UNIVERSITI PUTRA MALAYSIA

CULTURE OF MARINE MICROALGAE, Tetraselmis tetrathele (WEST) BUTCHER, IN ANNULAR PHOTOBIOREACTOR FOR APPLICATION IN FORMATION OF NANOCOSMECEUTICALS

NURUL FARAHIN BINTI ABD WAHAB

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By

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

September 2015

SPECIAL DEDICATION OF THIS GRATEFUL FEELING TO MY…..

Beloved father and mother;
Mr. Abd Wahab Ahmad & Mrs. Norsiah Harron

Loving brothers and sisters

and to those I loved for the understanding, encouragement and unconditional love and support throughout the course of this work.
I love you.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

CULTURE OF MARINE MICROALGAE, *Tetraselmis tetrathele* (WEST) BUTCHER 1959, IN ANNULAR PHOTOBIOREACTOR FOR APPLICATION IN THE FORMATION OF NANOCOSMECEUTICALS

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September 2015

Chairperson : Fatimah Md. Yusoff, PhD
Faculty : Institute of Bioscience

There has been a remarkable surge of interest on natural products and their application in the cosmeceutical industry. Cosmeceutical are cosmetic-hybrids intended to enhance health and beauty of the skin. Topical delivery of antioxidants from natural marine sources is one of the approaches used to reduce the reverse sign of skin aging. The marine prasinophyte *Tetraselmis tetrathele* is one of the important microalgae used as feed in aquaculture due to its high nutritional values and able to be mass produced because of its eurythermal and euryhaline characteristics. This indigenous microalga also contains bioactive compounds such as flavonoids, polyphenols and polyunsaturated fatty acids (PUFA), which makes it an appropriate raw material for various product developments in cosmeceutical industries. The antioxidant activity of *T. tetrathele* (UPMC-A0007) was determined by culturing it in f/2 media for 56 days in 120 L annular photobioreactor. Microalgae biomass was collected six times throughout the culture period to quantify total phenolic (TPC) and antioxidant contents. The antioxidant activities of *T. tetrathele*'s crude extract were determined by diphenylpicrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. Two groups of cell size; small sized-cells ($3.0\text{-}5.0\times10^{-11}$ g/cells) and big sized-cells ($5.5\text{-}8.0\times10^{-11}$ g/cells) were observed. The total phenolic content of small sized-cell (2.99 ± 0.14 mg GAE/g) was 1.6 times higher compared to big sized-cell. These results suggest that *T. tetrathele* could be a valuable source of phenolic content and antioxidant. The effective antioxidant production can be achieved by controlling the cell size in during culture process. Identification of phytochemical constituents was achieved by GC-MS analyses. Generally, six main chemical compounds identified were responsible for the bioactivity in both small sized-cells and big sized-cells.

Compositions from ternary phase diagrams were selected as pre-formulated emulsions. Topical nanocosmeceutical formulations from palm kernel oil esters (PKOEs) : 1% of crude extract *T. tetrathele*/Tween 80/water systems were chosen due to the presence of large isotropic liquid region which are suitable for the production of nanoemulsion. Particle size analysis showed that the mean particle sizes of these formulations (T1, T2 and T3) ranged from 102.3 to 249.5 nm. Zeta potential analysis for all emulsions

showed negative values from -33.2 to -71.7 mV. Stability studies showed that, after four hours of stirring at room temperature (25°C), the formulations were stable during centrifugation test at 4000 rpm for 15 minutes. In addition, T1, T2 and T3 were stable with no separation at different storage temperatures (4, 25 and 45°C) for the duration of eight weeks. However, between eight to ten weeks, only T1 and T2 were stable at 4, 25, and 45°C. This study illustrated that T1 and T2 formulations are considered to be the most suitable formulation for nanocosmeceutical product because they were stable after undergoing thaw cycles test, storage at room temperature (25°C) and 45°C for more than eight weeks. Moreover, the particle size ranged between 165 to 199 nm which resulted in low occurrence of Ostwald ripening.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGKULTURAN MIKROALGA MARIN, *Tetraselmis tetrathele* (WEST) BUTCHER 1959, DI DALAM FOTOBIOREAKTOR ANULUS UNTUK APLIKASI DALAM FORMULASI NANOKOSMESEUTIKAL

Oleh

NURUL FARAHIN BINTI ABD WAHAB

September 2015

Pengerusi : Fatimah Md. Yusoff, PhD
Fakulti : Institut Biosains

Terdapat peningkatan permintaan luar biasa terhadap produk yang berasaskan semulajadi dan juga aplikasinya dalam industri kosmeseutikal. Kosmeseutikal merupakan kosmetik-hibrid yang bertujuan untuk meningkatkan tahap kesihatan dan juga kecantikan kulit. Penghantaran antioksidan secara topical daripada sumber marin semulajadi adalah salah satu pendekatan bagi mengurangkan tanda penuaan. Prasinofit marin *Tetraselmis tetrathele* merupakan salah satu mikroalga penting yang digunakan sebagai makanan dalam akuakultur kerana ianya mempunyai kandungan nutrisi yang tinggi dan mudah untuk dihasilkan dalam kuantiti yang banyak kerana cirinya yang boleh bertoleransi dengan pelbagai suhu dan juga saliniti. Mikroalga asli ini juga mempunyai sebatian bioaktif seperti flavanoid, poliferol dan asid lemak tidak tepu (PUFA) yang menjadikannya sebagai bahan mentah yang sesuai dalam perkembangan pelbagai produk industri kosmeseutikal. Aktiviti antioksidan bagi *T. tetrathele* (UPMC-A0007) yang dikultur dalam media f/2 selama 56 hari di dalam 120 L fotobioreaktor anulus telah ditentukan. Biojisim mikroalga telah dikumpulkan sebanyak enam kali sepanjang tempoh pengkulturan bagi mengukur jumlah sebatian fenolik (TPC) dan kandungan antioksidan. Aktiviti antioksidan daripada ekstrak mentah *T. tetrathele* ini ditentukan oleh *diphenylpicrylhydrazyl* (DPPH), *ferric reducing antioxidant power* (FRAP) dan *2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)* (ABTS) esei. Dua kumpulan saiz sel; sel bersaiz kecil (3.0-5.0×10^{-11}g/sel) dan sel bersaiz besar (5.5-8.0×10^{-11}g/sel) telah dikenalpasti. Kumpulan sel bersaiz kecil (2.99 ± 0.14 mg GAE/g) menunjukkan 1.6 kali lebih tinggi kandungan sebatian fenolik berbanding kumpulan sel bersaiz besar. Keputusan ini menunjukkan bahawa *T. tetrathele* boleh dijadikan sebagai sumber bagi kandungan sebatian fenolik dan antioksidan. Penghasilan antioksidan secara efektif boleh dicapai dengan mengawal saiz sel semasa proses pengkulturan dijalankan. Pengenalpastian juzuk fitokimia telah dibuat menggunakan analisis GC-MS. Secara umumnya, enam sebatian kimia utama telah dikenalpasti bagi mewakili sebatian utama yang bertanggungjawab untuk aktiviti-biologi dalam kedua-dua saiz sel tersebut.

Komposisi daripada gambarajah fasa segitiga telah dipilih sebagai emulsi pra-formulasi. Formulasi nanokosmeseutikal yang berasaskan minyak ester isirong kelapa sawit (PKOE) : 1% daripada ekstrak mentah *T. tetrathele* / Tween 80 / air telah dipilih kerana gambarajah fasa segitiga menunjukkan kawasan isotropi yang besar, dan mencadangkan bahawa kawasan ini sesuai digunakan bagi penyediaan nanoemulsi. Analisis saiz partikel menunjukkan bahawa saiz zarah purata formulasi (T1, T2 dan T3) adalah antara 102.3 kepada 249.5 nm. Potensi zeta adalah antara -33.2 hingga -71.7 mV. Kajian kestabilan menunjukkan bahawa, selepas empat jam proses pengacauan pada suhu bilik (25°C), formulasi didapati masih stabil selepas proses pengemparan pada 4000 rpm selama 15 minit. Selain itu, kajian kestabilan di bawah suhu yang berbeza (4, 25 dan 45°C) juga menunjukkan bahawa T1, T2 dan T3 didapati stabil selama lapan minggu. Walau bagaimanapun, di antara lapan hingga sepuluh minggu, didapati hanya T1 dan T2 sahaja yang masih stabil pada suhu 4, 25 dan 45°C. Kajian ini menunjukkan bahawa formulasi T1 dan T2 dianggap sebagai formulasi yang paling sesuai bagi tujuan produk nanokosmeseutikal kerana tahap kestabilannya setelah menjalani ujian kitaran pencairan, penyimpanan pada suhu bilik (25°C) dan 45°C selama lebih daripada lapan minggu. Manakala purata saiz zarahnya antara 165 hingga 199 nm mengurangkan berlakunya proses kematangan *Ostwald*.

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I certify that a Thesis Examination Committee has met on 9 September 2015 to conduct the final examination of Nurul Farahin binti Abd. Wahab on her thesis entitled "Culture of Marine Microalgae, *Tetraselmis tetrathele* (West) Butcher, in Annular Photobioreactor for Application in Formation of Nanocosmeceuticals" 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 Thesis Examination Committee were as follows:

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Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Muta Harah binti Zakaria, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Norio Kurosawa, PhD
Professor
Soka University
Japan
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 16 February 2016

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

Fatimah Md. Yusoff, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairperson)

Mahiran Basri, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Mohamed Shariff Mohamed Din, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

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Supervisory Committee: Prof. Dr. Fatimah Md. Yusoff

Signature: ________________________________
Name of
Member of
Supervisory Committee: Prof. Dr. Mahiran Basri

Signature: ________________________________
Name of
Member of
Supervisory Committee: Prof. Dato' Dr. Mohamed Shariff Mohamed Din

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
Cm	Centimeter
°C	Degree celcius
GAE	Gallic Acid Equivalent
G	Gram
H	Hour
HLB	Hydrophilic Lipophilic Balance
LFCs	Lead Functional Components
µm	Micro meter
µM	Micro Molar
Ml	Mililiter
Mm	Milimeter
mV	Milivolt
Min	Minute
Nm	Nano meter
OD	Optical Density
O/W	Oil-in-Water
PKOEs	Palm Kernel Oil Esters
ROS	Reactive Oxygen Species
Rpm	Rotational per minute
S	Second
sp.	Species
SPSS	Statistical Package for the Social Sciences
TEAC	Trolox Equivalent Antioxidant Activity
TEM	Transmission Electron Microscopy
Tween 80	Polyoxyethylene Sorbitan Monostearate
v/v	Volume per volume
W/O	Water-in-Oil
w/w	Weight per weight
XG	Xanthan Gum

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of study

In recently years, the use of microalgae as a natural source for nutrition (Varfolomeev and Wasserman, 2011), food supplement (Graziani *et al.*, 2013), cosmetic (Balboa *et al.*, 2014) and pharmaceutical products (Pangestuti and Kim, 2011) has received increasing attention. Microalgae are unicellular photosynthethic microorganisms containing chlorophyll, and can be found in both saline and fresh water environments. They are diverse group of photosynthethic microorganisms that convert sunlight, water and carbon dioxide to synthesise chemical energy such carbohydrates, lipids and protein into algal biomass (Demirbas and Fatih, 2011; Gong *et al.*, 2011). These microorganisms are potentially source of diverse phytometabolic contents with various chemical structure (arachidonic acid, linolenic acid, sterol, α-tocopherol and phycobilin) and biological activities for many application purposes such as antioxidant, anticancer and protection to the skin (Servel *et al.*, 1994; Plaza *et al.*, 2009; de Jesus Raposo *et al.*, 2013). The chemical composition and productivity depend on growth phase, harvesting time, nutrients contents and light intensity. Due to that, microalgae biomass is able to provide additional physiological and pharmacological benefits for human health as a biochemical product. Recently, the market produces about 5000 t of microalgae dry matter/year and generates a turnover of aproximately US$ 1.25×10^9/year (El Gamal, 2010; Hajimahmoodi *et al.*, 2010).

Previous studies have reported that plants, fruits and macroalgae are well known for their high content of antioxidant properties with diverse chemical structure and biological activities (Kaur and Kapoor, 2002; Ismail *et al.*, 2004; Duan *et al.*, 2006; Zhang *et al.*, 2007; Kuda and Ikemori, 2009; Tsantili *et al.*, 2011; Lester *et al.*, 2012). However, higher plants and macroalgae have certain limitations such as time to grow, space, water resources and usage of herbicides. Furthermore, plant and macroalgae can be limiting resource in developing countries. These resources which become one of the main food sources in poverty stricken countries will be depleted in order to meet the application of biotechnology research such as pharmaceutical and cosmetic purposes (Balasubramanian *et al.*, 2011).

Microalgae have received increasing attentions as an alternative antioxidant source because they are the fastest-growing plants in the world and hence they have higher productivity compared to land plants (Chisti, 2007; Demirbas, 2010). Microalgae do not require high quality land and herbicides since they can grow in almost all aquatic environment even in sewage and salt water (Scott *et al.*, 2010). Microalgae can also produce antioxidant substances against oxidative and radical stressors. Therefore, several researchers tried to extract antioxidant compounds from various algae biomass (Li *et al.*, 2007; Goh *et al.*, 2010; Hajimahmoodi *et al.*, 2010; Custódio *et al.*, 2012; Manivannan *et al.*, 2012). Marine microalgae produce a wide variety of chemically active metabolites in their surroundings such as antioxidant activities as an aid to protect themselves against other settlings organisms (Servel *et al.*, 1994; Vo *et al.*, 2012). Therefore, marine microalgae are believed to be a promising supply to provide not only novel biologically active substances for the growth of pharmaceuticals but also essential compounds for human nutrition and aquaculture food mainly for live feeds for larval culture (Huerlimann *et al*., 2010; Pangestuti and Kim, 2011). Among marine microalgae, *Tetraselmis tetrathele* (West) Butcher 1959, is a green four-flagelated prasinophyte, which has good nutritive characteristics (especially in relation to polyunsaturated fatty acid composition) and thus it can be useful for health food, aquaculture and nutrition applications (de La Peña and Villegas, 2005; Natrah *et al*., 2007). *Tetraselmis tetrathele* could be a potential microalga for the future as it can be easily mass produced due to its euryhaline and eurythemal characteristics. Therefore, *T. tetrathele* is suitable for large scale production as it is necessary to consider its cost-effective production (Ronquillo *et al*., 1997).

The potential and invention of algal biomass became a reality in Germany in the 1940s, and towards the end of World War II. At that time, microalgae were grown in bigger quantities for various purposes, such as the production of lipids for energy using flue gasses, anti-microbial substances and the production of various bio-chemicals (Chaumont, 1993; Ugwu *et al*., 2008; Grobbelaar, 2012). Then, after industrialization has begun, mass cultivation of algae was implemented by some group of workers in Carnegie Institute at Washington for reduction of CO_2. Between early 1970s and late 1970s, this work was continued by many research groups, most notably in Stanford (USA), Tokyo (Japan), Essen (Germany), Israel, Czech Republic and Taiwan (Burlew, 1953). As a matter of fact, the intention of growing algae depended on the needs of the people nowadays.

Algae are grown either in open culture systems (lakes, ponds) or closed systems (photobioreactors). Closed systems offer better control over contamination, mass transfer, and other cultivation conditions even though the open pond systems seem to be favoured for commercial cultivation of microalgae at present due to their low capital costs (Li *et al*., 2008). Photobioreactor has been proposed as it gives the advantages of closed systems over open ponds ranging from laboratory to industrial scale such as high biomass productivity and and cell density, reduced contamination and better use of CO_2 (Shen *et al*., 2009). Photobioreactor is defined as a closed (mostly closed) vessel for phototrophic production where energy supplied lights. Commonly, laboratory-scale photobioreactors are artificially illuminated (either internally or externally) using fluorescent lamps or other light distributors. For example, closed photobioreactors have attracted much attention because they allow a better control of the cultivation conditions than open systems. With closed photobioreactors, higher biomass productivities are obtained and contamination can be simply prevented. With the availability of suitable culture systems, culture conditions such as nutrient availability, light intensity, pH, temperature and salinity can then be optimized for high quality and maximum biomass production (Singh and Sharma, 2012).

The term cosmeceuticals, coined by Dr. Albert Kligman, may be defined as a hybrid of drugs and cosmetics (Kligman, 2005). Cosmeceutical is a category of multifunctional products that rely on science and technology to deliver clinically proven active ingredients to the skin. Cosmeceutical-based products are formulated with pharmaceutical-type ingredients, have a unique ability to treat or beautify skin from inside out. Due to that, this product has the largest growing segments of skin care market based on the sales report and becomes makes the popular among consumers nowadays. In industry, the effectiveness of the products is one of the major concerns. The advancement of nanotechnology enables nanoemulsions to be used as a nanocarrier to effectively deliver the active components in the products to its targeted cells (Teo *et al*., 2010; Ng *et al*., 2013).

An emulsion is defined as a dispersion consisting of mixture of two insoluble materials; water and an oil phase stabilized against separation using an emulsifier surfactant (Abd Gani *et al*., 2011). It is system that most commonly used for cosmetics and pharmaceuticals. Microemulsions and nanoemulsions are two common types of colloidal dispersions that can be created from these components. These two kinds colloidal dispersion have some important differences eventhough there are many structural similarities. Nanoemulsions are composed of two phases but having extremely small size in the ranges of 20-500 nm (Mason *et al*., 2006; Solè *et al*., 2010; Bernardi *et al*., 2011). Due to extremely small size, nanoemulsions are becoming the subject of many studies on their wide range of potential uses and applications especially to be used as delivery system in cosmeceuticals. The characteristic of being absorbed by the skin makes nanoemulsions are sought after in the pharmaceutical industry (Mou *et al*., 2008; Salim *et al*., 2012a; Salim *et al*., 2012b; Ng *et al*., 2013).

Palm kernel oil ester (PKOE) is a long chain fatty acid synthesized from palm kernel oil, through enzymatic transesterification process. PKOE is rich in oleyl laurate, C30:1 (54.1%). Palm kernel oil ester will be used in this project as it has shown novel characteristics such as exhibiting superb behavior without the 'oily feeling', colourless and low viscocity. This statement had been approved by Salim *et al*. (2012a) that it is the best ingredient to be used in formulation of cosmeceutical and pharmaceutical industry. Since *T. tetrathele* (isolate UPM-A007) is an indigenous species, the combination between this species and the use of palm kernel oil ester to the nanoemulsions formulation, it will be add on the novel application of palm oil commodities in cosmeceutical industries in Malaysia.

1.2 Statement of problem

Microalgae are biologically diverse collection of microorganisms amenable to fermentation and mass culture. As well as cyanobacteria and nearly dozen eukaryotic classes, microalgae produce a wide array of compounds with biological activities (Arad and Yaron, 1992; El Gamal, 2010). Production of secondary metabolite by microalgae varies with environmental conditions. However, productivity of microalgae varies due to the differences in culture system, geographic locations, culture strategies (batch or continuous culture), algae species etc. Microalgae might become economic sources of new drug and other specialty chemicals when these processes are better understood. Moreover, production can be optimized in a controlled system.

Phytochemicals (e.g. phenolic acid, flavanoids and polyunsaturated fatty acids) are important nutrients produced by microalgae as secondary metabolites, especially when microalgae are in stress conditions. It may play a critical role in age-related disease or even cancer (Kobayashi *et al*., 1997; Rao *et al*., 2007; Sánchez-Saavedra and Voltolina, 2006). Hydrophilic and hydrophobic of bioactive compounds can present a problem in delivery for oil-in-water (o/w) systems. Nanoemulsion is kinetically stable (metastable dispersion state) and believed to be stable against creaming or sedimentation, flocculation and coalescence. However, nanoemulsion is also a very fragile system. Mechanical energy created to the mixture in the systems may be destroyed by spontaneous process such as coalescence or Ostwald ripening (Sonneville-Aubrun *et al*., 2009; Teo *et al*., 2010). Slightest sign of destabilization with ease to appear. They become opaque and creaming may be able to be seen as they are transparent and usually very fluid. Therefore, stability of the nanoemulsion is a critical factor to be analysed. The accomplishment of developing long time stability of cosmetic products (three years of shelf life) is often difficult and costly in the development of new formulation (Ng *et al*., 2013). The properties such as particle stability, rheology, appearance, colour, texture and shelf life will be affected by the size and polydispersity of nanoemulsion. (Bernardi *et al*., 2011).

Until now, there has been little attempt to prepare nanoemulsions using PKOE (Salim *et al*., 2012a). Currently there are no published reports on the use of palm kernel-based wax esters loaded with microalgae extracts as nano-delivery carrier in cosmeceuticals. *Tetraselmis tetrathele* is selected as the study organism because of its hardy characteristic, ease of culture, fast growing, good in nutrition and it is an indigenous species**.** The procedure for producing nanoemulsions comprised of nano-size droplets emulsions is difficult. On the other hand, one of the main problems with nanoemulsions is stabilization of developed system. The solubility and instability of actives in the nanoemulsions system also contribute to the difficulty in producing a stable nanoemulsions system.

Hence, the present study focused on the following objectives:

1. To mass produce *T. tetrathele* culture in annular photobioreactor.
2. To determine the phytochemical constituents of *T. tetrathele* extracts
3. To develop and characterize the physicochemical properties of palm kernel esters containing *T. tetrathele*-based nanoemulsions.

REFERENCES

Abd-Gani, S., Basri M., Abdul-Rahman, M., Kassim, A., Raja-Abd-Rahman, R., Salleh, A. and Ismail, Z. 2011. Engkabang Fat Esters for Cosmeceutical Formulation. *Journal of Surfactants and Detergents* 14: 227-233.

Abdullah, G. Z., Abdulkarim, M. F., Salman, I. M., Ameer, O. Z., Chitneni, M., Mahdi, E. S., Yam, M. F., Hameem, S., Basri, M., Sattar, M. A, and Noor, A. M. 2011. Stability studies of nano-scaled emulsions containing Ibuprofen for topical delivery. *International Journal of Drug Delivery* 3: 74-82.

Ahmad, K., Ho, C. C., Fong, W. K. and Toji, D. 1996. Properties of palm oil-in-water emulsions stabilized by nonionic emulsifiers. *Journal of Colloid and Interface Science* 181: 595-604.

Akhtar, N., Khan, B. A., Khan, M. S., Mahmood, T., Khan, H. M. S., Iqbal, M. and Bashir, S. 2011. Formulation development and moiturising effects of a topical cream of *Aloe vera* extract. *World Academy of Science, Engineering and Technology* 5: 1201-1209.

Akihisa, T., Yasukawa, K., Yamaura, M., Ukiya, M., Kimura, Y., Shimizu, N. and Arai, K. 2000. Triterpene alcohol and sterol ferulates from rice bran and their anti-inflammatory effects. *Journal of Agricultural and Food Chemistry* 48: 2313-2319.

Alberto-Vieira-Costa, J., Maria-Colla, L. and Fernando-Duarte-Filho, P. 2004. Improving *Spirulina platensis* biomass yield using a fed-batch process. *Bioresource Technology* 92:237-24.

Al-Rahman, I. R. A., Jawad, F. J., Alkateeb, E. H. and Abdulrasool, A. A. 2007. Preparation of Anise and Thyme Lotion for Topical Use. *Iraqi Journal of Pharmaceutical Sience* 16: 8-12.

Allard, B. and Templier, J. 2000. Comparison of neutral lipid profile of various trilaminar outer cell wall (TLS)-containing microalgae with emphasis on algaenan occurrence. *Phytochemistry* 54: 369-380.

Anjali, C. H., Sharma, Y., Mukherjee, A. and Chandrasekaran, N. 2012. Neem oil (*Azadirachta indica*) nanoemulsion- a potent larvicidal agent against *Culex quinquefasciatus*. *Pest management science* 68: 158-163.

Antelo, F. S., Costa, J. A. V., and Kalil, S. J. 2008. Thermal degradation kinetics of the phycocyanin from *Spirulina platensis*. *Biochemical Engineering Journal* 41: 43-47.

Arad, S. and Yaron, A. 1992. Natural pigments from red microalgae for use in foods and cosmetics. *Trends in Food Science and Technology* 3: 92-97.

Balasubramanian, S., Allen, J. D., Kanitkar, A. and Boldor, D. 2011. Oil extraction from *Scenedesmus obliquus* using a continuous microwave system-design, optimization, and quality characterization. *Bioresource Technology* 102: 3396-3403.

Balboa, E. M., Soto, M. L., Nogueira, D. R., González-López, N., Conde, E., Moure, A., Vinardell, M. P., Mitjans, M. and Domínguez, H. 2014. Potential of antioxidant extracts produced by aqueous processing of renewable resources for the formulation of cosmetics. *Industrial Crops and Products* 58: 104-110.

Barreira, J., Rodrigues, S., Carvalho, A. M. and Ferreira, I. C. F. R. 2013. Development of hydrosoluble gels with *Crataegus monogyna* extracts for topical application: Evaluation of antioxidant activity of the final formulations. *Industrial Crops and Products* 42: 175-180.

Bermejo-Román, R., Alvárez-Pez, J. M., Acién-Fernández, F. G. and Molina-Grima, E. 2002. Recovery of pure B-phycoerythrin from the microalga *Porphyridium cruentum*. *Journal of Biotechnology* 93: 73-85.

Bernardi, D. S., Pereira, T. A., Maciel, N. R., Bortoloto, J., Viera, G. S., Oliveira, G. C. and Rocha-Filho, P. A. 2011. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. *Journal of Nanobiotechnology* 9:44.

Bigogno, C., Khozin-Goldberg, I., Boussiba, S., Vonshak, A. and Cohen, Z. 2002. Lipid and fatty acid composition of the green oleaginous alga *Parietochloris incisa*, the richest plant source of arachidonic acid. *Phytochemistry* 60: 497-503.

Burlew, J.S. 1953. Algal culture. From Laboratory to Pilot Plant, Carnegie Inst. Washington Publ 600: 1.

Carballo-Cárdenas, E. C., Tuan, P. M., Janssen, M. and Wijffels, R. H. 2003. Vitamin E (α-tocopherol) production by the marine microalgae *Dunaliella tertiolecta* and *Tetraselmis suecica* in batch cultivation. *Biomolecular Engineering* 20: 139-147.

Chaumont, D. 1993. Biotechnology of algal biomass production: a review of systems for outdoor mass culture. *Journal of Applied Phycology* 5: 593-604.

Chini-Zittelli, G., Rodolfi, L., Biondi, N. and Tredici, M. R. 2006. Productivity and photosynthetic efficiency of outdoor cultures of *Tetraselmis suecica* in annular columns. *Aquaculture* 261: 932-943.

Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnology Advances* 25: 294-306.

Chu, S. P. 1942. The Influence of the Mineral Composition of the Medium on the Growth of Planktonic Algae: Part I. Methods and Culture Media. *Journal of Ecology* 30: 284-325.

Conforti, F., Sosa, S., Marrelli, M., Menichini, F., Statti, G. A., Uzunov, D., Tubaro, A., Menichini, F. and Loggia, R. D. 2008. In vivo anti-inflammatory and in vitro antioxidant activities of Mediterranean dietary plants. *Journal of Ethnopharmacology* 116: 144-151.

Cowan, A. K., Logie, M. R. R., Rose, P. D. and Phillips, L. G. 1995. Stress Induction of Zeaxanthin Formation in the β-Carotene Accumulating Alga *Dunaliella salina* Teod. *Journal of Plant Physiology* 146: 554-562.

Craft, B. D., Kerrihard, A. L., Amarowicz, R. and Pegg, R. B. 2012. Phenol-Based Antioxidants and the In Vitro Methods Used for Their Assessment. *Comprehensive Reviews in Food Science and Food Safety* 11: 148-173.

Custódio, L., Justo, T., Silvestre, L., Barradas, A., Duarte, C. V., Pereira, H., Barreira, L., Rauter, A. P., Albercío, F. and Varela, Jo. 2012. Microalgae of different phyla display antioxidant, metal chelating and acetylcholinesterase inhibitory activities. *Food Chemistry* 131: 134-140.

Davis, T. A., Volesky, B. and Mucci, A. 2003. A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research* 37: 4311-4330.

De Jesus Raposo, M. F., De Morais, R. M. S. C. and De Morais, A. M. M. B. 2013. Health applications of bioactive compounds from marine microalgae. *Life Sciences* 93: 479-486.

De La Noue, J. and De Paw, N. 1988. The potential of microalgal biotechnology: A review of production and uses of microalgae. *Biotechnology Advances* 6: 725-770.

De La Peña, M. R. and Villegas, C. T. 2005. Cell growth, effect of filtrate and nutritive value of the tropical Prasinophyte *Tetraselmis tetrathele* (Butcher) at different phases of culture. *Aquaculture Research* 36: 1500-1508.

Demirbas, A. 2010. Use of algae as biofuel sources. *Energy Conversion and Management* 51: 2738-2749.

Demirbas, A. and Fatih-Demirbas, M. 2011. Importance of algae oil as a source of biodiesel. *Energy Conversion and Management* 52: 163-170.

Demmig-Adams, B. and Adams, W. W. 2002. Antioxidants in photosynthesis and human nutrition. *Science* 298: 2149-2153.

Draelos, Z. D. 2001. Botanicals as topical agents. *Clinics in Dermatology* 19: 474-477.

Duan, X. J., Zhang, W. W., Li, X. M. and Wang, B. G. 2006. Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chemistry* 95: 37-43.

Durmaz, Y. 2007. Vitamin E (α-tocopherol) production by the marine microalgae *Nannochloropsis oculata* (Eustigmatophyceae) in nitrogen limitation. *Aquaculture* 272: 717-722.

El-Gamal, A. A. 2010. Biological importance of marine algae. *Saudi Pharmaceutical Journal* 18: 1-25.

Endar, V., Hutabarat, J. and Prayitno, B. 2013. Effect of using guillard and walne technical culture media on growth and fatty acid profiles of microalgae *Skeletonema* sp. in mass culture. *Journal of Coastal Development* 16: 50-56.

Farhadian, O., Yusoff, F. M. and Mohamed, S. 2008. Nutritional values of *Apocyclops dengizicus* (Copepoda: Cyclopoida) fed *Chaetocerous calcitrans* and *Tetraselmis tetrathele*. *Aquaculture Research* 40: 74-82.

Ferreira, M., Coutinho, P., Seixas, P., Fábregas, J. and Otero, A. 2009. Enriching Rotifers with "Premium" Microalgae. *Nannochloropsis gaditana*. *Marine Biotechnology* 11: 585-595.

Fidalgo, J. P., Cid, A., Torres, E., Sukenik, A. and Herrero, C. 1998. Effects of nitrogen source and growth phase on proximate biochemical composition, lipid classes and fatty acid profile of the marine microalga *Isochrysis galbana*. *Aquaculture* 166: 105-116.

Fung, A., Hamid, N. and Lu, J. 2013. Fucoxanthin content and antioxidant properties of *Undaria pinnatifida*. *Food Chemistry* 136: 1055-1062.

Gan, R., Kuang, L., Xu, X., Zhang, Y., Xia, E., Song, F. and Li, H. 2010. Screening of natural antioxidants from traditional Chinese medicinal plants associated with treatment of rheumatic disease. *Molecules* 15: 5988-5997.

Glencross, B. D. 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. *Reviews in Aquaculture* 1: 71-124.

Goh, S. H., Yusoff, F. M., and Loh, S. P. 2010. A Comparison of the Antioxidant Properties and Total Phenolic Content in A Diatom, *Chaetoceros* sp. and Green Microalgae, *Nannochloropsis* sp. *Journal of Agricultural Science* 2: 123-130.

Goiris, K., Muylaert, K., Fraeye, I., Foubert, I., Brabanter, J. and Cooman, L. 2012. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *Journal of Applied Phycology* 24:1477-1486.

Gong, Y., Hu, H., Gao, Y., Xu, X. and Gao, H. 2011. Microalgae as platforms for production of recombinant proteins and valuable compounds: progress and prospects. *Journal of Industrial Microbiology and Biotechnology* 38: 1879-1890.

Grampurohit, N., Ravikumar, P. and Mallya, R. 2011. Microemulsions for topical uses - A review. *Indian Journal of Pharmaceutical Education and Research* 45: 100-107.

Gracelin, D. H., Britto, A. J. and Kumar, B. J. R. 2013. Qualitative and quantitative analysis of phytochemicals in five Pteris species *International Journal of Pharmacy nad Pharmaceutical Sciences* 5: 105-107.

Graziani, G., Schiavo, S., Nicolai, M. A., Buono, S., Fogliano, V., Pinto, G. and Pollio, A. 2013. Microalgae as human food: chemical and nutritional characteristics of the thermo-acidophilic microalga *Galdieria sulphuraria*. *Food and Function* 4: 144-152.

Griffiths, T. W. 2010. Cosmeceuticals: coming of age. *British Journal of Dermatology* 162: 469-470.

Grobbelaar, J. U. 2012. Microalgae mass culture: the constraints of scaling-up. *Journal of Applied Phycology* 24: 315-318.

Grossi, V., Blokker, P. and Sinninghe-Damsté, J. S. 2001. Anaerobic biodegradation of lipids of the marine microalga *Nannochloropsis salina*. *Organic Geochemistry* 32: 795-808.

Guedes, A. C., Amaro, H. M., and Malcata, F. X. 2011. Microalgae as Sources of Carotenoids. *Marine Drugs* 9: 625-644.

Gunawan, E. R., Basri, M., Rahman, M. B. A., Salleh, A. B. and Rahman, R. N. Z. A. 2004. Lipase-catalyzed synthesis of palm-based wax esters. *Journal of Oleo Science* 53: 471-477.

Guschina, I. A. and Harwood, J. L. 2006. Lipids and lipid metabolism in eukaryotic algae. *Progress in Lipid Research* 45: 160-186.

Hajimahmoodi, M., Faramarzi, M., Mohammadi, N., Soltani, N., Oveisi, M. and Nafissi-Varcheh, N. 2010. Evaluation of antioxidant properties and total phenolic contents of some strains of microalgae. *Journal of Applied Phycology* 22: 43-50.

Hajji, M., Jarraya, R., Lassoued, I., Masmoudi, O., Damak, M. and Nasri, M. 2010. GC/MS and LC/MS analysis, and antioxidant and antimicrobial activities of various solvent extracts from *Mirabilis jalapa* tubers. *Process Biochemistry* 45: 1486-1493.

Hasui, M., Matsuda, M., Okutani, K. and Shigeta, S. 1995. In vitro antiviral activities of sulfated polysaccharides from a marine microalga (*Cochlodinium polykrikoides*) against human immunodeficiency virus and other enveloped viruses. *International Journal of Biological Macromolecules* 17: 293-297.

Herrero, M., Ibáñez, E., Cifuentes, A., Reglero, G. and Santoyo, S. 2006. *Dunaliella salina* microalga pressurized liquid extracts as potential antimicrobials. *Journal of Food Protection* 69: 2471-2477.

Huerlimann, R., De Nys, R. and Heimann, K. 2010. Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. *Biotechnology and Bioengineering* 107: 245-257.

Hung, L. C., Basri, M., Tejo, B. A., Ismail, R., Nang, H. L. L., Hassan, H. A. and May, C. Y. 2011. An improved method for the preparations of nanostructured lipid carriers containing heat-sensitive bioactives. *Colloids and Surfaces B: Biointerfaces* 87: 180-186.

Ibañez, E., Herrero, M., Mendiola, J. A., and Castro-Puyana, M. 2012. Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates. In: Marine Bioactive Compounds. Springer, p 55-98

Ip, P. F., Wong, K. H. and Chen, F. 2004. Enhanced production of astaxanthin by the green microalga *Chlorella zofingiensis* in mixotrophic culture. *Process Biochemistry* 39: 1761-1766.

Ismail, M., Phang, S. M., Tong, S. L. and Brown, M. T. 2002. A Modified Toxicity Testing Method Using Tropical Marine Microalgae. *Environmental Monitoring and Assessment* 75: 145-154.

Ismail, A., Marjan, Z. M. and Foong, C. W. 2004. Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry* 87: 581-586.

Jiang, L., Luo, S., Fan, X., Yang, Z. and Guo, R. 2011. Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO_2. *Applied Energy* 88: 3336-3341.

Kaur, C., and Kapoor, H. C. 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology* 37: 153-161.

Keng, P. S., Basri, M., Zakaria, M. R. S., Abdul Rahman, M. B., Ariff, A. B., Abdul Rahman, R. N. Z. and Salleh, A. B. 2009. Newly synthesized palm esters for cosmetics industry. *Industrial Crops and Products* 29: 37 - 44.

Khaiat, A. 2000. Botanical extracts. *Cosmetic Science and Technology Series* 1: 97-106

Kim, S. K., Ravichandran, Y. D., Khan, S. B. and Kim, Y. T. 2008. Prospective of the cosmeceuticals derived from marine organisms. *Biotechnology and Bioprocess Engineering* 13: 511-523.

Kligman, A. 2005. The Future of Cosmeceuticals: An Interview with Albert Kligman, MD, PhD. *Dermatologic Surgery* 31: 890-891.

Kobayashi, M., Kakizono, T., Nishio, N., Nagai, S., Kurimura, Y. and Tsuji, Y. 1997. Antioxidant role of astaxanthin in the green alga *Haematococcus pluvialis*. *Applied Microbiology and Biotechnology* 48: 351-56.

Kong, K. W., Mat-Junit, S., Aminudin, N., Ismail, A. and Abdul-Aziz, A. 2012. Antioxidant activities and polyphenolics from the shoots of *Barringtonia racemosa* (L.) Spreng in a polar to apolar medium system. *Food Chemistry* 134: 324-332.

Krohn, B. J., McNeff, C. V., Yan, B. and Nowlan, D. 2011. Production of algae-based biodiesel using the continuous catalytic Mcgyan® process. *Bioresource Technology* 102: 94-100.

Kuda, T. and Ikemori, T. 2009. Minerals, polysaccharides and antioxidant properties of aqueous solutions obtained from macroalgal beach-casts in the Noto Peninsula, Ishikawa, Japan. *Food Chemistry* 112: 575-581.

Kusuma, I.W., Kuspradini, H., Arung, E. T., Aryani, F., Min, Y. H., Kim, J. S., Kim, Y. U. 2011. Biological Activity and Phytochemical Analysis of Three Indonesian Medicinal Plants, *Murraya koenigii*, *Syzygium polyanthum* and *Zingiber purpurea*. *Journal of Acupuncture and Meridian Studies* 4: 75-79.

Lai, J. I., Yusoff, F. M. and Shariff, M. 2012. Large-scale culture of a tropical marine microalga *Chaetoceros calcitrans* (Paulsen) Takano 1968 at different temperatures using annular photobioreactors. *Pakistan Journal of Biological Science* 15: 635-640.

Lam, M. K. and Lee, K. T. 2012. Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnology Advances* 30: 673-690.

Latreille, B. and Paquin, P. 1990. Evaluation of emulsion stability by centrifugation with conductivity measurements. *Journal of Food Science* 55: 1666-1668.

Lecanu, L., Yao, W., Teper, G. L., Yao, Z. X., Greeson, J. and Papadopoulos, V. 2004. Identification of naturally occurring spirostenols preventing β-amyloid-induced neurotoxicity. *Steroids* 69: 1-16.

Lee, O. S., Kang, H. H. and Han, S. H. 1997. Oriental herbs in cosmetics: Plant extracts are reviewed for their potential as cosmetic ingredients. *Cosmetics and Toiletries* 112: 57-64.

Lester, G. E., Lewers, K. S., Medina, M. B. and Saftner, R. A. 2012. Comparative analysis of strawberry total phenolics via Fast Blue BB vs. Folin-Ciocalteu: Assay interference by ascorbic acid. *Journal of Food Composition and Analysis* 27: 102-107.

Li, H. B., Cheng, K. W., Wong, C. C., Fan, K. W., Chen, F. and Jiang, Y. 2007. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry* 102: 771-776.

Li, Y., Horsman, M., Wu, N., Lan, C. Q., and Dubois-Calero, N. 2008. Biofuels from Microalgae. *Biotechnology Progress* 24: 815-820.

Li, Y., Moheimani, N. R. and Schenk, P. M. 2012. Current research and perspectives of microalgal biofuels in Australia. *Biofuels* 3: 427-439.

Lin, Y., L., Wang, T. H., Lee, M. H. and Su, N. W. 2008. Biologically active components and nutraceuticals in the Monascus-fermented rice: A review. *Applied Microbiology and Biotechnology* **77**: 965-973.

Lintner, K., Mas-Chamberlin, C., Mondon, P., Peschard, O. and Lamy, L. 2009. Cosmeceuticals and active ingredients. *Clinics in Dermatology* 27:461-468.

Lorenz, R.T. and Cysewski, G. R. 2000. Commercial potential for Haematococcus microalgae as a natural source of astaxanthin. *Trends in Biotechnology* 18: 160-167.

Lourenço, S. O., Marquez, U. M. L., Mancini-Filho, J., Barbarino, E. and Aidar, E. 1997. Changes in biochemical profile of *Tetraselmis gracilis* I. Comparison of two culture media. *Aquaculture* 148: 153-168.

Lupo, M. P. 2005. Cosmeceutical Peptides. *Dermatologic Surgery* 31: 832-836.

Macías-Sánchez, M. D., Fernandez-Sevilla, J. M., Acién-Fernández, F. G., Cerón-García, M. C. and Grima, E. M. 2010. Supercritical fluid extraction of carotenoids from *Scenedesmus almeriensis*. *Food Chemistry* 123: 928-935.

Mahdi, E. S., Noor, A. M., Sakeena, M H., Abdullah, G. Z., Abdulkarim, M. F., Sattar and M. A. 2011. Formulation and in vitro release evaluation of newly synthesized palm kernel oil esters-based nanoemulsion delivery system for 30% ethanolic dried extract derived from local *Phyllanthus urinaria* for skin antiaging. *International journal of nanomedicine* 6: 2499-2512.

Manela-Azulay, M. and Bagatin, E. 2009. Cosmeceuticals vitamins. *Clinics in Dermatology* 27: 469-474.

Maisuthisakul, P., Suttajit, M. and Pongsawatmanit, R. 2007. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chemistry* 100: 1409-1418.

Manivannan, K., Anantharaman, P. and Balasubramanian, T. 2012. Evaluation of antioxidant properties of marine microalga *Chlorella marina* (Butcher, 1952). *Asian Pacific Journal of Tropical Biomedicine* 2: S342-S346.

Mariod, A. A., Ibrahim, R. M., Ismail, M. and Ismail, N. 2009. Antioxidant activity and phenolic content of phenolic rich fractions obtained from black cumin (*Nigella sativa*) seedcake. *Food Chemistry* 116: 306-312.

Martinez-Jeronimo, F. and Espinosa-Chavez, F. 1994. A laboratory-scale system for mass culture of freshwater microalgae in polyethylene bags. *Journal of Applied Phycology* 6: 423-425.

Mason, T. G. and Bibette, J. 1997. Shear rupturing of droplets in complex fluids. *Langmuir* 13: 4600-4613.

Mason, T. G., Wilking, J. N., Meleson, K., Chang, C.B. and Graves, S. M. 2006. Nanoemulsions: Formation, structure, and physical properties. *Journal of Physics Condensed Matter* 18: R635-R666

Mata, T. M., Martins, A. and Caetano, N. S. 2010. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews* 14: 217-232.

McClements, D. J. 1998. Food emulsions: principles, practice, and techniques. *CRC press,*

McClements, D. J. 2012. Nanoemulsions versus microemulsions: terminology, differences, and similarities. *Soft Matter* 8: 1719-1729.

Mendes, R. L., Reis, A. D. and Palavra, A. F. 2006. Supercritical CO_2 extraction of γ-linolenic acid and other lipids from Arthrospira (*Spirulina maxima*): Comparison with organic solvent extraction. *Food Chemistry* 99: 57-63.

Menke, S., Sennhenn, A., Sachse, J. H., Majewski, E., Huchzermeyer, B. and Rath, T. 2012. Screening of Microalgae for Feasible Mass Production in Industrial Hypersaline Wastewater Using Disposable Bioreactors. *Clean - Soil, Air, Water* 40: 1401-1407.

Miranda, M. S., Cintra, R. G., Barros, S. B. M. and Mancini-Filho, J. 1998. Antioxidant activity of the microalga *Spirulina maxima*. *Brazilian Journal of Medical and Biological Research* 31: 1075-1079.

Mirhosseini, H., Tan, C. P., Hamid, N. S. A. and Yusof, S. 2008. Effect of Arabic gum, xanthan gum and orange oil contents on ζ-potential, conductivity, stability, size index and pH of orange beverage emulsion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 315: 47-56.

Misra, H. P. and Fridovich, I. 1977. Purification and properties of superoxide dismutase from a red alga, *Porphyridium cruentum*. *Journal of Biological Chemistry* 252: 6421-6423.

Mohammady, N. W. 2004. Total, free and conjugated sterolic forms in three microalgae used in mariculture. *Zeitschrift fur Naturforschung* C 59: 619-624.

Moheimani, N.R. 2013. Long-term outdoor growth and lipid productivity of *Tetraselmis suecica*, *Dunaliella tertiolecta* and *Chlorella* sp. (Chlorophyta) in bag photobioreactors. *Journal of Applied Phycology* 25: 167-176.

Morganti, P., Sud, M. 2008. Cosmeceuticals. *Clinics in Dermatology* 26: 317

Moroney, J. V., Bartlett, S. G., and Samuelsson, G. 2001. Carbonic anhydrases in plants and algae. *Plant, Cell and Environment* 24: 141-153.

Mou, D., Chen, H., Du, D., Mao, C., Wan, J., Xu, H. and Yang, X.. 2008. Hydrogel-thickened nanoemulsion system for topical delivery of lipophilic drugs. *International Journal of Pharmaceutics* 353: 270-276.

Natrah, F., Yusoff, F. M., Shariff, M., Abas, F. and Mariana, N. 2007. Screening of Malaysian indigenous microalgae for antioxidant properties and nutritional value. *Journal of Applied Phycology* 19: 711-718.

Ng, S. H., Woi, P. M., Basri, M., Ismail, Z. 2013. Characterization of structural stability of palm oil esters-based nanocosmeceuticals loaded with tocotrienol. *Journal of nanobiotechnology* 11: 1-27.

Nichols, B. W., Wood, B. J. B. 1968. The occurrence and biosynthesis of gamma-linolenic acid in a blue-green alga,*Spirulina platensis*. *Lipids* 3: 46-50.

Okamoto, O. K., Asano, C. S., Aidar, E. and Colepicolo, P. 1996. Effects of cadmium on growth and superoxide dismutase activity of the marine microalgae, *Tetraselmis gracilis* (prasinophyceae) *Journal of Phycology* 32: 74-79.

Olaizola, M. 2003. Commercial development of microalgal biotechnology: from the test tube to the marketplace. *Biomolecular Engineering* 20: 459-466.

Onofrejová, L., Vašíčková, J., Klejdus, B., Stratil, P., Mišurcová, L., Kráčmar, S., Kopecký, J. and Vacek, J. 2010. Bioactive phenols in algae: The application of pressurized-liquid and solid-phase extraction techniques. *Journal of Pharmaceutical and Biomedical Analysis* 51: 464-470.

Otero, A. and Fábregas, J. 1997. Changes in the nutrient composition of *Tetraselmis suecica* cultured semicontinuously with different nutrient concentrations and renewal rates. *Aquaculture* 159: 111-123.

Othman, A., Ismail, A., Abdul-Ghani, N. and Adenan, I. 2007. Antioxidant capacity and phenolic content of cocoa beans. *Food Chemistry* 100: 1523-1530.

Pangestuti, R. and Kim, S. K. 2011. Biological activities and health benefit effects of natural pigments derived from marine algae. *Journal of Functional Foods* 3: 255-266.

Pelah, D., Sintov, A. and Cohen, E. 2004. The effect of salt stress on the production of canthaxanthin and astaxanthin by *Chlorella zofingiensis* grown under limited light intensity. *World Journal of Microbiology and Biotechnology* 20: 483-486.

Petkov, G. and Garcia, G. 2007. Which are fatty acids of the green alga *Chlorella*? *Biochemical Systematics and Ecology* 35: 281-285.

Phatarpekar, P. V., Sreepada, R. A., Pednekar, C. and Achuthankutty, C. T. 2000. A comparative study on growth performance and biochemical composition of mixed culture of *Isochrysis galbana* and *Chaetoceros calcitrans* with monocultures. *Aquaculture* 181: 141-155.

Plaza, M., Herrero, M., Cifuentes, A. and Ibáñez, E. 2009. Innovative Natural Functional Ingredients from Microalgae. *Journal of Agricultural and Food Chemistry* 57: 7159-7170.

Plaza, M., Santoyo, S., Jaime, L., García-Blairsy R. G., Herrero, M., Señoráns, F. J. and Ibáñez, E. 2010. Screening for bioactive compounds from algae. *Journal of Pharmaceutical and Biomedical Analysis* 51: 450-455.

Pulz, O. and Gross, W. 2004. Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology* 65: 635-648.

Rao, A. R., Dayananda, C., Sarada, R., Shamala, T. R. and Ravishankar, G. A. 2007. Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. *Bioresource Technology* 98: 560-64.

Reis, A., Mendes, A., Lobo-Fernandes, H., Empis, J. A., Novais, J. M. 1998. Production, extraction and purification of phycobiliproteins from *Nostoc* sp. *Bioresource Technology* 66: 181-187.

Renaud, S. M., Thinh, L.V., Lambrinidis, G. and Parry, D. L. 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture* 211: 195-214.

Rezaee, M., Basri, M., Raja Abdul Rahman, R. N. Z., Salleh, A. B., Chaibakhsh, N. and Fard-Masoumi, H. R. 2014. A multivariate modeling for analysis of factors controlling the particle size and viscosity in palm kernel oil esters-based nanoemulsions. *Industrial Crops and Products* 52: 506-511.

Rice-Evans, C., Miller, N., and Paganga, G. 1997. Antioxidant properties of phenolic compounds. *Trends in Plant Science* 2: 152-159.

Rise, M., Cohen, E., Vishkautsan, M., Cojocaru, M., Gottlieb, H. E. & Arad, S. M. 1994. Accumulation of Secondary Carotenoids in *Chlorella zofingiensis. Journal of Plant Physiology* 144: 287-292.

Rodolfi, L., Zittelli, G.C., Bassi, N., Padovani, G., Biondi, N., Bonini, G. and Tredici, M.R. 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering* 102: 100-112.

Rodriguez-Garcia, I. and Guil-Guerrero, J. L. 2008. Evaluation of the antioxidant activity of three microalgal species for use as dietary supplements and in the preservation of foods. *Food Chemistry* 108: 1023-1026.

Ronquillo, J., Matias, J., Saisho, T. and Yamasaki, S. 1997. Culture of *Tetraselmis tetrathele* and its utilization in the hatchery production of different penaeid shrimps in Asia. *Hydrobiologia* 358: 237-244.

Ruiz-Ruiz, F., Benavides, J. and Rito-Palomares, M. 2013. Scaling-up of a β-phycoerythrin production and purification bioprocess involving aqueous two-phase systems: Practical experiences. *Process Biochemistry* 48: 738-745.

Saavedra, M. P. S. and Voltolina, D. 1994. The chemical composition of *Chaetoceros* sp. (Bacillariophyceae) under different light conditions. C*omparative Biochemistry and Physiology Part B: Comparative Biochemistry* 107: 39-44.

Salim, N., Basri, M., Rahman, M. B. A., Abdullah, D. K. and Basri, H. 2012. Modification of palm kernel oil esters nanoemulsions with hydrocolloid gum for enhanced topical delivery of ibuprofen. *International journal of nanomedicine* 7: 4739-4747.

Salim, N., Basri, M., Rahman, M. B. A., Abdullah, D. K., Basri, H., Salleh, A. B. 2012. Phase behaviour, formation and characterization of palm-based esters nanoemulsion formulation containing ibuprofen. *Journal of Nanomedicine and Nanotechnology* 2: 2-5.

Santoyo, S., Rodríguez-Meizoso, I., Cifuentes, A., Jaime, L., García-Blairsy, R. G., Señorans, F. J. and Ibáñez, E. 2009. Green processes based on the extraction with pressurized fluids to obtain potent antimicrobials from *Haematococcus pluvialis* microalgae. *LWT - Food Science and Technology* 42: 1213-1218.

Satoh, M., Karaki, E., Kakehashi, M., Okazaki, E. Gotoh, T. and Oyama, Y. 1999. Heavy-metal induced changes in nonproteinaceous thiol levels and heavy-metal binding peptide in *Tetraselmis tetrathele* (Prasinophyte). *Journal of Phycology* 35: 989-994.

Sánchez-Mirón, A., Contreras-Gómez, A., García-Camacho, F., Molina-Grima, E. and Chisti, Y. 1999. Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae. *Journal of Biotechnology* 70: 249-270.

Sánchez-Saavedra, M. D. P. and Voltolina, D. 2006. The growth rate, biomass production and composition of *Chaetoceros* sp. grown with different light sources. *Aquacultural Engineering* 35: 161-165.

Scherholz, M. L. and Curtis, W. R. 2013. Achieving pH control in microalgal cultures through fed-batch addition of stoichiometrically-balanced growth media. *BMC biotechnology* 13: 1-15.

Scott, S. A., Davey, M. P., Dennis, J. S., Horst, I., Howe, C. J., Lea-Smith, D. J. and Smith, A. G. 2010. Biodiesel from algae: challenges and prospects. *Current Opinion in Biotechnology* 21: 277-286.

Servel, M. O., Claire, C., Derrien, A., Coiffard, L. and De Roeck-Holtzhauer, Y. 1994. Fatty acid composition of some marine microalgae. *Phytochemistry* 36: 691-693.

Shen, Y., Yuan, W., Pei, Z. J., Wu, Q. and Mao, E. 2009. Microalgae mass production methods. *Transactions of the ASABE* 52: 1275-1287.

Singh, R. N. and Sharma, S. 2012. Development of suitable photobioreactor for algae production- A review. *Renewable and Sustainable Energy Reviews* 16: 2347-2353.

Simões, S. I., Tapadas, J. M., Marques, C. M., Cruz, M. E. M., Martins, M. B. F. and Cevc, G. 2005. Permeabilisation and solubilisation of soybean phosphatidylcholine bilayer vesicles, as membrane models, by polysorbate, Tween 80. *European Journal of Pharmaceutical Sciences* 26: 307-317.

Solè, I., Pey, C. M., Maestro, A., González, C., Porras, M., Solans, C. and Gutiérrez, J. M. 2010. Nano-emulsions prepared by the phase inversion composition method: Preparation variables and scale up. *Journal of colloid and interface science* 344: 417-423.

Sonneville-Aubrun, O., Simonnet, J. T. and L'Alloret, F. 2004. Nanoemulsions: a new vehicle for skincare products. *Advances in Colloid and Interface Science* 108-109:145-149.

Sonneville-Aubrun, O., Babayan, D., Bordeaux, D., Lindner, P., Rata, G. and Cabane, B. 2009. Phase transition pathways for the production of 100 nm oil-in-water emulsions. *Physical Chemistry Chemical Physics* 11: 101-110.

Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, An. 2006. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering* 101: 87-96.

Srilatha, B. 2011. Nanotechnology in agriculture. *Journal of Nanomedicine and Nanotechnology* 2: 1-5.

Stahl, W., Schwarz, W. and Sies, H. 1993. Human serum concentrations of all-trans beta-and alpha-carotene but not 9-cis beta-carotene increase upon ingestion of a natural isomer mixture obtained from *Dunaliella salina* (Betatene). *The Journal of nutrition* 123: 847-851.

Stachurski, J. and MichaŁek, M. 1996. The effect of the ζ-potential on the stability of a non-polar oil-in-water emulsion. *Journal of Colloid and Interface Science* 184: 433-436.

Suali, E. and Sarbatly, R. 2012. Conversion of microalgae to biofuel. *Renewable and Sustainable Energy Reviews* 16: 4316-4342.

Sulaiman, A., Basri, M., Bakar-Salleh, A., Abdul-Rahman, R. N. Z. R. and Ahmad, S. 2005. Phase behavior of oleyl oleate with nonionic surfactants. *Journal of dispersion science and technology* 26: 689-691.

Tadros, T., Izquierdo, P., Esquena, J. and Solans, C. 2004. Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science* 108: 303-318.

Teo, B. S. X., Basri, M., Zakaria, M. R. S., Salleh, A. B., Rahman, R. N. and Rahman, M. B. A. 2010. A potential tocopherol acetate loaded palm oil esters-in-water nanoemulsions for nanocosmeceuticals. *Journal of nanobiotechnology* 8: 1-11.

Tsantili, E., Konstantinidis, K., Christopoulos, M. V., and Roussos, P. A. 2011. Total phenolics and flavonoids and total antioxidant capacity in pistachio (*Pistachia vera* L.) nuts in relation to cultivars and storage conditions. *Scientia Horticulturae* 129: 694-701.

Ugwu, C. U., Aoyagi, H. and Uchiyama, H. 2008. Photobioreactors for mass cultivation of algae. *Bioresource Technology* 99: 4021-4028.

Varfolomeev, S. D. and Wasserman, L. A. 2011. Microalgae as source of biofuel, food, fodder, and medicines. *Applied Biochemistry and Microbiology* 47: 789-807.

Valentão, P., Trindade, P., Gomes, D., Guedes de Pinho, P., Mouga, T., and Andrade, P.B. 2010. *Codium tomentosum* and *Plocamium cartilagineum*: Chemistry and antioxidant potential. *Food Chemistry* 119: 1359-1368.

Vega, J. M. P., Roa, M. A. C. and del Pilar Sánchez, M. 2010. Effect of culture medium and nutrient concentration on fatty acid content of *Chaetoceros muelleri*. *Rev. Latinoam Biotechnol Amb Algal* 1: 6-15.

Vijayabaskar, P., Vaseela, N. and Thirumaran, G. 2012. Potential antibacterial and antioxidant properties of a sulfated polysaccharide from the brown marine algae *Sargassum swartzii*. *Chinese Journal of Natural Medicines* 10: 421-428.

Viyoch, J., Klinthong, N. and Siripaisal, W. 2003. Development of oil-in-water emulsion containing Tamarind fruit pulp extract I. Physical characteristics and stability of emulsion. *Naresuan University Journal* 11: 29-44.

Vo, T. S., Ngo, D. H. and Kim, S. K. 2012. Marine algae as a potential pharmaceutical source for anti-allergic therapeutics. *Process Biochemistry* 47: 386-394.

Volkman, J. K., Barrett, S. M. and Blackburn, S. I. 1999. Eustigmatophyte microalgae are potential sources of C_{29} sterols, C_{22}-C_{28} n-alcohols and C_{28}-C_{32} n-alkyl diols in freshwater environments. *Organic Geochemistry* 30: 307-318.

Volkman, J. K., Kearney, P. and Jeffrey, S. W. 1990. A new source of 4-methyl sterols and 5α(H)-stanols in sediments: prymnesiophyte microalgae of the genus Pavlova. *Organic Geochemistry* 15: 489-497.

Wangensteen, H., Samuelsen, A. B. and Malterud, K. E. 2004. Antioxidant activity in extracts from coriander. *Food Chemistry* 88: 293-297.

Wijffels, R. H., Kruse, O. and Hellingwerf, K. J. 2013. Potential of industrial biotechnology with cyanobacteria and eukaryotic microalgae. *Current Opinion in Biotechnology* 24: 405-413.

Wikfors, G. H., Twarog, J. W. and Ukeles, R. 1984. Influence of Chemical Composition of algal food sources on growth of juvenile oysters, Crassostrea Virginica. The Biological Bulletin 167: 251-263.

Wikfors, G. H., Patterson, G. W., Ghosh, P., Lewin, R. A., Smith, B. C. and Alix, J. H. 1996. Growth of post-set oysters, *Crassostrea virginica*, on high-lipid strains of algal flagellates *Tetraselmis* spp. *Aquaculture* 143: 411-419.

Wu, J. and Zhong, J. J. 1999. Production of ginseng and its bioactive components in plant cell culture: current technological and applied aspects. *Journal of Biotechnology* 68: 89-99.

Yaakob, Z., Ali, E., Zainal, A., Mohamad, M. and Takriff, M. S. 2014. An overview: biomolecules from microalgae for animal feed and aquaculture. *Journal of Biological Research-Thessaloniki* 21: 1-10.

Zhang, D., Xue, S., Sun, Z., Liang, K., Wang, L., Zhang, Q. and Cong, W. 2014. Investigation of continuous-batch mode of two-stage culture of *Nannochloropsis* sp. for lipid production. *Bioprocess and Biosystems Engineering* 37: 2073-2082.

Zhang, W. W., Duan, X. J., Huang, H. L., Zhang, Y. and Wang, B. G. 2007. Evaluation of 28 marine algae from the Qingdao coast for antioxidative capacity and determination of antioxidant efficiency and total phenolic content of fractions and subfractions derived from *Symphyocladia latiuscula* (Rhodomelaceae). *Journal of Applied Phycology* 19: 97-108.

Zittelli, G., Rodolfi, L., Biondi, N. and Tredici, M.R. 2006. Productivity and photosynthetic efficiency of outdoor cultures of *Tetrasemis suecica* in annular columns. *Aquaculture* 261: 932-943.

Zhu, W. and Gao, J. 2008. The Use of Botanical Extracts as Topical Skin-Lightening Agents for the Improvement of Skin Pigmentation Disorders. *Journal Investigative Dermatology Symposium Proceedings* 13: 20-24.