



Dringo rhizome (*Acorus calamus* L.): A potential source high β -asarone

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ABSTRACT

Dringo plant (*Acorus calamus* L.) is a medicinal plant with the main chemical components (major compound) is asaron (β -asarone) are commonly found in a rhizome, but did not rule these compounds are also present in other parts including leaves. β -asarone have high activity against *Microsporium gypseum* as antimicrobial, *Trichopyton rubrum*, and *Penicillium marneffeii* and have anti-carcinogenic effects. This study aimed to determine the contents of β -asarone most of the leaves and rhizomes of plants dringo (*A. calamus*) by TLC-densitometry method. The leaves and rhizomes of each extracted by maceration method using *n*-hexane solvent. TLC performed on a standard profile and the sample was eluted with the eluent *n*-hexane: ethyl acetate (8: 2), then the TLC profiles scanned by TLC-densitometry at a wavelength of 313 nm maximum. The results showed levels of β -asaron on plant rhizomes dringo (*A. calamus*) leaves more than a ratio of 1: 0.58. β -asarone contained in the rhizome as 2743.49 mg / mL, while the leaves as much as 1593.63 mg / mL.

Keywords: β -asarone, Antimicrobial, *A. calamus* L., *n*-hexane extract, TLC-densitometry

INTRODUCTION

Dringo (*A. calamus*) is a tropical plant of the family Araceae. It is a semi-aquatic plant of aquatic habitats in temperate to subtemperate regions such as Asia and Americas [1]. This plant contains a large number of metabolic form of secondary metabolites such as alkaloids, flavonoids, phenolics, and terpenoids or isoprenoids which has often been associated with medicinal and pharmacological properties of the plants [2]. Some major compounds found in dringo are asaronee (β -asarone), α -patchoulen, β -caryophyllen, humulen, metal-eugenol, elemicine, cis-Ocimen, generally located in rhizome. However, they can also be found in other parts including the leaves [3].

Research about *A. calamus* had reported that the major active compound dringo is asarone (β -asarone) which is generally located in its rhizome and found in leaves as well.

According to the above, this research will be about comparison study of β -asarone in rhizome and leaves of plants dringo (*A. calamus*).

EXPERIMENTAL SECTION

The leaves and rhizomes of plants dringo (*A. calamus*) were obtained from the District Tiroang, Pinrang, South Sulawesi Province-Indonesia.

Sample Extraction by Maceration

The crude of leaves and rhizome of dringo (*A. calamus*) each weighed as much as 10 grams, put in maceration container, then n-hexane added as much as 200 mL until the crude is completely covered. The crude let soaked for three days in covered container and protected from the light with stirred once in a while. The crude strained after three days. Each of the strained results be collected and did the next experiment.

Identification of β -asarone by TLC

N-hexane extract of the leaves and rhizomes of plants dringo (*A. calamus*) compared to standard β -asarone by means spotted on TLC plates and then the plates were eluted with n-hexane : ethyl acetate (8: 2) [2].

Determination of Content β -asarone by TLC-Densitometry

a. Preparation of standard solution of β -asarone

Comparative β -asarone pipetted 5 ml and then diluted with 5 mL of n-hexane at a concentration of 1000 ppm. From a concentration of 1000 ppm were made varying concentrations of 100, 200, 300, and 400 ppm respectively, then the concentration of n-hexane paid back by up to 1 mL.

b. Determination of β -asarone on samples

TLC plates were made with a size of 10 x 10 cm. Variations in the concentration of the standard solution that was created by using a micropipette spotted as many as 1 mL, then each of the aqueous extract of leaves and rhizomes of plants dringo (*A. calamus*) 5 mL pipette subsequently spotted by using a micropipette as much as 1 mL on the same TLC plate with a comparison and replication is performed three times. The plates were eluted in the chamber containing n-hexane: ethyl acetate (8: 2). Separate stain was observed with 254 nm UV light and measured by TLC-densitometry at a wavelength of 313 nm maximum, an analysis of the results of the scan [4].

RESULTS AND DISCUSSION

Sample Extraction by Maceration

The extraction of 10 grams of leaves and rhizome dried dust of plants dringo (*A. calamus*) each obtained by each of 155 mL and 165 mL.

Table 1: Results of leaves and rhizome extracts of dringo plants (*A. calamus*) were extracted by maceration using n-hexane

Samples	Sample weight (gram)	Eluent (mL)	Liquid extract (mL)
Leaves	10	200	155
Rhizome	10	200	165

The extract obtained is the result of extraction by maceration method, this method is selected because it uses bulbs that have a soft texture and can easily dissolve the active substance content of the sample in the eluent. In addition, this method is also used because the equipment is simple and easily cultivated. The solvents used were n-hexane, which is a polar solvent that has a low level (non-polar) making it easier to pull the compound β -asarone non-polar too, as well as the principle of 'like dissolve like'.

Identification of β -asarone by TLC

Extract each sample, then in the identification of the content of β -asarone qualitatively using TLC. Extract the sample and comparative standard β -asarone each spotted on TLC plates and Rf values obtained. Results Rf values obtained for β -asarone comparator 100 ppm is 0.65. Comparative e 200 ppm, 300 ppm, and 400 ppm and the leaves extract of samples had the same Rf value is 0.64. Leaves extract II and III as well as the rhizome extract I have the same Rf value is 0.65. Rhizome extract II and III have Rf values respectively 0.66 and 0.67, as shown in figure 1 below:

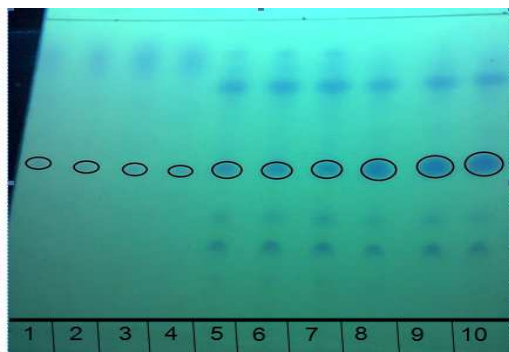


Figure 1. TLC profile at 254 nm UV light. 1-4 β -asarone standard, 5-7 leaves extract and 8-10 rhizome extract

The results of the qualitative identification by TLC indicates that the sample contains β -asarone with purple-blue stain and parallel to the standard comparator β -asarone after detection at 254 nm UV lamp. The differences obtained Rf values can be influenced by factors saturation chamber during elution.

Content Determination with TLT-Densitometry

Quantitative analysis of a compound that has been separated can be instrumental analysis method based on the analyte in the form of electromagnetic radiation with a spot on the plate. Determination of a compound able to use the tool TLC-densitometry by means of stains that have been separated on the plate is inserted into the device and then be titrated based on rapport Area Under Curves (AUC) of each stain on the plate.

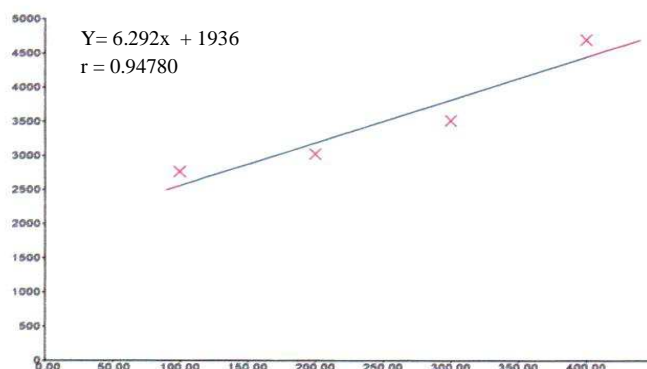


Figure 2. Graph standard curve β -asarone by TLC-Densitometry

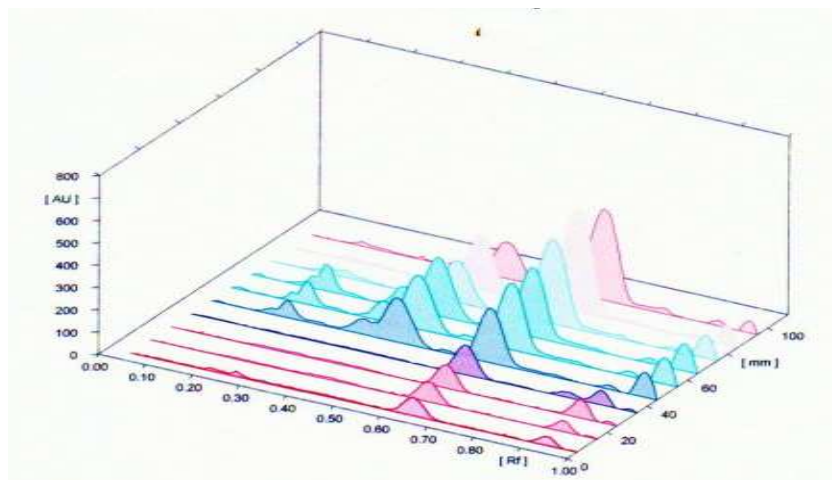


Figure 3. Profile of the results of two-dimensional TLC-Densitometry β -asarone

Densitometry is the measurement of the absorption properties of a substance or flourosensi directly on a thin layer chromatogram using a tool with a single or dual light source, either by light reflected by transmitted or spotting on the plates. In figure 2 shows the standard curve of β -asarone. In the standard curve obtained by linear equations used to calculate the levels of β -asarone on each sample of leaves and rhizomes of plants dringo (*A. calamus*).

Table 2: Results of the calculation of the average levels of β -asarone on leaves and rhizomes of plants dringo (*A. calamus*) by TLC-densitometry

Samples	Repetitions	Wide area	Content β -asarone ($\mu\text{g}/\mu\text{L}$)	Average content β -asarone ($\mu\text{g}/\mu\text{L}$)
Leaves	I	9945.07	1272.89	1593.63
	II	12685.96	1708.51	
	III	13258.55	1799.51	
Rhizomes	I	17440.22	2464.11	2743.49
	II	21659.84	3134.74	
	III	18494.22	2631.63	

In this research, the levels of β -asarone the rhizomes obtained as 2743.49 mg / mL and the leaves as much as 1593.63 mg / mL as shown in Table 2. This shows that the levels of β -asarone the rhizome more than the levels of β -asarone contained in the leaves, with a ratio of 1: 0.58. β -asarone also called cis-isomers of 2,4,5-trimethoxypropenylbenzene a constituent of calamus oils (oils evaporate) derived from plants dringo rhizomes (*A. calamus*). β -asarone been reported to have high activity as an anti-microbial and as anti-carcinogenic, so dringo rhizomes can be used as a source of β -asarone potential.

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