International Journal of Medical & Pharmaceutical Sciences
DOI: https://doi.org/10.31782/IJMPS.2024.141201

Research Article

IJMPS
Section: Pharmaceutical Sciences

Development and Evaluation of Anti-Acne Topical Gel Formulation Enriched with Herbal Extracts

Madhukar Shende*, Ravi Khatri

Department of Pharmaceutical Sciences, Sardar Patel University, Balaghat 481331, Madhya Pradesh, India

ABSTRACT

Aim: This study aims to formulate and evaluate herbal gel preparations incorporating various extracts (aqueous, methanol, ethyl acetate, and petroleum ether) of *Bougainvillea glabra* to explore their potential as alternative treatments for acne, addressing the increasing resistance associated with conventional antimicrobial agents.

Methodology: The herbal gel formulations (F1-F4) were prepared using *Bougainvillea glabra* extracts and subjected to a comprehensive evaluation, including physical characteristics, washability, skin irritation potential, spreadability, pH, viscosity, extrudability, swelling index, and accelerated stability, using standard protocols. Antibacterial efficacy was assessed by determining the minimum inhibitory concentration (MIC) against *Escherichia coli* and *Bacillus subtilis*, and the results were compared with a marketed herbal product and the standard drug Clindamycin.

Results: Among the formulations, F2 exhibited optimal properties across the evaluated parameters. The extraction yield of *Bougainvillea glabra* was 11.21%. Antibacterial testing revealed that the aqueous extract exhibited minimal activity, while the methanol and ethyl acetate extracts showed moderate activity. The petroleum ether extract demonstrated the highest antibacterial efficacy among the extracts but remained less effective than Clindamycin. Formulations (F1-F4) mirrored the trends observed with the extracts but showed reduced antibacterial activity compared to the marketed herbal product.

Conclusion: The herbal gel formulations containing *Bougainvillea glabra* extracts offer a potential alternative for acne treatment, with F2 showing the most promising results. While the petroleum ether extract demonstrated notable antibacterial activity, further optimization and in vivo studies are warranted to enhance their therapeutic potential and compare their efficacy with established products.

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, a well-established link exists between testosterone levels and sebum production. The condition is initiated by androgen-induced enlargement of the sebaceous glands, resulting in excessive sebum secretion. Within these glands, steroid-metabolizing enzymes convert dehydroepiandrosterone sulfate (DHEAS) into dihydrotestosterone (DHT). Additionally, testosterone is further metabolized into the more potent DHT by two isoforms of the enzyme 5-α-reductase, type-1 and type-2, which are distributed in tissues such as the sebaceous glands, dermal papillae, genitourinary tissues, and hair follicles, as well as the scalp and chest. The quest for effective acne

Corresponding Author:
Mr. Madhukar Shende, Research Scholar, Department of Pharmaceutical Sciences, Sardar Patel University, Balaghat 481331, Madhya Pradesh, India; Email: madhushende1@gmail.com

ISSN: 2231-2188 (Print) **ISSN:** 2231-685X (Online)

Received: 30.09.2024 **Revised:** 03.11.2024 **Accepted:** 24.11.2024 **Published:** 08.12.2024

treatment methods remains a critical focus in pharmaceutical and personal care industries. The prolonged use of antibiotics poses a significant risk of fostering antibiotic-resistant bacteria. This resistance emerges from various factors, including the inherent interactions between bacteria and antibiotics, making the need for alternative treatment approaches increasingly urgent. Medicinal plants have garnered attention as viable alternatives due to their potential to address antibiotic resistance and reduce treatment costs, offering promising avenues for tackling acne and related challenges.

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

Plant Extraction

The extraction process employed the Soxhlet apparatus. Collected plant material was shade-dried for a predetermined period and ground into fine powder. A total of 100 g of the powdered material, divided into smaller portions, underwent continuous hot extraction using a Soxhlet extractor with solvents such as water, methanol, ethyl acetate, and petroleum ether in appropriate ratios. The process was carried out at temperatures ranging from 50°C to 75°C across 32 cycles. Post-extraction, the solvents were removed under controlled temperature and reduced pressure using a rotary vacuum evaporator.

Gel Formulation Development

The gel was prepared with 1% w/w *Bougainvillea glabra* extract, 1% w/w carbopol 940, 0.4% w/w triethanolamine, ethanol, and distilled water. The extract was dispersed into a mixture comprising 24.1% w/w distilled water and 18.72% w/w ethanol, which was gradually added to hydrated carbopol 940 containing 6.25% w/w ethanol and 49.3% w/w distilled water. The mixture was stirred continuously until a gel began to form. At this stage, dimethyl sulfoxide (DMSO), serving as a permeation enhancer, was added promptly. The gel was then left to set for optimal consistency (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

A 0.5 g sample of the formulated gel was applied to a 6 cm^2 area of normal, hairless skin and subsequently covered with a semi-occlusive bandage for one hour. After the application period, the bandage was removed, and any remaining gel was thoroughly wiped off. The treated area was then examined visually for signs of irritation, such as rashes or redness. This assessment was conducted daily over a span of seven days, and the observations were categorized using a grading system.

Spreadability

The spreadability of the polyherbal dermal gel was evaluated based on its slip-drag properties. The procedure involved placing 2 g of the gel formulation on a glass slide and covering it with another slide equipped with a hooked edge. A weighted object was placed on the slides to eliminate trapped air and create a consistent film between them. Excess gel was carefully removed from the edges. The top slide was then dragged with a force of 50 g, and the time required for the slide to move a distance of 6 cm was recorded. The spreadability (S) was calculated using the formula:

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

M = weight applied to the top slide (20 g)**; L** = distance moved by the slide (6 cm)**; T** = time taken (seconds) to separate the slides

pH Measurement

The pH of the dermal gel formulations was measured using a calibrated digital pH meter. For each test, 1 g of the gel was dissolved in 25 mL of distilled water. The glass electrode was immersed in the solution until a stable reading was achieved. Each formulation's pH was determined three times, and the average value was recorded to ensure accuracy.

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

The antibacterial activity of various extracts and formulations was assessed using a modified agar well diffusion technique. Nutrient agar plates were inoculated with 0.2 mL of a 24-hour broth culture of *Bacillus subtilis*, while soybean casein digest media plates were seeded with 0.2 mL of a 24-hour broth culture of *Escherichia coli*. After inoculation, the plates were allowed to dry for 1 hour. Sterile 8 mm borers were used to create equidistant wells in each plate. Subsequently, 0.5 mL of the test solutions, including extracts, the prepared herbal gel formulation (F2), clindamycin (standard drug), and a marketed herbal product, were introduced into the wells. The plates were incubated at 37°C ± 1°C for 24 hours, after which the diameter of the inhibition zones (in mm) was measured to determine antibacterial efficacy. Each experiment was performed in triplicate to ensure accuracy, and the average values were recorded. Dimethyl sulfoxide (DMSO) served as a negative control in this evaluation (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 clumps.

Spreadability

The spreadability of the herbal gel formulations (F1-F4) was observed to range between 13.76–17.81 g.cm/sec, indicating that the gels can be easily spread with minimal shear force. A comparative analysis of spreadability and viscosity showed an inverse relationship; as the viscosity of the formulation increased, the spreadability significantly decreased.

pH

The pH values of the herbal gel formulations (F1-F4) were measured between 6.1 and 6.6, which closely align with the natural pH of human skin, ensuring compatibility and minimal irritation (**Table 2**).

Extrudability

The prepared herbal gels exhibited good extrudability, with the amount of gel extruded varying from moderate (++) to excellent (+++). However, as the viscosity of the gel increased, the extrudability was reduced, making it less convenient to dispense the gel from a collapsible tube.

Viscosity

Viscosity plays a crucial role in determining the pharmaceutical characteristics of the gel, including spreadability, extrudability, and ease of dispensing. The viscosity of the herbal gel formulations (F1-F4) was found to be in the range of 49,500–55,000 cps. Rheological analysis revealed that increasing torque led to higher shear stress, resulting in a reduction in viscosity, reflecting the shear-thinning behavior of the gels.

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.

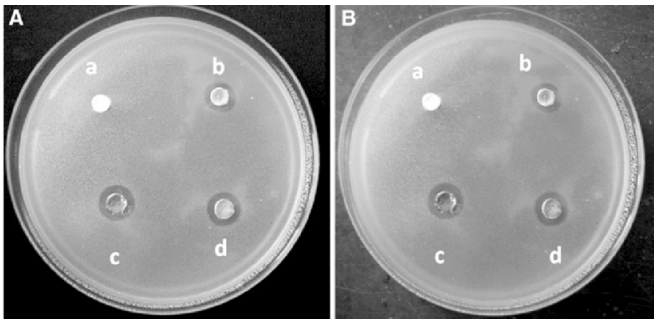

Figure 1. Pictures of microbial plates (A) *E. coli* (B) *B. subtilis*. (a) *Bougainvillea glabra* aqueous extract (b) *Bougainvillea glabra* methanol extract (c) *Bougainvillea glabra* ethyl acetate extract (d) *Bougainvillea glabra* petroleum ether extract.

Table 2: 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.

CONFLICT OF INTEREST

No conflict of interest is declared.

FUNDING INFORMATION

No agency provided funding.

ETHICAL STATEMENT

No animal was employed in this study.

ACKNOWLEDGEMENT

No acknowledgement is provided.

AUTHORS' CONTRIBUTION

Ravi Khatri: Analysis, Plagiarism correction, Revision

Madhukar Shende: Concept, Checking, Editing, Grammar correction, Literature Review, Formatting, Writing manuscript

REFERENCES

1. A. Vats and P. Sharma, "Formulation & evaluation of topical anti acne formulation of coriander oil," International Journal of Pharmacy and Pharmaceutical Science Research, vol. 2, no. 3, pp. 61–66, 2012.
2. A. J. Hayes and B. Markovic, "Toxicity of Australian essential oil Backhousia citriodora (Lemon myrtle). Part 1. Antimicrobial activity and in vitro cytotoxicity," Food and Chemical Toxicology, vol. 40, no. 4, pp. 535–543, 2002.
3. H. Azimi, M. Fallah-Tafti, A. A. Khakshur, and M. Abdollahi, "A review of phytotherapy of Acne vulgaris: perspective of new pharmacological treatments," Fitoterapia, vol. 83, no. 8, pp. 1306–1317, 2012.
4. C. Hsu, T.-H. Tsai, Y.-Y. Li,W.-H.Wu, C.-J. Huang, and P.-J. Tsai, "Wild bitter melon (Momordica charantia Linn. var. abbreviata Ser.) extract and its bioactive components suppress Propionibacterium acnes-induced inflammation," Food Chemistry, vol. 135, no. 3, pp. 976–984, 2012.
5. J. Saising and S. P. Voravuthikunchai, "Anti Propionibacterium acnes activity of rhodomyrtone, an effective compound from Rhodomyrtus tomentosa (Aiton) Hassk. leaves," Anaerobe, vol. 18, no. 4, pp. 400–404, 2012.
6. S. Fu, C. Sun, X. Tao, and Y. Ren, "Anti-inammatory effects of active constituents extracted from Chinese medicinal herbs against Propionibacterium acnes," Natural Product Research, vol. 26, no. 18, pp. 1746–1749, 2012.
7. V. Patil, A. Bandivadekar, and D. Debjani, "Inhibition of Propionibacterium acnes lipase by extracts of Indian medicinal plants," International Journal of Cosmetic Science, vol. 34, no. 3, pp. 234–239, 2012.
8. M. Kanlayavattanakul and N. Lourith, "Therapeutic agents and herbs in topical application for acne treatment," International Journal of Cosmetic Science, vol. 33, no. 4, pp. 289–297, 2011.
9. R. K. Chaudhuri and F. Marchio, "Bakuchiol in the management of acne-affected skin," Cosmetics & Toiletries Magazine, vol. 126, no. 7, pp. 502–510, 2011.
10. T. Mahmood, N. Akhtar, B. A. Khan, H. M. S. Khan, and T. Saeed, "Outcomes of 3% green tea emulsion on skin sebum production in male volunteers," Bosnian Journal of Basic Medical Sciences, vol. 10, no. 3, pp. 260–264, 2010.
11. Shivhare, R. S., Kamble, M. A., Mahapatra, D. K., Ingole, A. R., & Baheti, J. R. (2018). Development of mosquito repellant gel formulations from various natural volatile oils: comparative study with the marketed formulation odomos®. Journal of Drug Delivery and Therapeutics, 8(6), 106-110.
12. Roy, S., Bose, S., Sarkar, D., Mandal, S., Sarkar, S., & Mandal, S. K. (2020). Formulation and evaluation of anti-acne gel containing Murraya koeinigii extract. International Journal of Current Pharmaceutical Research, 108-113.
13. Prasad, S. B., & Bist, M. (2018). In vitro anti acne activity of methanolic extract of dried fruit of Embelia ribes. International Journal of Pharmaceutical Quality Assurance, 9(02), 148-152.