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Synergistic modification of polyvinyl alcohol and natural phospholipids: Nanoliposomal carrier for *Sonneratia caseolaris* L. delivery and therapeutic care

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ABSTRACT

The synergistic modification of the basic formulation for delivering natural active compounds aims to enhance stability, prevent degradation, and potentially influence their interaction with skin cells. Nanoliposomes are an effective delivery system, capable of preserving the integrity of the compounds and supporting targeted skin distribution. The system's effectiveness will be evaluated for its antioxidant and anti-acne properties using the ethyl acetate fraction of *Sonneratia caseolaris* L. leaf extract. Liposomes were prepared by thin-layer hydration and then reduced and uniformed in size by ultrasonic and mini extruder into nanoliposome preparations. The preparation was evaluated descriptively and statistically based on all physical characteristics and stability tests of NANO-SERF-SCs preparation. The NANO-SERF-SCs produced were categorized as a small unilamellar vesicle with a particle size of 199.7 nm ± 3.61 with an even particle size distribution and spherical shape. The encapsulation efficiency obtained was 76.74% ± 2.80%. NANO-SERF-SCs were also stable under freeze-thaw cycle conditions, 25 times dilution, and 30 days of storage, with acceptable physical characteristics. The results of antioxidant testing using DPPH and ABTS methods show that NANO-SERF-SCs belong to the class of strong antioxidants with IC_{50} values close to the IC_{50} value of each positive control used. The results of the antimicrobial test using disc-diffusion showed that the NANO-SERF-SCs have anti-acne effectiveness that falls into the strong category because it has inhibition in the 10–20 mm range. The ethyl acetate fraction of *S. caseolaris* L. leaf extract can be formulated into a nanoliposome preparation and has good antioxidant and anti-acne activities.

1. INTRODUCTION

Acne scars occur due to the inflammatory reaction of acne that causes blood vessels to dilate. However, the dilated blood vessels sometimes cannot disappear when the inflammatory response has subsided. As a result, acne scars can be challenging to remove, annoying, and lower self-confidence [1]. Medical treatment of acne and acne scars is usually adequate. Still, it has some drawbacks, such as side effects, time required for optimal results, allergic reactions or sensitivities, routine use of topical medications, not always suitable for all types of acne, symptomatic treatments, and acne scars that do not disappear completely [2]. Mangroves are unique wetland ecosystems, one of which is (*Sonneratia caseolaris* L.) or the people of Kalimantan, usually called the Rambai Sungai plant. The leaves of Rambai Sungai have chemical content such as steroids, triterpenoids, saponins, flavonoids, tannins, and alkaloids, which are often processed by the community as traditional medicine made in the form of cold powder and are believed by the community for generations to overcome acne and acne scars [3]. However, the direct use of natural ingredients has limitations, which often fail in the clinical phase due to low bioavailability [4].

Nanotechnology-based drug delivery systems can incorporate different types of drugs into nano-carrier systems,

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increasing their solubility and stability and improving the pharmacokinetic profile of plant-derived drug molecules [5–7]. The non-toxicity of phospholipids and the ability to encapsulate different compounds, such as hydrophilic, lipophilic, and amphiphilic, make liposomes a promising option for better skin drug delivery [8–9]. However, most reports on conventional liposomes describe the local effect as the accumulation of vesicles in the stratum corneum or upper epidermis layer. To overcome this limitation, nanoparticle technology can promote absorption beyond the stratum corneum or epidermis [9]. This can increase their bioavailability using novel lipid vesicles with apparent membrane elasticity, such as flexible and elastic deformable liposomes and liposomes containing propylene glycol or polysorbate [10].

Microbes characterized by resistance to various drugs are a growing source of concern. This is due to the indiscriminate use of antibiotics to control a disease, as well as the increasingly limited use of synthetic antioxidants due to their toxicity and health risks. Therefore, discovering new antioxidant and antimicrobial agents of natural origin is urgent, and plants can be a good source. This study aims to develop and characterize the NANO-SERF-SCs: Nanoliposomal for the ethyl acetate fraction of *S. caseolaris* L. leaf extract delivery and care. The formulation incorporates polysorbate 20 along with synthetic and natural phospholipids encapsulated within polyvinyl alcohol (PVA) to enhance liposomal stability Figure 1. Particle size was subsequently reduced and homogenized using an ultrasonic probe and mini-extruder. This modification enables more effective and efficient delivery of small molecules with high sensitivity and low toxicity, capable of overcoming disruptions in the lipid bilayer of the stratum corneum through interactions between nanoliposomal vesicles and the skin's elastic components, thereby facilitating improved permeation pathways. According to Hua [11], conventional liposomes typically accumulate only in the upper layers of the skin. Additionally, Cao *et al.* [12] reported that conventional liposomes, composed solely of pure lipid layers, exhibit limited colloidal stability and particle size distribution. Therefore, the present formulation enhances liposomal membrane elasticity, allowing deeper penetration into the skin layers to target pustules, thereby increasing the bioavailability and efficacy of the active compounds. Furthermore, the use of mangrove extract, rich in bioactive compounds, offers a natural alternative for treating acne scars with a lower potential for side effects compared to conventional therapies. Stabilized by PVA and supplemented with polysorbate 20, this delivery system achieves greater stability and efficiency, presenting a novel nano-based approach utilizing natural materials that can improve clinical outcomes in acne scar treatment. This research is expected to provide new insights into the design of delivery systems for *S. caseolaris* L. extract, while simultaneously enhancing the value of local cultivation to improve national competitiveness.

2. MATERIALS AND METHODS

2.1. Materials

The plant material used in this study was the leaf of *S. caseolaris* L. (commonly known as Rambai Sungai), collected from the coast of the Kendilo River, Tanah Grogot Sub-district, Paser District, East Kalimantan, Indonesia. Lipoid Ultraspheres® (a finely dispersed emulsion formulated with vitamins A, C, and E, using non-genetically modified soybean phosphatidylcholine as the base material) and Lipoid Phytosolve® (a finely dispersed emulsion formulated with vitamin E and utilizing phospholipids from non-genetically modified soybeans as the primary raw material source) were obtained from Lipoid GmbH, a phospholipid and lipid-based

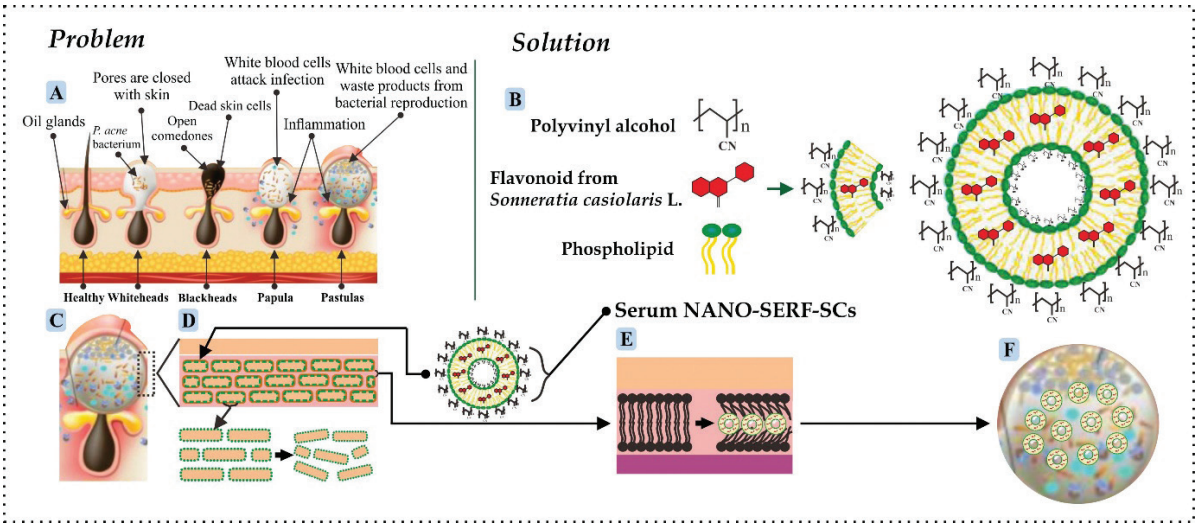

Figure 1. Modification of the delivery system and possible mechanisms for the delivery of NANO-SERF-SCs preparations into the skin membrane. (A) Mechanism of acne growth. (B) NANO-SERF-SCs design prepared in this study. (C) Formation of Pastula, a place for white blood cells and waste products from bacterial reproduction. (D) The NANO-SERF-SCs sediment enters through the tough skin membrane. (E) Overcoming the disruption of the lipid bilayer in the stratum corneum by the interaction of nanoliposome vesicles with elastic constituents and the stratum corneum, resulting in the opening of a pathway for enhanced permeation. (F) NANO-SERF-SCs sediment penetrates the target within the pastulas.

ingredient manufacturer for the pharmaceutical and cosmetic industries, based in Ludwigshafen, Germany. Other analytical-grade chemicals used in this study included: ascorbic acid (CAS No. 50-81-7), ethanol (CAS No. 64-17-5), potassium persulfate (CAS No. 7727-21-1), chloroform (CAS No. 67-66-3), methanol (CAS No. 67-56-1), blood agar, nutrient agar, nutrient broth, and polysorbate 20 (CAS No. 9005-64-5), all purchased from Merck, a pharmaceutical and chemical company headquartered in Darmstadt, Germany. Polyvinyl alcohol (PVA, CAS No. 9002-89-5), Trolox (CAS No. 53188-07-1), 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, CAS No. 30931-67-0), and 2,2-diphenyl-1-picrylhydrazyl (DPPH, CAS No. 1898-66-4) were obtained from Sigma-Aldrich, a research chemicals and reagents company based in St. Louis, Missouri, USA. Phosphate-buffered saline (PBS, pH 7.4) was obtained from Oxoid, a supplier of reagents for clinical and industrial laboratories based in Basingstoke, UK. Sterile 96-well plates (Biologix 07-6096) were procured from Biologix, a laboratory equipment company based in Lenexa, Kansas, USA. The bacterial strain *Staphylococcus epidermidis* Food and Nutrition Culture Collection (FNCC) 0048 was obtained from the FNCC, Gadjah Mada University, Indonesia. The strain was cultured on nutrient Agar (NA) or Tryptic Soy Agar (TSA) under aerobic conditions at 35°C–37°C for 18–24 hours. *Propionibacterium acnes* American type culture collection (ATCC) 11827 was acquired from the ATCC and cultured on reinforced clostridial medium, Brucella blood agar, or anaerobic blood agar under anaerobic conditions at 37°C for 48–72 hours. *Staphylococcus aureus* ATCC 25923, also obtained from ATCC, was cultured on Mannitol salt agar, Mueller–Hinton agar, NA, or TSA under aerobic conditions at 35°C–37°C for 18–24 hours. All bacterial strains were supplied by IndiLab, a biotechnology company based in Surabaya, East Java, Indonesia.

2.2. Ethyl acetate fraction of *S. caseolaris* L. leaf extraction

Five hundred grams of dry powder of *S. caseolaris* L. leaves were macerated using 5 l of 70% ethanol and ethyl acetate (2:1) for 24 hours (a combination of three extractions). The filtrate resulting from the extract was then evaporated using a rotary evaporator. The evaporated extract was put into an oven at 40ºC until a thick extract was obtained [3]. A total of 10 g of concentrated extract from the maceration process was dissolved in 100 ml of distilled water. The solution was then partitioned by adding 100 ml of n-hexane, shaken in a separatory funnel, and allowed to stand until there were two layers (distilled water at the bottom and n-hexane at the top). The two layers formed were then separated, and the n-hexane layer was taken. Adding n-hexane solvent formed in the water is repeated until the n-hexane becomes clear. The distilled water layer was then fractionated again using an ethyl acetate solvent. The fractionation results were evaporated using a rotary evaporator at 40°C until a concentrated extract was obtained [13].

2.3. Qualitative analysis of ethyl acetate fraction content of *S. caseolaris* L. leaf extraction by tube preaction method

Phytochemical testing aims to identify bioactive compounds contained in the extract. To detect the presence of alkaloids, 1 mg of extract was added to a test tube and treated with 2 drops of Bouchardat reagent; the formation of a brown–black colour indicates a positive result for alkaloids [14]. In a separate test, 1 mg of extract was mixed with 2 drops of Dragendorff reagent, and the appearance of a red to orange precipitate also indicates the presence of alkaloids [15]. Another confirmation test was conducted by adding 2 drops of Wagner's reagent to 1 mg of extract, where the formation of a brown precipitate further supports the presence of alkaloid compounds [15]. For the polyphenol test, 2 drops of $FeCl_3$ solution were added to a test tube containing 1 mg of extract. The formation of a blue–black colour indicates the presence of polyphenols [16]. The flavonoid test was carried out by mixing 1 mg of extract with magnesium powder, then adding 1 drop of HCl 2N and 70% ethanol, shaken until mixed, and left until the solution separated. If a reddish–yellow colour and precipitate appear, this indicates the presence of flavonoids [14]. In the saponin test, 1 mg of extract is mixed with enough hot water, and then 1 drop of HCl 2N is added. The presence of stable foam indicates the content of saponins [17]. The tannin test is carried out with a procedure similar to the polyphenol test: adding 2 drops of $FeCl_3$ solution into a test tube containing 1 mg of extract. The formation of a dark blue–black colour indicates the presence of tannins [14]. The quinone test is carried out by adding 2 drops of 5% KOH solution into a test tube containing 1 mg of extract. If the colour changes to reddish yellow, this indicates the presence of quinones [17]. The steroid test uses 2 drops of Liebermann–Bouchard reagent, and a greenish colour change indicates the presence of steroids [14]. The terpenoid test is carried out by dripping 1 mg of extract into Liebermann–Bouchard reagent. If a purple or orange colour forms, this indicates the presence of triterpenoids [15].

2.4. Preparation of NANO-SERF-SCs

The thin-layer hydration method is the simplest method for preparing liposomes. This method dissolves the lipids in volatile solvents such as chloroform and methanol [9]. The ingredients in phase 1 were homogenized and treated using a rotary evaporator to evaporate the Table 1 solvent. After the sample was seen to form a thin layer on the wall of the round-bottom flask, the sample was incubated at room temperature to remove all the solvent remaining on the sample for 30 minutes. The sample is back in the rotary evaporator for 30 minutes with the exact mechanism without being conditioned using a vacuum, and adding the ingredients in phase 2 Table 1. After the liposome preparation is formed, it is put into the refrigerator at 2°C–8°C for 10–15 minutes. Next, the suspension of the formed liposomes was down-sized using ultrasonic (130 W, 20 kHz, USA) at 70% power in an ice bath for 5 minutes (3 cycles of 2 minutes sonication and 3 minutes rest to allow cooling of the sample), and finally uniformed the particle size using a 0.1 µm polycarbonate membrane mini extruder (3 cycles). The nanoliposome preparation formed was purified using SPE (solid phase extraction). Samples taken as much as 3 ml were placed in the tube solutions within and then flowed through the 0.2 µm polycarbonate membrane assisted by the SPE push, and further characterization was carried out Figure 2 [9,18,19].

2.5. Evaluation of physical characteristics of NANO-SERF-SCs

An organoleptic test was conducted to visually observe the sample based on its color, shape, and odor. The sample was placed into a 100 ml beaker and evaluated using human sensory perception [20]. Particle size, polydispersity index value, and potential zeta value were observed using Malvern Panalytical's PSA (Particle size analyzer) – Zetasizer Pro. One millimetre of the sample was put into the cuvette, then into the PSA holder, and then observed for particle size and polydispersity index value with three readings. As for the zeta potential value, the sample was put into a special zeta cuvette, put into the PSA holder, and then observed with three readings. Morphological nanoliposome observation was carried out using Transmission electron microscopy (TEM) JEOLJEM-1010. Five hundred microliters of sample solution were placed on a grid of electrical mesh, absorbed using filter paper with the help of a vacuum, and then observed [9,8,21,22]. Evaluation of the encapsulation efficiency of ethyl acetate fraction nanoliposomes from *S. caseolaris* L. leaf extract with flavonoid content in NANO-SERF-SCs was performed by centrifuging 2 ml sample aliquots at 5,000 rpm for 30 minutes at 4°C to separate the supernatant from the precipitated nanoliposomes [9]. Then, 1 ml of the sample was dissolved in 10 ml of ethanol to obtain a concentration of 1,500 ppm. From the solution, 1 ml was pipetted, and added 1 ml of 2% $AlCl_3$ solution and 1 ml of 120 mM potassium acetate. The sample was then incubated for 1 hour at room temperature [23]. After method validation, analysis was performed using a UV/VIS Spectrophotometer to measure the amount of encapsulated and non-encapsulated flavonoids. The absorption spectra were measured in the

Table 1. Formulation of NANO-SERF-SCs.

Ingredient	Quantity
Ethyl acetate fraction of *S. caseolaris* L. leaf extract.	200 mg
Lipoid Ultraspheres®	2 ml
Lipoid Phytosolve®	1 ml
Polyvinyl Alcohol	2 ml
Polysorbate 2	1 ml
Chloroform	5 ml
Methanol	5 ml
PBS (pH 7.4)	20 ml

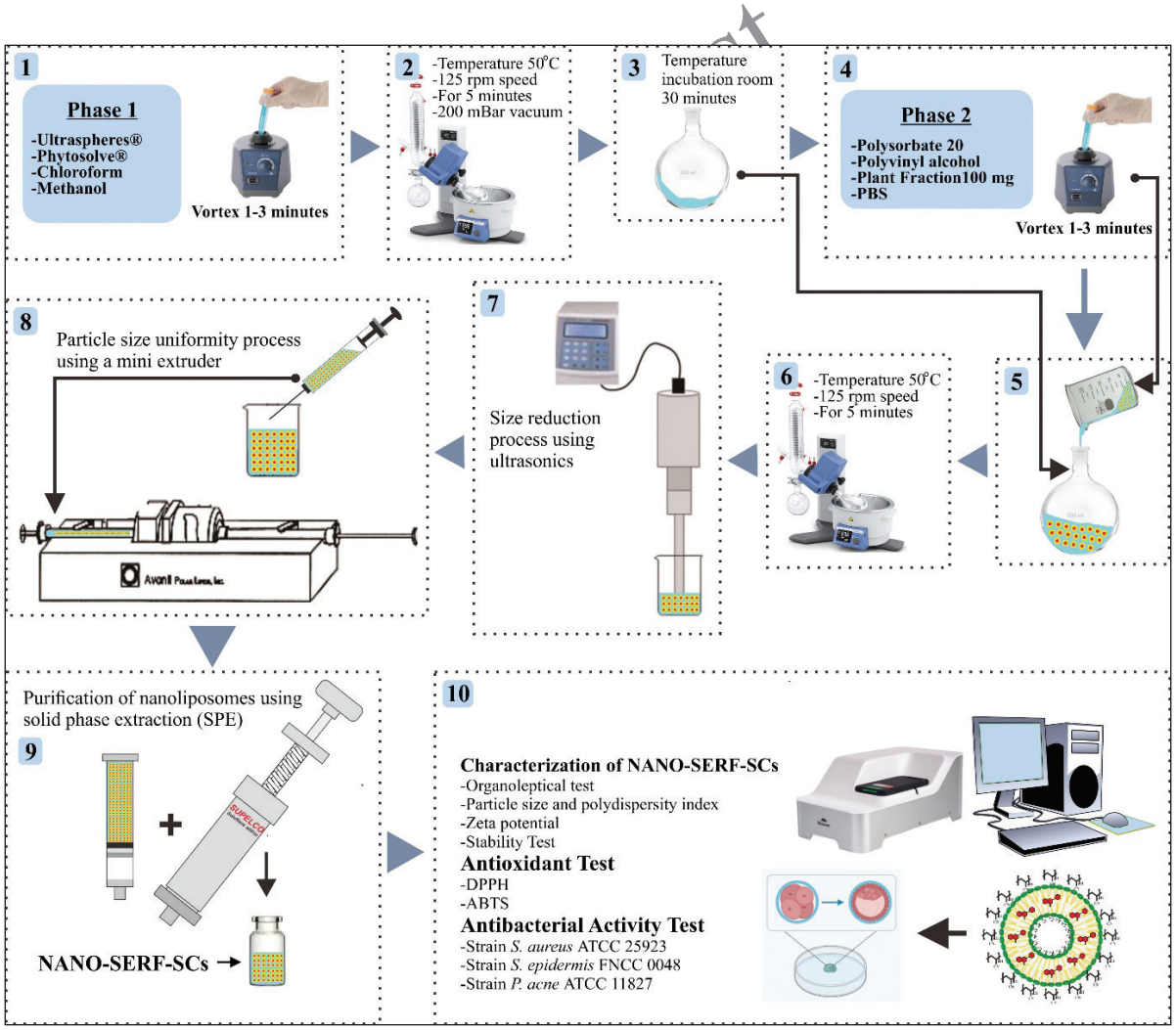

Figure 2. Preparation of ethyl acetate fraction nanoliposomes from *S. caseolaris* L. leaf extract in this study [9].

400–450 nm range, with the maximum wavelength at 435 nm. Encapsulation efficiency was calculated as the percentage of alkaloids encapsulated in the NANO-SERF-SCs using the equation:

$$EE\ (\%) = \frac{Total\ drug - Free\ drug\ in\ supernatant}{Total\ drug} \times 100$$ [9]

2.6. Stability test of NANO-SERF-SCs

The freeze-thaw cycle test was conducted by storing the NANO-SERF-SCs in a refrigerator at −20°C for 8 hours, then transferring them to room temperature (25°C) for 8 hours. This process was repeated for 6 cycles in 2 days. The evaluation was done to observe changes in particle size, polydispersity index, zeta potential, and encapsulation efficiency. To test durability, 1 ml of the sample was dissolved with PBS solvent (pH 7.4) in the ratios of 25, 50, 100, and 250 times, and then each was evaluated with the same parameters. The NANO-SERF-SCs preparation was sealed in a trilaminate bag and stored in a refrigerator at 2°C–8°C for 1 month. Every week, the samples were evaluated with the same parameters to detect any changes in the characteristics of the preparation [9].

2.7. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) antioxidant assay

Forty microliters of NANO-SERF-SCs were added into 40 µl of DPPH methanol solution (0.1 mM) in a 96-well plate. The mixture was shaken vigorously and incubated at 25°C for 30 minutes, and the absorbance was measured at 517 nm. The DPPH radical scavenging activity of the preparation was expressed as mg ascorbic acid equivalent per g (mg AAE/g dw) of a sample using the standard equation, plotted at various concentrations of NANO-SERF-SCs 100.00, 50.00, 25.00, 12.50, 6.25, and 3.13 ppm, whereas ascorbic acid 6.00, 5.00, 4.00, 3.00, 2.00, and 1.00 ppm [24].

2.8. 2,20-Azino-bis-3ethylbenzothiazoline-6-sulfonic Acid (ABTS) radical scavenging assay

Five millilitres of ABTS solution (7 mmol/l) was mixed with 88 µl of 140 mM potassium persulfate solution to produce ABTS+. The mixture was placed in the dark at room temperature for 16 hours. Then, the prepared ABTS+ solution was diluted with analytical-grade ethanol to obtain an initial absorbance of 0.7 at 734 nm. Then, 10 µl of NANO-SERF-SCs or standard was mixed with 290 µl of the diluted ABTS solution in a 96-well plate and incubated at room temperature for 6 minutes in the dark. Then, the absorbance was measured at 734 nm. The antioxidant ability was expressed as mg Trolox equivalent per g (mg AAE/g dw) of a sample using the standard equation, plotted at various NANO-SERF-SCs concentrations of 1,000.00, 500.00, 250.00, 125.00, 62.50, and 31.25 ppm. While Trolox 250.00, 125.00, 62.50, 31.25, 15.63, and 7.81 ppm [24].

2.9.Antibacterial activity test of NANO-SERF-SCs

Prior to the antibacterial assay, all equipment, including pipettes, spreaders, test tubes, and Petri dishes were sterilized using an autoclave at 121°C for 15–20 minutes. NA, NB, and Blood Agar media were prepared according to standard compositions and sterilized using the same method. After sterilization, the liquid media were cooled to approximately 45°C–50°C, poured into sterile Petri dishes, and allowed to solidify inside a laminar air flow cabinet to maintain aseptic conditions. Subsequently, *Staphylococcus epidermidis* FNCC 0048 and *S. aureus* ATCC 25923 were subcultured by inoculating colonies from frozen stocks onto Nutrient Agar and incubated aerobically at 35°C–37°C for 18–24 hours. For *P. acnes* ATCC 11827, subculturing was performed on Blood Agar and incubated in an anaerobic box at 37°C for 48–72 hours to maintain an oxygen-free environment. Following incubation, bacterial colonies were suspended in Nutrient Broth or sterile physiological saline, homogenized, and adjusted to match the turbidity of a 0.5 McFarland standard to ensure a uniform bacterial cell density. These bacterial suspensions were then evenly spread over the surface of Nutrient Agar (for *S. epidermidis* and *S. aureus*) or Blood Agar (for *P. acnes*) using sterile spreaders inside the Laminar Air Flow cabinet. Once the media surfaces were absorbed, sterile paper discs containing methanol as a negative control, chloramphenicol at 2 mg/ml as a positive control, and the NANO-SERF-SCs formulations at concentrations of 1 ppm, 5 ppm, and 10 ppm were placed separately onto the agar surfaces. The Petri dishes were subsequently incubated under appropriate conditions: aerobically at 35°C–37°C for 18–24 hours for *S. epidermidis* and *S. aureus*, and anaerobically at 37°C for 48–72 hours for *P. acnes*. After incubation, the inhibition zones formed around the discs were measured automatically using a Scan-500 device to evaluate the antibacterial efficacy of the tested formulations [21,25,26].

3. RESULTS AND DISCUSSION

3.1. Results of qualitative analysis of ethyl acetate fraction content of *Sonneratia caseolaris* L. leaf extraction

The results obtained from the extract of *S. caseolaris* L. leaves from 252.54 grams of dry powder of *S. caseolaris* L. leaves macerated using 5 l of 70% ethanol and ethyl acetate as much as (2: 1) obtained a thick extract weighing 35.93 g with a percentage yield of 14.22%. The extract yield is calculated by finding the weight percentage (b / b). The percentage yield of a good thick extract is at least 10%.

- Weight of dry powder : 252,54 g
- Weight of condensed extract : 24,10 g

$$\%\ Yields = \frac{Weight\ of\ Condensed\ Extract}{Sample\ Weight} \times 100\ \%$$

$$= \frac{35{,}93\ g}{252{,}54\ g} \times 100\ \%$$

$$= 14{,}22\ \%$$

The results of the fractionation of *S. caseolaris* L. leaf extract with the split funnel method obtained a thick fraction weighing 1.97 g. The calculation data of the resulting thick fraction is as follows:

- Weight of condensed extract : 5 g
- Weight of fraction : 1,97 g

$$\% \text{ Faction} = \frac{\textit{Weight of Condensed Faction}}{\textit{Sample Weight}} \times 100\ \%$$

$$= \frac{1.97\ \text{g}}{5\ \text{g}} \times 100\ \%$$

$$= 39{,}4\ \%$$

The results obtained in the qualitative analysis of the ethyl acetate fraction of *Sonneratia caseolaris* L leaf extract Figure 3, Hasil skrining fitokimia terhadap fraksi etil asetat ekstrak daun *S. caseolaris* L. menunjukkan keberadaan berbagai golongan senyawa metabolit sekunder yang ditandai dengan perubahan warna maupun pembentukan endapan sebagai respons terhadap reagen tertentu Table 2.

Alkaloid testing using Bouchardat's reagent yielded a positive result, indicated by a dark brown color change, suggesting the presence of alkaloid compounds in the fraction. Dragendorff's reagent also produced a positive result, evidenced by the formation of an orange precipitate, further confirming the presence of alkaloids. However, Wagner's reagent test gave a negative result, as no precipitate formed and the solution appeared turbid with a yellowish–orange color, indicating that alkaloids were not detected with this method possibly due to differences in reagent sensitivity. The polyphenol test using $FeCl_3$ solution resulted in a dark bluish–black color change, a positive indicator of polyphenolic compounds. Flavonoids were also detected through the use of magnesium powder combined with 2N HCl and 70% ethanol, which led to the appearance of a yellow color and the formation of a residue, indicating a positive reaction. A positive response was likewise observed in the saponin test using 2N HCl, marked by the formation of stable froth, a characteristic feature of saponins. Meanwhile, the tannin test using $FeCl_3$ showed a deep black coloration, indicating the presence of tannins. Testing for quinones with 5% KOH revealed a color change from yellow to red, which is a typical indicator of quinone compounds. Additionally, steroid and terpenoid detection using the Liebermann–Burchard reagent showed positive results, indicated by greenish and brownish color changes, respectively. Overall, these qualitative phytochemical screening results demonstrate that the ethyl acetate fraction of *S. caseolaris* L. leaf extract contains various bioactive compounds, including alkaloids, polyphenols, flavonoids, saponins, tannins, quinones, steroids, and terpenoids. The diversity of these compounds supports the pharmacological potential of this plant as a natural source for the development of herbal medicines [14–17].

3.2. Results NANO-SERF-SCs physical characteristics test

The organoleptic test results obtained from the NANO-SERF-SCs formulation showed a greenish–yellow color resembling milk with a faint fatty odor, a slightly thick viscosity, and no phase separation or color irregularities after 24 hours of storage Figure 4A. These characteristics indicate good initial physical stability of the formulation. This stability is supported by the combination of PVA and natural phospholipids, which theoretically contribute to the formation of stronger, more uniform nanoliposomal structures capable of effectively encapsulating active compounds. Although this interaction is supported by the literature and appears to enhance encapsulation efficiency and particle stability in the current formulation, it is important to note that this study does not include a direct quantitative comparison with control formulations, such as liposomes without PVA or those containing only one type of phospholipid. Therefore, these findings should be regarded as an initial exploration of a combination-based formulation strategy. Further comparative studies are needed to validate the

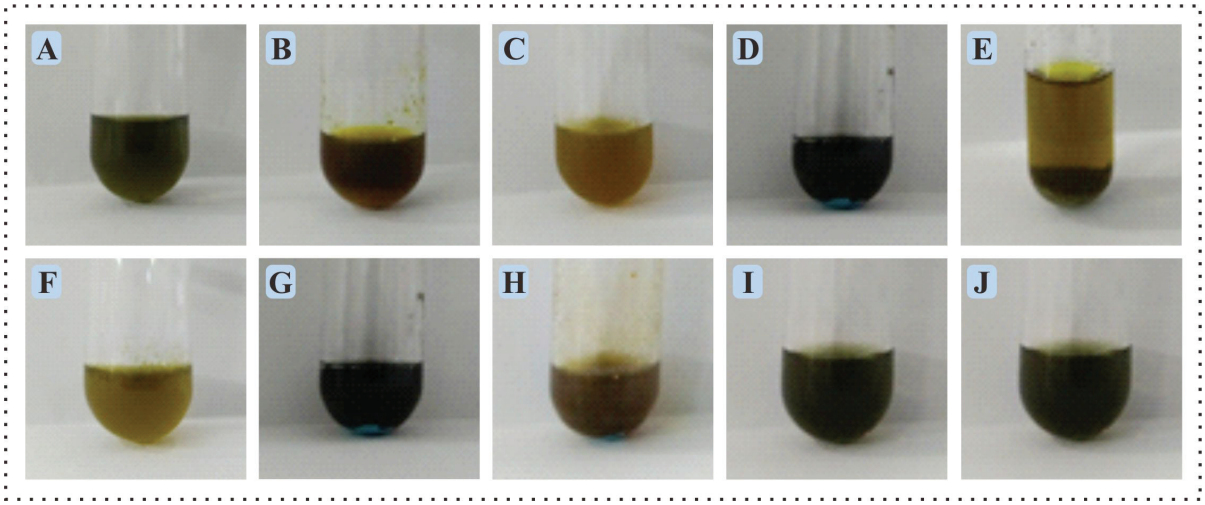

Figure 3. The results obtained in the qualitative analysis of ethyl acetate fraction of *S. caseolaris* L. leaf extract. (A) Alkaloid test with Bouchard at reagent. (B) Alkaloid test with dragendrof reagent. (C) Alkaloid test with Wagner reagent. (D) Polyphenol test with FeCl3 reagent. (E) Flavonoid test with Magnesium powder + 2N HCl + 70% ethanol. (F) Saponin test with 2N HCl reagent. (G) Tannin test with FeCl3 reagent. (H) Quinone test with 5% KOH reagent. (I) Steroid test with Liberman bouchard reagent. (J) Terpenoid assay with Liberman bouchard reagent.

contribution of each component to the overall performance of this delivery system.

The particle size measurement results of the NANO-SERF-SCs preparation in Table 3 show good results and are by the good nanoliposome size range of 100–300 nm [9]. NANO-SERF-SCs was formed based on a manipulation technique involving a combination of thin-layer hydration method and bottom-up technique, which is the manipulation of the formation of phospholipids into a series of liposome matrices that are further reduced using ultrasonic and uniformed in size into nanoliposomes by passing the liposome suspension through a polycarbonate membrane with a size of 0.2 µm with the help of a mini extruder [9]. This explains the formation of natural phospholipid particles assembled into a liposome matrix, which is then reduced and homogenized into nanoliposomes by ultrasound and extrusion as a protective matrix and carrier encapsulating the ethyl acetate fraction of *S. caseolaris* L. leaf extract [8,9].

Table 2. Results of qualitative analysis of ethyl acetate fraction content of *S caseolaris* L. leaf extraction by tube preaction method.

Golongan Senyawa	Reagen yang Digunakan	Hasil Uji
Alkaloid	Bouchardat	Positif
	Dragendorff	Positif
	Wagner	Negatif
Polifenol	$FeCl_3$	Positif
Flavonoid	Serbuk magnesium + HCl 2N + etanol 70%	Positif
Saponin	HCl 2N	Positif
Tanin	$FeCl_3$	Positif
Kuinon	KOH 5%	Positif
Steroid	Liebermann-Burchard	Positif
Terpenoid	Liebermann-Burchard	Positif

The particle size distribution of NANO-SERF-SCs can be seen in Figure 4B, illustrating that the resulting NANO-SERF-SCs composition has a uniform particle size and shows an even distribution of particles. The uniform particle distribution results are evidenced by the particle deformation observed since the beginning of peak formation at each test repetition. At the same time, the even distribution of particles is confirmed by the polydispersity index value obtained with a result of 0.170 PDI ± 0.01. A good polydispersity index value indicates long-term stability and particle size distribution in the formulation. The polydispersity index represents the particle size distribution, where the polydispersity index value ranges from 0 (for a very uniform particle size sample) to 1 (for a highly polydispersed sample with many particle size populations) [9].

The zeta potential value of NANO-SERF-SCs was −11.56 mV ± 0.5 Table 3. The negative value indicates that the nanoliposomes have a negative charge on their surface. This charge comes from the ethyl acetate fraction component, which may contain polar compounds or specific ions bound to the nanoliposome surface [27]. The values obtained indicate that the nanoliposomes have sufficient negative charge to provide some

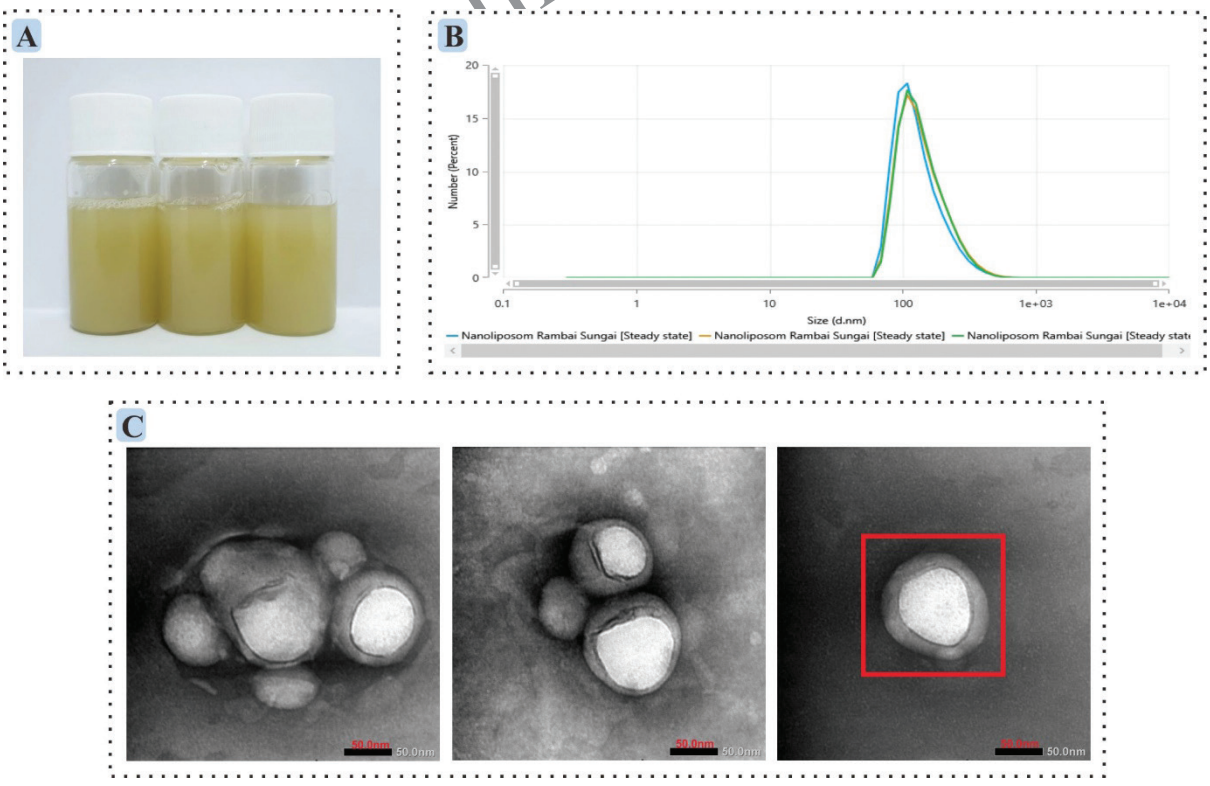

Figure 4. Evaluation of physical attributes of NANO-SERF-SCs. (A) Preparation image of NANO-SERF-SCs. (B) Particle size distribution graph with three readings. (C) morphology test preparation observed using TEM.

Table 3. Results of particle size, polydispersity index and zeta potential.

	Particle size (nm)	Polydispersity index (PDI)	Zeta potential (mV)
Test results	199.7 ± 3.61	0.170 ± 0.01	-11.56 ± 0.5

Description: Average result of 3 replications ± Standard deviation.

stability, but may not be high enough to prevent accumulation completely [28]. In general, zeta potentials lower than ±30 mV indicate that the system tends to be less stable because the attractive forces between particles are weaker, so aggregation is possible. In this case, the value of −11.56 mV indicates that the nanoliposomes have moderate stability, and the particles may have the potential to agglomerate over a long period without additional stabilization [28]. The expected potential zeta value, which should be more damaging than −30 mV or more positive than +30 mV, is considered good electrostatistical stability [9]. Zeta potential can be affected by several factors, such as pH, ion concentration in the solution, and the composition of the active ingredients contained in the extract. This value also indicates the chemical interaction between the components in the extract and the liposomal vesicles [28]. Morphological test results using TEM showed that the observed NANO-SERF-SCs preparation had a spherical globule shape with a stable single-layer structure. The nanoliposome particles did not aggregate, indicating that the nanoliposome formulation was homogeneous and stable. Figure 4C shows some nanoliposomes that appear denser or more compressed but retain their spherical shape. This could indicate the stability and formation of the desired structure in the nanoliposome formulation [29]. These results also confirm the shape of NANO-SERF-SCs with SUV (Small unilamellar vesicle) type with a size range of 20–200 nm, which is by the particle size results obtained, which is 199.7 ± 3.61; this is in accordance with the desired size for pharmaceutical or biotechnological applications [30]. The absence of aggregation between particles indicates good stability, which is essential in avoiding the formation of large particles that can reduce the efficiency and control of active ingredient release [31]. This spherical and monolayer structure provides advantages in applications of controlled drug delivery, gene therapy, and other products as it allows for more even distribution in biological systems [32]. Overall, these TEM results show that the nanoliposome manufacturing process has been performed well.

The results of the encapsulation efficiency test on the NANO-SERF-SCs preparation that encapsulates the ethyl acetate fraction of *S. caseolaris* L. leaf extract with flavonoid content were determined using the UV-Vis spectrophotometric method. As a comparison, quercetin was used, which was previously tested by running a quercetin solution at a wavelength range of 400–450 nm. As a result, the maximum wavelength of quercetin was 435 nm. The standard curve obtained showed the equation y = 0.0534x—0.0909 with a value of R^2 = 0.9982. Based on the data recorded in Table 4, about 76.74% ± 2.80% of flavonoids were successfully encapsulated in the nanoliposomes. The concentration of flavonoids in the nanoliposome sample

Table 4. Encapsulation efficiency test results on NANO-SERF-SCs preparations that encapsulate the ethyl acetate fraction of *S. caseolaris* L. leaf extract with flavonoid content.

Sampel	Consentration in sample	Concentration in supernatant	EE (%)
NANO-SERF-SCs	0.107 ± 0.004	0.025 ± 0.003	76.74 ± 2.80

Description: Average result of 3 replications ± Standard deviation.

was recorded as 0.107 ± 0.004, while the concentration of unencapsulated flavonoids in the supernatant was 0.025 ± 0.003. The encapsulation efficiency was calculated by comparing the flavonoid concentration in the nanoliposome sample with the total amount of flavonoids (including those encapsulated and those in the supernatant), resulting in an encapsulation efficiency (EE) value of 76.74% [9]. These results indicate that most of the flavonoids were successfully retained in the nanoliposomes, indicating that this nanoliposome formulation is effective in encapsulating flavonoids and has potential for applications in the pharmaceutical and therapeutic fields.

Several important factors can explain the success of flavonoids retained in NANO-SERF-SCs preparations. As a hydrophobic polymer, PVA can form a matrix that can hold active ingredients [33]. At the same time, amphiphilic phospholipids can interact with predominantly hydrophobic flavonoids and form a stable lipid layer [34]. This synergism between PVA and phospholipids creates a solid and stable structure that effectively encapsulates flavonoids. Phospholipids form a lipid layer surrounding the flavonoids [34], while PVA provides additional stability to the matrix and prevents the loss of active ingredients [33]. In addition, flavonoids, as polyphenolic compounds, interact with the hydrophobic part of phospholipids, which causes flavonoids to be trapped in such lipid matrices [35]. PVA also stabilizes by forming a protective layer around the nanoliposomes, keeping the particles stable and reducing agglomeration [33]. This synergistic modification allows the nanoliposome matrix to survive various environmental conditions, such as pH or temperature changes, while retaining the flavonoids inside. Thus, the combination of PVA and phospholipids provides better stability and enhances the ability of nanoliposomes to encapsulate and stabilize flavonoids, making them suitable for pharmaceutical or therapeutic applications.

Furthermore, to comprehensively evaluate the system's ability to retain and deliver flavonoids effectively, it is important to assess the release profile and pharmacokinetic behavior of the encapsulated compounds. A controlled and sustained release profile is desirable to ensure prolonged therapeutic activity and minimize dosing frequency [36]. Based on the physicochemical properties of PVA and phospholipids, the NANO-SERF-SCs system is expected to exhibit a biphasic release pattern—an initial burst release followed by a sustained diffusion-driven release phase. This pattern reflects the gradual release of flavonoids from the stable matrix, indicating that the nanoliposomes can release their contents in a controlled manner under physiological conditions [37]. Additionally, pharmacokinetic evaluation is essential to confirm whether the

encapsulated flavonoids demonstrate improved bioavailability and systemic circulation time compared to free flavonoids. Parameters such as Cmax, Tmax, Area under the curve (AUC), and half-life are critical in this context. The PVA-phospholipid system is expected to provide a protective barrier against enzymatic degradation and harsh gastrointestinal conditions, potentially resulting in higher AUC values and prolonged half-life [38]. Together, these release and pharmacokinetic properties would further affirm the capability of NANO-SERF-SCs not only to encapsulate but also to preserve and optimize the therapeutic potential of flavonoids.

3.3. Results of NANO-SERF-SCs stability test

The freeze-thaw cycle stability test results on the NANO-SERF-SCs preparation showed that the nanoliposome system remained stable despite exposure to extreme temperature changes. The measured particle size of 229.87 nm ± 1.04 nm indicates no significant aggregation or fusion, so the liposome structure is maintained. The polydispersity index value of 0.31 PDI ± 0.01 indicates a relatively homogeneous particle size distribution. This suggests that the formulation retained the particle size without drastically changing after freeze-thaw cycles. This stability can be attributed to the combination of polyvinyl alcohol and natural phospholipids, forming a stable nanoliposome structure. Polyvinyl alcohol, being a hydrophilic polymer, forms a steric layer around the nanoliposome vesicles that prevents coalescence or fusion of particles during freeze-thaw cycles [33]. When the temperature decreases drastically, the aqueous phase in the nanoliposome system can freeze, causing osmotic pressure that can damage the vesicle structure [39]. However, polyvinyl alcohol and phospholipids with stable hydrocarbon chains can maintain the nanoliposome structure so that the particle size is maintained [33–34]. The obtained zeta potential value of −11.43 ± 0.11 mV indicates that the system's stability is more influenced by the steric stabilization mechanism than electrostatic. Although, under ideal conditions, systems with zeta potential above ±30 mV have higher electrostatic stability [28], the stability of this system is maintained despite the relatively low potential zeta value. This is due to the effect of polyvinyl alcohol, which forms a protective layer and reduces inter-particle interactions that could lead to aggregation [33]. Although the electrostatic force is not very high, stability is maintained through the steric mechanism of polyvinyl alcohol [28,33]. The encapsulation efficiency of 68.79% ± 0.99% indicates that the vesicle structure remains intact without leakage of active ingredients despite exposure to extreme temperatures. Natural phospholipids form a stable bilayer that confines the active ingredients within the nanoliposome system. If there is an imbalance in the structure, for example, due to the pressure of ice crystallization, the encapsulation efficiency may decrease [40]. However, the stability of the polyvinyl alcohol-reinforced phospholipid bilayer ensures that the active ingredients are preserved [33–34].

Overall, the post-freeze-thaw cycle stability of NANO-SERF-SCs was achieved thanks to steric stabilization by polyvinyl alcohol, strong phospholipid bilayer, and stable particle size distribution. This combination ensures the formulation remains stable despite its low zeta potential, and the active ingredients are trapped in the nanoliposome vesicles. This makes it suitable for pharmaceutical applications, especially in nanoliposome-based drug delivery.

The results of the dilution resistance stability test on the NANO-SERF-SCs preparation showed that increasing the dilution level significantly impacted particle size, polydispersity index, zeta potential, and encapsulation efficiency. At the initial condition without dilution, the particle size of about 199.7 nm ± 3.61 reflected the stability of the liposomal system. However, with an increase in dilution from 25 to 250 times, the particle size increased significantly, even exceeding 600 nm ($p < 0.0001$) Figure 5A. The initially low polydispersity index indicates a homogeneous particle distribution. Still, starting at 50 times dilution, this index increased significantly ($p < 0.001$) and continued to increase at 100 to 250 times dilution ($p < 0.0001$), indicating increasing particle size heterogeneity. Figure 5B. This decrease in particle size and increase in polydispersity index is likely due to the destabilization of the phospholipid bilayer structure in the nanoliposome system [41]. High dilution reduces the concentration of phospholipids per unit volume, disrupting the hydrophilic–hydrophobic balance [9]. The phospholipids that form the nanoliposome bilayer have a specific stability limit in maintaining the vesicle structure. When too dilute, the number of phospholipid molecules

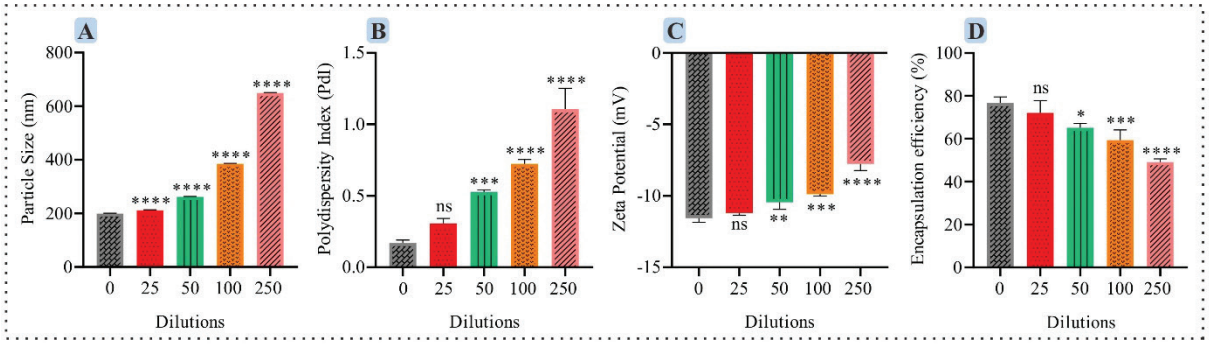

Figure 5. Observation graph of durability stability test of NANO-SERF-SCs against 25, 50, 100, and 250 times dilution. (A) particle size, (B) polydispersity index, and (C) zeta potential. (D) Encapsulation efficiency. Samples were analyzed using One-Way ANOVA with 95% confidence and a post-hoc test to determine significant differences in each group. n: 5; ns: not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

available to maintain membrane integrity decreases, causing nanoliposomes to be prone to bilayer fusion and rupture [9,42]. In addition, weakening the interaction of stabilizers such as PVA also contributes to nanoliposome instability, as PVA forms a protective layer that prevents aggregation [33]. High dilution reduces the concentration of PVA, weakens the steric effect that maintains the distance between particles, and increases coalescence between particles, making it easier for vesicles to collide and combine. This makes the nanoliposomes more susceptible to environmental changes, such as surface tension fluctuations, which further deteriorates their structural stability [33]. Electrostatic stability measured through zeta potential showed significant changes after dilution. At 25 times dilution, there was no significant change, but at 50 times dilution ($p < 0.01$), there was a considerable decrease and further decreased at 100 and 250 times dilution ($p < 0.0001$) Figure 5C. This decrease indicates that the electrostatic forces between particles are weakening. Zeta potential in colloidal systems plays a role in maintaining the repulsive force between particles, which prevents aggregation [9]. At initial conditions, the zeta potential was −11.56 mV ± 0.5, which although low, was still sufficient to maintain the stability of the system through the steric mechanism of PVA [33]. However, after 50 times or more dilution, the zeta potential value becomes increasingly hostile, indicating particle instability and a tendency to agglomerate. This decrease also shows a change in the interaction between phospholipids in the bilayer, which can trigger structural instability [34]. Encapsulation efficiency also decreased with dilution. In the initial condition, the encapsulation efficiency was 76.74% ± 2.80%, indicating the active ingredients were well trapped in the vesicles. However, after 50 times dilution, there was a significant decrease ($p < 0.05$), which was even more drastic at 100 to 250 times dilution ($p < 0.0001$) Figure 5D. This decrease in encapsulation efficiency is caused by the change in osmotic pressure due to high dilution that disrupts the osmotic balance between the internal and external phases, triggering uncontrolled water ingress or egress [43]. This leads to strain on the bilayer membrane, vesicle rupture, and leakage of active ingredients.

Overall, the stability of NANO-SERF-SCs was still good, up to 25 times the dilution level. Still, it began to degrade at 50 times the dilution level and became increasingly unstable with further dilution. The leading cause of this instability is the weakening of the interaction between phospholipids and stabilizers, which leads to an increase in particle size, a decrease in zeta potential, an increase in polydispersity, and leakage of active ingredients.

The 1-month storage stability test results at 2°C–8°C showed that the particle size of the NANO-SERF-SCs preparation remained stable at weeks 1 and 2 without significant changes. However, at week 3, the particle size began to increase significantly ($p < 0.01$), and at week 4, there was a drastic jump ($p < 0.0001$) Figure 6A. The polydispersity index also began to increase significantly from week 1 ($p < 0.001$) and continued to increase until week 4 ($p < 0.0001$) Figure 6B. The increase in particle size and polydispersity index after week 2 indicates aggregation or fusion between nanoliposome vesicles, most likely triggered by the phase change of the phospholipid bilayer during storage [29,44]. Phospholipids that form nanoliposome walls can undergo structural changes due to thermodynamic disturbances and energy fluctuations [34,45]. At 2°C–8°C, phospholipids undergo a phase transition from gel to liquid, weakening the intermolecular bonds in the membrane so that vesicles are more likely to coalesce and particle size increases [46]. Increasing particle size heterogeneity also indicates an increasingly non-uniform distribution [44]. This allows some unstable vesicles to break up or fuse to form larger particles. Factors such as thermal disruption, differences in initial vesicle size, and imbalances in phospholipid composition contribute to the fusion or enlargement of vesicles, while others remain small or fragmented. The zeta potential value at week 1 showed no significant change, but at week 2, it began to decrease, indicating a weakening of the repulsive force between particles. This decrease became more pronounced in weeks 3 and 4 ($p < 0.01$) Figure 6C. The decrease in zeta potential after week 2 indicates a reduction in the repulsive forces between particles, facilitating the formation of aggregates [9]. Stability in colloidal systems such as nanoliposomes depends on electrostatic and steric mechanisms. If the zeta potential falls below the stability threshold (±30 mV), the repulsive forces between particles are not strong enough to prevent aggregation [28]. From the beginning, the low potential zeta value progressively

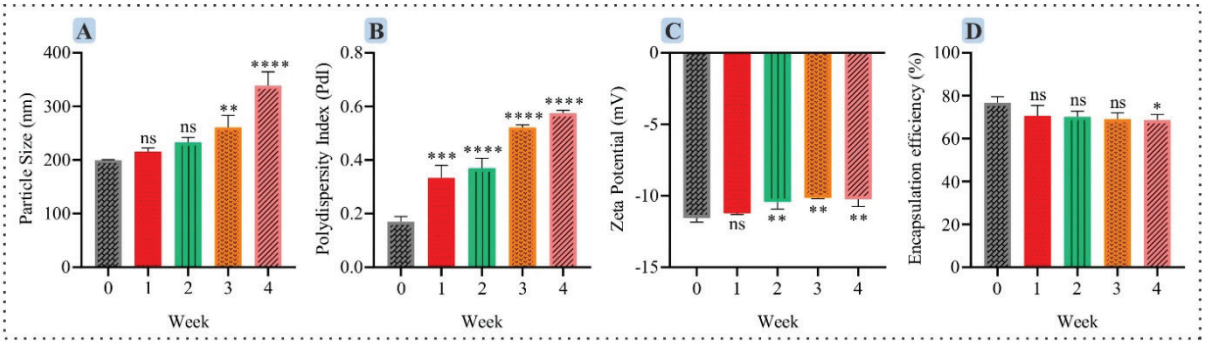

Figure 6. Stability test observation graph of NANO-SERF-SCs during the 2–8°C storage period from week 0 to week 4. (A) particle size, (B) polydispersity index, and (C) zeta potential. (D) Encapsulation Efficiency. Samples were analyzed using One-Way ANOVA with 95% confidence and a post-hoc test to determine significant differences in each group. n: 5; ns: not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

decreased, causing more obvious particle aggregation after week 3. The encapsulation efficiency showed no significant change until week 3; however, at week 4, there was a decrease ($p < 0.05$) Figure 6D. The leading cause of this leakage is the destabilization of the phospholipid bilayer, which can result from the effects of oxidative degradation or hydrolysis of phospholipids, especially if there is no addition of antioxidants or stabilizers in the formulation [47, 48].

Overall, changes in the stability of the NANO-SERF-SCs preparation most likely resulted from a combination of weakening of the phospholipid bilayer structure, decreased electrostatic stability (zeta potential), increased aggregation due to inter-particle attractive forces, and leakage of active ingredients due to rupture of some vesicles. To overcome this, it is necessary to optimize the formulation by adding stabilizers such as cholesterol to improve membrane stability or consider the lyophilization (freeze drying) method to make the preparation more durable in storage.

3.4. Results of DPPH and ABTS antioxidant assay

The working principle of the DPPH method involves the interaction of hydrogen atoms from antioxidant compounds with free electrons on radical compounds. This process causes a change from a free radical (*diphenylpicrylhydrazyl*) to a non-radical compound (*diphenylpicrylhydrazine*). The main indication of this reduction process is the change in colour of the solution, which initially turns purple to yellow due to the reduction by the antioxidant [49]. In determining antioxidant activity using the DPPH method, the IC_{50} parameter is defined as the sample concentration required to capture 50% of the DPPH radicals [50]. Table 5 presents the results of antioxidant testing by the DPPH method, where the standard equation of ascorbic acid obtained a linear regression with slope: 28.105; intercept: 33.652; R2: 0.9911 at a maximum λ of 517 nm, which showed an IC_{50} value of 1.789 ppm. At the same time, the NANO-SERF-SCs obtained a linear regression with slope: 29.428, intercept: 30.625, R^2: 0.9625 at a maximum λ of 517 nm, which showed an IC_{50} value of 15.483 ppm. A lower IC_{50} value indicates more potent antioxidant activity. Antioxidant testing using the DPPH method showed that NANO-SERF-SCs have powerful antioxidant activity with an IC_{50} value close to ascorbic acid as a comparison.

The principle of testing antioxidant activity with the ABTS method is based on the colour change of ABTS cations to measure the capacity of antioxidants that react directly with ABTS cation radicals [51]. ABTS cation itself is a nitrogen-based radical that has a characteristic blue–green colour. When reduced by antioxidant substances, this compound turns into a non-radical form and changes from coloured to colourless [52]. The ABTS method is susceptible to light; the ABTS cation formation process requires an incubation time of 12–16 hours under dark conditions for optimal results [53]. Table 6 presents the results of antioxidant testing by the ABTS method, where the Trolox standard equation obtained a linear regression with slope: 11.865; intercept: 30.959; R^2: 0.9918 at a maximum λ of 734 nm, which showed an IC_{50} value of 4.977 ppm. The NANO-SERF-SCs obtained a linear regression with slope: 14.469, intercept: 3.6336, R^2: 0.9737 at maximum λ 734 nm, which showed an IC_{50} value of 24.644 ppm. These findings indicate that NANO-SERF-SCs have potent antioxidant activity, with an IC_{50} value close to the IC_{50} value of the Trolox comparator, both less than 50 ppm.

Table 5. Results of NANO-SERF-SCs antioxidant testing using the DPPH method.

No	Sample	LN concentration (ppm)	Inhibition (%)	IC_{50} (ppm)
1	Ascorbic acid	1.792	92.454 ± 1.383	1.789
		1.609	80.622 ± 2.572	
		1.386	68.454 ± 1.970	
		1.099	56.924 ± 1.385	
		0.693	47.647 ± 1.261	
		0.000	40.723 ± 2.067	
2	NANO-SERF-SCs	4.605	100.420 ± 0.510	15.483
		3.912	95.345 ± 0.965	
		3.219	64.504 ± 1.042	
		2.526	35.126 ± 0.634	
		1.833	18.252 ± 1.146	
		1.139	9.765 ± 1.349	

Description: Average result of 3 replications ± Standard deviation

Table 6. Results of NANO-SERF-SCs antioxidant testing using the ABTS method.

No	Sample	LN Concentration (ppm)	Inhibition (%)	IC_{50} (ppm)
1	Trolox	5.521	98.034 ± 1.096	4.977
		4.828	87.891 ± 1.926	
		4.135	79.125 ± 0.520	
		3.442	70.318 ± 0.828	
		2.749	62.823 ± 0.863	
		2.055	57.266 ± 0.914	
2	NANO-SERF-SCs	6.908	99.659 ± 3.528	24.644
		6.215	95.741 ± 0.651	
		5.521	85.428 ± 23.178	
		4.828	74.853 ± 1.665	
		4.135	65.928 ± 2.909	
		3.442	49.456 ± 3.009	

Description: Average result of 3 replications ± Standard deviation

The antioxidant test results of the DPPH and ABTS methods showed that NANO-SERF-SCs had more potent antioxidant activity than the one used in the comparison. This confirms the content of secondary metabolites present in the ethyl acetate fraction of *Sonneratia caseolaris* L. leaf extract, containing flavonoids, tannins, terpenoids, alkaloids, and saponins, has potent antioxidant activity due to their unique chemical structure that allows effective interaction with free radicals, as well as the ability to inhibit the oxidation process [54,55]. Flavonoids, for example, have phenolic rings that can donate hydrogen atoms or electrons to free radicals, stabilizing them and preventing cell damage [56]. In addition, flavonoids can also bind to metal ions that trigger free radical formation,

reducing the amount of free radicals formed [57]. Tannins, as polyphenolic compounds, have many hydroxyl (OH) groups that can donate protons (H+) to neutralize free radicals, as well as astringent properties that help protect body tissues from oxidative damage [58]. Terpenoids protect cell membranes from oxidation with their complex carbon structure and lipophilic properties. Their structure allows interaction with free radicals and stops the chain of oxidative reactions [59]. Alkaloids, which have amine groups, can play a role in free radical capture and protect DNA and enzymes from oxidative damage [60]. Saponins, with sugar and aglycone moiety structures, can interact with free radicals and stabilize cell membranes, reducing oxidative damage to lipid membranes [61].

3.5. Results of evaluating the antimicrobial potential of NANO-SERF-SCs using disc diffusion test

The results of antibacterial activity using the agar diffusion method with three replicates compared the inhibition zone formed against *S. epidermidis* FNCC 0048, *P. acnes* ATCC 11827, and *S. aureus* ATCC 25923 with the treatment control (NANO-SERF-SCs concentrations of 10 ppm, 5 ppm, and 1 ppm), positive control (Chloramphenicol 2 mg/ml), and solvent control (Methanol). These results can be seen in Table 7. Each obtained a clean and clear inhibition zone from the three concentrations as a parameter. Figure 7 shows the diameter of the largest inhibition zone of each strain at a concentration of 1 ppm for *S. epidermidis* FNCC 0048 of 11.78 mm ± 0.72, a concentration of 5 ppm for *P. acnes* ATCC 11827 of 13.15 mm ± 2.93, and a concentration of 5 ppm for *S. aureus* ATCC 25923 of 13.18 mm ± 4.71. These lts shows how-SERF-SC has anti-acne effectiveness that falls into the strong category because it has inhibition in the 10–20 mm [62]. This proves that NANO-SERF-SCs have potent antibacterial activity in treating and preventing acne growth.

The flavonoids, tannins, terpenoids, alkaloids, and saponins contained in the ethyl acetate fraction of *S. caseolaris* L. leaf extract are secondary metabolites that act through various mechanisms, including damaging bacterial cell membranes, disrupting bacterial protein and DNA synthesis, and inhibiting enzymes important in bacterial metabolism [63]. Due to their diverse chemical structures and mechanisms of action, these secondary metabolites have great potential as natural antibacterial agents to address infections caused by *P. acnes*, *S. aureus*, and *S. epidermidis* [64]. The inhibition zone values indicate that the ethyl acetate fraction of *S. caseolaris L.* leaf extract encapsulated in the nanoliposomal system retains its antibacterial activity against acne-causing bacteria. However, it should be noted that these results are still preliminary and insufficient to comprehensively indicate clinical effectiveness. Determination of the minimum inhibitory concentration and minimum bactericidal concentration, along with validation through *in vivo* testing, are necessary in future studies to support the therapeutic potential of NANO-SERF-SCs as an anti-acne agent. Although the test results show antibacterial activity of the NANO-SERF-SCs formulation against *S. epidermidis*, *P. acnes*, and *S. aureus*, it is important to note that this testing did not include a control group with empty nanoliposomes (without the extract). Therefore, it cannot be conclusively determined whether the observed inhibition zones are solely attributed to the activity of the ethyl acetate fraction of *S. caseolaris L.* leaf extract or if there is a contribution from the delivery system. Further studies are needed to clarify the role of each component, including both the active extract and the nanoliposomal matrix, through the use of a vehicle-only control group in bioassays. In this antibacterial activity test, chloramphenicol was used as a positive control due to its broad-spectrum antibacterial properties. However, it should be noted that chloramphenicol is not a standard clinical agent specifically used in acne therapy. Therefore, the comparison results with NANO-SERF-SCs in this study should be regarded as an initial approach. The use of more clinically relevant positive controls, such as clindamycin or benzoyl peroxide, should be considered in future studies to more accurately evaluate the formulation's effectiveness against acne pathogens. Interestingly, the inhibition zone against *S. aureus* at a concentration of 5 ppm (13.18 mm) appeared larger compared to 10 ppm (8.78 mm), deviating from the expected dose-response relationship. This discrepancy could be attributed to biological variability between replicates, as reflected in the relatively large standard deviation at 5 ppm. Additionally, it may be influenced by uneven diffusion

Table 7. Results of antimicrobial potency evaluation using disc diffusion.

Conc. (ppm)	Strain	Zone of inhibition (mm)			Average
		1	2	3	
10	*Staphylococcus epidermidis* FNCC 0048	11.85	9.55	10.50	10.63
	Propionibacterium acnes ATCC 11827	10.65	11.90	12.15	11.57
	Staphylococcus aureus ATCC 25923	9.80	6.95	9.60	8.78
5	*Staphylococcus epidermidis* FNCC 0048	11.60	9.70	8.65	9.98
	Propionibacterium acnes ATCC 11827	11.05	11.90	16.50	13.15
	Staphylococcus aureus ATCC 25923	11.90	18.40	9.25	13.18
1	*Staphylococcus epidermidis* FNCC 0048	11.25	12.60	11.50	11.78
	Propionibacterium acnes ATCC 11827	12.15	11.50	12.30	11.98
	Staphylococcus aureus ATCC 25923	11.45	8.30	8.25	9.33
Positive control (Chloramphenicol 2 mg/ml)	*Staphylococcus epidermidis* FNCC 0048	18.30			
	Propionibacterium acnes ATCC 11827	19.95			
	Staphylococcus aureus ATCC 25923	19.50			
Solvent control (Methanol)	*Staphylococcus epidermidis* FNCC 0048	8.15			
	Propionibacterium acnes ATCC 11827	-			
	Staphylococcus aureus ATCC 25923	-			

Notes: The volume of sample and solvent control is 20 µl; The volume of positive control is 10 µl; The Sample exerts a PARTIAL inhibitory effect.

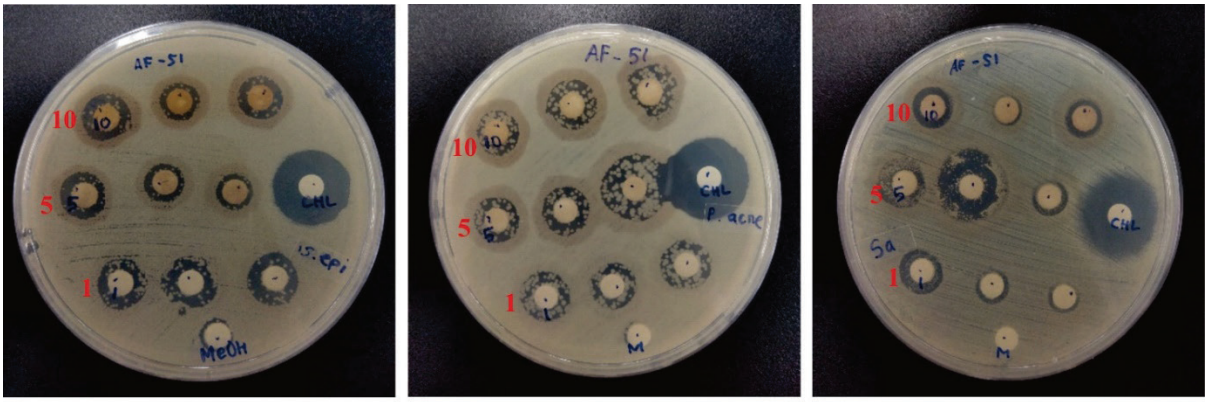

Figure 7. Zone of inhibition for NANO-SERF-SCs.

of the sample in the medium or the potential aggregation of the formulation at higher concentrations, which reduces the availability of the active compounds. This irregularity is noted as one of the limitations of the study, and further research is needed to confirm the consistency of the dose-based antibacterial effects, particularly against *S. aureus*.

4. CONCLUSION

The resulting NANO-SERF-SCs had a particle size of 199.7 nm ± 3.61 with an even particle size distribution and spherical shape. The encapsulation efficiency obtained was 76.74% ± 2.80%. NANO-SERF-SCs were also stable under freeze-thaw cycle conditions, 25 times dilution, and 30 days storage, with acceptable physical characteristics. Antioxidant test results using DPPH and ABTS methods showed that NANO-SERF-SCs were strongly antioxidant, with IC_{50} values close to the positive control. Evaluation of antimicrobial potential through disc diffusion test showed that NANO-SERF-SCs have strong antiacne effectiveness, with inhibition in the 10–20 mm range.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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