DETERMINATION OF ANNONACYN COMPOUND BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ON THE EXTRACT OF Annona muricata LINN SEED FOR PESTICIDE FORMULA

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ABSTRACT

Determination of annonacyn grade, a main insecticide compound has been done in the ethanolic and ethyl acetate extracts produced by soxhletation, and ethyl acetate extract produced by fractionation of ethanolic extract to find out the most active extract to be used as raw material of pesticide formulae. Analysis method used the reverse phase high performance liquid chromatography with column of Novapack ODS C-18 (waters; 3.9 x 150 nm), mobile phase was the mixture of acetonitrile-water. The result of this research showed the optimum condition as follows: the mobile phase was acetonitrile-water (60:40), flow rate of 0.2 mL min^{-1}, injection volume of 5.0 μL, detector UV, wavelength (λ) at 220 nm with AUFS of 1.00. The limit detection of annonacyn was 0.01 μg, annonacyn grade in ethanolic extract produced by soxhletation: 0.0405 $\pm$ 0.0021%, in ethyl acetate extract produced by soxhletation: 0.0293 $\pm$ 0.0009%, in ethyl acetate extract produced by fractionation of ethanolic extract: 0.1003 $\pm$ 0.0018%. The precision and accuracy of annonacyn in this research were respectively obtained 8.89% - 1.92%, and 4.91% - 7.28%, at the concentration of 5.00 μg mL^{-1} – 25.00 μg mL^{-1}. The sensitivity was 0.75 $\leq b \leq$ 1.13.

Keywords: *Annona muricata Linn, Annonacyn, HPLC.*

INTRODUCTION

The most active main compound of the tetrahydrofuran of acetogenin Annonaceae is annonacyn [1]. Qualitative and quantitative evaluation by high performance liquid chromatography (HPLC) of acetogenin mixture could be done by using ultraviolet, refractometry, and evaporation light scattering detectors, respectively. Frequently, column used μ-Bondapack C-18 (10 μm) or Novapack ODS C-18 with acetonitrile-water (80:20) and (70:30) as eluent [2]. This system has succeeded to separate two epimeric of acetogenin, rolliniastatin-1 and rolliniastatin-2. The eluent system of acetonitrile-water (80:20) has been done to separate acetogenins in methanolic extract of Holland jackfruit seed (*Annona muricata*) while the 70:30 system of acetonitrile-water was done to separate the acetogenins standard mixture which showed by the present of sharp and symmetry peaks and the annonacyn still have the fastest retention time (Fig 1).

In the previous paper [3], annonacyn has been identified qualitatively by ultraviolet and infrared spectra, and HPLC. In this paper, I wish to discuss the determination of annonacyn grade by HPLC on the extract of *A. muricata* Linn seed.

Based on the polarities, chromatography system consist of normal phase and reverse phase chromatography [4]. Phase normal chromatography system is the interaction of polar compounds with the polar of column filler material, and the mobile phase is non polar or semi-polar such as hexane and methylene chloride. The mobile phase used is the less polar than stationary phase. The reverse phase chromatography used the stationary phase of non polar and the mobile phase is polar [5]. The reverse phase chromatography is usually used to separate polar substances. The most polar substance will have shortest retention time, while the less polar components will be hold longer because they have more high affinity than column [6].

EXPERIMENTAL SECTION

Material

Solvents used were proanalysis quality. The seeds of Holland jackfruit (*A. muricata*) were obtained from PT. Ultra Jaya Milk Indo Bandung, dried and grinded to give flour. The standard compound was annonacyn ($C_{35}H_{64}O_7$).

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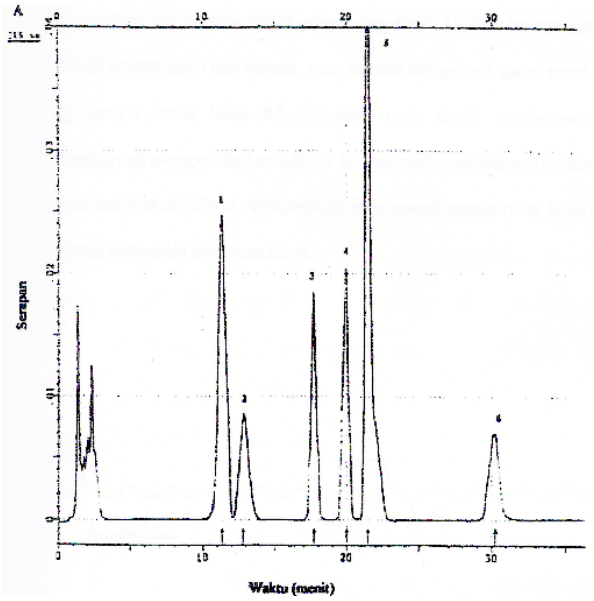

Fig 1 Chromatogram of methanolic extract of the Holland jackfruit seed, RP-HPLC, μ-Bondapack C-18 10 μm column (4.6 x 250 mm), eluent of acetonitrile-water (70:30), flow rate 1.5 mL min^{-1}, detector UV, injection volume 20 μL. (1. annonacyn, 2. annonacynon, 3. corossolin, 4. corossolon, 5. murisolin, and 6. solamin) [2]

Instrumentation

Chromatographic instrument was RP-HPLC Waters 501, ultraviolet detector (waters-490 E programmable multi wavelength detector), column Novapack ODS C-18 (3.9 x 150 nm) and wavelength at 220 nm, eluent of acetonitrile-water, where the acetonitrile was HPLC quality, and water was aquadest high purity. The data was processed by use baseline 810 chromatography work station.

Procedure

Preparation of sample

The first extract was ethanolic extract producing by soxhletation. The seeds flour of *A. muricata* (150 g) was extracted in soxhlet apparatus by hexane and ethanol 95% successively. The ethanolic filtrate was evaporated to obtain ethanolic extract.

The second extract was ethyl acetate extract that produced by soxhletation. The seed flour of *A. muricata* (100 g) was extracted in soxhlet apparatus by hexane and ethyl acetate successively. The ethyl acetate filtrate was evaporated to obtain ethyl acetate extract.

The third extract was ethyl acetate extract producing by fractionation of ethanolic extract. The ethanolic extract (1.5 g) was diluted in water (100 mL) then extracted by ethyl acetate. The ethyl acetate filtrate was added Na_2SO_4, then filtered and evaporated to obtain ethyl acetate extract produced by fractionation.

Preparation of standard compound

Annonacyn was diluted in methanol to obtain the following concentrations: 5; 10; 15, 20, 25 μg mL^{-1}.

Analysis by High Performance Liquid Chromatography

The experiment of compound standard involve maximum wavelength by HPLC, determination of purity on the optimum condition using Novapack C-18 (3.9 x 150 nm) column, composition and flow rate of eluent were arranged by baseline 810, injection volume were 5, 10, 15, and 20 μL. Detection system was done by arranging the wavelength at detector UV by HPLC.

Preparation of standard curve was done by using standard solution, and linearity test was also done. Based on the relationship of concentration and aboundement, it could be made the equation of its linear regression as: $Y = a \pm bx$; where Y = standard aboundement; a = intercept; b = slope; and x = concentration. Parameter analysis involved limit detection, sensitivity, precision and accuracy.

Determination of annonacyn grade on the sample

Sample Preparation. The extract of A. muricata seed was diluted in methanol, pipette and diluted again until injection, then filtered by using Millipore filter paper. Before injection, the extract filtered again by sample filter. Determination of annonacyn grade was done by injecting the sample on HPLC operated at optimum condition and then the quantitative identification was done on the produced chromatogram.

*Qualitative analysis (Co injection)***.** Qualitative analysis was done before quantitative analysis to know the present of annonacyn in sample [7]. The qualitative analysis was done by adding the annonacyn standard into sample then eluted to HPLC at the obtained optimum condition. On the chromatogram would be resulted retention time and abound width equivalent with peak area, that automatically measured by instrument. Annonacyn compound in sample could be identified by the change of peak area if it compared with the chromatogram that not added standard solution. Confirmation of the present annonacyn in sample was done by changing the polarity of mobile phase [8].

RESULT AND DISCUSSION

Determination of the HPLC Optimum Condition of standard compound.

Experiment on standard annonacyn compound by HPLC was aimed to observe the

chromatography profile of standard annonacyn compound, which will be used to determine by HPLC the annonacyn compound in the extract of *A. muricata* seed, qualitatively and quantitatively. Optimum separation was done by several experiments on standard annonacyn compound which injected to HPLC with several compositions of mobile phase, flow rate, injection volume, and wavelength. Variation of the mobile phase composition was done by using the mixture of polar solvent, acetonitrile and water at the comparison: 60:40; 70:30; 80:20; 85:15; 90:10 respectively. The best result was obtained at the eluent composition of 60:40, while retention time was 5.21 minute. The flow rate was tested at the 5, 10, 15, 20 μL and obtained the optimum injection volume was 5 μL.

Optimum separation was obtained by using the following conditions: mobile phase acetonitrile-water (60:40), flow rate 0.2 mL min^{-1}, injection volume 5 μL, detector UV at wavelength 220 nm with AUFS sensitivity of 1.00.

The samples extraction as result of the seed *A. muricata* Linn.

Ethanolic extracts produced by soxhletation obtained as much 3.75 g, ethyl acetate extracts produced by soxhletation obtained as much 2.61 g, and ethyl acetate extracts by fractionation from ethanolic extract produced by soxhletation obtained as much 0.32 g.

Analytical Performance

These performances covered several parameters i.e. calibration curve, detection limit, sensitivity, accuracy and precision. The calibrate curves were made by used the date chromatogram with grooving to concentration of the standard compound. From calculation result obtained by that detection limit for the annonacyn compound at this research was 0.01 μg. The sensitivity was $0.75 \leq b \leq 1.13$. Result of accuracy is 8.98 – 1.92 and precision is 4.91 – 7.28.

The results of the qualitative analysis and rate determination of each extracts

From the experimental results (Fig 2), as the reality it was observed that the extract of sample seed of *A. muricata* contains the annonacyn compound. The existence of annonacyn compound in the extract sample can be seen from chromatogram with the existence of wide change in culminate at retention time of 5,53 minute (ethanolic extracts produced by soxhletation); 5.40 minute (ethyl acetate extracts produced by soxhletation); and 5.66 minute (ethyl acetate extracts produced by

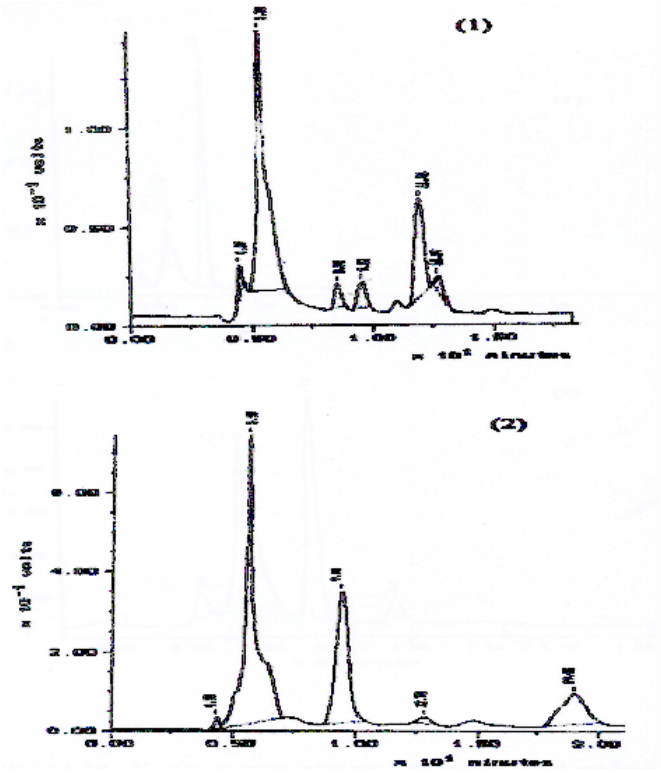

Fig 2 Chromatogram ethanolic extracts of the soxhletation samples of seed AML, the moving phase of the acetonitryl : water (60:40), flow rate is 0.2 mL/min, injected volume is 5,0 μL, detector UV, wavelength (λ) at 220 nm: (1) The extract sample of 5 ppm without annonacyn standard solution (2) The extract sample of 5 ppm with annonacyn standard solution

fractionation of ethanolic extracts), as compared to by chromatogram of extract sample before the addition of annonacyn standard compound.

The overflows of sample without standard compound were 3,766; 3,655; 3,688 volt second , and the rate annonacyn in sample may be calculated as x from regression equation of annonacyn curve (correction of result). The estimation of the level using the formula, $x \pm tS/\sqrt{n}$. As the result, the rate of annonacyn is 0.0405% ± 0.0021%, its mean at confidence level 95% population Brown's [9] the compounds exists between 0.0384% until 0.0426%.

The overflows of sample without standard compound were 2,655; 2,665; 2,701 volt second . As the results, the rate of annonacyn is 0.0293% ± 0.0009%, meaning at confidence level 95% population Brown's [9] exists between 0.0284% until 0.0302%.

The overflows of sample without standard compound were 9,210; 9,111; 9,204 volt second. As the result, the rate of annonacyn is 0.1003% ± 0.0018%, that is in 95% confidence level population

mean Brown's [9] exists between 0.0982% until 0.1021%.

Statistical test to compare the significant differences in the results of annonacyn compound toward ethanolic extracts (S-1), ethyl acetate extracts (S-2), and ethyl acetate extracts from ethanolic extracts (S-3), using t-test of each couple of sample extracts (S-1 and S-2 ; S-1 and S-3; S-2 and S-3) in interval confidence of 95%, db=5, based on the average value and deviation standard from every sample extracts couple is that annonacyn compound which obtained from sample extracts S-1 and S-2 are significantly differ, whereas [t-count]= 2.154 > t-table$_{(0.5;5)}$ =1.476. Therefore, for S-1 and S-3, S-2 and S-3 the annonacyn compound obtained are significantly differ, whereas [t-count] for S-1 and S-3 = 1.740 > t-table$_{(0.5;5)}$ =1.476, and [t-count] for S-2 and S-3 = 1.742 > t-table$_{(0.5;5)}$ =1.476.

CONCLUSION

1. The analysis of the compound of annonacyn extracts from *A. muricata* Linn seed can be conducted with the method of reversed phase HPLC are column of Novapack ODS-C18 (3.9x150 mm), show the optimum condition as follows. The mobile phase : mixture of acetonitril:water (60:40), flow rate: 0.2mL.min^{-1}, injection volume : 5.0 μL, detector UV, operated wavelength (λ) at 220 nm with AUFS of 1.00.
2. The rate of the annonacyn in the ethanolic extracts obtained by of soxhletation was 0.0405% ± 0.0021%, in ethyl acetate extract produced by soxhletation: 0.0293% ± 0.0009%, in ethyl acetate extract produced by fractionation of ethanolic extract: 0.1003% ± 0.0018%.

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