EVALUATION OF 1D AND 2D GEL-BASED PROTEOME FROM FRESH LEAF OF *Moringa oleifera*

NUR ATIQAH BINTI RAMLY

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Universiti Teknologi Malaysia

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ABSTRACT

Moringa species is one of the most useful trees in tropics and subtropics of Asia and Africa. Almost all parts of *Moringa* are edible and have been consumed as vegetable. Furthermore, due to low amounts of proteins in plant tissues the optimization of protein extraction and to establish a robust protocol for two-dimensional gel electrophoresis (2-DE) along with downstream processing are important step. Moreover, the problem of unidentified protein and protein spots from the leaves of *M. oleifera* can be overcome by applying two dimension gel electrophoresis. In this study, two conventional methods and two kits were evaluated on fresh leaf of *Moringa oleifera* to distinguish the most suitable protein extraction method. Besides, the protein pattern and spots distribution were determined from 1D and 2D proteomics respectively. From SDS-PAGE it was shown that protein extraction using QB method was the best method, resulting in more protein bands which allowed detection of 24 bands approximately. In addition, from two dimensional gel electrophoresis it was revealed that method 2 (2000 V) was the best method compared to method 1 (1000 V) as it gives better spot distribution and reproducible where the protein spots were distributed mainly within the pH range of 4-7.

ABSTRAK

Spesies *Moringa* adalah salah satu daripada pokok yang paling berguna di kawasan tropika dan subtropika Asia dan Afrika. Hampir semua bahagian *Moringa* boleh dimakan dan telah digunakan sebagai sayur-sayuran. Tambahan pula, disebabkan jumlah protein yang rendah dalam tisu tumbuhan pengoptimuman pengeluaran protein dan untuk mewujudkan protokol yang mantap untuk elektroforesis gel dua dimensi (2-DE) bersama-sama dengan pemprosesan hiliran adalah langkah penting. Selain itu, masalah protein yang tidak dikenali dan bintik protein dari daun *M. Oleifera* boleh diatasi dengan menggunakan gel elektroforesis dua dimensi. Dalam kajian ini, dua kaedah konvensional dan dua kit telah dinilai pada daun *Moringa oleifera* untuk membezakan kaedah pengekstrakan protein yang paling sesuai. Selain itu, corak protein dan bintik pengedaran ditentukan dari 1D dan proteomik 2D masing-masing. Daripada SDS-PAGE ia menunjukkan bahawa pengeluaran protein dengan menggunakan kaedah QB adalah kaedah terbaik, menyediakan lebih banyak corak protein yang membenarkan pengesanan lebih kurang 24 jalur. Di samping itu, dari gel elektroforesis dua dimensi ia telah mendedahkan bahawa kaedah 2 (2000 V) adalah kaedah terbaik berbanding dengan kaedah 1 (1000 V) kerana ia memberi pengedaran bintik yang lebih baik dan boleh di reproduksi di mana taburan bintik protein adalah dalam julat pH 4-7.

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LIST OF SYMBOLS

A_{595}	-	Absorbance at 595 nanometer
β	-	Beta
°C	-	Degree celcius
G	-	Gram
kDa	-	Kilo Dalton
µg	-	Microgram
µg/mL	-	Microgram per milliliter
µg/g	-	Microgram per gram
µL	-	Microliter
mg	-	Milligram
mL	-	Milliliter
mM	-	Milli Molar
M	-	Molar
%	-	Percent
V	-	Voltage
v/v	-	Volume per volume
w/v	-	Weight per volume

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LIST OF ABBREVIATIONS

2-DE	-	Two dimensional electrophoresis
APS	-	Ammonium persulfate
BSA	-	Bovine serum albumin
CHAPS	-	3-((3-Cholamidopropyl)dimethylammonia)-1-Propanesulfonic acid
CBB	-	Coomassie Brilliant Blue
DNA	-	Deoxyribonucleic acid
DTT	-	Dithiothreitol
EDTA		Ethylenediaminetetraacetic acid
et al	-	And friends
HCl	-	Hydrochloric acid
IAA	-	Indole-3-acetic acid
IEF	-	Isoelectric focusing
IPG	-	Immobilised pH gradient
KPO_4		Potassium phosphate
QB	-	Quenching buffer
RNA	-	Ribonucleic acid
Rpm	-	Rotary per minute
SDS	-	Sodium Dodecyl Sulfate
SDS-PAGE	-	Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis
SEM	-	Standard Error Mean
TCA	-	Trichroloacetic acid
TEMED	-	Tetramethylethylenediamine
PVPP	-	Polyvinylpolypyrrolidone
Rubisco	-	Ribulose bisphosphate decarboxylase/oxygenase

CHAPTER 1

INTRODUCTION

1.1 Background of study

The plant called *Moringa oleifera* is belonging to the Moringacaea which has a medium size tree. The family consists of the single genus called *Moringa*. It is known as multipurpose tree widely distributed in some countries such as India, Bangladesh, Nigeria, Philippines, Singapore and Malaysia (Okuda *et al.,* 2001). As reported in Ramachandran *et al.,* (1980), this plant grows well in almost all types of soils except stiff clays though sandy loams are the best. Previously studies showing that extract from various parts of this plant such as flowers, bark, roots and leaves have been consumed by the public as nutritional supplements and foods and other products (Jung *et al.,* 2015). It has been recorded by Bijina *et al.,* (2011) as high level of protease inhibitor activity after ammonium sulfate fractionation.

Proteomics is the study of protein properties in a cell, tissue or serum. It is one of the most efficient methods used to examine protein mixture extracted from cells, tissues or other biological samples with high-resolution two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) which, in combination with mass spectrometry (MS), provides a central tool for the identification of specific gene products and characterization of proteome-wide profiles (Jung *et al.,* 2006; Cánovas

et al., 2004; Ephritikhine *et al.,* 2004; Görg *et al.,* 2004; Newton *et al.,* 2004; Rose *et al.,* 2004; Wittmann-Liebold *et al.,* 2006).

The most important method used in molecular biology is to extract biomolecules such as DNA, RNA and protein. It is known as starting point for downstream processes and product development including diagnostic kits. There are few parameters such as detergent lysis, shearing force, treatment with ionic salt (salting out), and rapid changes in pressure for protein extraction which aimed to weaken and break the membranes surrounding the cell (Watson *et al.,* 2004).

According to Jorrin *et al.,* (2007), two-dimensional electrophoresis (2-DE) is one of the most great and efficient methods in order to study gene expression at the protein level. By using this technique, proteins are separated by molecular weight and isoelectric point (Rabilloud *et al.,* 2010). In some issues, protein extraction from plant tissues is difficult due to the existence of non protein contaminants specific to the plant such as lipids, organic acids, pigments, polyphenols and terpenes (Wang *et al.,* 2003).

1.2 Problem statement

In this regard, proteomic analysis of *Moringa oleifera* leaves tissues involves a number of practical challenges. This might be due to the presence of sulphur, phosphorus, potassium and magnesium compounds (Kinsella, 1970). Such contaminants are particularly problematic for 2-DE, resulting in horizontal and vertical streaking, smearing, and a reduction in the number of distinctly resolved protein spots (Jellouli *et al.,* 2010).

Currently, proteomics approach is acknowledged as a powerful strategy to analyze protein complexity and therefore, gain a better understanding of physiological responses in a target living organism. As reported in Chen *et al.,*

(2011), the average number of protein spots observed in two dimensional gel electrophoresis using phenol extraction method and urea/thiourea method were higher than with TCA/acetone method. Additionally, the distribution effect of protein spots from the phenol extraction method was far better than the other two methods.

However, in general, the phenol extraction method may be more applicable in 2-DE analysis compared with TCA/acetone and urea/thiourea methods. In this study, the experiment is conducted to distinguish the most suitable protein extraction buffer for *M. oleifera* as compared to the previous study. Moreover, the problem of unidentified protein and protein spots from the leaves of *M. oleifera* can be overcome by applying two dimension gel electrophoresis. Hence, proteomics approach is able to be used to identify the protein properties. There is no work has been reported on protein extraction of *M. oleifera* and no protein information on this plant.

1.3 Objectives

The objectives of this study are as follows:

i. To determine the best protein extraction method for *Moringa oleifera* leaves.
ii. To determine one dimensional protein electrophoretic pattern from *Moringa oleifera* leaves.
iii. To determine the protein spot distribution and pattern from 2D proteomics of *Moringa oleifera*.

1.4 Significance of work

This project aims to develop an optimized method for analysis of total protein from *Moringa oleifera*. There is no specific method that would be suitable for all plants since there are various methods available to extract proteins. Testing of

different buffers and methodologies are essential in achieving good quality protein which would be lead for further proteomics work. Furthermore, there is no such work has been stated either on this plant or the protein extraction of *M. oleifera.* Thus, this study could perform as a platform for preliminary protein identification.

1.5 Scope of work

The objective of this study is to differentiate the extraction buffers which are most suitable for *Moringa oleifera*. Two conventional and two kits were used to extract the protein. In this work, protein obtained was quantified using Bradford assay and its quality by comparative bands in SDS-PAGE in each extraction method. Then, two different methods of IEF for two-dimensional electrophoresis were performed to clarify which method resulted in better protein spot distribution.

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