The Potential of Donkey's Ear Abalone (*Haliotis asinina* Linnaeus, 1758) as an Antibacterial Agent and Its Effect on Blood Cholesterol Reduction in Mice (*Mus musculus* Linnaeus, 1758)

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ABSTRACT

Donkey's ear abalone (*Haliotis asinina* Linnaeus, 1758) is rich in essential nutrients and harbors bioactive compounds with antibacterial properties, potentially aiding in cholesterol reduction. This study focused on assessing antibacterial activity against *Staphylococcus aureus* (ATCC25923) and *Escherichia coli* (ATCC25922), determining the amino acid composition, screening for bioactive phytochemical compounds, and evaluating the effect of abalone meat extract on blood cholesterol in male house mice (*Mus musculus* Linnaeus, 1758) using an in vivo method. Results indicated significant antibacterial activity of *H. asinina* methanol extract (20% concentration) against *S. aureus*, while ethyl acetate extract (10% and 20% concentrations) showed inhibition zones against both *S. aureus* and *E. coli*. The n-hexane extract demonstrated antibacterial activity at a 10% concentration on both organisms, and at a 20% concentration against *S. aureus*. Phytochemical screening revealed the presence of saponins and alkaloids in the methanol extract. Amino acid analysis identified arginine (7,827.15 mg·kg^{-1}) as the highest essential amino acid and glutamic acid (14,803.9 mg·kg^{-1}) as the highest non-essential amino acid. Moreover, the methanol extract significantly reduced cholesterol levels in male house mice across various doses (100, 200, and 300 mg·kg^{-1}).

Keywords: Abalone, Antibacterial, Cholesterol reduction, *Haliotis asinina*

INTRODUCTION

Infectious diseases primarily caused by pathogenic bacteria like *Staphylococcus aureus* and *Escherichia coli* are commonly treated with antibiotics, which interfere with the growth or kill the bacteria by disrupting metabolism (Mancuso *et al*., 2021). Incorrect antibiotic use can lead to negative health effects and contribute to multi-drug resistance (Fernández *et al*., 2016). Marine organisms such as abalones have the potential as an alternative agent of new antibacterial. Abalon extracted with ethyl acetate has been reported to have antimicrobial activity and anthelmintic activity and contains secondary metabolite compounds such as alkaloids, terpenes, and flavonoids (Tortorella *et al*., 2021).

Cholesterol is a vital element necessary for regulating chemical processes in the human body (Luo *et al*., 2020). However, excessive cholesterol can cause hypercholesterolemia and fat accumulation on the walls of blood vessels, thereby triggering symptoms of atherosclerosis and a decrease in proteins in the body's tissue (Wong *et al*., 2016). Donkey's ear abalone (*Haliotis asinina* Linnaeus, 1758) is a natural biota that shows potential in reducing blood cholesterol. Besides being a source of beneficial omega-3 fatty acids, protein, vitamins, and minerals, abalone also possesses bioactive sulfate polysaccharide, peptides, glycoproteins, chitosan, and carotenoids, identified as compounds with antioxidant, anticoagulant, and antimicrobial effects (Zoysa, 2013). The latest

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study on *H. asinina* by Sari *et al*. (2020) revealed the presence of flavonoids, saponins, alkaloids, and phenols in methanol extracts of its meat and viscera. Moreover, marine organisms such as sea cucumber and *Stichopus hermanii* are known to contain EPA and DHA which have demonstrated the ability to reduce triglyceride levels in house mice *(Mus musculus* Linnaeus, 1758) (Pringgenies *et al*., 2012). Nonetheless, this health advantage has yet to be investigated in donkey's ear abalone. Therefore, this study was conducted to investigate: (1) the effects of *H. asinina* extract against pathogenic bacteria, *Staphylococcus aureus* and *Eschericia coli*; (2) its amino acid profile; and (3) the profile of its secondary metabolite compounds and their effect on blood cholesterol levels in house mice.

MATERIALS AND METHODS

Sample collection

Thirty pieces of adult *Haliotis asinina* with a size range 6–8 cm were collected at Tapulaga Beach, Konawe District, Southeast Sulawesi Province, Indonesia. The abalone samples were cleaned with water until free from dirt and attached substrate. Afterward, the abalone was dissected to separate the flesh, mucus, and viscera from the shell, with each component placed in a Ziplock plastic bag. Subsequently, the samples were carefully stored in a cool box to prevent degradation and transported to the Laboratory of Diponegoro University, Semarang. The specific part of the abalone intended for used is the flesh or meat.

Sample extraction and evaporation

Extraction by maceration involves a sequential extraction using three different solvents: n-hexane as a nonpolar solvent, ethyl acetate as a semipolar solvent, and methanol as a polar solvent. An 80 g sample was placed in an Erlenmeyer flask, mixed with 1,000 mL of n-hexane (1:12.5 w/v), and allowed to stand at room temperature for 24 h. After 24 h, the filtrate was separated using filter paper and evaporated using a rotary evaporator at 42 °C. The extraction process continued by re-soaking the abalone sample with 1,000 mL ethyl acetate, standing for within 24 h, followed by filtration using filter paper and evaporation. In the final step, the abalone sample was soaked in 1,000 mL of methanol, left to stand for 24 h at room temperature, and then evaporated using rotary evaporator (Burgess *et al*., 2003).

Preparation of test extract concentration

The antibacterial test involved testing extract concentrations of 10%, 20%, and 30%, along with a positive and negative control. The methodology for creating these concentration followed the approach outlined by Sernita *et al*. (2016). To prepare the 10% concentration, 0.1 g of the abalone extract was dissolved in 1 mL of distilled water. Similarly, the 20% concentration was achieved by dissolving 0.2 g of the extract in 1 mL of distilled water, and the 30% concentration was created by dissolving 0.3 g of the extract in 1 mL of distilled water.

For the positive control, a 1% chloramphenicol solution was prepared by dissolving 0.5 g of chloramphenicol powder in 50 mL of distilled water, mixed well until homogeneous. This solution was utilized to measure the inhibition zone diameter of the chloramphenicol antibiotic. As for the negative control, distilled water was used to evaluate whether the solvent had any impact on the antibacterial test results and served as a baseline for comparison.

Antibacterial test procedure

The antibacterial activity test was conducted using the paper disc or diffusion disk method. This method was chosen for its consideration of various factors, such as the solubility of the extract. It ensures that the extract is soluble in the solvent, optimizes the concentration of the extract in the solvent to achieve a sufficient antimicrobial effect, and facilitates proper absorbtion in the paper disc. The test procedure followed the methodology outlined by Ariyani *et al*. (2018). Two pathogenic bacteria used in this test are *S. aureus* (ATCC25923) and *E. coli* (ATCC25922). Prior to the test, the pathogenic bacteria were inoculated into the NA (Nutrient Agar) media for 24 h. Subsequently, they were inoculated into the NB (Nutrient Broth)

media and placed in a shaker to ensure homogeneity. The antibacterial test was performed with two replications. The pathogenic bacteria were evenly inoculated onto the MHA (Mueller Hinton Agar) media evenly using a sterilized cotton swab. Paper discs, loaded with the extract with various concentrations, along with negative and positive controls (20 µL of the substance), were placed on the MHA media. The test media were then incubated for 72 h and observed every 24 h.

Phytochemical screening

Phytochemical screening aims to qualitatively analyze the content of secondary metabolite compounds contained in a sample (Prayoga *et al*., 2019). The screening of flavonoid compounds involves adding 25 mg of magnesium (Mg) powder and three drops of 37% concentrated HCl to a 50 mg solution of *H. asinina* methanol extract dissolved in 5 mL of distilled water. Alkaloid compounds screening test was carried out using Dragendorff's reagent. Methanol extract of H. asinina as much as 50 mg was dissolved into 5 mL of methanol and 1 mL of Dragendorff reagent was dripped into the solution. The steroid test was performed using Liebermann-Burchard reagent, which reacted with a 50 mg methanol extract of *H. asinina* dissolved in 5 mL of methanol then added 1 mL of Liebermann-burchard reagent. The phytochemical screening test of tannin compounds was performed by reacting the methanol extract of abalone *H. asinina* with 1% $FeCl_3$ solution. A 50 mg crude abalone methanol extract was dissolved in 5 mL of methanol, and 1 mL of 1% $FeCl_3$ solution was added until a color change occurred. Saponin test is done by shaking the methanol extract that has been diluted with 5 mL distilled water, and the solution was shaken for 10 s. A positive reaction is indicated by the formation of stable froth that does not disappear within±10 m and then concentrated HCl is added.

Amino acid analysis

The samples for amino acid analysis comprised the abalone, *H. asinina*. High Performance Liquid Chromatography (HPLC) was used in this process and the samples were prepared by Kjeldahl method. This was followed by acid and base hydrolysis process on the samples. The hydrolyzed samples were then ready for analysis using standard-procedure HPLC. For sample preparation, 0.6 g of the abalone extract were weighed and 5 mL of 6N HCI were added. The hydrolysis was done at 110 °C for 22 h. The resulting hydrolysate was cooled to room temperature. After using 6 N NaOH to neutralize the pH to 7, distilled water was added to reach a volume of 10 mL. To this solution, 300 µL of orthophalaldehyde (OPA) dissolved in 40 µL of the sample solution was added. The mixture was then injected into an HPLC. Amino acid analysis using HPLC involves converting protein into amino acids in order to be detected by the chromatograph. Chromatographic conditions included the use of a C18 column, a temperature of 37 °C, a mobile phase of 60% acetonitrile, a fluorescence detector, a flow rate of 1 $mL \cdot min^{-1}$, and an injection volume of 5 µL. The recorder saves the results once the detector identified the amino acids. The recorded data are then displayed on the computer in retention time and area graphs, with the peaks representing the properties of each amino acid.

Preparation of test animals

The test animals used are male house mice aged 2–3 months with a body weight of 20–25 g. The preparation of test animals was conducted according to Shinta and Sudyanto (2016) with some modifications. There are 15 test animals, distributed across five testing groups. Prior to the experiment, the test animals were acclimatized for seven days in cages, during which they are provided with standard feed and water. Before the experiment, it is essential to ensure that the acclimatized mice are healthy and that their weight does not change more than 10%.

Treatment of experimental animals

The animals were treated according to Umami *et al*. (2016). Group 1 served as the negative control, where they were given a regular diet with high cholesterol (PTU 0.01% and quail egg yolk) and water, without any additional treatment. Group 2 was a positive control, received the same standard diet with high cholesterol (PTU 0.01% and quail egg yolk) along with simvastatin suspension. Group 3 received a low-dose treatment consisting of a regular

diet, high cholesterol diet (PTU 0.01% and quail egg yolk) and abalone extract at 100 mg·kg^{-1} BW. Group 4 received a moderate dose of a regular diet, high cholesterol (PTU 0.01% and quail egg yolk) and abalone extract at 200 mg·kg^{-1} BW. Group 5 was given a high dose, with a regular diet, high cholesterol (PTU 0.01% and quail egg yolk), and abalone extract at 300 mg·kg^{-1} BW. The initial cholesterol levels of each mouse, or day 0, were measured. Then, they were given a high-cholesterol and high-sugar diet for 14 days, after which their cholesterol levels were measured again. Mice induced with a high cholesterol and high sugar diet are then given abalone extract therapy with different concentrations or doses for another 14 days.

Determination of cholesterol levels with digital checks

The determination of cholesterol levels followed the methodology outlined by Umami *et al*. (2016). Cholesterol levels were assessed using the GCU Easy Touch instrument. Blood samples were collected from mice through the tail vein. The tip of the mice's tail was sterilized with an alcohol swab and then cut using a needle. The first drop of blood from the mice was discarded, and the second drop was used to measure cholesterol levels. This procedure was performed on all animals across all test groups.

RESULTS AND DISCUSSION

Extraction

In this study, *Haliotis asinina* was extracted in successive steps using three different solvents: n-hexane, ethyl acetate, and methanol. The extraction method used was maceration. The results obtained from the extraction process are presented in Table 1.

The extraction results showed that the n-hexane, ethyl acetate, and methanol extracts had a paste-like form and a dark green colour. The use of a rotary evaporator during the concentration process can influence the final texture, resulting in a paste-like form. Specifically, the methanol extract displayed the highest weight, measuring 6.86 g, with a yield of 8.57%. In comparison, the ethyl acetate extract weighed 0.98 g with a yield of 1.22%, and the n-hexane extract weighed 2.07 g and a yield of 2.58%. The yield is determined by the ratio between the initial weight and the weight of the extract obtained. As outlined by Wijaya *et al*. (2018), these products are expressed in a percentage (%), and the higher the yield value, the more extract value obtained. The results further showed that *H. asinina* samples have a high content of polar compounds since they have the highest weight on the methanol extract.

Antibacterial activity test

The *H. asinina* extracts obtained from the three different solvents, namely n-hexane, ethyl acetate and methanol, were used to conduct an antibacterial activity test against pathogenic bacteria *S. aureus* and *E. coli*. The three extracts of *H. asinina* were tested using different concentrations. The antibacterial activity test was carried out over a period of 72 h. A positive result of the antibacterial test was characterized by a clear zone occured around the disc paper. The results of the antibacterial activity test, with the measurement of the precise area, are shown in Table 2.

The antibacterial tests revealed that the extractions using each of the three solvents possess antibacterial activities against *S. aureus*, with the ethanol extract at 20% concentration showed the highest inhibition zone. However, the 10% and 30% methanol extracts along with the 30% n-hexane

Table 1. Extraction result of donkey's ear abalone, *Haliotis asinina*.

Solvent	Weight of extract (g)	Percentage of yield (%)	Form	Color	Odor
N hexane	2.07	2.07	Paste	Dark green	Fishy
Ethyl acetate	0.98	0.98	Paste	Dark green	Fishy
Methanol	6.86	6.86	Paste	Dark green	Fishy

Table 2. Antibacterial activity test results of donkey's ear abalone (*Haliotis asinina*) extract.

Pathogenic bacterial	Observation time	Inhibition diameter (mm±SD)							
		M10	M20	M30	E10	E20	N10	N20	N30
Staphylococcus aureus	24 h	-	3.80±5.37^{a}	-	3.60±0.28^{a}	5.10±0.99^{b}	0.20±0.28^{c}	0.18±0.25^{c}	-
	48 h	-	3.75±5.30^{a}	-	3.73±0.11^{a}	5.06±1.19^{b}	0.15±0.21^{c}	0.30±0.42^{c}	-
	72 h	-	3.05±4.31^{a}	-	3.15±0.49^{a}	5.05±2.05^{b}	0.10±0.14^{c}	-	-
Escherichia coli	24 h	-	-	-	3.15±0.07^{a}	4.05±0.07^{b}	0.55±0.78^{c}	-	-
	48 h	-	-	-	3.85±0.07^{a}	4.50±0.42^{b}	0.90±1.27^{c}	-	-
	72 h	-	-	-	3.60±0.14^{a}	4.35±0.07^{b}	0.40±0.57^{c}	-	-

Note: The results for each bacterium are not significantly ($p>0.05$) different across observation time; Mean±SD in each row superscripted with different lowercase letters are significantly ($p<0.05$) different. M, E and N = methanol, ethyl acetate and n-hexane extract, respectively and the following numbers (10, 20, and 30) represent concentrations of each extraction.

extract, showed no inhibition zone against this bacterium. Slighly different results were observed for *E.coli*, where all concentrations of the methanol extract did not form a clear zone, and neither did the n-hexane extracts at 20% and 30%. Among all treatments, the 20% ethanol extract showed the largest inhibition zone against *E. coli* although the size was less than 5 mm which indicated weak activity (Sun *et al*., 2021).

Ethyl acetate extract exhibits superior antibacterial activity due to its semi-polar nature, enabling the dissolution of both polar and nonpolar compounds. This characteristic imparts a broad spectrum of compounds, and notably, antibacterial agents derived from *H. asinina* tend to be semi-polar (Oktavianawati *et al*., 2023). According to the antibacterial findings from methanol and n-hexane extracts, it appears that the concentration of 30% lacks sufficient antibacterial activity. Exploring lower doses, such as 10% and 20% in ethanol extract, may offer more comprehensive insights into the compound's effectiveness in inhibiting bacterial growth at a reduced level. Upon phytochemical testing, the methanol extract of *H. asinina* contains alkaloid and saponin compounds. The antibacterial mechanism of alkaloid compounds is to disrupt the constituent components of peptidoglycan in bacterial cells. This disruption prevents the formation of cell wall layer, and consequently disrupt the synthesis of peptidoglycan. As a result, cell formation is (Yan *et al*., 2021). Saponin is used by abalone in high concentration as a self-defense mechanism against predators. Additionally, it serves as an antimicrobial compound. According to Sari *et al*. (2020), the methanol extract of *H. asinina* contained approximately 6.85 $\mu g \cdot mL^{-1}$ of saponin. Abalones also produce antibacterial peptides, also known as antimicrobial peptides (AMPs) or natural defense peptides (defensins). AMPs can damage bacterial cell membranes, inhibit protein synthesis, or damage bacterial DNA. Certain amino acids may play a role in the interaction with the bacterial cell membrane. For instance, amino acids with hydrophobic properties may cause antibacterial peptides to invade the bacterial membrane, damage the integrity of the membrane, and ultimately kill the bacteria (Seo *et al*., 2016).

Phytochemical test

The phytochemical screening results showed that the *H. asinina* methanol extract contained alkaloid and saponin compounds (Table 3). The alkaloid test, utilizing the Mayer reagent, indicated the presence of alkaloid compounds through the formation of yellow to light brown precipitates. This observation aligns with Miranti and Silviyani (2022), who explained that the reaction involves the nitrogen in alkaloid reacting with the K+ metal ion of potassium tetraiodomercurate (II), leading to the precipitation of the potassium-alkaloid complex.

commonly found in polar solvents. This characteristic is consistent with Akinpelu *et al*. (2014), who highlighted that saponins exhibit soapy properties and can form foam. Saponins are known for their diverse applications, including as antioxidants, anticancer agents, and for their potential in managing conditions such as hypercholesterolemia, hyperglycemia, and anti-inflammatory. The surfactant properties of saponins can disrupt bacterial cell membranes, causing leakage and bacterial death, suggesting a potential antibacterial effect.

Research indicates that saponins may also bind to bile acids, inhibiting the absorption of cholesterol and increasing its excretion, thereby potentially lowering cholesterol levels. Alkaloids, on the other hand, exhibit antibacterial activity by

Table 3. Phytochemical screening results of methanol extract of donkey's ear abalone (*Haliotis asinina*).

Phytochemical compounds	Observation	Results
Alkaloid	Yellowish to orangish precipitate	+ 
Saponin	Presence of stable foam	+ 
Tannin	No color change	- 
Flavonoid	No orange color formed	- 
Steroid	No color change	- 

Note: + and - denote the precence and absence of the compound in the extract, respectively

impeding bacterial growth or causing damage to bacterial cell structures (Rai and Yamazaki, 2022). Certain types of alkaloids may play a role in reducing cholesterol levels by affecting lipid metabolism or inhibiting enzymes involved in cholesterol synthesis. However, it's worth noting that tannin, flavonoid, and steroid tests on the methanol extract of *H. asinina* yielded negative results, as evidenced by the absence of colour change and sedimentation during the test.

Amino acid profile of Haliotis asinina

Simplo and duplo in results means that quantitative analysis is performed once and twice for the same sample. It aims to get the certainty of the analysis results. If both measurements provide data that is significantly different, it would be difficult to determine which analysis result is correct. Therefore, a re-measurement must be carried out. However, if the two measurements produce data that is nearly the same, the results of the analysis carried out can be assured and the respective data can be averaged as the result of the analysis (Westerink *et al*., 2020). The amino acid test results (Table 4) showed that arginine is the highest essential amino acid content in *H. asinina*, measuring 7,827.15 mg·kg^{-1}. Additionally, other most elevated essential amino acids are leucine, lysine and proline, with results of 5,953.68 mg·kg^{-1}, 5,756.41 mg·kg^{-1}, and 5,707.94 mg·kg^{-1}. Arginine is formed in the liver and processes to increase growth hormone, lymphocyte production and male fertility. These are essential amino acids commonly found in aquatic animals, known as high-protein food. Arginine is also known as a precursor for the synthesis of nitric oxide (NO), a molecule that has antimicrobial effects. Nitric Oxide may play a role in defense against bacterial infection through various mechanisms (Wu *et al*., 2021; Kim *et al*., 2022). As stated by Ibiza and Serrador (2008), Nitric oxide inhibits bacterial replication by binding directly to double-stranded DNA, causing deamination and damage, and by interfering with zinc metalloproteins involved in DNA synthesis. Nitric oxide also impairs bacterial function, by interfering with heme-containing

Table 4. Amino acid profile of donkey's ear abalone (*Haliotis asinina*).

No	Amino acids	Unit	Results		Average
			Simplo	Duplo	
	Essential amino acid				
1	L-Histidine	mg·kg^{-1}	1,263.60	1,276.41	1,270.05±9.05
2	L-Leucine	mg·kg^{-1}	5,908.08	5,999.29	5,953.68±64.49
3	L-Phenylalanine	mg·kg^{-1}	1,932.36	1,981.71	1,957.03±34.89
4	L-Isoleucine	mg·kg^{-1}	3,027.78	3,060.85	3,044.31±23.38
5	L-Valine	mg·kg^{-1}	3,488.90	3,548.73	3,518.81±42.30
6	L-Threonine	mg·kg^{-1}	3,671.80	3,714.97	3,693.38±30.52
7	L-Arginine	mg·kg^{-1}	7,775.71	7,878.59	7,827.15±72.74
8	L-Proline	mg·kg^{-1}	5,707.94	5,789.02	5,748.48±57.33
9	L-Lysine	mg·kg^{-1}	5,712.17	5,800.65	5,756.41±62.56
	Non-essential amino acid				
10	L-Aspartat acid	mg·kg^{-1}	9,665.50	9,789.94	9,727.72±87.99
11	L-Alanine	mg·kg^{-1}	7,132.58	7,237.36	7,184.97±74.09
12	L-Thyrosine	mg·kg^{-1}	1,479.43	1,509.57	1,494.5±21.31

bacterial enzymes and oxidizing bacterial lipids. Arginine can affect lipid metabolism and may contribute to lowering blood cholesterol levels. Arginine may increase the activity of the enzyme lipoprotein lipase, which plays a role in the breakdown of lipoproteins (Hadi *et al*., 2019; Lu *et al*., 2022; Szlas *et al*., 2022).

The amino acid test results for *H. asinina* showed that the predominant amino acids are non-essential, including glutamic acid, glycine, and aspartic acid. The respective concentrations of these amino acids are as follows: glutamic acid at 14,803.9 mg·kg^{-1}, glycine at 10,420.8 mg·kg^{-1}, and aspartic acid at, 9,727.72 mg·kg^{-1}. These non-essential amino acids serve crucial functions in living organisms. Gianto *et al*. (2017) highlighted the significance of glutamic acid in contributing aroma and taste characteristics to food ingredients. Glutamic acid contains hydrogen groups that, when substituted with sodium, form monosodium glutamate, giving a very high savoury taste. Moreover, glutamic acid may have antimicrobial effects through certain derivatives or compounds. For example, peptides or proteins containing glutamic acid residues could contribute to the antimicrobial activity of specific proteins. Additionally, glutamic acid is a precursor for the synthesis of glutathione an antioxidant crucial in cellular defense against oxidative stress. Inbaraj *et al*. (2011) demonstrated the effectiveness of γ -glutamic acid against *S. enteritidis*, *S. aureus*, and *E. coli* using the MIC method. This compound also demonstrated potential in reducing total cholesterol and LDL cholesterol levels in human clinical studies. Results were consistent in high fat fed rats, where γ-PGA, a derivative, reduced cholesterol levels by inhibiting lipogenic enzyme activity (Park *et al*., 2011). Shifting focus to glycine, an amino acid with a role as an inhibitor of neurotransmitters in the central nervous system (CNS), it plays a crucial role in inhibiting processes that causes brain stiffness, such as observed in multiple sclerosis (Carretero *et al*., 2023).

Effects of Haliotis asinina ethanol extract on cholesterol levels in house mice

Testing was done to determine the effects of different concentrations (100, 200, and 300 mg·kg^{-1}) of an extract from *H. asinina* on cholesterol levels in mice. The results of the test are shown in Table 5.

The results showed that feeding house mice with high cholesterol diet without additional treatment significantly ($p = 0.032$) increased their blood cholesterol levels. After consuming a high-fat meal, the cholesterol esters present in the food undergo hydrolysis, leading to the formation of cholesterol. This cholesterol, along with other lipids and unesterified cholesterol, is subsequently absorbed by the intestine. As a consequence, there is an increase in the total blood cholesterol levels. The absorbed cholesterol is incorporated into chylomicrons, along with the newly synthesized cholesterol (Nakano *et al*., 2019). The mice fed with the same diet plus *H. asinina* extract significantly lowered blood cholesterol regardless of concentration. Notably, the mice received simvastatin suspension

Table 5. Effects of different concentrations (100, 200, and 300 mg·kg^{-1}) of donkey's ear abalone (*Haliotis asinina*) extract on cholesterol levels in male house mice.

Concentration of the extract	Average cholesterol levels (mg·dL^{-1})			
	Pre-test	Post-test	Difference	*p*-value*
positive control (K+)	127.33±3.51	117.0±27.51	-10.33±24	0.065
negative control (K-)	81.0±8.54	116.0±4.00	+35.0±4.54	0.032
100 mg·kg^{-1} (T1)	149.3±17.50	109.0±21.00	-39.3±3.50	0.011
200 mg·kg^{-1} (T2)	118.66±25.06	114.67±25.06	-3.9±0.00	0.008
300 mg·kg^{-1} (T3)	118.33±29.50	111.0±23.51	-7.33±5.99	0.023

Note: *Paired t-test, significant if $p<0.05$; $n = 3$

which served as a positive control did not show change of blood cholesterol. This may be attributed to the inhibitory effect of simvastatin over HMG-CoA reductase which is involved in the synthesis of cholesterol. HMG-CoA reductase inhibitors prevents the liver from synthesizing cholesterol and consequently lowers levels of total cholesterol and low-density lipoproteins (LDL) in blood (Ndlovu *et al*., 2023).

Among three concentrations of the abalone extracts, T1 (100 mg·kg^{-1}) caused the highest decrease of blood cholesterol. This may occur due to the optimization of doses. Some substances or compounds exhibit biological responses that closely follow a sigmoid dose-response pattern. In other words, increasing the dose will increase the response until it reaches a saturation point where increasing the dose does not give a significant increase in response. In these cases, a small dose would already achieve the maximal level of response in mice (Yates and Mistry, 2023). The proper mice metabolism *in vivo* is crucial for enhancing drug efficacy and facilitating slow release mechanisms. Controlling mice metabolism through adjustment of physicochemical properties is essential for enhancing sustained release and therapeutic effects. Premature metabolism can lead to early drug leakage, leading to tissue toxicity and non-sustained release. However, excessively slow metabolism may cause long-term accumulation, causing adverse effects. Some nanomaterials and their metabolites can be biologically active and small doses may be more easily absorbed and efficiently processed by the mice (Bicker *et al*., 2020; Zhang *et al*., 2020).

Methanol solvent was used to extract abalone in the anti-cholesterol test because methanol has characteristics as a more polar solvent compared to ethyl acetate. Active components in abalone that have anti-cholesterol effects are generally polar or have a higher affinity for polar solvents, therefore methanol is used in this process. In addition, methanol also has a lower boiling point than ethyl acetate, so compounds extracted with methanol are likely to have better solubility in the solvent. *H. asinina* methanol extract contains saponin and alkaloid compounds that can act as cholesterol reducers. Saponins can reduce LDL cholesterol levels or known as bad cholesterol. Saponins inhibit the absorption of cholesterol from the intestine by forming complexes with cholesterol in the intestine and helping to remove it through feces (Moundras *et al*., 1997). As a result, the overall intake of cholesterol into the bloodstream is subsequently reduced. Alkaloid compounds have several kinds, one of which is berberine. Alkaloids are known to increase HDL cholesterol and lower LDL cholesterol (Kong *et al*., 2010). Amino acids, particularly arginine and glutamic acid, may play a role in reducing cholesterol, as suggested by a study conducted by Park *et al*. (2011). These findings indicate that the elevated levels of these amino acids could potentially contribute to the observed cholesterol-lowering effects.

CONCLUSION

This study was implemented to determine the antibacterial activity of *H. asinina* meat extract and its effect on cholesterol reduction in house mice. The ethyl acetate extract of *H. asinina* at concentrations of 10% and 20% showed antibacterial activity against *S. aureus* and *E. coli* while the methanol extract showed antibacterial activity at a concentration of 20% against *S. aureus*, while n-hexane extract showed antibacterial activity at 10% concentration against both *S. aureus* and *E. coli*, with 20% concentration specifically against *S. aureus*. HPLC analysis of amino acid composition revealed that the abalone extract contained all of the essential amino acids with the highest level in arginine (7,827.15 mg·kg^{-1}). The highest non-essential amino acid in *H. asinina* was glutamic acid (14,803.9 mg·kg^{-1}). *H. asinina* methanol extract contained alkaloids and saponins. Furthermore, the methanol extract demonstrated a cholesterol-reducing effect in male house mice with the best reduction observed at 100 mg·kg^{-1}. In conclusion, these results not only provide valuable insights into the bioactivity of *H. asinina* meat extract but also lay the groundwork for future studies aimed at harnessing its therapeutic and nutritional potential. The multifaceted properties of the extract make it a promising candidate for further exploration in the fields of medicine and nutrition.

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