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In vitro anticancer and antimicrobial activities of copper-zinc superoxide dismutase purified from *Cellana rota* snail

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Abstract

The antioxidant, antimicrobial and anticancer activities of molluscan gastropods were elucidated in numerous previous studies. One of the most important antioxidant enzymes is superoxide dismutase (SOD) was homogeneously purified and characterized from *Cellana rota* snail crude extract. The purification of *C. rota* SOD was carried out through chromatographical separation on anion exchanger and size-exclusion resins. The specific activity of the purified SOD was 520.7 units mg^{-1} protein, representing 6.6 folds over the crude extract. The purified SOD was found 180-kDa monomeric as detected by gel filtration and SDS PAGE. It was greatly enhanced with $CuCl_2$ and $ZnCl_2$ and strongly inhibited with KCN and H_2O_2, proposing it as a Cu/Zn-SOD enzyme that termed *Cellana rota* copper-zinc superoxide dismutase (*Cr*Cu/Zn-SOD). The antimicrobial screening of *Cr*Cu/Zn-SOD indicated its antibacterial capacity against *E. coli*, *S. typhi* and *S. aureus* with MIC value of 0.78 µg/mL for the three pathogens. Moreover, it showed a MIC value of 1.56 µg/mL against the fungus *A. niger* compared to 5 µg/mL nystatin. *Cr*Cu/Zn-SOD showed potent *in vitro* toxicity towards adenocarcinomic human alveolar basal epithelial cells A549, human colorectal carcinoma Caco2 and human hepatocellular carcinoma HepG2 cancer cells with 2.451, 3.933 and 10.5 µg/mL IC_{50} values respectively. These preceding results suggest that *Cr*Cu/Zn-SOD from *C. rota* snail may have the potential to serve as a substrate for future antimicrobial and anticancer drug development.

Keywords: *Cellana rota*, superoxide dismutase, purification, characterization, antimicrobial, anticancer

1. Introduction

The marine environment is known for its tremendous biodiversity, with mollusks representing 25% of the identified species [1-3]. Mollusks are mostly sessile organisms lacking adaptive immune responses. They depend on primitive immune response known as "innate immunity" that is composed primarily of phagocytic cells (hemocytes), and some physiological components [4]. Yet, mollusks managed to establish themselves in hostile marine environments in the presence of viruses, bacteria, and predators [2, 5]. Mollusks must therefore be equipped with an efficient chemical defense and humoral elements such as antioxidant enzymes and antimicrobial peptides [6]. The chemical diversity of mollusks provided them with a vast reservoir of new bioactive substances with unusual and distinct chemical properties. Indeed, natural molluscan products may act as antioxidants and anti-cancer factors, as well as play a preventative role in tumour management, underpinning their promise in the discovery of novel and effective pharmaceuticals [7-9]. The capability of marine organisms to overcome oxidative stress is one of the most important factors that affect their survival in marine environments. The antioxidant enzymes produced by these organisms to overcome oxidative stress, due to infection, salinity, and other stressors, have unique characteristics compared to their terrestrial counterparts. Thus, it can be useful in the

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food industry and be a potential source for biotechnological applications [10].

Amongst the enzymatic antioxidative system are superoxide dismutases (SODs), metalloenzymes present in all life kingdoms that constitute the first defensive line against injuries caused by reactive oxygen species [11, 12]. SODs catalyze the superoxide anions (O_2^-) dismutation into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), decreasing O_2^- levels that damage the cells at high concentrations [13]. SODs can be distributed into four different classes according to the metal cofactors present in their active sites: copper-zinc SOD (Cu/Zn-SOD), iron SOD (Fe-SOD), manganese SOD (Mn-SOD), and nickel SOD (Ni-SOD) [14]. Different studies have reported the therapeutic potentials and physiological significance of SODs [15]. They can work as anti-inflammatory agents and block precancerous cell variations. SOD is utilized in personal care products and cosmetics as an antioxidant and antiaging factor due to its ability to minimize the damage effects of free radicals on skin. Thus, it prevents fine lines, age spots and wrinkles, aids the healing of wounds; makes scar tissues soft, keeps from UV radiations; and diminishes other aging troubles. SODs have a significant role in various human disorders such as red blood cells-concerned disorders, malignant breast illness, cystic fibrosis, amyotrophic lateral sclerosis, steroid-sensitive nephrotic syndrome, neuronal apoptosis, cancer, and AIDS [16]. SODs have numerous commercial applications in the clinical nutrition and pharmaceutical industries [17]. SODs are used as antioxidant drugs in a variety of diseases and have been shown to have anti-inflammatory and anti-viral properties. Intra-articular injection of SOD helps in treating joint diseases [18-20]. Oral administration of active SOD prevented tumor progression via scavenging the inflammatory superoxide anions [21]. High SOD levels protect against neurotoxicity, neurobehavioral impairments generated by acute ischemia and neurobehavioral damages that accumulate through aging [22]. Low SOD levels have been associated with some degenerative disorders like fibromyalgia, cancer, diabetes, Alzheimer's, multiple sclerosis, and Parkinson's [23-25].

Cellana rota (Gmelin, 1791) is a member of the Nacellidae family of marine gastropod snails that commonly seen in Egypt's Red Sea [26]. The antimicrobial activity of *C. rota* crude solvent extract against different biofilm forming bacteria was reported [27]. In the present study, Cu/Zn-SOD from *C. rota* snail was purified, characterized and tested for its potential antimicrobial efficacy and cytotoxic activity against human hepatocellular carcinoma HepG2, adenocarcinomic human alveolar basal epithelial cells A549 and human colorectal carcinoma Caco2 cell lines.

2. Methods

2.1. Sample Collection

Cellana rota snails were compiled from the region of Ain El-Sokhna, Red Sea, Egypt, on October 2020 [26]. Snails were cleaned with dH_2O, shells were crushed and the soft tissues were dissected and kept at -20°C till utilized in this analysis.

2.2. Chemicals

Xanthine sodium salt, xanthine oxidase enzyme, cytochrome C from horse heart, nitroblue tetrazolium (NBT), sephacryl S-300, dimethyl sulfoxide (DMSO), diethylaminoethyl cellulose (DEAE-cellulose), crystal violet, phenazine methosluphate (PMS) and trypan *blue* dyes were purchased from Sigma chemical (St. Louis, Mo., USA). Fetal bovine serum, Roswell Park Memorial Institute 1640 Medium (RPMI-1640), Dulbecco's Modified Eagle Medium (DMEM), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer solution, gentamycin, L-glutamine and 0.25% typsin-EDTA were purchased from Lonza AG manufacturing company (Swiss). All other chemicals were of analytical grade.

2.3. Protein determination

Proteins were estimated throughout the different purification steps via the dye binding assay method utilizing bovine serum albumin (BSA) as a standard [28].

2.4. Assay of superoxide dismutase

The reaction mixture of SOD assay comprises 1.0 mL 0.02 M K-phosphate buffer at pH 7.8, 0.1 mM EDTA, 0.01 mM cytochrome C, 0.05 mM sodium xanthine and 21 mU xanthine oxidase enzyme and monitored at 550 nm. One SOD unit is the enzyme amount minimizes cytochrome C reduction by 50% [29].

2.5. Staining of SOD activity

The native gels were flooded after electrophoresis in 50 mL 0.1 M Tris-HCl, pH 8.6, comprising 20 mg NBT and traces of PMS. After that, gels are exposed to daylight till achromatic zone appears on a bluish background, denoting the SOD activity [30].

2.6. Purification of snail superoxide dismutase

All experimental proceedings were carried out at 4°C unless mentioned otherwise. Tissues of *C. rota* snails were grinded in 0.02 M K-phosphate buffer at pH 7.6 utilizing a Teflon pestled homogenizer. The homogenized mixture was centrifuged for 30 min at 10000 rpm to eliminate all insoluble materials and cell debris and obtain the filtrate as a crude extract. The crude extract was fractioned through DEAE cellulose anion column (120 x 24 mm) after

equilibrating it with the phosphate buffer at pH 7.6. Elution of SOD enzyme was carried out by applying a NaCl linear gradient (0.0–0.5 M) dissolved in the equilibrating buffer with 1 mL/min inflow rate. All fractions were investigated for enzyme activity, and those having SOD activity were collected and concentrated by lyophilization. This concentrate with SOD activity was then fractioned again via Sephacryl S-300 column (1420 x 17.5 mm) that was equilibrated and run with the previous phosphate buffer with 0.5 mL/min inflow rate.

2.7. Electrophoresis analysis

7% Native-PAGE and 12% SDS-PAGE gel electrophoresis were carried out [31-33]. Gels were stained for protein using coomassie brilliant blue R-250 dye.

2.8. Effect of cations and inhibitors

SOD type was determined and affirmed by carrying out the effect of cations and inhibition assays. The assay was performed in the presence of various cations and specific SOD inhibitors, with a control test without cations or inhibitors taken as 100% activity [34].

2.9. Determination of antimicrobial activity

All antimicrobial activity tests were performed in triplicates. The sample was prepared at 100 µg/mL concentration of *Cr*Cu/Zn-SOD. 50 µL aliquots of the enzyme were infiltrated on 5 mm filter paper discs (Whattman No. 1) and dried under sterilized conditions at room temperature. Paper discs were put on agar dishes seeded with test microorganisms and incubated for 24 h at the convenient temperature for every microbe. The bacterial and fungal organisms grew on nutrient agar, while fungal strains grew on PDA media (DSMZ 130). Culture of every microbe was diluted with sterile dH_2O (10^7 - 10^8) CFU/mL. Inoculated agar dishes were incubated in an upright position for 24 h at 37°C for bacteria and 48 h at 30°C for fungi. After incubation, the inhibition zones diameter was measured versus three Gram –ve bacteria (*Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* ATCC 25955 and *Salmonella typhi* ATCC6539), one Gram +ve bacteria (*Staphylococcus aureus* NRRL B-767) and two pathogenic fungal strains (*Candida albicans* ATCC 10231 and *Aspergillus niger* NRRLA-326) [35]. All experiment microbes were acquired from the culture collection center, Microbial Chemistry Department, NRC, Egypt.

2.10. Minimum inhibitory concentration

To determine the MIC, the test was performed in 96-well flat polystyrene plates. Serial concentrations of the tested sample (100, 50, 25.5, 12.5, 6.25, 3.12, 1.56, 0.78 µg/mL final concentrations) were added to 150 µL LB broth, then adding 10 µL bacterial culture suspension (log phase), and incubation at 37°C overnight. The positive antibacterial effects of *Cr*Cu/Zn-SOD were monitored after incubation as clearance in wells. The pathogen without any treatment was taken as a control and the absorbance was monitored after about 20 h at 600 nm.

2.11. Biofilm inhibitory activity

*Cr*Cu/Zn-SOD biofilm inhibitory activity against 4 clinical microbes (*P. aeruginosa*, *S. aureus*, *E. coli* and *B. subtilis*) was carried out utilizing the MTP assay in 96 flat bottom well polystyrene plate. Each well was filled out with 180 µL LB broth (10 g/L tryptone, 5 g/L yeast extract and 10 g/L NaCl) followed by inoculation with 10 µL of the overnight bacterial culture, addition of 10 µL *Cr*Cu/Zn-SOD sample and incubation for 24 h at 37 ºC. After incubation, the well contents were removed and the floating bacteria were eliminated through washing by 200 µL phosphate buffer saline pH 7.2. For staining, 0.1% (w/v) crystal violet was added for 1 h and then wash each well with 200 µL dH_2O and keep the plate in the laminar flow for drying. The dried plate was washed with 95% ethanol and OD was measured at 570 nm utilizing absorbance microplate reader [36].

2.12. Evaluation of cytotoxic effects

Human hepatocellular carcinoma HepG2, adenocarcinomic human alveolar basal epithelial cells A549 and human colorectal carcinoma Caco2 were obtained from ATCC via the holding company for biological products and vaccines (VACSERA; Cairo, Egypt) and employed for this study and staurosporine was utilized as a control. The cell lines were freshly cultivated as monolayers in RPMI-1640 medium supplemented with 1% glutamine, 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin, incubated at 37°C in 5% CO_2 incubator and cytotoxicity of *Cr*Cu/Zn-SOD was estimated via MTT assay [9]. The relative cell viability was expressed as a percentage and calculated from the following relation:

$$\text{Cell viability \%} = \frac{\text{Absorbance of treated samples (A570)}}{\text{Absorbance of untreated samples (control; A570)}} \times 100$$

3. Results

3.1. Purification of superoxide dismutase from *Cellana rota* snail

A typical purification scheme for SOD from the *C. rota* snail is presented (Table 1). Elution profile of *C. rota* snail crude extract on DEAE-cellulose resin (Fig. 1a) displayed two SOD peaks: one minor SOD peak obtained with 0.02 M K-phosphate buffer and a

major SOD peak obtained with 0.1 M NaCl that designated *Cr*Cu/Zn-SOD. The *Cr*Cu/Zn-SOD fractions were combined, concentrated and applied on a Sephacryl S-300 resin (Fig. 1b) that results in one SOD peak. The specific activity of *Cr*Cu/Zn-SOD was increased to 512.7 U/mg protein, representing 6.6 folds and 26.5% recovery (Table 1). The molecular weight of *Cr*Cu/Zn-SOD was determined using its elution volume from the Sephacryl resin (Fig. 1b) which was previously calibrated with the standard proteins: 440-kDa ferritin, 240-kDa catalase, 150-kDa alcohol dehydrogenase, 67-kDa serum albumin, and 17-kDa myoglobin. The molecular weight of the native *Cr*Cu/Zn-SOD was found as 180 kDa.

3.2. Electrophoretic analysis of purified *C. rota* superoxide dismutase

Electrophoretic analysis of samples from different purification steps of *Cr*Cu/Zn-SOD on 7% native gel showed single band for the protein and SOD isoenzyme patterns (Fig. 2a). Electrophoresis on SDS gel compared with standard markers showed *Cr*Cu/Zn-SOD as 180 kDa protein (Fig. 2b).

3.3. Effect of cations and inhibitors

The action of ions and specific inhibitors on *Cr*Cu/Zn-SOD activity was investigated after princubation with each reagent at 37°C. $CoCl_2$, $CuCl_2$, $MgCl_2$, and $ZnCl_2$ increased the activity of *Cr*Cu/Zn-SOD while $CaCl_2$, $MnCl_2$, KCN, H_2O_2, NaN_3 and SDS inhibited it, suggesting this SOD as a Cu/Zn-SOD isoform (*Cr*Cu/Zn-SOD) (Table 2).

Table 1 A typical purification scheme of *Cellana rota* snail superoxide dismutase (*Cr*Cu/Zn-SOD)

Purification steps	Total protein (mg)	Total Activity (unit)	Specific Activity	Yield (%)	Fold Purification
Crude extract	69.7	5508	79.0	100	1.0
0.05 M NaCl DEAE-Cellulose fraction	14.7	2695	183.3	48.9	2.3
Sephacryl S-300 fraction	2.8	1458	520.7	26.5	6.6

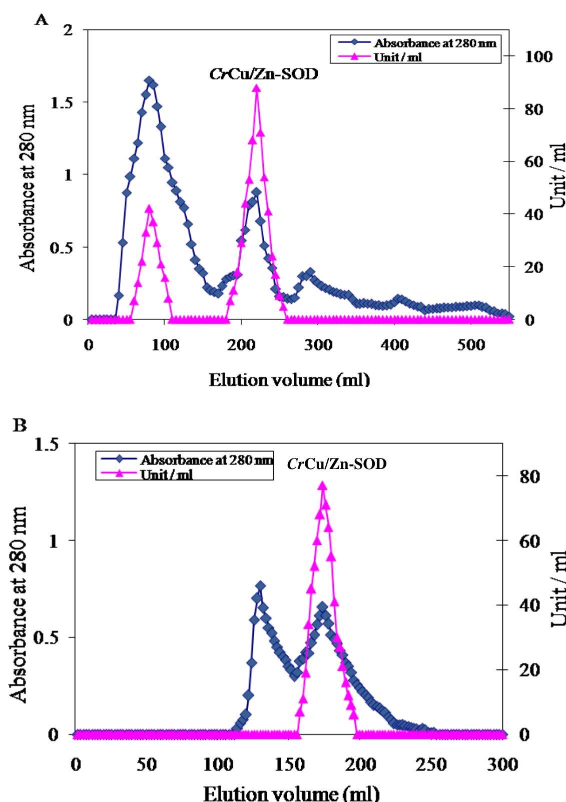


Fig. (1): (a) A typical elution profile for the *Cellana rota* snail crude extract on DEAE-cellulose column (12 x 2.4 cm) previously equilibrated with 0.02 M K-phosphate buffer pH 7.6. (b) Typical elution profile for the chromatography of the concentrated pooled DEAE-cellulose fractions *Cr*Cu/Zn-SOD on Sephacryl S-300 column (142 x 1.75 cm) previously equilibrated with 0.02 M K-phosphate buffer pH 7.6.

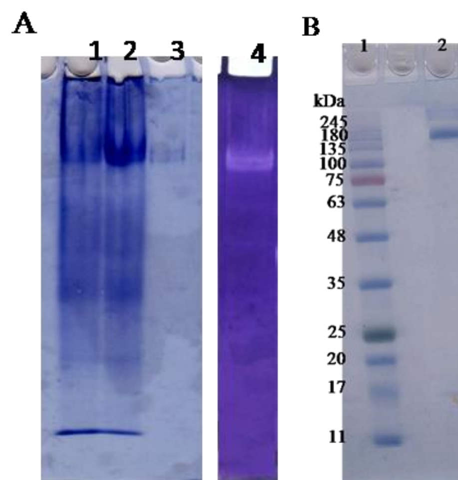


Fig. (2): (a) Electrophoretic analysis of protein and SOD isoenzyme patterns of *Cr*Cu/Zn-SOD on 7% native PAGE: (1) *Cellana rota* snail crude extract, (2) DEAE-cellulose fraction, (3) *Cr*Cu/Zn-SOD purified fraction and (4) *Cr*Cu/Zn-SOD purified fraction isoenzyme pattern. (b) Molecular weight determination of *Cr*Cu/Zn-SOD on 12 % SDS-PAGE: (1) Molecular weight marker proteins and (2) *Cr*Cu/Zn-SOD purified fraction.

3.4. Antimicrobial activity of *Cr*Cu/Zn-SOD

The results of the antimicrobial screening of *Cr*Cu/Zn-SOD revealed its efficient antimicrobial capacity. The antimicrobial inhibition zones presented in Table 3 indicate that, *Cr*Cu/Zn-SOD enzyme yielded a comparable antibacterial capacity to ciprofloxacin against *E. coli*, *P. aeruginosa*, *S. typhi* and *S. aureus*. The antifungal activity against *C. albicans* and *A. niger* was, however, more robust than the control drug, nystatin (Table 3).

The MIC values also confirmed the antimicrobial potency of *Cr*Cu/Zn-SOD. The enzyme showed a MIC value of 0.78 µg/mL against each of *E. coli*, *S. typhi* and *S. aureus*. The fungus *A. niger* was more sensitive to *Cr*Cu/*Zn-SOD,* as it showed a MIC value of 1.56 µg/mL compared to 5 µg/mL for nystatin, the control drug (Table 4).

The *Cr*Cu/Zn-SOD showed considerable biofilm inhibition activity against the tested microbes. The highest biofilm inhibition was 85%, observed with *E. coli*, while the lowest inhibition was 73% noted with *P. Aeruginosa*, compared to 98% inhibition for ciprofloxacin (Fig. 3).

3.5. Cytotoxic Effects

The *in vitro* cytotoxic impacts of *Cr*Cu/Zn-SOD against HepG2, A549 and Caco2 cell lines were examined utilizing the MTT assay and compared with a positive control drug, staurosporine. *Cr*Cu/Zn-SOD showed potent toxicity towards A549, Caco2 and HepG2 cell lines, with 2.451, 3.933 and 10.5 µg/mL IC_{50} values respectively, compared to 9.979, 5.469 and 6.33 µg/mL for the control drug. A dose-dependent decrease in cell viability was observed. The enzyme also showed strong cytotoxic activity against HepG2 cell line with 10.5 µg/mL IC_{50} value (Table 5 and Fig. 4).

Table (2): Effect of divalent cations and specific inhibitors on *Cr*Cu/Zn-SOD

Reagent (5mM)	Residual activity (%)
Control	100.0
$CaCl_2$	88.8
$CoCl_2$	118.0
$CuCl_2$	148.0
$FeCl_2$	108.0
$MgCl_2$	128.0
$MnCl_2$	77.8
$NiCl_2$	104.6
$ZnCl_2$	121.0
Potassium cyanide (KCN)	18.2
Hydrogen peroxide (H_2O_2)	37.5
Sodium azide (NaN_3)	88.6
Sodium dodecyl sulphate (SDS)	67.4

Table (3): Antimicrobial inhibition zones (mm) of *Cr*Cu/Zn-SOD against various bacteria and fungi

Control	Bacteria				Fungi	
	E. coli	*P. aeruginosa*	*S. typhi*	*S. aureus*	*C. albicans*	*A. niger*
*Cr*Cu/Zn-SOD	24.00 mm	23.00 mm	23.00 mm	25.00 mm	19.00 mm	21.00 mm
Ciprofloxacin	19.00 mm	18.00 mm	-	18.00 mm	-	-
Nystatin	-	-	-	-	18.00 mm	17.00 mm

Table (4): Minimum inhibitory concentration (MIC) of *Cr*Cu/Zn-SOD and standard drugs against various pathogenic microbes

Compound	MIC (µg/mL)					
	E. coli	*P. aeruginosa*	*S. typhi*	*S. aureus*	*C. albicans*	*A. niger*
*Cr*Cu/Zn-SOD	0.78	1.56	0.78	0.78	3.12	1.56
Ciprofloxacin	0.39	0.39	-	0.50	-	-
Nystatin	-	-	-	-	0.39	5.0

Table (5): In vitro cytotoxicity IC_{50} (µg/ml) of *Cr*Cu/Zn-SOD against human cancer cells

Sample	HepG2	Caco2	A549
*Cr*Cu/Zn-SOD	10.5 ± 0.59	3.933 ± 0.23	2.451 ± 0.14
Staurosporine	6.33 ± 0.37	5.469 ± 0.69	9.979 ± 0.59

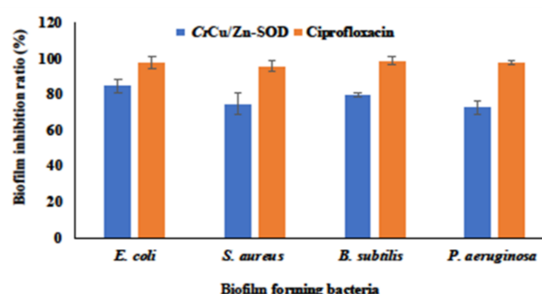
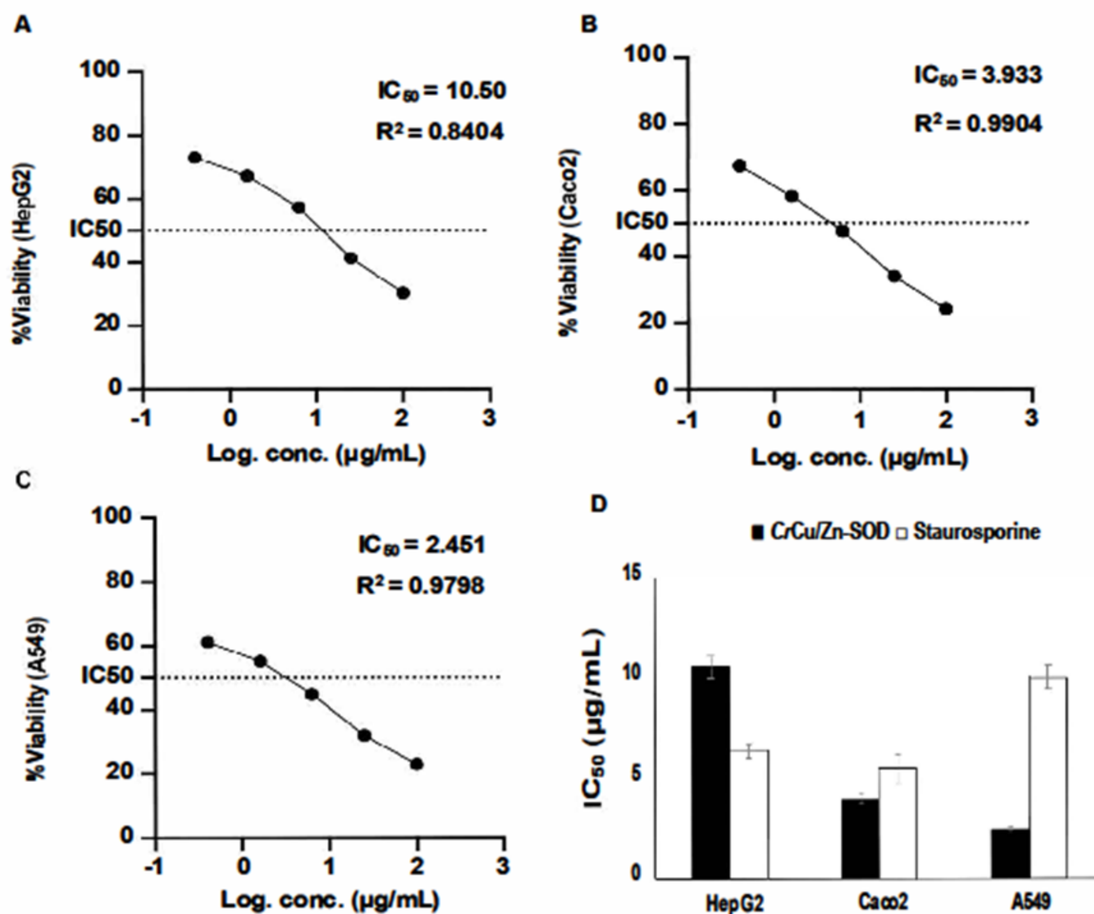


Fig. (3): *Cr*Cu/Zn-SOD biofilm inhibitory activity against different bacteria.



F ig. (4): Cytotoxic effects of *Cr*Cu/Zn-SOD against different human cancer cell lines after 24 h of treatment. (a) HepG2: Human hepatocellular carcinoma; (b) A549: Adenocarcinomic human alveolar basal epithelial cells; (c) Caco2: Human colorectal cell carcinoma (X-axis: Log concentrations of the extract from 0.4 to 100 μg/mL and Y-axis: the percentage of cell viability). (d): The comparison of average IC_{50} of the *Cr*Cu/Zn-SOD versus staurosporine as a positive control. Data represent the mean ± SD of three independent experiments.

4. Discussion

Marine natural products exhibit a wide range of pharmaceutically relevant bioactivities, including anticancer, antibiotic, antiviral, antimicrobial and antioxidant properties [37]. Copper/zinc superoxide dismutases (Cu/Zn-SODs) are essential for the protection of various tissues from oxidative stress [38]. The decline in the expression of this enzyme has been manifested in numerous pathological signs and diseases. This present study summarizes a simple SOD purification procedure from *C. rota* snail crude extract. The SOD separation is carried out via chromatography on an anionic DEAE-cellulose column and gel filtration using Sephacryl S-300 resin. SODs from shrimp muscle tissue [39], *Leishmania infantum* [40], camel tick larvae [41], hens' eggs [42] and mangrove trees [43] were purified with similar procedures. In the present study, chromatography on a DEAE-cellulose column separated two SOD peaks; the major of which was eluted with 0.1 M NaCl and designated *Cr*Cu/Zn-SOD. The Sephacryl S-300 chromatography of *Cr*Cu/Zn-SOD showed it as a monocular SOD enzyme peak and derived its molecular weight as 180 kDa. *Cr*Cu/Zn-SOD specific activity was increased to 520.7 units/mg protein, representing 6.6 folds and a 26.5% yield. Various purification folds and yield percentages for SOD have been reported: 103.7 folds and 70% yield for *Leishmania peruviana* and 101.6 folds and 86% yield for *Leishmania amazonensis* [40]; 28.5 folds and 53% yield from yeast [44] and 29.5, 17.7 and 13.3 folds with 34.9%, 20.1% and 9.4% yields from tick larvae [41].

The *C. rota* SOD (*Cr*SOD) enzyme is represented as one protein band that agrees with SOD activity band, affirming *Cr*SOD purity. The molecular weight of *Cr*SOD is obtained from the gel filtration column as 180 kDa, which was further confirmed by SDS PAGE. Many SOD molecular weights were stated; 67 kDa, 40 kDa and 90 kDa for *H. dromedarii* SODs [41]; 110–140 kDa for bacteria SOD [45]; 125 kDa for yeast SOD [46] and (130 kDa, 155 kDa and 205 kDa) for SOD of mussel, *Mytilus edulis* [47]. It has already been demonstrated that several reagents can

be employed for identification of various SOD types. For example, cyanide inhibits Cu/Zn-SOD and H_2O_2 inhibits Fe-SOD, whereas Mn-SOD is resistant to both reagents. $ZnCl_2$, $CuCl_2$, $MgCl_2$, and $CoCl_2$ activated *Cr*SOD, while $MnCl_2$ and $CaCl_2$ inhibited its activity, emphasizing the sharing of copper and zinc ions in *Cr*Cu/Zn-SOD activity. Zn^{+2} was involved in SOD in shrimp muscle tissue [39]. SOD categories are determined by inhibition sensitivity to KCN and H_2O_2 [34, 48]. Involvement of Cu^{+2} and Zn^{+2} ions in SOD activity was proven via inhibition with KCN and H_2O_2 [49], since Cu/Zn-SOD isoenzymes are susceptible to both KCN and H_2O_2 [50, 51]. Inhibition of SOD activity with cyanide identified Cu/Zn-SOD isoforms in the mussel, *Mytilus edulis* [47]. Similar Cu/Zn-SOD isoforms were also identified in other marine mollusks [52, 53].

The antimicrobial screening of *Cr*Cu/Zn-SOD revealed its efficient antibacterial capacity against *E. coli*, *S. typhi*, *S. aureus* and *P. Aeruginosa* respectively in comparison with the control drug Ciprofloxacin. Furthermore, the antifungal activity against *C. albicans* and *A. niger* was more robust than the control drug, nystatin with the *A. niger* showed high sensitivity to *Cr*Cu/Zn-SOD. Moreover, the purified enzyme showed considerable biofilm inhibition activity against the four tested bacteria especially *E. coli*. The antimicrobial activity of marine natural substances was reported for different snails [53, 54]. This *Cr*Cu/Zn-SOD's antimicrobial activity could be attributed to its antagonistic interaction with putative bacterial SODs. Bacteria commonly have Fe-SOD and/or Mn-SOD in their cytoplasm, which have very similar protein structures [55]. The inhibition of bacteria by *Cr*Cu/Zn-SOD may also be related to its binding with lipopolysaccharides (LPS), which are main ingredients of Gram-negative bacteria outer membrane [56]. In support for this speculation, an extracellular superoxide dismutase (extracellular SOD) was discovered from the oyster *Crassostrea gigas* with an amino acid sequence containing an LPS-binding motif and an affinity for *E. coli* [51]. Furthermore, it was found that Cu/Zn-SOD from the Pacific abalone *Haliotis discus hannai* was up-regulated following LPS stimulation, leading to oxidative stress [52]. Destruction of the protective bacterial membrane makes it vulnerable to foreign stressors, including *Cr*Cu/ Zn-SOD, that might enter bacterial cells and increase the creation of ROS, leading to the destruction of several essential metabolic enzymes, escalating cell membrane damage, and changing the morphological and physiological characteristics of the cells.

The *Cr*Cu/Zn-SOD showed potent toxicity towards A549 and Caco2 cell lines, with IC_{50} values lower than that of the control drug. The enzyme also showed strong cytotoxic activity against the HepG2 cell line. The mechanism of *Cr*Cu/Zn-SOD-mediated cytotoxicity observed in the present study is not clear. It is unlikely that it restored the redox homeostasis of the tumor cells because oxidative stress is implicated in the killing of tumor cells. In a multiple myeloma cell line, overexpression of Cu/Zn-SOD correlated with resistance to anticancer drugs and cancer progression. However, the pharmacological inhibition of Cu/Zn-SOD increased the drug's cytotoxicity in both drug-sensitive and drug-resistant cell lines [57]. In fact, targeting Cu/Zn-SOD was proposed as a therapeutic target for cancer [58]. It was indicated that, some anticancer drugs enhance Cu/Zn-SOD activity [59]. Cu/Zn-SOD from *C. rota* may trigger overproduction of ROS species in tumor cells through binding to some surface cell receptors as well as diminishing the endogenous production of SODs. Another possible pathway may be due to its binding to DNA and subsequent inhibition of the expression of vital genes in the cancerous cells. Previous studies suggested that SOD might be a DNA binding protein linked to the regulation of gene expression [60, 61]. Cu/Zn-SOD can influence the amount of intracellular ROS or bind to DNA to regulate the expression of oncogene genes. Through interactions between DNA and Cu/Zn-SOD, SOD depletion can help cancer cells resist both apoptosis and anti-cancer treatment [62].

Conclusion

In summary, this is the first study that reports the SOD enzyme from the *Cellana rota* snail and presents an easy purification method for a Cu/Zn-SOD that could be fundamental for preventing oxidative stress. This SOD enzyme showed potent antimicrobial and anticancer activity, which makes it an important natural agent and a candidate drug for treating lung, colon and liver cancers. This distinct *Cr*Cu/Zn-SOD activity could be attributed to the challenging physical and biological environmental conditions in which *C. rota* lives, which encourage these snails to evolve defence mechanisms and special physiological abilities in order to adapt to and survive in such conditions.

Abbreviations: *Cr*Cu/Zn-SOD; *Cellana rota* copper-zink superoxide dismutase, BSA; Bovine serum albumin, PAGE; Polyacrylamide gel electrophoresis, MIC; Minimum inhibitory concentration, MTP; Microtitre plate, IC_{50}; The half maximal inhibitory concentration.

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Authors' contributions

HMM, MRH and MAI designed the study. MRH, RMG, REMA and KMZ collected and processed samples. HMM, MSH, AAH, MRH and DAD conducted the experiments. HMM, MRH and MAI analyzed data and wrote the manuscript: All authors read, revised and approved the final manuscript.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All data and materials are available.

Competing interests

No potential conflict of interest was reported by the authors.

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