IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 11, Issue 11 Series. I (November 2021), PP. 01-07
www.iosrphr.org

Sunscreen Activityof Carotenoid Fraction, Norbixin, and Bixinfrom*Bixaorellana* Linn. Seeds

Yuvianti Dwi Franyoto[1*], Erna Rizkyana[1],Lia Kusmita[1], Mutmainah[1], IkaPuspitaningrum[1]

[1]*STIFAR College of Pharmaceutical Sciences YayasanPharmasi Semarang, LetjendSarwoEdhieWibowo KM 1, Semarang, Central Java, Indonesia.*

Received 05November 2021; Accepted 20November 2021

Abstract: The use of synthetic sunscreens can cause contact dermatitis, so natural sunscreens are preferred. Kesumbakeling (annatto) contains carotenoids such as norbixin, which has conjugated double bonds that cause photoprotection. This study aims to determine the potential of the carotenoid fraction and norbixin as an active ingredient in sunscreen by measuring the SPF, %TE and %TP values using UV-Visible Spectrophotometry. The results showed that the yield of the carotenoid fraction was 3,73± 0.33% with 16,66 ± 0.49% of norbixin content and 84,57 ± 0,54% of bixin content. The identification of norbixin produced a bluish-green color and a single spot on TLC with an Rf of 0,82 with a spectral pattern at λ 457,30; 486,30 and 567,70 nm. The identification of bixin produced a *cornflower-blue* color and a single spot on TLC with an Rf of 0,37 with a spectral pattern at λ 352,00; 458,00 dan 486,70 nm. The carotenoid fraction produced SPF values with minimum (100 and 150 ppm), moderate (200 and 250 ppm) and extra protection (300 ppm). The %TP values provided extra protection (50–100 ppm) and sunblock category (150–300 ppm). Norbixin compound produced SPF values with minimum (200 ppm), moderate (300 ppm), extra (400 and 500 ppm) and maximum protection (600 ppm). The results of %TP provided extra protection (100 ppm) and sunblock category (200–600 ppm). Bixin compound produced SPF values with minimum (300 and 400 ppm), moderate (500, 600and700 ppm) and extra protection (800, 900 and 1000ppm). The results of %TP provided extra protection (100–300 ppm) and sunblock category (400–1000 ppm). Both the carotenoid fraction, norbixin and bixin compound from *Bixaorellana* seeds have the ability as a sunscreen.

Keywords:*Bixaorellana*, Bixin, Carotenoid fraction, Norbixin, Sunscreen

I. INTRODUCTOION

Ultraviolet radiation such as UV-A (320 – 400 nm) and UV-B (290 – 320 nm) can cause pigmentation and erythema, so it is ideal to use sunscreen as protection[1]. However, commercial sunscreens contain many synthetic ingredients such as PABA *(para-aminobenzoic acid)*and*oxybenzone* which causes contact dermatitis[2].So that research is carried out on sunscreens made from natural ingredients such as the kesumba rivet plant *(Bixa orellana)*.The part of the kesumba rivet (annatto) seed has a red membrane that contains a lot of apokarotenoids such as bixin and norbixin[3]. These compounds have conjugated double bonds that are photoprotective[4]. The purpose of this study was to determine the potential of carotenoid fractions as well as bixin and norbixin compounds as active ingredients in sunscreens. The parameter to be measured is the SPF value, %TE and %TP. These three parameters can be calculated by in vitro method using UV-Visible Spectrophotometry [5].

II. MATERIALS AND METHODS

2.1 Plant material Collection and Authentication

The sample used was wet kesumba rivet seeds obtained from Salatiga, Central Java, Indonesia. The research was carried out at the STIFAR biology laboratory of the Pharmasi Foundation, Semarang.

2.2 Chemicals

The chemical agents such ashexane, etanol, KOH 0,5%, H_2SO_4 concentrated, alumunium foil, *silica gel for column* (Merck Si-60 0,2 – 0,5 mm), TLC plate (Merck TLC Silica gel F_{254}), bixin standard (Sigma-Aldrich), N_2 gas and reagents for phytochemical screening.

2.3 Preparation and Fractionation of crude extract of plant material

The carotenoid fraction was obtained by weighing the wet seeds of kesumba rivet and then extracted with acetone (fast maceration) until the seeds were colorless[6]. Then the acetone extract was filtered and evaporated at 30°C. The evaporation results were partitioned with petroleum ether in a separatory funnel with a ratio of acetone: petroleum ether: water (10:5:1). After separating, the petroleum ether phase (top layer) is accommodated, added Na2SO4, filtered and re-evaporated. After that it is stored and dried with N2 gas[7]. Furthermore, phytochemical screening of the carotenoid fraction was carried out[8,9,10].

2.4 Norbixin and BixinContent estimation

Analysis of % norbixin content was measured by dissolving 0.1 g of carotenoid fraction in 100 ml KOH 0,5% then diluted 100x. The solution was measured absorbance at λ 482 nm[11].

Analysis of % bixin content was measured by dissolving 0.1 g of carotenoid fraction in 100 ml of acetone then diluted 100x. The solution was measured absorbance at λ 502 nm.

The calculation of % bixin can be calculated by the formula:

$$\%\ \boldsymbol{bixin} = \frac{\boldsymbol{A}}{\mathbf{2870}}\ \boldsymbol{x}\ \frac{\mathbf{100000}}{\boldsymbol{sample\ weight\ (mg)}}\ \boldsymbol{x}\ \mathbf{100}$$

Description

In calculating 0.1 gram of sample is considered equivalent to 1 gram of sample.

A = Absorbance obtained

100000= Constanta

2870 = Absorbance constant (E1%1 cm) bixin in acetone on λ 502 nm

Bixin and norbixin compounds were obtained by separation using column chromatography. The stationary phase used is silica gel Si-60 with acetone as the mobile phase : hexane (1:1 v/v). The eluate was collected and identified using the color test with H_2SO_4, stain on TLC with stationary phase of silica gel F254 and acetone mobile phase: heksana (1:1 v/v) and see the spectrum pattern using Spectrophotometry UV-Visible in λ 300 – 600 nm.

2.5 In Vitro Sun Protection Factor Evaluation

The sunscreen ability test was carried out on the carotenoid and norbixin fractions. Determination of the SPF value using the equation Mansur et al. from absorbance measurements every 5 nm interval at λ 290 – 320 nm[12].

$$\boldsymbol{SPF = CF\ x \sum_{290}^{320} EE\ (\lambda)\ x\ I\ (\lambda)\ x\ absorbance(\lambda)}$$

Description

CF = Correction factor (10)

EE = Erythema spectrum of effect

I = Intensity of the light spectrum

The value of EE x I is a constant of 290 – 320 nm every5 nm which has been specified Sayre et al[13].

Furthermore, the value of %TE and %TP can be determined by the method Balsam and Sagarinfrom transmittance measurements every 5 nm interval at λ 290 – 320 nm (%TE) and λ 320 – 375 nm (%TP)[14]. The transmission value (T) is obtained from the equation A = - log T.

$$\%\ \boldsymbol{erythema} = \frac{\boldsymbol{\Sigma\ Ee}}{\boldsymbol{\Sigma\ Fe}} = \frac{\boldsymbol{\Sigma\ (T\ x\ Fe)}}{\boldsymbol{\Sigma\ Fe}}$$

$$\%\ \boldsymbol{pigmentation} = \frac{\boldsymbol{\Sigma\ Ee}}{\boldsymbol{\Sigma\ Fe}} = \frac{\boldsymbol{\Sigma\ (T\ x\ Fe)}}{\boldsymbol{\Sigma\ Fe}}$$

Description

T = Transmission value

Fe / Fp = Erythema flux / Pigmentation flux

Ee / Ep = Amount of erythema flux / pigmentation flux transmitted by sunscreen

ΣFe / ΣFp = Total amount of UV light energy that causes erythema/pigmentation

Fe and Fp values are constants of λ 290 – 375 nm every 5 nm refers to Abdassah et al[5].

2.6 Statistical Analysis

The data obtained were analyzed by One Way ANOVA using SPSS Version 16.0 program. The normality test was carried out by the Shapiro-Wilk test while the homogeneity test was carried out with Levene's Test.

III. RESULTS AND DISCUSSION

Analysis of % norbixin content in the carotenoid fraction obtained was 16,66± 0,49%.. These levels meet the requirements of the 15 – 20% norbixinrange[11]. Meanwhile, in the analysis of % levels of bixin in the carotenoid fraction was 84,57± 0,54%. These results are in accordance with the literature where the levels of bixin is ± 80% [7]. Furthermore, the bixin and norbixin compounds were separated by column chromatography, which resulted in 69 vials with the selected norbixin fraction being fraction 2 to fraction 5 and bixin fraction from fraction 21 to fraction 30. The norbixin fraction tends to be yellow to orange in color while the bixin fraction tends to be more concentrated from orange to red. Identifying color with H_2SO_4 produces a bluish green color (blue-green) in the norbixin fraction and cornflower-blue color in the bixin fraction as in Figure 1. This is clarified by the TLC results in Figure 2. The results of the identification of norbixin showed a single yellow stain with an Rf of 0.82. The spectral pattern of norbixin in Figure 3 also shows three typical carotenoid peaks with λ 457,30 nm; 486,30 nm and 567,70 nm. These results are similar to norbixin in the study of Noppe et al[15]. Meanwhile, the results of the identification of bixin were compared with the bixin standard (Sigma-Aldrich). The TLC results for bixinRf 0.37 have similarities with the bixin standard Rf 0.38. The results of the bixin spectrum in Figure 4 also show the same pattern in λ 352,00 nm; 458,00 nm and 486,70 nm which is almost the same as the standard.

Fig 1:Color test identification

a. Norbixin **b) Bixin**

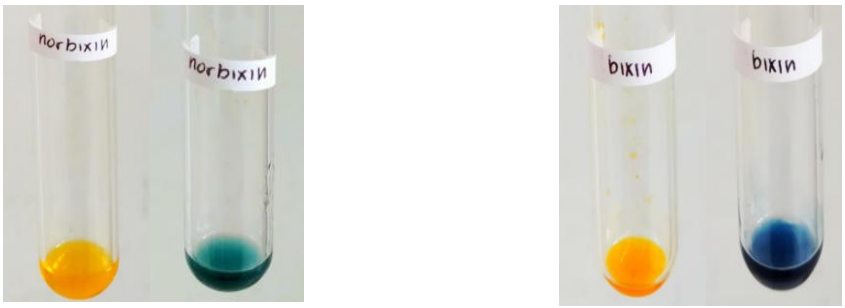
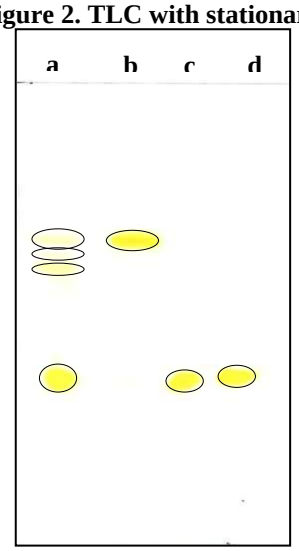


Figure 2. TLC with stationary phase silica gel F254 and mobile phase aceton : hexane (1:1 v/v)



Description:

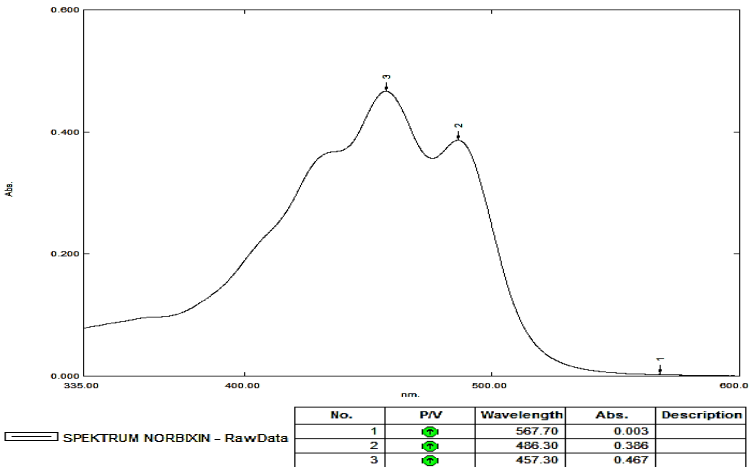
a. carotenoid fraction:Rf1 0,82; Rf2 0,78; Rf3 0,73; Rf4 0,37

b.norbixinRf 0,82

c.bixinRf 0,37

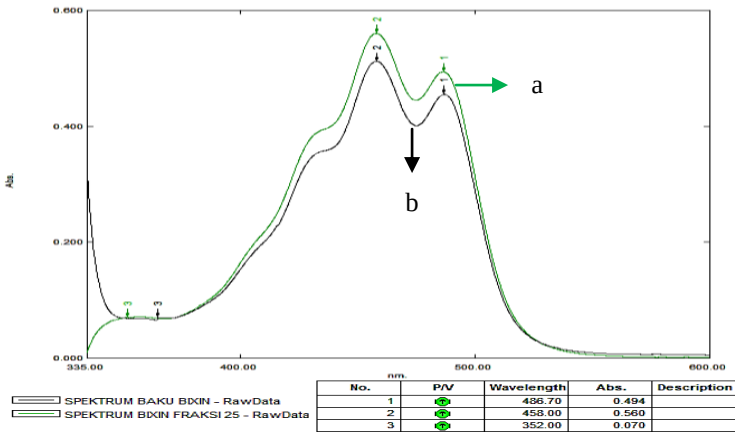
d.bixinstandartRf 0,38

Figure 3. Spectrum norbixin



No.	P/V	Wavelength	Abs.	Description
1		567.70	0.003	
2		486.30	0.386	
3		457.30	0.467	

Figure 4. Spectrum bixin(a) and bixinsatndart (b)



No.	P/V	Wavelength	Abs.	Description
1		486.70	0.494	
2		458.00	0.560	
3		352.00	0.070	

The SPF value is the ratio of the UV ray energy required to cause minimal erythema on protected skin with unprotected skin. Erythema is a reddish reaction caused by UV rays, especially UV-B (290 – 320 nm). The SPF value that has been obtained from the absorbance measurement is then classified into 5 categories. From the calculation of the SPF of the carotenoid fraction in Table 1, it is found that at a concentration of 50 ppm it has not been able to provide protection as a sunscreen. Sunscreen activity starts to show at concentrations of 100 ppm and above. Concentrations of 100 ppm and 150 ppm provide minimal protection. Concentrations of 200 ppm and 250 ppm provide moderate protection and a concentration of 300 ppm is included in the category of extra protection. While the norbixin compound at a concentration of 100 ppm has not been able to provide sunscreen protection, but at a concentration of 200 ppm it can provide minimal protection. Furthermore, a concentration of 300 ppm is in the moderate protection category, a concentration of 400 ppm and 500 ppm is in the extra protection category and a concentration of 600 ppm is in the maximum protection category. In the compound bixin, the ability of new sunscreens is seen at concentrations of 300 ppm and 400 ppm which can provide minimal protection. Furthermore, concentrations of 500 ppm to 700 ppm are in the medium protection category and concentrations of 800 ppm to 1000 ppm are in the extra protection category.

The %TE value describes the number of UV rays that are transmitted after hitting the sunscreen so that it can cause erythema (redness) so that the measurement is carried out at λ UV-B (290 – 320 nm). While the %TP value describes the amount of UV rays that are passed on after hitting the sunscreen so that it can cause pigmentation (dark spots) so that measurements are made on λ UV-A (320 – 375 nm). The %TE and %TP values that have been obtained from transmittan measurements are then classified into 4 categories[5]. The carotenoid fraction provides extra protection at concentrations of 50 and 100 ppm, and the rest is in the sunblock category (150, 200, 250 dan 300 ppm). The norbixin compound produces extra protection at a concentration of 100 ppm and the rest is in the sunblock category (200, 300, 400, 500 dan 600 ppm). While the bixin compound produces extra protection at concentrations of 100 ppm to 300 ppm and the rest is in the sunblock category (400, 500, 600, 700, 800, 900 dan 1000 ppm) as shown in Table 2. At the transmittance value, the higher the concentration, the more solute and the more concentrated the solution. Less light is passed which causes %T to

be small so the graph is inversely proportional.

Table 1. SPF value of carotenoid fraction, norbixin and bixinfrom *Bixaorellana*

Sample	Concentration	SPF	Protection
Carotenoid fraction	50 ppm	1.36 ± 0.04	–
	100 ppm	2.27 ± 0.04	Minimal
	150 ppm	3.19 ± 0.10	Minimal
	200 ppm	4.23 ± 0.08	Moderate
	250 ppm	5.14 ± 0.07	Moderate
	300 ppm	6.06 ± 0.01	Extra
Norbixin	100 ppm	1.67 ± 0.02	–
	200 ppm	3.10 ± 0.03	Minimal
	300 ppm	4.59 ± 0.02	Moderate
	400 ppm	6.23 ± 0.02	Extra
	500 ppm	7.71 ± 0.06	Extra
	600 ppm	9.26 ± 0.06	Maximal
Bixin	100 ppm	1.30 ± 0.001	–
	200 ppm	1.95 ± 0.001	–
	300 ppm	2.37 ± 0.003	Minimal
	400 ppm	3.20 ± 0.002	Minimal
	500 ppm	4.11 ± 0.004	Moderate
	600 ppm	4.79 ± 0.003	Moderate
	700 ppm	5.58 ± 0.002	Moderate
	800 ppm	6.42 ± 0.002	Extra
	900 ppm	7.17 ± 0.002	Extra
	1000 ppm	7.97 ± 0.002	Extra

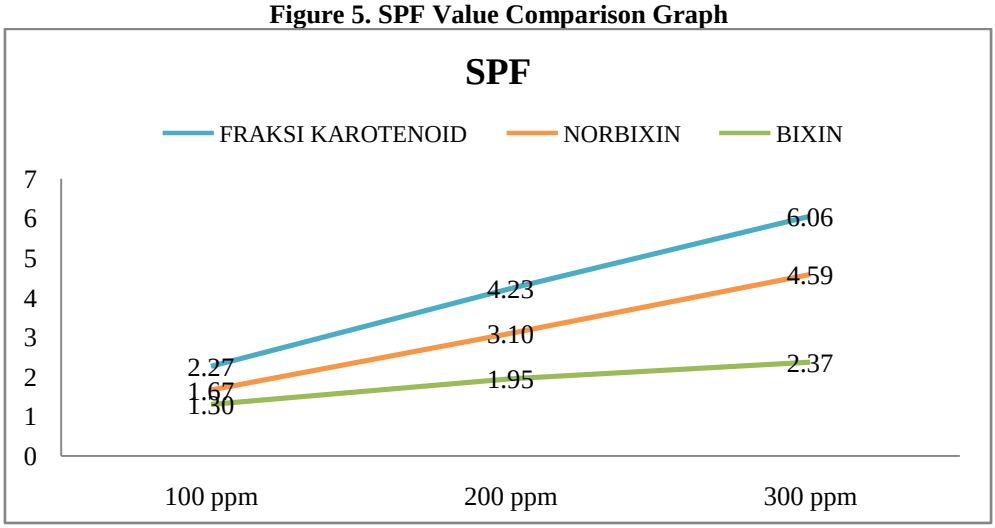
Table 2. %TE dan %TP value of carotenoid fraction, norbixin and bixin from *Bixaorellana*

Sample	Concentration	%TE	%TP	Category
Carotenoid fraction	50 ppm	73.52 ± 0.03	68.61 ± 0.14	*Extra protection*
	100 ppm	58.08 ± 0.12	49.46 ± 0.06	*Extra protection*
	150 ppm	46.48 ± 0.28	36.69 ± 0.24	*Sunblock*
	200 ppm	35.83 ± 0.13	26.26 ± 0.10	*Sunblock*
	250 ppm	28.24 ± 0.18	19.65 ± 0.15	*Sunblock*
	300 ppm	22.53 ± 0.13	14.79 ± 0.08	*Sunblock*
Norbixin	100 ppm	66.22 ± 0.16	58.92 ± 0.16	*Extra protection*
	200 ppm	47.13 ± 0.13	37.05 ± 0.09	*Sunblock*
	300 ppm	33.40 ± 0.24	23.38 ± 0.23	*Sunblock*
	400 ppm	22.77 ± 0.22	14.19 ± 0.19	*Sunblock*
	500 ppm	16.09 ± 0.20	9.24 ± 0.14	*Sunblock*
	600 ppm	11.15 ± 0.15	5.84 ± 0.09	*Sunblock*
Bixin	100 ppm	72.17 ± 0.25	76.01 ± 0.18	*Extra protection*
	200 ppm	61.30 ± 0.17	59.94 ± 0.11	*Extra protection*
	300 ppm	55.98 ± 0.17	48.60 ± 0.11	*Extra protection*
	400 ppm	45.84 ± 0.10	37.83 ± 0.08	*Sunblock*
	500 ppm	36.63 ± 0.09	29.41 ± 0.05	*Sunblock*
	600 ppm	31.20 ± 0.12	23.54 ± 0.09	*Sunblock*
	700 ppm	25.87 ± 0.03	18.51 ± 0.02	*Sunblock*
	800 ppm	21.16 ± 0.08	14.87 ± 0.05	*Sunblock*
	900 ppm	17.74 ± 0.11	11.71 ± 0.07	*Sunblock*
	1000 ppm	14.57 ± 0.03	8.95 ± 0.04	*Sunblock*

In statistical analysis with SPSS, the results of the normality test with Shapiro-Wilk showed a significance value of more than 0.05 so that the data were normally distributed. Furthermore, homogeneity test with Levene's Test showed that the data of the carotenoid fraction was homogeneous with the SPF value

(0,182), %TE (0,181) and %TP (0,095). Norbixin compounds showed homogeneous data with SPF value (0,274), %TE (0,642) and %TP (0,275). Bixin compounds also showed homogeneous data with SPF value (0,406), %TE (0,096) and %TP (0,174). In the results of One Way ANOVA both have a significance value of 0.000 so that there are differences in each concentration group.

Figure 5. SPF Value Comparison Graph



The structure of carotenoids can absorb light because they have conjugated double bonds so that they experience resonance when exposed to UV rays. Carotenoid components also act as antioxidants (radical scavengers) by reducing (quencher) singlet oxygen ($1O_2$) due to the presence of a chromophore group. Singlet oxygen ($1O_2$) plays a role in produce UV-A-induced oxidative stress[16]. The carotenoid fraction with a concentration of 100 ppm was able to provide activity as a sunscreen, while the new norbixin compound could provide activity as a sunscreen at a concentration of 200 ppm and bixin compound at a concentration of 300 ppm. Bixin and norbixin there is only a single compound, while in the carotenoid fraction there are still several compounds other than carotenoids that can provide sunscreen activity such as flavonoids. If seen in Figure 5, the bixin compound gave the lowest SPF value at the same concentration compared to the carotenoid fraction and norbixin compound. The bixin compound is likely to be degraded. Satyanarayana et al. states that bixin is easily oxidized and produces a degradation product in the form of a yellow C-17 polyene called Mc Keowncompound[3].

IV. CONCLUSION

Bixin and norbixin compounds can be isolated from the carotenoid fraction of kesumba rivet seeds with the identification of norbixin bluish green using sulfuric acid, yellow stains on TLC with Rf 0.82 and typical carotenoid spectral patterns with λ 457,30 nm; 486,30 nm and 567,70 nm. While the results of the color bixin identification cornflower-blue, TLC results with Rf 0.37 and the spectrum pattern at λ352,00 nm; 458,00 nm and 486,70 nm which is almost the same as the bixin standard. The carotenoid fraction with a concentration of 100 ppm has shown the ability as a sunscreen with an SPF value 2,24 ± 0,02 (minimal protection) and value %TE 58,08 ± 0,12 and %TP 49,46 ± 0,06 (extra protection). The new 200 ppm concentration of norbixin showed its ability as a sunscreen with an SPF value 3,10 ± 0,03 (minimal protection) and value %TE 47,13 ± 0,13 and %TP 37,05 ± 0,09 (sunblock). Meanwhile, the new 300 ppm concentration of bixin showed the ability to act as a sunscreen with an SPF value 2,37 ± 0,003 (minimal protection) and value %TE 55,98 ± 0,17 and %TP 48,60 ± 0,11 (extra protection).

ACKNOWLEDGEMENTS

This research was partly funded by the Ministry of Research, Technology, and Higher Education, Indonesia for financial support to this research through "PEKERTI" research grant scheme (Number 26/E1/KPT/2020).

REFERENCES

[1]. Hassan I, Dorjay K, Sami A dan Anwar P., 2013. Sunscreens and Antioxidants as Photo-protective Measures: An update. *Our Dermatology Online*, **4**(3): 369–374. https://dx.doi.org/10.7241/ourd.20133.92

[2]. Cefali LC, Ataide JA, Moriel P, Foglio MA dan Mazzola PG., 2016. Plant-based active photoprotectants for sunscreens. *International Journal of Cosmetic Science*, **38**: 346–353. https://dx.doi.org/10.1111/ics.12316

[3]. Satyanarayana A, Prabhakara Rao P dan Rao D., 2003. Chemistry, processing and toxicology of annatto (Bixa orellana L.). *J Food Sci Technol*, **40**(2): 131–141.
[4]. Alwis D, Chandrika U dan Jayaweera P., 2021. Photostability of apocarotenoids on surface of TiO2 semiconductor nanoparticles. *Journal of Photochemistry and Photobiology A: Chemistry*, **407**: 113061.https://dx.doi.org/10.1016/j.jphotochem.2020.113061
[5]. Abdassah M, Aryani R, Surachman E dan Muchtaridi M., 2015. In-vitro assessment of effectiveness and photostability avobenzone in cream formulations by combination ethyl ascorbic acid and alpha tocopherol acetate. *Journal of Applied Pharmaceutical Science*, **5**(6): 070–074.https://dx.doi.org/10.7324/JAPS.2015.50611
[6]. Britton G, Liaaen-Jensen S dan Pfander H., 2009. *Carotenoids: Nutrition and Health Volume 5*. Birkhäuser Basel. https://dx.doi.org/10.1007/978-3-7643-7501-0
[7]. Kurniawati PT, Soetjipto H dan Limantara L., 2007. Antioxidant and antibacterial activities of Bixin pigment from Annatto (Bixa orellana L.) seeds. *Indonesian Journal of Chemistry*, **7**(1): 88–92.
[8]. Abayomi M, Adebayo AS, Bennett D, Porter R, Campbell JS dan Dawkin G., 2014. Phytochemical Testing and In vitro Antibacterial Activity of Bixa orellana (Annatto) Seed Extract. *British Journal of Pharmaceutical Research*, **4**(11): 1387–1399. https://dx.doi.org/10.9734/BJPR/2014/10925
[9]. Ahmed S, Moni BM, Ahmed S, Gomes DJ dan Shohael AM., 2020. Comparative phytochemical, antioxidant, and antibacterial study of different parts of Doigota plants (Bixa orellana L.). *Bulletin of the National Research Centre*, **44**(95): 1–10.https://dx.doi.org/10.1186/s42269-020-00349-1
[10]. Harborne JB., 1987. *Phytochemical Methods.* Chapman & Hall.University of Reading. https://dx.doi.org/10.1007/978-94-009-5570-7
[11]. Smith, J. 2006. Annatto extracts-chemical and technical assessment. *Chem Tech Assess Manual*, 1–21.
[12]. Mansur J, Breder M, Mansur M dan Azulay RD., 1986. Determination of sun protection factor by spectrophotometric methods. *An Bras Dermatol*, **61**: 121–124.
[13]. Sayre RM, Agin PP, LeVee GJ dan Marlowe E., 1979. A comparison of in vivo and in vitro testing of sunscreening formulas. *Photochemistry and Photobiology*, **29**: 559–566. https://dx.doi.org/10.1111/j.1751-1097.1979.tb07090.x
[14]. Balsam MS dan Sagarin E., 1972. *Cosmetics: Science and Technology*. Wiley-Inter Science. Michigan University.
[15]. Noppe H, Abuin Martinez S, Verheyden K, Van Loco J, Beltran R dan Brabander H., 2009. Determination of bixin and norbixin in meat using liquid chromatography and photodiode array detection. *Food Additives and Contaminants*, **26**: 17–24. https://dx.doi.org/10.1080/02652030802322564
[16]. Britton G, Liaaen-Jensen S dan Pfander H., 2009. *Carotenoids: Nutrition and Health Volume 5*. Birkhäuser Basel. https://dx.doi.org/10.1007/978-3-7643-7501-0

Yuvianti Dwi Franyoto, et. al. "InVitro Sunscreen Capability Testing Of Carotenoid Fraction, Norbixin, and BixinfromBixaorellana Linn. Seeds." *IOSR Journal of Pharmacy (IOSRPHR)*, 11(11), 2021, pp. 01-07.