Phytochemical, TLC Profile, and Antioxidant Activity of Malinau Endemic Plant of Tabar Kedayan (Aristolochia papilifolia Ding Hou) Root Fractions

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Phytochemical, TLC Profile, and Antioxidant Activity of Malinau Endemic Plant of Tabar Kedayan (*Aristolochia papilifolia* Ding Hou) Root Fractions

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Abstract : Tabar Kedayan (*Aristolochia papilifolia* Ding Hou) is Malinau endemic plant as a traditional medicine by Dayak community. However, the scientific data from this plant is limited. This study aimed to know the secondary metabolite groups, TLC profile and antioxidant activity in the fraction of this plant. The dried sample (one kg) was macerated with ethanol for 2 x 24 hour. The obtained extract (80 gram) was partitioned using liquid-solid extraction with ethyl acetate, then ethyl acetate soluble fraction (22.4 gram) was conducted fractionation using vacuum liquid chromatography. Each fraction was analyzed of preliminary phytochemical, TLC profile, and antioxidant activity (qualitative and quantitative). Based on the results, had obtained data, such as preliminary phytochemical was showed the groups of alkaloid and steroid were present in fractions A, B, and C. Phenol groups was present only in the fraction F, while the fraction C, D, E, and F was present flavonoid. The TLC profile had demonstrated the ability of fluorescent components of based on the different properties of compounds contained in each fraction. Antioxidant activity determination of ethyl acetate soluble fraction from Tabar Kedayan (*A. papilifolia* Ding Hou) roots using DPPH (2,2-diphenyl-1-picrylhydrazyl) method with a qualitative activity (TLC autography method) was fraction C, and a quantitative activity (IC_{50} value) was 139.11 µg/ml.

Keywords: *Aristolochia papilifolia* Ding Hou, preliminary phytochemical, 2,2-diphenyl-1-picrylhydrazyl, IC_{50}, TLC autography.

Introduction

Malinau is one of the districts in North Kalimantan Province, Indonesia. Most areas of Malinau is still a primary forest with abundant biodiversity. One of the species used as medicine by the local community derived from the genus of Aristolochia (family Aristolochiaceae)[1].

The genus of Aristolochia comprises about 500 species, and the species of this genus has efficacy to be used as a medicine. This genus is a vines shrub and most widely spread in Asia, Africa, North and South America [2,3,4]. Many plants of this genus have efficacy as an anti-asthmatic, anti-inflammatory[5], anticancer, analgesic, infections diseases, cardiovascular[6], nephrotoxicity[7], antioxidant, antimicrobial, and insect repellant or antiphenom[8,9]. Some research was reported from this genus to contain secondary metabolites such as

flavonoid[5], alkaloid[10,11], tannic acid, fixed oil, nitrophenanthrene carboxylic acid, resin[12], and aristolochic acids[7].

Tabar Kedayan (*A. Papilifolia*) is a Malinau endemic plant widely traditionally used by Dayak community as a traditional medicine, such as detoxifying, neutralizing venom (particularly the insect and snake), and all sorts of venomous animal bites, food poisoning, diarrhea, toothache and can also be used to neutralizing alcohol in the human body (alcoholic). However, scientific data from this species (mainly secondary metabolites and activity potential) has not reported. Therefore, this study aimed to know the secondary metabolite groups, TLC profile and antioxidant activity in the fraction of Tabar Kedayan (*A. Papilifolia*).

Experimental

Material

Plant

Tabar Kedayan (*A. Papilifolia*) root was collected from Malinau, North Kalimantan, Indonesia. Moreover, this plant was identified at Laboratory of Dendrology, Faculty of Forestry, Mulawarman University, Samarinda, East Kalimantan, Indonesia and the voucher specimen was deposited at Pharmaceutical Research & Development Laboratory of FARMAKA TROPIS, Faculty of Pharmacy, Mulawarman University, Samarinda, East Kalimantan, Indonesia.

Chemical and equipment

The chemical was used in this study such as; aqua DM, n-hexane, ethyl acetate, ethanol were purchased from SmartLab Indonesia, Indonesia. DPPH (2,2-diphenyl-1-picrylhydrazyl) was purchased from Sigma-Aldrich, Germany. Silica gel GF 60, silica gel GF 254 and TLC plate were purchased from Merck, Germany. The equipment was used such as; Water bath (Memmert®), the rotary evaporator (Buchi®), Chamber (Camaq®), hot plate (Stuart®), vortex (Health®), and Spectrophotometry UV-Visible Double Beam (Dynamica®).

Extraction and Fractionation Process

The extraction process carried out by extracting The dried sample (1 kg) using maceration method with ethanol based on standard conventional extraction procedures. The extracts obtained (80 grams) then partitioned using solid-liquid extraction methods with ethyl acetate. The ethyl acetate soluble extract (22.4 grams) carried out fractionation using a vacuum liquid chromatography method with a solvent combination (n-hexane and ethyl acetate) in a ratio of 5: 5, 4: 5, 3: 5, 2: 5, 1: 5, and 0: 5, respectively.

Phytochemical Screening

Each fraction was analyzed using chemical reagent for preliminary analysis of various groups of phytoconstituents such as alkaloids, flavonoids, steroid, phenolic, and polyphenols by using standard procedure[13,14,15].

TLC Profile

The TLC profile determination performed based on the literatures[14], with slight modification. The stationary phase using TLC plate (10x20 cm) and the mobile phase using an eluent comparison based on the polarity levels was used in this study. The retention factor (Rf) value was a data of TLC profile.

Antioxidant Activity

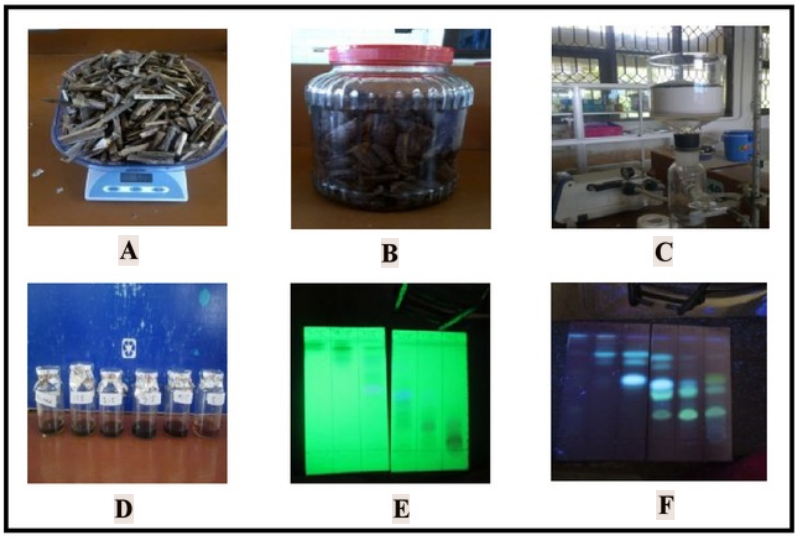
Determination of antioxidant activity conducted using DPPH free radical scavenging assay (qualitative and quantitative assay) as the standard procedures[16,17,18,19] with slight modification. For qualitative activity, each solution of extract sample was spotted (5 x 2 µg/ml) d on TLC-plates (silica gel thin layer chromatography) and were spread using a mobile phase (appropriate solvent systems). The plates developed with 0.1 % DPPH in methanol and placed in the dark condition for 30 minutes, and the color changes (yellow on purple background) were noted. For quantitative activity, the sample solution was used for experimental at 30 times dilution. The reaction mixture contained two mL of 40 µg/mL DPPH and two mL of sample solution. The DPPH free radical scavenging activity of the sample was quantified using spectrophotometry at 517 nm wavelength. Results were reported as the IC_{50} (the amount of antioxidant necessary to decrease by 50% the initial DPPH concentration).

Results and Discussion

Extraction and Fractionation Processes

Tabar Kedayan (*Aristolochia papilifolia* Ding Hou) is a native plant of Kalimantan, particularly Malinau region. This plant is a snub, root russet, edged, elongated and usually grown in the tropical rainforests. The local communities take advantage of this plant as a traditional medicine from generation to generation. The roots can be used in the form of thin slices and mixed with warm water.

The fresh samples sorted, washed, chopped, and dried. The dried samples were extracted by maceration using ethanol. Subsequently, the crude extract was partitioned using solid-liquid extraction method using ethyl acetate. The ethyl acetate soluble extract (the ethyl acetate extract) was fractioned with vacuum liquid chromatography method using n-hexane: ethyl acetate eluent (a ratio of 5: 5, 4: 5, 3: 5, 2: 5, 1: 5, and 0: 5, respectively). Extraction and fractionation process (shown in **Figure 1**).



Where: A was the dried root sample of Tabar Kedayan (*Aristolochia Papilifolia* Ding Hou); B was maceration processes; C was vacuum liquid chromatography procedures; D was the prepared sample ready for analysis; E was the TLC spot at 254 nm wavelength, and F was the TLC spot at 366 nm wavelength.

Figure 1 Extraction and Fractionation processes

Phytochemical analysis

Preliminary phytochemical was performed on all the ethyl acetate soluble fraction including fraction A, B, C, D, E, and F. The content of secondary metabolites examined, namely alkaloids, flavonoids, steroids, polyphenols, and phenolic (shown in **Table 1)**.

Table 1 Results of preliminary phytochemical of ethyl acetate soluble fraction

No	Secondary Metabolite	Ethyl Acetate Soluble Fraction					
		A	B	C	D	E	F
1	Alkaloid	(+)	(+)	(+)	(-)	(-)	(-)
2	Polyphenolic	(-)	(-)	(-)	(-)	(-)	(-)
3	Flavonoid	(-)	(-)	(+)	(+)	(+)	(+)
4	Steroid	(+)	(+)	(+)	(-)	(-)	(-)
5	Phenolic	(-)	(-)	(-)	(-)	(-)	(+)

where: (+)= Present, (-) = Absent

Based on **Table 1** shows that the groups of alkaloid and steroid were present in fractions A, B, and C. Phenol groups was present only in the fraction F, while the fraction C, D, E, and F was present flavonoid.

TLC Profile

TLC profile of the ethyl acetate soluble fraction (including fraction A, B, C, D, E, and F) shown in **Table 2**. TLC profile at UV 254 nm wavelength was detected spots are different in each of the fractions, namely four spots (fraction A), seven spots (fraction B), one spot (fraction C), one spot (fraction D and E), and two spots (fraction F). Profile TLC at UV 366 nm wavelength was also present with the spot differently on each of the fractions, namely three spots (fraction A and F), five spots (fraction B and C), seven spots (fraction D), and six spots (fraction E). Moreover, profiles TLC at visible wavelength using 10% H_2SO_4 spray was detected four spots (fraction B), three spots (fraction A, C, and D), and one spot (fraction E and F).

Table 2 TLC Profile of Ethyl Acetate Soluble Fraction

Ethyl acetate soluble fraction	Total Spot and Rf value			
	Spot	UV (254nm)	UV (366nm)	H_2SO_4 Spray
Fraction A (5:5)	1	-	0,51	-
	2	-	0,62	0,62
	3	0,74	-	0,69
	4	0,82	-	-
	5	0,85	-	-
	6	0,92	0,92	0,92
Fraction B (4:5)	1	0,28	-	
	2	0,32	0,31	-
	3	-	0,38	-
	4	-	0,51	-
	5	-	0,62	0,62
	6	-	0,69	0,69
	7	0,74	-	0,74
	8	0,82	-	-
	9	0,85	-	-
	10	0,92	-	-
	11	0,97	-	0,97
Fraction C (3:5)	1	-	0,54	-
	2	-	0,71	-
	3	-	0,77	0,77
	4	-	-	0,88
	5	0,92	-	-
	6	-	0,97	0,95
	7	-	0,98	-
Fraction D (2:5)	1	0,31	0,31	-
	2	-	0,46	-
	3	-	-	0,49
	4	-	0,54	0,54
	5	-	-	0,62
	6	-	0,71	-
	7	-	0,77	-
	8	-	0,97	-
	9	-	0,98	-
Fraction E (1:5)	1	0,31	0,31	-
	2	-	0,51	0,49
	3	-	0,57	-
	4	-	0,71	-
	5	-	0,77	-
	6	-	0,98	-

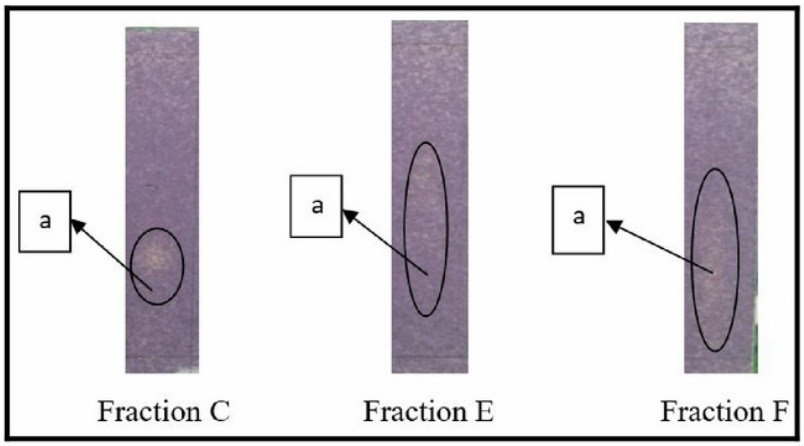
Fraction F (0:5)	1	0,38	0,34	-
	2	0,52	-	0,54
	3	-	0,66	-
	4	-	0,85	-

In UV 254 nm, the TLC plates will fluoresce while the sample will appear dark because the power of interaction between UV light with a fluorescence indicator contained on the TLC plates. Fluorescence is the emission of light emitted by the components contained in each spot when the excitation of the electrons undergo basic energy level to a higher energy level and then return to its original state while releasing energy. While at UV 366 nm has the opposite fluoresce because the power of interaction between UV light with chromophore groups are bound by auxochromes that existed at the spots. Meanwhile, the detection principle of a spot at a visible wavelength using 10% H_2SO_4 spray reagent was based on the ability of reagents damage the chromophore groups of spot components, so that the wavelength will be shifted toward the longer (UV into VIS) and the spot components become visible. The ability of fluorescent components based on the different properties of compounds contained in each fraction.

Antioxidant Activity

1. Qualitative antioxidant activity using TLC autography methods

In the present study, a qualitative test for the antioxidant activity of ethyl acetate soluble fractions using DPPH method. Determination of antioxidant activity using DPPH method is a simple, rapid method and does not require many reagents. The measurement results with DPPH samples demonstrated the ability of antioxidants in general and not based on the radical inhibitory. The spots of each fraction were sprayed using 0.1% DPPH solution, with a presence of yellow spots whitish after 30 minutes showed a positive antioxidant. The results showed the fraction that has antioxidant activity was a fraction C, E, and F (shown in **Figure 2**). At the fraction C, the presence of round yellow spots at the center of the TLC plate, the results indicate that the separation of compounds in this fraction showed the best separation of compound groups.



Where a is a positive reaction of DPPH free radical scavenging with the color changes of spot (yellow on purple background) meant that sample has an antioxidant activity

Figure 2 Results of the antioxidant activity of the ethyl acetate soluble fraction after spraying 0.1 % DPPH (only shown the fraction has a positive reaction)

2. Quantitative antioxidant activity using determination of IC_{50} of DPPH free radical scavenging

The antioxidant activity determination with DPPH free radical scavenging was used fraction C. The IC_{50} value was obtained using linear regression equation that states the relationship between the sample concentration with the symbol (X) and the DPPH free radical scavenging activity with the symbol (Y) of a

measurements replication series. The equation result was obtained Y = 0.292X + 9.38 with coefficient correlation (R^2) = 0.998 (shown in **Figure 3**). A substance (active compound) has the antioxidant properties when the IC_{50} value of less than 200 µg/ml[18,20]. Antioxidant activity determination of ethyl acetate soluble fraction (fraction C) from Tabar Kedayan (*A. papilifolia* Ding Hou) roots using DPPH method with IC_{50} value was 139.11 µg/ml (showed in **Table 3**). At a concentration of µg/ml demonstrated the concentration of ethyl acetate soluble fraction could inhibit oxidation process by 50 percent.

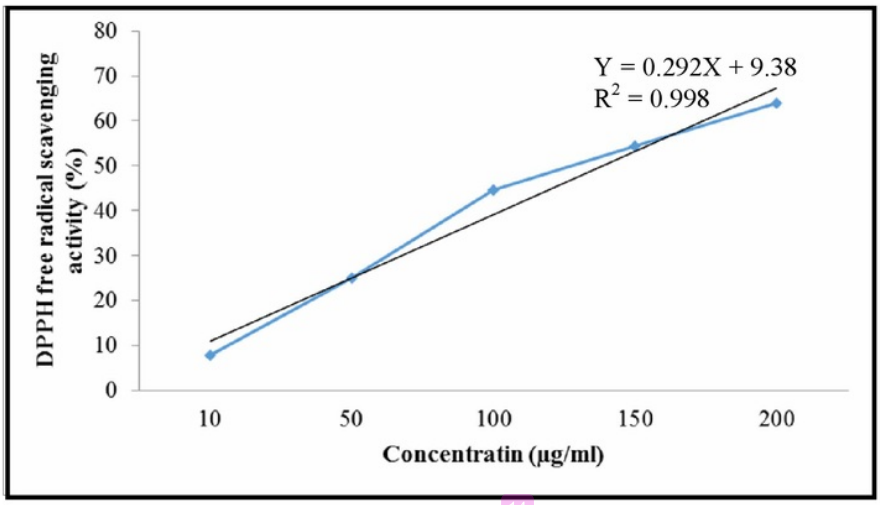


Figure 3 Effect of antioxidant activities against concentration of ethyl acetate soluble fraction

Table 3 Results of measurement and determination of a DPPH free radical scavenging assay (IC_{50}) from ethyl acetate soluble fraction (fraction C)

No.	Concentration	Approximately absorbance	DPPH free radical scavenging activity (%)	Linear regression equation	IC_{50} (µg/ml)
1	10 ppm	0,339	7,95	Y= 0,292x + 9,38 R^2= 0,98	**139,11**
2	50 ppm	0,276	25,12		
3	100 ppm	0,204	44,55		
4	150 ppm	0,168	54,29		
5	200 ppm	0,133	63,84		

Conclusion

Based on the above results, the fraction C was an active fraction from Tabar Kedayan (*A. papilifolia* Ding Hou) roots and was analyzed (including preliminary phytochemical and TLC profile). The results are preliminary data related to the potential as a source of natural medicine and are first information to explore these plants in further study.

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Conflict of Interest

We declare that we have no conflict of interest

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7