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by Darus Sa'adah Johanis Paransa 4

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Exploration study of the pigment types of stone crab (*Grapsus albolineatus*) as pharmaceutical and cosmetics Ingridients

Darus Saadah J. Paransa[1]*, Desy M.H. Mantiri[1], James J.H. Paulus[1], Cysca Lumenta[1], Medy ompie[1], Suria Darwisito[1], Carolus Paruntu[1], Alfread Kase[2] and Anggreni D.N. Rupidara[2]

[1]Fisheries and Marine Science Study Program of Sam Ratulangi University, Manado, North Sulawesi, Indonesia
[2]Fisheries and Marine Science Study Program of Artha Christian University, Kupang, East Nusa Tenggara, Indonesia

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ABSTRACT

Grapsus albolineatus is a type of crustacean on the rocky coast of tropical regions like Indonesia. Some of the distribution areas of this crab species are the rocky coastline of, Tanawangko and Manggatasik Village of, Manado City, North Sulawesi. *Grapsus albolineatus* is known to have a high content of carotenoid pigment. Carotenoid pigments are useful for human needs, such as immune substances, and various industrial raw materials such as pharmaceutical, cosmetic and foodstuff industries. In this study, identification of the pigment type was performed on the carapace, gonad, blood, epidermal layer, and hepatopancreas using Thin Layer Chromatography method. The identification of pigments on the carapace was using Column Chromatography and High-Performance Liquid Chromatography (HPLC) methods. The results showed that *Grapsus albolineatus* was identified at the D molting stage. In the Thin Layer Chromatography analysis, there were found some pigment types* namely β-Carotene, Echinenone, Canthaxanthin, Cryptoxanthin, Adonirubin, Astaxanthin and Astacen. Meanwhile, the separation by Column Chromatography method showed the results of pigments such as β-Carotene, Canthaxanthin, Adonirubin, and Fucoxanthin. The concentrations of pigments contained in the carapace, epidermal layer, hepatopancreas, gonad and blood were respectively 6.88 mg/g, 210.47 mg/g, 454.0 mg/g, 433.33 mg/g, and 153 mg/g. The pigment content of each organ of *G. albolineatus* crabs was 6.95 mg (the carapace), 7.26 mg (epidermal layer), 7.07 mg (hepatopancreas), 34.94 mg (gonad), and 5.86 mg (hemocyanin).

Key words *: Pigment, Carotenoid, Crustacea, TLC, CC*

Introduction

One of the organisms that become the potential resource of North Sulawesi Province, Indonesia is crabs. The coastal morphology of North Sulawesi is dominated by mangrove coastal waters androcky coastal waterswhich are the habitats for crabs to survive. Bliss and Mantel (1985) found that *Scylla serrata* crabs survive by adapting to mangrove areas. Moreover, according to Majchacheep (1989), there are many types of *Grapsus albolineatus* found in rocky coastal waters. Both types of crabs contain carotenoid pigments in the external and internal organs. Carotenoid pigments in these two types of crabs can be used as pharmaceutical and cosmetic raw materials.

Corresponding author's email: darusparansa@unsrat.ac.id

Based on the results of studies by Paransa *et al.* (2000a) and Paransa *et al.* (2000b), in female and male *S. serrata* crab extracts, there were found same types of pigments, namely β-carotene, zeaxanthin ester, astaxanthin ester, and free phoenicoxanthin and astaxanthin. Both female and male *S. serrata* crabs were obtained in the Coastal Waters of Likupang Village, North Minahasa Regency, North Sulawesi Province, Indonesia. Paransa *et al.* (2002) also discovered that in the total extract of *G. albolineatus* crabs captured in the coastal waters of Kalasey Beach, Minahasa Regency, North Sulawesi, Indonesia which is dominantly rocky, there were found some types of pigments, namely β-carotene, echinenone, canthaxanthin in the internal organs (hemocyanin, hepatopancreas, and epidermal layer) and some other types of pigments, namely free phoenicoxanthin and astaxanthin, were found in the external organ of carapace. Furthermore, according to Paransa (2005) and Paransa *et al.* (2008), the type of major carotenoid pigment, namely astaxanthin was found separately in the extracted carapace, epidermal layer, hepatopancreas, hemocyanin, and gonad of male *G. albolineatus* crabs captured in the Coastal Waters of Malalayang Beach, Manado, North Sulawesi, Indonesia. The analysis of the carotenoid pigments of *Scylla seratta* and *G. albolineatus* crabs was done using Thin Layer Chromatography (TLC) analysis method with semipolar developer solutions.

Mantiri and Kepel (1999) stated that the carotenoid pigments as an ingredient for natural dyes can be used as immune substances and raw materials for pharmaceutical, cosmetic, and foodstuff industries. Additionally, the results of Naguib's (2000) study suggest that astaxanthin pigments have an antioxidant activity that is ten times stronger than â-carotene, canthaxanthin, lutein and zeaxanthin pigments. Based on the results of several previous studies above, the carapaces of stone crabs (*G. albolineatus*) contain astaxanthin pigments which can be used as a source of natural colouring agents. Utilization of natural dyes from the extraction of coastal resources of North Sulawesi, Indonesia can support the industrial sector. This research was enhanced by utilizing purer materials. Thus, the analysis of carotenoid pigments was necessarily conducted through the chromatography column stage.

Research Method

The crab (*G. albolineatus*) samples were taken at night during the high tide of the coastal waters ofTanawangko Village, Minahasa Regency, North Sulawesi, Indonesia. The organism species of the samples were then identified and then continued with the molting stage determination. The next stage was taking the internal and external organs, including Carapace (C), Epidermal Layer (EL), Hepatopancreas (H), Gonad (G), and Hemocyanin (D) to be extracted through the grinding process in acetone and petroleum ether solutions. The concentration and quantity of carotenoid pigments in the total pigment extract were analyzed according to Britton *et al.* (1995).

The types of pigments in Carapace (C), Epidermal Layer (EL), Hepatopancreas (H), Gonad (G), and Hemocyanin (D) were determined using Thin Layer Chromatography (TLC) and maximum UV-Vis spectrophotometric absorption analysis methods with a length wave ranging from 380 – 550 nm (Britton *et al.*, 2004). The TLC analysis was done using a semi-polar developer according to Mantiri (1997). The content of the total pigment extract of the carapace organ was analyzed using the separation of Chromatography Column (CC) and the maximum UV-Vis spectrophotometric absorption analysis methodaccording to Britton *et al.* (1995). The CC analysis was undertaken using the dry method principle using a semipolar developer and silica gel powder as a stationary phase (Mantiri, 1997).

The separation of carotenoids using HPLC was achieved in a mixture of methanol: acetone: water gradient (80: 5: 15) and 0.8 mL minute water rate. After 10 minutes, the water content is reduced linearly then the acetone increased into 20% at 50 minutes after injection. The composition of the gradient mixture is methanol: acetone (80:20).

Results and Discussion

Sample Identification

The sample identification was aimed to determine the species of the samples obtained from the sampling location. The carapace morphological form of the stone crabs taken from the location had blackish green stripe patterns and the anterior part between the eyes was divided into four blackish green lobes. The first pair of walking legs was the shortest with a line of setae that appeared on the ventral boundary of the walking leg which amounted to eight legs

(four pairs) with no pair of swimming legs. This type of crabs was characterized by a pair of purple claws (as seen in Figure 1). Based on Carpenter (19[illegible]), crabs living on the rocky shoreare identified as *Grapsus albolineatus* species (Latreille in Milbe[illegible] 1812) and mostly male. The uropod part of *G. albolineatus* crabs (Latreille in Milbert, 1812) used in this research was observed under a microscope with 10x magnification, and based on Skinner (1962), this belongs to the D moulting stage or also known to be at the premolt stage. In the premolt stage, a new rigid exoskeleton would be formed.

Analysis of Carotenoid Pigment Type and Content

The concentrations of pigments contained in the carapace, epidermal layer, hepatopancreas, gonad and blood were respectively 6.88 mg/g, 210.47 mg/g, 454.0 mg/g, 433.33 mg/g, and 153 mg/g. The pigment content of each organ of *G. Albolineatus* crab was 6.95 mg (carapace), 7.26 mg(epidermal layer), 7.07 mg (hepatopancreas), 34.94 mg (gonad), and 5.86 mg (blood). Types of pigments contained in each organ of *G. albolineatus* crabs (Latreille in Milbert, 1812) can be seen in the table below.

The distribution of pigment types in each organ of *G. albolineatus* crabs (Latreille in Milbert, 1812) presented in the table above shows that the first fraction produced yellow color identified as a β-carotene pigment, contained in the gonad, hemocyanin, and hepatopancreas organs. The second fraction appeared to be orange-colored identified as echinenone pigments, contained in the hepatopancreas, epidermal layer, and carapace organs. Meanwhile, in the third fraction,

Fig. 1. Male Stone Crab or *Grapsus albolineatus* (Latreille in Milbert, 1812) Obtained from the Coastline of Tanawangko Village, Minahasa Regency, North Sulawesi when at the D Molting Stage

Table 1. Results of Maximum UV-Vis Spectrophometric Absorptionand TLC Separation Analysis Using PE and Acetone Developer Solutions (80:20) from Total Pigment Extract of *G. albolineatus* crabs (Latreille in Milbert, 1812)

F	Rf	Color - TLC	Wave Length (nm) - Maximum Spectrophotometer Absorption	Pigment Type	Description				
					G	D	H	EL	C
1	1.00	Yellow	427, 452, 478	β-Carotene	*	*	*		
		Reddish	-	-				*	*
2	0,91	Orange	458	Echinenone			*	*	*
3	0,82	Reddish	468	Canthaxanthin			*	*	*
4	0,64	Orange	425,450, 476	β- cryptosanthine	*	*	*	*	*
5	0,58	Yellow	462	Adonirubine	*	*	*	*	*
6	0,54	Reddish	470	Astaxanthin	*	*	*	*	*
7	0,48	Yellow	475	Astacen	*	*	*		
8	0,41	Yellow	427	-					
9	0,22	Reddish Yellow	452	-	*	*	*	*	*

Description
F = Fraction
Rf = *Retention factor*
G = Gonad
D = Hemocyanin
H = Hepatopancreas
EL = Epidermal Layer
C = Carapace

canthaxanthin pigments were identified with a reddish color, contained in the hepatopancreas, epidermal layer, and carapace organs. The fourth fraction showed orange color identified as cryptoxanthin pigments, found in the gonad, hemocyanin, hepatopancreas, epidermal layer, and carapace organs. The fifth fraction also produced yellow color identified as adonirubin pigments, contained in the gonad, hepatopancreas, epidermal layer, and carapace organs. In the sixth fraction, the pigments identified were astaxanthin with a reddish color, contained in the gonad, hemocyanin, hepatopancreas, epidermal layer, and carapace organs. The last or seventh fraction produced yellow color identified as astacin pigments, found in the gonad, hemocyanin and hepatopancreas organs.

According to Makalalag *et al.* (2017), female *G. albolineatus* crabs contain β-carotene, echinenone, canthaxanthin, adonirubin, astaxanthin, and astacin in which the quantity of the pigments is concentrated in the gonad and hemocyanin organs. Meanwhile, Paransa (2002) explained that in the total pigment extract of male *S. serrata* crabs, the carapace and epidermal layer contain some types of pigments covering β-carotene, and free zeaxanthin ester and astaxanthin, as well as have antibacterial activities. Moreover, Thamin *et al.* (2006) found that β-carotene, echinenone, astaxanthin diester, astaxanthin monoester and free astaxanthin were contained in the total pigment extract of carapace and epidermal layer organs of male *G. albolineatus* crabs obtained from the Coastal Waters of Malalayang Dua Beach, Manado, North Sulawesi, Indonesia. His study also shows that astaxanthin and astaxanthin diester pigments of the extracted carapace organ have antibacterial activities from *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella paratyphi B.* On the other side, the results of the study by Paransa *et al.* (2014) suggest that β-carotene and Lutein pigments of the extracted *Sargassum polycystum* brown algae (C. Agardh) have antibacterial activity from *Escherichia coli, Klebsiella pneumonia, Salmonellaparatiphy b* and *Staphylococcus aureus*.

The pigment metabolic flow of *G. albolineatus* crabs (Latreille in Milbert, 1812) is formed through two metabolic pathways. The first pathway is the

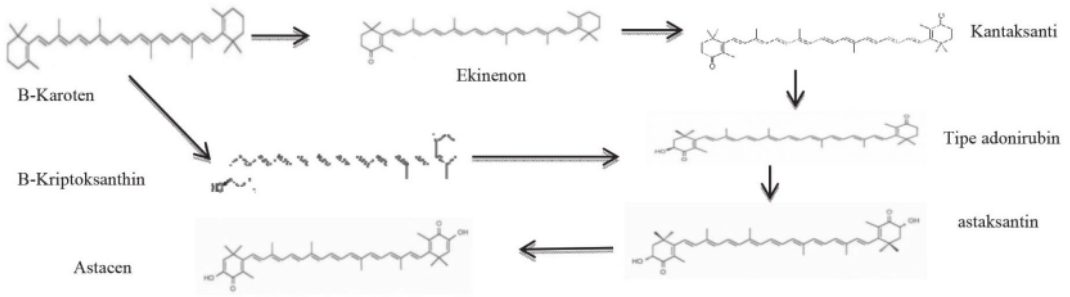

Fig. 2. Forecast of Pigment Metabolic Cycles of *G. albolineatus* Crabs (Lamarck, 1888)

Table 2. Results of Maximum UV-Vis Spectrophometric Absorption and CC Separation Analysis with Hexane and Acetone Developer Solutions from Total Pigment Extracts of *G. albolineatus* Crab (Latreille in Milbert, 1812)

Stage I-CC Separation			Stage II-CC Separation		
BAND	WAVELENGTH (nm)	PIGMENT TYPE	BAND (nm)	WAVE LENGTH	PIGMENT TYPE
JKP1	424, 448 485	β- Carotene	-	Not Continued	-
JKP2	465	Canthaxanthin	JKP2P1	460	Echinenone
			JKP2P2	482	Astasen
JKP3	475	Adonirubine	JKP3P1	424,449, 479	Meso-zeaxanthin
			JKP3P2	423,448, 476	Diatoksanthin
			JKP3P3	-	-
JKP4	459, 478	Fucoxanthin	JKP4P1	425,449,476	β- cryptoxanthin
			JKP4P2	468	Astaxanthin

addition of carbonyl groups on one side of β-carotene pigments to form echinenone pigments. Moreover, the echinenone pigments receive carbonyl groups in other cyclic chains to form canthaxanthin pigments, to subsequently create astaxanthin and astacin pigments. In the next metabolic pathway, β-carotene pigments receive hydroxyl groups to form β-cryptoxanthin pigments and subsequently adinorubin pigments. Furthermore, major pigments, generally consisting of astaxanthin and astacin pigments, are formed in crustaceans (Mantiri *et al*., 1999).

Based on the results of the study by Mantiri *et al*. (1999), there is a pigment metabolic pathway formed in *Panulirus versicolor* lobster (Latreille, 1804), starting from β-carotene pigments which subsequently form echinenone, canthaxanthin and astaxanthin pigments in a series. The pigment metabolism occurs in the hemocyanin, hepatopancreas, epidermal layer, carapace and gonad organs.

Determination of Pigment Types Using Column Chromatography

The total carapace pigment extract of *G. albolineatus* crabs (Latreille in Milbert, 1812) captured in the research location was separated through the stage I-CC separation by using semipolar developers of hexane and acetone (80:20). The extract resulted from the CC separation was analyzed using UV-Vis spectrophotometer absorption. Then, the pigment types of the formed maximum UV-V is spectrophotometric absorption peak was identified according to Britton *et al*. (2004). The results of the stage 1-CC separation formed four bands identified as β-carotene, canthaxanthin, adonirubin, and fucoxanthin pigments. The results of Column Chromatography analysis using semipolar developer solutions are shown in the table below.

The extract of canthaxanthin pigments contained echinenone and astacin pigments. Both pigment types were found through further separation using the same semipolar developer solutions. Moreover, through the CC separation, adonirubin pigments were found in the third band of the total pigment extract of carapaces of *G. albolineatus* crabs (Latreille in Milbert, 1812). The extract of adonirubin pigments was further separated using CC, resulting in meso-zeaxanthin and diatoxanthin pigments. Meanwhile, from the further CC separation of the fucoxanthin pigment extract, two bands were found, each of which was identified using maximum UV-Vis spectrophotometric absorption analysis method. The results showed that the two bands represented β-Cryptoxanthin and Astaxanthin pigments. Overall, the total carapace pigment extract resulted from the Stage II-CC separation showed the same pigment types with that resulted from TLC separation, namely echinenone, β-cryptoxanthin, astaxanthin and astacin. In conclusion, the pigments obtained from crustaceans which are utilized as natural dyes are generally taken from the carapace organ. The results of the separation of KCKT in the isolation extract of second fraction from TLC (Figure 3) are identified as canthaxanthin and Meso-zeaxanthin.

Carotenoids have a good effect on human health, serving as a precursor to vitamin A, antioxidants, and anticancer (Jaswir *et al.*, 2011). In humans, carotenoids can function as antioxidants (Yeum *et al.*, 2011). Carotenoids are known to work as well as tocopherols (vitamin E) which can stabilize reactive oxygen when photooxidative stress occurs (Stahl *et al.*, 2000). The use of carotene compounds by humans increases from year to year because carotenoids positively affect human health, such as re-

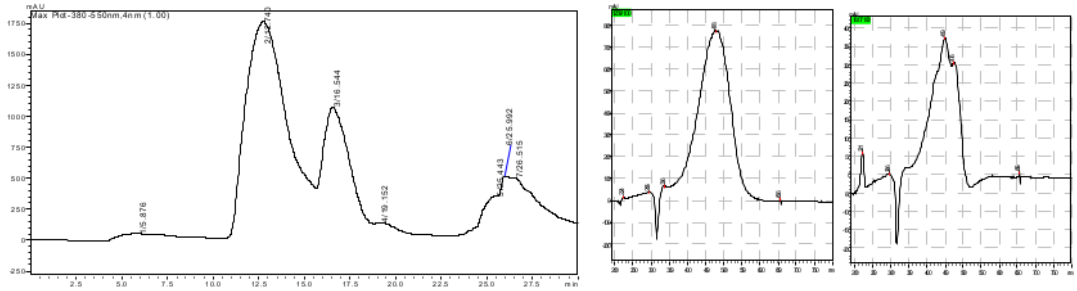

Fig. 3. HPLC chromatogram profile from KLT extract 2nd fraction of Grapsus albolineatus crab (Latreille in Milbert, 1812) with methanol, acetone and water solvent. The second and third peaks were identified as canthaxanthin and Meso-zeaxanthin pigments

ducing cancer risk and improving immune system function due to the role of antioxidants (Das *et al.*, 2005).

Conclusion

Grapsus albolineatus is one of crab in the rocky coastline of Tanawangko and Manggatasik Village of Manado city, North Sulawesi. Grapsusalbolineatus have high content of carotenoid pigment. Each organ of *Grapsus albolineatus* have different contentration of carotenoid. The concentration of pigment contained in carapace was 6.88 mg/g, epidermal layer 210.47 mg/g, hepatopancreas 454.0 mg/g, gonad 433.33 mg/g, and blood 153 mg/g. The pigment content of each organ *G. albolineatus*, ie at 6.95 mg carapace, epidermal layer 7.26 mg, hepatopancreas 7.07 mg, gonad 34.94 mg, and blood 5.86 mg. Carotenoid pigment of *Grapsusalbolineatus* are consist of β-Carotene, Echinenone, Cantaxanthin, Cryptoxanthin, Adonirubin, Astaxanthin and Astacene. Carotenoid is a color pigment that have some benefit as a raw material of industry, pharmacy, or food additive.

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