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Physical Characteristics and Antibacterial Activity of Leaf Extract Gel *Bougainvillea glabra* against *Staphylococcus aureus* and *Escherichia coli*

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Abstract
Bacterial resistance to conventional antibiotics has driven the search for natural antibacterial agents. Paper flower (Bougainvillea glabra) leaves contain secondary metabolites such as flavonoids, tannins, saponins, alkaloids, and steroids/terpenoids that are potentially antibacterial, yet their application in topical gel preparations and the accompanying stability evaluation remain scarcely studied. This study aimed to formulate a gel from Bougainvillea glabra leaf ethanol extract and to evaluate its physical characteristics, stability, and antibacterial activity against Staphylococcus aureus and Escherichia coli. The extract was obtained by maceration with 96% ethanol, yielding 10.05%, and was formulated into four gels containing 0% (F0), 5% (F1), 10% (F2), and 20% (F3) extract. Physical evaluation showed that all formulas remained homogeneous and stable throughout six freeze-thaw cycles, with pH 6.19-7.35, viscosity 3,353-8,506 cPs, spread ability 5.43-7.21 cm, and adhesion time 2.34-8.61 seconds. Disc diffusion assays showed that F3 produced the largest inhibition zones, 9.85±1.33 mm against Staphylococcus aureus and 9.20±0.56 mm against Escherichia coli, although still lower than the positive control. Statistical analysis indicated that increasing extract concentration did not significantly change antibacterial activity among formulas. The Bougainvillea glabra leaf extract gel exhibited favorable physical characteristics and shows potential as a natural topical antibacterial candidate.

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1. INTRODUCTION

Bacterial infections remain a major public health problem in many countries, particularly due to increasing resistance to conventional antibiotics *Staphylococcus aureus, a* Gram-positive bacteria and *Escherichia coli* As Gram-negative bacterium, are the two microorganisms most frequently isolated in skin and soft tissue infections, including open wounds and chronic wounds exposed to environmental contamination. These two bacteria are known to form biofilms and exhibit increased resistance to various antibiotic classes, making their control a challenge in modern skin infection therapy.

The increasing reliance on synthetic antibiotics has driven the development of alternative antimicrobial agents based on natural ingredients. One plant with the potential to be developed as a source of antibacterial compounds is the paper flower (*Bougainvillea glabra*), especially the leaves. Phytochemical analysis reported that the leaves of *Bougainvillea glabra* contain secondary metabolites such as flavonoids, tannins, saponins, alkaloids, and phenolic compounds, with a total phenolic content of 27.68 mg GAE/g extract and a total flavonoid content of 31.76 mg RE/g extract under optimum conditions. These contents underlie the antibacterial, antioxidant, and anti-inflammatory activities of this plant.

Several previous studies have reported antibacterial activity of *Bougainvillea glabra*. Literature review shows that the flowers and leaves of paper flowers have antibacterial potential against several skin pathogens, including *Staphylococcus aureus* and *Escherichia coli*, but it is still in the form of a literature review without any development of the preparation. Testing the antibacterial activity of pure leaf extracts of *Bougainvillea glabra* against *Staphylococcus aureus produced* an inhibition zone of 26.2-27.3 mm at a concentration range of 25-100%, with the highest effectiveness at a concentration of 75%. In the bacterium *Escherichia coli*, the paper flower leaf dip preparation showed an inhibition zone of 8.95-12.45 mm, with a concentration of 15% providing the highest activity, although still classified as weak-moderate. The formulation of the ethanol extract gel of the flower (not the leaf) of *Bougainvillea glabra at* a concentration of 20% it was also reported to produce an inhibition zone of 15.22-17.67 mm against *Staphylococcus aureus,* accompanied by the fulfillment of the evaluation of the physical quality of the gel, but not accompanied by long-term stability testing such as*cycling test* [6].

These findings indicate that research on the effectiveness of antibacterial *Bougainvillea glabra.* Most studies still focus on pure extracts or simple preparations such as dips, while development of topical gel preparations that provide more intensive contact between the active ingredient and the skin surface is still limited, especially from the leaves. Furthermore, there has not been much research specifically evaluating the physical characteristics and stability of Bougainvillea glabra leaf gel preparations using test *cycling* (*freeze-thaw*) while linking it to antibacterial activity against Gram-positive and Gram-negative bacteria in the same study.

This study aims to formulate a gel from the ethanol extract of leaves of *Bougainvillea glabra at* several concentration variations, evaluate the physical characteristics and stability through test *cycling (freeze-thaw)* for six cycles, and determine the concentration of extract that provides the most effective antibacterial activity against *Staphylococcus aureus* and *Escherichia coli through* the disc diffusion method. The results of this study are expected to provide scientific data regarding the potential of the leaves of *Bougainvillea glabra* as an alternative topical antibacterial active ingredient based on natural ingredients.

2. RESEARCH METHODS

2.1 Research Design

This research is an experimental laboratory study with a quantitative approach, conducted from March to May 2026 at the Pharmaceutical Laboratory and Microbiology Laboratory of Duta Bangsa University, Surakarta. Variations in the concentration of ethanol extract of leaves *Bougainvillea glabra* formulated in a gel preparation, then tested for its antibacterial activity against *Staphylococcus aureus* And *Escherichia coli* in vitro based on the diameter of the inhibition zone formed.

2.2 Materials and Samples

The main sample is leaves of *Bougainvillea glabra.* Fresh specimens were obtained from Tanjung Village, Nguter District, Sukoharjo Regency, and were determined at the Functional Implementation Unit (UPF) of the Army Health Strategic Reserves of Dr. Sardjito General Hospital, Tawangmangu, as the species *Bougainvillea glabra* Choisy (family Nyctaginaceae). Microbiological test material in the form of pure culture: *Staphylococcus aureus* and *Escherichia coli standardized* to a McFarland equivalent of 0.5 ($\pm 1.5\times10^8$ CFU/mL). The gel formulation ingredients included Carbopol 940, propylene glycol, triethanolamine (TEA), methylparaben, propylparaben, and peppermint oil. Each treatment was replicated three times using a purposive sampling technique based on the criteria of pure culture, optimal growth (18-24 hours), and freedom from contamination.

2.3 Extraction and Standardization

Leaf simplicia of *Bougainvillea glabra were* dried under direct sunlight for 5 days until a yield of 16.96% of the simplicia was obtained. Extraction was carried out by the maceration method using 96% ethanol solvent at a simplicia-solvent ratio of 1:10, followed by remaceration

for 2 days. The macerate was then concentrated using a rotary evaporator at 55°C until a thick extract with a yield of 10.05% was obtained. The extract was then standardized through drying loss tests, water content, ash content, and ethanol-free test, as well as screening for phytochemical content (alkaloids, flavonoids, saponins, tannins, and steroids/terpenoids).

2.4 Gel Formulation

The gel was formulated based on the modification of the reference formula [6] into four extract concentrations, namely F0 (0%, base/negative control), F1 (5%), F2 (10%), and F3 (20%), with a fixed composition of Carbopol 940 0.5% (gelling agent), propylene glycol 5% (humectant), triethanolamine 0.4% (alkalizing agent), methyl paraben 0.05% and propyl paraben 0.02% (preservative), peppermint oil 0.1% (fragrance), and 50 g of distilled water (Table 1). The gel preparation began with the development of Carbopol 940, followed by mixing methyl paraben in propylene glycol and propyl paraben in hot distilled water; then the extract was added and ground until homogeneous, and ended with the addition of triethanolamine and peppermint oil until a homogeneous gel was formed.

Table 1. Formulation of Bougainvillea glabra leaf extract gel

Material	Function	F0 (%)	F1 (%)	F2 (%)	F3 (%)
B. glabra leaf extract	Active ingredients	0	5	10	20
Carbopol 940	Gelling agent	0,5	0,5	0,5	0,5
Propylene glycol	Humectant	5	5	5	5
Triethanolamine (TEA)	Alkalizing agent	0,4	0,4	0,4	0,4
Methyl paraben	Preservative	0,05	0,05	0,05	0,05
Propylparaben	Preservative	0,02	0,02	0,02	0,02
Minyak paper mint	Fragrance	0,1	0,1	0,1	0,1
Aquadest	Solvent	ad 50	ad 50	ad 50	ad 50

2.5 Physical Evaluation and Stability Test

The physical evaluation of the gel included organoleptic tests, homogeneity, pH (pH meter), viscosity (Brookfield spindle viscometer number 4, 60 rpm), spread ability (gradual loading of 50-300 grams), and adhesiveness (load of 1 kg for 5 minutes). The stability test was carried out using the method *cycling test* (*freeze-thaw*) for six cycles, namely alternating storage at low temperature (4°C) and high temperature (40°C) for 24 hours per cycle, with physical parameter observations in each cycle.

2.6 Antibacterial Activity Test

Antibacterial activity was tested using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA) medium. Paper discs with a diameter of 5 mm were soaked in each test solution (F0, F1, F2, F3, and positive control), placed on the medium that had been inoculated with test bacteria, and then incubated at 37°C for 24 hours. The diameter of the inhibition zone was measured using a caliper based on the average vertical and horizontal diameters minus the disc diameter.

2.7 Data Analysis

Physical characteristic data were analyzed descriptively. Inhibition zone diameter data were analyzed using the Shapiro-Wilk normality test and Levene's homogeneity test, followed by One-

Way ANOVA and Post Hoc Tukey HSD tests using IBM SPSS Statistics version 25.0 to determine differences in antibacterial activity between formulas and against positive controls.

3. RESEARCH RESULTS AND DISCUSSION

3.1 Research Results

Plant determination confirms that the leaf sample used is the species *Bougainvillea glabra Choisy* from the Nyctaginaceae family. The drying process of the crude drug yielded a yield of 16.96%, while maceration extraction using 96% ethanol produced a thick extract with a yield of 10.05%. The resulting extract was blackish green, had a distinctive odor, a bitter taste, and was declared ethanol-free.

The results of the standardization of the simplex and extract (Table 2) showed that all non-specific parameters were within the required limits, namely drying loss and water content below 10%, and ash content below 6%. The results of phytochemical screening (Table 3) showed positive results for all groups of compounds tested, namely alkaloids, flavonoids, saponins, tannins, and steroids/terpenoids.

Table 2. Results of standardization of simplicia and Bougainvillea glabra leaf extract

Parameter	Simple Ingredients (%)	Extract (%)
Drying shrinkage	1,60 ± 0,48	4,45 ± 1,44
Water level	8,63 ± 0,50	7,10 ± 0,46
Ash content	4,50 ± 0,65	5,70 ± 0,64
Ethanol free	-	Negative (ethanol free)

Table 3. Results of phytochemical screening of Bougainvillea glabra leaf extract

Active compounds	Reagent	Observation result	Conclusion
Alkaloid	Mayer / Dragendorff / Wagner	White/orange-brown / brown sediment	Positive
Flavonoid	Mg powder + concentrated HCl	Orange color change	Positive
Saponin	Ethanol 96% + distilled water	Stable bus	Positive
Land	FeCl3 5%	Blackish green color change	Positive
Steroid/terpenoid	Lieberman-Burchard	Blue-green color change	Positive

Physical evaluation showed that all formulas (F0-F3) had a homogeneous texture without lumps and a distinctive mint aroma, with the color becoming increasingly intense as the extract concentration increased. A summary of the results of pH, viscosity, spread ability, and adhesiveness in the initial cycle (cycle 0) and the end of the stability test (cycle 6) is presented in Table 4. The pH value increased with increasing extract concentration, while viscosity and adhesiveness decreased, and spread ability increased. The values of these four parameters were relatively stable from cycle 0 to cycle 6 in all formulas.

Table 4. Physical characteristics and stability of Bougainvillea glabra leaf extract gel (cycle 0 and cycle 6)

Formula	pH (cycle 0)	pH (cycle 6)	Viscosity cPs (cycle 0)	Viscosity cPs (cycle 6)	Spread power cm (cycle 0)	Spread power cm (cycle 6)	Second adhesion force (cycle 0)	Second adhesion force (cycle 6)
F0	6,19± 0,05	6,21± 0,06	8.506± 307	6.298± 96	5,43± 0,13	5,44± 0,19	8,61± 0,25	6,96± 0,17
F1	6,38± 0,05	6,35± 0,09	5.332± 124	3.135± 73	5,56± 0,09	6,02± 0,13	4,45± 0,35	4,13± 0,54
F2	6,66± 0,03	6,66± 0,11	3.836± 71	3.130± 74	6,67± 0,15	6,78± 0,18	3,70± 0,10	3,62± 0,28
F3	7,35± 0,04	7,37± 0,07	3.456± 116	2.311± 293	7,22± 0,08	7,12± 0,06	2,34± 0,26	2,17± 0,22

The antibacterial activity test using the disc diffusion method (Table 5, Figure 1) showed that all gel formulas were able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. Formula F3 (20%) provided the largest average inhibition zone among the test gel preparations, namely 9.85±1.33 mm against *Staphylococcus aureus* and 9.20±0.56 mm against *Escherichia coli*, but still smaller than the positive control (24.17±0.83 mm and 22.50±0.98 mm).

Table 5. Results of the antibacterial activity test using the disc diffusion method

Formula	S. aureus inhibitory zone (mm)	Category	E. coli inhibitory zone (mm)	Category
F0	1,13 ± 0,36	Weak	1,13 ± 0,38	Weak
F1	7,15 ± 1,03	Currently	5,20 ± 0,22	Currently
F2	5,83 ± 0,29	Currently	5,07 ± 0,14	Currently
F3	9,85 ± 1,33	Currently	9,20 ± 0,56	Currently
Control (+)	24,17 ± 0,83	Very strong	22,50 ± 0,98	Very strong

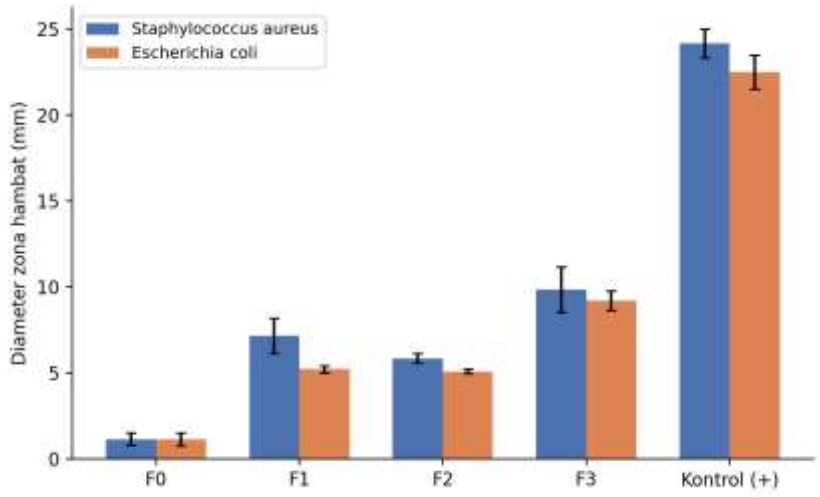

Figure 1. Comparison of the diameter of the inhibition zone of Bougainvillea glabra leaf extract gel against Staphylococcus aureus and Escherichia coli

3.2 Discussion

The physical characteristics of gels F0 to F3 showed good quality, with a homogeneous texture without lumps and a distinctive mint aroma in all formulas (Table 4). The pH value increased with increasing extract concentration, from 6.19 (F0) to 7.35 (F3). This trend is consistent with the weak alkaline nature of some of the alkaloid content in the ethanol extract of leaves of *Bougainvillea glabra*. The pH value obtained remains within the acceptable range for topical preparations, namely 4.5-8, so that it is physiochemically safe for use on the skin [7].

Increasing the extract concentration actually decreased the gel viscosity value, from 8,506 cPs (F0) to 3,353-3,456 cPs (F3). This decrease occurred because the ethanol extract of the leaves of *Bougainvillea glabra carries* an additional liquid component that dilutes the three-dimensional network density of Carbopol 940 as a gelling agent. The phenomenon of decreased viscosity due to the addition of liquid active ingredients has also been widely reported in other herbal gel formulations based on Carbopol [8]. However, all formulas in this study remained within the viscosity range required for topical preparations (500-10,000 cPs), so as not to interfere with the comfort of application.

Spread ability increased with decreasing viscosity, from 5.43 cm (F0) to 7.21-7.22 cm (F3), in line with the usual inverse relationship between viscosity and spread ability of semisolid preparations. Spread ability values from F0 to F2 were within the ideal range (5-7 cm), while F3 slightly exceeded this upper limit. The wider spread ability of F3 provides the advantage of greater application coverage on the skin surface, but its more liquid consistency risks reducing the preparation's contact time with the skin if not balanced by adequate adhesion.

In contrast, the adhesion strength decreased consistently with increasing extract concentration, from 8.61 seconds (F0) to 2.34 seconds (F3). This decrease indicates that the addition of the extract weakened the gel's adhesion to the skin surface, in line with the decrease in its viscosity. The shorter adhesion strength of F3 potentially reduces the duration of contact between the antibacterial compound and the skin surface, potentially lowering its therapeutic efficacy in real-world applications than projected from in vitro data alone.

Test results: *cycling tests* showed that all formulas remained homogeneous and maintained their characteristic aroma and color during six alternating storage cycles at 4°C and 40°C. The pH, viscosity, spread ability, and adhesiveness values in the sixth cycle did not differ much from the initial cycle (Table 4), which indicates that the leaf extract gel of *Bougainvillea glabra* has good physical stability against extreme temperature fluctuations. This stability complements the data not yet available in gel formulation research on *Bougainvillea glabra previously*, which has not evaluated the resistance of the preparation to long-term temperature changes [6].

Disc diffusion tests showed that all gel formulas were able to inhibit the growth of *Staphylococcus aureus* And *Escherichia coli*, with a pattern of increasing inhibition zones that is not completely linear with respect to extract concentration (Table 5) *Staphylococcus aureus*, the inhibition zone increased from F0 (1.13 mm) to F1 (7.15 mm), decreased at F2 (5.83 mm), then increased again at F3 (9.85 mm); a similar pattern also occurred at *Escherichia coli* This non-linear phenomenon indicates that the antibacterial activity of the gel is not solely determined by the extract dose, but also by the kinetics of the release of the active substance from the gel matrix. The higher viscosity of F2 compared to F1 (Table 4) is thought to have inhibited the rate of diffusion of the active compound into the agar medium, so that the inhibition zone of F2 was actually smaller than that of F1 even though the extract concentration was higher.

These results are in line with research on the antibacterial activity of pure leaf extracts of *Bougainvillea glabra* against *Staphylococcus aureus*, which also reported that increasing concentration is not always directly proportional to the area of the inhibition zone, with maximum activity achieved at a concentration of 75% (27.3 mm) which actually decreased at a concentration of 100% (26.5 mm) [4]. The inhibition zone of the pure extract is much larger than the results in the gel preparation of this study, which is normal because the active compounds in the free extract can diffuse directly without the hindrance of the gelling agent matrix, whereas in the gel preparation the active compounds must first be released from the base before diffusing into the agar medium. Compared with the flower extract gel formulation (*Bougainvillea glabra* a concentration of 20%, which produced an inhibition zone of 17.67 mm against *Staphylococcus aureus*[6], the F3 results in this study (9.85 mm) were relatively lower. This difference was likely influenced by the differences in the plant parts used (leaves compared to flowers), the composition of the gel base, and the volume of the preparation absorbed by the test disc.

The gel preparation consistently showed a larger zone of inhibition against *Staphylococcus aureus* compared to *Escherichia coli in* all formulas. This difference is related to the cell wall structure of the two bacteria: *Staphylococcus aureus, a* Gram-positive bacteria have a thick peptidoglycan layer but is relatively permeable to polar phytochemical compounds, whereas *Escherichia coli, as* a Gram-negative bacterium, it has an outer membrane composed of lipopolysaccharides, which functions as an additional barrier against the penetration of antibacterial compounds. Nevertheless, F3 still showed relatively balanced activity against both bacteria (9.85 mm against *Staphylococcus aureus* and 9.20 mm against *Escherichia coli*), which indicates that at the highest concentration, the diffusion barrier due to the outer membrane of *Escherichia coli* can be offset by a larger number of active compounds.

The antibacterial activity of the gel is thought to be supported by the flavonoid, tannin, and saponin content that were positively identified in the phytochemical screening of the extract (Table 3). Flavonoids work by increasing the permeability of bacterial cell membranes and disrupting nucleic acid synthesis, tannins precipitate cell proteins and inhibit bacterial enzymes, while saponins damage membrane integrity by reducing surface tension [9]. The combination of the working mechanisms of these compounds supports the formation of an inhibition zone around the test disc, although the magnitude of the final effect is still influenced by the ability of the gel matrix to release active compounds into the medium.

Statistical analysis using the Shapiro-Wilk and Levene tests showed that the data were normally distributed and homogeneous ($p>0.05$), so the One-Way ANOVA test was continued. The ANOVA results showed a significant difference in at least one treatment group ($p<0.05$). Further Tukey HSD post hoc tests showed that Formulas 0, 1, 2, and 3 were not significantly different from each other ($p>0.05$), while all formulas were significantly different from the positive control ($p<0.05$).

Thus, although F3 descriptively has the highest average inhibition zone, increasing the extract concentration from F1 to F3 has not been statistically proven to provide a significant difference in antibacterial activity between formulas; the difference detected in the ANOVA test was mainly due to the higher activity of the positive control compared to all extract gel formulas. The

variability in the standard deviation values for each formula was also influenced by technical factors in the disc diffusion test, such as agar medium thickness, disc absorption capacity, and inoculum density [10], which explained some of the deviation range in the data beyond the variation in the formulation itself. This finding confirms that the antibacterial effectiveness of the leaf extract gel of *Bougainvillea glabra* in the concentration range of 5-20% is not completely linearly dependent on the dose, but is also influenced by the physical characteristics of the preparation such as viscosity and the ability to release the active substance from the gel matrix.

All gel formulas showed statistically significantly lower antibacterial activity than the positive control clindamycin (24.17 mm for *Staphylococcus aureus* and 22.50 mm for *Escherichia coli*). Although F3 was the most optimal gel formula in this study, its effectiveness did not match or exceed the positive control, so the potential of this gel is more appropriately positioned as a natural antibacterial candidate companion rather than as a replacement for standard topical antibiotic therapy. This study also has limitations because the antibacterial activity test was only carried out in vitro without an active substance release test, skin irritation test, or post-storage antibacterial activity test, so further research is needed before this preparation is considered for topical application in humans.

4. CONCLUSION

Ethanol extract gel of leaves *Bougainvillea glabra* at a concentration of 0-20% has good physical characteristics and remains stable for six *freeze-thaw cycles*, with pH, viscosity, spread ability, and adhesiveness that meet topical preparation standards. Increasing the extract concentration affects the physical characteristics of the gel, namely increasing pH and spread ability but decreasing viscosity and adhesiveness. All gel formulas are proven to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli in* vitro. Formula F3 (20%) provided the largest average inhibition zone, namely 9.85±1.33 mm against *Staphylococcus aureus* and 9.20±0.56 mm against *Escherichia coli*, but still below the positive control. Statistical analysis showed that increasing the extract concentration from 5% to 20% did not produce a significant difference in antibacterial activity between formulas, so variations in concentration within this range have not been statistically proven to determine the antibacterial effectiveness of the gel. These results indicate that the leaf extract gel of *Bougainvillea glabra* has the potential to be developed as a topical antibacterial preparation based on natural ingredients, but its safety and effectiveness in humans still require further in vivo testing and skin irritation tests before it can be applied clinically.

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