Characterization of Rhynchophorus, sp. Exoskeleton

Misran Lawani [1, 2)], Jantje Pelealu [3)], Redsway T. D. Maramis [3)], and Lucia C. Mandey [3)]
[2)] The Faculty of Agriculture, Khairun University, Ternate, North Maluku, Indonesia
[3)] The Faculty of Agriculture, Sam Ratulangi University, Manado, North Sulawesi, Indonesia

***Abstract*——** **Exoskeleton was one of biological composite materials that have not been widely studied. The study was conducted to find out the chemical characteristics and the microstructure of *Rhynchophorus*, sp. exoskeleton. The results of the study showed that the exoskeleton (i.e., exoskeleton powder of dorsal or tergum parts, which were pronotum and elytra, ventral or sternum parts, horns, snouts and legs) of the *Rhynchophorus*, sp. contained 27.063% water, 0.990% ash, 44.577% protein, 3.940% fat, and 23.430% carbohydrate. There were eight elements of the exoskeleton of the beetle, which were three main elements in huge quantity, including Carbon (C) 42.307%, Nitrogen (N) 26.147%, and Oxygen (O) 29.730%, and five other elements in small quantity, including Phosphorus (P) 0.227%, Sulphur (S) 0.243%, Potasium (K) 3.312%, Copper (Cu) 0.413%, and Zirconium (Zr) 0.637%. The cross-section view of the exoskeleton (elytra) of the *Rhynchophorus*, sp. showed that there were four laminar areas, including epicuticle, exocuticle, mesocuticle, and endocuticle.**

***Index Term*——** **Characterization, exoskeleton, cuticle, Rhynchophorus, sp., elytra**

INTRODUCTION

The majority of natural and biological exoskeleton materials was complex. The complex structure that has been constantly increasing through hundreds year evolution, inspired scientists in designing new material [1]. Cuticle of insects was a very good and multifunctional composite material. It was strong and light and many scientists of various disciplines have long been being interested in the material [2, 3].

The cuticle of insects was biological composite material abundantly found on earth and it has not been widely studied. The material of arthropoda exoskeleton had in general various properties though it usually consisted of limited organic materials, especially biomacromolecular chitin, protein, catechol, minerals, fat and water. It is light but it had strong structure that enabled the insects to fly, to jump, to attach musculature, to serve as guard and waterproof [4].

The exoskeleton of the insects or the cuticle represented covering guard of their entire body and secure structure for foregut, hindgut and tracheae attachment. It represented 25% of dry weight of their entire body. It was complex extracellular biocomposite secreted by epidermis and consisted of some functional layers, including waterproofing envelope, high protein content epicuticle and high chitin content procuticle [5, 3].

The cuticle was often considered as arthropoda skin with many functions. It supported and protected internal organs against chemical and mechanical damage, while at the same time functioned as skeleton, precisely exoskeleton on which moving musculature attached [6]. Not only it supported insects, but also it gave them form, functioned as moving parts, was waterproof and provided the insects with various mechanical tricks [3]. Its surface was highly hydrophobic and hence prevented water and other external bodies from entering into the body of the insects [7].

There were many arthropoda exoskeletons indicative of extraordinary material properties with multifunctional capabilities. Such multifunctions resulted from the fact that the cuticle must serve all of the functions of skin and skeleton. Since almost all of adult insects could fly, the cuticle skeleton must be efficient and light. The exoskeleton consisted of chitin fiber with high rigidity and was bound by protein with other additional components. Its main material was chitin fiber associated with protein and provided with lipid, pigment, inorganic material, polyphenol, and water [8, 3, 7]. Arthropoda used the chitin along with the protein as essential elements of the cuticle to form exoskeleton [9].

A widely accepted theory suggested that the cuticle of insects was a compound of chitin fiber and protein matrix in a laminar structure [3]. The chitin represented general constituent of the exoskeleton of the insects [10]. It was also the case of elytra, wings and Coleoptera covering membranous back wings, folded under front wings during rest time. Elytra were “hard” and highly sclerite [2].

The cuticle consisted of three layers, which were covering layer, epicuticle and procuticle [11]. The structure of the cuticle of insects has been analyzed. Study results showed that the surface of the cuticle of dung beetles had laminar composite structure [3].

Usually, the exoskeleton of beetles consisted of four parts, including epicuticle, exocuticle, mesocuticle, and endocuticle. The most outer part was epicuticle and it was the thinnest part of 1 μm in thickness and consisted of lipid and protein. It served the function of difussion guard and was not load-bearing. Other parts of the exoskeleton were exocuticle, mesocuticle, and endocutile that had load-bearing capacity. The complex hiearchy of the microstructure of the exoskeleton was one of the important factors in finding out its characteristics [8].

The knowledge of the characteristics of the exoskeleton could be useful as the basis in selecting and making use of materials, as consideration in engineering tools, processing and storing techniques, and in developing products for diversification and creation of various new products. Based on its characteristics, the exoskeleton of beetles had a potential as both macro- and micromolecular compound sources and could be used as raw materials in producing chitin and chitosan. The study was conducted to find out the chemical characteristics and the microstructure of the exoskeleton of *Rhynchophorus*, sp. The taxonomic position of the beetle was Class: insecta, Order: Coleopthera, Family: *Curculionidae* and Subfamily: *Rhynchophorinae* [12, 13].

MATERIALS AND METHOD

Materials

The primary material used in the study was the exoskeleton of adult *Rhynchophorus*, sp. The beetles were collected from decaying sago logs by directly catching them or using hand picking method in sago plantation and processing area in Poso district of Central Sulawesi province (N: 1º39'17.532", E: 120º40'59.7216") and then kept in a container. The parts of the exoskeleton used in the study included dorsal (tergum) pronotum and elytra, ventral (sternum), horns, snouts and legs.

Other materials included plastics, scissors, air tight containers, filtering paper, mineral-free water, and chemicals (*GR for analysis*, *Merck* KGaA, 64271 Darmstadt, Germany) such as potassium sulphate (K_2SO_4), coppersulphate ($CuSO_4$), sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), hydrochloric acid (HCl), boric acid (H_3BO_3), methyl red ($C_{15}H_{15}N_3O_2$), ethanol (C_2H_6O), bromocresol green ($C_{12}H_{14}Br_4O_5S$), and petroleum ether (C_7H_7BrMg).

Proximate Analysis

Sample Preparation

The collected beetles *Rhynchophorus,* sp. were left in hunger for 48 hours in a container to reduce the content of digestive tract. Subsequently, they were euthanized by separating their heads from their abdomen with scissors. The internal parts of the exoskeletons were removed. The parts of the exoskeletons were collected and crushed using blender into fine powder.

Sample Analysis

Proximate analysis of the exoskeleton of the *Rhynchophorus*, sp. was made to water content, ash, and fat using "gravimetric" method, to protein content using "Kjeldah"; carbohydrate content using "by different" method and Official Methods of Analysis of AOAC International [14].

Electron Scanning Microscope and Energy Dispersive X-Ray Spectrometer (SEM+EDS)

Sample Preparation: Analysis of Microstructures and Elements

The collected beetles *Rhynchophorus*, sp. were left in hunger for 48 hours to reduce the content of digestive tract, put in an air tight container that was packaged in plastic bag, euthanized by freezing them in a freezer, liquefied in ambient temperature and dried at 50ºC for 48 hours [15]. The internal parts of the exoskeleton were then removed. They were collected and crushed using blender into fine powder and finally sieved using sieve of 25 mesh.

Sample Preparation: Cross-Section Analysis

The collected beetles *Rhynchophorus*, sp. were put into an air-tight container and left to die. Subsequently, they were dried at 50ºC for 48 hours. The elytra of the beetles were separated from the bodies of the beetles. Samples were cut in transverse manner into pieces of the dimension of 5 mm length, 5 mm width, and 1-10 mm thickness.

Sample Analysis

Sample was analyzed using SEM + EDS of the type of JSM-6510LA, JEOL, Tokyo, Japan. Acquisition parameters included: *Instrument*, 6510 (LA); *acc. Voltage*, 20.kv; *probe current*, 1.0000 nA; PHA mode,: T3; *real time*, 51.34 - 51.63 sec; *live time*, 50.00 sec, *dead time*, 2-3%; *counting rate*, 3061 - 3471 cps; and *energy range of* 0-20 keV. It was mixed on adhesive tape and then encapsulated using platinum coating with sputter coater [15]. Scanning was conducted on three surface areas at different magnifications to obtain clear image.

Data Analysis

The preparation and the determination of the sample were repeated three times. The resulting data of the analysis were reported in a table as mean ± standard deviation. Analysis of the data was made using software IBM SPSS Statistics version 24.

RESULTS AND DISCUSSION

Proximate Analysis

The results of the proximate analysis of the exoskeleton (i.e., the powder of the dorsal and tergum part of the exoskeleton, which were pronotum and elytra, ventral and sternum parts, horns, snouts and legs) of the *Rhynchophorus*, sp. were summarized in Table I.

Table I
The means of the contents of proximate exoskeleton

Component	Mean ± Sd.
Water (%)	27.063 ± 0.380
Ash (%)	0.990 ± 0.066
Protein (%)	44.577 ± 1.396
Fat (%)	3.940 ± 0.076
Carbohidtrate (%)	23.430 ± 1.841

*) *The mean resulting from three times determination ± standard deviation (Sd.)*

The results of the proximate analysis showed that the exoskeleton of the *Rhynchophorus,* sp. contained biomacromolecular compound such as water, ash, protein, fat and carbohydrate with different chemical composition. The biggest quantity of components took place to protein (43.64-45.05%) followed by water (27.06-31.84%), carbohydrate (22.99-24.43%), fat (0.50-3.94%), and ash (0.84-0.99%).

Organic matrix of honey bees consisted of 23%-32% chitin, 35%-45% protein, 30-40% melanine, and 3% mineral compound. Organic matrix of silk worm consisted of about 20% chitin and other components such as protein, minerals, and fat. The exoskeleton of cockroaches contained about 30%-37% chitin in dorsal abdomen, ventral abdomen, metathoracic legs, mesothoracic part, prothoracic part, pronotum, heads, about 29%-25% chitin in genitalia, dorsal thorax, ventral thorax, antenna, cerci, and about 19% chitin in forewings and hindwings [16].

The materials of the exoskeleton of the arthropoda had in general various characteristics though they consisted of limited organic materials, especially biomacromolecular chitin and protein, cathechol, minerals, fat, and water. The composition and the structure of the organic matrix of insects are different [4, 16].

Electron Scanning Microscope and Energy Dispersive X-Ray Spectrometer (SEM + EDS)

Microstructures and Elements

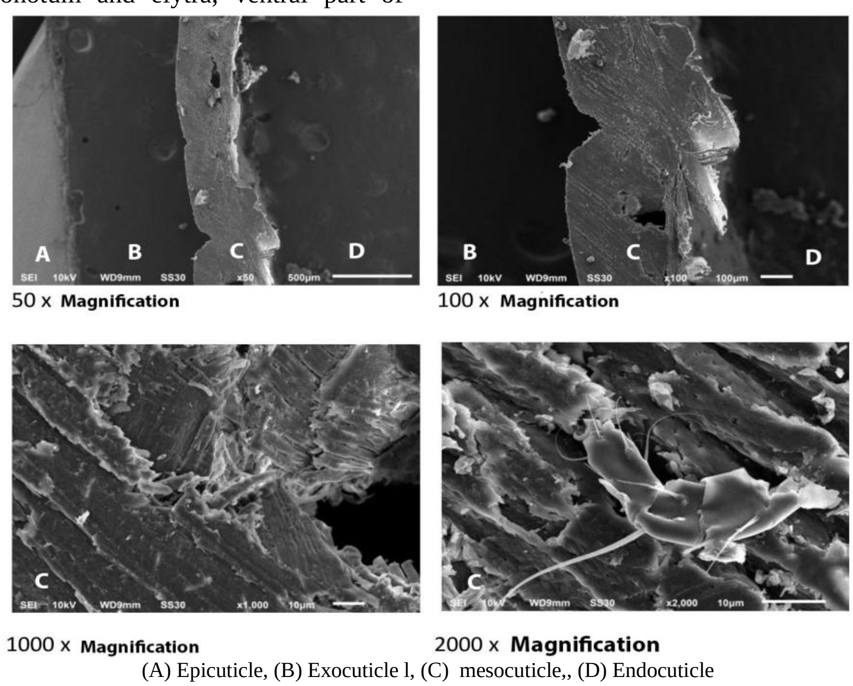
The results of SEM + EDS of the three surface areas of the exoskeleton (i.e., exoskeleton powder of dorsal part or targum, which were pronotum and elytra, ventral part of sternum, horns, snouts and legs) of the *Rhynchophorus,* sp. indicated the microstructure of the surface and the elements that formed the exoskeleton as illustrated in Figure 1. The recapitulation of the results of the SEM + EDS of the three surface areas of the exoskeleton of the *Rhynchophorus,* sp. was summarized in Table 2.

It was clearly observed in Table 2 that there were eight elements identified in the exoskeleton of the *Rhynchophorus,* sp., including three primary elements in huge quantity (mass, %), which were 42.307% carbon (C), 26.147% nitrogen (N), and 29.730% oxygen (O), and five remaining elements, which were 0.227% phosphorus (P), 0.243% sulphur (S), 3.312% potassium (K), 0.413% copper (Cu), and 0.637% zirconium (Zr). The exoskeleton (elytra) of *Coleopteron Semicrematus*, sp. (burnt beetle) was found to contain calcium (Ca) and silicon (Si). sulpfur (S) compound, manganese (Mn) and phosphorus (P) in small quantity (trace) [17].

The mass (%) as indicated in Table 2 was the mass of an object that was not influenced by its location. The weight of an object changed depending on its location on earth and drastically changed when it was moved from earth to moon. Though weighing was commonly used for one to find out the weight of an object, the weighing was essentially measuring its mass [18, 19].

The Cross-Section of Elytra

The SEM of the photograph of the cross section of the exoskeleton (elytra) of the *Rhynchophorus,* sp. with different magnifications was illustrated in Figure 2.

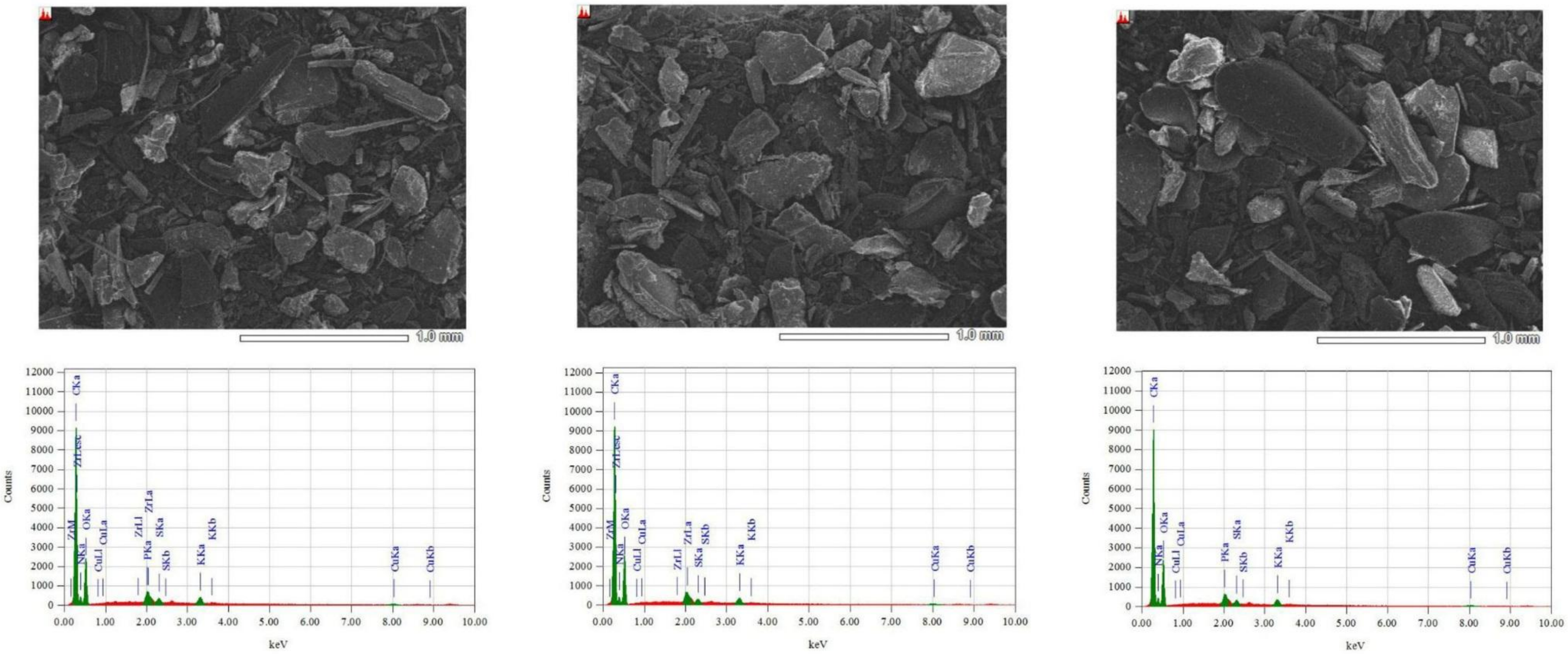


(A) Epicuticle, (B) Exocuticle l, (C) mesocuticle,, (D) Endocuticle

Fig. 2. The SEM of the micrograph of the cross section of the elytra of the *Rhynchophorus,* sp.

It was clearly observed in Figure 2 that the longitudinal section of the exoskeleton (elytra) of the *Rhynchophorus,* sp. had laminar structure consisting of epicuticle, exocuticle, mesocuticle, and endocuticle. Typical exoskeleton (integument) had laminar structure [6]. The study of the exoskeleton (elytra) of *Popilia japonica* showed that there were similar morphologies in all of the locations of the study, including different laminar areas of epicuticle, exocuticle, mesocuticle, and endocuticle [8].

The exoskeleton of beetles ussually consisted of four parts, including epicuticle, exocuticle, mesocuticle, and endocuticle. The most outer part was epicuticle and the thinnest one (ussually less than 1 μm) and consisted of lipid and proteint. It served the function of hindering difussion and was not load-bearing. Other parts of the exoskeleton (i.e., exocuticle, mesocuticle, and endocuticle) had load-bearing capacity. The primary material was chitin fiber and associated with protein along with lipid, pigment, inorganic material, and water [8, 20].



ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.0670

Element	(keV)	Mass%	Sigma	Atom%	Compound	Mass%	Cation	
C K	0.277	42.47	1.86	48.63				45.90
N K	0.392	25.81	8.12	25.34				29.88
O K	0.525	29.62	8.03	25.46				20.32
P K	2.013	0.30	0.29	0.13				0.64
S K	2.307	0.26	0.26	0.11				0.57
K K	3.312	0.32	0.30	0.11				0.62
Cu K	8.040	0.41	0.87	0.09				0.67
Zr L	2.042	0.80	0.59	0.12				1.37
Total		100.00		100.00				

ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.0721

Element	(keV)	Mass%	Sigma	Atom%	Compound	Mass%	Cation	
C K	0.277	42.46	0.09	48.66				46.21
N K	0.392	25.61	0.27	25.17				29.63
O K	0.525	29.89	0.22	25.72				20.53
S K	2.307	0.22	0.01	0.09				0.47
K K	3.312	0.27	0.01	0.10				0.52
Cu K	8.040	0.43	0.02	0.09				0.70
Zr L	2.042	1.11	0.02	0.17				1.90
Total		100.00		100.00				

ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.0703

Element	(keV)	Mass%	Sigma	Atom%	Compound	Mass%	Cation	
C K	0.277	41.99	0.09	47.80				46.31
N K	0.392	27.02	0.28	26.37				31.46
O K	0.525	29.68	0.23	25.36				19.70
P K	2.013	0.38	0.01	0.17				0.80
S K	2.307	0.25	0.01	0.11				0.52
K K	3.312	0.29	0.01	0.10				0.54
Cu K	8.040	0.40	0.02	0.09				0.63
Total		100.00		100.00				

Fig. 1. The SEM + EDS of the exoskeleton of the *Rhynchophorus*, sp. in the three surface areas

Table II
The SEM + EDS of the exoskeleton of the *Rhynchophorus,* sp.

Elemen	(keV)	Massa (%)	Sigma	Atom (%)	Kation
		Mean ± Sd.*)	Mean ± Sd.*)	Mean ± Sd.*)	Mean ± Sd.*)
C	0.277	42.307 ± 0.274	0.680 ± 1.022	48.363 ± 0.488	46.140 ± 0.214
N	0.392	26.147 ± 0.763	2.890 ± 4.529	25.627 ± 0.649	30.323 ± 0.993
O	0.525	29.730 ± 0.142	2.827 ± 4.506	25.513 ± 0.186	20.187 ± 0.433
P	2.013	0.227 ± 0.200	0.100 ± 0.165	0.100 ± 0.089	0.480 ± 0.423
S	2.307	0.243 ± 0.021	0.093 ± 0.144	0.103 ± 0.012	0.520 ± 0.050
K	3.312	0.293 ± 0.025	0.107 ± 0.167	0.103 ± 0.006	0.560 ± 0.053
Cu	8.040	0.413 ± 0.015	0.303 ± 0.491	0.090 ± 0.000	0.667 ± 0.035
Zr	2.042	0.637 ± 0.573	0.203 ± 0.335	0.097 ± 0.087	1.090 ± 0.981

*) *The mean Resulting from three times determination ± standard deviation (Sd.)*

CONCLUSION

The characteristics of the exoskeleton of the *Rhynchophorus*, sp. were 27.063% water content, 0.990% ash content, 44.577% protein content, 3.940% fat content, and 23.430% carbohydrate content. There were eight elements found in the exoskeleton, including 42.307% Carbon (C), 26.147% Nitrogen (N), 29.730% Oxygen (O), 0.227% Phosphorus (P), 0.243% Sulphur (S), 3.312% Potasium (K), 0.413% Copper (Cu), and 0.637% Zirconium (Zr). The cross-section of the exoskeleton (elytra) of the beetle *Rhynchophorus*, sp. indicated that there were four laminar areas, including epicuticle, exocuticle, mesocuticle, and endocuticle.

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