DETERMINATION OF PROTEIN CONTENT IN SELECTED PLANTS METHANOL EXTRACT

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CERTIFICATION

This is to certify that we have read this research paper entitled **'Determination of Protein Content in Selected Plants Methanol Extract'** by Iman Najla binti Thairun Aris (D18A0045), and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT 55204 – Research Project.

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My family

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Thank You

DEDICATIONS

In the name of Allah, The Most Beneficent, The Most Merciful, and The Most Gracious I humbly dedicate this thesis to my all-time beloved family, supportive friends & members of Faculty of Veterinary Medicine, Universiti Malaysia Kelantan. A special feeling of gratitude to my loving parents, Haniza and Thairun Aris, whose words of inspiration and prodding to perseverance echo in my ears. My sisters, Anis and Ayuni, have never left my side and are very special.

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List of Abbreviations

BSA	Bovine serum albumin
NSA	Non-structural carbs
CMC	Critical micellar concentration
SDS	Sodium dodecylsulfate

ABSTRACT

An abstract of the research paper was presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement for the course DVT 55204 – Research Project.

Protein levels and ileal digestibility is critical in animal feeding. It is closely attributed to the animal's performance, which includes growth and efficiency. Plant-based proteins have consistently been one of the prime sources of protein that the livestock have ingested throughout the course of many years. The amount of protein in various plants can be determined by analyzing the protein content using selected plant extracts. This is a preliminary study to assess the protein content by using methanol extract of selected plants. *Alpinia galanga, Curcuma* spp.*, and Musa* spp. leaves were used in this study. Using the Bradford method, *Musa* spp. leaves have the highest concentration of protein content, which is 261, 670 ng/mL, followed by *A. galanga*, which has 67, 670 ng/mL protein content, and *Curcuma* spp. has the lowest protein concentration, which is -20, 000 ng/mL. When the protein value is determined, the most suitable plant can be incorporated into the animal's feed thus it will fulfill the daily nutritional requirements of the animals.

Keywords: Plant Extract, Protein Content, Methanol Extract

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Tahap protein dan penghadaman ileal adalah kritikal dalam pemakanan haiwan. Ia berkait rapat dengan prestasi haiwan, yang merangkumi pertumbuhan dan kecekapan. Protein berasaskan tumbuhan secara konsisten menjadi salah satu sumber protein utama yang telah dimakan oleh haiwan ternakan bertahun-tahun. Jumlah protein dalam pelbagai tumbuhan boleh ditentukan dengan menganalisis kandungan protein menggunakan ekstrak tumbuhan terpilih. Ini adalah kajian awal untuk menilai kandungan protein dengan menggunakan ekstrak metanol tumbuhan terpilih. A*lpinia galanga, Curcuma* spp*.,* dan daun *Musa* spp. telah digunakan dalam kajian ini. Menggunakan kaedah Bradford, daun *Musa* spp. mempunyai kepekatan kandungan protein yang paling tinggi, iaitu 261 670 µg/mL, diikuti oleh *A. galanga,* yang mempunyai kandungan protein 67 670 µg/mL, dan *Curcuma* spp. mempunyai kepekatan protein yang paling rendah, iaitu -20 000 µg/mL. Apabila nilai protein ditentukan, tumbuhan yang paling tinggi kepekatan proteinnya boleh dimasukkan ke dalam makanan haiwan lantas ia akan memenuhi keperluan pemakanan harian haiwan.

Kata kunci: Plant Extract, Protein Determination, Methanol Extract

1.0 INTRODUCTION

Proteins and other components are required as building blocks for animal tissues and organs. Therefore, proteins in animal diets are essential for tissue development and repair. Proteins are typically the most expensive component of an animal's diet and may, regrettably, never be substituted with other nutrients. The term "plant-based proteins" refers to proteins that can be found in plant-based foods including whole grains, legumes, and nuts (Qin et al., 2022). Since their nutrient digestibility and fecal quality are comparable to those of a meat diet, these plant protein sources are alternatives substitutes for animal protein sources in extruded pet foods (Yamka et al., 2004).

As for the livestock, plant proteins have been crucially incorporated into the animal's feed. Unfortunately, not all types of plant can provide the livestock's daily protein needs. The protein level analysis is needed to determine the protein value in different types of plants. Plant-based proteins such as legumes (soybeans) and grains (corn) have always been utilized heavily in the feed mill industry. However, in light of the significant rise in worldwide demand for animal products, as well as the rise in global demand for plant-based and animal-based sources of protein, it is important to note that the demand for animal products is expected to continue to significantly increase (Henchion et al., 2017). There is no doubt that feed protein supply, sources, and alternatives beyond legumes (soybeans) and grains (corn) will need to be increased. Hence, there is significant potential for enhancing food security and supply by providing better protein feed to the livestock if we can diversify the feed protein source for the animal.

1.1 Research problem

Protein for animal diets can be sourced from a wide variety of different sources, and there are several opportunities for substitution and diversification. In present, plant proteins especially corn and soy are very high in demand to be the main diet for the feed mill industry. Due to the fact that Malaysia imports the majority of these plant proteins, it will be vital to address any future shortages or undersupplies to prevent adverse effects on the livestock and jeopardize the economic stability. Therefore, further study on alternative protein sources, particularly on the copious local plants, should be done in order to prevent extremely protein-dependent diets from mainly containing soy and corn.

1.2 Research questions

1.2.1 What is the protein level of the selected plant with methanol extract?

1.3 Research hypothesis

1.3.1 Each plant methanol extract will have its own protein quantity value.

1.4 Objectives

1.4.1 To determine protein level in selected plant extract by using methanol as the solvent.

2.0 LITERATURE REVIEW

2.1 Importance of Diversifying the Protein Source of Farm Animal's Feed

Protein sources for animal diets are many and diverse, with several prospects for future diversification and substitution. It was emphasized that microbial protein, which is digested in the small intestine of ruminants, is an important nutritional source of amino acids because it promotes a healthy and balanced diet. Amino acids are employed primarily as building blocks for protein synthesis, as well as precursors for glucose and fatty acid synthesis, and are therefore essential for maintenance, growth, health, reproduction, and lactation (Alvarado, 2019). There are two types of protein that are included in the daily feed of the livestock which are the plant-based protein and animal-based protein, for instance the soybean meal and the fishmeal. According to the Food and Agriculture Organization (2016), fish meal is a great by-pass protein source for ruminants and a decent source of quality protein for monogastric animals (De Angelis et Al., 2021). Compared with other sources of plant protein and cereals, fishmeal can also provide a good nutritional source of calcium and phosphorus in animal diets. When it comes to producing animal feed, soybeans remain the most sought-after and valued source of high-quality vegetable protein. Due to its high crude protein content (44–50%) and well-balanced amino acid composition, soybean meal, a by-product of oil extraction, is an excellent substitute for maize meal in the formulation of animal feed. In high performance monogastric diets, a significant amount of inclusion (30–40%) is used (Food and Agriculture Organization, 2016).

In addition, between 1995 and 2020, the demand for meat products will rise globally by 58%. By 2020, there will be an increase in milk consumption from 568 to 700 million tonnes, a potential increase in meat consumption to 300 million tonnes, and an estimated 30% increase in egg supply. (Food and Agriculture Organization, 2016). Thus, more research on alternative sources of protein especially plant protein is needed before it can be implemented in feed formulation of the livestock.

2.1.1 Common plant protein in animal's feed

Over time, soybean meal has surpassed all other protein sources as the norm in modern intensive livestock farming. Moreover, according to Cutrignelli et al. (2022), the proteins from this source are well-balanced with the non-structural carbs and have a low rumen degradation rate (NSC). High amounts of protein (380 g/kg dry matter) and lipid (200 g/kg dry matter) in soybeans (*Soja hispida*) result in high energy density (1.1–1.2 UFL/kg dry matter). However, the cost and availability of soybean meal are significantly tied to the price development of agricultural commodities on the global market (Jezierny et al., 2010). We also need to take into account how genetically modified (GM) soybeans affect the environment. Even while no direct evidence that it may represent a probable threat for health has been revealed for several years, a number of publications have lately been published with controversial outcomes, and this is causing the public to become increasingly concerned about eating GM foods. As a result of this demand for plant-based protein, there has been a rise in the consumption of grain legumes as a potential alternative protein source.

The main reason grain legumes are grown is for their highly nutritious, mature seeds that are high in protein and energy. Grain legumes such as lupins, fava beans, and peas have ripe, dry seeds that are either utilized as an animal feed element or consumed by humans (Singh et al., 2007). For optimal growth, legume crops require less nitrogen fertilizer than other kinds of cultivated plants, and the use of legumes in crop rotation systems lowers the requirement for nitrogen fertilizer in crop production (López-Bellido et al., 2005). These legumes are beneficial in agriculture because they lessen the amount of nitrogenous fertilizer applied to fields, which in turn helps to lessen pollution. Compared to fava beans (301 g/kg dry matter) and peas (246 g/kg dry matter), lupins have more crude protein (324-381 g/kg dry matter) than the other grain legumes. The crude protein level of fava beans, peas, and lupins (*Lupinus albus)* is between 45% and 55% higher than that of soybean meal (Degussa, 2006).

2.1.2 *Musa* spp. leaves as ruminant feed

The use of alternative feeds for ruminants is a method aimed at lowering feed costs and overcoming the problem of pasturage scarcity that occurs during critical times. In Malaysia, banana tree is considered one of the most important fruit trees, with a large planted area and total annual output. However, the leaves that remain after the fruit is harvested are not being used and discarded. The byproducts of banana processing can be used as a fresh, ensiled, or dry feed for ruminants and monogastric animals. Hence, the ability of ruminants to efficiently use fiber makes it possible to explore agricultural by-products and wastes from tropical crops, like banana, which may also provide energy and protein. Banana byproducts can be given to ruminants and monogastric animals as fresh, dried, or ensiled diets. The highest concentration of crude protein is found in the leaves, followed by fruit peel and pseudostem. Pseudo stem and leaf both provide moderate levels of fiber, albeit this is more than can be said about banana peels (Teixeira et al., 2021). The alternative feed products such as using banana leaves are one way for the farmers to cut costs associated with feed by making use of resources that are readily available in the local area whilst providing enough nutrition for their livestock.

2.2.2 *Curcuma* spp. as part of livestock feed

Turmeric rhizome (Curcuma longa/Zingiberaceae), mainly known as turmeric, is a popular spice, food preservative, and colouring agent that has biological and medicinal properties. (Chattopadhyay et al., 2004; Akbarian et al., 2012). Additionally, it is essential for traditional therapeutic uses as an antibacterial and anti-carcinogenic agent (Azuine & Bhide, 1992; Panpatil et al., 2013). Active components found in *C. longa* include curcumin, demethoxycurcumin, bisdemethoxycurcumin, and tetrahydrocurcuminoids (Wuthi-Udomler et al., 2000). The primary bioactive component in *C. longa* that exerts the biological activity is curcumin (Nouzarian et al., 2011). It has roughly 69.4% carbs, 5.1% fat, 6.3% protein, 3.5% minerals, and 13.1% moisture contents (Chattopadhyay et al., 2004; Dono, 2018).

The anti-inflammatory, antioxidant, antiviral, antifungal, anticarcinogenic, and hypocholesteric properties of turmeric's bioactive components include both volatile and non-volatile phytochemicals that are less hazardous. (Ammon et al., 1992; Miquel et al., 2002). Thus, in recent years, turmeric has been studied for its potential as a substitute for antibiotic growth promoters in animal feed rather than its protein content. More research needs to be done on turmeric to be one of the protein sources in animal feed.

2.2.3 *Alpinia galanga* in livestock feed

Alpinia galanga, often known as lengkuas, belongs to the Zingiberaceae family (Noman et al., 2020). Although its exact origin is uncertain, *A. galanga* is believed to have originated from Indonesia. Due to its strong therapeutic value, *A. galanga* has now spread throughout much of South and Southeast Asia. *A. galanga* consist of glycosides, carbohydrates, resins, magnesium, phosphate and sulfate. The leaf oil contained 1, 8-cineole, -pinene and camphor as major constituents. Root of *A. galanga* contains 3 different compounds namely galangin, campheride, and analpinin. Volatile essential oil is obtained that has a pleasant odor and it contains 7 chemical components viz methyl cinnamate, cineole, 1-camphene, 1- borneol, methyl chavicol, cargene and -pinene. According to Dan-Zhang et al. (2019) demonstrated the most potent activity against various strains of *Staphylococcus aureus* that were resistant to antibiotics, and it is deemed suitable for use as an antibiotic substitute in animal feed. Other than that, Poh & Noor (2000) discovered that galangal extract might serve as a potential natural source of antioxidants for meat and meat products.

There are a lot of benefits from incorporating *A. galanga* into the animal feed, which more studies can be done on the plant to make it as one of the alternative proteins for the livestock. For example, there is one study on the impact of *A. galanga* inclusion in diet on broiler chicken growth performance and some psychological parameters showed that treatment groups supplemented with varying amounts of *A. galanga* outperformed the control group in terms of productivity, economic efficiency, and production index (Elnaggar et al., 2021). The results obtained in this study corresponded with the results of

Viveros et al. (2011) found that these galangal rhizome components stimulate digestive enzymes and promote overall digestion, resulting in increased body weight.

2.2 Protein Determination and Quantification

Ultraviolet (UV) and visible spectroscopy-based spectrophotometric protein quantification assays provide a rapid method for estimating a protein's quantity in respect to a standard or by employing an assigned extinction coefficient. Protein quantitation and protein assays are essential for measuring the protein quantity in a sample. Spectrophotometric protein quantification assays are techniques that quickly estimate the concentration of a protein in relation to a standard or utilizing an assigned extinction coefficient using UV and visible spectroscopy. In order to provide information on how to analyze protein concentration using UV protein spectroscopy measurements, conventional and common dye-based absorbance measurements, there are several methods that are described: The fluorescent dye-based assays and the Biuret, Lowry, and Bradford assays: Assays for detergent partition and amine derivatization (Martina & Vojtech, 2015).

It is critical to have standardized analytical methods that are used to estimate the amount of protein in a diet. Therefore, in the food industry, various methods have been used to determine and quantify protein content in the food. The Kjeldahl method, Dumas method, direct measurement methods employing UV-spectroscopy, and refractive index measurement are the most often used methods for evaluating protein content in foods (Hayes, 2020).

2.2.1 UV Spectrophotometric method in protein quantification

UV and visible spectroscopy-based spectrophotometric protein quantification assays provide a rapid method for estimating a protein's quantity in respect to a standard or by employing an assigned extinction coefficient. The amount of protein can be precisely quantitated by UV absorbance if the molar extinction coefficient of the protein is known and the protein is pure and free of UV-absorbing nonprotein components such as bound

nucleotide cofactors, heme, or iron-sulfur centers, as described by the Beer-Lambert law. Proteins exhibit a distinctive UV absorption spectrum at about 280 nm, which is predominantly caused by the aromatic amino acid tyrosine and tryptophan. Nonetheless, this approach will produce inaccurate results if the main sequence has none or only a small number of these amino acids.

Besides, it is frequently used in research projects in the fields of forensic investigation, medicine, and pharmacy because of its quick and simple analysis capabilities. However, there are also disadvantages which are the stray light of UV-Vis spectrophotometer that is caused by the faulty equipment design and other factors could influence spectra measurement accuracy of the absorption in substance, because the stray light will decrease linearity range and reduce the absorbency of substance it measures. Additionally, the quality of the detector circuit and electronic circuit in spectrometers will determine how much noise is linked into the measurement signal, diminishing measurement accuracy and lowering the instrument's sensitivity (Wu, 2015).

2.2.2 Bradford Method in Protein Quantification

The Bradford method is popular since the results are revealed after 5 minutes; nevertheless, it is worthless for proteins with a very low arginine concentration. It is based on an absorbance shift of the dye Coomassie Brilliant Blue G-250, in which the red form of the dye changes into its blue form in the presence of acid to bind to the protein being measured (Noble & Bailey 2009). The basic mechanism of the assay is the binding of the dye to arginine, histidine, phenylalanine, tryptophan, and tyrosine residues at acidic pH (de Moreno et al., 1986), and hydrophobic interactions (Fountoulakis et al., 1992). Upon binding protein, a metachromatic shift from 465 to 595 nm is observed due to stabilization of the anionic form of the dye. The interaction with arginine residues accounts for the majority of the observed signal, resulting in the wide protein to protein variation characteristic of Bradford assays (Noble & Bailey 2009).

Each protein has a different chromogenic rate. The chromogenic process can be impaired by surfactant contamination. Other than that, the disadvantage of this test is that it interferes with many compounds. Interference, defined as the creation of colour by substances other than the analyte of interest, is a prevalent issue in indirect colorimetric tests (Martina & Vojtech, 2015).

2.2.3 Lowry method in protein quantification

The Lowry assay and other preparations with improved test performance are based on a two-step method (Lowry et al., 1951). For a while, it was the method of choice for precisely determining proteins in cell fractions, chromatography fractions, enzyme preparations, and other samples. The method is based on both the Biuret reaction, in which the peptide bonds of proteins react with copper under alkaline conditions to produce Cu^{+}, which reacts with the Folin reagent, and the Folin-Ciocalteau reaction, which is poorly understood but in essence phosphomolybdotungstate is reduced to heteropoly-molybdenum blue by the copper-catalyzed oxidation of aromatic amino acids. The reactions produce a bright blue colour, which is dependent on the tyrosine and tryptophan concentration (Waterborg, 2009). The reduction of the Folin-Ciocalteu reagent is measured as a blue colour at 750 nm (Noble & Bailey 2009). The assay demonstrates protein sequence variation because intensity of the color is caused by tyrosine, tryptophan, and, to a lesser extent, t cystine, cysteine, and histidine residues as well as the reduced copper-amide bond complex (Peterson, 1977; Wu et al., 1978).

The Lowry assay is highly sensitive and widely used. However, it is a complex process that requires extensive preparation. Due to the fact that the chromogenic reaction is a reduction process, contamination of the reduction material might affect the quantitative determination, and the chromogenic rate of each protein may vary.

3.0 MATERIALS AND METHODS

3.1 Sample collection

Curcuma spp. and *Alpinia galangal* were acquired from Pasar Siti Khadijah in Kota Bharu, Kelantan. The *Musa* spp. leaves were obtained from a household farmer who sells bananas in Kota Bharu. To keep them from being contaminated, the plants were maintained in a sealed plastic bag.

3.2 Sample preparation

A. galangal were peeled and cut. The leaves of *Musa* spp. were cut into smaller pieces. These plants then weighed at 3 kg and were air dried at a constant room temperature. Afterwards, they were freeze dried. After that, the plants were grounded into powder form using a mechanical grinder. Lastly, the powder form of the plant was kept in a sterile beaker.

3.3 Maceration Technique for Plant Extraction

The powdered form of *A. galangal* was dissolved with methanol. Then, it was filtered using Whattman's Filter Paper into another separate beaker. The maceration was done 3 times as the residue on the Whatman's filter paper was filtered again using Methanol. Then, the extracted plant in the beaker was placed into the rotary evaporator to help separate the extract from the methanol. Then, a crude extract was obtained. Similar steps were done for *Curcuma* spp. and *Musa* spp. leaves.

3.4 Preparation of plant sample for protein determination

The crude extract obtained is weighed at 0.05 g and was pipette into a micro centrifuge tube. After that, sterile water was added to dilute the crude extract. The tube was then centrifuged for 10 minutes to ensure the crude extract had dissolved with the sterile water.

3.4.1 Protein Content Determination

3.4.1.1 Preparation of Bovine Serum Albumin (BSA) Standard Curve

Bovine Serum Albumin (BSA) Solution (2 µg/ml) was diluted into 500 µg/ml, 400 µg/ml, 300 µg/ml, 200 µg/ml, 100 µg/ml and 0 µg/ml. The BSA solution was then diluted with sterile water. The calculation used to diluted the BSA solution was by using the formula:-

$$M_1V_1 = M_2V_2$$

A total of 50 µl of Bradford reagent was then added to each of the diluted 1000 µl BSA samples in a dark room. In total there were 6 concentrations of diluted BSA solution with concentration of 500 µg/ml, 400 µg/ml, 300 mg/ml, 200 µg/ml, 100 µg/ml and 0 µg/ml. Then, it was incubated for 10 minutes in a dark room as the Bradford Reagent is light sensitive. The same procedure was performed for *A. galangal, Curcuma* spp. and *Musa* spp. leaves methanol extract in which the 50 µl of Bradford reagent was added into the 1000 µl plant sample and was also incubated in the dark room for 10 minutes.

After incubation, using the spectrophotometer, the blank column (0 µg/ml of BSA Sample) was inserted first to get the standard absorbance. Then, followed by 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml of the diluted BSA sample. A standard curve was prepared. The absorbance was set at 595 nm.

4.0 RESULTS

Table 4.1 showed absorbance obtained for the standard curve prepared for bovine serum albumin (BSA). As the BSA concentration increased, the absorbance also increased.

Table 4.1. Absorbance for different concentration of BSA at 595 nm

BSA Concentration (µg/ml)	**Absorbance at 595 nm**
0	0.000
100	0.096
200	0.382
300	0.498
400	0.587
500	0.714

From the absorbance obtained, a standard curve was plotted (Figure 4.1). The standard curve was used to determine protein content in the samples by using the equation from the graph.

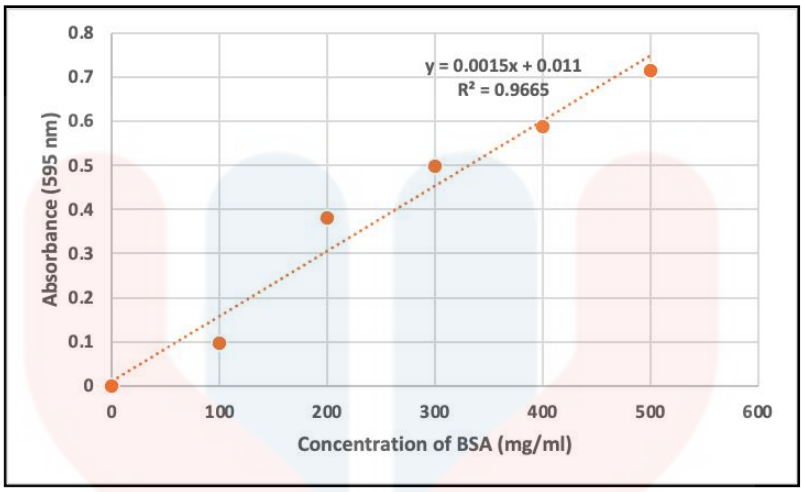

Figure 4.1. Standard curve of BSA at 595 nm

Table 4.2 showed concentration of protein in methanol crude extract of *Alpinia galanga, Curcuma* spp., *Musa* spp. leaves. From the results, Pisang (*Musa* spp.*)* leaves showed the highest protein concentration with 261 670 ug/ml while Kunyit *(Curcuma* spp.*)* showed the lowest with -20 000 ug/ml.

Table 4.2. Concentration of protein in methanol crude extract of *Alpinia galanga, Curcuma* spp., and *Musa* spp. leaves.

Plant Methanol Extract Sample	First Absorbance (595 nm)	Second Absorbance (595 nm)	Average Absorbance (595 nm)	Concentration of protein (ng/mL)
Lengkuas (Alpinia galanga)	0.106	0.119	0.1125	67 670
Kunyit (Curcuma spp.)	-0.055	0.017	-0.019	-20 000
Pisang (Musa spp. leaves)	0.396	0.411	0.4035	261 670

5.0 DISCUSSION

Based on the results of plant protein of methanol extract, there are significant differences of protein level between these three types of the plants. In this case, *Musa* spp. leaves have the highest concentration of protein content, which is 261 670 µg/mL, followed by *A. galanga*, which has 67 670 µg/mL protein content, and *Curcuma* spp. has the lowest protein concentration, which is -20 000 µg/mL. This result was acquired by utilizing the Bradford method for protein quantitation.

From the absorbance and result there is a positive and negative value of the protein quantification, which is greatly influenced by the reference's absorbance, in this case 0 µg/mL of BSA solution, which was used as the blank measurement. In this instance, the absorbance is negative for *Curcuma* spp. is because the absorbance of the reference used in the blank measurement at the particular frequency is higher than the absorbance. Using a more sensitive assay, if possible, might be beneficial. Otherwise, to precisely calculate the concentration, we must either concentrate the sample or prepare a new sample. Moreover, different proteins have various dye-binding capabilities and hence produce different results in the experiment. When the molecular weight is lower than the detection limit of the Bradford method, which is roughly 3000-5000 Daltons, a different quantification method should be used. One such method is the bicinchoninic acid assay (BCA assay), which substitutes the Folin-reagent Cioalteu's described for the Lowry method with bicinchoninic acid to produce a protein assay with improved sensitivity and tolerance to interfering compounds (Smith et al., 1985). In the Bradford method, the dye binds to both basic and aromatic amino acid residues, which results in an absorbance shift. It is easily distinguished by a color shift from brown to blue. By making comparisons to a reference protein, most frequently BSA, we may determine the protein's concentration.

There are a number of variables that can affect the measurement, including temperature, wavelength, detergents, and even the type of cuvettes we used. The reason why there is a difference of high and low value of the protein concentration in the plant protein is because

the method used to prepare the sample can alter the protein concentration. The fresh plant sample should be processed shortly after being harvested, but since the plant sample was purchased at a market, the information about the storage technique and harvest conditions prior is unknown. In addition, if the extraction is going to be done within 5-10 days, store the fresh plant sample at 2°C, or otherwise keep it frozen (at -20°C) until extraction. Proteins are heat labile, so it is preferable to evaluate their protein content, for example in this case, *Musa* spp. leaves when they are young and actively growing rather than when they are older and have been exposed to heat or sun for an extended period of time. Other than that, the Bradford protein test, in contrast to other protein assays, is less vulnerable to interference from numerous compounds that may be present in protein samples. Elevated detergent concentrations are notable exceptions. Sodium dodecylsulfate (SDS), a common detergent, may be present in protein extracts since it is used to lyse cells by destroying their membrane lipid bilayer. For higher SDS concentrations, critical micellar concentration (CMC) significantly associates with the green form of coomassie dye, causing the equilibrium to shift toward producing more of the green form, increasing absorbance at 595 nm regardless of protein presence.

A single protein solution would most likely produce different findings if measured using multiple methods because they are based on different principles. Several different methods exist for quantifying proteins besides the Bradford method utilized here. These include the Kjeldahl method and Lowry method. The choice of an appropriate approach will depend on the type of proteins present in the sample, the purity of the extracts, the required sensibility and accuracy, the desired speed, and the fact that there is no such thing as a perfect method; each method has benefits and limits (Boyer, 1986).

In this study, the Bradford method was chosen due to its conspicuous advantages such as (i) the use of a single reactive, (ii) the rapidity of the reaction, (iii) a high stability of the protein-dye complex, (iv) a high reproducibility, and (v) the occurrence of minimal interferences (Nuria & Tamayo, 2001). It is based on an absorbance shift of the dye Coomassie Brilliant Blue G-250 in which under acidic conditions the red form of the dye is converted into its blue form to bind to the protein being assayed (Noble & Bailey 2009).

There are, however, detergent-compatible Bradford reagents. The Bradford assay is dependent on the protein sequence. The dye will not bind to the protein as effectively if the protein does not have a sufficient amount of arginine and/or aromatic residues, which underestimates the protein concentration thus can lead to erroneous results. Due to the fact that the Bradford test primarily measures the concentration of arginine and hydrophobic amino acid residues, the concentration-absorbance curve might be affected by the amino acid composition of the protein. Proteins vary in their amino acid composition, hence it is important to employ a standard (such BSA, or Bovine Serum Albumin) whose protein is chemically similar to the protein being analyzed.

Another method that can be used for quantifying protein in the plant is the Kjeldahl method. The great precision and reproducibility as well as the ease of application of the Kjeldahl method have established it as a norm for evaluating nitrogen and protein concentrations in food and feeds especially the milk composition in the livestock. This technique exclusively detects ammonium and nitrogen attached to organic compounds (such as proteins, amino acids, and nucleic acids) in the sample. Compounds with nitrogen in azo or nitro groups, or in ring structures (quinoline, pyridine, nitrate, and nitrite, etc), are unfavorable for this procedure. As a result of their molecular structures, the nitrogen in these molecules cannot be transformed to ammonium sulfate using the Kjeldahl process. Consequently, if the plant sample methanol extract contains quinoline alkaloids or pyridine alkaloids, which are extremely prevalent nitrogen-containing chemicals in plants, it may go undetected and produce false results.

The Lowry method combines the biuret reagent with the Folin-Ciocalteau phenol reagent, which reacts with the tyrosine and tryptophan residues in proteins. This results in a bluish color that can be measured between 500 and 750 nm, depending on the required sensitivity. In addition, the Lowry test has been modified to lower its susceptibility to interfering chemicals, extend its dynamic range, and increase the speed and hence stability of color production (Peterson, 1979). A small peak around 500 nm can be used to assess protein concentrations with high concentrations, whereas a large peak around 750 nm can be used to determine protein concentrations with low concentrations. Compared to the biuret

method, this method is more sensitive to low protein concentrations. Nevertheless, it also has its own disadvantages which the Lowry method is considerably more time-consuming than the Bradfords or Bicinchoninic Acid (BCA) assays since it involves more steps and reagents. Other than that, after the Folin reagent is being added, it is only reactive for a limited amount of time. Furthermore, once the protein sample has interacted with the dye, it can no longer be utilized in further assays. Although both the Bradford method and the Lowry method are colorimetric assays, they employ distinct principles and rely on different reagents, with the former relying on interactions between basic amino acid residues (primarily arginine, lysine and histidine) (Lowry et al., 1951) while the latter assay works under alkaline settings by reducing Cu^{2+}, which then binds to protein to form a Cu^{+} peptide complex. (Smith et al., 1985), hence, when selecting a method for protein measurement, it is critical to take into account the target protein's composition and buffers to get the accurate results.

6.0 CONCLUSION AND RECOMMENDATION

To conclude, *Musa spp*. leaves have the highest concentration of protein content, which is 261 670 ng/mL, followed by *A. galanga*, which has 67 670 ng/mL protein content, and *Curcuma* spp. has the lowest protein concentration, which is -20 000 ng/mL. This result was accomplished by employing the Bradford method, which is using methanol as the solvent to determine the protein level in the selected plants.

It is recommended that the plant sampling method be done in a way that will not alter the protein amount of the plant, as the results and outcomes of the protein quantification value are influenced by the methodologies employed in this study and by the plant sampling method before the plant extraction. For instance, the procedure of harvesting must be performed properly in order to preserve the protein content of the plant. The temperature when storing the plant before performing the experiment must be adequate and crucial to maintain its protein content thus it will prevent the protein denaturation. Lastly, if a plant such as *Curcuma* spp. has an absorbance value lower than the standard, it may be necessary to conduct a Bicinchoninic Acid (BCA) assay instead of the Bradford method for a more comprehensive protein measurement. Using both Bradford method and BCA assay for protein quantification will produce more conclusive results. Lastly for further recommendation, the replication of plant extraction can be done by using different solvents such as ethanol and hexane which the results of the protein analysis can be compared.

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