Characterizing Monomers by Alpha G Fibers in Phenolic Compounds

by

Ahmed Omar Mohammed Basharahil

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Dissertation submitted in partial fulfillment of
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32610, Bandar Seri Iskandar,

Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

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Approved by,

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(Dr. Asna M. Zain)

UNIVERSITI TEKNOLOGI PETRONAS
BANDAR SERI ISKANDAR, PERAK
September 2015

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

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AHMED OMAR MOHAMMED BASHARAHIL

ABSTRACT

Diabetes mellitus has been spreading rapidly in the current era. This research aims to discover a new treatment of diabetes involving the beta-Glucan represented by Oat and phenolic compounds extracted from two different sources namely Vernonia Amygdalina and Archidendron Jiringa. Both Beta-Glucan and Phenolic compounds are inserted into each other in a process called intercalation to make up a new compound which has different properties. The phenolic compounds will be extracted using water bath shaker and the extracted sample will be tested using HPLC to quantify the phenolic compound using Gallic acid as a standard. The Vernonia Amygdalina, Archidendron Jiringa and Oat will be mixed in a water base solution and to be given to diabetic patients in order to test the effectiveness of the blood glucose reduction. The testing shows that there is a blood glucose reduction of 25% for non-diabetic patients and 19% for diabetic patients.

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CHAPTER 1

INTRODUCTION

1.1 Background

The project investigates the phenolic compounds and Beta-Glucan as inhibitors to convert the more complex sugar into a simple one. Below will discuss in-short the components and processes involved in the study.

Antioxidants are compounds which may delay or prevent the oxidation of molecules by eliminating the initiation of oxidizing chain reactions. The antioxidant activity of phenolic compounds is because of their re-dox characteristics, which may act as an important role in neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

Glucans are polycaccharides composed of D-glucose monomers linked by glycosidic bonds. There are many different forms, each with different biological activities.

There are two types of Glucans that can be either Alpha (α) or beta (β) glucans. Both Alpha (α) and beta (β) glucans are only differentiated by stereochemistry. Alpha glycosidic bonds are formed in an axial position while beta glycosidic bonds are formed in an equatorial position. Numbering of both alpha and beta glucans relate to the number of the carbon atoms on which the glycosidic bond is formed. Thus, in a

beta-1,3 glucan, the glycosidic bonds are formed at the first and third carbons in the glucose ring.

Glucans may be found in nature in different forms. According to different studies, Glucans is proven to be effective in eliminating some diseases especially different types of cancer (Ma et al., 2010).

The combination between both beta-glucans and phenolic compounds is done through intercalation process. Intercalation is the inclusion of molecules into compounds with a layered structure. Some common examples of intercalation is the insertion of potassium metal or lithium metal into graphite for the purpose of yielding KC_8 and LiC_6. In graphite intercalated compounds (GIC), useful properties such as superconductivity can be obtained through the intercalation of atomic or molecular species in between the grapheme layers in graphite.

1.2 Problem Statement

Diabetes Mellitus (DM) is a serious disease caused by a flaw in the glucose homeostasis system. Roughly 171 million people worldwide suffer from diabetes and 3.2 million deaths have been reported yearly by the World Health Organization. Beta-Glucans and phenolic compounds are proven to be effective in eliminating some diseases including diabetes. Using intercalation process we can study the effectiveness of both combination in reducing Blood Glucose level.

1.3 Objectives

This study aims to:

1. Optimize the extraction for phenolic compound from two plant species based on Gallic Acid standard solution using water bath shaker and HPLC.
2. To test the effectiveness of the beta-glucan and phenolic compound combination in reducing blood glucose level.

1.4 Scope of study

This Study will focus on:

1. The extraction of the phenolic compounds from Vernonia Amygdalina and Archidendron Jiringa.
2. The measurement of the phenolic content of the sample extracted using HPLC.
3. The preparation of the dosage of both phenolic compounds for intercalation with Beta-Glucans.
4. Inducing the dosage combination on patients affected with diabetes mellitus and non-patients.
5. Testing the effect of the combination in reducing the blood glucose level.

CHAPTER 2

LITERATURE REVIEW

2.1 Phenolic compounds as antioxidant

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants (Randhir, Lin, & Shetty, 2004). These compounds, one of the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in plants. These compounds play an important role in growth and reproduction, providing protection against pathogens and predators (Bravo, 1998), besides contributing towards the colour and sensory characteristics of fruits and vegetables (Alasalvar, Grigor, Zhang, Quantick, & Shahidi, 2001).

Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Benavente-Garcia, Castillo, Marin, Ortuno, & Del Rio, 1997). Phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits and vegetables (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993). The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity (Heim, Tagliaferro, & Bobilya, 2002). Phenolic compounds could be a major determinant of antioxidant potentials of foods (Parr & Bolwell, 2000), and could therefore be a natural source of antioxidants.

Structurally, phenolic compounds comprise of an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds (Bravo, 1998). Despite this structural diversity, the group of compounds are often referred to as polyphenols. Most naturally occurring phenolic compounds are present as conjugates with mono- and polysaccharides, linked to one or more of the phenolic groups, and may also occur as functional derivatives such as esters and methyl esters (Harborne, 1989). Though such structural diversity results in the wide range of phenolic compounds that occur in nature, phenolic compounds can basically be categorized into several classes (Harborne, 1989). Of these, phenolic acids, flavonoids and tannins are regarded as the main dietary phenolic compounds (King & Young, 1999). Phenolic acids consist of two subgroups, i.e., the hydroxybenzoic and hydroxycinnamic acids (Fig. 2.1). Hydroxybenzoic acids include gallic, p-hydroxybenzoic, protocatechuic,

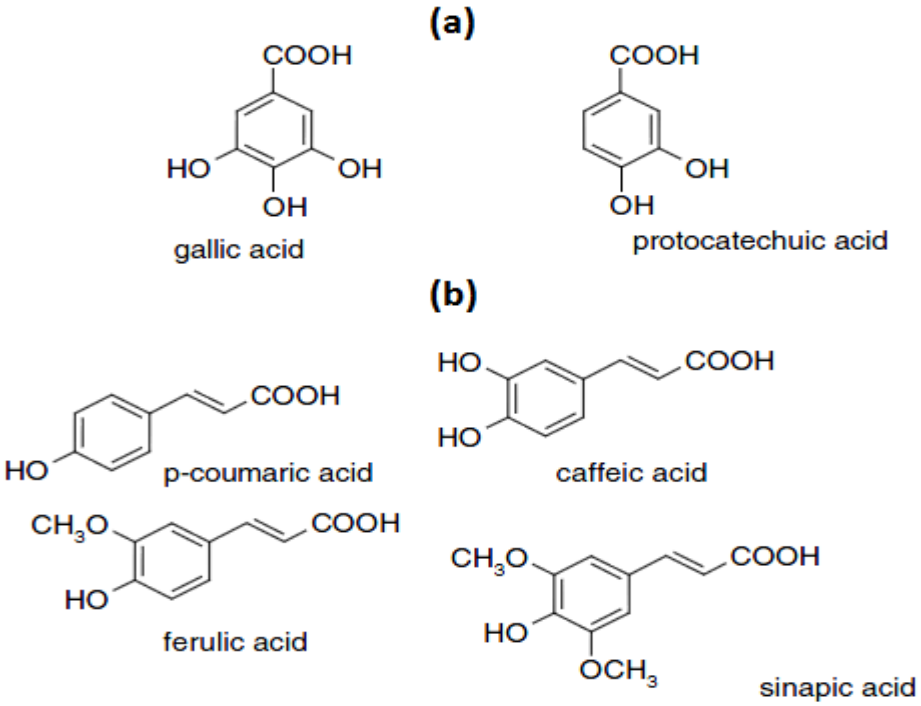


FIGURE 2.1 Examples of hydroxybenzoic (a) and hydroxycinnamic (b) acids.

There are wide variations between the total phenolics contents of the different fruits or vegetables, or even for the same fruits or vegetables reported by different

authors. These differences may be due to the complexity of these groups of compounds, and the methods of extraction and analysis (Bravo, 1998). For example, phenolic compounds present in fruits are found in both free and bound forms, but as the latter are often excluded from analyses, the total phenolics contents of fruits are often underestimated (Sun, Chu, Wu, & Liu, 2002). Besides, phenolics contents of plant foods depend on a number of intrinsic (genus, species, cultivars) and extrinsic (agronomic, environmental, handling and storage) factors (Toma´s-Barbera´n & Espı´n, 2001). Species differences are also pronounced, it is well known that the phenolics content of some fruits, i.e., banana, litchi (lichee), mango, and persimmon is considerably lower than that of berries and grapes. Asami, Hong, Barrett, and Mitchell (2003) reported that organically grown strawberries were found to have higher phenolics content than conventionally grown crops, though another study could not establish such a correlation (Ha¨kkinen & To¨rro¨nen, 2000). Processing and storage may have varying impacts on different phenolic compounds, as seen in berry processing where myricetin and kaempferol were found to be more prone to losses than quercetin (Ha¨kkinen et. al., 2000).

2.2 Glucans

Glucans are polycaccharides composed of D-glucose monomers linked by glycosidic bonds. There are many different forms, each with different biological activities. Alpha (α) and beta (β) glucans are differentiated by stereochemistry. Alpha glycosidic bonds are formed in an axial position while beta glycosidic bonds are formed in an equatorial position. Numbering of both alpha and beta glucans relate to the number of the carbon atoms on which the glycosidic bond is formed. Thus, in a beta-1,3 glucan, the glycosidic bonds are formed at the first and third carbons in the glucose ring.

Glucans are commonly ingested, and injected in some medical procedures. Glucans are also commonly inhaled, since all airborne fungal spores have cell walls containing primarily beta glucan. Glucan-containing airborne particles tend to be in

the large particle fraction of indoor (and probably also outdoor) aerosols (Chen & Hildeman 2009). The vast majority of fungal glucan exposure occurs outdoors. Crawford et al. (2009) measured 1,3 β d glucan indoors (geometric mean 1.0 ng/m3, range 0.81-1.2) and outdoors (geometric mean 7.34 ng/m3, range 6.1-8.9). In a similar study, Lee et al. (2006) found geometric mean concentrations indoors of 0.92 ng/m3 and outdoors of 6.44 ng/m3. The geometric mean indoor/outdoor ratio was 0.14.

Exposure to glucans is natural and occurs throughout life. They have been shown to be immunostimulants, and may contribute to the early development of the immune system in newborns. Glucans have been widely studied as anti-cancer agents (Ma et al., 2010). In the laboratory, positive effects on lung cancer (Zhong et al., 2009), leukemia (Gao et al., 2007), melanoma (Kamiryo et al., 2005), prostate cancer (Fullerton et al., 2000), and many others have been found. Glucan oral supplements have also been studied in relation to their effect on glucose metabolism and diabetes. They are also advertised as an aid to weight loss, however Beck et al. (2010), found no enhancement of weight loss by oak glucans. Talbott and Talbott (2010) found positive effects of beta-glucan supplements on respiratory disease (fewer symptoms), vigor, tension, fatigue and confusion.

There is considerable controversy about the negative health effects of exposure to beta-glucans. Some evidence has been found that inhalation of glucans can be irritating. Inhalation challenges have resulted in stimulation of inflammatory cells (Beijer et al., 2002), however this group used glucan from the polypore fungus, Grifola frondosa (not a common exposure source), and the concentrations used were quite high compared to reported concentrations in air (28.1 ng/m3, range 17.1-44.9). Bodin et al. (2009) also used chamber exposures to study irritant effects of dust alone, dust with added glucan, and dust with added aldehydes. Only those with nasal hyperreactivity from some previous cause reacted to the exposures. In another chamber study (Bonlokke et al., 2006), the same group measured small changes in nasal volume and other parameters with all exposures. They considered glucan to have a stimulatory effect when other particles are present. They and others (Young et al., 2003), noted

that particulate glucan has a stronger inflammatory effect than soluble glucan. In the laboratory, Holck et al. (2007) used several glucans in lymphocyte culture and found that the glucans increased histamine release only when combined with antigen (dust mite).

On the other hand, several research groups report no health related effects associated with inhalation of indoor glucans (Codispoti et al., 2010). This lack of effect was confirmed by Stuurman et al. (2008) in bakers with much higher exposure to glucans than are found in residential environments.

Overall then, while there is laboratory evidence for a role of β glucans in inflammation, the effects appear to be limited to relatively high exposures and to occur in those already experiencing hyperactivity from other causes.

2.3 Intercalation Process

Looking at the aspect of intercalation, we can observe that molecules with two dimensional networks show reactivity of the intercalation nature such as simple ions, organic species, coordination compounds or organometalics can be fitted into the interlayer region. The host-guest relation between intercalated molecules result in changes in the products chemical, catalytic, electronic optical and mechanical properties. Some compounds have the remarkable ability to adsorb through intercalation, cationic or neutral molecules between the layers. Examples of compounds like these are lamellar solids such as clay minerals which consist of tetrahedral and octahedral sheets, joined together by oxygen atoms. An octahedral sheet in between two layers of tetrahedral sheets is the most common structure for cationic clays. A charge deficiency on the layers is caused by the substitution of structural cations by others with lower valence electrons. This is neutralized by the adsorbed species forming an intercalated compound. (Constantino, Barbosa, Bizeto and Diaz, 1999)

Anionic clays known better as Layered double hydroxides (LDHs), represent a category of layered materials with unique properties which allow it to be used as a heterogeneous catalysis. These materials are also widely used in adsorption and decontamination processes, polymer processing and pharmacy. LDHs can act also a host inorganic structure for preparation of hybrid materials with interesting physical and chemical properties. In the polymer industry, the matrix of the material can considerably improve its properties such as tensile strength, heat and chemical resistance, gas permeability, as well as fire retardation. LDHs are usually prepared by co-precipitation, when a solution containing M_{II} and M_{III} metal cations in comparable proportions reacts with an alkaline solution. LDHs have a weak bonding between interlayer anions and hydroxide sheets, thus, anions can be exchanged under suitable conditions. Another alternative procedure is rehydration of mixed oxides obtained after thermal decomposition of a LDH precursor containing volatile interlayer anions (e.g., CO_3^{2-}or NO^{3-}). The mixed oxides prepared at moderate calcination temperatures (400 –600 °C) can be rehydrated in aqueous solutions. The rehydration reaction results in the intercalation of anions from the solution resulting in the reconstruction of the layered LDH structure. This phenomenon known as reconstruction is often applied for the intercalation of various anions into the LDH hosts. For intercalation of polymer molecules into LDH structure, all methods mentioned above, i.e., co-precipitation, anion exchange, and rehydration can be used. Direct co-precipitation can be used to prepare the LDHs intercalated with water soluble. Most polymers are hydrophobic and their compatibility with hydrophilic LDH filler is improved by modifying inorganic layers with surfactants. An intercalation of LDHs with bulky organic anions makes easier their exfoliation in some solvents or melts and improves generally the dispersion of hydroxide nano-sheets in the resulting polymer nano-composites. (Kovanda et. al., 2009)

2.4 Extraction of Phenolic Compounds

The extraction of phenolic compounds from their respective plant tissues has been successfully achieved through the use of an array of solvents such as ethanol,

methanol, ethyl ethers and ethyl acetates. In the food industry, ethyl acetates are used primarily due to its efficiency in extracting phenols. Additionally, because of its low polarity, it extracts phenols that are already dissolved in the lipid fraction of the food. Its ability to be removed and reused is due to the fact that it has a low boiling point. (Bonilla, Mayen, Merida and Medina, 1998) Solvent extractions are the most commonly used procedures to prepare extracts from plant materials due to their ease of use, efficiency, and wide applicability. It is generally known that the yield of chemical extraction depends on the type of solvents with varying polarities, extraction time and temperature, sample-to-solvent ratio as well as on the chemical composition and physical characteristics of the samples.

Traditional spectrophotometric assays provide simple and fast screening methods to quantify classes of phenolic compounds in crude plant samples. However, due to the complexity of the plant phenolics and different reactivity of phenols toward assay reagents, a broad spectrum of methods is used for assay of the constituents, leading to differing and often non-comparable results. In addition to that, the methods are quite prone to interferences and consequently often result in over- or underestimation of the contents. Modern high-performance chromatographic techniques combined with instrumental analysis are the "state of art" for the profiling and quantification of phenolic compounds. Gas chromatographic (GC) techniques have been widely used especially for separation and quantification of phenolic acids and flavonoids. The major concern with this technique is the low volatility of phenolic compounds. Prior to chromatography, phenolics are usually transformed into more volatile derivatives by methylation, conversion into trimethylsilyl derivatives, etc.

HPLC currently represents the most popular and reliable technique for analysis of phenolic compounds. Various supports and mobile phases are available for the analysis of phenolics including anthocyanins, proanthocyanidins, hydrolysable tannins, flavonols, flavan-3-ols, flavanones, flavones, and phenolic acids in different plant extract and food samples. Moreover, HPLC techniques offer a unique chance to analyze simultaneously all components of interest together with their possible derivatives or degradation products.

CHAPTER 3

METHODOLOGY

3.1 Specimen and Chemicals

Below is a list of materials and chemicals that have been used in this experiment:

1. *Vernonia Amygdalina* as a source of phenolic compound (shown in figure 3.1 a).
2. *Archidendron Jiringa* as a source of phenolic compound (shown in figure 3.1 b).
3. Ethanol.
4. Methanol.
5. Active Oat 35 as a source of Beta-Glucan with a concentration of 35% beta-glucan (shown in figure 3.1 c)
6. Phosphoric acid.

(a)

(b)

(c)

FIGURE 3.1 Specimen used for the preparation of blood glucose control (a) Vernonia Amygdalina, (b) Archidendron Jiringa and (c) Active Oat 35.

3.2 Tools and Equipment

The table 3.1 shows the list of tools and equipment as well as their purpose of use.

TABLE 3.1 List of Tools and Equipment

Tools/Equipment	**Purpose**
Oven	To dry peels
Grinder	To grind peels for phenolic extraction
Volumetric Flask (100mL)	To prepare Gallic acid.
Weighing balance	To weigh the sample.
Conical Flask (100mL 3 pieces)	To dilute the peels with ethanol
Measuring Cylinder (10mL)	To measure volume of extraction agent
Water Bath Shaker (Innova 2000)	To separate phenolic compounds from solution
Filter paper (No. 41 Whatman) and filter funnel	Filtration of solids from phenolic compounds
Syringe (2500µL)	To take small amounts of sample into test tubes for HPLC use
HPLC	To measure the quantity of extracted phenolic compounds.

3.3 Extraction of Phenolic Compound

Organic solvent extraction is the method used to extract phenolic in this experiment. Chemical procedures are used to detect the presence of total phenolic, while chromatographic techniques are utilized to identify and quantify individual phenolic compounds.

3.3.1 Sample Preparation

Four samples namely: *Vernonia Amygdalina, Archidendron Jiringa, Vernonia Amygdalina* and *Archidendron Jiringa and Beta-Glucan* have been prepared for extraction purpose. Steps below describe sample preparation:

1. 400 mg of Sample A (*Vernonia Amygdalina*) was dried and grinded.
2. Put 20 ml of 50% ethanol and 50% de-ionized water solution to the sample.
3. The sample is extracted using water bath shaker at 130rpm for 18 hr at 60 ^{o}C.
4. The sample extracted is then filtered using solvent filtration system with filter paper (Whatman, No. 41, 20-25 µm particle retention) to prepare for HPLC usage.
5. The total phenolic content in the extract was determined using HPLC
6. Re-do Steps 1 - 4 for samples B (*Archidendron Jiringa*) , C (200 mg *Vernonia Amygdalina* and 200 mg *Archidendron Jiringa*) and D (*Beta-Glucan)*

3.3.2 Gallic Acid Standard Preparation

Gallic acid has been used as standard to quantify the content of phenolic compound in the sample using HPLC. We prepare stock solution of 0.5 g of GA in 100 ml methanol in order to prepare five standard solutions of 5 ppm, 50ppm, 100ppm, 250ppm and 500ppm in 100 ml methanol then the standard solution was filtered using Nylon membrane 0.45 m µm for HPLC analysis.

3.4 Gantt Chart

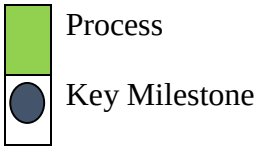
3.4.1 FYP I Gantt Chart

Table 3.2 shows the targeted timeline for Final Year Project 1 (FYP 1) course of the project entitled, Characterizing monomers by alpha G Fibers in Phenolic Compounds. The week 1 is starting from 18 May 2015.

TABLE 3.2 Gantt chart for FYP 1

Week / Detail	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Selection of Project Title														
Preliminary Research Work														
Submission of Extended Proposal														
Start of Project Work														
Proposal Defence														
Project Work Continues														

Submission of Interim Draft Report												●	
Submission of Interim Report													●



3.4.2 FYP 2 Gantt chart

Table 3.3 shows the targeted timeline for Final Year Project 2 (FYP II) course of the project entitled, Characterizing monomers by alpha G Fibers in Phenolic Compounds.

TABLE 3.3 Gantt Chart for FYP II

Week / Detail	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Project Work Continues														
Simulation Work														
Submission Progress Report														
Pre-SEDEX														
Submission of Draft final report														
Submission of dissertation and Technical Paper														
Viva														

Submission Project Dissertation (Hard Bound)														

3.5 Key Milestones

Table 3.4 shows the Key milestones for FYP 1 and FYP 2

TABLE 3.4 Key Milestones

FYP 1	
Milestone	**Date**
Preliminary Research on The Topic And Starting To Write The Extended Proposal Defense	Week7
Submission of Extended Proposal Defense	Week 7
Proposal Defense Presentation	Week 8
Submission of Interim Report	Week 13
FYP 2	
Preparation of Sample	Week 5
Extraction of Sample	Week 6
Preparation of Standard	Week 7
Quantification using HPLC	Week 8
Second Sample Preparation and Quantification using HPLC	Week 9
Third Testing sample and standard Using HPLC	Week 11
Dosing on Humans	Week 12
Submission of Draft Final Report	Week 13
Submission of Project Dissertation	Week 15

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Phenolic Compound Extraction Result:

The extraction is basically performed by using HPLC analysis to determine the quantity of phenolic compound extracted from *Vernonia Amygdalina*, *Archidendron Jiringa*, *mixture of Vernonia Amygdalina* and *Archidendron Jiringa and Beta-Glucan*.

The standard curve is determined by preparing 5ppm, 50ppm, 250ppm and 500ppm of Gallic acid stock. Figure 4.1 below shows the calibration curve for HPLC analysis at 1.717 minutes. The best fit line is obtained from the standard curve and the equation of the best fit line is

$$y = 2025x + 11421 \qquad (1)$$

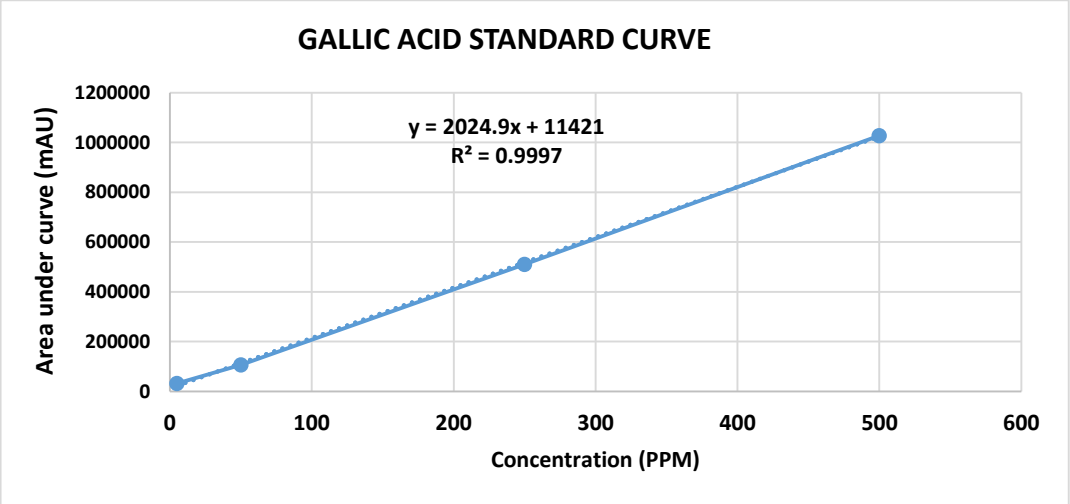


FIGURE 4.1 Standard curve for Gallic Acid

From the equation 1 that we obtained from the standard curve, we will be able to specify the exact amount of the Gallic acid presents in the sample:

$$y = 2025x + 11421 \qquad (1)$$

For Pithecellobium jiringa, it was found that at the same retention time as that of standard curve, we will have an area of 48932 mAU*s, applying the equation 1, we will have:

$$x = \frac{48932 - 11421}{2025} = 18.525\ ppm$$

TABLE 4.1 Concentration of the sample

Plant Species	**Area (mAU)**	**Ethanol /Water Fraction**	**Concentration (ppm)**
Vernonia Amygdalina	-	50%	-
Pithecellobium jiringa	48932	50%	18.525
Vernonia Amygdalina & Pithecellobium jiringa	35964	50%	12.121
Beta-Glucan	23854	50%	6.140

As we can see from Table 4.1, *Pithecellobium jiringa* has the highest Gallic acid concentration among other species.

4.2 Dose Response Study

The dose is a combination of the three components namely Vernonia Amygdalina, Archidendron Jiringa and Oat with defined quantity of 833 mg of each component. The combination is well mixed in a water based solvent of 175 ml pure water. The dose is then given to respondents to examine the effectiveness of the combination in reduction of blood glucose.

4.2.1 Dose Response Study (Non-Diabetic Patient)

Figure 4.2 shows the result of the test for 3 non-diabetic patients after preparing the dose and gave it to three respondents:

FIGURE 4.2 Dose Response Study for non-diabetic patients

The test result above shows clearly the effectiveness of the combination of both beta-glucan and phenolic compounds in controlling the blood glucose level for non-diabetic patients. There is a blood glucose reduction of almost 25 %.

4.2.2 Dose Response Study (Diabetic Patient)

For diabetic patients, the results for the combinations were as shown in figure 4.3 for only one respondents:

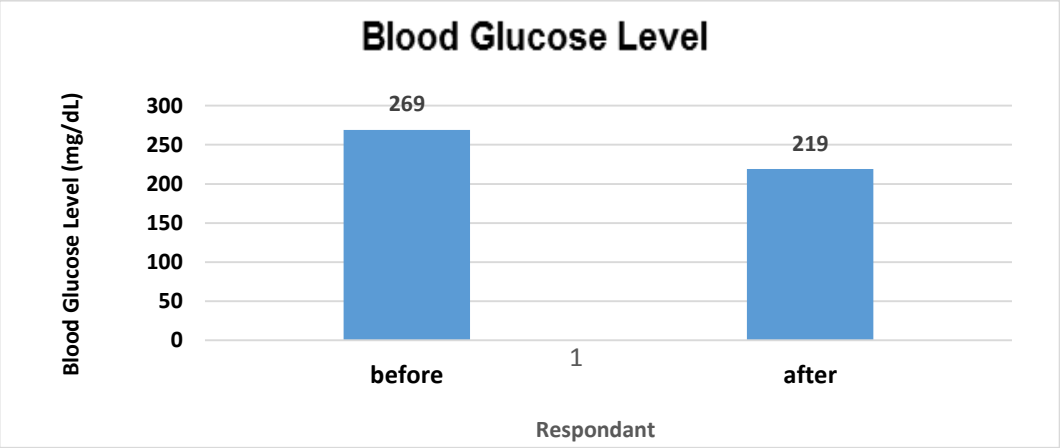


FIGURE 4.3 Dose Response Study (Diabetic Patients)

The test result shows a blood glucose reduction of 19 % compare to 25% using melformin diabetic medication. This result is clearly indicating the effectiveness of the combination in the blood glucose reduction.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

As a conclusion, this project is important as it deals with two compounds that are used for the health of human beings. Both compounds namely beta- glucan and phenolic compounds are existing in the nature. The scope of this project is to find the contribution of the beta-glucans and phenolic compounds in reducing the blood glucose.

The project is within capability of a final year student to be executed with the help and guidance from the supervisor and the coordinator. The time frame is also feasible and the project can be completed within the time allocated.

The result for extraction shows that Archidendron jiringa has the highest Phenolic compounds content of Gallic acid with 18.525 ppm compared to Vernonia Amygdalina and Oat.

For non-diabetic patients, the combination of phenolic compounds and beta-glucan shows significant reduction of 25 % blood glucose level. For diabetic patients, the combination of phenolic compounds and beta-glucan shows significant reduction of 19 % blood glucose level

5.2 Recommendation for future work

The study of combination of phenolic compounds and beta-glucans is still in its preliminary stages. The study is proven to be effective in reducing blood glucose level but there are many factors should be considered in order for the study to be solid proven. The testing has to be involved with more respondents so that the result will be practical to be commercialized. The study of the negative effects of the combination on human beings also should be considered as a part of any upcoming researches. Moreover, the perfect timing for the dosage is to be investigated as well. Extraction of phenolic may be optimized by having standards other than Gallic acid standard. Also, different extraction methods should be used in order to compare the best method in yielding the highest phenolic compound extracted from the plant sample. Other than that, different plant samples should be tested for the best combination with beta-glucans.

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