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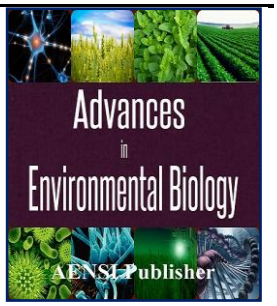

The Optimization Of Eluent Chromatography Thin Layer 2-D For The Purification Of Isolat Alkaloid Of Pulai Having Potention As Anti-Toxoplasma

[1]A. Suryadinata, [1]B. Fauziyah and [2]F. Rahmawati

[1]Lecturer in Pharmacy Department, State Islamic University of Maulana Malik Ibrahim Malang, Indonesia
[2] Graduate from Pharmacy Department, State Islamic University of Maulana Malik Ibrahim Malang, Indonesi

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ABSTRACT

There was a research having purpose to get pure alkaloid from pulai leaf in a way of chromatography thin layer two dimensions using 2 variants of eluent KLT 2-D. the result of this research is the best profile of chromatography thin layer obtained from eluent chloroform: methanol: ammonia (85: 15: 1) and also from KLT. There was 13 stains where the orange colored alkaloid is on the 8th and 9th stain/ spot. The profile of chromatography thin layer 2-D variant eluent 1 is from phase 1 (stain 1) and phase 2 (stain 2) whereas variant eluent 2 is from phase 1 (3 stains) and phase 2 (1 stain). According to the profile, the best eluent that will be used is variant eluent 2, that is eluent phase 1: chloroform: etil asetat (60:40) and eluent phase 2: chloroform: methanol: ammonia (85: 15:1).

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INTRODUCTION

Pulai is one of plants used as a medicine. Pulai was used as the medicine of ailment such as diarrhea, fever, a cough, diabetes, malaria and kidney stones [2]. Almost all parts of it can be used for medicine such as stem, root, rhizome, flower, fruit, and seed [8]. The part of pulai that is specifically used as antioksidan and antidiabetes is the leaf [9] [10].

The research about use and bioactivity benefit from pulai had been investigated by Misra et al[7]. It is about phytochemical test and activity as anti-bacterial in the root, leaf, and rod plant of pulai by using variant liquid hexane, benzene, isopropanol, ethyl acetate, methanol and water. Phytochemical analysis from sample variant 3 extract showed some compounds produced such as alkaloid, carbohydrates, terpenoids, steroids and saponins. A research from Luo [6] stated that pulai extract using ethanol, then being fractionated using petroleum ether, ethyl acetate and water produce alkaloid compound.

Chemical content from pulai leaf extract with n-hexane solvent is alkaloid, saponin and steroid. There is terpenoid in pulai stem extract. There are alkaloid, terpenoid and steroid in pulai leaf extract with ethyl acetate solvent whereas terpenoid is found in pulai stem extract, and also there is alkaloid, terpenoid, steroid and saponen in the pulai root extract. There is alkaloid, steroid and saponin in the pulai leaf exratct with methanol and alkaloid, terpenoid, steroid and saponin in the pulai stem and root extract [7]. Khyade [4] stated that pulai leaf contains some compounds such as acubin / iridoids, coumarin, Phlobatannin, phenolics, alkaloids, flavonoids, saponins, tannins, and steroids.

Abraham [1] did extraction in pulai leaf with ethanol solvent produced alkaloid extract. Then, separation of alkaloid with KLT produced best eluent that is ethyl acetate: methanol: water (6:4:2) that had been identified as dragendorff reagent, where the research was obtained 6 stains in the 6th stain as alkaloid, shown by Rf 0.76 and the color is pale orange in the observation under UV lamp set with emission wavelength in 254 nm and 366 nm.

Hasibuan [3] used KLT 2-D to the purification using movement phase I: chloroform: methanol: ammonia (85:15:1) and movement phase II: chloroform: ethyl acetate (60:40), silent phase is silica gel F254 and produced 1 stain after sprayed with dragendorff reagent that is red orange (Rf 0.69). It showed isolate alkaloid has pure.

Kusrini [5] did separation of alkaloid from tempuyung leaf *(Sonchus arvensis Linn)* and identify it with dragendorff. After that, it was being analyze use KLT 2-D with movement phase using eluent ethyl acetate:

Corresponding Author: B. Fauziyah, Lecturer in Pharmacy Department, State Islamic University of Maulana Malik Ibrahim Malang, Indonesia
E-mail: bhefha@gmail.com

ethanol: n-hexane (2:1:30) and chloroform: acetone: methanol (20:3:2). From the result of preparative KLT is obtained 6 stains and the 6th stain identify alkaloid that is shown by Rf 0.77 colored light blue in the observation under UV lamp 365 nm. Whereas KLT 2-D in UV lamp with wavelength 365 nm produced a single stain colored blue expected isolate alkaloid have pure.

Methodology:

The equipment used are oven, mortar, hammer, blender, mesh screen 80 mesh, filter paper, analytical balance, desiccators, vaporizer cup, lid 500 mL Erlenmeyer, hot plate, magnetic stirrer, 500 mL separating funnel, glass stirrer, spatulas, scissors, aluminum foil, Büchner filter, shaker, rotary evaporator, 500 ml glass beaker ; 250 mL ; 100 mL, glass funnels, test tubes, pipette measure 10 mL and 5 mL, suction ball, micro pipette size of 10-100 mL, micro pipette 100-1000 mL size, measuring flask 1 L, 100 mL volumetric flask, fume hood, glasses vials, pipette, capillary tube, GF254 TLC plate, and UV light, pH meter.

The main material used in this research is part of plant that is pulai leaf (*Alstonia scholaris* (L) R. Br) found in Pasuruan, Jawa Timur. Chemical materials used in this research are ethanol solvent (C_2H_5OH) p.a, ethyl acetate ($C_4H_8O_2$) p.a, sulfuric acid (H2SO4 2 %), ammonium hydroxide (NH4OH 25 %), chloroform (CHCl3) pa, acetone (CH3COCH3) pa, methanol (CH 3 OH) pa, pa n - hexane, diethyl ether pa. Other materials used are distilled water, meyer reagents, reagent dragendroff, hydrochloric acid (HCl 1 %) and N2 gas .

This research is using experimental test in laboratory. The sample of pulai leaf was taken, cleaned from dust and dirt, washed, dried, coarsely grounded, then determined the water standard. After that the pulai leaf is being refined with blender then sifted using sieve with a range of 80 mesh until the soft powder is obtained (80 mesh), then determined again the water standard until less then 10%. The amount sample of each powder is being extracted maceration with using ethanol solvent 95% until pale filtrate is obtained.

The filtrate is being concentrated by using rotary evaporator vacuum in 50 ℃ degree and flew N_2 gas. A thick extract that is obtained is continued to be alkaloid fractionated before being fractionated with ethanol extract 95%, do phytochemical testing to know alkaloid compound group by using reagent. Then, do separation to the alkaloid with KLT analytic based on multiple compounds of eluent and reagent used. Eluent which give best separation will be used to separate with KLT preparative. The isolate obtained from KLT preparative is being tested the purification using KLT with eluent compound and KLT 2-D. in conclusion, explanation above can be written as follow:

1. Sample preparation
2. Water standard testing
3. Maceration extraction alkaloid compound of pulai leaf (*Alstonia scholaris* L.R.Br)
4. Phytochemical testing rough extract with reagent test
5. Alkaloid compound identification with analytical chromatography thin layer (KLT)
6. Separation of alkaloid compound with preparative chromatography thin layer (KLT)
7. Purity testing of alkaloid isolate with KLT 2-D

RESULTS AND DISCUSSION

From the extraction stage using ethanol produced extract tight with green colored in compact texture. Then, that extract in this research is called ethanol extract obtained is 43.5738 %. The result of extraction can be shown as picture 1 and the calculation of rendemen result is in table 1

Fig. 1: The result of maceration extract of pulai leaf with ethanol

Advances in Environmental Biology, 9(23) October 2015, Pages: 237-241

Conclusion:

Table 1: The calculation of rendemen result of ethanol extract of pulai leaf

Solvent	Ekstract color	Sample's weight	Extract's weight	Rendemen
Ethanol 95 %	Dark Green	50 g	6,7641 g	13,5282 %
		50 g	6,9415 g	13,883 %
		50 g	8,0813 g	16,1626 %
Total	150 g	21,7869 g	43.5738%	

From the ethanol extract obtained, do alkaloid extraction process using chloroform. The result obtained is shown in picture 2. From 20 grams of ethanol extract, obtained rough extract alkaloid 0.388%. Then, separate with analytical chromatography thin layer using 5 types of eluent. The purpose is to determine the right and best eluent in separating alkaloid from pulai leaf. From those five eluent, it is obtained that the best eluent in obtained form eluent chloroform: methanol: ammonia (85:15:1). The picture of analytical KLT result with the best eluent is shown in the following.(pic3)

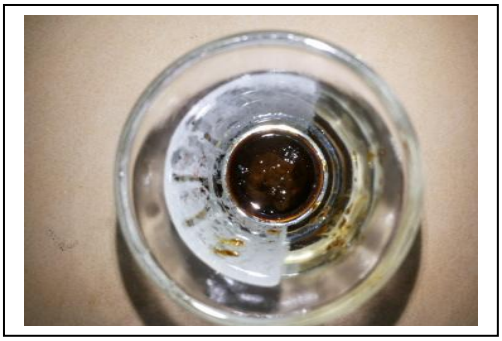
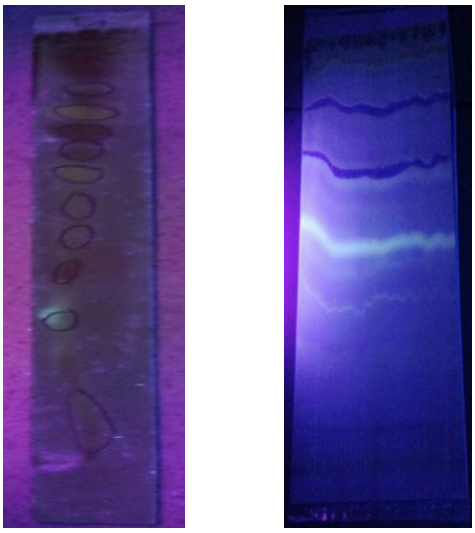


Fig. 2: The result of alkaloid extraction from ethanol etract

Table 2: The calculation of rendemen result of ethanol extract of pulai leaf

Solvent	Extract's color	Extract's weight (g)	Rendemen (%) (b/b)
Chloroform	Chocolate	0,5821 g	0,388 %



a b

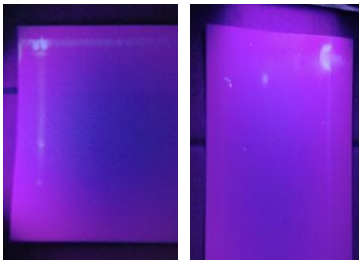
Pic. 3: The result of chromatography thın ıayer a) analytical KLT b) preparative KLT

Preparative KLT is done by using the best eluent that is obtained from analytical KLT. By using eluent chloroform, methanol and ammonia (85:15:1), it is obtained 13 spots of stains in the result of preparative KLT. The stain that shows the existence of alkaloid is the stain with orange colored as in the 8^{th} and 9^{th} stains. Then, both stain are being scraped to be refined with KLT 2-D. the 13^{th} stain or spot that is obtained is shown in table 3 whereas the result of preparative KLT is shown in picture 3b. the result of preparative KLT in stain 8 and 9 that is that is being scraped, separate with KLT 2-D using 2 variants eluent.

Table 3: the result of preparative KLT with best eluent of analtical KLT

Stain	Rf Score	The color of stain	
		before reagen given	After reagen given
1	0,083	Purple	Purple
2	0,244	Saffron	Brownish Purple
3	0,344	Yellow	Brownish Purple
4	0,433	Light Blue	Purple
5	0,477	Bright Green	Bright Green
6	0,561	Yellowish Green	Purple
7	0,6	Light Blue	Brownish Purple
8	0,655	Orange	Chocolate
9	0,822	Orange	Chocolate
10	0,855	Green	Brownish Purple
11	0,872	Purple	Brownish Purple
12	0,916	Yellow	Yellow
13	0,933	Light Green	Yellow

The first variant using eluent phase 1: chloroform: methanol: ammonia (85:15:1) and eluent phase 2: chloroform: ethyl acetate (60:40). Whereas the second variant using eluent phase 1: chloroform: ethyl acetate (60:40) and eluent phase 2: chloroform: methanol: ammonia (85:15:1). The result showed that from variant 1 is obtained one spot from phase 1 and two spots from phase 2. On the other hand, in the 2nd variant, it is obtained 3 spots from phase 1 and 1 spot from phase 2. The pictures each are shown in picture 4a and 4b.



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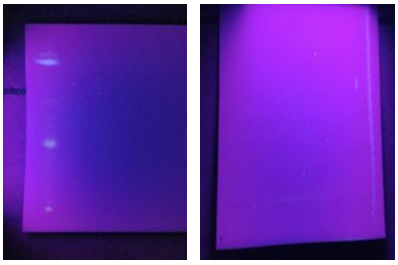

b

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