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Analysis of zoochemical from *Meretrix casta* (Mollusca: Bivalvia) extracts, collected from Rameswaram, Tamil Nadu, India and their pharmaceutical activities

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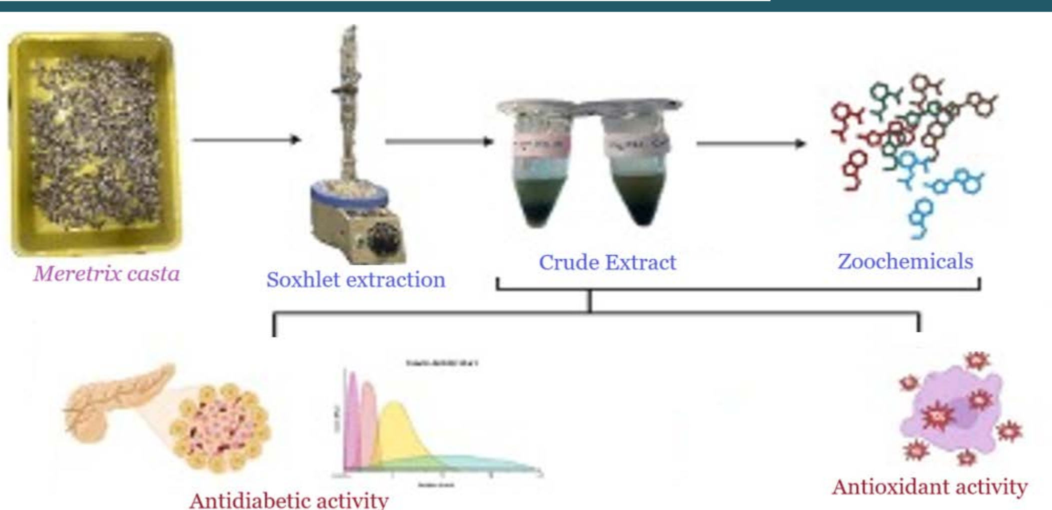
Article

ABSTRACT



The marine ecosystem's diverse animal species offer a unique opportunity to discover marine-derived natural products. While numerous invertebrates have been studied, research on Indian marine invertebrates, especially *Meretrix casta*, remains limited. This study explores the zoochemical composition of ethyl acetate and methanolic extracts from *Meretrix casta* off Rameswaram, Tamil Nadu, India, and evaluates their bioactive potential, focusing on antioxidant properties, glucose uptake in yeast cells, and alpha-amylase activity. The results reveal the presence of alkaloids, flavonoids, polyphenols, sterols, terpenoids, and cardiac glycosides in both extracts, highlighting their bioactive potential. Although their antioxidant capacity is slightly lower than ascorbic acid, the extracts demonstrated significant alpha-amylase inhibition, suggesting their potential in blood sugar regulation and diabetes management. These findings underscore the therapeutic potential of *M. casta* in developing anti-diabetic compounds, warranting further pharmacological exploration.

Keywords: Marine natural products, Marine Ethnomedicine, Marine Pharmacognosy, Therapeutic exploration, Marine Anti-diabetic Agents

INTRODUCTION

Zoochemicals, compounds of natural origin derived from animal byproducts, have garnered significant attention in the pharmaceutical realm. These compounds, if isolated and studied in detail, hold the potential to address various health issues, offering unique solutions inspired by nature to humanity's medical problems.[1] The marine milieu, a treasure trove for pharmaceutical prospecting, is a testament to its potential.[2] Marine organisms are an excellent resource for producing a vast spectrum of compounds, each showing different biological properties.[3] Our research on Indian marine invertebrates, particularly *Meretrix casta*, contributes to this body of knowledge, highlighting these organisms' importance in natural products and drug development. This research expands our understanding of marine biodiversity and inspires further exploration and innovation in anti-diabetic compounds.[4]

Molluscs, the fascinating marine organisms found and described by Linnaeus in 1758,[5] have carved a niche for some of their molluscan members among these. These beings range from the silent abyss of the deep seas to the dynamic shorelines and vibrant tropics, showing a good range of ecological and evolutionary adaptations. This diversity is reflected in their survival capacity and the many biologically active compounds they developed over very

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long periods.[6] For instance, deep-sea snails, living in the ocean's dark depths where light hardly passes through, have created unique compounds to cope with this mysterious environment.[7] Shoreline clams, subjected to alternation of immersion and emersion, have their biochemical arsenal attuned to tolerate such changes.[8] Valid for form, tropical cephalopods, masters in disguises and locomotion, have compounds assisting them in their complex behaviours and interactions.[9] Their extended evolutionary history, massive habitat range, and varied physiological complexities make molluscs a goldmine of various potential biopharmaceutical compounds.[10] Marine bivalve Mollusca species of *Meretrix casta* are known to exhibit a wide range of bioactive compounds like proteins, peptides, fatty acids, minerals, and so on, to endow lots of therapeutic properties that have attracted significant attention in pharmaceutical research through several approaches.[11]

This clam showed significant free radical scavenging activity, making it suitable for combating oxidative stress-related diseases. Furthermore, its antimicrobial contributions will make it a good candidate for developing new antibiotics, mainly due to the rise in antibiotic resistance.[12] In drug resistance, developing new antibiotics is essential, as it helps fight against antibiotic-resistant bacteria that combat infections that are no longer responsive to the current treatment. The clam extracts may contain bioactive compounds that exhibit antimicrobial activity, which could be developed as new antibiotics or potentiate the available ones in the market[13]. The increasing prevalence of diabetes across the globe demands discoveries of compounds that are radical and will either fight or manage the disease.[14]

While several assays are available for bioactivity assessment, our research provides comprehensive insights into the modulatory interactions of zoochemical constituents with antioxidant ability and glucose uptake. This approach offers a more encompassing perspective of the molluscs' therapeutic prospects, extending beyond diabetes management to various other oxidative stress-related conditions. Our study is explorative, aiming to elucidate the zoochemical profile of *M. casta*, explore their antioxidant properties, and evaluate their potential in promoting glucose uptake in yeast cells - an in-vitro anti-diabetic activity. This research is a significant step towards bridging the knowledge space in the field of natural sources of anti-diabetic compounds, paving the way for future therapeutic innovations and inspiring further exploration in this area[15]. The potential of *M. casta* as a source of bioactive compounds offers hope for developing new and effective treatments for oxidative stress-related diseases and diabetes.

Materials and Methods

The animal model, *Meretrix casta,* a yellow clam used for the present study, was collected 300 meters from the coastal bank of Rameswaram, Tamil Nadu, India.

The coastal region of Rameswaram, situated around 9.2876° N latitude and 79.3129° E longitude, serves as a vital habitat for various marine species, notably *M. casta*. These clams prefer intertidal zones characterized by sandy or muddy substrates, prevalent along the Rameswaram coast.

Collection and Identification of *Meretrix casta: M. casta* samples were collected live by hand-picking from the above coastal region during July 2023. The species were identified using morphological characteristics based on the field guide[16] and the World Registry of Marine Species (WoRMS). After establishing the species identity, the gathered specimens were kept in suitable solvents and brought to the laboratory for additional testing. Initially, the shells were carefully removed from the *M. casta* specimens using a sharp blade. Following shell removal, the flesh samples were thoroughly cleansed by rinsing with tap water several times to remove surface contaminants and debris, followed by a wash with distilled water.

Preparation of crude extract

About 100 ml of ethyl acetate and methanol were taken, and the whole flesh sample was homogenised for about 48 hours to ensure adequate extraction of the bioactive chemicals present in *M. casta*. After homogenisation, samples were centrifuged at 1000 RPM (9490Xg) (OS20-S, D Lab, Romania) for twenty minutes at room temperature. Meticulously collected supernatant obtained from ethyl acetate extract (EA) and methanol extract (ME) was concentrated under reduced pressure by using a rotary evaporator (RE 100-S, D Lab, Singapore). The pooled concentrated extracts were stored separately at 4°C until further analysis was performed.[17]

Qualitative Analysis of Zoochemicals

1. Tests for Alkaloids: The presence of alkaloids is determined by the formation of a yellow precipitate[18]. To 10mg of both the extracts (EE and ME), 2 mL of 2% hydrochloric acid was added and boiled at 95°C for 15 min in a water bath. After boiling, the mixture was filtered using Whatman filter paper, and the filtrate was collected. Then, Then, 1 mL of the filtrate was treated with 2 drops of 1% picric acid.

2. Flavonoids Tests: To carry out this test, 10 mg of extract was mixed with 5 mL of ethanol and a piece of magnesium ribbon was added to it. Subsequently, 5 drops of concentrated hydrochloric acid were added and the reaction was allowed to proceed for 10 min and observed for the developed colour. The development of a pink or red colour indicated the presence of flavonoids.[18]

3. Polyphenols tests: For polyphenols, 10 mg of the extracts were mixed with 2 mL of distilled water and two drops of a 5% ferric chloride solution and allowed to react. The presence of polyphenols is confirmed if a brown precipitate is formed.[18]

4. Test for Saponins: To detect saponins, 10 mg of both extracts were dissolved in 5 mL of distilled water. The solution was then boiled in a water bath at 70°C. The presence of saponins was indicated by the formation of hexagonal foam.[18]

5. Test for Sterols: A solution of chloroform and glacial acetic acid (1 mL each) was prepared cold at 0°C. To this, one drop of concentrated sulphuric acid was added followed by 5 mL of the extracted suspension. The blue, green, red, or orange colour denotes the presence of sterols.[18]

6. A test for terpenoids: For terpenoids, 1-2 mg of the extracts was dissolved in 1 mL of chloroform. This was added with 1 mL of a concentrated solution of sulphuric acid. The appearance of two separate red or yellow phases shows the presence of terpenoids and sterols.[18]

7. Tests for Cardiac Glycosides: A mixture of 1 mL glacial acetic acid, two drops of concentrated sulfuric acid and two drops of 5% ferric chloride was added to the 10 mg of the extracts. If after addition a green-blue shade had developed, this would confirm the presence of cardiac glycosides.[18]

Quantitative Analysis of Zoochemicals

Estimation of flavonoids

Total flavonoid content was estimated by the aluminum chloride method[19]. Firstly, 0.25 mL of both extracts was aliquoted separately in test tubes which were mixed with 1.25 mL of distilled water and incubated for 5 minutes at room temperature. Following this, 0.3 mL of 10% aluminium chloride and 2 mL of 5% sodium nitrite were added to the mixture. This mixture was incubated in a dark room for 45 minutes and the absorbance was taken at 510 nm using a spectrophotometer (UV-1800, Shimadzu, Japan). Quercetin was made use of as a standard for comparison and results were expressed in equivalent units of it.

Estimation of Phenolic compounds

The determination of total phenolic content followed the method reported by Singleton et al.[19] using Folin-Ciocalteu's reagent (FCR). To 1 mL of extract, 1 mL of 1:10 v/v diluted FCR was added. Then, 5 mL of 9% sodium carbonate solution was added. The volume then was adjusted to the total in the tubes 10 mL with distilled water. The mixture was mixed well and left for 90 minutes at room temperature in the dark. Absorbance was taken using a spectrophotometer at 750 nm. A calibration curve was referenced with gallic acid and the phenolic contents were expressed as milligram of gallic acid per gram of dry weight.

Estimation of Tannin

To 1 mL of extract, 5.5 ml of distilled water, 0.5 mL of FCR and 1 ml of 35% sodium carbonate were added for the estimation of tannin content. The mixture was diluted to a total volume of 10 mL using distilled water and then incubated for half an hour[19]. The absorbance of this reaction mixture was measured at 700 nm using a spectrophotometer, using tannic acid as a reference standard and measured in milligram per gram of dry weight[19].

Estimate of Alkaloid

For the estimation of alkaloids, 0.2g extract was added to 25 mL of distilled water and 10 mL of diluted ammonium. After this, 20 mL of chloroform was added. The chloroform layer after formation was separated carefully, evaporated and got redissolved in distilled water of the residue. To the redissolved extract, 20mL of 0.1N hydrochloric acid was added, and this got titrated against 0.1N sodium hydroxide. A few drops of methyl orange were used as an indicator. The equivalence of 0.1N of sodium hydroxide to 0.03384g atropine was inferred from the quantity of alkaloids[19].

Antioxidant tests

2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

The extract's free-radical scavenging was determined by the use of DPPH (2,2-diphenyl-1-picrylhydrazyl) assay[20]. The DPPH solution was freshly prepared in methanol at 0.004% w/v and 1 mL of it was taken. To this, add 3 ml of sample dissolved up to a concentration of 100 µg/ml in methanol. The mixture was allowed to stand at room temperature for 20 minutes in the dark. After incubation, the mixture was vortexed thoroughly followed by taking its absorbance at 517 nm with spectrophotometer. In this experiment, the reference compound is ascorbic acid while methanol served as blank control. The following formula was used to calculate the percentage of DPPH free radical scavenging activity from the absorbance value obtained

$$\text{percent inhibition} = (A_0 - A_1)/A_0 \ X\ 100$$

Where A_0= Absorbance of control. A_1= Absorbance of the sample

Ferric Reducing Antioxidant Power Assay

The antioxidant ability of the extracts was evaluated against the Ferric Reducing Antioxidant Power (FRAP) assay[21]. To begin with, 1 mL of extract was taken and thoroughly mixed with phosphate-buffered saline (PBS) 2.5 mL and potassium ferricyanide solution 2.5 mL from 1%. This particular mixture was incubated at 50°C for 20 minutes to activate the required chemical reactions. The mixture was cooled then to room temperature. Following this, 10% trichloroacetic acid (TCA) (2 mL) was added to this cooled mixture, precipitating proteins as well as other macromolecules. Then, centrifugation was done at 860 g for ten minutes. The clear supernatant was then carefully decanted following centrifugation. Distilled water (2.5 mL) and ferric chloride solution (0.1%,0.5 mL) were subsequently added to this supernatant. Finally, the absorbance of the reaction mixture was measured by a spectrophotometer using a wavelength of 700 nm. A standard curve with known concentrations of ascorbic acid was generated for quantification and interpretation of results. Later, these absorbance values were compared to that standard curve in order to calculate the equivalent amount of ascorbic acid present in terms of antioxidant capacity of that extract subjected to experimentations

Glucose Uptake Assay by Yeast Cell

Cirillo[22] established a methodology based on which a glucose uptake assay was designed using baker's yeast suspension. The 1% w/v solution of baker's yeast was prepared by dissolving the yeast in distilled water and allowing it to stand still overnight at room temperature (25°C). The yeast solution was then centrifuged at 1680 g for 5 minutes. By repeating this step with distilled water, clear supernatant is obtained after several times of centrifuging. The clear liquid was also diluted with distilled water in a 1:9 ratio to produce 10% v/v yeast cell suspension. Additionally, this extract was mixed with dimethyl sulfoxide (DMSO) tube and it contained different concentrations of glucose (5, 10 and 25 mM), then incubated for 10 min at 37°C. After incubation, the glucose-extract mixture received 100 µL of the yeast suspension that were thoroughly mixed; then further incubated at the same temperature for about an additional sixty minutes so as to initiate the reaction. These reaction mixtures were then centrifuged at 1370 g for five minutes. This spectrophotometer subsequently determined residual glucose concentration in the samples, and absorbance was measured at wavelength λ=520 nm. At the same wavelength, the

absorbance of the control was also checked as a reference. The overall increase in glucose uptake was determined using a formula.

$$\% \text{ Glucose Uptake} = (A_0 - A_1)/A_0 \text{ X } 100$$

Where A_0= The absorbance of control. A_1= The absorbance of sample

ALPHA-AMYLASE INHIBITORY ASSAY

Phosphate buffer with 4 Units/mL of α-amylase was used in the enzymatic assay for α-amylase inhibition[23]. Meanwhile, a 1% w/v starch solution in Phosphate Buffered Saline (PBS) was prepared and heated until it became clear in a water bath. To perform the assay, a sterile microcentrifuge tube containing 400 µl of the starch solution, 160 µl of distilled water and 40 µl of extract were mixed. The reaction was initiated by adding an α-amylase solution of 200 µl. The mixture was then incubated at a room temperature controlled to be at 25°C for 3 minutes. After that, another microcentrifuge tube containing 100 µl of the 3,5-Dinitrosalicylic acid (DNS) reagent was pipetted with a sample of the reaction mixture consisting of about 200 µl. This tube was placed in a water bath where its temperature ranged between 85°C and 90°C and incubation period for 15 min. The resulting-coloured sample after incubation was diluted by adding carefully to approximately 700 µl of distilled water and absorbance was taken at 540 nm using UV-Vis spectrophotometer. Based on the absorbance values, the inhibitory activity of the extract against α-amylase was computed and articulated as a percentage inhibition, derived using the equation.

$$\% \ \alpha - \text{amylase Activity} = (A_0 - A_1)/A_0 \text{ X } 100$$

Where A_0= The absorbance of control. A_1= The absorbance of sample

RESULTS AND DISCUSSION

ZOOCHEMICAL COMPOUNDS

Our study found that ethyl acetate and methanolic extracts of *M. casta* contain various bioactive compounds, including alkaloids, flavonoids, polyphenols, sterols, terpenoids, and cardiac glycosides. These compounds were identified based on the characteristic colours they develop upon identification tests (Fig. 1). Our findings are in line with those reported by Joenilo et al.[24] and Sreejamole and Radhakrishnan[25] in the crude methanolic extract of green mussel, *Perna viridis*. However, our study revealed that flavonoids and cardiac glycosides were also present in EE and ME of the edible mussel *M. casta*. Alkaloids in molluscs highlight their ecological defensive strategies against predators and suggest potential therapeutic applications such as novel analgesics, antitumor agents, or antibiotics.[26] The detection of flavonoids and polyphenols in molluscs, such as *M. casta*, hints at either unique metabolic pathways or the possibility of these compounds being accumulated from their diet, possibly from algae or other consumed microorganisms like bacteria, fungi, and plankton. Their inherent antioxidant nature could be repurposed for therapeutic interventions[27]. Saponins in some marine molluscs serve as their protective shield against predators and have promising pharmaceutical applications due to their immune-modulatory and antifungal attributes[28]. While cholesterol is the predominant sterol in molluscs, aiding in maintaining cell membrane fluidity, other cholesterol-derived compounds might present bioactive properties.[29] Terpenoids, especially those sourced from marine molluscs, often act as defensive metabolites. Some marine molluscs' sequestering terpenoids from dietary sources, like sponges, underscores their potential anti-inflammatory and antibacterial properties.[30] Lastly, the intriguing presence of cardiac glycosides in molluscs suggests unique metabolic pathways or potential dietary accumulation. Known for their effect on human cardiac muscle contractility, these compounds open doors to therapeutic avenues.[31]

Zoochemicals Test	Observation		Result
Alkaloids		Yellow Turbidity	+
Flavonoids		Pinkish Red Colour	+
Polyphenols		Brown Precipitate	+
Saponins		No Froth	-
Sterols		Blue Green Colouration	+
Terpenoids		Red Colour	+
Cardiac Glycosides		Blue Green Colouration	+

Figure 1: Qualitative analysis of Zoochemicals from *M. casta* extract. + = Present **-** = Absent

Sterols, including cholesterol, are notably abundant in Bivalvia. Their main role is to defend against pathogenic bacteria, particularly during reproductive periods, and to safeguard the gastrointestinal mucosal lining.[32] *Paphia malabarica*, a yellow-footed bivalve clam, has also been found to contain terpenoids, namely isopimarane norditerpenoids, which act as precursors in the manufacture of sterols and steroids in bivalve species.[33] Quantitative analysis of Zoochemicals from *M. casta* extracts is

shown in Table 1. The total alkaloid content in the EE of *M. casta* was found to be 22.56 ± 1.95 mg AE/g, while in the ME, it was 21.43 ± 1.95 mg AE/g, and no significant differences could be observed. However, the total flavonoid content was significantly higher in EE of *M. casta* (71.52±5.57 mg QE/g) compared to ME (43.94±3.27 mg QE/g). Flavonoids, known for their antioxidant properties and potential health benefits, are a promising area for further research[34]. Bivalves, being organisms that primarily rely on filter-feeding and detritus consumption, obtain a diverse array of zoochemicals within their system, including terpenoids, flavonoids, and alkaloids, which are derived from sources such as algae and plankton[35]. The EE of *M. casta* had a total tannin content of 17.87 ± 0.16 (mg TAE/g), while the ME contained 19.50 ± 0.04 (mg TAE/g). Tannins are polyphenolic compounds known for their astringent properties and potential health effects. The EE and ME of *M. casta* showed similar total phenolic compound content of 21.73±0.18 (mg GAE/g) and 21.55±0.18 (mg GAE/g), respectively. There are detectable amounts of polyphenols and saponins in the crude methanolic extract of *P. viridis*. The saponins may have originated from the diatoms *Coscinodiscus spp*. And *Phaeodactylum spp.* that *M. casta* consumed as a feeding diet.[36]

Table 1: Quantitative analysis of Zoochemicals from EE and ME of *M. casta*

Zoochemicals	Ethyl acetate Extract (mg/g)	Methanolic Extract (mg/g)
Alkaloids (mg AE/g)	22.56 ± 1.95	21.43 ± 1.95
Flavonoids (mg QE/g)	71.52 ± 5.57	43.94 ± 3.27
Phenols (mg GAE/g)	21.73 ± 0.18	21.55 ± 0.18
Tannin (mg TAE/g)	17.87 ± 0.16	19.50 ± 0.04

ANTIOXIDANT ACTIVITY OF *M. CASTA* EXTRACTS

DPPH RADICAL SCAVENGING ACTIVITY

In the marine ecosystem, various bioactive substances with considerable potential for therapy are available. Marine organisms are especially notable among sea creatures because of their unique ways of processing energy and adapting to the tough conditions of the ocean. Their special traits help them survive and thrive in challenging environments[37]. The DPPH radical scavenging activity of ascorbic acid (at standard), EE, and ME at different concentrations (20, 40, 60, 80, and 100 µg/mL) are presented in Figure 1. Accordingly, ascorbic acid has the highest inhibition percentage at all concentrations, indicating its high antioxidant capacity. Both the ethyl acetate and methanolic extracts show a concentration-dependent increase in DPPH scavenging activity but do not exceed that of the standard at any tested concentration. The methanolic extract is consistently more active than the ethyl acetate, suggesting that the methanol solvent may have extracted more potent antioxidant compounds from the source material. At 100 µg/mL, both extracts exhibit substantial antioxidant activity with a narrowing gap in activity between them at higher concentrations (Fig 2). Marine animals like *Spongia officinalis* and *Hippospongia communis* have developed potent antioxidants. These substances combat environmental stress by neutralizing reactive oxygen species, chelating metal ions, and inhibiting stress-inducing enzymes. This adaptation showcases the complex biochemical strategies for survival in harsh marine environments[38,39]. Marine algae are primary producers in the ocean with solid antioxidants like phycocyanin and astaxanthin. These antioxidants are transferred through the food chain to animals like shrimps, crabs, and certain fish species. Echinoderms, such as sea cucumbers and sea urchins, also exhibit antioxidant properties, with *Holothuria atra* showing significant activity due to phenolic and flavonoid compounds.[40–42]

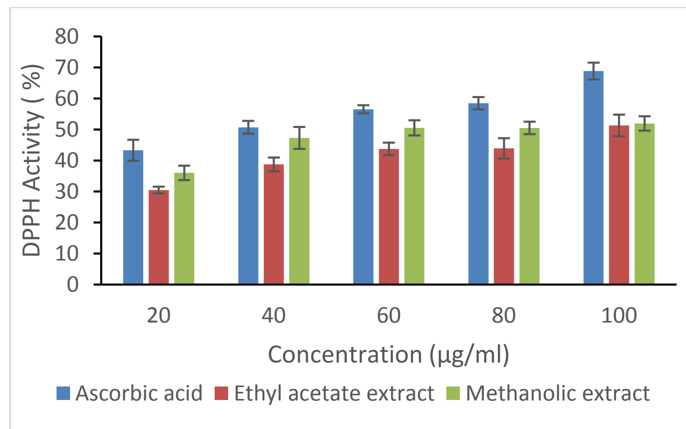

Figure 2: DPPH radical scavenging activity of *M. casta* extracts

Marine molluscs, such as *M. casta*, are no exception. Several species of marine molluscs, including oysters and clams, are documented to contain potent antioxidants. Their antioxidant potential can be attributed to their diet, as they often feed on phytoplankton rich in antioxidants and other bioactive compounds[43]. The EE and ME of *M. casta* as an antioxidant were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Both the extracts of *M. casta* scavenged DPPH radical in a concentration-dependent manner (Figure 2). The highest scavenging activity for both extracts occurred at a concentration of 100 µg/ml (EE: 51.32%; ME: 51.94%). The theory is that antioxidants' impact on DPPH may be due to their capacity to deliver hydrogen.

Nevertheless, it should be noted that ascorbic acid exhibited scavenging activity of 68.85%, while the extract had lower potency for DPPH radical scavenging. The findings suggest that crude tissue extract from green mussels has significant DPPH neutralizing capacity.[44] It was revealed during the study that the extract could give protons to dyes and function as a quencher of radicals. Besides, the presence of natural extracts with antioxidant effects can be attributed to their capacity to donate hydrogen. The change in color

of the solution to violet under spectrophotometry at a wavelength of 517 nm indicates that DPPH accepts electrons from antioxidant chemicals[45]. A recent study on the methanolic extract of *P. viridis* showed significant radical scavenging properties against DPPH radicals[46]. Kruk et al. found a direct link between phenolic content and the power to fight free radicals.[47]

Ferric reducing antioxidant power assay

Various marine organisms, including molluscs, seaweeds, and certain fishes, have emerged as reservoirs of antioxidant compounds. These marine-derived antioxidants, ranging from proteins, peptides, and lipids to polysaccharides, have gained traction due to their potential therapeutic benefits, especially in counteracting oxidative stress-induced diseases[48]. Several studies have underscored the unique antioxidant profiles of marine animals, often attributing their potency to the challenging and diverse marine habitats they thrive in, which necessitates robust antioxidative defense mechanisms[49]. The current study used the ferric reducing antioxidant power (FRAP) assay to assess the antioxidant capacity. The fundamental concept of this technique depends on the analyte's capacity to facilitate the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+})[50]. Examining the FRAP assay data, we observe an ascending trend in the antioxidative response as the concentration increases, which is common in such assays. The graph delineates that ascorbic acid consistently exhibits superior absorbance readings, underscoring its robust antioxidative properties.

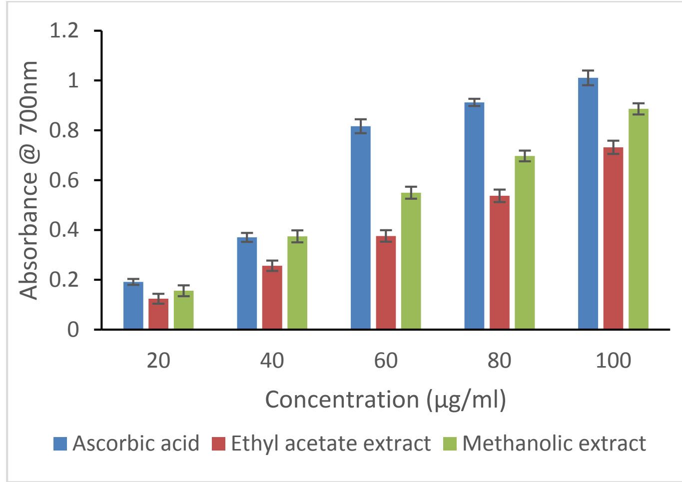

Figure 3: Ferric-reducing activity of *M. casta* extracts.

At the 100 µg/mL concentration, ascorbic acid's absorbance peaks, reflecting its high electron-donating capability, essential for neutralizing free radicals. Comparatively, the extracts derived from ethyl acetate and methanol manifest an incremental rise in FRAP values, yet neither reaches the benchmark set by ascorbic acid. Notably, at the 100 µg/mL concentration, the methanolic extract's absorbance approaches that of ascorbic acid, a hint that at sufficient concentrations, the methanolic extract could offer a substantial antioxidant effect. This rise might suggest that methanol is adept at solubilizing antioxidant biochemicals from the source material.

While ascorbic acid remains a potent antioxidant, the data suggest a potential for the extracts, mainly when used in higher quantities. The ranking of the reduction activity potential of extracts can be determined as follows: ascorbic acid exhibits the highest potential, followed by ME and EE.

The radical scavenging capacity of *P. viridis* extracts may be attributed to the presence of reducing sugars and trace elements. These findings demonstrate a strong correlation with previous studies of a similar nature.[51]

There is a positive correlation between absorbance and FRAP values, whereby an increase in absorbance corresponds to an increase in FRAP values. The antioxidant activity of *M. casta* extracts can be explained by their ability to donate hydrogen, which may be helped by the presence of phenolic compounds, bioactive peptides, alkaloids, reducing sugars, and trace elements in the extracts.[52]

Effect of *M. casta* Extracts on Glucose Uptake Capacity by Yeast Cells.

For several reasons, the Glucose Uptake Assay that employed yeast cells is a vital tool for scientific research. Firstly, it is essential in metabolic studies since it offers insights into how yeast cells can absorb glucose, which is crucial for their energy production and growth. Secondly, regarding drug development, the assay assists in screening potential agents or drugs that may influence glucose uptake, which can be particularly relevant concerning diabetes research.[53] As indicated by Figures 4, 5, and 6, the findings showed that extracts from *M. casta* enhanced glucose molecules' transport through the plasma membrane of yeast cells. Initial concentrations of 5 mM and 10 mM of *M. casta* extracts resulted in similar glucose uptake as that in the presence of metronidazole (Figure 4 & 5). However, no literature has been documented about glucose uptake activity in any marine organisms. In contrast to *M. casta* extracts, metronidazole slightly affected the glucose absorption rate of yeasts at a concentration of 25 mM (Figure 6). Moreover, when the concentration was increased from 20 mg/mL to 100 mg/mL, it accounted for approximately twenty percent of the observed sugar level. It reached nearly sixty percent, respectively (Fig. 4).

This implies that increasing the concentration of *M. casta* extract enhances the ability of yeast cells to absorb a greater quantity of glucose from their environment. The data indicate a clear relationship between the glucose concentration in the solution and the rate at which yeast cells absorb it. Specifically, lower glucose concentrations lead to a more rapid uptake by the yeast cells. This suggests that *M. casta* extracts may facilitate a more efficient glucose transport mechanism, which becomes particularly effective at lower glucose concentrations. Consequently, these findings highlight the potential of *M. casta* extracts to modulate glucose absorption dynamics in yeast cells, providing a basis for further exploration into their mechanisms and applications in metabolic regulation.[54] Additionally, yeast cells may exhibit distinct mechanisms of glucose absorption compared to other eukaryotic or human cells. While human cells often rely on specific transport systems such as the phosphotransferase enzyme system, yeast cells may utilize facilitated diffusion for glucose transport across their membranes.

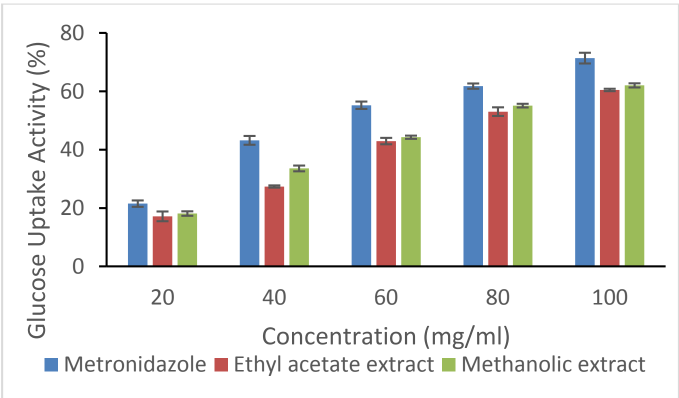

Figure 4: Stimulation of glucose uptake in yeast cells by *M. casta* extract at 5mM

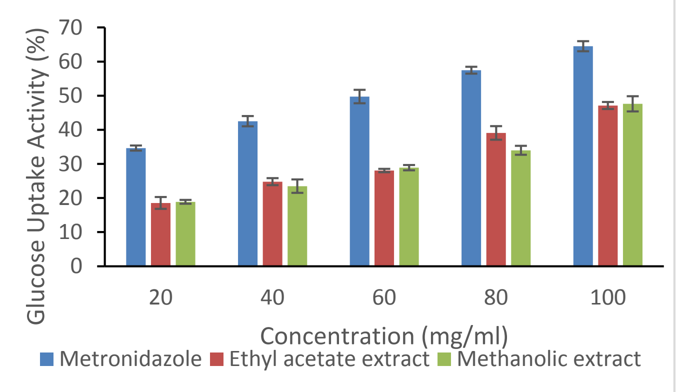

Figure 5: Stimulation of glucose uptake in yeast cells by *M. casta* extract at 10mM

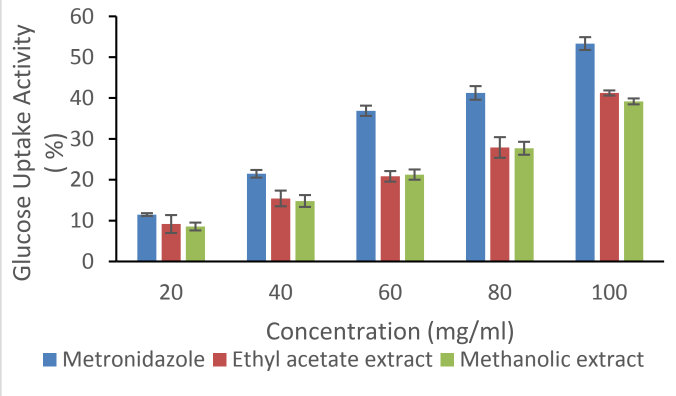

Figure 6: Stimulation of glucose uptake in yeast cells by *M. casta* extract at 25mM

This implies that increasing the concentration of *M. casta* extract enhances the ability of yeast cells to absorb a greater quantity of glucose from their environment. The data indicate a clear relationship between the glucose concentration in the solution and the rate at which yeast cells absorb it. Specifically, lower glucose concentrations lead to a more rapid uptake by the yeast cells. This suggests that *M. casta* extracts may facilitate a more efficient glucose transport mechanism, which becomes particularly effective at lower glucose concentrations. Consequently, these findings highlight the potential of *M. casta* extracts to modulate glucose absorption dynamics in yeast cells, providing a basis for further exploration into their mechanisms and applications in metabolic regulation.[54] Additionally, yeast cells may exhibit distinct mechanisms of glucose absorption compared to other eukaryotic or human cells. While human cells often rely on specific transport systems such as the phosphotransferase enzyme system, yeast cells may utilize facilitated diffusion for glucose transport across their membranes. This process involves glucose molecules moving through specific carrier proteins embedded in the yeast cell membrane, allowing for efficient uptake without the need for energy input. Understanding these differences in glucose absorption mechanisms is crucial for accurately interpreting the effects of *M. casta* extracts and could provide insights into their potential applications across various biological systems[54]. Several factors can impact glucose assimilation by yeast cells, including intracellular glucose concentration and subsequent glucose metabolism. If internal glucose is rapidly converted into other metabolites, the intracellular glucose concentration will decrease, promoting further glucose uptake by the cells. This transformation process ensures a gradient that facilitates continuous glucose absorption. Efficient metabolic pathways that convert glucose into various by-products can thus enhance the overall capacity of yeast cells to assimilate glucose. These dynamics underscore the importance of intracellular metabolic processes in regulating glucose uptake and highlight how *M. casta* extracts might influence these pathways to optimize glucose absorption and utilization in yeast cells. Understanding these factors is crucial for developing strategies to leverage natural extracts in metabolic regulation and biotechnological applications[55].

Similarly, higher glucose metabolism and facilitated diffusion may contribute to the enhanced glucose absorption observed in yeast cells in the presence of *M. casta* extracts. Exploring the in vivo activities of these natural extracts, which could potentially enhance glucose uptake in muscle cells and adipose tissues, is a promising research direction. It is hypothesized that the extracts may bind to glucose, facilitating its transport across cell membranes and promoting further metabolism. This mechanism could play a significant role in improving glucose homeostasis, offering potential therapeutic benefits for conditions like diabetes and metabolic syndrome. Understanding the molecular interactions and pathways through which *M. casta* extracts facilitate glucose uptake will be essential for developing new strategies to optimize metabolic health[56].

Effect of *M. casta* extracts on Alpha-Amylase Inhibitory Activity

The α-Amylase Inhibitory Assay is critically important in multiple research and clinical fields due to its diverse applications. Primarily, this assay is instrumental in diabetes management as it evaluates the efficacy of compounds or natural substances in inhibiting α-amylase, an enzyme crucial for carbohydrate digestion. By inhibiting pancreatic alpha-amylase, the breakdown of oligosaccharides into absorbable monosaccharides at the intestinal brush border is delayed. This mechanism effectively reduces postprandial hyperglycemia, as the slower conversion of carbohydrates into glucose leads to a more controlled release of

glucose into the bloodstream. Therefore, the α-Amylase Inhibitory Assay not only aids in identifying potential therapeutic agents for diabetes but also contributes to understanding how various substances can modulate glucose metabolism and absorption. This assay's role is vital for developing strategies to manage and treat diabetes, highlighting its significance in both research and practical healthcare applications.[57]. Phenolic substances, including flavonoids and phenolic acids, are known to form covalent bonds with alpha-amylase, thereby affecting its enzymatic activity. These interactions can alter the enzyme's structure and function, inhibiting its ability to catalyze the breakdown of carbohydrates. By binding to specific sites on the alpha-amylase molecule, phenolic compounds can interfere with the enzyme's active site or induce conformational changes that reduce its catalytic efficiency. This inhibition mechanism is particularly relevant in the context of managing postprandial hyperglycemia, as it slows down the digestion of carbohydrates into glucose. Consequently, phenolic substances offer potential therapeutic benefits for diabetes management and underscore the importance of studying their interactions with digestive enzymes like alpha-amylase[58]. Compounds capable of forming quinones or lactones can enhance the inhibition of alpha-amylase by reacting with nucleophilic groups on the enzyme. These reactive intermediates can covalently bind to amino acid residues within the enzyme's active site or other critical regions, leading to modifications that disrupt its normal function. Quinones and lactones, due to their electrophilic nature, readily interact with nucleophilic groups such as sulfhydryl, amino, and hydroxyl groups on the enzyme. This interaction can result in the formation of stable enzyme-inhibitor complexes, thereby effectively reducing the enzyme's ability to catalyze the breakdown of carbohydrates. Understanding these molecular interactions provides valuable insights into designing and developing more effective alpha-amylase inhibitors, which can be utilized for therapeutic purposes, particularly in managing diabetes by controlling postprandial blood glucose levels.[59]

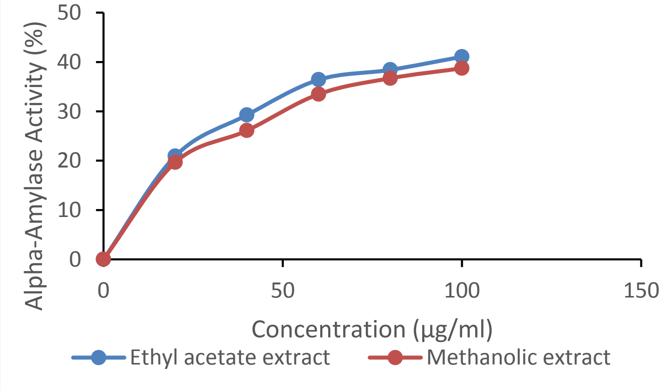

Figure 7: Percentage α-amylase inhibition of the *M. casta* extracts

Figure 7 illustrates the proportion of alpha-amylase inhibition by the crude extracts of *M. casta*. The data indicate that at a concentration of 100 µg/mL, the extracts were able to inhibit the enzyme by approximately 40%. This suggests that the extracts contain bioactive components with significant alpha-amylase inhibitory activity. These findings underscore the potential of *M. casta* extracts in modulating carbohydrate digestion and managing postprandial blood glucose levels. The presence of compounds such as phenolic substances, which can interact with and inhibit the enzyme, likely contributes to this effect. Understanding the specific bioactive constituents and their mechanisms of action could provide further insights into their potential therapeutic applications, particularly in the context of diabetes management. It is postulated that these extracts operate by alternative processes, similar to previous therapeutic interventions for treating Diabetes mellitus, and partially by inhibiting alpha-amylase.[60,61]

Conclusion

In conclusion, this study delves into the bioactive compounds found in both the ethyl acetate and methanolic extracts of *Meretrix casta*, emphasizing their potential health advantages. The extracts exhibit a rich composition of zoochemicals, encompassing flavonoids, cardiac glycosides, sterols, alkaloids, polyphenols, terpenoids, and tannins, underscoring their nutritional and therapeutic significance. Noteworthy antioxidant properties are evident, demonstrated by their ability to counteract free radicals (as observed in the DPPH test) and reduce ferric ions (as indicated by the FRAP test), albeit with a slightly lower efficacy than ascorbic acid. Furthermore, the extracts display promising outcomes in facilitating yeast cell glucose uptake, suggesting a potential role in regulating glucose metabolism. Additionally, their inhibitory effect on alpha-amylase activity suggests a potential application in managing postprandial hyperglycemia, a critical aspect of diabetes management. However, it is crucial to emphasize that further research is imperative to comprehensively evaluate their medicinal effectiveness and safety for human consumption and to explore other potential applications of these compounds.

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Conflict of Interest Statement

Authors declare that there is no conflict of interest for publication of this work

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