

Conference Paper

Measurement of Alkaloids Achyranthes Aspera Linn Level Using Thin Layer Chromatography Method and High-Performance Liquid Chromatography

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Abstract

One of the plants with the potential to be developed into a raw material for anti-breast cancer drugs is jarong (*Achyranthes aspera* linn). Allegedly alkaloid of *Achyranthes aspera* linn leaves showed anticancer activity in vitro in mice myeloma cells is extremely potent. In vivo study showed that the alkaloid *Achyranthes aspera* linn causes apoptosis and healing in breast cancer cells in mice induced by benzopyrene. Alkaloids of *Achyranthes aspera* linn leaves as anticancer been listed on Incestual Property Rights (IPR) since October 12, 2012, with number P00201299839. This study aims to prove the existence of alkaloids in the leaves of *Achyranthes aspera* linn using thin layer chromatography (TLC), determine the level of alkaloids in the leaves alkaloid fraction of *Achyranthes aspera* using High-Performance Liquid Chromatography (HPLC) and alkaloid fraction dose as an anticancer and dosing for the study. Alkaloid qualitative measurement results with thin layer chromatography showed alkaloid in orange. Alkaloid levels using HPLC has a retention time of 3.482 minutes, a wavelength of 254 nm, there are 13 kinds of chemical substances with the major components amounted to 52.36% alkaloids, the other component is the green substance with a peak area of 13.0624%, while other chemical substances in a very small peak area of between 0.428% until 8.3598%. Alkaloid fraction dose of *Achyranthes aspera* used in the study as anticancer is controlled the dose of 0 mg/kg, 1 treatment dose of 60 mg/kg, 2 treatment dose of 75 mg/kg and 3 treatment dose of 90 mg/kg.

Keywords: Alkaloids, *Achyranthes aspera* linn, TLC, HPLC.

1. Introduction

Achyranthes aspera linn, plant known as jarong, Jarongan or remek getih, pulet or puluten empirically has been used for thin out the births of drinking leaves juice after

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Received: 03 October 2017

Accepted: 10 October 2017

Published: 29 November 2017

Publishing services provided
by Knowledge E

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doing sexual intercourse. Pregnant women are not allowed to drink the leaves juice is due to be able to cause a miscarriage. The preliminary study conducted by [9] used the leaves juice of *Achyranthes aspera* can cause changes in the estrus cycle in mice that extension phase of proestrus and diestrus. [10] conducted a study of *Achyranthes aspera* leaves ethanol extract given to female mice mated with a dose of 300 mg/kg bw orally cause a decline in the number of pregnant mice, the site of implantation, the number of corpus luteum and the number of fetus conceived during a period of pregnancy. Alkaloid as antimitotic and antitelomerase have the ability to bind to tubulin, which is a protein that compiles microtubules by inhibiting or blocking the polymerization of protein into microtubules causing the destruction of microtubules resulting cells will stop dividing so that will be followed by the occurrence of cell death (apoptosis) [1, 4, 5, 9, 10].

Some alkaloid found in plants known to work as an antimitotic and has been used in the society as an anticancer drug such as vinblastine, vincristine contained in the leave of tapakdara plants (*Catharanthus rose*), paclitaxel (taxol) derived from the bark of the Yew (type of pine) and etoposide contained in the leaves of mandrake plants (*Podophyllumpellatum*) type of papaya trees and colchicine derived from *Colchicum autumnale* plants known to have an effect as antimitotic and now used to inhibit the growth of cancer cells [7]. From that information, the *Achyranthes aspera* plants are necessary to study the magnitude of the fraction of alkaloids contained in leaves extract which can be used as an anticancer drug. This study aims to prove the existence of alkaloids and measure the alkaloid fractions of *Achyranthes aspera* leaves.

2. Materials and Methods

Making alkaloid fraction extract of *Achyranthes aspera* with standardization and validation extracts based on [2]. First, Proving the existence of alkaloids in *Achyranthes aspera* linn extract using thin layer chromatography (TLC). Then, Determining the level of alkaloid fraction extract of *Achyranthes aspera* using HPLC method of [2] and determining the alkaloid fraction dose anticancer.

2.1. Extraction and Determination of Alkaloids in Alkaloid Fraction Extract of *Achyranthes aspera* linn Leaves

The extraction process was done by [2] include the maceration process with n-hexane, methanol-tartaric acid, alkalization process with ammonium hydroxide and fractionation process with chloroform.

2.2. Determination of Alkaloid Fraction Dose of *Achyranthes aspera* linn Leaves

Based on [8] for drugs that were not yet known the effective dose using the dose comparisons as follows: 0 mg / kg.bw; 3 mg / kg.bw; 10 mg / kg.bw; 30 mg / kg.bw; 100 mg / kg.bw; 300 mg / kg.bw; 1000 mg / kg.bw; 3000 mg / kg.bw; 10,000 mg / kg.bw and so on. From the dose given can be known the effective dose which give significant effects of a drug substance to be studied. Then the dose above created a logarithm is $\log 0 = 0.00$; $\log 3 = 0.48$; $\log 10 = 1.00$; $\log 30 = 1.48$; $\log 100 = 2.00$; $\log 300 = 2.48$; $\log 1.000 = 3.00$; $\log 3.000 = 3.48$; $\log 10,000 = 4.00$ and so on. From the effective dose above will be determined the lower limit and upper limit can still provide significant effects of the investigational drug material. From the dose calculation, the alkaloid fraction of *Achyranthes aspera* leaves used in the study were: dosage control (control) = 0 mg / kg, 1 treatment dose (P1) = 60 mg / kg, 2 treatment dose (P2) = 75 mg / kg and 3 treatment dose (P3) = 90 mg / kg.

3. Results

3.1. Extraction and Determination of Alkaloids in Alkaloid Fraction Extract of *Achyranthes asperalinn* leaves

From 5 kg of wet leaves after extraction with n-hexane and methanol obtained methanol extract as much as 400.01 grams, or about 8.00% of wet leaves. Then separation by acidification and alkalization fractionation with chloroform then evaporated, then dried in a desiccator, then weighed, gained as much as 100.01 grams of alkaloid fraction extract. Results of the extraction stage can be seen in Table 1:

TABLE 1: The extraction stage results of *Achyranthes aspera* linn leaves.

<i>Achyranthes aspera</i> linn leaves	Weight (gram)	%
Wet leaves	5000,00	100
Dry leaves	4080,00	81,6
Fine powder	4015,00	80,3
Methanol extract	400,01	8,00
Dried chloroform fraction extract	100,01	2,00

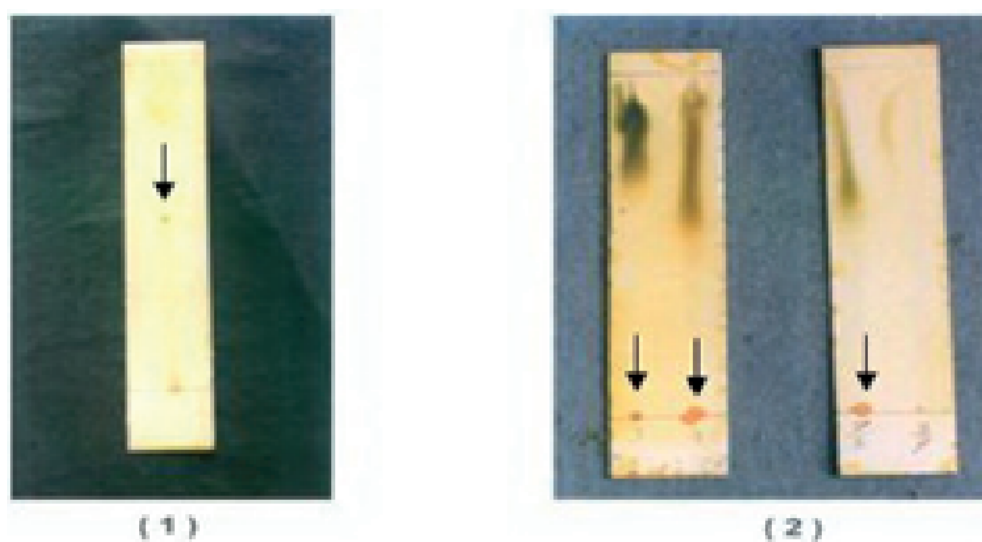


Figure 1: Alkaloid qualitative measurement results with thin layer chromatography. Orange color (arrows) showed the presence of alkaloids. (1) Eluent chloroform: methanol in ratio 1: 5. (2) Eluent ethyl acetate: methanol: water in ratio 7: 4: 2.

3.2. Determination of The Presence of Alkaloids in *Achyranthes aspera* linn leaves

To determine the presence of alkaloids tested by thin layer chromatography (TLC), using stationary phase silica gel aluminum while mobile phase using chloroform and methanol at a ratio of 5: 1. As a comparison using more polar mobile phase is a mixture of ethyl acetate: methanol: water with a ratio of 7: 4: 2. By using a mobile phase of chloroform and methanol a ratio 5: 1, the alkaloid retained on retardation factor (Rf) of 0.55. While using the mobile phase ethyl acetate: methanol: water in ratio 7: 4: 2, the alkaloid retained in position 0. It means that the use of more polar mobile phase showed alkaloid contained in *Achyranthes aspera* linn leaves was non-polar.

3.3. Measurement of Alkaloids in Alkaloid Fraction Extract of *Achyranthes aspera* linn leaves

Measuring levels of alkaloids contained in the extract alkaloid fraction leaves *Achyranthes aspera* L using High-Performance Liquid Chromatography (HPLC) at a wavelength of 254 nm, it is known there is 13 spacious top of the chart obtained from a printout of detector used. This means there are 13 kinds of chemical substances contained in the alkaloid fraction.

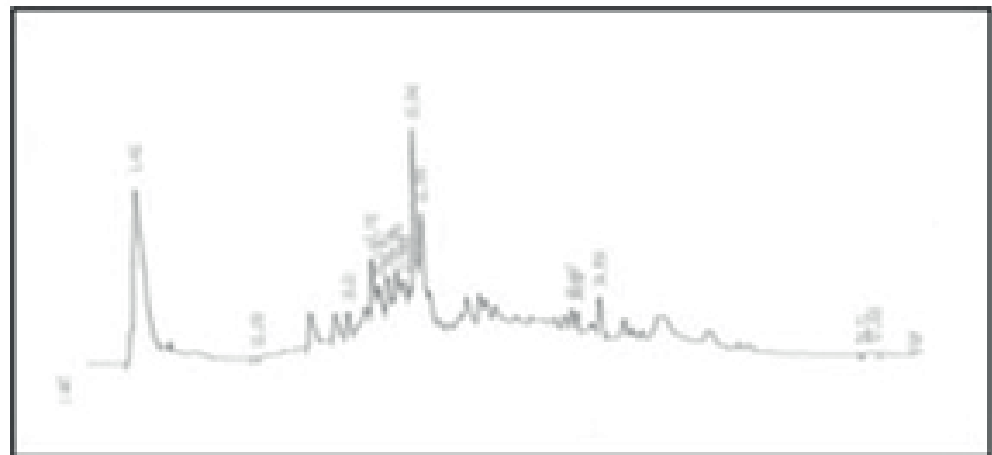


Figure 2: Print out the curve chromatography alkaloids in with HPLC method.

Alkaloid Fraction	Time (minute)	Total (%)
1	3,482	52,3636
2	18,21	1,8737
3	19,795	5,5786
4	20,173	3,1878
5	20,772	3,8871
6	21,398	3,9026
7	21,868	2,196
8	22,542	13,0642
9	22,998	8,3598
10	33,167	1,2382
11	33,52	1,0507
12	39,958	2,8699
13	57,333	0,428

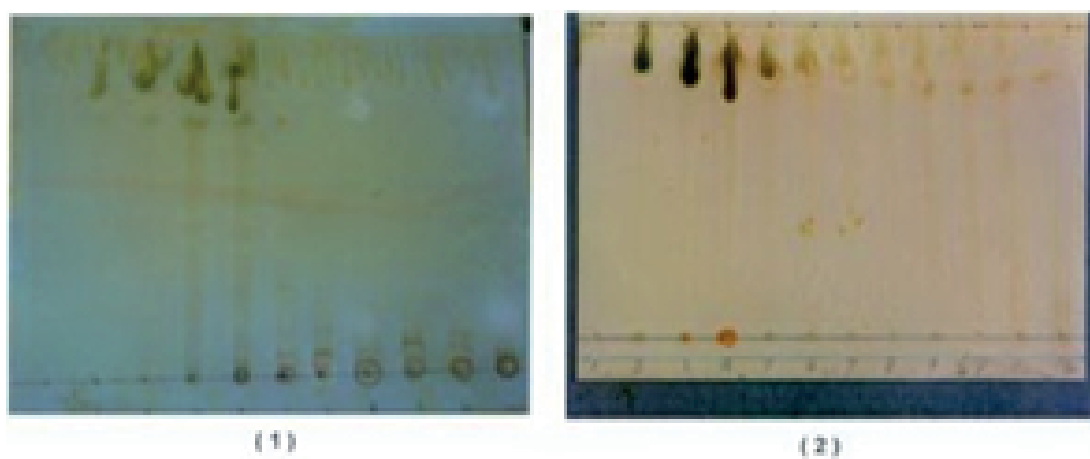


Figure 3: TLC test of alkaloid *Achyranthes aspera* fractionation result in 12 concentration gradient chloroform and methanol. (1) The mobile phase chloroform: methanol in ratio 5: 1. (2) The mobile phase ethyl acetate: methanol: water ratio 7: 4: 2.

4. Discussion

From the thirteen kinds of chemical substances was a major component of alkaloid with a peak area of 52.36%, the other component is the green substance with a peak area of 13.0624%, while the other chemicals in very small peak area of between 0.428%-8.3598%, but this substance has not been done the identification of the type of material contained in it. Based on the discovery of some investigators to the composition of chemical substances contained in *Achyranthes aspera* linn leaves were chlorophyll compound, class of terpenoids, flavonoids, saponins fatty substances leaves and some derivative of glucose. All types of material in addition to the fraction of alkaloids should be degraded and also dissolved in a more polar solvent in the stage of making an alkaloids fraction extract. The result was an analysis of a peak area from each component can be seen in Figure 2.

5. Conclusion

Achyranthes aspera linn using thin layer chromatography proved to contain alkaloid fractions in orange. Levels of *Achyranthes aspera* linn alkaloid fractions using HPLC with a retention time of 3.482 minutes, a wavelength of 254 nm containing the main component with a peak area of 52.36%, green substance with a peak area of 13.0624%, while the other chemicals in very small peak area of between 0.428 %-8.3598%. Alkaloids fraction extract of *Achyranthes aspera* linn leaves used in the study was a dose control of 0 mg/kg, 1 treatment dose of 60 mg/kg 2 treatment dose of 75 mg/kg and 3 treatment dose of 90 mg/kg.

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