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Anti-acne activity and nanoemulsion formulation of a combination of lime peel (*Citrus aurantifolia* (Christm.) Swingle) extract and essential oil

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ABSTRACT: Acne is a common skin disorder closely associated with bacterial infection, particularly *Cutibacterium acnes* and *Staphylococcus epidermidis*. Lime peel contains flavonoids, saponins, phenolics, tannins, and essential oils that exhibit antibacterial potential. This study aims to evaluate the anti-acne activity of a combination of lime peel extract and essential oil and to formulate it into a nanoemulsion dosage form. An experimental study was conducted using the disk diffusion method to assess anti-acne activity. The inhibition zone was measured after 24-hour incubation at 37 °C. Nanoemulsions were then prepared through ultrasonic processing and characterised using PSA and TEM. The results demonstrated that the extract–essential oil combinations at ratios 1:1, 1:2, and 2:1 produced greater inhibition zones than the single agents, indicating a synergistic effect. The nanoemulsion formulated with Alkamuls CRH 40, glycerin, and VCO produced particles <100 nm with a polydispersity index of 0.072–0.141, although the zeta potential values (−14.1 to −16.7 mV) indicated suboptimal stability. Overall, the combination of lime peel extract and essential oil exhibited significant anti-acne activity and has the potential to be developed as a topical nanoemulsion-based preparation.

KEYWORDS: Antibacterial; *Citrus aurantifolia;* ultrasonic; particle size distribution; zeta potensial.

INTRODUCTION

Acne is one of the most prevalent skin disorders affecting the Indonesian population. According to Sifatullah (2021), approximately 85% of adolescents have experienced acne. In Southeast Asia, the prevalence of acne ranges from 40% to 80% of the population [1]. Meanwhile, Yusuf et al. (2020) reported that in Indonesia, acne ranks third among dermatological conditions among outpatients in hospitals and dermatology clinics. The highest prevalence was recorded among individuals aged 14–17 years and among males aged 16–19 years [2].

The concept of "back to nature" has become increasingly popular among the public. With rapid advancements in science and technology, traditional medicine continues to coexist alongside modern pharmacotherapy. One of the key reasons behind the growing preference for natural treatments is the adverse side effects associated with long-term usage of synthetic drugs, including skin irritation and bacterial resistance, prompting a shift toward safer therapeutic alternatives [3].

Lime (*Citrus aurantifolia*) has long been utilised in traditional medicine; however, its peel is frequently discarded as waste, although it contains bioactive constituents with therapeutic potential. Lime peel is known to contain flavonoids, phenolics, tannins, saponins, and essential oils, with *limonene* as a major constituent that have been reported to exhibit antibacterial and anti-acne effects [3], [4]. Optimising the delivery of these bioactive compounds is necessary, including by formulating them into a nanoemulsion system. Nanoemulsions offer superior performance as delivery systems due to their larger surface area compared to microemulsions, which enhances the penetration and bioavailability of active compounds in topical preparations. Based on the considerations above, this study was conducted to evaluate the anti-acne activity and nanoemulsion formulation of a combination of lime peel extract and essential oil (*Citrus aurantifolia*) as a topical anti-acne preparation.

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MATERIALS AND METHODS

Materials

Lime peel (*Citrus aurantifolia* (Christm.) Swingle), Essential oil of *Citrus aurantifolia* (Christm.) Swingle (Happygreen, Indonesia), *Cutibacterium acnes* ATCC 6919, *Staphylococcus epidermidis* ATCC 12228, nutrient agar (Merck, Germany), nutrient broth (Merck, Germany), Virgin Coconut Oil (Inbi, Indonesia), glycerin (Chemcheed, USA), Alkamuls CRH 40 (PEG-40 Hydrogenated Castor Oil) (Syensqo, Belgium), hesperidin, 70% ethanol (Hayuda, Indonesia), dimethyl sulfoxide (DMSO), quercetin (Sigma, USA).

Preparation of extract and essential oil *Citrus aurantifolia* (Christm.) Swingle

In this study, the sample used was lime peel (*Citrus aurantifolia* (Christm.) Swingle) from the Rutaceae family. The plant material was obtained from the Balai Penelitian Tanaman Rempah dan Obat (Balittro), Bogor, West Java. The plant determination was carried out at the Herbarium Depokensis (UIDEP), Biota Collection Room,Universitas Indonesia, and assigned specimen number 038/UN2.F3.11/PDP.02.00/2025. The peel was separated from the fruit and cut into small pieces. The peel was air-dried and later extracted with 70% ethanol by maceration. The maceration process was carried out for three days at room temperature, protected from direct sunlight, with occasional stirring. The resulting extract was concentrated using a rotary evaporator at 50–60 °C [5]. The essential oil was obtained commercially from CV. Mikaya Makmur Sejahtera Surabaya (Happy Green Lime Essential Oil).

Phytochemical screening

Flavonoid test

100 mg of the sample was added to 10 mL of water and heated to boiling for 5 minutes, then filtered. To 5 mL of the filtrate, 0.1 g of magnesium, 1 mL of HCl $_{(p)}$, and 2 mL of amyl alcohol were added. The mixture was shaken vigorously. A positive result is indicated by the appearance of red, yellow, or orange color in the amyl alcohol layer [6].

Saponin test

500 mg of the extract was placed into a test tube, followed by the addition of 1 mL of warm distilled water, and shaken vigorously for approximately 1 minute. The presence of saponins was indicated by the formation of foam with a height of 1–10 cm. After the addition of one drop of 2 N hydrochloric acid, the foam did not disappear [6].

Tanin test

500 mg of the extract was weighed and transferred into a beaker, followed by the addition of 50 mL of hot water. The mixture was stirred and filtered. The obtained filtrate was divided into three test tubes, and 10 drops of 1% $FeCl_3$ were added to each. The formation of a dark blue or greenish-black color indicated the presence of tannins [6].

Phenolic test

1 g of the extract was mixed with 20 mL of 70% ethanol. Subsequently, 1 mL of the obtained extract was treated with 2 drops of 1% ferric chloride ($FeCl_3$) solution. The appearance of red, purple, blue, green, or bluish-green coloration indicated the presence of phenolic compounds in the extract [6].

Total flavonoid extract

Determination of total Flavonoid of *Citrus aurantiofolia* (Christm.) Swingle using Spektrofotometer UV-Vis. A sample of lime peel extract (Citrus aurantifolia (Christm.) Swingle) was dissolved in 70% ethanol. Then, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water were added. The mixture was shaken until homogeneous and allowed to stand for 30 minutes. Quercetin was used to compare, which had been made in concentrations of 150, 125, 100, 75, and 50 ppm. İts absorbance is read at 431 nm wavelength using UV-Vis spectrophotometer.

Antiacne activity test extract, essential oil, and combination

Antibacterial activity against *Staphylococcus epidermidis* and *Cutibacterium acnes* was evaluated by measuring the inhibition zone diameter using the disk diffusion method. Nutrient agar (NA) was prepared in

Petri dishes, and bacterial suspensions were standardized to 0.5 McFarland (≈10^8 CFU/mL), then uniformly spread onto the agar surface using a sterile swab. The test samples consisted of lime peel extract at concentrations of 2.5%, 5%, 7.5%, 10%, 12.5%, and 15%, and lime peel essential oil at concentrations of 5%, 7.5%, 10%, 20%, 22.5%, and 25%. Each sample was applied to sterile paper disks, which were then placed onto the inoculated agar plates. Chloramphenicol was used as the positive control, while 1% DMSO served as the negative control. All plates were incubated at 37 °C for 24 hours. The inhibition zones around each disk were measured, and the presence of a clear zone indicated antibacterial activity. The obtained data were statistically analyzed using ANOVA [6]. As this study primarily focused on nanoemulsion development and characterization, MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) determinations were not performed and are recommended for future studies to quantitatively evaluate antibacterial potency.

Preparation of nanoemulsion combining extract and essential oil

Table 1. Nanoemulsion formula.

Material	Formula (%)			Function
	F1	F2	F3	
Lime Peel Extract	10	10	10	Active ingridient
Lime Peel Essential Oil	20	20	20	Active ingridient
Virgin coconut oil	5	5	5	Oil phase
Alkamuls CRH 40	20	15	10	Surfactant
Glycerin	20	20	20	Co-surfactant
Aq dest ad	100	100	100	Solvent

The extract was dissolved in glycerin (mass 1), and the essential oil was dissolved in VCO (mass 2). Alkamuls were dissolved in water to 100 mL, then homogenised (mass 3). Mass 1 and mass 2 were mixed and stirred until homogeneous, followed by the gradual addition of mass 3 under homogenization at 1500 rpm and 25 °C for one hour. Particle size reduction was carried out using a Hielscher Ultrasonic Homogeniser (UP200St, amplitude 40%, 60 watts, 4 minutes, pulse on 30 seconds/off 5 seconds, 26 kHz) at temperatures below 25 °C [7].

Characterization of nanoemulsion

Characterisation of nanoemulsion included organoleptic evaluation, polydispersity index, emulsion type, particle size, and zeta potential using a Particle Size Analyser (PSA). Morphological observations of nanoemulsion droplets were performed using a FEI Tecnai G2 20 S-Twin (high tension 200 kV) transmission electron microscope (TEM).

▪ RESULTS

Extract lime peel (*Citrus aurantifolia* (Christm.) Swingle)

Extraction of lime peel (*Citrus aurantifolia* (Christm.) Swingle) was perfomede using the kinetic maceration method to prevent degradation of heat-sensitive compounds. The dried peel was then extracted using 70% ethanol at room temprature, protected from direct sunlight with occasional stirring and solvent removal using a rotary vacuum evaporator to obtain a concentrated extract. The extraction process yielded 19.5% and a native DER value of 5.128 [8].

Phytochemical screening

The phytochemical screening results showing the secondary metabolites identified in the lime peel extract are presented in Table 2. The screening confirmed the presence of flavonoids, saponins, phenolics, and tannins [6].

Table 2. Phytochemical screening.

Test	Reagent	Result	Description
Flavonoid	Mg + HCl(p) + Amilalkohol (shaken)	+	Orange color formed in the amyl alcohol layer
Saponin	Hot H_2O (shaken)	+	Foam did not disappear after the addition of 2N HCl
Phenolic	70% ethanol + 1% $FeCl_3$	+	Blue-green color is formed
Tannin	1% FeCl3	+	Greenish-black color is formed

Total flavonoid extract

Table 3. Total flavonoid of lime peel extract (*Citrus aurantifolia* (Christm.) Swingle).

Sample	Anti-acne Activity (mm) ± SD		Total Flavonoid (mgQE/g Extract±SD)
	Replication	*Absorbance Value*	
800 ppm	1	0.353	64.25
	2	0.344	62
	3	0.358	63.91
		Average	63.91 ± 1.21

The total flavonoid content was determined by calculating the absorbance values using the linear regression equation of the quercetin standard curve. Based on this calculation, the flavonoid content in lime peel extract (*Citrus aurantifolia* (Christm.) Swingle) was found to be 63.91±1.21 mg QE/g.

Antiacne activity test extract

The results of the anti-acne activity test of lime peel extract (*Citrus aurantifolia* (Christm.) Swingle) indicated antibacterial activity, demonstrated by the formation of inhibition zones. The anti-acne evaluation was performed by dissolving the extract in 1% DMSO and preparing six concentrations (2.5%, 5%, 7.5%, 10%, 12.5%, 15%). DMSO 1% was used as a diluent because it has no bactericidal effect. The positive control (+) used was chloramphenicol tablets dissolved in 1% DMSO, while the negative control (–) used was 1% DMSO. DMSO was selected as the diluent because it is capable of dissolving both polar and non-polar compounds and does not exhibit bactericidal activity [6].

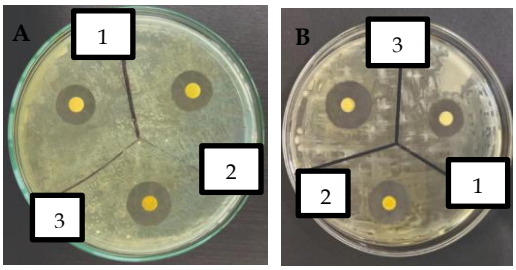

Figure 1. Results of Anti-Acne Activity Test of Extract; (A) *Cutibacterium acnes*; (B) *Staphylococcus epidermidis*; (1) 10%; (2) 12.5%; (3) 15%.

Table 4. Anti-acne activity of extract.

Sample	Concentration (%)	Anti-acne activity (mm) ± SD		Antibacterial strength
		Cutibacterium acnes	*Staphylococcus epidermidis*	
Extract	2.5	0	0	No activity
	5	0	0	No activity
	7.5	0	0	No activity
	10	11.51±1.65	11.81±0.53	Strong
	12.5	11.61±1.34	15,09±0,65	Strong
	15	12.38±0.70	18.62±0.56	Strong
Positive control	-	41.50	26.95	Very Strong
Negative control	-	0	0	No activity

Statistical analysis using Two-Way ANOVA showed that extract concentration, test bacterial species, and their interaction significantly affected inhibition zone diameter ($p<0.05$).

Antiacne activity test essential oil

The results of the anti-acne activity test of lime peel essential oil (*Citrus aurantifolia* (Christm.) Swingle) indicated antibacterial activity, demonstrated by the formation of inhibition zones.

Table 5. Anti-acne activity of essential oil.

Sample	Concentration (%)	Anti-acne activity (mm) ± SD		Antibacterial strength
		Cutibacterium acnes	*Staphylococcus epidermidis*	
Extract	5	0	0	No activity
	7.5	0	0	No activity
	10	0	0	No activity
	20	13.74±2.90	10.63±1.13	Strong
	22.5	11.76±3.11	11.46±0.43	Strong
	25	13.90±2.72	12.34±0.63	Strong
Positive control	-	41.50	26.95	Very strong
Negative control	-	0	0	No activity

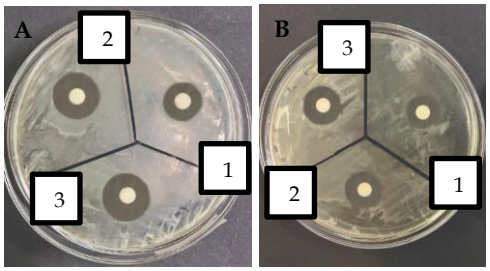

Figure 2. Results of Anti-Acne Activity Test of Essential Oil; (A) *Cutibacterium acnes*; (B) *Staphylococcus epidermidis*; (1) 20%; (2) 22.5%; (3) 25%.

Statistical analysis using Two-Way ANOVA indicated no significant differences ($p> 0.05$) in the effect of essential oil concentration, bacterial species, or their interaction on inhibition zone diameter.

Antiacne activity test combination extract and essential oil

The combination of extract and essential oil showed increased antibacterial activity against both *C. acnes* and *S. epidermidis* compared with the individual ingredients, indicating a synergistic effect among the active compounds [9]. The extract-essential oil combinations (1:1, 1:2, and 2:1) were prepared in 1% (v/v) DMSO. A 1% DMSO solution was used as the negative control and showed no antibacterial activity against the tested bacterial strains.

Table 5. Anti-acne activity of combination of lime peel extract and essential oil.

Combination	Activity Antibactery (mm)±SD	
	Cutibacterium acnes	*Staphylococcus epidermidis*
Extract : Essential oil (1:1)	16.46±3.03	18.93±3.85
Extract : Essential oil (1:2)	18.41±3.44	18.98±4.62
Extract : Essential oil (2:1)	16.98±6.56	17.43±3.40

One-Way ANOVA analysis for both bacteria demonstrated a significant difference ($p<0.05$) between single agents and the combination, confirming a synergistic interaction. However, no significant differences were observed among the ratios 1:1, 2:1, and 1:2 ($p>0.05$).

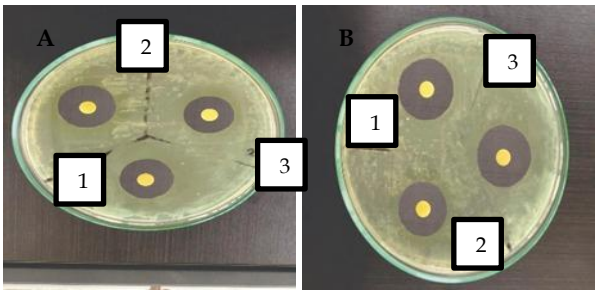

Figure 3. Results of Anti-Acne Activity of Extract–Essential Oil Combination; (A) *Staphylococcus epidermidis*; (B) *Cutibacterium acnes*; (1) 1:1; (2) 1:2; (3) 2:1.

Preparation and characterization of nanoemulsion combining extract and essential oil

The characterization results demonstrated that all formulations produced oil-in-water (O/W) nanoemulsions with particle sizes below 100 nm. Among the tested formulations, F1 exhibited the smallest particle size (57.41 nm), the lowest polydispersity index (PDI = 0.072), and good physical stability without phase separation. In contrast, F2 and F3 showed larger particle sizes (67.43 and 92.84 nm, respectively) and experienced phase separation during stability evaluation. Although all formulations showed comparable zeta potential values, F1 demonstrated the most favorable physicochemical characteristics, including smaller particle size, narrower particle-size distribution, and superior stability. Therefore, F1 was selected as the optimal nanoemulsion formulation for further development into a serum and subsequent evaluation of its anti-acne activity.

Figure 5. Nanoemulsion of lime peel extract–essential oil combination.

Table 6. Nanoemulsion Characterization.

Formula	Parameter test					
	Organoleptic		Emulsion type	Particle size (nm)	Particle size distribution (PDI)	Zeta potential (mV)
Formula 1 (F1)	Color	Dark brown (++)	O/W	57.41	0.072	-14.5
	Aroma	Characteristic				
	Clarity	Not clear				
	Stability	Stable				
Formula 2 (F2)	Color	Dark brown (+)	O/W	67.43	0.076	-14.1
	Aroma	Characteristic				
	Clarity	Not clear				
	Stability	Two-phase separation				
Formula 3 (F3)	Color	Dark brown	O/W	92.84	0.141	-16.7
	Aroma	Characteristic				
	Clarity	Not clear				
	Stability	Two-phase separation				

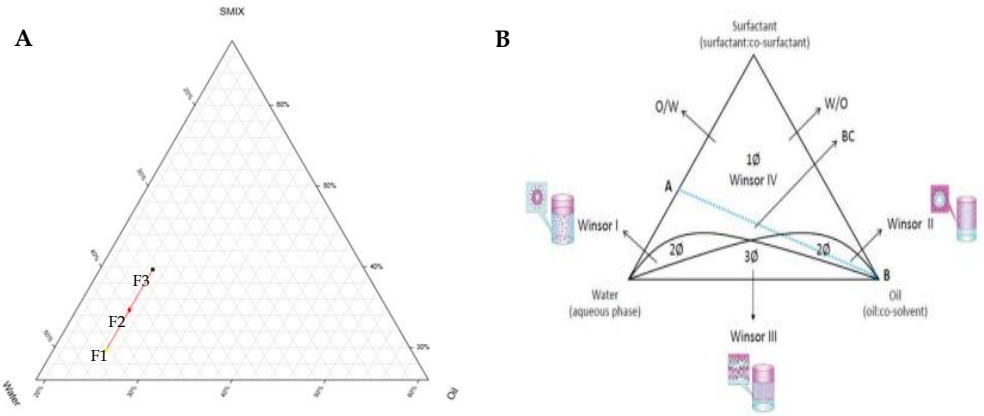

Figure 4. Pseudo-Ternary Diagram of Nanoemulsion; (A) Nanoemulsion of Extract–Essential Oil Combination; (B) Classification of Winsor Systems [10].

Based on the pseudo-ternary phase diagram (Figure V.7), the formulation was dominated by the aqueous phase (35–45%) and smix (30–40%), forming an O/W (Winsor I) system for Formula 1 and a borderline Winsor IV system for Formula 2, while Formula 3 was categorised as Winsor IV. Prajapati et al. (2023) stated that smix concentrations ≥ 20% are required to achieve optimal stability and increased viscosity [11].

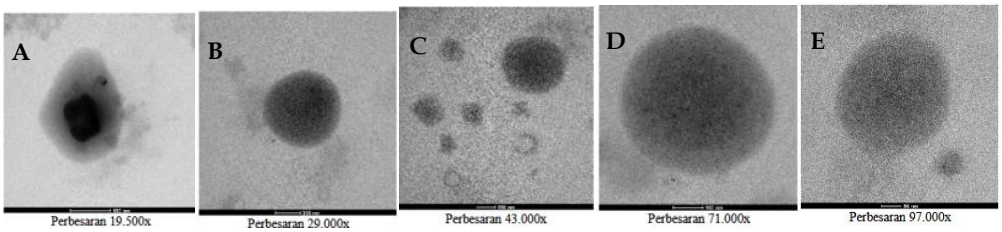

Figure 6. TEM Characterization Results: (A) 19,000×; (B) 29,000×; (C) 43,000×; (D) 71,000×; (E) 97,000× magnification.

DISCUSSION

Preparation of extract and essential oil *Citrus aurantifolia* (Christm.) Swingle

The lime peel (*Citrus aurantifolia* (Christm.) Swingle) was extracted using a maceration method (cold extraction) to prevent the degradation or loss of bioactive compounds during the extraction process [12]. The hydroalcoholic solvent enabled efficient extraction of polar to semi-polar compounds, while protection from light and occasional stirring enhanced stability and extraction efficiency. Solvent removal using a rotary vacuum evaporator under reduced pressure ensured the concentration of the extract without degrading active constituents.

Phytochemical screening

The phytochemical screening confirmed the presence of flavonoids, saponins, phenolics, and tannins in the lime peel extract. The formation of an orange color in the amyl alcohol layer following the Mg–HCl (Shinoda) reaction indicates the presence of flavonoids due to the reduction of flavonoid compounds. The persistence of foam after shaking and its disappearance upon acid addition confirms saponins, which are known for their surfactant properties. The green coloration with $FeCl_3$ indicates the presence of phenolic compounds through complex formation with ferric ions, while the dark coloration observed in the tannin test further supports the presence of tannins. These secondary metabolites are well known to contribute to antibacterial activity, supporting the observed bioactivity of the extract [6], [13].

Total flavonoid extract

The determination of total flavonoid content was carried out using the UV–Vis spectrophotometric method. This method is based on the principle of color formation through complexation reactions. In this assay, flavonoid compounds react with aluminum chloride ($AlCl_3$) to form stable complexes involving the keto group at C-4 and the hydroxyl groups at C-3 or C-5 in flavone and flavonol structures. The addition of aluminum chloride facilitates the formation of acid-stable complexes with ortho-dihydroxyl groups present on ring A or B of flavonoid compounds. This complexation results in a measurable increase in absorbance, which is directly proportional to the flavonoid concentration [14].

Antiacne activity test extract

The inhibition zone observed is attributable to secondary metabolites such as flavonoids, saponins, phenolics, and tannins, each of which exhibits distinct antibacterial mechanisms. These compounds can damage bacterial cell walls, alter membrane permeability, denature proteins, and inhibit enzymatic activity, ultimately causing cell death [15]. The 10% concentration demonstrated strong antibacterial activity and was considered sufficiently effective as the optimal formulation because it represents the lowest concentration that exhibited significant antibacterial activity against *Cutibacterium acnes* and *Staphylococcus epidermidis.* Although higher concentrations (12.5% and 15%) showed slightly increased inhibition zones, the improvement was not substantial. Therefore 10% was considered sufficient and more efficient for further formulation studies.

Antiacne activity test essential oil

Anti-acne of lime peel essential oil against *Cutibacterium acnes* and *Staphylococcus epidermidis* was only detected at 20%, 22.5%, and 25%. For *C. acnes*, concentrations of 20–25% produced strong inhibition zones ranging from 11.76 –13.90 mm. For *S. epidermidis*, the inhibition zones ranged from 10.63 to 12.34 mm. The antibacterial effect of the essential oil is supported by the presence of compounds such as limonene, β-pinene, γ-terpinene, α-pinene, α-terpineol, and α-terpinene, which act by disrupting bacterial cell walls and membranes, and by inhibiting biofilm formation and ATP synthesis [16], [17].

Antiacne activity test combination

The combination of lime peel extract and essential oil at ratios of 1:1, 1:2, and 2:1 was prepared by dissolving the samples in dimethyl sulfoxide (DMSO) as a solvent to obtain the desired concentrations. Stock solutions were initially prepared and subsequently diluted for antibacterial testing. The final concentration of DMSO in all test solutions was maintained below 1% (v/v) to ensure that it did not interfere with bacterial growth. A DMSO control was also included to confirm that the solvent had no inhibitory effect on the tested bacteria. The combination of extract and essential oil showed increased antibacterial activity against both *C. acnes* and *S. epidermidis* compared with the individual ingredients effect among the active compounds [11].

The highest anti-acne activity for *C. acnes* was found in the 1:2 ratio (18.41±3.44 mm), and for *S. epidermidis* in the 1:2 ratio (18.98±4.62 mm). The combination's ability to inhibit acne-causing bacteria is attributed to its secondary metabolites [18]. Since antibacterial activity was evaluated using the disk diffusion method, synergistic interactions could not be confirmed. Therefore, the observed effect is reported as enhanced antibacterial activity, while further FICI (Fractional Inhibitory Concentration Index) analyses are required to verify the interaction between the extract and essential oil.

Preparation and characterization of nanoemulsion combining extract and essential oil

Sonication was performed after the nanoemulsion preparation to obtain nanometer-sized particles. This method uses ultrasonic waves to convert electrical signals into physical vibrations, producing cavitation that breaks down droplets in the liquid sample. Cavitation helps reduce droplet size and improve nanoemulsion homogeneity. Frequency, sonication duration, and irradiation power are key parameters that determine the success of ultrasonic emulsification [19], [20]. Alkamuls CRH 40 (PEG-40 Hydrogenated Castor Oil) is a non-ionic surfactant with a yellowish-white paste consistency at 20 °C and liquid form at 30 °C, with pH 6–7 and HLB 14–16. It is stable in water and alcohol and can be sterilised with autoclaving at 121 °C. In nanoemulsion formulation, Alkamuls CRH 40 is advantageous because it can solubilise lipophilic compounds and essential oils, facilitating the formation of O/W emulsions and nano-sized droplets. Glycerin was used as a cosurfactant due to its high aqueous solubility, which enables it to migrate into the surfactant's polar region and lower interfacial tension. This reduction in surface tension enhances droplet breakdown during homogenization. VCO (Virgin Coconut Oil) was selected as the oil phase due to its Medium-Chain Triglyceride (MCT) content, which facilitates the formation of more stable and clearer nanoemulsions than Long-Chain Triglycerides (LCT).

These findings are consistent with previous studies, which state that particle size < 100 nm and PDI < 1 reflect a homogeneous droplet distribution. PDI values close to zero indicate greater uniformity, and distribution is strongly influenced by stirring, energy input, and temperature. F1 exhibited a smaller particle size compared to F2 and F3. This is attributed to the higher concentration of CRH 40 surfactant in F1 relative to F2 and F3. An increase in surfactant concentration reduces the interfacial tension between particles, thereby facilitating the formation of smaller droplet sizes. The use of surfactants has been shown to decrease the particle size of the resulting nanoemulsion by stabilizing the oil droplets formed at the oil–water (O/W) interface. In addition, the presence of a cosurfactant further contributes to the reduction of particle size [21]. The zeta potential values of −14.1 to −16.7 mV indicate suboptimal stability, with a potential risk of particle flocculation. The negative values were influenced by the free fatty acid content of VCO, whereas good physical stability is typically achieved at zeta potentials of ±30 mV [22]–[25]. Formula 1 was found to be stable, no visible phase separation, creaming, or aggregation was observed immediately after preparation, indicating initial physical stability of the system. Therefore, Formula 1 was selected for the preparation of a nanoemulsion containing a combination of lime peel extract and essential oil, as well as for subsequent anti-acne activity testing.

▪ CONCLUSION

This study evaluated the anti-acne activity of a combination of lime peel extract and lime peel essential oil, along with the formulation of the combination into a nanoemulsion dosage form. Both the extract and essential oil demonstrated antibacterial activity against *Cutibacterium acnes* and *Staphylococcus epidermidis*, and the combination showed a synergistic effect, producing greater inhibition zones than the single agents. The nanoemulsion formulated using Alkamuls CRH 40, glycerin, and VCO produced particle sizes below 100 nm with a homogeneous droplet distribution, although the zeta potential value (−14.1 to −16.7 mV) indicated suboptimal physical stability. Overall, the combination of lime peel extract and essential oil has promising potential to be developed as an effective topical anti-acne nanoemulsion preparation.

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Conflict of interest statement: Fill in this section according to the signed conflict of interest statement when submitting your article. If there is no conflict of interest to be declared by any of the authors, write "The authors declared no conflict of interest" in the manuscript.

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