CHAPTER 1: INTRODUCTION

1.1 SEAWEEDS

Seaweeds are macroscopic and multicellular algae which are found on the shallow fringes of the ocean and can be classified into three broad groups based on pigmentation. There are about 1200 species of Chlorophyta, 1750 species of Phaeophyta and 6000 species of Rhodophyta. Brown algae are usually large, and range from the giant kelp that is often 20 m long, to thick, leather-like seaweeds from 2-4 m long, to smaller species 30-60 cm long. Green algae and red algae are smaller in size, generally ranging from a few centimetres to about a metre in length (McHugh, 2003). A number of algae are plant-like in their appearances; having stem, root and leaf analogs in the form of anchoring holdfasts, stipes and blades. The similarities in macroscopic structure between seaweeds and land plants are attributed to parallel evolution (Graham and Wilcox, 2000).

1.2 USES OF SEAWEEDS

Macroalgae are ecologically and biogeochemically important in modern ecosystems. The biomass of seaweeds is useful for animal and human nutrition, various industrial applications, bioremediation, and bioconversion for energy. Tseng (1981) reported that 107 genera and 493 species of seaweeds have economic value. From more than 20,000 species of algae, 60 have been explored and used commercially for the production of phycolloids, raw materials for feed and fertilizer, fiber diet for direct human consumption, cosmetics and in medicinal or pharmaceutical use. Seaweed utilization is possibly as old as humankind, and current global usage of seaweeds supports a multi-billion dollar industry. The total annual production of food products for human consumption from seaweeds is estimated at almost US$ 5.5 to 6 billion (McHugh, 2003).

Seaweeds contain a notable amount of proteins, vitamins, minerals and trace elements. Hence, the demand of seaweeds for production of health food products has increased dramatically. Mineral contents of seaweeds can account for up to 36% of dry mass (Dharmananda, 2002). Some of the major constituents in seaweeds are:

Iodine: The highest iodine content is found in the brown algae with dry kelp having iodine ranging from 1500-8000 parts per million (ppm) dry weight (DW). Red and green algae have lower contents, about 100- 300 ppm DW, but still higher than in land plants.

Calcium: Apart from iodine, one of the richest plant sources of calcium is seaweed, but its calcium content relative to dietary requirements is much lower when compared to iodine.

Protein: The protein fraction of seaweeds is variable according to species and seasonal variation. Red and green seaweeds (10-47% DW) contain higher protein content than brown seaweeds (3-15% DW). The red seaweed Porphyra tentera was reported to contain high protein levels (47% DW) (Fleurence, 1999).

Vitamins: In general, algae have high amount of vitamin A and E. The content of vitamin C and Niacin is about the same as in all groups of algae. However, the concentrations of vitamin B1, B2, pantothenic acid, folic and folinic acids are higher in green and red algae compared with brown algae (Prud'homme and Trono, 2001). Other vitamins are also present, including B12, which is not found in most land plants (Dharmananda, 2002). Red and brown algae are rich in carotenes (provitamin A) ranging from 20 to 170 ppm. Vitamin C content which ranges from 500- 3000 ppm is also found in considerable amounts in the red and brown algae (Dharmananda, 2002).

Fatty acids: Although seaweed lipids have a higher proportion of essential fatty acids than land plants, they have very low lipid contents, ranging from 1-5% DW. Green algae which have the closest structure to higher plants, have a much higher oleic and alpha- linoleic acid (Dharmananda, 2002).

Fiber: There is a high range of fiber content in seaweeds (1- 5% DW). The soluble fiber fraction accounts for 51-56% of total fibers in green (ulvans) and red algae (agars, carrageenans and xylans) and for 67-87% in brown algae (laminaria, fucus, and others) (Dharmananda, 2002).

1.2.1 As Food and Food Supplements

Seaweeds are also used for direct human consumption. Seaweeds as a staple item of diet have been used in Japan, Korea and China since prehistoric times. Seaweeds accounted for more than 10% of the Japanese diet until relatively recently, and seaweed consumption reached an average of 3.5 kg per household in 1973, a 20% increase in 10 years (Indergaard, 1983). Seaweeds may bring colour, flavour, texture and chewiness which make them delicacies (Arasaki and Arasaki, 1983; Madlener, 1977; Trono and Ganzon-Fortes, 1988). Examples of seaweeds consumed by humans are *Caulerpa lentillifera*, *Alaria esculenta*, *Porphyra* spp., *Gracilaria* spp., *Monostroma* spp., *Enteromorpha* spp., *Laminaria japonica*, *Undaria pinnatifida*, *Chondrus crispus* and *Hizika fusiforme* (McHugh, 2003). Many species are eaten by coastal societies (Phang, 1994). As mentioned before, seaweeds are rich in protein and contain essential amino acids which are participating in important metabolic processes such as energy and enzyme production (Demazel, 2008). Seaweeds contain high amounts of simple and complex carbohydrates as well, which can be considered as a source of additional fuel, in particular the sulphated complex carbohydrates are thought to enhance the immune system's regulatory response (Baba *et al*., 1988). Seaweeds also contain important fatty acids such as omega-3 and omega-6 which play a key role in energy production as well.

1.2.2 Phycocolloid Production

Traditionally, extra-cellular polysaccharides of seaweeds have been exploited, which are called phycocolloids (Chan, 2003). The most important phycocolloids: agar, alginate and carrageenan, are extracted from various red and brown seaweeds. Phycocolloids are high molecular weight polysaccharides which are polymers of sugar units (Pereria *et al.*, 2009). They are the main structural components of seaweed cell walls and may be involved in recognition mechanisms between seaweeds and pathogens (Potin *et al.*, 1999). Phycocolloids are used extensively in industries with a total phycocolloid production of approximately 55,000 tonnes, giving a value of US$ 585 million (McHugh, 2003).

Agar and carrageenan, the commercially important phycocolloids are mainly extracted from Rhodophyta while alginate is extracted from Phaeophyta. The wide uses of these phycocolloids are based on their gelling, viscosifying and emulsifying properties (Karina *et al.*, 2006). The structures of these phycocolloids are shown in figure 1.1.

1.2.2.1 Alginate

Alginic acid is a phycocolloid extracted from brown algae (Phaeophyceae) especially from the species of *Ascophyllum, Durvillaea, Ecklonia, Laminaria, Macrocystis* and *Sargassum* (McHugh, 2003). Alginic acid or alginate is a family of linear polysaccharids. Comprising of a non-regular arrangement of 1, 4 linked β-D-mannuronic and α-L- guluronic acid (Andrade *et al*., 2004). It is applied as low-price viscosifiers or thickeners in a wide range of products and is used primarily in frozen desserts where they regulate the formation of ice crystals and help to provide a smooth, creamy body in products. Alginate is the most commonly used stabilizer and emulsifier in the manufacturing of dairy products. The extensive use of alginate in non-food industries includes its use in paint, cotton textile, vulcanite fibre, plastics, glass production, linoleum, imitation leather, weather-proof products and etching industries (Prud'homme and Trono, 2001).

1.2.2.2 Carrageenan

The generic name for the family of natural water-soluble sulphated galactans is carrageenan which has an alternating backbone consisting of α (1-4) -3, 6- anhydro- D-galactose and β (1-3)-D galactose (Goncalves *et al*., 2002). Carrageenans used in many dairy products, many types of gums used in low fat products and also in processed foods. Carrageenan is solely extracted from *Kappaphycus alvarezii* (Doty) *Eucheuma denticilatum* and *Hypnea* with at least 75% of commercial production from the tropical regions. Generally, it can be used as stabilizing, thickening, suspending and gelling agents in food production. It is also utilized in non-food products: cosmetics, toothpaste, solid gel-type air fresheners and textile paints (Prud'homme and Trono, 2001).

1.2.2.3 Agar

Agar commands a high price in the world market (Kain and Destombe, 1995). Commercially, the species of Rhodophycean genera which are the sources of agar belong to *Gelidium*, *Gracilaria* and *Pterocladia* (McLachlan, 1985). Agar can be used for food applications (e.g. baked goods, candles), cosmetic industry (e.g. sun cream, perfumed deodorant sticks), pharmaceutical use (e.g. smooth laxative) and bacteriological and other biotechnology applications, especially in gel electrophoresis (Van der Meer and Patwary, 1995).

Agar is a seaweed galactan which contains α (1–4)-3, 6-anhydro-L-galactose and β (1–3)-D-galactose residues and is used in the canning of low calorie foods and in the pharmaceutical industry (Karina *et al*., 2006).

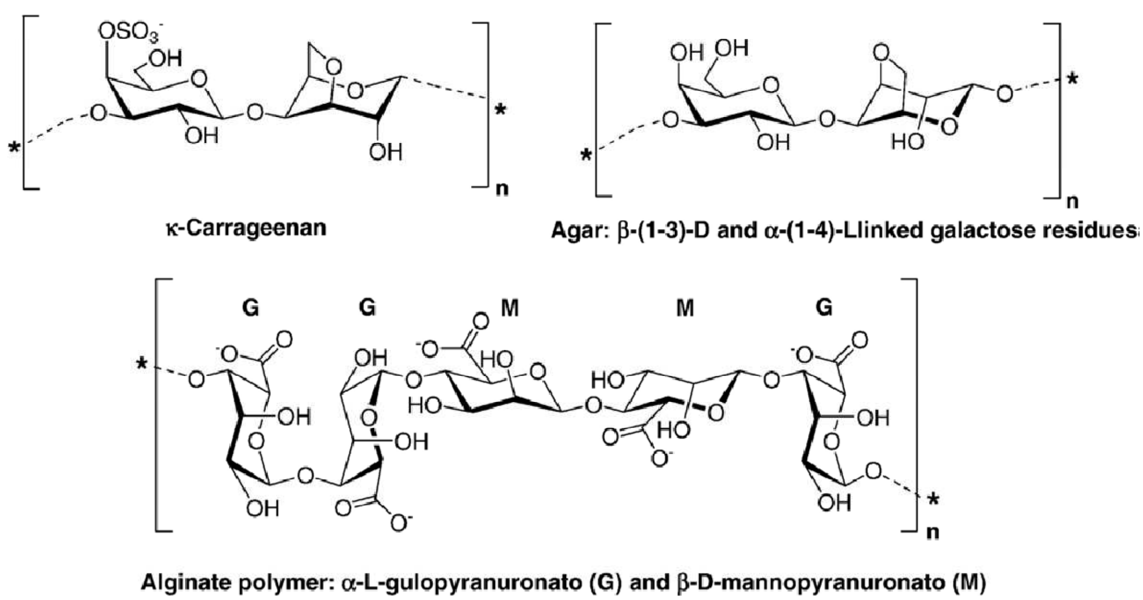

Figure 1.1: Molecular structures of agar, κ-carrageenan and alginate polysaccharides (Adapted from Karina *et al*., 2006)

1.2.3 Medical and Pharmaceutical Uses

The earliest record of seaweed utilization for medicinal purposes originates from the Chinese ‘Materia Medica’ of Shing-nung dating from 2700 B.C (Hoppe, 1979). In many areas primarily the coastal countries, numerous algae are used as medicaments. They are used in folk medicine against goiter, nephritic diseases, helminthes and catarrh. Various red algae have been used in the Mediterranean as sources of dying agents and as anthelmintic and other health remedies since pre-Christian times. *Caulerpa* spp. contains caulerpin and caulerpicin which functions as mild anaesthethics, therefore, has clinical values (Prud’homme and Trono, 2001). *Digenea* (Ceramiales; Rhodophycota) produces effective vermifugal properties (Arasaki and Arasaki, 1983; Michanek, 1979). *Corallina* is extensively used in bone-replacement therapy. *Chondrus crispus* (Irish Moss) was recommended as a health remedy in Ireland at the beginning of the 19th century (Mitchell and Guiry, 1983). Compounds with biological activities have been discovered in marine bacteria, invertebrates, and algae (Mayer and Lehmann, 2000).

1.2.3.1 Antiviral and Antibiotic Activities

Sulphated polysaccharides from red algae have been shown to have antiviral activity towards viruses which are responsible for human infectious disease. It is necessary for antiviral polysaccharides to have very low cytotoxic activities to mammalian cells. However, different researchers have reported that the antiviral activity of sulphated polysaccharides have a direct relationship with the degree of sulphation and the molecular weight of the macroalgae (Witvrouw and De Clercq, 1997; Lu¨scher-Mattli, 2000; Haslin *et al*., 2001).

1.2.4 Use as Cosmetics

Algal products of gelling substances (agar, alginates, carrageenans), seaweed flour and ground seaweed have been used in the cosmetic industries. Sachets containing ground seaweed are used for immersion in a bathtub; bath salts containing seaweed pastes are used in thalassotherapy (Arasaki and Arasaki, 1983). Alginate and carrageenan have been show to enhance the skin moisture retention properties of the product (Mc Hugh, 2003).

1.2.5 Use as Biofilters

In the middle of the seventies, seaweeds were used for treating the effluent for enclosed mariculture (Haines, 1975; Ryther *et al*., 1975; Roels *et al*., 1976; Langton *et al*., 1977; Harlin *et al*., 1979). The early results led to increased research (Vandermeulen and Gordin, 1990; Cohen and Neori, 1991; Neori *et al*., 1991; Haglund and Pedersén 1993; Buschmann *et al*., 1994, 1996; Jiménez *et al*., 1994; Krom *et al*., 1995; Neori, 1996; Noeri *et al*., 1996), confirming that wastewater from intensive and semi-intensive mariculture is a good nutrient source for seaweed production, and that integration with seaweeds significantly reduces the loading of dissolved nutrients to the environment. Seaweeds are good biofilters as they can remove nutrients and other pollutants from the wastewater (Primavera, 1993; Flores-Nava, 1995; Lin 1995). As an example, *Gracilaria* has been shown to be efficient biofiltration agents in highly fertilized waters associated with aquaculture areas (Gao and McKinley, 1994; Buschmann *et al*., 1996).

1.2.6 As Fertilizer and Soil Conditioner

Another usage of seaweeds is their biotransformation into compost (Potoky and Maz´e, 1988; Vallini *et al*., 1993, Eyras *et al*., 1998) providing a simple and economically suitable biotechnology for using large quantities of macroalgae (Cuomo *et al*., 1995).

Utilization of seaweeds as a fertiliser has continued from past centuries till now where seaweeds are produced commercially as fertilisers and, soil conditioners, and as well as a source of livestock feed. Seaweeds can be used for entrapping fertilisers in runoff from farms; when harvested, the algae itself is enriched enough to be used as fertiliser (Demazel, 2008).

Manual harvesting of drift seaweeds, largely members of the *Phaeophyta,* has been carried out since ancient times for spreading on fields as a fertilizer and for soil conditioning, particularly in maritime parts of Europe (Prud'homme and Trono, 2001). Liquid extracts of marine brown algae are marketed for agriculture and horticulture purposes. A great deal of these extracts is prepared from dried *Ascophyllum nodosum* meal or from drift seaweed. Since plants can easily absorb the soluble potash and other mineral elements in seaweeds, the plants show great increase in crop yields, resistance of plants to frost, increased uptake of inorganic constituents from the soil and resistance to stress conditions (Blunden, 1977).

1.2.7. As Fuel Sources

The concept of marine farms as a source of fuel was experimented in the United States (Bird *et al*., 1990; Chynoweth *et al*., 1987; Flowers and Bird, 1990; Neushul, 1987; North, 1980, 1987). It is possible to use algae for making biodiesel, bioethanol, biobutanol, and by some considerations can produce extensive amounts of vegetable oil, in comparison with terrestrial crops which are grown for the same purpose.

Hans Gaffron, a researcher from Germany, in 1939, observed that *Chlamydomonas reinhardtii* (a green algae), would sometimes switch from the production of oxygen to the production of hydrogen. So algae can be grown to produce hydrogen and the biomass can later be burned to produce heat and electricity.

1.2.8 Role of Algae in Pollution Control

Seaweeds also have potential use in wastewater treatment to reduce the total nitrogen- and phosphorus-containing compounds from sewage and some agricultural wastes before release of these treated waters into rivers or oceans. Algae are used in wastewater treatment facilities to reduce the requirement of toxic chemicals which already used for sewage treatment. Some seaweed is used as suitable biomonitors to study the environment contamination (Munda, 1982; COST 48, 1987; Munda and Hundnik, 1991 and references therein; Marcomini *et al*., 1993; Haritonidis and Malea, 1995, 1999; Sfriso *et al*., 1995; Malea and Haritonidis, 1999a, b, 2000), where heavy metal concentrations in macroalgae are used to monitor the level of bioavailable metals in estuarine and sea shore areas. The genera of macroalgae most extensively used for monitoring heavy metal contamination of water are *Fucus*, *Enteromorpha*, *Laminaria* and *Ulva*.

1.2.9 As Animal Fodder

Seaweed meal is produced only from fresh seaweeds and used as supplementary diet in animal feed for cattle, farm animals, fish and abalone. In Europe, the production of seaweeds as seaweed meal is valued at US$5 million annually (McHugh, 2003). The meal produced from dried seaweed can be used as a feed supplement. Cattle and sheep sometimes are allowed to forage on suitable shores, or have their diet supplemented with seaweeds harvested from the sea by farmers.

1.3 IMPORTANCE OF SEAWEED TAXONOMIC STUDIES

Taxonomy is defined as the classification of organisms based on their evolutionary relationships (Keeton and Gould, 1986). There are at least four major approaches to reconstruct the evolutionary history or phylogeny of a group species, namely classical evolutionary taxonomy, phenetics (numerical taxonomy), cladistics and molecular taxonomy. All aspects of biological and environmental science rely on taxonomy and systematics. There is a lot of life of earth is still unnamed and undescribed. Extinction and loss of biodiversity can be prevented unless the species and their relationships are known.

Relationships among organisms are visualized as evolutionary trees, which are also known as cladograms or phylogenetic trees. A phylogenetic tree consists of two components which are branching order (showing group relationships) and branch length (showing amount of evolution). Phylogenetic trees of species and higher taxa are used to study the evolution of traits (e.g., molecular characteristics) and the distribution of organisms (biogeography).

The use of molecular taxonomy is growing rapidly, both because gene sequences provide excellent tools for solving difficult taxonomic problems, and because technological advances are making it ever easier to obtain gene sequences. However, molecular data have not solved all taxonomic problems associated with algae (Janson and Hayes, 2006). DNA sequencing is an ideal selection for scientists to establish a molecular systematic since the DNA sequences are the actual gene sequences. DNA genomes are valuable reservoirs of taxonomy characters for phylogenetic analysis (Coleman and Goff, 1988).

Analysis of molecular sequences can be used to resolve the evolutionary relationships and taxonomic position for species that have few distinct morphological characteristics. Molecular analyses primarily employed nuclear small subunit rDNA (18S) and plastid rbcL data, as well as data on intron gain, complete genome sequencing and mitochondrial sequences (Lewis and McCourt, 2004). DNA genomes in particular, are valuable reservoirs of taxonomic characters potentially important for phylogenetic analysis (Coleman and Goff, 1991). Comparison of protein coding genes can provide valuable information, but generally only if they are homologous genes, i.e., they share the same evolutionary history (Mathieson, Norton and Neushul, 1981).

Some taxonomic entities of the genus *Caulerpa* are highly distinctive in their morphology and have well-defined boundaries (e.g. *C. filicoides* Yamada, *C. verticillata* J. Agardh), whereas other entities exhibit extreme morphological plasticity that results in an unstable classification of varieties and formae (Ohba *et al*., 1992; Prud'homme van Reine *et al*., 1996; Pillmann *et al*., 1997; Fama` *et al*., 2002). The morphological plasticity within *Caulerpa* species has been ascribed to environmental factors such as substratum, wave exposure, water currents, depth, light intensity, season, and grazing pressure (Svedelius, 1906; Borgesen, 1907; Peterson, 1972; Calvert, 1976; Ohba and

Enomoto, 1987; Ohba *et al*., 1992; Carruthers *et al*., 1993; Meinesz *et al*., 1995; Gacia *et al*., 1996; Collado-Vides and Robledo, 1999; Collado-Vides, 2002).

Correct identification of the genus *Caulerpa* is essential for exploitation and cultivation purposes. Since Lamouroux's original description in 1809, some 70 species of *Caulerpa* have been described. *Caulerpa* species are chiefly defined on the basis of their assimilator morphology (Weber-van Bosse, 1898; Svedelius, 1906; Børgesen, 1907; Nizamuddin, 1964; Calver, 1976; Ohba and Enomoto, 1987; Coppejans and Meinesz, 1988; Coppejans, 1992; Coppejans and Prud'homme van Reine, 1992).

Caulerpa racemosa is one of the most taxonomically problematic species of the genus due to its great morphological plasticity (Prud'homme van Reine *et al*., 1996). It can be defined as a species complex comprising many varieties or morphological forms. The pattern of variation in this species has lead to the description of numerous taxa, such as *C. racemosa* var. *macrophysa*, *microphysa*, *peltata*, *laetevirens*, and *turbinata* (Eubank, 1946; Peterson, 1972). According to Verlaque *et al*. (2000) and Durand *et al*. (2002), three different morphological or genetic varieties of *C. racemosa* coexist in the Mediterranean.

In addition to *Caulerpa racemosa*, another species of *Caulerpa*, *Caulerpa serrulata* (Forsskål) J. Agardh is also highly variable and according to Silva *et al*. (1996), a total of three varieties namely, *Caulerpa serrulata* var. *serrulata*, *Caulerpa serrulata* var. *boryana* and *Caulerpa serrulata* var. *hummii* in which the formal varities, *Caulerpa serrulata* var. *serrulata* exists in three forma: namely, *Caulerpa serrulata* var. *serrulata* forma angusta; *Caulerpa serrulata* var. *serrulata* forma lata; *Caulerpa serrulata* var. *serrulata* forma spiralis. There is yet any study to determine whether these different varieties or forma is due to genetic factor or plasticity.

There are a number of reasons why seaweeds are an interesting study subjects. One of the reasons is they are used widely as food, ingredients in cosmetics and fertilizers, and in hydrocolloid production. Besides, seaweeds are of ecological importance because they help in supplying oxygen to the sea and act as one of the primary producers in the marine food chain. Meanwhile, some seaweeds have the ability to remove heavy metals from the water and can potentially be used in biomonitoring and in the bioremediation of pollutants. When seaweeds are exposed to many environmental stresses, they tend to possess excellent survival strategies. Lastly, each of the three groups of seaweed has a unique life-cycle and physiology in which it can becomes the study subject for scholars.

1.4 OBJECTIVES OF RESEARCH

Collection of *Caulerpa serrulata* was carried out at Pulau Redang, Terengganu. Two taxa or varieties of *Caulerpa serrulata* were found. Based on morphological features, the two taxa fulfilled the description for *Caulerpa serrulata* var. *serrulata* and *Caulerpa serrulata* var. *boryana*.

A possible hypothesis for the existence of the two varieties is that they could be two distinct species and the two forms are genetically based, while another possibility will be the two varieties are to the highly plasticity of the seaweeds. Thus, one of the aims of this project is to examine the following hypotheses:

H_0: The *Caulerpa serrulata* var. *boryana* and *Caulerpa serrulata* var. *serrulata* are similar species

H_1: The *Caulerpa serrulata* var. *boryana* and *Caulerpa serrulata* var. *serrulata* are not similar species.

The objectives of this study are as follows:

1. To identify, differentiate and describe species based on morphological characteristics.

2. To understand the molecular relationship between the two taxa of *Caulerpa serrulata* based on *tuf*A marker.