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Original Article

THE OPTIMIZATION OF ELUTING CONDITION OF SOLID PHASE EXTRACTION METHOD FOR α-MANGOSTIN PURIFICATION IN MANGOSTEEN PERICARP EXTRACT

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ABSTRACT

Objective: To obtain the optimum condition of eluent selection for purification of α-mangostin in mangosteen pericarp extract with solid phase extraction (SPE) method.

Methods: The analysis was conducted by SPE cartridge octadecylsilane (ODS)-5, solvents used were methanol for conditioning, aqua bidestilated for washing and three different eluents for eluting: methanol, ethanol and ethyl acetate.

Results: Recovery value for each eluent were 32.4741% for methanol, 46.6130% for ethanol and 33.6383% for ethyl acetate. Comparison of alpha-mangostin level in the mangosteen pericarp extract for each eluent i.e. 14.4578% for methanol, 10.0598% for ethanol, and 14.2898 % for ethyl acetate while compared to the extract without SPE was 21.7934%.

Conclusion: Ethanol had the highest recovery value, compared to methanol and ethyl acetate.

Keywords: α-mangostin, Eluent, Optimization, Solid phase extraction

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INTRODUCTION

Mangosteen pericarp extract from *Garcinia mangostana* has been studied for its pharmacological effects such as activity against tumor [1], antioxidant [2], anti-inflammatory agent [3] and anti-bacterial agent [4]. One of the active compounds which have the highest number in mangosteen extract is alpha-mangostin [5]. Beside of alpha-mangostin, the result of mangosteen extraction followed by the possibility of many impurities and other secondary metabolites such as tannin, anthocyanin and other xanthone derivates [6, 7]. All of those impurities may interfere with both qualitative and quantitative analysis of the target compound.

Solid phase extraction (SPE) is a widely applied method for purification of extract sample. SPE is used in the separation process to overcome limitations in analyzes such as the concentrations of the analyte is very low or complex sample matrix which can cause blocked columns. Therefore, SPE can minimize "column killers" and extend the life of the high-performance liquid chromatography (HPLC) columns [8-12].

One of the factors that determine the success of the SPE method is the type of eluent used because it will affect the number of target compounds to elute and release from solid phase matrix [13]. The aimed of this study was to develop the new method for obtaining the optimum condition of SPE method for purification of α-mangostin in *Garcinia mangostana* ethanol extract using three types of solvent, i.e. methanol, ethanol and ethyl acetate. Further, our study can be used as the basic of the extraction of alpha-mangostin from dosage forms and biological samples for analysis.

MATERIALS AND METHODS

Chemicals and reagents

Standard α-mangostin was purchased from Chengdu Biopurify. Mangosteen pericarp from Tasikmalaya was macerated with 70% ethanol then standardized according to parameters of General standard parameters of medicinal plant extracts by Indonesian Food and Drug Regulatory Agency [14]. The chemicals and solvents used in this experiment were methanol pro-HPLC from Merck, high purity aqua bidestilated from IPHA Laboratories, ethanol, ethyl acetate and acetic acid from Merck.

Preparation of alpha-mangostin standard solution

Accurately weigh of 10 mg of alpha-mangostin were dissolved in methanol add to 100 ml, obtain the standard solution with a concentration of about 100 µg/ml which was subsequently diluted to 5 µg/ml.

Preparation of mangosteen extract solution

Weigh of 10 mg of mangosteen extract were also dissolved in methanol add to 100 ml to obtain the extract concentration of about 100 µg/ml. Then the solution was diluted to obtain a concentration of 50 µg/ml and filtered through millipore 0.45 µm.

Optimization of SPE condition

SPE cartridge ODS-5 octadecyl (Whatman®) was activated by let through 3 ml of methanol using the vacuum. The cartridge was kept wet. 1 ml of sample solution was inserted into the cartridge. The filtrate was re-inserted into the cartridge to optimize the desired analyte retained in the cartridge. Then the cartridge was washed with 2 ml of aqua bidestilated. Circulate the air through SPE tube for 3-5 min until the cartridge was dry. Alpha-mangostin was eluted 2 times with 1 ml of eluent (the condition I with methanol, the condition II with ethanol and the condition III with ethyl acetate) so the final volume was 2 ml. This procedure was performed for a 5 µg/ml standard solution of alpha-mangostin and 50 µg/ml of mangosteen extract solution.

Mobile phase preparation

Methanol and acetic acid 1% with a ratio 95:5 was sonicated for 15 min to remove the air bubbles then filtered through millipore 0.45 µm.

Analysis of alpha-mangostin content using HPLC

The alpha-mangostin standard solution and mangosteen extract solution result of the three different SPE condition and mangosteen extract solution without SPE preparation was injected to HPLC with the condition [15,16]:

Column: Enduro C-18 (diameter 4,6 mm and length 250 mm)

Flow rate: 1.0 ml/min

Detector: UV 246 nm

Mobile phase: Methanol: acetic acid 1% (95:5)

Injection volume: 10 µL

The concentration of alpha-mangostin was calculated by measuring the area under the curve to the linear regression equation and recovery value measured with the equation:

$$\text{Recovery (\%)} = \frac{a}{b} \times 100\ \%$$

where: a is recovery concentration of standard solution and b is recovery concentration [17].

RESULTS AND DISCUSSION

SPE cartridge ODS-5 has been used as stationary phase in this study. The selection of sorbent based on its interaction mechanism between sorbent and targeted compound also its properties, where ODS sorbent is used for a semi to non-polar target compound so it can be used for alpha-mangostin [18]. Beside that, in the previous study by Chitchumroonchokchai *et al.* (2012), ODS is used for extraction of xanthone in a urine sample in which alpha-mangostin is one of the xanthone group compound [19].

This study was conducted to see the best eluent in eluting alpha-mangostin with SPE method. The eluent that has been used are methanol, ethanol and ethyl acetate. Methanol was selected because alpha-mangostin soluble in methanol, while ethanol and ethyl acetate were selected because their polarity is close to methanol. It is expected that the compounds remaining in the SPE cartridges can be eluted or pushed out entirely by the eluent which has the same polarity.

The best condition of SPE to alpha-mangostin purification can be seen from percent recovery of analyte for each eluent. The higher value of percent recovery shows the higher number of alpha-mangostin which eluted so that eluent which has the highest value of percent recovery is the best for eluting alpha-mangostin in this method because the possibility of sample loss is minimum. The recovery value was obtained by comparing the concentration of alpha-mangostin standard solution the result of SPE with the standard concentrations of alpha-mangostin that used in the SPE process. Results of recovery for each eluent used can be seen in the levels of alpha-mangostin detected by HPLC. Levels of alpha-mangostin obtained by entering the value of AUC to the equation y = 0.6952x+0.3625 with regression value R^2 = 0.9975 (fig. 1).

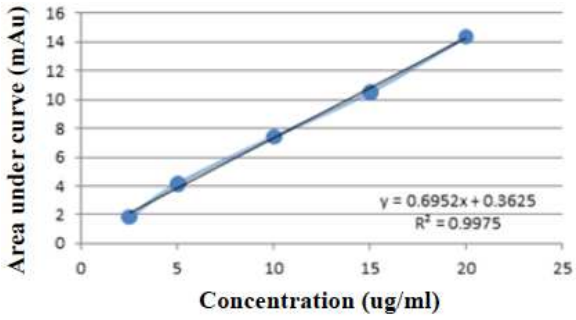

Fig. 1: Calibration curve of alpha-mangostin

Based on the data of recovery can be seen that the three eluents used are not optimum in eluting the alpha-mangostin compounds in the SPE, but ethanol produces the best percent recovery compared to methanol and ethyl acetate although the percent recovery is not high at 46.6130% (table 1). Lower recovery value that can be caused due to the eluents are not able to break the bonds between the alpha-mangostin with sorbent so the alpha-mangostin was not eluted perfectly. Seen from the three types of eluent used, ethanol is a solvent having the strongest ability to break the bond between analyte and sorbent than methanol and ethyl acetate. Ethanol having index polarity 4.3, lower than methanol with an index polarity 5.1 and ethyl acetate 4.4. The low recovery can also be caused due while the process of conditioning sorbent was dry causing a decrease in retention of samples on the sorbent.

Table 1: The results of recovery each eluent

Eluent	Standard level (µg/ml)	Standard SPE result (µg/ml)	Recovery (%)
Methanol	5	1.6237	32.4741
Ethanol	5	2.3306	46.6130
Ethyl Acetate	5	1.6819	33.6383

When compared with the results of sample extracts with SPE for each eluent, that produces the higher levels of alpha-mangostin if use methanol in the elution process. The possibility of the high level of alpha-mangostin detected because the other components in the extract that is soluble and get carried away with methanol causing an accumulation of absorption.

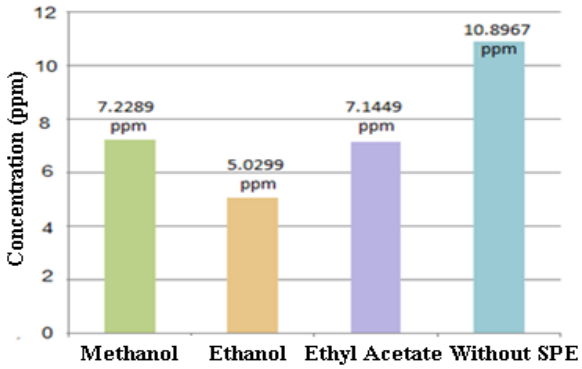

Fig. 2: Comparative diagram of SPE alpha-mangostin level

Fig. 2 shows that the alpha-mangostin levels produced from the extract samples prepared with SPE were much lower when compared to the extract samples without SPE. It can be caused by loss of alpha-mangostin in the process of SPE. In addition, this may be due also because the amount of solvent used for elution is less. When compared with the study by Walsh *et al.* (2007), the amount of eluent used in eluting isoflavones in a urine sample is 7 ml of methanol; Seo *et al.* (2005), used 10 ml of n-hexane-ethyl acetate (27:75, v/v) to elute growth hormone from dried meat extract samples; Nurabadi *et al.* (2015) used 12 ml of ethanol to elute ferulic acid from the extracted sample [20-22].

CONCLUSION

Ethanol has the highest recovery value (46.6130 %), compared with methanol (32.4741 %) and ethyl acetate (33.6383 %). Levels of alpha-mangostin in mangosteen extract of SPE with eluent methanol 14.4578%, ethanol 10.0598% and ethyl acetate 14.2898% whereas when compared with extract without SPE alpha-mangostin levels in mangosteen peel of 21.7934%.

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CONFLICTS OF INTERESTS

Declared none

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