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Research Article

In-vitro study of potential antioxidant activities of mangosteen and its nanoemulsions

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ABSTRACT

Reactive Oxygen Species (ROS) are the major key component in cellular processes and participate in vital functions within cell signaling pathways, regulation of gene expression, and cellular metabolism. Mangosteen peel, rich in powerful antioxidants to prevent oxidative damage due to ROS, is believed to have potential health benefits. Nanoemulsion, a liquid dispersion with small droplets, can have significant applications in drug delivery due to their enhanced bioavailability and stability so which can be used to optimize the application of mangosteen peel extract. The aim of this research is to examine and compare the antioxidant potential of mangosteen peel compound and nanoemulsion (*Garcinia mangostana* L.). The mangosteen peel nanoemulsion was characterized by analysis using particle size analysis, zeta potential analysis and transmission electron microscopy. The antioxidant activity of several Mangosteen Nanoemulsion (MN) and isolate (MI) concentrations were analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), hydrogen peroxide (H_2O_2), nitric oxide (NO), and ferric reducing antioxidant power assays (FRAP). MN can be formulated and the average particle size of MN was 200.9 nm. The average zeta potential of MN was -69.36 mV. MN also has strong antioxidant activity. MI has higher antioxidant activities than MN. This study shows that the stability of MN and its potential as an antioxidant can be studied further in the field of pharmaceutical formulations.

Keywords: Antioxidant, *Garcinia mangostana* L., nanoemulsions

INTRODUCTION

Reactive Oxygen Species (ROS) are produced through metabolic activity present in nearly all types of cells. ROS have been mainly recognized as signaling molecules in response to abiotic and biotic stress-related events. These reactive molecules are produced in response to a variety of environmental and biological factors, and they play crucial roles in cell signaling pathways, regulation of gene expression, and cellular metabolism (Mhamdi and Van Breusegem, 2018). Antioxidants play a role in preventing oxidative damage due to ROS. Antioxidants can be found in a variety of sources. Among the most plentiful sources of antioxidants is present in higher plants and their various constituents, which contain numerous beneficial compounds (Shahidi and Zhong, 2015). Mangosteen, also known as *Garcinia mangostana* L., is a tropical fruit that is native to Southeast Asia. Mangosteen peel, one of the parts

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of the mangosteen, is highly valued for its bioactive compounds including polyphenols and xanthones, that have been found to have strong antioxidant properties (Suttirak and Manurakchinakorn, 2014). These compounds are known to play an essential function in the body's neutralization of damaging free radicals, leading to cellular damage and playing a role in the onset of various diseases. According to earlier studies, it was reported that mangosteen peel extract exhibits antioxidant properties as determined by the 2,2-diphenyl-1-pycrylhydrazyl (DPPH) test (Kusmayadi *et al*., 2019; Widowati *et al*., 2014). This exotic fruit has been extensively studied for its antioxidant activity and has shown promise in the prevention and treatment of a variety of health conditions. As such, mangosteen has gained popularity as a functional food and dietary supplement and is increasingly being recognized for its potential to support overall health and wellness (Ansori *et al*., 2022).

Nanoemulsion can be used to optimize the application of mangosteen peel extract because nanoparticles can help the absorption and penetration of the emulsion. Nanoemulsions are a type of two-phase liquid dispersion that is made up of two liquids that are not soluble in each other, either oil in water (O/W) or water in oil (W/O), which are stabilized by a special type of surfactant called an amphiphilic surfactant. These specialized emulsions are comprised of extremely small droplets that are ultrafine in nature. Small droplet characteristics can be analyzed by Particle Size Analysis (PSA), composition and concentration of droplet, Zeta Potential Analysis (ZPA), interfacial tension, and polydispersity (Sneha and Kumar, 2022). It can provide a range of beneficial properties, including differential drug loading, viscoelasticity, and unique visual characteristics. Nanoemulsions have potential in various applications, especially in drug delivery because of their unique properties. They can improve the bioavailability and effectiveness of therapeutic agents, as well as provide enhanced stability and controlled release. These properties make nanoemulsions a potential approach for the enhancement of novel drug delivery systems that can counter certain limitations of traditional drug delivery methods (Singh *et al*., 2017). Nanoemulsions are classified into 2 based on the droplet size, including up to 500 nm (milky) and 50-200 nm (transparent) (Sneha and Kumar, 2022). The advantages of nanoemulsion compared to macroemulsion are small droplet size, large surface area, high stability, better conclusivity, has drug carrier properties, and can form films on the skin (Li *et al*., 2018). Other studies also state that nanoemulsions are more stable kinetically than microemulsions, and the formation process depends on the droplet particles being dropped. (Yukuyama *et al*., 2016). The primary objective of this research is to analyze and compare the antioxidant capabilities of mangosteen peel nanoemulsion (*G. mangostana* L.) (MN) and mangosteen isolate (MI).

MATERIALS AND METHODS

Extract and isolate preparation

The maceration process was carried out for the extraction of mangosteen (*G. mangostana* L.) peel using a modified method (Widowati *et al*., 2014). The mangosteen peel was extracted using 96% ethanol for 24 hours, soaking three times. The extraction process was conducted repeatedly twice. The macerate was set apart from the residue and then evaporated at room temperature. Maserate was washed with hot water three to five times, then dried. Crude was added with 1:1 methylene chloride, stirred, and filtered, the residue and filtrate were separated. Then the residue was added to 1:2 chloroform, stirred, and filtered, the residue and filtrate were separated. The filtrate was then evaporated at room temperature until isolates were obtained.

Nanoemulsion preparation

Mangosteen peel nanoemulsion preparation was modified from Ahmad *et al.* (2019). For phase one, dissolve 0.05 gram of mangosteen in virgin coconut oil (VCO) 70 gram by heating at a temperature of 35°C, assisted by using a sonicator for 60 minutes with a maximum temperature of 65°C. For phase two, surfactant (sucrose monoester 1750) and cosurfactant (glycerol) were put into a glass beaker and stirred until cream formed. Lastly, mix phase one into phase two little by little until it runs out while still stirring on a hotplate with the heat of 50°C until a cream is formed, the stirring process is carried out for approximately 30 until 40 minutes (Ahmad *et al*., 2019).

Evaluation of nanoemulsion preparations

Evaluation of mangosteen nanoemulsion preparations was carried out through analysis of purity with a refractometer,

stability analysis with a centrifugator, and analysis with a spectrophotometer (Patil *et al*., 2021). Spectrophotometer measurements were made by measuring the nanoemulsion dispersion (made with a ratio of 1:100 aquadest) at a wavelength of 350-650 nm (Analytic Jenna). The sample's absorbance is determined similarly to the blank's absorbance. The transmittance percentage is calculated using the following equation:

$$\% T = 10^{-a}$$

Annotation:

T = Transmittance

A = Absorbance

Nanoemulsion characterization by PSA, ZPA and Transmission Electron Microscopy (TEM)

To determine the size of the particles and zeta potential, a dynamic light scattering particle size analyzer was used (Horiba SZ-100) for analysis, a cleaned cuvettt containing 10 mL of sample was put into the sample container (Atun *et al*., 2020). Meanwhile, to see the morphology of the nanoemulsion was done with TEM (HT7700).

Antioxidant activity by DPPH assay

In 96-well plates, 50 µl of MN and MI samples were added with 200 µl of DPPH (0.077 mmol; Sigma Aldrich, D9132). In the blank, 250 µl of DMSO was added, while of DPPH was added to the control with the same total volume. Incubated in the dark at 25°C for 30 min and measured with a microplate reader (Thermo Scientific) at 517 nm. The percentage of DPPH activity is obtained through the formulation below:

$$\% \text{ DPPH scavenging activity} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100$$

Additionally, the median inhibitory concentration (IC_{50}) for DPPH activity was determined (Rasmussen *et al*., 2020; Jadid *et al*., 2017; Widowati *et al*., 2022).

Antioxidant activity by ABTS assay

In 96-well plates, 2 µL of MN and MI samples were loaded with 198 µL of ABTS reagent (Sigma A1888-2G). A total of 200 µL ABTS was used as a control and 200 µL DMSO was used as a blank. Plates were incubated for 6 min (37°C). Absorbance was measured at 745 nm (Widowati *et al*., 2014, 2022; Prahastuti *et al*., 2020). The percentage of ABTS reduction activity is determined using the equation below:

$$\% \text{ ABTS reducing activity} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100$$

Antioxidant activity by H_2O_2 assay

Into the 96-well plates (sample well and control well), 12 µL of ferrous ammonium sulfate (1mM, Sigma Aldrich, 7783859) was added. Then, to the samples and blanks, 60 µL of MN and MI samples at different final concentrations were added, followed by H_2O_2 (3 µl, 5mM; Merck, 1.08597.1000) to the samples. In the blank and control, 90 µL and 63 µL sample solvent (DMSO) were added, respectively. Incubated for 5 minutes (dark place, at 25°C) after adding H_2O_2. After this, 1,10-phenanthroline (75 µL) was added to the control and sample mixture, which was then incubated for another 10 min (dark place, at 25°C) and measured at 510 nm (Prahastuti *et al*., 2020; Widowati *et al*., 2022). The percentage to determine the proportion of scavenging activity can be seen in the following equation:

$$\% H_2O_2 \text{ scavenging activity} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100$$

Antioxidant activity by NO assay

At 96-well-plates, the MN and MI samples were added with 10 mM sodium nitroprusside (106541, Merck) in phosphate-buffered saline (PBS) (Gibco, 1740576). Then, Griess reagent [a mixture of 0.1% N-(1-napthyl) ethylenediamine dihydrochloride (Sigma Aldrich, 22248), 2% H_3PO_4 (Merck 100573), and 1% sulfanilamide (Merck 111799)] was added. Then incubated at 25°C (2 h). Samples were measured at 546 nm (Prahastuti *et al*., 2020). The percentage of activity from scavenging of NO can be seen in the following equation:

$$\% \text{ NO scavenging activity} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100$$

Antioxidant activity by FRAP assay

In 96-well plates, 7.5 µL of different concentrations of MN and MI samples and solutions of FRAP (142.5 µL) (a mixture of acetate buffer (10 mL of 300 mM, pH 3.6) and ferric chloride hexahydrate (1 mL of 20 mM; Merck, 1.03943.0250, USA) in distilled water, and 2,4,6-Tris(2-

pyrid) yl)-s-triazine (TPTZ) (1 mL, 10 mM; Sigma Aldrich, 3682-35-7) in 40 mM HCl) was added. Then 150 µL of FRAP was added into the control sample, while DMSO was added to the blank sample, both having the same total volume. The mixture was then incubated at 37°C for 30 min. Subsequently, the absorbance was measured at 760 nm. Ferrous sulfate (in distilled water) was used for standardization (Prahastuti *et al*., 2020; Widowati *et al*., 2020).

Statistical analysis

Statistical analysis was performed using SPSS software (version 20.0), presenting the results as mean ± standard deviation. The normality and homogeneity of the data were assessed using the Saphiro Wilk test and Levene test, respectively. If the data was not normally distributed, the Kruskal-Wallis and Mann-Whitney U test were employed to examine group differences. When the data followed a normal distribution, analysis used One Way ANOVA with Tukey HSD Post-Hoc, and Independent Samples test was applied. Statistically significant differences were considered at a p-value ≤0.05 (Widowati *et al*., 2014, 2020).

RESULTS AND DISCUSSION

Stability test of mangosteen nanoemulsion

The MN is stable for 76 days. According to the analysis of MN dispersion at a wavelength of 650 nm, the recorded absorbance value was 0.108 nm, so the transmittance value obtained was 77.9%. From the results obtained, the nanoemulsion preparation had a level of clarity close to that of water (100%). While the purity value of the MN on the refractometer measurement showed a value of 1,333 nD and stability analysis through centrifugation results at 3800 rpm for 30 minutes did not show the separation of the oil and solid phases (Figure 1).

Characterization of mangosteen nanoemulsion preparations

PSA of mangosteen nanoemulsion

Based on the results of PSA measurements, the particle size of the MN was 200.9 nm, this indicates that the MN produced meets the requirements, where nanoemulsions have a size of 50-500 nm (Septiyanti *et al.,* 2022) with a polydispersity index (PDI) value of 0.361.

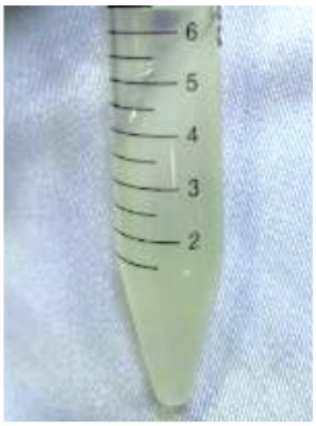

Figure 1: Stability analysis of mangosteen nanoemulsion by centrifugation

ZPA of mangosteen nanoemulsion

Based on the ZPA measurement results, the MN had an average zeta potential value of -69.36 ± 1.02 mV. The zeta potential value indicates the surface charge of the particle. A good particle charge indicates a strong repulsive force resulting in a stable dispersion and the smaller the possibility of aggregation forming. These results indicate that the potential value for the MN formulation is stable.

TEM of mangosteen nanoemulsion

Figure 2 shows the results of morphological analysis of MN using TEM at magnifications of 20000, 40000 and 80000 times. In the figure, it can be seen that the morphology of MN is in the form of a liquid sphere covered with a thick line (black arrow). This thick line is an emulsifier that protects the oil, while the phase that fills the thick line is the oil phase (a mixture of VCO and mangosteen isolate).

DPPH activity of mangosteen and mangosteen nanoemulsion

This assay is based on the formation of a non-radical compound form 2,2-diphenyl-1-picrylhydrazine. These non-radical compounds react with alcoholic solutions of DPPH in the presence of a hydrogen-providing antioxidant (DPPH-H). Usually, DPPH samples changed their purple color to

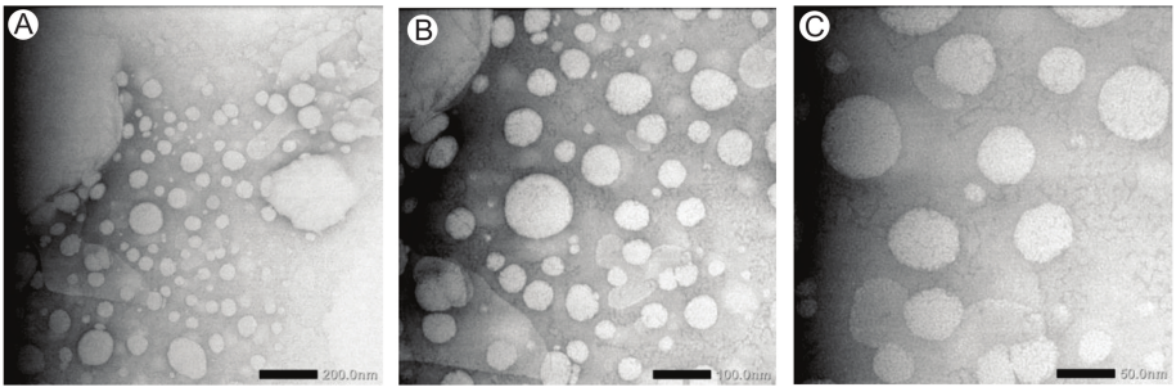

Figure 2: TEM characterization of mangosteen nanoemulsion at 20000X (A) 40000X (B) and 80000X (C) magnification

greenish yellow because antioxidants neutralize DPPH free radicals (Widowati *et al.*, 2020). The DPPH scavenging activity of mangosteen isolates and nanoemulsions is shown in Figure 3. In the DPPH assay, the greatest level of activity exhibited was at a concentration of MN 20 µg/mL (62.83 ± 0.66%), while for MI it was at a concentration of MI 20 µg/mL (81.25 ± 1.37%). The ability of these samples to scavenge free radicals increases as their concentration increases. In this study, MN and MI had antioxidant activity with an IC_{50} value of 9.88 ± 0.06 µg/mL and 8.06 ± 0.14, respectively (Table 1). When compared to the IC_{50} value of the nanomulsion form, the MI had a smaller IC_{50} value which indicates that the DPPH scavenging activity is higher than MN. This is also represented in the histogram Figure 3.

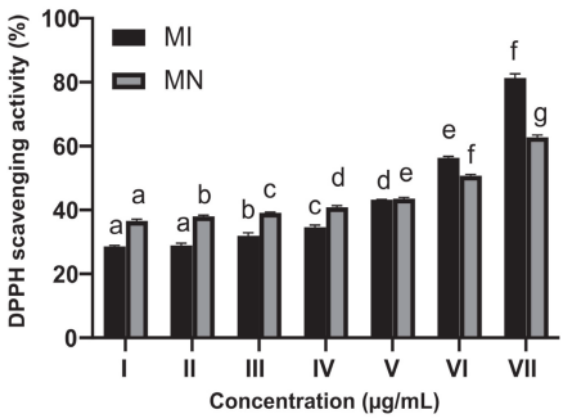

Figure 3: Percentage of DPPH scavenging activity in mangosteen and mangosteen nanoemulsion

*Data are presented as mean ± standard deviation. For each treatment, the assay was performed in triplicate. The different superscript (a, b, c, d, e, f, g) marks significant difference among various concentrations of MI ($P < 0.05$, Independent Samples Test) and MN ($P < 0.05$, Tukey's HSD test). Roman numeral I-VII represents the final concentration of MN and MI. I: 0.31 µg/mL; II: 0.63 µg/mL; III: 1.25 µg/mL; IV: 2.50 µg/mL; V: 5.00 µg/mL: VI: 10.00 µg/mL; VII: 20.00 µg/mL

Table 1: IC_{50} value of DPPH scavenging activity of mangosteen and mangosteen nanoemulsion

Sample	Equation	R^2	IC_{50} Value (µg/ml)
MI	y = 2.688x + 28.337	1.00	8.06 ± 0.14
MN	y = 1.2978x + 37.18	0.99	9.88 ± 0.06

ABTS assay of mangosteen and mangosteen nanoemulsion

ABTS reducing activity refers to the ability of a substance to neutralize or remove free radicals generated from ABTS molecules. This test is frequently utilized for assessing the antioxidant capacity of a sample or substance, where a reduction in absorbance at a certain wavelength indicates reduction of ABTS radicals by antioxidants. The percentage of ABTS reduction activity follows the concentration of the sample tested. Figure 4 illustrates the reducing activity of mangostin isolate and its nanoemulsion form at a p-value of less than 0.05. This showed clearly that the greater concentration of the test sample elevated the reduction activity of ABTS, both in MI (86.07 ± 0.06%) and MN (41.52 ± 0.53%). When the two are compared, MI had higher activity than MN, this is also shown in the IC_{50} value of MI which has a smaller value than MN (Table 2).

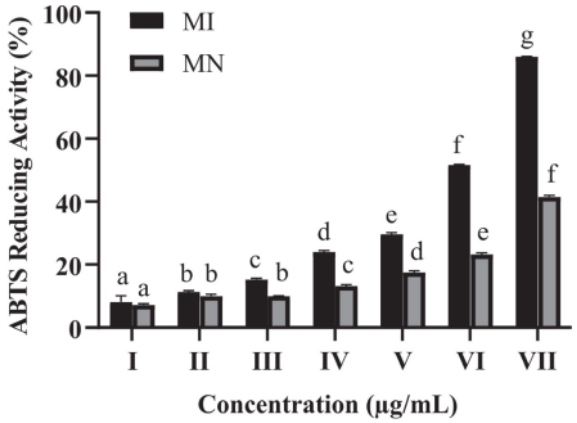

Figure 4: Percentage of ABTS reducing activity in mangosteen and mangosteen nanoemulsion

*Data are presented as mean ± standard deviation. For each treatment, the assay was performed in triplicate. The different superscript (a, b, c, d, e, f, g) marks significant difference among various concentrations of MI ($P < 0.05$, Mann Whitney U) and MN ($P < 0.05$, Tukey's HSD test). Roman numeral I-VII represents the final concentration of MN and MI. I: 0.23 µg/mL; II: 0.47 µg/mL; III: 0.94 µg/mL; IV: 1.88 µg/mL; V: 3.75 µg/mL: VI: 7.50 µg/mL; VII: 15.00 µg/mL

Table 2: IC_{50} value of ABTS reduction activity of mangosteen and mangosteen nanoemulsion

Sample	Equation	R^2	IC_{50} Value (µg/ml)
MI	y = 5.1624x + 10.317	0.99	7.68 ± 0.03
MN	y = 2.2017x + 8.1281	0.99	19.02 ± 0.36

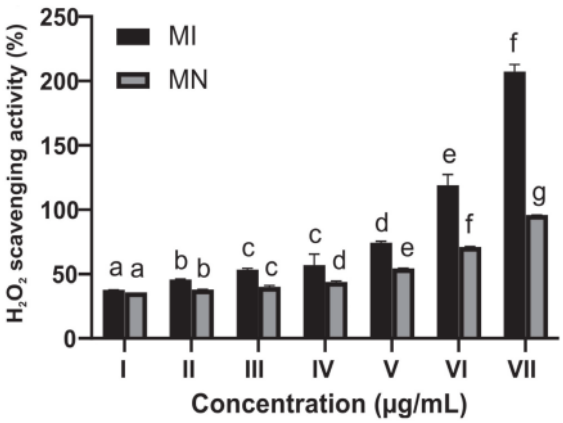

Figure 5: Percentage of H_2O_2 scavenging activity in mangosteen and mangosteen nanoemulsion

*Data are presented as mean ± standard deviation. For each treatment, the assay was performed in triplicate. The different superscript (a, b, c, d, e, f, g) marks significant difference among various concentrations of MI ($P < 0.05$, Mann Whitney U) and MN ($P < 0.05$, Tukey's HSD test). Roman numeral I-VII represents the final concentration of MN and MI. I: 6.25 µg/mL; II: 12.50 µg/mL; III: 25.00 µg/mL; IV: 50.00 µg/mL; V: 100.00 µg/mL: VI: 200.00 µg/mL; VII: 400.00 µg/mL

Table 3: IC_{50} value of H_2O_2 scavenging activity of mangosteen and mangosteen nanoemulsion

Sample	Equation	R^2	IC_{50} Value (µg/ml)
MI	y = 0.4198x + 37.413	0.99	29.92 ± 6.61
MN	y = 0.1532x + 36.843	0.99	85.88 ± 1.34

H_2O_2 assay of mangosteen and mangosteen nanoemulsion

Hydrogen peroxide is a precursor to hydroxyl radicals that can cause damage to the cells. Hydrogen peroxide can also inactivate some enzymes by oxidizing important thiol (-SH) groups. Figure 5 shows that the H_2O_2 scavenging activity in the MN and MI samples showed a significant increase depending on the high concentration of the samples. The histogram clearly showed that the highest concentration showed highest scavenging activity at MN (95.51 ± 0.13%) and MI (207.49 ± 5.37%). When MN and MI are compared, the nanoemulsion form of mangosteen isolate was lower than the isolate form, this is confirmed in Table 3 which shows that the IC_{50} value of MI is lower than that of MN.

NO assay of mangosteen and mangosteen nanoemulsion

NO scavenging activity refers to a substance's ability to neutralize or remove NO from a solution. It is an important property for substances with potential therapeutic applications, as it can help regulate NO levels in the body and prevent diseases associated with excessive NO production. The NO scavenge activity of MN and MI is shown in Figure 6. In this assay, the most significant activity was observed at an MN concentration of 133.33 µg/mL (59.08 ± 0.31%), while for MI it was at a concentration of MI 33.33 µg/mL (74.33 ± 1.00%). The capacity of MN and MI to scavenge free radicals grows in direct correlation with the rising concentration in the sample. From the results of this study, MN and MI had antioxidant activity with their

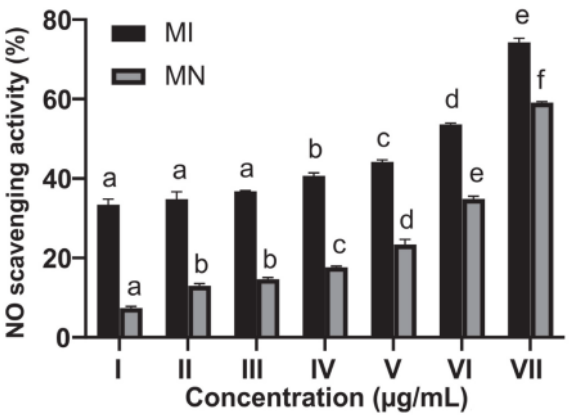

Figure 6: Percentage of NO scavenging activity in mangosteen and mangosteen nanoemulsion

*Data are presented as mean ± standard deviation. For each treatment, the assay was performed in triplicate. The different superscript (a, b, c, d, e, f) marks significant difference among various concentrations of MI ($P < 0.05$, Independent Samples Test) and MN ($P < 0.05$, Tukey's HSD test). Roman numeral I-VII represents the final concentration of MN and MI. Variation of MI concentration: I: 0.52 µg/mL; II: 1.04 µg/mL; III: 2.08 µg/mL; IV: 4.17 µg/mL; V: 8.33 µg/mL: VI: 16.67 µg/mL; VII: 33.33 µg/mL. Variation of MN concentration: I: 2.08 µg/mL; II: 4.17 µg/mL; III: 8.33 µg/mL; IV: 16.67 µg/mL; V: 33.33 µg/mL: VI: 66.67 µg/mL; VII: 133.33 µg/mL

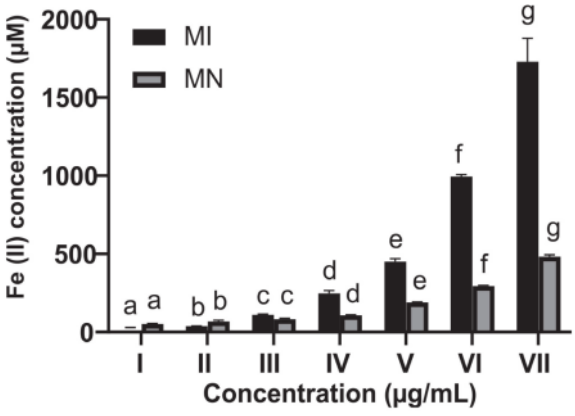

Figure 7: Percentage of Fe (II) Concentration in mangosteen and mangosteen nanoemulsion

*Data are presented as mean ± standard deviation. For each treatment, the assay was performed in triplicate. The different superscript (a, b, c, d, e, f, g) marks significant difference among various concentrations of MI ($P < 0.05$, Independent Samples Test) and MN ($P < 0.05$, Mann Whitney U). Roman numeral I-VII represents the final concentration of MN and MI. I: 1.56 µg/mL; II: 3.13 µg/mL; III: 6.25 µg/mL; IV: 12.50 µg/mL; V: 25.00 µg/mL: VI: 50.00 µg/mL; VII: 100.00 µg/mL

Table 4: IC_{50} value of NO scavenging activity of mangosteen and mangosteen nanoemulsion

Sample	Equation	R^2	IC_{50} Value (µg/ml)
MI	y = 1.2092x + 34.006	0.99	13.23 ± 0.01
MN	y = 0.3677x + 10.416	0.99	107.66 ± 0.55

respective IC_{50} values shown in Table 4. When compared to the IC_{50} value of the nanomulsion form, the MI has a smaller IC_{50} value which indicates that the H_2O_2 scavenging activity is higher. This is also represented in the histogram Figure 6.

FRAP assay of mangosteen and mangosteen nanoemulsion

The FRAP method works by utilizing the antioxidant's capacity to transfer electrons and convert Fe^{3+} ions to Fe^{2+} ions in the presence of TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), which leads to the development of a vivid blue Fe^{2+}-TPTZ complex (Joseph *et al.*, 2016). The most effective FRAP activity of nanoemulsion containing mangosteen was at 50 µg/mL concentration present in the study with the most effective activity of FRAP on MN and MI was at a concentration of 100 µg/mL. The FRAP activity of this sample increased significantly with increasing concentrations of MN (482.87 ± 11.65 µM Fe(II)/µg sample) and MI (1728.66 ± 150.08 µM Fe(II)/µg sample). If the two are compared, the activity value of FRAP MI presented on the histogram (Figure 7) shows a higher value compared to MN, this is also comparable to the IC_{50} value of MN which displays a greater value than MI.

The main focus of this research is to assess the antioxidant potential of mangosteen isolate nanoemulsion through the utilization of multiple assays, including the DPPH, ABTS, H_2O_2, NO, and FRAP assays and their comparison to mangosteen isolate. The nanoemulsion made in this study consisted of a mixture of two phases, the oil phase (Virgin Coconut Oil (VCO) and mangosteen) and the emulsifier/surfactant and co-surfactant phases (sucrose monoester 1750 and glycerol) using a sonicator. VCO was chosen as the oil phase in the nanoemulsion formula because it is better at maintaining the stability of the nanoemulsion compared to olive oil (Atun *et al.*, 2020). This study incorporates co-

surfactants to reinforce the surfactant anchorage, as they help reduce the surface tension between the two phases. This addition of amphiphilic short-chain molecules aims to achieve a surface tension nearing the value to zero, thereby enhancing the flexibility of the interfacial film. (Rachmawati *et al*., 2015). In addition, the sonication process significantly helps in reducing the droplet size of the nanoemulsion and promotes the formation of smaller and more uniform droplets (Branco *et al*., 2020).

This is supported by the results of the evaluation of the nanoemulsion in this study which showed that the mangosteen nanoemulsion was stable after 76 days of storage which was also shown from the results of particle size, polydispersity index, refractive index, transmittance, and centrifugation (Figure 1). The success of nanoemulsion formation in this study was confirmed by the measurement results with PSA which showed that the particle size entered the required range (50-500 nm). The particle size of 200.9 nm and the monodispersity form shown in the PSA results will lead to easy solubility without changing the droplet size distribution (Ranjan *et al*., 2016). The polydispersity index value shown in the results of this study shows an acceptable value (Danaei *et al*., 2018). In addition, the zeta potential value in this study also showed a value below 30 mV. This implies that the electrostatic stability of the mangosteen nanoemulsion formulation is ensured because the droplets carry a robust electric charge, leading to the dominance of repulsive forces among the droplets in the nanoemulsion system (Salvia-Trujillo *et al*., 2015). PSA and ZPA results were also confirmed morphologically by TEM analysis which showed that the particle size in this study was quite uniform and homogeneous (Figure 2).

In this study, mangosteen isolate was used as a mixture of the oil phase formula. It is well known that there are various structural compositions of isolates isolated from mangosteen peel (Suttirak and Manurakchinakorn, 2014) are confirmed that to exhibit strong antioxidant activity (Moongkarndi *et al*., 2014). The results of this study also confirmed that mangosteen isolate and nanoemulsion samples had antioxidant activity through DPPH, ABTS, H_2O_2, NO, and FRAP assays (Figure 3-7). However, in contrast to the mangosteen isolate, the antioxidant activity for the entire assay was classified as very strong compared to the nanoemulsion form (Table 1-4), although in the nanoemulsion form, the antioxidant activity was still relatively strong (Haerani *et al.,* 2019). This is because the amount of mangosteen isolate in the nanoemulsion is less than when measuring the form of the isolate directly. On the contrary, some research indicates that increased surfactant concentrations can aid in isolates dissolution within the oil phase, resulting in elevated antioxidant activity. The radical scavenging activity of isolate nanoemulsion is subject to variation based on the concentrations of oil and surfactant. This variation occurs due to their impact on the level of dissolved isolates, which serves as an antioxidant (Joung *et al*., 2016).

CONCLUSION

The nanoemulsion in this study was stable, which was shown through the results of the evaluation and characterization results. Mangosteen has a higher antioxidant activities than nanoemulsion, although the mangosteen nanoemulsion is still classified as having a strong antioxidant activities. Overall, the results of this study report that the mangosteen nanoemulsion has the potential as a pharmaceutical formulation preparation.

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Conflict of interest

The authors declare no conflict of interest

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