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The antioxidant potential of the New Zealand surf clams

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ABSTRACT

The antioxidant action of three New Zealand surf clams was evaluated for the first time. Aqueous (*cd*) and ethanolic extracts from Diamond shell – *Crassula aequilatera,* Storm shell – *Mactra murchisoni,* and Tua tua – *Paphies donacina* were studied for their antioxidant potentials using two *in vitro* assays. The ethanolic extracts were further fractioned into four parts; petroleum ether (*pe*), ethyl acetate (*ea*), n-butanol (*nb*), and the final aqueous fraction (*w*). Comparing among all fractions tested, the *ea* fraction of *P. donacina* showed the strongest free radical scavenging power, with a radical scavenging activity of 76.14% at 20 μg/mL. The *ea* fraction of *C. aequilatera* had the highest copper reducing activity with an absorbance of 1.596 at 20 μg/mL. Results from this study suggest that some bioactive compounds with significant antioxidant effects may exist in the New Zealand surf clams, and could potentially reduce oxidative stress to deliver health benefits or to produce functional foods.

1. Introduction

The wide diversity of marine organisms contained in the world's oceans offers a rich source of natural products (Wijesekara, Pangestuti, & Kim, 2011) and, as such, is regarded as the largest remaining reservoir of natural molecules to be evaluated for drug activity. Marine-derived bioactive peptides have been shown to possess many physiological functions including antihypertensive action or angiotensin-I-converting enzyme (ACE) inhibition (Je, Park, Byun, Jung, & Kim, 2005). Extensive research has shown the usefulness of the surf clams, both nutritionally and medically. Clams are important recreational and commercial resource in many countries (McLachlan et al., 1996).

Individuals are exposed to oxidants, both endogenous and exogenous, since the moment of conception. Reactive oxygen and nitrogen species are generated *in vivo* and cause damage to DNA, lipids, proteins and other bio-molecules (Halliwell, 1996). DNA damage can occur, for example, when hydroxyl radical, a highly reactive oxygen species (ROS), reacts with DNA bases by adding to double bonds of DNA bases and by abstracting an H atom from the methyl group of thymine and each of the C–H bonds of 2́-deoxyribose (Cooke, Evans, Dizdaroglu, & Lunec, 2003). Antioxidants are, therefore, needed to prevent the formation and to oppose the actions of oxidants. Antioxidants can be consumed in the diet and are synthesised *in vivo* in humans. Synthetic antioxidants have been invented, tested for acute toxicity and proposed as an addition to naturally occurring antioxidants. Unfortunately, recent reports have revealed that these synthetic antioxidants may be associated with toxic and carcinogenic effects (Zhang et al., 2010). As a part of society's demand for a better lifestyle and increased longevity, consumers have developed an increasing interest towards consuming 'nutraceuticals' and functional foods rich in natural bioactive compounds (Fung, Hamid, & Lu, 2013). In an attempt to address this interest, there has been a dramatic increase in the number of investigations aimed at identifying dietary compounds from natural sources which may be effective in preventing diseases caused by oxidative damage (Tierney, Croft, & Hayes, 2010).

Research on surf clams have been carried out in different parts of the world, as it is known to exhibit a variety of biological activities. It has been reported that the extracts of certain molluscs like abalone, oyster and clams have shown both antibacterial and antiviral activities (Lin-rui, 2012). An extract from the edible clam *Mercenaria mercenaria* prevented the development of transplanted

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sarcoma 180 and Krebs-2-ascites tumors in Swiss mice (Schmeer & Huala, 1965). Chang, Li, Sun, Yang, and Sun (2012) reported that polysaccharides from the Chinese surf clam (*Mactra chinensis*) have scavenging activity against superoxide anion and hydroxyl free radicals in a concentration-dependent way. Furthermore, the aqueous and alcoholic extracts from *Mactra veneriformis* showed antioxidant potentials using various *in vitro* assays (Luan, Wang, Wu, Jin, & Ji, 2011). It has also been reported to show detoxification, cyst elimination, protecting the body cells from mutation and decrepitude (Leng, Liu, & Chen, 2005), as well as decreasing blood sugar and lipid levels. Surf clam extracts have also shown to have hepatoprotective, antihypertensive, antineoplastic, antimicrobial, hypocholesterolemic, and antiulcer effects (Lin, Tsai, Hung, & Pan, 2010; Ramasamy & Balasubramanian, 2012).

Surf clams, like most seafood, contain hydrophilic or lipophilic antioxidant compounds, such as carotenoids, free amino acids and polyunsaturated fatty acids. These antioxidant compounds are most likely responsible for the therapeutic activities of surf clams (Luan et al., 2011).

There are seven main species of surf clams in New Zealand: Tua tua – *Paphies donacina* (PDO), Diamond shell – *C. aequilatera* (SAE), Trough shell – *Mactra discors* (MDI), Storm shell – *Mactra murchisoni* (MMI), Moon shell – *Dosinia anus* (DAN), Fine dosinia – *Dosinia subrosea* (DSU) and Frilled venus – *Bassina yatei* (BYA). Four out of those seven are given more attention: PDO, SAE, MMI and DAN (Ministry for Primary Industries., 2012). None of the New Zealand surf clams has been studied for their antioxidant potential despite being a popular export to Asian seafood market. The present study was undertaken to evaluate, for the first time, the antioxidant potentials of three most harvested and exported New Zealand surf clam species, Diamond shell (*C. aequilatera*), Storm shell (*M. murchisoni*) and Tua tua (*P. donacina*).

2. Materials and methods

2.1. Clam collection and maintenance

Clams were obtained from Cloudy Bay Clams Ltd (Fig. 1). All water used in this process is USFDA approved and regularly monitored to maintain the highest possible quality. Prior to export, the water is cooled to below 10 °C as required by New Zealand Food Safety Authority. The clams are packed into Polystyrene boxes (to regulate temperature) and freighted dry to the end user – Auckland University of Technology (AUT).

2.2. Chemicals and reagents

Methanol, n-Butanol, Petroleum spirit and Ethyl acetate were purchased from Global Science (Auckland, New Zealand) while ethanol was purchased from Thermofisher (Auckland, New Zealand) and were all of HPLC grade. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) and Neocuproine ⩾98%, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock cultures of DPPH were prepared in methanol. All other reagents, solvents and chemicals used were obtained from AUT Applied Sciences laboratory and were of analytical grade.

2.3. Preparation of extracts and fractions

Frozen clams were prepared. The flesh was taken out by shucking the shells with a knife. The clam juice was drained and the frozen flesh was thawed at room temperature. The flesh was laid onto plastic trays and then it was dried in a hot air oven at 60 °C to constant weight for 4 days. Flesh was turned two to three times daily to prevent it from sticking.

Dried sample was then pulverised at low speed in a laboratory blender (model: 800 W Sunbeam Multiblender-Pro blender). Milled clam flesh was transferred into glassware, weighed and recorded. Sample was stored in the dark at room temperature until when it was needed.

All clam extraction and measurements were carried out in dim light to reduce any possibility of oxidation. Extraction method from a previous study was adopted (Luan et al., 2011).

2.3.1. Aqueous extraction

Dried, pulverised clam sample was extracted in distilled water and stirred constantly using a magnetic bar at room temperature for an hour. At the end of one hour the solvent was filtered out using a Whatman No. 1 (Diameter: 9 cm, Pore size: 11 μm) filter paper (Thermofisher, New Zealand) to remove all solids. This procedure was repeated at least 7 times and all supernatant was combined. Solvent was concentrated and stored at −20 °C until use. The extract was named '*cd*'.

2.3.2. Solvent extraction

Dried, pulverised flesh was extracted several times (one hour per operation) with ethanol absolute and stirred using a magnetic bar at room temperature for an hour. This process was repeated until the solvent was colourless. All solvents from each extraction process were combined and this was filtered using a Whatman No.

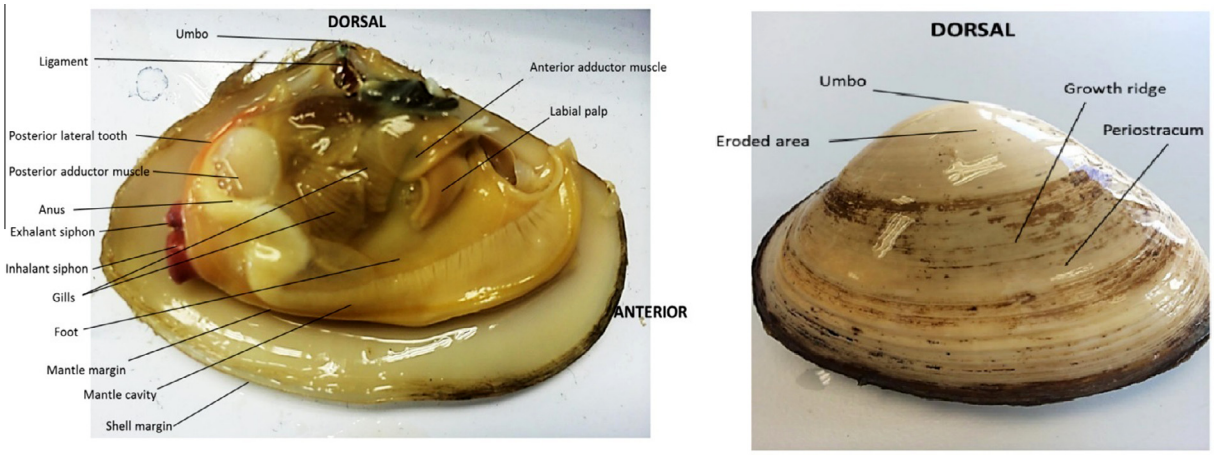

Fig. 1. New Zealand surf clam structure.