

Antioxidant substances and properties of coquina clam (*Donax variabilis*) of the coastal waters of Luuk Buhi, Tongsinah, Bongao, Tawi-Tawi, Philippines

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Abstract. The total flavonoid and total phenolic contents are significant indicators of the antioxidant activity, as they help inhibit oxidation and reactions triggered by oxygen, peroxides, or free radicals. The total flavonoid and total phenolic contents and antioxidant activities of the coquina clam *Donax variabilis* collected from Luuk Buhi, Tongsinah, Bongao, Tawi-Tawi, Philippines were determined using Aluminum Chloride complex forming assay for the total flavonoid contents, Folin-Ciocalteu reagents with analytic grade gallic acid as the standard for the total phenolic contents, and the antioxidant activities were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. The results showed that the highest phenolic content was found in the methanol extract, with 0.29 ± 0.007 mg GA g^{-1} , followed by ethyl acetate extract with 0.22 ± 0.007 mg GA g^{-1} . The highest flavonoid content was present in the methanol extract with 0.16 ± 0.022 mg QE g^{-1} , followed by the ethyl acetate extract with 0.09 ± 0.036 mg QE g^{-1} . The strongest free radical scavenging activity (DPPH) was recorded for the methanol extract, 0.067 ± 0.001 mg Trolox g^{-1} , followed by the ethyl acetate extract with 0.045 ± 0.001 mg Trolox g^{-1} . The results of the radical cation decolorization power (ABTS) assay showed the methanol extract with 0.043 ± 0.001 mg Trolox g^{-1} and ethyl acetate extract with 0.038 ± 0.001 mg Trolox g^{-1} . The results suggest that coquina clam has strong antioxidant potential and could be a source of natural antioxidant compounds that could be used in the pharmaceutical industries.

Key Words: free radical scavenging, marine bivalves, natural antioxidants, oxidative stress, Tawi-Tawi coast.

Introduction. Marine organisms, particularly invertebrates such as mollusks, crustaceans, and algae, are rich reservoirs of natural antioxidant compounds, which have been explored for therapeutic applications in counteracting oxidative stress and inflammation, both of which are recognized as major contributors to chronic conditions such as cancer, cardiovascular diseases, and neurodegenerative disorders (Tabakaeva et al 2020). In particular, bivalve mollusks have attracted growing attention due to their bioactive peptides, polysaccharides, and phenolic compounds that exhibit antioxidant and anti-inflammatory properties. Polysaccharides derived from bivalves have been shown to possess notable antioxidant, immunomodulatory, anticoagulant, and anticancer activities (Tan et al 2023). Likewise, protein hydrolysates from bivalves such as Manila clam (*Ruditapes philippinarum*) contains antioxidant peptides exhibiting potent scavenging activity (Jin et al 2024). Studies on bivalves reveal potent antioxidant activity; for example, enzymatic and acid hydrolysates from *Anadara broughtonii* demonstrated strong DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging abilities and effectively stabilized lipids in food systems, suggesting their potential as natural antioxidants (Tabakaeva et al 2020).

However, while many marine bivalves have been studied extensively, some species remain largely underexplored. One of these is the coquina clam (*Donax variabilis*), a

species of small edible saltwater bivalve belonging to the family Donacidae, commonly known as the bean clams. Coquina clam is typically found in the upper intertidal zone of sandy beaches, where it plays a critical ecological role as a filter feeder and serves as a food source for shorebirds and marine animals, and serves as host in complex host-parasite epibiont systems (Cobb et al 2011; McElroy et al 2023). Its shell, ranging from 15 to 25 mm in length, is noted for its vibrant color variation, from almost white, through yellow, pink, orange, red, purple to brownish and blueish, with or without the presence of darker rays, contributing to its ecological distinctiveness and visual appeal (Luna & To 2014). Their local abundance is also closely tied to beach ecosystem health, thriving in undisturbed, naturally formed sandy shorelines and being highly sensitive to habitat alteration like beach nourishment (Cobb et al 2011; Paris et al 2023).

Biologically, coquina clams are characterized by rapid growth and remarkably short lifespans, with a total lifespan of only 3 to 5 months, which is significantly shorter than previously estimated one- to two-year lifespans (Jones et al 2004). Despite its brief individual lifespans, population recruitment events occur approximately every eight months, indicating a high turnover rate and environmental sensitivity (Cobb et al 2011). In environments lacking moving water, these clams struggle to feed effectively and survive only briefly, as they depend on water currents for filter-feeding nutrients. As most mollusks, the coquina clam is host to a variety of parasitic trematodes (McElroy et al 2023). Recent molecular evidence has clarified that it functions as a facultative intermediate host for multiple trematodes, contradicting previous assumptions of obligatory host relationships, highlighting its complex ecological interactions (Hill-Spanik et al 2021).

Despite its ecological importance and promising biological characteristics, the antioxidant potential of coquina clam remains insufficiently characterized. Recent investigations have uncovered promising bioactivities: a purified polysaccharide from coquina clam exhibited anticancer effects by inducing reactive oxygen species-mediated apoptosis in A549 lung cancer cells (Sahayanathan et al 2020). Similarly, crude protein extracts especially from its mantle showed significant antiproliferative effects against multiple cancer cell lines, including A549 ($IC_{50} \approx 100 \mu\text{g mL}^{-1}$), MCF-7, and HT-29 (Sahayanathan et al 2018). Further, polysaccharides isolated from coquina clam displayed selective cytotoxicity toward various cancer cell lines (e.g., MDA-MB-231, HeLa, HepG2, HT-29), with IC_{50} values ranging from 200 to 400 $\mu\text{g mL}^{-1}$ (Padmanaban et al 2022).

However, these studies have primarily focused on its anticancer activity, and few have examined its antioxidant profile in detail, particularly its radical-scavenging capacity and content of phenolic or flavonoid compounds. Understanding the antioxidant activity of coquina clam could provide insights into its potential application in nutraceuticals or functional food development, particularly as interest in marine-derived antioxidants continues to grow.

The purpose of this study is to address this knowledge gap by evaluating the total phenolic content (TPC), total flavonoid content (TFC), and radical scavenging activity using DPPH and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assays in extracts obtained from coquina clam collected from the coastal waters of Luuk Buhi, Tongsinah, Bongao, Tawi-Tawi, Philippines. Through this, the study aims to generate baseline data on the species' antioxidant properties and contribute to the broader exploration of marine mollusks as alternative sources of bioactive compounds with therapeutic potential.

Material and Method. The study was conducted from March to May 2024. Samples of coquina clam were collected from the coastal waters of Luuk Buhi, Tongsinah (Figure 1), located in the municipality of Bongao, province of Tawi-Tawi within the Bangsamoro Autonomous Region in Muslim Mindanao (BARMM). The area features coral reefs and seagrass beds, providing a natural habitat for coquina clam. The chemicals and reagents used in the study were methanol, ethyl acetate, NaNO_2 , AlCl_3 , NaOH , FC reagent, Na_2CO_3 , ABTS reagent and DPPH reagent. All the chemicals used in the study were analytical grade and were obtained from Mindanao State University Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), Sanga-Sanga, Bongao, Tawi-Tawi, Philippines.

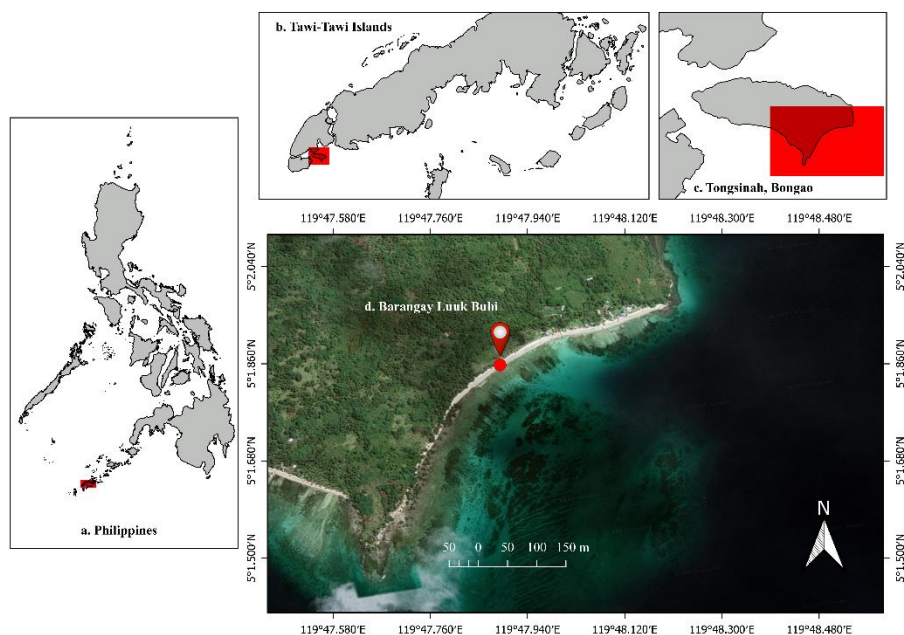


Figure 1. Map of the sampling site at Barangay Luuk Bui, Tongsinah, Bongao, Tawi-Tawi.

Collection and processing of samples. Live specimens of coquina clam were collected manually during low tide. After collection, the clams were rinsed with clean seawater to remove sand and debris, then thoroughly washed with distilled water. The soft tissue was carefully separated from the shells and homogenized using a blender. The homogenized tissue was air-dried under shade for 48 hours and further dried in an oven at 40°C until a constant weight was achieved. The dried tissue was then ground into fine powder and stored in airtight containers at 4°C until further use.

Methanol extraction procedure. A 25.00 g portion of the powdered coquina clam tissue was soaked in 95% methanol. The mixture was kept at room temperature for 48 hours, then filtered using Whatman No. 1 filter paper. The residue was re-extracted with an additional 100 mL of methanol for one hour and filtered again. The combined filtrates were stored in a refrigerator at 4°C until analysis (Parkash et al 2015).

Ethyl acetate extraction procedure. Another 25.00 g sample of the powdered tissue was separately extracted using 200 mL of ethyl acetate following the same procedure in Parkash et al (2015). Initial soaking was done for 48 hours, filtration, re-soaking with 100 mL of ethyl acetate for one hour, and final filtration. The combined filtrates were stored in a refrigerator at 4°C until analysis.

Total phenolic content (TPC). The Folin-Ciocalteu method from Villabeto et al (2021) was used to quantify the total phenolic content for both the methanol and ethyl acetate extracts. Using a 25 mL vial, a 0.5 mL aliquot of each extract was mixed with 4.5 mL of distilled water, followed by 0.5 mL of Folin-Ciocalteu reagent and 10 mL of 7% sodium carbonate (Na_2CO_3). The Folin-Ciocalteu reagent was prepared with 0.0166 g of gallic acid monohydrate dissolved in absolute methanol and diluted to 50 mL. Then, 2.5 mL distilled water was added to make a 12.5 mL solution. The solution was incubated for 90 minutes under room temperature, and then the absorbance was read at 750 nm using a Perkin Elmer Lambda 25 UV/VIS spectrophotometer. TPC was expressed in mg gallic acid equivalent per gram (mg GA g^{-1}). All the experiments were performed in triplicates.

Total flavonoid content (TFC). Total flavonoid content was determined via the aluminum chloride complex-forming assay. In a clean vial, 1 mL of 1000 ppm extract was mixed with 5 mL of absolute methanol, followed by 0.3 mL of 5% sodium nitrite (NaNO_2). After 5

minutes at room temperature, 0.6 mL 10% aluminum chloride (AlCl₃) was added. After another 6 minutes, 2 mL of 1 mM sodium hydroxide (NaOH) and 1.10 mL of absolute methanol were added. The mixture was incubated for 20 minutes at room temperature, and the absorbance then was read at 510 nm using again a Perkin Elmer Lambda 25 UV/VIS spectrophotometer. Results were expressed as mg quercetin equivalents per gram (mg QE g⁻¹). All assays were performed in triplicates (Pekal & Pырzyska 2014; Matic' et al 2017).

DPPH radical scavenging assay. Antioxidant activity was assessed using the DPPH method following protocols from Gulcin & Alwasel (2023). A 0.2 mL aliquot of 1000 ppm extract was added to 5.8 mL of 0.01 mM DPPH solution. The DPPH solution was prepared by dissolving 0.0250 g of Trolox in absolute ethanol and diluting to 100 mL in a volumetric flask. The mixture was incubated in the dark for 30 minutes at room temperature. The absorbance was read at 517 nm using a Perkin Elmer Lambda 25 UV/VIS spectrophotometer.

ABTS radical cation decolorization assay. The ABTS scavenging activity was measured following the method of Irondi et al (2017) with slight modifications. A 0.2 mL aliquot of 1000 ppm extract was combined with 5.8 mL of the ABTS reagent, prepared by dissolving 0.0250 g of Trolox in absolute ethanol and diluting to 100 mL in a volumetric flask. The mixture was then incubated for 6 minutes at room temperature, and the absorbance was then read at 734 nm using a Perkin Elmer Lambda 25 UV/VIS spectrophotometer.

Results and Discussion. The antioxidant profile of coquina clam extracts obtained using methanol and ethyl acetate is presented in Table 1. Results revealed that the methanol extract exhibited notably higher values across all parameters which supports that a more polar solvent like methanol enhances the extraction of phenolic and flavonoid compounds responsible for antioxidant activity (Mohammed et al 2022; Vladkova et al 2022).

Table 1

Antioxidant substances and properties of *D. variabilis*

Solvent	TPC (mg GA g ⁻¹)	TFC (mg QE g ⁻¹)	DPPH (mg Trolox g ⁻¹)	ABTS (mg Trolox g ⁻¹)
Methanol	0.29±0.007	0.16±0.022	0.067±0.001	0.043±0.001
Ethyl acetate	0.22±0.007	0.09±0.036	0.045±0.001	0.038±0

Total phenolic content. The presence of phenolic compounds was first investigated through phytochemical screening using ferric chloride test, and quantified via Folin-Ciocalteu assay, with analytical-grade gallic acid as the standard, since this is the first study specifically focused on its biochemical components. Upon the addition of few drops of ferric chloride solution to the sample, a red coloration developed, confirming the presence of phenolic compounds. As in many studies on various marine species, coquina clams are also rich in phenolic compounds. Several studies reported the presence of phenolic compounds in clams. In the study of Ramasamy & Balasubramanian (2012), phenolics and other various bioactive compounds were detected in the marine clam *Anadara granosa* which exhibited antimicrobial activity.

Phenolic compounds from marine organisms are far less studied than those from terrestrial sources due to their structural diversity and variability and the need for powerful analytical tools (Mateos et al 2020). The TPC of the methanol extraction of coquina clam was 0.29±0.007 mg GA g⁻¹, while the ethyl acetate extract was 0.22±0.007 mg GA g⁻¹. In the study by Joy et al (2016), TPC measured at 5 mg mL⁻¹ in two edible bivalve clams were recorded as 88.62 mg GA g⁻¹ for *Paphia malabarica* and 73.87 mg GA g⁻¹ for *Villorita cyprinoides*. According to Tomsone et al (2012), the solvent polarity is a very significant factor in extraction yield. The higher the polarity of the solvent, the better the solubility and extraction efficiency. Polyphenols including phenolic acids are believed to confer numerous health benefits (Catarino et al 2021). In marine species, these compounds play significant ecological and chemical defenses, contributing to survival and resilience against

environmental stressors (Mannino & Micheli 2020). Marine phenolics are also known to provide protection against pathogens and predators, fouling organisms, oxidative stress, inflammation and cancer prevention (Catarino et al 2021; Matulja et al 2022).

Total flavonoid content. The presence of flavonoids was first investigated through phytochemical screening using tannic acid test and quantified using the aluminum chloride complex forming assay. Upon the addition of tannic acid solution to the extract, a yellow-colored precipitate formed, confirming the presence of flavonoid compounds. Flavonoids are low-molecular-weight secondary metabolites that can be found abundantly in marine organisms (Kumar & Pandey 2013). The highest TFC in coquina clams was recorded in the methanol extract 0.16 ± 0.022 mg QE g⁻¹, followed by the ethyl acetate extract at 0.09 ± 0.036 mg QE g⁻¹. In comparison, freshwater clam (*Corbicula fluminea*) TFC measured in ethyl acetate extract was 43.84 mg QE g⁻¹ Villabeto et al (2021).

Several flavonoids have been reported to exhibit a variety of pharmacological activities including antioxidant, antimicrobial, antitumor, anticoagulant, and antidiabetic effects (Yang et al 2019; Villabeto et al 2021), as well as demonstrating its potential as bioactive compounds of pharmaceutical and nutraceutical importance (Chakraborty & Joy 2020). These properties support the clam's potential as potent antioxidant source. This group of compounds has been commonly detected in marine bivalves, including *P. malabarica*, *T. granosa*, *C. fluminea*, and *Donax cuneatus* (Nazeer et al 2012; Eswar et al 2015; Yang et al 2019; Villabeto et al 2021).

Scavenging activity (DPPH) assay. Marine organisms that produce secondary metabolites often exhibit radical scavenging activity, and the extract of coquina clam demonstrated potent antioxidant activity when tested using the DPPH scavenging assay method. Antioxidants are compounds that scavenge free radicals by interrupting radical chain reactions or preventing the formation of reactive oxidative species in the first place, which in turn protects the cells from oxidative damage (Chaudhary et al 2023; Kozlov et al 2024; Tumilaar et al 2024). The presence of antioxidants in aquatic organisms may influence photochemical reactions and support the health and resilience of aquatic life (Jia 2025). In this study, the methanol extract of the coquina clam showed the strongest free radical scavenging activity, with a value of 0.067 ± 0.001 mg Trolox g⁻¹, followed by the ethyl acetate extract at 0.045 ± 0.001 mg Trolox g⁻¹. These findings are consistent with Joy et al (2016), who also reported a significant DPPH radical scavenging activity of 0.76 mg Trolox g⁻¹ in two bivalves' species, *P. malabarica* and *V. cyprinoides*. These results indicate that coquina clam contains bioactive compounds with antioxidant properties, and that methanol, a more polar solvent, is more effective in extracting these compounds in coquina clams. This observed antioxidant potential of coquina clam extracts signals its relevance as a promising natural source of bioactive compounds, which could further support the investigation into its therapeutic and nutraceutical application.

ABTS radical cation decolorization assay. The ABTS assay is based on the interaction between an antioxidant and the pre-generated ABTS⁺ radical cation, which can be quantitatively detected via the bleaching effect on its absorption maxima, typically at 414, 417, 645, 734, and 815 nm (Cano et al 2023; Munteanu & Apetrei 2021). In this study, the coquina clam extract displayed its highest ABTS radical scavenging activity in the methanol extract at 0.043 ± 0.001 mg Trolox g⁻¹, followed by ethyl acetate extract at 0.038 ± 0 mg Trolox g⁻¹. These results show that coquina clam possess antioxidant potential, however, their activity is comparatively lower than that reported for other edible bivalves. For instance, Joy et al (2016) documented substantially higher ABTS radical scavenging activities of 1.27 mg Trolox g⁻¹ in *P. malabarica* and 1.41 mg Trolox g⁻¹ in *V. cyprinoides*. The lower values observed in coquina clams can be attributed to species-specific differences in bioactive compound composition, extraction efficiency, or environmental factors influencing metabolite production (Istomina et al 2021; Villabeto et al 2021; Jing et al 2023). Nonetheless, the presence of a consistent radical scavenging activity supports the role of coquina clams as a source of natural antioxidants, albeit at a lower value compared to other bivalves.

Conclusions. The results of the study show that the extracts of *Donax variabilis* are a potential source of natural antioxidants which appears to be largely influenced by their total phenolic and total flavonoid contents. These findings suggest that, although the observed antioxidant levels were relatively modest compared to other bivalves, the species may still serve as a supplementary natural source of antioxidants with possible applications in food and pharmaceutical industries. It is recommended that further study should be conducted, particularly on the quantitative determination of total phenolics, total flavonoids, and antioxidant activities of the crude extracts of *D. variabilis* to better establish its potential health and industrial benefits.

Conflict of interest. The authors declare that there is no conflict of interest.

Acknowledgements. The authors are grateful to the College of Education of Mindanao State University - Tawi-Tawi College of Technology and Oceanography (MSU-TCTO) for the encouragement in the preparation and presentation of this paper. They also wish to acknowledge the chancellor of the university for her support.

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Received: 06 June 2025. Accepted: 28 August 2025. Published online: 16 November 2025.

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How to cite this article:

Tangon E., Pon M. M. R., Pajiji J. A., Eldani N. A. S., Alawi F. M. A., Sala R. U., 2025 Antioxidant substances and properties of coquina clam (*Donax variabilis*) of the coastal waters of Luuk Buhi, Tongsinah, Bongao, Tawi-Tawi, Philippines. *AACL Bioflux* 18(6):2488-2495.