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Review: Analysis and Benefit of Shells Content of Freshwater and Land Snails from Gastropods Class

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Abstract: Gastropods are the largest class of phylum mollusks. Members of this class include land snails, sea snails, freshwater snails, land snails, and limpet. In some countries, snails are often processed to be food. Along with the high consumption of snail meat, their shells' disposal rate, which can become waste on land and waters, is also high. This study aimed to determine the analysis method and benefits of the chemical compound content of several freshwater and land snail shells from the gastropod class. The method in this review was literary search from national and international journals. The result is known that the snail shell contains major chemical compounds, namely $CaCO_3$, chitin, and chitosan. In addition, there are other minor compounds, such as minerals zinc, iron, copper, phosphorus, manganese, sodium, potassium, and proximate data. These compounds can be used as biomaterials that are useful for the world of health. Instruments for analysis of chitin and chitosan compounds can use FTIR spectrophotometry and mineral compounds of $CaCO_3$ also; other minerals can use AAS and XRF.

Keywords: chitin; chitosan; calcium carbonate; benefits; analysis; heavy metal; analysis proximate.

1. Introduction

Gastropods are the largest class of *phylum Mollusca*. This class includes land snails, sea snails, freshwater snails, land snails, and limpets [1]. In some countries, freshwater snails and ground snails are often managed for their meat as food. In France, food processing from this snail is known as escargot. European states such as France, Portugal, Sardinia, and Spain provide this food as a menu "hors-d'oeuvre" or appetizer. Markets around the Cameroon area and restaurants in other parts of Nigeria and Africa are even experiencing increased demand for snail meat availability in their diet [2-4]. Furthermore, for the Asian region, there are China, Hong Kong, Japan, Thailand, Taiwan, and Indonesia, which often manage snail meat into food [5-8].

The snails' meat is high in protein and low in fat [3, 9-11]. Along with the high consumption of snail meat, the level of disposal of its shells which can become waste on land and waters, is also high [2,3], or some use it as animal feed and accessories (bracelets, necklaces, and wall ornaments or aquariums), but these efforts have not yet too high economic uses.

Based on several studies, it has been conducted to determine the potential value of the benefits of snail shell waste in the health world, including can be useful as hydroxyapatite [12,13], collagen [14], active medicinal substances such as antibacterial, antimicrobial,

antioxidant, pharmaceutical additives such as stabilizers, emulsifiers, thickening agents in the food industry [8,15-19], absorbers of compounds metals [20,21], and water bioindicators of polluting iron compounds [22]. This is because the snail shell contains active chemical compounds such as chitin $(C_8H_{13}NO_5)n$, which is the main organic material for the manufacture of chitosan $(C_6H_{11}NO_4)n$ [23,24], calcium carbonate ($CaCO_3$). The shell's main constituent ingredients [25,26], other mineral content, and the composition of proximate compounds [27,28]. These compounds can be used as biomaterials that are useful for the world of health. Biomaterials themselves are substances derived from natural or synthetic sources used as medical devices and can interact with biological systems [29].

The content of active chemical compounds snail shells that can be found in nature varies depending on the source, such as the influence of the living place's mineral content, protein, and microorganisms. Seeing the great potential of the use of snail shell waste, writing a review of this journal aims to provide some information regarding the active chemical compound content of several gastropod-class freshwater snail shells that can be used as a reference basis to be made into biomaterials for raw materials for pharmaceutical drug preparations.

2. Materials and Method

This review's writing is compiled based on studies related to the analysis of active chemical compounds and the benefits contained in freshwater snail shells and gastropod class soil. The materials used are primary data, namely international and national journals, and secondary data sources taken from research reports and scientific articles. The number of literature reviewed is 55 journals. The search references in review journals presented here are taken from Science Direct, Google Scholar, Elsevier, Pubmed PMC, BioOne, and other journal websites. The research was conducted in Indonesian and English. The keywords used in the literature search included concentration chitin or chitosan of gastropods, the concentration of calcium carbonate of gastropods, shell freshwater snail, biomaterials, analysis, benefits, eaten snails.

3. Results and Discussion

3.1. Analysis method.

A series of analysis processes can determine the content of active chemical compounds found in snail shells. The components of the proximate composition, mineral, chitin and chitosan compounds in the snail shells have different analysis processes, from treatment to analysis using instruments. In addition, chitin and chitosan compounds based on research on gastropod class snail shells from different species have other differences: solvent concentration, type of solvent, temperature, and time. And for $CaCO_3$ compounds, there are differences in temperature and time during the calcination process. The analysis method and the chemical compound content obtained can be seen in detail in Tables 1 and 2.

Table 1. Identification of chemical compounds in snail shell of gastropods class.

Chemical Compounds	Species Name	Test Conditions				Identification Method (Instrument)	Ref.
		Treatment	Solvent	Temperature	Time		
Chitin	*Achatina achatina*	Deproteinized	1N NaOH	Boiled	10 minutes	FTIR	[30]
		Demineralised	6N HCl	20 °C	10 minutes		
		Decolorization	-	-	-		
		Deproteinized	1N NaOH	Boiled	10 hour	FTIR	[30]

Chemical Compounds	Species Name	Test Conditions				Identification Method (Instrument)	Ref.
		Treatment	Solvent	Temperature	Time		
	Archachatina marganita	Demineralised	6N HCl	20 ºC	10 minutes		
		Decolorization	-	-	-		
	Achatina fulica	Deproteinized	3.5% NaOH	65 ºC	2 hour	FTIR	[31]
		Demineralised	1N HCl	40 ºC	30 minutes		
		Decolorization	0.315% NaOCl	40 ºC	1 hour		
	Nerita crepidularia	Deproteinized	1N NaOH	80 ºC	24 hour	FTIR	[14]
		Demineralised	2N HCl	25-30 ºC	24 hour		
		Decolorization	-	-	-		
	Pila ampullacea	Deproteinized	3.5% NaOH	60-70 ºC	4 hour	FTIR	[32]
		Demineralised	1.5M HCl	60-70 ºC	4 hour		
		Decolorization	-	-	-		
	Pomacea canaliculata	Deproteinized	2M NaOH	25-30 ºC	2 hour	FTIR and XRD	[33]
		Demineralised	2M HCl	25-30 ºC	2 hour		
		Decolorization	-	-	-		
Chitosan	*Achatina achatina*	Deacetylation	50% NaOH	100 ºC	4 hour	FTIR	[30]
	Archachatina marginata	Deacetylation	50% NaOH	100 ºC	4 hour	FTIR	[30]
	Achatina fulica	Deacetylation	60% NaOH	110 ºC	1 hours	FTIR	[31]
	Nerita crepidularia	Deacetylation	40% NaOH	110 ºC	6 hours	FTIR	[14]
	Pila ampullacea	Deacetylation	50% NaOH	100-110 ºC	4 hours	FTIR	[32]
	Pomacea canaliculata	Deacetylation	50% NaOH	140 ºC	2 hours	FTIR and XRD	[33]

Table 2. Identification of mineral content in snail shell of gastropods class.

Chemical Content	Species Name	Test Conditions			Method of Analysis (Instrument)	Ref.
		Treatments	Temperature	Time		
$CaCO_3$	*Achatina achatina*	Calcination	400-600 ºC	4 hours	FTIR and XRD	[34]
	Archachatina marginata	Calcination	320-670 ºC	3 hours	EDX and XRF	[35]
	Achatina fulica	Calcination	50 ºC	48 hours	AAS	[36]
	Lanistes varicus	Calcination	400-600 ºC	4 hours	FTIR and XRD	[34]
	Bellamya Javanica	Calcination	1000 ºC	2 hours	AAS	[37]
	Pila ampullicea	Calcination	470 ºC	2 hours	XRF	[38]
	Pomacea canaliculata	Calcination	900 ºC	3 hours	AAS and UV-VIS	[39]
Other Minerals	*Achatina achatina*	-	-	-	AAS	[27-28, 40]
	Archachatina marginata					
	Achatina fulica					
	Limicolaria sp					

* AAS (Atomic Absorption Spectrophotometer); EDX (Energy Dispersive X-Ray Spectroscopy); FTIR (Fourier Transform Infrared); XRD (X-Ray Diffraction); XRF (X-Ray Fluorescence); UV-VIS (UV Visible)

3.1.1. Chitin and chitosan compounds.

Analysis of chitin and chitosan compounds needs to be treated first, namely the separation of other compounds, such as protein and mineral content, and acetyl groups' removal to transforming chitin compounds into chitosan. Two ways can be used, namely chemically or enzymes. These methods involve deproteinized, demineralized, decolorization, and deacetylation processes that differ if the chemical method uses chemical solvents and the enzyme method, using microbes [41,42].

The method for treating chitin and chitosan compounds used is the chemical method. At the stage of the process of several gastropod class snail shells showed the difference in solvent concentration, temperature, and time used by each researcher. The process of deproteinization, demineralization, decolorization, and deacetylation can be done by stirring, heating at a high temperature >100 ºC, and soaking alone without stirring. Research conducted by Kaewboonruang *et al.* [33] showed that the stirring process had better and optimum results compared to high-temperature heating and soaking without stirring. The same result was shown in a study by Maya *et al.* [31] that the greatest yield chitin and chitosan production of *Achatina fulica* shells used reflux plus water bath and magnetic stirrer.

The deproteinization process aims to remove protein, and demineralization removes the main minerals $CaCO_3$ and $Ca_3(PO_4)_2$ (small amounts) contained in the shells of snails [24]. According to Sugita *et al.* [41], the efficiency of deproteinization and demineralization depends not only on the concentration of base (deproteinized), acid (demineralized), and temperature but also on the species of chitin source. In addition, based on the study of Oyekunle *et al.* [43] Regarding the process during demineralization, it shows that the smaller particle size of the snail shell powder is 300-600 μm, making the process of decreasing the Ca^{2+} concentration faster in the first 5 minutes. The chemical deacetylation process can be done by using a strong base of NaOH or KOH. But the use of KOH can break the strong hydrogen bonds between chitin. Therefore in research, the use of NaOH solvent is more often used. Long deacetylation time with high temperature will cause a decrease in the yield and molecular weight of chitosan [41].

Instrument analysis for these two compounds can be seen in Table 1. Most of the instruments used are spectrophotometry Fourier Transform Infrared (FTIR). This method can be used simultaneously, reduces the risk of contamination due to a long work process, and minimizes the use of solvents in the analysis, and can be used for qualitative and quantitative analysis.

3.1.2. Mineral compounds.

The snail shell contains many mineral compounds, with the major compound being $CaCO_3$. The compounds' method treats $CaCO_3$ mainly through a calcination process at high temperatures ranging from 320-1200 ºC. Mineral $CaCO_3$ is present in three phases, namely aragonite, calcite, and vaterite. Each of these phases has different properties. At room temperature, calcite is a stable phase, while vaterite and aragonite are metastable phases that can be transformed into a stable phase (calcite). These materials can be made as biomaterials for medical applications [38]. Thermodynamically calcite is a polymorph of $CaCO_3$, the most stable at pressure and room temperature. Aragonite will change to calcite at a temperature of 380-470 °C [38]. It is known that the optimum temperature of the total $CaCO_3$ phase conversion of calcite is in the range 380-600 °C, but just like chitin and chitosan compounds, the efficiency of $CaCO_3$ is also influenced by the species [41].

Analysis of instruments for mineral compounds can be seen in Table 2. Most of the instruments used are FTIR, AAS (Atomic Absorption Spectrophotometer), and XRF (X-Ray Fluorescence). AAS is particularly appropriate for the analysis of mineral and metal compounds at low concentrations [44]. XRF is a method of analysis that does not destroy the sample [45]. These three instruments are effective for analyzing qualitative and quantitative data on mineral and metal compounds.

3.2. Chemical compounds' content and benefits.

3.2.1. Chitin and chitosan content.

Analysis of the content of chitin and chitosan compounds from several gastropod class snail shell species is shown in Table 3. Chitin is the second-largest polysaccharide in nature after cellulose. Chitin is a poly [2-acetamido-2-deoxy-β- (1,4)-D-glucopyranose] compound with the molecular formula $(C_8H_{13}NO_5)n$. Through a chemical process that involves deproteinization, demineralization, decolorization, and deacetylation of chitin compounds transformed into chitosan $(C_6H_{11}NO_4)$ n by removing acetyl groups [23,41].

Chitin and chitosan in the health sector are useful as antibacterial agents. The results of the chitosan test from the shell *Achatina fulica* show that at an optimum concentration of 500 ppm an inhibitory power occurs of 34.33 mm against *Staphylococcus aureus* [8] and test data with a concentration of 300-700 ppm for diabetic ulcer patients caused by bacteria *Staphylococcus aureus* also show a sensitive reaction [46]. Another study also showed that if the cotton cloth coated with chitosan acetate *Achatina fulica*, the longer the immersion, the greater the activity against bacteria *Staphylococcus aureus* [47]. Based on the research of Yuvaraj *et al.* [48], $CaCO_3$ from *Pomacea canaliculata* combined with chitosan from *Periplaneta americana* has antimicrobial benefits. The results showed that the combination of these two compounds could inhibit the growth of *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus licheniformis.* And as a single compound, these two compounds also have potential as antimicrobials.

In the industrial world, chitosan is useful as a bioabsorbent. The research by Poerwati *et al.* [49] states that chitosan from the shell *Achatina fulica* can remove the color from liquid waste from the dyeing of the textile industry with a percentage of 88.05% with stirring for 72 hours. Chitosan from the shell of *Achatina fulica* also has the ability to adsorb methylene blue dye by 85.05% with a contact time of 6 hours [18]. In addition to absorbing the chitosan color from this species, it is useful as a heavy metal absorber. At the size of 250 microns, chitosan can absorb heavy metals by 95.27%, and at the size of 355 microns, 96.18% with a mass of 9 grams of chitosan. The optimum adsorption capacity was obtained in chitosan measuring 250 microns [24]. As well as studies were done by Nitase *et al.* [50], chitosan *Pila ampullaceal* has an adsorption capacity of 0.355 mg g^{-1} with a contact time between adsorbent and adsorbate for 240 minutes.

The following data for chitin and chitosan are listed in percentage values shown in Table 3.

Table 3. Chitin and chitosan content in snail shells of gastropod class.

Species Name	Chitin (%)	Chitosan (%)
Land Snail		
Achatina achatina	Unknown	46.37[30]
Achatina fulica	13.42[31]	67.16[31]
Archachatina marginata	Unknown	35.85[30]
Freshwater Snail		
Nerita crepidularia	35.43[14]	44.29[14]
Pila ampullacea	46.41[32]	39.83[32]
Pomacea canaliculata	1.99[33]	42.56[33]

3.2.2. Mineral content.

Analysis of the mineral compound of $CaCO_3$ from several species of gastropod class snail shells is shown in Table 4 and other minerals Table 5. CaCO3 compound is commonly used in the pharmaceutical world as an antacid because of its ability to neutralize stomach acid. Research conducted by Siregar *et al.* [39] makes hydroxyapatite biomaterial [$Ca_{10}(PO_4)_6(OH)_2$] sourced from $CaCO_3$ from snail shell waste *Pomacea canaliculata.*

Hydroxyapatite itself is a biomaterial that is used for bone graft with bone implants which is useful for repairing damaged tissues and broken bones. Currently, two types of hydroxyapatite morphology have been found, namely hydroxyapatite dense and porous. Porous hydroxyapatite has advantages due to its good pore conditions for nutrient transport, tissue infiltration, and vascularization [39]. The analysis showed that the ratio of calcium and phosphorus for hydroxyapatite was dense and porous 1.677 and 1.673, respectively, so that the hydroxyapatite approached the standard hydroxyapatite. The compressive strength of hydroxyapatite dense and porous is 19.61 MPa and 9.807 MPa, respectively, so hydroxyapatite is porous more effective in nutrient infiltration to repair bone damage [39]. Not only as a bone graft, hydroxyapatite is also useful for the removal of Pb (II) [51].

Calcium carbonate can also be converted into calcium phosphate as research from Bonou *et al.* [52] using the shells of species, *Achatina achatina* calcium phosphate compounds can be useful as bone repair surgery. According to the results of other studies also stated that $CaCO_3$ from the species *Pomacea canaliculata* can be useful as an absorber of heavy metal cardium [53] and bichromate ion from the species *Pila ampullacea* [54].

Table 4. Calcium Carbonate Content in Snail Shells of Gastropod Class

Species Name	$CaCO_3$ (%)[a] (mg/g)[b]
Land Snail	
Achatina achatina	98.5[a] [34]
Achatina fulica	48.11-48.92[b] [36]
Archachatina marginata	81[a] [35]
Laniste varicus	98.75[a] [34]
Freshwater Snail	
Bellamya javanica	67.80[a] [37]
Pila ampullacea	93,438[a] [38]
Pomacea canaliculata	48.02[a] [39]

Table 5. Other Mineral Content in Snail Shell of Gastropods Class

Species Name	Zinc (mg/l)	Cooper (mg/l)	Manganese (mg/l)	Iron (mg/l)[a] (mg/100 g)[b]	Phospor (mg/100 g)	Sodium (mg/ 100 g)	Potassium (mg/100 g)	Ref.
Land Snail								
Achatina achatina	9.85	6.47	4.31	251.23[a]	-	-	-	[27]
Achatina fulica	8.02	5.51	16.98	37.04[a]	-	-	-	[27]
Archachatina marginata	2.50	5.33	6.71	57.45[a]	-	-	-	[27]
Limicolaria sp	6.30	4.46	1.99	208.58 [a]	-	-	-	[27]
Freshwater Snail								
Bellamya bengalensis	-	-	-	300.10[b]	1680.56	200.89	40.83	[40]
Melania tuberculata	-	-	-	280.38[b]	1440.98	230.08	50.72	[40]

Analysis of other mineral compositions in the shells of the snails showed that all species had different levels. The sample *Archatina archatina* has the highest amount of iron value, which is 251.23 mg/l, as shown in Table 5. Iron compounds are one of the most abundant metals on earth, which is ranked 9 and has benefits, such as iron (III) chloride as a coagulant

in wastewater treatment especially in metal removal [27,28]. Data regarding levels of mineral compounds from gastropod snail shells can be seen in Table 4 and Table 5 is listed in mg/l or mg/100g.

3.2.3. Proximate composition.

In the gastropod snail shell, the data on the levels of proximate compounds that are owned is insufficiently large or called minor content data. An analysis of the various proximate compositions of different snail species is shown in Table 4. NFE (Nitrogen Free Extract) is known as a dissolved carbohydrate. The analysis shows that *Archatina archatina* has the highest carbohydrate value, one of which is influenced by the size of the snail, the largest compared to other types listed in the table. The high content of NFE can be added to several food ingredients to increase the carbohydrate content [27,55].

The ash content aims to determine the carbon compounds and inorganic components in the form of salts and oxides in the shells of snails. Carbon can be useful as an absorber for gaseous precursors that produce odors, odors in wastewater, and canned garbage [27,55].

The fiber content of each species increases the snail shell's strength, which affects the hardness of the snail shell [27,28]. Besides that, the environment can be useful as a medium for absorbing waste [55]. Data regarding the proximate composition of gastropod sp snail shells, which can be seen in Table 6.

Table 6. Proximate Composition in Snail Shell of Gastropods Class

Species Name	Protein (%)	Fiber (%)	Fat (%)	Ash (%))	NFE (%)	Ref.
Land Snail						
Achatina achatina	0.12	4.06	0.79	2	93.04	[28]
Archachatina marginata	0.42	3.37	0.75	10	5.46	[28]
Achatina fulica	0.30	3.96	0.38	10	85.36	[28]
Limicolaria sp	0.23	4.14	0.48	13	82.15	[28]

4. Conclusions

The shells of freshwater and land snails from the gastropod class contain a wide variety of chemical compounds. Based on the results of several studies in snail shells, they contain major chemical compounds, that is, $CaCO_3$, chitin, and chitosan. Where the highest content of these compounds came from *Lanistes varicus* for $CaCO_3$ with concentration 98.75%, chitin content from *Pila amppulacea* was 46.41%, and from the deacetylation process of chitin, chitosan compounds were obtained, with the highest content come from the species *Achatina fulica* which was 67.16%. Also, there are other minor compounds, such as minerals zinc, iron, copper, phosphorus, manganese, sodium, potassium, and proximate data. These compounds can be used as biomaterials that are useful in the world of health. Instruments for the analysis of chitin and chitosan compounds can use FTIR spectrophotometry and mineral compounds $CaCO_3$ and other minerals. can use AAS and XRF

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Conflict Interest

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