

## SEA CUCUMBER AS ANTICANCER AGENTS AND ITS DEVELOPMENT FOR FUNCTIONAL FOOD PRODUCTS

### *Teripang sebagai Agen Antikanker dan Pengembangannya untuk Produk Pangan Fungsional*

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Article history:

Received: 10 April 2014; Revised: 18 Juli 2014; Accepted: 25 Juli 2014

#### ABSTRACT

Indonesia is popularly known as sea cucumber (teripang) exporter in the form of dried teripang. Commonly known as *beche-de-mer* or *gamat*, sea cucumber has long been used as medicine and food by Asian and Middle East people. Recent study has shown that sea cucumbers contain active compounds that show potential health benefits and other biological properties such as antibacterial and antifungal products, anticoagulants, antihypertensives, immuno modulation, inhibitor of osteoclastogenesis. It was reported that sea cucumber possesses aphrodisiacs, potentially improve immunity, anticancer and anticoagulation. Sea cucumber is also rich in collagen as a component of connective tissue which can further be converted into smaller molecule and act as bioactive substances. This review presents the potential of sea cucumber as a functional food especially to prevent cancer and strategy to develop sea cucumber-based functional food by enzymatic hydrolysis and *in vivo* study.

**Keywords:** anticancer agent, sea cucumber, enzymatic, *in vivo*

#### ABSTRAK

Indonesia populer sebagai negara eksportir teripang dalam bentuk teripang kering yang pada umumnya dikenal sebagai *beche-de-mer* atau *gamat*, teripang telah lama digunakan sebagai obat dan makanan oleh masyarakat di Asia dan Timur Tengah. Studi terbaru menunjukkan bahwa teripang mengandung senyawa aktif yang berpotensi memiliki manfaat kesehatan dan fungsi biologis lainnya seperti produk antibakteri dan antijamur, antikoagulan, antihipertensi, modulasi sistem imun, inhibitor osteoklastogenesis. Dilaporkan pula bahwa teripang berpotensi sebagai aprodisiaka, dan berpotensi meningkatkan imunitas, antikanker dan antikoagulan. Teripang juga kaya akan kolagen sebagai komponen jaringan pengikat yang lebih lanjut dapat diubah menjadi molekul lebih kecil yang dapat berfungsi sebagai komponen bioaktif. Tulisan ini menyajikan potensi teripang sebagai pangan fungsional terutama untuk pencegahan kanker serta strategi penelitian untuk mengembangkan pangan fungsional berbasis teripang melalui hidrolisis teripang secara enzimatik dan studi *in vivo*.

**Kata Kunci:** agen antikanker, teripang, enzimatik, *in vivo*

### 1. Introduction

In the last few decades, functional foods offer a new and practical approach to achieve optimal health through natural products with physiological benefits reducing the risk of various chronic diseases. Ashwell (2001) has defined functional food as foods intended to be consumed as part of normal diet and contains biological active components that potentially enhance health or reduce risk of disease. Most of available

functional foods can be used as therapeutic agents (Bordbar et al., 2011), either indirectly or directly from natural sources. Sea cucumber is one of marine-derived natural sources. According to the updated trade and catch data available, Asia and Pacific are the two top sea cucumbers producer regions. It is estimated that the combined harvestable catches of sea cucumber for the Asia-Pacific regions are in the range of 20,000 to 40,000 ton per year. The medicinal properties of these animals are ascribed to the

presence of functional components with promising multiple biological activities (Bordbar et al., 2011).

Sea cucumbers belong to the phylum *Echinodermata* (thorn-skinned animals). They are usually soft-bodied echinoderms comprised of a diverse group of flexible, elongated, worm-like, with leathery skin and a supple body which looks like cucumber. Sea cucumbers tend to live in the sea floor of the deep sea (Tuwo, 2004). Sea cucumbers, commonly known as *beche-de-mer*, or gamat, has long been used as medicine and food by Asian and Middle East people. However, edible sea cucumber species with high economic value have only been found from the family *Aspidochirotae* genus *Holothuria*, *Muellaria*, and *Stichopus*. Moreover, the genus *Holothuria*, *Muellaria*, and *Stichopus* are only found in Indonesian seas (Tuwo, 2004).

Information on the potential health benefits and other biological properties of the sea cucumber, has been growing rapidly in modern biomedical research, especially in the field of nutraceutical, such as antibacterial and antifungal products (Aryantina, 2008), anticoagulants (Mulloy et al., 2000), antihypertensives (Zhao et al., 2007), immuno modulation (Gowda et al., 2009) and inhibitor of osteoclastogenesis (Kariya et al., 2004). There are a series of other bioactive and antiagent substances discovered in sea cucumbers, such as triterpene glycosides, enzymes, amyloses, fatty acids and cytotoxins. Their medical potential use includes increasing immunity, resisting tumor and anticoagulation, protecting nerve tissue, relieving pain and resisting epiphyte as well as contributing to immunopotential, anticancer and anticoagulation. Recently, extract of sea cucumber has been reported to have the ability to reduce the growth of cancer, there by opening new perspectives for the development of functional foods and pharmacology from marine organisms (Guadalupe et al., 2012).

Sea cucumbers was also reported as *aphrodisiacs* due to their bioactive compounds, such as triterpene glycosides (saponins), chondroitin sulfate, glycosaminoglycans, polysaccharides sulfate, plant sterols (glycosides and sulfates), phenolics, peptides, collagen, cerbrosida, and lectins (Bordbar et al., 2011). Sea cucumbers are rich in proteins that contain essential amino acids, mainly glycine, glutamic acid and arginine. Glycine may stimulate the production and release of IL-2 and B cell antibody which contribute to the increasing of phagocytosis. Moreover, glycine and glutamic acid play a role as an essential component to synthesize glutathione that lead to stimulating NK cell activation and proliferation. Arginine may improve immune cells by promoting the activation and proliferation of T cells (Bordbar et al., 2011). Amino acid components of sea cucumbers have an important

role in the regulation of immune function. Most (70%) of the body wall of sea cucumber protein consists of collagen (Saito et al., 2002). Collagen is a component of connective tissue that can further be converted into gelatin, so that it can be developed as a functional food based on the bioactive substances (Bordbar et al., 2011).

## 2. Anticancer

According to the National Cancer Institute (2012), the colon cancer death ranks third in the world. Whereas the highest incidence rate of lung cancer is found in men, and for breast cancer, the highest incidence rate is in women. The WHO estimates that in 2030 there will be increase in cancer patients in Indonesia until seven times (DEPKES, 2010). Cervical cancer is the first ranks for cancer mortality in Indonesia (DEPKES, 2010), followed with other cancers, such as colon cancer that ranks seventh in Indonesia. Although colon cancer is not the first leading cause of mortality, most of colon carcinomas are caused by diets, besides various exogenous/endogenous carcinogenic compounds. The colon cancer may ameliorat when risk factors such as genetic, life-style, and environmental factors are modified. Diet has been epidemiologically shown to be closely associated with human colorectal cancer. Chemoprevention of cancer using antimutagens and anticarcinogens present in foods or natural products has been suggested by various studies, offering the most effective means for preventing human colon cancer (Naveena et al., 2010).

Cancer is a disease caused by DNA damage that makes mutations in vital genes involved in controlling cell division. Cell division is a physiological process that occurs on the tissues. The balance between proliferation and programmed cell death are maintained under normal circumstances, usually in the form of apoptosis, cells tightly regulate both processes. Specific mutations in DNA causes cancer through interfering with the programming process of cell division. Carcinogenesis is the process of normal cells turn into cancer cells (Figure 1). It is characterized by the development of changes in the cellular and genetic level, which reprogram the cell to undergo uncontrolled division, thus formed a malignant mass (tumor) that spread into distant locations (Guadalupe et al., 2012).

The actual prevalence of cancer may be avoided through diet as an alternative prevention and treatment of cancer, by consuming natural ingredients (Zakaria, 2001) that can be from marine biological resources. Recent research reported that sea cucumbers have bioactive compounds against certain cancers. Tian

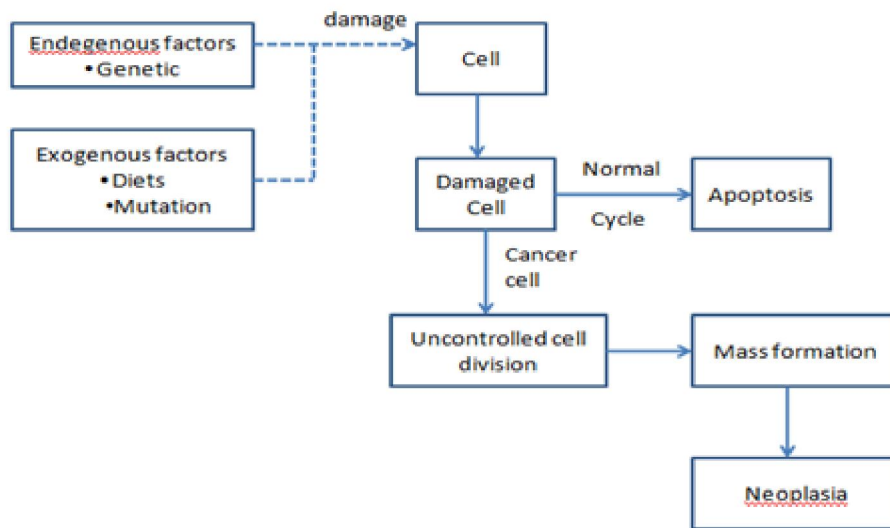


Figure 1. The process of normal cells turn into cancer cells (Picture courtesy of Guadalupe et al., 2012).

et al. (2005) have conducted the *in vivo* and *in vitro* study on philinopside E compound (PE) of sea cucumbers as anti-angiogenic agent. Their research showed that the presence of *in vitro* inhibition by the sea cucumber compound, through proliferation, adhesion, migration, tube formation and apoptosis in human umbilical vein endothelial cells (HUVECs) and human microvascular endothelial cells (HMECs). They assessed through an *in vitro* trial angiogenesis inhibition potential of the compound using different assays. Furthermore, they also used *in vivo* to examine the PE-inhibition activity on the physiological angiogenesis using chorioallantoic membran (CAM) assays. The researchers was using western blotting technique to appraise the efficacy of PE on the vascular endothelial growth factor (VEGF) attributing biosignal in HMECs. The results revealed that PE considerably inhibited the proliferation of HMECs and HUVECs, with  $IC_{50}$  of  $2.22 \pm 0.31 \mu\text{M}$  and  $1.98 \pm 0.32 \mu\text{M}$ , respectively and induced endothelial cell apoptosis at amounts less than  $2 \mu\text{M}$ , showing a concentration-dependent suppression of cell migration and cell adhesion as well as tube formation in HUVECs and HMECs. Similarly, in an *in vivo* CAM assay, PE (5 nM/egg) showed suppression of spontaneous angiogenesis, and exhibited growth inhibition considerably in experimental mouse (sarcoma 180 and hepatoma 22) models. The PE takes an important role as an efficient anti-angiogenic agent, for suppressing the active (phosphorylated) forms of vascular endothelial growth factor receptors involved in the endothelial cell survival, adhesion, proliferation and migration of the endothelial cells. The PE compound was able to inhibit the proliferation of

HMECs and HUVECs based on *in vivo* study (Tian et al., 2005).

Philinopside A, from sea cucumber (*Pentacta quadrangulari*), was tested against angiogenesis and tumor growth by Tong et al. (2005) both *in vitro* and *in vivo* models. It possessed dual cytotoxic and antiangiogenic effect that might be attributed to its inhibitory potential for receptor tyrosine kinases (RTKs). The results indicated that philinopside A substantially inhibited the proliferation, migration and tube formation of human microvascular endothelial cells (HMECs). The inhibition depends on the dose applied, with average  $IC_{50}$  values of  $1.4 \pm 0.17$ ,  $0.89 \pm 0.23$  and  $0.98 \pm 0.19 \mu\text{M}$ , for proliferation, migration and formation of HMECs. About 2–10  $\mu\text{M}$  philinopside A restrained the formation of new microvessels in cultured rat aortas using rat aortas culture assay which mimics angiogenesis process *in vivo*.

Philinopside A compound (2–10 nmol/egg) inhibited angiogenesis using chick embryo chorioallantoic membrane assay. Furthermore, philinopside A demonstrated strong anti-tumor activities both *in vitro* and *in vivo*. Philinopside decrease mouse sarcoma 180 tumor volume by stimulating apoptosis of tumor and tumor-associated endothelial cells examined using immuno fluorescent analysis. An examination of the effects of philinopside A on the angiogenesis-related receptor tyrosine kinases (RTKs) indicated that philinopside A generally inhibited all tested RTKs, including vascular endothelial growth factor (VEGF) receptor, fibroblast growth factor (FGF) receptor-1, platelet-derived growth factor (PDGF) receptor- $\alpha$  and epithelial growth factor (EGF) receptor, with  $IC_{50}$  values ranging from 2.6–4.9  $\mu\text{M}$ .

Those results implied that philinopside A can be proposed as a candidate for anti-cancer agent that possesses dual cytotoxic and anti-angiogenic effects because of its inhibitory effects on RTKs (Tong et al., 2005)

The results showed that philinopside A significantly inhibited the proliferation, migration and tube formation of human microvascular endothelial cells (HMECs) in a dose-dependent manner, with average  $IC_{50}$  values of  $1.40 \pm 0.17$ ,  $0.89 \pm 0.23$  and  $0.98 \pm 0.19$   $\mu$ M, respectively. Rat aortas culture assay provided a close imitation of *in vivo* angiogenesis process and 2–10  $\mu$ M philinopside A suppressed the formation of new microvessels in cultured rat aortas. Philinopside A compound (2–10 nmol/egg) inhibited angiogenesis in chick embryo chorioallantoic membrane assay. In addition, philinopside A showed strong anti-tumor activities both *in vitro* and *in vivo*. Philinopside reduced mouse sarcoma 180 tumor volume by inducing apoptosis of tumor and tumor-associated endothelial cells through immuno fluorescent analysis. An examination of the effects of philinopside A on the angiogenesis-related receptor tyrosine kinases (RTKs) showed that philinopside A broadly inhibited all tested RTKs, including vascular endothelial growth factor (VEGF) receptor, fibroblast growth factor (FGF) receptor-1, platelet-derived growth factor (PDGF) receptor- $\alpha$  and epithelial growth factor (EGF) receptor, with  $IC_{50}$  values ranging from 2.6–4.9  $\mu$ M. Their results suggested that philinopside A is a promising anti-cancer agent that possesses dual cytotoxic and anti-angiogenic effects due to its inhibitory effects on RTKs.

The anticancer activity of three triterpene glycosides, intercedensides A, B, and C isolated from the sea cucumber (*Mensamaria intercedens*) have been evaluated by Zou et al. (2006). Their structures have been elucidated using spectroscopic analysis

(NMR and ESIMS) and chemical transformations. Intercedensides A (1) and C (3) have a conjugated double bond (22Z,24-diene) in the side chain of the aglycon. Intercedenside B (2) has two  $\alpha$ -D-xylose and two sulfate groups in the carbohydrate chain. All three glycosides showed significant cytotoxicity against 10 human tumor cell lines with effective doses 50 ( $ED_{50}$ ) in the range 0.6–4.0  $\mu$ g/ml. Intercedenside A (1) showed significant *in vivo* antineoplastic activity against mouse Lewis lung cancer and mouse S180 sarcoma. Intercedensides A-C merit further study as potential anticancer agent (Zou et al., 2006).

Silchenko et al. (2007) also studied the anticancer activity of three new triterpeneoligoglycosides, namely okhotosides B1, B2, and B3, isolated from sea cucumber *Cucumaria okhotensis*, along with the known compounds frondoside A (4), frondoside A<sub>1</sub>, cucumarioside A<sub>2-5</sub>, and koreoside A (Figure 2). 2-D NMR and MS analysis are used to elucidate the structures of okhotosides B1-3 on the basis of spectroscopic data established. Compounds 1–3 were moderately toxic against HeLa tumor cells. Frondoside A (4) compound showed more potent cytotoxicity against THP-1 and HeLa tumor cell lines with the  $IC_{50}$  values of 4.5 and 2.1  $\mu$ g/ml, respectively. It decreased both the AP-1-dependent transcriptional activities induced by UVB, EGF, or TPA in JB6-LucAP-1 cells and the EGF-induced NF- $\kappa$ B-dependent transcriptional activity in JB6-LucNF- $\kappa$ B cells at doses of about 1  $\mu$ g/mL. At the same doses, it increased the p53-dependent transcriptional activity in non activated JB6-Lucp53 cells and inhibited the colony formation of JB6 P<sup>+</sup> Cl 41 cells activated with EGF ( $INCC_{50} = 0.8$   $\mu$ g/mL). Their result concluded that compounds 1–3 were moderately toxic against HeLa tumor cells, but Frondoside A showed more cytotoxic effect against THP-1 and HeLa tumor cell lines. Similarly, a novel

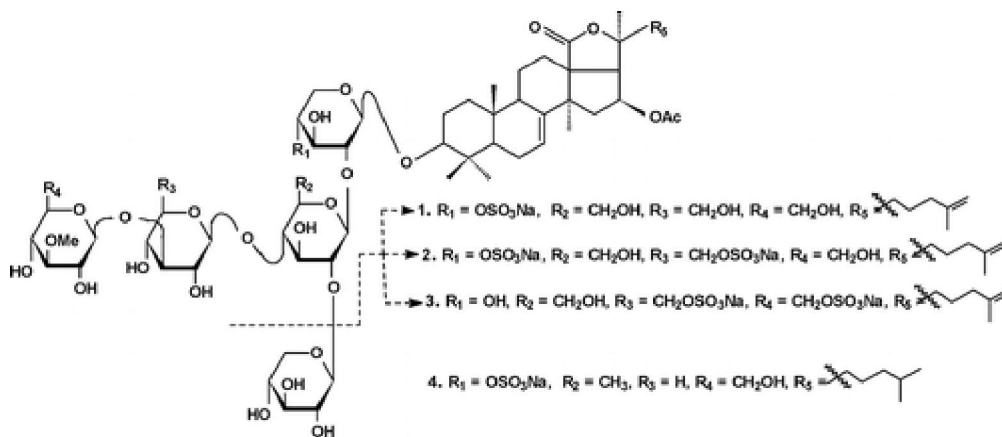


Figure 2. The structure of compounds frondoside A (4), frondoside A<sub>1</sub>, cucumarioside A<sub>2-5</sub>, and koreoside A (Silchenko et al., 2007).

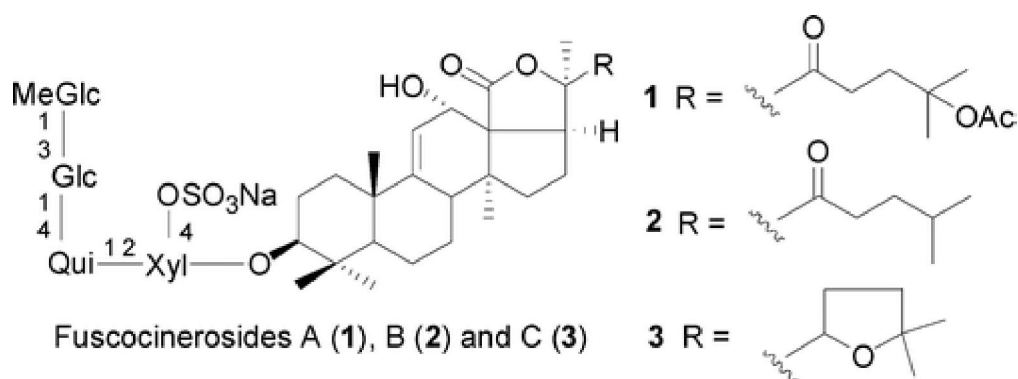


Figure 3. The structure of compounds fuscocinerosides A (1), B(2), and C(3) (Zhang et al., 2006).

triterpenoid, frondoside A, derived from an Atlantic sea-based sea cucumber species namely *Cucumaria frondosa* has showed effective growth inhibitory function against human pancreas cancer cells. The proliferation inhibition potential was followed by the magnitude of marked apoptosis. Frondoside A was supposed to induce apoptosis through mitochondrial and cascade activation pathways.

Zhang et al. (2006a) used the bioassay-guided fractionation of the active *n*-BuOH extract of the sea cucumber *Holothuria fuscocinerea*, resulting in the isolation of three new triterpene glycosides, fuscocinerosides A (1), B (2), and C (3) (Figure 3), along with two known glycosides, pervicoside C (4) and holothurin A (5), as active compounds causing morphological abnormality of *Pyricularia oryzae* mycelia. Compounds 1-5 possess the same tetrasaccharide moiety, 3-*O*-methyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -D-quinovopyranosyl-(1 $\rightarrow$ 2)-4-*O*-sodiumsulfato- $\alpha$ -D-xylopyranosyl, linked to C-3 of holostane triterpene aglycones that differ in their side chains and 17-substituents. Their structures were elucidated by extensive spectral studies as well as chemical evidence. All glycosides showed *in vitro* cytotoxicity against two human tumor cell lines (Figure 3). Zhang et al. (2006b) have also isolated three new sulfated triterpene glycosides from extract sea cucumber, *i.e.*, violaceusides I, II, and III (1-3, resp.), as active compounds causing morphological abnormality of *Pyricularia oryzae* mycelia. Their structures were elucidated by spectroscopic methods including 2D-NMR and MS experiments, as well as chemical evidence. Compounds 1-3 displayed the similar structural characteristics for the existence of a 16-oxo group in the holostane-type triterpene aglycone with the C(7)=C(8) bond, except the differences in the side chains and the tetrasaccharide moieties.

Compound 1 has one sulfate group, while the others are equipped with disulfated glycosides. The entire glycosides demonstrated considerable cytotoxicities against human gastric cancer MKN-45 and human colon cancer HCT-116 cells *in vitro* (Zhang et al., 2006b).

Compounds 1-3 showed the same structural features, *i.e.*, the presence of a 16-oxo group in the holostane-type triterpene aglycone with the C(7)=C(8) bond, but differ in the side chains and the tetrasaccharide moieties. Compound 1 possesses one sulfate group, while 2 and 3 are disulfated glycosides. All the glycosides showed significant *in vitro* cytotoxicities against human gastric cancer MKN-45 and human colon cancer HCT-116 cells (Zhang et al., 2006).

From the same compounds, Zou et al. (2006) have found six new triterpene glycosides, intercedensides D<sup>h</sup>I (1-6) (Figure 4). These compounds were isolated from the whole bodies of the sea cucumber *Mensamria intercedens* Lampert that was found in the South China Sea. Their structures were elucidated by extensive spectroscopic analysis (NMR and ESIMS) and chemical methods. Intercedensides D (1), E (2), G (4), and H (5) have a conjugated double bond system (22Z,24-diene) in the aglycon side chain, while intercedensides F (3) and I (6) have only a single double bond (24, 25) in this same chain. Intercedensides D<sup>h</sup>H (1-5) manifested significant cytotoxicity (ED<sub>50</sub> 0.96-5.0  $\mu$ g/ml) against 10 human tumor cell lines, (Figure 4). Two new triterpene glycosides, hillasides A (1) and B (2), were isolated from the sea cucumber *Holothuria hilla* Lesson, together with one known glycoside holothuria B (3). Their structures were deduced by extensive spectral analysis and chemical evidences. The presence of conjugated double bonds [22E,24-diene] in the

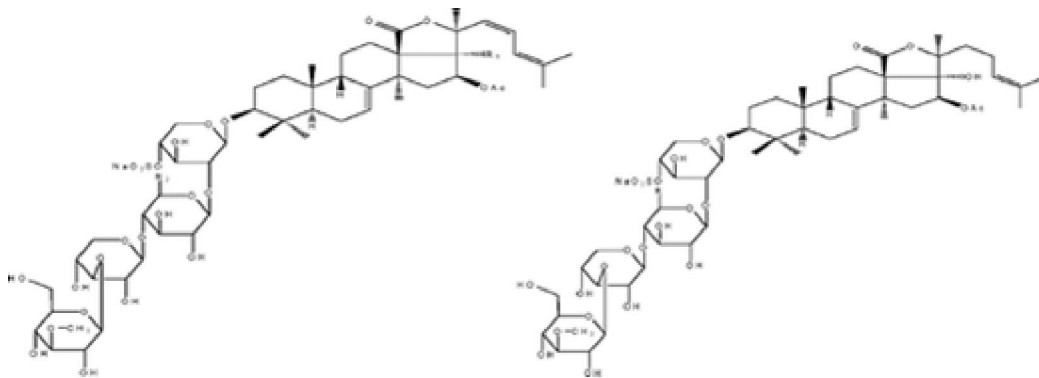


Figure 4. The structure of compounds intercedensides D'I (1-6) (Zou et al., 2006).

aglycone of 1 is a rare structural feature among sea cucumber glycosides. The two glycosides presented significant cytotoxicity against eight human tumour cell lines (A-549, MCF-7, IA9, CAKI-1, PC-3, KB, KB-VIN and HCT-8) with  $IC_{50}$  in the range of 0.1–3.8  $\mu\text{g/ml}$  (Wu et al., 2011).

In Ogushi's et al. (2006), sea cucumbers (*Stichopus japonicas*) were treated with hot water at 98 °C for 60 min to prepare extracts that were used to assess their effect on the proliferation and  $\text{H}_2\text{O}_2$  susceptibility of human colon adenocarcinoma Caco-2 cells. The growth of Caco-2 cells was significantly inhibited by sea cucumber extracts in a dose dependent manner. High molecular weight sea cucumber extract at a concentration of 0.108 mg/ml could inhibit the cell proliferation. No growth was observed at 1.04 mg/ml of the hot water extract after 96h incubation. Cell damage by sea cucumber extract was evident above 1mg/ml.  $\text{H}_2\text{O}_2$  showed concentration-dependent cytotoxicity to Caco-2 cells. In addition, co-administration of sea cucumber extracts intensified the  $\text{H}_2\text{O}_2$  cytotoxicity. Ogushi et al (2006) used phosphatidylserine translocation (APO Percentage Assay kit) for the induction of apoptosis, terminal deoxynucleotide transferase-mediated dUTP-biotin nick-end labeling (TUNEL), and DNA fragmentation as a DNA ladder.

Althunibat et al. (2009) have investigated the effect of aqueous and organic extracts from three sea cucumber species (*Holothuria leucospilota*, *Holothuria scabra*, *Stichopus chloronotus*), on the growth of two human cancer cells namely A549 (human non-small lung carcinoma) and C33A (cervical cancer cells) using MTT assay. Of the extracts tested, only *S. chloronotus*-derived extract showed antiproliferative activity against the tested cancer cell lines. Conversely, aqueous extract (AE) from *S. chloronotus* exhibited more toxicity against C33A cells ( $IC_{50} = 10.0 \mu\text{g/ml}$ ) than A549. Whereas AE produced from *H. leucospilota* and *H. scabra* revealed no notable action

on the growth of the cancer cells within the concentrations limits employed. Sea cucumber extracts produced by the organic solvents inhibited the growth of both the cell lines (A549 and C33A) to varying degrees. The organic extract (OE) from *H. scabra* species given greater antiproliferative action against A549 and C33A cells with  $IC_{50}$  values of 15.5  $\mu\text{g/ml}$  and 3.0  $\mu\text{g/ml}$ , respectively. Furthermore. The OE from *S. chloronotus* presented more cytotoxicity against C33A cells ( $IC_{50} = 6.0 \mu\text{g/ml}$ ) but little action against A549 cells ( $IC_{50} = 21.0 \mu\text{g/ml}$ ). Stress oxidative and degenerative diseases that can be cured with the presence of the phenols and flavonoids, come from the antiproliferative and anticancer of sea cucumber extracts (Bordbar et al., 2011).

Antiproliferative and anticancer functionality of sea cucumber extracts might be ascribed to the presence of considerable amounts of total phenols and flavonoids which were valued as effective antioxidants to protect from oxidative stress and degenerative diseases including certain cancers. Janakiram et al. (2010) examined the chemopreventive effects of frondanol A, a glycolipid isolated from sea cucumber *Cucumaria frondosa*, against azoxymethane-induced rat colon carcinogenesis. They used ACF (aberrant colonic crypt foci) as an efficacy marker to assess the proliferation expression levels during this study. Besides, the growth-inhibitory and apoptotic effects of frondanol A over concentration range of 10–120  $\mu\text{g/ml}$  using HCT-116 cell line (Janakiram et al., 2010).

Based on the proliferative activity of water extracts of *Stichopus variegatus* on spinal astrocytes cell lines, Patar et al. (2012) evaluated the dose-dependant effect of *Stichopus variegatus* water extracts (SVWE) on spinal astrocytes. The extracts were prepared in four different concentrations of 0.1; 1.0; 5.0 and 10.0  $\mu\text{g/ml}$ . The Epidermal Growth Factor (EGF) at 10.0 ng/ml was used as the positive control. The proliferation assay was performed using [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-

2H-tetrazolium] (MTS) assay kit. The proliferation was showed as percentages of surviving spinal astrocytes after 72 h treatments. Their study showed that the SVWE has proliferative effect on rat spinal astrocytes in a dose-dependant manner. At the concentration of 10 µg/ml SVWE has significantly increased the spinal astrocytes proliferation over a period of 72 h ( $p < 0.01$ ). At the concentration of 5 µg/ml, the astrocytes proliferation was increased only at 72 h ( $p < 0.05$ ). The  $EC_{50}$  of SVWE was 5.18 µg/ml. The Malaysian SVWE ( $EC_{50} = 5.18$  µg/ml) showed potential as a growth promoting agent to promote proliferation of spinal astrocytes at the concentration of 5.0 and 10.0 µg/ml (Patar et al. 2012). Patar et al. (2012) revealed that isolates of sphingoid cerberosides sea cucumber (*Stichopus variegatus*) also has cytotoxic effects against human colon cancer cell lines. Cerberosides isolates have characteristics such as a branched alkyl chain at C17 to C19 and the double bond of 1 to 3. Sphingoid sea cucumbers exhibited potent cytotoxic activity against cancer cells (DLD-1, WiDr and Caco-2 cells) that can reduce cancer cell survival, but dependent on the concentration. Compounds were tested whether it triggers morphological changes such as condensed chromatin fragments of compounds that can increase the activity of caspase-3. Sphingoid can reduce cell viability by causing apoptosis (Patar et al., 2012). This suggests that sea cucumber sphingoid can serve as a functional food component for anticancer.

Himaya et al. (2010) demonstrated that the ethyl acetate solvent fraction of the sea cucumber *Stichopus japonicus* (SCEA-F) significantly inhibited the productions of NO and  $PGE_2$  by inhibiting iNOS and COX-2 at their protein and gene levels. The production and the gene transcription of pro-inflammatory cytokines were also inhibited. The responsible molecular signaling for these inhibitory actions was found to be through suppression of ERK and p38 MAPK. Their results indicated that SCEA-F inhibits LPS-induced inflammatory response through blocking of MAPK signaling pathway in murine macrophages, thus demonstrated its *in vitro* anti-inflammatory potential. Therefore it could be suggested that SCEA-F could be effectively used in functional food preparations.

Roginsky et al. (2010) investigated the effects and mechanism of Frondanol-A5P, a polar extract from *Cucumaria frondosa*, on growth inhibition and apoptosis of S2013 and AsPC-1 human pancreatic cancer cells. The effects of Frondanol-A5P on proliferation, cell cycle, expression of cell cycle proteins and p21<sup>waf1</sup>, phosphorylation of MAP kinases, annexin V binding, and caspase-3 activation were examined. The result showed that Frondanol-A5P

inhibited proliferation and induced G<sub>2</sub>/M phase cell cycle arrest in both cell lines with decreased expression of cyclin A, cyclin B, and cdc25c. Frondanol-A5P induced phosphorylation of stress-activated protein kinase and Janus kinase (SAPK/JAK) and p38 mitogen-activated protein kinase (MAP) within 5 minutes. Frondanol-A5P markedly increased the expression of p21<sup>waf1</sup> messenger RNA and protein at 3 hours in both cell lines. This effect was reduced by the p38 kinase inhibitor, SB203580. Frondanol-A5P markedly increased annexin V binding and activated caspase-3.

Li et al. (2009) carried out *in vivo* study on the potential of echinoside isolated from sea cucumber. Their study revealed the anticancer effects and mechanisms of action of the tested compound. Anticancer effects of echinoside A were evaluated *in vitro* and *in vivo*. TUNEL (Terminal deoxynucleotidyl transferase mediated dUTP nick-end-labeling) and DNA fragmentation assays were applied to examine its ability to induce apoptosis. Echinoside A restrained the development of tumors in both mouse models and human prostate carcinoma xenograft using nude mouse models. There are two special features demonstrated by Echinoside A in the way of restraining the noncovalent binding of Top2 $\alpha$  to DNA. The first is done by play a role in competition with DNA for the DNA-binding site of the enzyme, while the other feature is conducted mostly by getting involved with Top2 $\alpha$ -mediated prestrand passage cleavage/religation equilibrium during the poststrand passage phase. These characteristics made Echinoside A different from other common Top2 $\alpha$  inhibitors, therefore this compound stimulated DNA double-strand split in a Top2 $\alpha$ -dependent way.

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Echinoside A inhibited the growth of tumors in mouse models and human prostate carcinoma xenografts in nude mouse models. Echinoside A showed unique characteristics of inhibiting the noncovalent binding of Top2 $\alpha$  to DNA by competing with DNA for the DNA-binding domain of the enzyme

and of interfering predominantly with the Top2 $\alpha$ -mediated prestrand passage cleavage/religation equilibrium over with the poststrand passage one. These features distinguish echinoside A from other known Top2 $\alpha$  inhibitors. As a result, echinoside A induced DNA double-strand breaks in a Top2 $\alpha$ -dependent manner.

### 3. Peptide from Sea Cucumber as Anti Cancer Agent

Peptides with biological activity released during digestion or food processing plays an important role in the regulation of metabolism and modulation. In the parent proteins, peptides in protein are still not be in active form. However, after being released into peptides, then they become active peptides. Bioactive peptides usually have a length of 2-20 amino acid residues. Several studies have reported peptides with a length of more than 20 amino acid residues, known as the apoptosome and activation of caspase cascade initiation (Lang-Hong et al. 2011; Lee et al., 2006).

Protein in the sea cucumber has a complete range of amino acids, both essential amino acids and non-essential amino acids, as has recently been reported by Chen (2006) and Nurjanah (2008) that showed the presence of, almost all essential amino acids except tryptophan in the sand sea cucumber *Holothuria scabra*. The differences were only found in the amino acids phenylalanine, tryptophan, asparagine and glutamine. Chen (2005) reported the absence of phenylalanine in *Holothuria*, *Actinopyga*, and *Thelenota*.

Protein hydrolyzate from marine sources obtained by enzymatic digestion was an important source of bioactive peptides and it has been proven to be a good source of antioxidant peptides. Purified peptide showed cytotoxic effects on some cancer cells such as HeLa, AGS, and DLD-1, suggesting its potential development and application in cancer therapy. Antioxidants are known to be beneficial for human health because it can protect the body from the harmful effects of molecules known as ROS (reactive oxygen species). ROS can attack membrane lipids, proteins and DNA. This ROS can be a causative factor in the development and progression of many different human diseases such as cancer. The reduction of high ROS may prevent the occurrence of cancer, because the inhibition of oxidative stress may lead to reduced genetic changes such as mutations and chromosomal rearrangements that play an important role in the initiation of carcinogenesis (Bordbar et al., 2011).

Apoptosis (programmed cell death) is one of the main mechanisms of cell death in response to cancer therapy. Apoptosis is also a naturally occurring process

and evolution of cells that are no longer useful geared to death. Apoptosis plays a role and fundamental processes in development, physiology, and homeostasis. Deregulation, loss of pro-apoptotic signals or no anti-apoptotic signals can cause a variety of pathological conditions such as cancer initiation, promotion and progression or treatment failure. Apoptosis normally does not trigger inflammatory or immune response; and therefore apoptosis of cancer could become a way for the treatment of cancer (Lang-Hong et al. 2011). Modulation of apoptotic pathways and selective induction of apoptosis by chemical agents tend to be a promising approach for cancer therapy (Lang-Hong et al. 2011). For example as shown in Figure 5, in mammals, there are two major signaling systems that lead to caspase activation, the extrinsic death receptor pathway and the intrinsic mitochondrial pathway (Lang-Hong et al., 2011).

There has been an increasing evidence that the anticancer peptides from the marine sources have cytotoxicity that can trigger apoptosis by targeting many cellular proteins, and may induce apoptosis process by both intracellular and extracellular pathways. In most normal cells, proto-oncogene encodes a protein that sends signals to the nucleus to stimulate cell division. Signal transduction proteins takes place in several stages called transduction cascade. This involves a cascade of signaling molecules to the membrane receptor protein intermediates that carry signals into the cytoplasm and in the nucleus of the cell transcription factors that activate genes for cell division. At every stage of the factors or proteins will enable the next phase (Hartwell, 2007; Robbins & Kumar, 2007).

Wang et al. (2010) made the gelatin from the body wall of sea cucumber (*Stichopus japonicus*) hydrolyzed with flavourzyme. Low-molecular-weight gelatine hydrolyzate (LMW-GH) of 700-1700 Da have been produced using ultrafiltration membrane bioreactor system. Chemiluminescence analysis revealed that the LMW-GH capture free radicals, high-concentration-dependent, IC<sub>50</sub> values for superoxide and hydroxyl radicals were 442 and 285 mg/ml, respectively. LMW-GH inhibited melanin synthesis and tyrosinase activity in B16 cells. Furthermore, LMW-GH primarily increased intracellular glutathione (GSH), which can suppress melanogenesis. LMW-GH antioxidation activity, holds the potential of being used as a valuable ingredient in function food, cosmetics and pharmaceuticals or nutraceuticals.

Research of Perez-Vega et al. (2013) revealed that the *Isostichopus badionotus*'s flour was hydrolysed with the new simulated *in vitro* gastrointestinal digestion with digestive enzymes; pepsin and a pepsin–Corolase PP-mixture. It has capability as



Figure 5. Apoptosis induction pathways of marine-derived anticancer peptides. Picture courtesy of Lang-Hong et al. (2011).

ACE-inhibitors, radical scavenging activity, iron reduction capacity and cytotoxic effects against colorectal cancer cells HT-29. The hydrolysed product were fractions containing peptides <3000 Da capable as ACE-inhibitors, an effect augmented with combined action of gastric (pepsin) and intestinal enzymes ( $IC_{50} = 0.038 \pm 0.004$  mg/ml). Depending on the hydrolysis method, low and higher molecular weight peptides from sea cucumber have cytotoxic capacity against colorectal HT-29 cells due to their antioxidant activity..

Hydrolyzate contains a multi functional peptide that is resistant to digestive enzymes, greatly increasing the probability of meeting the diverse biological functions. Sea cucumbers *Isostichopus badionotus* also found to have high concentrations of several amino acids (eg Glysin, Arginine and Alanine), which may play an important role in the physiological effects such as reducing serum levels of total cholesterol. Peptides present in protein hydrolysates and have biological activity depending on molecular weight and amino acid sequence (Guadalupe et al., 2012). Sea cucumbers hydrolysates and ultrafiltered fractions are potential ingredients for functional food.

Protein of sea cucumber meat contains 70% collagen and 37% amino acid (Saito et al. 2002; Patar et al. 2012). Hydrolysis of protein is the method used to obtain peptides and amino acids from protein foods that have better biological activity such as antioxidant, antihypertensive, antimicrobial and antiproliferative than the parent material, i.e protein. This hydrolysis process breaks peptide bonds and produce smaller peptides or free amino acids. Enzymatic hydrolysis

of protein is a commonly used method. Some proteolytic enzymes used to obtain the hydrolyzate include, *alcalase, trypsin, pepsin, chymotrypsin, pancreatin, pepsin, and thermolysin*. Several studies have shown that enzymatic hydrolysis likely increased the antioxidant activity of hydrolysates produced by increasing radical scavenging activity. Bioactive peptides showing potential antioxidant and anticancer activities as well as immunostimulatory and antiproliferative effects (Jun et al., 2004; Picot et al., 2006) are found in marine protein hydrolysates and collagen fibers, which can serve as a protein supplement in food. Antioxidants are potential active compounds that are suitable for the prevention and treatment of diseases associated with active oxygen species, especially cancer (Bordbar et al., 2011). Not only peptide but also collagen can be used as a cancer preventive agent in the gastrointestinal tract (Lee et al., 2006; Lang-Hong et al., 2011).

#### 4. Strategy Development of Functional Food Based on Sea Cucumber

It is very important to develop strategies for exploiting the potential of sea cucumbers as functional food with anticancer activity. Sea cucumbers are rich in bioactive compounds, especially peptides and collagen; and therefore further studies, especially *in vivo* experiments, are very important to understand their physiology effect in humans and mechanism as anti colon cancers. Xiukun et al. (2010) reported that in *in vivo* situation, apoptosis acts to eliminate potentially deleterious cells without causing such

adverse effects such as inflammatory response and ensuing scar formation.

Enzymatic hydrolysis of food proteins is considered as an efficient way to recover potent bioactive peptides, since several peptides obtained by this process have different bioactivities and these may represent a potential approach to anticancer drugs (Guadelupe et al., 2012). The diverse range of natural environments where the sea cucumber live in combination with the efficient hydrolysis method applied will contribute to the discovery of a wide variety of sea cucumber-derived natural products. Furthermore, this offers an exciting opportunity to find novel peptides from these organisms, since the study of marine animal-derived peptides is still in its infancy. Compared with the peptides found from other sources, marine-derived peptides are reported to be more diverse (Chen et al., 2006). Many cyclic peptides or depsipeptides have been found in marine organisms such as sea cucumber. These marine peptides seem to be very useful and promising for biomedical research. There is no doubt that the diversity of marine peptides, in terms of chemical structure and mode of action, may provide a rich source for designing very specific and potent new pharmaceutical compounds against a wide variety of diseases (Chen et al., 2006). Another potential exploitation of sea cucumber is developing functional food products, such as ingredients that are needed for the substitution or fortification of food.

Based on the description above, future research should be directed towards enzymatic hydrolysis of sea cucumber protein and *in vivo* study. Flour from the sea cucumber *Stichopus variegatus* could be hydrolyzed using gastrointestinal protease to obtain several peptides. *In vivo* study could subsequently be conducted to investigate the enzymatic process in the gastrointestinal tract. The expected result could provide insight into the anticancer effect and mechanism of action of the resulting peptides. It was reported by WHO (2012) that diets have been epidemiologically shown to be closely associated with human colorectal cancer.

The sea cucumber *Stichopus variegatus* is suggested to be a good source of functional food, because some of the most prevalent marine components used in food, such as glycosides, amino acids, collagen, gelatin, and peptides, can be obtained from this invertebrate. Sea cucumber is normally processed as flour that can be applied as food ingredients or supplements, cereal-based product (e.g. breads, breakfast cereal and cake), flavor binding, snack foods and gelatin product. The approach could include delivery of the protected bioactive ingredient to their target site and release under certain trigger

factor, such as enzymes, pH, salts, temperature (Chen et al., 2006). Mechanical and thermal processes commonly applied for food processing are size-classification, heating and evaporation using vacuum-drying, flour-milling, and mixing (Chen et al., 2006). The temperature used should be set up under 70 °C to prevent ingredient from denaturation, oxidation and Maillard reaction during processing and storage. It is necessary to investigate (Chen et al., 2006). The formulation of each ingredient, the sensory, and the understanding of the relation between bioactive ingredient release to meet specific needs of food applications.

## 5. Conclusions

Finding functional food products represents one of the major current challenges for pharmacology and medicine. Therefore, extensive research efforts have been focused on identifying new potential bioactive compounds from natural resources. Development of products based on marine protein hydrolysates and collagen still remain largely unexplored. Studies on peptides obtained from protein hydrolysates and collagen have revealed that these molecules have antioxidant and antiproliferative activities, suggesting their potential development as anticancer drugs. However, more research should be conducted to better understand their mode of action on the cell cycle arrest or apoptosis of cancer cell lines. Nevertheless, there is a need for strategies to develop and produce these compounds. This could be achieved by utilization of sea cucumber as functional food products.

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