TYPE AND AMOUNT OF GLUCOSINOLATES IN THE LEAVES OF *Moringa oleifera*

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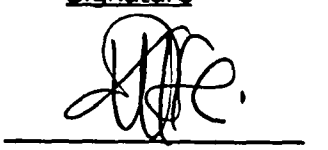

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ABSTRACT

Glucosinolates and their hydrolysis product isothiocyanates are actively researched due to their anti cancer properties as well as their high potential usage in the biomedical field. This study was done to detect and identify the presence of glucosinolates, particularly the glucomoringin (GMG), in the leaves of the locally grown *Moringa* oleifera by identifying its derived isothiocyanate, glucomoringin isothiocyanate (GMG-ITC). The hydrolysis was carried out in optimum condition resulting in the presence of isothiocyanate which was subsequently extracted using dichloromethane. Consequently, the identification and analysis of the isothiocyanate was conducted using Gas Chromatography-Mass Spectrometry (GC-MS). Besides that, this study was also done to quantify the amount of glucosinolate identified in the leaves of *M. oleifera.* Quantification of the amount of glucosinolate present was done using the High Performance Liquid Chromatography (HPLC) method. The GC-MS results showed the occurrence of a minor isothiocyanate, benzyl isothiocyanate in the sample extracts. This observation proves the presence of a minor glucosinolate, benzyl glucosinolate in the leaves sample of the locally grown *M. oleifera.* However, the GC-MS was unable to identify GMG-ITC due to its low volatility. On the other hand, result of HPLC proves the presence of minor glucosinolate, benzyl glucosinolate in the *M. oleifera* leaves sample with the amount of 0.3154 g. Even though future research is needed to confirm these findings, this study helps provide a preliminary database on the glucosinolates present in the locally grown *M. oleifera* which can contribute to the biomedical field as well as the local production of pharmaceuticals products.

ABSTRAK

TAJUK: JENIS DAN AMAUN GLUKOSINOLAT DALAM DAUN Moringa oleifera

Glukosinolat dan isotiosionat telah dikaji secara aktif kerana kewujudan sifat anti kanser serta potensinya yang tinggi dalam bidang bioperubatan. Kajian ini dijalankan untuk mengenalpasti kehadiran glukosinolat khususnya glucomoringin (GMG) dalam daun spesis Moringa oleifera tempatan. Hal ini dilakukan dengan mengenalpasti isotiosianat hasil terbitan dari GMG iaitu glucomoringin isotiosianat (GMG-ITC). Proses hidrolisis dijalankan dalam keadaan optimum yang mana menghasilkan isotiosianat. Sejurus itu, isotiosianat ini diekstrak menggunakan diklorometana. Pengenalpastian dan analisis isotiosianat ini telah dilakukan dengan menggunakan Gas Chromatography-Mass Spectrometry (GC-MS). Selain itu, kajian ini juga bertujuan untuk membuat pengkuantitian jumlah glukosinolat yang terdapat di dalam daun M. oleifera menggunakan kaedah High Performance Liquid Chromatography (HPLC). Dapatan GC-MS menunjukkan kehadiran satu isotiosianat kecil dalam ekstrak sampel iaitu benzil isotiosianat. Dapatan ini membuktikan kehadiran benzil glukosinolat di dalam sampel. Walaubagaimanapun, kaedah GC-MS didapati gagal mengenalpasti kehadiran GMG-ITC disebabkan sifat kemeruapannya yang rendah. Selain itu, hasil kaedah HPLC membuktikan kehadiran benzil glukosinolat di dalam daun sampel M. oleifera dengan amaun sebanyak 0.3154 g. Walaupun kajian lanjutan harus dilakukan bagi mengesahkan dapatan kajian ini, kajian ini boleh memberikan satu pangkalan data awal tentang kehadiran glukosinolat dalam spesis M. oleifera terdapat di Malaysia, yang mana boleh menyumbang kepada bidang bioperubatan dan penghasilan produk farmaseutikal tempatan.

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LIST OF SYMBOLS AND ABBREVIATIONS

μ/m	micrometre
mA	milliampere
°C	degree Celsius
cm	centimetre
g	gram
g/mol	gram per mol
g/cm^3	gram per centrimetre cube
M	molarity (mol L^{-1})
mL	mililiter
min	minute
mm	millimetre
nm	nanometre
ppm	part per million
rpm	revolution per min
t_R	retention time
V	volume (L)
$C_{14}H_{17}NO_{10}S_2$	Benzyl glucosinolate
CH_2Cl	Dichloromethane
CH_3OH	Methanol
N_2	Nitrogen
Na_2SO_4	Sodium Sulphide
Na_3PO_4	Sodium Phosphate
NaCl	Sodium Chloride
GC-MS	Gas chromatography- Mass Spectrometer

BGLS	Benzyl glucosinolate
GMG	Glucomoringin
GMG-ITC	Glucomoringin isothiocyanate
HPLC	High Performance Liquid Chromatography
M. oleifera	*Moringa oleifera*

CHAPTER 1

INTRODUCTION

1.1 Background of study

Nature has never failed in providing us with our daily needs and has always been a primary object of studies by researchers for its potential application in the human life. It is highly possible that the knowledge obtained from studying for instance the natural compounds available in nature (carbohydrates, protein and other biomolecules) can never be satiated since there are always amazing discovery to stumble upon. This helps researchers to better understand in full what great wonder that the living organisms holds. Continuous study and observation on nature has enabled advanced technology especially in the field of biochemistry, medicine and industrial chemistry.

Observation in the last few decades has seen explosive growth in the field of herbal medicine which immensely helps the growth of biochemistry and its application in pharmaceuticals in general. Herbal medicine is preferred in both developing and developed countries due to its natural origin and lesser side effects (Barret *et al.*, 1999). *Moringa oleifera*, a special tree highly regarded since it is believed that all of its parts have beneficial properties will be the focus in this study. Its glucosinolate, found naturally especially in *Brassica* plant is therefore taken into concern in this study not only for its traditional purposes such as for treating fever, abnormal blood pressure, tuberculosis but also for its chemo-preventive properties (Hayes *et al.*, 2008; Fahey., 2005).

Most plant species normally contains more than one glucosinolates and its concentration might differ significantly across varieties. Not only that, difference of its content and composition also can also be noted among the cultivars, plant individuals and also parts of the plant. These differences are caused by various factors such as genetics, environmental condition and also the plant nutrients itself. Moving on, even though a plant may contain many types of glucosinolates, only a few types (one to four) will predominate (Rosa *et al.*, 1997). Hence, the distributions of glucosinolates are not the same where quality and quantity among the single plant parts are taken into concern. For example, the leaves contains a different type of glucosinolates in a different quantity compares to the flower and other parts of the same single plant. Report by Carlson *et al.* (1985) has come up with a conclusion that seeds contain higher concentration of glucosinolates compared to the other edible parts of a plant. Bartoszek (2002) on the other hand explained the difference of glucosinolates content in a plant is due to the growing environment in which the plant is exposed to. Glucosinolates are broken down through the aid of a hydrolyzing agent known as the enzyme myrosinase. Example of the hydrolysis product of glucosinolates includes the number of bioactive breakdown properties such as isothiocyanates, organic cyanides, oxazolidinethiones and ionic thiocyanate (Moreno *et al.*, 2006).

Moringa oleifera has also been traditionally used over the years for traditional purposes. However, it's chemo-preventive or the anti-cancer properties are the main reason why *M. oleifera* is steadily gaining interest among the researchers. The anti-cancer properties are found in the breakdown products of the glucosinolates, which is well known among the researches and also users due to the vast amount of reports stating that the breakdown products of glucosinolates are vastly reported to be exhibiting anti-carcinogenic properties. This fact is supported by an article published by Chalkier and Du (1997) where they reported that the breakdown products not only show cancer-preventive properties but also biological activities which includes plant interaction with pathogen.

Study by Fahey *et al.* (2001) also includes the fact that a number of *in vitro* and *in vivo* studies have stated that one the degradation compound of glucosinolate, isothiocyanates affect many steps of cancer development including modulation of

phase I and phase II enzyme induction, reduction of *helicobacter* infections, induction of apoptosis, control of the cell cycle and modulating cell signaling.

In this study, the glucosinolates extracts from *M. oleifera* leaves were identified and its amount determined. Therefore, this introduction chapter helps provide an overview of the glucosinolates of *M. oleifera*, as well as stating the objectives of the study. Further details on the research findings of glucosinolates and the *M. oleifera* will be discussed in the literature reviews in Chapter Two.

Next, Fahey *et al.* (2001) also stated that there are more than 120 known glucosinolates that has been successfully identified to date which belongs to 16 families of dicotyledonous angiosperms which includes the *Cruciferae*, *Capparidaceae*, *Resedaceae* and *Moringaceae* angiosperms. The β-D-thioglucose group, a common core structure which is shared by glucosinolates linked to a sulfonated aldoxime moiety. Consequently, glucosinolates exhibits a wide range of structural diversity due to its different side chains and modification. There are three major categories of glucosinolates that are aliphatic glucosinolates derived from methionine, indolic glucosinolates from tryptophan and also aromatic glucosinolates from phenylaline (Chen *et al.*, 2001).

Researchers believe that glucosinolates, one of a group of phytochemicals can protect and prevent consumers from contracting chronic diseases, are found in generous amount in *M. oleifera* (Fahey *et al.*, 2001). The said glucosinolate from *M. oleifera* when reacted with the enzyme myrosinase will be broken into various chemicals such as nitriles and isothiocyanates after receiving damages. These chemicals, with nitrile as an exception, appear to be anti-carcinogenic. Therefore, intake of *M. oleifera* can traditionally help reduce the probability of having chronic disease.

1.2 Objectives of study

The objectives of this study are:

1. To identify the type of glucosinolates present in *M. oleifera* leaves.
2. To determine the amount of glucosinolates in the leaves of *M. oleifera*.

1.3 Scope of study

The identification types of the glucosinolate present in *M. oleifera* were based on the R-group (side group) of the products after the hydrolysis of the parent glucosinolates. They were analysed using GC-MS, where the structure of said R-group of the possible glucosinolate hydrolysis products are analysed based on the mass- spectrometry spectra and compared to the known glucosinolates. HPLC on the other hand was used to identify the amount of glucosinolate in the leaves of *M. oleifera* by comparison of the standard benzyl glucosinolate. The preparation for the standard benzyl glucosinolate is shown in Chapter Three (Methodology) of this thesis.

1.4 Relevance of study

The study on *M. oleifera* is interesting and can be further researched due to several reasons. One of the reasons is that although there are many studies of glucosinolate extraction on various *Brassica* vegetables, the same study on *M. oleifera* is still relatively new, especially in the locally grown *M. oleifera*. Therefore, by studying the locally grown *M. oleifera*, it can help in the profiling of this magical plant. This study also can provide a preliminary database on the glucosinolates present in the local *M. oleifera*. Last but not least, it can immensely contribute to the biomedical field and also in the local production of local pharmaceutical products since there is constant demand of it among Asians and Malaysian to be exact.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Fruits and vegetables are widely consumed and human beings as consumers in this context had undeniably felt the health benefits, although the knowledge of which parts of them that brings about the health goodness is relatively unknown. Fortunately, ongoing scientific research is on the way to establish the fact that these benefits are due to the presence of phytochemicals in these plants. By successfully identifying these parts, researchers will be able to extract it and used it widely and commercially in the biomedical field. One of the phytochemicals that has been made known is the core subject in this study which is the glucosinolates. Glucosinolates are an important phytochemical since according to Das *et al.* (2000) and Shapiro *et al.* (2001), not only can it cure traditional ailments, it has also been proven to have certain chemo preventive properties that can reduce the risk of cancer. A report by Fahey *et al.* (2001) also supported the claim as extensive studies on glucosinolates also found that they have important benefits regarding fungicidal, bacteriocidal, rematocidal and allelopathic properties. Due to the boom of demand of herbal, organic medicine, recent research interest is also taking in concern the bioactivity of glucosinolate metabolites.

In this literature review, information on the main glucosinolates available in plants, biography of *M. oleifera;* main sample plant in this study and its glucosinolates properties are outlined. The information regarding this plant includes the

structures, types, distribution, biosyntheses, chemo prevention properties of glucosinolates and also findings on its glucosinolates.

2.2 *Moringa oleifera*

Moringa oleifera is one of the vegetables of the *Brassica* order which belongs to the family *Moringaceae*. According to Khawaja *et al.*, (2010), the *Moringaceae* is a single genus family with 13 known species. Of the known species, the sample plant used in the study which is *M. oleifera* is the most widely known. The common name of *M. oleifera* is drumstick tree or horseradish tree, which is considered a small native tree originated from the sub-Himalayan religions of North West India. Nowadays, as shown in Figure 2.1, it is indigenous to many regions in Africa, Arabia, South East Asia, the Pacific and South America. *M. oleifera* has always been traditionally used to cure ailments and consumed as vegetables among these regions. Therefore, it is not a surprise that it has high health benefits. People who consumed this plant also would go and described *M. oleifera* as a 'miracle tree' since almost every part of it can be used. Miracle or not, it is undeniable that *M. oleifera* possess an innate ability in helping to cure various ailments and also some chronic diseases such as cancer.

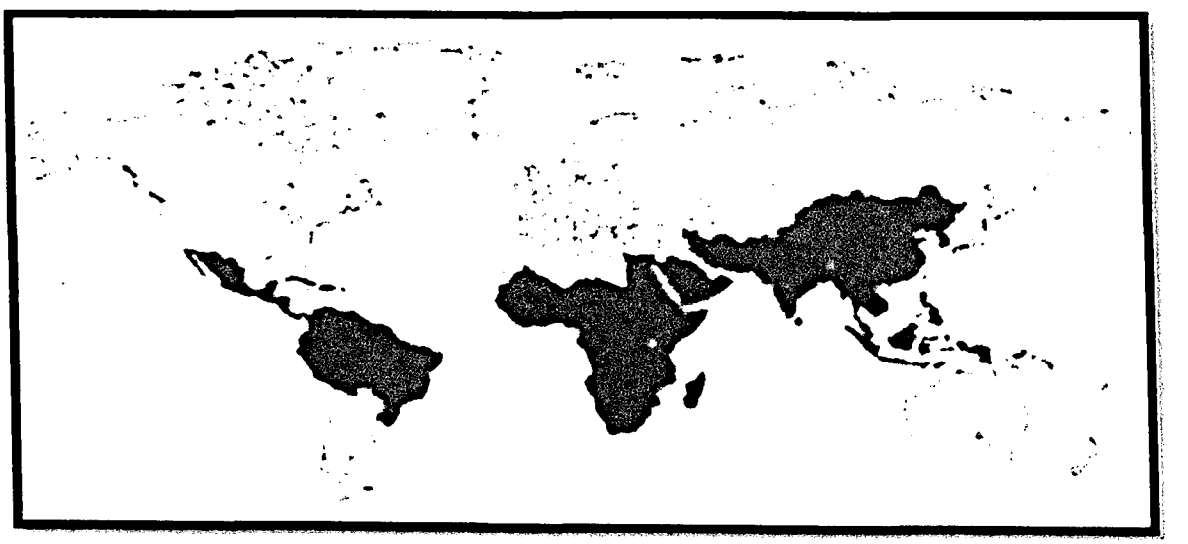

Figure 2.1 Regions highlighted in green shows where local *Moringa oleifera* are mostly grown. (Source: Trees for Life, 2011)

2.2.1 Taxonomy of *Moringa oleifera*

The scientific classification of the Moringa oleifera is illustrated in Table 2.1. The botanical description of *Moringa oleifera* in various languages which supported the fact that it is widely known and consumed is shown on Table 2.2.

Table 2.1 Scientific classification of *Moringa oleifera*

Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Order	*Brassicales*
Family	*Moringaceae*
Genus	*Moringa*
Species	*Moringa oleifera Lam.*

Table 2.2 Botanical description of *Moringa oleifera*

Latin	*Moringa oleifera*
Sanskrit	Subhanjana
English	Drumstick tree, Horseradish
Chinese	La ken
French	Morungue
Arabian	Rawag
Telugu	Mulaga
Malay	Meringgai, Semunggai, Kachang Kelur

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