JURNAL PHARMA BHAKTA

FORMULATION AND ACTIVITY TEST OF ANTI ACNE GEL BASED ON RAMANIA LEAF EXTRACT (*Bouea macrophylla* Griffith) AGAINST *Cutibacterium acnes* BACTERIA

Muhammad Fadhil Kamil[1]*, Wahyudin Bin Jamaludin[1], Fitriyanti[2]

[1]Department of Pharmacy, Universitas Borneo Lestari,
[2]Department of Midwifery, Universitas Borneo Tarakan

*Corresponding Author: fitriyantihudari@gmail.com

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ABSTRACT

Background: Acne is an inflammatory condition of the pilosebaceous unit involving infection by Cutibacterium acnes. Ramania leaves (*Bouea macrophylla*) contain flavonoids, saponins, and tannins with known antibacterial properties**. Objective:** This study aimed to formulate a methanolic extract gel of Ramania leaves and evaluate its physical and organoleptic qualities as well as its antibacterial activity against *Cutibacterium acnes*. **Method:** The extract was obtained via maceration and formulated in a carbopol-based gel with concentrations of 5%, 7.5%, 10%, 15%, and 20%. Physical evaluations included organoleptic testing, homogeneity, pH, viscosity, adhesiveness, and spreadability. Antibacterial activity was tested using the well diffusion method. **Results:** showed all gel formulas met standard physical and organoleptic criteria. The 5% extract gel demonstrated the largest inhibition zone against *Cutibacterium acnes* (22.8 mm ± 2.06). Concentrations above 10% showed decreased effectiveness, likely due to saturation or physical instability**. Conclusion:** The 5% ramania leaf extract gel is the most effective and stable formulation for topical anti-acne application.

ABSTRAK

Latar belakang: Jerawat merupakan peradangan unit pilosebasea yang melibatkan infeksi Cutibacterium acnes. Daun Ramania (Bouea macrophylla) mengandung flavonoid, saponin, dan tanin yang bersifat antibakteri. **Tujuan:** Penelitian ini bertujuan memformulasikan gel ekstrak metanol daun Ramania serta mengevaluasi mutu fisik, organoleptik, dan aktivitas antibakterinya terhadap *C. acnes.* **Metode:** Ekstrak diperoleh melalui maserasi, lalu diformulasikan dalam basis karbopol dengan konsentrasi 5%, 7,5%, 10%, 15%, dan 20%. Evaluasi mutu fisik meliputi uji organoleptik, homogenitas, pH, viskositas, daya lekat, dan daya sebar. Aktivitas antibakteri diuji dengan metode difusi sumuran. **Hasil:** menunjukkan seluruh formula gel memenuhi kriteria mutu fisik dan organoleptik. Gel konsentrasi 5% menghasilkan zona hambat terbesar (22,8 mm ± 2,06) terhadap *C. acnes*. Konsentrasi di atas 10% menunjukkan penurunan efektivitas, diduga akibat saturasi atau ketidakstabilan

fisik. **Simpulan:** Gel ekstrak daun Ramania konsentrasi 5% merupakan formulasi paling efektif dan stabil sebagai sediaan topikal antijerawat.

INTRODUCTION

Acne (acne vulgaris) is a common chronic inflammatory skin disease, particularly in adolescents who experience increased sebum production due to hormonal changes. One of the main causes of acne is infection with the bacteria *Cutibacterium acnes* (*C. acnes*), which thrives in clogged, sebum-filled hair follicles. This bacterium breaks down triglycerides into free fatty acids, triggering irritation and producing enzymes and proteins that exacerbate skin inflammation (Pariury et al., 2022).

Acne therapy generally involves the use of antibiotics such as tetracycline, erythromycin, and clindamycin. However, long-term use can lead to bacterial resistance, which is a serious problem globally, including in Indonesia (Ariani et al., 2020). This has prompted the search for safer and more sustainable alternative treatments.

Herbal plants are a potential solution because they contain bioactive compounds such as flavonoids, saponins, and tannins, which have antibacterial and anti-inflammatory activities. One promising local plant is Ramania (*Bouea macrophylla*), which is traditionally used in Kalimantan medicine. Methanol extract of Ramania leaves has been shown to have various pharmacological activities as a potent antibacterial against *C. acnes*, with an inhibition zone reaching 20.90 mm (Fitriyanti et al., 2024).

Gels are effective topical preparations because they are easy to apply, comfortable to use, and allow for even distribution on the skin. Therefore, this study aimed to formulate and test the activity of an anti-acne gel based on Ramania leaf extract against *C. acnes* as a safer and more natural treatment alternative.

METHOD

Equipment

The equipment used included a digital scale (Ohaus®), pH meter (Hanna®), rotary evaporator (IKA® RV 10), incubator (Memmert®), oven (Memmert®), laminar air flow (LAF) (Esco®), autoclave (All American®), spirit lamp, loop needle, mortar and pestle, petri dish, porcelain dish, spatula, dispersion tester, and laboratory glassware such as test tubes, measuring cylinders, Erlenmeyer flasks, and beakers.

Materials

The materials used were: Ramania leaf extract (*Bouea macrophylla*), methanol (PT. Brataco Chemical, Indonesia), 70% ethanol, 96% ethanol (CV Multi Kimia Raya), distilled water (PT. Brataco Chemical), carbopol 940, triethanolamine, propylene glycol (CV Indo Kimia Pratama), nipagin, 2% clindamycin (Kimia Farma®), *Cutibacterium acnes* ATCC 6919 bacteria, Mueller Hinton Agar (MHA) media (Oxoid®), McFarland

solution 0.5 (visual standard), gelatin, $FeCl_3$ 1%, NaCl 0.9%, NaOH 10%, and well diffusion antibacterial activity test kit.

Research Procedures

Plant Collection and Identification

Samples of Ramania (*Bouea macrophylla* Griffith) leaves were collected from the Banjarmasin area, South Kalimantan, in October 2024. The leaves selected were mature, healthy, and physically undamaged. After collection, the leaves were washed under running water, drained, and then air-dried in a shaded area. Plant identification was carried out by the Faculty of Mathematics and Natural Sciences Laboratory, Lambung Mangkurat University, Banjarbaru, South Kalimantan.

Preparation of Simplicia

The dried leaves are ground using a blender, then sieved using a 60-mesh sieve to obtain simplicia powder with uniform particle size (Katno, 2008); (Astuti, et al., 2024).

Making Extracts

A total of 500 g of powdered medicinal plants were extracted using the maceration method using technical methanol as the solvent at a ratio of 1:10 (medicinal plant:solvent). The soaking process was carried out for 24 hours, with the first 6 hours being stirred using a macerator, and the remaining 18 being left to stand. The extraction was carried out twice (remaceration) to obtain maximum yield. The filtrate was filtered and evaporated using a rotary evaporator at low temperature until a thick extract was obtained (Asworo *et al.,* 2023).

Gel Formulation

The extract was incorporated into a carbopol 940 gel base with five concentration variations: 5%, 7.5%, 10%, 15%, and 20%. The formulas contained methylparaben as a preservative, TEA (triethanolamine) as a pH adjuster, and propylene glycol as a cosolvent and humectant. All formulas used distilled water as a solvent. The following anti-acne gel formulations based on methanol extract of Ramania leaves are shown in Table 1.

Table 1. Anti-acne Gel Formulation of Methanol Extract of Ramania Leaves

Material	Function	F1	F2	F3	F4	F5
Ramania Leaf Extract	Active substance	5 ml	7,5 ml	10 ml	15 ml	20 ml
Carbopol	Thickener	1 g	1 g	1 g	1 g	1 g
Trietanolamin	pH adjuster	0,1 g	0,1 g	0,1 g	0,1 g	0,1 g
Methylparaben	Preservative	0,1 g	0,1 g	0,1 g	0,1 g	0,1 g
Propilen Glikol	Humektan	10%	10%	10%	10%	10%

Material	Function	F1	F2	F3	F4	F5
Aquadest	Solvent	q.s ad 100 ml	q.s ad 100 ml	q.s ad 100 ml	q.s ad 100 ml	q.s ad 100 ml

Physical and Organoleptic Evaluation

Organoleptic

Observation of the form, odor, and color of the preparation (Ansel, 1989).

Homogeneity

Observation by smearing the preparation on a glass slide to ensure there are no coarse grains (Surini and Amirtha, 2018).

pH

The pH test is performed using a pH meter. One gram of the gel preparation is weighed and dissolved in 10 mL of distilled water. The pH meter is then inserted into the solution and left for a few moments until the display shows no change (constant). The pH of the preparation should be between 4.5 and 6.5. If it is too acidic, it can cause skin irritation, while if it is too alkaline, it can cause scaly skin (Warnida et al., 2017)**.**

Spread Power

Measured using two glass plates and a load of 50 g, the distribution diameter was observed (Grag et al., 2002).

Stickiness

A 0.25 g gel was placed on a slide and pressed with a 1 kg load for 5 minutes. The slide was then mounted on a test apparatus. The test apparatus was given an 80 g load and the gel release time from the slide was recorded (Ismirani et al., 2016).

Viscosity

The viscosity of a preparation is measured by placing a sample in a viscometer. The spindle size and rotation speed are adjusted, then the device is turned on, and the viscosity of the gel is read. Record the results from the viscometer when the number indicated by the viscometer is constant (Septiani et al., 2011). The viscosity value of a gel preparation is 3000-50000 cPs (SNI 16-4380-1996).

Antibacterial Activity Test

Sterilization

Glassware and media were sterilized using an autoclave at 121°C for 15 minutes. Loop needles and tweezers were sterilized by annealing (Kumesan et al., 2013).

Media Preparation

MHA (34 g/L) was dissolved in distilled water, sterilized, and then used for solid agar and agar slants.

Bacterial Rejuvenation

Cutibacterium acnes (ATCC 6919) colonies were inoculated onto agar slants and incubated for 24 hours at 37°C (Anggraini et al., 2013).

Control

Gel base without extract was used as a negative control, while 2% clindamycin gel was used as a positive control.

Activity Test

Paper discs were soaked in each gel formula for 30 minutes. The bacterial suspension was inoculated onto MHA, and then the discs were placed on top. The plates were incubated for 24 hours at 37°C. The zones of inhibition formed were measured in millimeters. The test was performed three times (triplicate).

Data analysis

Organoleptic data are presented descriptively. Quantitative data from pH, spreadability, viscosity, adhesiveness, and inhibition zone tests were analyzed using: One-Way ANOVA for normally distributed and homogeneous data, Kruskal-Wallis for non-normal or non-homogeneous data, Mann-Whitney as a two-group follow-up test, and descriptive statistics to present the mean and standard deviation values.

RESULT

Ramania Determination

The results of the determination indicate that the ramania leaf plant used belongs to the Anacardiaceae family, genus Bouea, and species *Bouea macrophylla* Griffith. The identification process was conducted at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, under certificate number 248/LB.LABDASAR/XI/2024.

Preparation of Ramania Leaf Simplicia

Ramania leaves were selected based on their healthy morphology, washed, dried in the shade, then ground and sieved through a No. 60 mesh screen. This process produced a dry simplicia powder used as an extraction material.

Ramania Leaf Extraction

A total of 350 grams of ramania leaf powder was extracted using the maceration method with 70% methanol for 24 hours (6 hours of stirring, 18 hours of standing). The maceration method was chosen because it does not involve excessive heating, thus maintaining the stability of active compounds that could potentially be destroyed by heating (Nurhasnawati, 2017). Furthermore, research results (Fitriyanti et al., 2024) showed that ramania leaf extract obtained through the maceration method had superior antibacterial activity compared to the soxhletation method. At a concentration of 50%, the extract produced by maceration had an inhibition zone of 19.28 mm against Staphylococcus aureus, which was larger than the soxhletation extraction, which only produced an inhibition zone of 10.7 mm at the same concentration. The extract yield is presented in Table 2.

Table 2. Yield Of Ramania Leaf Extract

Powder weight (g)	Extract weight (g)	Yield (%)
350	43,6	12,48

Phytochemical Screening of Ramania Leaf Extract

The methanol extract was tested using specific reagents to identify bioactive compounds. These results align with studies by Hairunnisa et al. (2022) and Conitaty (2022), which reported the presence of flavonoids, saponins, tannins, and phenols associated with antibacterial activity against C. acnes. The screening test results are presented in Table 3.

Table 3. Phytochemical Screening Results of Ramania Leaf Extract

Compund	Reagent	Result	Information
Flavonoid	Concentrated HCl + Mg powder	Positive	Orange color is formed
Alkaloid	Dragendorff	Positive	Orange precipitate forms
	Mayer		White precipitate forms
Steroid	Kloroform	Positive	The bottom layer is red
Triterpenoid	H_2SO_4 concentrated	Positive	The undercoat is reddish brown
Saponin	Distilled water	Positive	Stable foam lasts >10 minutes
Fenol	$FeCl_3$ 1%	Positive	The color of the solution becomes green
Tanin	$FeCl_3$ 1%	Positive	The color of the solution becomes dark green
Kuinon	NaOH 1%	Negative	There is no color change to red

Physical Quality of Ramania Leaf Extract Gel Preparations

Five gel formulas with extract concentrations of 5%, 7.5%, 10%, 15%, and 20% were tested for physical quality. The organoleptic test results of the Ramania leaf extract gel are shown in Table 4. Homogeneity, pH, viscosity, and adhesion data are shown in Table 5.

Table 4. Organoleptic test results of ramania leaf extract gel

Formula	Form	Colour	Smell
F1	Semi-solid	Green	Etanol + herb
F2	Semi-solid	Green	Etanol + herb
F3	Semi-solid	Orange	Etanol + herb
F4	Semi-solid	Orange	Etanol + herb
F5	Semi-solid	Brown	Etanol + herb

Table 5. Results of homogeneity, pH, Viscosity, Spreadability, and Adhesiveness Tests of Gel Preparations

Formula	Homogeneity	pH	Viscosity Average ± SD	Spreadability	Adhesiveness Average ± SD
F1	Homogen	6,24	25.750 ± 0.66	6,03	3,73 ± 0,30
F2	Homogen	6,44	24.833 ± 0.76	5,60	3,58 ± 0,41
F3	Homogen	6,86	19.500 ± 0.50	5,00	3,02 ± 0,78
F4	Homogen	7,31	17.667 ± 0.28	5,80	2,26 ± 0,92
F5	Homogen	7,52	14.333 ± 0.76	6,03	2,02 ± 0,29

The pH value shows a physiological range (4.5–6.5) to slightly alkaline, Viscosity decreases with increasing extract concentration

Antibacterial Activity Test of Ramania Leaf Extract Gel

The results of the inhibition test against *Cutibacterium acnes* showed that all formulas exhibited antibacterial activity, with the best results demonstrated at a concentration of 5% (Table 9). These results support the finding that flavonoids and tannins in the extract play a role in antibacterial activity. The decrease in inhibition at higher concentrations is suspected to be due to precipitation of the active compounds or a viscosity-pH imbalance. This aligns with the literature by Hairunnisa et al. (2022) and Budhi et al. (2020), which indicates that antimicrobial effectiveness depends on the stability of the preparation and the solubility of the active compounds. The average inhibition zone diameter of the Ramania Leaf Methanol Extract gel against *C. acnes* is shown in Table 5.

Table 5. Average Inhibition Zone of Ramania Leaf Methanol Extract Gel against *C. acnes*

Concentration (%)	Average (mm)	Categories
Formula 1	20,63	Very Strong
Formula 2	18,16	Strong
Formula 3	20,10	Very strong
Formula 4	14,76	Strong
Formula 5	11,17	Strong
Control (+)	28,57	Very Strong
Control (-)	0	-

DISCUSSION

The results of antibacterial testing using the well diffusion method showed that the ramania leaf extract gel had inhibitory activity against C. acnes. The measured inhibition zone decreased with increasing extract concentration in the gel. The formula with 5% extract produced a small inhibition zone (approximately 22.8–18.7 mm), while

the highest extract concentration produced a smaller inhibition zone (approximately 10.8–11.8 mm). The positive control (2 µg clindamycin antibiotic) produced a much larger inhibition zone (approximately 27.8–29.6 mm), while the negative control (aquadest base gel) did not produce an inhibition zone (0 mm). Statistical analysis (one-way ANOVA) showed a significant difference between the inhibition zones of the lowest extract formula and the negative control ($p < 0.05$), indicating a real antibacterial effect on the extract gel.

These results align with the report by Hairunnisa et al. (2022) who found that methanol extract of ramania leaves can inhibit bacterial growth, although the inhibition zone is relatively small. In Hairunnisa's (2022) study, a concentration of 2.048 mg/mL only produced a zone of ~3.33 mm (weak category), while the highest concentration of 32.760 mg/mL produced a zone of ~8.48 mm (moderate category) against *Escherichia coli*. Similarly, Conitaty (2022) reported that methanol extract of ramania leaves only produced weak inhibition of *Staphylococcus aureus* growth at various concentrations.

This comparison shows a similar pattern: Bouea leaf extract exhibits antibacterial activity, but quantitatively it is moderate to weak compared to synthetic antibiotics. Although the inhibition zone is relatively small, its presence is statistically significant and provides scientific evidence that ramania leaf extract can suppress the activity of C. acnes. This antibacterial activity is likely influenced by the flavonoids, tannins, and alkaloids in the extract, which are known to neutralize bacterial cell walls or damage cell membranes. The fact that *Bouea macrophylla* seed extract in the study by Worrapan et al. (2022) also showed clear antibacterial activity against C. acnes further confirms that the Bouea genus contains active anti-acne compounds. Thus, these results support the hypothesis that ramania leaf extract can be developed as a herbal anti-acne agent. The main scientific significance lies in verifying the antibacterial activity of this natural extract: although the effect is still moderate, this study confirms the potential of *Bouea macrophylla* in herbal-based acne therapy. This approach aligns with previous findings and emphasizes the importance of exploring local biological resources as an alternative therapy with minimal side effects.

CONCLUSION

Ramania leaf extract gel at various concentrations met the physical and organoleptic quality standards for topical preparations. All formulations demonstrated antibacterial activity against Cutibacterium acnes, with the best effectiveness demonstrated at a concentration of 5% (22.8 mm inhibition zone). Concentrations above 10% showed decreased inhibition and stability. Therefore, a concentration of 5% was declared the optimal formula, effective and stable for use as a natural-based anti-acne gel preparation.

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