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PROXIMATE COMPOSITION AND BIOACTIVITIES OF CORN SILK (STIGMA MAYDIS) FOR POTENTIAL COSMECEUTICAL APPLICATION

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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

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Cosmetics are products that are used to improve the appearance and health of the skin. In order to enhance the appearance and provide a healthy skin, many ingredients that act as antioxidant, whitening and anti-ageing agent are included in the product. However, the source and safety of the ingredients has become the issues among Muslim consumers as there are many products in the market contained dubious ingredients. Some of the products contained lard as substitute oil because it is cheaper and readily available and some of the chemical-based cosmetics products may cause skin problems to the users. As such, the *halal* and natural-based cosmetic ingredients are an important research area to be studied as it could provide an alternative for consumers especially for Muslims. Therefore, the aims of this study were to determine the proximate composition and to evaluate the antioxidant, whitening, and anti-ageing properties of three types of corn silk which are sweet corn (SC), baby corn (BC) and pearl corn (PC) extracts for potential cosmeceutical application. A study on proximate compositions of SC, BC, and PC powder was conducted and the results showed that BC had the highest moisture (16.5%) and fat content (1.5%), while PC had the highest protein content (20.3%). PC and SC have approximately the same amount of crude fibre which is 21.06 and 21.83%, respectively. However, there is no significant difference was observed among the three samples in ash content. The ash was further analysed for their mineral content which is calcium (Ca), sodium (Na), magnesium (Mg), potassium (K), iron (Fe), and zinc (Zn). The results showed that PC contained the highest amount of Ca (0.17%), K (2%), Fe (0.0097%), and Zn (0.0094%), while SC contained the highest amount of Na (0.026%). Same amount of Mg was observed among SC and PC which is 0.14%. The total phenolic content (TPC) of the extracts are measured using Folin-Ciocalteu reagent and the total flavonoids content (TFC) was determined using aluminium chloride ($AlCl_3$) colorimetric assay. The results of the extracts for TPC and TFC ranged from 6.0 to 20.4 mg GAE/g extract and 1.0 to 58.4 mg

RE/g extract, respectively. The antioxidant activity of the samples was then evaluated by using three different assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, metal ion chelating capacity, and β-carotene-linoleate bleaching activity. The SC extract exhibited the strongest activity on all antioxidative assays as compared with BC and PC extracts. The *in vitro* enzymatic assay was conducted on corn silk extracts to evaluate their whitening and anti-ageing properties. The results showed that all extracts exhibited a low activity for tyrosinase inhibition at all concentrations which ranged from 15.1 to 26.3%. The anti-elastase and anti-matrix metalloproteinase-1 (MMP-1) assay was assessed for anti-ageing properties and the results indicated that SC extract had the highest activity for both assays which is 67.1% and 49.5%, respectively at 20 mg/mL concentration. In conclusions, this study revealed that SC extracts could be a potentially used as alternative ingredient for *halal* cosmetic products that have antioxidants and anti-ageing properties. Further studies are required to optimize the potential of SC extracts in cosmeceuticals.

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KOMPOSISI PROKSIMAT DAN BIOAKTIVITI SUTERA JAGUNG (*STIGMA MAYDIS*) UNTUK POTENSI APLIKASI KOSMESUTIKAL

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Kosmetik ialah produk yang digunakan untuk memperbaiki penampilan dan kesihatan kulit. Untuk menyerlahkan penampilan dan menghasilkan kulit yang sihat, pelbagai bahan digunakan sebagai agen antioksida, pemutihan dan anti-penuaan di dalam produk kosmetik. Walaubagaimanapun, sumber dan keselamatan bahan-bahan tersebut sering menjadi isu di kalangan pengguna Islam berikutan terdapat banyak produk di pasaran mengandungi bahan-bahan yang meragukan. Sebagai contoh, terdapat produk yang menjadikan lemak babi sebagai minyak pengganti untuk menghasilkan produk kosmetik kerana ianya lebih murah dan mudah diperolehi. Selain itu, produk kosmetik yang berasaskan bahan kimia akan menyebabkan masalah kulit kepada pengguna. Sehubungan itu, kajian mengenai bahan-bahan kosmetik dari sumber yang halal dan semulajadi merupakan bidang yang penting kerana ia dapat memberikan pilihan kepada pengguna terutama pengguna yang beragama Islam. Oleh yang demikian, objektif kajian ini adalah untuk menentukan komposisi proksimat dan menilai ciri-ciri antioksidan, pemutihan, dan anti-penuaan bagi tiga jenis sutera jagung iaitu jagung manis (SC), jagung muda (BC) dan jagung mutiara (PC) sebagai potensi dalam aplikasi kosmeseutikal. Kajian komposisi proksimat ke atas serbuk sutera jagung (SC, BC, PC) telah dijalankan dan keputusan menunjukkan bahawa serbuk BC mempunyai peratusan kelembapan (16.5%) dan lemak (1.5%) paling tinggi manakala PC mempunyai kandungan protein (20.3%) yang paling tinggi. PC dan SC mempunyai jumlah serat mentah yang hampir sama iaitu 21.06 dan 21.83%. Walaubagaimanapun, tiada perbezaan yang ketara diperhatikan bagi kandungan abu untuk ketiga-tiga sampel tersebut. Kemudian, abu sutera jagung dianalisis untuk menentukan kandungan mineral seperti Kalsium (Ca), Natrium (Na), Magnesium (Mg), Kalium (K), Zat besi (Fe), dan Zink (Zn). Keputusan kajian menunjukkan bahawa PC mengandungi jumlah kandungan Ca (0.17%), K (2%), Fe (0.0097%), dan Zn (0.0094%) yang paling

tinggi, manakala SC mengandungi jumlah kandungan Na (0.026%) yang paling tinggi. Jumlah kandungan Mg yang sama dapat diperhatikan bagi sutera jagung SC dan PC iaitu 0.14%. Jumlah kandungan fenolik (TPC) daripada ekstrak diukur menggunakan reagen Folin-Ciocalteu manakala jumlah kandungan flavonoid (TFC) ditentukan dengan menggunakan kaedah kolorimetrik Aluminium klorida ($AlCl_3$). Julat keputusan kandungan fenolik dan flavonoid masing-masing adalah diantara 6.0 hingga 20.4 mg bersamaan Asid galik (GAE)/g ekstrak dan 1.0 hingga 58.4 mg bersamaan Rutin (RE)/g ekstrak. Aktiviti antioksidan bagi semua ekstrak dinilai dengan menggunakan tiga kaedah yang berbeza iaitu aktiviti perencatan radikal 2,2-Diphenyl-1-picrylhydrazyl (DPPH), keupayaan memerangkap ion logam, dan aktiviti pelunturan β-karotena-linoleate. Keputusan menunjukkan bahawa ekstrak SC menghasilkan aktiviti yang tertinggi bagi semua kaedah antioksidan berbanding ekstrak BC dan PC. Kaedah enzim *in vitro* telah dijalankan ke atas semua ekstrak sutera jagung bagi menentukan ciri-ciri pemutihan dan anti-penuaan. Keputusan kajian menunjukkan bahawa semua ekstrak menghasilkan peratusan aktiviti yang rendah untuk perencatan enzim tyrosinase pada semua kepekatan iaitu antara 15.1 hingga 26.3%. Kaedah anti-elastase dan anti-matriks metalloproteinase-1 (MMP-1) telah digunakan untuk menentukan ciri anti-penuaan dan keputusan menunjukkan bahawa pada kepekatan 20 mg/mL, ekstrak SC memberikan peratusan aktiviti tertinggi bagi kedua-dua ujian tersebut iaitu masing-masing adalah 67.1 dan 49.5%. Kesimpulannya, kajian ini mendedahkan bahawa ekstrak SC berpotensi untuk dijadikan bahan alternatif bagi menghasilkan produk kosmetik yang halal dan mempunyai ciri-ciri antioksidan dan anti-penuaan. Walaubagaimanapun, kajian lanjut diperlukan untuk memaksimumkan potensi ekstrak SC di dalam produk kosmeseutikal.

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Thank you.

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
AF	Acetic ether
AGEs	Advanced glycation end products
$AlCl_3$	Aluminium chloride
AOAC	Association of Official Analytical Chemists
ATN	Acute tubular necrosis
BC	Baby corn
BChE	Butyrylcholinesterase
BF	Butanol fraction
BG	Blood glucose
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BUN	Blood urea nitrogen
Ca	Calcium
Cg	Carrageenan
COX-2	Cyclooxygenase-2
DNA	Deoxyribonucleic acid
DCS	Dry corn silk
DE	Dry extract
DMSO	Dimethyl Sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
EF	Ethanol fraction
EGCG	Epigallocatechin gallate
EtOAc	Ethyl Acetate
EtOH	Ethanol
FCS	Flavonoids corn silk
Fe	Iron
FRAP	Ferric reducing antioxidant power
FST	Force swimming test
FW	Fresh weight
GAE	Gallic acid equivalent
GC-MS	Gas chromatography- mass spectrometry
GR	Glutathione reductase
GM	Gentamicin
GPX	Glutathione peroxidase
GSDS	Glutathione disulfide
GSH	Glutathione
HCl	Hydrochloric acid
HDL-C	High-density lipoprotein cholesterol
HNO_3	Nitric acid
H_2SO_4	Sulphuric acid
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
K	Potassium
LDL-C	Low-density lipoprotein cholesterol
L-DOPA	L-3,4-dihydroxyphenylalanine

LPO	Lipid peroxidation
LPS	Lipopolysaccharide
MDA	Malondialdehyde
Mg	Magnesium
MMP-1	Matrix metalloproteinases-1
MMPs	Matrix metalloproteinases
Na	Sodium
NaOH	Sodium hydroxide
NNGH	N-Isobutyl-N-(4-methoxyphenylsulfonyl) glycyl hydroxamic acid
$^{\cdot}OH$	Hydroxyl radical
O_2^-	Superoxide anion
OGTT	Oral glucose tolerance test
PC	Pearl corn
PF	Petroleum ether
PO CS	Polysaccharides corn silk
RE	Rutin equivalent
ROS	Reactive oxygen species
S.A.N.A	*N*-succ-$(Ala)_3$-nitroanalide
SC	Sweet corn
SO	Superoxide
SOD	Superoxide dismutase
STZ	Streptozotocin
TC	Total cholesterol
TDF	Total dietary fibre
TFC	Total flavonoids content
TG	Triglycerides
TNF	Tumor necrosis factor-α
TPC	Total phenolic content
TST	Tail suspension test
WF	Water fraction
Zn	Zinc

CHAPTER 1

INTRODUCTION

Increasing number of reactive oxygen species (ROS) in human body are caused by the exposure to sunlight, pollution and radiation (Szydłowska-Czerniak *et al*., 2008). The increasing in ROS could enhance melanin biosynthesis melanocyte and resulted in hyperpigmentation diseases (Wang *et al*., 2006). The UV exposures are able to cause wrinkle formation to the skin due to the alteration of connective tissue by free radicals (Thring *et al*., 2009). Therefore, the ability of antioxidant to inhibit lipid oxidation and scavenge free radicals is greatly important in decreasing the oxidative damage in order to prevent skin aging and hyperpigmentation. Thus, the antioxidant agents are needed in cosmetic ingredients to reduce or delay the process of skin aging as well as maintaining the healthy skin (Chermahini *et al*., 2011). The growing awareness among consumer regarding the toxicity of synthetic antioxidants had shifted them to use natural antioxidants which safer and contain some bioactive compounds (Liu *et al*., 2011a). However, for Muslim consumers, the source of the cosmetic ingredients become an issue as they are prohibited to apply or consume any porcine-based and/or animal-based products that are not slaughtered according to *Shariah* (Islamic) law. Furthermore, the cosmetic products must not harm or hazardous to the consumer in order to become *halal* compliance and to fulfill the *toyyiban* aspect as we know many chemical-based products in the market use hydroquinone as one of the ingredients which can cause skin cancer. For these reasons, the searches of natural antioxidants are an important study to provide an alternative for Muslim consumers.

Phenolic acids are simple phenols derived from benzoic and cinnamic acid that can be found in free or complexes forms (Marina *et al*., 2009). These phenolics are important in counteract the ROS and reduce the effect of oxidative stress (Kim *et al*., 2012). Some phenolics possess the tyrosinase inhibitory effect for depigmentation of the skin (Palanisamy *et al*., 2011). These compounds have been found in many plants and shows high correlation with antioxidant capacity (Keser *et al*., 2013). Fruits and vegetables are well reported to contain of phenolic compounds which play a major role as antioxidants (Othman *et al*. 2007).

Several fruits and vegetables are consumed as processed products, hence, many by-products and wastes generated during the processing. For example, industrial processing of tomatoes contributed 10-30% wastes of their weight which consists part of pulp, seeds and skin (Savatovic *et al*., 2012). These wastes consist of valuable compounds that may contribute to high antioxidant activity. Meanwhile, grape skins, seeds and stems are by-products generated in grape juice and wine processing; and these wastes contained many polyphenol compounds (Keser *et al*., 2013).

Corn silk (*Stigma maydis*) is the stigmas of maize female flowers that are found inside the husks. It becomes waste materials when baby and matured corn were processed into food since the utilization of corn in food industry is broad. It was estimated that about 50,000 tonnes of corn silk waste was discarded each year (The estimation is calculated based on the total corn silk weight is equal to the weight of the young baby corn and the number of small holders planting corn as a source of baby corn in the country) (Nurhanan *et al*., 2012). Traditionally, corn silk has been used as therapeutic remedy for inflammation of urinary bladder and prostate as well as irritation in urinary bladder (Wan Rosli *et al*., 2008). It is rich in phenolic compound, particularly flavonoids that are known to significantly affect human health (Liu *et al*., 2011a). The biological activities of corn silk are well reported in the literatures such as antioxidant activity (Nurhanan *et al*., 2012; Liu *et al*., 2011a; Ebrahimzadeh *et al*., 2008; Maksimovic *et al*., 2003), antidiabetic (Zhao *et al*., 2012), antidepressant (Zhao *et al*., 2012; Ebrahimzadeh *et al*., 2009), and anti-inflammatory (Wang *et al*., 2011b). Corn silk also is use as food additive to improve nutrient content and physical characteristic of chicken and beef patties (Wan Rosli *et al*., 2011a, Wan Rosli *et al*., 2011b). These studies of corn silk provide and information on the potential use of corn silk as antioxidative, whitening and anti-ageing agent in cosmetic products. Therefore, it is necessary to carried out a research work of corn silk on cosmeceutical field since there is no study regarding the potential of corn silk as cosmetic ingredients.

1.1 Problem statements

The status of existing cosmetic products becomes an issue among Muslims due to their concern about the sources and the safety of the products. The antioxidant, anti-aging, and skin whitening products that available in the market are not necessary *halal* compliance. In addition, the ingredients used are mostly derived from chemical-based products that cause skin and eye allergic effect to the users, thus, it does not fulfilling the toyyiban aspect of *halal* standard requirements. Therefore, it is important to find an alternative ingredients containing antioxidants, anti-aging and whitening properties from natural sources as well as meeting the *halal* compliance.

1.2 Significance of the study

In this study, corn silk can be potential alternative ingredient containing antioxidant, anti-aging and whitening properties from natural sources, hence, it can be developed to produce safe and *halal* antioxidant, anti-ageing and whitening for cosmeceutical products. In addition, the utilization of corn silk could also reduce the waste generated by corn processing industries.

1.3 Objectives

The objectives of the study were as follows:

i) To determine the proximate compositions of corn silk from pearl, sweet, and baby corn.
ii) To evaluate the bioactivities (antioxidants, whitening and anti-ageing) of corn silk extracts from pearl, sweet, and baby corn for potential cosmeceutical applications.

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