Purification of Curcumin Derivate (1,5-bis(4'-hydroxy-3'-methoxyphenyl)-1,4-pentadien-3-on) Using Chromatotron

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Abstract

Curcumin derivate with IUPAC name 1,5-bis(4'-hydroxy-3'-methoxyphenyl)-1,4-pentadien-3-on is also known as Gamavuton-0 (GVT-0) reported has an activity as anti-inflammation and anti-cancer. GVT-0 can be synthesized using vanillin and acetone as starting material by aldol condensation. Purification process of GVT-0 after synthesis usually was done using maceration. This method resulting un-pure GVT-0, therefore, another separation technique of Chromatotron was chosen. This research aims to investigate the effectiveness of Chromatotron towards the purity of GVT-0. The study was started by GVT-0 synthesis. The synthesis was using 4.4 mole of vanillin and 1 mole acetone as the raw material. Microwave irradiation was used as the energy source. Isolation of GVT-0 using hot water maceration. Further, the purification of GVT-0 was conducted using Chomatotron. First step of purification using Chomatotron was preparing best ratio between hexane and chloroform as solvent to remove vanillin. Further, the best combination of solvent was used to separate all the vanillin remaining in the bulk of GVT-0. Last step after all vanillin was removed, the GVT-0 remining in the silica was elucidated using chloroform. The purity was evaluated using Melting-point analysis and Thin Layer Chromatography (TLC) with four different mobile phases. Based on the data of melting point and TLC, a pure GVT-0 was obtained. The Chomatotron may be recommended for purification of GVT-0, however this method consumes a lot of organic solvent that may need to be improved in the future with more environmentally process.
***Keywords**: Chromatotron, GVT-0, melting point, TLC.*

INTRODUCTION

Cancer is a disease caused by free radicals (Singh et al., 2018). Cancer arises due to the abnormal development of human tissue cells (Raflizar & Nainggolan, 2010). An increase in free radical compounds in the body followed by a decrease in antioxidant compounds in the body lead organ cell damage (Balasubramanyam et al., 2003).

One of the compounds that have anticancer activity is curcumin, which can be extracted from *Curcuma sp* plants (Muti'ah, 2015). Curcumin has been proven in preclinical studies of in vivo and in vitro approaches to regulate transcription factors, growth factors, inflammatory cytokines, protein kinases and enzymes (Raflizar & Nainggolan, 2010; Sardjiman et al., 1997; Valko et al., 2015). However, curcumin has stability problem that is unstable in the high pH and low light stativity (Tønnesen, 2002). In addition, the bioavailability of curcumin is low (Sabet et al., 2021; Siviero et al., 2015). Fast metabolism and low absorption are the reasons curcumin is needed to be developed as a modern drug.

One of the analogues of curcumin is Gamavuton-0 or 1,5-bis(4'-hydroxy-3'-methoxyphenyl)-1,4-pentadiene-3-on. GVT-0 has only one carbonyl group. GVT-0 has a shorter carbon chain compared to curcumin. GVT-0 have better stability than the parent compound. Sardjiman et al (1997) stated that the activity of GVT-0 as an antioxidant showed the same results as its leading compound, curcumin (Sardjiman et al., 1997).

TLC analysis for bulk GVT-0 resulting from the synthesis obtained two spots when elucidated using chloroform: ethyl acetate at ratio 5 to 1. First spot has the same R_f as

vanillin which is 0.72 and the second spot has the same R_f as GVT-0, which is 0.5. Based on this analysis, it can be interpreted that in the bulk of GVT-0 still containing starting material, i.e. vanillin (Harimurti et al., 2019).

Chromatotron or centrifugal chromatography is one type of chromatographic methods. This method uses the separation technique of centrifugal force and gravitational force (Móricz & Vovk, 2016). The working principle of this method is the same as other chromatographic methods, but this method will more quickly separate the desired component from the solvent. This is because the chromatotron method uses centrifugal force (Atun, 2014).

RESEARCH METHODOLOGY

Materials

Food grade of vanillin and analytical grade of HCl 37% were obtained from Bratachem. Acetone analysis grade, ethanol analysis grade, chloroform analysis grade, hexane analysis grade, Silica gel GF 254 for TLC, and ethyl acetate analysis grade were obtained from Merck.

Methods

Synthesis of GVT-0

The synthesis of GVT-0 was using starting material vanillin and acetone at mole ratio of 4.4:1. HCl 37% was used as a catalyst as much as 55 µl. The synthesis of GVT-0 was conducted under microwave irradiation for 2 minutes and a power of 650 watts in the kitchen microwave tool. Vanillin was weighed as much as 9.1114 grams and dissolved in ethanol. Then 10 ml of acetone was acidified using 55 µl of HCl. One (1) ml of acidified acetone was taken to be mixed into the dissolved vanillin. Then the mixture is put in the microwave for reaction process (Harimurti et al., 2019).

GVT-0 Isolation

Isolation of GVT-0 was using hot distilled water at temperature of 95°C to remove raw material vanillin remain in the synthesized mixture. Hot distilled water was mixed into the synthesized mixture, then directly filtered using filter paper and the bulk results were taken from paper filter. Further, an amount of hot water then passed into the bulk remain in the paper filter for flushing. Separate yellowish GVT-0 then collected and put in porcelain pot for overnight slow drying in the oven at 50°C, to remove remining water.

Purification of GVT-0 using Chromatotron

Silica gel GF 254 was used as the stationer phase. Silica gel was prepared for 24 hours before use by adding of 0-10^{o}C distilled water in the powder of Silica gel. Different polarity of mobile phases was used, that are combination of hexane and chloroform at ratio 6 to 4 (based on the optimization of solvent) and chloroform only. The combination of the solvent was used to remove vanillin and the chloroform was used to elucidate GVT-0 form the silica gel during the purification. The best ratio of hexane and chloroform was found from optimization proses. The optimization of hexane and chloroform was done by trying to vary of ratio between hexane and chloroform (10:0, 7:3, 6:4, 4:6, and 3:7, respectively) to separate GVT-0 and vanillin using TLC (**Figure 1**). After the best ratio solvent was found than used to remove vanillin using Chromatotron. The elution technic was done in polarity gradient technique, that means the separation was done in different polarity of eluent. The 2.5 grams of bulk was dissolved in 5 ml of chloroform, then put into the Chromatotron for elution. The elution result was collected time to time to obtain the pure GVT-0. The liquid containing GVT-0 then evaporated to obtain the pure GVT-0.

Purity analysis of purified GVT-0

The qualitative analysis was used Thin Layer Chromatography (TLC) and melting point analysis. The TLC analysis was done using three (3) different eluents, that is butanol : acetic acid : ethanol (6:1:5); butanol:

acetic acid: water (7:0,5:0,5); and ethyl acetate : butanol : acetic acid (4:3:1).

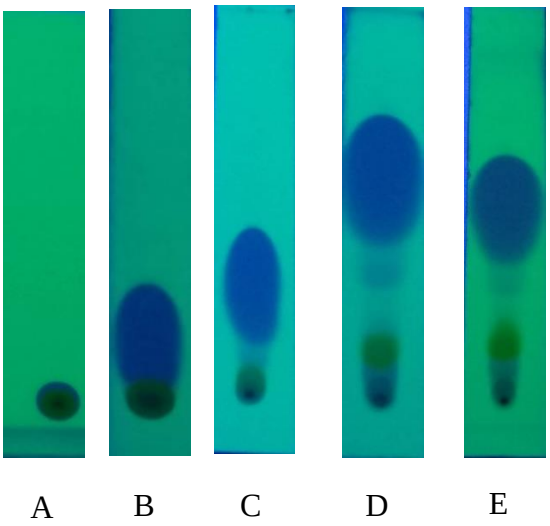

Figure 1. TLC analysis for optimization mobile phase, where A. Hexane; B. Hexane : chloroform (7:3) ; C. Hexane : chloroform (6:4) ; D. Hexane : chloroform (4:6) ; E. Hexane : chloroform (3:7)

RESULT AND DISCUSSION

Synthesis of GVT-0

The synthesis of GVT-0 was carried out under microwave irradiation. The use of a microwave is based on the use of a tool that is easy and fast to perform synthesis resistance, in addition, the use of microwaves can be energy efficient and more environmentally friendly when compared to conventional methods (Harimurti et al., 2019). Synthesis was done using starting material of vanillin and acetone at the mole ratio of 4.4:1. The synthesis was done for 2 minutes under microwave irradiation. The result of the synthesis is a brownish yellow liquid.

Isolation of GVT-0

Hot distilled water at 95°C was used as a solvent in the isolation of GVT-0. The use of hot distilled water is intended to separate the starting material vanillin and any by product which is still present in the synthesized liquid. This isolation stage was carried out twice using filter paper and a separating funnel. After isolation, GVT-0 was found to be an average of 5.7495 grams. This isolation using hot distilled water producing not pure GVT-0. This can be seen in **Figure 2**, that during testing using TLC, the bulk of GVT-0 after isolation using hot distilled water still containing raw material vanillin and unknown by-product. The Rf GVT-0 was found to be 0.5 and the Rf of starting material was found to be 0.72. Under the UV light at 254 nm, GVT-0 give yellow color and vanillin give blue spot. Many spots were also found in the TLC plat, this finding indicate there may some unknown by products was produced during the synthesis. This is in accordance with previous research, which is reported that in the TLC analysis GVT-0 has an Rf of 0.5 and any other spots were found (Harimurti et al., 2019).

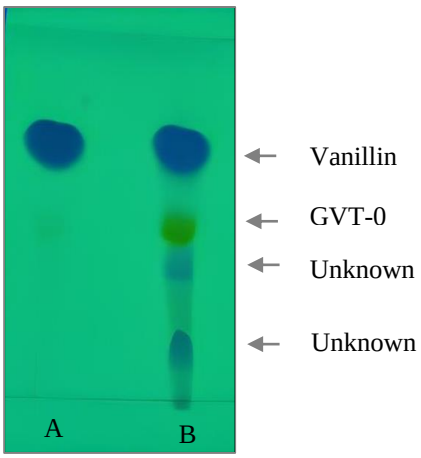

Figure 2. TLC analysis of bulk GVT-0, A = vanillin and B = bulk GVT-0

Purification of GVT-0 using Chromatotron

Purification that carried out using a gradient elution technique namely Chromatotron was done by another researcher for bioactive compound (Agrawal & Desai, 2015). This technique requires several different mobile phases to separate a compound. The main purpose of this gradient system is to change the polarity of the mobile phase so that it can be adjusted to the desired compound (Jandera & Churáček, 1981).

The prepared bulk was put into a Chromatotron for elution. The first mobile phase selected was hexane: chloroform (6:4). This mobile phase was used to bind vanillin first to leave the Chromatotron.

Vanillin that comes out of the Chromatotron was accommodated in an Erlenmeyer. The gradient technique is used to remove the compound except GVT-0 from the bulk. The removing process was stopped when the solvent that was used does not contain any material. This condition can be approved by a qualitative analysis using TLC, when the plat of TLC does not appear any spot that mean all the compounds except GVT-0 were removed.

The next step is elution of GVT-0 from the Chromatotoron plat. The process was conducted using chloroform. The chloroform was chosen since this solvent able to dissolve the GVT-0. The GVT-0 was then collected and evaporated to obtain the GVT-0.

Purity evaluation of GVT-0

The purity evaluation was conducted using melting point analysis and TLC analysis using three different solvents. The three solvents were used to make sure the purity of GVT-0, when the elution is conducted in many different solvents with different polarity and give single spot, that can be conclude the compound is pure. The mobile phase used was butanol : acetic acid : ethanol (6:1:5) ; butanol : acetic acid : water (7:0,5:0,5); and ethyl acetate : butanol : acetic acid (4:3:1). The results obtained from the multi-eluent purity test can be seen in **Figure 3**. Based on the results of multi-eluent TLC, a single spot was obtained in each mobile phase. It can be said that the purified GVT-0 is purely by TLC.

Another purity test performed is melting point. Melting point is one of the important characteristics of each compound. Melting point is a test to see the temperature at which a change from solid to liquid occurs in a compound. Melting point is an important physical constant in synthesized compounds (Yalkowsky & Alantary, 2018).

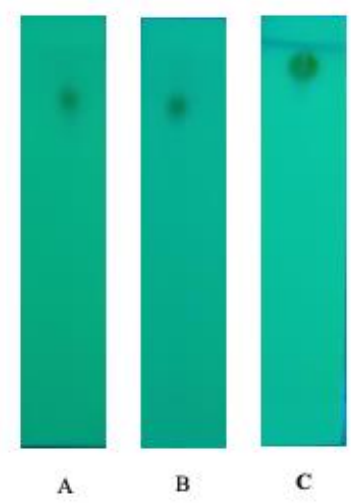

Figure 3. The TLC analysis using multi-eluent of purified GVT-0
(A : butanol : acetic acid : ethanol (6:1:5) ; B : butanol : acetic acid : water (7:0,5:0,5) ; C : Ethyl acetate : butanol : acetic acid (4:3:1))

The melting point of GVT-0 with the IUPAC name 1,5-bis(4'-hydroxy-3'-methoxyphenyl)-1,4-pentadien-3-on is 98-99 °C (Safitri et al., 2018). The measurement of melting point from the purified GVT-0 was started at 87.9 °C and totally melt at 89.8 °C.

The different temperature of initial and final melting of GVT-0 was 1.9°C. This is still following the standard melting point analysis of a pure compound is between 1-2 °C (Safitri et al., 2018; Yalkowsky & Alantary, 2018). Thus, the purified GVT-0 can be said to be pure based on its melting point analysis.

CONCLUSIONS

Chromatotron is effective for purifying and separating curcumin derivative compounds GVT-0 from raw material and other by product occur during the synthesis. The resulting GVT-0 was concluded pure by TLC and melting point after separation using Chromatotron by showing a single spot-on TLC with three eluents and the initial to final melting point analysis was found to be 1.9 °C.

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