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The Marine Mollusc *Turbo radiatus* Glutathione S-Transferase Isolation and Biochemical Characterization: Antibacterial Effect and Cytotoxicity Against Different Cancer Cell Lines

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ABSTRACT

Glutathione S-transferases (GSTs) in aquatic organisms are extensively utilized as biomarkers for monitoring environmental contamination due to their ability to detoxify numerous pollutants. In this study, a GST enzyme from marine snails *Turbo radiatus* (TrGST) was purified through successive chromatographic separations on DEAE-cellulose, Sephacryl S-300, and glutathione-sepharose columns. TrGST was purified with 181.7-folds, 47.4% recovery and a specific activity of 194.4 Umg^{-1}. Molecular weight of TrGST, as determined by gel filtration, was 47 kDa. The SDS-PAGE analysis revealed TrGST as a single band of 23.4 kDa, indicating a homodimer protein of two identical subunits. The isoelectric point (pI) of TrGST was located at pH 6.1. The purified TrGST had *Km* values of 1.67 and 0.55 mM for CDNB and GSH with corresponding *Vmax* values of 0.72 and 0.4U/ mg, respectively. TrGST exhibited maximal activity at pH 8.4. The ions Co^{2+} and Mg^{2+} increased TrGST activity, while Mn^{2+}, Cu^{2+}, and Fe^{2+} suppressed it. TrGST was strongly inhibited by quercetin, lithocholic acid, cumene hydroperoxide, hematin, and triphenyltin chloride, with triphenyltin chloride being the most potent inhibitor showing noncompetitive inhibition with a Ki value of 0.125µM. TrGST revealed significant inhibition against certain bacterial strains; however, no inhibition was observed against others, indicating selective antimicrobial effects. TrGST exhibited strong cytotoxic effects against prostate cancer (PC3) and hepatocellular carcinoma (HepG2) cell lines, while showing weak activity against the colon cell line HCT116 and no effect on lung carcinoma cells (A549). The obtained results indicated that the TrGST protein may have the potential to serve as a tool for developing antibacterial and anti-cancer drugs in the future.

INTRODUCTION

Marine organisms account for nearly half of the world's total biodiversity, with mollusks containing the second largest number of species, including snails and bivalves **(Eghianruwa *et al.*, 2019)**. Snails are ubiquitous in aquatic environments and are

commonly used as bio-indicators for pollutants due to their ability to accumulate heavy metals and other toxicants **(Itziou & Dimitriadis, 2011; Baurand *et al.*, 2014; Bo *et al.*, 2015)**. The increase in industrialization and urbanization has potentially proliferated the contamination of aquatic ecosystems through sewage discharge in addition to industrial and agricultural waste **(Bille *et al.*, 2015)**. Pollutants entering aquatic bodies from various sources can disrupt the balance between free radicals and antioxidant defense mechanisms, leading to oxidative stress when free radical levels exceed antioxidant capacity. Antioxidant defenses, which include both non-enzymatic and enzymatic mechanisms, protect cells from free radical damage by restoring them to normal levels. Changes in antioxidant levels in aquatic organisms can indicate exposure to pollutants, providing sensitive biochemical markers and early warning tools for environmental pollution **(Bebianno *et al.*, 2007)**. Enzymatic antioxidant defenses include superoxide dismutases, catalases, glutathione peroxidases, and glutathione S-transferases (GSTs) **(Zheng *et al.*, 2013; Wang *et al.*, 2014)**. GSTs are a superfamily of metabolic enzymes involved in detoxifying foreign substances such as carcinogens, pollutants, mutagens, pesticides, drugs, and endogenous toxicants **(Tu & Akgul, 2005; Li *et al.*, 2015)**. GST catalyzes conjugation process of these chemicals to GSH, yielding water-soluble products and easier to excrete **(Sheehan *et al.*, 2001; Blanchette *et al.*, 2007; Whalen *et al.*, 2010)**.

Mollusks are sessile organisms lacking adaptive immune responses; depend on innate immunity composed of phagocytic cells and physiological components to be established in hostile marine environments against viruses, bacteria, and predators **(Rodríguez *et al.*, 2021; Weng *et al.*, 2022)**. Consequently, mollusks have developed efficient chemical defenses and humoral elements, including antioxidant enzymes and antimicrobial peptides **(Shen *et al.*, 2019)**. The chemical diversity in mollusks provides a vast reservoir of new bioactive substances with distinct chemical properties, acting as antioxidants and anti-cancer factors and playing a preventative role in tumor management **(Khalifa *et al.*, 2019; Habib *et al.*, 2022, 2023)**. The competitively exigent and aggressive environment around marine organisms result in various types of substances comprising polysaccharides, vitamins, polyunsaturated fatty acids, minerals, enzymes, bioactive peptides and antioxidants **(Pavlicevic *et al.*, 2020)**. Marine bioactive substances have prospective biological activities with unique structural characteristics which makes them safer to be used in some current synthetic drugs **(Suleria *et al.*, 2016)**. Marine natural products own immunomodulator, antibacterial, antifungal, antioxidant, anti-inflammatory, antimicrobial, anticancer, analgesic, neuroprotective and anti-malarial features. They have the ability of controlling carcinogenesis through activation of macrophages, induction of apoptosis and prevention of DNA oxidative damage **(Boopathy & Kathiresan, 2010; Malve, 2016)**. *Turbo radiatus* (Gmelin, 1791) is a marine gastropod snail in the family Turbinidae, frequently found in the shallow waters of the Red Sea in Egypt **(Abu El-Einin *et al.*, 2021)**. These snails are highly regarded for

human consumption due to their high nutritional content **(Ab Lah *et al.*, 2017)**. The flesh of *Turbo brunneus* has been investigated for its potential as a source of bioactive compounds **(Tamil & Selvaraj, 2015)**.

The aim of this study was to isolate and characterize GST enzyme of *T. radiatus* snails and to investigate its biochemical, antimicrobial, and cytotoxic properties, paving the way for exploring biologically active molecules from marine sources, which are considered largely untapped sources of potential novel medicinal leads. A similar study on Cu/Zn-SOD from *Cellana rota* snail highlighted the importance of marine mollusks in providing bioactive compounds with therapeutic potential **(Habib *et al.*, 2023)**. This current research builds on these findings to explore the capabilities of GST from *T. radiatus*, further emphasizing the significant role of marine gastropods in biomedical research.

MATERIALS AND METHODS

Materials and methods

Sample collection

T. radiatus snails were acquired from the Ain El-Sokhna shores, Red Sea, Egypt, in October 2020 **(Abu El-Einin *et al.*, 2021)**. In the laboratory, snails were subjected to washing with distilled water, crushing the shells, and dissection of soft tissues before storing at -20°C till further analysis.

Chemicals

1-Chloro-2,4-dinitrobenzene (CDNB), diethylaminoethyl cellulose (DEAE-cellulose), glutathione sepharose 4 fast flow, reduced glutathione (GSH), nitroblue tetrazolium chloride (NBT), sephacryl S-300, phenazine methosulphate (PMS), cumene hydroperoxide, lithocholic acid, hematin, triphenyltin chloride, and marker proteins for size exclusion were obtained from Sigma Chemical Co (St. Louis, Missouri, USA). All other chemicals were of analytical grade.

Protein determination

Proteins were estimated using Bradford assay **(Bradford, 1976)**.

GST activity assay

GST activity assay was carried out in a 1.0ml reaction consisting of 0.1 M Na-phosphate buffer pH 6.5, comprising 1.0 mM GSH, 1.0 1 mM CDNB, and enzyme aliquots. At 340nm, the optical density increase was followed for 3 minutes at 37°C, using 9.6 mM^{-1} cm^{-1} as extinction coefficient for CDNB. GST activity unit (U) is defined as the amount of GST enzyme conjugating 1.0µmol CDNB with GSH per minute **(Habig *et al.*, 1974)**.

Purification of *T. radiatus* GST

DEAE-cellulose chromatography

All purification proceedings were done at 4°C unless stated otherwise. *T. radiatus* tissues were homogenized in Na-phosphate buffer, pH 7.0 utilizing a Teflon pestle, and centrifuged for half an hour at 12,000 *x g* for getting rid of cell debris and obtaining the supernatant as a crude extract. Loading of this crude extract on a column of DEAE-cellulose (12 x 2.4cm) that had been equilibrated with the homogenizing buffer was carried out. The elution of proteins from the column was achieved using sodium chloride gradient prepared in homogenizing buffer, and the column flowing rate was set at 1.0ml/ min. Fractions showing GST activity were collected and concentrated by lyophilization for further purification.

Size exclusion chromatography

The concentrated fractions from DEAE-cellulose were furthermore applied onto a column of sephacryl S-300 (142 x 1.75 cm) that had been equilibrated and eluted with the previous Na-phosphate buffer, with the column flowing rate set at 0.5ml/ min. Fractions holding GST activity were collected and concentrated by lyophilization for the next purification step. For determining molecular mass of the purified GST enzyme, calibration of the column with molecular size standard proteins was carried.

Glutathione sepharose affinity chromatography

The concentrated fractions from sephacryl S-300 were applied to a glutathione sepharose affinity column (7.8 x 1.5cm) that had been equilibrated with Na-phosphate, buffer of pH 7.0. The elution of proteins from the column was achieved using 0.05 M Tris/HCl, pH 8.0, including 0.01 M GSH at 30ml/ h flowing rate.

Electrophoretic analysis

Proteins were separated using 7% Native-PAGE for patterns **(Smith, 1969)** and 12% SDS-PAGE for determining the subunit molecular mass **(Weber & Osborn, 1969; Laemmli, 1970)**. Isoelectric focusing (IEF)-PAGE was utilized to locate the pI value of the purified GST **(O'Farrell, 1975; Ubuka *et al.*, 1987)**. All gels were stained with the reagent of Coomassie brilliant blue.

Effect of pH on GST activity

The action of pH on GST activity has been identified utilizing buffers of pH 5.6 - 8.0 (Na-phosphate) and pH 8.0 - 9.8 (Tris/HCl) at 37°C.

GST enzyme kinetics

Values of *Km* and *Vmax* for the purified TrGST enzyme were deduced from the extracted Lineweaver-Burk plot when testing the enzyme with increasing concentrations of CDNB substrate (0.1 - 2mM) at 1.0mM GSH constant concentration or increasing GSH concentrations (0.1 - 2 mM) at 1.0 mM CDNB constant concentration at 37°C in Na-phosphate of pH 6.5.

Effect of ions and inhibitors on TrGST activity

To study their effect on enzyme activity, the cations (Ca^{2+}, Co^{2+}, Ni^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, and Fe^{2+}) and some different inhibitors have been incubated with TrGST for 5 minutes at 37°C, and then the activity of purified GST enzyme was measured.

Antimicrobial activity of TrGST

The antimicrobial activity of TrGST was tested against six bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli* 0157 ATCC 700728 and *Escherichia coli* ATCC 8739), and two fungi (*Candida albicans* and *Aspergillus niger*) **(Alhadrami *et al.*, 2021)**. This examination was done in 96-well polystyrene flat bottom plates by incubating 10µl TrGST (5µg/ ml final concentration), lysogeny broth (80µl) and bacterial culture suspension (10µl) overnight at 37°C. Antibacterial effects were indicated by well clearance, with the absorbance measured at 600nm utilizing a microplate reader (Spectrostar Nano, BMG-LABTECH, Germany). All experimental strains were acquired from the culture collection center, Microbial Chemistry Dept., NRC, Egypt.

Cytotoxic effect of TrGST on human cell lines

Cytotoxic activity of TrGST was evaluated in the Bioassay-Cell Culture Lab., NRC, Egypt, utilizing MTT test for cell viability to be assessed **(Mosmann, 1983)**. Cells were cultured in RPMI 1640 medium for HePG2, MCF7 and HCT116and DMEM medium for PC3 and A549, and then supplemented with 1% mixture of antibiotic-antimycotic and 1% L-glutamine at 37°C under 5% CO_2. Seeding of the cells at 10 x 10^3 cells/well was carried out in plates of 96 wells, followed by incubation for 24 hours. After incubation, mediums were replaced with fresh media (without serum) containing various TrGST concentrations (0.78 – 100µg/ ml). After 48 hours, 2.5µg/ ml MTT salt was added, followed by further incubation of cells for other 4 hours. This reaction was terminated, and the formazan was dissolved using 200µl 10% SDS, then overnight incubated at 37°C, and the optical density was monitored at 595nm utilizing a reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA). Viability was calculated, and statistical significance was determined using SPSS 11 software. IC_{50} and IC_{90} values were calculated by Probit analysis.

RESULTS

Purification of *T. radiatus* GST

The purification scheme of *T. radiatus* snail TrGST illustrated a significant enhancement in specific activity and purification through successive steps. Starting with the crude extract, the total protein was 122.5mg with a specific activity of 1.07, yielding 100% and serving as the baseline with a fold purification of 1.0. The DEAE-cellulose fraction step reduced the total protein to 61.2mg and increased the specific activity to 1.46, achieving a yield of 68.1% and a fold purification of 1.36 (Fig. 1a). Further purification with sephacryl S-300 resulted in a total protein of 21.2mg and a specific activity of 3.5, with a yield of 57.0% and a fold purification of 3.27 (Fig. 1b). The final GSH-sepharose fraction step markedly increased the specific activity to 194.4, despite the total protein dropping to 0.32mg, yielding 47.4% and achieving a fold purification of 181.7 (Fig. 1c). This step demonstrated the most significant purification level, highlighting the efficiency of the GSH-Sepharose fraction in purifying TrGST (Table 1).

Table 1. A typical purification scheme of *T. radiatus* snail TrGST

Purification steps	Total protein (mg)	Total activity (unit)	Specific activity	Yield (%)	Fold purification
Crude extract	122.5	131.2	1.07	100	1.0
DEAE-cellulose fraction	61.2	89.3	1.46	68.1	1.36
Sephacryl S-300 fraction	21.2	74.8	3.5	57.0	3.27
GSH-sepharose fraction	0.32	62.2	194.4	47.4	181.7

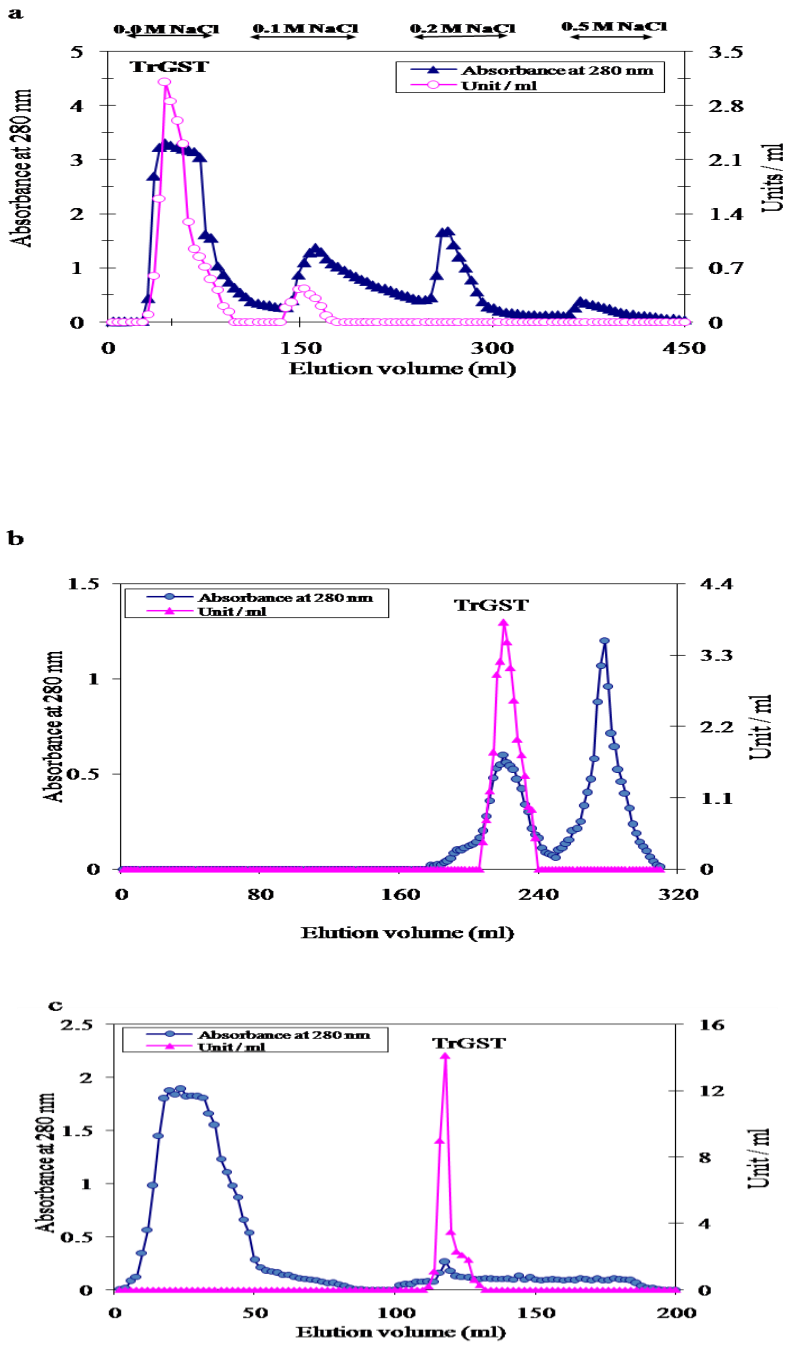

Fig. 1. (a) Pattern of *T. radiatus* snail crude extract chromatography on DEAE-cellulose column (10 x 2.8cm). **(b)** Chromatography of TrGST on sephacryl S-300 column (142 cm x 2.4cm). **(c)** Chromatographic pattern of TrGST sephacryl S-300 concentrate on GSH-sepharose column (8 x 1.4cm)

Electrophoretic analysis of TrGST

Electrophoresis of the crude extract and TrGST fractions from DEAE-cellulose, sephacryl S-300, and glutathione sepharose on 7% native-PAGE revealed a single protein band (Fig. 2a). The subunit mass of denatured TrGST was located at 23.4 kDa compared with known molecular weight markers on 12% SDS-PAGE (Fig. 2b). Apparent pI (isoelectric point) of TrGST was located at pH 6.1 using isoelectro-focusing PAGE (Fig. 2c).

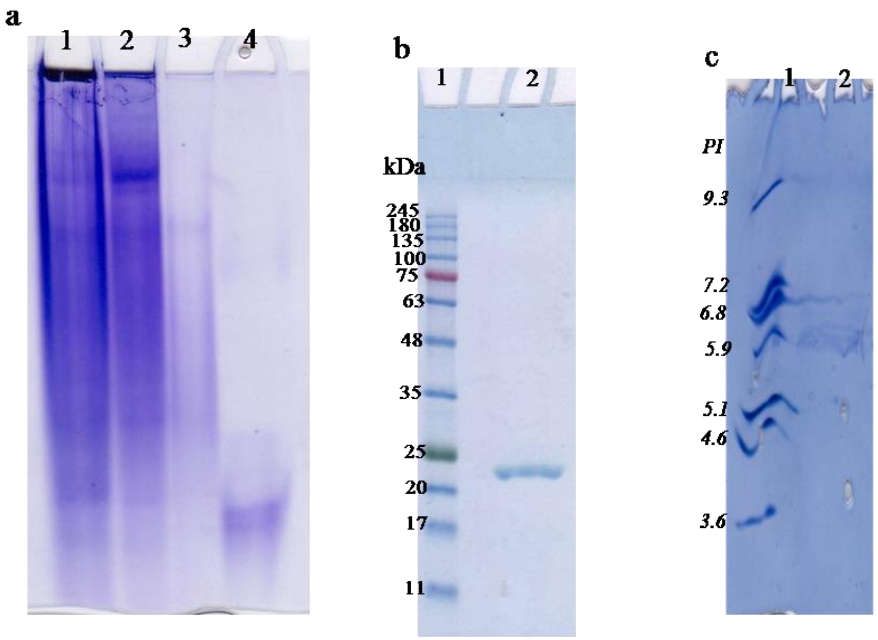

Fig. 2. Electrophoresis of TrGST purification steps on native 7% PAGE: (1) Snail extract, (2) DEAE cellulose TrGST, (3) Sephacryl S-300 TrGST and (4) GSH-sepharose TrGST.(b) Electrophoresis of TrGST on 12% SDS PAGE; (1) Marker proteins and (2) TrGST. (c) Electrophoresis of TrGST on isoelectric-focusing PAGE: (1) Marker proteins and (2) TrGST

Optimum pH and enzyme kinetics of TrGST

Effect of pH on TrGST activity was evaluated using Na-phosphate and Tris/HCl buffers with CDNB as a substrate. The highest TrGST activity was observed at pH 8.4 (Fig. 3a). Kinetic analysis of TrGST with varying concentrations of CDNB and GSH showed *Km* and *Vmax* values of 1.67 mM and 0.72U/ mg, respectively, for CDNB (Fig. 3b), and 0.55 mM and 0.4U/ mg, respectively, for GSH (Fig. 3c).

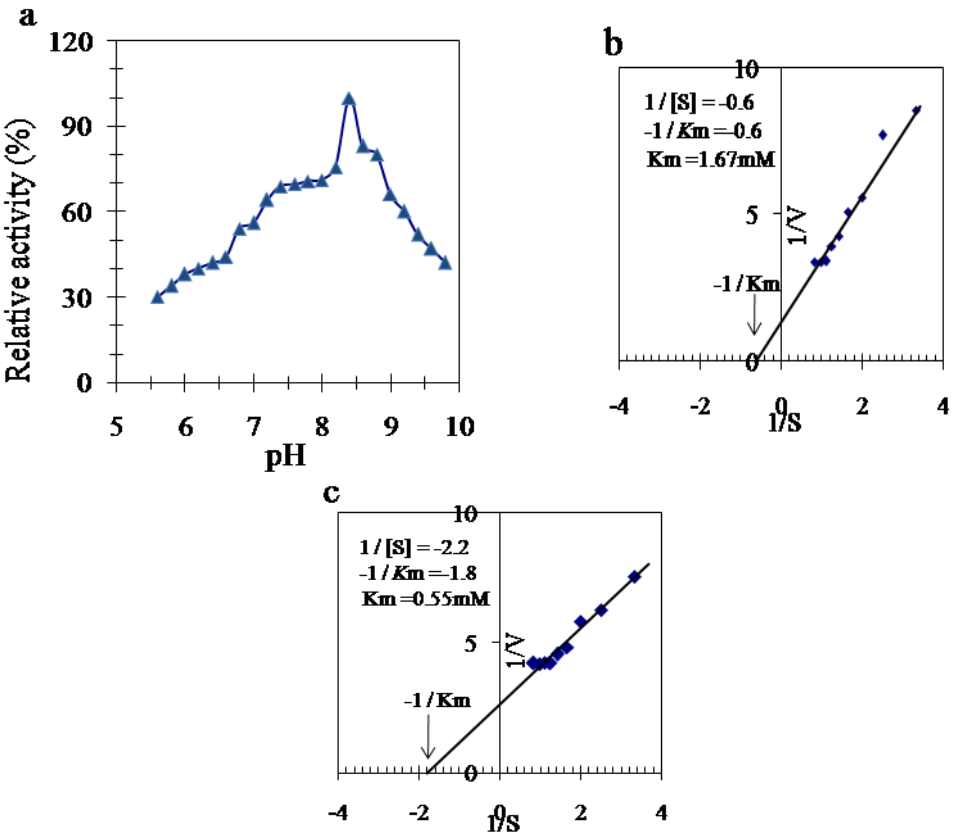

Fig. 3. (a) Effect of pH on TrGST utilizing Na-phosphate, pH 5.7 - 8.0 and Tris/HCl, pH 8.0 - 9.2. **(b)** Lineweaver-Burk plot of TrGST reaction velocity utilizing various CDNB concentrations at a constant 1.0 mM GSH. **(c)** Lineweaver-Burk plot of TrGST reaction velocity utilizing various GSH concentrations at a constant 1.0 mM CDNB

Effect of divalent ions and inhibitors on TrGST activity

The impact of divalent cations on purified TrGST revealed significant variations in relative activity. For $CaCl_2$, the relative activity was 98.6% at 2.0 mM and increased to 104.4% at 5.0 mM. $CoCl_2$ maintained 100.0% activity at 2.0 mM and increased to 112.6% at 5.0 mM. $CuCl_2$ showed a significant decrease in activity, with 69.4% at 2.0 mM and 38.6% at 5.0 mM. $FeCl_2$ exhibited even lower activity, with 56.0% at 2.0 mM and 15.0% at 5.0 mM. $MgCl_2$ enhanced activity to 106.5% at 2.0 mM and 109.8% at 5.0 mM. $NiCl_2$ showed a relative activity of 101.6% at 2.0 mM but decreased to 92.3% at 5.0 mM. $ZnCl_2$ displayed a similar pattern, with 100.2% activity at 2.0 mM and 92.3% at 5.0 mM. $MnCl_2$ demonstrated a relative activity of 92.0% at 2.0 mM and dropped to 56.0% at 5.0 mM. The highest relative activity was observed with $CoCl_2$ at 5.0 mM (112.6%), indicating a strong enhancement effect, while the lowest activity was seen with $FeCl_2$ at 5.0 mM (15.0%), indicating a strong inhibitory effect (Table 2).

The impact of inhibitors on purified TrGST revealed varying degrees of inhibition at specific concentrations. Quercetin at a 2 mM concentration showed an inhibition of 86.0%, while cumene hydroperoxide at the same concentration exhibited an inhibition of 62.1%. Hematin at 2 mM showed an inhibition of 37.27%, and lithocholic acid at 2 mM resulted in 73.7% inhibition. Triphenyltin chloride at 1.0 µM exhibited the highest inhibition at 96.7%. The control group had 0.0% inhibition, serving as a baseline. The highest inhibition was observed with triphenyltin chloride (96.7%), indicating its strong inhibitory effect on TrGST, while the lowest inhibition was observed with hematin (37.27%) among the tested inhibitors (Table 3).

Table 2. Effect of divalent cations on the purified TrGST

Reagent	Concentration (mM)	Relative activity (%)
Control	-----	100.0
$CaCl_2$	2.0	98.6
	5.0	104.4
$CoCl_2$	2.0	100.0
	5.0	112.6
$CuCl_2$	2.0	69.4
	5.0	38.6
$FeCl_2$	2.0	56.0
	5.0	15.0
$MgCl_2$	2.0	106.5
	5.0	109.8
$NiCl_2$	2.0	101.6
	5.0	92.3
$ZnCl_2$	2.0	100.2
	5.0	92.3
$MnCl_2$	2.0	92.0
	5.0	56.0

Table 3. Effect of inhibitors on the purified TrGST

Inhibitor	Concentration	Inhibition (%)
Control	---	0.0
Quercetin	2.0 mM	86.0
Cumene hydroperoxide	2.0 mM	62.1
Hematin	2.0 mM	37.27
Lithocholic acid	2.0 mM	73.7
Triphenyltin chloride	1.0 µM	96.7

Kinetics of TrGST inhibition with triphenyltin chloride

The inhibition of TrGST activity by different concentrations of triphenyltin chloride exhibited a linear relationship until 50% inhibition (I_{50}) was reached at 0.2µM, with maximum inhibition at 1.0µM (Fig. 4a). A Hill plot of log Vi/Vmax–Vi values *vs.* log [I] values for triphenyltin chloride yielded a slope of 0.88 (Fig. 4b). Lineweaver-Burk plots indicated noncompetitive inhibition, with a Ki value of 0.125µM (Fig. 4c, 4d).

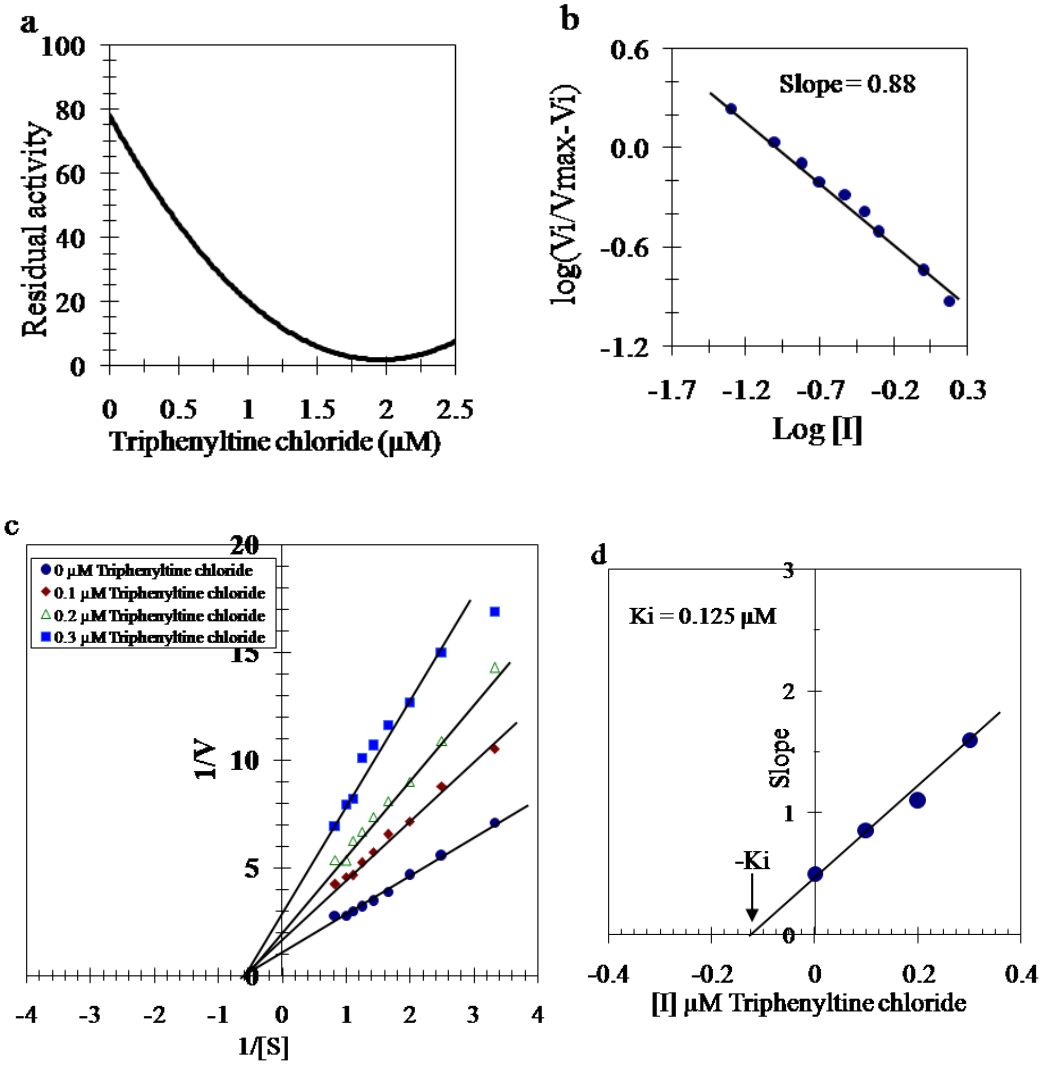

Fig. 4. (a) TrGST inhibition with triphenyltin chloride increasing concentrations. **(b)** Hill plot of TrGST inhibition with triphenyltin chloride. **(c)** TrGST type of inhibition with triphenyltin chloride. **(d)** TrGST inhibition constant (*Ki*) for triphenyltin chloride

Antimicrobial activity of TrGST

The antimicrobial activity of TrGST demonstrates varying inhibition ratios against different microbes. *Staphylococcus aureus* ATCC 25923 showed an inhibition ratio of 34.2%, while *Listeria monocytogenes* ATCC 7644 exhibited 0.0% inhibition. *Pseudomonas aeruginosa* TCC 90902 had the highest inhibition ratio of 45.8%, indicating strong antimicrobial activity. *Salmonella typhimurium* ATCC 14028 and *Candida albicans* both showed 0.0% inhibition, indicating no antimicrobial effect. *Escherichia coli* 0157 ATTCC 700728 showed an inhibition ratio of 37.2%, and *Escherichia coli* ATCC 8739 (lot 03801105) had an inhibition ratio of 25.2%. *Aspergillus niger* also exhibited 0.0% inhibition. The highest inhibition ratio of 45.8% was observed against *Pseudomonas aeruginosa*, highlighting TrGST's significant antimicrobial activity against this microbe, whereas the lowest inhibition ratios of 0.0% were observed against *Listeria monocytogenes*, *Salmonella typhimurium*, *Candida albicans*, and *Aspergillus niger*, indicating no antimicrobial effect (Fig. 5).

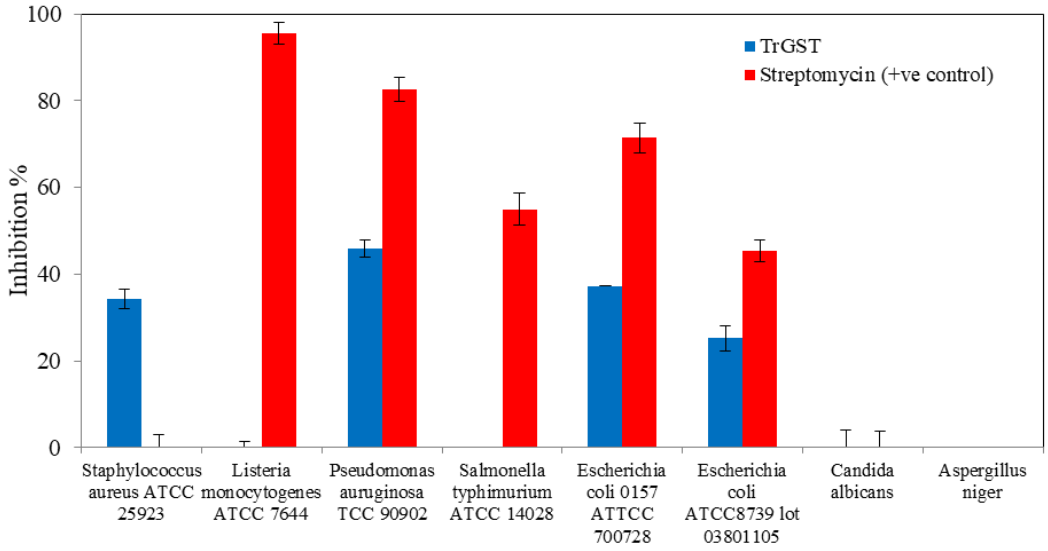

Fig. 5. Antimicrobial impacts of TrGST against various bacterial and fungal microorganisms

Cytotoxicity evaluation of TrGST

The cytotoxicity results of the enzyme TrGST extracted from the sea snail *T. radiatus* against the HepG2, HCT116, PC3, and MCF7 cancer cell lines show a dose-dependent decrease in cell viability. The calculated IC_{50} values for TrGST were 28.9µg/ ml for HepG2, 50.1µg/ ml for HCT116, 22.2µg/ ml for PC3 and 37.0µg/ ml for MCF7. In comparison, the IC_{50} values for the reference drug doxorubicin were 21.6µg/ ml for

HepG2, 37.6µg/ ml for HCT116, 23.8µg/ ml for PC3 and 26.1µg/ ml for MCF7. Notably, TrGST exhibited the best result against the PC3 cell line with an IC_{50} value of 22.2µg/ ml indicating a strong cytotoxic effect similar to that of doxorubicin (Table 4 & Fig. 6).

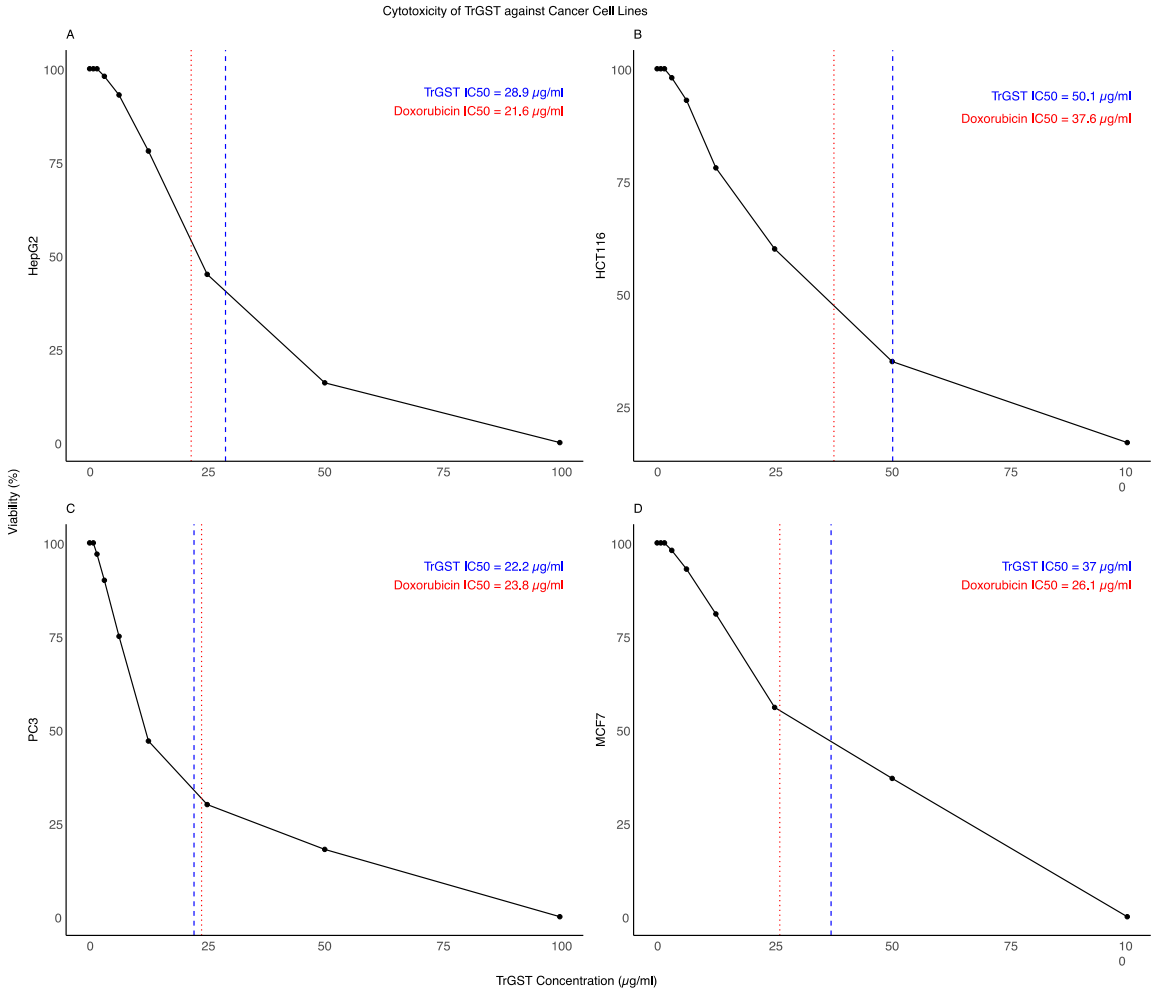

Fig. 6. Effect of TrGST on the viability of different human cancer cell lines: **(a)** HEPG2. **(b)** HCT116. **(c)** PC3. **(d)** MCF7

Table 4. *In vitro* cytotoxicity IC_{50} and IC_{90} (µg/ml) of TrGST against human cancer cells

Cell line	IC_{50} µg/ml		*Tr*GST IC_{90} µg/ml
	Doxorubicin	*Tr*GST	
HEPG2	21.6	28.9	48.6
HCT116	37.6	50.1	93.7
A549	28.3	--	--
MCF7	26.1	37.0	63.5
PC3	23.8	22.2	46.3

DISCUSSION

Snails are renowned for their ability to accumulate high amounts of toxic pollutants, making them ideal organisms for bio-monitoring in aquatic environments as convenient bio-indicators of contaminants. Antioxidants play critical roles in responding to pollutants, with GST being one of the most important oxidative stress biomarkers, invaluable in environmental toxicological studies **(Itziou & Dimitriadis, 2011)**. The GST enzyme from *T. radiatus* was successfully purified using three successive chromatographic steps: DEAE-cellulose anion exchanger, sephacryl size-exclusion, and GSH-sepharose affinity resins. The GSH-Sepharose affinity column revealed a unique GST activity peak eluted with 10 mM GSH, termed TrGST (Fig. 1c). This purification resulted in a significant increase in specific activity to 194.4Umg^{-1}, representing 181.7-fold purification with 47.4% recovery (Table 1). Similar purification procedures have been employed for GSTs from various sources, such as German cockroach **(Yu & Huang, 2000)**, non-biting midges Chironomidae larvae **(Yuen & Ho, 2001)**, filarial worms **(Ahmad *et al.*, 2008)**, and blueberry fruits **(Balc *et al.*, 2019)**.

The molecular weight of TrGST was estimated from its elution volume on the sephacryl S-300 column to be 47 kDa. SDS-PAGE analysis of denatured TrGST revealed a subunit molecular weight of 23.4 kDa, indicating that TrGST is a homodimer protein consisting of two identical subunits. This homodimer structure has been reported in various sources, including German cockroach **(Yu & Huang, 2000)**, marine snail *Atactodea striata* **(Yang *et al.*, 2003)**, and the grasshopper **(Adewale & Afolayan, 2006)**. The homogeneity of TrGST was confirmed through two electrophoretic analyses on native and isoelectric focusing PAGEs, showing it as a single protein band with an apparent pI value of 6.1 (Fig. 2a, c). Comparable pI values have been reported for GSTs from midge larvae and snails **(Yuen & Ho, 2001; Yang *et al.*, 2003)**.

TrGST exhibited maximum activity at pH 8.4, similar to other GSTs from *H. longicornis* tick, midge larvae, and camel tick **(Yuen & Ho, 2001; Hernandez *et al.*, 2018; Masoud *et al.*, 2023)**. The kinetic parameters of TrGST revealed Km values of 1.67 mM for CDNB and 0.55 mM for GSH, with corresponding Vmax values of 0.72 and 0.4Umg^{-1}, respectively. These values are consistent with those reported for GSTs from

other sources, such as cockroach **(Yu & Huang, 2000)** and tick larvae **(Masoud *et al.*, 2023)**.

The effect of divalent ions on TrGST activity showed that Co^{2+} and Mg^{2+} ions enhanced enzyme activity, while Mn^{2+}, Cu^{2+}, and Fe^{2+} ions inhibited it (Table 2). This observation aligns with findings in quail liver and tick larvae GSTs, where Co^{2+} increased activity and Cu^{2+} acted as an inhibitor **(Taysi & Temel, 2021; Masoud *et al.*, 2023)**. Inhibitor studies demonstrated that TrGST was strongly inhibited by quercetin, cumene hydroperoxide, lithocholic acid, hematin, and triphenyltin chloride, with triphenyltin chloride being the most potent inhibitor, exhibiting noncompetitive inhibition with a Ki value of 0.3µM. These findings are consistent with previous studies on GST inhibition by similar compounds in different organisms **(Yu & Huang, 2000; Ahmad *et al.*, 2008; Masoud *et al.*, 2023)**.

The antimicrobial activity of TrGST was evaluated against various microorganisms, revealing significant inhibition against several bacterial strains, notably *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with inhibition ratios of 34.2 and 45.8%, respectively. This suggests that TrGST possesses substantial antimicrobial properties, particularly against Gram-negative bacteria. However, no inhibition was observed against *Listeria monocytogenes*, *Salmonella typhimurium*, *Candida albicans*, and *Aspergillus niger*, indicating a selective antimicrobial effect.

The cytotoxicity evaluation demonstrated that TrGST exhibited strong cytotoxic effects against PC3 and HepG2 cell lines, with IC_{50} values of 22.2 and 28.9µg/ ml, respectively, comparable to doxorubicin. In addition, TrGST moderately affected MCF7 cells, with an IC_{50} value of 37.0µg/ ml, while showing a weak activity against HCT116 and no effect on A549.

Comparing these findings with results from a similar study on copper-zinc superoxide dismutase (Cu/Zn-SOD) from *Cellana rota* snails **(Habib *et al.*, 2023)**, Cu/Zn-SOD also demonstrated substantial antimicrobial and cytotoxic activities. Cu/Zn-SOD exhibited considerable antimicrobial efficiency against *Pseudomonas aeruginosa* (39.7%) and *Staphylococcus aureus* (32.4%), values that are comparable to the antimicrobial effects of TrGST. However, Cu/Zn-SOD displayed a broader antimicrobial spectrum, showing activity against additional strains such as *Escherichia coli* and *Bacillus subtilis*. Regarding cytotoxicity, Cu/Zn-SOD from *Cellana rota* showed IC50 values of 25.3µg/ ml for HepG2 and 24.8µg/ ml for PC3, which are similar to the IC50 values observed for TrGST (28.9µg/ ml for HepG2 and 22.2µg/ ml for PC3). This suggests that both enzymes exhibit comparable cytotoxic effects against these cancer cell lines. However, Cu/Zn-SOD also demonstrated significant cytotoxicity against A549 cells, unlike TrGST, which had no effect on this cell line.

CONCLUSION

In conclusion, this is the first study to report the GST enzyme from the *T. radiatus* snail and to present an efficient purification method for TrGST. The enzyme demonstrated potent antimicrobial activity and cytotoxicity to different cancer cell lines, making it a potential promising candidate for treating various cancers, including prostate and liver cancers, while requiring more investigations. The distinct activity of TrGST can be attributed to the challenging physical and biological environmental conditions in which *T. radiatus* thrives, necessitating the evolution of robust defense mechanisms. and physiological adaptations. These results emphasize the capability of marine snails as a beneficial source of bioactive molecules with significant therapeutic and pharmaceutical applications.

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Ethics approval
Applicable (Registration Number: 1-6-4)

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

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Authors' contributions
Every one of the authors shared in experimental design, practical work, writing, discussions and comments of the manuscript.

REFERENCES

Ab Lah, R.; Smith, J.; Savins, D.; Dowell, A.; Bucher, D. and Benkendorff, K. (2016). Investigation of nutritional properties of three species of marine turban snails for human consumption. Food Sci. Nutr., 5(1):14-30. https://doi.org/10.1002/fsn3.360.

Abu El-Einin, H.A.; Gad El-Karim, R.M.; Habib, M.R.; Zayed, K.M. and Ali, R.E.M. (2021). Identification of the gastropod snails and shells collected from Ain

El-Sokhna region, Red Sea, Egypt. Egypt. J. Aquat. Biol. Fisher., 25:101-117. https://doi.org/10.21608/EJABF.2021.172857

Adewale, I.O. and Afolayan, A. (2006). Studies on glutathione transferase from grasshopper (Zonocerus variegatus). Pestic. Biochem. Phys., 85:52-59. https://doi.org/10.1016/j.pestbp.2005.10.004

Ahmad, R.; Srivastava, A.K. and Walter, R.D. (2008). Purification and biochemical characterization of cytosolic glutathione-S-transferase from filarial worms Setaria cervi. Comp. Biochem. Physiol., 151:237-245. https://doi.org/10.1016/j.cbpb.2008.03.019

Alhadrami, H.A.; Orfali, R.; Hamed, A.A.; Ghoneim, M.M.; Hassan, H.M.; Hassane, A.S.I.; Rateb, M.E.; Sayed, A.M. and Gamaleldin, N.M. (2021). Flavonoid-Coated Gold Nanoparticles as Efficient Antibiotics against Gram-Negative Bacteria-Evidence from In Silico-Supported In Vitro Studies. Antibiotics (Basel), 10(8):968. https://doi.org/10.3390/antibiotics10080968.

Balc, N.; Turkan, F.; Sakiroglu, H.; Aygun, A. and Sen, F. (2019). Purification and characterization of glutathione S-transferase from blueberry fruits (*Vaccinium arctostaphylos* L.) and investigated of some pesticide inhibition effects on enzyme activity. Heliyon. 5:e01422. https://doi.org/10.1016/j.heliyon.2019.e01422.

Baurand, P-E.; Capelli, N.; Scheifler, R. and De Vaufleury, A. (2014). An assessment of the embryotoxicity of cadmium in the terrestrial mollusk *Cantareus aspersus*: From bioaccumulation to impacts at different levels of biological organization. Ecotoxicol. Environ. Saf., 110:89-94. https://doi.org/10.1016/j.ecoenv.2014.08.017.

Bebianno, M.J.; Lopes, B.; Guerra, L.; Hoarau, P. and Ferreira, A.M. (2007). Glutathione S-tranferases and cytochrome P450 activities in Mytilus galloprovincialis from the South coast of Portugal: Effect of abiotic factors. Environ Int 33(4):550-558. https://doi.org/10.1016/j.envint.2006.11.002.

Bille, L.; Binato, G.; Cappa, V.; Toson, M.; Pozza, P.D.; Arcangeli, G.; Ricci, G.; Angeletti, A. and Piro, R. (2015). Lead, mercury and cadmium levels in edible marine molluscs and echinoderms from the Veneto Region (north- western Adriatic Sea- Italy). Food Control, 50:362-370. https://doi.org/:10.1016/j.foodcont.2014.09.018

Blanchette, B.; Feng, X. and Singh, B.R. (2007). Marine glutathione S-transferases. Mar. Biotechnol., 9 (5):513-542. https://doi.org/10.1007/s10126-007-9034-0.

Bo, L.; Wang, D.; Li, T.; Li, Y.; Zhang, G.; Wang, C. and Zhang, S. (2015). Accumulation and risk assessment of heavy metals in water, sediments, and aquatic organisms in rural rivers in the Taihu Lake region, China. Environ. Sci. Pollut. Res., 22:6721-6731. https://doi.org/10.1007/s11356-014-3798-3.

Boopathy, N.S. and Kathiresan, K. (2010). Anticancer drugs from marine flora: An overview. J. Oncol., 1:214186. https://doi.org/10.1155/2010/214186

Bradford, M.M. (1976). A rapid and sensitive method for quantization of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal. Biochem., 72: 248-254. https://doi.org/10.1006/abio.1976.9999.

Eghianruwa, Q.A.; Osoniyi, O.R.; Maina, N. and Wachira, S. (2019). Bioactive peptides from marine molluscs – A Review. IJBCRR, 27(4):1-12. https://doi.org/10.9734/ijbcrr/2019/v27i430130

Habib, M.R.; Hamed, A.A.; Ali, R.E.M.; Zayed, K.M.; Gad El-Karim, R.M.; Sabour, R.; Abu El-Einin, H.M. and Ghareeb, M.A. (2022). *Thais savignyi* tissue extract: Bioactivity, chemical composition, and molecular docking. Pharm. Biol., 60(1):1899-1914. https://doi.org/10.1080/13880209.2022.2123940

Habib, M.R.; Masoud, H.M.M.; Helmy, M.S.; Hamed, A.A.; Gad El-Karim, R.M.; Ali, R.E.M.; Zayed, K.M. and Ibrahim, M.A. (2023). In vitro anticancer and antimicrobial activities of copper-zinc superoxide dismutase purified from Cellana rota snail. Egypt. J. Chem., 66(12):1-10. https://doi.org/10.21608/EJCHEM.2023.200467.7736.

Habig, W.H.; Pabst, M.J. and Jakoby, W.B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Hernandez, E.P.; Kusakisako, K.; Talactac, M.R.; Galay, R.L.; Hatta, T.; Matsuo, T.; Fujisaki, K.; Tsuji, N. and Tanaka, T. (2018) Characterization and expression analysis of a newly identified glutathione S-transferase of the hard tick *Haemaphysalis longicornis* during blood-feeding. Parasite. Vector., 11:91. https://doi.org/10.1186/s13071-018-2667-1

Itziou, A. and Dimitriadis, V.K. (2011). Introduction of the land snail *Eobania vermiculata* as a bioindicator organism of terrestrial pollution using a battery of biomarkers. Sci. Total Environ., 409(6):1181-1192. https://doi.org/10.1016/j.scitotenv.2010.12.009.

Khalifa, S.; Elias, N.; Farag, M.A.; Chen, L.; Saeed, A.; Hegazy, M.F.; Moustafa, M.S.; **Abd El-Wahed, A.; Al-Mousawi ,S.M.; Musharraf, S.G.; Chang, F.R.; Iwasaki, A.; Suenaga, K.; Alajlani, M.; Goransson, U. and El-Seedi, H.R.** (2019). Marine natural products: a source of novel anticancer drugs. Mar. Drugs, 17(9):491. https://doi.org/10.3390/md17090491.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature, 227: 680-685. https://doi.org/10.1038/227680a0

Li, H.; Yang, Z.; Huan,g Q. and Li, Y. (2015). Molecular cloning and characterization of a sigma-class glutathione S-Transferase from the freshwater mussel *Hyriopsis cumingii*. Microbiol. Immunol., 59:219-230. https://doi.org/10.1111/1348-0421.12250.

Malve, H. (2016). Exploring the ocean for new drug developments: Marine pharmacology. J. Pharm. Bioall. Sci., 8(2):83-91. https://doi.org/10.4103/0975-7406.171700.

Masoud, H.M.M.; Helmy, M.S.; Darwish, D.A. and Ibrahim, M.A. (2023). Purification, characterization, and enzyme kinetics of a glutathione S transferase from larvae of the camel tick *Hyalomma dromedarii*. JGEB, 21:28. https://doi.org/10.1186/s43141-023-00486-w

Mosmann, T. (1983). Rapid colorimetric assays for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65:55-63. https://doi.org/10.1016/0022-1759(83)90303-4.

O'Farrell, P.H. (1975). High resolution two-dimentional electrophoresis of proteins. J. Biol. Chem., 250:4007-4021.

Pavlicevic, M.; Maestri, E. and Marmiroli, M. (2020). Marine bioactive peptides-an overview of generation, structure and application with a focus on food sources. Mar. Drugs, 18(8):424. https://doi.org/10.3390/md18080424.

Rodríguez, A.A.; Otero-González, A.; Ghattas, M. and Ständker, L. (2021). Discovery, optimization, and clinical application of natural antimicrobial peptides. Biomed., 9(10):1381. https://doi.org/10.3390/biomedicines9101381

Sheehan, D.; Meade, G.; Foley, V.M. and Dowd, C.A. (2001). Structure, function and evolution of glutathione transferases: Implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem. J., 360 (Pt 1):1-16. https://doi.org/10.1042/0264-6021:3600001.

Shen, Y.; Huang, Z.; Liu, G.; Ke, C. and You, W. (2019). Hemolymph and transcriptome analysis to understand innate immune responses to hypoxia in Pacific abalone. Comp. Biochem. Physiol. Genom. Proteom., 30:102-112. https://doi.org/10.1016/j.cbd.2019.02.001.

Smith, I. (1969). Acrylamide gel disc electrophoresis. In "Electrophoretic techniques" (Edited by Smith, I.), 365-515, Academic press, New York.

Suleria, H.A.R.; Gobea, G.; Mascia, P. and Osborne, S.A. (2016). Marine bioactive compounds and health promoting perspectives; innovation pathways for drug discovery. Trends Food Sci. Technol., 50:44-55. https://doi.org/10.1016/j.tifs.2016.01.019

Tamil, M.P. and Selvaraj, D. (2015). Analysis of bioactive constituents from the flesh of *Turbo brunneus* (Roding, 1798) by GCMS. Int. J. Fish. Aquat. Stud., 3(1):257-259.

Taysi, M.Ş. and Temel, Y. (2021). Glutathione S-transferase: Purification and characterization from quail (*Coturnix japonica*) liver and the impact of some metal ions on enzyme activity. BioNanoSci., 11:91-98. https://doi.org/10.1007/s12668-020-00811-4

Tu, C.P. and Akgul, B. (2005). Drosophila glutathione S-transferases. Methods Enzymol., 401:204-226. https://doi.org/10.1016/S0076-6879(05)01013-X.

Ubuka, T.; Masuoka, N.; Yoshida, S. and Ishino, K. (1987). Determination of isoelectric point value of 3-mercaptopyruvate sulfurtransferase by isoelectric

focusing using ribonuclease A-glutathione mixed disulfides as standards. Anal. Biochem., 167: 284-289. https://doi.org/10.1016/0003-2697(87)90165-5.

Wang, X.; Liu, Z.; Wang, W.; Yan, Z.; Zhang, C.; Wang, W. and Chen, L. (2014). Assessment of toxic effects of triclosan on the terrestrial snail (*Achatina fulica*). Chemosphere 108:225-230. https://doi.org/1016/j.chemosphere.2014.01.044.

Weber, K. and Osborn, M. (1969). The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. J. Biol. Chem., 244: 4406-4412. https://doi.org/10.1016/S0021-9258(18)94333-4

Weng, N.; Meng, J.; Huo, S.; Wu, F. and Wang, W.X. (2022). Hemocytes of bivalve mollusks as cellular models in toxicological studies of metals and metal-based nanomaterials. Environ. Pollut., 312:120082. https://doi.org/10.1016/j.envpol.2022.120082.

Whalen, K.E.; Lane, A.L.; Kubanek, J. and Hahn, M.E. (2010). Biochemical warfare on the reef: the role of glutathione transferases in consumer tolerance of dietary prostaglandins. PLoS One, 5(1):e8537. https://doi.org/10.1371/journal.pone.0008537.

Yang, H.; Zeng, Q.; Nie, L.; Zhu, S. and Zhou, X. (2003). Purification and characterization of a novel glutathione S-transferase from Atactodea striata. Biochem. Biophys. Res. Com., 307:626-631. https://doi.org/10.1016/s0006-291x(03)01221-x.

Yu, S.J. and Huang, S.W. (2000). Purification and characterization of glutathione S-transferases from the German cockroach, Blattella germanica (L.). Pestic Biochem Phys 67:36-45. https://doi.org/10.1006/pest.1999.2472

Yuen, W.K. and Ho, J.W. (2001). Purification and characterization of multiple glutathione S-transferase isozymes from Chironomidae larvae. Comp. Biochem. Physiol. A, 129:631-640. https://doi.org/10.1016/s1095-6433(01)00327-0.

Zheng, S.; Wang, Y.; Zhou, Q. and Chen, C. (2013). Responses of oxidative stress biomarkers and DNA damage on a freshwater snail (*Bellamya aeruginosa*) stressed by ethylbenzene. Arch. Environ. Contam. Toxicol., 65(2):251-259. https://doi.org/10.1007/s00244-013-9899-z.