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Research Article

Formulation and Evaluation of Herbal Anti-Acne Cream Using *Millingtonia hortensis* Flower Extract

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Abstract

The present study aimed to develop and evaluate a herbal anti-acne cream utilising the flower extract of *Millingtonia hortensis* as a natural remedy for acne treatment. This study focuses on the extraction and evaluation of bioactive phytoconstituents such as saponins, alkaloids, glycosides, terpenoids, tannins, phenols and flavonoids, which are recognised for their antioxidant, antimicrobial, moisturising and skin toning properties. Additionally, the research incorporated the evaluation of antibacterial properties for the development of a stable topical product, ensuring its safety, compatibility and therapeutic effectiveness. The antioxidant potential was determined by the DPPH radical scavenging assay. The formulated cream was evaluated for physicochemical parameters, including colour, odour, pH, viscosity, spreadability, loss on drying, phase separation and stability. Invitro antibacterial activity was assessed using the well diffusion method. All formulations F6 exhibited optimum characteristics and satisfactory quality control results. The developed formulation demonstrated anti-acne activity comparable to the marketed herbal preparations, Himalaya Clarina Anti-Acne cream. It was discovered that the herbal antiacne cream formulation was stable, secure and efficient when used topically. As a promising natural source for cosmetic and dermatological preparations, Millingtonia hortensis is highlighted in the study. Additionally, the proposed formulation may be a sustainable for synthetic antibacterial compounds found in traditional goods.

Keywords: Millingtonia hortensis flower, antioxidant, Anti-acne cream. Propionibacterium acne

1. INTRODUCTION:

The chronic inflammatory condition known as acne vulgaris affects the pilosebaceous unit and is characterised by inflammation and infection of the sebaceous glands. Approximately 75% of people between the ages of 11 and 30 are affected, making it one of the most common dermatological problems in the world.[1] Acne can manifest clinically as a variety off lesions, such as papules, pustules, nodules and cysts. Severe cases of acne can cause sinus tract formation and substantial scarring. A key factor in the aetiology and development of acne is microbial colonisation. Along with other microbes like *Staphylococcus epidermis, Staphylococcus aureus and Candida albicans*[2], *Cutibacterium acnes* is often linked to the inflammatory lesions by producing poisons, pro-inflammatory mediators and enzymes. These microorganisms fuel inflammation, lowering the microbial burden and stopping the spread. Anti-acne cream varies according to the active substances or ingredients it contains, as acne is a complex disorder that involves inflammation, follicular hyperkeratosis, *Cutibacterium acnes* proliferation, and excessive sebaceous production. In order to reduce outbreaks and improve skin texture, anti-acne treatments unclog pores, eliminate germs that cause acne, reduce inflammation and normalise skin cell turnover. [3]

It has been observed that a variety of bioactive phytoconstituents are present in different parts of *Millingtonia hortenisis*. The chemical constituents like lapachol, Beta-sitosterol and paulownin are known to be present in the roots [4]. The plant's medicinal qualities are influenced by the presence of Betasterol, tannins and bitter ingredients in the bark and heartwood. According to phytochemical analysis of leaves, Beta-carotene and flavonoids like hispidulin and dinatin are present. [5] These substances have anti-inflammatory and antioxidant properties are widely known. The flowers are particularly rich in flavonoids and glycosides. Compounds such as scutellarein and aa novel glycoside, scutellarein-5-galactoside, have been isolated from fresh flowers[6]. Overall, the presence of these phytoconstituents supports the medicinal and cosmetic potential of *Millingtonia hortensis,* particularly due to their antioxidant, antimicrobial and anti-inflammatory properties. [7]

Millingtonia hortensis has numerous medicinal uses, which have long been recognised in the Ayurvedic and Unani systems of medicine, such as antihypertensive and diuretic properties, as well as lipid-lowering, antispasmodic, antiulcer, and hepatoprotective properties.

2. MATERIALS AND METHODS:

Millingtonia hortensis flowers were collected in September and October in Davangere (Karnataka)and Hyderabad (Telangana), India, and taxonomically verified by trained botanists. The flowers were dried in the shade at room temperature for approximately 20 days and ground into powder using a blender. The powdered material was either stored for later use or subjected to extraction. For microscopic evaluation, dried petals were ground and examined under a powder microscope using 90% ethanol and 1% phloroglucinol in concentrated hydrochloric acid for staining.

Figure 1: Millingtonia hortensis flowers

Figure 2: Millingtonia Hortensis Flower Extract

Extraction procedure:

Millingtonia hortensis flowers were washed, air-dried at room temperature, the midrib was removed, and then ground to a fine powder. The powder was extracted sequentially with petroleum ether (60–80 °C) and then with a mixture of methanol and water (1:1).The extract was concentrated by evaporating the solvent under vacuum. Both were tested at a concentration of 500 µg/ml, and the hydroalcoholic extract showed positive activity, while the petroleum ether extract was inactive.(08)The aqueous extracts of the flowers were then subjected to qualitative phytochemical testing using standard procedures to identify bioactive components[8].

Antioxidant activity from DPPH assay:

Method:

Preparation of sample from Flower extract:

100mg of Ethyl acetate and Ethanol extract of *Millingtonia hortensis* is dissolved in 50 ml of methanol.[9]

Preparation of DPPH solution:

22 mg of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is dissolved in 100 ml of methanol in a volumetric flask and dissolved well and it is called stock solution. Then, 18 ml of the stock solution is makeup with methanol up to 100 ml.[10]

DPPH Preparation of standard ascorbic acid solution:

50mg of ascorbic acid is dissolved in 50 ml of methanol.

Procedure:

The antioxidant activities were evaluated using DPPH (Sigma-Aldrich, Germany; M.W. 394.32) as a free radical. A 1 mg/ml solution of the plant extract in methanol was created. A solution of 6 x 10^-5 mol/L DPPH in methanol was also prepared. To this, 0.1 ml of the plant extract was combined with 3.9 ml of the DPPH solution. The reduction in absorbance at 517 nm was measured at one-minute intervals for up to 15 minutes, or until a plateau was observed. Initially, a blank sample was prepared that contained the same quantities of methanol and DPPH solution, which served as the control. Ascorbic acid was utilized as the standard[11]. The experiment was executed in triplicate. The free radical scavenging activity was calculated using the following equation:

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance Of control)] x 100 [12]

Antibacterial activity:

The extract was screened for antibacterial activity by the agar well diffusion method against bacterial strains. *Propionibacterium acne (MTCC 1951*) was used as a bacterial strain. By this method minimum inhibitory concentration (MIC) was found out using Azithromycin as a standard drug. All the stock solutions were prepared. The fresh culture of bacteria is obtained by inoculating bacteria into peptone media. This culture was mixed with nutrient agar media and poured into petri plates by following aseptic techniques and autoclaved for 121^{0}C for 15 lbs and, cooled and poured on sterilised petri plates and allowed for solidification. After solidification, inoculate the test pathogenic organism, make a lawn

streak/spread, and later make the well and introduce the different concentrations of standard drug and extract. Incubate the nutrient agar plates at 35^{0}C for 24 hours. The incubated plates were observed for growth inhibition activities. After the incubation period, petriplates were observed for a zone of inhibition by using a Vernier scale. The results were evaluated by comparing the zone of inhibition shown by the extract with standard drug. The results are the mean value of the zone of inhibition measured in millimetres of two sets.[13-16]

Formulation of anti-acne cream:

An anti-acne oil cream was prepared using stearic acid, cetyl alcohol, and liquid paraffin dissolved in the oil phase and heated to 75 °C in a water bath.

The aqueous phase containing glycerol, methylparaben and triethanolamine was prepared, separately and heated to the same temperature. After reaching 75°C, the aqueous phase was slowly added to the oil phase. Continuous agitation was maintained during mixing to ensure proper emulsification. Stirring was continued until the mixture cooled and a stable cream was obtained.[17]

Table 1: List of chemicals with grade and suppliers

Sl.No	Materials	Suppliers
1	DPPH	Himedia laboratories pvt.ltd
2	Stearic acid	S.D Fine chem Ltd Mumbai
3	Cetyl alcohol	S.D Fine chem Ltd Mumbai
4	Liquid paraffin	LOBA Chemie Pvt.Ltd
5	Glycerin	LOBA Chemie Pvt.Ltd
6	Methyl paraben	S.D Fine chem Ltd Mumbai
7	Triethanolamine	S.D Fine chem Ltd Mumbai
8	Ethanol	LOBA Chemie Pvt.Ltd

Table 2: Formulation of antiacne cream from *Millingtonia hortensis*

Ingredients	F-1	F-2	F-3	F-4	F-5	F-6
Plant extract	0.5ml	1ml	1.5ml	2ml	2.5ml	3ml
Steric acid	5gm	5gm	5gm	5gm	5gm	5gm
Cetyl alcohol	2gm	2gm	2gm	2gm	2gm	2gm
Liquid paraffin	2ml	2ml	2ml	2ml	2ml	2ml
Glycerin	2.6ml	2.6ml	2.6ml	2.6ml	2.6ml	2.6ml
Methyl paraben	0.028gm	0.028gm	0.028gm	0.028gm	0.028gm	0.028gm
Triethanolamine	0.028	0.028	0.028	0.028	0.028	0.028
Distilled water	25ml	25ml	25ml	25ml	25ml	25ml

Evaluation parameters:

- **Physical evaluation**

Physical parameters such as colour, appearance & consistency were checked visually.

- **Washability**

Formulations were applied on the skin & then ease & extent of washing with water were checked manually.

- **pH**

pH of 1% aqueous solution of the formulation was measured by using a calibrated digital pH meter at constant temperature

- **Globule diameter**

With the help of projection microscope, SIPCON SP, 585. The average will be taken for particle size range 19.91 µm,14.04µm so concluded that is uniformity, no agglomeration of the emulsion and smooth in nature.[18]

- **Greasiness**

Here the cream was applied on the skin surface in the form of smear and checked if the smear was oily or

grease-like. According to the results, we can say that all three formulations were non-greasy.

- **Spreadability**

The spreadability was expressed in terms of time in seconds taken by two slides to slip off from the cream, placed in between the slides, under certain load. Lesser the time taken for separation better the spreadability. Two sets of glass slides of standard dimension were taken. Then one slide of suitable dimension was taken and the cream formulation was placed on that slide. Then other slider was placed on the top of the formulation. Then a weight or certain load was placed on the upper slide so that the cream between the two slides was pressed uniformly to form a thin layer. Then the weight was removed and excess of formulation adhering to the slides was scrapped off. The upper slide was allowed to slip off freely by the force of weight tied to it. The time taken by the upper slide to slip off was noted.

SPREADABILITY= M×l÷t

Where, m= Standard weight, which is tied to or placed over the upper slide. (30g) l= Length of a glass slide (7.5cm)

t= Time taken in seconds.

- **Phase separation**

Prepared cream was kept in a closed container at room temperature away from light. then phase separation was checked for 24 hours for 30 days. Any change in the phase separation was observed.

- **Washability**

Washability test was carried out by applying a small amount of cream on the hand and then washing it with tap water.

3. RESULTS AND DISCUSSION:

Results:

1. UV-Visible spectroscopic Analysis of Ethanolic Millingtonia hortensis extract

A UV spectrophotometric analytical method was successfully developed for Quercetin by using water as the solvent. Showed an absorption band in the range of 260-280nm, which is closely corresponds to the quercetin. This indicates that Quercetin is a major constituent present in the Millingtonia hortensis extract.

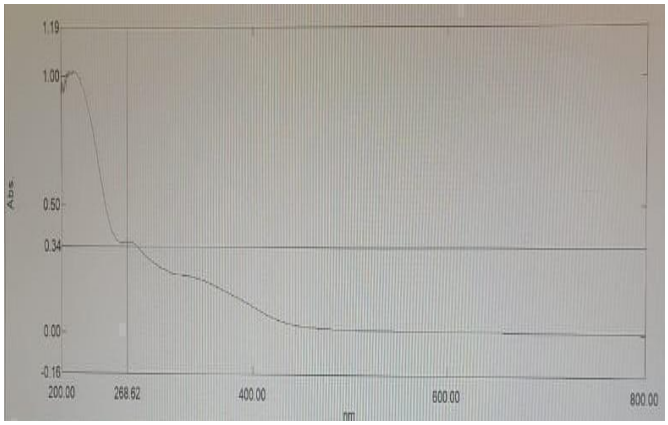

Figure 3: UV spectrum of Quercetin

2. Phytochemical screening

Qualitative phytochemical analysis of the flower extract of Millingtonia hortensis revealed the presence of all the essential phytoconstituents. (16)

Table 3: Phytochemical constituents with inference

Phytochemical constituents	Inference
Alkaloids	++
Flavonoids	++
Phenols	++
Tannins	++
Terpenoids	++
Cardiac glycosides	++
Proteins and amino acids	++
Carbohydrates	--
Phytosterols	++

3. Anti-oxidant activity by DPPH assay:

Figure 4: Serial dilutions of test sample, standard and blank samples

The flower extract of Millingtonia hortensis showed concentration-dependent activity in the DPPH assay. The percentage of radical scavenging activity increased from 17.58% at 10µg/mL to 68.7% at 40µg/mL. Approximately 50% inhibition was observed at 30 µg/mL, indicating a good IC_{50} value and strong free radical scavenging potential.

Table 4: Calculation of % Radical Scavenging and IC50 from DPPH Assay

Concentration	Absorbance of control	Absorbance of sample	%RSA	IC_{50}
10 µgm	0.687 λmax	0.565	17.58	5.353523
20 µgm	0.687 λmax	0.445	35.22	11.31513
30 µgm	0.687 λmax	0.34	50.5	17.27674
40 µgm	0.687 λmax	0.215	68.7	23.23835

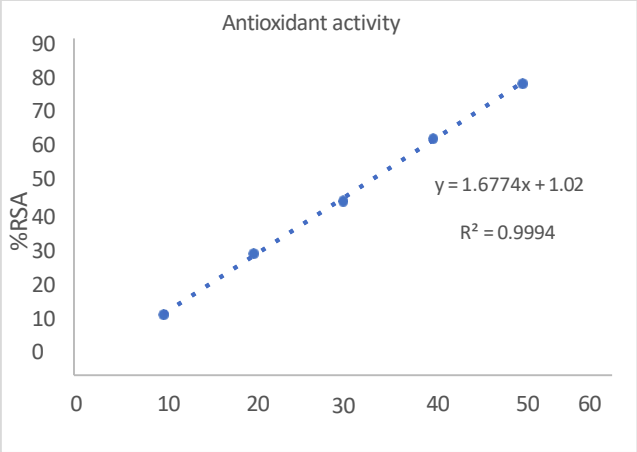

Figure 5: DPPH graph

4. Evaluation parameters

Six different formulations of anti-acne cream were prepared using Millingtonia hortensis extract, and each was evaluated for quality control parameters such as pH, washability, Globule diameter, greasiness, phase separation, spreadability, and stability. The results indicated that all formulations met the required standards, with the F3 formulation showing optimal performance in terms of consistency, skin compatibility, and ease of application. Irritancy tests confirmed that these formulations are safe for topical use, showing no signs of skin irritation or adverse reactions.

Table 5: Evaluation parameters of F3 formulation

Formulation	Washability	pH	Globule diameter(µm)	Greasiness	Spreadbility (g/cm²)	Phase separation
F1	Easily Washable	6.02	19.91	No greasiness	7.95	No Phase separation
F2	Easily Washable	6.03	14.04	No greasiness	8.37	No Phase separation
F3	Easily Washable	6.12	13.05	No greasiness	7.12	No Phase separation
F4	Easily Washable	6.45	16.08	No greasiness	8.43	No Phase separation
F5	Easily Washable	6.57	13.61	No greasiness	9.45	No Phase separation
F6	Easily Washable	6.55	12.05	No greasiness	9.60	No Phase separation

5. Antibacterial activity

The table shows the antibacterial activity of different concentrations (0.5-3.0), HAAC (Herbal Anti-Acne Cream). MHE (*Millingtonia hortensis* flower extract), and standard azithromycin against the test organism. Formulation F3 showed good antibacterial activity against Propionibacterium acne (MTCC 1951), which is evident from the zone of inhibition. Whereas other formulations showed activity in a dose-dependent manner.

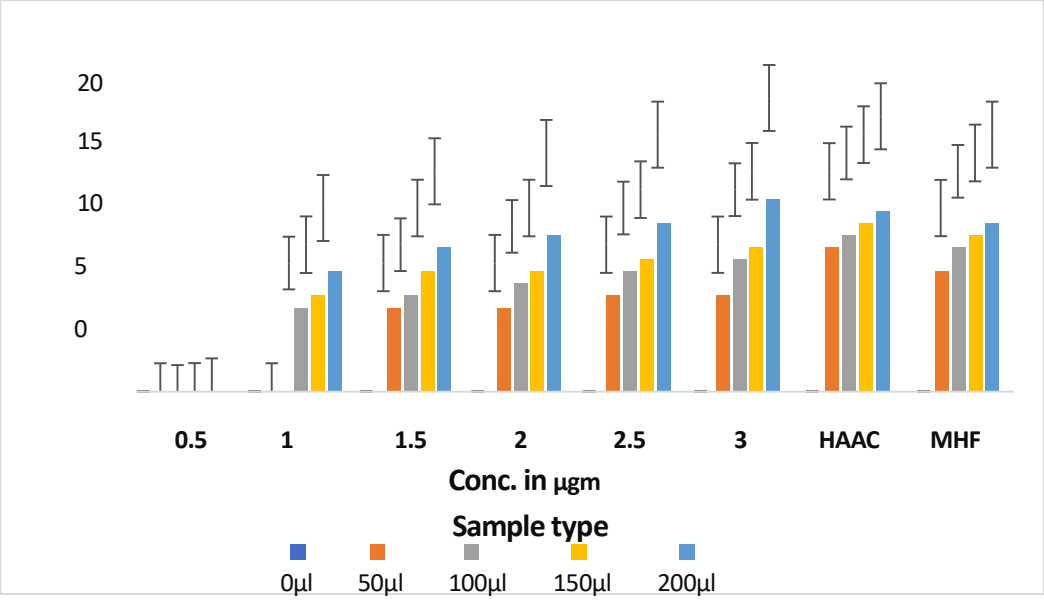

Figure 6: Antibacterial activity against *Propionibacterium acne*

Sample Name	ZOI (mm)					
	0 µg/mL	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	30* µg/mL
0.5	-	-	-	-	-	24
1.0	-	-	7	8	10	24
1.5	-	7	8	10	12	25
2.0	-	7	9	10	13	25
2.5	-	8	10	11	14	24
3.0	-	8	11	12	16	24
HAAC	-	12	13	14	15	24
MHE	-	10	12	13	14	25

Table 6: Antibacterial activity against *Propionibacterium acne*

DISCUSSION:

Phytochemical screening: Various qualitative chemical tests can be carried out to determine the composition of an ethanolic extract. The following tests were performed on extracts to determine the presence of various phytoconstituents. Scavenging activity of the plant extract of *Millingtonia hortensis* on DPPH radical was evident. The antioxidant activity for different concentrations of plant extract of *Millingtonia hortensis* was screened, and was found to be a good radical scavenger with the percentage of RSA 68.7. Six different formulations of anti-acne cream were prepared using *Millingtonia hortensis* extract, and each was evaluated for quality control parameters such as pH, washability, Globule diameter, greasiness, phase separation, spreadability, and stability. The results indicated that all formulations met the required standards, with the F3 formulation showing optimal performance in terms of consistency, skin compatibility, and ease of application. Irritancy tests confirmed that these formulations are safe for topical use, showing no signs of skin irritation or adverse reactions. The results confirm that the formulated Herbal Anti-Acne Cream (HAAC) possesses significant antibacterial activity in a dose-dependent manner. While its activity is lower than the standard azithromycin, HAAC demonstrated comparable and slightly improved performance over the crude extract (MHE), indicating successful incorporation of active

phytoconstituents into the formulation. Therefore, the developed herbal cream shows promising anti-acne potential and can be considered a safe and effective natural alternative for topical antibacterial therapy.

CONCLUSION:

The present study successfully demonstrated the formulation and evaluation of a herbal anti-acne cream containing the flower extract of *Millingtonia hortensis.* Phytochemical screening confirmed the presence of bioactive constituents, including flavonoids, phenols, tannins, terpenoids, alkaloids, and glycosides, which contribute to significant antioxidant activity (68% DPPH radical scavenging). The formulated creams complied with physicochemical quality parameters showing acceptable pH, good spreadability, absence off phase separation, non-greasiness, and stability with formulation F3 exhibiting optimal performance. Furthermore, the formulation F6 demonstrated notable antibacterial activity against *Cutibacterium acnes* (MTCC 1951), supporting its anti-acne potential. Overall, the developed herbal cream was found to be safe, stable and effective, highlighting *Millingtonia hortensis* as a promising natural alternative for topical anti-acne therapy.

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