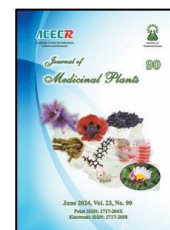




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### Research Article

## Pomegranate (*Punica granatum* L.) peel-based topical nanoemulgel for skin infection: formulation and antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*

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ARTICLE INFO	ABSTRACT
<p><b>Keywords:</b></p> <p>Pomegranate peel</p> <p>Nanoemulgel</p> <p>Topical</p> <p>antibacterial activity</p> <p><i>S. aureus</i></p> <p><i>S. epidermidis</i></p>	<p><b>Background:</b> Utilizing pomegranate peel as an antibacterial agent in topical formulations presents an opportunity for optimization through innovative drug delivery systems, notably encapsulating extracts and fractions within a nanoemulgel. <b>Objective:</b> This study aimed to formulate ethanol extract and ethyl acetate fraction of pomegranate peel into nanoemulgels and assess their antibacterial activity against skin disease-causing bacteria. <b>Methods:</b> The methodology encompassed extraction, formulation, testing, and antibacterial assays involving maceration and fractionation using ethanol and ethyl acetate solvents. The physical properties and antibacterial efficacy of the nanoemulgels were evaluated. <b>Results:</b> Nanoemulsions derived from pomegranate peel ethanol extracts and ethyl acetate exhibited promising attributes, demonstrating 98.27 % and 98.77 % transmittance levels and zeta potentials of 0.18 mV and 0.32 mV. The nanoemulgel with ethanol had a pH of <math>6.62 \pm 0.02</math>, <math>6.86 \pm 0.01</math>, <math>6.3 \pm 0.01</math> in 0.5 %, 1 %, and 1.5 % concentrations. For nanoemulgels with ethyl acetate, the pH levels for concentrations 0.5 %, 1 %, and 1.5 % are <math>6.58 \pm 0.00</math>, <math>6.80 \pm 0.01</math>, and <math>6.94 \pm 0.01</math>, respectively. These nanoemulgels displayed consistent odour, colour, and homogeneity characteristics, highlighting their suitability for topical application. The adhesion, spreadability, and viscosity assessments showed concentration-dependent variations, influencing effectiveness and user comfort. Notably, these nanoemulgels displayed substantial potential as antimicrobial agents against <i>S. aureus</i> and <i>S. epidermidis</i> bacteria in inhibitory assays, signalling promise for addressing skin infections. <b>Conclusion:</b> Overall, the study underscores the potential of nanoemulgels derived from pomegranate peel extracts as a natural alternative for topical antimicrobial therapy against skin infections.</p>

### 1. Introduction

Natural antimicrobial agents sourced from various origins, including plants, animals, bacteria, algae, and fungi, have garnered increased interest as alternatives to antibiotics

[1]. Antibiotics derived from natural sources are deemed to exhibit superior effectiveness and may render bacteria less prone to developing resistance due to their ability to target multiple bacterial factors simultaneously. However, the

**Abbreviations:** VCO, Virgin Coconut Oil; Rpm, Rotation per Minute; PEG, Polyethylene Glycol

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synergistic effects of these mechanisms compared to standard synthetic antibiotics have yet to be thoroughly evaluated in clinical studies. Several examples of mono and multi-extract combinations show synergistic effects based on multi-target action mechanisms [2]. Furthermore, natural products can enhance the anti-biofilm effects of antimicrobial compounds, providing potential for combined therapy [3]. Natural molecules displaying significant antioxidant and antibacterial activities hold great promise for developing novel antibacterial agents.

Addressing the escalating issue of antibiotic resistance necessitates the exploration of such natural alternatives. Previous studies have substantiated the antimicrobial efficacy of extracts derived from pomegranate peel against a spectrum of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, among others [4-8] *Staphylococcus aureus* and *Staphylococcus epidermidis*, both Gram-positive bacteria commonly associated with human skin infections, were chose for their respective prevalence and clinical significance [9,10]. The observed antimicrobial effects of pomegranate peel extracts have been associated with constituents like punicalagin, tannins, and phenolic compounds [11-13]. These constituents disrupt bacterial cell walls, inhibit enzyme activity, and cause oxidative damage to bacterial cells, thereby exerting their antimicrobial effects. Moreover, the demonstrated antioxidant properties of pomegranate peel are closely interlinked with its antimicrobial characteristics [14, 15]. The versatility of pomegranate peel's antimicrobial potential has been explored across diverse domains, from food preservation, packaging, to dental care products [16, 17].

Harnessing pomegranate peel extract as a topical antibacterial agent necessitates a tailored

drug delivery system for dermal application. Nanoemulsions, with their enhanced solubility for poorly soluble compounds like pomegranate peel extracts, offer stability and fine-tuned droplet sizes [18] However, their low viscosity limits optimal skin penetration. To solve this problem, mixing nanoemulsions with a hydrogel matrix creates a nanoemulgel system that stays on the skin longer by getting around the limitations of viscosity.

Nanoemulgel formulations have demonstrated improved antimicrobial activity compared to plain extracts, as evidenced by their enhanced antimicrobial activity against various microorganisms [19, 20]. Their unique structure allows for better penetration and retention in the skin, leading to more effective delivery of bioactive compounds. Studies have shown that nanoemulgels can significantly enhance the stability, bioavailability, and controlled release of active ingredients, which are crucial for the treatment of skin infections [21, 22]. Additionally, nanoemulgels are known to provide better patient compliance due to their pleasant texture and ease of application [23].

Given these advantages, our study aims to develop a nanoemulgel formulation for the topical application of pomegranate peel extracts and evaluate its antimicrobial activity against *S. aureus* and *S. epidermidis*. By leveraging the benefits of nanoemulgels, we seek to create a more effective and user-friendly treatment for skin infections.

## 2. Materials and Methods

### 2.1. Research tools and materials

The tools utilized in this study include an analytical balance (Ohaus-Germany), Freeze dryer (Christ Alpha 1-2 LO plus), Particle Size Analyzer (PSA) (HORIBA SZ-100), adhesive strength tester, spreadability tester, RION

viscometer (Viskotester VT-06E), UV-Vis spectrophotometer (Genesys 10S UV-VIS), incubator (Mettler), stirrer (Thermo Scientific Cimarec), magnetic stirrer (Thermo Scientific Cimarec), refrigerated cabinet (Polytron), pH stick (Suncare universal test paper), glassware (Pyrex).

Research Materials: Pomegranate peel, ethanol (Bratacho), ethyl acetate (Bratacho), methanol (Bratacho), toluene (Bratacho), *S. aureus* ATCC 25923, *S. epidermidis* ATCC12228, Carbopol 940 (Bratacho), VCO, Tween 80 (Bratacho), PEG 400 (Bratacho), Aqua PI (Ikapharmindo), NaOH 0.1 N, glycerin (Bratacho), methylparaben (Bratacho). The pomegranate peel used in this study was identified with herbarium code KM.04.02/2/1989/2022, determined at the Laboratory of the Center for Research and Development of Medicinal Plants and Traditional Medicines (B2TOOT) Tawangmangu, Central Java, Indonesia.

## 2.2. Extraction stage

Pomegranate peel powder was obtained from fresh pomegranate from Surakarta region, Central Java, Indonesia. The powder underwent maceration using 96 % ethanol for 5 days. For process optimization, a remaceration process was conducted. The ethanol extract obtained from maceration was then evaporated using an evaporator, followed by drying the extract using a water bath. A portion of the dried ethanol extract from pomegranate peel was used for the nanoemulgel formulation, while another portion was fractionated using ethyl acetate solvent. The fractionation output was subsequently

evaporated using an evaporator and dried on a water bath. The resulting fraction is then ready for formulation [24].

## 2.3. Formulation stage

### 2.3.1. Preparation of nanoemulsion formulation of ethanol extract and ethyl acetate fraction of pomegranate peel

The production of nanoemulsion requires ethanol extract and ethyl acetate fraction of pomegranate peel (active ingredients), VCO (oil), Tween 80 (surfactant), PEG 400 (cosurfactant), and injection-grade aqua pro (water phase) [25]. The composition is explained in Table 1 and 2. The nanoemulsion is prepared by first dissolving the ethanol extract and ethyl acetate fraction of pomegranate peel in half the amount of tween with the assistance of a stirrer until dissolved. Next, half the amount of PEG is added while stirring at a speed of 600 rpm for 10 minutes, maintaining the temperature at 60 °C. Subsequently, VCO is slowly added while continuously stirring at 700 rpm for 10 minutes, still keeping the temperature at 60 °C. The remaining tween is then added while continuously stirring with the stirrer at a speed of 800 rpm for 10 minutes. Afterward, the remaining PEG is added, stirring for the same duration and speed at 60 °C. In the final stage, the water phase is slowly added drop by drop into the mixture, carefully observing the rotation and temperature of the stirrer. The resulting nanoemulsion is then transferred into bottles and left to settle for 24 hours to obtain a clear solution.

**Table 1.** Preparation of nanoemulsion formulation of ethanol extract of pomegranate peel

Ingredients	Nanoemulgel Ethanol Extract of Pomegranate Peel		
	I	II	III
Ethanol Extract	0.5 %	1 %	1.5 %
VCO	1 %	1 %	1 %
Tween 80	7 %	7 %	7 %
PEG 400	2 %	2 %	2 %
Aqua pro injectie	ad 100 %	ad 100 %	ad 100 %

**Table 2.** Preparation of nanoemulsion formulation of ethyl acetate fraction of pomegranate peel

Ingredients	Nanoemulgel Ethyl Acetate Fraction of Pomegranate Peel		
	I	II	III
Ethanol Extract	0.5 %	1 %	1.5 %
VCO	1 %	1 %	1 %
Tween 80	7 %	7 %	7 %
PEG 400	2 %	2 %	2 %
Aqua pro injectie	ad 100 %	ad 100 %	ad 100 %

### 2.3.2. Incorporation of nanoemulsion of ethanol extract and ethyl acetate fraction of pomegranate peel into a hydrogel matrix

**Table 3.** Composition of gel base formulation

Material	Concentration / Amount
Carbopol 940	1 %
NaOH 0.1 N	10 %
Glycerin	5 %
Methylparaben	0.2 %
Triethanolamine	qs
Aquadest ad	100 mL

### 2.4. Method of gel base preparation

The ingredients utilized for the gel base preparation were presented in Table 3. Initially, Carbopol was dispersed in distilled water and allowed to stand for 24 hours. Subsequently, the dispersed Carbopol was mixed gradually with 0.1 N NaOH while stirring until it achieved homogeneity. Then, triethanolamine was added drop by drop until a neutral pH was achieved. Finally, glycerin and methylparaben were added and stirred until uniformity was attained.

The subsequent step involved the incorporation of the nanoemulsion derived from the ethanol extract of pomegranate peel and the ethyl acetate fraction of pomegranate peel into

the gel base. The ratio between nanoemulsion and the gel base was (50:50). The gel base was mixed with the nanoemulsion until it became homogeneous [26]

### 2.5. Testing stage

#### 2.5.1. Physical properties testing of the formulation

**a.** Nanoemulsion Formulation Characterization: Nanoemulsion characterization was conducted based on parameters such as percent transmittance, droplet size, and zeta potential. The percent transmittance was measured using a UV-Vis spectrophotometer at a wavelength of 650 nm with distilled water as a blank. Droplet size and zeta potential of the nanoemulsion were determined using a Particle Size Analyzer (PSA-HORIBA SZ-100) through dynamic light scattering at a scattering angle of 90° [27].

**b.** Organoleptic Evaluation of Nanoemulgel: The physical observation of the nanoemulgel formulation derived from ethanol extract and ethyl acetate fraction of pomegranate peel included assessment of color, odor, homogeneity, and consistency [27].

c. pH Testing: The pH of the nanoemulgel formulation derived from ethanol extract and ethyl acetate fraction of pomegranate peel was measured using an Ohaus pH meter. The measurement was repeated three times [27].

d. Viscosity Test: The viscosity of the nanoemulgel was measured using a RION viscometer with rotor no. 1 and rotor no. 2. The gel sample was placed into a test pot, and the rotor was positioned in the middle of the pot containing the nanoemulsion gel sample. The measurement was recorded once the instrument stabilized. Each measurement was performed three times [27].

e. Adhesive Strength Test: 0.25 grams of nanoemulgel was placed on one object glass and adhered to another glass slide, followed by the application of a 1 kg weight for 5 minutes. The object glass was mounted on a testing apparatus, and an 80-gram weight was released to measure the time taken for detachment. This test was performed three times [27].

f. Spreadability Test: 0.5 grams of the nanoemulgel was weighed and placed at the center of a petri dish with a millimeter block. Another petri dish was placed on top as the initial load for 1 minute. The gel spread diameter was measured on four sides of the petri dish. The test was repeated by adding a 50-gram load every 1 minute until reaching a load of 300 grams. This test was performed three times [27].

## 2.6. Antibacterial testing

The antibacterial activity of the nanoemulgels was assessed using the disc diffusion method. This method evaluates the potency of the antibacterial action by measuring the diameter of the inhibition zone around each disc. The testing focused on the effectiveness of pomegranate peel ethanol extract nanoemulgel and ethyl acetate fraction nanoemulgel against *Staphylococcus*

*aureus* and *Staphylococcus epidermidis*, both of which are Gram-positive bacteria, prevalence on human skin and their well-known role in various skin infections. *S. aureus* is a major pathogen responsible for a wide range of skin conditions, from minor infections like impetigo to severe infections such as abscesses and cellulitis [10]. *S. epidermidis*, although typically less virulent, is an opportunistic pathogen known to cause infections, especially in individuals with compromised immune systems or those with indwelling medical devices [9]. These bacteria are commonly found on the skin, making them relevant targets for evaluating the efficacy of topical antibacterial treatments.

## 2.7. Preparation of nanoemulgel samples

Nanoemulgel formulations containing different concentrations of pomegranate peel ethanol extract (0.5 %, 1 %, and 1.5 %) and ethyl acetate fraction (0.5 %, 1 %, and 1.5 %) were prepared. The concentrations were selected based on preliminary study that indicated suitable pH for subcutaneous human skin while also maintaining acceptable physical properties and user comfort for topical applications [28]. The samples were then sterilized and ready for antibacterial testing.

## 2.8. Disc diffusion method

1. Culture Preparation: Bacterial cultures of *S. aureus* and *S. epidermidis* were grown in nutrient broth at 37 °C until they reached the logarithmic phase. The cultures were then adjusted to a turbidity equivalent to 0.5 McFarland standard.

2. Agar Plates Preparation: Sterile Mueller-Hinton agar plates were prepared and allowed to solidify. The surface of each agar plate was inoculated uniformly with the bacterial suspension using a sterile swab.



3. Application of Nanoemulgel Samples: Sterile filter paper discs (6 mm in diameter) were impregnated with 20 µL of each nanoemulgel sample. The discs were then placed on the inoculated agar plates, ensuring even spacing between them.

4. Controls:

- Positive Control: Chloramphenicol (standard antibiotic) discs were used as the positive control to compare the inhibition zones.

- Negative Control: Discs impregnated with gel base alone were used as the negative control to ensure the observed effects were due to the active ingredients in the nanoemulgel.

5. Incubation: The agar plates were incubated at 37°C for 24 hours.

6. Measurement of Inhibition Zones: After incubation, the diameter of the inhibition zones around each disc was measured in millimeters (mm) using a caliper. The measurements were taken in triplicate to ensure accuracy.

2.9. Data analysis

The collected data were analyzed utilizing SPSS version 23. To summarise the data, means, standard deviations, and tables were employed. The data of viscosity, spreading ability, and adhesive properties of the nanoemulgel formulation were analyzed using one-way ANOVA and Mann-Whitney tests.

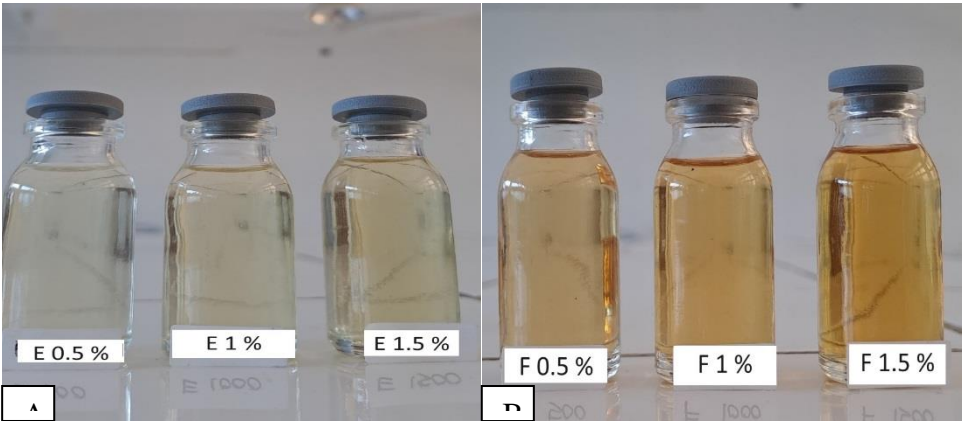
3. Results

**Table 4.** Test Results of Transmittance Percentage, Particle Size Distribution, and Zeta Potential of Nanoemulsions

Sample	Transmittance percentage (%)	Particle size distribution (nm)	Zeta potential (mV)
Pomegranate Peel Ethanol Extract Nanoemulsion	98.27 ± 0.11	14.20 ± 0.53	0.18
Pomegranate Peel Ethyl Acetate Fraction Nanoemulsion	98.77 ± 0.35	14.37 ± 0.99	0.32

3.1. Formulation of ethanol extract and ethyl acetate fraction of pomegranate peel

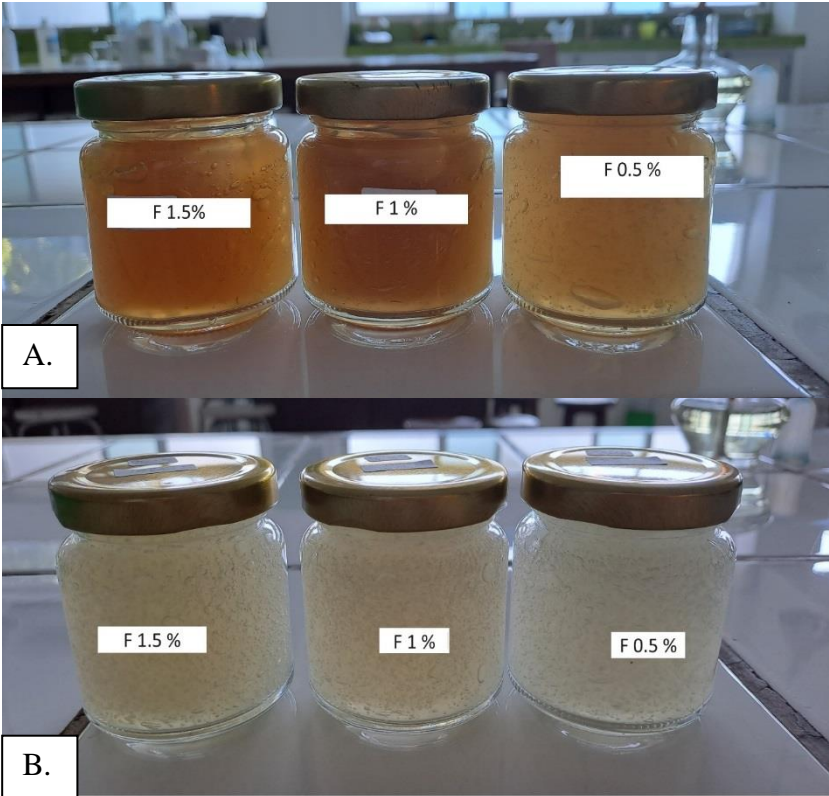
The results of formulating nanoemulsions from both ethanol extract and ethyl acetate fraction of pomegranate peel demonstrated clear outcomes with transmittance values approaching 100%, as observed in Table 4. The transmittance values of the nanoemulsions for ethanol extract and ethyl acetate fraction of pomegranate peel were 98.27 % and 98.77 %, respectively. Higher transmittance percentages (nearing 100 %) indicate clearer and more transparent nanoemulsion formulations, a significant parameter for nanoemulsion [29]. Particle size distribution analysis revealed particles below 100 nm for both nanoemulsions, falling within the nano-sized range of 1 – 100 nm [30]. In this study, nanoemulsion formulation results observed in Figure 1. Zeta potential measurements for the ethanol extract and ethyl acetate fraction nanoemulsions were 0.18 mV and 0.32 mV, respectively. The low zeta potential values were attributed to the use of non-ionic surfactant tween 80, lacking charges on its hydrophobic groups, resulting in oil droplet surfaces less prone to charge [31]. Zeta potential represents the surface potential in the nanoemulsion film layer, creating electrical forces between oil droplets to prevent coalescence. Values between -30 mV and +30 mV indicate a high level of nanoemulsion stability [32].



**Fig 1.** **A.** Nanoemulsion of the Ethanol Extract of Pomegranate Peel. **B.** Nanoemulsion of the Ethyl Acetate Fraction of Pomegranate Peel

Organoleptic evaluations of the nanoemulgel formulations from ethanol extract and ethyl acetate fraction of pomegranate peel involved assessments of consistency, odor, color, and homogeneity. Both nanoemulgel formulations exhibited similar characteristics in odor (distinctive pomegranate peel scent), clear color

(pale yellow to dark yellow), and consistent consistency due to identical gelling agent concentrations as observed in Figure 2. Both nanoemulsion formulations displayed good homogeneity, evidenced by uniform mixing of all constituents.



**Fig. 2.** **A.** Nanoemulgel of the Ethanol Extract of Pomegranate Peel. **B.** Nanoemulgel of the Ethyl Acetate Fraction of Pomegranate Peel

3.2. pH testing of ethanol extract and ethyl acetate fraction of pomegranate peel

The aim of pH measurement in the nanoemulgel is to ensure the safety of the gel when applied to the skin surface. The pH measurement results of nanoemulgel from ethanol extract of pomegranate peel at concentrations of 0.5 %, 1 %, and 1.5 % were  $6.62 \pm 0.02$ ,  $6.86 \pm 0.01$ , and  $6.93 \pm 0.01$ , respectively. Meanwhile, for nanoemulgel from

ethyl acetate fraction of pomegranate peel at concentrations of 0.5 %, 1 %, and 1.5 %, the pH values were  $6.58 \pm 0.00$ ,  $6.80 \pm 0.01$ , and  $6.94 \pm 0.01$ , respectively (Table 5). With pH values approaching 7, both nanoemulgel formulations indicate their safety for skin use. Products with excessively low or high pH values may disrupt the skin's pH balance, causing damage to the skin barrier. Such damage can lead to increased skin sensitivity, irritation, and other skin issues [33].

Table 5. pH test results

Sample	pH
	Mean $\pm$ SD
Pomegranate Peel Extract Nanoemulgel 0.5 %	$6.62 \pm 0.02$
Pomegranate Peel Extract Nanoemulgel 1 %	$6.86 \pm 0.01$
Pomegranate Peel Extract Nanoemulgel 1.5 %	$6.93 \pm 0.01$
Pomegranate Peel Ethyl Acetate Nanoemulgel 0.5 %	$6.58 \pm 0.00$
Pomegranate Peel Ethyl Acetate Nanoemulgel 1 %	$6.80 \pm 0.01$
Pomegranate Peel Ethyl Acetate Nanoemulgel 1.5 %	$6.94 \pm 0.01$

The pH measurement results of both nanoemulgels show values approaching 7, falling within the normal human skin pH range. Nanoemulgels with nearly neutral pH are expected to reduce the risk of skin irritation or side effects when applied. Statistical analysis indicates no significant difference in pH between nanoemulgels from ethanol extract and ethyl acetate fraction of pomegranate peel at various concentrations. This suggests that neither the extract nor the fraction significantly affected the pH value of the nanoemulgels.

3.3. Adhesion test

The adhesion parameter or adhesion in the nanoemulgel formulation represents its ability to strongly and stably adhere to the skin surface or the application area. As indicated in Table 6, based on statistical analysis, there was a difference in adhesion among the formulations,

demonstrated by a p-value of 0.026 ( $P < 0.05$ ). This signifies that the differing concentrations of pomegranate peel ethanol extract in each formulation resulted in varying adhesion. In Table 7, based on statistical analysis, there was a difference in adhesion among the formulations, indicated by a p-value of 0.025 ( $P < 0.05$ ). This implies that the differing concentrations of pomegranate peel ethyl acetate fraction in each nanoemulgel formulation led to different adhesion levels. Good gel adhesion is defined as if the gel can adhere for more than 1 second [34]. The nanoemulgel formulation from pomegranate peel ethanol extract at concentrations of 0.5 %, 1 %, and 1.5 % exhibited good adhesion, lasting for more than 1 second. The observed decrease in the intensity of adhesion at the 1.5 % ethanol concentration compared to the 1 % ethanol concentration could be attributed to higher concentrations of ethanol



could prompt viscosity and solubility changes or the active ingredients, impacting the overall influence interaction between the gel base and adhesive quality of the formulation.

Table 6. Adhesion test results of pomegranate peel extract nanoemulgel

No	Sample	Adhesion (s)
		Mean ± SD
1	Pomegranate peel extract nanoemulgel 0.5 %	1.87 ± 0.01
2	Pomegranate peel extract nanoemulgel 1 %	2.73 ± 0.01
3	Pomegranate peel extract nanoemulgel 1.5 %	2.32 ± 0.03

Table 7. Adhesion test results of pomegranate peel ethyl acetate nanoemulgel

No	Sample	Adhesion (s)
		Mean ± SD
1	Pomegranate peel ethyl acetate nanoemulgel 0.5 %	2.47 ± 0.01
2	Pomegranate peel ethyl acetate nanoemulgel 1 %	2.53 ± 0.01
3	Pomegranate peel ethyl acetate nanoemulgel 1.5 %	2.85 ± 0.01

3.4. Spreadability test

Spreadability in nanoemulgels refers to the formulation's ability to spread evenly and easily on the skin or the desired area. Good spreadability is crucial for topical products, including nanoemulgels, as it influences user comfort and the effectiveness of active ingredient distribution over the application area. Statistical analysis of the spreadability of pomegranate peel ethanol extract nanoemulgel showed significant results with a p-value of 0.025 ( $P < 0.05$ ), indicating that different concentrations resulted in variations in spreadability (Table 8). The variations in spreadability may be influenced by

different ethanol concentrations, which can alter the properties of the interfacial layer. This change can affect the interactions between droplets and solvents, or between droplets themselves, leading to variations in viscosity [35]. The highest spreadability was observed in the 0.5 % concentration. However, the statistical analysis of the spreadability of pomegranate peel ethyl acetate fraction nanoemulgel indicated a p-value of  $0.980 > 0.05$ , suggesting no difference in spreadability among different concentrations of the ethyl acetate fraction nanoemulgel (Table 9).

Table 8. Spreadability test results of pomegranate peel extract nanoemulgel

No	Sample	Adhesion (s)
		Mean ± SD
1	Pomegranate peel extract nanoemulgel 0.5 %	6.8 ± 0.82
2	Pomegranate peel extract nanoemulgel 1 %	5.21 ± 0.71
3	Pomegranate peel extract nanoemulgel 1.5%	6.30 ± 0.88

**Table 9.** Spreadability test results of pomegranate peel ethyl acetate nanoemulgel

No	Sample	Adhesion (s)
		Mean $\pm$ SD
1	Pomegranate peel ethyl acetate nanoemulgel 0.5 %	6.27 $\pm$ 0.84
2	Pomegranate peel ethyl acetate nanoemulgel 1 %	6.17 $\pm$ 1.02
3	Pomegranate peel ethyl acetate nanoemulgel 1.5 %	6.27 $\pm$ 0.84

### 3.5. Viscosity test

Statistical results of the viscosity test for pomegranate peel ethanol extract nanoemulgels showed a p-value of  $0.158 > 0.05$ , indicating no significant difference in viscosity among the three pomegranate peel extract nanoemulgels (Table 10). Similarly, the statistical results for pomegranate peel ethyl acetate fraction nanoemulgels displayed a p-value of  $0.105 > 0.05$ , indicating no significant difference in viscosity among the three ethyl acetate fraction nanoemulgels (Table 11). This occurs because both the pomegranate peel ethanol extract and ethyl acetate fraction nanoemulgels use the same ratio of gelling agent composition and have the same mixing ratio between nanoemulsion and gel base (50:50).

### 3.6. In vitro antibacterial activity

In the test assessing antibacterial effects through the disc diffusion method, the potency of the antibacterial action is demonstrated by the size of the inhibited area or the transparent zone. The test of inhibitory activity of ethanol extract nanoemulgel from pomegranate peel against the growth of *S. aureus* and *S. epidermidis* bacteria

aimed to evaluate the effectiveness of ethanol extract nanoemulgel from pomegranate peel in inhibiting the growth of *S. aureus* and *S. epidermidis*, which are Gram-positive bacteria often causing infections in humans.

Results from the inhibitory test of pomegranate peel ethanol extract nanoemulgels against *S. aureus* bacteria demonstrated significant differences in inhibition among various tested concentrations (Table 12). Statistical tests showed a significant difference in inhibition within the three nanoemulgels concentrations (p-value  $0.08 < 0.05$ ), as well as significant differences in several concentration group pairs. These results indicate that the inhibition of *S. aureus* growth can be influenced by the concentration of nanoemulgels used.

The inhibitory test results of pomegranate peel ethanol extract nanoemulgels against *S. epidermidis* demonstrate antimicrobial potential at various tested concentrations (Table 13). Statistical tests indicate significant differences in inhibition among the five sample concentrations (p-value  $0.009 < 0.05$ ), showing significant differences in several concentration group pairs.

**Table 10.** Viscosity test results of pomegranate peel extract nanoemulgel

No	Sample	Viscosity (Cps)
		Mean $\pm$ SD
1	Pomegranate peel extract nanoemulgel 0.5 %	1491 $\pm$ 191.85
2	Pomegranate peel extract nanoemulgel 1 %	1269 $\pm$ 176.98
3	Pomegranate peel extract nanoemulgel 1.5 %	1139 $\pm$ 210.16

**Table 11.** Viscosity test results of pomegranate peel ethyl acetate nanoemulgel

No	Sample	Viscosity (Cps)
		Mean $\pm$ SD
1	Pomegranate peel ethyl acetate nanoemulgel 0.5 %	1332 $\pm$ 139.99
2	Pomegranate peel ethyl acetate nanoemulgel 1 %	1287 $\pm$ 37.51
3	Pomegranate peel ethyl acetate nanoemulgel 1.5 %	1131 $\pm$ 94.65

**Table 12.** Antibacterial activity assay of pomegranate peel extract nanoemulgel against *S. aureus*

Sample concentration (%)	Average diameter of inhibition zone (mm)		
	Pomegranate peel extract nanoemulgel	Positive control (Chloramphenicol)	Negative control (gel base)
0.5	0.00 $\pm$ 0.00 <sup>*)**</sup>		
1	2.04 $\pm$ 0.01 <sup>*</sup>	20.91 $\pm$ 0.02	0.00 $\pm$ 0.00
1.5	2.23 $\pm$ 0.15 <sup>*</sup>		

<sup>\*)</sup> differs significantly from Chloramphenicol ( $P < 0.05$ )

<sup>\*\*)</sup> does not differ significantly from gel base ( $P > 0.05$ )

**Table 13.** Antibacterial activity assay of pomegranate peel extract nanoemulgel against *S. epidermidis*

Sample concentration (%)	Average diameter of inhibition zone (mm)		
	Pomegranate peel extract nanoemulgel	Positive control (Chloramphenicol)	Negative control (gel base)
0.5	2.07 $\pm$ 0.02 <sup>*</sup>		
1	2.50 $\pm$ 0.10 <sup>*</sup>	25.47 $\pm$ 0.00	0.00 $\pm$ 0.00
1.5	2.70 $\pm$ 0.10 <sup>*</sup>		

<sup>\*)</sup> differs significantly from Chloramphenicol ( $P < 0.05$ )

<sup>\*\*)</sup> does not differ significantly from gel base ( $P > 0.05$ )

Thus, these results serve as an important basis for the development of pomegranate peel ethanol extract nanoemulgels as a natural alternative in effectively and sustainably treating bacterial infections caused by *S. epidermidis*. The inhibitory test results of pomegranate peel ethanol extract nanoemulgels against *S. epidermidis* demonstrate interesting potential in developing natural antimicrobial therapy. The inhibition diameters obtained at various nanoemulgels concentrations indicate inhibitory effects on *S. epidermidis* bacterial growth.

Statistical results also provide evidence of significant differences in inhibition among some concentration groups and compared to the positive control chloramphenicol and negative control gel base. This indicates that the

concentration of nanoemulgels affects its inhibitory effectiveness against bacteria.

In this analysis, the greatest inhibition diameter was seen in the positive control chloramphenicol, showing strong ability in inhibiting *S. epidermidis* bacterial growth. However, pomegranate peel ethanol extract nanoemulgels also demonstrate significant antimicrobial activity with significant inhibition diameters at 1 % and 1.5 % concentrations.

The results of the inhibition test of pomegranate peel ethyl acetate fraction nanoemulgel against *S. aureus* bacteria indicate an interesting and potential antimicrobial agent (Table 14). Statistical analysis shows a significant difference in inhibition between the five samples ( $p\text{-value } 0.008 < 0.05$ ). Statistical

tests also reveal significant differences between several concentration groups.

This analysis demonstrates that the pomegranate peel ethyl acetate fraction nanoemulgel holds potential as an antimicrobial agent against *S. aureus* bacteria. Significant inhibition diameters at concentrations of 1% and 1.5% display increased effectiveness in hindering bacterial growth. Although the positive control, chloramphenicol, exhibited greater inhibition compared to the nanoemulgel, employing the ethyl acetate fraction of pomegranate peel as the active ingredient offers a potentially more sustainable and effective natural alternative against *S. aureus* bacterial infection.

The inhibition test results of the pomegranate peel ethyl acetate fraction nanoemulgel against *S. aureus* bacteria demonstrate promising potential as an antimicrobial agent. Different concentrations of the nanoemulgel were tested to

evaluate its effectiveness in inhibiting *S. aureus* bacterial growth.

At a concentration of 0.5 %, there was no significant inhibition against *S. aureus* bacteria, with an inhibition diameter of  $0.00 \pm 0.00$  mm. However, at concentrations of 1 % and 1.5 %, there was a significant increase in inhibition, with inhibition diameters of  $2.07 \pm 0.06$  mm and  $3.13 \pm 0.01$  mm, respectively. These findings indicate that the higher the concentration of the pomegranate peel ethyl acetate fraction nanoemulgel, the stronger its ability to inhibit *S. aureus* bacterial growth.

The results of the inhibition test of pomegranate peel ethyl acetate fraction nanoemulgel against *S. epidermidis* bacteria show promising antimicrobial effects (Table 15). Statistical analysis indicates a significant difference in inhibition among the five samples ( $p\text{-value } 0.008 < 0.05$ ). The statistical test also reveals significant differences among several concentration groups.

**Table 14.** Antibacterial activity assay of pomegranate peel ethyl acetate nanoemulgel against *S. aureus*

Sample concentration (%)	Average diameter of inhibition zone (mm)		
	Pomegranate peel ethyl acetate nanoemulgel	Positive control (Chloramphenicol)	Negative control (gel base)
0.5	$0.00 \pm 0.00^{**}$		
1	$2.07 \pm 0.06^*$	$20.91 \pm 0.02$	$0.00 \pm 0.00$
1.5	$3.13 \pm 0.01^*$		

\*) differs significantly from Chloramphenicol ( $P < 0.05$ )

\*\*) does not differ significantly from gel base ( $P > 0.05$ )

**Table 15.** Antibacterial activity assay of pomegranate peel ethyl acetate nanoemulgel against *S. epidermidis*

Sample concentration (%)	Average diameter of inhibition zone (mm)		
	Pomegranate peel ethyl acetate nanoemulgel	Positive control (Chloramphenicol)	Negative control (gel base)
0.5	$2.07 \pm 0.02^*$		
1	$2.57 \pm 0.02^*$	$25.47 \pm 0.00$	$0.00 \pm 0.00$
1.5	$2.70 \pm 0.10^*$		

\*) differs significantly from Chloramphenicol ( $P < 0.05$ )

\*\*) does not differ significantly from gel base ( $P > 0.05$ )

#### 4. Discussion

Nanoemulsions formulated from extracts of pomegranate peel using ethanol and ethyl acetate

demonstrated encouraging properties, with transmittance levels of 98.27 % and 98.77 %, and zeta potentials measuring 0.18 mV

and 0.32 mV, respectively. The pH values of ethanol-derived nanoemulgels were  $6.62 \pm 0.02$ ,  $6.86 \pm 0.01$ , and  $6.93 \pm 0.01$  for concentrations of 0.5 %, 1 %, and 1.5 %, while those of ethyl acetate-derived nanoemulgels were  $6.58 \pm 0.00$ ,  $6.80 \pm 0.01$ , and  $6.94 \pm 0.01$  for the same concentrations.

These nanoemulgels exhibited consistent characteristics in terms of odor, color, and homogeneity, underscoring their suitability for topical application. The evaluations of adhesion, spreadability, and viscosity revealed concentration-dependent variations, impacting both efficacy and user comfort. Importantly, the nanoemulgels exhibited significant antimicrobial activity against *S. aureus* and *S. epidermidis* bacteria in inhibitory assays, suggesting their potential for treating skin infections. The pomegranate peel contains several bioactive compounds, including punicalagins, ellagic acid, and flavonoids, which have been documented for their antimicrobial properties [4, 13]. These compounds exert their effects by disrupting bacterial cell walls, inhibiting nucleic acid synthesis, and interfering with bacterial enzymes and proteins essential for survival.

Nanoemulsions enhance the bioavailability and stability of these bioactive compounds, improving their penetration through the bacterial cell wall and the human skin barrier [29, 30]. The small droplet size of nanoemulsions increases the surface area for interaction with bacterial cells, facilitating more effective delivery of the antimicrobial agents directly to the site of infection [29]. Additionally, the stability provided by the nanoemulsion formulation protects the bioactive compounds from degradation, ensuring sustained antimicrobial activity [22].

It's important to note that the inhibition capacity of the pomegranate peel extracts nanoemulgels are still lower than the positive control (chloramphenicol). Nevertheless, this discovery remains promising because the nanoemulgel utilizes natural substances from pomegranate peel, showing potential as a safer and more sustainable antimicrobial alternative. The reduced risk of side effects and the potential for lower likelihood of developing resistance compared to synthetic antibiotics are significant advantages of using natural extracts.

While our study demonstrated the antimicrobial potential of pomegranate peel extracts within a gel formulation, the inclusion of methylparaben as a preservative may have influenced the results. The absence of a paraben-free control limits our ability to distinguish the specific effects of the pomegranate peel extracts from those of the preservative. Moreover, To accurately determine the size and morphology of the nanoemulsions, Transmission Electron Microscopy (TEM) is needed. Future research should include paraben-free controls and use TEM to accurately evaluate the antimicrobial efficacy of pomegranate peel extracts, the nanoemulsion characteristics. And to isolate their effects from any contributions made by preservatives.

Moreover, it's crucial to note that these results stem from in vitro tests, and further research, including clinical trials in humans, is needed to validate the efficacy and safety of the pomegranate peel extracts nanoemulgel in clinical treatment. With a comprehensive scientific approach, this nanoemulgel has the potential to become an innovative solution in addressing antibiotic resistance issues and contributing to bacterial infection care in a more sustainable and effective manner.



## 5. Conclusion

In summary, the formulation of nanoemulsions from pomegranate peel extracts demonstrated promising attributes, showing high transmittance values near 100 % and particle sizes within the nano-range. Their pH levels aligned with human skin pH, ensuring safe application without disrupting skin balance. Evaluation tests highlighted consistent attributes for odor, color, and homogeneity, indicating their potential for topical use.

Tests for adhesion, spreadability, and viscosity revealed varying results based on concentrations, emphasizing their impact on effectiveness and user comfort. Notably, these nanoemulgels exhibited significant potential as antimicrobial agents against *S. aureus* and *S. epidermidis* bacteria in inhibitory tests, albeit requiring further mechanistic understanding.

These findings underscore the potential of pomegranate peel extract-derived nanoemulgels as a natural alternative in topical antimicrobial therapy for skin infections. Although demonstrating promise in vitro, further research, including clinical trials, is essential to validate

their efficacy and safety in practical skin infection treatments. Leveraging nanoemulsion technology and natural extracts, these formulations hold promise in revolutionizing topical antimicrobial treatments for skin infections, offering a safer and more sustainable approach in combating bacterial infections. Continued exploration of these nanoemulgels presents a significant opportunity to advance topical solutions for skin infections and contribute to improving dermatological healthcare practices.

## Authors's contribution

R.P and F.R.S.P designed and drafted the manuscript; R.P and S.W conducted the study; F.R.S.P and S.W conducted data analysis.

## Conflict of interest

The authors declare no conflict of interest.

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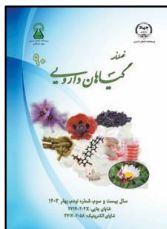
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## فصلنامه گیاهان دارویی

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مقاله تحقیقاتی

# ژل نانو امولسیون موضعی بر پایه پوست انار (*Punica granatum* L.) برای عفونت پوست: *Staphylococcus aureus* و *Staphylococcus epidermidis*

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دانشکده پزشکی، دانشگاه محمدیه سوراکارتا، سوراکارتا، ۵۷۱۶۲، جاوه مرکزی، اندونزی

اطلاعات مقاله	چکیده
گل واژگان:	مقدمه: استفاده از پوست انار به عنوان یک عامل ضد باکتری در فرمولاسیون های موضعی، فرصتی را برای
پوست انار	بهینه سازی از طریق سیستم های نوآورانه داروسازی، بویژه از طریق انکپسوله کردن عصاره ها و فراکشن ها در یک
نانو امولژل	ژل نانو امولسیونه فراهم کرده است. هدف: این مطالعه با هدف فرموله کردن عصاره اتانولی و فراکشن اتیل استاتی
موضوعی	پوست انار به ژل نانو امولسیونه و ارزیابی فعالیت ضد باکتریایی آنها در برابر باکتری های مولد بیماری پوستی
فعالیت ضد باکتریایی	انجام شد. روش بررسی: این روش شامل استخراج، فرمولاسیون، آزمایش و سنجش های ضدباکتریایی شامل
استافیلوکوکوس	خیساندن و فراکشن سازی با استفاده از حلال های اتانول و اتیل استات بود. خواص فیزیکی و اثر ضد باکتریایی
اورئوس	ژل نانو امولسیونه مورد ارزیابی قرار گرفت. نتایج: نانو امولسیون های مشتق شده از عصاره های اتانولی پوست انار
استافیلوکوک	و اتیل استات ویژگی های امیدوارکننده ای را نشان دادند که سطوح انتقال ۹۸/۲۷ و ۹۸/۷۷ درصد و پتانسیل زتا
اپیدرمیدیس	۰/۱۸ میلی ولت و ۰/۳۲ میلی ولت را نشان دادند. ژل نانو امولسیونه با اتانول دارای pH ۶/۶۲ ± ۰/۰۱، ۶/۸۶ ± ۰/۰۱، ۶/۳ ± ۰/۰۱ در غلظت های ۰/۵، ۱ و ۱/۵ درصد بود. برای ژل نانو امولسیونه دارای اتیل استات، سطوح pH
	برای غلظت های ۰/۵، ۱ و ۱/۵ درصد به ترتیب ۶/۵۸ ± ۰/۰۰، ۶/۸۰ ± ۰/۰۱ و ۶/۹۴ ± ۰/۰۱ است. این ژل ها
	بو، رنگ و ویژگی های یکدستی را نشان می دهند که مناسب بودن آنها برای کاربرد موضعی را برجسته می کند.
	ارزیابی های چسبندگی، پخش پذیری و ویسکوزیته تغییرات وابسته به غلظت را نشان داد که بر اثربخشی و راحتی
	کاربر تأثیر می گذارد. قابل ذکر است، این ژل های نانو امولسیونه پتانسیل قابل توجهی را به عنوان عوامل ضد
	میکروبی در برابر نشان دادند. باکتری های <i>Staphylococcus aureus</i> و <i>Staphylococcus epidermidis</i> در
	سنجش های مهارتی، نویدی را برای رسیدگی به عفونت های پوستی می دهند. نتیجه گیری: به طور کلی، این مطالعه
	بر پتانسیل ژل های نانو امولسیونه مشتق شده از عصاره پوست انار به عنوان یک جایگزین طبیعی برای درمان ضد
	میکروبی موضعی در برابر عفونت های پوستی تأکید می کند.

مخفف ها: VCO، روغن نارگیل تصفیه نشده (بکر)؛ Rpm، دور بر دقیقه؛ PEG، پلی اتیلن گلیکول

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