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Phytochemical composition, total phenolic content and antioxidant activity of *Anadara granosa* (Linnaeus, 1758) collected from the east coast of South Sumatra, Indonesia

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Abstract:

Anadara granosa is a species of the class bivalve commonly found on the east coast of South Sumatra as a fishery commodity. This species has not been widely studied as a source of new bioactive compounds that have antioxidant abilities. This study aims to analyze the antioxidant ability of *A. granosa* against DPPH radicals and its phytochemical profile qualitatively. Samples were taken at the fishing port of Sungsang Village, South Sumatra, Indonesia. Furthermore, the samples were extracted using ethanol as a solvent and tested for antioxidants against DPPH radicals, total phenol analysis, and preliminary phytochemical test. Based on the antioxidant test results, the IC_{50} value of the ethanolic extract of *A. granosa* was 85 g/ml with ascorbic acid 2 g/ml as a comparison. Then, the ethanol extract contained a total of 10.7057 mgGAE/g phenol and the results of the phytochemical test contained bioactive compounds of alkaloids, steroids, flavonoids, saponins, and tannins. The ethanolic extract of *A. granosa* contained bioactive compounds, which were reported to have potent antioxidant activity. The results of this study were expected to be important information in the latest report of the antioxidant activity of *A. granosa* species and contributed to the development of marine natural products.

Keywords: *Anadara granosa*, Antioxidant, Bioactive compounds, Phytochemical composition, Total phenolic content

Introduction:

Benthic communities are found in the ecosystem of the east coast of South Sumatra. The east coast area dominated by mangrove ecosystems becomes a good habitat for benthic community life [1]. The diversity and distribution of benthic organisms are supported by the high food sources and the availability of living places on the surface of vast mud subsrates [2]. The environment on the east coast of South Sumatra has a wide muddy landscape because it is influenced by the dynamics of the waters of major rivers such as the Musi River and Banyuasin River and the sea waters of the Bangka Strait which tend to carry a considerable sedimentation factor. The area is overgrown by dense mangroves with fluctuating environmental physical-chemical dynamics [3,4]. Natural fluctuations in the coastal environment cause only benthic organisms, especially certain mollusks, to survive such as marine shellfish and marine gastropods organisms.

Anadara granosa is a group of the family Arcidae, a class of bivalves, mollusk phylum known for a long time [5]. Marine mollusks have been reported to have biological activity because they contain a variety of bioactive compounds, such as gastropod groups in mangrove vegetation [6] and coral reef ecosystem [7], and the marine bivalves group [8]. Bioactive compounds produce essential biological activities in the form of antioxidant abilities that are attracting attention today. Antioxidants aim to capture free radicals that enter

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the body's systems [9,10], while free radicals can cause common diseases to cancer [11]. Some bioactive compounds have good antioxidant abilities, such as alkaloids, terpenoids, and phenols [12,13]. Phenols are a group of compounds that have properties as potent antioxidants [14]. The components of phenolic compounds released from the extract are responsible for antioxidant activity. The mechanism of action of these antioxidants is done by trapping free radicals and metal ions [15].

A. granosa has been consumed by the general public as healthy seafood. This species is a high-protein food because it contains several minerals such as protein and zinc [16]. Some of the benefits of *A. granosa* are believed to cure and prevent some types of diseases. The biological activity research *of A. granosa* is still focused on its antimicrobial capabilities [17]. However, the antioxidant abilities of this species are still not well reported. The application of bioactive compounds with antioxidant activity in food products and medicines can have health effects on the body. Natural compounds such as flavonoids and phenolics can be used in food additives as nutritional enhancers, cytostatic drugs, and beauty products in pharmaceutical products.

The study aims to analyze the antioxidant abilities of *A. granosa* extracted with ethanol solvents. In addition, qualitative assessment of phytochemical content and quantitative assessment of phenol content were also measured. This study is significant to report the antioxidant activity of ethanol extract of *A. granosa* against the free radical 2,2-diphenyl-1-picrylhydrazyl.

Materials and Methods:

Sample collection

A sample of *A. granosa* was taken in March 2021. The samples were obtained from Sungsang Village, South Sumatera caught from the Banyuasin waters. Next, the sample was carried out using a cooling box during the trip to the laboratory. The sampling location is presented in Fig. 1.

Figure 1. Sampling location map

Preparation of extractions

A. granosa was taken from Sungsang Village, which came from fishers. The sample collected was cleaned using flowing water. The samples were separated between the shell and meat. The sample was put in the oven at 40 °C ± 30 min so that the meat was not too wet. The sample dried with the heat of the sun. After drying, the sample was smoothed using a blender. Maceration was carried out for two days and filtered using filter paper. Maceration required each of 200 g of meat. Maceration used ethanol solvents with a ratio of 1: 4 (b/v). The sample was concentrated using a rotary evaporator until it became a paste or crude extract at 40 °C. The next step was to make a parent solution

for the concentration dilution used in the antioxidant test.

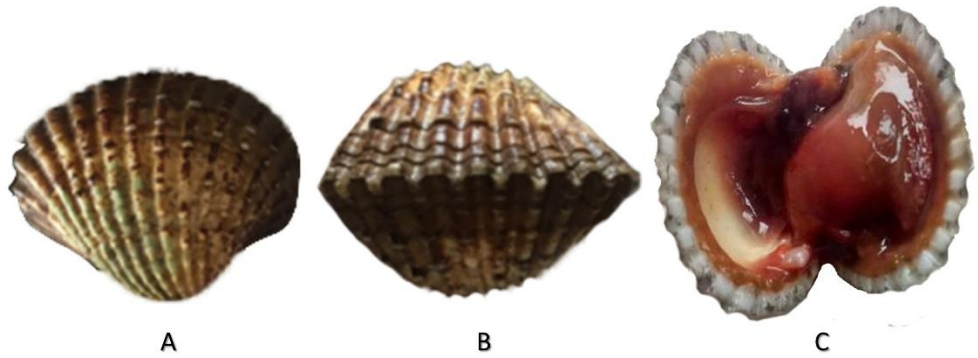

Figure 2. Morphology of *A. granosa*, A: Upper shell; B: Front shell; C: Meat in shell

Preliminary phytochemical

Qualitative tests of extract phytochemicals referred to [18], included alkaloids, flavonoids, saponins, tannins, and steroids tests. Extract of 2.5 mg was prepared for each test and then mixed in each test solution formula. Preparation of phytochemical tests using several different solutions in each test included, the alkaloids test preparing the meyer and dragendoff reagents, flavonoids test using a mixture of 2% NaOH in alkaline reagent test, saponins test mixing HCl solution in foam test, Tannins test using 1% FeCl solution, and steroids test prepared acetic anhydride solution $(CH_3CO)_2O$ and H_2SO_4. The phytochemical test was classified as a conventional qualitative test based on the color change in the liquid mixture.

Determination of total phenolic content

Total phenol of extract analysis was using the Folin-Ciocalteau method was remarked in detail in a previous study [19].

Determination of antioxidant activity

Antioxidant activity was analyzed by the diphenyl-1-picrylhydrazyl (DPPH) method [9,20]. Extract weighed as much as 0.2 g and added 100 ml of ethanol, so that parent solution can be produced with the concentration of 2000 μg/ml. DPPH 0.1 mM solution was made by weighing 0.002 g of DPPH crystals into 50 mL of ethanol and obtaining a solution with a concentration of 40 μg/ml, then shaken to dissolve the DPPH powder. The test concentrations used were 200, 400, 600, 800, and 1000 μg/ml diluted from a parent solution of 2000 μg/ml. Next, as many as 1.5 ml of test solution and comparison was reacted by 1.5 ml solution DPPH 0.1 mM in the test tube.

DPPH solution mixed with ethanol and extracted was homogeneous and incubated for 30 min in a dark place. After that, absorbance measurements were taken using a UV-Vis spectrophotometer at a wavelength of 517 nm. Levels of antioxidant strength with DPPH method based on the strong criteria 50 - 100 μg/ml, very strong < 50 μg/ml, moderate 101 - 250 μg/ml, weak 251 - 500 μg/ml, inactive > 500 μg/ml [21].

Results:

Morphology of *A. granosa*

A. granosa had a thick, rough, and contorted shell on the surface. The shape of the shell was round, slightly oval, consisting of symmetrical shell, had a pallial line on the complete inner shell and a striped outer pallial line, while the inner shell had a smooth texture with shiny white color. The primary color of the clam was brownish-red, and the flesh was blood red. The largest measurement of the individual length of *A. granosa* was obtained at 5.97 cm long and 4.36 cm wide, while the smallest individual had a length of 4 cm with a width of 3.42 cm.

Extraction of *A. granosa*

Extraction resulted in a yield of crude extract. The higher the percentage value of the extract yield, the more compounds contained in the extract. Crude extract *A. granosa* was blackish brown. It happened because the sample in the form of meat came from animals. The fishy smell contained in a sample was due to the sample derived from shellfish meat. The measurement process of each frish weight (FW), dry weight (DW), smooth weight (SW), extract weight (EW), and percentage of the water content of *A. granosa* is presented in Table 1.

Table 1. Percentage of depreciation

Sample	Sample weight (g)		Depreciation percentage (%)	Weight percentage (%)
	Frish	**Dry**		
A. granosa	1500	227	84.8	15.2

Solution	Extract weight (g)		Depreciation percentage (%)	Extract percentage (%)
	Dry powder	**Crude extract**		
Ethanol extract of *A. granosa*	220	29.46	86.6	13.4

Sample *A. granosa* had taken frish weight 1500 g, and after a few days of drying, the dry weight of *A. granosa* became 227 g with a percentage of the decreased water content of 84.8 %. The sample was smoothed by 220 g, and after the extraction process was obtained, the extract weight was 29.46 g.

Preliminary Phytochemical of *A. granosa* extract

Identification of phytochemical content was helpful for grouping bioactive compounds in *A. granosa* extracts. Phytochemical screening of *A. granosa* ethanol extract obtained in this study showed that flavonoid compounds, saponins, tannins, alkaloids, steroids, and tannins were present in polar solvent testing. Phytochemical tests were conducted to determine the compounds contained in the extract so that the group of compounds that caused antioxidant activity. The result of the phytochemical test is presented in Table 2.

Table 2. Preliminary phytochemical test of *A. granosa* extract

Phytochemical	Analysis results	Analysis type
Tannins	+	Qualitative
Saponins	+	Qualitative
Flavonoids	+	Qualitative
Alkaloids	+	Qualitative
Steroids	+	Qualitative

Based on Table 2, a group of bioactive compounds analyzed on phytochemical tests was declared active in extracts. In ethanol extract, *A. granosa* contained alkaloids, steroids, flavonoids, saponins, and tannins. Some of these bioactive compounds were known to have a working system as good antioxidants.

Total phenolic determination of *A. granosa* extract

Determination of the content of phenolic compounds in ethanol extract was a quantitative measurement. Measurement of total phenol used the method of adding Folin-ciocalteu reagent to the test solution as shown in Table 3.

Table 3. Quantitative measurement of total phenol *A. granosa* extract

Sample	Unit	Analysis results	Sample condition
A. granosa ethanol extract	mgGAE/g	10.7057	Green crude extract

In this study, the total phenol content of the ethanol extract of *A. granosa* was tested in the form of green crude extract. Based on Table 3, the analysis of the total phenol content in the ethanol extract was 10.7057 mgGAE/g. This result reported that *A. granosa* had quite good phenolic compounds. In addition, this measurement was quite useful for quantitatively validating the presence of a group of bioactive derivatives of phenolic compounds as reported in the qualitative results in Table 2.

Determination of antioxidant activity

Antioxidant potential was quantitatively expressed by the percentage value of free radical inhibition and IC_{50} value from *A. granosa* extract. The percentage of inhibition expressed the percentage value of the extract in inhibiting free radicals at a given concentration. IC_{50} value

expressed the concentration of extract in inhibiting free radicals by 50%. The percentage results of inhibition and average absorbance extract *A. granosa* and ascorbic acid were presented in Table 4.

Table 4. Inhibition percentage and average absorbance extract of *A. granosa* and ascorbic acid

Concentration (μg/ml)	Ascorbic Acid		*A. granosa*	
	Abs	% Inh	Abs	% Inh
200	0.023	96.058	0.172	69.502
400	0.018	97.234	0.154	72.891
600	0.011	98.133	0.126	79.253
800	0.005	99.170	0.101	81.259
1000	0.001	99.516	0.047	91.286

Abs: Absorption; % Inh: % Inhibition

Based on the calculation results of the percentage of inhibition obtained results as presented in Table 4. Extract of *A. granosa* had a percentage value of inhibition > 50 %, which could be interpreted that extract *A. granosa* had the potential as an antioxidant. Ascorbic acid was an antidote to radicals so well that it was used as a control and as a comparison. Antioxidant activity expressed in value IC_{50}.

Table 5. The IC_{50} value of ascorbic acid and extract of *A. granosa*

Sample	Formula	R^2	IC_{50}	Category
Ascorbic acid	y = 0.9048x + 4.6098	0.9101	2 μg/ml	Very strong
A. granosa	y = 1.2989x + 1.7934	0.9805	85 μg/ml	Strong

The small IC_{50} indicated that its antioxidant activity improved, while a sizeable IC_{50} value indicated its antioxidant activity was lower. The value of the linear regression equation and IC_{50} of *A. granosa* extract and ascorbic acid can be seen in Table 5.

Discussion:

The size of the shellfish found in this study was included in the size taken for commercial use. Shellfish shells had colors and shapes that varied depending on the type, food, and habitat [3,22]. *A. granosa* lived by immersing themselves in sandy and muddy beaches [23]. *A. granosa* was found on a muddy substrate at the estuarine with sloping coastal topography up to a depth of 20 m. *A. granosa* was an infauna that was living by immersing itself in shallow waters under the surface of mud [24].

The resulting extract was good enough to become a paste or crude extract [25]. Extraction was done at a low temperature so that the sample could not be too hot and the compound was not damaged. Polar compounds would only dissolve in polar solvents such as ethanol, methanol, butanol, and aqueous. Non-polar compounds would also dissolve in non-polar solvents such as ether, chloroform, and hexane [26]. Polar solvents tend to be able to extract more bioactive compounds.

The antioxidant activity was affected by the content of its bioactive components, namely flavonoids, alkaloids, and phenols [12,13]. Alkaloids could function as an antibacterial, were the most significant secondary plant compound [27,28]. Steroids compounds tend to have antibacterial and anti-inflammatory functions [29]. Saponins were a glycoside form of sapogenin so that they would be polar. Saponins compounds were surface-active and could cause foam if shaken in water [30]. Tannins were secondary metabolite compounds that were efficacious as astringents, antidiarrheal, and inhibit free radicals [31]. Flavonoids had functioned as antioxidants [32]. Flavonoids act as antioxidants that could prevent cardiovascular disease [33]. Flavonoids could act as exogenous antioxidants that could dampen the activity of free radicals derived from sun exposure and air pollution. Phenol compounds such as flavonoids were the most potent compounds that played an active role as antioxidants [34].

The total phenol obtained was directly proportional to the results of antioxidants. High phenol content could produce good antioxidant activity [35]. Phenol content was commonly found in plants, mollusks, and marine organisms. The content of phenols in some types of shellfish came from microalgae and other tiny organisms that were filtered or entered the digestive system of these shellfish [36].

DPPH radicals included organic nitrogen compounds that were widely commercialized to assess antioxidant abilities in warding off free radicals. DPPH radicals could accept hydrogen atoms (electrons) to become more stable molecules with simple mechanisms [37]. In this study, the

percentage of radical inhibition of *A. granosa* was measured using ethanol extract. Based on comparisons between three types of polar solvents; methanol, ethanol, and water, ethanol extracts were more significant than water and less significant than methanol [38]. However, ethanol solvents were safer from the safety element than methanol solvents because they contained methane elements.

Antioxidant activity using DPPH radicals in Table 5 indicated that the IC_{50} value from *A. granosa* extract was 85 μg/ml. The antioxidant activity of *A. granosa* extract was relatively strong but not more vital when compared to the antioxidant activity of ascorbic acid as a comparison, which was 2 μg/ml, classified as an antioxidant with potent inhibition activity was adjusted to the criteria [21].

Some of the biological activities of the shellfish group had been evaluated by researchers. The antioxidant activity of the species *P. viridis* in the same solvent produced an IC_{50} of 154.3 μg/ml [38]. The razor clams of the family group Solenidae once measured their antioxidant activity by resulting in 489.56 μg/ml of IC_{50} with weak categories [39]. The antioxidant activity found from other bivalve species showed no powerful antioxidants. That indicates that the presence of bioactive compounds in *A. granosa* was better as an antioxidant. Apart from being a food source, variations in the biochemical content in marine organisms could be influenced by geographic location, marine environmental conditions, water quality, and extraction techniques also significantly affect the content of bioactive organisms [40–42].

This study aims to investigate the capabilities of one of the commercial bivalves, *A. granosa.* The ability of antioxidants shown by *A. granosa* through ethanol extract in this study could be the latest and essential report as an evaluation material for utilizing bivalve species in the pharmaceutical field.

Conclusion:

The IC_{50} value of ethanol extract of *A. granosa* showed a value of 85 μg/ml and belonged to the strong antioxidant category. Based on preliminary phytochemical results, *A. granosa* extract contained several bioactive compounds such as alkaloids, steroids, flavonoids, saponins, and tannins. The results of the total phenol test analysis of 10.7057 mgGAE/g indicate the good antioxidant ability of *A. granosa*.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Sriwijaya.

Authors' contributions:

R., G.D., F., W.A.E.P., and A.A. was contributed to the design of the research and supervised the findings of this work. N. developed the theory and performed the computations. R.Y.N., and M. were contributed to the analysis of the results and to the writing of the manuscript. All authors discussed the results and contributed to the final manuscript.

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التركيب الكيميائي النباتي والمحتوى الفينولي الكلي والنشاط المضاد للأكسدة في *Anadara granosa* (Linnaeus، 1758) التي تم جمعها من الساحل الشرقي لجنوب سومطرة ، إندونيسيا

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الخلاصة:
Anadara granosa هو نوع من ذوات الصدفتين يوجد عادة على الساحل الشرقي لجنوب سومطرة كسلعة سمكية. لم يتم دراسة هذا النوع على نطاق واسع كمصدر للمركبات النشطة بيولوجيًا الجديدة التي لها قدرات مضادة للأكسدة. تهدف هذه الدراسة إلى تحليل القدرة المضادة للأكسدة للفطر *A. granosa* ضد جذور DPPH وخصائصه الكيميائية النباتية نوعيا. تم أخذ العينات في ميناء الصيد بقرية سونج سانج ، جنوب سومطرة ، إندونيسيا. علاوة على ذلك ، تم استخراج العينات باستخدام الإيثانول كمذيب واختبارها لمضادات الأكسدة ضد جذور DPPH وتحليل الفينول الكلي والاختبار الكيميائي النباتي الأولي. بناءً على نتائج اختبار مضادات الأكسدة ، كانت قيمة IC_{50} للمستخلص الإيثانولي من 85 *A. granosa* جم / مل مع حمض الأسكوربيك 2 جم / مل على سبيل المقارنة. ثم احتوى مستخلص الايثانول على ما مجموعه 10.7057 ملجم GAE / جم فينول ونتائج الاختبار الكيميائي النباتي احتوت على مركبات نشطة بيولوجيا من القلويات والستيرويدات والفلافونويد والصابونين والعفص. يحتوي المستخلص الإيثانولي من *A. granosa* على مركبات نشطة بيولوجيًا ، والتي تم الإبلاغ عن أن لها نشاطًا قويًا كمضاد للأكسدة. كان من المتوقع أن تكون نتائج هذه الدراسة معلومات مهمة في التقرير الأخير للنشاط المضاد للأكسدة لأنواع *A. granosa* وساهمت في تطوير المنتجات الطبيعية البحرية.

الكلمات المفتاحية: *Anadara granosa* ، مضادات الأكسدة ، المركبات النشطة بيولوجيا ، التركيب الكيميائي النباتي ، المحتوى الفينولي الكلي