Isolation of Proteins and Oligopeptides with Potential Antimicrobial Properties

Nor Shazliana Binti Ghazalee
(35059)

Bachelor of Science with Honours
(Resource Biotechnology)
2015
DECLARATION OF ORIGINAL WORK

This declaration is made on the 9th day of 2018.

Student's Declaration:

Nor Shahidzah Binti Ghazalee, B35059, Faculty of Resource Science and Technology (Resource Biotechnology) hereby declare that the work entitled, "Isolation of Proteins and Polysaccharides with Enhanced Properties" is my original work. I have not copied from any other students' work or from any other sources with the exception where due reference or acknowledgement is made explicitly in the text, nor has any part of the work been written for me by another person.

[Signature]

9/10/2018
Date submitted

Name of the student (Matric No.)

Supervisor's Declaration:

I, Assoc. Prof. Dr. Hadi Hashim, (SUPERVISOR'S NAME), hereby certify that the work entitled, "Isolation of Proteins and Polysaccharides with Enhanced Properties" was prepared by the aforementioned or above mentioned student, and was submitted to the "FACULTY" as a Bachelor Degree of Resource Biotechnology (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work.

[Signature]

Received for examination by: ________________________ Date: ________________________

(Name of the supervisor)
I declare this Project/Thesis is classified as (Please tick (✓)):

☐ CONFIDENTIAL (Contains confidential information under the Official Secret Act 1972)*
☐ RESTRICTED (Contains restricted information as specified by the organisation where research was done)*
✓ OPEN ACCESS

I declare this Project/Thesis to be submitted to the Centre for Academic Information Services (CAIS) and uploaded into UNIMAS Institutional Repository (UNIMAS IR) (Please tick (✓)):

✓ YES
☐ NO

Validation of Project/Thesis

I hereby duly affirmed with free consent and willingness declared that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abide interest and rights as follows:

- This Project/Thesis is the sole legal property of Universiti Malaysia Sarawak (UNIMAS).
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis of academic and research only and not for other purpose.
- The Centre for Academic Information Services has the lawful right to digitise the content to be uploaded into Local Content Database.
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis if required for use by other parties for academic purposes or by other Higher Learning Institute.
- No dispute or any claim shall arise from the student himself/herself neither a third party on this Project/Thesis once it becomes the sole property of UNIMAS.
- This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student himself/herself without first obtaining approval from UNIMAS.

Student's signature ____________ (Date) ________________________________
Supervisor's signature: ____________ (Date) ________________________________

Current Address:
No. 6, Jln 7/6, Bandar Tasik Puteri, 81800 Rawang, Selangor.

Notes: * If the Project/Thesis is CONFIDENTIAL or RESTRICTED, please attach together as annexure a letter from the organisation with the date of restriction indicated, and reasons for confidentiality and restriction.
ACKNOWLEDGEMENT

I would like to express my deepest appreciation to my supervisor, Assoc Prof. Dr. Hasnain Bin Md. Hussain, who had received me as his final year students to carry out the final year project. Besides, he also continuously conveyed a spirit of adventure and excitement in completing this research. He also provided financial support in providing all the necessary equipment and chemicals in the lab. Without his financial support and spirit, my project would be so complicated to be done.

I would like to thank all post-graduate students in proteomic lab as they always assist and teach me the laboratory works and skills. Their time sacrificing to teach me were really appreciated. Without their guidance and persistent helps, this dissertation cannot be completed successfully.

Besides, I am really grateful that I able to meet with UNIMAS plant specialist, Mr Hidir Bin Marzuki. He helped me in identified and confirmed the scientific name of all my plant samples which were used in this project. His information had assisted me a lot to understand more about my sample and experiment.

Last but not least, a very big thankful given to my parents as they were always be there when I am in stressed and troubled especially during the critical time in completing this project. Thank you for all your loves, concerns, motivations and supports.
DECLARATION

I hereby certify that this material, which submit for assessment on the programme of study leading to the award of Bachelor Degree is totally my own work, that I have exercised reasonable care to ensure the work is original and no portion of this work referred to the thesis that has been submitted.

(Nor Shazliana Binti Ghazalee)  
Resource Biotechnology,  
Department of Molecular Biology,  
Faculty of Resource Science and Technology,  
Universiti Malaysia Sarawak
TABLE OF CONTENTS

LIST OF ABBREVIATIONS

LIST OF TABLES AND FIGURES

ABSTRACT

1.0 Introduction

2.0 Literature Review

2.1 Medicinal plant

2.2 Proteins and oligopeptides

2.3 Isolation and extraction of proteins and oligopeptides

2.4 Analysis of proteins and oligopeptides

2.5 Antimicrobial properties of proteins

2.6 Inhibition zone

2.7 Bradford assay

2.8 Partial purification

3.0 Materials and Methods

3.1 Sampling site and collection

3.2 Protein extraction buffer method

3.3 SDS-PAGE

3.4 Bradford assay

3.5 Disc diffusion assay

3.6 Ammonium sulphate partial purification

4.0 Results

4.1 Sample used based on traditional medicine application

4.2 SDS-PAGE

4.3 Bradford assay

4.4 Disc diffusion assay

4.4.1 Antibacterial assay

4.4.2 Antifungal assay

5.0 Discussion

5.1 Background of plant sample used
5.2 Pre-test by using TCA-acetone extraction
5.2 Extraction method testing
5.3 Plant sample 1
5.4 Plant sample 2
5.5 Plant sample 3
5.6 Principle behind main techniques

6.0 Conclusion

7.0 Recommendations

8.0 References
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>μl</td>
<td>Microliter</td>
</tr>
<tr>
<td>TCA</td>
<td>Trichloroacetic acid</td>
</tr>
<tr>
<td>2ME</td>
<td>2-Mercaptoethanol</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulphate-polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>V</td>
<td>Volt</td>
</tr>
<tr>
<td>mL</td>
<td>Millilitre</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium persulfate</td>
</tr>
<tr>
<td>TEMED</td>
<td>Tetramethylethylenediamine</td>
</tr>
<tr>
<td>mg/mL</td>
<td>Milligram per millilitre</td>
</tr>
<tr>
<td>Na₂S₂O₃</td>
<td>Sodium thiosulfate</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>MDRO</td>
<td>Multi-drug resistant organism</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
</tbody>
</table>
List of Tables and Figures

a) List of tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Sample used in the experiment.</td>
<td>12</td>
</tr>
<tr>
<td>Table 2</td>
<td>Protein concentration of sample 1.</td>
<td>17</td>
</tr>
<tr>
<td>Table 3</td>
<td>Protein concentration of sample 2.</td>
<td>17</td>
</tr>
<tr>
<td>Table 4</td>
<td>Protein concentration of sample 3.</td>
<td>18</td>
</tr>
<tr>
<td>Table 5</td>
<td>Protein concentration of purified protein sample.</td>
<td>18</td>
</tr>
<tr>
<td>Table 6</td>
<td>Comparison of protein concentration.</td>
<td>19</td>
</tr>
</tbody>
</table>

a) List of figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Sample 1</td>
<td>13</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Sample 2</td>
<td>13</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Sample 3</td>
<td>14</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Protein bands from different extraction method.</td>
<td>14</td>
</tr>
<tr>
<td>Figure 5</td>
<td>Protein bands of sample 1.</td>
<td>15</td>
</tr>
<tr>
<td>Figure 6</td>
<td>Protein bands of sample 2.</td>
<td>15</td>
</tr>
<tr>
<td>Figure 7</td>
<td>Protein bands of sample 3.</td>
<td>16</td>
</tr>
<tr>
<td>Figure 8</td>
<td>Protein bands of purified protein sample.</td>
<td>16</td>
</tr>
<tr>
<td>Figure 9</td>
<td>Replicate 1 for antibacterial test of sample 1 by using different (a). extraction buffer</td>
<td>19</td>
</tr>
<tr>
<td>Figure 9</td>
<td>Replicate 2 for antibacterial test of sample 1 by using different (b). extraction buffer.</td>
<td>20</td>
</tr>
<tr>
<td>Figure 10</td>
<td>Replicate 1 for antibacterial test of sample 1, 2, and 3 towards (a). B. subtilis.</td>
<td>20</td>
</tr>
<tr>
<td>Figure 10</td>
<td>Replicate 2 for antibacterial test of sample 1, 2, and 3 towards (b). B. subtilis.</td>
<td>21</td>
</tr>
</tbody>
</table>
Figure 11  Replicate 1 for antibacterial test of sample 1, 2 and 3 towards \( E.\) \( \textit{coli}. \)  
(a).

Figure 11  Replicate 2 for antibacterial test of sample 1, 2 and 3 towards \( E.\) \( \textit{coli}. \)  
(b).

Figure 12  Antibacterial test of proteins that present in 20% and 40% concentration solution Antibacterial test was done towards \( B.\) \( \textit{subtilis}. \)  
(a).

Figure 12  Antibacterial test of proteins that present in 60% and 80% concentration solution Antibacterial test was done towards \( B.\) \( \textit{subtilis}. \)  
(b).

Figure 13  Replicate 1 for antifungal test of sample 1, 2 and 3 towards \( A.\) \( \textit{flavus}. \)  
(a).

Figure 13  Replicate 2 for antifungal test of sample 1, 2 and 3 towards \( A.\) \( \textit{flavus}. \)  
(b).

Figure 14  Protein bands from TCA-acetone extraction.
ABSTRACT

The purpose of this project was to isolate proteins and oligopeptides from selected plants that possess antimicrobial properties. This was because in some instances, antibiotics that supposedly could kill the bacteria cannot longer carry out its function anymore towards the multi-drug resistance organisms (MDROs). Thus, this research was conducted with intention to find more sources of antimicrobial drug in order to combat the multi-drug resistance bacterial infection problems. The samples were extracted for its proteins by using protein buffer extraction method. The presence of proteins obtained were confirmed and separated based on their mass by using SDS-PAGE. Then, disc diffusion assay was conducted to determine the antimicrobial properties of the proteins towards both bacteria and fungi. Bradford assay was carried out to determine protein concentration available in the protein crude. Lastly, the positive antimicrobial proteins were undergo ammonium sulphate partial purification to determine the specific proteins that responsible in carry out the antimicrobial properties. By the end of this study, proteins and oligopeptides from three selected samples were successfully isolated with only proteins from Gelung-ge sample, Senna alata exhibited antimicrobial properties towards gram positive bacteria. Inhibition zone was successfully showed in disc diffusion assay.

Keywords: MDROs, SDS-PAGE, disc diffusion assay, inhibition zone, partial purification

ABSTRAK


Kata kunci: Organisma yang mempunyai daya tahan terhadap pelbagai ubat, SDS-PAGE, cakera resapan assay, zon perencatan, pembersihan separa.
1.0 Introduction

Nowadays, multi-drug resistance bacterial infection cases become increasing with the advance of global. Thus, scientists especially those who are in medical, biotechnology and pharmaceutical field progressively conduct researches to overcome this problem. This study is one of the efforts in finding more sources of antimicrobial drug to combat the multi-drug resistance bacterial infection problems by using selected plants. Plant was used because plant contains a lot of protein especially in protein bodies inside cell walls (Martinez-Maqueda et al., 2013). Most of antimicrobial peptides that had been found before come from plants and they had similar structure and function with human antimicrobial peptides. Therefore, plant is one of the good sources to find antimicrobial peptides for development of new antibacterial drug.

In this experiment, plant samples were obtained at Bidayuh village in Serikin. Based on traditional knowledge, people in the village used this plant for several diseases and wounds. They used it by grinding and directly apply on the diseased area. Plants that were utilised by boiling it first were not taken as a sample in this study because boiling could denature the proteins structure of the plants. During boiling process which were around 100°C, structure of proteins started to changed and formed unstable conformation. As a consequence, the proteins functions were disturbed (Daniel et al., 1996). Medicinal plants which used boiling technique showed that it might use another exists components in the plant instead of protein in order to cure the particular disease. The aim of this study was to isolate proteins in the plant leaves and to determine the antimicrobial properties of the proteins. The formation of inhibition zone determined the presence of antimicrobial peptides.

This experiment was conducted by using protein buffer extraction method, SDS-PAGE, Bradford assay, disc diffusion assay and ammonium sulphate partial purification method in order to achieve the objectives of this study. Protein buffer extraction method is
a technique to extract or isolate the total protein soluble from leaves of the plant. This technique used 0.1 M Tris-HCl solution to precipitate proteins and effectively stop the protease actions in plant tissues (Ranjan et al., 2012). While SDS-PAGE is one dimension electrophoresis which responsible to separate proteins from other components based on their mass. SDS-PAGE was used to confirm the presence of proteins in the extraction solution. In order to determine the presence of antimicrobial properties and diameter of inhibition zone, disc diffusion assay was used. Disc diffusion method was subjected to test for antibacterial properties and also for antifungal properties. Bradford assay was used to identify the concentration of protein available in the protein sample. Lastly, the ammonium sulphate partial purification was done in order to identify the specific protein that exhibited the antimicrobial properties.

By the end of this study, proteins in the plant leaves were successfully be isolated and also the antimicrobial properties of the extracted proteins was successfully determined. The formation of inhibition zone by disc diffusion assay determined the presence of antimicrobial peptides.

Therefore, the objectives of this project were to:
1- Isolate proteins and oligopeptides from plants
2- Identify the antimicrobial properties of the proteins and determine diameter of inhibition zone.
2.0 Literature Review

2.1 Medicinal plant

Traditional medicine is the use of knowledge, beliefs, skills and experiences of each culture to carry out the therapeutics activity towards several illnesses (Benzie & Wachtel-Galor, 2011). Most of traditional medicines come from medicinal plant especially herbs. Medicinal plant is important as it is the primary sources of health service for ancient people especially those who live in rural and poor areas (World Health Organization, 1998). Knowledge of these traditional medicines has been passed from one generation to other generations and this field has developed recently even in modern society. This is because people still searching for natural alternative medicine instead of synthetic chemical drugs. Medicinal plants contribute huge percentage in pharmaceutical market in this world. More than 50% of drugs in this world were made from natural products and its actives ingredients (Wyk & Wink, 2004).

Benefits of these medicinal plants had been known and revealed through scientific research and novel search. Scientific studies also help to give understanding on how do the biological activity of these medicinal plants work (Wyk & Wink et al., 2004). Therefore, this study is one of the approaches in screening for a new source of compounds to formulate a new antimicrobial drug. Potential medicinal plant used in this research is selected randomly from Bidayuh village in Sarawak based on traditional knowledge and experiences of the local people.
2.2 Proteins and oligopeptides

Protein is one or more large strain of amino acids and its existence is crucial to ensure body function properly (Campbell, 2009). Oligopeptide is one type of proteins which made up of relatively small number of amino acids which usually consist of 2 to 20 amino acids. Proteins are produced as a product of central dogma which is replication, transcription and translation process of genome (Crick, 1970).

Plant consists of small amount of proteins but huge proportion of secondary compounds such as storage polysaccharides, lipid and phenolic compounds (Faurobert et al, 2007, Wu et al., 2014). The phenolic compound is made up of around 8000 types of other components which gathered and soluble in vacuoles of the plant. This plant phenolic occupies a large significant volume of the plant instead of proteins (Wu et al., 2014). Thus, to identify and isolate proteins or proteome from plant are quite difficult.

Proteome is a total amount of proteins that presence in a particular cell, tissue or organism (Rehm, 2006). Proteins are responsible in most of physiological processes in particular organism. Life sign of cell is depends on the function and concentration of its proteins (Rehm et al., 2006). There are various types and functions of proteins. Of the various types, one of them is the one that possess antimicrobial properties. Their functions are killing and inhibiting the growth of multi-drug resistant bacteria.

2.3 Isolation and extraction of proteins and oligopeptides

Extraction technique includes cell disruption steps, protein solubilisation steps, and protein enrichment steps. In this research, extraction of proteins was conducted by using TCA-acetone extraction. Cell disruption step is used to break plant cell walls and protein enzymes in order to release proteins and remove components which may interfere in the assay and analysis. While protein solubilisation steps are used to separate proteins from unwanted
elements. This step is important to reduce proteolysis and modification of proteins. Lastly, the purpose of protein enrichment step is to raise the quantity of proteins and gain distinguishable fractions of desired protein (Martinez-Maqueda et al., 2013).

2.4 Analysis of proteins and oligopeptides

Proteins that had been isolated and extracted were analysed by using a type of electrophoresis. Most of analysis study of protein used SDS-PAGE for its electrophoresis process because SDS-PAGE was able to give beautiful band in only 30 to 45 minutes for each gel run (Rehm et al., 2006). SDS-PAGE is a type of 1 dimension electrophoresis (Costas et al., 1989). It uses polyacrylamide gel and SDS in conducting the electrophoresis Function of SDS-PAGE is to separate substances especially proteins or nucleic acid based on their molecular weight (Sattayasai, 2012) SDS is a detergent which responsible to denature the proteins into linear structure and give negative charges to the proteins proportional to its mass (Nowakowski, 2014).

Polyacrylamide gel was used instead of agarose gel. In order to ensure the polymerization of polyacrylamide is successful, ammonium persulfate and buffer was prepared freshly. It was incubated at 40°C. This was crucial for good polymerization. Before loading the sample, the sample solution was heated first at 95°C for 5 minutes in test buffer. This helped solubilisation of protein sample as proteases were inhibited and the tertiary structures were denatured and unfolded. But, heated at 40°C instead of 95°C was encouraged to prevent latter formation (Rehm et al., 2006).
2.5 Antimicrobial properties of proteins

Protein with antimicrobial properties refers to protein that able to stop the growth of microbes by killing or inhibit the microbes. This protein is important to kill pathogens and multidrug-resistant organisms that may cause various types of diseases. The study of this antimicrobial protein is one of efforts to find drug which can overcome this problem.

Based on previous research, antibiotic could carry out 2 biological mechanisms against bacteria which are either induces death of cell or inhibit growth of cell. Physical interaction between drug and targeted bacteria were involved in inducing cell death pathway. The biochemical, molecular and ultrastructure of bacteria were modified and lead to death of cells. Example of this type of antibiotic is penicillin which had been discovered in 1929 (Kohanski et al., 2010)

Another example of research that had been conducted to obtain antimicrobial protein was done towards Cratoxylum formosum plant. Stem bark of C. formosum was extracted for its gum and this gum was investigated for its antimicrobial properties towards several strains of Streptococcus mutans. In this study, the C. formosum was tested by using agar diffusion method and two-fold broth dilution method. The antimicrobial properties of C. formosum was proved by showing inhibition zone at range 9.11 mm to 11.5 mm and minimum inhibitory concentration (MIC) value from 48 μg/ml to 97 μg/ml (Suddhasthira et al., 2006). The presence of inhibition zone and MIC value are two significant characteristics in determining the antimicrobial properties of the sample.

Antifungal is one of antimicrobial properties. Antifungal agent is a component or substances that have ability to kill or inhibit growth fungal and yeast. The first antifungal formulated was fluconazole and itraconazole in the early 1990s. Several years later, second generation of antifungal was formulated as most of the fungal especially non-albican
Candida species and moulds were resistant towards first generation antifungal agent. Fungal are continuously evolve in order to adapt and survive. Thus, finding new sources of antifungal agent is crucial to prevent the spread of fungal infections. Fungal infection can be classed into two groups which are superficial and invasive fungal infections (IFIs). Dermatophytes and yeast are the main agents which causing superficial antifungal expression. Whereas, IFIs could causes life-threatening diseases. Thus, finding new sources of broad spectrum antifungal agent is important as conducting early treatment is a strategy to reduce fatality rate (Chen & Sorrell, 2007).

2.6 Inhibition zone

One of the characteristics of protein which has the antimicrobial properties is showing inhibition zone when conducting qualitative screening method. Inhibition zone is a clear area that surrounds the proteins sample when tested with certain types of bacteria. The inhibition zone indicates the antimicrobial activities that protein capable to carry out (Aliamadi et al., 2011). This property was tested by using disc diffusion assay. The width of the inhibition zone formed showed the strength of antibacterial action carried out by the protein (Roy et al., 2010). In this project, the protein sample was tested by using Escherichia coli, Bacillus subtilis and Aspergillus flavus.

2.7 Bradford assay

Bradford assay is used for determining the concentration of protein present in the sample solution. Bradford assay is simpler, faster and more sensitive compared to Lowry assay. It gives a quick and accurate result as it has low interference potential by non-protein components including the common reagents. Coomassie Blue G250 was used in this method to bind at protein sample. The unbound Coomassie Blue is presence in four different ionic forms. Three of them are cationic red, cationic green and anionic blue. They work well in
acidic solution. The cationic red and green have maximum absorbance at 470 nm and 650 nm respectively. Anionic blue on the other hand has maximum absorbance at 590 nm. The anionic blue is the one that bind to the protein sample. Thus, in order to detect the concentration of protein, amount of blue dye is measure through the absorbance of the solution at 595 nm (Kruger, 2009).

2.8 Partial purification

In this project, ammonium sulphate precipitation was being used to carry out partial purification. Ammonium sulphate precipitation utilized salting-out technique. The strength of ammonium sulphate salt is that it has high solubility properties and high ionic strength. However its solubility strength slightly changes with temperature. Besides, this method did not need high cost as ammonium sulphate is not expensive. Sulphate ions used in this process was responsible in salting-out the protein from the solution. Sulphate ions prevented formation of hydrogen bonding between proteins and water. On the other hand, it facilitated the aggregation of proteins among themselves by forming bond with the proteins and tighten the binding complex. Concentration of sulphate ions and proteins coprecipitated depends on their binding and protein conformation (Rachana et al, 2014; Grodzki & Berenstein, 2010).
3.0 Materials and Methods

3.1 Sampling site and collection

Plant samples were obtained. The samples were collected at Bidayuh village in Serian previously. Based on traditional knowledge of the local people, the plant samples could be used as treatment for several diseases and wounds. There were three types of plant samples obtained which are Gelung-ge (*Senna alata*), Ulok tangom (*Blechnum orientale*) and Daun akar belan (*Merremia peltata*).

3.2 Protein Extraction Buffer Method

Total protein extraction method was carried out following the standard protocol as described by Ranjan et al. (2012) with some modification. Cell disruption was done by slightly dipped the plant sample in liquid nitrogen and ground in a mortar and pestle until formed fine powder. Then, 0.5 g of the plant powder obtained was transferred into a new seal-rite 1.5 mL microcentrifuge tube. Next, 1.0 mL of extraction buffer which contained of 0.1 M Tris-HCl, pH 8.0 was added into the same microcentrifuge tube. The solution formed was stored at 4°C for overnight. Centrifugation at 10,000 rpm for 15 min was conducted after finishing the incubation. The supernatant was transferred into new seal-rite 1.5 mL microcentrifuge tube while the pellet formed was discarded. The supernatant was centrifuged again at 10,000 rpm for 10 min to remove the remaining debris. The supernatant obtained was transferred into new microcentrifuge tube and the pellet form was discarded.

3.3 SDS-PAGE

SDS-PAGE was conducted by mixing protein crude with sample buffer. This sample buffer contained 2 Mercaptoethanol (2ME). Negative control also was prepared by using sample buffer only. The mixture was heated at 95°C for 5 min. Then, electrophoresis was conducted
by using 12% SDS-PAGE with 120 V. The sample solutions and negative control were loaded into respective well. One well of the gel was provided for standard protein ladder. When tracking dye reached 1 cm above the end of gel cast, the electrophoresis was stopped. The result was seen after the gel undergo staining and destaining process (Aliahmadi et al., 2011).

3.4 Bradford Assay

In conducting Bradford assay, standard protocol as described from Bradford (1976) was followed. Bradford reagent which consisted of 10 mg Coomassie Brilliant Blue G-250, 5 mL of 95% ethanol and 10 mL 85% phosphoric acid were prepared. The Bradford reagent then was filtered through filter paper into new aluminium foil-coated bottle. Standard protein curve were constructed based on data obtained from standard protein assay. Standard protein assay was done started from 0.0 mg/mL until 10 mg/mL by varying the volume of bovine serum albumin solution and pH 8, 0.1 M Tris-HCl solution. Next, the protein sample assay was prepared by mixing the sample solution with 10 mL of Bradford reagent. Lastly, all these standard protein solutions and protein sample solutions were vortexed and inverted for 2 minutes. The absorbance data at 595 nm were obtained and recorded.

3.5 Disc Diffusion Assay

Disc diffusion assay was carry out for antibacterial and antifungal susceptibility test. Firstly, the concentration of bacteria and fungus used were standardized. The bacterial used were *E. coli* and *B. subtilis* while fungus used was *A. flavus*. Bacterial were tested at 550 nm wavelength until obtained the reading of 0.168 absorbance while fungus was tested at 450 nm wavelength until obtained reading of 0.600 absorbance. Then, bacteria were poured onto the Mueller Hinton Agar plate and fungus onto Potato Dextrose Agar respectively. These microorganisms were spread evenly throughout the petri dish by streaking with sterile cotton.
bud. Sterile filter paper discs were placed on the agar. 10 μl of each protein sample was pipetted onto particular disc. Negative control of this assay was conducted by using autoclave distilled water. Positive control was done by using ampicillin for antibacterial and ketoconazole for antifungal. The Mueller Hinton Agar were placed in incubator at 37 °C for 24 hours. Whereas, Potato Dextrose Agar was placed in room temperature for 72-96 hours. The presence of inhibition zone were observed and recorded. These procedure were conducted as described by Chandrasekaran and Venkatesalu (2004) but with some modification.

3.6 Ammonium Sulphate Partial Purification

In this study, partial purification was done by using ammonium sulphate technique. Protein sample which gave positive result in antimicrobial test was subjected to this partial purification. The protein sample was mixed with ammonium sulphate into different fractionated concentration which were 20%, 40%, 60% and 80% concentration. In each step, the solution formed was centrifuged at 10,000 rpm for 15 min in 4 °C. The protein-contained pellet formed was stored at -20 °C for further uses. While, the supernatant formed was transferred into new microcentrifuge tube and started to proceed with the next concentration (Ahmed et al., 2013)
4.0 RESULTS

In this project, three samples were selected to be used. All of them were selected based on their traditional applications. All of these samples then were extracted for their proteins and were further analysed by using SDS-PAGE, Bradford assay and disc diffusion assay in order to isolate the antimicrobial proteins. Proteins sample which showed inhibition zone when tested towards disc diffusion assay was undergo partial purification by using ammonium sulphate precipitation to isolate more specific protein which had the antimicrobial properties.

4.1 Samples that were chosen based on traditional medicine application

Table I Sample used in the experiment. All of these samples were selected as their preparation method in traditional application did not use procedure which denatured the proteins available in the samples. Thus, the treatment might use proteins available in the sample to carry out the treatment and this study was conducted to confirm the concept and directly isolated the antimicrobial proteins.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Traditional name</th>
<th>Scientific name</th>
<th>Uses</th>
<th>Preparation for traditional treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ulok tangom</td>
<td><em>Blechnum</em></td>
<td>Stomach ache</td>
<td>Used as poultice</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>orientale</em></td>
<td></td>
<td>and applied on stomach</td>
</tr>
<tr>
<td>2</td>
<td>Gelung-ge</td>
<td><em>Senna alata</em></td>
<td>Ringworm</td>
<td>Grinding and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>directly applied on infected area.</td>
</tr>
<tr>
<td>3</td>
<td>Daun akar</td>
<td><em>Merremia peltata</em></td>
<td>Sores and belan</td>
<td>Smoked over a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>flame and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>directly applied on infected area.</td>
</tr>
</tbody>
</table>
1. Sample 1 (Ullok tangom)

![Sample 1](image1)

*Figure 1 Sample 1*

2. Sample 2 (Gelung-ge)

![Sample 2](image2)

*Figure 2 Sample 2*