The Optimization of Maltodextrin and Arabic Gum in the Microencapsulation of Aqueous Fraction of Clinacanthus nutans Using Simplex Lattice Design

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ABSTRACT
The aqueous fraction of Clinacanthus nutans leaf extracts contains flavonoids which known had antioxidative properties. To improve acceptability, this viscous and bitter aqueous fraction was microencapsulated using maltodextrin and Arabic gum. This research aims to discover the effectivity of maltodextrin and Arabic gum and the concentrations for optimum microencapsulation. Optimization design was done using Design Expert with simplex lattice design with ratios of 1:0; 0.75:0.25; 0.5:0.5; 0.25:0.75 and 0:1. The evaluations done to the results were microcapsule yield, moisture content, flow rate, and antioxidant activity. The optimum ratio of maltodextrin and Arabic gum was obtained at 0.806:0.194 with 1.49% moisture content, flow rate 4.375 g/s and antioxidant activity at the value of 842,499 ppm. The result of one-sample T-test showed that the prediction result of Design Expert did not differ significantly from the experiment result. From the data, it was concluded that the resulting equation was valid.

Keywords: aqueous fraction, Clinacanthus nutans, microencapsulation, maltodextrin, Arabic gum.

INTRODUCTION
Currently, there are many research on plant antioxidants because the common synthetic ones such as BHA and BHT was suspected to be carcinogenic. Clinacanthus nutans is one of the plants commonly found in Indonesia and has potential as antioxidants. Clinacanthus nutans is a shrub commonly used as hedgerows in Indonesia. In traditional medicines, this plant is used as antiabeticics, antioxidants, antiinflammation, analgetics, antiviral, and also for wound treatment.

The leaves of Clinacanthus nutans contain alkaloids, triterpenoids/stereoids, glycosides, tannins, saponins, and flavonoids. The isolates of Clinacanthus nutans methanolic leaf extracts contains six C-glycosides flavons, namely shaftoside, isomollupentin 7-O-β-glukopyranoside, orientin, isoorientin, vitexin, and isovitexin. The flavonoids are known to acts as antioxidants by capturing free radicals. The aqueous fraction of Clinacanthus nutans leaf extracts had value of EC₅₀ 532.24 ppm, showing antioxidants properties.

The usage of Clinacanthus nutans leaf extracts fractions has several shortcomings, amongst them is its viscosity that gave difficulties in formulation and its astringent and bitter taste that made it hard to use orally. To overcome these shortcomings, microencapsulation can be used. Microencapsulation is a process of microscopic encapsulation of drug particles with specific coat that result in better physical and chemical properties of those particles. Microencapsulation intends to protect sensitive components, reduce the loss of nutritions, and converts liquids to solids. The choice of microencapsulation methods depends on its applications and specific parameters, such as desired particle size, physicochemical properties of the core and the coating, release mechanism, process cost, etc. Freeze drying is one of drying method for encapsulation which had advantages in preserving the quality of the drying products, especially for heat-sensitive materials. Encapsulation can be attained as homogenous core in matrix solution which then was co-lyophilized, resulting in irregular shapes. Freeze drying is a suitable method for encapsulation of antioxidants to preserve their properties because antioxidants are easily damaged by heat and light.

The choice of coating materials for microencapsulation of aqueous fraction of Clinacanthus nutans leaf extracts was based on the usage of combination of Arabic gum and maltodextrin for microencapsulation of grape anthocyanins and on encapsulation of Berberis vulgaris extracts using Arabic gum and maltodextrin in 1:3 ratio. The combination of maltodextrin and Arabic gum as encapsulant is expected to form stable microcapsule and protect the aqueous fraction of Clinacanthus nutans because of the film-forming properties of Arabic gum and the ability of maltodextrin to protect microcapsules from oxidation. This research intended to find the optimum
concentrations of microencapsulants of the aqueous fraction of Clinacanthus nutans.

MATERIALS AND METHODS

Tools and Materials

Materials that were used on this research were Clinacanthus nutans leaves, analytical grade ethanol 96% (Merck), analytical grade methanol (Merck), analytical grade n-hexane (Merck), analytical grade ethyl acetate (Merck), DPPH, maltodextrin (Dextrose Equivalent 10,6), Arabic gum (Tic Gams), and distilled water. Tools used were separation funnels, rotary evaporator (Buch Rotavapor R-200), freezer, freeze dryer (Thermo Scientific Powerdry LL 1500), moisture analyzer (Mettler Toledo HE53), flow funnels, ovens, UV-visible spectrophotometer (Shimadzu), SEM (Scanning Electron Microscopy).

Methods

Extraction dan Fractionation of Clinacanthus nutans Leaves

Two hundred grams of Clinacanthus nutans leaf powder was macerated with two liters of ethanol 96% for two days. Diluted extracts was separated from the solvent using rotary vacuum evaporator at 40°C. The extract then gradually fractionated using liquid-liquid partition in separation funnels with water, ethyl acetate, and n-hexane as solvents. The water fraction then was evaporated using rotary vacuum evaporator at 80°C.

Microencapsulation of Aqueous Fraction of Clinacanthus nutans Leaf Extracts

Maltodextrin and Arabic gum in various ratio (see Table 1) were suspended in distilled water and the to the mixture the aqueous fraction of Clinacanthus nutans leaf extracts was added. The resulting mixture was frozen for 24 hours and then dried using freeze-dried in the temperature -100°C. The dry samples were pulverized and then were sifted using 24 mesh sieve. The resulting microcapsules were kept in tightly closed container and protected from light.

The Evaluation of Microcapsule Characteristics

Microcapsule yield

The yield was calculated by comparing the weight of microcapsule to the total active and coating materials. The percentage of the microcapsule yield was calculated using Equation 1:

\[
\text{Yield} = \frac{\text{Wt} - \text{Wo}}{\text{Wo}} \times 100%
\]

\[
\text{Wo} = \text{Wt} - \text{Wo} x 100% .................................................(1)
\]

Filter papers and residues were dried in oven at 105°C for three hours and then were cooled down and weighed. The solubility percentage then was calculated using equations 2 and 3.

Solubility percentage = 100% - residue percentage .................................................(2)

\[
\text{Residue percentage} = \frac{\text{weight of filter paper and residue - weight of filter paper}}{\text{weight of sample}} \times 100%
\]

.................. (3)

Flow Rate

Flow rate was measured by weighing 100 g microcapsules, and then inputting those microcapsules into a closed-end funnel. The cover at the end of the funnel then was opened and then the microcapsules was let to flow until there weren’t any microcapsules remaining in the funnel. The flow time was recorded as the time the microcapsules needed to flow from the time the cover was opened until all the granules flowed out.

Antioxidant Activity Assay with DPPH Method

Antioxidant activity was evaluated by solving 250 mg microcapsule in 50 ml of methanols and diluting the solution to 1000,1500, 2000, 2500 and 3000 ppm. From those solutions, 2 ml were taken and 4 ml DPPH solution was added. The mixtures then were incubated at room temperature in dark condition for 30 minutes. The lowering of absorbancy was measured with spectrophotometer at 517 nm. The negative controls were made without any samples. From the resulted absorbancy, percentage of inhibition and EC\text{50} value were calculated. Percentage of inhibition was calculated using Equation 4.

Table 1: Microcapsule Formulation of Aqueous Fraction Clinacanthus nutans Leaf Extracts.

<table>
<thead>
<tr>
<th>Materials</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabic gum</td>
<td>1</td>
<td>0.75</td>
<td>0.50</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>0</td>
<td>0.25</td>
<td>0.50</td>
<td>0.75</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 1: Microcapsules of aqueous fraction of Clinacanthus nutans leaf extract.
RESULTS AND DISCUSSION
The concentration range of maltodextrin and Arabic gum which can be used as encapsulant are were v from the total mixture. The ratio of aqueous fraction to the encapsulants was 1:10. The microencapsulation results can be seen at Figure 1. The result of physical characteristics and antioxidant test can be seen at table 2.

Microcapsule yield was within the range of 28.15 ± 0.49 % to 59.07 ± 1.47%. Freeze-drying works by freezing the material and reducing the pressure around it and adding sufficient heat to the surrounding environment to allow water to sublime, therefore there are no other material other than water undergoes sublimation, even if the yield is less than 100%. Moisture content tests were done to find the moisture content of the microcapsules. High moisture content result in damages of microcapsules and this damage influences the stability of microcapsules themselves. The moisture content of the aqueous fraction of Clinacanthus nutans leaf extracts microcapsules was found to be relatively low, within the range of 1.32 ± 0.12% to 2.06 ± 0.13%. This low moisture content also can improve the flow rate of the granules. According to Design Expert version 10.0 Trial analysis results, it was shown that maltodextrin (M) and Arabic gum (G) each contributes significantly to the moisture content. On the other hand, the M-G interaction affected significantly in lowering moisture content. It was proven from the prob>F value smaller than 0.05 (<0.0001) according to Equation 6. The addition of Arabic gum can increase moisture content because Arabic gum contains a large amount of shorter hydrophilic groups, therefore it easily binds water molecules in the air. (Mahdavi, et al., 2016).

\[ Y = 1.28 \text{ (M)} + 2.08 \text{ (G)} - 0.22 \text{ (MG) (5)} \]

Flow rate Test was done to find the capability of microcapsules to dissolve in water. The number of solubility value of the aqueous fraction of Clinacanthus nutans leaf extracts microcapsules was within the range of 95.17 ± 0.22 % to 97.97 ± 0.52%. The solubility was affected by the moisture content, in which the low moisture content enable the microcapsule to disperse easily in water. According to the analysis results of Design Expert version 10.0 Trial, maltodextrin (M), Arabic gum (G), and MG interaction give significant effect with the prob>F value smaller than 0.05 (<0.0001) according to equation 7.

\[ Y = 98.00 \text{ (M)} + 95.24 \text{ (G)} - 1.69 \text{ (MG) (6)} \]

According to equation 7, it can be seen that the solubility will increase along the increase of the amount of maltodextrin dan Arabic gum. It was shown by the positive constants value. It can be seen from the equation that the addition of maltodextrin affected the increase of solubility since maltodextrin itself has high solubility9. Flow Rate Test was done to find the capability of certain amount of material to flow in certain time. High flow rate indicates that the material has good capability of flowing. Free-flowing microcapsules are highly desired because it makes measurement easier and ensures the homogeneity of composition and weight is all packages. The average flow rate of the resulting microcapsules was 4.03 g/s. A preparation can be said having good flow rate if it’s flow rate is less than ten seconds11. According to analysis results of Design Expert version 10.0 Trial, maltodextrin (M), Arabic gum (G), and MG interaction give significant effects, with prob>F less than 0.05 (<0.0011) according to Equation 8.

\[ Y = 4.42 \text{ (M)} + 3.92 \text{ (G)} - 1.15 \text{ (MG) (7)} \]

According to Equation 8, the value of flow rate will increase along the increase the maltodextrin because maltodextrin is a free-flowing material9. Antioxidant activity test. The test was done quantitatively using DPPH method. The principle of this method is the measurement of the inhibition synthetic free radicals DPPH in polar organic solvents such as methanol by donating a hydrogen atom to stabilize purple DPPH radical to become yellow DPPH-H^+9. The result then was compared to the antioxidant activity of the aqueous fraction of Clinacanthus nutans leaf extract. According to the test, the EC50 value of the microcapsules was within the range of 70.3 ± 23.7 to 91.9 ± 80.48 ppm while the antioxidant activity of aqueous fraction of Clinacanthus nutans leaf extract was 532.24 ppm6. This reduction of

<table>
<thead>
<tr>
<th>Test</th>
<th>Formula</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcapsule yield (%)</td>
<td></td>
<td>28.15</td>
<td>34.20</td>
<td>53.48</td>
<td>59.07</td>
<td>40.05</td>
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<tr>
<td>Moisture content (%)</td>
<td></td>
<td>1.32</td>
<td>1.34</td>
<td>1.67</td>
<td>1.87</td>
<td>2.06</td>
</tr>
<tr>
<td>Solubility (%)</td>
<td></td>
<td>97.97</td>
<td>97.16</td>
<td>95.90</td>
<td>95.84</td>
<td>95.17</td>
</tr>
<tr>
<td>Flow rate (g/s)</td>
<td></td>
<td>4.46</td>
<td>4.00</td>
<td>3.89</td>
<td>3.90</td>
<td>3.89</td>
</tr>
<tr>
<td>Antioxidant activity (ppm)</td>
<td></td>
<td>919.98</td>
<td>886.87</td>
<td>703.37</td>
<td>981.78</td>
<td>919.88</td>
</tr>
</tbody>
</table>

Note: The result above were the mean of four replications

\[ \text{Percentage of inhibition} = \frac{(\text{Negative control absorbancy} - \text{Sample absorbancy})}{\text{Negative control absorbancy}} \times 100\% \]

\[ Y = 1.15 \text{ (M)} + 3.92 \text{ (G)} - 4.42 \text{ (MG) (5)} \]
Figure 4: The Optimized microcapsules

Figure 2: Profile of microcapsules of aqueous fraction of *Clinacanthus nutans* leaf extract according to the Simplex Lattice Design with (a) flow rate (b) moisture content (MC) (c) solubility (d) antioxidant activity.

Figure 3: Formula optimum *Design Experts*.

antioxidant activity probably caused by the possibility of not all the fraction had been encapsulated. Although there was a reduction of antioxidant activity, the microcapsule can give protection to the aqueous fraction of *Clinacanthus nutans* leaf extract during the storage because the active components are coated so their contact with air and light is reduced. According to the analysis result of *Design Expert version 10.0 Trial*, neither maltodextrin nor Arabic gum did not give significant effect to the antioxidant activity with the prob>F value larger than 0.05 (0.1331) which
Table 3: The experiment result compared to the theoretical value.

<table>
<thead>
<tr>
<th>Response</th>
<th>Actual value</th>
<th>Prediction value</th>
<th>Significance</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>4.375 g/s</td>
<td>4.141 g/s</td>
<td>0.088</td>
<td>Did not differ significantly</td>
</tr>
<tr>
<td>Moisture content</td>
<td>1.495 %</td>
<td>1.39 %</td>
<td>0.007</td>
<td>Differed significantly</td>
</tr>
<tr>
<td>Solubility</td>
<td>97.47 %</td>
<td>97.203 %</td>
<td>0.572</td>
<td>Did not differ significantly</td>
</tr>
<tr>
<td>EC₅₀</td>
<td>842.499 ppm</td>
<td>856.371 ppm</td>
<td>0.010</td>
<td>Differed significantly</td>
</tr>
</tbody>
</table>

Figure 5: The micrograph of aqueous fraction of Clinacanthus nutans leaf extract microcapsules (a) 100x magnification (b) 1000x magnification (c) 2000x magnification

CONCLUSION
Based on the experiment result, it could be concluded that aqueous fraction of Clinacanthus nutans leaf extract microcapsules could be made with the ratio of 0.804 part maltodextrin and 0.194 part Arabic gum. The resulting microcapsules had 1.49% moisture content; 97.47 % solubility; 4.375 g/s solubility; 842.499 ppm antioxidant activity.

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