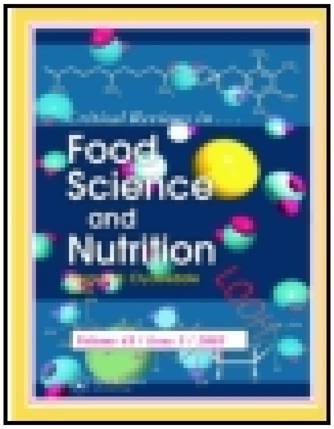

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Therapeutic Potential of Abalone and Status of Bioactive Molecules: A Comprehensive Review

H. A .R Suleria[ab], P. P. Masci[a], G. C. Gobe[a] & S. A. Osborne[b]

[a] School of Medicine, University of Queensland, Centre for Kidney Disease Research, Translational Research Institute, Brisbane 4102, Australia

[b] CSIRO Agriculture Flagship, 306 Carmody Rd, St Lucia, QLD 4067, Australia

Accepted author version posted online: 26 Jun 2015.

To cite this article: H. A .R Suleria, P. P. Masci, G. C. Gobe & S. A. Osborne (2015): Therapeutic Potential of Abalone and Status of Bioactive Molecules: A Comprehensive Review, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2015.1031726

To link to this article: http://dx.doi.org/10.1080/10408398.2015.1031726

Therapeutic Potential of Abalone and Status of Bioactive Molecules: A Comprehensive Review

H.A.R Suleria[1, 2], P.P. Masci[1], G.C. Gobe[1] and S.A. Osborne[2]

[1]School of Medicine, University of Queensland, Centre for Kidney Disease Research, Translational Research Institute, Brisbane 4102, Australia

[2]CSIRO Agriculture Flagship, 306 Carmody Rd, St Lucia, QLD 4067, Australia

Corresponding Author

Hafiz Ansar Rasul Suleria

PhD Fellow

School of Medicine

The University of Queensland, Australia

Email: hafiz.suleria@uqconnect.edu.au

ABSTRACT

Marine organisms are increasingly being investigated as sources of bioactive molecules with therapeutic applications as nutraceuticals and pharmaceuticals. In particular, nutraceuticals are gaining popularity worldwide owing to their therapeutic potential and incorporation in functional foods and dietary supplements. Abalone, a marine gastropod, contains a variety of bioactive compounds with anti-oxidant, anti-thrombotic, anti-inflammatory, anti-microbial and anti-cancer activities. For thousands of years different cultures have used abalone as a traditional functional food believing consumption provides health benefits. Abalone meat is one of the most precious

 ACCEPTED MANUSCRIPT

commodities in Asian markets where it is considered a culinary delicacy. Recent research has revealed that abalone is composed of many vital moieties like polysaccharides, proteins and fatty acids that provide health benefits beyond basic nutrition. A review of past and present research is presented with relevance to the therapeutic potential of bioactive molecules from abalone.

Keywords: Marine foods, nutraceuticals, abalone and drug discovery

 ACCEPTED MANUSCRIPT

BACKGROUND

Abalone, a marine gastropod, is a single-shelled, herbivorous, reef-dwelling mollusc widely distributed throughout temperate and tropical coastal waters. Up to 100 species of abalone have been classified with an ecologically diverse distribution; larger species commonly inhabit temperate water, while smaller species favour tropical habitats (Estes *et al.*, 2005). Although all species are economically important, just over 20 species have been identified as commercially significant with the majority of these being larger species (except *Haliotis diversicolor supertexta*) harvested from wild stocks.

Marine organisms currently being investigated for therapeutic potential include sponges, tunicates, bryozoans, molluscs, bacteria, cyanobacteria, macroalgae (seaweeds) and microalgae. These organisms have produced a variety of bioactive molecules targeted to the treatment of a range of diseases including asthma, atherosclerosis and heart disease (Kim and Pallela, 2012). In 2003, over 650 new compounds were isolated from marine organisms including phytoplankton, green, brown and red algae, sponges, coelenterates, bryozoans, tunicates, echinoderms and molluscs (Blunt *et al.*, 2005).

Abalone, a good source of proteins, lipids, essential fatty and amino acids (Latuihamallo *et al.*, 2015; Lou *et al.*, 2012), has long been considered a functional food with different cultures believing consumption provides health benefits (Li *et al.*, 2013). Recent research has revealed that abalone is composed of many bioactive molecules like polysaccharides, proteins and fatty acids that provide health benefits beyond basic nutrition. The biological activities assigned to abalone molecules include anti-oxidant, anti-thrombotic, anti-inflammatory, anti-microbial and anti-cancer and are reviewed with relevance to therapeutic potential.

 ACCEPTED MANUSCRIPT

A comprehensive review of the therapeutic potential of bioactive molecules from abalone must also consider supply of raw material; as such a review of the world abalone markets and commercial supply is presented. Specifically, the size, distribution and current trends in the world abalone markets are detailed along with the range of commercially available abalone products. This review also considers how the world abalone market is supplied through the contributions by abalone farming and harvesting from wild stocks.

World abalone supply

In the past forty years the world abalone supply has increased five-fold owing primarily to increased abalone farming practices. In the 1970's global supply was limited to 20,370 t comprised mostly harvested wild stocks (Gordon and Cook, 2013). During this period abalone farming was almost non-existent with legal harvest estimated to account for 20,000 t. The remaining 370 t included farm production and illegal harvesting of wild stocks. By 2013 however, farm production of abalone was estimated to be 103,464 t (You, 2014; Park, 2014) with legal harvests of wild stocks estimated to be less than 8,000 t (Gordon and Cook, 2013).

China and Korea are the two largest producers of farmed abalone in the world. There were more than 300 functional abalone farms in China in 2013 (You, 2014), amongst these a few are renowned all over the world because of their efficient production. In 2004, 60% of the abalone farms in China were land-based, however by 2010 more than 95% were sea-based. Korea is also a major supplier of abalone to the world market where farm production increased more than 60 times in the last 10 years and was estimated to be 4500 t in 2007, 6288 t in 2010, and is predicted to reach more than 10,000 t by 2015 (Park, 2014). Approximately 85% of abalone farmed in

ACCEPTED MANUSCRIPT

Korea is exported to Japan largely influencing the world market. *H. discushannai* is one of the most famous species sold at very high prices in the Japanese market.

There are a few small abalone farms in Europe but the market range is comparatively low compared to Asia. Abalone farms are also located in the UK, Ireland, the Channel Islands, Spain and France (Cook, 2010). Some abalone farms are present in Chile, Japan, South Africa, Australia, Taiwan, New Zealand, Mexico, Thailand and the Philippines that will help to increase the production of abalone in near future (Gordon and Cook, 2013; Korpov *et al.*, 2000).

With respect to the legal harvest of wild stocks, Australia supplies more than 50% of the world market. Recently two of the world's major wild abalone fisheries, USA and South Africa, were decommercialised leaving Japan and New Zealand as the other major producers of wild harvest abalone (Cook, 2010).

World abalone market and commercial products

Demand for abalone in China, the world's biggest consumer of the product, has significantly decreased over the past few years and this has resulted in a general fall in prices. Similarly in Japan, the recent downturn in the national economy has resulted in a decreased demand for luxury foods (Cook, 2014).

Live, fresh and frozen abalone

Prior to the recent economic downturn, Japan was regarded as the largest consumer of live, fresh and frozen abalone (Oakes and Ponte, 1996). Japan is still a highly lucrative market for high end abalone products and provides a bench mark regarding quality and consumer preference

 ACCEPTED MANUSCRIPT

standards. The traditional Japanese fisheries are of considerable cultural significance as they define the taste and quality preferences for the consumers of premium quality abalone products.

Premium quality live abalone is harvested and sold to be frequently used in Sushi and other conventional Asian recipes. The standard minimum size recommended for a premium product intended for sale in Japanese markets should be around 9 cm and is sold by kilograms (9-10 individuals/kg). Comparatively smaller sized (6.5 cm, 60-85 g, 15-20 individuals/kg) premium quality specimens command excellent prices for Chinese, European, Indonesian and Korean cuisines, as they are ideal for a variety of conventional dishes and hors d'oeuvres (Oakes and Ponte, 1996).

Abalone fillets

Restaurants in the USA prefer abalone to be served in the form of fillets or 'steak', where a process tenderizes the meat producing slices with a white coloration and delicate flavour range. The conventional base for abalone consumption is primarily nestled in California, where it was regarded as a regional delicacy and was subject to a booming fisheries industry until 1970s. Over harvesting, poaching and non-sustainable production practices drove prices to such an extent that abalone is now regarded as an expensive and high end restaurant food (Oakes and Ponte, 1996).

Canned abalone

Canned abalone is generally regarded as a low priced product however it represents the largest market segment by volume. Hong Kong and China are inarguably the largest importers of canned abalone. Mainland China remains the largest consumer nation for abalone where nearly all abalone consumed is canned. Outside of China, canned abalone is not considered a premium

ACCEPTED MANUSCRIPT

product. However, in China canned abalone is regarded as highly prestigious and is presented as a sign of respect during banquets and traditional celebrations (Oakes and Ponte, 1996).

Abalone farming

With commercial demand for abalone increasing over the past few decades, wild abalone stocks have suffered massive decline due to over fishing, illegal poaching, diseases and climate change (Byrne *et al.*, 2010). Abalone farming has provided a means to meet increasing commercial demand reducing the pressure on wild stocks by providing ~90 % of the global abalone market (Cook, 2014).

It's reported that both wild-caught and farmed abalone attract premium market prices with the aim of abalone farming to provide an alternative to a dwindling supply of wild abalone. During farming practices, one of the objectives is to provide abalone products comparable to wild-caught with respect to both physicochemical and sensorial attributes. However, significant differences have been found between farmed and wild-caught *H.rubra* (blacklip) abalone. In a study by Cochet *et al.* (2013) wild-caught blacklip abalone were firm, chewy, higher in aroma, flavour and aftertaste and larger in size when compared to farmed abalone. Furthermore, wild-caught and farmed abalone also varied with respect to chemical composition; it might be due to change in growing conditions as well as growing techniques (Cochet *et al.*, 2013).

Farmed abalone products are sold in various international markets; as a result farmed abalone is considered good quality and a reliable source that can produce abalone products in all seasons. During the farming of abalone, it is important to pay careful attention to appearance, taste and texture of the meat. By considering and maintaining the aforementioned parameters the final product will be suitable for international markets. In Asian markets the most liked abalone are actually grown on farms, they are sold live and their texture is maintained for a long period of time during shipping, ensuring suitability for various products including sushi.

Abalone and other marine organisms as sources of bioactive molecules

Marine organisms are increasingly being investigated as sources of bioactive molecules with therapeutic applications in the nutraceutical and pharmaceutical industries. Molecules with anti-oxidant, anti-thrombotic, anti-microbial, anti-inflammatory and anti-cancer activity have broad appeal. People are moving towards more simplified, natural and convenient ways of living where marine food sources are gaining special attention. This shift is contributing to the development of marine pharmaceuticals and nutraceuticals including functional foods and supplements, however this research can also be used to highlight the health benefits of consuming marine sources (Kim and Pallela, 2012).

Anti-oxidant perspectives

In the pursuit of marine anti-oxidants, bioactive molecules with anti-oxidant capacity have been isolated from different marine species including fish (Je *et al.*, 2009), squids (Lin and Li, 2006), shrimps (Guerard *et al.*, 2007), echinoderms (Mamelona *et al.*, 2010) and bivalve molluscs (Dong *et al.*, 2010). Specifically, marine-derived polysaccharides with sulphate groups have demonstrated stronger radical scavenging capacity compared to neutral polysaccharides (Tsiapali *et al.*, 2001; Qi *et al.*, 2005; Xing *et al.*, 2005). High radical scavenging abilities have been reported in sulphated polysaccharides extracted from alga *Porphyra haitanesis* (Zhang *et al.*, 2003), *Ulva pertusa* (Qi *et al.*, 2005), and *Laminaria japonica* (Wang *et al.*, 2008; Zhao *et al.*,

ACCEPTED MANUSCRIPT

2008) and are possibly due to sulphate groups acting as electrophiles promoting the abstraction of intramolecular hydrogen. It was further suggested by Tsiapali *et al.* (2001) that the radical scavenging ability of carbohydrates can be increased by the effect of two electrophile substrates *i.e.* sulphate and phosphate groups. However, sulphation is not the only factor involved in the radical scavenging ability of polysaccharides as there is no strict correlation between sulphation and radical scavenging capacity (Zhang *et al.*, 2003; Wang *et al.*, 2008).

Abalone has recently been investigated as a source of anti-oxidant molecules and is composed of many vital moieties like polysaccharides, proteins and fatty acids. Zhu *et al.* (2008) prepared crude polysaccharides (CPs) from abalone viscera with the ability to act as hydroxyl radical scavenging agents, reducing agents as well as chelating agents. Furthermore, abalone are hydrolysed on a commercial scale for the production of food grade proteases, however these hydrolysates also have 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radical scavenging abilities, reducing power, and ferrous ion chelating capacity. These positive activities have contributed to abalone hydrolysates gaining special attention in drug discovery research.

Furthermore, it is also postulated by Li *et al.* (2008) that the radical scavenging ability of abalone is also related to conjugated polysaccharides implying involvement by peptides. Structural studies including molecular weight and monosaccharide composition analysis also show positive correlations between radical scavenging capacity and polysaccharide content (Tsiapali *et al.*, 2001). Accordingly, it is further reported from Saiga *et al.* (2003) that a strong relationship exists between specific amino acid residues and anti-oxidant capacity of protein hydrolysates. It is generally suggested that peptides that contain high amounts of histidine, proline, alanine, valine,

ACCEPTED MANUSCRIPT

methionine, and leucine exhibit a strong anti-oxidant capacity (Chen *et al.*, 1996; Guo *et al.*, 2009).

Free amino acids (FAA) are produced in abundance as a result of protein hydrolysis. Generally, anti-oxidant activity in peptides is greater than amino acids because peptides can enhance free radical scavenging ability, metal chelation and activity of aldehyde adduction (Saiga *et al.*, 2003). Extensive hydrolysis of protein also produces significant amounts of small peptides and it is well recognized that short peptides also contribute to the anti-oxidant activity in protein hydrolysates (Peñta-Ramos and Xiong, 2002; Wu *et al.*, 2003; Li *et al.*, 2008). Wu *et al.* (2003) proposed that a 1.4 kDa peptide from a fish muscle hydrolysate produces greater anti-oxidant ability than 0.9 or 0.2 kDa peptides. It was also proposed that very small sized peptides (below 0.5 kDa) may lower the anti-oxidant ability of protein hydrolysates, indicating that the degree of hydrolysis may affect overall anti-oxidant capacity.

Conversely, other studies suggested that there is no relationship between anti-oxidant activity and protein content, molecular weight or polysaccharide content (Zhu *et al.*, 2008; Lin and Meyers, 2009). Moreover, Wang *et al.* (2008) opined that there is no single factor influencing the anti-oxidant activity of polysaccharides, indeed the effect on anti-oxidant activity may involve a combination of factors. Regardless of the precise mechanism involved, anti-oxidant activity has been demonstrated in extracts prepared from abalone foot/muscle (*H. discus hannai Ino*) and viscera (*H. rubra*) demonstrating that abalone contains bioactive molecules with potential as anti-oxidant therapeutics.

 ACCEPTED MANUSCRIPT

Anti-thrombotic and anti-coagulant perspectives

Abalone and other marine organisms are being investigated as sources of anti-thrombotic molecules because they are rich sources of structurally diverse bioactive components that may possess nutraceutical and pharmaceutical attributes (Harnedy and FitzGerald, 2012). Fresh and processed abalone products are sold as both food and medicine in Hong Kong, Singapore, and elsewhere in Southeast Asia. In China, where food and medicine have long been considered more or less the same thing, abalone is highly valued for its healing powers (Kim and Pallela, 2012).

Thrombosis often leads to severe health related disorders like heart attack and stroke. Numerous studies reveal the risk factors for thrombosis are: abnormally high blood lipids, high blood glucose, elevated plasma fibrinogen, hypertension and cancer insurgence (Leopold and Loscalzo, 2009). In the last few decades, prevention and treatment of thrombosis have gained special attention with drugs like heparin and warfarin frequently used for anti-thrombotic therapy. There are two main classes of anti-thrombotic drugs; anti-coagulant and anti-platelet. Anti-coagulant drugs mostly reduce the formation of fibrin, prevent clot formation and slow down the process of blood clotting while anti-platelet drugs mostly inhibit platelet clumping thereby stopping clot formation (Sikka and Bindra, 2010). Heparin and warfarin both are anti-coagulant and ant-platelet drugs, heparin is administered intravenously and frequent laboratory monitoring is needed to prevent unwanted and sometimes life-threatening bleeding (Franchini and Mannucci, 2009). Warfarin, an antagonist of Vitamin K, is also limited by frequent monitoring and dose adjustment to prevent serious bleeding. Heparin induced thrombocytopenia (HIT) is also one of the serious complications of heparin therapy particularly in cardiac subjects. Onset of HIT is

 ACCEPTED MANUSCRIPT

treated by immediate discontinuation of heparin sometimes accompanied by intravenous saline to flush out all the heparin (Ahmed *et al.*, 2007). There is an ongoing need to find alternative anti-coagulants that can help to minimize or reduce these serious side effects.

Several studies have been published based on the pharmaceutical importance of abalone. Recently, Li *et al.* (2011) isolated a glycosaminoglycan-like polysaccharide, denoted as AAP, from abalone and conducted *in vitro* investigations on its anti-coagulant activity. It was concluded that AAP can prolong the activated partial thromboplastin time (aPTT) and thrombin time (TT). *In vitro* anti-coagulant assays consist of thrombin time (TT), prothrombin time (PT) and activated partial thrombin time (aPTT) and these indicate anti-coagulant activity with respect to the intrinsic and extrinsic pathways in the blood coagulation cascade. TT is also called as thrombin clotting time (TCT) and reflects the time taken to form a clot in plasma. PT actually reflects the extrinsic pathway of the coagulation cascade; it refers to the anti-coagulation status of patients treated with oral anti-coagulants. aPTT is another laboratory test and it monitors the effect of parenteral anti-coagulants reflecting changes in the intrinsic pathway of the blood coagulation cascade (Sikka and Bindra, 2010).

Thrombosis can be prevented and treated simply by inhibiting platelet aggregation since platelets play an important role in thrombus development (Jang *et al.*, 2013). Li *et al.* (2013) prepared different abalone extracts from viscera and muscles of *H.discus hannai Ino*; these extracts were prepared by using aqueous and ethanolic treatments as well as enzyme digestions. According to the findings, the aqueous extract showed the lowest platelet aggregation ability in comparison to other extracts in a dose-dependent mode. However, other publications (Kim and Lee, 2006; Gadi *et al.*, 2009) proposed that platelet aggregation is not considered to be the best indicator for thrombosis.

Different types of sulphated polysaccharides that have been obtained from visceral portions and gonads of abalone, require a higher degree of purification as reported by Zhu *et al.* (2010). Polysaccharides from pleopods of abalone are comprised of 1-, 1,4-, 1,6-, 1,4,6-linked glucose and in accretion 1-, 1,3-, 1,6-, 1,4,6-linked galactose (Zhu *et al.*, 2009). She *et al.* (2002) also proposed that sulphated polysaccharides from abalone pleopods are comprised of galactose, glucose, fructose and xylose in a quotient of 11.5:3.7:1.0:1.0. The acidic polysaccharide content has not been fully determined however several glycosaminoglycan-like structures have been defined.

Glycosaminoglycans (GAG) are the major components of acidic polysaccharides mainly found in animal tissues (Ashie and Pedersen, 2005). Uniquely sulphated polysaccharides are found in marine sources and have a complex structure composed of galactose, fucose, glucuronic acid, galactosamine in quotient of 1:2.94:2.37 and 2.14 respectively. The quantitative ratio of sulphate was about 5.5% with an average molecular weight (MW) of 56.2 kDa (Li *et al.*, 2011). The chemical structure of AAP, a carbohydrate isolated by Li *et al.* (2011) contains galactosamine and glucuronic acid backbone linked to sulphated-fucose, and galactose, considered to be similar to the fucosylated chondroitin sulphate present in the sea cucumber (Chen *et al.*, 2011). In abalone even though AAP is linked with galactose to fucose branch, it is still considered a chondroitin-like polysaccharide (Li *et al.*, 2011).

According to the Li *et al.* (2013), water extracts of abalone viscera improve PT, aPTT, and TT more as compared to other abalone extracts prepared with different solvents. Activity was weaker than a heparin control requiring 2-times and 5-times more concentration (µg/mL) to maintain the same activity as heparin. The anti-coagulant action of these molecules was also observed in the presence of anti-thrombin III mediated thrombin inhibition using chromogenic assay. Other research also demonstrated that GAG-like molecules isolated from different sea cucumbers did not show a prominent effect on PT but efficiently improved the aPTT and TT (Chen *et al.*, 2011). Li *et al.* (2013) also conducted experimental modelling in rats and concluded that abalone water extract can increase the tail bleeding time and aPTT while no effect was observed on PT and platelet number. Modern research regarding the abalone extracts provides evidence that its anti-thrombotic activity is due to polysaccharides, however further investigation and clarification is required (Li *et al.* (2013).

Several studies have demonstrated abalone viscera, gonads and pleopods to be sources of potent anti-thrombotic and anti-coagulant polysaccharides highlighting the therapeutic potential of abalone in coagulation control.

Anti-inflammatory perspectives

Different nutraceutical products are currently being used as therapies to treat and prevent inflammation and pain associated with arthritis. In the past evidence based research has been focussed on New Zealand green lipped mussel (NZGLM), cartilage of shark (SC) and several fish oils that are rich sources of omega 3 fatty acids (Murphy *et al.*, 2003; Su *et al.*, 2004), proteins and GAG (Alencar et al., 1995). Consumption of these bioactive ingredients has been linked with lowered clinical symptoms of inflammation associated with arthritis.

Several studies suggest that the major action of NZGLM and SC is the significant prevention of interleukin-1 (IL-1)-induced prostaglandin-E2 (PGE2) production and IL-1-induced GAG synthesis; whereas abalone extract has been found to significantly inhibit IL-1-induced nitric oxide (NO) production (Pearson *et al.*, 2007). Basically, these marine sources contain bioactive molecules that influence three important pathways of the catabolic cycle of arthritis and impart relief from pain and other degradative symptoms associated with disease.

It is further reported that SC and NZGLM have both an anti-inflammatory and chondro-preventive role. In research conducted by Pearson *et al.* (2007), post-absorptive accumulation of dietary GAGs was observed within the synovial space of Sasha's EQ (SEQ) horses in both the arthritis induced (IL-1) and control (saline-injected) groups consistent with significant decreases in inflammation or pain in the SEQ horses group as compared to the control group. Moreover, dogs provided dietary NZGLM (Bright, 2010) exhibited significant resistance to cartilage degradation by IL-1 due to the provision of glucosamine and chondroitin - the major bioactive constituents of SC (Dechant *et al.*, 2005).

A two year human clinical trial investigating the effect of glucosamine and chondroitin on symptoms associated with osetoarthritis (Glucosamine/Chondroitin Arthritis Intervention Trial or GAIT) reported no important differences when compared to a placebo. However, the study showed beneficial trends in response to glucosamine. Furthermore, it was also reported consumption of glucosamine and chondroitin was not associated with any adverse effect (Sawitzke *et al.*, 2010).

Anti-microbial peptide

Anti-microbial peptides (AMPs) provide protection against invading pathogens like bacteria, fungi, envelope viruses, protozoans and other parasites (Bulet *et al.*, 2004; Cruz *et al.*, 2014) that can create various life threating disorders in humans. AMPs play vital role in the host's defence system; they are mostly cationic and hydrophobic molecules with molecular mass of less than 10 kDa. They develop strong interactions with anionic membranes (Reddy *et al.*, 2004). AMPs are biochemically diverse and may possess anti-cancer properties, wound curative effects as well as extensive specificity against microorganisms (Cho *et al.*, 2009).

Histone proteins are reported to have high anti-microbial activity in humans (Kim *et al.*, 2002), fish (Birkemo *et al.*, 2003) and amphibians (Park *et al.*, 1996). The first anti-microbial activity was verified in 1958 from calf thymus (Hirsch, 1958) while the first histone H2A-derived AMP from Asian toad called Buforin I. Moreover, several other histone-derived AMPs were isolated from fish including *Parasilurus asotus, Salmon salar, Oncorhynchus mykiss,* and *Hippoglossus hippoglossus*, respectively. However, only a few studies report histone-derived AMPs from invertebrates such as scallop *Chlamys farreri* (Li *et al.*, 2007) and pacific white shrimp (Richards *et al.*, 2001). Furthermore, limited data is available on AMP from abalone and includes only two sequences of defensin from *Haliotis discus discus* (FJ864724) and *H. discus hannai* (ABF69125).

AMPs play a vital role to protect the immune system against various pathogenic microorganisms. In one of the latest studies involving *H. discus discus* (disk abalone), AMPs were expressed from histone 2A (H2A) full-length cDNA (De Zoysa *et al.*, 2009). Abhisin, a designed anti-microbial peptide, represented 40 amino acids of the N terminal of H2A. Abhisin showed the typical properties of AMPs comprising a higher proportion of hydrophobic residues (~27%) with 2.82 kcal/mol protein binding potential with a net positive charge (+13) (De Zoysa *et al.*, 2009). Abhisin had ~80% similarity with buforin I peptide of histone H2A. Moreover, De Zoysa *et al.* (2009) reported that abhisin (250 mg/mL) showed growth inhibition characteristics against both gram positive (*Listeria monocytogenes*), and negative (*Vibrio ichthyoenteri*) bacteria with stronger activity reported against gram positive bacteria. Furthermore, 50 µg/mL abhisin applied to different cancer cell lines show reduced THP-1 leukemia cancer cell viability by 25%, however it did not show any effect on normal kidney (Vero) cells. It's possible that abalone can be used for synthesizing different anti-microbial compounds, like abhisin, further demonstrating the therapeutic benefit of bioactive molecules from abalone.

Anti-cancer perspectives

Abalone has long been used as a valuable food source in East Asian countries. Although the therapeutic importance of abalone has been reported through *in vitro* and *in vivo* studies, there is little evidence about the potential anti-tumour effects of abalone visceral extract. A water extract of abalone has anti-tumour effects in mice (Uchida *et al.*, 1987) however the mechanisms involved in the anti-tumour effects have not been fully clarified (Lee *et al.*, 2010).

Specifically the investigation into the anti-cancer potential of abalone is focussed on anti-neoplastic substances (Shimizuan, 1983). These anti-neoplastic constituents can be further divided into two sub classes as direct and indirect on the basis of their functionality. Uchida *et al.* (1987) have suggested that the water extract obtained from the glycoprotein fractions of *H. discus hannai* have anti-tumor activity mediated through the host's responses. Recent literature also highlights the importance of abalone viscera as a source of chemo-preventive molecules with the potential against many type of cancers (Zhu *et al.*, 2009), especially from abalone water extract (Toyosawa *et al.,* 2007). However, the mode of action has not been fully clarified and is still a debatable issue.

In a recent study by Wang *et al.* (2014), three polysaccharides, AAP, AVAP I, and AVAP II, were isolated from abalone extract and assessed for their effects on proliferation of a human liver carcinoma cell line (HepG2). It was concluded that all the fractions stimulated cell growth albeit at different concentrations. Conversely, in a study by Lee *et al.* (2010) where abalone visceral extract was provided orally to mice with induced breast cancer (BALB/c, 4T1 mammary carcinoma), abalone extracts showed remarkable inhibition in tumor progression by suppressing the levels of Cox-2, EGF, VEGF, and FGF in primary tumors as well as metastatic lesions. Moreover, abalone visceral extract had the ability to proliferate and promote cytolytic activity of CD8+ T cells. The treatment of primary tumor cells with the abalone extract inhibited growth and tumor metastasis by modulating expression of cyclo-oxygenase-2 (COX-2), angiogenic factors and metalloproteinases. However, further research should be conducted to determine the molecules responsible for the anti-tumor activity of abalone visceral extract, and the mode of action involved.

Conclusions

Abalone has long been used as a valuable food source in Asian countries. Southeast Asia along with Japan and China are the major consumer regions for abalone. Abalone and other marine sources like shark cartilage and fish are being widely used as over the counter preventions and treatments for various life-threatening disorders. These marine sources are rich sources of structurally diverse bioactive components that may possess several nutraceutical and pharmaceutical attributes. Many studies have demonstrated abalone to be a source of valuable bioactives with anti-thrombotic, anti-coagulant, anti-inflammatory, anti-oxidant and anti-cancer activities. Although the therapeutic importance of abalone has been reported through *in vitro* and *in vivo* studies, little is known about the actual mechanisms involved, and mode of action of abalone bioactives.

 ACCEPTED MANUSCRIPT

REFERENCES

Ahmed, I., Majeed, A., and Powell, R.. (2007). Heparin induced thrombocytopenia: diagnosis and management update. *J Postgrad Med*. **83**: 575-582.

Alencar, J.W., Pessoa, J. C., Craveiro, A. A., Machado, M. I., and Matos, F. J. (1995). Chemical composition of shark cartilage. *Revista Brasileira de Ciências Farmacêuticas*. **76**: 7–8.

Ashie, I., and Pedersen, B.P. (2005). Method for extraction of glycosaminoglycan from animal tissue. *Google Patents*.

Birkemo, G.A., Luders, T., Andersen, O., Nes, I.F., and Nissen-Meyer, J. (2003). Hipposin, a histone-derived antimicrobial peptide in Atlantic halibut (Hippoglossus hippoglossus L.). *Biochimica et biophysica acta*. **1646**: 207-215.

Blunt, J.W., Copp, B.R., Munro, M.H.G., Northcote, P.T., and Prinsep, M.R. (2005). Marine natural products. *Natural Product Reports*. **22**: 15-61.

Bright, D. (2010). Nutraceutical composition and methods of use. *Google Patents*.

Bulet, P., Stocklin, R., and Menin, L. (2004). Anti-microbial peptides: from invertebrates to vertebrates. *Immunological reviews*. **198**: 169-184.

Byrne, M., Ho, M., Wong, E., Soars, N.A., Selvakumaraswamy, P., Shepard-Brennand, H., Dworjanyn, S.A., and Davis, A.R. (2010). Unshelled abalone and corrupted urchins: development of marine calcifiers in a changing ocean. Proceedings of the Royal Society of London: *Biological Sciences*. **278** (1716): 2376-2383.

Chen, H.-M., Muramoto, K., Yamauchi, F., and Nokihara, K. (1996). Antioxidant Activity of Designed Peptides Based on the Antioxidative Peptide Isolated from Digests of a Soybean Protein. *Journal of Agricultural and Food Chemistry*. **44**: 2619-2623.

Chen, S., Xue, C., Yin, L.a., Tang, Q., Yu, G., and Chai, W. (2011). Comparison of structures and anticoagulant activities of fucosylated chondroitin sulfates from different sea cucumbers. *Carbohydrate Polymers.* **83**: 688-696.

Cho, J.H., Sung, B.H., and Kim, S.C. (2009). Buforins: Histone H2A-derived antimicrobial peptides from toad stomach. Biochimica et Biophysica Acta (BBA) - *Biomembranes.* **1788**: 1564-1569.

Cochet, M., Brown, M., Kube, P., Fluckiger, M., Elliott, N., and Delahunty, C. (2013). Sensory and Physicochemical Assessment of Wild And Aquacultured Green and Black Lip Abalone (*Haliotis laevigataandHaliotis rubra*). *Journal of Shellfish Research.* **32**: 81-88.

Cook, P.A. (2010) Developments and Trends in Worldwide Abalone Farming and Implications for the European Industry. *Aquaculture Europe*. **30**: 5-8.

Cook, P.A. (2014) The Worldwide Abalone Industry. *Modern Economy*. **5**: 1181-1186.

Cruz, J., Ortiz, C., Guzman, F., Fernandez-Lafuente, R., and Torres, R. (2014). Antimicrobial peptides: promising compounds against pathogenic microorganisms. *Current medicinal chemistry.* **21**: 2299-2321.

De Zoysa, M., Nikapitiya, C., Whang, I., Lee, J.-S., Lee, J., 2009. Abhisin: A potential antimicrobial peptide derived from histone H2A of disk abalone (*Haliotis discus discus*). *Fish & shellfish immunology*. **27**: 639-646.

Dechant, J.E., Baxter, G.M., Frisbie, D.D., Trotter, G.W., and McIlwraith, C.W. (2005). Effects of glucosamine hydrochloride and chondroitin sulphate, alone and in combination, on normal and interleukin-1 conditioned equine articular cartilage explant metabolism. *Equine veterinary journal*. **37**: 227-231.

Dong, X.-P., Zhu, B.-W., Zhao, H.-X., Zhou, D.-Y., Wu, H.-T., Yang, J.-F., Li, D.-M., and Murata, Y. (2010). Preparation and in vitro antioxidant activity of enzymatic hydrolysates from oyster (*Crassostrea talienwhannensis*) meat. *International Journal of Food Science & Technology* **45**: 978-984.

Estes, J.A., Lindberg D.R., and C. Wray. (2005). Evolution of large body size in abalones (Haliotis): patterns and implications. *Paleobiology*. **31**(4): 591–606.

Franchini, M., Mannucci, P.M., 2009. A new era for anticoagulants. European Journal of Internal Medicine 20, 562-568.

Gadi, D., Bnouham, M., Aziz, M., Ziyyat, A., Legssyer, A., Legrand, C., Lafeve, F.F., and Mekhfi, H. (2009). Parsley extract inhibits in vitro and ex vivo platelet aggregation and prolongs bleeding time in rats. *Journal of Ethnopharmacology*. **125**: 170-174.

Gordon, H.R. and Cook, P.A. (2013) World Abalone Supply, Markets and Pricing: 2011 Update. *Journal of Shellfish Research*. **32**: 5-7.

Guerard, F., Sumaya-Martinez, M.T., Laroque, D., Chabeaud, A., and Dufossé, L. (2007). Optimization of free radical scavenging activity by response surface methodology in the hydrolysis of shrimp processing discards. *Process Biochemistry*. **42**: 1486-1491.

Guo, H., Kouzuma, Y., and Yonekura, M. (2009). Structures and properties of antioxidative peptides derived from royal jelly protein. *Food Chemistry*. **113**: 238-245.

Harnedy, P.A., and FitzGerald, R.J. (2012). Bioactive peptides from marine processing waste and shellfish: A review. *Journal of Functional Foods.* **4**: 6-24.

Hirsch, J.G. (1958). Bactericidal action of Histone. *The Journal of Experimental Medicine*. **108**: 925-944.

Jang, J.Y., Kim, T.S., Cai, J., Kim, J., Kim, Y., Shin, K., Kim, K.S., Park, S.K., Lee, S.P., Choi, E.K., Rhee, M.H., and Kim, Y.B. (2013). Nattokinase improves blood flow by inhibiting platelet aggregation and thrombus formation. *Laboratory animal research*. **29**: 221-225.

Je, J.-Y., Lee, K.-H., Lee, M.H., Ahn, C.-B. (2009). Antioxidant and antihypertensive protein hydrolysates produced from tuna liver by enzymatic hydrolysis. *Food Research International*. **42**: 1266-1272.

Kim, H.S., Cho, J.H., Park, H.W., Yoon, H., Kim, M.S., and Kim, S.C. (2002). Endotoxin-neutralizing antimicrobial proteins of the human placenta. *J Immunol*. **168**: 2356-2364.

Kim, M.-S., Lee, K.-A. (2006). Antithrombotic activity of methanolic extract of Umbilicaria esculenta. *Journal of Ethnopharmacology*. **105**: 342-345.

Kim, S.K., Pallela, R., 2012. Medicinal foods from marine animals: current status and prospects. *Advances in food and nutrition research*. **65**: 1-9.

Korpov, K.A., Haaker, P.L., Taniguchi, I.K. and Rogers-Bennett, L. (2000) Serial Depletion and Collapse of the Californian Abalone (Haliotis spp.) Fishery. *Canadian Special Publication on Fisheries and Aquatic Science*. **130**: 11-24.

Latuihamalloa, M., Irianab D., and Apituleya, D. (2015). Amino Acid and Fatty Acid of Abalone Haliotis Squamata Cultured in Different Aquaculture Systems. P*rocedia Food Science*. **3**: 174–181.

Lee, C.G., Kwon, H.K., Ryu, J., Kang, S., Im, C.R., II Kim, J., and Im, S.H. (2010). Abalone visceral extract inhibit tumor growth and metastasis by modulating Cox-2 levels and CD8+ T cell activity. *BMC complementary and alternative medicine*. **10**: 60.

Leopold, J.A. and Loscalzo, J. (2009). Oxidative risk for atherothrombotic cardiovascular disease. *Free Radical Biology and Medicine*. **47**: 1673-1706.

Li, C., Song, L., Zhao, J., Zhu, L., Zou, H., Zhang, H., Wang, H., and Cai, Z., (2007). Preliminary study on a potential antibacterial peptide derived from histone H2A in hemocytes of scallop Chlamys farreri. *Fish & shellfish immunology*. **22**: 663-672.

Li, G., Chen, S., Wang, Y., Xue, Y., Chang, Y., Li, Z., Wang, J., and Xue, C. (2011). A novel glycosaminoglycan-like polysaccharide from abalone Haliotis discus hannai Ino: Purification, structure identification and anticoagulant activity. *International journal of biological macromolecules*. **49**: 1160-1166.

Li, J., Tong, T., Ko, D.O., and Kang, S.G. (2013). Antithrombotic potential of extracts from abalone, Haliotis Discus Hannai Ino: In vitro and animal studies. *Food Science and Biotechnology*. **22**: 471-476.

Li, X.X., Han, L.J., and Chen, L.J. (2008). In vitro antioxidant activity of protein hydrolysates prepared from corn gluten meal. *Journal of the Science of Food and Agriculture*. **88**: 1660-1666.

Lin, A.Y.M., and Meyers, M.A. (2009). Interfacial shear strength in abalone nacre. *Journal of the Mechanical Behavior of Biomedical Materials*. **2**: 607-612.

Lin, L., and Li, B.F. (2006). Radical scavenging properties of protein hydrolysates from Jumbo flying squid (*Dosidicus eschrichitii Steenstrup*) skin gelatin. *Journal of the Science of Food and Agriculture*. **86**: 2290-2295.

Lou, Q.M., Yu-Ming W. and Xue. CH. (2012). Lipid and fatty acid composition of two species of Abalone, haliotis discus hannai ino and haliotis Diversicolor Reeve. *Journal of Food Biochemistry*. **37**: 296–301.

Mamelona, J., Saint-Louis, R., and Pelletier, É. (2010). Nutritional composition and antioxidant properties of protein hydrolysates prepared from echinoderm byproducts. *International Journal of Food Science & Technology*. **45**: 147-154.

Murphy, K.J., Mann, N.J., and Sinclair, A.J. (2003). Fatty acid and sterol composition of frozen and freeze-dried New Zealand Green Lipped Mussel (Perna canaliculus) from three sites in New Zealand. *Asia Pacific journal of clinical nutrition*. **12**: 50-60.

Oakes, F.R., and Ponte, R.D. (1996). The abalone market: Opportunities for cultured abalone. *Aquaculture*. **140**: 187-195.

Park, C. (2014) Pers. Comm.

Park, C.B., Kim, M.S., and Kim, S.C. (1996). A novel antimicrobial peptide from Bufo bufo gargarizans. *Biochemical and biophysical research communications.* **218**: 408-413.

Pearson, W., Orth, M.W., Karrow, N.A., Maclusky, N.J. and Lindinger, M.I. (2007). Anti-inflammatory and chondroprotective effects of nutraceuticals from Sasha's Blend in a cartilage explant model of inflammation. *Molecular nutrition & food research*. **51**: 1020-1030.

Peñta-Ramos, E.A. and Xiong, Y.L. (2002). Antioxidant Activity of Soy Protein Hydrolysates in a Liposomal System. *Journal of Food Science*. **67**: 2952-2956.

Pham-Huy, L.A., He, H., and Pham-Huy, C. (2008). Free Radicals, Antioxidants in Disease and Health. *International Journal of Biomedical Science*: *IJBS*. **4**: 89-96.

Qi, H., Zhang, Q., Zhao, T., Chen, R., Zhang, H., Niu, X., and Li, Z. (2005). Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from Ulva pertusa (Chlorophyta) in vitro. *International journal of biological macromolecules*. **37**: 195-199.

Reddy, K.V.R., Yedery, R.D., Aranha, C. (2004). Antimicrobial peptides: premises and promises. *International Journal of Antimicrobial Agents*. **24**: 536-547.

Richards, R.C., O'Neil, D.B., Thibault, P., Ewart, K.V. (2001). Histone H1: an antimicrobial protein of Atlantic salmon (Salmo salar). *Biochemical and biophysical research communications*. **284**: 549-555.

Saiga, A., Tanabe, S., and Nishimura, T. (2003). Antioxidant Activity of Peptides Obtained from Porcine Myofibrillar Proteins by Protease Treatment. *Journal of Agricultural and Food Chemistry*. **51**: 3661-3667.

She, Z.G., Hu, G.P., and Wu, Y.W. (2002). Study on the methanolysis of the sulphated polysaccharide Hal-A from Haliotis diverisicolor Reeve. *Chineese Journal of Organic Chemistry*. **22**: 367–370

Shimizu, Y., and Kamiya, H. (1983). Bioactive Marine Biopolymers. In: Scheuer, P.J. (Ed.), Marine Natural Products. Academic Press, pp. 391-427.

Sikka, P., and Bindra, V.K. (2010). Newer antithrombotic drugs. *Indian Journal of Critical Care Medicine*. **14**: 188-195.

Su, X.Q., Antonas, K.N., and Li, D. (2004). Comparison of n-3 polyunsaturated fatty acid contents of wild and cultured Australian abalone. *International journal of food sciences and nutrition*. **55**: 149-154.

Toyosawa, T., Sasaki, M., Y. Kato, H. Yamada, and Terada S. (2007). Development of A Novel Serum-free Cryopreservative Solution. *Cell Technology for Cell Products*. **3**: 225–231.

Tsiapali, E., Whaley, S., Kalbfleisch, J., Ensley, H.E., Browder, I.W., and Williams, D.L. (2001). Glucans exhibit weak antioxidant activity, but stimulate macrophage free radical activity. *Free Radical Biology and Medicine*. **30**: 393-402.

Uchida, H., Sasaki, T., Uchida, N.A., Takasuka, N., Endo, Y., and Kamiya, H. (1987). Oncostatic and immunomodulatory effects of a glycoprotein fraction from water extract of abalone, Haliotis discus hannai. *Cancer Immunol Immunother*. **24**: 207-212.

Wang, J., Zhang, Q., Zhang, Z., and Li, Z. (2008). Antioxidant activity of sulfated polysaccharide fractions extracted from Laminaria japonica. *International journal of biological macromolecules.* **42**: 127-132.

Wang, Y.M., Wu, F.J., Du, L., Li, G.Y., Takahashi, K., Xue, Y., and Xue, C.H. (2014). Effects of polysaccharides from abalone (Haliotis discus hannai Ino) on HepG2 cell proliferation. *International journal of biological macromolecules*. **66**: 354-361.

Wu, H.C., Chen, H.M., and Shiau, C.Y. (2003). Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomber austriasicus). *Food Research International*. **36**: 949-957.

Xing, R., Liu, S., Guo, Z., Yu, H., Wang, P., Li, C., Li, Z., and Li, P. (2005). Relevance of molecular weight of chitosan and its derivatives and their antioxidant activities in vitro. *Bioorganic & medicinal chemistry*. **13**: 1573-1577.

You, W.W. (2014) Pers. Comm.

Zhang, Q., Yu, P., Li, Z., Zhang, H., Xu, Z., and Li, P. (2003). Antioxidant activities of sulfated polysaccharide fractions from Porphyra haitanesis. *Journal of Applied Phycology*. **15**: 305-310.

Zhao, X., Xue, C.H., and Li, B.F. (2008). Study of antioxidant activities of sulfated polysaccharides from Laminaria japonica. *Journal of Applied Phycology*. **20**: 431-436.

Zhu, B.W., Wang, L.S., Zhou, D.Y., Li, D.M., Sun, L.M., Yang, J.F., Wu, H.T., Zhou, X.Q., and Tada, M. (2008). Antioxidant activity of sulphated polysaccharide conjugates from abalone (Haliotis discus hannai Ino). *European Food Research and Technology*. **227**: 1663-1668.

Zhu, B.W., Zhou, D.y., Li, T., Yan, S., Yang, J.F., Li, D.M., Dong, X.P., and Murata, Y. (2010). Chemical composition and free radical scavenging activities of a sulphated polysaccharide extracted from abalone gonad (*Haliotis Discus Hannai Ino*). *Food Chemistry*. **121**: 712-718.

Zhu, B.W., Zhou, D.Y., Yang, J.F., Li, D.M., Yin, H.L., and Tada, M. (2009). A neutral polysaccharide from the abalone pleopod, Haliotis discus hannai Ino. *European Food Research and Technology*. **228**: 591-595.