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Mineral Composition of the Cockle (*Anadara granosa*) Shells of West Coast of Peninsular Malaysia and It's Potential as Biomaterial for Use in Bone Repair

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Abstract: The study was conducted to determine the composition of mineral content of cockle (*Anadara granosa*) shells from 3 major cockle cultivation of West Coast of Malaysia. Three samples of cockle shells from three different sources were evaluated to determine the content of 12 macro-and micro-elements (Calcium (Ca), Carbon (C), Magnesium (Mg), Sodium (Na), Phosphorus (P), Potassium (K), Ferum (Fe), Copper (Cu), Nickel (Ni), Zink (Zn), Boron and Silica (Si)). For convenience and ease of reference, Ca and C were combine into one unit (Calcium Carbonate, CaC) while Mg, Na, P and K was evaluated individually and Fe, Cu, Ni, Zn, B and Si were evaluated as one group (others). Analysis of elements content was done using inductively Coupled plasma, Auto Analyzer, an Atomic Absorption Spectrophotometer and Carbon Analyzer. Results in this study revealed that the mineral compositions of cockle shells from 3 different sources in West Coast of Peninsular Malaysia were consistence almost in all the samples. The percentage of CaC comprises of more than 98.7% of the total minerals content of the cockle shells of the 3 sources. About 1.3 % of the composition are comprises of Mg, Na, P, K and others (Fe, Cu, Ni, B, Zn and Si).Overall, the minerals composition of cockle shells of West Coast of Peninsular Malaysia are as followed: CaC 98.7 %, Mg 0.05%, Na 0.9%, p 0.02 and others 0.2%.

Key words: Cockle (*Anadara granosa*) shells, mineral compositions, biomaterial, corals

INTRODUCTION

Anadara granosa or locally known as 'kerang' in Malaysia is a bivalve belonging to the family *Arcidae*. It is indigenous to the intertidal mudflats bordering the coastal regions of many South East Asian countries particularly Indonesia, Malaysia and Thailand. In South East Asian region, *Anadara granosa* is also an important protein source. *Anadara granosa* is by far, the most important species cultured in Malaysia. In Malaysia, cockle aquaculture occupies an area of 4272.01 ha, with the annual output of approximately 80000 t (Annual Fisheries Statistics, 2001). Major cockle cultivations in Malaysia are in Juru, Penang, Matang, Perak and Kuala Selangor, Selangor.

A number of studies have been done on the consumable aspect of cockle, but very limited study has been conducted on the cockle shell itself. Usually these shells are removed and thrown away after getting the mussels. Recent report study by Zuki *et al*. (2004) revealed that the mineral composition of *Anadara granosa* is almost similar to that of coral. Thus, the finding suggests the possibility of using cockle shell as alternative biomaterials for bone substitute in managing bone defects. However, further evaluation needs to be carried out to evaluate the consistency of mineral composition of cockle shells in different places. Thus, the present study was undertaken to determine the mineral composition of cockle shell (*Anadara granosa)* from 3 major cockle cultivation areas in West Coast of Peninsular Malaysia.

MATERIALS AND METHODS

Collection of samples: Cockle (*Anadara granosa)* or kerang ware collected from three different places along theWest Coast of Peninsular Malaysia namely KualaSelangor, Selangor, Klebang, Malacca and Kuala Juru, Penang.

Samples preparation: The cockles were washed and scrubbed free of dirt. Then, they were boiled for 5 min and

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cooled at room temperature before the contents of the cockles were removed leaving behind the shells. The shells were washed thoroughly with clean water and then oven dried at 50°C for three days. The cockles were ground using Blendor ® until they turned into powder form. The powder from the shells was then, sieved at 850 μm after which they were packed into McCartney bottles and sterilized for one hour in the oven at 105°C. The dry-sterilized cockle powder was then subjected to biochemical test.

For Carbon (C) detection, approximately 2g samples were taken directly from the prepared samples. For detection of other elements (Ca, Mg, Fe, Cu, Ni, Zn, B, Si, Na, P and K), the samples initially has to go through the dry ashing process prior to evaluations. Approximately 2g of samples were weighed in porcelain dish and placed in a muffle furnace. The temperature was increased gradually to 300°C and maintained for 1 h until the smoke disappeared. The temperature was further increased to 500°C and maintained for 5 h until a whitish or grayish ash has formed, after which samples were taken out and left to be cooled at room temperature. Once the ash has cooled, a pipette was used to moisturize the ashes with 2 mL of distilled water. It was then added with 2 mL of concentrated hydrochloric acid and steamed on a hot plate. Subsequently, 10 mL of nitric acid were added in which they were then dissolved with water for 1 h in a water bath at a temperature of 100°C. These dissolved materials were then placed in a 100 mL volumetric beaker. The porcelain dish was rinsed several times to ensure all dissolved materials were totally collected into the volumetric beaker in which distilled water was topped up to 100 mL mark. These solutions were then shake and filtered through no. 2 filter paper.

Samples analysis: Carbon content was detected by using Carbon Analyzer. For the Ca, Mg, Fe, Cu, Ni, Zn and Si, the aliquot prepared was utilized for analysis by using Inductively Coupled Plasma (ICP) machine. The elements content of P and K were obtained through an Auto Analyzer machine while the Atomic Absorption Spectrophotometer machine was used for analyzing the Na. Three groups of samples (Kuala Selangor, Selangor; Klebang, Malacca; Kuala Juru, Penang) each with 3 replications each were arranged in a Completely Randomized Design. The mineral content was expressed in Parts Per Million (PPM) and converted to percentage for ease and convenience in comparisons with other studies. The Ca and C element were combined and expressed as one unit. Whereas the elements of Fe, Cu, Ni, Zn, B and Si were combined together and termed as 'others' based on the fact that their compositions in the samples were too small to be counted individually.

Statistical analysis: All the data were analyzed using one way ANOVA.

RESULTS

The study revealed that the composition of CaC, Na and K in the cockle shells collected from 3 different areas was not significantly different. The composition of CaC made up about 98.7% of the total mineral composition in all the samples (Fig. 1). The remaining 1.3% comprised of Mg, Na, P, K and others (Fe, Cu, Ni, B, Zn and Si) (Fig. 2 and 3). The Mg content of cockle shells taken from Malacca (0.0437 ± 0.0020) showed significant different ($p<0.05$) as compared to those from Penang (0.0476 ± 0.0006) and Kuala Selangor (0.0477 ± 0.0014). Meanwhile, the percentage of P of cockle shells shows significant different ($p<0.05$) between the three areas (Table 1). The composition of other minerals (Fe, Cu, Ni, B and Zn) showed no significant different ($p>0.05$) between the three areas except for Si (Table 2). The cockle shells from 3 places contained around 0.15% iron and small quantity of Cu, Ni, B and Zn (< 0.01%). Overall, the minerals compositions of cockle shells of West Coast of Peninsular Malaysia were as followed: CaC 98.7%, Mg 0.05%, Na 0.9%, p 0.02 and others 0.2%.

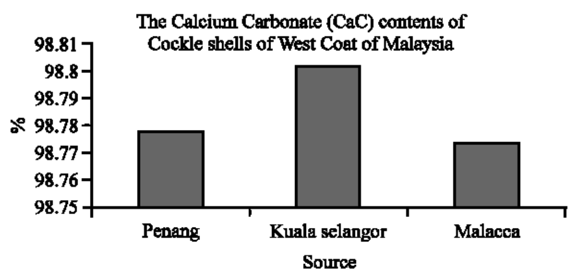

Fig. 1: Calcium and Carbon (CaC) composition of cockle shells from Kuala Juru, Penang, Kuala Selangor, Selangor and Klebang, Malacca

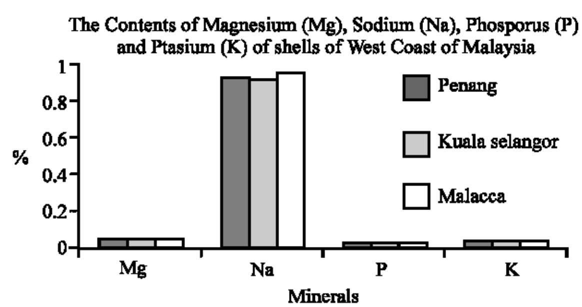

Fig. 2: Mg, Na, P and K composition of cockle shells from Kuala Juru, Penang, Kuala Selangor, Selangor and Klebang, Malacca

Table 1: The content (percentage) of major minerals of cockle shells of West Coast of Malaysia

Sources/Minerals	Ca C	Mg	Na	P	K	Others
Penang	98.7770±0.0229[a]	0.0476±0.0006[b]	0.9192±0.0344[a]	0.0183±0.0003[c]	0.0398±0.0018[a]	0.1981±0.1051[a]
Kuala selangor	98.8007±0.1463[a]	0.0477±0.0014[b]	0.9076±0.1410[a]	0.0176±0.0002[a]	0.0392±0.0015[a]	0.1871±0.1067[a]
Malacca	98.7834±0.0612[a]	0.0437±0.0020[a]	0.9386±0.0473[a]	0.0178±0.0004[bc]	0.0380±0.0016[a]	0.1894±0.1830[a]

[abc]means with different superscript within a column were significantly different at $p<0.05$. n = 3

Table 2: The content (percentage) of other minerals of cockle shells of West Coast of Malaysia

Sources/Minerals	Fe	Cu	Ni	Zn	B	Si
Penang	0.15348±0.003597[a]	0.00188±0.00005[a]	0.00431±0.00058[a]	0.00120±0.00008[a]	0.00127±0.00012[a]	0.03600±0.00721[c]
Kuala selangor	0.14985±0.008661[a]	0.00190±0.00020[a]	0.00405±0.00013[a]	0.00127±0.00026[a]	0.00123±0.00005[a]	0.02884±0.00177[ab]
Malacca	0.15766±0.018647[a]	0.00185±0.00030[a]	0.00428±0.00076[a]	0.00125±0.0008[a]	0.00120±0.000001[a]	0.02312±0.00050[a]

[abc]means with different superscript within a column were significantly different at $p<0.05$. n = 3

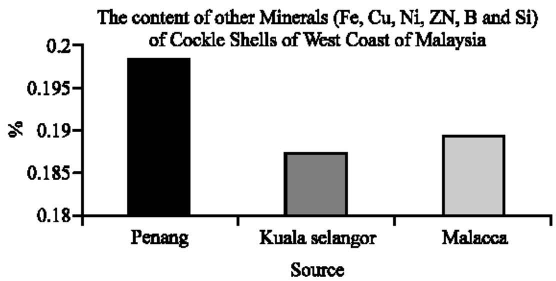

Fig. 3: Other elements composition of cockle shells from Kuala Juru, Penang, Kuala Selangor, Selangor and Klebang, Malacca

DISCUSSION

Corals has been used as biomaterials for orthopedic applications especially the mandibulofacial surgery. Corals minerals composition comprises of>97% of Ca, 0.05-0.2 % Mg, Na<1% and p <0.05% (Demers *et al*., 2002). The inorganic content of bone comprised primarily of calcium phosphate and calcium carbonate, with small quantities of magnesium, fluoride and sodium (Kalfas, 2002). The weight of dry bone is made up of 65-75% of inorganic calcium phosphate and 30-35% of organic matrix of fibrous protein and collagen (Copenhaver *et al*., 1978; Recker, 1992; Muschler *et al*., 1990). From these data, its can be conclude that minerals composition is one of the important criteria to look for in developing an alternative bone substite.

The results of this study is in accordance with the previous study by Zuki *et al*. (2004) who conducted a comparative study of mineral composition of the cockle shells (*Anadara granosa*), hard clamp shells (*Meretrix meretrix*) and corals (*Porites* sp.). The only difference is the percentage of Mg which was found to be higher (0.20%) in their study, while in this study the composition of Mg is less than 0.1%. Thus, it is suggested that cockle shell has potential to be used as biomaterial for bone repair based on their minerals composition.

Bone grafts prepared from corals have been shown to be useful in the treatment of bone defects (Guillemin, *et al*., 1987; Vuola, 2001). The structure of the commonly used coral (*Porites* sp.) is similar to that of cancellous bone and its initial mechanical properties resemble those of bone (Holmes, 1979). The exoskeleton of coral contained high calcium carbonate and was shown to be biocompatible, osteoconductive and biodegradable at variable rates depending on the exoskeleton porosity, the implantation site and the species. Although not osteoinductive or osteogenic, coral grafts act as an adequate carrier for growth factors and allow cell attachment, growth, spreading and differentiation (Chiroff *et al*., 1975). When applied appropriately and when selected to match the resorption rate with the bone formation rate of the implantation site, natural coral exoskeletons have been found to be an impressive bone graft substitutes (Demers *et al*., 2002). However, the use of coral exoskeleton in creating a new issue since coral is an endangered species. This will disturb the ecology of corals reef and may lead to extinction. Thus, the principles of applications of coral for bone substitute might be applied to cockle and further study is needed to proove it.

Anadara granosa is a bivalve and can tolerate harsh environment such as water pollution in its environment. From previous studies, they were known to have ability to accumulate metals (Darracot and Watling, 1975). Study by Noordin (1994) revealed that cockles commonly consumed by people in South East-Asia particularly in Malaysia, contain trace minerals which are useful and toxic in nature. The level of inorganic pollutants has been proved to affect the size of the cockle's body and weight (Noordin, 1994) and study by Alzieu *et al*. (1989) revealed that toxic has an effect on bivalve in the form of shells deformation. Beside that availability of foods and ambient temperature also has an impact to growth and body mass of bivalves (Beukema, 1974a, 1994b). In Peninsular Malaysia, cockles are reared only at the West Coast (Malacca Straits) and the areas are facing a great pollution problem (DOE, 1994). For example, the Juru area in Penang is one of the most polluted coastal areas in Malaysia (Yahya and Leong, 1987). The discharge of domestic waste and industrial effluent from a man-made canal have lead to the decline in fisheries and affect the growth of cockles and those reared close to the discharge point suffered high mortality (Din and Ahmad, 1995).

Due to this issue it is important to further evaluate the composition of the minerals of the cockle shells from a few places to identify if the differences of minerals composition of cockle shells exist due to demographic factor. Based on the results of this study, there was no significant difference between the minerals composition of cockle shells between the three areas. Thus, this may suggest that the mineral composition of cockle shells were not influenced by the environment and water quality.

CONCLUSION

The minerals profile of cockle shells from 3 different places of West Coast of Peninsular Malaysia revealed uniform percentage of all the minerals composition evaluated. The percentage of CaC comprises of more than 98% of the cockle shells content and this suggests that the cockle shell has potential to be a material for the development of biomaterial for orthopedic applications.

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