Antibacterial Effectiveness Of Formulations Nanoemulsion

Cratoxylum Glaucum Korth. Extract

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Abstract. Cratoxylum glaucum Korth. is a members of the family Hypericaceae which are spread in Indonesia, one of which is found in the forest of the Bangka Belitung Islands. Cratoxylum glaucum Korth is often used by the people of Bangka Belitung as a flavoring ingredient from ancient times until now it is still consumed and utilized by people around Bangka Belitung. One source of natural antioxidants is the top of the crust. The effort of increasing its activity is very strong, so in this study we try to develop extraction of Cratoxylum glaucum made in the form of nanoemulsion. This study aims to determine the comparison of Cratoxylum glaucum extract in nanoemulsion preparations with extracts as antibacterial. Nanoemulsion is made with the composition of Virgin Coconut Oil (VCO) as an oil phase, tween 80 as surfactant and aquades as a water phase with a ratio of 2.5: 10: 25. The nanoemulsion extract of Cratoxylum glaucum have a size 422.9 nm and PI value 0.493. The antibacterial activity of nanoemulsion formulation has inhibitory growth power in moderate categorized Staphylococcus aureus bacteria, whereas in the extract of Cratoxylum glaucum it has the inhibitory growth of active Staphylococcus aureus bacteria.

1. Introduction

Cratoxylum glaucum are one of the members of the family Hypericaceae which are spread in Indonesia, one of which is found in the forest of the Bangka Belitung Islands. Cratoxylum glaucum Korth. plants are known by the people of Bangka Belitung as endemic plants, usually given the name “Pucuk Idat”. The species Cratoxylum glaucum is often used by the people of Bangka Belitung as a flavoring ingredient from ancient times, until now it is still consumed and utilized by people around Bangka Belitung .

Cratoxylum glaucum are widely used such as the use of the roots, stems, leaves and bark as traditional medicine to facilitate breastfeeding, tighten the skin, treat diarrhea, anticancer, antibacterial, antiviral and other diseases. The major secondary metabolites of Cratoxylum glaucum Korth. include anthraquinone and xanthones. The major metabolites of Cratoxylum glaucum Korth. has similarities to the major metabolites of mangosteen (Garcinia mangostana) which have bioactivity as an antioxidant [1].

The antioxidant activity of Cratoxylum glaucum Korth. ethyl acetate extract showed very strong activity with an IC₅₀ value of 32.212 µg / mL [2]. The effort of increasing its activity is very strong, so
In this study we try to develop extraction of *Cratoxylum glaucum* Korth. to made in the form of nanoemulsion. The use of nanoemulsion in the drug delivery system is called Self-Nanoemulsifying Drug Delivery System (SNEDDS). SNEDDS is a corporation of oil, surfactants, cosurfactants and active substances when mixed with water to form oil / water type nanoemulsions [3]. Nanoemulsion is a new drug delivery system having a size range between 50 - 1000 nm [4]. The term nanoemulsification is said to be a stable, clear preparation of two insoluble liquids, such as oil and water, stabilized by the interface film of surfactant molecules [4].

The advantage of nanoemulsion is to have a good surface area compared to macroemulsion so that it is more effective as a carrier system. The small size of nanoemulsion particles can penetrate easily through the skin layer and increase skin penetration in the drug inserted [5]. In addition nanoemulsions preparations are used as an alternative to make small droplet sizes can facilitate penetration and absorption across bacterial membranes, with this measure nanoemulsion has a clear and transparent physical form and increases the effectiveness of the *Cratoxylum glaucum* Korth as antibacterial. Therefore, in this study, the formulation of the *Cratoxylum glaucum* Korth. acetone extract of nanoemulsion which has high stability and small droplet size as an antibacterial effectiveness against *Staphylococcus aureus*.

2. **Research methods**

2.1 **Sample Preparation**

Leaves of *Cratoxylum glaucum* Korth. are obtained from Kimak Village, Pemali District, Bangka Regency. Then the sample is dried under the sun. The dried sample is ground into dry powder. Furthermore, sifted and macerated.

2.2 **Extraction**

Dry powder leaf of *Cratoxylum glaucum* Korth. was taken 50 grams macerated with acetone solvents as much as 500 mL for 3 x 24 hours. After that the obtained filtrate was concentrated with rotary evaporator vacuum until the concentrated extract of Aseton was obtained.

2.3 **Preparation of Nanoemulsion Acetone Extract Chratoxylum glaucum Korth.**

*Cratoxylum glaucum* Korth. extract was made by varying the addition of acetone extracts by 0.01, 0.02, 0.03, 0.04, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75 g which is suspended with an emulsifier. Each extract will be suspended in virgin coconut oil, with magnetic stirring. Then added with tween 80, added with distilled water and stirring using homogenizer at a speed of 8,000 for 30 minutes. Then measurements were made of physical stability and percent transmittance [6]. Furthermore, the product nanoemulsion product was analyzed by particle size analyzer (PSA).

2.4 **Antibacterial Activity Testing**

Antibacterial testing was carried out by the inhibitory zone method. 50 μL were taken from each test solution with a concentration of 20%, 50% and 80% as well as a positive control solution and a negative control. Then put into a hole that has been formed. After that, it was incubated for 24 hours at 37°C. Measurements are made by looking at the inhibition zone formed.

3. **Result and Discussion**

3.1 **Physical Stability of Nanoemulsion**

One of the physical stability tests was carried out by measuring nanoemulsion transmittance. The percent transmittance test obtained showed that the more extracts used, the smaller the yield value of transmittance was obtained because the extracts added with more concentrations would produce more concentrated colors or the mixture of oil and surfactant would be incorporated by extract. Good nanoemulsion has a clear or clear visual appearance with a high percent transmittance value. The addition
of *Cratoxylum glaucum* depends on the solubility in the system used [6],[7]. PH testing decreased in the concentration range of 0.075 - 0.1 to be more acidic. this shows that the more extracts, the smaller or acidic pH values will be obtained [8].

### Table 1. Physical Stability Test Results of Nanoemulsion *Cratoxylum glaucum*

| Formula | Concentration of Extract (gr) | pH  | Transmittance (%) | Density         |
|---------|-------------------------------|-----|-------------------|-----------------|
| E1      | 0.01                          | 6   | 69.2              | 0.99138         |
| E2      | 0.02                          | 6   | 58.07             | 0.98990         |
| E3      | 0.03                          | 6   | 44.06             | 0.98778         |
| E4      | 0.04                          | 6   | 43.56             | 0.98909         |
| E5      | 0.05                          | 6   | 37.26             | 0.98915         |
| E6      | 0.075                         | 6   | 25.17             | 0.98830         |
| E7      | 0.1                           | 5   | 10.68             | 0.99204         |
| E8      | 0.25                          | 5   | 1.29              | 0.99009         |
| E9      | 0.5                           | 5   | 0.043             | 0.99122         |
| E10     | 0.75                          | 5   | 0.014             | 0.98958         |

### 3.2. Particle Size Analysis of Nanoemulsion Formulations

Nanoemulsion measurement using PSA was done three times. The results of the analysis of the size of nanoemulsion extract of *Cratoxylum glaucum* extract are shown in Figure 1. The particle size analyzer results showed that nano size was formed from formulation E1 which consisted of 0.01 gr extract with the ratio of Virgin Coconut Oil (VCO) as an oil phase, tween 80 and aquades 2.5: 10: 25. From the results of the PSA it shows that the size formed by the size of nanoemulsion <10 nm normalized intensity distribution 12-20%.

![Figure 1](image.png)

**Figure 1.** Graph of particle size analyzer results (normalized intensity distribution) of formulation nanoemulsion *Cratoxylum glaucum* Korth.

The results of droplet size and the value of the polydispersity index (PI) shown in Table 2. PI which can help provide information about the stability and uniformity of nanoemulsion sizes. The results
showed that the droplets size of *Cratoxylum glaucum* extract nanoemulsion was 422.9 nm. The nanoemulsion size ranges from 50-1000 nm [4]. So, the results of the droplet size obtained in this study met the nanoemulsion preparation criteria. Furthermore, the results of the PI value indicate the homogeneity or stability of nanoemulsion. The smaller the value of PI, the size of the droplet will be more uniform and homogeneous [9]. The Good PI value ranged from 0.01 - 0.7. The results of the PI value of this study amounted to 0.493. So that the *Cratoxylum glaucum* extraction nanoemulsion formulation is included in the general nanoemulsion criteria.

| Table 2. Analysis of nanoemulsion of *Cratoxylum glaucum* extract using Particle Size Analyzer |
|------------------------------------------------------|
| E1 | Diameter (nm) | PI |
| Average | 422.9 | 0.493 |

3.3. Antibacterial Activity

Based on the results of Antibacterial testing obtained showed that the results of the thick extract of *Cratoxylum glaucum* had a greater inhibitory value than in the form of nanoemulsion for testing *Staphylococcus aureus* bacteria. The extract of *Cratoxylum glaucum* which is used in testing is completely pure or without any comparison of mixtures whereas in the form of nanoemulsion the extract ratio is different. The level of inhibition of bacterial growth was categorized as <5 mm weak, medium 5-10 mm, 10-19 active and 20 mm very active [10] (Table 3). Size of nanoemulsions particularly effective delivery systems for *Cratoxylum glaucum* extract to increase antibacterial efficacy. This is because the small size of the nanoemulsion makes it easy for the extract to interact with the surface of the cell membrane [11].

| Table 3. Antibacterial activity of *Staphylococcus aureus* |
|----------------------------------------------------------|
| No | Extract | Concentration % | Inhibition (mm) |
| 1. | Extract | 20% | 14.025 |
| 2. | Extract | 50% | 15.275 |
| 3. | Extract | 80% | 15.215 |
| 4. | E1 | 20% | 9.57 |
| 5. | E1 | 50% | 7.865 |

4. Conclusion

Based on the research carried out, a formulation of nanoemulsion extract of *Cratoxylum glaucum* have a size 422.9 nm and PI value 0.493. Composition of formulation nanoemulsion in this study consists of 0.01 g (2%) extract with the ratio of VCO: tween 80: aquades 2.5: 10: 25. The antibacterial activity of nanoemulsion formulation has inhibitory growth power in moderate categorized *Staphylococcus aureus* bacteria, whereas in the extract of *Cratoxylum glaucum* it has the inhibitory growth of active *Staphylococcus aureus* bacteria.

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