ORAL SOLUTION FORMULATION OF *Garcinia mangostana* L RIND EXTRACT AS ANTIOXIDANT USING COSOLVENCY METHOD

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Abstract

*Garcinia mangostana* L rind contains xanthones which are not soluble in water. One of the type of xanthones is $\alpha$-mangosteen that has a high antioxidant activity. To improve its solubility, in this research it was conducted by cosolvent addition method. The xanthones were extracted from mangosteen rind using 70% ethanol and then formulated into oral solution by dissolving the extract with various cosolvents and addition of sorbitol and water soluble violet dye. The composition cosolvent were PEG 400-glycerol (20:20, 20:40, 40:20, 40:40). The clarity, pH and density of all the oral solution were fulfill the requirement. Oral solution with cosolvent of PEG 400-glycerol (40:40) has the highest content of $\alpha$-mangosteen.

Keywords: Mangosteen rind, $\alpha$-mangosteen, antioxidant, cosolvency

Background

*Garcinia mangostana* L or Mangosteen fruit rind is discarded by consumers or can be termed as agricultural waste. Mangosteen fruit rind contains xanthones which have anti-oxidant, anti-proliferation and anti-microbials activity that are not found in any other fruit. Mangosteen rind extract has been shown to have powerful antioxidant activity. IC value of 50% ethanol extract of mangosteen rind is 9.26 mg / L with the resulting yield of 18.99% (Mardawati et al. 2008)

It has been found more than 40 types of xanthones, including $\alpha$-mangosteen and $\gamma$-mangosteen have the ability to prevent various diseases and to stop inflammation by inhibiting the production of Cox-2 enzyme that causes inflammation. Xanthones also useful to prevent the growth of cancer cells and tumors. Xanthones have poor solubility in water. Solubility is one of the important physicochemical properties that have to be considered preparation of solution. To produce soluble active substances need for additional supporting materials and appropriate methods to improve the solubility. One of the appropriate methods to enhance the solubility of xanthones in mangosteen rind extract is cosolvency method.
Various composition of the mixed PEG 400 and glycerin with the percentage ratio of PEG 400- glycerin (20:20), (20:40), (40:20), (40:40). PEG 400 is cosolvent which can increasing the solubility more optimally than some others, inert and can be used as a preservative, while glycerin is used to inhibit crystallization, giving a sweet taste and improve solubility.

**Methods**

**Plant raw material**
Ripened *G. mangostana* L fruit was obtained from a local fruit farm at Tasikmalaya. Taxonomic authentification was performed by Herbarium Bogoriensse LIPI, Bogor. The fruit rinds were separated from the edible part and the rinds were chopped before drying at 40°C then grinded to be powder.

**Chemical and reagents**
α-mangostin reference compound was purchased from Chengdu Biopurify Phytochemicals Ltd. DPPH reference compound were acquired from Sigma. Additional material consisting of 70% sorbitol, glycerin, PEG 400 were examined based on monographic examination of each ingredient according to Farmakope Indonesia IV.

**Preparation of the fruit rind extracts**
Extract were prepared by the maceration method at room temperature using 70% ethanol (1:4) at 220 rpm at 3X24 hs. Ethanolic extract was concentrated by vacuum rotavapor at 40°C 120 mBar. This extract were examined by organoleptic, flavonoid identification, α-mangosteen content by UV spectrophotometric methods (Pothitirat 2008) and antioxidant activity using DPPH method.

**Solubility analysis**
About 500 mg extract of mangosteen rind was put in erlenmeyer which already contains a certain volume of solvent (100 mL) consisting of 70% ethanol- PEG 400 (50:50), and placed on a shaker for 2 h. Absorption of this solution was measured at a wavelength of 320 nm.
**Oral Solution Composition**
Table 1. showed the oral solution composition of four formula.

**Evaluation of Oral Solution**
All of the oral solution were examined of their colour, odor, clarity, pH, density and xanthones content. Clarity assay was performed using Farmakope Indonesia IV method.

**Analysis of dissolved xanthones**
Measuring of dissolved xanthones content in formulas I-IV formula was performed at a wavelength of 320 nm using a blank formula of I-IV without extract.

**Results**

**Extraction**
Brown concentrate solution with pH of 5.00 was obtained. The percentage of crude extract to the dried raw material was 7.12 %.

**Identification of xanthones**
Xanthones is a yellow pigment phenol and its color reaction is the same as flavonoids. Material testing is termed positive for xanthones if formed of red, orange or yellow in amyl alcohol layer (upper layer) as Fig 1.

**Anti-oxidant activity of extract**
Trials of antioxidant activity of the mangosteen fruit rind extract was conducted using the DPPH method. The absorbance was measured at a wavelength of 517 nm using vitamin C as positive reference.

The anti-oxidant test results showed that the higher the concentration of the extract, the higher the percentage of inhibition. This is due to the dissolved xanthones the higher the antioxidant content that also impact on the level of inhibition of free radicals by such anti-oxidants. The IC$_{50}$ of mangosteen rind extract was 16.98 ppm (Fig. 2) while the IC$_{50}$ of vitamin C was 5.0972 ppm (Fig. 3)

**Solubility Analysis**
Solubility analysis was conducted as a preliminary analysis to determine cosolvent concentration that will be used in the formula. Measurement of absorbance was
performed at a wavelength of 320 nm using 10 mL of PEG 400-ethanol 70% (50:50) as blank. After 2 hours the absorbance was 0.6862

**Evaluation of Oral solution**

Based on the results of organoleptic oral solution dosage with different composition of cosolvent of four formula, various composition does not affect the colour and odor. Formula II and IV are sweetish while formula I and III that had lower level of glycerin were bitter. Clarity scale is used to determine the level of clarity of a stock which is compared with equivalent suspension that made fresh. The scale is 1, which means the highest dosage in a clear state and the lowest is 5, which means preparation in very turbid. **Table 3.** shows that all of the formula have the clarity scale of 1.

**Density and pH**

The density of Formula I, II, III and IV are 1.1349 g/mL, 1.1617 g/mL, 1.1505 g/mL and 1.1752 g/mL respectively. Formula I, II, III and IV have pH of 5.83; 5.36; 5.34 and 5.61 respectively.

**Assay of α-mangosteen content**

Assay of xanthan content in four formula was performed using UV spectrophotometric method. In this research xanthan content was calculated as α-mangosteen. The results of these could be seen in **Table 4.**

**Discussion**

Determination of specific gravity is meant to look at the weight ratio of substances to water with the same volume and temperature. For the preparation of solutions approached terms of specific gravity specific gravity of water is 1.0. Weighting of the data type of each formula above with different concentrations of supporting materials can be said has not changed and is eligible dosage oral solution specific gravity specific gravity of water is approaching. All formulas have the clarity scale. They contains ingredient that can be dissolved in water. Formula I, II, III dan IV also have the density that close to water. Supporting
materials especially PEG 400 and glycerin with different level don’t affect the density.

pH evaluation performed at room temperature for four different formulas with no difference in pH results significantly, although there are differences in the concentration of PEG 400 and glycerin kosolven of each formula, this is due to the formula kosolven have almost the same pH range. The magnitude of the pH of the fourth formula is still in the acidic pH xanthones.

To determine the xanthone level, formula I was measured after 2 times of dilution while the formula II, III and IV performed with 4 times dilution. From the measurement results it can be seen that formula IV with PEG 400-glycerin (40:40) gave the greatest absorbance. The greatest level of xanthone was found in formula IV. This is supported by the value of dielectric constant ($\varepsilon$) formula IV which had $\varepsilon$ value close to ethanol 70% = 41.01 ($\varepsilon$ formula I = 59.1; formula II = 51.7; formula III = 45.6 formula IV = 38.2)

**Conclusion**

Mangosteen rind extract has antioxidant activity with IC$_{50}$ of 16.98 ppm

Cosolvency could enhance the solubility of xanthone.

All of the formula were fulfill the requirement based on sensory evaluation, pH, density and clarity.

Formula IV has the highest level of xanthones (calculated as $\alpha$-mangosteen)

**Acknowledgement**

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### Tables and Figures

**Table 1. Oral solution composition**

<table>
<thead>
<tr>
<th>Formula</th>
<th>weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Mangosteen rind extract</td>
<td>0.017</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>20</td>
</tr>
<tr>
<td>PEG 400</td>
<td>20</td>
</tr>
<tr>
<td>Glycerin</td>
<td>20</td>
</tr>
<tr>
<td>Violet dye</td>
<td>0.0015</td>
</tr>
<tr>
<td>Aquadest until</td>
<td>100</td>
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</table>

**Table 2. Organoleptic test result of oral solution**

<table>
<thead>
<tr>
<th>Formula</th>
<th>Form</th>
<th>Colour</th>
<th>Odor</th>
<th>Taste</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>Liquid</td>
<td>Dark violet</td>
<td>Odorless</td>
<td>Bitter</td>
</tr>
<tr>
<td>II</td>
<td>Liquid</td>
<td>Dark violet</td>
<td>Odorless</td>
<td>Sweetish</td>
</tr>
<tr>
<td>III</td>
<td>Liquid</td>
<td>Dark violet</td>
<td>Odorless</td>
<td>Bitter</td>
</tr>
<tr>
<td>IV</td>
<td>Liquid</td>
<td>Dark violet</td>
<td>Odorless</td>
<td>Sweetish</td>
</tr>
</tbody>
</table>

**Table 3. The results of clarity test**

<table>
<thead>
<tr>
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<th>1x</th>
<th>2x</th>
<th>3x</th>
</tr>
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<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Description clarity scale:
1. same as the equivalent suspension 1 (clear)
2. same as the equivalent suspension 2 (somewhat clear)
3. same as the equivalent suspension 3 (somewhat turbid)
4. same as the equivalent suspension 4 (turbid)
5. same as the equivalent suspension 5 (very turbid)
### Table 4. Absorbance of formula I- formula IV

<table>
<thead>
<tr>
<th>Formula</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.5724</td>
<td>0.4895</td>
<td>0.5645</td>
<td>0.6995</td>
</tr>
<tr>
<td>Dilution</td>
<td>2x</td>
<td>4x</td>
<td>4x</td>
<td>4x</td>
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<tr>
<td>Xanthone level calculated as α-mangosteen</td>
<td>17.596</td>
<td>30.095</td>
<td>34.706</td>
<td>43.006</td>
</tr>
</tbody>
</table>

![Fig 1. Identification result of xanthones](image1)

![Fig 2. Plot % inhibition vs concentration of extract](image2)
Fig 2. Plot % inhibition vs concentration of vitamin C

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