UNIVERSITI PUTRA MALAYSIA

WOUND HEALING AND ANTIOXIDANT PROPERTIES OF EUCHEUMA COTTONII EXTRACT ON SPRAGUE DAWLEY RATS

SAMANEH GHASEMI FARD
FSTM 2009 31
WOUND HEALING AND ANTIOXIDANT PROPERTIES OF *EUCHEUMA COTTONII* EXTRACT ON SPRAGUE DAWLEY RATS

SAMANEH GHASEMI FARD

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2009
WOUND HEALING AND ANTIOXIDANT PROPERTIES OF
EUCHEUMA COTTONII EXTRACT ON SPRAGUE DAWLEY RATS

By

SAMANEH GHASEMI FARD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for Degree of Master of Science

October 2009
DEDICATIONS

To the infinite source of wisdom and understanding…

“God does not play dice”

Albert Einstein

To my brother, Ashkan, who is not with us but he is alive forever in my heart

To my father who has supported me unconditionally in this journey

To my mother for having faith in me before I learned to have faith in myself

To my sister and brother, who I am alive because of them
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

WOUND HEALING AND ANTIOXIDANT PROPERTIES OF *Eucheuma cottonii* EXTRACT ON SPRAGUE DAWLEY RATS

By

SAMANEH GHASEMI FARD

October 2009

Chair: Suhaila Mohamed, PhD

Faculty: Food Science and Technology

Wounds and their treatment are a big burden on the healthcare system, both in terms of cost, time and energy of care required. The lost in productivity and decreased quality of life is immeasurable. This study reports on the potential wound healing and antioxidant properties of oral consumption of ethanolic and aqueous extracts of *Eucheuma cottonii*. Two cm diameter excision of skin wound model was used, with honey (100 mg/kg body weight) as positive control and untreated normal rats as negative control groups. Both extracts significantly ($P<0.05$) increased the rate of wound contraction, better than honey. The extracts decreased lipid peroxidation in the plasma and increased erythrocyte antioxidant enzyme activities (superoxide dismutase) and reduced glutathione compared to both the positive and negative control groups. The ethanolic extract was more effective than the aqueous extract by 20%. Histopathological wound tissue observations showed both extracts significantly
reduced scars, enhanced epithelization, hair follicle growth and tissue granulation compared to both control groups. The HPLC results revealed that *E. cottonii* possessed several antioxidant compounds, which may be responsible for the wound healing acceleration. This is the first report showing that oral consumption of tropical seaweed extracts could enhance wound healing.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

CIRI-CIRI PENYEMBUHAN LUKA DAN ANTIOKSIDA DARIEKSTRAK *Eucheuma cottonii* PADA TIKUS SPALGE DAWLEY

Oleh

SAMANEH GHAEMI FARD

October 2009

Pengerusi: Suhaila Mohamed, PhD

Fakulti: Sains dan Teknologi Makanan

Luka yang teruk dan rawatannya telah menjadi beban kepada sistem rawatan kesihatan dari segi kos, masa dan tenaga. Kehilangan produktiviti dan pengurangan kualiti di dalam hidup tidak dapat dinafikan lagi. Pengurangan ini melaporkan kesan pengambilan ekstrak ethanolik dan akues *Eucheuma cottonii* terhadap potensinya untuk mempercepatkan penyembuhan luka. Luka berdiameter 2 cm telah dilakukan ke atas tikus sebagai model. Madu (100 mg/kg berat badan) telah digunakan sebagai kawalan positif dan tikus yang normal dan tidak diberikan rawatan dijadikan sebagai kawalan negatif. Kedua-dua ekstrak menunjukkan peningkatan yang bermakna terhadap kadar pengecutan luka berbanding madu. Ekstrak ini telah mengurangkan peroksidaan lipid di dalam plasma dan meningkatkan aktiviti enzim antioksidan (superoxide dismutase) dan reduced glutathione di dalam darah berbanding kedua-dua kumpulan kawalan positif dan negatif. Ekstrak etholik lebih efektif berbanding
ekstrak akues sebanyak 20%. Pemerhatian luka secara histopatologi terhadap kedua-dua esktrak menunjukkan pengurangan jelas terhadap parut, peningkatan epithelisasi dan pertumbuhan folikel rambut dan granulasi tisu berbanding kedua-dua kumpulan kawalan. Keputusan HPLC menunjukkan *E. cottonii* mempunyai beberapa bahan antioksidan yang dapat mempercepatkan penyembuhan luka. Ini adalah laporan yang pertama yang menunjukkan pengambilan rumpai laut secara oral dapat mempercepatkan penyembuhan luka.
ACKNOWLEDGEMENTS

Although I cannot express in words the extent of my gratitude, I would like to thank my advisor Prof. Dr. Suhaila Mohamed from whom I have learned a great deal, not just scientifically but also personally. It goes without saying that this dissertation would not have been possible without her guidance and keen advice. Her ability to keep the big picture in sight has pulled me back on track on numerous occasions. I truly appreciate the long discussions we have had where she helped infuse clarity into my own thoughts and ideas. Most of all, I have to thank her for teaching me how to communicate and present myself as a scientist and for showing me, by example, what it takes to be a successful scientist/person.

I would also like to thank the members of my thesis committee, Dr. Kharidah Muhammad and Dr. Goh Yong Meng for taking the time and effort to come to my thesis committee meetings and for offering very helpful advice and suggestions to keep my research as focused as possible.

I would also like to thank Dr. Ajwad Awad Mohammed and Professor Karim Alwan AL-Jashamy for technical support in histopathological evaluation.

In addition, my deep appreciation goes to all of my lab members and friends who were the biggest support throughout my years as a graduate student, Rosalina Tan Roslan Tan, Fatemeh Shamsabadi, Mahsa Motshakeri, Mehdi Javadi, Maslia Manja B.Z and Anyanji Victor Uchenna.
I would like to acknowledge Dr. Gururaj Bagal Kotkar in the Institute of Bioscience (IBS) and Mr. Abd. Halim bin Abd. Rahman at the faculty of Food Science and Technology for their valuable assistance in HPLC. I would like to thank Mr. Azman Bin Abu Yamin whom I had bothered with many technical questions in the past few years. My gratitude to Mr. Kufli Che Noor for his help in animal study.

I would like to thank my parents, Tahereh and Aliakbar for their consistent support and love. They have shared all the bad and good times together with me in my life.

Finally, I gratefully acknowledge the support for this work provided by the Ministry of Higher Education Fundamental Research Grant Scheme under Grant No: 91030.
I certify that a Thesis Examination Committee has met on 15 October 2009 to conduct the final examination of Samaneh Ghasemi Fard on her thesis entitled "Wound Healing and Antioxidant Properties of Eucheuma cottonii extract on Sprague Dawley rats" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee were as follows:

Faridah Abas, PhD  
Lecturer  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Chairperson)

Fauziah Othman, PhD  
Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Internal Examiner)

Azizah Abdul Hamid, PhD  
Associate Professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Internal Examiner)

Ayub Mohd Yatim, PhD  
Associate Professor  
Faculty Science and Technology  
Universiti Kebangsaan Malaysia  
(External Examiner)

BUJANG KIM HUAT, PhD  
Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia  

Date: 24 December 2009
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Suhaiala Mohamed, PhD**  
Professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Chairman)

**Goh Yong Meng**  
Lecturer  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Sharifah Kharidah Bt Syed Muhammad**  
Associate professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Member)

---

**HASANAH MOHD/GHAZALI, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia  

Date: 14 January 2010
DECLARATION

I declare that the thesis is my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

__________________________________________
SAMANEH GHASEMI FARD

Date: 18 June 2009
TABLE OF CONTENTS

DEDICATIONS
ABSTRACT
ABSTRAK
ACKNOWLEDGEMENTS
APPROVAL
DECLARATION
LIST OF TABLES
LIST OF FIGURES
LIST OF ABBREVIATIONS

CHAPTER

1 INTRODUCTION
  1.1 General introduction
  1.2 Objectives

2 LITERATURE REVIEW
  2.1 Seaweeds
    2.1.1 Description
    2.1.2 Compounds in seaweeds
    2.1.3 Biological effects
  2.2 Free radical in wound healing
  2.3 Wound healing
    2.3.1 Normal wound healing
    2.3.2 Nutritional support for wound healing
    2.3.3 Botanical medicines for wound healing
  2.4 Hair follicle
    2.4.1 Hair follicle structure
    2.4.2 Morphological stages of hair cycle
    2.4.3 Nutritional support for hair follicle growth
    2.4.4 Botanical medicines for hair follicle
  2.5 High pressure liquid chromatography (HPLC)
    2.5.1 Flavonoids analysis using HPLC

3 MATERIALS AND METHODS
  3.1 Chemicals and reagents
  3.2 Sample preparation
  3.3 Preparation of sample extract
  3.4 Animal subjects
  3.5 Wound creation and treatments
  3.6 Sampling and wound healing evaluation
    3.6.1 Wound contraction
    3.6.2 Period of epithelization
3.7 Sampling and biochemical assay
  3.7.1 Plasma and red blood cells (RBC) preparation 29
  3.7.2 Catalase (CAT) activity measurement 30
  3.7.3 Superoxide dismutase (SOD) activity measurement 30
  3.7.4 Reduced glutathione (GSH) assay 32
  3.7.5 Malondialdehyde (MDA) level determination 32
3.8 Determination of flavonoids 33
  3.8.1 Chemicals and reagents 33
  3.8.2 Sample preparation and HPLC procedure 34
3.9 Statistical analysis 35

4 RESULTS AND DISCUSSION 36
  4.1 Body weight 36
  4.2 Wound healing 38
    4.2.1 Wound contraction 38
    4.2.2 Period of epithelization 42
    4.2.3 Histopathology of wound 43
      4.2.3.1 Re-epithelization 43
      4.2.3.2 Granulation tissue development 52
  4.3 Hair follicle growth and hair cycle 54
    4.3.1 Percentage of hair growth 54
    4.3.2 Counting hair follicles 59
    4.3.3 Sebaceous glands 62
  4.4 Analysis of antioxidants in blood 63
    4.4.1 Catalase (CAT) activity 63
    4.4.2 Superoxide dismutase (SOD) activity 66
    4.4.3 Reduced glutathione (GSH) activity 69
  4.5 Malondialdehyde (MDA) level 72
  4.6 Antioxidants in ethanolic extract of seaweed 74
    4.6.1 Flavonoids 74

5 CONCLUSION 82

REFERENCES 84
BIODATA OF STUDENT 99
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>4.10</td>
<td></td>
</tr>
</tbody>
</table>

3.1 Gradient program used for the separation of seaweed flavonoids 35
4.1 Variation in body weights of rats fed with *E. cottonii* extracts during the experimental study (in grams) 37
4.2 The wound area (mm$^2$) of different groups of rats fed with *E. cottonii* extracts over a period of 15 days 40
4.3 The wound healing (%) of different groups of rats fed with *E. cottonii* extracts over a period of 15 days 41
4.4 Period of epithelialization (day) of rats fed with *E. cottonii* extracts 42
4.5 Number of hair follicle, area size of hair bulb, and length of hair follicle around the wound in rats treated with various extractions of *E. cottonii* at day 15 60
4.6 Catalase activity (k/mg protein) in erythrocytes rats fed with *E. cottonii* extracts over a period of 15 days 65
4.7 Superoxide dismutase activity (U/mg protein) in erythrocytes rats fed with *E. cottonii* extracts over a period of 15 days 68
4.8 Reduced glutathione (µg/g protein) in erythrocytes rats fed with *E. cottonii* extracts over a period of 15 days 71
4.9 Malondialdehyde level (µM/g protein) in erythrocytes rats fed with *E. cottonii* extracts over a period of 15 days 73
4.10 Compounds identified in Er.t by HPLC 76
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>The sequence of events during normal wound healing</td>
<td>10</td>
</tr>
<tr>
<td>2.2</td>
<td>The blood components spill into the site of injury</td>
<td>12</td>
</tr>
<tr>
<td>2.3</td>
<td>Impact of nutrients on the different phases of wound healing</td>
<td>13</td>
</tr>
<tr>
<td>2.4</td>
<td>Hair follicle structure</td>
<td>15</td>
</tr>
<tr>
<td>3.1</td>
<td><em>Eucheuma cottonii</em></td>
<td>23</td>
</tr>
<tr>
<td>3.2</td>
<td>The sequence of extraction method</td>
<td>25</td>
</tr>
<tr>
<td>3.3</td>
<td>Photograph showing excision wound</td>
<td>27</td>
</tr>
<tr>
<td>3.4</td>
<td>Evaluation method of wound healing</td>
<td>28</td>
</tr>
<tr>
<td>4.1</td>
<td>Photomicrographs of epidermis in Er.t group</td>
<td>46</td>
</tr>
<tr>
<td>4.2</td>
<td>Photomicrographs of epidermis in E70 group</td>
<td>47</td>
</tr>
<tr>
<td>4.3</td>
<td>Photomicrographs of epidermis in Wr.t group</td>
<td>48</td>
</tr>
<tr>
<td>4.4</td>
<td>Photomicrographs of epidermis in W70 group</td>
<td>49</td>
</tr>
<tr>
<td>4.5</td>
<td>Photomicrographs of epidermis in HNY group</td>
<td>50</td>
</tr>
<tr>
<td>4.6</td>
<td>Photomicrographs of epidermis in WTR group</td>
<td>51</td>
</tr>
<tr>
<td>4.7</td>
<td>Granulation tissue of the wounded skin treated orally with Er.t (A), E70 (B), Wr.t (C), W70 (D),</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>HNY (E) and WTR (F) (H&amp;E stain; ×400)</td>
<td></td>
</tr>
<tr>
<td>4.8</td>
<td>Photograph showing the hair growth in Er.t group at the 15 day post treatment</td>
<td>56</td>
</tr>
<tr>
<td>4.9</td>
<td>Photograph showing the hair growth in E70 group at the 15 day post treatment</td>
<td>56</td>
</tr>
<tr>
<td>4.10</td>
<td>Photograph showing the hair growth in Wr.t group at the 15 day post treatment</td>
<td>57</td>
</tr>
</tbody>
</table>
4.11 Photograph showing the hair growth in W70 group at the 15 day post treatment

4.12 Photograph showing the hair growth in HNY group at the 15 day post treatment

4.13 Photograph showing the hair growth in WTR group at the 15 day post treatment

4.14 Micrographs of hair follicle after 15 days oral treatment in different treatment groups (H&E stain; ×40)

4.15 Light microscopy of sebaceous glands in ethanolic extract group (a) and negative control group (b) at 15 day post treatment (H&E stain; ×100)

4.16 Chromatogram of five flavonoids standards

4.17 Flavonoids in ethanolic extract of *E. cottonii*

4.18 Co-elution of putative catechin (1), rutin (3) and quercetin (5) peaks in ethanolic extract of *E. cottonii* with standard compounds added

4.19 Structure of the flavonol quercetin showing features important in defining the classical antioxidant potential of flavonoids

4.20 Hypothesis of the links between the working mechanisms of flavonoids and their effects on disease
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHA</td>
<td>Butylated hydroxyanisole</td>
</tr>
<tr>
<td>BHD</td>
<td>Butylated hydroxytoluene</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated hydroxytoluene</td>
</tr>
<tr>
<td>CAT</td>
<td>Catalase</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper</td>
</tr>
<tr>
<td>DP</td>
<td>Dermal papilla</td>
</tr>
<tr>
<td>DS</td>
<td>Dermal sheath</td>
</tr>
<tr>
<td>DTNB</td>
<td>5, 5'-dithio- bis-(2-nitrobenzoic acid)</td>
</tr>
<tr>
<td><em>E. cottonii</em></td>
<td><em>Eucheuma cottonii</em></td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin &amp; Eosin</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>HCL</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HF</td>
<td>Hair follicle</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>IRS</td>
<td>Inner root sheath</td>
</tr>
<tr>
<td>kDa</td>
<td>KiloDalton</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>O$_2^{−}$</td>
<td>Superoxide anion</td>
</tr>
<tr>
<td>OH$^\cdot$</td>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>ORS</td>
<td>Outer root sheath</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
</tbody>
</table>

XV
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
</tr>
<tr>
<td>PhOH</td>
<td>Phenolic antioxidants</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SSD</td>
<td>Silver sulfadiazine</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive substances</td>
</tr>
<tr>
<td>TBHQ</td>
<td>Tert-butyl hydroquinone</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Transforming growth factor beta</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet spectroscopy</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial cell growth factor</td>
</tr>
</tbody>
</table>
CHAPTER ONE
INTRODUCTION

1.1 General introduction

Wounds are unavoidable events of life and might arise due to any agent that induces injury or stress and wound has been a menace the world over. Healing is a survival mechanism and represents an attempt to maintain normal anatomical structure and function. Treatment is therefore aimed at minimizing the undesired consequences. Wound healing management is a complicated and expensive program. Research on drugs that improve wound healing is developing in modern biomedical sciences. Several drugs obtained from plant sources are known to improve healing of different wound types. Some of these drugs have screened scientifically for evaluation of their wound healing activity in different pharmacological models and patients, but the potential of many herbal agents used traditionally, remains unexplored (Sandeep et al., 2009).

Hair has many useful biological functions, including protection from the elements and dispersion of sweat gland products. It also has psychosocial importance in our society and patients with hair loss (alopecia) often suffer tremendously (Paus and Cotsarelis, 1999). The demand for drugs that alter hair growth and appearance has led to multibillion dollar industries; also synthetic based product may cause human health hazard with several side effects, therefore investigation on plant extracts in order to find natural products are effective for these purposes (Rathi et al., 2008).
In order to find an effective natural product, that possesses both wound healing and hair re-growth properties, *Eucheuma cottonii* was chosen as one of the edible tropical seaweeds. It was obtained from Sabah area of Malaysia and was studied as a novel source of variety compounds (Matanjun *et al*., 2008) that is necessary for both properties, like polyphenols, vitamin C, α-tocopherol, minerals and protein. The seaweeds are used worldwide for many medicinal purposes.

There are reports in the literature of sulphated polysaccharides as antiviral substances and fucoidans as anticoagulant, antithrombotic, anti-inflammatory and antitumoral. Also there are a clear understanding of the mechanisms of action of flavonoids, either as antioxidants or modulators of cell signalling, and the influence of their metabolism on these properties are key to the evaluation of these potent biomolecules as wound healing (Williams *et al*., 2004).

Flavonoids have certain health effects and their antioxidant, radical scavenging, anti-mutagenic and anti-carcinogenic properties are well known (Middleton *et al*., 2000). The therapeutic applications of flavonoids on inflammation have previously been reported. Inflammation is important in many serious diseases. Therefore, intake of flavonoids is very important in the management of wound repair (Havsteen, 2002).
1.2 Objectives

With the above background, this study has the following objectives:

1. To evaluate the efficacy of seaweed extracts for wound healing and hair follicle growth in normal rats.

2. To study blood antioxidant activities of seaweed extracts.

3. To identify the flavonoids responsible for wound healing and hair follicle growth by HPLC.
CHAPTER TWO
LITERATURE REVIEW

2.1 Seaweeds

2.1.1 Description

Seaweeds (algae) are not true plants. They do not have flowers, any clearly marked steam or leaves. They do not have true roots but has held fast, which does not absorb food but simply attaches the plant firmly to a stone or rock. All seaweeds at some stage in their life cycles are unicellular, as spore or zygotes, and may be temporarily plank tonic (Guiry, 1998).

There are over 9,000 species of seaweeds which can be organized into three major types: green (Chlorophyta), brown (Phaeophyta) and red (Rhodophyta). Species of the genera Caulerpa, Durvillea, Laminaria, Monostroma, Nereocystis, Oedogonium, Porphyra, Rhodymenia, Sargassum, and Spirogyra are particularly commonly used as food in different parts of the world. Red is the most species-rich group (6,000) followed by brown (2,000) and green (1,200) (Guiry, 1999).

Seaweeds have been consumed in Asia since ancient times. Consumption of brown (66.5%), red (33%) and green algae (5%) is high in Japan and China compared to other Asian countries (Dawes, 1998). McHugh (2003) showed other countries, such as the Republic of Korea, the United States of America, South America, Ireland, Iceland, Canada and France have significantly increased
consumption, production and marketing of seaweeds. Approximately one million tonnes of wet seaweeds were harvested in 35 countries as a source of food, polysaccharides, fertilizer, fuel and cosmetics annually (Ruperez and Saura-Calixto, 2002). More recently marine algae have been utilized in Japan as raw materials in the manufacture of many seaweed food products such as jam, cheese, wine, tea, soup and noodles (Nisizawa et al., 1987) and in the western countries, mainly as a source of polysaccharides (agar, alginates, carrageenans) for the food and pharmaceutical industries. Seaweeds as a food in Malaysia are not as common as in countries like Japan and China. At present, seaweed is only consumed in certain coastal areas especially along the east coast of peninsula Malaysia, where it is occasionally eaten as a salad dish (Wong and Peter, 2000).

Seaweeds are a valuable food source as they contain protein, lipids, vitamins and minerals (Norziah and Ching, 2000; Sa'nchez-Machado et al., 2004). Seaweeds are not only a useful food source to humans, whole plants and seaweed mixes have been used in animal nutrition (Chapman and Chapman, 1980; Robledo and Freile-Pelegrin, 1997), poultry feed (Briand, 1991) and fish feed (McHugh, 2003). Some countries like Hong Kong used seaweeds as animal feeds or fertilizers, especially among the coastal villagers (Hodgkiss and Lee, 1983). However, very few of the world’s available seaweed species are used commercially. This may be because they cannot be harvested or cultivated on a commercially viable scale, or because their composition simply makes them unsuitable.