RESEARCH ARTICLE

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Isolation and Characterization of Superoxide Dismutase from *Tectus dentatus* Marine Snail with Antimicrobial and Antitumor Activities

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Abstract

Objective: The aim of this study is to reports the purification, biochemical characterization, and biological activities of superoxide dismutase (SOD) isoenzymes from the marine snail *Tectus dentatus* (*TdSOD*). **Methods:** The enzyme was purified through DEAE-cellulose ion-exchange and Sephacryl S-300 size-exclusion chromatography, yielding two distinct isoenzymes, *Td*SOD1 and *Td*SOD2. *Td*SOD1 was further purified to homogeneity with a 6.9-fold purification and a specific activity of 658.3 U/mg. **Results:** Electrophoretic analyses confirmed the enzyme's purity and revealed a native molecular weight of approximately 180 kDa, composed of subunits around 90 kDa. *Td*SOD1 showed optimal activity at pH 7.8 and was strongly activated by Zn^{2+} and Cu^{2+}, while inhibitors such as KCN and H_2O_2 significantly reduced its activity. Functionally, *TdSOD1* demonstrated notable antimicrobial activity, especially against *Candida albicans* (97.3% inhibition) and Gram-negative bacteria including *Pseudomonas aeruginosa* (61.7%) and *Escherichia coli* (60.2%). Lower activity was observed against *Klebsiella pneumoniae* (39.1%) and *Staphylococcus aureus* (2.9%). In anticancer assays, *Td*SOD1 exerted a dose-dependent cytotoxic effect on MCF-7 and MDA-MB-231 breast cancer cell lines, with an IC_{50} of 33.42 µg/mL for MDA-MB-231 cells. **Conclusion:** These results suggest that *Td*SOD1 possesses promising antimicrobial and anticancer potential, supporting its future exploration as a multifunctional therapeutic agent.

Keywords: Marine snails-*Tectus dentatus*- Superoxide dismutase- Purification- characterization- Antimicrobial

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Introduction

Marine ecosystems are a rich source of bioactive compounds, many of which have been harnessed for therapeutic applications. Among marine invertebrates, mollusks have garnered attention due to their production of diverse secondary metabolites with significant pharmacological properties, including antimicrobial and anticancer activities [1, 2]. Reactive oxygen species (ROS), such as superoxide anions (O_2^-), are byproducts of normal cellular metabolism, while low levels of ROS play important roles in cell signalling; however, their excessive accumulation can cause oxidative stress, damage cellular components and contribute to the pathogenesis of many diseases including cancer, neurodegeneration, cardiovascular disorders, and ageing [3-6]. Organisms have evolved antioxidant defence mechanisms to mitigate ROS-induced damage, with superoxide dismutase (SOD) being a pivotal enzyme in this defence system [7]. SOD facilitates the conversion of superoxide radicals into oxygen and hydrogen peroxide, helping to shield cells from oxidative harm [3, 8, 9]. Marine snails, like many other aerobic organisms, possess several types of (SOD) enzymes that play critical roles in defending against oxidative stress [10]. The primary SOD isoforms identified in marine snails include copper/zinc SOD (Cu/Zn-SOD), manganese SOD (Mn-SOD), and, to a lesser extent, iron SOD (Fe-SOD). Cu/Zn-SOD is typically found in the cytosol and extracellular fluids, and its expression in marine gastropods such as *Onchidium struma* has been shown to increase in response to environmental stressors like heavy metals [11]. Mn-SOD is localized in the mitochondria and is essential for neutralizing superoxide radicals generated during aerobic respiration,

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mitochondrial SOD activity has been inferred from broader molluscan research [12]. Fe-SOD, although more common in bacteria and plants, has been detected in certain invertebrate marine species, suggesting it may also be present in some marine snails [13]. The therapeutic potential of SOD extends beyond its antioxidant capacity [14, 15]. Studies have demonstrated that SOD exhibits antimicrobial properties, effectively inhibiting the growth of various bacterial and fungal pathogens [16, 17]. For instance, SOD purified from the marine snail *Cellana rota* displayed significant antibacterial activity against *Escherichia coli, Salmonella typhi, and Staphylococcus aureus*, as well as antifungal activity against *Aspergillus niger* [18]. In addition to its antimicrobial effects, SOD has shown promise as an anticancer agent [19, 20].The study on *C. rota* SOD revealed its cytotoxic effects on human cancer cell lines, including A549 (lung carcinoma), Caco-2 (colorectal adenocarcinoma), and HepG2 (hepatocellular carcinoma), indicating its potential role in cancer therapy [21]. Similarly, mucin extracted from the terrestrial snail *Eremina desertorum* was found to enhance the expression of antioxidant enzymes and Tumor suppressor genes in HepG2 and Caco-2 cells, further supporting the anticancer potential of snail-derived compounds [22]. The emerging significance of SOD in infectious diseases, especially concerning COVID-19, has been highlighted by recent findings [23], which suggest that alterations in SOD activity could help predict disease severity and outcomes. Despite these promising findings, research on SOD from *Tectus dentatus*, a marine gastropod, remains limited. Therefore, in this study superoxide dismutase was isolated from the soft tissues of *T. dentatus* marine snail and identified, as well its antimicrobial and antitumor activities were evaluated.

Materials and Methods

Sample collection

Tectus dentatus snails were collected from Ain El-Sokhna shore, Red Sea, Egypt ((latitude: 29º28'18.2"N and longitude: 32º27'12.6"E, May, 2023), transferred to laboratory and cleaned utilizing distilled water, shells were crushed and the soft tissues were dissected out and kept at -20°C till utilized in the experimental tests [24].

Chemicals

All chemicals were obtained from reputable suppliers as follows: xanthine sodium salt, xanthine oxidase enzyme, cytochrome C (from horse heart), nitroblue tetrazolium (NBT), Dimethyl sulfoxide (DMSO), phenyl methosulfate (PMS), phenylmethylsulfonyl fluoride (PMSF), 1,4-dithiothreitol (DTT), 1,10-phenanthroline, trypan blue dye, bovine serum albumin (BSA), blue dextran, crystal violet, Sephacryl S-300, DEAE-cellulose, and gel filtration molecular weight marker kits were purchased from Sigma Co. SDS molecular weight marker proteins were acquired from Pharmacia Co. Meanwhile, cell culture media and supplements including DMEM, RPMI-1640, fetal bovine serum, HEPES buffer solution, gentamicin, and L-glutamine were obtained from Lonza, Belgium.

Methods

SOD activity assay

The superoxide dismutase (SOD) activity is typically measured by assessing its ability to inhibit the reduction of cytochrome C. This process is based on the scavenging of superoxide anions, which are generated by the xanthine-xanthine oxidase system. In this assay, a reaction mixture is prepared containing 1.0 mL of a 0.02 M potassium phosphate buffer (pH 7.8), supplemented with 0.1 mM EDTA, 0.01 mM cytochrome C, and 0.05 mM sodium xanthine. The reaction is triggered by the addition of 21 mU of xanthine oxidase, which catalyzes the oxidation of sodium xanthine, leading to the formation of superoxide anions. These anions reduce cytochrome C, causing a shift in absorbance, which can be measured at 550 nm. The level of SOD activity is determined by its ability to inhibit this reduction, with one unit of activity corresponding to the amount of enzyme that results in 50% inhibition of the cytochrome C reduction rate [3].

Staining of SOD activity on polyacrylamide gels

The staining of superoxide dismutase (SOD) activity is often performed using the method described by Weisiger and Fridovich, [8]. In this method, the activity of SOD following electrophoresis is assessed by using a reaction mixture containing phenazine methosulphate (PMS) and nitroblue tetrazolium salt (NBT), which generates superoxide anions. These anions then reduce NBT, producing formazan, which causes a colour change. When SOD is present, it scavenges the superoxide anions, preventing NBT reduction and forming achromatic zones on the gel. These clear zones appear where the superoxide radicals have been neutralized by the SOD enzyme, contrasting with the blue colour of the surrounding gel. The gels are incubated with the buffered mixture of NBT and PMS, and then exposed to sunlight for several minutes, allowing the achromatic zones to develop, indicating areas of SOD activity.

Purification of T. dentatus snail superoxide dismutase

Preparation of snail crude extract

All experimental proceedings were carried out at 4°C unless mentioned otherwise, and the snail extract was prepared according to [25]. 7 g of *T. dentatus* snail tissues were mixed with 2 volumes of 0.02 M K-phosphate buffer at pH 7.4 and homogenized utilizing a Teflon pestled homogenizer. The homogenate was then centrifuged at 10000 x g for 30 min to remove insoluble materials and cell debris and obtaining the filtrate (15 ml) as a crude extract.

Chromatography on DEAE-cellulose column

The procedure was carried out as described by Sheehan and FitzGerald, [26]. Crude extract of the *T. dentatus* snail was subjected to ion-exchange chromatography using a DEAE-cellulose column (12 cm x 2.4 cm i.d.), which had been pre-equilibrated with 0.02 M potassium phosphate buffer at pH 7.4. Elution of the proteins bound to the column was achieved by applying a stepwise gradient of sodium chloride (NaCl), ranging from 0 to 1 M, in the equilibration buffer. The elution process was conducted at a flow rate of 60 mL per hour. Fractions of 5 mL were collected throughout the process, and those containing superoxide dismutase (SOD) activity were identified and pooled based on their activity profiles.

Chromatography on Sephacryl S-300 column

The procedure was carried out as described by Ó'Fágáin et al, [27]. The material from the peak containing superoxide dismutase (SOD) activity was concentrated and subsequently applied to a Sephacryl S-300 column (142 cm x 1.75 cm i.d.) for further purification. The Sephacryl S-300 column was pre-equilibrated with a 0.02 M potassium phosphate buffer at pH 7.4, and the proteins were eluted at a flow rate of 30 mL per hour. Fractions of 2 mL were collected throughout the elution process for further analysis.

Electrophoretic analysis

Gel electrophoresis was performed using 7% polyacrylamide gel electrophoresis (PAGE), following the method of Smith [28]. SDS-PAGE was carried out with12% polyacrylamide gel, based on the method of Laemmli [29]. The molecular weights of the purified superoxide dismutase (SOD) enzymes were determined using SDS-PAGE, according to the method described by Weber and Osborn [30]. Proteins were stained using Coomassie Brilliant Blue (R-250) at a concentration of 0.25%. To detect SOD activity, the gel was stained according to the protocol of Weisiger & Fridovich [31], allowing for the visualization of SOD activity.

Protein determination

Protein concentrations during the purification steps were measured spectrophotometrically using the Bradford method [32].

Effect of pH

The optimum pH for activity of purified *Td*SOD was carried out as described by Ibrahim et al, [33] utilizing buffer 20 mmol L-1 pH 6.0 to 9.0 (Na-phosphate and Tris-HCl buffer).

Effect of cations

The effect of divalent cations on TdSOD activity was measured after 5 mM for each cation pre- incubation at 37˚C as described by Ibrahim et al. [33]. Activity without added cations was taken as 100% activity.

Effect of inhibitors

The effect of divalent cations on TdSOD1 activity was assessed after pre-incubation with 5 mM of each cation at 37°C as described by Ibrahim et al., [33]. Activity without inhibitors cations was taken as 100%.

Antimicrobial activity determination

The antimicrobial activity of purified *Td*SOD was tested against a panel of microbial strains, including four bacterial species; *S. aureus, K. pneumoniae, P. aeruginosa, and E. coli*, and one fungal species, *C. albicans*. All bacterial strains were cultured in nutrient broth at 37 °C for 18–24 hours, while *Candida albicans* was grown in Sabouraud dextrose broth under the same conditions [34].

Antitumor activity determination

Cell Culture

MCF-7 and MDA-MB-231 cell lines were purchased from the Cell Bank of Type Culture Collection of the Vacsera, Giza, Egypt. Dulbecco's Modified Eagle's Medium (DMEM, Hyclone) was supplemented with 1% Penicillin-Streptomycin Solution (GP3108, Genview) and 10% fetal bovine serum (FBS, A0500-3010, Cegrogen Biotech). Cell lines were incubated at 37 °C in a humidified incubator containing 5% CO2/95% air (v/v) [2]

Cell viability measurement

Assessment of cell viability was carried out using the MTT assay [35]. MCF-7 and MDA-MB-231 cells were seeded in 96-well plates (5 × 10^3 cells/well) and incubated for 24 h at 37°C with 5% CO_2. Cells were then treated with varying concentrations of SOD for another 24 h. After treatment, 10 µL of MTT solution (5 mg/mL) was added and incubated for 4 h. The resulting formazan crystals were solubilized with 100 µL DMSO. Plates were shaken for 10 min in the dark to ensure full dissolution. Absorbance was read at 570 nm using (BioTek Instruments, USA). All experiments were performed in triplicate.

Statistical analysis

Statistical analyses were performed by GraphPad Prism 8 software. The results are representative of at least three independent experiments performed in triplicate and are expressed as the means ±SD. The data were analyzed using the student's t-test and data were considered significant when $P<0.05$ [36].

Results

Purification of superoxide dismutase from T. dentatus snail

The purification process of *T. dentatus* superoxide dismutase (Td SOD) isoenzymes was carried out through a series of chromatographic steps, resulting in progressive increases in specific activity and purification fold. Starting from the crude extract, which contained 64 mg of total protein and 6150 units of SOD activity (specific activity of 96.1 U/mg), two distinct SOD isoenzymes were separated using DEAE-cellulose ion-exchange chromatography (Figure 1a). The first isoenzyme, *Td*SOD1, was eluted with 0.0 M NaCl, yielded 2665 units in 14.4 mg of protein, corresponding to a 43.3% recovery, a specific activity of 185.1 U/mg, and a 1.93-fold purification (Table 1). The second isoenzyme, *Td*SOD2, was eluted with 0.2 M NaCl, exhibited 1556 total units in 11.4 mg of protein, with a 24.9% recovery and specific activity of 136.5 U/mg (1.6-fold purification). Further purification of *Td*SOD1 using Sephacryl S-300 size-exclusion chromatography (Figure 1b) produced 2.3 mg of protein with 1514 units of activity, achieving a 24.6% recovery, a specific activity of 658.3 U/mg, and a 6.9-fold purification compared to the crude extract (Table 1). The molecular weight of *Td*SOD1 was determined from gel filtration column elution volume

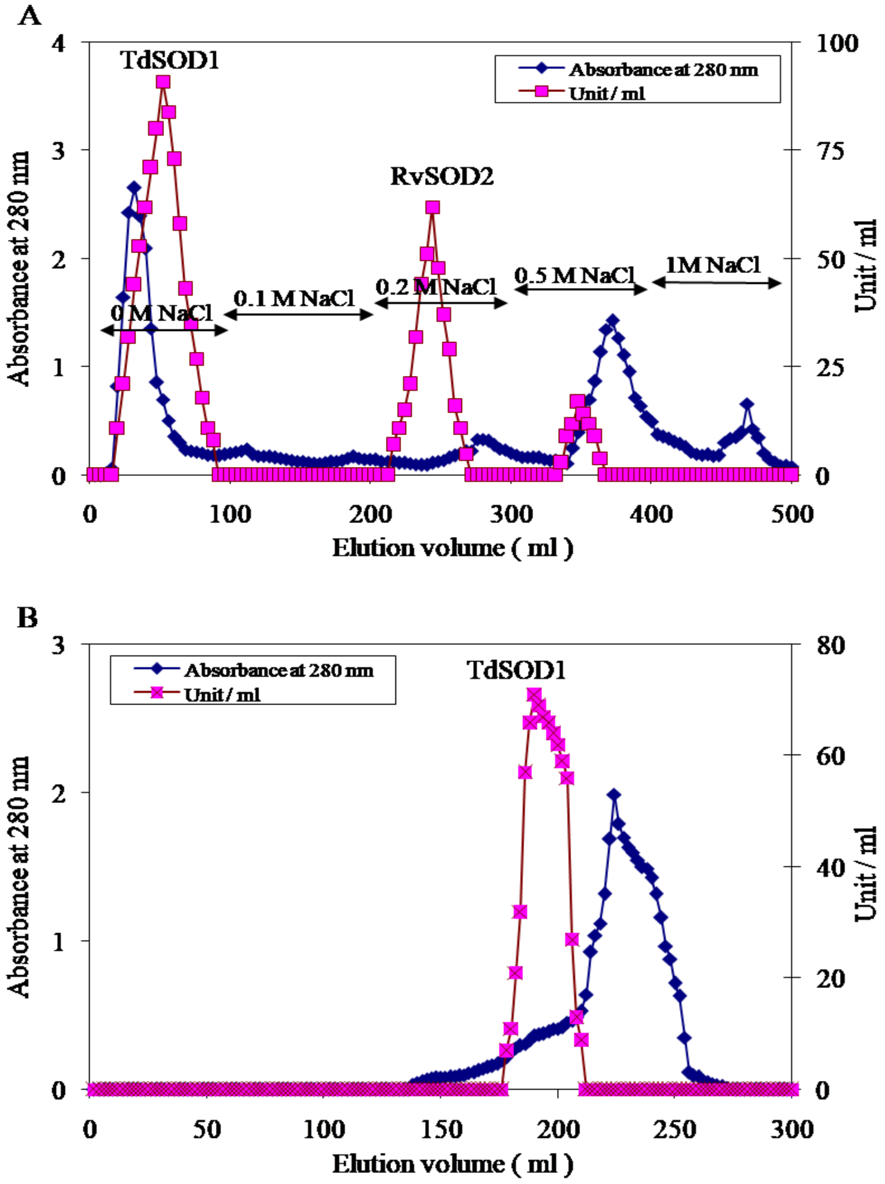

Figure 1. (A) A typical elution profile for the *Tectus dentatus* snail crude extract on DEAE-cellulose column (12 cm x 2.4 cm i.d.) previously equilibrated with 0.02 M K-phosphate buffer pH 7.4. (B) Typical elution profile for the chromatography of the concentrated pooled DEAE-cellulose fraction *Td*SOD1 on Sephacryl S-300 column (142 cm x 1.75 cm i.d.) previously equilibrated with 0.02 M K-phosphate buffer pH 7.4.

to be 180 ± 2.3 kDa (Figure 2c).

Electrophoretic analysis

The purification steps: including the crude extract, DEAE-cellulose fraction, and Sephacryl S-300 fraction were analyzed by electrophoresis using 7% native PAGE. A single distinct protein band (Figure 2a) was observed corresponding to the SOD activity band (Figure 2b), indicating the progressive purification and homogeneity of the *Td*SOD1 preparation. Further analysis using SDS-PAGE revealed that the purified *Td*SOD1 molecular weight was 180 kDa consisting of two identical subunits with an estimated molecular weight of approximately 90 ± 1.7 kDa each, as determined by comparison to standard

Table 1. A Typical Purification Scheme of *Td*SOD Isoenzymes

Purification step	Total proteins (mg)	Total units	Recovery (%)	Specific activity	Fold purification
T. dentatus crude extract	64	6150	100	96.1	1
DEAE-cellulose fractions					
0.0 M NaCl (*Td*SOD1)	14.4	2665	43.3	185.1	1.93
0.2 M NaCl (*Td*SOD2)	11.4	1556	24.9	136.5	1.6
Sephacryl S-300 fractions					
*Td*SOD1	2.3	1514	24.6	658.3	6.9

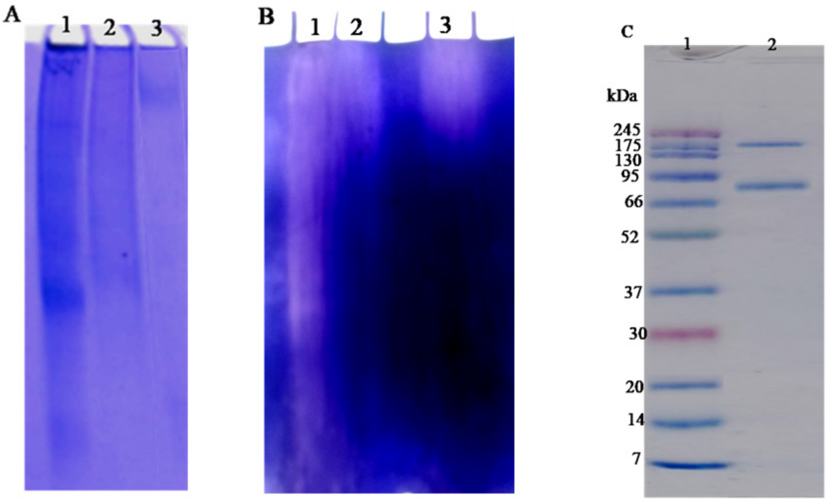

Figure 2. Electrophoretic Analysis of *Tectus dentatus* Superoxide Dismutase 1 (*Td*SOD1) Purification Steps on 7% Native PAGE; (a) Protein patterns, and (b) SOD isoenzyme patterns: (1) crude extract, (2) DEAE-cellulose fraction and (3) Sephacryl S-300 fraction. (c) Subunit molecular weight determination of *T. dentatus* superoxide dismutase 1 (*Td*SOD1) on 12 % SDS-PAGE: (1) Molecular weight marker proteins and (2) denatured purified *Td*SOD1.

Table 2. Effect of Divalent Cations on the Purified *Td*SOD1

Reagent (5 mM)	Residual activity (%)
Control	100.0
$CaCl_2$	37.6
$CoCl_2$	108.8
$CuCl_2$	153.1
$FeCl_2$	3.2
$MgCl_2$	13.5
$MnCl_2$	76.5
$NiCl_2$	21.9
$ZnCl_2$	186.7

* These values represent % of the control and the means of triplicate experiments

protein markers (Figure 2c).

Effect of pH

The optimum activity of purified *Td*SOD1 was carried out utilizing buffers (Na-phosphate and Tris-HCl) pH 6.0 to 9.0. The highest *Td*SOD1 activity was recorded at pH 7.8 (Figure 3a).

Effect of cations

The effect of various divalent cations on the residual activity of purified *Td*SOD1 was evaluated by incubating the enzyme with 5 mM of each cation. The control sample, without any divalent cation, showed 100% enzyme activity. Among the tested cations, $ZnCl_2$ exhibited the most significant enhancement, increasing enzyme activity to 186.7% of the control. $CuCl_2$ also strongly activated *Td*SOD1, with residual activity reaching 153.1%. $CoCl_2$ provided mild activation, with residual activities of 108.8%. In contrast, $MnCl_2$, $NiCl_2$, $CaCl_2$, $MgCl_2$, and $FeCl_2$ showed inhibitory effects with residual activities of 76.5%, 21.9%, 37.6%, 13.5%, and 3.2%respectively. These results suggest that zinc and copper are the most effective divalent cations in enhancing the activity of *Td*SOD1 (Table 2).

Effect of inhibitors

The effect of various inhibitors on the activity of purified *Td*SOD1 was assessed by incubating the enzyme with 5 mM of each inhibitor. The control sample showed no inhibition (0.0%). Potassium cyanide (KCN) caused a significant inhibition, reducing enzyme activity by 67.5%, suggesting that *Td*SOD1 is highly sensitive to cyanide, likely due to its interaction with the enzyme's active site. Hydrogen peroxide (H_2O_2) also resulted in substantial inhibition (53.6%), indicating that oxidative stress can severely impair *Td*SOD1activity. DL-Dithiothreitol (DTT), a reducing agent, led to a the most significant inhibition of 76.5%, which may be attributed to its interference with the disulfide bonds essential for *Td*SOD1's structure and function. Other inhibitors such as Sodium azide (NaN3), β-Mercaptoethanol, and Potassium dichromate caused moderate inhibition, with activity reductions of 8.1%, 21.8%, and 25.7%, respectively. (EDTA), which chelates metal ions, reduced activity by 13.5%. (SDS), a detergent, caused minimal inhibition (3.2%), while 1,10-phenanthroline, a metal chelator, led to slight inhibition (6.8%). These results suggest that *Td*SOD is particularly vulnerable to inhibitors that target the enzyme's active site, disrupt metal ion cofactors, or cause oxidative damage, with KCN and H_2O_2 being the most potent inhibitors (Table 3).

Antimicrobial activity

The antimicrobial activity of purified *Td*SOD1 was evaluated against a panel of bacterial and fungal pathogens compared with standard antibiotics ciprofloxacin (5 µg) and nystatin (10 µg). *Td*SOD1 exhibited strong antifungal activity against *C. albicans*, achieving 97.3% inhibition, which was comparable to the activity of nystatin (98.9%). Among the tested bacterial strains, *Td*SOD1 showed the highest inhibitory effect against *P. aeruginosa* (61.7%)

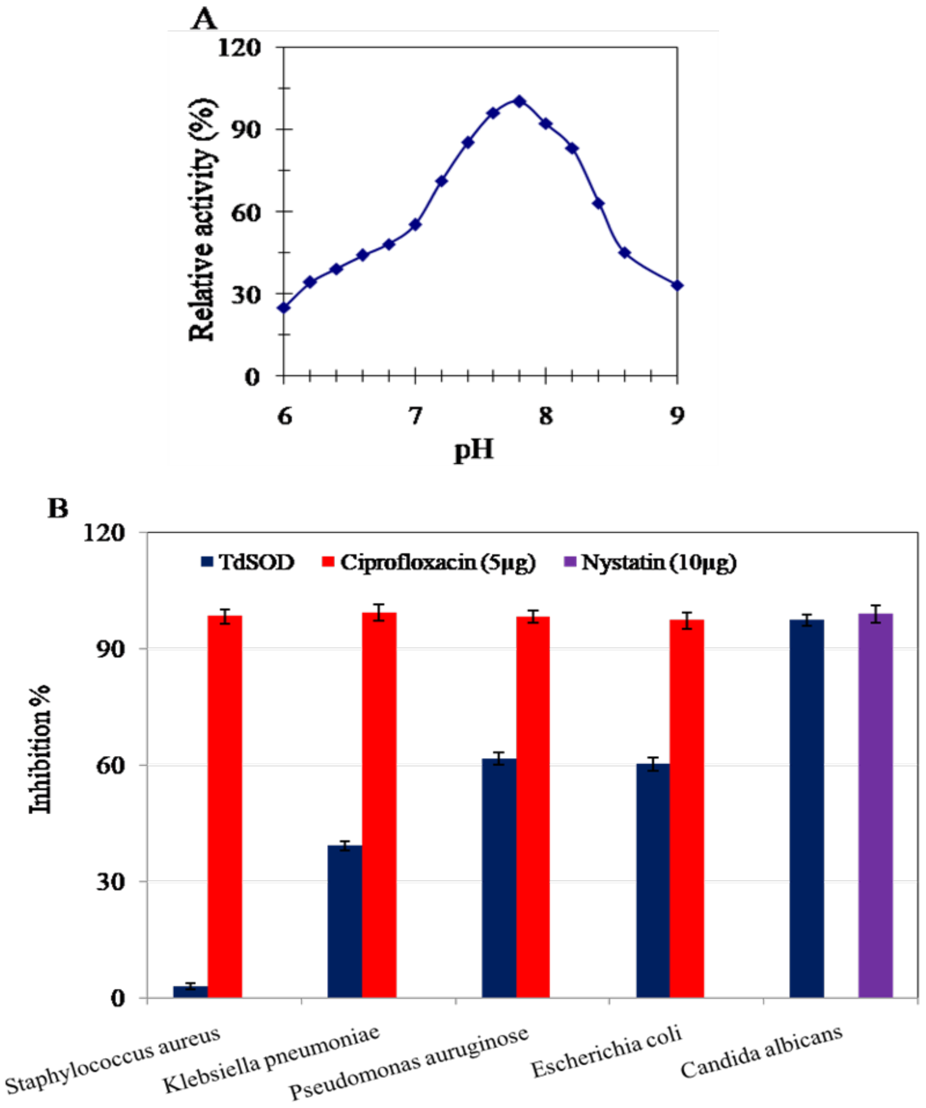

Figure 3. (a) The optimum pH for activity of purified *Tectus dentatus* superoxide dismutase 1 (*Td*SOD1) was carried out utilizing buffer 20 mmol L-1 pH 6.0 to 9.0 (Na-phosphate and Tris-HCl buffer). (b) Antimicrobial activity of purified *T. dentatus* superoxide dismutase 1 (*Td*SOD1).

Table 3. Effect of Inhibitors on the Purified *Td*SOD1

Inhibitor (5 mM)	Inhibition %
Control	0.0
Potassium cyanide (KCN)	67.5
Hydrogen peroxide (H_2O_2)	53.6
Sodium Azide (NaN_3)	8.1
Sodium dodecyl sulphate (SDS)	3.2
Ethylenediamine tetraacetic acid (EDTA)	13.5
DL-Dithiothreitol (DTT)	76.5
β-Mercaptoethanol	21.8
Potassium dichromate	25.7
1,10-Phenanethroline	6.8

* These values represent % of the control and the means of triplicate experiments

and *E. coli* (60.2%), followed by *K. pneumoniae* (39.1%). Minimal inhibition was observed against *S. aureus,* with only 2.9% inhibition. In contrast, ciprofloxacin demonstrated high efficacy against all bacterial strains, with inhibition rates exceeding 97%, while nystatin was ineffective against bacteria. These results suggest that *Td*SOD1 possesses selective antimicrobial activity, particularly against Gram-negative bacteria and fungal pathogens (Figure 3b).

Antitumor activity

The effect of *Td*SOD1on the viability of MCF-7 and MDA-MB-231 breast cancer cells was evaluated using the MTT assay, as shown in (Figure 4). Both cell lines were treated with increasing concentrations of *Td*SOD1 (a Cu/Zn SOD isoenzyme) (ranging from 0 to 100µg/mL), and cell viability was expressed as a percentage relative to untreated control cells. The results revealed a dose-dependent cytotoxic effect of *Td*SOD1 on both cell lines. In MCF-7 cells, a sharp decline in cell viability was observed at low concentrations, with viability dropping

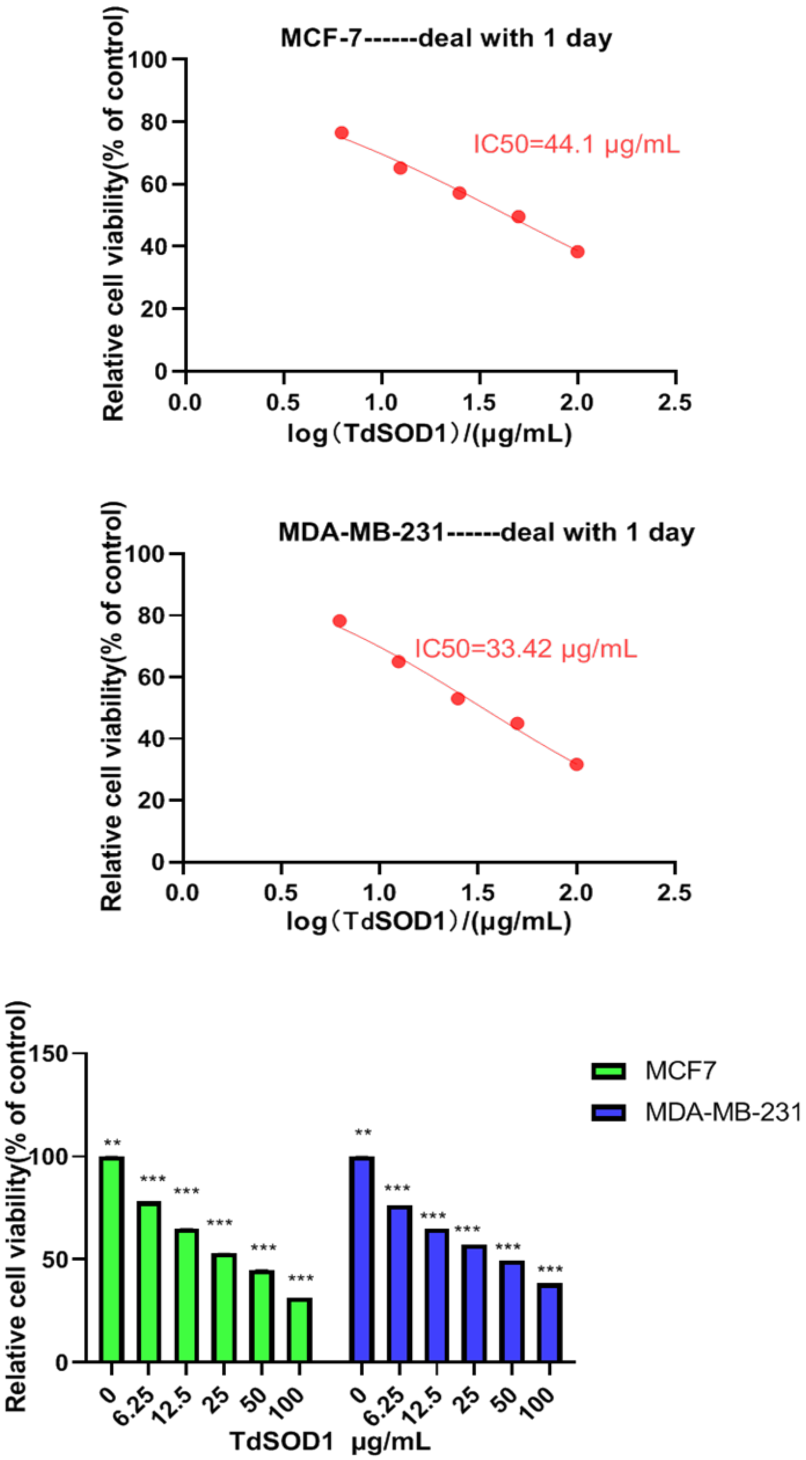

Figure 4. The Effect of *Tectus dentatus* Superoxide Dismutase 1 (*Td*SOD1) on the Viability of MCF-7 and MDA- MB-231 Breast Cancer Cells was Evaluated Using the MTT Assay.

below 60% at approximately 25µg/mL. This downward trend continued with increasing concentrations, ultimately reducing viability to about 35% at 100µg/mL, indicating a strong cytotoxic effect. Similarly, MDA-MB-231 cells exhibited a dose-dependent decrease in viability, with the half-maximal inhibitory concentration (IC_{50}) calculated at 33.42µg/mL. At higher concentrations, *Td*SOD1 significantly reduced cell viability, further confirming its cytotoxic potential against MDA-MB-231 cells. These findings suggest that *Td*SOD1 exerts potent cytotoxic activity in a dose-dependent manner on both MCF-7 and MDA-MB-231 breast cancer cell lines.

Discussion

Interest in marine mollusks has grown exponentially over the past several decades, driven by the discovery of a wide range of health-promoting compounds they contain. These bioactive substances have positioned marine mollusks as a valuable source for the expanding nutraceutical and pharmaceutical markets, as reflected in rapid increase of marine organism-based products currently available [21, 2, 37]. The exploration of marine-derived SODs is of particular relevance [38, 39]. *T. dentatus*, a marine mollusk, has not been extensively studied for its enzymatic activities. In this context, the present study focused on isolating and characterizing SOD from this species, assessing its activity in the presence of divalent cations and inhibitors, and exploring its antimicrobial and antitumor potential. Previous studies have highlighted the promising roles of marine SODs in various biotechnological applications, including their antioxidant, antimicrobial, and anticancer activities [40, 41]. The purification of SOD from *T. dentatus* (*Td*SOD1) was successfully carried out in the present study leading to isolation of two distinct isoenzymes, *Td*SOD1 and *Td*SOD2, from the crude extract, each with different levels of activity and specificity (Figure 1a). *Td*SOD1 was the more active isoenzyme, with a higher specific activity and fold purification, suggesting it may be the primary isoform responsible for the enzymatic function in *T. dentatus*. In contrast, *Td*SOD2 exhibited lower specific activity and recovery, which could indicate a lower abundance of this isoform in *T. dentatus*. Different SODs from different sources have been isolated similarly [42, 33, 43]. Purification of SOD from *T. dentatus* (*Td*SOD1) was closely monitored using 7% native PAGE electrophoresis at each purification step. The results confirmed the successful isolation and enrichment of the target enzyme throughout the different stages, from crude extract to final purification by Sephacryl S-300 chromatography. Native PAGE analysis confirmed the successful purification of *Td*SOD1 from *T. dentatus*, with a single protein band corresponding to the SOD activity band in the final Sephacryl S-300 fractions. The molecular weight of *Td*SOD1 was 180 kDa and the enzyme is likely a dimer consisting of two 90 kDa subunits. The dimeric nature of *Td*SOD1 is consistent with the molecular structure of many SOD enzymes, which often function through multimerization to achieve optimal enzymatic activity [44-46].

The optimal pH for enzymatic activity is a critical parameter that provides insight into the physiological conditions under which the enzyme functions most effectively. In this study, the results revealed that *Td*SOD1 exhibited the highest activity at pH 7.8, suggesting that this is the optimal pH for the enzyme's activity. Likewise, the optimal pH for SOD activity in tick larvae was 7.8. [33], and at pH 8.0 in pearl millet [47] and tea [48].The effect of divalent cations on the activity of the purified *Td*SOD1 revealed that the most notable activators were CuCl2 and ZnCl2, both of which significantly enhanced *Td*SOD1 activity. The presence of CuCl2 resulted in 153.1% of the control activity, indicating that copper ions play a critical role in the enzyme's activity. Similarly, $ZnCl_2$ showed an even greater enhancement, with residual activity reaching 186.7% of the control. This suggests that Cu^{2+} and Zn^{2+} ions may be essential cofactors for *Td*SOD1 or significantly improve its catalytic efficiency. These results align with findings from other studies, where copper and zinc are integral components of many Cu/Zn-SODs, facilitating the dismutation of superoxide radicals. Cu^{2+} and Zn^{2+} ions were required for SOD activity from *C. rota* snail [18] and $ZnCl_2$ was required for SOD activity of the shrimp muscle tissue [49]. On the other hand, $CaCl_2$ reduced enzyme activity indicating a possible inhibitory effect of calcium ions on *Td*SOD1. FeCl2 caused the most substantial inhibition, suggesting that Fe^{2+} may interfere with the enzyme's structure or function, potentially by disrupting the active site. MgCl2 and NiCl2 also reduced enzyme activity. SOD can be classified based on their metal cofactors, and the differentiation between SOD classes is often achieved through selective chemical inhibitors, such as hydrogen peroxide (H_2O_2) and potassium cyanide (KCN). These inhibitors exhibit distinct effects on different SOD isoenzymes, depending on the metal ion in the enzyme's active site. *Td*SOD1 was highly sensitive to inhibition by KCN, with 67.5% inhibition. This finding is consistent with the characteristics of Cu/Zn-SOD isoenzymes, which are well known for their sensitivity to KCN. The cyanide ion binds to the copper or zinc cofactor at the enzyme's active site, preventing the enzyme from catalyzing the dismutation of superoxide radicals. This inhibition suggested that *Td*SOD1 likely belongs to the Cu/Zn-SOD class, which is characterized by the presence of copper and zinc in its active site [8]. In addition to KCN, *Td*SOD1 also exhibited significant inhibition by H_2O_2 (53.6%), another common inhibitor of Cu/Zn-SODs. The presence of H_2O_2 can lead to oxidative damage at the enzyme's metal center, further inhibiting its activity. This characteristic inhibition by H_2O_2 is another key feature of Cu/Zn-SODs and further supports the classification of *Td*SOD1 as a Cu/Zn-containing isoenzyme [50, 18]. DL-Dithiothreitol (DTT), a reducing agent, also potently inhibited *Td*SOD1, likely by reducing disulfide bonds that are important for maintaining the enzyme's structural integrity. This suggests that *Td*SOD1 may have critical disulfide linkages that contribute to its stability and function like to SOD of *Radix lethospermi* seed [51].

The antimicrobial activity of *Td*SOD1 isolated from *T. dentatus* demonstrated selective and promising bioactivity against a range of microbial pathogens. Notably, *Td*SOD1 exhibited potent antifungal activity against *Candida albicans*, achieving 97.3% inhibition, which is comparable to the standard antifungal agent nystatin (98.9%). This strong effect suggests that *Td*SOD1 may interfere with fungal oxidative stress defence mechanisms, potentially disrupting cell membrane integrity or metabolic processes. Among bacterial strains, *Td*SOD1 showed higher efficacy against Gram-negative bacteria, particularly *P. aeruginosa* (61.7%) and *E. coli* (60.2%), followed by *K. pneumoniae* (39.1%). The relatively higher susceptibility of Gram-negative bacteria may be attributed to their thinner peptidoglycan layer and greater sensitivity to oxidative stress induced by SOD activity. In contrast, the Gram-positive *S. aureus* was minimally affected (2.9%), likely due to its thicker cell wall and more robust antioxidant defence systems. Compared to ciprofloxacin, a broad-spectrum antibiotic, *Td*SOD1 exhibited moderate antibacterial activity, suggesting it may not act as a direct bactericidal agent but rather exert its effects through oxidative stress modulation. The lack of antibacterial activity by nystatin and lack of antifungal activity by ciprofloxacin further support the selective nature of *Td*SOD1's antimicrobial spectrum. These findings indicate that *Td*SOD1 may serve as a potential antimicrobial agent, especially against fungal infections and Gram-negative bacterial pathogens. Similarly, SOD from *C. rota* snail displayed efficient antimicrobial activity against *E. coli, S. typhi, P. aeruginosa, S. Aureus, C. albicans* and *A. niger* [18].

The *Td*SOD1 was screened for its antitumor activity; the findings from the MTT assay demonstrated that *Td*SOD1 exerts a significant dose-dependent cytotoxic effect on both MCF-7 and MDA-MB-231 breast cancer cell lines. This cytotoxicity is evident from the marked reduction in cell viability with increasing concentrations of *Td*SOD1. Notably, MCF-7 cells displayed a higher sensitivity to *Td*SOD1 at lower concentrations. A sharp decline in viability below 60% at just 25 µg/mL. In contrast, MDA-MB-231 cells, which are resistant to many therapies, also showed substantial sensitivity, with an IC_{50} of 33.42 µg/mL. While the decline in viability was more gradual compared to MCF-7, the results still indicate that *Td*SOD1 can overcome resistance mechanisms in these cells at higher concentrations. The dose-dependent nature of the response in both cell lines highlights the potential of the Cu/Zn SOD isoenzyme (*Td*SOD1) as a therapeutic agent or adjunct in breast cancer treatment. *Td*SOD1 may modulate signalling pathways involved in cell proliferation and survival. Overall, the results support the hypothesis that *Td*SOD1 possesses strong anti-proliferative properties against diverse breast cancer subtypes. In comparison to other investigations, C. rota snail SOD displayed potent toxicity against A549, Caco2 and HepG2cell lines [18]. Also, an enzyme from Turbo radiates snail with GST activity exhibited comparable cytotoxic impacts against PC3, HepG2 and MCF7 cell lines [52].

In conclusion, the present study successfully purified and characterized superoxide dismutase (*Td*SOD1) from the marine snail *Tectus dentatus*, revealing it as a high-molecular-weight Cu/Zn-dependent isoenzyme with significant biological activity. *Td*SOD1 demonstrated strong antioxidant properties, optimal enzymatic activity at physiological pH, and marked sensitivity to metal cofactors and specific inhibitors, indicating its structural and functional dependence on metal ions. Importantly, *Td*SOD1 exhibited substantial antimicrobial activity, particularly against *Candida albicans* and Gram-negative bacteria, as well as potent dose-dependent cytotoxic effects on MCF-7 and MDA-MB-231 breast cancer cells. These findings highlight the potential of *Td*SOD1 as a multifunctional bioactive compound with promising therapeutic applications in antimicrobial and anticancer treatments.

Author Contribution Statement

All authors contributed to the experimental design, hands-on work, discussions, and comments on the manuscript.

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Declarations
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Applicable (Registration Number: 1-6-4)

Availability of data and material
All data and materials are available.

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Conflict of interest
The authors state that there is no conflicts of interest.

References

1. Ciavatta ML, Lefranc F, Carbone M, Mollo E, Gavagnin M, Betancourt T, et al. Marine mollusk-derived agents with antiproliferative activity as promising anticancer agents to overcome chemotherapy resistance. Med Res Rev. 2017;37(4):702-801. https://doi.org/10.1002/med.21423.
2. Zayed KM, Ghareeb MA, Habib MR, El-Einin HMA, Ali RE, El-Karim RMG, et al. Biological activity, chemical profiling and molecular docking of tissue extracts of the sea snail trochus erithreus. J Appl Pharm Sci. 2023;13(6):199-210. https://doi.org/10.7324/JAPS.2023.119765
3. McCord J, Fridovich I, Mccord JM, fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969;244:6049-55.
4. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408(6809):239-47. https://doi.org/10.1038/35041687.
5. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44-84. https://doi.org/10.1016/j.biocel.2006.07.001.
6. Rasheed Z. Superoxide dismutase: Challenges, opportunities, and promises for clinical translation. Int J Health Sci (Qassim). 2024;18(3):1-3.
7. Ludewig M, Götz K-P, Romero-Oliva C, Landaverde P, Chmielewski FM. Response of superoxide dismutase (sod) to homogeneous and heterogeneous food sources in bumblebees (bombus terrestris) and honeybees (apis mellifera). Physiologia. 2023;3:272-80. https://doi.org/10.3390/physiologia3020019.
8. Fridovich I. Superoxide radical and superoxide dismutases. Annu Rev Biochem. 1995;64:97-112. https://doi.org/10.1146/annurev.bi.64.070195.000525.
9. Chen Y, Li B, Li K, Lin Y. Superoxide dismutase nanozymes: Current status and future perspectives on brain disease treatment and diagnosis. Chemical communications (Cambridge, England). 2024;60. https://doi.org/10.1039/d3cc06288k.
10. Zang Y, Chen J, Li R, Shang S, Tang X. Genome-wide analysis of the superoxide dismutase (sod) gene family in zostera marina and expression profile analysis under temperature stress. Peer J. 2020;8:e9063. https://doi.org/10.7717/peerj.9063.

11. Wang WX, Rainbow PS. Comparative approaches to understand metal bioaccumulation in aquatic animals. Comp Biochem Physiol C Toxicol Pharmacol. 2008;148(4):315-23. https://doi.org/10.1016/j.cbpc.2008.04.003.
12. Levanon D, Almagor H, Fridovich I. Purification and characterization of superoxide dismutase from Saccharomyces cerevisiae. Archives of Biochemistry and Biophysics. 1985; 237(2), 341–347
13. Campbell W.H, Laudenbach DE. Characterization of iron-superoxide dismutasefrom the cyanobacterium anacystis nidulans. Archives of biochemistry and biophysics. 1986;246(1):1–9.
14. Liu Y, Wang W, Yang J, Zhou C, Huang Q, Zhang H. The antioxidative effects of superoxide dismutase on chronic kidney disease in vivo and in vitro. Oxidative medicine and cellular longevity. 2018.
15. Zheng M, Liu Y, Zhang G, Yang Z, Xu W, Chen Q. The applications and mechanisms of superoxide dismutase in medicine, food, and cosmetics. Antioxidants (Basel). 2023;12(9). https://doi.org/10.3390/antiox12091675.
16. Frye KA, Sendra KM, Waldron KJ, Kehl-Fie TE. Old dogs, new tricks: New insights into the iron/manganese superoxide dismutase family. J Inorg Biochem. 2022;230:111748. https://doi.org/10.1016/j.jinorgbio.2022.111748.
17. Khan W, Khan L, Chen D, Chen F. Comparative analyses of superoxide dismutase (sod) gene family and expression profiling under multiple abiotic stresses in water lilies. Horticulturae. 2023;9:17. https://doi.org/10.3390/horticulturae9070781.
18. Habib M, Masoud H, Helmy M, Hamed A, Gad R, Ezzat R, et al. In vitro anticancer and antimicrobial activities of copper-zinc superoxide dismutase purified from cellana rota snail. Egypt J Chem. 2023. https://doi.org/10.21608/ejchem.2023.200467.7736.
19. Zhao H, Zhang R, Yan X, Fan K. Superoxide dismutase nanozymes: An emerging star for anti-oxidation. J Mater Chem B. 2021;9(35):6939-57. https://doi.org/10.1039/D1TB00720C.
20. Mohideen K, Chandrasekaran K, Kareema M, Jeyanthikumari T , Dhungel S, Ghosh S. Assessment of antioxidant enzyme superoxide dismutase (sod) in oral cancer: Systematic review and meta-analysis. Dis Markers. 2024;2024:2264251. https://doi.org/10.1155/2024/2264251.
21. Habib MR, Hamed AA, Ali REM, Zayed KM, Gad El-Karim RM, Sabour R, et al. Thais savignyi tissue extract: Bioactivity, chemical composition, and molecular docking. Pharm Biol. 2022;60(1):1899-914. https://doi.org/10.1080/13880209.2022.2123940.
22. Atta SA, Ibrahim AM, Megahed FAK. In-vitro anticancer and antioxidant activities of eremina desertorum (forsskal, 1775) snail mucin. Asian Pac J Cancer Prev. 2021;22(11):3467-74. https://doi.org/10.31557/apjcp.2021.22.11.3467.
23. Chu J, Hua L, Liu X, Xiong H, Jiang F, Zhou W, Wang L, Xue G. Superoxide dismutase alterations in COVID-19: implications for disease severity and mortality prediction in the context of omicron variant infection. Frontiers in Immunology. 2024 Feb 22;15:1362102.
24. Abu Eleinin HM, Gad R, Habib M, Zayed K, Ezzat R. Identification of the gastropod snails and shells collected from ain el-sokhna region, red sea, egypt. Egypt J Aquat Res. 2021;25:101-17.
25. Oida S, Sone M, Sasaki S. Purification of swine kidney alkaline phosphatase by immunoaffinity chromatography. Anal Biochem. 1984;140(1):117-20. https://doi.org/10.1016/0003-2697(84)90141-6.
26. Sheehan D, FitzGerald R. Ion-exchange chromatography. Methods Mol Biol. 1996;59:145-50. https://doi.org/10.1385/0-89603-336-8:145.
27. Ó'Fágáin C, Cummins PM, O'Connor BF. Gel-filtration chromatography. Methods Mol Biol. 2017;1485:15-25. https://doi.org/10.1007/978-1-4939-6412-3_2.
28. Smith J. Polyacrylamide gel electrophoresis: A new method for protein separation. J Biochem Biophys Methods. 1969;6(3):142–50.
29. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage t4. Nature. 1970;227(5259):680-5. https://doi.org/10.1038/227680a0.
30. Weber K, Osborn M. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J Biol Chem. 1969;244(16):4406-12.
31. Weisiger RA, Fridovich I. Superoxide dismutase: organelle specificity. Journal of Biological Chemistry. 1973;248(10):3582-92.Https://doi.org/10.1016/s0021-9258(18)50801-4.
32. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1):248-54. https://doi.org/https://doi.org/10.1016/0003-2697(76)90527-3.
33. Ibrahim MA, Mohamed MM, Ghazy AH, Masoud HM. Superoxide dismutases from larvae of the camel tick hyalomma dromedarii. Comp Biochem Physiol B Biochem Mol Biol. 2013;164(3):221-8. https://doi.org/10.1016/j.cbpb.2013.01.002.
34. Alhadrami HA, Orfali R, Hamed AA, Ghoneim MM, Hassan HM, Hassane ASI, et al. Flavonoid-coated gold nanoparticles as efficient antibiotics against gram-negative bacteria-evidence from in silico-supported in vitro studies. Antibiotics (Basel). 2021;10(8). https://doi.org/10.3390/antibiotics10080968.
35. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63. https://doi.org/10.1016/0022-1759(83)90303-4.
36. Bailey NTJ. Estimation, standard errors and confidence limits. In: Statistical methodsin biology. Cambridge university press; 1995. pp24-30
37. Ali RE, Zayed KM, Hamed AA, Habib MR, Abu ElEinin HA, Ghareeb MA, Gad El-Karim RM. Bioactive potential and chemical characterization of Planaxis sulcatus (Phylum: Mollusca, Family: Planaxidae) solvent extracts. Advances in Traditional Medicine. 2024 Sep;24(3):859-70
38. Mayer a. M.S, Rodríguez A.D, Berlinck R.G.S. Marine natural products. Natural product reports. 2010;27(1):97-106.
39. Kim JS, Kim H, Yim B, Rhee JS, Won EJ, Lee YM. Identification and molecular characterization of two cu/zn-sods and mn-sod in the marine ciliate euplotes crassus: Modulation of enzyme activity and transcripts in response to copper and cadmium. Aquat Toxicol. 2018;199:296-304. https://doi.org/10.1016/j.aquatox.2018.03.020.
40. Mitsui Y, Mizuta S, Kudo T. Purification and characterization of a manganesesuperoxide dismutase from a marine bacterium, vibrio alginolyticus. Appl Environ Microbiol. 2004;70(2):1069-76.
41. Rajesh K, Rajendra M, Subramanian S. Characterization and antioxidant potential of superoxide dismutase isolated from spirulina platensis. J Appl Phycol. 2012;24(1):101-10.
42. Longoni SS, Marín C, Sánchez-Moreno M. Excreted leishmania peruviana and leishmania amazonensis iron-superoxide dismutase purification: Specific antibody detection in colombian patients with cutaneous leishmaniasis. Free Radic Biol Med. 2014;69:26-34. https://doi.org/10.1016/j.freeradbiomed.2014.01.012.

43. Zeinali F, Homaei A, Kamrani E. Identification and kinetic characterization of a novel superoxide dismutase from avicennia marina: An antioxidant enzyme with unique features. Int J Biol Macromol. 2017;105:1556-62. https://doi.org/10.1016/j.ijbiomac.2017.07.054.
44. Aydemir T, Tarhan L. Purification and partial characterisation of superoxide dismutase from chicken erythrocytes. Turk J Chem. 2001;25:451-9.
45. Oztürk-Urek R, Tarhan L. Purification and characterization of superoxide dismutase from chicken liver. Comp Biochem Physiol B Biochem Mol Biol. 2001;128(2):205-12. https://doi.org/10.1016/s1096-4959(00)00300-6.
46. Qin X, Zhang M, Qin J, Yuan S, Hou Y, Liu J. Two-step purification of cu,zn-superoxide dismutase from pumpkin (cucurbita moschata) pulp. Sep Purif Tech. 2012;87:79–83. https://doi.org/10.1016/j.seppur.2011.11.025.
47. Babitha MP, Prakash HS, Shekar shetty H. Purification and partial characterization of manganese superoxide dismutase from downy mildew resistant pearl millet seedlings inoculated with sclerospora graminicola. Plant Sci. 2002;163(4):917-24. https://doi.org/10.1016/S0168-9452(02)00238-8.
48. Vyas D, Kumar S. Purification and partial characterization of a low temperature responsive mn-sod from tea (camellia sinensis (l.) o. Kuntze). Biochem Biophys Res Com. 2005;329(3):831-8. https://doi.org/10.1016/j.bbrc.2005.02.051.
49. Yao CL, Wang AL, Wang WN, Sun RY. Purification and partial characterization of mn superoxide dismutase from muscle tissue of the shrimp macrobrachium nipponense. Aquaculture. 2004;241:621-31. https://doi.org/10.1016/j.aquaculture.2004.08.023.
50. He N, Li Q, Sun D, Ling X-P. Isolation, purification and characterization of superoxide dismutase from garlic. Biochem Eng J. 2011;38:33-8. https://doi.org/10.1016/j.bej.2007.06.005.
51. Haddad NIA, Yuan Q. Purification and some properties of cu,zn superoxide dismutase from radix lethospermi seed, kind of chinese traditional medicine. J Chromat B Anal Technol Biomed Life Sci. 2005;818(2):123-31. https://doi.org/10.1016/j.jchromb.2004.12.010.
52. Masoud HMM, Helmy MS, Hamed AA, Gad El-Karim RM, Ali REM, Zayed KM, Habib MR. The marine mollusc turbo radiatus glutathione s-transferase isolation and biochemical characterization: Antibacterial effect and cytotoxicity against different cancer cell lines. Egypt j aquat biol fish. 2024;28(6):1239-58. https://doi.org/10.21608/ejabf.2024.396336.