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The Effect of Extraction Methods of Bawang Dayak (Eleutherine Palmifolial. MERR) Against TLC Profiles and Sunscreen Activities

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Abstract : A research has been conducted on the effect of extraction methods of Bawang Dayak (*Eleutherine palmifolia* (L.) Merr) against TLC profiles and sunscreen activity. The study aims to determine the effect of extraction methods against yield, TLC profile, and sunscreen activities.The Sample was extractedusing several extraction methods (maceration, reflux, and soxhlet)with different polarity solvent(n-hexane, ethyl acetate, ethanol, dan methanol) and single solvent (methanol), respectively. Yield value obtainedmethanol extract (single solvent) are 8.027% maceration, 6.943% reflux, and 10.694%soxhlet.N-hexane extract are 1.572% maceration, 1.251% reflux, 2.146% reflux. Ethyl acetate extract were 2.141% maceration, 0.966% reflux 1.681% soxhlet. Ethanol extract are 3.537% maceration, 3.280% reflux, 3.974% soxhlet. Methanol extract are 1.999% maceration, 2.553% reflux, 2.682% soxhlet.Furthermore, the determination of the TLC profile from all methods using comparisons N-hexane: ethyl acetate among other;N-hexane extract was 6-7 spots (3:1), ethyl acetate extract was 9 spots(2:1), methanol extract (single solvent) was 8 spots (2:1). Determination TLC profile using comparisons chloroform:methanoleluent namely; ethanol extract was 9 spots (7:1), methanol extract was 5 spot (2:1), and methanolextract (single solvent)was 8 spots (5:1).Sunscreen activityfor ethyl acetate extract (using soxhlet methods)categorizedassun blockand otheras extra protective.

Keywords : *Eleutherine palmifolia* (L.) Merr, extraction methods, TLC Profile, sunscreen activity.

Introduction

Bawang Dayak (*Eleutherinepalmifolia* (L.) Merr, synonyms *E. americana* (L.) Merr.) from the family Iridaceae, a traditional herbal medicine which is used by most people in the area of Borneo in the fresh form.This plant is a kind of onions that grow wild in the Kalimantan forest. It used as an antiemetic, diuretic, and laxative[1], astringent, gastrointestinal infections, diabetes mellitus, hypertension, and cysts[2].Besides, it has activity as an alpha-glucosidase inhibitory[3], Antidermatophyte, antimelanogenesis[4,5], antimitotic[6], and antioksidan[7].Some of the compounds have been isolated from this plant such as A, B, and C[8],elecanicin, eleutherol, isoeleutherol, eleutherin, isoeleutherin, hong-conin, eleutherin-one[1-7] as well as anthraquinones[4,9,10,11].

In previous studies have obtained sunscreen activity by determining the percentage value of erythema and pigmentation transmission from Bawang Dayak (*E.palmifolia.* Merr) extract using methanol solvent[12]. However, research continues to be developed to enrich the knowledge of the extraction method selection, because until recently had never done the study on the effects of the extraction method towards sunscreen activity.In order tofurther research, it developed in the direction of other extraction methods using a variety of solvents to obtain optimal activity.

Experimental

Plant

Bulb of Bawang Dayak (*Eleutherine falmifolia* Merr.) were collected from the area of Tenggarong, Kutai Kartanegara, East Kalimantan, Indonesia, and were identified by Dendrology Laboratory of Forestry Faculty, Mulawarman University, Samarinda, East Kalimantan, Indonesia and the voucher spesimen was deposited at Pharmaceutical Research & Development Laboratory of FARMAKA TROPIS, Mulawarman University. The bulb was washed thoroughly with tap water. The dried materials were separately cut into small pieces and powdered using a grinder and were stored in air tight bottle.

Extraction Procedure

a. Extraction using Methanol (single solvent)

The extraction process conducted by extracting a sample using maceration, reflux, and soxhlet methodswith methanol solvent. Each obtained extract was evaporated using a rotary evaporator to obtain a dry extract.

b. Extraction using difference of solvent polarity levels

The extraction process conducted by extracting a sample using maceration, reflux, dan soxhlet methodswith a difference of solvent polarity levels successively N-hexane, Ethyl Acetate, Ethanol, and Ethanol. Each obtained extract was evaporated using a rotary evaporator to obtain a dry extract.

TLC Profile Determination

Determination of a TLC profile conducted by using a thin layer chromatography method with the TLC plate as a stationary phase and an eluent comparison based on the polarity level as the mobile phase.The data of TLC profile are in the form of retention factor (Rf) value of each spot generated on the extract.Rf value obtained from the following equation:

$$Rf = \frac{Distance\ from\ baseline\ travelled\ by\ solute}{Distance\ from\ Baseline\ travelled\ by\ solvent}$$

Sunscreen Activityusing Erythema and Pigmentation transmittance Measurement

Determination of sunscreen activity performed by the method spectrophotometric in vitro by calculating the percentage of erythema and pigmentation transmission from several concentrations of each test sample is based on the Transmittance measurement method[13,14]. The basisof previous studies that the lowest concentration able to provide sunscreen activity at 10 ppm[12]. Each extract is made in the concentration of 10 ppm and the absorbance is measured using a UV-Vis spectrophotometer at a wavelength that can cause erythema and pigmentation namely 292.5 to 372.5 nm. The absorbance value (A) obtained was used to calculate the transmission value (T) using the formula A =-log T. The erythema transmission (Te) is calculated using the formula Te= T x Fe, where Fe is the erythema flux value at a specific wavelength. The amount of erythema flux forwarded by sunscreen (Ee) is calculated using the formula Ee= $\sum$(T x Fe). The erythema transmission percentage calculated using the formula:

$$\textbf{The erythema transmission percentage} = \frac{Ep}{\Sigma Fe} \times 100\% = \frac{\Sigma(T \times Fe)}{\Sigma Fe} \times 100\ \%$$

The pigmentation transmission (Tp) calculated using the formula Tp = T x Fp, where Fp is the pigmentation fluxvalue at a specific wavelength. The number of pigmentation fluxforwarded by sunscreen (Ep) is calculated using the formula Ep = ∑(T x Fp). The pigmentation transmission percentage calculated using the formula:

$$\textbf{The pigmentation transmission percentage} = \frac{Ep}{\Sigma Fp} \times 100\% = \frac{E\Sigma(T \times Fp)p}{\Sigma Fp} \times 100\%$$

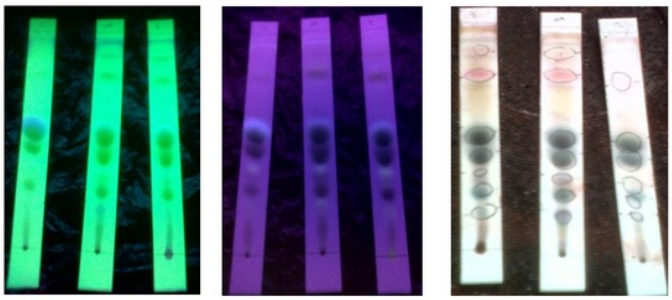
Results and Discussion

Extracts Yield

An extract yield obtained from each extraction method was based on the difference of solvent polarity levels. It was obtained by extracted using Maceration method (1.572% n-hexane extract, 2.141% ethyl acetate extract, 3.537% ethanol extract, and 1.999% methanol extract), Soxhlet method (2.146% n-hexane extract, 1.681% ethyl acetate extract, 3.974% ethanol extract, and 2.682% methanol extract), and Reflux method (1.251% n-hexane extract, 0.966% ethyl acetate extract, 3.280% ethanol extract, and 2.553% methanol extract). Whereas,the extract yield obtained for the direct extraction using methanol solvent(8.027% Maceration method, 10.694%Soxhlet methods, and the reflux of 6.943%).

Effect of Extraction Method towards TLC Profile

Determination of Rf value from TLC result (distance from baseline travelled by a solvent is 7.5 cm) for each extract was obtained. The appropriate solvent usage greatly affects spot/stain generated from the polarity level of the eluent is very influential on the extract polarity to be analyzed by TLC.



R M S R M S S M R
254 nm 366 nm 10% H_2SO_4

Figure 1 N-hexane extract: Reflux (R), Maceration (M), and Soxhlet (S) using n-hexane:ethyl acetate eluent comparison (3:1)

Determination of TLC profiles on the N-hexane extracts (shown in **Figure 1** and **Table 1**) obtained by the maceration, reflux andsoxhlet method with eluent proper comparison is using n-hexane: ethyl acetate in a 3: 1ratio. Separation spot/stain obtained is very good and based on these three methods obtained Rf value is almost identical, it indicates that the compounds are attracted to the solvent n-hexane has a resemblance.However, there were stains disappear off the Rf value range from 0.8 to 0.9 on the reflux method, it is due to the possibility the direct heating effect that causes the compound to disappear or evaporate so undetected.

Table 1 N-Hexane Extract (UsingN-hexane:Ethyl acetate eluent; 3:1)

Spot	Rf Value		
	Soxhlet	Reflux	Maceration
1	0.147	0.187	0.160
2	0.240	0.240	0.240
3	0.307	0.307	0.307
4	0.373	0.427	0.387
5	0.467	0.507	0.480
6	0.733	0.773	0.760
7	0.853	-	0.880

Table 2 Ethyl Acetate (using N-hexane:ethyl acetate eluent; 2:1)

Spot	Rf Value		
	Soxhlet	Reflux	Maceration
1	0.107	0.107	0.107
2	0.240	0.227	0.227
3	0.400	0.373	0.360
4	0.467	0.453	0.427
5	0.560	0.533	0.520
6	0.640	0.600	0.600
7	0.720	0.667	0.680
8	0.787	0.747	0.760
9	0.853	0.840	0.853

Determinationof TLC profile for ethyl acetate extract (obtained from the maceration, reflux, and soxhlet method)conductedusing an eluent comparison n-hexane:ethyl acetatewitha 2:1 ratio (shown in **Figure 2**). Separation of spots (component) obtained by this comparison is very well based on its Rf value (**Table 2**),the third of the methods had the same relative Rf value, so that the possibility of the compound was attracted on extracts from each method are also the same.

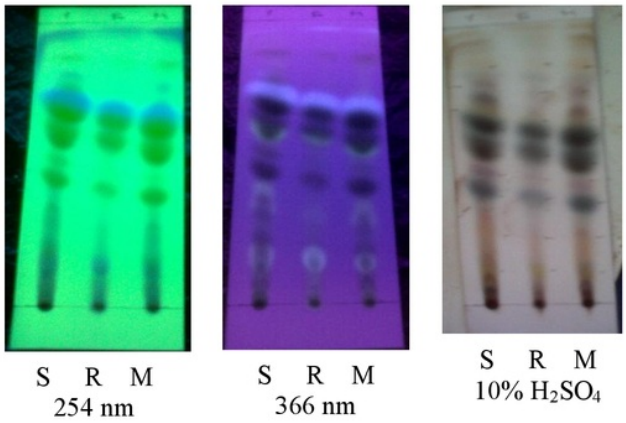


Figure 2Ethyl acetate extract: Reflux (R), Maceration (M), and Soxhlet (S) using n-hexane:ethyl acetate eluent comparison (2:1)

The ethanol extract (can be seen in **Figure 3**) both obtained from a reflux, maceration, and soxhlet method was conducted using an eluent proper comparison chloroform: methanol with a ratio of 7:1. Separation spot obtained with this excellent comparison based on the value of Rf obtained (shown in **Table 3**), whereby

the three methods have the same relative spot (component). Separation spot based on the level of a certain polarity and the separation based on the polarity level of each compound (spot) which is in the extract.

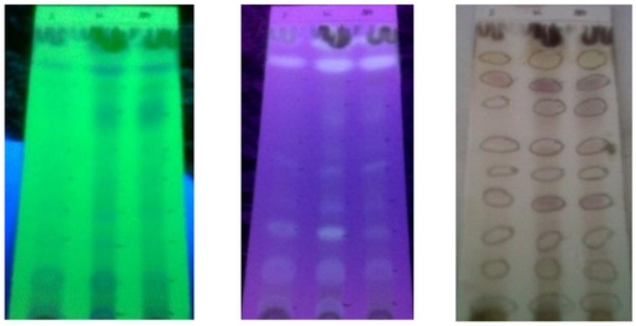


Figure 3Ethanol extract; Reflux (R), Maceration (M), and Soxhlet (S) using chloroform:methanol eluent comparison (7:1)

Table 3 Ethanol Extract (usingchloroform:methanol eluent; 7:1)

Spot	Rf Value		
	Soxhlet	Reflux	Maceration
1	0.053	0.047	0.053
2	0.133	0.147	0.147
3	0.227	0.240	0.240
4	0.347	0.360	0.360
5	0.447	0.453	0.453
6	0.533	0.547	0.547
7	0.680	0.680	0.680
8	0.773	0.773	0.773
9	0.853	0.867	0.867

Table 4 Methanol Extract (usingchloroform:methanol eluent; 2:1)

Spot	**Rf Value**		
	Soxhlet	**Reflux**	**Maceration**
1	0.147	0.160	0.147
2	0.253	0.253	0.267
3	0.547	0.547	0.533
4	0.667	0.667	0.680
5	0.787	0.787	0.800

The Methanol extract (can be seen in Figure 4) either obtained from a reflux, maceration, and soxhlet methods was performed by using the eluent appropriate comparison of chloroform:methanol in the ratio 2:1. Separation spot/stain obtained with this comparison very good based on the Rf value (shown in **Table 4**), where each separate spot evenly on the TLC plate. And spot stain obtained from these three methods have relatively similar properties. A ratio of 2: 1 obtained showing that methanol extract has a polarity which is higher than the compounds in ethanol extract.

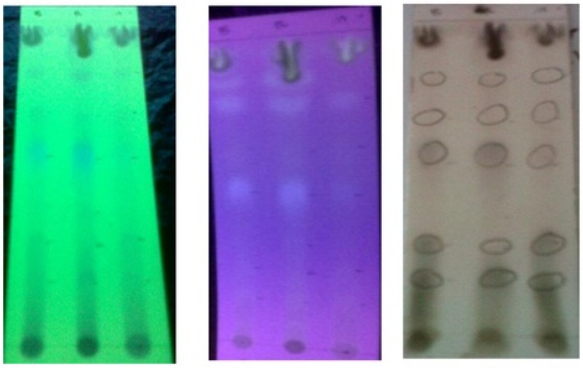


Figure 4Methanol extract: Reflux (R), Maceration (M), and Soxhlet (S) using chloroform:methanol eluent comparison (3:1)

Table 5 Methanol Extract (single solvent) using n-hexane:ethyl acetate; 2:1

Spot	Rf Value		
	Soxhlet	Reflux	Maceration
1	0.173	0.187	0.173
2	0.427	0.413	0.413
3	0.587	0.580	0.587
4	0.653	0.653	0.653
5	0.693	0.693	0.693
6	0.747	0.747	0.747
7	0.827	0.840	0.827
8	0.907	0.920	0.907

Tabel 6 Methanol Extract (single solvent) using chloroform:methanol eluent; 5:1

Spot	Rf Value		
	Soxhlet	Reflux	Maceration
1	0.107	0.120	0.133
2	0.160	0.160	0.173
3	0.227	0.233	0.253
4	0.360	0.373	0.427
5	0.453	0.480	0.520
6	0.520	0.547	0.573
7	0.653	0.667	0.693
8	0.800	0.800	0.827

The methanol extract with a single solvent (shown in **Figure 5** and **Figure 6**) both obtained from reflux, maceration, and soxhlet method was performed using two comparison eluent right namely n-hexane: ethyl acetate in a ratio of 2: 1 and chloroform: methanol in the ratio 5: 1. The use of two different eluent comparison is because the ability methanol attracts polar and non-polar compounds. The use of n-hexane: ethyl acetate to attract nonpolar compound and spot stain obtained can be split by either the ratio of 2: 1, while the use of chloroform: methanol is expected to attract polar compounds and spot stain obtained can be separated well at a ratio of 5 : 1. Spot obtained from both comparisons eluent has the same relative similarity of the three extraction methods.

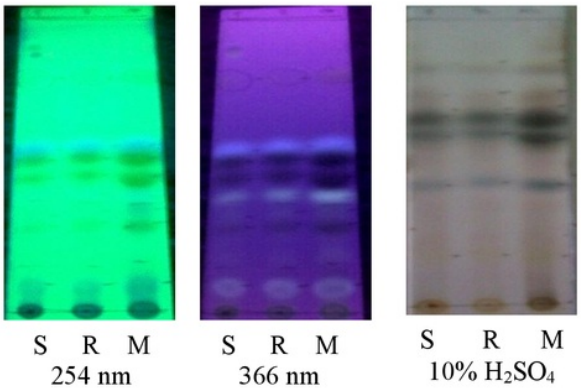


Figure 5 Methanol extract (single solvent): Reflux (R), Maceration (M), and Soxhlet (S) using n-hexane:ethyl acetate eluent comparison (3:1)

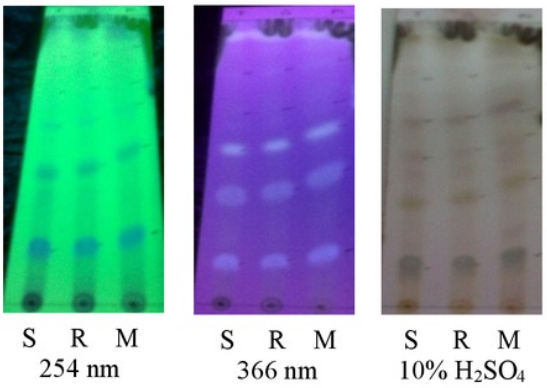


Figure 6 Methanol extract (single solvent): Reflux (R), Maceration (M), and Soxhlet (S) using chloroform:methanol eluent comparison (5:1)

Sunscreen Activity using Erythema and Pigmentation transmittance Measurement

Determination of sunscreen activity performed using the in vitro spectrophotometric method by calculating the percentage of erythema and pigmentationtransmission of the Bawang Dayak (*E.palmifolia*Merr) extract. In this study, the sunscreen activity test is confined to the determination of the percentage of transmission of erythema and pigmentation of each extract (10 ppm). The sample was analysed using a spectrophotometer set at a wavelength of 292.5 to 372.5 nm in the ranges every 5 nm and then determined the percentage of transmission of erythema and pigmentation.

The suntan products can be divided into several groups based on the percentage of erythema and pigmentation transmission at a wavelength of 292.5 to 372.5 nm include a total block or sunblock (ranges of <1% erythema range and <40% pigmentation), extra protective (1-6% erythema and 41-86% pigmentation ranges), Regular Suntan (ranges of 6-12% erythema and 45-86% pigmentation), and Fast Tanning (10-18%erythemaand 45-86% pigmentation ranges)[15,16,17,18]. In general, each of the resulting extracts has sunscreen activity in spite of having a percentage of erythema and pigmentation were different(can be seen in **Table 7**). The difference is probably due to the influence of each extraction methods so that there are differences in the number of compounds contained in extracts led to the activity generated is also different.The ethyl acetate extract (Soxhlet method) has the best activity and categorized as a sun block (total block), whereas other extracts activity is categorized as an extra protective.

Tabel 7Erythema and Pigmentation transmittance Measurement

Extract	Soxhlet		Reflux		Maceration	
	Te (%)	Tp (%)	Te (%)	Tp (%)	Te (%)	Tp (%)
N-hexane	2.78	2.94	3.13	3.23	5.45	5.36
Ethyl Acetate	0.72	0.55	1.59	1.40	1.38	1.22
Ethanol	3.95	3,52	5.63	5.34	1.76	1.59
Methanol	5.82	5.55	2.05	1.93	4.19	4.08
Methanol (C)	1.33	1.18	3.14	2.94	2.38	2.27

Te : erythema transmittance Tp: pigmentation transmittance
Methanol (C) : Extraction using single solvent

Conclusion

In the present study, the ethyl acetate extract (Soxhlet method) has the activity of the most powerful and categorized as a sun block (total block), although the use of other methods did not affect completely different in terms of profile KLT and sunscreenactivity, but in other termsfor instance consideration of solvent, time, principles, properties of the compound remain a consideration in the selection of appropriate extraction method.

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