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Research Article

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Development of Anti-Acne Topical Gel Formulation Containing Herbal Extract

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ABSTRACT

Systemic and topical antimicrobials are effective in the treatment of inflammatory acne vulgaris; however, widespread use of these agents is becoming increasingly associated with the emergence of resistant pathogens raising concerns about microorganism resistance and highlighting the need for alternative non-antimicrobial agents for the treatment of acne. Several multinational companies like Lakme Cosmetics Ltd., Lotus Ltd., Ayur Healthcare Ltd., Patanjali Ayurveda, etc. have started developing anti-acne products which contain multiple extracts of common Indian plants. The study aims to develop herbal gel formulations containing *Bougainvillea glabra* extract (aqueous, methanol, ethyl acetate, and petroleum ether) for the treatment of acne. The formulations were comprehensively evaluated for Physical evaluation, Washability, Skin irritation test, Spreadability, pH, Viscosity, Extrudability, Swelling index, and Accelerated stability studies as per the standard methods. Amongst all the formulations studied, batch F2 was found optimum for all the parameters. The extraction yield was found to be 11.21%. *Bougainvillea glabra* aqueous extract showed low anti-bacterial activity whereas both *Bougainvillea glabra* methanol extract and *Bougainvillea glabra* ethyl acetate extract expressed moderate anti-bacterial activity with average MIC value against *E. coli* and *B. subtilis*. In contrast to them, the petroleum ether extract of *Bougainvillea glabra* presented highest anti-bacterial activity, however, the activity was less pronounced than the standard drug Clindamycin. The formulations (F1-F4) represented alike results with that of the extracts and expressed lesser activity as compared to the marketed herbal product.

Keywords: *Bougainvillea glabra*, Formulations, Acne, Characterization, Antimicrobial, Extract

INTRODUCTION

Acne is a multifactorial chronic inflammatory illness of the pilosebaceous follicles that is characterised by androgen-induced sebum hyperplasia, altered follicular keratinization, hormonal imbalance, immunological hypersensitivity, and bacterial (*Propionibacterium acnes*) colonisation. Although acne does not have the urgency of a life-threatening disease and does not impede general fitness, it has long-term consequences that may be significant, leaving cutaneous and emotional scars that last a lifetime. It affects a person's confidence, inflicting physical, social, and psychological harm, as well as lowering self-esteem and generating emotional pain. Although there is almost no death with this condition, there is typically significant physical and psychological morbidity. According to statistics, roughly 85 percent of young adults aged 12–25 years old, approximately 8% of people aged 25–34 years old, and 3% of adults aged 35–44 years old suffer from acne at some point in their lives.

In acne vulgaris, there is a known relationship between testosterone levels and sebum production. The initial cause of the development of acne is androgen-induced sebaceous gland enlargement, which leads to excessive sebum production. Steroid metabolising enzymes in the sebaceous glands convert dehydroepiandrosterone (DHEAS) to dihydrotestosterone (DHT). Furthermore, testosterone is converted to the more active DHT by two kinds of 5-α-reductase isozymes, type-1 and type-2, which are found in the scalp, chest, sebaceous glands, genitourinary tissue, and dermal papillae, as well as hair follicles.

The hunt for acne-fighting methods is still a significant research and development project in the pharmaceutical and personal care sectors. The use of antibiotics indefinitely increases the danger of developing anti-biotic-resistant bacteria, which is self-evident. Antibiotic resistance is caused by a variety of factors, including the nature of bacteria's interactions with antibiotics. As a result, there are ample reasons to seek out and find alternate solutions

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to these issues. Medicinal plants have been researched as potential remedies for ailments to combat antibiotic resistance and excessive treatment costs. Numerous studies have shown that employing medicinally powerful plant actives to combat bacteria growth and the inflammatory response might be a viable option. The presence of 2,50,000-5,00,000 plant species provides a huge opportunity for testing phytotherapeutic compounds that may be used to treat acne. Traditional herbal remedies are a fascinating and mostly untapped source of novel medication research. Traditional treatments and natural items have a lot of promise for finding bioactive lead molecules and developing them into medications to treat acne vulgaris. With an ongoing quest for new physiologically active botanical compounds, an effort is also being made to list the prospective possibilities from traditional medical systems for the treatment of acne.

The present study deals with developing *Bougainvillea glabra* extract loaded gel formulations (F1-F4) and their further pharmaceutical evaluations as well as testing antimicrobial activity against *E. coli* and *B. subtilis*.

MATERIALS AND METHODS

Plants and Extraction

Procurement of Plant

Bougainvillea glabra plant was procured from Rajkishore Nagar area of Bilaspur, Chhattisgarh, India.

Authentication of Plant

The plant was authenticated from Department of Botany, Guru Ghasidas Vishwavidyalaya (A Central University), Bilaspur, Chhattisgarh, India.

Extraction of plants

The extraction was done through Soxhelation process. The plant material was collected, dried in the shade for a specified period, and powdered suitably. The dried powder (100 g, divided into multiple smaller amounts) was subjected to continuous hot Soxhlet extraction with various solvents (aqueous, methanol, ethyl acetate, and petroleum ether) in desired ratio at a temperature of 50-75°C during 32 cycles. The solvent was removed under reduced pressure and controlled temperature using a rotary vacuum evaporator.

Formulation development

The gel was formulated using *Bougainvillea glabra* extract (1% w/w), carbopol 940 (1% w/w), triethanolamine (0.4% w/w), ethanol and distilled water. *Bougainvillea glabra* extract was dispersed in a mixture of 24.1% w/w distilled water and 18.72% w/w ethanol, which was added drop wise into the hydrated carbopol 940 containing 6.25 % w/w ethanol and 49.3% w/w distilled water. The content was stirred continuously until to form the gel. As the gel started forming, DMSO which acts as a permeation enhancer was added immediately, and further, the gel was allowed to set (**Mahajan et al., 2017**) (**Table 1**).

Table 1: Formulation Chart

INGREDIENTS	F1	F2	F3	F4
Bougainvillea glabra aqueous extract	1 g	-	-	-
Bougainvillea glabra methanol extract	-	1 g	-	-
Bougainvillea glabra ethyl acetate extract	-	-	1 g	-
Bougainvillea glabra petroleum ether extract	-	-	-	1 g
Carbapol 940	1 g	1 g	1 g	1 g
Triethanolamine	0.4 mL	0.4 mL	0.4 mL	0.4 mL
Ethanol	24.5 mL	24.5 mL	24.5 mL	24.5 mL
DMSO	2 mL	2 mL	2 mL	2 mL
Distilled Water	74.1 mL	74.1 mL	74.1 mL	74.1 mL

Evaluation parameter

The formulations were comprehensively evaluated for Physical evaluation, Washability, Skin irritation test, Spreadability, pH, Viscosity, Extrudability, Swelling index, and Accelerated stability studies as per the methods/protocols given by **Shivhare et al., 2019**.

Physical appearance

The formulated polyherbal gel was visually evaluated for color, appearance, and transparency. The smoothness of the gel was estimated by rubbing the formulation between the fingers to observe the smoothness, clumps, roughness, and homogeneity.

Washability

The washability of formulations was examined by applying the gel on the skin and then evaluating the ease and the extent of washing it with distilled water and manually observing the effect.

Skin irritation test

The formulated gel in the quantity of 0.5 g was applied to the normal hairless skin at an area of 6 cm^2 and then covered with a semi-occlusive bandage for the duration of 1 hr. After the application time, the bandage was removed, the applied gel was scrapped off completely, and the area was visually inspected for any rashes or similar symptoms. The test was done for a period of 7 days. The results were expressed in terms of grades.

Spreadability

Based on the principle of the slip-drag feature of the polyherbal dermal gel, the spreadability was determined. The protocol involved taking 2 g of formulation and placing it on a ground slide and sandwiching it by an analogous glide slide, having a hook attached. A heavy mass was applied to the slides to remove the entrapped air so as to form a uniform film between the slides. The excess gel content was scrapped off from the edges. Following it, the top slide was made to drag 50 g intensity. The time needed by the top slide to cover a distance of 6 cm was determined from the formula:

$$S = M \times L / T$$

where, M = weight tied to the upper slide (20 g); L = length of glass slide (6 cm); T = time taken (sec) to separate the glide slides from each other

pH

The pH of the dermal gel was evaluated with the digital calibrated pH meter. 1 g of the formulation was dissolved in 25 mL of distilled water and the glass electrode was dipped into it until constant reading obtained. The pH measurement was performed thrice for each formulation and the average reading was noted.

Viscosity

The viscosity of the formulation was determined by using the Digital Brookfield Viscometer using spindle no. 6 at 10 rpm and temperature of 25±1°C. A sufficient quantity of gel was filled in appropriate wide mouth container in such way that it should sufficiently allow to dipped the spindle and allowed to settle over 30 min before the measurements.

Extrudability

The extrudability of the prepared formulation was determined by first filling the gels (100 g) into a capped collapsible aluminum tubes and sealed by using manual ointment sealing machine. The tubes (containing different formulations) was placed in between two slides and properly clamped. It was followed by placing 500 g weight over the slides and ultimately opening the cap where the extruded ribbon length was noted after 10 min.

Swelling index

The creaming index was determined by adding 5 mL of emulsion in plastic pots, immediately after preparation. After 4 hr, the volume of the formed cream was measured and creaming percentage was determined accordingly. The swelling index of the prepared dermal polyherbal gel was determined by taking 2 g of gel in a beaker containing 10 mL of distilled water. After 1 hr, the swelled formulation was removed from the beaker and was put on a petridish. The content was re-weighed and the swelling index was estimated from the formula:

$$Si = Wt – Wo / Wo$$

where, Wt = weight of swollen at t time; Wo = original weight of gel at zero time

Accelerated stability studies

The optimized formulation was subjected to accelerated stability study (40°C±2°C temperature; 75%±5%, relative humidity) for the duration of 90 days. The prepared gel formulation was kept in a PVC container and covered with a black foil. The above-mentioned critical parameters were evaluated again.

Anti-acne activity

Selection of bacterial samples

Bacillus subtilis and *Escherichia coli* were collected from Om Patholab, Bilaspur and performed accordingly.

Anti-acne property

Modified agar well diffusion method was used to detect the antibacterial activities of different extracts and formulations. In this method, each nutrient agar plates were planted with 0.2 mL of 24 hr broth culture of *Bacillus subtilis*, soybean casein digest media plates were seeded with 0.2 mL each of 24 hr broth culture of *Escherichia coli*. The plates were dried for 1 hr. In each of the plates, equidistant wells were excavated with a sterile 8 mm borer. Into each plate, 0.5 mL of solutions of extract, prepared herbal gel formulation (F2), clindamycin (standard drug), and marketed herbal formulation was introduced. The plates of were incubated at 37°C±1°C for 24 hr. The diameter of the zones of inhibition (in mm) was measured for evaluating the antibacterial activity. The experiment was repeated three times and the mean was recorded. Dimethyl sulfoxide (DMSO) was employed as negative control (Roy et al., 2020).

Determination of minimum inhibitory concentration (MIC)

The MIC is defined as the lowest concentration of the compound to inhibit the growth of microorganisms. The extract was dissolved in DMSO to make a concentration of 100 mg/mL in order to determine the relative minimum inhibitory concentration values. The extract was then diluted with DMSO to make different concentrations. To prepare different concentrations, the extract was diluted with DMSO. Then, 100 µL of these extracts was added to each cup individually. All the test was repeated in duplicates and the mean was recorded (Prasad and Bist, 2018).

Statistical analysis

All the data was represented in Mean ± SD. Data was analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests using Sigmastat® software. The

group means was considered significantly significant when p-value is < 0.05.

RESULTS AND DISCUSSION

Extraction yield

The extraction yield was found to be 11.21%.

Characterization of herbal gel formulation

Physical appearance

The formulated gel formulations were visually evaluated for color, appearance, and transparency. All the formulations were semi-transparent, semi-solid, off-white to colored, and greasy. The smoothness of the ointment formulations were estimated by rubbing the formulation between the fingers to observe the smoothness, clumps, roughness, and homogeneity where it was found that the developed herbal gel formulations (F1-F4) were greasy to some extent but were completely free from any particles or clump or aggregations.

Spreadability

The herbal gel formulations (F1-F4) presented the spreadability in the range of 13.76-17.81 g.cm/sec which reflected that the ointment formulation can be easily spread by a small amount of shear. A relative study of spreadability and viscosity revealed that with an enhancement of formulation viscosity, the spreadability reduces significantly.

pH

The pH of the herbal gel formulations (F1-F4) was found to be in the range of 6.1-6.6 which lies in the close proximity with pH range of the skin **(Table 2)**.

Extrudability

The formulated herbal gel preparations displayed a notable extrudability with a large volume of extrudes ranging from ++ to +++. With an increase in the viscosity of the formulation, the extrudability decreases alongside and prevents easy extrusion from the collapsible tube.

Viscosity

Viscosity is an imperative factor which influences pharmaceutical properties such as spreadability, extrudability, pourability attribute from the container, etc. The viscosity of the herbal gel formulations (F1-F4) lies in the range of 49500-55000 cps. The rheological study indicated that with an increase in the torque, the shear stress extensively increases which results in a decrease in the formulation viscosity.

Washability Test

A brilliant washability attribute has been observed for all the developed herbal gel ointment formulations. The formulation F1 had the best washing characteristics whereas the least washability attribute was observed in formulation F3.

Skin Irritancy

After the application of herbal gel formulations (F1-F4) for the 7 days, the skin irritation test highlighted no striking signs and symptoms of rashes, edema, erythema or any inflammation.

Swelling index

The swelling index was observed in the range of 106-115%. The swelling index signified the matrix nature of the gel formulation which facilitates a controlled release of the drug material.

Stability Test

On subjecting the optimized formulation (F2) at accelerated conditions (40±2°C and 75±5% RH) for 90 days, no substantial disparity in the pH, viscosity, spreadability, swelling index, extrudability, and physical appearance were detected. A change in pH by 0.3 unit, viscosity by 1000 cps, and spreadability by 1.14 g.cm/sec have been noticed considerably. However, no changes in the physical appearance, translucency, and smoothness have been seen after the study. In overall, the formulation remained stable for the 3 months duration and is expected to remain in his original form for a longer duration in tropical and sub-tropical regions.

Table 2: Characterization of developed herbal gel formulations

Characteristics	F1	F2	F3	F4
Spreadability (g.cm/sec)	13.76	17.81	15.66	16.37
pH	6.3	6.6	6.1	6.5
Extrudability	+++	++	+++	+++
Viscosity (cps)	55000	49500	52300	51900
Swelling index (%)	112	113	109	115
Washability	Best	Medium	Lowest	Medium
Skin Irritancy	No irritation	No irritation	No irritation	No irritation

Antimicrobial study

The results of antibacterial screening of herbal extract, gel formulations, standard drug, and marketed herbal formulation are shown in **Table 2**. *Bougainvillea glabra* aqueous extract showed low anti-bacterial activity whereas both *Bougainvillea glabra* methanol extract and *Bougainvillea glabra* ethyl acetate extract expressed moderate anti-bacterial activity with average

MIC value against *E. coli* and *B. subtilis* (**Figure 1**). In contrast to them, the petroleum ether extract of *Bougainvillea glabra* presented highest anti-bacterial activity, however, the activity was less pronounced than the standard drug Clindamycin. The formulations (F1-F4) represented alike results with that of the extracts and expressed lesser activity as compared to the marketed herbal product.

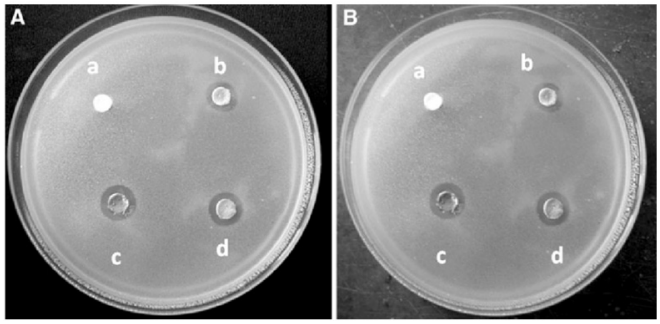

Figure 1: Pictures of microbial plates (A) *E. coli* (B) *B. subtilis*. (a) *Bougainvillea glabra* aqueous extract (b) *Bougainvillea glabra* methanol extract (c) *Bougainvillea glabra* ethyl acetate extract (d) *Bougainvillea glabra* petroleum ether extract.

Table 3: Anti-microbial activity of herbal extract, gel formulations, standard drug, and marketed herbal formulation.

Components	*E. coli*	*B. subtilis*
Bougainvillea glabra aqueous extract	13.6 ± 1.17*** (12.5)	11.7 ± 1.99*** (12.5)
Bougainvillea glabra methanol extract	16.9 ± 1.43*** (12.5)	13.5 ± 1.63*** (12.5)
Bougainvillea glabra ethyl acetate extract	14.2 ± 1.91*** (12.5)	15.8 ± 1.81*** (12.5)
Bougainvillea glabra petroleum ether extract	19.3 ± 1.33*** (12.5)	17.6 ± 1.47*** (12.5)
Clindamycin #	29.9 ± 1.57 (6.25)	28.1 ± 1.15 (6.25)
F1	22.4 ± 1.37*** (25)	20.2 ± 1.47*** (25)
F2	24.1 ± 1.72*** (25)	23.6 ± 1.69*** (25)
F3	20.7 ± 1.44*** (25)	21.9 ± 1.31*** (25)
F4	23.9 ± 1.73*** (25)	22.3 ± 1.52*** (25)
Marketed herbal formulation	24.8 ± 0.96 (12.5)	25.4 ± 0.88 (12.5)

All values represent mean ± SEM of n = 3; ***p<0.001. Zone of inhibition of test compounds against microbes are measured in mm. Values inside the bracket represents the minimum inhibitory concentration (MIC). # Standard reference for anti-bacterial activity

CONCLUSION

The extraction yield was found to be 11.21%. *Bougainvillea glabra* aqueous extract showed low anti-bacterial activity whereas both *Bougainvillea glabra* methanol extract and *Bougainvillea glabra* ethyl acetate extract expressed moderate anti-bacterial activity with average MIC value against *E. coli* and *B. subtilis*. In contrast to them, the petroleum ether extract of *Bougainvillea glabra* presented highest anti-bacterial activity, however, the activity was less pronounced than the standard drug Clindamycin. The formulations (F1-F4) represented alike results with that of the extracts and expressed lesser activity as compared to the marketed herbal product. It can be concluded that the gel prepared from *Bougainvillea glabra* is effective as well as safe to be used as a topical preparation for treating acne.

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