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Enzyme Assays on Antidiabetic Properties of *Tebu Gajah* (*Albizia myriophylla*) Dried Jelly

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ABSTRACT

Tebu Gajah is a plant with high potential as a traditional medicine in Southeast Asia and is believed to have some antidiabetic properties due to a high phytochemical composition, especially phenols and flavonoids. The objective of this research paper was to identify and determine the α-amylase inhibitory property of the *Tebu Gajah* dried jelly extract in different concentrations and then compare the α-amylase inhibitory property of the extract with the α-amylase inhibitory property of the pure plant extract. The inhibition strength will be further determined by comparing the IC50. *Tebu Gajah* pure extract and dried jelly were crudely extracted with 70% ethanol. These extracts were further treated with 10, 5, 2.5, 1.25, and 0.625 mg/mL. α-Amylase inhibition was performed using the iodine-starch colorimetric system where the absorbance value was measured at 620 nm. The % inhibition, strength of inhibition (IC50), and % inhibition correlation were determined. The results showed that in all *Tebu Gajah* samples, inhibitory activity was dose-dependent. Pure extract exhibited the best inhibitory value of 72.09 % at 10mg/mL, then dried jelly extract 2 and 1 with values 56.83 % and 54.29 %, respectively. Even with reduced inhibitory potency due to the presence of the transformation process of dried jelly, it was observed that both forms of the jelly are efficient inhibitors of the enzymes. The level of the extracts and percentage inhibitions showed a positive correlation, which was more pronounced in the dried extracts. Consequently, there was a conclusion drawn to imply that the dried form of *Tebu Gajah* jelly has the ability to inhibit a-amylase in a functional food in regulating starch and glucose. However, more research would be needed in phytochemicals and in vivo studies concerning its use in antidiabetics.

1. Introduction

Tebu Gajah (Albizia myriophylla) is a popular Malay traditional medicine plant, found in most of the Southeast Asian nations, and commonly utilized to treat fever, inflammations, urinary tract infections, and diabetes. The phytochemical analyses show that the plant is enriched with high concentrations of polyphenols, flavonoids, and other bioactive compounds that have antidiabetic potential. These compounds have been demonstrated to prevent the action of a-amylase and a-glucosidase, which breaks carbohydrates down into smaller units, maltose, maltotriose, and dextrins, which are further disintegrated to glucose through the activity of intestinal enzymes, including maltase and isomaltase [11]. As a-amylase triggers the process of starch digestion, its inhibition can

slow down the process of the release of glucose in the blood, which is why a-amylase inhibitors are relevant in postprandial glucose regulation and the treatment of diabetes [13]. Enzyme assays are basic biochemical tools employed to measure enzyme activity, inhibition kinetics and bioactivity of plant-derived compounds. According to previous research, *Tebu Gajah* extract can be used as a natural alternative to Acarbose synthetic antidiabetic medications, which may reduce the postprandial hyperglycemia rate with a smaller number of gastrointestinal side effects [3]. The rapid prevalence of diabetes around the world is increasing and it is under stress to have safe and effective alternative therapies. Despite the common understanding that natural products are potential carbohydrate-digestive enzyme inhibitors [13], the scientific data on the antidiabetic effect of Tebu Gajah (*Albizia myriophylla*) is insufficient, even though the latter has been used traditionally long before [3]. Early phytochemical evidence suggests that the plant has flavonoids, saponins and phenolic acids that are linked to enzyme inhibition, antioxidant and insulin sensitization. There is however little research on its a-amylase inhibitory activity, especially in processed forms like dried jelly. Thus, the proposed study will assess the a-amylase inhibitory property of the Tebu Gajah dried jelly extract by comparing the inhibitory activity at various concentrations of the extract to the pure extract, percent inhibition of a-amylase at various concentrations, and strength of inhibition by analyzing its enzyme activity in the presence of the jelly extract.

2. Methodology

2.1 Preparation of Tebu Gajah Dried Jelly Crude Extract (Maceration using Ethanol)

The Tebu Gajah dried jellies were collected and made by the other students in order to determine the antidiabetic qualities. The ground Tebu Gajah dried jellies were powdered using a blender to increase the number of areas where they can be extracted. 50 g of powdered jelly was weighed in an analytical balance and 50 g of powdered jelly was put in a clean beaker. To extract it, 500 mL of 70 % ethanol was subsequently put into the powdered jelly in a 1:10 (w/v) proportion. The mixture is wrapped and left to soak at room temperature (between 48 to 72 hours). This period was done through shaking of solution after every 12 hours or by putting the solution in a shaker in order to experience continuous agitation to ensure that there is sufficient solute-solvent interaction. The mixture was then filtered using a funnel lined with filter paper to drain the solid residue. This filtration was repeated again in case of necessity to achieve clear filtrate. The product is filtered and then moved to petri dishes and allowed to dry in a fume hood at room temperature until all solvent is removed. The difference between the weight of the crude extract and the weight of the empty petri dish is used to calculate the weight of the crude extract.

2.2 Serial Dilution of Tebu Gajah Crude Extract Solution

Five clean experimental tubes Coded A, B, C, D and E. The pure solution of Tebu Gajah extract in another tube. To make the serial dilutions, 1.5 mL of the stock solution (10 mg/mL) was set in Tube A. This tube will be the pure sample and it will be the highest level of the dilution series. Tube A solution was added to Tube A (0.5 mL) and phosphate buffer (1.0 mL) was added. It was thoroughly combined up to the concentration of 5 mg/mL. Then, 0.5 mL of Tube B was immediately transferred to Tube C and 1.0 mL of buffer was added to the mixture. The mixture was gently mixed up to reach a final concentration of 2.5 mg/mL. Serial dilution This step was repeated where 0.5 mL of the contents of Tube C was added to Tube D and 1.0 mL of buffer added to dilute the contents so that the final concentration was 1.25 mg/mL. Finally, 0.5 mL of Tube D was transferred to Tube E and mixed with 1.0 mL of buffer producing the weakest dilution in the series of 0.625 mg/mL. Each tube

was then shaken to homogeneity after dilution and the next step carried out. The entire solutions were made and kept in the chiller awaiting to be utilized in enzyme assays. All prepared concentration of each tube listed as table 1 below.

Table 1
Prepared concentration of each tube

Tube	Concentration (mg/mL)
A	10
B	5
C	2.5
D	1.25
E	0.625

2.3 Solutions Preparation (α-Amylase Enzyme, 1% Soluble Starch, Iodine Reagent)

The a-amylase enzyme solution was made by adding 0.5 mL (500 uL) of enzyme into a 50 mL volumetric flask before adding a phosphate buffer to dilute it to volume (49.5 mL). The mixture was then mixed softly in order to have a uniform distribution without excessive mixing which would lead to denaturation of the enzyme. Enzyme solutions were prepared and kept on ice during enzyme assays in a closed container at 4°C temperature to retain the activity and the rest of the enzyme solution was kept at 4°C and consumed within a few days. The 1% soluble starch solution was made by adding 0.2 g of soluble starch to 2 mL of cold distilled water in order to develop a slurry. Individually, 18 mL of distilled water was warmed to 60-70°C and more slowly added to the slurry with constant stirring up until a clear solution was achievable. The solution was cooled down to room temperature, then diluted with 50 mL of distilled water and mixed completely and then refrigerated at 4°C for up to 1-2 days [11]. The iodine solution was made by adding 0.332g of potassium iodide (KI) to 5-6mL of distilled water and adding 0.254g of iodine crystals gradually with constant stirring until the thick dark brown solution was obtained. The mixture was poured into a 10 mL volumetric flask, diluted to volume with distilled water, swirled, and put in an amber bottle at 4°C to avoid light degradation [11].

2.4 α-Amylase Inhibition Assay (Iodine-Starch Method)

In order to establish the enzyme assay, 3 different preparations were conducted, which include the blank, control and sample reaction. In a clean test tube, a blank was prepared by combining 1.0 mL of soluble starch solution (1%), and 2.0 mL of phosphate buffer (pH 7.2). 2-3 drops of 0.1 M iodine solution were then added to it. This mix contains no enzyme or extract and is used to correct any background absorbance. In the control, which determines the full activity of a-amylase enzyme in the absence of an inhibitor, 1.0 mL of a 1% soluble starch solution was combined with 1.0 mL of a-amylase solution and 1.0 mL of phosphate buffer (pH 7.2). The mixture was left to incubate at the optimum temperature of 37°C between 15 and 30 minutes. After the incubation, 2-3 drops of 0.1 M iodine solution were then put into the tube in order to test the amount of unhydrolyzed starch in the tube.

To investigate the inhibitory effect of *Tebu Gajah* dried jelly extract, the sample preparations were investigated. 1.0 mL of 1% soluble starch solution, 1.0 mL of a-amylase and 1.0 mL of the *Tebu Gajah* dried jelly extract at different concentrations (A,B,C,D,E) were added to each sample tube. The sample groups were incubated at 37°C between 15 to 30 minutes with the same conditions as the control. After incubation, 2-3 drops of 0.1 M iodine were put into each sample tube. A spectrophotometer was used to analyze all the tubes blank, control and sample. To identify the

quantity of starch that remained after the process, absorbance was taken at 620 nm in order to observe the colour, which indicates the action of enzyme and any inhibitory influence of the extract on a-amylase [9].

2.5 Calculation of Inhibition Percentage and Inhibition Strength

The percentage inhibition of α-amylase was calculated using formula :

$$\left(\frac{Absorbance\ of\ sample - Absorbance\ of\ control}{Absorbance\ of\ blank - absorbance\ of\ control}\right) \times 100 \quad (1)$$

Formula 1 determines the extent to which the inhibitor reduces enzyme activity relative to the total activity range. The difference between the sample and control absorbance represents the reduction in enzyme activity caused by the inhibitor, while the difference between the blank and control reflects the maximum enzyme activity within the assay system. The calculated value is expressed as a percentage to indicate the inhibitory effect. Subsequently, inhibition percentages were plotted against the concentrations of the jelly extract to generate an inhibition curve. The inhibition strength was determined using the IC_{50} value, defined as the concentration of extract required to inhibit 50% of α-amylase activity. The IC_{50} was identified from the concentration corresponding to 50% inhibition on the curve, where a lower IC_{50} indicates stronger inhibitory potency.

2.6 Statistical Analysis

Once the data is collected, the data is analyzed for correlation to ensure the data is significant or not. Correlation analysis was used to measure the strength and direction of the relationship between two continuous variables. The correlation coefficient (r-value) shows whether the relationship is positive, negative, or weak. Therefore, correlation provides a clearer understanding of the experiment. It shows how one variable changes in relation to another. Therefore, correlation in providing strong evidence to support the interpretation and conclusion of the experiment's findings.

3. Results

3.1 Effect of Concentrations on Absorbance Value (Amount of remaining starch)

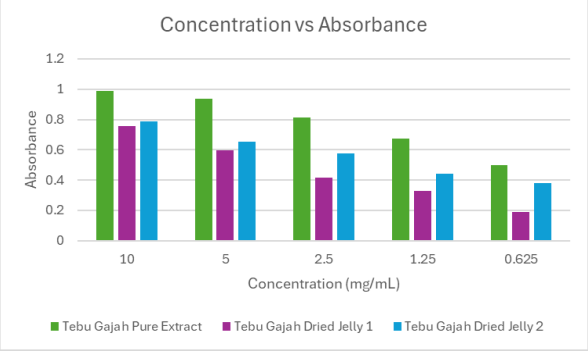

Fig. 1. Concentration vs absorbance (remaining starch)

Figure 1 shows the concentration dependent a-amylase inhibitory activity of *Tebu Gajah* (*Albizia myriophylla*) samples (Pure Extract, Dried Jelly 1 and Dried Jelly 2) when tested at 0.625-10 mg/mL. In the iodine-starch test, the greater the absorbance, the more starch will remain undigested and the greater will be the inhibition of the enzyme. The control sample was associated with the lowest

absorbance (0.046), which means maximum starch hydrolysis and a complete a-amylase activity. The Pure Extract had the highest absorbance at all concentrations (0.986 at 10 mg/mL), which is the strongest inhibitor. Dried jelly formulations also exhibited an inhibitory effect that could be measured, and at lower and intermediate concentrations, Dried Jelly 2 exhibited a slightly stronger absorbance than Dried Jelly 1. Even though there was a reduction in the inhibitory activity in processing into jelly relative to the Pure Extract, the remaining activity of *Tebu Gajah*-based products confirms the potential of these products as functional foods in the regulation of starch digestion and postprandial glucose to meet Objective 1.

3.2 α-Amylase Inhibition Percentage (%)

The α-amylase inhibitory activity of *Tebu Gajah* extract was evaluated at various concentrations to determine its potential effect on carbohydrate-digesting enzymes. The inhibition percentage was calculated using Equation in 2.4. The results (Figure 2) show that Pure *Tebu Gajah* extract exhibited the highest α-amylase inhibition across all concentrations, ranging from 34.82% at 0.625 mg/mL to 72.09% at 10 mg/mL.

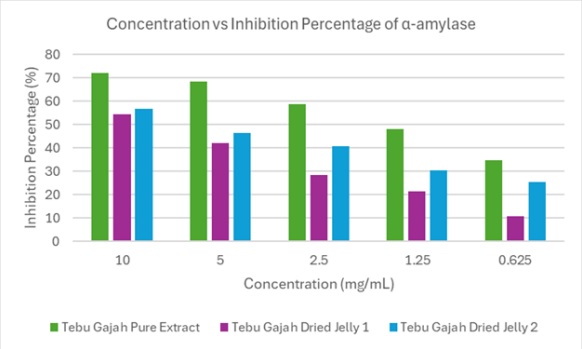

Fig. 2. Concentration vs inhibition percentage

This study compared the a-amylase inhibitory property of *Tebu Gajah* dried jelly extracts at 0.625-10mg/mL of concentration. These findings (Figure 2) revealed a distinct dose-dependent inhibition with the higher the concentration, the more the inhibitory effect. The Pure Extract showed the greatest inhibition of 72.09% at the highest concentration of 10 mg/mL, followed by the Dried Jelly 2 (56.83) and 1 (54.29) at 10 mg/mL. This tendency indicates that bioactive molecules present in *Tebu Gajah* react with the enzyme and inhibit the hydrolysis of starch. The inhibitory capacity of *Tebu Gajah* is in the moderate category of plant-based a-amylase inhibitors as compared to literature reports. Spondias mahagoni is an example of plant extract with higher inhibition (approximately 77.5%), and others have much lower levels [9]. Pharmaceutical inhibitors such as acarbose usually reach greater levels of inhibition (80-90%) [19], but the moderate effects of *Tebu Gajah* could be beneficial since there is a risk of side effects in the gastrointestinal system caused by stronger inhibitors. Probably the factor causing the inhibition is due to the phytochemicals which include phenolic compounds and flavonoids which may bind to enzyme active or allosteric sites and decrease catalytic activity. On the whole, the *Tebu Gajah* dried jelly extracts show concentration-dependent a-amylase inhibition, which justifies the potential of these products in natural functional foods to modulate the digestion of starch and control the postprandial glucose level.

Table 2
Correlation between concentration and inhibition percentage

Sample	Correlation Coefficient (r)	p-value
Pure Extract	0.852	0.0668
Tebu Gajah Dried Jelly 1	0.957	0.0106
Tebu Gajah Dried Jelly 2	0.951	0.0129

Table 2 presents the correlation analysis demonstrating the concentration-dependent inhibition activity of *Tebu Gajah* samples. The Pure Extract showed a strong positive correlation (r = 0.852) between concentration and inhibition activity, although it was not statistically significant (p = 0.0668), possibly due to the small sample size or variation in bioactive compound composition. In contrast, both processed samples showed very strong and statistically significant correlations: Dried Jelly 1 (r = 0.957, p = 0.0106) and Dried Jelly 2 (r = 0.951, p = 0.0129). These findings indicate that increasing extract concentration enhances the inhibitory activity, likely due to higher levels of phenolic and flavonoid compounds. Overall, the correlation analysis confirms that concentration plays a significant role in determining the bioactivity of *Tebu Gajah*-based products.

3.3 Inhibition Strength (IC_{50})

In order to measure the inhibitory strength of *Tebu Gajah* extracts further, half-maximal inhibitory concentration (IC50) was calculated. IC50 is the concentration of extract that can prevent 50% of the α-amylase activity and it is commonly employed to compare the efficacy of enzyme inhibitors. When the value of IC50 is low, the inhibition is greater, which is an indication of greater bioactivity of the extract. In this experiment, the degree of inhibition of α-amylase by Pure *Tebu Gajah* extract and two dried jelly extracts at different concentrations was used to obtain the IC50 values. These findings give a quantitative determination of the strength of the inhibition and can be used to directly compare the extracts between themselves and with the standard controls or those of literature-reported plant inhibitors and to be able to evaluate their potential as useful functional antidiabetic agents.

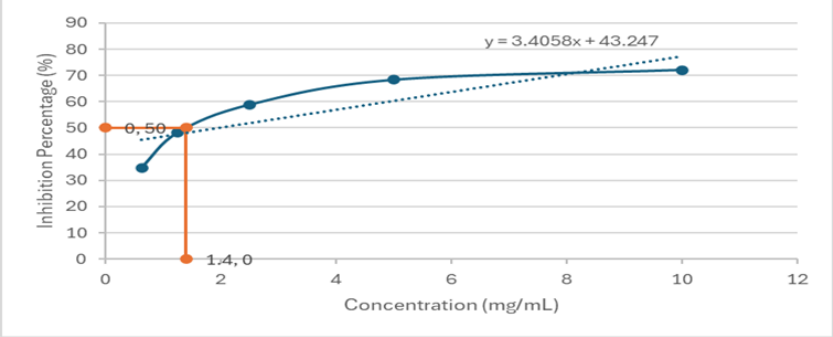

Fig. 3. IC_{50} graph of *Tebu Gajah* pure extract

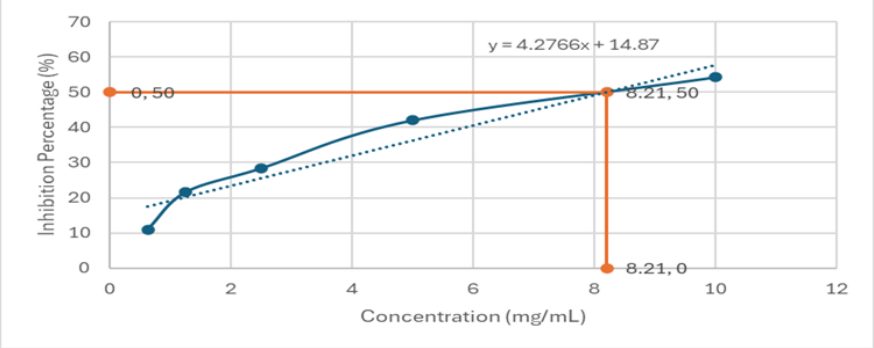

Fig. 4. IC_{50} graph of *Tebu Gajah* dried jelly 1

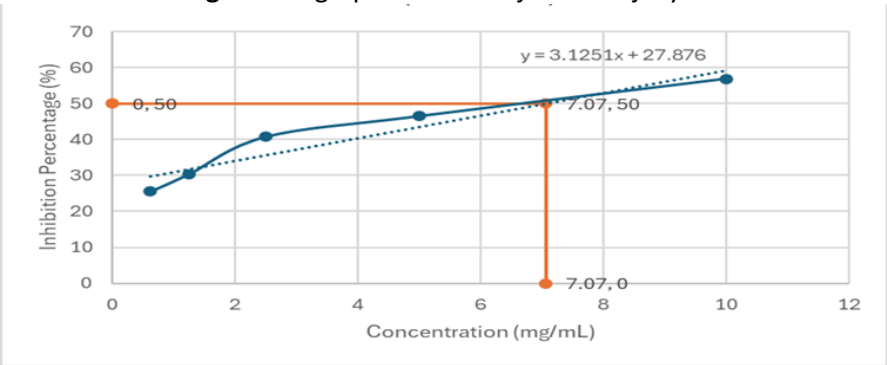

Fig. 5. IC_{50} graph of *Tebu Gajah* dried jelly 2

The strength of *Tebu Gajah* samples was determined with the help of the concentration of the sample which inhibits 50 percent of the enzyme activity as the IC50 value. The lower the IC50 the stronger the action of the inhibition. The Pure Extract was the most potent with the IC50 value of 1.47 mg/mL (Figure 3), which implies that the extract contains a high level of active phytochemicals including phenolic acids and flavonoids that make it bioactive. Comparatively, Dried Jelly 2 and Dried Jelly 1 had a lower IC50 value of 7.07mg/mL and 8.25mg/mL, respectively (Figures 4 and 5), which implies less inhibitory potential. This loss can be explained by the degradation or dilution of bioactive compounds during processing, such as heat, oxygen, dehydration, and the introduction of food substances such as sugars. But slightly more activity was observed in Dried Jelly 2 than in Dried Jelly 1 indicating that phytochemical stability can be affected by the processing conditions and formulation. On the whole, the findings show that minimum processing leaves more bioactive compounds and leads to a more potent biological activity. These results emphasize the need to maximize the processing procedures in order to preserve the health-promoting qualities of *Tebu Gajah*-based businesses.

4. Conclusions

In conclusion, this research conducted a comparison of the a-amylase inhibitory activity of *Tebu Gajah* (*Albizia myriophylla*) in pure extract and dried jelly preparations under iodine-starch assay. The findings revealed a distinct concentration-dependent inhibition of a-amylase in all the samples suggesting the existence of bioactive compounds that have the ability to control the digestion of starch. The Pure Extract showed the most significant inhibitory activity as shown by the increasing absorbance values, the percentage inhibition and the lowest IC50 (1.47 mg/mL) value among the samples. This indicates that the pure extract has a greater amount of active phytochemicals like phenolic and flavonoid compounds. The dried jelly formulations still had a-amylase inhibitory activity, which was less effective than the pure extract. Such a decrease can be explained by processing

conditions like heat exposure, dehydration, oxidation and dilution in formulation additives that may diminish the stability and concentration of bioactive compounds. Dried Jelly 2, however, showed a little more inhibition as compared to Dried Jelly 1 which showed that formulation and processing conditions can help to preserve phytochemicals. Correlation analysis has also revealed that there was a strong positive relationship between extract concentration and percentage of inhibition especially in the dried jelly samples thus proving that higher concentration boosts inhibitory activity. In general, these results indicate that *Tebu Gajah* has potential as a natural a-amylase inhibitor and has prospects as a functional food ingredient in the regulation of starch digestion and postprandial glucose.

On the basis of these results, a follow-up research is suggested in order to justify the development and use of *Tebu Gajah* as a functional antidiabetic ingredient. Future research on this topic should include a thorough phytochemical profiling through the applications of analytical methods including Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC) or Liquid Chromatography-Mass Spectrometry (LC-MS) to determine and quantify the identity of the specific bioactive compounds that result in the observed inhibitory effect. Determining the connection between these compounds and the inhibition of enzymes would improve the processes of extraction and processing and facilitate the standardization of formulations. Moreover, further research must involve the development of functional foods by optimizing formulas of dried jelly, sensory analysis on consumer acceptability of the product and shelf-life research to establish the stability of bioactive compounds in storage. Their product formulation should also be standardized and additional biological testing, such as in vivo tests, should be conducted to confirm the antidiabetic nature of Tebu Gajah and facilitate its use in functional foods and nutraceutical products.

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