PROTEIN PROFILE AND ANTIOXIDANT ACTIVITY OF *Moringa oleifera* SEED

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DEDICATION

This dissertation is dedicated to my father, my pillar of strength who always supports, encourage and believe that I can reach many more milestones. Also, to my mother, who always keeps me going without giving up on me with her unconditional love, trust and support.

DAD & MUM, THIS IS FOR YOU

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ABSTRACT

Moringa oleifera is one of the common plants found in different parts of the world with medicinal and nutritional properties. Many studies have proved the edibility of all parts of the tree such as leaves, pods, and seeds which have the ability to treat and prevent diseases making it a highly valued plant. To date, no proteomics and antioxidant studies are available on different parts of the fresh and naturally sun-dried seeds which are the whole seed, seed and seed coat. Hence, this study focuses on the One-dimensional (1D) electrophoretic pattern of the fresh and dry seed in three different parts determined using Sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Protein identification was further analysed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analysis for the seed samples with the highest band count. Next, antioxidant activity was carried out for the protein extract using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and Ferric Reducing Antioxidant Power (FRAP) assay. As for the crude extract, antioxidant activity was tested using FRAP and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Based on the electrophoretic pattern obtained, there were approximately 16-29 bands in all the samples in which the dry seed had the highest band count followed by the fresh seed. As for the protein identification, a total of 328 proteins for the fresh seed and 326 proteins for the dry seed was identified. The proteins were further classified according to their functional groups based on GoMapMan database. The highest functional groups for both samples were protein biosynthesis, followed by metabolism and RNA biosynthesis. In terms of antioxidant activity, the dry seed coat had the highest antioxidant value for protein extract tested on both assays. Meanwhile, for the crude extract, the dry whole seed resulted in the highest antioxidant value for DPPH assay while the dry seed coat had the highest FRAP assay. Overall, the seed coat exhibits good antioxidant activity in both protein and crude extract. The findings obtained provide information on the proteins and antioxidant activity found in fresh and dry seed of *Moringa oleifera.* Thus, this information could work as a platform for further analysis on the protein identification of other seed parts influenced by environmental conditions. In addition, the information could be used for comparisons with commercialized *Moringa* seed products for better understanding on the effect of drying method on the nutrient content of plant seeds.

ABSTRAK

Moringa oleifera merupakan salah satu tumbuhan yang boleh dijumpai di pelbagai negara di serata dunia dengan ciri-ciri perubatan dan nutrisi. Banyak kajian telah membuktikan bahawa semua bahagian pokok ini seperti daun, buah, dan biji mempunyai keupayaan untuk merawat dan mencegah penyakit menjadikannya tumbuhan yang sangat berharga. Setakat ini, tiada lagi kajian proteomik dan antioksidan pada bahagian yang berbeza untuk benih segar dan benih yang dikeringkan secara semula jadi iaitu keseluruhan benih, benih dan kulit benih. Oleh itu, kajian ini memberi tumpuan kepada elektroforasi 1-dimensi (1D) benih segar dan kering dalam ketiga-tiga bahagian yang berlainan menggunakan gel elektroforasis *Sodium dodecyl-sulfate polyacrylamide* (*SDS-PAGE*). Identifikasi protein telah dianalisa menggunakan *Liquid Chromatography-Tandem Mass Spectrometry* (*LC-MS/MS)* untuk sampel benih yang mempunyai bilangan jalur yang terbanyak. Seterusnya, aktiviti antioksidan telah dijalankan untuk ekstrak protein menggunakan kaedah *2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)* (*ABTS)* dan *Ferric Reducing Antioxidant Power (FRAP)*. Untuk ekstrak mentah, aktiviti antioksidan telah dianalisa menggunakan kaedah *FRAP* dan *2,2-Diphenyl-1-picrylhydrazyl* (*DPPH)*. Berdasarkan corak elektroforasi, terdapat 16-29 jalur yang dikenal pasti dalam semua sampel di mana sampel benih kering mempunyai jalur yang terbanyak diikuti benih segar. Untuk identifikasi protein, 328 protein untuk benih segar dan 326 protein untuk benih kering telah dikenal pasti. Protein yang diidentifikasi seterusnya diklasifikasikan mengikut kumpulan berfungsi berdasarkan pangkalan data *GoMapMan*. Kumpulan berfungsi tertinggi untuk kedua-dua sampel adalah biosintesis protein, diikuti oleh metabolisme dan biosintesis RNA. Kulit benih didapati mempunyai nilai antioksidan yang tertinggi dalam ekstrak protein untuk kedua-dua kaedah. Untuk ekstrak mentah, keseluruhan benih kering mempunyai nilai antioksidan yang tertinggi untuk kaedah *DPPH* manakala kulit benih kering mempunyai nilai *FRAP* yang tertinggi. Secara keseluruhan, kulit benih mempunyai aktiviti antioksidan yang baik untuk kedua-dua ekstrak protein dan ekstrak mentah. Hasil kajian yang diperolehi dalam kajian ini memberi maklumat mengenai protein dan aktiviti antioksidan yang terdapat dalam buah segar dan kering *Moringa oleifera*. Oleh itu, maklumat ini dapat menjadi landasan untuk analisis lanjut mengenai identifikasi protein untuk bahagian benih yang lain yang dipengaruhi oleh keadaan persekitaran. Selain itu, maklumat itu boleh digunakan untuk perbandingan dengan produk benih *Moringa* yang dikomersialkan untuk pemahaman yang lebih baik mengenai kesan kaedah pengeringan pada kandungan nutrien benih tumbuhan.

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LIST OF ABBREVIATIONS

1D	-	1-Dimensional
ABA	-	Abscisic acid
ABTS	-	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
APS	-	Ammonium persulfate
ATP	-	Adenosine triphosphate
BITC	-	Benzyl isothiocyanate
BSA	-	Bovine serum albumin
ACN	-	Acetonitrile
DNA	-	Deoxyribonucleic acid
DPPH	-	2,2-Diphenyl-1-picrylhydrazy
DTT	-	Dithiothreitol
EDTA	-	Ethylenediaminetetraacetic acid
FAO	-	The Food and Agricultural Organization
$FeSO_4.H_2O$	-	Iron (II) sulphate
FRAP	-	Ferric Reducing Antioxidant Power
GA	-	Gibberellic acid
HCl	-	Hydrochloric acid
IAA	-	Iodoacetamide
KCl	-	Potassium chloride
MS	-	Mass spectrometry
LC-MS/MS	-	Liquid chromatography-tandem mass spectrometry
NCBI	-	National Center for Biotechnology Information
NH_4CHO_3	-	Ammonium bicarbonate
OPP	-	Oxidative Pentose Phosphate Pathway
PEITC	-	Phenethyl isothiocyanate
PMSF	-	Phenylmethylsulphonyl fluoride
PR	-	Pathogen related
RNA	-	Ribonucleic acid
ROS	-	Reactive oxygen species
RSA	-	Radical scavenging activity

SDS-PAGE	-	Sodium dodecyl-sulphate polyacrylamide gel electrophoresis
SEM	-	Standard error mean
TCA	-	Tricarboxylic acid
TEMED	-	Tetramethylethylenediamine
TPTZ	-	2,4,6-Tripyridyl-S-Triazine
Uniprot	-	Universal Protein Resources
WHO	-	World Health Organization

LIST OF SYMBOLS

β	-	Beta
°C	-	Degree Celsius
%	-	Percent
cm	-	Centimetre
g	-	Gram
Da	-	Dalton
kDa	-	Kilodalton
kg	-	Kilogram
L	-	Litre
M	-	Molar
µL	-	Microliter
µM	-	Micromolar
µg	-	Microgram
mg	-	Milligram
mL	-	Millilitre
m/z	-	Mass per charge
nm	-	Nanometer
rpm	-	Rotation per minute
V	-	Volume
v/v	-	Volume per volume
w/v	-	Weight per volume

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CHAPTER 1

INTRODUCTION

1.1 Problem Background

Malaysia being one of the megadiverse countries with highest endemism have over 2000 plants discovered to possess properties beneficial to the human health due to their nutritive value and abundant bioactive compounds (Bakar *et al.*, 2018). They are currently being actively researched in depth globally to identify the nutritional contents and benefits to the complex human body. Among the many traditional medicinal plants discovered today, *Moringa oleifera* originating from the family of Moringaceae, is one of the well-known and widely distributed species that is rich with nutrition serving as a source of food and medicine. Daba (2016) reported that the World Health Organization (WHO) addressed *Moringa oleifera* as one of the food sources that helps treat malnutrition. Besides that, this tree is also actively being explored today due to its potential to treat diseases such as cancer, anaemia, diabetes, asthma, malaria and skin diseases. Most parts of the tree consisting of leaves, roots, barks, fruit, flower, seed and pods are edible and consumable (Ramachandran *et al.*, 1980).

Many studies have stated that various parts of the well adapted tree have been discovered to be rich with nutritional content and antioxidants. Studies on the nutrient composition on the fresh and dry leaves, leaf powder, pods and seeds confirmed that the *Moringa oleifera* is abundant with nutrients and antinutrients such as vitamins (B1, B2, B3, C, E), calcium, magnesium, iron, proteins and carbon. Besides that, many medicinal plants including *Moringa oleifera* contain great antioxidant potential which will be helpful to reduce oxidative stress and treat human diseases. Antioxidants are important to the antioxidant system in human body to scavenge free radicals especially if the sources are natural and not synthetic. Based on research by Fitriana *et al.* (2016), the leaves of the *Moringa oleifera* was stated to

have phenolic compounds such as kaempferol and gallic acid which act as a natural antioxidant sources and prevent diseases. Makkar and Becker (1997) that studied different parts of the tree for their antiquality and nutrient factors concluded that the leaves of the trees have high amount of essential amino acids and true protein with low antinutritional factors. Hence, *Moringa oleifera* is suggested to work well as a protein supplement (Gopalakrishnan *et al.*, 2016). Additionally, the presence of various anticancerous agents mainly isothiocyanate, glucosinolates, niazimicin and glycoside compounds that was reported to play a role in anticancerous activity makes it a potential medicine to combat the cancer (Berkovich *et al.,* 2013). Other phytochemical actions that *Moringa oleifera* possess includes antimicrobial, anti-inflammatory, cardiovascular and anti-infertility activity (Anwar *et al.,* 2007).

Throughout the years, each part of *Moringa oleifera* tree is being thoroughly studied to analyse the nutritional and medicinal benefits they carry upon. The plant has been repeatedly reported to have antioxidant compounds, making it a research subject for further discovery of potential compounds and benefits for human consumptions. Besides that, repeated statement on its high nutrient content including protein makes this plant as another target to study their protein content. Also, antioxidant analysis on the seed of this tree are little known till now as most of the researches take interest in the leaves of *Moringa oleifera*. In addition, no proteomic studies have been conducted on *Moringa oleifera* seeds till date which is important for better understanding on the protein identity and its functions as it is crucial to contemplate the content of essential amino acids and its digestibility when consuming them. Thus, this research aims to identify the protein profile and antioxidant activity in *Moringa oleifera* seeds.

1.2 Problem Statement

Moringa oleifera is a plant well known as a traditional food source with natural nutritional properties. Current researches further prove the benefit of the tree in terms of medical line in which each part of the tree carries a specific function such as possessing high protein content, antioxidant activity and anticancer agents (Anwar

et al., 2007). Over the years, researchers have identified phytochemical constituents on the seeds regardless of it being commercialised seed, dried using different methods or in different forms such as raw, germinated or fermented flour. In addition to that, the seeds also possess anti-inflammatory, anti-cancer, hepatoprotective and anti-asthmatic activity and has potential to treat diseases such as epilepsy gout, rheumatism, sexually transmitted diseases and Crohn's disease (Paikra, 2017; Daba, 2016). These makes the seeds of the *Moringa oleifera* equally important as other parts of the tree. Currently, the fresh pods and dry seeds are being commercialised for daily consumption and for culinary purposes. However, the differences the fresh and dry seed carry in terms of its pharmacological actions and phytochemical constituents remain unknown. To date, proteome analysis and antioxidant activity is being investigated on the seeds of *Moringa oleifera*. However, this study aims to focus more on the difference in protein content and antioxidant activity in the protein and crude extract of fresh and naturally sun dried *Moringa oleifera* seed. Thus, the outcome of this research will accelerate further work in studying the *Moringa oleifera* seeds for better understanding and more discovery in the benefits they carry regardless of it being fresh or dry.

1.3 Research Objectives

The objectives of the research are:

(a) To determine the protein content and the electrophoretic pattern from fresh and dry seed of *Moringa oleifera*.

(b) To identify the proteins from the fresh and dry seed of *Moringa oleifera*.

(c) To determine the antioxidant activity of the protein and crude extracts of *Moringa oleifera* seeds.

1.4 Significance and Contribution of This Study

To date, no studies have been conducted to compare the fresh and dry seeds especially in terms of whole seed, seed and seed coat. This is important because it can provide us information on the nutritional value each part of the seed carries in two different conditions. Also, no preliminary one-dimensional (1D) protein analysis has been carried out on the seeds of *Moringa oleifera.* Thus, this project focuses on obtaining the electrophoretic pattern of the fresh and dry seed. Besides that, the identity of protein found in the fresh and dry seeds will also provide better understanding on the protein function the seed carries which can be further researched to be used as a potential tool to prevent or diagnose diseases. This project also aims to focus on the different value the protein and crude extract possess. In summary, this project was conducted to study the antioxidant activity and protein profile in the *Moringa oleifera* seeds which can serve as a platform to study the nutritional and medicinal properties of the seeds.

1.5 Scope of Study

The scope of this study was to obtain the protein content in the fresh and dry seed of *Moringa oleifera* and compare the protein and crude extract values. The protein was extracted using method by Sheoran *et al.,* 2009. Meanwhile, the crude was extracted using 80% methanol (Akowuah *et al.,* 2008). The extracted protein was analyzed in terms of quantity by Bradford assay and quality via Sodium dodecyl- sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Further analysis on the seed protein extract was carried out to identify the proteins using Liquid chromatography–tandem Mass Spectrometry (LC-MS/MS) analysis. The antioxidant activity of the seeds protein and crude extract was determined using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Ferric Reducing Antioxidant Power (FRAP) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assays.

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