Validation of UV-Vis Spectrophotometric Method for Catechin Determination in Kawangkoan Peanut Skin Extract Nanoemulsion as an Antioxidant

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Abstract: *Kawangkoan peanut skin is a rich source of catechins, potent natural antioxidants with potential applications in pharmaceutical and cosmetic formulations. Reliable analytical methods are required to quantify catechin in complex delivery systems such as nanoemulsions. This study aimed to validate a UV-vis spectrophotometric method for catechin determination using a vanillin–H_2SO_4 reagent and to evaluate the antioxidant activity and physicochemical properties of a nanoemulsion containing Kawangkoan peanut skin extract. Method validation included linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). Antioxidant activity was evaluated using DPPH and ABTS assays. The method showed good linearity over the concentration range of 3–15 µg/mL (R = 0.9991), with LOD and LOQ of 0.4800 and 1.600 µg/mL, respectively. The nanoemulsion was clear, stable, and nanosized, with a particle size of 12.3 nm, zeta potential of −35 mV, and catechin entrapment efficiency of 74.77%. The nanoemulsion exhibited strong antioxidant activity. These results demonstrate that the validated UV-vis method is suitable for routine catechin analysis and support the potential application of catechin-loaded nanoemulsions as functional antioxidant ingredients.*

Keywords: *Kawangkoan peanut skin; catechin; nanoemulsion; antioxidant activity; UV-vis method validation*

■ INTRODUCTION

Peanuts are an important food crop in the global food sector. However, its processing generates by-products, including peanut skin waste. Peanut skin contains beneficial antioxidant compounds, including procyanidins, catechin, epicatechin, resveratrol, and anthocyanidins [1-3]. Catechins have been widely explored for use in pharmaceutical, nutraceutical, and cosmetic formulations owing to their ability to scavenge free radicals and inhibit oxidative stress pathways. However, their practical applications are often limited by low stability, poor water solubility, and susceptibility to degradation by pH, light, and oxygen [4]. The development of effective delivery systems is crucial for enhancing the physicochemical stability and bioavailability of catechin as an antioxidant. Thus, natural antioxidant compounds extracted from plant sources are often encapsulated in advanced delivery systems to enhance stability, solubility, and bioavailability. Lipid-based nanoformulations, including nanoemulsions, solid lipid nanoparticles (SLNs), and liposomes, have demonstrated improved antioxidant protection and delivery efficiency. In contrast, hydrogel systems provide extended release and physical stabilization of encapsulated bioactives [5-7].

Among various formulation strategies for antioxidant products, nanoemulsion systems have proven highly promising and are widely used to enhance the effectiveness of antioxidant compounds in delivering therapeutic benefits. Incorporating catechins into a nanoemulsion system can improve dispersibility, protect against degradation, and enhance functional performance. Nanoemulsions help maintain catechin

stability against degradation and preserve its content over time [8]. In addition, nanoemulsions not only maintain stability but can also enhance the biological potential and absorption of catechin [9]. In nanoemulsion systems, the presence of surfactants, oils, and other formulation components may interfere with analyte detection; therefore, analytical method validation is essential to demonstrate that the method is suitable for its intended purpose. Analytical method validation has been successfully developed for 4-*n*-butylresorcinol nanoparticles [10], resveratrol nanoemulsions [11] and vitamin A nanoemulsions [12] using the HPLC method. Besides that, flavonoid quercetin in nanoemulsion matrices can be quantified using UV-vis spectrophotometry if the method is properly validated, resulting in high accuracy (recovery 99.7–100.1%) and precision (RSD ≤ 2%) [13] reported that UV-vis spectrophotometric methods have been developed for pharmaceutical quality control [14] and applied to the determination of catechins [15-16]. One technique that can be developed for UV-vis spectrophotometry is the colorimetric technique using a vanillin–acid reagent (vanillin–H_2SO_4). This technique is simple, sensitive, and effective for the qualitative and semi-quantitative analysis of flavan-3-ol compounds such as catechin and proanthocyanidins [17-18]. The catechin–vanillin reaction produces a characteristic brick-red color, detectable at 500 nm by UV-vis spectrophotometry [18]. To ensure analytical reliability, the UV-vis spectrophotometric method used for catechin quantification in delivery systems must be validated. According to the International Council for Harmonization (ICH Q2(R1)) guidelines, the key parameters that must be evaluated include linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ), which serve as the basis for assessing the performance of analytical methods [19]. Furthermore, physicochemical characterization and bioactivity evaluation of catechin-loaded nanoemulsions are crucial to ensure their stability and effectiveness as antioxidant delivery systems. These evaluations included visual appearance, clarity, transmittance, turbidity, entrapment efficiency, particle size, size distribution, zeta potential, and radical scavenging activities using DPPH and ABTS assays. Previous studies have shown that formulation parameters (particle size, surfactant type/concentration) significantly affect the kinetic stability, encapsulation efficiency, and maintenance of antioxidant activity of nanoemulsions during storage [20]. Therefore, this study aims to validate the UV-vis spectrophotometric method for determining catechin content using the vanillin–H_2SO_4 reagent, and to characterize the antioxidant activity and physicochemical properties of nanoemulsions formulated with Kawangkoan peanut skin extract. This validation and characterization provide a scientific basis for the development of catechin-loaded nanoemulsions as promising antioxidant candidates for pharmaceutical applications.

■ EXPERIMENTAL SECTION

Materials

Kawangkoan peanut skin (Minahasa Regency, North Sulawesi, Indonesia), acetone (Technical grade, China), distilled water (Bratachem, Indonesia), catechin p.a. (Sigma Aldrich, USA), vanillin p.a. (Merck, Germany), sulfuric acid (Merck, Germany), lecithin (Himedia, India), tween 80 (Merck, Germany), DPPH (Sigma Aldrich, USA), ABTS (Sigma Aldrich, USA), potassium persulfate (Sigma Aldrich, USA), ethanol p.a. (Merck, Germany), ascorbic acid (Merck, Germany), and deionized water (PT. Ikapharmindo Putramas tbk., Indonesia) were used in this work.

Instrumentation

UV-vis spectrophotometer (Shimadzu UV-1800, Japan) with a double-beam detector configuration with a 1 cm pathlength quartz glass cuvette, hotplate stirrer (Four E's Scientific, China), sonicator (James Ultra 8060D-H Ultrasonic Cleaner, United Kingdom), centrifuge (Clements GS 150, Australia), waterbath (Julabo TW12, Germany), PSA and ZPA (Nano Particle Analyzer Horiba SZ-100, Japan), and rotary evaporator (Hahnvapor HS-2005S-N, Korea).

Procedure

Preparation of the acetone extract

Kawangkoan peanut skin was extracted using the maceration technique. Dried and powdered peanut skin was placed in an appropriate extraction vessel and mixed with acetone as the solvent. The mixture was stirred continuously with a magnetic stirrer at room temperature to facilitate diffusion of the bioactive compounds into the solvent. After the maceration period, the extract was filtered to remove plant residue. The filtrate was then concentrated using a rotary evaporator to obtain the acetone extract of Kawangkoan peanut skin.

Nanoemulsion formulation of skin peanut extract

Nanoemulsions were produced with slight modifications. A sample of catechin extract (1%), representing the aqueous phase, was dissolved in analytical-grade ethanol (10%) and Tween 80 (9.5%) in 79% deionized water. The aqueous phase was subsequently heated to 70 °C and stirred. The oil phase containing 0.5% lecithin was then added dropwise to the aqueous phase under continuous magnetic stirring until a nanoemulsion formed. Particle size reduction was performed using a sonicator for 1.5 h to obtain a transparent catechin nanoemulsion (20 mL) with a yellow appearance [21].

Characterization of peanut skin extract in nanoemulsion

Organoleptic test. This test aimed to evaluate the color and physical appearance of the nanoemulsion as part of its characterization [22].

Turbidity and percent transmittance. Turbidity of the nanoemulsions was measured instrumentally by absorbance at 600 nm in a 1 cm cuvette using a spectrophotometer [23]. The turbidity value was calculated using Eq. (1);

$$\text{Turbidity } (\text{cm}^{-1}) = \frac{2.303 \times \text{abs at 600 nm} \times \text{dilution factor (1)}}{\text{the path length of the cuvette (cm)}} \quad (1)$$

Transmittance measurements were performed using a UV-vis spectrophotometer at 650 nm, with distilled water as a blank, to determine the transparency of the nanoemulsion [24].

Physical stability. To assess the nanoemulsion's stability, three types of stability tests were conducted: centrifuge, freeze-thaw, and thermal. To perform the centrifugation test, 5 mL of each sample was placed in a centrifuge tube and centrifuged at 3000 rpm for 30 min [25]. The freeze-thaw stability of the nanoemulsions was assessed using a previously described method [26]. Specifically, the nanoemulsion was frozen at 20 °C for 24 h, then thawed at room temperature, and its stability was monitored. Thermal stability was evaluated by subjecting the emulsion to heat treatment in water baths at 25, 37, and 85 °C for 30 min, followed by cooling to 25 °C before further observation [27].

Particle size and zeta potential analysis. A total of 2 mL of the nanoemulsion formulation was placed in a cuvette, and the sample was then placed in the holder. The next step was to select the menu for nanoparticles (nm) and zeta potential (mV) [28].

Preparation of a standard stock solution of catechin

A catechin standard stock solution was applied by accurately weighing 1 mg of standard catechin and then placing it in a 10.0 mL volumetric flask. The volume was adjusted to absolute ethanol to dissolve catechin.

Determination of the maximum wavelength of catechin

The determination of catechin content was carried out based on the catechin–vanillin colorimetric reaction [17]. An aliquot of the catechin standard stock solution was reacted with 3 mL of ice-cold vanillin reagent (1% vanillin in 70% H_2SO_4), added slowly to prevent premature color formation. The reaction mixture was then adjusted to a final volume of 1.7 mL with distilled water to obtain a catechin concentration of 6 μg/mL. The solution was thoroughly mixed and allowed to stand at room temperature for 15 min to ensure complete color development. The absorbance of the resulting orange-colored complex was recorded over 400–800 nm using a UV-vis spectrophotometer, with vanillin reagent and distilled water as the blank.

Validation of method development

The UV-vis spectrophotometric method was validated in accordance with the International Conference on Harmonization (ICH) guidelines, which include assessments of linearity, precision, accuracy, LOD, and LOQ.

Linearity. The catechin standard stock solution was diluted with 3 mL of ice-cold vanillin (1% vanillin in 70% H_2SO_4) to avoid immediate color development. The contents were brought to 1.85, 1.7, 1.55, and 1.4 mL with distilled water to achieve catechin concentrations of 3, 6, 9, 12, and 15 µg/mL. The solutions were shaken thoroughly and allowed to stand for 15 min to complete the reaction. The absorbance of the solution was measured at the maximum wavelength for catechin. The standard calibration curve was obtained by plotting absorbance vs. a series of catechin concentrations, using a UV-vis spectrophotometer with vanillin reagent and distilled water as the blank.

LOD and LOQ. The LOD and LOQ were calculated from the calibration curve using the standard deviation and slope. These parameters were determined using Eq. (2) and (3);

$$\mathrm{LOD} = 3.3 \times \sigma / s \quad (2)$$

$$\mathrm{LOQ} = 10 \times \sigma / s \quad (3)$$

where σ represents the standard deviation of the y-intercepts of the regression line, and s denotes the slope of the calibration curve.

Accuracy. The absorbance of catechin solutions at concentrations of 6, 9, and 12 µg/mL was measured using a UV-vis spectrophotometer at the maximum absorption wavelength of the compound. Each measurement was performed in triplicate. Accuracy was evaluated based on the percentage recovery (% recovery).

Precision. The precision of the analytical method was assessed through intraday and interday evaluation. The intraday precision was determined by measuring the absorbance of catechin solutions at 6, 9, and 12 µg/mL in triplicate on a single day. In contrast, the interday precision was evaluated by performing the same measurements across three separate days. Relative standard deviation (RSD) was calculated for each assessment.

Catechin determination

A total of 40 µL of the 1% sample nanoemulsion (equivalent to 0.4 mg of nanoemulsion/mL system) was diluted with 3 mL of ice-cold vanillin reagent (1% w/v vanillin prepared in 70% H_2SO_4), and added slowly to prevent premature color formation. The reaction mixture was then adjusted to a final volume of 5 mL with distilled water, homogenized by gentle shaking, and allowed to stand for 15 min at room temperature to ensure complete color development. The absorbance of the resulting solution was measured at the maximum absorption wavelength (λ_{max}) of catechin. Vanillin reagent and distilled water without a sample were used as the blank. Quantification was performed using a catechin calibration curve (y = ax + b), and the results were expressed as mg CE/g sample. This unit reports the amount of vanillin-reactive flavan-3-ol compounds, calculated using catechin as the reference standard, providing a more specific and standardized expression of the total flavan-3-ol content.

Encapsulation efficiency

The encapsulation efficiency was determined by comparing the catechin concentrations in the nanoemulsion with those in the original Kawangkoan peanut skin extract using Eq. (4) [29].

$$\%\mathrm{EE} = \frac{\mathrm{catechin}_{\mathrm{total}} \times \mathrm{catechin}_{\mathrm{free}}}{\mathrm{catechin}_{\mathrm{total}}} \times 100\% \quad (4)$$

Determination of antioxidant activity by the DPPH method

The DPPH radical scavenging activity of the nanoemulsions was evaluated using a previously established method [30] with slight modifications. Briefly, 1 mL of each sample solution was mixed with 3 mL of ethanol p.a., then 1 mL of 0.4 mM DPPH solution was added. The mixture was vortexed for 1 min and incubated in the dark at 25 °C for 15 min to allow the reaction to proceed. The absorbance was then measured at 517 nm, with the nanoemulsion formulation (ethanol, tween 80, and lecithin) without extract used as the blank. Antioxidant activity was expressed as the IC_{50} value, calculated from the linear regression curve of percentage inhibition versus sample concentration. Percentage inhibition was calculated using Eq. (5).

$$\%\mathrm{Inhibition} = \frac{\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{sample}}}{\mathrm{Abs}_{\mathrm{control}}} \times 100\% \quad (5)$$

Determination of antioxidant activity by the ABTS method

The ABTS radical scavenging activity of the nanoemulsions was evaluated using a previously

established method [30] with slight modifications. The $ABTS^{+}$. The solution was prepared by dissolving 28.406 mg ABTS and 14 mg potassium persulfate in 20 mL of distilled water, and the mixture was allowed to stand at room temperature for 16 h. The solution was subsequently diluted with distilled water to a final volume of 100 mL. A total of 1 mL of the sample solution was mixed with 3 mL of distilled water and 1 mL of ABTS reagent. The mixture was vortexed for 1 min, then incubated in the dark at 25 °C for 10 min. The absorbance was measured at 730 nm, with the nanoemulsion formula (ethanol, tween 80, lecithin) without extract used as the blank. Antioxidant capacity was expressed as the IC_{50} value, determined from a linear regression of percent inhibition versus sample concentration. The percentage inhibition was calculated using Eq. (5).

Statistical analysis

All data are presented as the mean ± SD based on at least three replicates for each sample.

■ RESULTS AND DISCUSSION

Formulation of Nanoemulsion of Kawangkoan Peanut Skin Acetone Extract

According to the qualitative analysis of catechin compounds in the aqueous, methanol, ethanol, and acetone extracts [31]. Catechin was detected only in the acetone extract, as indicated by the formation of a brick-red color when reacted with the vanillin–H_2SO_4 reagent. The presence of catechin in the acetone extract was confirmed using a spiking test with a catechin standard. A shift in the maximum absorbance wavelength of catechin to 535 nm has been reported in a previous study [31]. A bathochromic shift in the maximum wavelength of catechin to approximately 568 nm has also been reported, with the sample solution exhibiting a red coloration [32]. Therefore, the acetone extract was incorporated into the nanoemulsion formulation.

The type and concentration of surfactants play a critical role in catechin entrapment in the nanoemulsion formulation of kawangkoan peanut skin acetone extract containing catechin. Tween 80 is a hydrophilic, nonionic surfactant commonly used to reduce the droplet size of nanoemulsions. Soy lecithin, a liquid lipid that functions as a surfactant capable of forming a stable nanostructure, further enhanced the stability of the system. Therefore, the combination of Tween 80 and lecithin can be utilized as an effective carrier system for catechin.

Nanoemulsion Characteristic

Organoleptic test

The acetone extract of Kawangkoan peanut skin was formulated as a water-based nanoemulsion. A transparent catechin nanoemulsion with a yellow appearance is shown in Fig. 1(a).

Turbidity and percent transmittance

The color of an emulsion is primarily determined by its scattering and absorption efficiencies. Scattering is influenced by droplet characteristics and concentration,

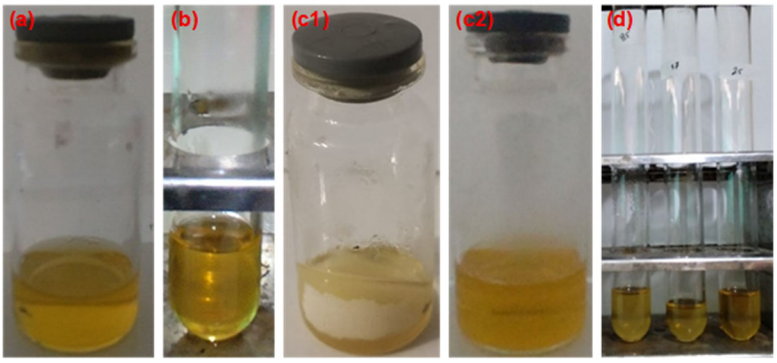

Fig 1. (a) Acetone extract of Kawangkoan peanut skin in nanoemulsion with a yellow appearance; (b) results of the nanoemulsion centrifugation test; (c) the result of the freeze-thaw test of the nanoemulsion: (c1) freeze condition and (c2) post-thaw condition; and (d) the result of the thermal test of the nanoemulsion

which affect the emulsion's turbidity, opacity, and brightness. Absorption efficiency depends on the properties and concentration of the coloring agent. As shown in Fig. 1, freshly prepared nanoemulsions appeared yellow. The turbidity of the nanoemulsions, measured using a UV-vis spectrophotometer, indicated a value of 0.33 cm^{-1} for a 0.5 wt.% oil-in-water nanoemulsion. From visual observations, emulsions can be considered optically transparent when the turbidity at 600 nm is less than approximately 0.05 cm^{-1} for a 0.1 wt.% oil-in-water nanoemulsion [33]. However, the results obtained show better turbidity values compared to those of nanoemulsion-based edible films loaded with vitamin D3 and *Cordia myxa* mucilage [34], functional nanoemulsion using pomelo peel essential oil and curcumin [35], and vanilla nanoemulsions [23], which have values of 0.954 and 1.646; 1.03–7.15 and 9.31–23.22 cm^{-1}, respectively. These findings suggest that the turbidity of diluted emulsions, as a function of wavelength, can be used to estimate droplet size. The relationship between droplet size and turbidity is inversely proportional. Nanoemulsions exhibit optical transparency when most droplets are below approximately 80 nm, corresponding to a specific turbidity of < 0.5 cm^{-1}%vol^{-1}. Reducing droplet size decreases light scattering intensity, resulting in lower turbidity. However, turbidity is also affected by particle size distribution, as the presence of larger droplets can significantly increase light scattering [33].

The transmittance percentage is closely related to the overall clarity of the nanoemulsion system [36]. A nanoemulsion is deemed transparent if its percent transmittance is greater than 99% [37], namely, 100% (close to the aquadest percent transmittance) [38]. Higher transmittance values indicated better uniformity and stability [24]. The %transmittance of the nanoemulsions, measured using a UV-vis spectrophotometer, was 87.50%. The transmittance value of 87.5% indicates that the nanoemulsion possesses good optical clarity. However, it does not fully match the transmittance values of water near 90–100%, suggesting that the nanoemulsion formulation produces a clear, transparent dispersion with nanometer-sized particles [39]. This value suggests that the formulation still exhibits some light scattering, likely owing to slightly larger droplets or a broader droplet size distribution than in an ideal nanoemulsion. However, a transmittance level above 80% is generally regarded as acceptable for nanoemulsion systems and reflects the formation of a relatively transparent and stable dispersion [38]. Transmittance values above 85% suggest an optimal nano-sized droplet distribution, which contributes to their improvement. Additionally, the increased clarity and stability of these formulations indicate successful emulsification, making them promising candidates for further development of topical formulations [24]. Therefore, while the sample did not achieve the maximum clarity of aquadest, a transmittance of 87.5% still demonstrates that the system maintains nano-sized droplets and meets the criteria for a clear nanoemulsion.

Physical stability

Physical stability can be reviewed by comparing the physical properties of the preparation before and after the centrifugation test, thermal cycling, and real-time methods [40], and freeze-thaw cycles [41]. Physical instability parameters, such as phase separation, sedimentation, creaming, and breaking, can be observed by centrifugation [42]. Therefore, centrifugation was used to assess phase separation in the nanoemulsions. The presence of observable phase separation indicates physical instability of the nanoemulsion formulation. Based on these results, the formulation showed no signs of instability (Fig. 1(b)), indicating that the nanoemulsion component was present at optimal concentrations and supporting the formulation's physical stability. In addition to centrifugation testing, physical stability can be evaluated using freeze–thaw tests. The results of the physical stability test of the nanoemulsions at low and high temperatures showed that the nanoemulsions remained clear and transparent (stable), with no color change or phase separation. The results of this test are shown in Fig. 1(c).

Nanoemulsions are inherently thermodynamically unstable, and exposure to elevated temperatures promotes energy uptake by droplets, which may lead to demulsification, droplet coalescence, and flocculation within the system [43]. Therefore, thermal stability tests were performed at 25, 37, and 85 °C. A temperature

range of 25–85 °C was chosen to represent typical storage conditions (25 °C) and an accelerated thermal-stress environment (up to 85 °C) to induce potential physical changes. Elevated temperatures are commonly used in stability testing to accelerate degradation and reveal early signs of instability, such as color alteration and phase separation, which can be observed through organoleptic evaluation [44]. As shown in Fig. 1(d), the nanoemulsions remained clear and transparent (stable) throughout the test, with no color change or phase separation.

Particle size and zeta potential

Physical characterization of the nanoemulsion was initially conducted by measuring the particle size, polydispersity index (PDI), and zeta potential using a PSA. These parameters are essential indicators for evaluating the droplet size distribution, homogeneity, and colloidal stability of nanoemulsion systems, because they directly influence the stability of the formulation during storage and under stressful conditions [45]. Dynamic light scattering (DLS) analysis showed that the nanoemulsion exhibited an average particle size of 12.3 nm with a PDI of 0.179 (Fig. 2(a)), indicating a relatively narrow particle size distribution. PDI indicates the uniformity of particle size distribution in nanoemulsion systems. PDI values near zero indicate nearly monodisperse systems with highly uniform particle sizes. PDI values below 0.2 indicate a narrow and homogeneous size distribution, while values between 0.2 and 0.3 are still considered acceptable for lipid-based nanocarriers, reflecting relatively good homogeneity and physical stability [46].

The measured zeta potential of the acetone extract of Kawangkoan peanut skin in nanoemulsion was −35 mV (Fig. 2(b)), suggesting high colloidal stability. Zeta potential is an important indicator of colloidal stability. According to Tamboli and Tade [47]. Zeta potential values greater than +30 mV or less than −30 mV indicate sufficient electrostatic repulsion between particles, resulting in good physical stability. In contrast, zeta potential values within the range of −30 to +30 mV suggest weaker repulsive forces and a higher tendency for particle aggregation, unless additional stabilization mechanisms are present. Furthermore, the strongly negative surface charge of the acetone extract of Kawangkoan peanut skin in the nanoemulsion may facilitate interactions with positively charged regions on the cell membrane, thereby enhancing cellular uptake

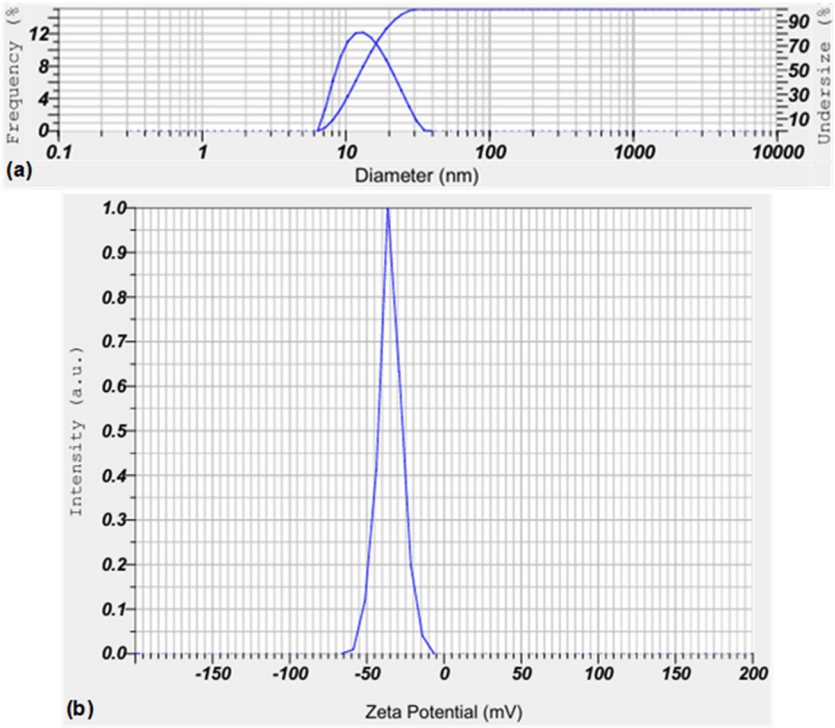

Fig 2. (a) Particle size distribution and polydispersity index of nanoemulsion and (b) zeta potential of nanoemulsion

via endocytosis. The results obtained in this study are comparable and demonstrate physicochemical characteristics consistent with previously reported catechin nanoemulsions prepared using lecithin and Tween 80, which achieved small particle sizes, narrow size distributions, and high zeta potential values [8,29].

Maximum Wavelength of Catechin

The maximum wavelength of catechin obtained from absorbance scanning in the range of 400–800 nm was 510 nm (Fig. 3(a)), which differs from the previously reported value of 500 nm [17]. Therefore, 510 nm was selected as the quantification wavelength for subsequent catechin analysis in this study.

Validation of Method Development

Method validation is a verification process conducted through laboratory analytical testing to generate data demonstrating the reliability of an analytical method derived from a specific procedure [48]. The validation parameters evaluated in this study included linearity, LOD, LOQ, accuracy, and precision.

Linearity

Linearity describes the ability of an analytical method to produce test results directly proportional to analyte concentration within a specified range, as demonstrated using appropriate statistical approaches. In this study, linearity was evaluated by constructing calibration curves obtained from a series of standard solutions of varying concentrations, and measuring their absorbance at 510 nm using UV-vis spectrophotometry. The resulting calibration curves (Fig. 3(c)) followed a linear regression model ($y = 0.0416x + 0.1173$) and showed excellent linearity, with a correlation coefficient (R) value of 0.9991. These R values exceeded the acceptance criteria for method validity, where a correlation coefficient ≥ 0.999 was considered indicative of good linearity [49]. The strong correlation, with R values close to 1, confirms a robust linear relationship between absorbance and analyte concentration, demonstrating that the analytical method is suitable for quantitative analysis within the tested concentration ranges.

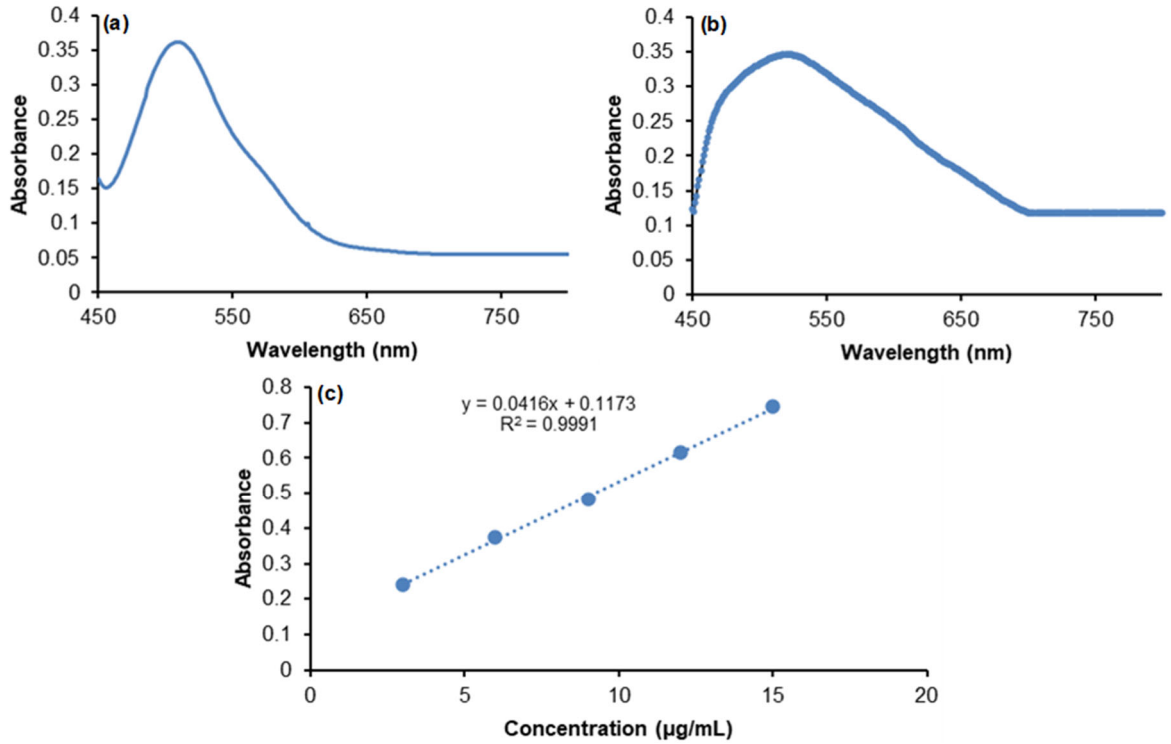

Fig 3. (a) UV-vis spectrum in catechin standard and (b) catechin in nanoemulsion; and (c) catechin calibration curve at the wavelength 510 nm

LOD and LOQ

The LOD refers to the lowest concentration of an analyte that can be detected in a sample, although it may not be quantified with adequate precision. The LOQ is defined as the lowest concentration that can be quantitatively determined with acceptable accuracy and precision. In this study, the LOD and LOQ were calculated statistically using the standard deviation of the response (or y-intercept) and the slope of the calibration curve, which were 0.4800 and 1.600 μg/mL, respectively. The obtained values indicate that the method exhibits good sensitivity, with LOD and LOQ in the low-concentration range, demonstrating its capability to reliably detect and quantify small amounts of analyte.

Compared with HPLC-based methods, the LOD and LOQ values obtained are generally higher. This difference is expected, as HPLC typically provides superior sensitivity through chromatographic separation prior to detection, thereby enhancing selectivity, improving the signal-to-noise ratio, and minimizing matrix interference. Quality control data of catechins in green tea leaf waste by HPLC reported LOD and LOQ values for major catechins were found in the lower 0.25 to 0.6 μg/mL range, demonstrating high analytical sensitivity [8]. Similarly, the study entitled Quality control data of catechins in Oolong tea leaf waste by HPLC also reported low detection and quantification limits (0.03–0.18 μg/mL), confirming HPLC's capability to accurately determine catechin levels at trace concentrations [29].

Nevertheless, the UV-vis spectrophotometric method developed in this study demonstrates satisfactory analytical performance for routine quantitative analysis. Although it may not reach the ultra-trace sensitivity of HPLC, the method remains reliable, cost-effective, and sufficiently sensitive for applications within the established working concentration range. Therefore, the obtained LOD and LOQ values confirm that the method is suitable for accurate and reproducible quantification of the analyte at low concentration levels [48].

Accuracy

Accuracy reflects the degree of closeness between the measured value and the true concentration of an analyte and is commonly expressed as the percentage recovery. The recovery value (%) in this study met the acceptance criteria according to the Association of Analytical Chemists AOAC because the %recovery value was discovered in the range of 98.438–99.902% (Table 1). These results indicate that the method employed has adequate accuracy, as it can detect analytes with high agreement with the true concentration values. The percentage recovery values approaching 100% at all concentration levels demonstrated that the analyte could be reliably recovered with minimal loss or matrix interference. This confirmed that this method is suitable for catechin analysis.

Precision

Precision refers to the closeness of the agreement between individual measurement results and the mean value when an analytical procedure is repeatedly applied to samples from a homogeneous system. It is commonly evaluated using RSD to identify potential variability in the analytical process. The analytical method was considered precise when the RSD was below 2%. In this study, both intraday and interday precision studies showed that the %RSD met the acceptance criteria of ≤ 2% (Table 2). This indicates that the instrument and analytical method used were stable and not significantly affected by random factors that could cause large variations in the measurement results. Therefore, the UV-vis spectrophotometric method used in this study shows good precision for determining catechin levels.

Catechin Content and Encapsulation Efficiency

Fig. 3(a) shows that the catechin compound exhibits a typical absorption band in the UV range, with a maximum at 510 nm. However, in the nanoemulsion formulation, a bathochromic shift occurred at the maximum wavelength of 520 nm (Fig. 3(b)). Fluorescence studies have reported that the entrapment of curcumin in nanoemulsions resulted in a shift of the

Table 1. Accuracy study

Concentration (μg/mL)	% Recovery
6	98.438
9	99.902
12	99.833

Table 2. Intraday and interday precision study

Intraday precision						
Concentration (μg/mL)		Mean concentration (μg/mL)		SD		%RSD
6		5.906		0.0027		0.274
9		8.991		0.0007		0.066
12		11.98		0.0002		0.021
Interday precision						
Concentration (μg/mL)	Concentration D-1 (μg/mL)	Concentration D-2 (μg/mL)	Concentration D-3 (μg/mL)	Mean concentration (μg/mL)	SD	%RSD
6	5.906	5.618	6.042	5.856	0.0080	0.803
9	8.991	8.751	8.054	8.695	0.0124	1.238
12	11.98	12.64	11.94	12.19	0.0126	1.257

maximum emission wavelength from 540 to 490 nm [50]. This result is expected in a colloidal environment and does not affect the compound's photochemical and photobiological characteristics. Moreover, a shift in the maximum wavelength observed by UV-vis spectrophotometry has been reported across different micellar systems, including microemulsions and nanoemulsions [51]. This indicates the method's sensitivity in detecting subtle changes in micellar systems. The results were as expected, given the physicochemical differences between the two emulsion types.

Based on these findings, the catechin content was determined. The catechin content encapsulated in the acetone extract nanoemulsion was used to determine the encapsulation efficiency relative to the catechin content in the original extract. In this context, the success of the catechin encapsulation process, which provides protection against degradation and controlled release, can be evaluated. Based on the measurement results, the amount of catechin encapsulated in the acetone extract nanoemulsion was 64.51 ± 0.332 mg CE/g sample, yielding an encapsulation efficiency of 74.77%. A high encapsulation efficiency indicates the carrier system's ability to retain the active compound effectively and minimize losses during the formulation process.

Catechin encapsulation using starch nanoparticles has been reported to yield efficiencies of 55.00–59.09%, while preserving antioxidant activity during *in vitro* digestion simulation [52]. Meanwhile, a significant increase in encapsulation efficiency to 86.5–88.2% has been reported using a combination of wet milling and ultrasonication in a β-glucan matrix, resulting in improved stability and biological activity compared to free catechin [53]. Therefore, encapsulation efficiency serves as a key indicator of formulation success in improving catechin stability.

Antioxidant Activity

The antioxidant activity of catechin can be evaluated using the DPPH and ABTS methods, which measure a compound's ability to scavenge free radicals (Table 3). However, catechin nanoemulsion formulations generally exhibit higher antioxidant activities than pure catechin extracts (52.832 ± 2.143 μg/mL for DPPH and 22.078 ± 5.690 μg/mL for ABTS) [31]. The acetone extract nanoemulsion showed DPPH antioxidant activity of 22.536 ± 2.330 μg/mL and ABTS activity of 15.086 ± 2.102 μg/mL. This enhancement was attributed to the smaller particle size and more homogeneous dispersion of catechin within the nanoemulsion system,

Table 3. Catechin content and antioxidant activity

Sample	Catechin content (mg CE/g sample) ± SD	Antioxidant activity			
		DPPH linear regression	DPPH-IC_{50} (μg/mL) ± SD	ABTS linear regression	ABTS-IC_{50} (μg/mL) ± SD
Aceton extract	255.65 ± 0.597	-	-	-	-
Nanoemulsion of acetone extract	64.51 ± 0.332	0.9991	22.536 ± 2.330	0.9939	15.086 ± 2.102

which increased the bioavailability and facilitated more effective interactions between the active compound and free radicals [54].

Catechin nanoemulsions have been shown to exhibit a significant improvement in antioxidant activity, as indicated by lower DPPH IC_{50} values (reflecting stronger activity) compared to unencapsulated catechin [55]. Catechin nanoemulsions demonstrate a significant improvement in antioxidant activity, as indicated by lower DPPH IC_{50} values (reflecting stronger activity) compared to unencapsulated catechin. Similarly, the ABTS assay results showed a consistent increase in activity, since $ABTS^+$ radicals are soluble in both aqueous and lipid phases, allowing better interaction with catechin dispersed in the nanoemulsion system. This is probably related to the different reagents used and the relatively poor selectivity of ABTS in reactions with hydrogen-atom donors (i.e., catechin) compared with DPPH [56]. This was influenced by differences in the dominant deactivation mechanisms between the two methods. Although both systems may involve single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms, ABTS radicals are more susceptible to the SET mechanism, whereas DPPH radicals are more inclined toward the HAT mechanism under appropriate conditions [57]. Therefore, although the ABTS assay is typically classified as a SET-based approach, the HAT mechanism is equally applicable [58].

■ CONCLUSION

The catechin nanoemulsion was successfully formulated and characterized, demonstrating suitable physicochemical properties and antioxidant activity. The analytical methods were properly validated, showing good linearity (high R^2), precision (low RSD), and reliability, confirming the accuracy of catechin quantification and antioxidant measurements. However, a bathochromic shift from 510 to 520 nm was observed, indicating changes in the microenvironment of catechin after incorporation into the nanoemulsion, suggesting physical interactions without structural alteration. Furthermore, differences in results of DPPH and ABTS assays are attributed to their distinct reaction mechanisms and solvent systems.

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■ CONFLICT OF INTEREST

The authors declare no conflict of interest.

■ AUTHOR CONTRIBUTIONS

Irma Antasionasti conceptualization, methodology, data curation, formal analysis, validation, visualization, writing original draft; Surya Sumantri Abdullah conducted resources, validation, writing review, and editing; Utami Sasmita Lestari conducted project administration, writing review, and editing. All authors agreed to the final version of this manuscript.

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