

**GLUCOSINOLATES IN WATERCRESS  
(*Nasturtium Officinale*) AND THEIR  
HYDROLYSIS PRODUCTS UNDER DIFFERENT  
CONDITIONS**



**NURAZILAH FARHANA BINTI ARIPIIN**

**UMS**  
UNIVERSITI MALAYSIA SABAH

**FACULTY OF SCIENCE AND NATURAL  
RESOURCES  
UNIVERSITI MALAYSIA SABAH  
2017**

**GLUCOSINOLATES IN WATERCRESS  
(*Nasturtium Officinale*) AND THEIR  
HYDROLYSIS PRODUCTS UNDER DIFFERENT  
CONDITIONS**

**NURAZILAH FARHANA BINTI ARIPIIN**



**UMS**

**THESIS SUBMITTED IN FULFILLMENT FOR  
THE DEGREE OF MASTER OF SCIENCE**

**FACULTY OF SCIENCE AND NATURAL  
RESOURCES  
UNIVERSITI MALAYSIA SABAH  
2017**

## DECLARATION

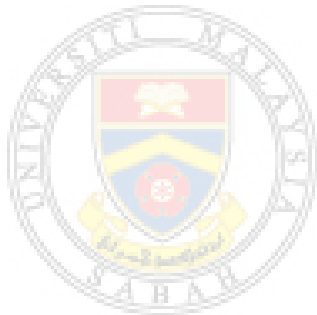
I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledge.

27 July 2017

.....

Nurazilah Farhana Bt. Aripin

MS1321016T



UMMS  
UNIVERSITI MALAYSIA SABAH

## CERTIFICATION

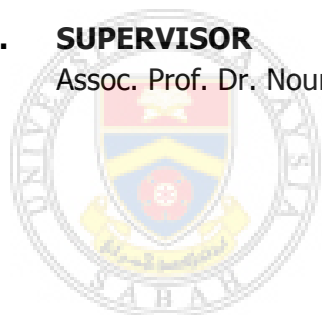
NAME : **NURAZILAH FARHANA BINTI ARIPIIN**  
MATRIC NO. : **MS1321016T**  
TITLE : **GLUCOSINOLATES IN WATERCRESS (*NASTURTIUM OFFICINALE*) AND THEIR HYDROLYSIS PRODUCTS UNDER DIFFERENT CONDITIONS**  
DEGREE : **MASTER OF SCIENCE (INDUSTRIAL CHEMISTRY)**  
VIVA DATE : **12<sup>TH</sup> MAY 2017**

### CERTIFIED BY;

**1. SUPERVISOR**

Assoc. Prof. Dr. Nourie Surugau

Signature



UMS  
UNIVERSITI MALAYSIA SABAH

## **ACKNOWLEDGEMENT**

Foremost, I would like to express my fully appreciation to my respective advisor Assoc. Prof Dr. Noumie @ Loumi Surugau of the Faculty of Science and Natural Resources, you have been a tremendous and a very helpful mentor for me. I would like to thank you for encouraging my research and also been patient enough to advise, guide and supervise me throughout my study journey. Her continuous encouragement provided me the necessary impetus to complete the research and publish this thesis.

Besides, I also would like to take this golden opportunity to forward my thanks to Mr, Recheidy, Mr. Taipin and Mdm. Norazyma, Faculty of Science and Natural Resources for their great help in handling analytical instruments that involve in my study.

Last but definitely not least, I would like to forward my greatest appreciation to my beloved parents, siblings and friend who have given me the opportunity to further my study and continuous given me encouragement and moral support all this while.

Nurazilah Farhana Aripin

27<sup>th</sup> July 2017

## ABSTRACT

Watercress or *Nasturtium officinale* is a cruciferous vegetable that commonly known as a rich source of gluconasturtiin or 2-phenyl ethyl glucosinolates (PEGLS), the type of glucosinolate that abundant in watercress. However, very few studies have been done on PEGLS content thus the first objective of this study is to quantify and qualify glucosinolates content (PEGLS and other GLS if any) in watercress. It is crucial to understand the hydrolysis of PEGLS because (and depending on conditions) phenylethyl nitrile (PEN) and / or phenylethylthiocyanate (PEITC) are possible hydrolysis products of PEGLS. Unlike PEITC, these other hydrolysis products are not known to possess anticancer properties. The production of PEITC and any other hydrolysis products are caused by endogeneous myrosinase activity hence, the second objective of this study is to study the myrosinase activity under different conditions. There are many factors that influence the hydrolysis of glucosinolates which are external factors such as temperature, pH, presence of redox agents, and intrinsic factors such as structure of the side-chain of GLS, myrosinase activity and protein specifiers etc. Hence, the hydrolysis products of watercress (PEITC and PEN) also being investigated through this study. The conditions studied for both myrosinase activity and hydrolysis products of watercress were temperature ( 25°C, 45°C and 65°C), pH ( 3, 7, 9), ascorbic acid and ferrous ions with similar concentration (2mM, 4mM, 6mM, 8mM, and 10 mM). Glucosinolates contents (extracted using deionized water) and myrosinase activity (crude myrosinase mixed with known concentration of sinigrin, as substrate) were analysed using HPLC. While GC used in this study was to profile the hydrolysis products that being extracted using dichloromethane after the hydrolysis of glucosinolates and enzyme myrosinase in the presence of water. The results showed that the amount of PEGLS in the watercress plant for 0.5 g is 0.21  $\mu\text{mol/g}$ . The optimum conditions of myrosinase activity showed at 45°C ( $1.23 \text{ mM min s}^{-1}$ ), pH 7 and 9 ( $1.36 \text{ mM min s}^{-1}$ ) and both 2mM concentration in ascorbic acid ( $0.67 \text{ mM min s}^{-1}$ ) and ferrous iron ( $1.44 \text{ mM min s}^{-1}$ ) . While for PEITC and PEN, all conditions showed the highest content of PEITC compare to PEN. For ascorbic acid and ferrous iron, the highest content of PEITC showed in the concentration of 2 mM which are 886.8 ppm and 756.1 ppm respectively while 25°C for temperature (601.1 ppm) and at pH 9 for pH conditions (561.1 ppm).The overall aim of this study is to identify the optimum conditions for the production of PEITC in watercress and the activity of the endogeneous enzyme myrosinase. These findings will be beneficial to provide information on how to optimize the uptake of this anticancer secondary metabolite from watercress.

## **ABSTRAK**

### **GLUKOSINOLAT DI DALAM SELADA AIR (*Nasturtium Officinale*) DAN PRODUK HIDROLISISNYA DI BAWAH FAKTOR KEADAAN YANG BERBEZA**

Selada air ataupun nama saintifiknya *Nasturtium officinale* adalah sejenis tumbuhan cruciferous yang terkenal sebagai sumber yang kaya dengan glukonasturtin atau 2-fenil etil glukosinolat (PEGLS), iaitu kandungan glukosinolat yang paling tinggi dalam selada air. Walau bagaimanapun, kajian yang dilakukan ke atas kandungan PEGLS di dalam selada air adalah terhad. Oleh itu, objektif pertama kajian ini adalah untuk mengenalpasti kepekatan kandungan glukosinolat (PEGLS dan GLS lain jika ada) dalam selada air. Ia adalah penting untuk memahami proses hidrolisis PEGLS kerana bergantung kepada faktor keadaan, produk hidrolisis PEGLS berkemungkinan feniletil nitril dan / atau feniletil isotiosianat. Tidak seperti PEITC, produk-produk hidrolisis lain tidak diketahui sekiranya mempunyai ciri-ciri anti-kanser. Penghasilan PEITC dan mana-mana produk hidrolisis lain adalah disebabkan oleh aktiviti mirosinas endogen dengan itu, objektif kedua kajian ini adalah untuk mengkaji aktiviti mirosinas dalam keadaan yang berbeza. Terdapat banyak faktor yang mempengaruhi hidrolisis glukosinolat di mana faktor-faktor luaran seperti suhu, pH, kehadiran agen redoks dan faktor-faktor intrinsik seperti struktur sampingan rangkaian GLS, aktiviti mirosinas dan protin spesifik dan lain-lain. Oleh itu, produk hidrolisis selada air (PEITC dan PEN) juga dikaji dalam kajian ini. Antara parameter yang digunakan dalam kajian ini bagi kedua-dua aktiviti mirosinas dan produk hidrolisis selada air adalah suhu (25 °C, 45 °C dan 65 °C), pH (3, 7, 9), asid askorbik dan ion ferus dengan kepekatan yang sama (2mM, 4mM, 6mM, 8mM, dan 10 mM). Kandungan PEGLS (diekstrak menggunakan air ternyahion) dan aktiviti mirosinas (ekstrak mirosinas bercampur dengan sinigrin yang diketahui kekuatannya, sebagai substrat) telah dianalisis dengan menggunakan instrumen HPLC. Manakala instrumen GC telah digunakan dalam kajian ini untuk memprofil produk hidrolisis PEGLS yang telah diekstrak menggunakan larutan diklorometana selepas hidrolisis glukosinolat oleh enzim mirosinas dengan kehadiran air. Hasil daripada kajian ini menunjukkan bahawa kepekatan PEGSL bagi 0.5 g serbuk selada air adalah 0.21  $\mu\text{mol} / \text{g}$ . Aktiviti mirosinas yang optimum telah dikenal pasti pada suhu 45 °C ( 1.23 mM min), pH 7 dan 9 (1.36 mM min ) dan pada kepekatan 2mM dalam asid askorbik (0.67 mM min) dan besi ferus (1.44 mM min ). Bagi PEITC dan PEN pula, semua keadaan menunjukkan kandungan tertinggi PEITC berbanding dengan PEN. Pada faktor asid askorbik dan ion ferus, kandungan tertinggi PEITC menunjukkan pada kepekatan 2 mM di mana masing-masing 886.8 ppm dan 756.1 ppm manakala 601.1 ppm pada suhu 25 °C dan 561.1 ppm pada pH 9. Oleh itu, skop kajian ini adalah untuk mengenal pasti keadaan optimum untuk penghasilan PEITC yang maksimum dalam selada air dan aktiviti enzim mirosinas endogen yang terlibat dengan proses hidrolisis glukosinolat. Oleh itu, keseluruhannya kajian ini akan memberi ilmu pengetahuan yang amat berguna mengenai cara untuk mengoptimalkan pengambilan metabolit sekunder iaitu PEITC dari selada air yang telah dibuktikan sebagai agen antikanser.

# TABLE OF CONTENTS

	Pages
<b>TITLE</b>	i
<b>DECLARATION</b>	ii
<b>CERTIFICATION</b>	iii
<b>ACKNOWLEDGEMENT</b>	iv
<b>ABSTRACT</b>	vi
<b><i>ABSTRAK</i></b>	vii
<b>TABLE OF CONTENT</b>	viii
<b>LIST OF TABLES</b>	ix
<b>LIST OF FIGURES</b>	xii
<b>LIST OF SYMBOLS</b>	xv
<b>LIST OF ABBREVIATIONS</b>	xvi
<b>LIST OF APPENDICES</b>	xvii
<b>CHAPTER 1: INTRODUCTION</b>	1
1.1 Introduction	1
1.2 Objectives	2
<b>CHAPTER 2: LITERATURE REVIEW</b>	5
2.1 Glucosinolates	5
2.1.1 Glucosinolates Discovery and Distribution in Plants	8
2.1.2 Glucosnolate Synthesis	12
2.2 Watercress	14
2.2.1 Cultivation and Classification	15
2.2.2 Morphology and Taxonomy of Watercress	15
2.3 Glucosinolate Hydrolysis Products	17
2.3.1 Isothiocyanate	18
2.3.2 Nitrile and Epithionitrile	23
2.4 Glucosinolate- Myrosinase System	24
2.5 Factors Influencing Glucosinolate Hydrolysis Products	27
2.5.1 Intrinsic Factors	27
2.5.2 Extrinsic Factors	27



2.6	Role of Glucosinolates in Human Health and Disease	29
2.6.1	Antimutagenic and Antiproliferic Activity	29
2.6.2	Anticarcinogenic of Isothiocyanates	31
<b>CHAPTER 3:</b>	<b>METHODOLOGY</b>	33
3.1.	Chemicals	33
3.2	Apparatus	35
3.3	Sampling and Sample Preparation	35
3.4	Standard Solution Preparation	36
3.5	Method Validation	37
3.5.1	Limit of Detection and Limit of Quantification	37
3.6	Glucosinolates Profiling in Watercress	36
3.6.1	Extraction of PEGLS	39
3.7	Determination of Myrosinase Activity in Watercress	39
3.7.1	Preparation of Myrosinase Extract	37
3.8	HPLC Analysis	40
3.9	Calculation of Myrosinase Activity	40
3.10	Glucosinolates Hydrolysis Products Profiling in Watercress	40
3.11	Quantitative Deyetermination of Phenethyl Isothiocyanate and Phenethyl Nitrile	41
3.12	Effect of Temperature, pH, Ascorbic Acid and Ferrous Ion Concentration	42
3.13	Identification and Quantification of GLS Hydrolysis Products	42
<b>CHAPTER 4:</b>	<b>RESULTS AND DISCUSSION</b>	43
4.1	Introduction	43
4.2	Method Validation	43
4.2.1	Response Linearity	44
4.2.2	Accuracy and Precision	47
4.2.3	Limit of Detection and Limit of Quantification	47
4.3	PEGLS Quantification in Watercress Samples	48
4.4	Myrosinase Activity	50
4.5	Glucosinolates Hydrolysis Products in Watercress Using	56

GC-MS Analysis	
4.5.1 Analysis of Autolysis Temperature	62
4.5.2 Analysis of pH	64
4.5.3 Analysis of Ascorbic Acid	65
4.5.4 Analysis of Ferrous Ion	66
<b>CHAPTER 5: CONCLUSION</b>	<b>69</b>
<b>REFERENCES</b>	<b>71</b>
<b>APPENDICES</b>	<b>82</b>



UMS  
UNIVERSITI MALAYSIA SABAH

## LIST OF TABLES

Table 2.1	Glucosinolates identified in varieties of <i>Brassica</i>	7
Table 2.2	Glucosinolates content in different types of <i>Brassicaceae</i> (mg/kg fresh weight)	11
Table 2.3	Taxonomy of watercress	16
Table 2.4	The antiproliferative activity of the varieties hydrolyzed glucosinolates products	30
Table 3.1	Chemicals	39
Table 4.1	LOD and LOQ values of Sinigrin, PEGLS, PEITC and PEN Standard compounds	48
Table 4.2	Glucosinolates hydrolysis products from watercress by GC/MS	60



UMS  
UNIVERSITI MALAYSIA SABAH

## LIST OF FIGURES

	Pages	
Figure 2.1	The most studied glucosinolates and their hydrolysis products implicated in human nutrition in each chemical classes : (A) aliphatic (B) aromatic (C) indole	5
Figure 2.2	Synthesis of glucosinolates	8
Figure 2.3	Glucosinolate structures and myrosinase-catalyzed degradation. General scheme of myrosinase-catalyzed degradation of glucosinolates (side chain indicated as R) in the absence and presence of the specifier proteins ESP and AtNSPs.	10
Figure 2.4	Structure of allyl isothiocyanate	13
Figure 2.5	The mechanism of hydrolysis of sinigrin. At a neutral pH of 7, close to what your mouth is, sinigrin is converted completely to allyl isothiocyanate. But if we change the pH to a more acidic 4, the intermediate aglycone (in the brackets) is converted into allyl cyanide.	17
Figure 2.6	Structure of benzyl isothiocyanate	18
Figure 2.7	Mechanism of hydrolysis of glucotropaeolin	18
Figure 2.8	Structure of sulforaphane	29
Figure 2.9	Hydrolysis of glucoraphanin into sulforaphane or sulforaphane nitrile	20
Figure 2.10	Structure of phenethylisothiocyanate	21
Figure 2.11	Reaction catalyzed by esp with terminal alkenyl and non alkenyl glucosinolates hydrolysis produce epithionitrile and simple nitrile	21
Figure 2.12	Glucosinolate-myrosinase system	22
Figure 2.13	Overall structure of plant myrosinase	23
Figure 2.14	Chemical reaction hydrolysis of PEGLS ( $C_{14}H_{17}NO_1OS$ ) to (PEITC)	24
Figure 4.1	Calibration Graph for Sinigrin Standard	44

Figure 4.2	Calibration Graph for PEGLS Standard	45
Figure 4.3	Calibration Graph for PEITC Standard	45
Figure 4.4	Calibration Graph for PEN Standard	46
Figure 4.5	Accuracy and precision measurement in 3 to 9 days interval.	47
Figure 4.6	HPLC Chromatogram For Sample Extract Of Watercress Sample With PEGLS Peak At 2.209 Min.	48
Figure 4.7	Myrosinase Activity In Watercress Under Different Temperature	50
Figure 4.8	Myrosinase Activity In Watercress At Different pH.	51
Figure 4.9	Myrosinase Activity In Watercress At Various Ascorbic Acid Concentration.	53
Figure 4.10	Myrosinase Activity In Watercress At Different Concentration Of Ferrous Ion (Fe 2+)	54
Figure 4.11	Expanded chromatograms of standard compounds PEITC (a) and PEN (b).	56
Figure 4.12	Chromatograms of glucosinolates hydrolysis products in watercress sample solutions.	57
Figure 4.13	Mass spectra of PEITC standard compound (a) and in watercress sample solution (b).	58
Figure 4.14	Mass spectra of PEN standard compound (a) and in watercress sample solution (b).	59
Figure 4.15	Enzymatic hydrolysis of glucosinolates	562
Figure 4.16	Effect Of Different Temperature On PEITC And PEN Formation In Watercress.	63
Figure 4.17	Effect Of Various pH On PEITC And PEN Formation In Watercress	64
Figure 4.18	Effect Of Different Concentration Of Ascorbic Acid On PEITC And PEN Formation In Watercress	65
Figure 4.19	Effect Of Various Concentration Of Ferrous Ion (Fe <sup>2+</sup> ) On PEITC And PEN Formation In Watercress.	67

## LIST OF SYMBOLS

$Fe^{2+}$	-	Ferrous ion
$Fe^{3+}$	-	Ferric ion
$^{\circ}C$	-	Celcius (temperature)
$Zn^{2+}$	-	Zinc ion
$Cu^{2+}$	-	Copper ion
$\mu m$	-	Micromolar
$mm$	-	Milimeter
$mg$	-	Miligram
$mL$	-	Militer
$mM$	-	Milimolar
$M$	-	Molarity
$V$	-	Volume
$n$	-	Number of mole
$R^2$	-	Coefficient relation
$M_1$	-	Initial concentration of stock solution used for dilution
$M_2$	-	Initial volume of stock solution that is need for dilution
$V_1$	-	Final concentration of standard solution needed for analysis
$V_2$	-	Final volume of stock standard needed for analysis
$SD$	-	Standard Deviation
$\mu L$	-	Microliter
$g$	-	Gram
$mM \text{ min}^{-1}$	-	Milimolar per minute
$Zn^{2+}$	-	Zinc ion

## LIST OF ABBREVIATIONS

BITC	Benzyl Isothiocyanate
BOP	N-nitrosobis (2-oxopropyl) amine
CHD	Coronary Heart Disease
DEN	Diethylnitrosamine
GNS	Gluconasturtiin
GLS	Glucosinolate
GC-MS	Gas Chromatography – Mass Spectra
HPLC-UV	High Performance Liquid Chromatography
ITC	Isothiocyanate
NNK	4-methylnitrosamino-1-3-pyridal-1-butanone
NBMA	N-nitrosobenzylmethylamine
PEGLS	Phenethyl Glucosinolate
PEITC	Phenethyl Isothiocyanate
PEN	Phenethyl Nitrile
PET	Phenethyl Thiocyanate
SNG	Sinigrin
TNBS	Trinitrobenzenesulfonic acid

## LIST OF APPENDICES

		Pages
Appendix A	Calculation of PEGLS concentration	82
Appendix B	PEGLS Standard chromatogram	87
Appendix C	NIST library matching assesment	89
Appendix D	PEGLS hydrolysis products chromatogram	109



UMS  
UNIVERSITI MALAYSIA SABAH



# CHAPTER 1

## INTRODUCTION

### 1.1 Introduction

Glucosinolates ( $\beta$ -thioglucoside-N-hydroxysulfates) are a group of secondary plant metabolites found almost exclusively in plants of the *Brassicales* order (Tian *et al.*, 2005) which includes the *Brassicaceae* family (Fahey *et al.*, 2001). Glucosinolates (GS) are sulphur-containing, water soluble phytochemicals which the structure consists of a  $\beta$ -D-glucopyranosyl moiety linked via a sulfur atom to an N-hydroximosulfate ester, and of a modified amino acid side chain (*R*) (Mohn *et al.*, 2007). More than 130 types of GS with varying side chains have been isolated from nature, but not all are present in edible plants (Fahey *et al.*, 2001). In cruciferous vegetables, the most commonly found GS are sinigrin, glucoraphanin, glucobrassicin and glucoiberin. Enzyme myrosinase (thioglucosidase, EC 3:2:3:1), which is present in plant cells, catalyses the hydrolysis of GS mainly into isothiocyanates (ITCs), nitriles and thiocyanates. Physical impacts on plant cells, such as cutting and heating, allow mixing of the enzyme and GS, thus hydrolysis occurs.

Consumption of cruciferous vegetables such as broccoli, cabbage, brussels sprouts, cauliflower and watercress has been associated with reduced incidence of cancer (Chan & Miskimins, 2012 ; Conaway *et al.*, 2002 & Gill *et al.*, 2007). In addition, dietary incorporation of Brassica vegetables has been observed to inhibit experimental carcinogenesis in laboratory animals (Brunelli *et al.*, 2010). The chemo-preventive properties of these vegetables are attributed to the ITCs i.e. the enzymatic degradation products of GS. In particular, aromatic ITCs such as indolyl-, phenyl-, benzyl-, phenethylITC (PEITC) and alkyl ITC such as sulforaphane (4-methylsulfinylbutyl ITC) have all demonstrated anticarcinogenic effects (Manesh *et*

*al.*, 2005 & Rosea *et al.*, 2000). It is widely reported that ITCs have inhibited liver, lung, colon, breast, ovary, prostate, bladder and pancreas cancers (Chan Miskimins, 2012 ; Xiao *et al.*, 2003; Srivasta & singh, 2004). The most convincing anticancer ITCs are sulforaphane, benzyl ITC (BITC) and PEITC (London *et al.*, 2000; Srivasta & Singh, 2004; Gupta *et al.*, 2014). This topic will be discussed further in Chapter 2.

Of all the edible cruciferous plants, watercress (*Nasturtium officinale*) is the least studied for its GS and GS hydrolysis products. Most of the previous reports on GS and ITCs are mainly focusing on the more commercial cruciferous vegetables such as broccoli, cauliflower and cabbages (Rosa *et al.*, 1997; Schonhof *et al.*, 2004 & Fahey *et al.*, 2001). Previous reports showed watercress contains PEITC which precursor is phenethyl GS (PEGLS) or its common name, gluconasturtiin (Williams *et al.*, 2009). PEITC is proven to restrain the growth of cancer cells (Gill *et al.*, 2007). Gupta *et al.* (2014) have published a comprehensive review on the anti-cancer effects of PEITC. Chapter 2 of this thesis will present a more thorough introduction and literature reviews on this topic.

In Sabah, watercress grow (either cultivated or survive as wild plant) as semi-aquatic plants near springs. It can be found growing in abundance as wild vegetable especially alongside slow running waterways in cold area such as in Kundasang, Ranau. Generally, watercress is widely consumed as soup, stir-fried and mixed with other foodstuffs or as salad. Also, it is normally sold at cheap price in the markets or supermarkets.

## **1.2 Problem Statement**

The formation of PEITC is easily affected by various factors such as temperature, pH and presence of additives (Eylen *et al.*, 2008). PEGLS is hydrolyzed into PEITC by the naturally-occurring enzyme myrosinase in watercress. This aspect needs to be systematically investigated because food preparation commonly involved cutting, heating and addition of other additives which may affect the PEITC formation. Currently, there are still scarce reports on the dynamic of hydrolysis of PEGLS in watercress under various external factors. Furthermore, beside PEITC, the other possible hydrolysis products of PEGLS are phenethyl nitrile (PEN) and/or phenethylthiocyanate (PET). The delicate aspect here is that PEN and PET are not

known to possess any therapeutic potential like PEITC. In fact some literatures mentioned that PET is toxic. Thus, it is imperative to ensure that the PEGLS is hydrolyzed into PEITC as optimum as possible.

### **1.3 Objectives**

The objectives of this current study are:

- i.) To extract and analyze the amount of GLS in watercress
- ii.) To study the effect of temperature, pH, and concentration of ascorbic acid and ferrous ions on the hydrolysis of GLS in watercress.
- iii.) To investigate the effects of temperature, pH, and concentration of ascorbic acid and ferrous ions of the myrosinase activity in watercress.

### **1.4 Scope of the Study**

The watercress samples used in this study were harvested fresh from Kg Melangkap in Kota Belud Sabah. These watercress grow as wild plant in shallow springs at the upstream of Panataran River which is located at the border of Mt Kinabalu Park (on the Kota Belud district side). Since watercress is a short shrubs, the whole part of the plant (except the roots) were used as samples. The selection of the watercress sample was not based on growth or development stage (i.e. the samples were mixed of matured and young watercress); healthy and free from insect attack were more emphasized during the sample collection. All the experiments were done using freeze-dried watercress powder after being freeze dried and grinded at Seaweed Research Laboratory.

The work presented here involves first, the extraction of PEGLS from the plant using 70 % methanol and analysed with HPLC-UV. The second part was investigation on the myrosinase activity under different conditions where the crude endogenous myrosinase extract from watercress was reacted with known concentration of pure sinigrin as substrate. The unreacted sinigrin in the extracts was then analysed using HPLC-UV. The third part was 30 mins of natural hydrolysis of GLS in the plant under similar conditions as the study on myrosinase activity. The resulted GLS hydrolysis products were then extracted using Dichloromethane (DCM) and analysed with GC-MS. Quantitation of the compounds

of interest was calculated using external standard calibration of the standard or authentic compounds.



## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Glucosinolates

Glucosinolates are organic anions that usually possess a  $\beta$ -D-thioglucoside grouping, and the differences between glucosinolates depend on the chemical nature of the side chain and significant effect upon the ultimate products of hydrolysis. Nowadays, more than 120 types of glucosinolates structure, which are having different only in their side chain (R-group) are known. Figure 2.1 shows the basic structure of glucosinolate.



**Figure 2.1 : Basic structure of glucosinolate, R groups are varies**

Source : Manchali *et al.*, 2012

They are classified into aliphatic, aromatic, and indolyglucosinolates based on oxime derivatives of amino acids (Hong *et al.*, 2011). For example, there are aliphatic glucosinolates (e.g., glucoraphanin and gluconapin), a phenyl glucosinolates (e.g., gluconasturtiin) and indole glucosinolates (e.g., glucobrassicin) in broccoli vegetables (Van Eylen *et al.*, 1997). Glucosinolates and their hydrolysis compound are obligate for the typical odor and taste in the brassica vegetables.

Most consumers having problems to deal with the brassica vegetables because of their bitter and pungent taste (Van Eylen *et al.*, 2007). Glucosinolate side-chains are characterized by a wide variety of chemical structures. Aliphatic glucosinolates derived from alanine, leucine, isoleucine, methionine and valine while aromatic glucosinolates derived from phenylalanine or tyrosine and indole glucosinolates from tryptophan (Cartea *et al.*, 2008). Table 2.1 gives an overview of the glucosinolates commonly found in Brassica vegetables (Velasco *et al.*, 2007). The most numerous glucosinolates are those containing either straight or branched carbon chains. Many of these compounds contain double bonds (olefins), hydroxyl or carbonyl groups or sulphur linkages. The largest single group (one-third of all glucosinolates) contain a sulphur atom in various states of oxidation such as methylthioalkyl-, methylsulphinylalkyl-, or methylsulphonylalkyl. The side chain of the glucosinolates is the basis for the structural heterogeneity and for the biological activity of the enzymatic and chemical breakdown products (Velasco *et al.*, 2007).



UMS  
UNIVERSITI MALAYSIA SABAH

**Table 2.1 : Glucosinolates identified in varieties of Brassica**

<b>Chemical class systematic name</b>	<b>Trivial name</b>
<b>Aliphatic</b>	
3-Butenyl	Gluconapin
4-Pentenyl	Glucobrassicinapin
2-(R)-2-Hydroxy-3-Butenyl	Progoitrin
2-(S)-2-Hydroxy-3-Butenyl	Glucoiberin
3-Methylsulphinylpropyl	Glucoibenerin
4-Methylsulphinylbutyl	Glucoraphanin
5-Methylsulphinylpentyl	Glucoalyssin
2-Hydroxy-4-Pentenyl	Gluconapoleiferin
4-Methylbiobutyl	Glucoerucin
<b>Indolyl</b>	
3-Iodylmethyl	Glucobrassicin
1-Methox-3-indolylmethyl	Neoglucobrassicin
4-Hydrox-3-indolylmethyl	4-Hydroxyglucobrassicin
4-Methoxy-3-indolylmethyl	4-Methoxyglucobrassicin
<b>Aromatic</b>	
2-Phenethyl	Gluconasturtiin
Benzyl	Glucotraepolin
p-hydroxybenzyl	Glucosinalbin

Source : Velasco *et al.*, (2007)

### 2.1.1 Glucosinolates discovery and distribution in plants

Nowadays, glucosinolates from the plant family Brassicaceae have been studied widely, whereas the plant family contains more than 350 genera and 3000 species. The research found that glucosinolate commonly can be detected in crucifer plants, but there also showed that one or more of the 120 known glucosinolate contained in about 500 species of non-cruciferous dicotyledenous (Fahey *et al.*, 2001). Due to the sharp taste of mustard seeds, the first study on the properties of glucosinolates and isothiocyanates were recorded at the beginning of 17<sup>th</sup> century to learn more about their chemical origin (Mohd Alnsour, 2013). In Figure 2.2, Gadamer (1897) had proposed the first general structure of glucosinolates at the end of 19<sup>th</sup> century, where he stated that the side chain or R group was linked to nitrogen not to the carbon atom of the 'NCS' group. In 1956, Ettlinger and Lundeen questioned the Gadamer structure due to the some inadequacies to explain certain properties of the compound and then proposed the new correct structure and the chemical synthesis of glucosinolates. Marsh & Waser (1970) supported their proposed structure by using X-ray crystallographic analysis of sinigrin to analyze the geometrical isomerism of the C=N bond. In other words, glucosinolates can be define as  $\beta$ -thioglucosidase N-hydroxysulfate (also commonly known as (Z)-(or cis)-N-hydroxyiminosulfate esters or S-glucopyranosyl thiohydroximate), with a R group (side chain) and a sulfur-linked  $\beta$ -D-glucopyranose moiety.

**Figure 2.2 : Structure of glucosinolate proposed by Gadamer(A) and Ettlinger (B)**

Source : Gadamer (1897) and Ettlinger & Lundeen (1961)