The Abundance of Bioactive Compound in Fingerroot Essential Oil Before and After Self Nanoemulsifying Drug Delivery System (SNEDDS)

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ABSTRACT

This research focuses on optimizing and stabilizing the essential oil of fingerroot (Boesenbergia rotunda), a plant native to Indonesia, using a self-nanoemulsifying drug delivery system (SNEDDS) technology. This essential oil possesses antimicrobial, anti-inflammatory, and antifungal properties, making it a potential alternative to synthetic antibiotics. However, its low bioavailability and volatility limit its usage. The study involved two stages: nanoemulsion creation by D-Optimal method using Design Expert software version 13 and stability analysis of bioactive compounds through gas chromatography-mass spectrometry (GC-MS). The optimized formula consisted of fingerroot essential oil, virgin coconut oil (VCO), Tween 80, and Polyethylene Glycol (PEG) 400 in percentages of 12.61%, 12.61%, 53.65%, and 21.12%, respectively. Prior to nanoemulsion, the essential oil contained 28.29% camphor and 27.13% 1,8-cineole, which are the highest bioactive compounds. After applying the SNEDDS technology, the camphor content decreased to 25.17%, while the 1,8-cineole content reduced to 13.05%. These findings indicate that while the concentration of bioactive compounds in fingerroot essential oil decreases, the oil itself facilitates its potential application in pharmaceutical and therapeutic fields as SNEDDS base, characterized by valuable particle size and polydispersity index (PDI).

Keywords: Essential Oil, Fingerroot, Nanoemulsion, SNEDDS

INTRODUCTION

Over the past few decades, the use of antibiotic growth promoters (AGPs) as growth stimulants for livestock has been strictly banned worldwide. In line with this, Indonesia has implemented regulations prohibiting the use of antibiotics through the Minister of Agriculture Regulation No. 14/Permentan/PK.350/5/2017. This prohibition has prompted researchers and stakeholders to seek alternative solutions to replace AGPs in livestock production. Furthermore, Indonesia’s average temperature and relative humidity range from 27 °C to 30 °C and 80%, respectively. These high temperatures and humidity levels significantly increase livestock susceptibility to parasitic and pathogenic bacterial infections (Hadi et al., 2013; Gopi, 2014).

Indonesia is rich in various tropical plants that offer potential and diverse health benefits, serving as a promising alternative to AGPs (Arniputri et al., 2007). Phytochemicals have emerged as viable alternatives derived from plant parts, including leaves, roots, and flowers. These phytochemicals exhibit functionalities akin to antibiotics, encompassing various effects, including antiparasitic properties, feed efficiency enhancement, growth promotion, and antimicrobial effects (Shah, 2015; Ujilestari et al., 2019). The presence of bioactive compounds in these plants is attributed to the pharmacological effects of these phytochemicals. Phytochemicals are typically classified into three main groups: herbs, spices, and essential oils (Shah, 2015). Notably, essential oils have demonstrated the
ability to enhance feed utilization efficiency in livestock, thus providing a potential substitute for AGPs. This effect can be attributed to the stimulation of digestive enzymes by essential oils, which, in turn, improves the breakdown of feed substrates into essential nutrients. As a result, increased nutrient availability facilitates greater molecular solvent, ultimately contributing to improved livestock production (El-Hack et al., 2022).

One plant with potential as a source of phytochemicals is fingerroot (Boesenbergia rotunda), native to Indonesia and found in other countries such as China, Sri Lanka, India, Malaysia, and Thailand. Fingerroot is harvested every 4–5 months and can grow throughout the year (Aiswarya, 2015; Baharudin et al., 2015). Fingerroot contains various bioactive compounds, including camphor, 1,8-cineole, camphene, trans geraniol, and linalool. The major bioactive compounds in fingerroot are camphor and 1,8-cineole, which have been reported as antimicrobial agents capable of inhibiting pathogenic bacteria such as Escherichia coli, Salmonella sp., and Staphylococcus aureus (Mikususanti et al., 2008; Chahyadi et al., 2014). However, the therapeutic benefits of fingerroot essential oil cannot be fully utilized due to its low bioavailability (solubility) and high volatility (evaporation) (Silalahi, 2017). Therefore, there is a need for technology that can enhance bioavailability and reduce the volatile nature of fingerroot essential oil to ensure its effective and efficient utilization as a potential substitute for synthetic antibiotics.

Nanoemulsion refers to an isotropic dispersed system of two immiscible liquids, namely oil in water (o/w) or water in oil (w/o), with an average particle size ranging from 100 to 500 nm (Joye & McClements, 2013; Abbas et al., 2014; Tsai et al., 2014; Kwagiruch et al., 2016). The objectives of utilizing nanoemulsion technology include protecting bioactive compounds from evaporation before reaching the small intestine for absorption, as well as delivering water-insoluble bioactive components of plants that can be easily dissolved in drinking water (Joye & McClements, 2013). However, nanoemulsions are inherently thermodynamically unstable and sensitive to various factors, such as dilution, sunlight, temperature, and pH changes (Hao et al., 2018; Liu et al., 2019; Saani et al., 2019). Achieving high kinetic stability through ultrasonication becomes crucial to enhance the performance of surfactant molecules that possess both lipophilic and hydrophilic properties and reduce particle size (Borai et al., 2018). The structural arrangement of nanoemulsion particles delivers the properties of non-polar compounds, such as oil into water or water into oil. In this arrangement, the semi-polar components are located on the outer part, which includes the amphiphilic core consisting of surfactants, cosurfactants, and water. Meanwhile, the non-polar components, represented by the lipophilic core, are readily soluble in water (Joye & McClements, 2013).

The D-optimal design combines surfactants, cosurfactants, and carrier oils to formulate SNEDDS. Fingerroot essential oil is volatile, so it is necessary to add a carrier oil, such as virgin coconut oil (VCO). VCO is commonly used as a carrier oil in nanoemulsion preparation due to its easy digestibility and non-interference with the composition of essential oils. In addition, VCO was selected as phase oil because VCO can bond with tween 80 (Anindhita et al., 2016; Ujilestari et al., 2019). The choice of surfactants is often based on their compatibility with the oil phase and ability to form stable nanoemulsions, such as Tween 80 (Sugumar et al., 2014; Chellapa et al., 2015; Halnor et al., 2018). Cosurfactants are selected based on their ability to enhance the surfactant’s performance. Short- and medium-chain alcohols such as butanol, pentanol, isopropanol, and propylene glycol can act as cosurfactants, reducing surface tension and increasing fluidity. They also influence water solubility and the oil phase through partitioning between these phases (Khataee et al., 2018). Considering that nanoemulsion consists of multiple components, it is essential to consider the quantity and stability of bioactive compounds within the nanoemulsion, both before and after SNEDDS, to ensure the effective utilization of nanoemulsion from fingerroot essential oil as an antimicrobial agent and a potential substitute for AGPs.

MATERIALS AND METHODS
The fresh fingerroot (Boesenbergia rotunda) used in this study was sourced from Boyolali, Central Java, Indonesia. Virgin coconut oil, provided by the Lansida Group, Yogyakarta, Indonesia, was utilized as a carrier oil in nanoemulsion preparation. Tween 80 from KAO Indonesia Chemical in Bekasi, Indonesia, and Polyethylene Glycol (PEG) 400 from Petronas Chemicals in Labuan, Malaysia, were employed as surfactants and cosurfactants, respectively. For the preparation of the artificial gastric fluid (AGF), a
mixture of 0.9% NaCl (Merck, Darmstadt, Germany), 37% HCl (Mallinckrodt, England), and distilled water (aquades) were combined. The method employed in this study was a trial by D-Optimal method using Design Expert software version 13 (State-Ease, Inc., Minneapolis, MN, USA) to prepare and optimize self-nanoemulsifying drug delivery systems (SNEDDS). The results of the first phase were statistically analyzed using a t-test. In the second phase of the study, the composition and stability of the essential oil nanoemulsion of fingerroot were determined using gas chromatography mass-spectrometry (GC-MS), which was analyzed descriptively.

Production of Fingerroot Essential Oil

The production of fingerroot essential oil was carried out following the procedure by Gakuubi (2016). Initially, 500 kg of fingerroot was thoroughly washed to remove impurities. The fingerroot was then finely chopped and left to air dry at room temperature (25 °C) for one day and one night. Afterward, the finely chopped fingerroot was subjected to steam distillation for six hours. The distillation process resulted in the separation of water, which primarily occurred approximately four hours after the initiation of the process. Following the initial four hours, the essential oil began to emerge. From 500 kg of fingerroot, approximately 500 mL of fingerroot essential oil was obtained through steam distillation.

Analysis of Bioactive Compound Content in Fingerroot Essential Oil

The chemical composition of fingerroot essential oil was analyzed at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta, Indonesia. A volume of 1 mL of a volume of 1 mL of fingerroot essential oil was subjected to identification using a gas chromatography-mass spectrometer (GC-MS). The analysis was performed using an Agilent HP-5MS column with a length of 30 m and a diameter of 0.25 mm. Helium was utilized as the carrier gas with a column pressure of 13.0 kPa. Qualitative testing was done by setting the column temperature to 50 °C and the injector temperature to 300 °C. The oven temperature program was set to start at 50 °C for five min, followed by an increased temperature to 240 °C, which was maintained for seven min. The identification of compounds in fingerroot essential oil, or retention index (RI), was obtained by comparing the results obtained with the Mass Spectral (MS) data system (Daning et al., 2022).

Preparation of SNEDDS (Self-Nanoemulsifying Drug Delivery System)

After determining the chemical composition of fingerroot essential oil, the preparation of fingerroot essential oil-based SNEDDS involved several stages, including formulation, optimization, and characterization. Each stage is described in the following sections.

SNEDDS formulation

The SNEDDS formulation entailed the development of a self-nanoemulsion using the water titration method. Virgin coconut oil (VCO) and fingerroot essential oil were utilized as the oil phase, while Tween 80 served as the surfactant, and PEG 400 was used as the cosurfactant. The fingerroot essential oil nanoemulsion preparation process involves magnetic stirring at a speed of 700 rpm for 15 min, followed by ultrasonication for 10 min. Subsequently, the mixture was immersed in a water bath at 45 °C for 15 min. The formulation of SNEDDS commences by determining the ratio of VCO and fingerroot essential oil as the oil phase. The ratio between fingerroot essential oil and VCO as the carrier oil on SNEDDS was 0.5:0.5, Tween 80 was 2, and PEG 400 was 1. Hence, the ratio composition of SNEDDS was 1:2:1. The appearance of the SNEDDS was employed as testing parameters. In this stage, Tween 80 and PEG 400 were combined at the same concentration ratio. In the oil phase, VCO was added to the Tween 80 and PEG 400 mixture. Subsequently, the fingerroot essential oil was gradually added dropwise to the mixture of VCO, surfactant, and cosurfactant to form the SNEDDS.

SNEDDS optimization

The optimization ratio of SNEDDS is determined by selecting the lowest amount of Tween 80 (in mL) that yields the highest transmittance value. The formula establishes a lower limit of 1:1:1 and an upper limit of 1:3:1. Once the upper and lower limits are set, the values of each component within the upper and lower limits are entered into the D-Optimal method using Design Expert software (Basalious et al., 2010). The lower limit values for oil, Tween 80, and PEG 400 are 33.33%, while the upper limit values are 20% for oil, 60% for Tween 80, and 20% for PEG 400. This results in 16 formulas (Table I).
The compositions of VCO, fingerroot essential oil, Tween 80, and PEG 400 are prepared according to the runs specified by the D-Optimal method using Design Expert software version 13 (Stat-Ease, Inc., Minneapolis, MN, USA), response parameters are transmittance and emulsification time.

The transmittance test evaluates the clarity level of the self-nanoemulsion drug delivery system (SNEDDS) containing the essential oil of fingerroot. The analysis is performed by measuring the transmittance at a wavelength of 650 nm using a UV/VIS spectrophotometer, with sterile water used as a blank. The SNEDDS formulation that produces the clearest combination with the lowest surfactant composition is selected as the optimal SNEDDS formulation (Gaur et al., 2014; Khan et al., 2015). To conduct the transmittance test, a 1 mL sample of the formulation is taken and diluted in 50 mL of purified water. Then, a blue tip transfers 200 µL of the diluted sample into a microplate. The transmittance percentage is measured using a UV/VIS spectrophotometer at a wavelength of 650 nm (Gaur et al., 2014; Khan et al., 2015; Ujilestari et al., 2019). The emulsification time test was conducted using gastric fluid, which was prepared by combining 2 mL of 37% hydrochloric acid and 2 grams of NaCl in distilled water. The artificial gastric fluid had a pH range of 2-3. A 1 mL formulation of SNEDDS was added, according to the optimized formula obtained from the optimization test, into the artificial gastric fluid, totaling 500 mL, at a predetermined temperature of 40 °C. Subsequently, the mixture was stirred using a magnetic stirrer at 100 rpm. Visual observations were made, and the emulsification time was recorded, following the method described by Parmar et al. (2011).

**SNEDDS characterization**

The characterization of SNEDDS involves particle size analysis and polydispersity index (PDI), which is performed through a particle size analyzer (PSA). PSA is employed to determine the size of emulsion droplets and their size distribution (Malvern MAL1244, DKSH Indonesia, Jakarta, Indonesia). The dynamic light scattering (DLS) method analyzes droplet size. The sample is diluted with water at a ratio of 1:1000 (v/v), and the mixture is stirred using a magnetic stirrer for five min. The size of the formulated nanoemulsion droplets and their polydispersity index (PDI) are analyzed and measured using PSA (Farouk et al., 2016).

**Analysis of Bioactive Compound in SNEDDS Containing Fingerroot Essential Oil**

The chemical composition of SNEDDS containing the fingerroot essential oil is analyzed at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta, Indonesia. Fingerroot essential oil (1 mL) is identified using a Gas Chromatography-Mass Spectrometer (GC-MS).

### Table I. Formulation design and observation results of emulsification time and transmittance for SNEDDS by D-Optimal method using Design Expert software version 13 (Stat-Ease, Inc., Minneapolis, MN, USA)

<table>
<thead>
<tr>
<th>Run</th>
<th>Oil</th>
<th>Surfactant</th>
<th>Co-Surfactant</th>
<th>Emulsification time (s)</th>
<th>Transmittance (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>33.33</td>
<td>39.50</td>
<td>27.16</td>
<td>38.57</td>
<td>89.46</td>
</tr>
<tr>
<td>2</td>
<td>33.33</td>
<td>46.67</td>
<td>20.00</td>
<td>26.47</td>
<td>91.06</td>
</tr>
<tr>
<td>3</td>
<td>33.33</td>
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<td>27.16</td>
<td>49.71</td>
<td>89.79</td>
</tr>
<tr>
<td>4</td>
<td>26.61</td>
<td>40.05</td>
<td>33.33</td>
<td>38.51</td>
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</tr>
<tr>
<td>5</td>
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<td>34.48</td>
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<td>28.24</td>
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</tr>
<tr>
<td>6</td>
<td>25.89</td>
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</tr>
<tr>
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<td>40.96</td>
<td>90.96</td>
</tr>
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<td>8</td>
<td>32.18</td>
<td>34.48</td>
<td>33.33</td>
<td>41.48</td>
<td>76.82</td>
</tr>
<tr>
<td>9</td>
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<tr>
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<td>20.00</td>
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<td>33.33</td>
<td>37.91</td>
<td>90.84</td>
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<tr>
<td>12</td>
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<td>20.00</td>
<td>27.42</td>
<td>90.48</td>
</tr>
<tr>
<td>13</td>
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<td>28.46</td>
<td>85.47</td>
<td>90.83</td>
</tr>
<tr>
<td>14</td>
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<td>20.00</td>
<td>44.46</td>
<td>91.37</td>
</tr>
<tr>
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<td>90.80</td>
</tr>
<tr>
<td>16</td>
<td>20.00</td>
<td>60.00</td>
<td>20.00</td>
<td>46.77</td>
<td>90.37</td>
</tr>
</tbody>
</table>
The column used is DB-5MS, with a length of 30 m and a diameter of 0.25 mm. The carrier gas is helium, with a column pressure of 24.7 kPa. The qualitative testing is performed by setting the column temperature to 50°C and the injector temperature to 300°C. The oven temperature program is initially set at 50°C for five min, then increased to 240°C and maintained for 17 min. The identification of compounds in Zedoary essential oil, or the Retention Index (RI), is obtained by comparing the results obtained with the Mass Spectral (MS) data system (Kassem et al., 2016).

**RESULTS AND DISCUSSION**

The optimization of VCO, fingerroot essential oil, Tween 80, and PEG 400 for SNEDDS, based on the D-Optimal method using Design Expert software version 13 (Stat-Ease, Inc., Minneapolis, MN, USA), with respective ratios of...
0.5, 0.5, 2, and 1 mL (Table I). The result was performed to determine the optimal transmittance and emulsification time values.

Based on the analysis results of the D-Optimal method using Design Expert software version 13 (StatEase, Inc., Minneapolis, MN, USA), it is evident that the data in the normal plot of residuals for the emulsification time response of SNEDDS containing fingerroot essential oil (Figure 1) are normally distributed and clustered around the diagonal line. This can be observed from the absence of potential outliers deviating significantly from the diagonal line. The clarity of a nanoemulsion can be determined by its transmittance value. The clearer the nanoemulsion, the closer the transmittance value is to 100 (Madan et al., 2014). The linear model of transmittance (Figure 1) indicates that the quantities of oil, Tween 80, and PEG 400 do not affect the transmittance response. The preferred color approaches red (Patel et al., 2011).

Therefore, ANOVA can be performed to investigate the indication of significant differences based on a probability of less than five percent (Kumari et al., 2013). Using a cubic model, the ANOVA statistical analysis was performed on the emulsification time of fingerroot essential oil (Table II). The results indicated a significant effect (p<0.05), suggesting that changes in the formulation of each SNEDDS sample would influence the emulsification time. The lack of fit (p>0.05) indicated no significant difference between the predictions generated by the D-optimization design and the actual experimental values in the field. A desirable emulsification time in the model is indicated by approaching the blue color, which signifies a shorter time required for the surfactant and co-surfactant to emulsify the oil in water (Patel et al., 2011). Using a linear model, the ANOVA statistical analysis was conducted on the transmittance values of fingerroot essential oil (Table II). The results indicated a non-significant effect (p>0.05), suggesting that changes in the formulation of each SNEDDS sample would not affect the transmittance values. The lack of fit (p>0.05) indicated no significant difference between the predictions generated by the D-Optimal method using Design Expert software version 13 and the actual experimental values in the field. Transmittance values above 90 percent are desirable, indicating that the emulsion is approaching a nano-sized state (Bali et al., 2011).

Verification of optimization results
The solution formula from the D-Optimal method using Design Expert software version 13 consisted of fingerroot essential oil, virgin coconut oil (VCO), Tween 80, and Polyethylene Glycol (PEG) 400 in percentages of 12.61, 12.61, 53.65, and 21.12%, respectively. The experimental results were verified using a t-test performed in IBM SPSS Statistics version 25, and the findings are presented in Table 3. Based on Table 3, it can be observed that all the p-values (p>0.05) indicate that there is no significant difference between the predicted and actual values for both the emulsification time and transmittance variables. This suggests that the optimal formulation has been successfully verified. Therefore, the optimal formula can be validated (Wulandari et al., 2016).

SNEDDS characteristics
Particle size and polydispersity index (PDI) (Figure 2) were determined from particle size analysis, yielding 22.92 nm and 0.402, respectively. Nanotechnology is a technology used to reduce the particle size of bioactive compounds for enhanced solubility and improved functionality as a drug within the stomach (Yuliasari et al., 2022). Particle sizes below 100 nm and a PDI less than 0.5 are considered stable (Wulandari et al., 2016).

The abundance of bioactive compounds in fingerroot essential oil nanoemulsion
The combination of VCO, fingerroot essential oil, Tween 80, and PEG 400 is “after SNEDDS,” while “before SNEDDS” only consists of Fingerroot essential oil. Fingerroot essential oil exhibits abundant bioactive compounds before and after SNEDDS formulation, as shown in Table 4. Identification results from Gas Chromatography-Mass Spectra (GC-MS) of fingerroot essential oil revealed the presence of six major bioactive compound components: camphor (28.29%), 1,8-cineole (27.13%), trans geraniol (12.13%), camphene (11.43%), 1,3,6-octatriene-3,7-dimethyl (5.56%), and two propanoic acids (5.12%). After the SNEDDS formulation, the identified amounts of these compounds were as follows: camphor (25.17%), 1,8-cineole (13.05%), trans geraniol (12.13%), camphene (5.30%), 1,3,6-octatriene-3,7-dimethyl (4.41%), and 2-propanoic acids (2.93%).

The results from GCMS analysis show a decrease in the abundance of fingerroot essential oil bioactive compounds by 17.47% after SNEDDS formulation based on GC-MS analysis, except for trans geraniol, which remained relatively stable.
The SNEDDS formulation contained 17.47% ethylene glycol as an organic compound used in the formulation with PEG 400, while 14.53% consisted of trace amounts of unidentified bioactive compounds. The combination of fingerroot essential oil, VCO, Tween 80, and PEG 400 in proportions of 12.61%, 12.61%, 53.65%, and 21.12%, respectively, resulted in camphor and 1,8-cineole amounts of 3.17% and 1.53% per 100 mL in the SNEDDS formulation.

Bioactive compounds in plants may experience reduced damage due to exposure to ultraviolet light, pH variations, or other chemical components from applying SNEDDS. SNEDDS minimizes damage during processing, storage, transportation, or digestion (Perez-Esteve et al., 2013). Therefore, a 17.47% reduction in bioactive compounds after SNEDDS formulation. SNEDDS can protect active compounds from external factors, enhance solubility, expand functionality, maintain the stability of active compounds, improve absorption, enhance uniformity of final food products, and modify physical and organoleptic properties (Pateiro et al., 2021).

**CONCLUSION**

The optimization of fingerroot essential oil SNEDDS was achieved by combining fingerroot essential oil, VCO, Tween 80, and PEG 400 in proportions of 12.61%, 12.61%, 53.65%, and

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**Table IV. Bioactive Compounds Before and After SNEDDS Formulation**

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Camphene</td>
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</tr>
<tr>
<td>2</td>
<td>1,8-cineole</td>
<td>27.13</td>
</tr>
<tr>
<td>3</td>
<td>1,3,6-octatriene-3,7-dimethyl</td>
<td>5.56</td>
</tr>
<tr>
<td>4</td>
<td>Trans geraniol</td>
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</tr>
<tr>
<td>5</td>
<td>Champor</td>
<td>28.29</td>
</tr>
<tr>
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<td>2-preponoic acid</td>
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</tr>
<tr>
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</tr>
<tr>
<td>8</td>
<td>Limonene</td>
<td>4.73</td>
</tr>
<tr>
<td>9</td>
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</tr>
<tr>
<td>10</td>
<td>1,3-butene-3,3-dimethyl</td>
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</tr>
<tr>
<td>11</td>
<td>Ethylene glycol</td>
<td>0</td>
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<td>12</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
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</tr>
</tbody>
</table>

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**Figure 2. Size and polydispersity index (PDI) of SNEDDS.**

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<td>10</td>
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<td>Ethylene glycol</td>
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<tr>
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<td>Unidentified</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
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</table>
21.12%, respectively. Based on GC-MS analysis, there was a decrease in the bioactive compounds before and after the SNEDDS formulation, amounting to 17.47 percent.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES


Dyanovita Al Kurnia


Abundance of Bioactive Compound in Fingerroot Essential Oil


