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Original Research Report

Superoxide Dismutase from Marine as a Cytotoxic Agent

Dwi Wulan Apriani[1*], Ana Indrayati[1], Wiwin Herdwiani[1], Hema Novita Rendati[2], Khairina Zulfah[2]

[1] *Faculty of Pharmaceutical, Setia Budi University. Surakarta, Indonesia.*
[2] *Borneo Citra Medika Health Polytechnic. Tanah Laut, Kalimantan Selatan, Indonesia.*

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***Corresponding Author:**
Dwi Wulan Apriani
Email:
dwiwulanapriani@gmail.com

Abstract: The prevalence of cancer in Indonesia has shown an increase from 1.4 per 1000 population in 2013 to 1.79 per 1000 population in 2018. 70% of our planet is covered by unparalleled air and marine biodiversity. Therefore, medical researchers have focused on the marine world which has great potential, in the last six decades (Sagar et al. 2010). To find out other uses of biota, it is necessary to study the SOD of marine biota that reacts with their metal cofactors, to determine the specific SOD of some biota, to determine the SOD cytotoxic activity of several marine biota, to determine the mechanism of SOD of marine biota as anticancer. This literature review was carried out in several stages: formulating problems, collecting, evaluating, analyzing, and synthesizing data. The data sources used are international journals from publishers such as Science Direct, Elseiver, and SpringerLink between 2010 and 2021. The literature results obtained for the SOD classification of marine biota based on metal cofactors are 1,092 articles. The articles obtained will be selected and 52 articles that meet the inclusion criteria will be reviewed. The results of the search for specific SOD activities from marine biota were obtained by 1,243 articles. 13 articles that meet the inclusion criteria and will be reviewed. The results of the search for SOD cytotoxic activity from marine biota obtained 2,199 articles. 7 articles that met the inclusion criteria will be reviewed. The search results for specific SOD activities from marine biota were 2,496 articles. 8 articles that meet the inclusion criteria and will be reviewed. The results showed that SOD from marine biota had cytotoxic activity.

Keywords: Cytotoxic, Free Radicals, Marine Life, SOD, Superoxide Dismutase.

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1. Introduction

Cancer is a disease caused by an abnormal genome, characterized by continuous proliferation signals, damage to growth suppressor genes, absence of cell death processes, uncontrolled cell replication, stimulation of angiogenesis, and invasion of surrounding tissue [1]. The prevalence of cancer in Indonesia showed an increase from 1.4 per 1000 population in 2013 to 1.79 per 1000 population in 2018. The highest cancer prevalence was in Yogyakarta province at 4.86 per 1000 population. Followed by West Sumatra with 2.47 per 1000 population, and Gorontalo with 2.44 per 1000 population [2].

Chemotherapy is a therapy that is often used in Indonesia in connection with the condition of late-diagnosed patients. Although chemotherapy gives many positive results, on the other hand, it causes many side effects such as nausea, vomiting, decreased red blood cells (RBC), decreased white blood cells (WBC/leukocytes), decreased platelet count, mucositis, hair loss, and peripheral nerve disorders [3]. Cancer cells that are multidrug-resistant (MDR) or resistant to various cancer drugs have also spurred researchers to find new anti-cancer drugs that can penetrate and kill these cancer cells [4][5]. The need to find safe and effective cancer drugs is necessary.

Antioxidants play a vital role in health, especially in reducing the effects of free radicals which can cause various bad effects on the body, including cell or tissue damage which is thought to trigger the emergence of cancer. Superoxide Dismutase (SOD) is an enzymatic antioxidant that protects cells from oxidative stress by catalyzing the dismutase of superoxide (O2-) into O2 and H2O2 [5]. SOD, which is an endogenous antioxidant, works quickly to convert free radicals into more stable compounds [6]. The production of free radicals exceeds the ability of intracellular antioxidants to neutralize them, so excess free radicals will cause oxidative stress and have the potential to cause cell damage [7]. Research by Balasubramanian R et al. (2007) methanol extract from Phyllanthus polyphyllu which has SOD activity shows promising in vitro cytotoxicity against human cancer cells MCF-7, HT-29, and HepG2. In MCF7 cells the IC50 value is 27 μM/ml, in HT-29 cells the IC50 value is 42 μM/ml, while in HepG2 the IC50 value is 38 μM/ml [8].

More than 60% of anticancer drugs come from natural resources. Compounds that are effective as anti-cancer drugs are mostly isolated from metabolite compounds produced by plants and to a small extent by marine plants and microorganisms [9]. 70% of our planet is covered by water and marine biodiversity is unrivaled. Therefore, medical researchers have focused on the marine world which has great potential, in the last six decades [10]. The long evolutionary history of marine biota causes marine biota to have very high molecular diversity. Various types of compounds with various bioactivities have been found from this biota, ranging from antibacterial, antifungal, antiviral, antiplasmodium and so on [11].

2. Literature Review

One of the causes of cancer is the presence of free radicals. Free radicals in certain amounts are needed by the body to help physiological processes by transferring electrons. However, if free radicals are present in excessive amounts, oxidative stress will occur, where there is an imbalance between the number of free radicals and intracellular antioxidants [12]. Increased oxidative stress can cause cell growth disorders. Abnormal cell growth can be seen morphologically from the nodules that form and anatomically it is characterized by the cell size exceeding normal size and experiencing a change in shape from the original. Apart from that, cell abnormalities are also characterized by cell necrosis. Cells that experience necrosis show chromatin clumping, organ swelling, cell membrane damage, and release of cell contents [13].

The main problem currently faced in cancer treatment is the toxicity of chemotherapy to normal tissue. Since it is known that some chemotherapy agents affect the synthesis of nucleic acids and proteins, both cancer cells and normal cells are destroyed. Inhibition of these normal cells is responsible for the undesirable side effects of cancer treatment. Cancer treatment is generally based on efforts to remove cancerous tissue or by killing cancer cells and minimizing the effects of treatment on surrounding normal cells. Currently, the main cancer treatments are surgery, radiotherapy, and chemotherapy, but all three types of treatment have shortcomings. Radiation treatment can kill local tumors but radiation will also kill surrounding normal cells. Most chemotherapy drugs such as taxol, 5-fluorouracil (5-FU), and adriamycin target cell division [14], but this chemotherapy can cause diarrhea and hair loss. Continuous treatment with chemical drugs can trigger organ damage which is a side effect of using chemical drugs. Besides, chemical drugs are relatively expensive, so it is necessary to use alternative treatment methods, for example, herbal therapy [15].

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Herbal therapy is currently widely used in plants because the raw materials for medicines can be reduced so that other alternatives are needed for cancer treatment, namely using ingredients from marine biota. The potential of marine biota as a source of new bioactive materials has been widely studied in recent years, although there has not been much research on terrestrial biota. The long evolutionary history of marine biota causes marine biota to have very high molecular diversity [11]. Showed that flour derived from seaweed contains SOD in the form of CU, ZN SOD [16]. Reported that five raphidophyte species contain SOD [7].

3. Methodology

This research uses the Systematic Literature Review (SLR) method by processing data qualitatively (meta synthesis). The SLR method follows the Preferred Reporting İtems for Systemic Reviews and Meta Analyses (PRISMA-2020) protocol. The data processed is a collection of Randomized Control Trial (RCT) experimental research results presented in the review. The SLR results will focus on collecting and analyzing SOD activity from marine biota.

4. Finding and Discussion

Most eukaryotic algae contain FeSOD or MnSOD or both [17]. Meanwhile, marine animals that have SOD mostly contain Zn/CuSOD [18]. From the entire literature, the detailed number of metal cofactors used in SOD research from marine biota can be seen in the image below.

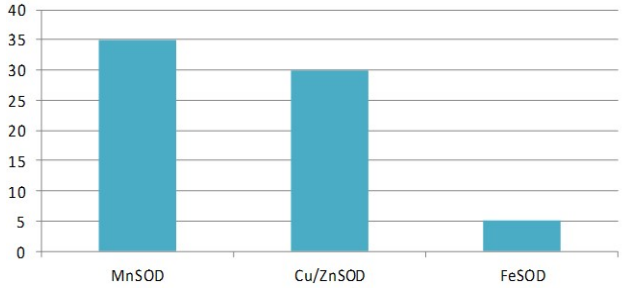

Figure 1. Number of Metal Cofactors from Marine Biota in the Literature Review

Table 1 shows the types of metal cofactors in marine biota

Table 1. Types of Metal Cofactors in Marine Biota

No	Marine Biota	Place/Source	Methode	Metal Cofactors	Ref.
1	*Zhikong Scallop Reveals*	China	Protparam tool	Cu/ZuSODs(1-6) Mn-SODs(7,8)	[18]
2	*Vibrio shiloi*	BCCMTM/LMG Bacteria collection, Laboratory for microbiology, University of Gent, Belgium	SDS-PAGE	Mn-SOD	[19]
3	*Lichen Microalgae*	Sammlung von algenkulturen at the university Gotinggen Germany	Elektroforesis	Mn-SOD Fe-SOD	[20]
4	*Red lip mullet*	Coastal areas Japan, China and Korea	real-time PCR (qPCR)	MuMnSOD	[21]
5	*Rimicaris exoculata*	The southern part of the Mid-Atlantic in depth 3059m	ESpript 3.0	Cu-ZnSOD	[22]
6	*Red lip mullet*	Coastal areas Japan China and Korea	ExPASy PROSIT	CuZnSOD	[23]
7	*Yellow drum fish*	Fujian province Ningde fisheries extension station China	BioEdit 7.0.9.	icCuZnSOD	[24]

No	Marine Biota	Place/Source	Methode	Metal Cofactors	Ref.
8	*Sterlet*	Fangshan state sturgeon farm, Beijing	DNAMAN 5.2.2	MnSOD	[25]
9	*Avicennia marina*	The port of Khamir is located on the northern coast of the Persian Gulf	The gel was incubated in various KCN and H2O2 solutions with various concentrations under normal experimental conditions.	CuZnSOD	[26]
10	*Prorocentru m minimum*	Korean Marine Microalgae Culture Center, University Pukyong National, Busan Korea	PROSITE, pI/Mw tool, and NCBI Conserved Domain Database	CuZnSOD	[27]
11	*Apostichopu s japonicus*	Dalian Pacific aquaculture Company	BLAST search program	CuZnSOD	[28]
12	*Cyanea capillata*	Sanmen Beach in Northeast China Province Zhejiang, China	BLASTx analysis	CuZnSOD1	[29]
13	*Litopenaeus vannamei*	Local shrimp farm near Hermosillo beach (Sonora, Mexico).	PCR	mMnSOD	[30]
14	*Perinereis nuntia*	Aquaculture farming in Yeosu, Korea South.	PCR	2Cu/Zn-SODs Mn-SOD	[31]
15	*Deep-sea sea cucumber*	Mariana Trench (11°47.975′ N, 142°6.906′ E) At a depth of 5567 m and described as a new species Benthodytes marianensis sp. Nov	ExPASy ProtParam too	Bm-Cu,Zn-SOD	[32]
16	*Halomonas sp*	The hssod gene (708 bp) from the Antarctic strain sea ice	metode 2.4	Cu/Zn SOD	[33]
17	*Tridacna squamosa*	Xanh Tuoi Tropical Fish Co. of Vietnam	PCR	CuZnSOD	[34]
18	*Litopenaeus vannamei*	Shrimp pond located at middle zone of the Gulf of California	PCR	mMnSOD	[35]
19	*Psychropote s longicauda*	Mariana Trench	SignalP 4.1, Scratch protein predictor, InterPro scanner and Swiss model server	Pl-Cu,Zn-SOD	[36]
20	*Sepiella maindroni*	Dongji cultural agriculture in Zhoushan, Zhejiang province, P.R. China	BLASTn program of NCBI	icCu/Zn-SOD	[37]
21	*Crassostrea hongkongen sis*	South China Sea coast	BLAST at NCB	ChMnSOD ChCuZnSOD	[38]
22	*Marsupeneus japonicus*		LASTX and BLASTP program	MjCu/ZnSOD	[39]
23	*Zostera marina*	Zona subtidal di Qingdao, Provinsi Shandong, China	Blast software	ZmMnSOD	[40]

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No	Marine Biota	Place/Source	Methode	Metal Cofactors	Ref.
24	*Chlamys farreri*	Farms in Qingdao, China,	EditSeq module in Lasergene program suite 14.0.0.88	CfmtMnSOD	[41]
25	*Neanthes succinea*	Aquarium facilities of the Department of Chemistry, College of Natural Sciences, Hanyang University in Seoul, South Korea.	PCR	Cu/Zn-SODMn-SOD	[42]
26	*Pseudosciae na crocea*	Dongji agricultural culture in Zhoushan, province Zhejiang, P. R. China	BLASTn program of NCB	inCu/Zn-SOD, Mn-SOD, and ecCu/Zn-SOD	[43]
27	*Metopus es*	Anaerobic upflow laboratory scale Sludge Blanket Reactor (UASB) processing plant waste.	Incubate samples for 1 hour with KCN (1 mM) or H2O2 (0.5mM)	MnSOD	[44]
28	*Mytilus galloprovinc ialis*	Shellfish cultivation farm Local	LAST algorithm	MgeMnSOD and MgeCuZnSOD	[45]
29	*Brachionus koreanus*	Uljin, South Korea	RT-PCR	Bk-Mn-SOD	[46]
30	*Ruditapes philippinarum*	Seongsan Beach is located on Jeju Island (Republic of Korea)	qRT-PCR	MnSOD (RpMnSOD) and Cu/ZnSOD (RpCu/ZnSOD)	[47]
31	*Debaryomces hansenii*	by the Centro de Investigaciones Biologicas del Noroeste (CIBNOR, Mexico).	BLAST	LpMnSOD LpicCu/ZnSOD	[48]
32	*Litopenaeus vannamei*	Universitas Sonora, DICTUS, Kino Bay Unit	RT-PCR	cMnSOD	[49]
33	*Mytilus coruscus*	Zhoushan, Zhejiang province, P. R. China	BLASTn program of NCBI	CuZnSOD MnSOD	[50]
34	*Eriocheir sinensis*	from local farms in Qingdao, China	Blast	cytMnSOD mtMnSOD	[51]
35	*Callinectes sapidus*	Blue crab hatching in Aquaculture Research Center, [Institute of Marine and Environmental Technology (IMET), Baltimore, Maryland]	ORF finder program	ecCuZnSOD-2	[52]
36	*Argopecten irradians*	Shellfish farming in Qingdao (prov Shandong, China)	PCR	Ai-icCuZnSOD, Ai-MnSOD Ai-ecCuZnSOD	[53]
37	*Pinctada fucata*	South China Sea Fisheries Research Institute in Xincun village,Hainan Province, China	MatGat software	PoMnSOD	[54]
38	*Scapharca broughtonii*	Qingdao Nanshan aquatic products market, Shandong Province, China	DNAStar 7.0	SbMnSOD	[55]
39	*Oplegnathus fasciatus*	Governing Provincial Marine and Fisheries Research Institute Alone (Jeju, Republic of Korea)	BLAS	cCu/ZnSOD	[56]
40	*Litopenaeus vannamei*	from the local shrimp farm Acuacultura Mahr, S.A. de C.V (La Paz, BC,Mexico)	NASIS v 2.5	cMnSOD	[57]
41	*Hippocamp us abdominalis*	Fish Cultivation Center Korean Sea Decorations on Jeju Island (Republic of Korea)	BLAST	CuZnSOD	[58]

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No	Marine Biota	Place/Source	Methode	Metal Cofactors	Ref.
42	*Oplegnathus fasciatus*	Governing Provincial Marine and Fisheries Research Institute Alone (Jeju, Republic of Korea)	Roche 454 Genome Sequencer FLX System	Of-mMnSOD	[59]
43	*Hippocamp us abdominalis*	Korean Ornamental Fish Breeding Center on Jeju Island (Republic of Korea)	BLAST	rHaMnSOD	[60]
44	*Portunus trituberculat us*	Local farm in Qingdao, China,	BLAST	cMnSOD	[61]
45	*Crassostrea gigas*	Local farming in Qingdao, Province Shandong, China	PCR	CgEcSOD	[62]
46	*Tegillarca granosa*	Yueqing, city Wenzhou, Zhejiang province, China	BLAST algorithm at NCBI	TgmMnSOD	[63]
47	*Haliotis diversicolor supertexta*	Commercial farms in Nan'ao, Shenzhen, China	BLAST	Cu/ZnSOD	[64]
48	*Paralichthys olivaceus*	Local advertising supplier (Nagasaki, Japan).	Edman degradation with a Procise Model 492 protein Sequencer	Mn-SOD	[17]
49	*Litopenaeus vannamei*	Local farm located in Chachoengsao province (East Thailand).	PCR	LvcMnSOD2	[65]
50	*Euplotes crassus*	Artificial sea water (Crystal Reef, Aquarium System, OH, USA),	Genetyx Version 7.0.3	Ec-Cu/Zn-SODs EcMn-SOD	[66]
51	*Nibea albiflora*	Ningde Fisheries Extension, Fujian Province, China.	Clustal W	NaEcSOD	[67]
52	*Ciona intestinalis*	Southern area Venice Lagoon, near Chioggia	sqRT-PCR	Cu,Zn SOD	[68]

Cytotoxic testing is the development of methods to predict the presence of new cytotoxic drugs, including those from natural ingredients that have the potential to act as anticancer agents. Based on the toxic properties of these compounds, cytotoxic properties are an absolute requirement for anticancer drugs [70]. Cancer cells are known to have high ROS content and become increasingly dependent on active antioxidants such as SOD to prevent excessive cell damage and apoptosis during tumor progression.

Cell growth and viability can be measured by several methods such as MTT, XTT, and SRB. MTT is a sensitive, quantitative, and colorimetric method used to measure cell viability, proliferation, and activation. This method is based on the capacity of the mitochondrial dehydrogenase enzyme in living cells to reduce MTT salts to water-insoluble blue or purple formazan [71].

The data obtained shows that SOD can be used as a new alternative cancer treatment. The anti-cancer mechanism of SOD is to have an apoptosis-inducing effect. Apoptosis is an intracellular suicide program that is carried out by activating caspases. The three mechanisms by which a cell carries out apoptosis are, the first is triggered by a signal that appears within the cell itself, the second is triggered by a death activation signal outside the cell that is bound to a receptor on the cell surface such as TNF-α, lymphotoxin and Fas ligand (FasL)., and the third is triggered by reactive oxygen species which harm cells [72].

SOD is a primary enzyme in the body because it can protect body cells from free radicals. Cell damage is triggered by molecules containing oxygen atoms which can produce free radicals or which are activated by radicals in the form of hydroxyl, superoxide and hydroxy peroxide radicals. Changes in macromolecules such as fatty acids in membrane lipids, proteins, and DNA cause major damage to cells [73].

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Table 2. Cytotoxic Activity

No	Marine Biota	Methode	Cell Line	Cancer Type	Concentr-Ation	Result	Ref
1.	*Ascophyllum Nodosum*, *Fucus Serratus* And *Fucus Vesiculosus*	MTT	Caco- 2	Colon	0.5 to 2.0 mg/ml	*Ascophyllum nodosum* : 1 mg/ml *Fucus serratus* : 0,5 mg/ml *Fucus vesiculosus* : 0,5 mg/ml	[74]
2.	D. *Sephen*	MTT	HeLa	Cervix	20 µg/mL	81%	[75]
			R aw 264.7	Cell mouse leukemia	100 µg/mL	150%	
3.	R. *Globostellata* And S. *Inconstans*	MTT	HeLa	Cervix	12, 25 µg/mL	110%	[76]
			HEK – 293	Kidney cancer	12, 25 µg/mL	115%	
4.	C. Inscriptus	MTT	HeLa-HPV 16	Cervix	20µg/ml	78%	[77]
5.	Gracilaria Lemaneiformis	MTT	HepG-2	Liver	0,2 mg\L	22%	[78]
6.	I.Zhanjiangensis	MTT	HepG2	Liver	0,25M	100%	[79]
7.	Padina Tetrastromatica, Caulerpa Racemosa And Turbinaria Ornata	MTT	MCF-7	Breast	100–500 µg/mL	0.21 ± 1.25 µg/ml	[80]

MnSOD increases mitochondrial outer membrane permeabilization and activates Bax. The mechanism of apoptosis via the classical intrinsic pathway is characterized by upregulating p53 and p21 gene expression and causing G0/G1 phase arrest. Mitochondrial outer membrane permeabilization (MOMP) is a critical event of the intrinsic pathway controlled by Bcl-2 family members and requires activation of Bax or Bak [81].

Explained that the apoptosis mechanism of MnSOD is by inducing the caspase 3 pathway [82]. An increase in caspase 3 can activate specific proteins in cells which will then induce the apoptosis process. Increasing the expression of the caspase-3 enzyme will increase PC-3 prostate cancer cell turnover in vitro via the intrinsic pathway. The reason for this is, theoretically the MnSOD enzyme resides in the mitochondria, which also mediates the intrinsic pathway of apoptosis. MnSOD can also induce apoptosis via death receptors, (Fas). After combining with the Fas ligand, it can combine with Fas-related protein with death domain (FADD), FADD can further convert caspase-8 zymogen into active caspase-8 and finally activate caspase-3 to induce apoptosis [83].

The p53 protein is the most studied tumor suppressor and acts in response to diverse forms of cellular stress to mediate antiproliferative processes, either G1 cell cycle arrest or apoptosis, depending on the cellular context and type of activating agent. In the process of apoptosis, the role of p53 in gene transactivation and transrepression is well established. The most studied p53 transactive genes in apoptosis are probably Bax, Puma, and Noxa [84]. Gene expression analysis shows that p53 protein-dependent apoptosis is preceded by the induction of 14 genes out of 7202 genes, called PIG, including proline oxidase (POX), and PIG-6 [85]. POX, localized to the inner mitochondrial membrane, is an enzyme that converts proline to pyrroline-5-carboxylate (P5C). P5C is the only intermediate that directly links the tricarboxylic acid and urea cycles to amino acid metabolism [86]. Overexpression of POX also causes apoptotic cell death in various types of cancer cells [87]. This mechanism can be seen in Figure 2.

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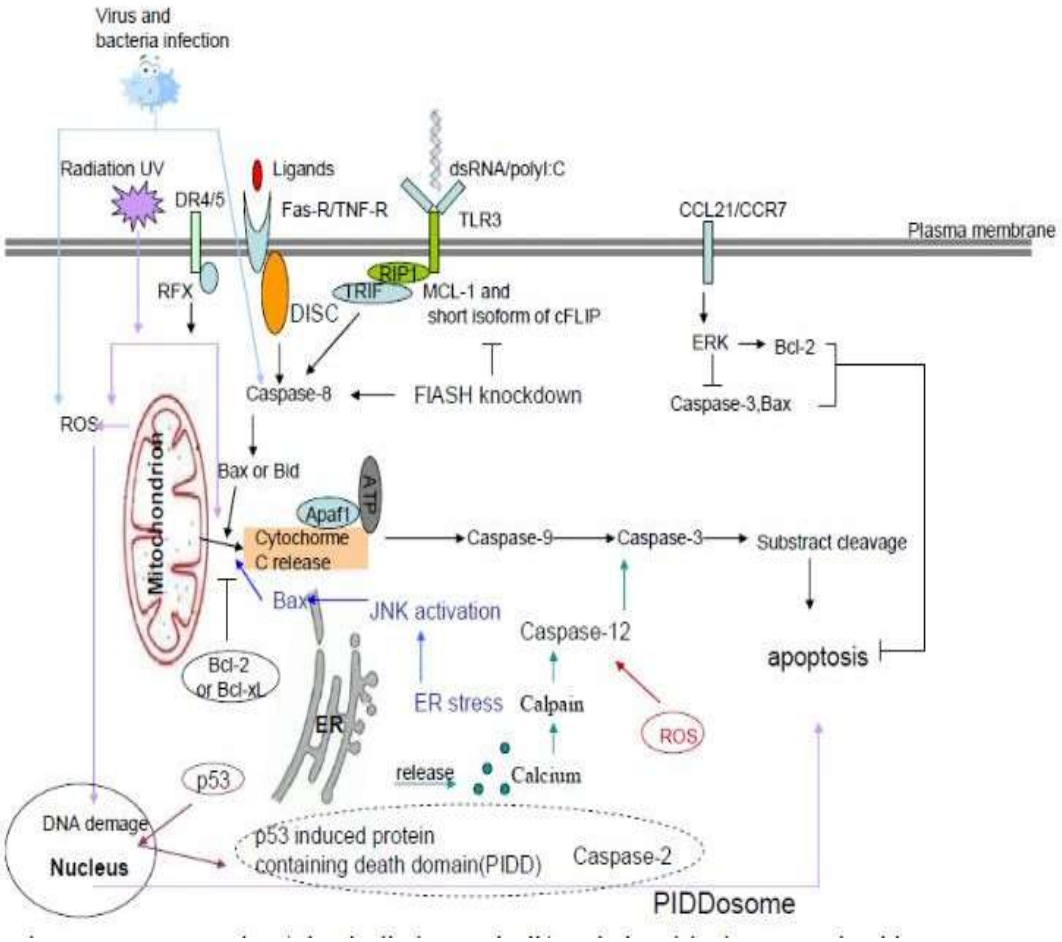

Figure 2. Mechanism of Apoptosis

Caspase Dependent Pathways (Extrinsic and Intrinsic). Mitochondria and nuclear organelles play an important role in this type of apoptosis. This organelle can connect different signals for caspase activation resulting in changes in reactive oxygen compounds, cytochrome c, and mitochondrial membrane potential. Apart from the mitochondrial pathway, external ligands can also activate ERK which is followed by a series of caspase activities [88].

Table 3. Cytotoxic Mechanisms of SOD Cofactors

No	SOD Cofactor	Cancer Cells	Types of Cancer Cells	Mechanism	Ref
1.	MnSOD	NB4, U937 and HL-60	NB4: acute promyelocytic leukemia U937 : human macrophages and HL-60 : colon	Apoptosis	[89]
2.	MnSOD	GC-823 and SGC-7901	GC-823: gastric cancer cells SGC-7901: gastric cancer cells	Apoptosis and proliferation	[90]
3.	MnSOD	K562	K562 : leukemia	Proliferation	[91]

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No	SOD Cofactor	Cancer Cells	Types of Cancer Cells	Mechanism	Ref
4.	CuZnSO	Human MM cell lines RPMI- 8226 (8226), MM.1S, and U266B1	RPMI-8226 (8226), MM.1S, and U266B1 : human myoma cancer	Apoptosis	[92]
5.	RMnSOD	A2780 and MCF-7	A2780: ovarian cancer and MCF-7: breast cancer	Proliferation	[93]
6.	EcSOD	MIA PaCa-2 and AsPC-1	MIA PaCa-2 and AsPC-1 : pancreatic cancer cells	Modulation of HIF-1α signaling and subsequent disruption of it angiogenesis	[94]

5. Conclusion

Based on the secondary data collected, it can be concluded as follows:

- SOD from marine biota has copper cofactors in the form of Cu/ZnSOD, MnSOD, and FeSOD.
- The marine biota that has the highest SOD levels is Rimicaris exoculata at 616 U/mg.
- SOD from marine biota has activity as a cytotoxic agent.

The four mechanisms of apoptosis of SOD copper ions from marine biota are by increasing expression through the caspase, Bax, and P53 pathway, inducing DNA damage, and inducing loss of mitochondrial membrane potential.

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