

Sacha inchi oil-based nanoemulsion of bay leaf (*Syzygium polyanthum*) and binahong leaf (*Anredera cordifolia*) extracts and in vitro release using a dialysis membrane

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ARTICLE INFO

Article history:

Received Mar 27, 2026

Revised Apr 2, 2026

Accepted Apr 9, 2026

Keywords:

Bay Leaf
Binahong Leaf
Flavonoids
In Vitro Release
Nanoemulsion

ABSTRACT

This study aimed to develop and characterize a nanoemulsion system containing combined extracts of bay leaf (*Syzygium polyanthum*) and binahong leaf (*Anredera cordifolia*) using sacha inchi oil, and to evaluate its in vitro release profile using a dialysis membrane method. The extracts were prepared by maceration using 96% ethanol and characterized through physicochemical and phytochemical analyses. Nanoemulsions were formulated using Cremophor RH40 and PEG 400, followed by homogenization and sonication. The formulations were evaluated for particle size, polydispersity index (PDI), zeta potential, and morphology. The results showed that the optimized formulation (NEC5) exhibited the smallest particle size (130.06 ± 56.66 nm), acceptable PDI (0.45 ± 0.12), and moderate zeta potential (-14.15 ± 4.88 mV), indicating good stability. Morphological analysis confirmed spherical and uniformly distributed droplets. In vitro release studies demonstrated an initial burst release followed by a more controlled release pattern, with the nanoemulsion showing more stable release compared to the extract. In conclusion, the nanoemulsion system successfully improved the physicochemical properties and controlled release behavior of the plant extracts, indicating its potential as an effective delivery system for plant-derived bioactive compounds.

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INTRODUCTION

The development of modern drug delivery systems continues to advance in response to the need to improve therapeutic effectiveness and overcome limitations associated with the physicochemical properties of active compounds. Many bioactive compounds exhibit promising pharmacological activities but are often limited by low solubility, poor stability, and low bioavailability, which can reduce their therapeutic effectiveness when formulated in conventional dosage forms (Vishwakarma et al., 2026). Therefore, the application of nanotechnology-based drug delivery systems has been widely explored as an effective strategy to enhance drug performance (Adepu &

Ramakrishna, 2021). Nanocarrier systems are known to improve solubility, enhance stability, and enable controlled release of active compounds, thereby increasing therapeutic efficiency (Liu et al., 2019).

One of the lipid-based nanotechnology systems that has gained considerable attention is the nanoemulsion system. Lipid-based nanocarriers are widely used because they can enhance drug delivery efficiency, improve penetration through biological membranes, and increase the bioavailability of active compounds (Jafar et al., 2024). Nanoemulsions are colloidal systems composed of oil, water, surfactant, and co-surfactant, with droplet sizes in the nanometer range, providing a large surface area and improving the solubility of lipophilic compounds (Adepu & Ramakrishna, 2021).

The small droplet size of nanoemulsions plays an important role in enhancing drug dissolution and absorption. Increased surface area facilitates better interaction between the dispersed phase and the surrounding medium, which contributes to improved drug release and bioavailability (Harwansh et al., 2019). In addition, nanoemulsion systems exhibit good physical stability and are capable of protecting bioactive compounds from degradation, making them suitable for delivering plant-derived compounds (Sadeq, 2020).

Indonesia is rich in medicinal plants that have long been used in traditional medicine, including bay leaf (*Syzygium polyanthum*) and binahong leaf (*Anredera cordifolia*). These plants contain various secondary metabolites such as flavonoids, alkaloids, tannins, and saponins, which contribute to their pharmacological activities (Kusriani et al., 2023). Flavonoid compounds, including kaempferol and quercetin, are particularly important due to their antioxidant and potential therapeutic properties. However, similar to many plant-derived compounds, these bioactive constituents often exhibit poor solubility and stability, which may limit their effectiveness in conventional formulations (Jafar et al., 2017).

To overcome these limitations, the development of nanoemulsion-based formulations using plant extracts is considered an effective strategy. The incorporation of bioactive compounds into lipid-based nanocarriers can enhance their dispersion in aqueous systems, improve stability, and increase penetration through biological membranes (Jafar et al., 2024). Additionally, appropriate selection of formulation components such as oil phase, surfactant, and co-surfactant is crucial in determining the physicochemical characteristics and stability of the system (Vishwakarma et al., 2026).

The oil phase plays a critical role in the successful formation of nanoemulsions, as it influences droplet formation, stability, and solubilization of lipophilic compounds. One of the oil phases that has demonstrated excellent performance is sacha inchi oil (SIO). This oil is rich in unsaturated fatty acids, particularly α -linolenic acid and linoleic acid, which contribute to improved fluidity and facilitate the formation of nanosized droplets during emulsification. A study by Sinsuebpol & Changsan, 2020 reported that nanoemulsion formulations based on sacha inchi oil exhibited favorable physicochemical characteristics, including a particle size of 148 nm, a polydispersity index of 0.210, and a zeta potential of -31.09 mV, indicating good stability and homogeneity. Therefore, the use of sacha inchi oil is considered advantageous in producing stable nanoemulsion systems (Muti et al., 2025).

Characterization of nano-sized lipid systems is an essential step to evaluate their quality and performance. Key parameters include particle size, polydispersity index, and zeta potential, which reflect system stability and homogeneity. Well-formulated lipid nanocarriers typically exhibit particle sizes below 1000 nm, low polydispersity index (<0.5), and sufficient zeta potential to prevent aggregation (Jafar et al., 2022). Furthermore, morphological analysis using Transmission Electron Microscopy (TEM) confirms the formation of spherical and uniformly distributed nanoparticles, which correlate with good stability and dispersion (Jafar et al., 2021).

Based on these considerations, this study aims to formulate and characterize a nanoemulsion system containing a combination of bay leaf (*Syzygium polyanthum*) and binahong

leaf (*Anredera cordifolia*) extracts using sachai inchi oil as the oil phase. In addition, the in vitro release profile of the nanoemulsion is evaluated using a dialysis membrane method to assess its potential in improving the release behavior of bioactive compounds compared to conventional extract systems (Suhandi et al., 2025).

RESEARCH METHOD

Instrument

The instruments used in this study included an analytical balance (Ohaus), magnetic stirrer (IKA), high-speed homogenizer (Ultra Turrax), and probe sonicator (Sonics Vibra Cell) for nanoemulsion preparation. Particle size, polydispersity index (PDI), and zeta potential were analyzed using a dynamic light scattering (DLS)-based particle size analyzer (Malvern Zetasizer). The morphology of the nanoemulsion droplets was observed using Transmission Electron Microscopy (TEM) (JEOL). For in vitro release evaluation, a dialysis membrane apparatus was used, accompanied by a magnetic stirrer to maintain constant agitation during the release study. The concentration of released active compounds was determined using a UV-Vis spectrophotometer. All instruments were calibrated prior to use to ensure the accuracy and reliability of the measurement results.

Materials

The materials used in this study included bay leaf (*Syzygium polyanthum*) extract and binahong leaf (*Anredera cordifolia*) extract as active ingredients. Sachai inchi oil (*Plukenetia volubilis* L.) was used as the oil phase. Surfactants such as Cremophor RH40 and co-surfactants such as polyethylene glycol 400 (PEG 400) were used to stabilize the nanoemulsion system. Distilled water (aquadest) was used as the aqueous phase. All materials used were of pharmaceutical or analytical grade and were obtained from reliable suppliers.

Research Procedures

- a. Preparation of Plant Extracts, bay leaf (*Syzygium polyanthum*) and binahong leaf (*Anredera cordifolia*) were collected and authenticated prior to use. The plant materials were cleaned, dried, and ground into powder to obtain simplicia. The powdered materials were extracted using maceration with 96% ethanol for 3 × 24 hours with occasional stirring. The filtrate was then collected and concentrated using a rotary evaporator at 50°C to obtain viscous extracts (Kusriani et al., 2023).
- b. Formulation and Optimization of Nanoemulsion, formulations were prepared by mixing surfactant (Cremophor RH40) and co-surfactant (PEG 400) in the aqueous phase, while the oil phase consisted of sachai inchi oil and a combination of bay leaf and binahong leaf extracts. The aqueous phase was gradually added to the oil phase under continuous stirring using a magnetic stirrer at 500 rpm and 50°C for 30 minutes to form a coarse emulsion (Jafar et al., 2021). The mixture was then subjected to probe sonication (70 kHz for 30 minutes) to obtain a transparent and stable nanoemulsion. Several formulations were prepared with varying compositions to determine the optimal formulation based on physical stability and clarity (Jafar et al., 2017).
- c. Characterization of Nanoemulsion, the nanoemulsion was characterized to determine its physicochemical properties. Particle size and polydispersity index (PDI) were measured using a particle size analyzer. Zeta potential was analyzed using a zeta analyzer to evaluate the stability of the system. Morphological observation of the nanoemulsion droplets was performed using Transmission Electron Microscopy (TEM) (Jafar et al., 2021). These parameters were used to assess the uniformity, stability, and quality of the nanoemulsion system.
- d. In Vitro Release Study Using Dialysis Membrane, the in vitro release study was carried out using a dialysis membrane method. The nanoemulsion sample was placed into a dialysis

membrane and immersed in a receptor medium under constant stirring. At predetermined time intervals, samples were withdrawn and replaced with fresh medium to maintain constant volume. The concentration of released active compounds was measured using a UV-Vis spectrophotometer, and the cumulative release percentage was calculated to evaluate the release profile of the nanoemulsion system (Suhandi et al., 2025).

RESULTS AND DISCUSSIONS

Preparation of Plant Extracts

The physicochemical characteristics of bay leaf (*Syzygium polyanthum*) and binahong leaf (*Anredera cordifolia*) simplicia are presented in Table 1. The results showed that all evaluated parameters, including loss on drying, moisture content, acid-insoluble ash, and extractive values, complied with the requirements of the Indonesian Herbal Pharmacopoeia (FHI). These findings indicate that the raw materials used in this study were of acceptable quality and suitable for further processing.

Table 1. Physicochemical characteristics of bay leaf and binahong leaf simplicia according to Indonesian Herbal Pharmacopoeia (FHI)

Parameter	Bay Leaf Result (% w/w)	FHI Requirement (%)	Binahong Leaf Result (% w/w)	FHI Requirement (%)
Loss on Drying	5.32 ± 1.63	< 10	5.08 ± 1.24	< 10
Total Ash Content	1.5 ± 0.0	< 2.5	4.0 ± 0.7	< 2.5
Acid-Insoluble Ash Content	1.0 ± 0.0	< 1.8	1.0 ± 0.0	< 1.8
Water-Soluble Extractive Value	16 ± 1	> 14.8	46 ± 2	> 13.5
Ethanol-Soluble Extractive Value	22.33 ± 1.52	> 19.9	28.25 ± 3.6	> 14.8
Moisture Content	5.0 ± 0.1	< 10	9.3 ± 0.5	< 10

The moisture content of both simplicia was below 10%, suggesting good stability against microbial growth and enzymatic degradation. Similarly, the acid-insoluble ash values met the standard limits, indicating minimal contamination from inorganic materials such as soil or sand. However, the total ash content of binahong leaf slightly exceeded the FHI requirement, which may be attributed to the presence of higher mineral content or residual inorganic matter (KEMENKES RI, 2022).

The extractive values in water and ethanol reflect the ability of the solvent to dissolve bioactive compounds. In this study, both plants showed relatively high ethanol-soluble extractive values, indicating the presence of semi-polar compounds such as flavonoids and tannins. Ethanol is widely used as an extraction solvent due to its effectiveness in extracting a broad range of secondary metabolites (Sidhartha et al., 2024a).

The extraction yield results are shown in Table 2. The yield of binahong leaf extract (14.4%) was higher than that of bay leaf extract (12.27%), suggesting a greater amount of extractable compounds in binahong leaf under the same extraction conditions. The maceration method using ethanol was effective in extracting phytochemical constituents, as it allows sufficient solvent penetration and diffusion of compounds from plant matrices (Sidhartha et al., 2024b).

Table 2. Extraction yield of bay leaf and binahong leaf extracts

Extraction Method	Plant Material	Sample Weight (g)	Extract Weight (g)	Extraction Yield (%)
Maceration	Binahong leaf	500	72.2616	14.4
Maceration	Bay leaf	500	61.3750	12.27

Phytochemical screening results (Table 3) revealed that both extracts contained alkaloids, flavonoids, quinones, tannins, and saponins, while steroids/triterpenoids were not detected. The presence of these secondary metabolites supports the potential pharmacological activity of the extracts. Flavonoids, in particular, are known for their antioxidant and antidiabetic properties, including the ability to modulate glucose metabolism and reduce oxidative stress (Sulfianti et al., 2023). Overall, the results demonstrate that both bay leaf and binahong leaf extracts possess good physicochemical quality and contain bioactive compounds that are suitable for further formulation into nanoemulsion systems (Sidhartha et al., 2024b).

Phytochemical Compound	Test Method	Bay Leaf Result	Binahong Leaf Result
Alkaloids	Mayer	(+)	(+)
	Dragendorff	(+)	(+)
Flavonoids	Mg + HCl	(+)	(+)
Quinones	NaOH	(+)	(+)
Tannins	FeCl ₃	(+)	(+)
	Gelatin	(+)	(+)
Saponins	Foam test	(+)	(+)
Steroids/Triterpenoids	Liebermann-Burchard	(-)	(-)

The LC-MS/MS chromatograms of bay leaf (*Syzygium polyanthum*) and binahong leaf (*Anredera cordifolia*) extracts showed multiple peaks at different retention times, indicating the presence of diverse secondary metabolites in both extracts. Differences in peak patterns suggest variations in their chemical composition (Alwie et al., 2021). The presence of flavonoids in both extracts is supported by phytochemical screening results. Bay leaf has been reported to contain flavonoids such as quercetin, while LC-MS/MS analysis identified compounds including delphinidin (m/z 303), malvidin (m/z 331), and flavan-3-ol (m/z 457), which are associated with antioxidant and α -glucosidase inhibitory activities (Alwie et al., 2021). Meanwhile, LC-MS analysis has confirmed the presence of kaempferol and its derivatives in binahong leaf extract, indicating that it is a potential source of kaempferol (Cid-Ortega & Monroy-Rivera, 2018).

Flavonoids such as quercetin and kaempferol are known to contribute to antidiabetic activity through inhibition of α -glucosidase and reduction of oxidative stress. However, definitive identification of compounds in this study requires further confirmation based on m/z values and fragmentation patterns. Therefore, the LC-MS/MS results mainly provide qualitative evidence supporting the presence of bioactive compounds (Alwie et al., 2021).

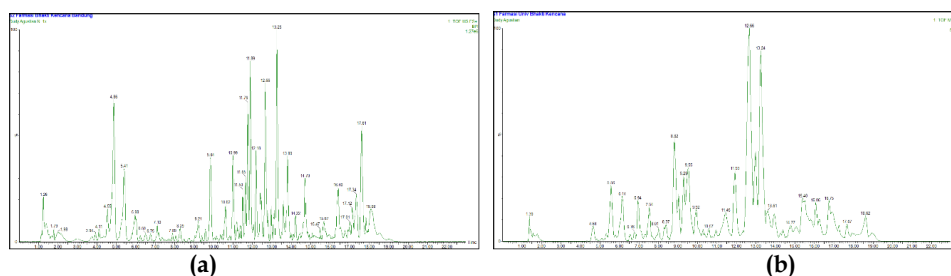


Figure 1. LC-MS chromatograms of plant extracts: (a) bay leaf (*syzygium polyanthum*) and (b) binahong leaf (*anredera cordifolia*)

Formula and Characterization of Nanoemulsion

Particle size is one of the most important parameters in evaluating nanoemulsion systems, as it directly affects physical stability and formulation performance. The measurement was carried out using the *Dynamic Light Scattering* (DLS) method based on Brownian motion of particles (Jafar,

2025). In general, nanoemulsions have droplet sizes in the range of 20–200 nm, which contribute to improved stability and enhanced solubility of lipophilic compounds (Khalid et al., 2023).

In this study, the particle size ranged from 130.06 nm to 617.55 nm (Table 4). NEC5 exhibited the smallest particle size, indicating more efficient emulsification and better dispersion of the oil phase. Smaller particle size increases the interfacial surface area, enhances dissolution, and prevents aggregation (McClements, 2015). In contrast, larger particle sizes observed in NEC3 and NEC4 may indicate less optimal surfactant–oil ratios, leading to droplet coalescence (Jafar et al., 2022).

Table 4. Composition and characterization of nanoemulsion formulations

Code	Bay Leaf Extract (%)	Binahong Leaf Extract (%)	Sacha Inchi Oil (%)	Cremophor RH40 (%)	PEG 400 (%)	Aquadest (mL)	Particle Size (nm)	PDI	Zeta Potential (mV)
NEC1	1	1	10	6.37	10	ad 100 mL	245.10 ± 87.16	0.24 ± 0.02	-17.93 ± 0.57
NEC2	1	1	5.5	28.18	10	ad 100 mL	139.35 ± 13.27	0.44 ± 0.03	-11.95 ± 4.50
NEC3	1	1	1	50	10	ad 100 mL	616.20 ± 507.70	0.61 ± 0.55	-9.97 ± 0.86
NEC4	1	1	3.25	39.09	10	ad 100 mL	617.55 ± 724.71	0.80 ± 0.29	-4.82 ± 7.31
NEC5	1	1	7.75	17.27	10	ad 100 mL	130.06 ± 56.66	0.45 ± 0.12	-14.15 ± 4.88

The polydispersity index (PDI) values ranged from 0.24 to 0.80. NEC1 showed the lowest PDI, indicating a more homogeneous system, while NEC4 exhibited the highest value, suggesting broader size distribution. A lower PDI (<0.5) is generally associated with better physical stability and uniformity of nanoemulsion systems (Danaei et al., 2018). The zeta potential values ranged from -4.82 to -17.93 mV. Although these values are below the typical threshold of ± 30 mV for electrostatic stability, nanoemulsions stabilized by nonionic surfactants such as Cremophor RH40 rely primarily on steric stabilization mechanisms (Gupta et al., 2016). This steric barrier helps prevent droplet aggregation and maintains system stability.

Morphological observation using TEM (Figure 2) showed spherical and uniformly distributed droplets, confirming successful formation of nanoemulsion systems. Spherical morphology with smooth surfaces is associated with improved stability and consistent dispersion ((Danaei et al., 2018); McClements, 2015).

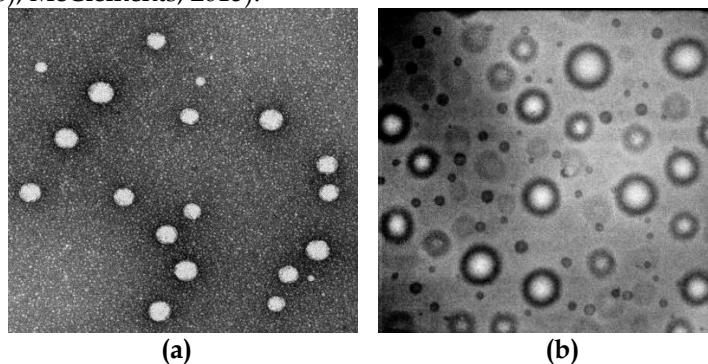


Figure 2. Morphology of nanoemulsion droplets observed by *Transmission Electron Microscopy* (TEM): (a) blank nanoemulsion and (b) nanoemulsion containing combined extracts of bay leaf and binahong leaf

These physicochemical characteristics strongly influence the release behavior of the system. Smaller particle size and larger surface area facilitate faster diffusion of active compounds across the dialysis membrane, while uniform distribution contributes to controlled and consistent release profiles (Jafar, 2025). Overall, the results indicate that the optimized nanoemulsion formulation, particularly NEC5, provides improved physicochemical properties and controlled release behavior, making it a promising system for delivering plant-derived bioactive compounds. Therefore, based on the overall evaluation of particle size, polydispersity index, and zeta potential, NEC5 was selected as the optimal formulation and was further used in the *in vitro* release study using the dialysis membrane method (Jafar et al., 2017).

The *in vitro* release profiles of the nanoemulsion (NEC5) and extract are presented in Table 5 and Figure 3. The study was conducted using the dialysis membrane method to evaluate the release behavior of active compounds under controlled conditions. The dialysis membrane method is widely used for evaluating drug release from nanoformulations because it allows separation between donor and receptor compartments, enabling diffusion of dissolved compounds across a semi-permeable membrane. This method simulates the release of active compounds into biological fluids and is considered suitable for assessing controlled release systems (Dudhipala et al., 2023; Suhandi et al., 2025).

Table 5. Comparison of *in vitro* release profiles of nanoemulsion (nec) and extract

Time (min)	Nanoemulsion (%)	SD	Extract (%)	SD
0	0.00	0.00	0.00	0.00
15	56.01	5.44	52.99	0.15
30	37.93	2.80	56.45	5.65
45	50.13	2.56	68.04	9.44
60	44.49	2.98	57.33	23.33
90	47.02	2.95	53.84	2.13
120	47.72	2.89	47.58	2.92
240	53.11	3.04	54.71	4.99
360	48.62	2.92	64.14	2.65
480	51.59	2.37	60.28	4.00

The results showed that both systems exhibited a rapid initial release phase followed by a more controlled release pattern. At 15 minutes, the nanoemulsion released 56.01%, slightly higher than the extract (52.99%). This initial burst release may be attributed to the diffusion of compounds located near the surface of nanoemulsion droplets, which is commonly observed in lipid-based delivery systems (Adepu & Ramakrishna, 2021). After the initial phase, the nanoemulsion demonstrated a more controlled and stable release profile compared to the extract. The nanoemulsion maintained relatively consistent release values, reaching 51.59% at 480 minutes, whereas the extract showed fluctuating release behavior. These fluctuations indicate a less controlled diffusion mechanism in the extract system due to the absence of a carrier matrix (Suhandi et al., 2025).

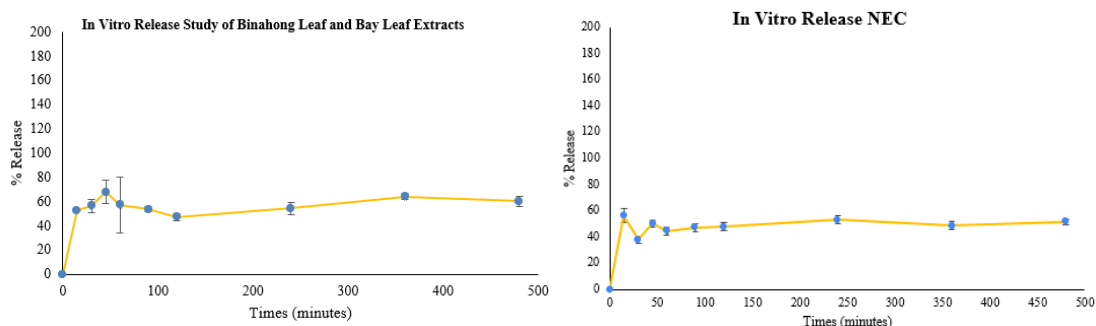


Figure 3. In vitro release profiles of (a) combined extracts of binahong leaf (*Anredera cordifolia*) and bay leaf (*Syzygium polyanthum*) and (b) nanoemulsion (NEC) containing the combined extracts, evaluated using the dialysis membrane method

The improved release behavior of the nanoemulsion can be attributed to the encapsulation of bioactive compounds within the oil phase, which regulates diffusion through the dialysis membrane. Lipid-based nanoformulations provide sustained release through a combination of diffusion and structural barrier effects, resulting in improved stability and prolonged release (Adepu & Ramakrishna, 2021; Suhandi et al., 2025). Overall, the nanoemulsion formulation (NEC5) exhibited a more controlled and stable release profile compared to the extract, indicating its potential to enhance the delivery and bioavailability of plant-derived bioactive compounds (Suhandi et al., 2025).

CONCLUSION

This study successfully formulated and characterized a nanoemulsion system containing combined extracts of bay leaf (*Syzygium polyanthum*) and binahong leaf (*Anredera cordifolia*) using sacha inchi oil. The results demonstrated that the physicochemical characteristics of the nanoemulsion were strongly influenced by the composition of oil and surfactant. Among the tested formulations, NEC5 was identified as the optimal formulation, exhibiting the smallest particle size, acceptable polydispersity index, and adequate zeta potential, indicating good stability. Furthermore, the nanoemulsion system showed spherical morphology and uniform droplet distribution, confirming successful formation of the nanosystem. The in vitro release study using the dialysis membrane method revealed that the nanoemulsion provided a more controlled and stable release profile compared to the extract. Overall, the developed nanoemulsion system has the potential to enhance the delivery, stability, and bioavailability of plant-derived bioactive compounds. Future studies are recommended to further evaluate its biological activity and in vivo performance to support its application in pharmaceutical formulations.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the Faculty of Pharmacy, Bhakti Kencana University, DKSH Indonesia, and RnD Bio Farma (Persero) for their invaluable support in providing research facilities and technical assistance that greatly contributed to the successful completion of this study. The authors also acknowledge the constructive feedback and valuable insights from colleagues during the preparation of this manuscript.

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