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Evaluation of anti-acne gel of *Piper crocatum* leaves ethanolic extract against *Propionibacterium acnes*

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ABSTRACT: The red betel leaf (*Piper crocatum* Ruiz & Pav.) exhibited antibacterial properties by inhibiting *Propionibacterium acnes,* known for causing acne. The study aimed to develop an anti-acne gel formulation using *Piper crocatum* leaves ethanolic extract and to evaluate its antibacterial efficacy against *Propionibacterium acnes*. The extract was formulated into three gel formulas, with concentrations of 15%, 20%, and 25%, respectively. Antibacterial activity was evaluated using the agar well diffusion method, with Clindamycin gel as the positive control. The inhibition diameters were analyzed statistically using One Way ANOVA and Post Hoc LSD tests at a 95% confidence level. The result showed that the gel met the physical test requirements. The inhibition zone of the samples was observed with an inhibition diameter of 7.98 mm, 8.06 mm, and 8.16 mm for 15%, 20%, and 30% extract of gel, respectively, compared to the positive control 27.60 mm ($p < 0,05$). The research findings indicate that anti-acne gel of *Piper crocatum* leaves ethanolic extract exhibit moderate antibacterial activity in an in vitro setting. Statistically significant differences in inhibition diameters were observed between each gel sample and positive control.

KEYWORDS: Antibacterial; gel; red betel leaf

INTRODUCTION

Acne is a dermatological concern arising from the hyperactivity of sebaceous glands, which leads to the obstruction of pores due to the accumulation of excess fat deposits. When it is infected by bacteria, it can cause inflammation [1]. Bacterial species such as *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* contribute to acne development. Strategies to mitigate acne include reducing sebum production, inflammation, and the number of colonies responsible for acne [2]. Antibacterial topical therapy is a viable solution, however, their prolonged use may induce skin irritation and resistance. Recently, the incorporation of green products in topical formulations has gained popularity. Plant extracts are widely acknowledged for their diverse advantageous properties, including antibacterial.

Red Betel leaf (*Piper crocatum* Ruiz & Pav.) a tropical plant renowned for its medicinal properties. It contains alkaloid, saponin, tannin, and flavonoid, which confer their antibacterial potential [3]. It also contains quercetin, which inhibits beta-lactamase, disrupts bacterial peptidoglycan synthesis, and enhances cell membrane permeability [4]. In previous study, demonstrated that gel containing quercetin as an active ingredients, exhibited an inhibition zone against *P. acnes* [5]. *Piper crocatum* Ruiz & Pav. leaves extract has demonstrated promising in inhibiting *Staphylococcus aureus, Staphylococcus epidermidis* [6], Methicillin Resistant *Staphylococcus aureus* (MRSA) [7], *Propionibacterium acnes* [8], *Streptococcus mutans* [9], and *Escherichia coli* [10]. The ethanolic extract of *Piper crocatum* leaves exhibit antibacterial activity against *P. acnes* with minimum inhibition concentration of 10% [11].

This study formulated *P. crocatum* Ruiz & Pav. leaves ethanolic extract into topical preparations, specifically as a gel. Gel is favored over ointment due to their potential for topical drug delivery. Gels are characterized by rapid absorption and lack an oily phase that could exacerbate the acne conditions [1]. Within topical preparations, the active substance must be released from the base and diffuse into the surface of skin tissue. Nevertheless, a more viscous base can impede the diffusion process and hinder optimal performance [12]. This study encompassed the formulation of gel preparations containing varying concentrations of *P.crocatum* Ruiz & Pav. leaves ethanolic extract (15%, 20%, and 25%), with subsequent assessment of their antibacterial activity against *P. acnes*. The selection of these concentration was informed by a previous study that indicated the antibacterial activity of the specified concentration against *P. acnes*.

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MATERIALS AND METHODS

Materials

The tools that used were glassware (PT. Iwaki Glass Indonesia, Sumedang, Indonesia), analytical weight scale (Ohaus, Shanghai, China), mixer (Philips, Netherland), rotary evaporator (Buchi R 300, Flawil, Switzerland), incubator (Biobase, Shandong, China), autoclaves (All American, USA), Lamina Air Flow (LAF), magnetic stirrer (Thermo, USA), and other supporting tools.

The materials were red betel leaves (*Piper crocatum* Ruiz & Pav.) as active ingredients that harvested in Pesanggaran District, Bayuwangi, Jawa Timur. Other materials utilized in this study include carbopol (PT. SABA, Denpasar, Indonesia), triethanolamine (TEA) (PT. SABA, Denpasar, Indonesia), glycerin (PT. SABA, Denpasar, Indonesia), propylene glycol (PT. SABA, Denpasar, Indonesia), methyl paraben (PT. SABA, Bali, Indonesia), aquadest, ethanol (PT.Brataco, Denpasar, Indonesia), nutrient agar media (NA), Mc. Farland 0.5, *P. acnes* bacterial, and Clindamycin gel (Surya Dermato Medica Laboratories, Surabaya, Indonesia).

Plant determination and extract preparation

The plant was identified in Karakterisasi Kebun Raya "Eka Karya" Bedugul, Bali-BRIN, Indonesia. The sample was shade dried and ground in the mixer. The powdered flowers (1000 g) were extracted by maceration with ethanol (1:5) for three days with regular shaking every eight hours. The extract was filtered and evaporated with rotary evaporator at 50 °C to obtain *P.crocatum* leaves ethanolic extract. The crude extract was stored at -20°C until further use.

Gel formulation

The gel formulation was listed in Table 1. Carbopol 940 as gelling agent was swelling into distilled water that had been heated. Carbopol 940, once adequately hydrated, was vigorously stirred and combined with glycerin, forming mixture 1. In a separate mortar, methyl paraben was mixed with propylene glycol (mixture 2). Mixture 2 was added gradually into the mixture 1, and mixed it properly. The extract was diluted with distilled water and TEA, with continuous stirring until it no longer adhered to the mortar. The gel base then added gradually into the extract and stirred until homogenous [13].

Table 1. The Formula of gel with *Piper crocatum* leaves ethanolic extract.

Materials	Negative control (%)	FI (%)	FII (%)	FIII (%)
Extract	-	15	20	25
Carbopol	1	1	1	1
TEA	1.2	1.2	1.2	1.2
Glycerin	10	10	10	10
Propylene glycol	15	15	15	15
Methyl paraben	0.2	0.2	0.2	0.2
Aquadest	ad 50 mL	ad 50 mL	ad 50 mL	ad 50 mL

Evaluation of gel preparations

The physical evaluation of gel preparations includes organoleptic, pH, adhesion, and spreadability test. The organoleptic test includes color, odor, and consistency [14]. The pH test involved dissolving 500 mg each gel in 5 mL of distilled water, followed by pH universal testing of the solution [15]. The adhesion test conducted by placing 0.5 g sample between two glass objects, applying a 65 g load for 5 minutes. Placed the glass object in the test device and recording the duration until detachment. The spreadability test consisted of placing a 0,5 g sample between two glass plates, applying loads of 50 g, 100 g, and 150 g, and measuring the diameter of the spread sample [16].

Antibacterial test

Antibacterial activity test was conducted using agar well diffusion method, with nutrient agar as the media. The turbidity of *P.acnes* suspension was compared with Mc. Farland 0.5 standard solution. If the suspension exhibited cloudiness, NaCl 0,9% was added to neutralize until the desired turbidity level was achieved. Subsequently, 1 mL of bacterial suspension poured into the sterilized petri dish, followed by 20 mL of preheated nutrient agar (NA) media. The rotating motion was applied to the petri dish to ensure thorough mixing, and it was then allowed to solidify. Once solidified, five wells were created in agar to accommodate the test sample comprising a negative control (gel base), FI, FII, FIII, and positive control (Clindamycin gel). The petri dish was subsequently incubated for 24 hours at 37°C under anaerobic conditions for *P.acnes* in an anaerobic jar inside the incubator. The clear zone surrounding the wells, indicating inhibition zones, were observed and measured using a caliper. The procedure was repeated three times [17].

Data analysis

The data of inhibition diameter was analyzed using One Way ANOVA and followed by Post Hoc LSD. The experimental procedures should be described in sufficient detail to enable others to repeat the experiments.

▪ RESULTS AND DISCUSSION

Physical evaluation of anti-acne gel of *Piper crocatum* leaves ethanolic extract

The organoleptic assessment involved the examination of shape, color, and odor of three formula of gel preparations. The organoleptic result is summarized in Table 2. The sample exhibited a gel-like consistency, displaying a dark greenish color and smells typical scent of the extract. The selection of Carbopol 940 as the gelling agent was intended to enhance the consistency of the gel formulations. The choice was based on its facile water dispersion and favorable viscosity. The action of carbopol hinges on its ability to bind the solvent to the structure of polymer, leading to cross-linking with the polymers, which causes the entrapment of water [12]. The combination of carbopol, propylene glycol, and glycerin had a noteworthy impact. Propylene glycol played a role in reducing the strength of the tissue structure by the presence of hydrogen bonds. It contributes to maintaining the gel viscosity, preventing water evaporation from the formulation, and preventing water absorption by the extract [18]. Glycerin has a mechanism to increase the surface tension, thereby elevating the viscosity preparations [5].

Table 2. Physical evaluation of anti-acne gel of *Piper crocatum* leaves ethanolic extract.

Test	Detail	FI (15%)	FII (20%)	FIII (25%)
Organoleptic	Color	DG	DG	DG
	Odor	SE	SE	SE
	Consistency	G	G	G
pH		6 ± 0.000	6.5 ± 0.000	6.5 ± 0.000
Adhesion (sec)		2.28 ± 0.380	2.60 ± 0.469	2.95 ± 0.167
Spreadability (cm)		6.97	6.60	6.30

Note : DG : Dark greenish; SE : Scent of extract; G : Gel

The pH assessment was conducted to ascertain the gel's acidity and ensure that the preparations fell within the acceptable pH range for skin (4.5 to 6.5) to prevent skin irritation [19],[20]. The pH test of the sample was presented in Table 2, where all of the formulations met the criteria for skin pH. Notably, an increase in the extract concentration led to an elevation in the pH of the gel preparations. Carbopol has a hydroxyl group that exhibits acid conditions within a pH range of 2,5 to 4,0. To achieve the desired pH levels, the addition of TEA as stabilizing agent was necessary. TEA facilitated the ionization of the carboxyl group in carbopol, creating a repulsive force that resulted in the formation of hydrogen bonds that are capable of absorbing and retaining water, resulting in an elevation of viscosity [21],[22]. Carbopol is stable and maintains its viscosity within the pH range of 6 to 11. However, its viscosity will decrease if the pH of gel falls below 3 or exceeds 12 [23].

The adhesion test was conducted to ensure the ability of the preparations to adhere effectively to the skin upon application. Enhanced adhesion of the gel to the skin improves the active substance's delivery, thereby increasing the effectiveness of the preparations [24]. The result of the adhesion test was listed in Table 2,

indicating that a higher concentration of extract, yielded the more viscous preparations, which required a longer time to adhere.

The spreadability test was carried out to determine the spreading properties of preparations when applied to the skin surface. A formulation's eases of dispersion on the skin is essential for user convenience. Optimal spreadability has a range between 5-7 cm [25]. An increased extract concentration reduced spreadability, inversely proportional to the viscosity. The decrease in dispersive ability was further evidenced by the higher viscosity levels in the gel preparations [20]. These results presented in Table 2, confirmed that all the formulations met the criteria of good spreadability range.

Antibacterial activities of anti-acne gel of *Piper crocatum* leaves ethanolic extract

Antibacterial activity test was conducted using three gel formulas as a sample, gel bases serving as the negative control, and Clindamycin gel as the positive control. All of the tests were replicated three times using distinct petri dishes. The agar well diffusion method was chosen based on its ability to accommodate a higher gel concentration, facilitating rapid diffusion into the medium, and consequently a more robust inhibitory effect. The antibacterial test was quantified by measuring the diameter of the inhibition zones around the well, as detailed in Table 3. The antibacterial activities were divided into four categories based on inhibition diameter, encompasses weak (< 5 mm), moderate (5-10 mm), strong (>10-20 mm), and very strong inhibition (>20-30 mm) [26]. Notably, all of the samples exhibited a moderate inhibitory effect on the growth of *P.acnes*, whereas Clindamycin gel as positive control showed very strong inhibition in 27.60 mm.

Table 3. Antibacterial activity of anti-acne gel of *Piper crocatum* leaves ethanolic extract.

Replication	Inhibition diameter (mm)				
	FI (15%)	FII (20%)	FIII (25%)	C (-)	C (+)
1	7.90	7.50	7.80	-	26.5
2	8.00	8.50	8.70	-	28.5
3	8.05	8.20	8.00	-	27.8
Mean	7.98 ± 0.07	8.06 ± 0.51	8.16 ± 0.47		27.60 ± 1.01

Note: Data expressed in mean±SD (n=3)

The negative control comprised a gel formulation without *P.crocatum* leaves ethanolic extract, serving as a correction factor due to the presence of preservatives (methylparaben) in the gel base. Nonetheless, the findings indicated that the negative control exhibited no antibacterial activity. Therefore it can be inferred that methylparaben which is included in the formula did not affect the inhibition against *P. acnes* [27]. The observed trend indicated a wider inhibition diameter as the extract concentration increased. This phenomenon can be attributed to the secondary metabolites within *P.crocatum* ethanolic leaf extract, including alkaloids, steroids, terpenoids, flavonoids, and tannins [11],[28]. Flavonoids were noted for their capacity to disrupt bacterial cell membranes by denaturing its protein bonds, thereby preventing bacterial cell replication and leading to cell lysis and death [20]. Terpenoids disrupt the function of cell membranes interfere with glucosyltransferase activity and cause bacterial death [9],[29]. Tannins inactivate microbial adhesin, enzymes, cell envelope transport protein, and complex with polysaccharides [30]. Alkaloids inhibit bacterial growth by interfering with the permeability of cell walls and membranes and also disrupt the component of peptidoglycan which makes up the cell wall of bacteria [9],[11].

Previous research showed that *P. crocatum* leaves ethanolic extract in exact concentration exhibited strong inhibitory effect against *P. acnes*. The differences observed attributed to the formulation of extract into gel preparations. The viscosity of gel was significantly influenced the diffusion of active ingredients. The more viscous gel can reduce the drug release rate, consequently resulting in a decrease in the antibacterial activity against bacteria [12]. The components of gel base were also identified as potential factors influencing drug release. Glycerin can increase the permeability of the active substance [31],[32], while propylene glycol has lipophilic properties, aiding in the penetration of quercetin through bacteria cell walls and acting synergistically to enhance the penetration of the active substance, thereby functioning as an antimicrobial component [5]. Both glycerin and propylene glycol contribute to the dehydration of skin membranes, that will increase the permeability of active substances from water-based formulations [33].

P. acnes growth optimally at pH range of 6-7, while the preparations maintained a pH level around 6-6.5. The gel has pH which compatible to skin pH, however, it showed the exact range in the growth of *P.acnes*.

This alignment resulted in a decrease in antibacterial activities of gel when compared to the extract. Statistical analysis was listed in Table 4. The result revealed that all the inhibition data of samples exhibited statistically significant differences when compared to the positive control ($p< 0.05$), but no significant differences were observed among the samples ($p>0.05$).

Table 4. Post Hoc LSD antibacterial activity.

Formula	Mean difference	95% CI		*p-value*
		Maximum	Minimum	
FI vs FII	0.083	1.245	1.078	0.873
FI vs FII	0.183	1.345	0.978	0.725
FI vs C+	19.616	20.778	18.455	0.000*
FII vs FIII	0.100	1.261	1.061	0.848
FII vs C+	19.533	20.695	18.371	0.000*
FIII vs C+	19.433	20.595	18.271	0.000*

Note : Data expressed in mean±SD (n=3) *p< 0.05

CONCLUSION

The ethanolic extrat of *Piper crocatum* leaves are can be formulated in gel preparations that meet the physical requirements of gel. Notably, preparations containing 15%, 20%, and 25% concentration of the extract exhibit moderate antibacterial activity against *P. acnes* with inhibition diameters of 7.98 mm, 8.06 mm, and 8.16 mm, respectively. These finding suggest that the *Piper crocatum* leaves ethanolic extract gel holds promising potential as anti-acne topical therapy in an in vitro setting.

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