UNIVERSITI PUTRA MALAYSIA

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

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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

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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

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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 DARI EKSTRAK *EUCHEUMA COTTONII* PADA TIKUS SPRAGUE DAWLEY

Oleh

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Pengerusi: Suhaila Mohamed, PhD

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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 sebgai 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.

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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.

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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

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LIST OF ABBREVIATIONS

BHA	Butylated hydroxyanisol
BHD	Butylated hydroxytoluene
BHT	Butylated hydroxytoluene
CAT	Catalase
Cu	Copper
DP	Dermal papilla
DS	Dermal sheath
DTNB	5, 5'-dithio- bis-(2-nitrobenzoic acid)
E. cottonii	*Eucheuma cottonii*
GSH	Glutathione
H&E	Hematoxylin & Eosin
H_2O_2	Hydrogen peroxide
HCL	Hydrochloric acid
HF	Hair follicle
HPLC	High pressure liquid chromatography
IRS	Inner root sheath
kDa	KiloDalton
MDA	Malondialdehyde
$O2^{\bullet-}$	Superoxide anion
$OH^{\bullet}$	Hydroxyl radical
ORS	Outer root sheath
PBS	Phosphate buffered saline

PDGF	Platelet-derived growth factor
PhOH	Phenolic antioxidants
RBC	Red blood cells
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SSD	Silver sulfadiazine
TBARS	Thiobarbituric acid reactive substances
TBHQ	Tert-butyl hydroquinone
TFA	Trifluoroacetic acid
TGFß	Transforming growth factor beta
UV	Ultraviolet spectroscopy
VEGF	Vascular endothelial cell growth factor

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, Nereocytstis, 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.