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Original Research Article

Formulation and characterization of antibacterial gel containing ethanol extract of oil palm leaves (*Elaeis guineensis* Jacq.)

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Abstract

Purpose: *To develop and characterize a gel formulation of ethanol extract from oil palm leaves as a treatment substitute for acne by preventing the growth of Staphylococcus epidermidis and Propionibacterium acne.*
Methods: *Gel formulations were prepared with 1 % (F1), 3 % (F2), and 5 % (F3) extract concentrations, and their physical properties, 12-week stability, skin irritability on volunteers, and antibacterial efficacy against Propionibacterium acnes and Staphylococcus epidermidis were investigated.*
Results: *The ethanol extract of oil palm leaves exhibited antibacterial activity, with minimum inhibitory concentration (MIC) of 0.10 mg/mL and inhibition zones of 7.23 mm against Propionibacterium acnes and 7.00 mm against Staphylococcus epidermidis. The formulated gel was homogeneous and stable for 12 weeks, had a pH of 5.0 – 6.2, and a viscosity of 2400 – 3400 cps with no incidence of skin irritation. Gel formulations with 1, 3, and 5 % extract showed inhibition zones of 12.00, 11.20, and 10.40 mm against P. acnes, and 11.60, 10.77, and 9.61 mm against Staphylococcus aureus, respectively.*
Conclusion: *Gel formulations containing 3 % (F2) and 5 % (F3) ethanol extract of oil palm leaves, respectively, demonstrate potent antibacterial activity against Propionibacterium acnes and Staphylococcus epidermidis. Further studies, including clinical investigations, may be required to assess the treatment efficacy of the formulations in vivo.*

Keywords: *Oil palm leaves, Gel, Antibacterial, Skin irritability, Propionibacterium acne, Staphylococcus epidermidis*

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INTRODUCTION

One of the most prevalent skin conditions among teenagers is acne. This affects persons of all ages, although prevalent amongst people between 12 to 24 years, accounting for 85 % of all cases [1]. Acne is often found on the face, neck, chest, and back. Even though acne does not have a fatal impact, it may affect people who care about their appearance by reducing self-confidence [2]. Acne is caused by increased activity of the sebum glands and may be exacerbated by bacterial infections. *Staphylococcus epidermidis, Staphylococcus aureus*, and *Propionibacterium acne* are bacteria that cause acne. Several other factors, such as genetics, hormones, food, cosmetics, stress, and other chemicals, may also be implicated [2].

Antibiotics, including erythromycin, clindamycin, and tetracycline, are useful in treating acne. However, improper antibiotic use results in resistance [2]. Long-term use of antibiotics leads to biofilm formation, which causes antibiotic resistance and offers protection from host defenses, thus altering the skin's natural microbiota [1]. Therefore, it is necessary to look for other alternatives that are used for acne treatment, such as plants with antibacterial activity [2]. In nature, many plants have antibacterial properties, including oil palm leaves (*Elaeis guineensis* Jacq.). Oil palm leaves contain flavonoids, alkaloids, saponins, tannins, steroids, terpenoids, and glycosides [3]. Many components of polyphenols, flavonoids, antioxidants, and catechins have been identified in the ethanol and aqueous extract of oil palm leaves [4]. Lutein chemicals, commonly referred to as flavonoids, are found in the leaves of oil palm plants. By preventing bacteria from synthesizing proteins and nucleic acids, luteolin has an antibacterial effect. It also exhibits strong antibacterial activity against gram-positive bacteria [4].

The gel is a pharmaceutical preparation widely used in the cosmetic field. Gels are comfortable to use, give a cool feeling to the skin, are not greasy, easy to apply, easy to wash, elegant, clear, elastic, do not clog pores and have good adhesion, as well as release active substances that are good [5]. This study, therefore, investigated the antibacterial activity of gel formulation of palm leaf (*Elaeis guineensis* Jacq.) ethanol extract against *Staphylococcus epidermidis* (*S. epidermidis*) and *Propionibacterium acne* (*P. acne*).

METHODS

Plant materials

Palm leaves (voucher no. 414/MEDA/2022) were collected from Asahan Regency, North Sumatra. The leaves used were green oil palm leaves from the first to fifth midrib (close to the fruit), which are in good condition due to their high flavonoid content.

Volunteers

Twelve participants met the following requirements: they were willing to participate, female, able-bodied, between the ages of 20 and 30, had no medical history connected to allergies at the time of testing, and were enrolled. Ethical approval was obtained from the Ethics Committee of Universitas Sumatera Utara (approval no. 980/KEPK/USU/2022) and complied with the guidelines of Declaration of Helsinki [6].

Extraction process

The oil palm leaves were cleaned, dried, and ground into fine powder to increase extraction efficiency. The powdered leaves were soaked in 96 % ethanol with occasional stirring for 5 days to get extract I and then continued for 2 days to get extract II. After maceration, both extracts were mixed and filtered to separate the solid residue from the liquid extract. The solvent was evaporated using a rotary evaporator to obtain a concentrated palm leaves ethanol extract (PLEE) [7].

Antibacterial assay for PLEE

The PLEE (2 g) was dissolved in 4 mL DMSO (Merck, USA) to obtain a concentration of 500 mg/mL, or 50 % (w/v). A concentration of 300 mg/mL (30 %), 200 mg/mL (20 %), 100 mg/mL (10 %), 50 mg/mL (5 %), 30 mg/mL (3 %), 10 mg/mL (1 %), 1 mg/mL (0.1 %), 0.75 mg/mL (0.075 %), 0.50 mg/mL (0.050 %), 0.25 mg/mL (0.025 %), and 0.1 mg/mL (0.01 %) were used to create a dilution.

Evaluation of antibacterial activity of PLEE against *P. acne* and *S. epidermidis*

The bactericidal properties of PLEE were evaluated at 0.1, 0.25, 0.50, 0.75, 1, 10, 30, 50, 100, 200, 300, and 500 mg/mL. The test was conducted in triplicate using paper discs and agar diffusion method. Mueller Hinton Agar (15 mL) media and 0.1 mL of inoculum (*Propionibacterium acne* (ATCC 1127) and *Staphylococcus epidermidis* (ATCC 12228)) were poured into sterile petri dishes, and the mixture was left to solidify. The paper discs were put on paper discs dripped with varying concentrations of diluted palm leaf ethanol extract. The strips were incubated for 24 h at 37°C. Thereafter, a calliper was used to measure the diameter of the inhibition area, also known as the clear zone, surrounding the paper disc [8].

Formulation of PLEE gel

Gel preparations of PLEE were formulated with three different concentrations and one blank, weighing 100 g (Table 1).

Preparation of PLEE

Carbopol was dissolved for 30 min with a portion of hot distilled water in a mortar until it swelled. Methylparaben was stirred in hot water until

dissolved in a glass beaker. After dissolution of the carbopol, TEA was added gradually while grinding to form a gel base, followed by methylparaben, which was added to the gel base and ground until a homogeneous preparation was obtained. Glycerin, a portion of propylene glycol, was added and the preparation was crushed in a separate mortar. The remaining propylene glycol was added to the gel base mixture and crushed until homogenous. The crushed extract and the base gel were mixed until homogeneous. Distilled water was added and mixed thoroughly to make up to 100 g [9].

Table 1: Formulation table of gel of PLEE

Material (g)	F0	F1	F2	F3
PLEE	-	1	3	5
Carbopol	1	1	1	1
Propylene glycol	10	10	10	10
Glycerine	5	5	5	5
Triethanol amine (TEA)	q.s.	q.s.	q.s.	q.s.
Methylparaben	0.1	0.1	0.1	0.1
Distilled water to	100 g	100 g	100 g	100 g

PLEE: Palm leaf ethanol extract, F0: gel without PLEE, F1: gel containing PLEE 1 %, F2: gel containing PLEE 3 %, F3: gel containing PLEE 5 %

Evaluation of gel preparations

Physical stability test

Visual inspection of shape, colour, and odour was used to assess the stability of the preparations [10]. Observations were made at room temperature on days 1, 4, 8, and 12 weeks.

Homogeneity test

Approximately 5 g of the preparation was spread on two pieces of plain glass. The preparation must exhibit a homogeneous composition with no visible coarse grains [10]. Observations were made at room temperature on day 1 and week 12.

pH test

The pH of the solution was measured with a pH meter. The instrument was first set up with a neutral pH standard buffer solution (pH 7.01) and an acidic pH buffer solution (pH 4.01) until it displayed the correct pH values. The electrode was rinsed with distilled water and dried with tissue paper. A 1 % sample solution was prepared by dissolving 1 g of the substance in 100 mL of distilled water. The electrode was immersed in the solution until a stable pH reading was obtained. Observations were made at room temperature on days 1, 4, 8, and 12th week in triplicate and the average was recorded.

Viscosity test

Viscosity was measured using an NDJ-8S viscometer. Spindle 3 at a speed of 3 rpm was used to measure the viscosity of 100 g of the gel. Determinations were done in triplicate and the average was taken.

Spreadability test

As much as 1 g of the preparation was placed on a 20 x 20 cm glass above graph paper, left for 60 s, and then diameter of the formed preparation was measured. Thereafter, it was covered with mica plastic weighing 50, 100, 150, and 200 g, and left for 60 s. Diameter of spread was measured in triplicate and the average was recorded [12].

Skin irritation test for volunteers

A skin irritation test was carried out by applying the preparation to the skin of the rear ear, which was done at a site left open, and the results were observed [13].

Antibacterial activity test of PLEE gel preparation against *P. acnes* and *S. epidermidis*

The PLEE gel was tested for antibacterial activity at F0 (control), F1 (1 %), F2 (3 %), and F3 (5 %). This test reproduced each well three times using diffusion. A sterile petri dish was used to solidify 0.1 mL of inoculum and 15 mL of Mueller-Hinton agar. Wells were produced with a well-stem tool in solidified agar. The PLEE gel, positive control (commercial acne spot treatment gel) and negative control (blank gel). The wells were incubated at 37 °C for 24 h. Thereafter, a caliper was used to assess the inhibition area (clear zone) [8]. Determinations were done in triplicate and the average was recorded.

Statistical analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 22 software (IBM, Armonk, NY, USA). A normality test was performed to identify statistical differences and compared using 2-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

RESULTS

Yield

The yield of the ethanol extract was 21.5 %.

Table 2: Results of antibacterial activity of PLEE

Concentration (mg/mL)	Diameter of inhibition area (mm)		Remark
	Propionibacterium acne	***Staphylococcus epidermidis***	
Control (+)	34.73±0.35	35.63±0.25	Control (+) > PLEE concentration of 500 – 0.05 mg/mL ($p < 0.05$) PLEE concentration 0.10 – 500 mg/mL > Control (-) ($p < 0.05$)
500	16.53±0.15[ab]	15.77±0.06[ab]	
300	14.67±0.06[ab]	14.57±0.15[ab]	
200	13.80±0.10[ab]	13.87±0.06[ab]	
100	13.10±0.10[ab]	13.40±0.10[ab]	
50	12.53±0.06[ab]	12.43±0.12[ab]	
30	11.77±0.15[ab]	11.80±0.10[ab]	
10	10.90±0.10[ab]	10.33±0.40[ab]	
1	9.50±0.10[ab]	8.80±0.20[ab]	
0.75	8.87±0.06[ab]	8.40±0.20[ab]	
0.50	8.37±0.06[ab]	8.13±0.06[ab]	
0.25	7.70±0.10[ab]	7.70±0.10[ab]	
0.10	7.23±0.06[ab]	7.00±0.10[ab]	
0.05	0	0	
Control (-)	0[a]	0[a]	

Positive control (+): Clindamycin 1 %, negative control (-): Dimethyl sulfoxide (DMSO); PLEE: Palm leaf ethanol extract, [a]$p < 0.05$ vs (+), [b]$p < 0.05$ vs (-)

Table 3: Gel preparation pH

Observation week	Mean pH of gel formulations			
	F0	F1	F2	F3
1	5.67±0.01	6.18±0.01	6.12±0.01	6.07±0.02
2	5.54±0.01	5.87±0.01	5.82±0.01	5.79±0.01
4	5.44±0.01	5.70±0.01	5.62±0.01	5.65±0.01
6	5.35±0.01	5.62±0.01	5.57±0.01	5.54±0.01
8	5.23±0.01	5.54±0.01	5.47±0.01	5.45±0.01
10	5.17±0.01	5.41±0.01	5.33±0.01	5.31±0.01
12	5.06±0.01	5.29±0.01	5.21±0.01	5.19±0.01

Table 4: Viscosity of gel formulations

Observation week	Mean viscosity of gel formulations			
	F0	F1	F2	F3
1	3347.33±5.03	3129.33±2.52	3130.00±4.58	3116.00±3.00
2	3134.33±3.06	3092.67±4.04	3106.00±3.00	3093.00±4.00
4	2983.00±4.00	3020.00±4.00	2992.33±3.51	2977.33±4.04
6	2891.33±4.51	2983.67±3.51	2962.00±3.61	2937.33±3.06
8	2761.33±4.51	2893.00±4.00	2893.00±4.00	2823.67±3.51
10	2648.33±4.16	2752.67±4.04	2795.67±3.51	2643.00±4.00
12	2585.33±3.51	2567.00±4.00	2559.33±3.51	2448.00±3.00

Antibacterial activity of PLEE

The PLEE had an area of inhibition diameter of 7.23 ± 0.06 mm and 7.00 ± 0.10 mm for *P. acnes* and *S. epidermidis*, respectively, with a minimum inhibitory concentration of 0.1 mg/mL (Table 2).

Characteristics of PLEE gel

Characteristics of 1 % (F1), 3 % (F2), and 5 % (F3) PLEE gel showed stable results throughout 12 weeks of storage at room temperature. The pH, viscosity, and spreading for all gels were still within acceptable ranges (Table 3 to Table 5).

Skin irritation

There was no adverse skin irritation (itchy rash, swollen and redness) observed throughout the evaluation period.

Table 5: Spreadability of gel formulations

Weight (g)	Spreadability (cm)			
	F0	F1	F2	F3
0	4.0	4.3	4.4	4.9
50	4.6	4.8	5.4	5.7
100	5.0	5.2	5.6	6.0
150	5.5	5.8	6.3	6.4
200	6.0	6.3	6.6	6.8

Table 7: Antibacterial activity of PLEE gels

Formulation	Inhibition zone diameter (mm)		Remark
	Propionibacterium acne	***Staphylococcus epidermidis***	
F0	0[a]	0[a]	F1, F2, F3 > F0 (p < 0,05)
F1	10.40±0.10[ab]	9.61±0.08[ab]	F3 > F1 and F2 (p < 0.05)
F2	11.20±0.10[ab]	10.77±0.31[ab]	
F3	12.00±0.10[ab]	11.60±0.10[ab]	
Control (+)	18.73±0.15	14.60±0.10	Control (+) > F0, F1, F2, F3 (p < 0.05)

F0 (gel without PLEE), F1 (gel containing PLEE 1 %), F2 (gel containing PLEE 3 %), F3 (gel containing PLEE 5 %), positive control (+), [a]P < 0.05 vs (+), p < 0.05 vs (-)

Antibacterial activity of PLEE gel

The blank gel showed no antibacterial activity. Gel formulations (F1, F2, and F3) demonstrated significantly higher inhibition zone diameter compared to controls (p < 0.05).

DISCUSSION

Antibacterial activity of the extract has been attributed to the presence of a wide array of secondary metabolites. Chemical analysis of ethanol extract from oil palm leaves verified the existence of secondary substances such as alkaloids, flavonoids, saponins, tannins, glycosides, and steroids/triterpenoids, each exhibiting distinct mechanisms for inhibiting bacterial growth [14]. Concentration of the ethanol extract gel formulation from palm oil leaves was established based on the inhibition zone diameter. Selected concentrations for PLEE gel were 10 mg/mL (1 %), 30 mg/mL (3 %), and 50 mg/mL (5 %). These concentrations produced strong inhibition zones, as previously revealed by Davis and Stout [15], with diameters of 10.90 mm, 11.77 mm, and 12.53 mm against *P. acne* and 10.33, 11.80 and 12.43 mm against *S. epidermidis*.

The gels were prepared based on the formula of Supomo *et al* [9], with modifications to the use of TEA (quantity sufficient). The gel preparation was light brownish-green to dark brownish-green with a characteristic odour. The reason for using carbopol as the gel base was because it has high stability and compatibility properties. Therefore, it may produce good and stable gel preparations, disperse quickly in water, and impart viscosity. Also, carbopol, when formulated, will form a gel with good spreadability, demonstrates a cold effect when used, is clear or transparent in appearance, does not clog skin pores, and is easily washed off with water [16].

The physical stability of the gel using organoleptic properties (odour, colour, texture) may be observed visually [10]. In this study, evaluation was carried out for 12 weeks and changes were observed every 2 weeks. The gel preparations were uniform because no coarse particles were discernible, similar to findings in a previous study [11]. The homogeneity test results for the gel preparations, including the blank (F0) and those supplemented with PLEE, indicated an absence of granular levels or compositional inconsistencies. The pH of PLEE gels gradually reduced weekly over the 12-week storage period. The results revealed that incorporation of the extract reduced the pH of the gels. The pH value of the preparation must conform to the range of human skin, specifically 4.5 - 6.5. The formulation must not be very acidic, as it may irritate the skin, nor excessively alkaline, as it might lead to dryness or flakiness. The pH of the gel may decline with prolonged storage due to degradation of the gel base, resulting in a modest reduction in pH [9].

Viscosity is also a measure of gel consistency. It has been established that resistance to flow increases with increasing viscosity [16]. The viscosity of the gel was reduced when PLEE was added. In contrast, the amount of extract used in the formula was different, so the preparation will be more acidic, decreasing the number of ionized carboxylic groups, and repulsion between groups occurs. Viscosity of PLEE gel was within the conventional values for optimal gel viscosity (2000-4000 cP). Also, possible reason why the viscosity is reduced may be due to the hygroscopic nature of carbopol absorbing the surrounding water vapor. Another factor that would cause the decline in the viscosity of the formulation is the temperature and conditions under which it was stored. Inadequate airtight packaging may result in the gel preparation absorbing external moisture, augmenting its water volume [17].

The spreadability of semisolid preparations describes the ability of the preparation to evenly spread on the skin, and it is a fundamental parameter to consider for topical preparations [18]. The requirement for good spreadability of gel preparations (semi-solid) is 5 – 7 cm. The composition of the formulation also influences

spreadability. Gelling agents and humectants are ingredients that significantly affect the spreadability of gel preparation. Spreadability of PLEE gels was within the requirements (5 – 7 cm). Good spreadability is necessary to spread the active substance over the application area (skin) evenly [9]. An irritation test was conducted to ascertain whether the formulation induces an allergic reaction or irritation. The irritant test of PLEE gels was conducted on 12 participants. Each formula underwent testing with three volunteers. The irritant test was conducted by applying a gel formulation to the skin behind the volunteer's ear. The irritation results of the PLEE gel preparation on 12 volunteers yielded negative outcomes, with no irritation reactions such as itching, redness, or swelling observed at the application site.

The antibacterial activity of PLEE gel was assessed using the well-diffusion method in solidified media. Putrajaya *et al* [19] categorized antibacterial activity into several classifications: weak (area of inhibition < 5 mm), moderate (inhibition area between 5-10 mm), strong (inhibition area between 10-20 mm), and very strong (inhibition area > 20 mm). The gel formulations exhibited concentration-dependent inhibition, with the highest inhibition observed in F3 (5%). Also, the inhibition zone diameter of PLEE gels was significantly higher compared to DMSO and lower compared to the positive control.

CONCLUSION

Gel formulations of PLEE were stable, non-irritant, and demonstrated antibacterial activity against *P. acne* and *S. epidermidis*. Further studies requiring clinical trials and investigations may be required to investigate the treatment efficacy *in vivo.*

DECLARATIONS

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Ethical approval

Ethical approval was obtained from the Ethics Committee of Universitas Sumatera Utara (approval no. 980/KEPK/USU/2022).

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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