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RESEARCH ARTICLE

Characterization of Carotenoid Extract and Bioactivity Membrane of the Seeds of Annato (*Bixaorellana*)

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Abstract

Bixa Orellana has a typical compound in the form of carotenoids. The types of carotenoids found in these compounds are bixin and norbixin. Both of these compounds have many benefits, one of which is as an antioxidant. Its nature as an antioxidant can also be used as a sunscreen. The purpose of this study was to determine the characteristics of carotenoid pigment in extract of the *B.orellana* sees membrane. Besides that, it is also to determine its biological activity in the form of antioxidants and sunscreen protectors. The TLC results showed 4 spot of carotenoids in the extract. Spot 1 identified as a bixin compound, because it has the same Rf as the bixin standard. HPLC result showed there were 5 peaks. The peak in tR of 9.046 was identified as norbixin and the peak in tR of 9.819 was identified as bixin. Carotenoid extract on the membrane of the seeds of *B. orellana* has antioxidant activity 16 times greater than standard bixin. Carotenoid extract at a concentration of 3.10 ppm had an SPF value of 6.27, including the extra protection category, and had a %TE value of 21.71% and Tp of 18.24% in the sunblock category.

Keywords: *Carotenoid, Antioxidant, Sunscreen, Bixin.*

Introduction

Bixaorellana is a small shrubby tree native in Central and South America [1]. These plants also thrive in Indonesia. This plant is one of the natural dye-producing plants [2]. The natural dyes produced by *B. orellana* are carotenoid pigments. There are 2 dominant and specific carotenoids produced namely bixin and norbixin.

According to [3] bixin and norbixin is the dominatpigmen in the *B.orellana* mostly found in the seed membrane. Bixin content in the seed membrane reaches 70-80 % and norbixin 20% [4]. These two pigments are often reffered to as annatto [5]. Annatto pigment is traditionally used by people as paint hair and bodies as insect repellant and protect from sunburn [6].The food industry also uses annatto as a dye, among them are the butter, margarine, cheese, biscuits, dairy product and chocolate [7]. This Pigment is also widely used as a dye in the cosmetic industry, seasoning and as a remedy in traditional medicine. The use of annatto dye is closely related to its activity, one of them as an antioxidant activity [8]. Antioxidants are compounds that can prevent chain reaction from free radical. Free radicals are the major cause of chronic and degenerative diseases such as, cancer, diabetes, hypertension, and coronary heart disease [9].The antioxidant mechanism of annatto is to quencher singlet oxygen [3, 8]. Singlet oxygen is very dangerous and reactive will be converted into triplet oxygen which is more stable.

Material and Methods

Material

Fresh plant material is collected from Salatiga, Central Java, Indonesia. Samples were collected in Februari 2020. The experiments were conducted at biology laboratory, STIFAR YayasanPharmasi Semarang.

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The acetone (P.A) used on the extraction and petroleum ether (P.A) used partitions. The standards bixin from Sigma-Aldrich Co. (St. Louis, USA). Acetonitrile of chromathographic grade from Merck.

Extraction Procedure

Pigment extraction is done with using the modified [10]. A total of 50 g of sample were dissolved with 250 mL acetone and added $CaCO_3$ 1 g, then stirred and filtered using Whatman paper. 2. Filtrates which is obtained is accommodated while the residue re-extracted using 250 mL aceton until all the pigment is removed. Extract is partitioned using petroleum ether, the petroleum ether is removed and added Na_2SO_4 then filtered and evaporated. The concentrated extract obtained is stored in a bottle and dried with N_2 gas.

Thin Layer Chromathography (TLC)

The extracts were analyzed using TLC silica gel GF 254 by spotting the sample on the plate. The TLC is then eluted with solution acetone:hexane (1:1 v/v). Pigment separation pattern is drawn and the Rf value is calculated.

High Performance Liquid Chromathography (HPLC)

HPLC analysis was performed Shimadzu HPLC equipped with photodiode array (PDA) detector. Column used is Cosmosil 5C-$_{18}$-MS-II (4,6 x 150 mm) (NacalaiTesque) and temperature at 30°C. Carotenoid extract was eluted with a gradient system using flow rate of 0.5 mL/menit. The solution used was A: 0.1% fomiat acid in H_2O and B: acetonitrile. The pigments were detected with a PDA detector and evaluated on 450 nm [11].

Antioxidant Activity

The antioxidant activity test was carried out using the DPPH method [8]. DPPH, a stable free radical at room temperature, produces a violet color in methanol. When the free radical reacts with an antioxidant, its free radical property is lost due to chain breakage and its color changes to light yellow [12].

Sun Screen Activity

Testing is done with determine sunscreen activity based on the value of SPF, %TE, and %TP value.

Sun Protection Factor (SPF)

The method for measuring the Sun Protector Factor (SPF) value is to use spectroscopy according to the [13]:

$$SPF = CV \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times abs(\lambda)$$

Which are:

CV = Correction value
EE = Spectrum of erythema effect
I = Spectrum of sun's intensity
Abs = Absorbance of sample

The sample solution is measured with a UV-Vis spectrophotometer on wavelengths 290-320 nm with the absorption value is recorded every 5 intervals.

Percentage erythema Transmission (Te) and Percentage Pigmentation Transmission (Tp)[14]

Sunscreen activity parameters is observed the percentage of erythema and the percentage of pigmentation. Sample measured its absorption using UV-Vis spectrophotometer at 292.5 – 375.5 nm. After the value is obtained absorption (A), then it can be done calculation of transmission (T) with using the formula:

$$A = -\log T$$

Erythema transmission (Te) is calculated using the formula:

$$Te = T \times Fe$$

Where Fe is the erythema flux its value at the wavelenght certain. Amount of erythema flux forwarded by sunscreen (Ee) calculated using the formula

$$Ee = \sum Te$$

Meanwhile %erythema transmission is calculated using the formula:

$$\%\ \text{Erythema transmission (Te)} = \frac{Ee}{\sum Fe} = \frac{\sum(T \times Fe)}{\sum Fe}$$

Pigmentation transmission (Tp) calculated using the formula

$$Tp = T \times Fp$$

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Where Fp is the pigmentation flux its value at the wavelength certain. The amount of pigmentation flux sunscreen (Ep) calculated using the formula:

$$\% \text{ Pigmentation transmission (Tp)} = \frac{\text{Ep}}{\sum \text{Fp}} = \frac{\sum(\text{T} \times \text{Fp})}{\sum \text{Fp}}$$

Result and Discussion

Annato extract was carried out by carotenoid identification using TLC, spectrophotometer, and HPLC. The identification results are as follows:

Thin Layer Chromathography (TLC)

TLC is the initial identification that is carried out. The results of TLC are shown in Figure 1.

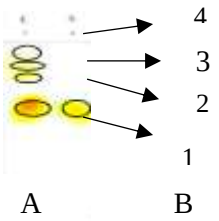


Figure 1: The result separation of *B.orellana* extract (a) and bixin standard (b) by TLC using silica gel GF_{254} and mobile phase hexane: acetone (1:1 v/v)

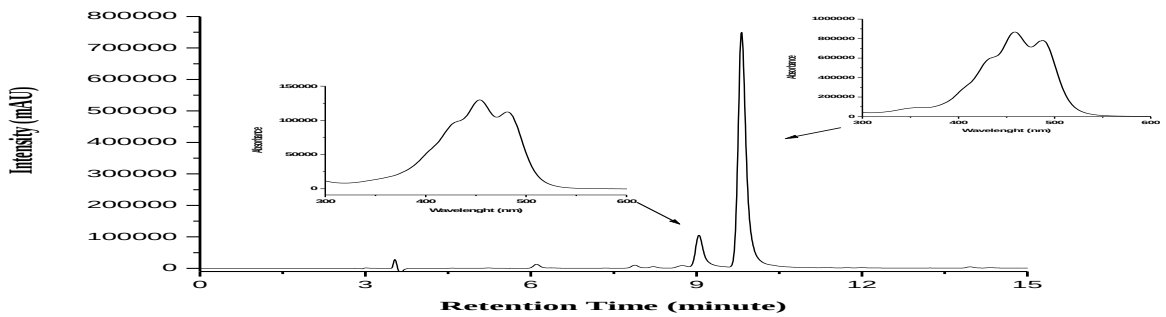
The identification of carotenoid from *B.orellana* extract is shown in table 1. Spot 1 has the same Rf as the bixin standard. So it can be concluded that spot 1 is a bixin compound. Spot 2 and 3 have a yellow color. According to [15] carotenoid pigments have a yellow, orange, to red color. So spot 2 and 3 are likely carotenoid pigments. To ensure that the resulting spots are carotenoid, further identification is needed using HPLC.

Table 1: Identification of *B. orellana* extract by Thin Layer Chromatography method

S.No	Spot colour	Rf	Identification
1	Orange	0.77	Bixin
2	Yellow	0.89	Carotenoid
3	Yellow	0.92	Carotenoid
4	Yellow	0.96	Carotenoid

High Performance Liquid Chromathography (HPLC)

The identification of the carotenoid extract was then carried out by HPLC analysis. HPLC analysis is more sensitive than TLC. The results of the HPLC analysis are shown in Figure 2 and Table 3.



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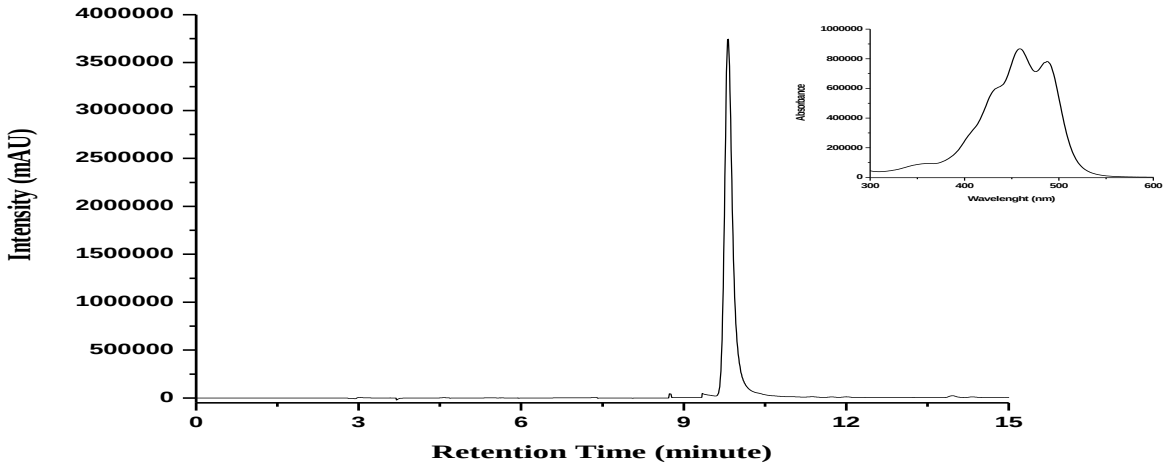

Figure 2: Kromatogram profile carotenoid extract of *B. orellana* seed membrane (a) and standard bixin (b) The detection was carried out at 450 nm

The results of the separation of the carotenoid extract of *B.orellana* seed membrane obtained 5 peaks showing a carotenoid spectrum pattern. The carotenoid spectrum has absorption at a wavelength of 300-600 nm [15, 16]. The peaks with the retention time of 9.819 show the bixin compound because it has the same retention time and spectrum pattern as the standard bixin. Bixin has absoption at 458 and 486 nm and nor bixin has absorption at 459 and 486 nm [11].

Table 3: Carotenoid identification of carotenoid extract on the membrane of the seeds of *B. Orellana*

Sample	tR	λ maks			Component	area %
Carotenoid extract	5.204		427		Carotenoid	4.83
	6.102		457	485	Carotenoid	4.33
	7.879		445	473	Carotenoid	4.15
	9.046	428	459	486	Nor bixin	14.29
	9.819	432	458	488	Bixin	72.4
Standartbixin	9.811		458	486	Bixin	99

The result of %area on the chromatogram shows bixin content is 72, 4%. The results are in accordance with the literature that the bixin content in the membrane of *B.orellana* seeds is 70-80% [3, 4]

Antioxidant Activity

The capacity of an antioxidant activity IAA based extract or compounds can divided into 4, namely IAA < 0.5 means low antioxidant activity, the IAA 0.5 – 1 means moderate antioxidant activity, IAA 1 -2 which means strong antioxidant activity and IAA>2 means very strong antioxidant activity [17]. The IC_{50} and IAA values of the extract and bixin standard are shown in Table 1.

Table 1: IC_{50} and IAA carotenoid extract and bixinstandart

Sample	IC50	IAA	Category
Extract	3.10 ppm	16.12	Very strong
Bixinstandart	49.25 ppm	1	Strong

IC_{50} value of extract is 16 times greater than standard bixin. These results probably contained other types of carotenoids in extract, example norbixin. Bixin and other carotenoids will work synergistically, resulting in higher antioxidant activity

Sunscreen activity

The sun screen activity uses an IC_{50} concentration (3.10 ppm). At this concentration the value of SPF, %TE and, %Tp were measured. The measurement results are shown in Table 2.

Table 2: Sun screen activity carotenoid extract at 3.10 ppm

	Value	Category
SPF	6.27	Extra
% Te	21.71	-
% Tp	18.24	Sun block

SPF values 6-8 are included in the extra protection category [18]. Based on the results of % TE and %TP of the carotenoid extract on the membrane of the seeds of *B. orellana*, it was included in the weak sun block category. The sun block is weak because the value obtained at % TE is not included in the category but for the % Tp value it is in the sun block category [19]

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Conclusion

Carotenoid extract of *B. orellana* seed membrane contains 5 types of carotenoid pigments. The most dominat pigment is bixin. The carotenoid extract has an antioxidant activity of 3.10 ppm. Its activity is 16 times greater than the standard bixin. In addition, carotenoid extract at a concentration of 3.10 ppm also has sunscreen activity with the sunblock category.

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