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Chemical Analysis, Physical Stability, and Antibacterial Activity of Nanoemulgel Hand Sanitizer Formulated with *Citrus aurantifolia* Essential Oil and Herbal Emollients

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Abstract

One of the most purchased hand sanitizer products by the public since the COVID-19 pandemic is hand sanitizer. However, alcohol-based hand sanitizer products often cause toxic effects and bacterial resistance. Therefore, alternative efforts are needed to replace antibacterial raw materials instead of alcohol, namely lime peel essential oils (*Citrus aurantifolia*) with *Aloe vera* and *Calendula officinalis emollients*. This study aims to evaluate the physicochemistry, physical stability, and antibacterial affectiveness of the a nanoemulgel handsanitizer formula composed of active lime peel essential oils supplemented with *Aloe vera* and *Calendula officinalis emollients*. The design of this research is an experiment. Research treatment: formula (F) hand sanitizer nanoemulgel F1 2%, F2 4%, and F6 6%. Essential oil component test using GC-MS method, SNEEDS physical stability includes centrifugation test, freeze-thawing, transmittance, particle size, polydispersity index (PI), potential zeta, physical stability of nanoemulgel hand sanitizer, including organoleptic, homogeneity, pH, and viscosity test, antibacterial efficacy test using hand swabbing method. The results: GC-MS analysis of the components of lime peel essential oils: α-pinene (8.87%), linalool (0.58%), and L-α-terpineol (8.98%). All SNEEDS formulas and nanoemulgels were stable during 6 weeks of storage. Formulas F2 4% and F3 6% reduced the percentage (%) of hand bacterial colonies to 74% and 75%. The results of the one-way ANOVA showed a significant decrease ($p < 0.05$) in the number of bacterial colonies after using nanoemulgel hand sanitizer, especially in the F2 and F3 formulas. The conclusion is that Formula 3 (F3 6%) hand sanitizer is recommended as the best candidate for the development of a nanoemulgel hand sanitizer based on lime essential oil, as it offers the most optimal combination of physical stability, chemical stability, and biological effectiveness.

Keywords: essential oils; lime fruit peel; Self-Nanoemulsifying Drug Delivery System (SNEEDS); hand sanitizer; nanoemulgel.

INTRODUCTION

Bacterial infections transmitted through hand contact remain one of the primary routes of spread in public health. According to the World Health Organization (WHO), one way to reduce the incidence of bacterial infections is to wash hands with soap or use hand sanitizer. (United Nations Children Fund (UNICEF) and World Health Organization, 2021). Alcohol-based hand sanitizers (typically ethanol or isopropanol ≥60%) effectively reduce the number of bacteria on hands. However, long-term use can cause skin irritation, dryness, and the risk of burns (Alhalwani et al., 2024). This encourages the development of non-alcoholic hand sanitizer formulations based on natural ingredients that are safer and more comfortable to use on hand skin.

One of the extracts of natural ingredients with antibacterial potential is lime essential oil (EO). Lime essential oil (*Citrus aurantifolia*) is known to contain d-limonene, citral, and linalool compounds that exhibit antibacterial activity (Husni et al., 2021). Another study reported that lime EO had an alcohol-comparable effectiveness of 78% against *Staphylococcus aureus* and *Escherichia coli* bacteria (Mohammed et al., 2024). Thus, lime essential oil has the potential to be an active ingredient in non-alcoholic hand sanitizer formulations.

Other natural ingredients are *Calendula officinalis* and *Aloe vera.* The use of *C. officinalis* as a component of hand sanitizer formula is due to its anti-inflammatory, antioxidant, and supportive skin tissue regeneration (Parente et al., 2012). Calendula oil is often used as a moisturizer and anti-irritant in topical preparations. Meanwhile, *Aloe vera* is widely known as a humectant and emollient that can increase skin moisture, reduce irritation, and add sensory comfort (Catalano et al., 2024). The combination of lime oil, calendula, and *Aloe vera* is expected to produce *a hand sanitizer* that is not only an effective antimicrobial, but also safe and comfortable to use on the skin of the hands.

However, using essential oils in conventional forms has limitations, including low water solubility, high volatility, and limited stability. Therefore, nanoemulsion technology is needed to reduce the size of oil droplets to the nanometer scale, increasing solubility, bioavailability, and stability (Omidian et al., 2025). The combination of nanoemulsion with gel base results in nanoemulgels that are easier to apply, clear, and allow for more stable release of active ingredients (Donthi et al., 2023). Therefore, nanoemulgel is an ideal formulation for essential oil-based hand sanitizers.

Several studies have reported the development of nanoemulgel-based hand sanitizers, including (Ibrahim et al., 2024), developing lemon peel extract nanoemulgel and demonstrating antimicrobial activity against skin pathogenic bacteria, Drais (2024) successfully developed nanoemulgel essential oil with high stability and potential as a skin sanitizer. However, the research generally only assesses aspects of physical stability or antimicrobial activity *in vitro*, without comprehensively evaluating the physicochemical characteristics and *in-use* tests on human skin.

This researcher aims to develop a Hand Sanitizer Nanoemulgel containing lime essential oil with *Aloe vera* and *Calendula officinalis* as emollients. Physical stability tests include: transmitter tests, particle size and polydispersion index, pH, viscosity, homogeneity, dispersability, and thermodynamic stability. In addition, this study also evaluated the number of bacteria on the hands before and after using Hand Sanitizer Nanoemulgel to assess its effectiveness directly in the community. This combination of physicochemical and microbiological data provides a comprehensive picture of the quality, stability, and biological activity of Hand Sanitizer Nanoemulgel lime essential oil products.

MATERIALS AND METHODS

Materials

Materials and tools include lime peel (*Citrus x aurantiifolia* (Christm.) Swingle), Aloe Vera Gel 99% organik Non Alkohol, Calendula Oil - 100% Pure Natural Oil, Hydroxypropyl Methyl Cellulose (HPMC) (Merck), Span 80 (Merck), Gliserol (Merck), 2,2-Diphenyl-1-Picrylhydrazil (DPPH) (sigma), Benzil alkohol (Merck), Phenol (Merck), Chloroform (merck), Quercetin (sigma), Nutrien Agar (Merck), Galic Acid (sigma), Polyethylene Glycol (PEG) 400 (Merck),

Essential Oil Extraction

40 kg of lime peel was placed in a steam distillation apparatus containing water (1:5 w/v ratio). The distillation time was 5 hours. A separator separated the essential oil (distillate) from the water. The essential oils were contained in tightly sealed bottles. The composition of the essential oils was tested using the gas chromatography–mass spectroscopy (GC-MS) technique.

Analysis of essential oil composition using the GC-MS method

Essential oil analysis was performed using Gas Chromatography–Mass Spectrometry (GC–MS) technique with Shimadzu GC/MS-QP2010 Ultra (serial number 020525101565SA). The capillary column was Rtx-5MS (30 m × 0.25 mm × 0.25 µm). The sample was injected in split mode, and the instrument was operated in electron ionization (EI) mode with an ionization energy of 70 eV. The carrier gas was helium (flow rate 1.69 mL/min). The column temperature program started at 50°C, then increased at a rate of 7°C/min to 180°C, and then increased again at a rate of 10°C/min to a final temperature of 280°C. The injection port temperature was set at 300°C, the ion source temperature at 200°C, and the interface temperature at 250°C. The analysis was performed in scan mode with a mass-to-charge ratio (m/z) range of 40–500, with a total analysis time of 28 minutes. Sample components were identified by comparing the retention index and mass fragmentation pattern of each compound to data available in the NIST (National Institute of Standards and Technology) library (Mohammed et al., 2024)

DPPH antioxidant analysis of essential oil lime peel and Calendula oil

Antioxidant activity test of essential oil lime peel and Calendula oil was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, modified from Baliyan et al. (2022) A total of 24 mg of DPPH powder was dissolved in 100 mL of methanol to make a stock solution. This solution was then filtered and adjusted to absorb approximately 0.97 at a wavelength of 517 nm using a UV-Vis spectrophotometer. Test samples of Aloe vera gel extract (dissolved in 80% methanol) and Calendula oil (dissolved in ethanol with the addition of 0.1% Tween-20 to homogenize) were prepared at several concentrations (10–1000 µg/mL). A total of 3 mL of DPPH solution was mixed with 100 µL of test sample solution in a test tube. As a control, 3 mL of DPPH solution was used, and 100 µL of methanol was added without a sample. All tubes were then incubated in the dark for 30 minutes at room temperature to prevent free radical degradation. After incubation, absorbance was measured at a wavelength of 517 nm. The percentage of antioxidant activity was calculated using the following formula:

$$\%\ \text{antioxidant activity} = \frac{(A_c - A_s)}{A_c} \times 100$$

Description:

Ac = absorbance of the control (DPPH solution without sample),

As = absorbance of the DPPH solution with the test sample.

The IC_{50} value (the concentration capable of inhibiting 50% of DPPH radicals) was then determined from the relationship curve between sample

concentration and percentage inhibition. The positive control used was ascorbic acid.

Formula SNEEDS (Self Nanoemulsifying Drug Delivery System) dan nanoemulgel hand sanitizer

The making of SNEEDS and nanoemulgel hand sanitiser is shown in Table 1.

Table 1. *SNEEDS* formula and nanoemulgel hand sanitizer.

Phase	Ingredients	Function	F1	F2	F3
	Lime essential oil	active compounds	2.00%	4.00%	6.00%
	Calendula oil	Emollient	0.50%	0.50%	0.50%
	Span 80	Surfactant	2%	2%	2%
	Cremophor RH 40	Surfactant	10%	10%	10%
Phase A	PEG 400	Co-surfactant	10%	10%	10%
	HPMC	gelling agent	1%	1%	1%
	Aloe vera gel	Emollient	5%	5%	5%
	Gliserol	Moisturized	3%	3%	3%
	Benzyl alcohol	Preservative	0.1%	0.1%	0.1%
Phase B	aquadest	Solvent	66.40%	64.40%	62.40%

Based on Table 1. The production of SNEEDS is carried out by weighing all ingredients according to their formulation percentages. Surfactants (Cremophor RH 40) and co-surfactants (PEG 400) are placed in a beaker, heated and stirred at 40–45 °C with a magnetic stirrer until a homogeneous and precise mixture is formed. Span 80 is added slowly in a surfactant–co-surfactant mixture. Calendula oil is included in the mix. Lime essential oil is added while stirring continuously until a homogeneous and clear mixture is obtained (an indication of phase A has been formed as a nanoemulsion pre-concentrate)—the homogeneous phase. A mixture is then stored in a tightly closed container at room temperature. (Drais, 2024).After obtaining stable SNEEDS, a nanoemulgel *hand sanitizer* formulation is made by weighing all ingredients. Phase A preparation involves mixing Cremophor RH 40 and Span until a homogeneous mixture is achieved, followed by the addition of lime peel essential oil and *Calendula oil* in a *mixer* (speed 1500 rpm for 10 minutes), followed by a nanoemulsion size test in this phase. Next, prepare for Phase B by developing HPMC with enough aquadest until it expands. Add the *Aloe vera* gel, glycerol, and benzyl alcohol to a mixer and mix until homogeneous. Insert phase A into phase B with a *magnetic stirrer* until homogeneous for 10 minutes (Ibrahim et al., 2024).

SNEEDS and Nanoemulgel Physical Stability Evaluation Test

SNEEDS physical stability evaluation tests include centrifugation (thermodynamics), Freeze Thawing, transmitters, Particle Size, Compressed Potential Index (PDI), and Potential Zeta (Annisa et al., 2023). The physical stability test of nanoemulgel hand sanitizer includes organoleptic, homogeneity, pH, and viscosity tests during 6 weeks of storage. (Muñoz et al., 2023).

Nanoemulgel Hand Sanitizer Efficacy Test

The efficacy test of hand sanitizer was carried out in the following way: respondents were asked to contaminate their hands. After that, the initial sample (baseline) was taken from both hands of each respondent by rubbing the palms and fingertips with sterile swabs moistened with Brain Heart Infusion (BHI) broth. Furthermore, each respondent was asked to pour 3 mL of hand sanitizer to clean their hands (*hand hygiene*) according to WHO procedures. After using hand sanitizer, a sample was retaken with a sterile cotton swab moistened with sterile BHI broth, then rubbed on both respondents' palms, interlodes, and fingertips. The swab was then spread on Nutrient Agar (NA) media (Oxoid, UK) and incubated aerobically at a temperature of 36°C for 1x24 hours (overnight) (Sommatis et al., 2023). After incubation, the percentage of bacterial reduction (R%) is calculated by the following equation:

$$R\ (\%)\frac{(ib-ia)}{ib}x100\%$$

R: Percentage reduction of bacteria
ib: number of bacteria before being given nanoemulgel hand sanitizer
ia: The number of bacteria after being given nanoemulgel hand sanitizer

Data Analysis

Data analysis uses IBM SPSS 25 statistics. Parametric data (ratio) was tested for normal distribution, homogeneity, and then one-way ANOVA (Sig<0.05). If significant, continue the *Post-Hoc* test to find groups with significant influence.

RESULTS AND DISCUSSION

Chemical composition of lime fruit peel essential oil

Based on the GC-MS analysis of lime peel essential oils, 41 chromatogram peaks were obtained. The visualization of the Chromatogram can be seen in Figure 1.

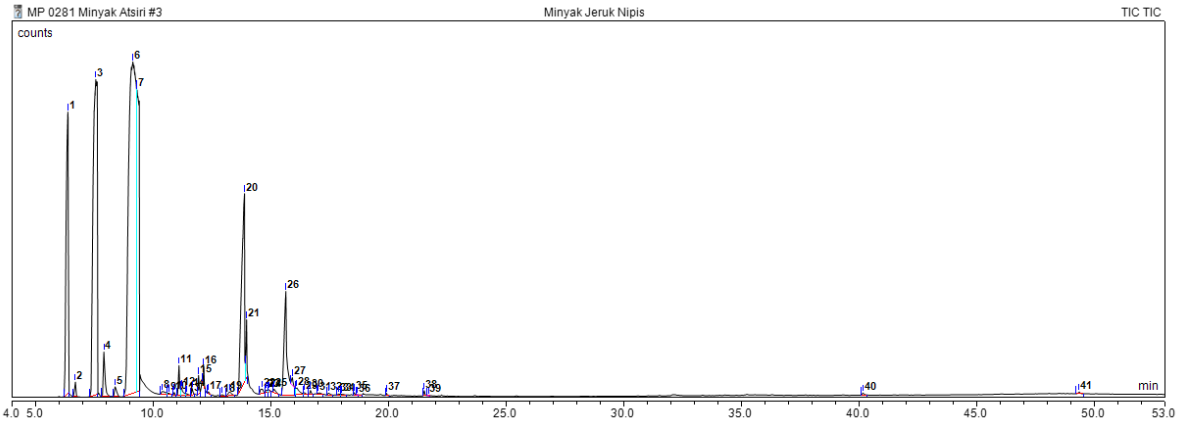

Figure 1. GC-MS chromatogram of the components of the essential oil of lime fruit peel.

Based on Figure 1. The compounds contained in lime oil are α-pinene (8.87%), linalool (0.58%), and L-α-terpineol (8.98%). The largest content is L-α-terpineol. GC-MS analysis results include the essential oil components of lime peel from Slovakia for α-terpineol (1.4%) and α-pinene (12.6%) (Galovičová et al., 2022), as well as linalool (0.64%) (Lin et al., 2019). The dominant compound almost always found in essential oils of the citrus genus (including *Citrus aurantifolia*) is *d-limonene* or monoterpenes, which give citrus fruits a fresh aroma. P-cymene*, γ-terpinene*, and *β-pinene* are found in small amounts. Based on the results of GC–MS, lime peel essential oils in this study were dominated by L-α-terpineol and α-pinene. This composition differs from the general profile of fresh citrus fruit peel, which is typically dominated by d-limonene (Li et al., 2022; Galovičová et al., 2022). The difference is likely due to the oxidation process of limonene during distillation or storage of the sample. An increase in oxygenated monoterpene compounds such as α-terpineol indicates limonene conversion due to exposure to heat and oxygen.

Value percentage (%) inhibition (DPPH Radical Scavenging Activity)

The antioxidant activity of lime peel and calendula essential oils was evaluated using the DPPH radical scavenging assay method to determine their ability to neutralize free radicals. The percent inhibition values (% inhibition) obtained at various concentrations (2%, 4%, and 6%) are presented in Table 2.

Table 2. Mean percentage value (%) inhibition (DPPH Radical Scavenging Activity).

Sample	Mean ± SD % inhibition		
	2%	4%	6%
Ascorbic acid (positive control)	$5.12^{a} \pm 0.86$	$13.82^{b} \pm 0.52$	$75.90^{c} \pm 0.37$
Lime fruit peel essential oil	$21.27^{a} \pm 0.71$	$22.50^{b} \pm 0.62$	$33.15^{c} \pm 0.66$
Calendula oil	$19.96^{a} \pm 0.22$	$22.61^{b} \pm 0.04$	$31.76^{c} \pm 0.37$

Superscript letters with different concentrations in each sample showed significant differences ($p<0.05$) (one-way ANOVA).

Based on Table 2. Antioxidant activity tests using the DPPH method showed that all samples could suppress free radicals that increased with increased concentration. Ascorbic acid, which was used as a positive control, showed the highest activity with an mean inhibition of 75.90% at a concentration of 6%. This indicates a powerful free radical inhibition potential because the simple phenolic structure of ascorbic acid acts as an efficient electron donor against DPPH radicals. (Jaganjac et al., 2021). Lime peel *Citrus aurantifolia* essential oil showed a significant increase in antioxidant activity ($p < 0.05$) from 21.27% at a concentration of 2% to 33.15% at a concentration of 6%. The IC_{50} value of 16.15% indicates that lime peel essence exhibits moderate antioxidant capabilities, with lower effectiveness compared to the ascorbic acid control yet still displays a clear *dose-dependent response* pattern. Essential oils of the genus *Citrus* generally have moderate antioxidant activity due to the dominance of non-oxygenating monoterpene compounds such as *d-limonene* and *α-pinene,* which are volatile and have limited radical inhibition capabilities compared to phenolic or flavonoid compounds. (Li et al., 2022). Calendula oil (*Calendula officinalis*) also showed a pattern of significant increase in % inhibition ($p < 0.001$), with a mean inhibition value of 31.76% at a concentration of 6% and an IC_{50} of 11.55%, meaning that the antioxidant activity was more potent than that of lime oil. A lower IC_{50} value indicates that the amount of oil needed to neutralize 50% of DPPH radicals is less. This is likely due to the presence of flavonoid glycosides, triterpenoids, and phenolic compounds in Calendula oil, which contribute to

increased hydrogen donor ability and free radical stabilization. These results are in line with the findings. Shahane et al. (2023) reported that Calendula extract and oil exhibited high antioxidant activity which was strongly correlated with total the phenolic compounds (TPCs) content.

Based on the IC_{50} value, the order of DPPH free radical inhibition is: Ascorbic acid (most potent) > Calendula oil (IC_{50} = 11.55%) > Lime oil (IC_{50} = 16.15%). Their chemical composition can explain the difference in activity between lime oil and Calendula. In lime oil, the dominant components are *limonene*, *β-pinene*, and *γ-terpinene*, which tend to provide moderate antioxidant effects because their hydrocarbon structure lacks proton donor groups. In contrast, Calendula oil's oxygenated α-tocopherol, quercetin, and triterpenoid content contribute significantly to the antioxidant effects through radical scavenging and chain-breaking mechanisms (Chen et al., 2023).

Overall, these results confirm that Calendula oil has better antioxidant potential than lime oil, although both are still less effective than ascorbic acid. The variation in activity is due to differences in species, geographical origin, and extraction methods. Water content, extraction temperature, and thermal degradation of monoterpenes during the distillation process can also cause variations in results between studies and between citrus essential oil-producing countries. (Li et al., 2022; Catalano et al., 2024).

Physical stability of SNEEDS (Self-Nanoemulsifying Drug Delivery System)

SNEEDS (Self-Nanoemulsifying Drug Delivery System) is an initial system of nanoemulgels that function to produce *preconcentrates*, which are homogeneous mixtures of oils, surfactants, and cosurfactants. These mixtures will spontaneously form nanoemulsions with water or water phases (such as gel bases) without the need for considerable energies (such as sonication or high-pressure homogenization). Therefore, SNEEDS is the initial stage of nanophase formation that ensures that the particle size is tiny (<100 nm) and stable, before the phase is mixed into the gel base to form a nanoemulgel ready for topical use. The manufacture of SNEEDS was carried out before nanoemulgels because it functions to produce a homogeneous, stable, and nano-sized droplet that can form nano-sized droplets spontaneously when mixed with water. This stage is essential to ensure the system's stability, the efficiency of releasing active substances, and the increased biological activity, such as antioxidants or antibacterials of lime and Calendula essential oils. The initial formulation of SNEEDS was prepared to ensure the physical stability of the nanosystem before it was developed into a nanoemulgel. The physical stability in this study encompasses thermodynamic tests (centrifugation), *freeze-thawing,* transmitters, particle size, index polydispersity, and nanoemulsion potential zeta. (R. A. Anindita et al., 2023)

Thermodynamic test (centrifugation) and freeze-thawing SNEEDS

The SNEEDS thermodynamic (centrifugation) test aims to observe the potential for phase separation and homogeneity of the mixture. This test ensures that all formulas remain stable without any good phase separation to proceed to the nanoemulgel formulation stage. In contrast, the freeze-thawing test evaluates physical stability against extreme temperature changes. This test aims to simulate varying storage conditions and assess the potential for phase separation, turbidity, or viscosity changes that may affect the system's stability. Each test cycle alternates storing the formulation at low (–20 °C) and high temperature (25–40 °C) for several days. The results of the thermodynamic and freeze-thawing SNEEDS tests are presented in Figure 2.

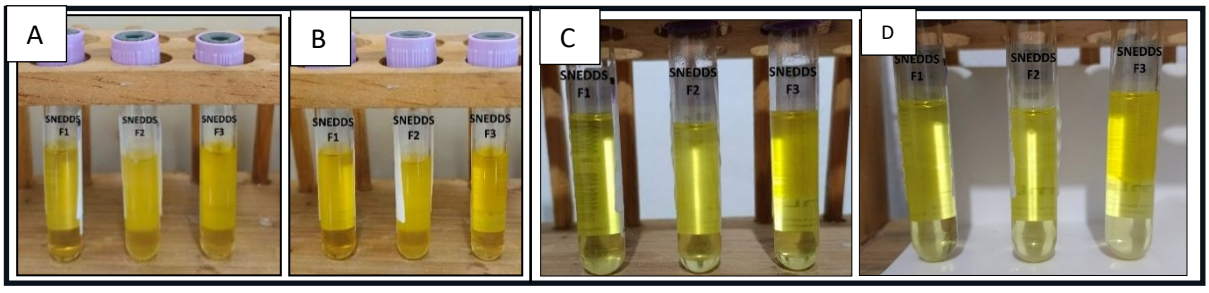

Figure 2. Centrifugation test results (A. Before centrifugation. B. After centrifugation). Freeze-thawing test results (C. Before freeze-thawing. D. After freeze-thawing).

Based on Figure 2, the centrifugation test of the SNEEDS F1 2%, F2 4%, and F3 6% formulas showed no discoloration, turbidity, or phase separation in the entire formula after the centrifugation process for 30 minutes at 5000 rpm, while the freeze–thawing test results for the whole of the formula showed good stability without turbidity or phase separation after three freezing and thaw cycles. This confirms that the SNEEDS system has sufficient thermal stability for the nanoemulgel formulation stage. The absence of phase separation or

turbidity after centrifugation tests indicates that the oil droplets in the system have enough interface defenses against high mechanical forces. If separation is found after centrifugation, the top layer appears to contain a separate oil phase, indicating that the surfactant–cosurfactant ratio is not optimal and that the droplet size is large enough to allow coalescence and creaming under centrifugal force. In contrast, a reasonably stable formulation (tiny droplet size, optimal surfactant/SMix, effective cosurfactant) can pass multiple freeze–thaw cycles without separation as the interface remains protected. (Pavoni et al., 2020).

Test of transmittance, particle size, polydispersity index, and potential Zeta

The optimized Self-Nanoemulsifying Drug Delivery System (SNEEDS) system was physically characterised to ensure the formation of a stable and homogeneous nano system. The main parameters analysed included transmittance value (%T), particle size (nm), *polydispersity index* (PDI), and *zeta potential*. The transmittance value is used to assess the system's clarity and indirectly indicates the level of dispersion of droplets in the continuous phase. Particle size and PDI reflect the homogeneity and kinetic stability of the system, while *zeta potential* describes the electrostatic stability associated with the repulsive force between droplets. Evaluating these four parameters provides a comprehensive picture of the stability and effectiveness of nanoemulsion formation. The results of testing the four characteristics are presented in Table 3.

Table 3. Mean of test results of transmitters, particle size, polydispersity index, and potential Zeta.

Variabel	F1	F2	F3
Transmittance (%)	99.049	97.529	91.096
Particle size (nm)	123.0	100.3	113.5
polydispersity index (PI)	0.487	0.450	0.381
Potential zetas (mV)	-35.0	-31.9	-42.0

Based on Table 3, the values of all SNEDDS F1-F3 formulations show >90%, meaning that the greater the concentration of essential oils, the smaller the transmitter value. It can be seen that SNEDDS F1 with an essential oil concentration of 2%, 4%, and 6% gives transmitter results of 99.049%, 97.529%, and 91.096%. Transmitter value requirements to determine the stability of nanoemulsion droplets if the value is >90% (Dewanti, Ariyadi, Martien, & Zuprizal, 2024);A transmitter value >90% indicates a clearly formed nanoemulsion system, an isotropic system, and a homogeneous system. When the transmittant value in the system is high (>90%), the uniformity level is high, the droplet size is tiny, and the dispersion is uniform. This high transparency indicates that the droplet size is relatively small and few particles give rise to a light/cloudy scattering. According to Huang et al. (2022), %T value between 97–100% for the nanoemulsion indicates that the droplets are in the range of <200 nm and the system is optically stable. These transmitter indicators will typically provide strong justification for determining the nanosystem before testing it using a more accurate particle size analyser tool. The blank used in the study is aquadest as a comparison because aquadest does not have particles that will block the transmission of light, so that later the light that will be passed will not be disturbed by the effect of light scattering, so that the value of the blank transmitter is usually 100% (Taiyeb et al., 2024).

The particle size shows that F2 has the smallest size (100.3 nm), F3 is slightly larger (113.5 nm), and F1 is 123 nm. All are below the 200 nm threshold, which is often used as a kinetically stable "nanoemulsion" criterion. (Xu et al., 2024). A smaller size (F2) will provide advantages: greater surface area, better kinetic stability, and more negligible light scattering (referring to high transmittants). The larger particle size of F1 and F3 may indicate that the surfactant/component ratio may be slightly less optimal than that of F2. The Polydispersity Index (PDI) shows the width of the particle size distribution; PDI values < 0.3–0.4 are generally considered homogeneous and stable; > values of 0.4–0.5 indicate a wider distribution. The results of this study showed that F3 = 0.381 (good), F2 = 0.450, and F1 = 0.487 (wider). The PDI F1 and F2 results are greater than 0.4, although the average size is small and has considerable variation in size. However, it can potentially increase the risk of coalition or size change during storage. Alhamdany et al. (2021) Said that PDI < 0.3–0.4 indicates better stability. Potential zetas indicate the surface charge of droplets and provide an idea of the electrostatic repulsion force between droplets. The greater the zeta potential, the more electrostatically stable the system. The general value that is considered sufficient for stability is > 30 mV (positive or negative) (Gurpreet & Singh, 2018). The result of this researcher's potential zeta at F1 = -35.0 mV, F2 = -31.9 mV, and F3 = -42.0 mV. All zeta values have a potential > 30 mV. This shows the potential for good electrostatic stability. The highest F3 value (-42 mV) indicates the most substantial interdroplet repulsion and has the highest potential for long-term stability. Therefore, although F3 has a slightly larger particle size than F2, it has the best potential zeta and the lowest PDI. This predicts that F3 is the optimal formulation for achieving a balance of size, homogeneity, and charge, making it the most suitable candidate for the next stage (nanoemulgel).

Physical stability of nanoemulgel hand sanitizer.

Based on the results of the physical stability of the Self-Nanoemulsifying Drug Delivery System (SNEDDS) system, formula F3 was selected as the best candidate to be further formulated into a nanoemulgel hand sanitizer (HS) preparation with formula 1 (F1) 2%, formula 2 (F2) 4%, and formula 3 (F3) 6%. Evaluation of the physical

stability of HS nanoemulgel preparations includes organoleptic tests, homogeneity, pH, viscosity, dispersibility, each of which provides an overview of the consistency, stability of the active components, and the convenience of application of the preparation on the skin.

Organoleptic and homogeneity tests

Organoleptic and homogeneity tests are an essential first step in evaluating the physical stability of nanoemulgel preparations, as these two parameters directly reflect the visual consistency, odour, texture, and uniformity of distribution of active and excipient components within the system. The results of the organoleptic and homogeneity tests can be seen in Figure 3.

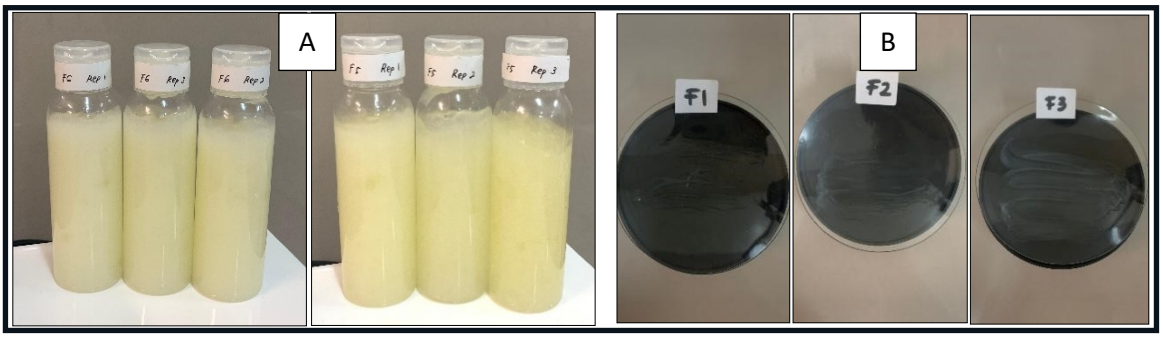

Figure 3. A. organoleptic. B. Homogeneity.

Based on Figure 3. Organoleptic and homogeneous tests of nanoemulgel hand sanitizer (HS) F1 2%, F2 3%, and F3 6%, until week 6 the preparation remained homogeneous with a bright yellow color, a typical lime smell, and a gel texture that remained thick and did not undergo phase separation or noticeable texture changes. Organoleptic examinations are performed to ensure that during storage, the preparation does not undergo any discolouration, odour, or consistency, which could indicate the occurrence of chemical degradation or physical instability (Bajaj et al., 2013) Homogeneity indicates the extent to which the nanoemulgel system maintains a uniform distribution of particles, whether from the oil, surfactant, or gel base phases. Inhomogeneity in nano-preparations can lead to droplet coalescing, creaming, or separation phases, which ultimately affects the effectiveness of the active ingredient as well as the comfort of use (Pavoni et al., 2020a). In nanoemulgel hand sanitizer formulations, the organoleptic stability also reflects the interaction between the nanoemulsion phase (SNEEDS) and the gel base, which determines the stability of viscosity, color, and gradual release of the active ingredient. Therefore, visual observation of color, the distinctive smell of lime essential oil, and the consistency of the gel are important indicators of the integrity of the system during storage (Sevinç-Özakar et al., 2022).

pH test, viscosity, dispersability

Table 4. Mean of pH and viscosity values of nanoemulgel hand sanitizer during 6-week storage.

Week	Mean ± SD	
Formula	**pH**	**viscosity (cps)**
F1 2%		
1	5,77[a]±0,02	19.333±94
2	5,56 [b] ±0,02	18.567±47
3	5,45 [c] ±0,02	16.533±94
4	5,37 [d] ±0,03	15.533 ±47
5	5,19 [e] ±0,12	14.433±47
6	4,90 [f] ±0,02	12.933±47
F2 4%		
1	5,55 [a] ±0,02	19.667±94
2	5,43 [b] ±0,02	18.400±82
3	5,35 [c] ±0,02	16.233±47
4	5,26 [d] ±0,17	15.233 ±47
5	4,93 [e] ±0,02	14.067±94
6	4,86 [f] ±0,17	12.100±82
F3 6%		
1	5,31 [a] ±0,05	19.733±47
2	5,23 [b] ±0,05	18.100±82
3	5,17 [c] ±0,05	15.933±47
4	5,04 [d] ±0,03	14.767 ±47
5	4,91 [e] ±0,01	13.767±47
6	4,85 [f] ±0,03	11.700±82

ANNOVA Test. The different superscripts in each formula showed significant differences ($p<0.05$).

Based on Table 4. Mean of pH on F1 2% decreased from 5.77 to 4.90, F2 4% decreased from 5.55 to 4.86, while F3 decreased from 5.31 to 4.85. ANOVA test results ($p < 0.05$): showed that the storage time of 6 weeks had significantly affected the average pH value. However, the pH value remains within the standard range

for topical preparations (4.5–6.5), indicating that it does not cause skin irritation (Fenny & Safitri, 2021). Mean of viscosity value (cps) of F1 decreased from 19.33 to 12.93, F2 from 19.67 to 12.10, and F3 from 19.73 to 11.70. ANOVA test results ($p < 0.05$)÷ showed a significant decrease in viscosity during 6-week storage. This decrease can be caused by gel-based dehydration (e.g. carbopol), or the instability of nanoemulsion droplets that affect the system's viscosity. However, F3 has the highest viscosity consistently. This shows that F3 has better nanoemulgel system stability than F1 and F2. High viscosity values are essential for maintaining the adhesion and contact time of the active ingredient in the skin (Aldeeb et al., 2024). Overall, all formulas still meet the stability standards of topical preparations as stated by the European Medicines Agency EMA (2022) which states that the pH should not change by more than ±1 unit and there should be no visual phase separation during storage. Decreased pH and viscosity during storage are typical characteristics in essential oil-based nanoemulgel systems resulting from electrostatic interactions between surfactants, cosurfactants, and gel bases (Pavoni et al., 2020b). Despite the decline, the F3 formula exhibits the best rheological and chemical stability, which can be attributed to higher concentrations of lime and calendula oil and a more homogeneous distribution of droplets.

Efficacy of Nanoemulgel Hand Sanitizer

In vivo biocide efficacy testing was based on Public Health England and Lambrechtsetal protocols. The agar standard plate count (SPC) method was used to determine the total number of bacteria for all samples, which were incubated at 37 ◦C for 24–48 hours. Table 5 shows the results of antibacterial tests before and after washing hands with nanoemulgel hand sanitizer can be seen in table 5.

Table 5. Mean number of bacterial colonies before and after washing hands with nanoemulgel hand sanitizer.

Treatment	Before (A)	After (B)	bacterial reduction (%)
F1 2%	93	44,5	52,15 [a]
F2 4%	101	12	74,16[b]
F3 6%	34	25,5	75[c]

ANNOVA one-way test: different superscripts showed significant differences (P<0.05) between treatment groups.

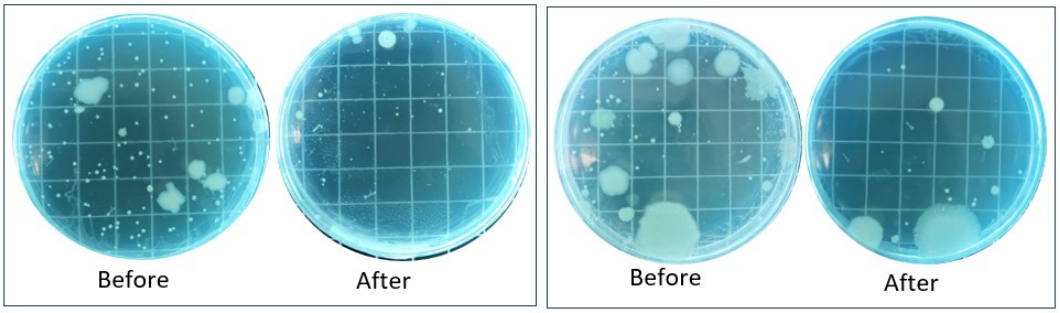

Figure 5. Antibacterial activity test of nanoemulgel hands sanitizer lime essential oil. Treatment before and after hand washing.

Based on Table 5 and Figure 5. all formulas were able to reduce the number of bacterial colonies, but F2 and F3 showed the highest antibacterial activity (74%–75%). Figure 5 shows a decrease in the number and size of colonies after using nanoemulgel hand sanitizer. This demonstrates that nanoemulgel hand sanitizer exhibits a bactericidal/bacteriostatic effect. Lime oil contains limonene, citral, and linalool, which can disrupt the integrity of lipid membranes and inhibit the respiration enzymes of microorganisms. Limonene and citral are lipophilic, allowing them to easily penetrate the cell walls of both Gram-positive and Gram-negative bacteria and cause denaturation of membrane proteins (Pavoni et al., 2020b). Increasing the concentration of essential oils from 2% (F1) to 6% (F3) generally increases antibacterial effectiveness, but F2 (4%) has already reached maximum effectiveness (74%). This indicates a saturation effect, where an increase in oil concentration no longer increases activity due to limited diffusion of active ingredients to bacterial cells. Results before and after, the number of colonies dropped significantly on F2 and F3. This proves that the nanoemulgel system works through a mechanism of direct contact between essential oil droplets and bacterial cells, causing cytoplasmic leakage and protein clumping (bacteriolysis) (Hosny et al., 2021).

Based on the results, there was a significant decrease in bacterial colonies ($p < 0.05$) after using nanoemulgel hand sanitizer, especially in the F2 and F3 formulas. Antibacterial activity increases with the concentration of lime essential oil, demonstrating the active role of limonene and citral compounds. Nanoemulgel is an effective delivery system because it extends the contact time of the active ingredient with the skin and improves

diffusion. Formulas F2 and F3 can be recommended as optimal candidates because they show antibacterial effectiveness >70% or as per the standard effectiveness of hand antiseptic preparations (Sommatis et al., 2023)

The advantages of this study include: analysis of essential oil components using the GC-MS method, formulation of SNEEDS and nanoemulgel handsanitizer, and the efficacy test of nanoemulgel hand sanitizer on the number of bacterial colonies in both palms. The limitation of this study is that tests have not been carried out on antibiotic-resistant bacteria such as *Staphylococcus aureus* and *Eschericia coli*

CONCLUSIONS

GC–MS analysis of lime peel essential oils showed the dominant components of L-α-terpineol and α-pinene. The self-nanoemulsifying drug delivery system (SNEEDS) formula F1 2%, F2 3%, and F3 4% are capable of forming dispersions with particle size (<150 nm), low polydispersibility index (<0.5), and stable negative potential zeta values (up to −42 mV). The formulas F1 2%, F2 4%, and F3 6% nanoemulgel Hand Sanitizer (HS) during 6-week storage were proven stable. The palm-based antibacterial efficacy test showed that the entire formula could significantly reduce the number of colonies ($p<0.05$) by a percentage of 52%-75%. Formula 3 (F3 6%) is recommended as the best candidate for developing a nanoemulgel hand sanitizer based on lime essential oil, as it has the most optimal combination of physical stability, chemical stability, and biological effectiveness. The implications of this result open up the opportunity to use lime peel essential oil as an alternative to antibacterial active ingredients in nano-based delivery systems that are environmentally friendly and have the potential to be developed on an industrial scale.

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REFERENCES

Aldeeb, R. A. E., Ibrahim, S. S. A., Khalil, I. A., Ragab, G. M., El-Gazar, A. A., Taha, A. A., Hassan, D. H., Gomaa, A. A., & Younis, M. K. (2024). Enhancing collagen based nanoemulgel for effective topical delivery of Aceclofenac and Citronellol oil: Formulation, optimization, in-vitro evaluation, and in-vivo osteoarthritis study with a focus on HMGB-1/RAGE/NF-κB pathway, Klotho, and miR-499a. *Drug Delivery and Translational Research*, *14*(11), 3250–3268. https://doi.org/10.1007/s13346-024-01548-3

Alhalwani, A., Husain, A., Saemaldahar, A., Makhdoum, F., Alhakami, M., Ashi, R., Wali, R., Alsharif, S., Khan, M. A., Jastaniah, N., & Fasfous, I. (2024). The impact of alcohol hand sanitizer use on skin health between healthcare worker: Cross-sectional study. *Skin Research and Technology*, *30*(1). https://doi.org/10.1111/srt.13527

Anindita, R. A., Uzia Beandrade, M., Kurnia Putri, I., & Dwi Nathalia, D. (2023). Penentuan Nilai Sun Protection Factor (SPF) Nanoemulgel Ekstrak Daun Keji Beling dan Kelor. *JURNAL BIOSHELL*, *12*(2), 127–133. https://doi.org/10.56013/bio.v12i2.2380

Anindita, R., Nathalia, D. D., Beandrade, M. U., Putri, I. K., Sari, A. E., & Setyodewi, E. (2024). Uji Nanoemulsi Ecoenzym Terhadap Bakteri Staphylococcus aureus. *Jurnal Ilmiah Biosaintropis (Bioscience-Tropic)*, *9*(2), 47–56. https://doi.org/10.33474/e-jbst.v9i2.553

Annisa, R., Mutiah, R., Yuwono, M., & Hendradi, E. (2023). Nanotechnology Approach-Self-Nanoemulsifying Drug Delivery System (SNEDDS). In *International Journal of Applied Pharmaceutics* (Vol. 15, Issue 4, pp. 12–19). Innovare Academics Sciences Pvt. Ltd. https://doi.org/10.22159/ijap.2023v15i4.47644

Bajaj, S., Singla, D., & Sakhuja, N. (2013). Stability Testing of Pharmaceutical Products. *Journal of Applied Pharmaceutical Science*, *2*(3), 129–138. https://doi.org/www.japsonline.com

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. *Molecules*, *27*(4). https://doi.org/10.3390/molecules27041326

Catalano, A., Ceramella, J., Iacopetta, D., Marra, M., Conforti, F., Lupi, F. R., Gabriele, D., Borges, F., & Sinicropi, M. S. (2024). Aloe vera—An Extensive Review Focused on Recent Studies. In *Foods* (Vol. 13, Issue 13). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/foods13132155

Dewanti, R., Ariyadi, B., Martien, R., & Zuprizal, Z. (2024). Self-nanoemulsifying black soldier fly (Hermetia illucens) oil drug delivery system: Optimization, formulation, and characterization. *Journal of Applied Pharmaceutical Science*, *14*(02), 192–199. https://doi.org/10.7324/JAPS.2024.153961

Donthi, M. R., Munnangi, S. R., Krishna, K. V., Saha, R. N., Singhvi, G., & Dubey, S. K. (2023). Nanoemulgel: A Novel Nano Carrier as a Tool for Topical Drug Delivery. In *Pharmaceutics* (Vol. 15, Issue 1). MDPI. https://doi.org/10.3390/pharmaceutics15010164

Drais, H. K. (2024). Development and Evaluation Essential Oils Nanoemulgel as Human Skin Sanitizer Using Novel Method.

Turkish Journal of Pharmaceutical Sciences, *21*(5), 456–462. https://doi.org/10.4274/tjps.galenos.2023.78006

Fenny, S., & Safitri, I. (2021). *Overview: Application of Carbopol 940 in Gel*.

Galovičová, L., Borotová, P., Vukovic, N. L., Vukic, M., Kunová, S., Hanus, P., Kowalczewski, P. Ł., Bakay, L., & Kačániová, M. (2022). The Potential Use of Citrus aurantifolia L. Essential Oils for Decay Control, Quality Preservation of Agricultural Products, and Anti-Insect Activity. *Agronomy*, *12*(3). https://doi.org/10.3390/agronomy12030735

Gurpreet, & Singh. (2018). Review of Nanoemulsion Formulation and Characterization Techniques. *Indian Journal of Pharmaceutical Sciences*, *80*(5), 781–789. www.ijpsonline.com

Hosny, K. M., Khallaf, R. A., Asfour, H. Z., Rizg, W. Y., Alhakamy, N. A., Sindi, A. M., Alkhalidi, H. M., Abualsunun, W. A., Bakhaidar, R. B., Almehmady, A. M., Abdulaal, W. H., Bakhrebah, M. A., Alsuabeyl, M. S., Kammoun, A. K., Alghaith, A. F., & Alshehri, S. (2021). Development and optimization of cinnamon oil nanoemulgel for solubility enhancement and evaluation of antibacterial, antifungal and analgesic effects against oral microbiota. *Pharmaceutics*, *13*(7). https://doi.org/10.3390/pharmaceutics13071008

Huang, C., Wang, C., Zhang, W., Yang, T., Xia, M., Lei, X., Peng, Y., Wu, Y., Feng, J., Li, D., & Zhang, G. (2022). Preparation, In Vitro and In Vivo Evaluation of Nanoemulsion In Situ Gel for Transnasal Delivery of Traditional Chinese Medicine Volatile Oil from Ligusticum sinense Oliv.cv. Chaxiong. *Molecules*, *27*(21). https://doi.org/10.3390/molecules27217644

Husni, E., Ramadani, S., & Irna Suryani, M. (2021). *Chemical Components and Antibacterial Activity of Essential Oil Extracted from Citrus x aurantifolia Peel*.

Ibrahim, F. M., Shalaby, E. S., El-Liethy, M. A., Abd-Elmaksoud, S., Mohammed, R. S., Shalaby, S. I., Rodrigues, C. V., Pintado, M., & Habbasha, E. S. El. (2024). Formulation and Characterization of Non-Toxic, Antimicrobial, and Alcohol-Free Hand Sanitizer Nanoemulgel Based on Lemon Peel Extract. *Cosmetics*, *11*(2). https://doi.org/10.3390/cosmetics11020059

Jaganjac, M., Tisma, V. S., & Zarkovic, N. (2021). Short Overview of Some Assays for Measuring Antioxidant Activity of Natural Products and Their Relevance in Dermatology. *Molecules*, *26*(2), 738–754. https://doi.org/10.3390/molecules

Li, Y., Liu, S., Zhao, C., Zhang, Z., Nie, D., Tang, W., & Li, Y. (2022). The Chemical Composition and Antibacterial and Antioxidant Activities of Five Citrus Essential Oils. *Molecules*, *27*(20). https://doi.org/10.3390/molecules27207044

Lin, L. Y., Chuang, C. H., Chen, H. C., & Yang, K. M. (2019). Lime (Citrus aurantifolia (Christm.) swingle) essential oils: Volatile compounds, antioxidant capacity, and hypolipidemic effect. *Foods*, *8*(9). https://doi.org/10.3390/foods8090398

Mohammed, I. O., Alrasheid, A. A., & Hussein Ayoub, S. M. (2024). GC-MS Analysis and Study of the Antimicrobial Activity of Citrus paradisi, Citrus aurantifolia, and Citrus sinensis Peel Essential Oils as Hand Sanitizer. *International Journal of Microbiology*, *2024*. https://doi.org/10.1155/2024/4957712

Muñoz, J., Alfaro-Rodríguez, M. C., Prieto-Vargas, P., Lobo, C., & Garcia, M. C. (2023). Preparation of Nanoemulgels Containing Lemon Essential Oil and Pectin: Physical Stability and Rheological Properties. *Applied Sciences (Switzerland)*, *13*(23). https://doi.org/10.3390/app132312662

Omidian, H., Cubeddu, L. X., & Gill, E. J. (2025). Harnessing Nanotechnology to Enhance Essential Oil Applications. In *Molecules* (Vol. 30, Issue 3). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/molecules30030520

Parente, L. M. L., Lino Júnior, R. D. S., Tresvenzol, L. M. F., Vinaud, M. C., De Paula, J. R., & Paulo, N. M. (2012). Wound healing and anti-inflammatory effect in animal models of Calendula officinalis L. growing in Brazil. *Evidence-Based Complementary and Alternative Medicine*, *2012*. https://doi.org/10.1155/2012/375671

Pavoni, L., Perinelli, D. R., Bonacucina, G., Cespi, M., & Palmieri, G. F. (2020a). An overview of micro-and nanoemulsions as vehicles for essential oils: Formulation, preparation and stability. In *Nanomaterials* (Vol. 10, Issue 1). MDPI AG. https://doi.org/10.3390/nano10010135

Pavoni, L., Perinelli, D. R., Bonacucina, G., Cespi, M., & Palmieri, G. F. (2020b). An overview of micro-and nanoemulsions as vehicles for essential oils: Formulation, preparation and stability. In *Nanomaterials* (Vol. 10, Issue 1). MDPI AG. https://doi.org/10.3390/nano10010135

Sevinç-Özakar, R., Seyret, E., Özakar, E., & Adıgüzel, M. C. (2022). Nanoemulsion-Based Hydrogels and Organogels Containing Propolis and Dexpanthenol: Preparation, Characterization, and Comparative Evaluation of Stability, Antimicrobial, and Cytotoxic Properties. *Gels*, *8*(9). https://doi.org/10.3390/gels8090578

Shahane, K., Kshirsagar, M., Tambe, S., Jain, D., Rout, S., Ferreira, M. K. M., Mali, S., Amin, P., Srivastav, P. P., Cruz, J., & Lima, R. R. (2023). An Updated Review on the Multifaceted Therapeutic Potential of Calendula officinalis L. In *Pharmaceuticals* (Vol. 16, Issue 4). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/ph16040611

Sommatis, S., Capillo, M. C., Maccario, C., Rauso, R., D'Este, E., Herrera, M., Castiglioni, M., Mocchi, R., & Zerbinati, N. (2023). Antimicrobial Efficacy Assessment and Rheological Investigation of Two Different Hand Sanitizers Compared with the Standard Reference WHO Formulation 1. *Gels*, *9*(2). https://doi.org/10.3390/gels9020108

Taiyeb, M., Hartati, H., Arwansyah, A., Muis, A., Mu, A., Rahman, A., & Salleh, L. (2024). Informatics in Medicine Unlocked Self-Nanoemulsifying Drug Delivery System (SNEDDS) formulation and molecular docking of mahogany seed extract (Swietenia mahagoni) as. *Informatics in Medicine Unlocked*, *47*(May), 101517. https://doi.org/10.1016/j.imu.2024.101517

Tarik Alhamdany, A., Saeed, A. M. H., & Alaayedi, M. (2021). Nanoemulsion and Solid Nanoemulsion for Improving Oral Delivery of a Breast Cancer Drug: Formulation, Evaluation, and a Comparison Study. *Saudi Pharmaceutical Journal*, *29*(11), 1278–1288. https://doi.org/10.1016/j.jsps.2021.09.016

United Nations Children's Fund (UNICEF) and World Health Organisation. (2021). *State of the World's Hand Hygiene: A Global Call to Action to Make Hand Hygiene a Priority in Policy and Practice*. World Health Organization.

Xu, F., Shi, Y., Li, B., Liu, C., Zhang, Y., & Zhong, J. (2024). Characterization, Stability and Antioxidant Activity of Vanilla Nano-Emulsion and Its Complex Essential Oil. *Foods*, *13*(5). https://doi.org/10.3390/foods13050801