. In this context, secondary metabolites derived from natural sources present significant potential for the creation of innovative anti-inflammatory agents **(Dunaway *et al.*, 2018).**

Natural products (NPs) sourced from various parts of plants, including stems, leaves, roots, fruits, and seeds, represent a rich reservoir of metabolites with significant

pharmacological implications in medicine **(Fernandes *et al.*,2023).** A multitude of studies has validated the effectiveness of NPs in treating diverse skin ailments. The escalating challenge of antibiotic resistance has further propelled research into NPs, driven by the urgent demand for new compounds exhibiting antibacterial and anti-inflammatory activities **(Mahdi *et al.*,2022).**NPs present a range of potential therapeutic options for various skin conditions, demonstrating considerable anti-inflammatory and antioxidant effects, as well as the ability to modulate immune responses in the skin. Various naturally occurring compounds are recognized by distinct membrane-bound immune receptors in the skin, which can initiate different immune responses beneficial for managing skin disorders **(Fernandes *et al.*,2023).**

The study the anti-inflammatory activity of individual as well as combination of extracts in a single dosage form which may show synergistic anti-inflammatory activity Polyherbalism offers a cost-effective approach to medication by shortening treatment durations and lowering individual expenses associated with anti-inflammatory and antimicrobial drugs. (**Suresh Kumar Dev *et al.*,2019)**that traditional Indian medicine favors complex herbal combinations over single-plant preparations, as the later often fail to yield the desired therapeutic outcomes. In contrast, polyherbal extracts demonstrate greater efficacy than their individual components due to their synergistic properties **(Tayyba Saher *et al.*, 2022).** The concept of polyherbal formulation (PHF) presents considerable potential increasing availability and lower costs of these formulations are driving their rising global popularity, especially in areas where expensive modern medical treatments are impractical. Polyherbal formulations are carefully designed by combining various herbs in precise ratios to optimize therapeutic effects while reducing the risk of toxicity. (**Muhammad Shahzad Aslam *et al.*,2016)**

Laurus nobilis (bay leaf) is a medicinal plant known for its antioxidant, antimicrobial, and anti-inflammatory properties. The leaves contain bioactive compounds such as flavonoids, phenolics, and essential oils, which contribute to skin protection, soothing effects, and prevention of microbial infections. These properties make Laurus nobilis a valuable ingredient in topical cosmetic formulations.

Cucumis sativus (cucumber) is widely recognized for its cooling, hydrating, and skin-refreshing effects. It is rich in water content, vitamins, minerals, and antioxidants, which help in maintaining skin moisture, reducing inflammation, and improving skin tone. Cucumber extract is commonly used in cosmetic products for its soothing and moisturizing properties.

Aloe vera is a well-known medicinal plant extensively used in dermatological and cosmetic preparations. It contains polysaccharides, vitamins, amino acids, and enzymes that provide excellent moisturizing, wound-healing, anti-inflammatory, and skin-protective effects. Aloe vera gel helps in enhancing skin hydration by retaining moisture and improving the skin barrier function.

The present study focuses on the formulation and evaluation of a herbal moisturizing cream containing *Laurus nobilis* leaf extract, *Cucumis sativus* extract, and Aloe vera gel. The aim of this study is to develop a stable, safe, and effective herbal moisturizing cream using natural ingredients and to evaluate its physicochemical properties, stability, spreadability, pH, homogeneity, and skin compatibility. The formulation is intended to provide effective skin hydration while minimizing the side effects associated with synthetic cosmetic products.

2. MATERIALS AND METHODS

The Materials and method pertaining to the current "Formulation and evaluation of herbal moisturizing cream containing *Laurus nobilis leaf, Cucumis sativus* and aleo vera "

Collection and extraction of Plants

The *Laurus nobilis leaf, Cucumis sativus* and Aloe vera samples were collected from market and grained using morter & pestle and 60% ethanol is used as a solvent. The obtained extract is filtered using whatmann filter paper and the purified extract is used for further analysis.

Antimicrobial activity

Medium preparation

For the cultivation of the bacterial strains, Nutrient broth medium (NBM) was prepared using 8% nutrient broth in double distilled water and agar-agar. It was subjected to autoclaving at 15 Ibs psi for 25–30 min s. Agar test plates were prepared by pouring 15 ml of NBM into petri dishes under aseptic condition and allowed to stand for room temperature for stabilization.

Bacterial cell cultures were maintained in peptone saline solution by regular sub-culturing and were incubated at 37OC for 24

Preparation of the bacterial inoculum

Stock cultures were maintained at 4° C on slopes of nutrient agar and potato dextrose agar. Active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of 50ml nutrient broth bacterial cultures were incubated with agitation for 24hours and at 37°c on shaking incubator for 3-5 days. Each suspension of test organism was subsequently stroke out on nutrient agar media and potato dextrose agar. Bacterial cultures then incubated at 37°c for 24 hours. Potato dextrose slant were incubated at 27°c for 3-5 days. These stock cultures were kept at 4°c. For use in experiments, a loop of each test organism was transferred into 50ml nutrient broth and incubated separately at 37°c for 18-20 hours for bacterial culture.

Antibacterial Activity

Agar Well Diffusion method

The antibacterial activity of polyherbal extract was determined by Well Diffusion method **(Bauer *et al.*, 1996)**. The 25 to 100 µl of extract was poured into the wells. After that, the plates were incubated at 37°C for 24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm. The tested cell suspension was spread on potato dextrose agar. Well were put into the agar medium using sterile forceps. Plant extract were poured on to wells. Then plates were incubated at 37°c for about 24 hours and control was also maintained. Zone of inhibition was measured from the clear zone in mm.

Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations or plant extracts against gram positive,gram negative bacteria. The extracts exhibited antibacterial activities against tested microorganisms.

Formulation of moisturizing cream

Stability of moisturising cream

Physical evaluation

Color, smell, texture, and condition were noted during the physical assessment. **(Swamini Kute *et al.*, 2024)**

pH determination

The pH of the gel was measured using a digital pH metre. To test the pH, 0.5 g of the sample was dissolved in 10 mL of distilled water **(Kalpana S. Patil *et al.*,2023)**

Spreadability

Two sets of a glass slide with standard dimension were taken. Polyherbal formulation cream was placed in between the two slides and sandwiched about the length of 60mm. Removed the adhered excess gel on the surface of the glass slides and Fixed to a stand without any disturbance. In the upper slide, 20 g weight was tied and noted the time taken for movement Of the upper slide to the distance of 60mm under the influence of weight.**(Ajinkya Mate *et al.*,2021)**

$$\text{Spreadability} = (\text{Weight} \times \text{Length}) / \text{Time}$$

Washability

The hand was given a small amount of cream and to see if it could be cleaned, tap water was Used.

Phase separation

The developed and commercialized creams were kept between 25 and 100 0 C in a sealed Jar away from light. Following that, phase Separation was examined for a period of 24h for 10 days.

Irritancy

Mark an area of 1 cm2 on the left dorsal Surface. Following the designated area's Application of the cream, the time was noted. It is then assessed erythema, oedema, and Irritation, if present, for a maximum of 24 Hours before being reported **(Swamini Kute *et al.,2024)***

Anti-inflammatory analysis of formulation cream

Protein Denaturation Assay

To Formulated anti-inflammatory cream is 0.5 g of the cream is diluted in 50ml of distilled water. From sample extract 10 – 50µL, 500 µL of BSA, to make upto 500 µL of phosphate buffer were added. The negative control is made by removing the extract from the above mixture. For 15 minutes, the mixture is incubated at 37°C. It is heated for 5 minutes at 70°C and then cooled. At 660 nm, absorbance is measured. **(Patil and samant*et al., 2023)***

$$\% \text{ of Inhibition} = \frac{\text{Abs of control} - \text{Abs of std}}{\text{Abs of control}} \times 100$$

MTT Assay

MTT cytotoxicity assay

The cytotoxicity of the polyherbal extract on Vero cells was determined by the MTT assay described by Mosmann (1983), with minor modifications. Cells were seeded in 96-well flat-bottom plates at a density of 1 × 10^4 cells per well in 100 µL of complete medium and left to attach for 24 h. The medium was then replaced with 100 µL of fresh medium containing different concentrations of the extract, and the plates were incubated for 24 hours. Untreated cells served as the negative control, cells treated with 0.1% DMSO served as the vehicle control, and wells containing medium alone were used as blanks. All treatments were set up in triplicate.

After the incubation period, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and the plates were incubated for a further 4 h at 37 °C in the dark. The medium was then carefully removed, and the purple formazan crystals were dissolved in 100 µL of DMSO. The plates were shaken gently for 10 min, and the absorbance was read at 570 nm with a reference wavelength of 630 nm in a microplate reader ([make and model]).

The percentage of cell viability was calculated as follows:

Cell viability (%) = [(OD of treated cells − OD of blank) / (OD of control cells − OD of blank)] × 100

The CC_{50} value (the concentration that reduced cell viability by 50%) was estimated from the dose-response curve by non-linear regression analysis using GraphPad Prism version. Cell morphology after treatment was also examined under an inverted phase-contrast microscope ([make/model]) at [magnification]. Following ISO 10993-5 (2009), a reduction in viability of more than 30% was considered a cytotoxic effect.

3. RESULT AND DISCUSSION

The Results and Discussion pertaining to the current study "Formulation and evaluation of herbal moisturizing cream containing *Laurus nobilis leaf, Cucumis sativus* and aleo vera"

Anti-microbial activity

Agar well diffusion method

The antibacterial activity of ethanolic extract of Laurus nobilis leaf, Cucumis sativus and aleo vera is found good against gram positive bacteria and gram negative bacteria shows the zone of inhibition against in both *Escherichia coli,* and *Staphylococcus aureus,* . The concentration of the sample was taken as 25, 50, 75, 100 µl of respectively. The highest antibacterial activity is seen at the highest concentration of 100 µl of the sample. The activity that is zone of inhibition increases with the increase in concentration of sample, shows that ethanolic extract *of Laurus nobilis leaf, Cucumis sativus* and *aleo vera* shows potent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli,* when compared with standard.

The agar diffusion test is a test of the antibiotics sensitivity of bacteria. It is widely used to determine the zone of inhibition of bacteria. Bacteria which are used to identify anti microbial activity are *Escherichia coli, Streptococcus aureus*

Table 1: Anti-bacterial activity

Name of the organism	Sample 25µl	Sample 50µl	Sample 75µl	Sample 100µl	Standard
Escherichia coli	12mm	16mm	18mm	13mm	20mm
Streptococcus aureus	12mm	14mm	16mm	18mm	14mm

The ethanolic extract of *Laurus nobilis leaf, Cucumis sativus* and *aleo vera Escherichia coli* are shown in the **Figure 1**



Figure 1: Result for Polyherbal extract against *Escherichia coli*

The ethanolic extract of *Laurus nobilis leaf, Cucumis sativus* and *aleo vera* against *Streptococcus aureus* are shown in the **Figure 2**



Figure 2: Result for Polyherbal extract against *Streptococcus aureus*

STABILIZATION OF CREAM

Physical evaluation

Colour, odour, and state were observed in the finished cream.

pH

The pH of the cream was measured using a pH meter. The pH value is closer to the skin's pH of 7.1.

Washability

The applied cream was washed in tap water.

Irritancy

The cream was applied to the skin and checked the irritability.

Phase separation

The cream is kept in a closed container at room temperature, away from sunlight, and observed for 24 Hours – 30 hours.

Table 2: Evaluation of cream

S.no	Parameter	Formulation 1	Formulation 2
1	Colour	Yellow	Pale green
2	Odour	Pleasant	Pleasant
3	State	Semi solid	Semi solid
4	pH	6.8	7.1
5	Washability	washable	washable
6	Irritant	nil	nil
7	Phase Separation	No phase separation	No phase separation

Spreadability

A spreadability test is done for all three formulations. The less time taken for the separation of both slides, The better the spreadability. According to the statement, the F3 had better speed

Table 3: Spreadability test

S.no	Formulation	Time (sec)	Spreadability
1	F1	7	2.3
2	F2	8	2.0

Anti-inflammatory activity

Inhibition protein denaturation

The effect of the formulated of anti-inflammatory cream on the Protein denaturation in bovin serum albumin. The result obtained indicates that the formulation showed Significant anti-inflammatory activity by reducing the protein Denaturation. The formulation at 0.5g inhibited 60% Protein denaturation which is nearly equal to the standard group treated With aspirin **vidyadharasuryadevara, *et al.*, 2021**

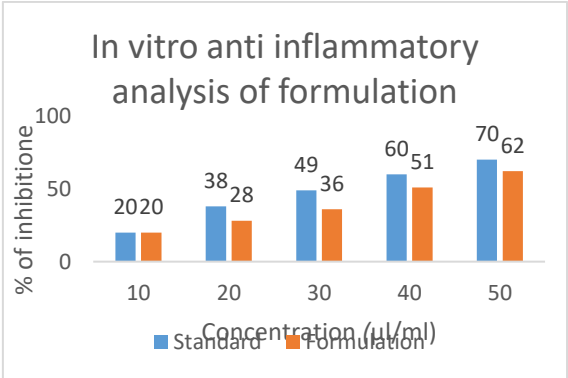


Figure 3: Estimation of Anti-inflammatory assay in formulated cream

MTT Assay

Table 4: MTT assay

Culture condition	% cell viability
Control	100
Sample A –12.5µg/ml	98.01
Sample A - 25 µg/ml	96.56
Sample A –50 µg/ml	94.63
Sample A - 100 µg/ml	92.88
Sample A - 150 µg/ml	91.61

The sample shows no cytotoxcity. While treated upto 150 µg/ml shows 91.61 % cell are viable. Control group shows 100 % cells are viable.

4. CONCLUSION

The polyherbal extract posses good anti-bacterial and anti-inflammatory activity, the polyherbal extract shows pharmacological activities it used to prepare a natural moisturizer. Stabilization analysis of moisturizer shows that stable with its original organeoleptic characteristics and in MTT cell line assay shows no cytotoxicity and 100 % cells are viable.

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