The effect of heating temperature on cytotoxicity and α-mangostin yield: Mangosteen pericarp juice and mangosteen extract

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Abstract. The pericarp of mangosteen (Garcinia mangostana L.) contains bioactive xanthones, with α-mangostin being the major component, has been known to possess antitumor, antiviral, and other pharmacological activities. In this study, the effect of elevated temperature during the preparation step of fresh mangosteen pericarp juice and mangosteen extract, on their α-mangostin yield and cytotoxicities was investigated. The cytotoxicity activity of fresh juice and mangosteen extract was investigated using the brine shrimp test. Heating the fresh pericarp mangosteen in water at 65°C for 30 minutes prior to blending produced a juice with higher α-mangostin yield and cytotoxicity compared to the traditional way of blending the juice at room temperature. Increasing α-mangostin yield of 9%-w/w due to heating was also observed when mangosteen extract was heated at 65°C, consistent with the increased cytotoxicity in terms of LC50 value. It is concluded that the effect of temperature on α-mangostin yield was in line with the temperature effect on cytotoxicity activity in all samples of pericarp juice and mangosteen extract in ethyl acetate fraction.

1. Introduction
Mangosteen tree (Garcinia mangostana Linn.) is a tropical evergreen tree with green leaves and dark purple fruits. The pericarp of the mangosteen fruit is traditionally used for treatment of skin infection, diarrhea, abdominal pain, infected wounds, and chronic ulcer[1]. Previous studies had shown that the pericarp of the mangosteen fruit is rich in xanthone compounds. The first group of xanthones isolated was the mangostin group with α-mangostin as the most abundant bioactive compound present[2]. The extract of the pericarp of mangosteen has pharmacological activities including anti-inflammatory, cytotoxic, antioxidant, antitumoral, neuroprotective, immunomodulatory, anti-allergic, antibacterial, and antiviral properties[3,4]. Satong-aun et al. dried mangosteen pericarp at three temperatures and obtained the highest α-mangostin extraction yield of 40.3% (w/w) after drying at 65°C[5]. Suttirak and Manurakchinakorn reported that the amount of xanthones in the pericarp and their free radical scavenging activities markedly decreased after the drying step[6]. They recommended drying temperature of 75°C to inactivate degradative enzymes such as polyphenol oxidase and also minimize thermal degradation of xanthones.
Traditionally, juices of mangosteen pericarp are consumed to get the benefits of xanthones that reside within. Even though the efficacy of mangostins is widely known and the mangostin yield in the pericarp as a function of heating temperature has been reported, cytotoxicity data of these juices have been reported yet. It is anticipated that heating the pericarp of mangosteen will not only change the α-mangostin yield of the juices, but also their cytotoxicities. Also of interest is the effect of heating temperature on mangostin yield and cytotoxicity of the extracts obtained from the mangosteen pericarp since they contain a large amount of bioactive compounds, and therefore, have potential to be used for anti-cancer medication. Therefore, the objectives of this research are to determine: (1) the effect of preparation method and heating temperature on mangostin yield and cytotoxicity of the fresh mangosteen pericarp juice; (b) the effect of heating of the pericarp on mangostin yield and cytotoxicity of ethyl acetate fraction obtained from the ethanolic extract of mangosteen pericarp.

2. Material and methods

2.1. Materials
Mangosteen pericarp was obtained from Solo, Central Java, Indonesia, and was identified as *G. mangostana* by Herbarium Bogoriense, Research Center for Biotechnology-Indonesian Institute of Sciences (LIPI). Sodium tripolyphosphate (food grade) was obtained from Brataco Chemical, Indonesia, calcium chloride and sodium alginites were obtained from Merck. The standard compound α-mangostin (98%) was obtained from Aktin Chemicals, China. Chitosan (medical grade; deacetylation degree of 93.6%; viscosity of 23.3 cp) was obtained from Biotech Surindo, Indonesia. α-amylase enzyme was purchased from Claricem Indonesia and β-glucosidase enzyme (from almonds; lyophilized powder; ≥6 U/mg) was purchased from Sigma-Aldrich. The brine shrimp eggs (*Artemia Salina* Linn.) were purchased from Red Top.

2.2. Preparation of samples with mangostin
There were two types of samples that were assayed to determine their mangostin yield and cytotoxicities: (a) fresh mangosteen pericarp juice prepared using five methods; (b) extract of mangosteen pericarp obtained from the ethanol extraction followed by fractionation using ethyl acetate.

2.2.1. Preparation of mangostin pericarp juice.
Samples of the fresh mangosteen pericarp juice were obtained using five preparation methods listed in Table 1. The thick and soft part of mangosteen rind was separated from its hard skin and then blended or boiled/heated in various ways. All preparation methods used a same ratio of pericarp pulp to water of 1:6 (w/w), i.e. 50 gram pulp in 300 ml water.

2.2.2. Preparation of mangosteen extract in ethyl acetate fraction.
The extraction of dry mangosteen pericarp was carried out using a modified procedure reported by Jung et al. [7]. The maceration was performed using ethanol 96% for 7 days with mangosteen powder to ethanol ratio of 1:3 (w/v) and periodic stirring of the mixture. The macerated mixture were filtrated and evaporated under reduced pressure. The crude ethanolic extract was then fractionated using a mixture of water and ethyl acetate in 1:1 volume ratio. The evaporation of the ethyl acetate fraction under reduced pressure produced mangostin powder/paste.

2.3. Cytotoxicity assay using brine shrimp test (BST)
The cytotoxicity assay was performed using the brine shrimp lethality test (BST) reported by Meyer et al. [8]. In this method, *in-vitro* lethality assay of *A. Salina* was used to detect the cytotoxicity of mangosteen extract in each sample [9]. Brine shrimp eggs were placed in seawater and aerated at room temperature (24-28° C) for 72 h, giving a large number of larvae. Total larvae alive were counted after a sample is exposed to a lamp for 24 h and the lethal concentration (LC50) was calculated.
2.4. Analysis of α-mangostin
The amount of α-mangostin in mangosteen pericarp juice and in the ethyl acetate fraction was determined quantitatively using UV spectrophotometry analysis (Spectroquant® Pharo 300, Merck). The juice was filtered prior to sampling at room temperature. The standard calibration curve was made based on the absorbance of α-mangostin standard solution (4-20 mg/L) obtained at the wavelength of 316 nm\textsuperscript{10}.

3. Result and discussion
The samples consisted of the mangosteen pericarp juice and ethyl acetate fraction of the mangosteen extract was prepared, and, the effect of the temperature imposed on the α-mangostin yield and the cytotoxicities of these samples were determined.

3.1. α-Mangostin yield of the fresh mangosteen pericarp juice
Table 1 shows the α-mangostin concentration in samples of fresh mangosteen pericarp juice obtained using different treatment methods. The results show that the juice preparation method affects the extraction yield of active compounds from the pericarp pulp. The traditional way to obtain the juice of mangosteen pericarp is by mixing with water in a blender. Other traditional techniques are by drying the pericarp and then boil it in water, or heat the pericarp at 65°C and mix it with water in a blender. It was found that the lowest concentration of α-mangostin in juice was obtained using the traditional way, i.e. mixing the soft part of the pericarp with water in the blender (treatment 1). In contrast, heating up the pericarp at 65°C for 30 min and followed by blending with water (treatment 3), produced a solution with the highest α-mangostin yield of 179 ppm, significantly higher than the yields obtained using other treatment. It can be explained that even though boiling pericarp mixture at 100°C for 30 min (treatment 2, 4, and 5) might degrade α-mangostin in the plant matrix, heating up at 65°C might not degrade it significantly.

Heating temperature of 65°C might only delay the action of the polyphenol oxidase enzyme to degrade the phenolic compounds present in the pericarp of mangosteen\textsuperscript{11}. This result, then indicates that heating the pericarp up to 65°C might enhance the solubility of α-mangostin in water, increase the diffusion of water in the mixture into the plant matrix, and at the same time delay the degradation action of the polyphenol oxidase enzyme. Al-Massarani et al. reported that during heating, plant extracts will degrade due to exposure to high temperature as well as to an enzyme called polyphenol oxidase that degrade the phenolic compounds in the extract\textsuperscript{11}. However, the results of this study showed differently.

Table 1. α-Mangostin concentration in fresh mangosteen pericarp juice and the corresponding %-lethality of A. salina.

| Treatment code | Treatment of the pericarp | α-mangostin yield (ppm) | %-lethality (w/w) | A. salina lethality (%) |
|----------------|---------------------------|-------------------------|-------------------|------------------------|
| 1              | Blended (with water)      | 56                      | 0.03              | 22                     |
| 2              | Blended and then boiled   | 88                      | 0.05              | 56                     |
| 3              | Heated and then blended   | 179                     | 0.11              | 86                     |
| 4              | Boiled only              | 66                      | 0.04              | 42                     |
| 5              | Dried and then boiled     | 106                     | 0.06              | 55                     |

Note: Juice blend is 50 g pericarp in 300 ml water; boiling in water at 100°C for 30 min; heating in water at 65°C for 30 min.

3.2. Cytotoxicity of mangosteen pericarp juice
Figure 1 shows the relationship between the concentration of α-mangostin in various juices and the %-lethality of A. salina that represent the cytotoxicity of the sample in BST assay.
Figure 1. The lethal percentage of *A. salina* from fresh juice samples with various concentrations of α-mangostin.

The BST results of the fresh mangosteen pericarp juice given in Table 1 show that sample receiving treatment 3 is the most cytotoxic with 86% lethality of *A. salina*, where the pulp of pericarp was first heated in water and then mixed in a blender. Juice made by only mixing the pulp with water in a juice blender (treatment 1) had the lowest cytotoxicity of 22% lethality. The positive correlation between concentration of α-mangostin and the cytotoxicity is shown in Fig 1, with the R-square around 0.94. This means that α-mangostin yield in juice significantly affect the cytotoxicity activity of pericarp extract juice. Sak reported the higher the concentration of phenolic compounds, such as α-mangostin, the higher the cytotoxicity[12].

3.3. Yield of α-mangostin in ethyl acetate fraction of mangosteen extract

The effect of heating temperature on the α-mangostin concentration in the extract samples is given in Table 2. Since the mangosteen extract was investigated in relation to its application for cancer medication via oral delivery system, the temperatures used were standard room temperature of 25°C, the body temperature of 37°C, and the heating temperature of 65°C to avoid degradation of α-mangostin[5]. The data show increased α-mangostin yields with increasing heating temperature of the mangosteen extract. The α-mangostin concentration in mangosteen extract heated at 65°C was around 50% (w/w), higher by 9% (w/w) compared to the concentration in the non-heated sample. The effect of temperature on the yield of α-mangostin in the mangosteen extract samples was similar with the result of that on pericarp juices as discussed previously.

![Graph showing the relationship between yield of α-mangostin and cytotoxicity assays of the mangosteen extract](image)

**Table 2. α-Mangostin yield and cytotoxicity assays of the mangosteen extract.**

| Temperature (°C) | α-mangostin yield (%-w/w) | *A. Salina* %-lethality* (%) | LC₅₀ of extract (ppm) |
|-----------------|---------------------------|-----------------------------|----------------------|
| 25              | 40.8                      | 57.0                        | 0.71                 |
| 37              | 45.1                      | 74.5                        | 0.51                 |
| 65              | 49.8                      | 97.1                        | < 0.1                |

*exposed to 1 ppm α-mangostin

The data obtained suggest that increased temperature of samples up to 65°C do not degrade the α-mangostin, which contradict with the result reported by Al-Massarani et al.[11]. It is known that the pericarp of mangosteen contained xanthones with structural molecules that are close to each other,
such as α-mangostin, γ-mangostin and Gartinin\(^{[10]}\). The hypothesis explaining this phenomenon is that the increased temperature of mangosteen extract might cause the shifting of functional group in other types of mangostin compounds onto the structure of α-mangostin, hence, the increased yield of α-mangostin.

3.4. Cytotoxicity of ethyl acetate fraction of mangosteen pericarp extract
The %-lethality of \(A.\) \(salina\) exposed to an extract sample containing 1 ppm α-mangostin given in Table 2 is shown in figure 2. The highest percentage of lethality (97.1%), corresponding to the lowest \(LC_{50}\) value (< 0.1 ppm), was obtained for the extract sample heated at 65°C. The potent bioactive compound becomes toxic when it is given in high doses, but it becomes a drug in low doses\(^{[8]}\). An extract sample having an \(LC_{50}\) value of less than 1000 ppm, is considered to be active as an anti-cancer agent\(^{[9]}\). Based on its \(LC_{50}\) value, the extract of mangosteen pericarp in ethyl acetate fraction has the potential to be used for anti-cancer medication.

![Figure 2. % Lethal of \(A.\) \(salina\) as function of log concentration of mangosteen extract at various temperatures.](image)

Figure 2 shows the positive correlation between the cytotoxicity and the concentration of α-mangostin in the extract, based on the data of % lethal \(A.\) \(salina\) for 1 ppm extract concentration. High lethality of \(A.\) \(salina\) was in line with high yield of α-mangostin in the extract.

![Figure 3. Effect of temperature on % lethal BST and α-Mangostin Yield in Extract](image)
Increasing temperature imposed on the extract sample increased the yield of α-mangostin as well as the cytotoxicity activity, represented as the %-lethality of A. salina in BST. The BST assay result of mangosteen pericarp extract in this study is similar to the result reported by Wang, Sanderson and Zhang and Xu et al.\textsuperscript{[13,14]}, that higher the α-mangostin in the extract of pericarp higher the cytotoxicity activity.

4. Conclusion
Heating the fresh pericarp mangosteen in water at 65°C for 30 minutes prior to blending produced a juice with higher α-mangostin yield and cytotoxicity compared to the traditional way of blending the juice at room temperature. Increasing α-mangostin yield of 9%-w/w due to heating was also observed when mangosteen extract was heated at 65°C, consistent with the increased cytotoxicity in terms of LC\textsubscript{50} values of 0.71 ppm at room temperature to < 0.1 ppm at 65°C. The results of this study indicate that the extract of mangosteen pericarp obtained from the ethyl acetate fraction of the ethanolic extract has the potential to be used for anti-cancer medication.

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