



## Evaluation on Bioactive Compounds, Antioxidant, Anti- $\alpha$ -Glucosidase and Anti-Acetylcholinesterase in Different Parts of *B. flabellifer* L.

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### ABSTRACT

*Borassus flabellifer* L. is a highly valuable medicinal plant, with all parts of the tree possessing potential therapeutic properties and diverse applications. This study seeks to comprehensively assess the phytochemical composition and bioactive potential of various parts of *B. flabellifer* L., including the endosperm, haustorium, leaves, male flowers, mesocarp, ripe pulp, exocarp, and sap. Specifically, this research investigates the phenolics, flavonoids, anthocyanins, carotenoids, tannins, antioxidant activities, and inhibitory effects on  $\alpha$ -glucosidase and acetylcholinesterase enzymes across various plant parts. The male flowers had the highest value of phenolic acids, flavonoids, anthocyanins, and tannins in the samples of all the different parts, while ripe pulp showed the highest value of carotenoids in all parts of *B. flabellifer* L. All sample parts of *B. flabellifer* L. possessed strong antioxidant capacity, with the male flowers demonstrating the highest DPPH, ABTS, and FRAP radical scavenging activity (IC<sub>50</sub> at 1.10mg/mL, IC<sub>50</sub> at 0.44mg/mL, 147.09mg of TE/100g sample). The male flowers of *B. flabellifer* L. also had the most activity for  $\alpha$ -glucosidase inhibitory with IC<sub>50</sub> at 0.75mg/mL, more than the positive control, acarbose, with IC<sub>50</sub> at 3.20mg/mL. The exocarp of *B. flabellifer* L. showed the highest acetylcholinesterase inhibitory activity with IC<sub>50</sub> at 157.82mg/mL. Seven phenolic acids (gallic acid, protocatechuic acid, vanillic acid, caffeic acid, coumaric acid, ferulic acid, and sinapic acid) and 3 flavonoids (catechin, rutin, and quercetin) were identified and quantified using HPLC. According to this study, *B. flabellifer* L. has the potential to be developed into dietary and pharmaceutical treatments for diabetes and Alzheimer's disease.

**Keywords:** Bioactive compounds, Antioxidant, Anti- $\alpha$ -glucosidase, Anti-acetylcholinesterase, *B. flabellifer* L.

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### INTRODUCTION

Plants are a source of natural product compounds, which contain a large number of bioactive compounds with therapeutic potential to treat various diseases and health conditions. Recently, there has been an increased interest in exploring native plants as a source of phytochemicals with antioxidant, antidiabetic, and neuroprotective activities (Anitha et al., 2023; Moise et al., 2024). Among these plants, *Borassus flabellifer* Linn., a type of palm tree and important economic crop, has gained attention as one of Thailand's local plants widely distributed throughout the country, especially in the southern region, due to its various benefits and medicinal

properties. *B. flabellifer* L., also known as palmyra palm in English and Ton Taan in Thai, is a medicinally important plant that is generally distributed and cultivated in tropical regions of Asian nations, including Bangladesh, India, Myanmar, Sri Lanka, Malaysia, and Thailand, and various regions of East Africa (Jamkhande et al., 2016; Kurian et al., 2017). It belongs to the family Arecaceae, usually grows to a height of 30-35 meters with a stem diameter of 1.5-3.0m, has a life span of more than 120 years, and can be used for a variety of benefits including food, beverages, fiber, animal feed, medicine, and wood (Basava Prasad et al., 2023; Subramanian et al., 2024). Because of the presence of various bioactive compounds, *B. flabellifer* L. tree is used in traditional medicine as remedies for treating a myriad

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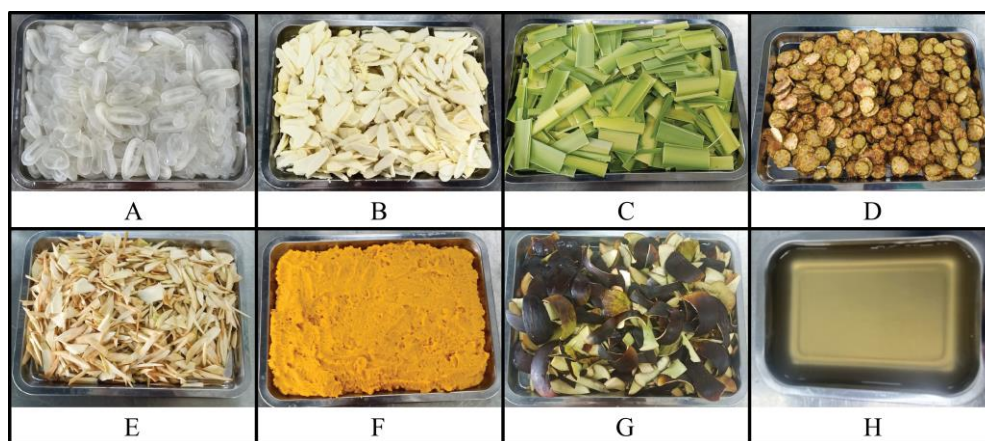
array of diseases, in which different parts of *B. flabellifer* L. tree, such as roots, stem, flower, fruit, sprout, leaves, and seed embryo, exhibit unique medicinal value (Basava Prasad et al., 2023; Abu et al., 2024). There have been studies on the nutritional and medicinal properties of various parts of the *B. flabellifer* L., such as the endosperm, which was reported as a beneficial source of carbohydrate, fiber, sodium, potassium, zinc, and phytochemical content (Rahman et al., 2021). The haustorium is a great source of macronutrients such as carbohydrates, protein, and fiber and micronutrients such as potassium and phosphorus, calcium, iron, and sodium (Basava Prasad et al., 2022). The sap is rich in nutrients and bioactive components such as polyphenols, antioxidants, flavonoids, and volatile compounds and has potential as an antidiabetic (Sarkar et al., 2023). The mesocarp and exocarp provide nutrients such as insoluble fiber, protein, ash, saponin, tannins, and phenolic compounds, and simple sugars (fructose, mannose, galactose, and glucose) and have antioxidant properties, with the exocarp demonstrating superior radical scavenging activity and reducing power than those of the mesocarp (Rodiah et al., 2019). Palm jaggery and palm honey have antidiabetic, antioxidant, anti-hyperglycemic, and insulinogenic characteristics (Manivannan et al., 2024). Palm syrup contains protein, phenolic content, and various vitamins, with vitamin E being the most abundant (Le et al., 2021). The male flowers have antioxidant, anti-inflammatory, and antibacterial effects (Tunit et al., 2022). The roots comprise dietary elements, phytochemicals, and bioactive compounds (carbohydrates, terpenoids, flavonoids, coumarins, alkaloids, tannins, saponins, cardiac glycosides, and proteins) (Arirudran et al., 2022). The antioxidant activity of several plant species is interesting due to their preventive properties against oxidative stress in the development of many chronic diseases, such as cancer, hepatitis, cardiovascular disorders, neurodegenerative conditions, and ageing (Sultana et al., 2023; Zongo et al., 2024). Oxidative stress is caused by an imbalance between the accumulation of reactive oxygen species (ROS) and the body's antioxidant defenses, resulting in cellular damage and tissue malfunction (Caturano et al., 2023). Natural antioxidants, including phenolic compounds, carotenoids, vitamins, and trace elements, would help prevent oxidation reactions (Kashtiban et al., 2024). Type 2 diabetes mellitus (T2DM) is becoming more prevalent worldwide. One treatment approach to manage type 2 diabetes is controlling carbohydrate digestibility by inhibiting  $\alpha$ -glucosidase, an essential enzyme that catalyses the final stage of carbohydrate digestion in the small intestine (Li et al., 2022; Kashtoh and Baek, 2022). Since plants all contain phytochemicals that can inhibit glucosidase enzymes and control blood sugar levels, many plants have been studied for their antioxidant and antidiabetic activities, and blood sugar-lowering effects (Niaz et al., 2021). Plant-based bioactive compounds, including polyphenols, alkaloids, flavonoids, coumarins, and terpenoids, exhibit superior antidiabetic efficacy compared to non-plant-based hypoglycemic agents (Tresina et al., 2022). Alzheimer's

disease (AD), the predominant form of dementia accounting for 70% of all dementia cases, is a progressive neurodegenerative disorder and a major health concern among the aging population worldwide (Rawat et al., 2024). Inhibition of acetylcholinesterase, a key enzyme involved in the breakdown of the neurotransmitter acetylcholine, is considered a promising treatment for Alzheimer's disease (Gajendra et al., 2024). Plants are currently gaining attention, since plants contain phytochemicals with a lot of potential to mitigate the risk of Alzheimer's disease, and phytochemicals from plants are safe to consume. Several plants from different families have been documented to have a range of bioactive chemicals such as galantamine, curcumin, and silymarin which are used for the treatment of Alzheimer's disease (Koul et al., 2023). The phytochemicals well-known for their cardiogenic properties and antimicrobial properties, such as alkaloids, flavonoids, glycosides, phenolic compounds, tannins, terpenes, saponins, steroids, and sterols, are present in *B. flabellifer* L. (Alam et al., 2022; Behera, 2022). Phenolic compounds present in various parts of the *B. flabellifer* L. plant, such as flavonoids and tannins, act as primary (chain breaking) antioxidants and have biological activities and pharmacological functions, including antimicrobial, anti-inflammatory, antiallergic, anticancer, and antineoplastic activity (Jamkhande et al., 2016; Behera, 2022). Different portions of *B. flabellifer* L., such as leaves, roots, pulp, flowers, sap, and fruit fibers, have been shown to be effective in treating diabetes (Kavatagimath et al., 2016; Akter et al., 2020; Leida et al., 2020) and possess anthelmintic, antioxidant, antiarthritic, antibacterial, anticancer, antidiabetic, antifungal, anti-inflammatory, anti-acetylcholinesterase, antipyretic, diuretic, hypersensitivity, immunomodulatory, and wound healing properties (Shanmugalingami et al., 2021; Abraham et al., 2021). Nonetheless, no research has examined the efficacy of antioxidants, acetylcholinesterase, and  $\alpha$ -glucosidase inhibitory in various parts of *B. flabellifer* L. Therefore, the aim of this study was to evaluate the bioactive compounds, antioxidant activities,  $\alpha$ -glucosidase inhibitory activities, and acetylcholinesterase inhibitory activities in each part of Thai *B. flabellifer* L., including endosperm, haustorium, leaves, male flowers, mesocarp, ripe pulp, exocarp, and sap.

## MATERIALS & METHODS

### Plant Material and Extraction Methodology

In the study, *B. flabellifer* L. samples (endosperm, haustorium, leaves, male flowers, young mesocarp, ripe pulp, exocarp, and sap) (Fig. 1) were collected from Phetchaburi province, Thailand, air-dried at 50°C, and ground into fine powder. The ethanolic extracts (70% ethanol) were performed following the procedure described by Nefzi et al. (2022) with minor modifications. One gram of dried powder was suspended with 10mL 70% ethanol, stirred with a magnetic stirrer, and kept at 4°C in the dark for 24h. The extracts were then filtered and placed at 4°C in the dark for future utilization.



**Fig. 1:** *B. flabellifer* L. (A) endosperm, (B) haustorium, (C) leaves (D) male flowers, (E) mesocarp, (F) ripe pulp, (G) exocarp, and (H) sap

## Bioactive Compounds

### Total Phenolics

Total phenolic content in each *B. flabellifer* L. extract was determined using Folin-Ciocalteu reagent following the methodology of Kupina et al. (2019) and Sansenya et al. (2021) with a minor modification. One milliliter of each extract was combined with 5mL of Folin-Ciocalteu reagent and then incubated in the dark for 5min at room temperature ( $30\pm 2^{\circ}\text{C}$ ). After that, the mixture was added with 4mL of 7.5% sodium bicarbonate ( $\text{Na}_2\text{CO}_3$ ) solution, further incubated for 30min, and measured at 765nm using a UV-visible spectrophotometer. The result was expressed in  $\mu\text{g}$  equivalents of gallic acid per 1g of dried sample ( $\mu\text{g}$  GAE/g).

### Flavonoids

The total flavonoid content of all *B. flabellifer* L. samples was quantified based on the aluminum chloride colorimetric technique as described by El Atki et al. (2020) with some modifications. 0.6mL of each extract of *B. flabellifer* L. was combined with 2.4mL of distilled water and 0.3mL of 5%  $\text{NaNO}_2$ . After incubating for 5min, the mixture was added with 0.3mL of 10%  $\text{AlCl}_3$ , and incubated again for 5min. Furthermore, the mixture was added with 1mL of 1M NaOH and 1.4mL of distilled water, incubated once again for 15min, and then measured at a wavelength of 415nm with a UV-Vis spectrophotometer. The outcome was quantified in  $\mu\text{g}$  equivalents of quercetin per 1g of dried sample (mg QE/g).

### Anthocyanins

The total Anthocyanin content was analyzed using the pH differential method as described by Anggraini et al. (2019), with minor changes. Each extract was diluted with 0.025M potassium chloride buffer (pH 1.0) and 0.4M sodium acetate buffer (pH 4.5). After 15min at ambient temperature, the absorbance of each dilution at 510 and 700nm was assessed utilizing distilled water as the blank. The anthocyanin content of each dilution was calculated and reported as milligrams of cyanidin-3-glucoside (C3G) per gram of dry weight (DW).

### Tannins

The total concentration of tannins content in *B. flabellifer* L. samples was quantified using a spectrophotometric method using Folin-Ciocalteu (FC)

reagent according to Chandran & Indira (2016). 0.1mL of each sample extract was combined with 7.5mL of distilled water, 0.5mL of Folin-Ciocalteu reagent, and 1mL of 35% sodium carbonate solution and allowed to incubate at room temperature for 30min. The absorbance of each sample at 450nm was determined as tannic acid equivalents (mg TA/g extract) using a standard curve of tannic acid.

### Total Carotenoids

The total carotenoid concentration in each segment of *B. flabellifer* L. was analyzed using a spectrophotometer (Suwanaruang, 2022) with absorbance at wavelengths of 470, 645, and 663nm. The quantification of carotenoids in each sample was calculated based on the equation described by Suwanaruang (2022).

## Estimation of Radical Scavenging Activities

### DPPH Radical Scavenging Ability

The DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay was conducted as detailed by Zhou et al. (2018) with slight modifications. Specifically, each sample extract (10 $\mu\text{L}$ ) was combined with 0.1mM DPPH (195 $\mu\text{L}$ ) and incubated in a well of a 96-well microtiter plate at room temperature for 30min in the dark, and the absorbance was measured at 517nm. The DPPH scavenging capacity of each extract was represented as  $\text{IC}_{50}$  (mg/mL) utilizing GraphPad Prism V5.0 software (GraphPad Software, San Diego, CA, USA).

### ABTS Radical Scavenging Ability

The ABTS assay was determined based on the methodology of Zhou et al. (2018) and carried out in a 96-well microtiter plate. Each extract (10 $\mu\text{L}$ ) was combined with ABTS radical solution (195 $\mu\text{L}$ ), mixed well, and incubated at room temperature for 30min in the dark, and the absorbance was measured at 734nm.  $\text{IC}_{50}$  values for the ABTS scavenging capacity of each extract were evaluated using GraphPad Prism V5.0 software.

### FRAP Radical Scavenging Assay

The FRAP assay was conducted as detailed by Liao & Banbury (2015) with slight modifications. The FRAP solution was prepared by mixing 1mL of TPTZ (10mmol/L), 1mL of  $\text{FeCl}_3$  (20mmol/L), and 10mL acetate buffer (pH 3.6), with the mixture held at  $37^{\circ}\text{C}$ . 50 $\mu\text{L}$  of each sample was combined with 250 $\mu\text{L}$  of the FRAP

solution in a well of a 96-well microtiter plate, incubated for 10min in the dark at 30°C, and then measured at 593nm. Trolox served as a positive control, and results were expressed as milligrams of Trolox equivalents/g of extract (mg TE/g extract)

### Enzyme Inhibitory Activities

#### Inhibition Assay for $\alpha$ -glucosidase Activity

The  $\alpha$ -glucosidase inhibitory activity was assessed following the method of Telagari & Hullatti (2015) with minor changes. 40 $\mu$ L of each sample extract was mixed with 10 $\mu$ L of  $\alpha$ -Glucosidase (1UN/mL) and 40 $\mu$ L of 3mM p-nitrophenyl- $\alpha$ -D-glucopyranoside in phosphate buffer (pH 6.9). The mixture was thereafter incubated at 37°C for 30min and added with 100 $\mu$ L of 1M Na<sub>2</sub>CO<sub>3</sub>. The absorbance of the mixture was subsequently assessed at a wavelength of 405nm. The  $\alpha$ -glucosidase inhibition of the extracts was estimated by IC<sub>50</sub> values (mg/mL) using GraphPad Prism V5.0 software.

#### Inhibition Assay for Acetylcholinesterase Activity

The anticholinesterase activity of the samples was conducted following the method of Ghribia et al. (2014) with slight modification with the dopachrome technique with a 96-well microplate reader. In a well of a 96-well microtiter plate, 5 $\mu$ L of each sample was combined with 45 $\mu$ L of acetylcholinesterase, incubated at 37°C for 15min, and subsequently added with reactive mix solution (1 $\mu$ L of acetylthiocholine iodide (ATCI), 0.5 $\mu$ L of DTNB, and 154 $\mu$ L of Tris-HCl buffer). The absorbance of the combination was recorded at a wavelength of 405nm at 10min. The inhibition of  $\alpha$ -acetylcholinesterase of each sample was calculated and determined as IC<sub>50</sub> (mg/mL) using GraphPad Prism V5.0 software.

### Determination of Phenolic Acids and Flavonoids by HPLC

Determination of phenolic acids (gallic acid, protocatechuic acid, vanillic acid, caffeic acid, coumaric acid, ferulic acid, and sinapic acid) and flavonoids (catechin, rutin, and quercetin) was carried out using HPLC by the method of Butsat & Siriamornpun (2010) and Zhang et al. (2015), respectively. The reversed-phase Phenomenex C18 column (4.6mm $\times$ 250mm) packed with 5 $\mu$ m diameter particles was utilized. The mobile phase of phenolics was Acetic acid 1% (phase A) and Acetonitrile (phase B), while flavonoids utilized a formic acid solution (pH 2.5, phase A) and methanol (phase B). A sample injection value of 10 $\mu$ L was set at a flow rate of 1mL, with absorbance measured at a wavelength of 280nm.

### Statistical Analysis

Each sample of *B. flabellifer* L. was analyzed in triplicate, and the results were provided as mean $\pm$ standard deviation (SD). The data were subjected to one-way analysis of variance (ANOVA), specifically Duncan's New Multiple Range Test (DMRT), for comparing mean differences test at a significant level of 5% ( $P < 0.05$ ).

## RESULTS & DISCUSSION

### Bioactive Compounds

The quantity of each bioactive compound (phenolic compounds, flavonoids, anthocyanins, carotenoids, and tannins) from the endosperm, haustorium, leaves, male flowers, mesocarp, ripe pulp, exocarp, and sap of *B. flabellifer* L. are showed in Table 1. The phenolic compounds, flavonoids, anthocyanins, carotenoids, and tannins of *B. flabellifer* L. samples ranged from 7.49-64.89mgQE/g sample, 22.85-338.92mg of catechin/g sample, 0.98-47.04mg/100g, 0.01-10.54mg/100g, and 1.26-136.63mgTA/100g, respectively. The male flowers showed a significantly high bioactive compound content value and the highest amount of phenolic acids, flavonoids, anthocyanins, and tannins among all the parts, whereas the ripe pulp showed the highest amount of carotenoid content with a significant value ( $P < 0.05$ ). The phenolic content found in the different parts of *B. flabellifer* L. was in the following order: male flowers > exocarp > endosperm > leaves > ripe pulp > mesocarp > sap > haustorium. Phenolic compounds are secondary metabolites of plants characterized by the presence of various phenol groups and possess high antioxidant activities, which means any plant extracts with a high polyphenol content will have a high antioxidant effect as well (Yun & Seo, 2023). Nevertheless, the amount of phenolic content in the plants depends on numerous environmental factors such as genetics, age, vegetation period, etc. (Hofmann et al., 2020). Rodiah et al., (2019) reported that the mesocarp and exocarp of *B. flabellifer* L. have low total phenolic content, while the exocarp has a higher total phenolic content than the mesocarp. Our experiments show corresponding results, with the exocarp having significantly ( $P < 0.05$ ) higher phenolic content (33.82mgQE/g sample) compared to the mesocarp (13.16mg QE/g sample). Flavonoids, a group of polyphenolic compounds, are plant-derived secondary metabolites, found in all plant parts, including leaves, fruits, seeds, roots, and bark (Saberi et al., 2022). Among the parts of *B. flabellifer* L., male flowers had the highest total flavonoid content (338.92mg catechin/g sample). The

**Table 1:** Bioactive compounds from different parts of *B. flabellifer* L.

|              | Total phenolics<br>(mg QE/g sample) | Total flavonoids<br>(mg of catechin /g sample) | Anthocyanins<br>(mg/100g) | Carotenoids<br>(mg/100g) | Tannins<br>(mg TA/100 g) |
|--------------|-------------------------------------|--|---------------------------|--------------------------|--------------------------|
| Endosperm    | 22.49 $\pm$ 1.35c                   | 101.06 $\pm$ 0.74c                             | 2.24 $\pm$ 1.44d          | 0.06 $\pm$ 0.01f         | 3.30 $\pm$ 0.31c         |
| Haustorium   | 7.49 $\pm$ 0.19g                    | 47.96 $\pm$ 0.94f                              | 2.15 $\pm$ 0.45d          | 0.20 $\pm$ 0.05e         | 1.26 $\pm$ 0.02c         |
| Leaves       | 21.49 $\pm$ 0.51c                   | 76.65 $\pm$ 1.61e                              | 19.76 $\pm$ 2.82b         | 7.72 $\pm$ 0.10b         | 5.56 $\pm$ 0.10c         |
| Male Flowers | 64.89 $\pm$ 1.20a                   | 338.92 $\pm$ 1.09a                             | 47.04 $\pm$ 1.99a         | 1.11 $\pm$ 0.02d         | 136.63 $\pm$ 5.73a       |
| Mesocarp     | 13.16 $\pm$ 0.38e                   | 26.89 $\pm$ 1.56g                              | 3.41 $\pm$ 0.61d          | 0.19 $\pm$ 0.02e         | 2.57 $\pm$ 0.29c         |
| Ripe Pulp    | 15.16 $\pm$ 0.51d                   | 22.85 $\pm$ 1.49h                              | 0.98 $\pm$ 0.45d          | 10.54 $\pm$ 0.08a        | 3.12 $\pm$ 0.15c         |
| Exocarp      | 33.82 $\pm$ 0.84b                   | 126.89 $\pm$ 1.64b                             | 7.90 $\pm$ 0.18c          | 1.71 $\pm$ 0.00c         | 19.35 $\pm$ 1.69b        |
| Sap          | 9.49 $\pm$ 0.38f                    | 90.70 $\pm$ 0.90d                              | 2.54 $\pm$ 0.17d          | 0.01 $\pm$ 0.00f         | 3.17 $\pm$ 0.10c         |

Values (mean $\pm$ SD) with different letters in a column indicate a significant difference ( $P < 0.05$ ).

total flavonoid content in various parts had a positive correlation to total phenolic content in the following descending order: male flowers > exocarp > endosperm > sap > leaves > haustorium > mesocarp > ripe pulp. Anthocyanins are one of the polar compounds of flavonoids that provide red, purple, and blue color in fruits and vegetables (Warnasih & Hasanah, 2018). Anthocyanins in the male flower were the highest (47.04mg/100g) followed by the leaves (19.76mg/100g) and the exocarp (7.90mg/100g), respectively. Carotenoids are a large group of hydrocarbons and xantofiles that contribute to the red, orange, or yellow color of plants (Warnasih & Hasanah, 2018). The highest content of carotenoids was present in ripe pulp (10.54mg/100g), followed by leaves (7.72mg/100g) and exocarp (1.71mg/100g), respectively. Tannins is a polar polyphenol compound found naturally in vegetables exhibiting antioxidant activities. Tannins were found to be in very high concentrations in the male flowers (136.63mg TA/100 g). Lou et al. (2018) reported that tannins exhibited more reducing power, DPPH, and ABTS radical scavenging activities than TBHQ, showed higher cellular antioxidant activity than gallic acid in the PBS wash protocol, and had the  $\alpha$ -amylase inhibitory activity beyond that of acarbose.

### Antioxidant Activity

The DPPH, ABTS, and FRAP radical scavenging activities of the *B. flabellifer* L. sample are given in Table 2. The male flower of *B. flabellifer* L. exhibited the highest DPPH, ABTS, and FRAP radical scavenging activity ( $IC_{50}$  at 1.10mg/mL,  $IC_{50}$  at 0.44mg/mL, 147.09mg of TE/100g sample), followed by exocarp ( $IC_{50}$  at 17.09mg/mL,  $IC_{50}$  at 2.57mg/mL, 17.33mg of TE/100g sample) and leaves ( $IC_{50}$  at 40.43mg/mL,  $IC_{50}$  at 5.61mg/mL, 6.89mg of TE/100g sample), respectively. The result indicates that the male flower of *B. flabellifer* L. has the greatest antioxidant activity, proportional to the amount of polyphenol. Generally, plant parts with higher antioxidant effects have

higher polyphenol content as well. Barbosa and Nueva (2019) reported a positive correlation between antioxidant activity and total phenolic content in the plant parts of *H. conoidea*, attributing the high total antioxidant activity predominantly to the phenolic compounds found in the leaves of *H. conoidea*.

### Enzyme Inhibition Studies

The  $\alpha$ -glucosidase inhibitory and anticholinesterase activity from different parts of *B. flabellifer* L. are shown in Table 3. The male flower of *B. flabellifer* L. exhibited the most significant  $\alpha$ -glucosidase inhibitory activity ( $IC_{50}$  at 0.75mg/mL), followed by exocarp ( $IC_{50}$  at 55.24mg/mL) and leaves ( $IC_{50}$  at 62.37mg/mL), respectively. In addition, the inhibitory effect of the male flowers was greater than that of the positive control, acarbose, which expressed the  $IC_{50}$  at 3.20mg/mL.  $\alpha$ -Glucosidase is an enzyme required for hydrolyzing starches and disaccharides to glucose for intestinal absorption, and has been used as a target for the treatment of type 2 diabetes (Assefa et al., 2019; Kittiwisut et al., 2021). Inhibition of this enzyme function delays carbohydrate absorption after a meal, leading to a reduction of postprandial blood sugar (Assefa et al., 2019). Various parts of the Palmyra tree have shown potent  $\alpha$ -glucosidase inhibitory activity and antidiabetic activity in animal models due to the presence of the components that exhibited  $\alpha$ -glucosidase inhibition and various bioactive chemicals such as flavonoids and phenolic acids (Dej-adisai et al., 2017; Abu et al., 2024). The male flowers have demonstrated antidiabetic potential in alloxan-induced diabetic rats, in which the antioxidant potential may be attributed to the presence of flavonoids and triterpenoids (Kavatagimath et al., 2016). Palm sugar, fruit pulp, immature endosperm, germinated endosperm, sap, palm jaggery, and palm honey had an antidiabetic effect on alloxan-induced diabetic rats (Leida et al., 2020; Rahman et al., 2021; Manivannan et al., 2024). Fresh sugar palm fruits were also found to have inhibitory activity

**Table 2:** DPPH, ABTS, and FRAP radical scavenging activities from different parts of *B. flabellifer* L.

|              | DPPH radical scavenging activity<br>( $IC_{50}$ mg/mL) | ABTS radical scavenging activity<br>( $IC_{50}$ mg/mL) | FRAP radical scavenging activity<br>(mg of TE/ 100g sample) |
|--------------|--|--|---|
| Endosperm    | 175.78±0.94a   | 33.91±1.19c  | 2.04±0.20de   |
| Haustrorium  | 165.20±1.90b   | 163.80±2.30a   | 0.26±0.05f  |
| Leaves       | 40.43±1.43f  | 5.61±0.12f   | 6.89±0.31c  |
| Male Flowers | 1.10±0.10h   | 0.44±0.04h   | 147.09±2.55a  |
| Mesocarp     | 119.40±1.04c   | 25.59±0.69e  | 2.24±0.15d  |
| Ripe Pulp    | 116.80±1.10d   | 31.41±1.11d  | 2.79±0.24d  |
| Exocarp      | 17.09±0.20g  | 2.57±0.12g   | 17.33±0.53b   |
| Sap          | 80.31±0.99e  | 48.72±1.02b  | 0.53±0.08ef   |
| Trolox       | 0.164±0.01h  | 0.107±0.02h  | -   |

Values (mean±SD) with different letters in a column indicate a significant difference ( $P<0.05$ ).

**Table 3:**  $\alpha$ -Glucosidase inhibitory and anticholinesterase activity from different parts of *B. flabellifer* L.

|               | $\alpha$ -glucosidase inhibitory $IC_{50}$ (mg/mL) | anticholinesterase activity $IC_{50}$ (mg/mL) |
|---------------|--|---|
| Endosperm     | 909.05±5.01b                                       | >1,000a                                       |
| Haustrorium   | >1,000a  | 561.88±3.48c                                  |
| Leaves        | 62.37±0.92d  | 356.14±2.82d                                  |
| Male Flowers  | 0.75±0.01g   | 918.52±6.76b                                  |
| Mesocarp      | >1,000a  | >1,000a                                       |
| Ripe Pulp     | >1,000a  | >1,000a                                       |
| Exocarp       | 55.24±1.17e  | 157.82±2.03e                                  |
| Sap           | 368.99±2.27c                                       | >1,000a                                       |
| Acarbose      | 3.20±0.08f   |   |
| Physostigmine |  | 0.02±0.00e                                    |

Values (mean±SD) with different letters in a column indicate significant difference ( $P<0.05$ ).

**Table 4:** Phenolic contents from different parts of *B. flabellifer* L.

|              | Gallic acid<br>(µg/g) | Proto-catechuic acid<br>(µg/g) | Vanillic acid<br>(µg/g) | Caffeic acid<br>(µg/g) | Coumaric acid<br>(µg/g) | Ferulic acid<br>(µg/g) | Sinapic acid<br>(µg/g) |
|--------------|-----------------------|--------------------------------|-------------------------|------------------------|-------------------------|------------------------|------------------------|
| Endosperm    | 31.49±0.16c           | 9.55±0.83d                     | 4.96±0.00d              | 7.37±0.51d             | 3.19±0.04e              | 5.97±0.06c             | 6.18±0.37e             |
| Haustorium   | ND                    | 6.07±0.05f                     | 5.60±0.15cd             | 5.29±0.07e             | 3.39±0.03de             | 5.11±0.01c             | 8.30±0.05de            |
| Leaves       | ND                    | 12.17±0.40b                    | 8.85±1.28a              | 8.92±0.41c             | 21.92±0.34a             | 14.76±2.69b            | 60.68±2.64a            |
| Male Flowers | 224.89±0.74a          | 10.33±0.26c                    | 5.32±0.36cd             | 38.27±0.65a            | 19.23±0.75b             | 28.07±6.50a            | 36.92±1.08b            |
| Mesocarp     | 8.32±0.13d            | 7.39±0.25e                     | 5.67±0.16cd             | 11.81±0.14b            | 3.82±0.12de             | 5.35±0.06c             | 6.48±0.20e             |
| Ripe Pulp    | 2.07±0.02e            | 12.49±0.12b                    | 8.31±0.02a              | 7.66±0.06d             | 3.62±0.12cd             | 5.59±0.06c             | 17.80±0.13c            |
| Exocarp      | 63.14±0.75b           | 7.81±0.05e                     | 6.20±0.10bc             | 8.85±0.09c             | 4.87±0.07c              | 5.80±0.02c             | 8.26±0.30de            |
| Sap          | 2.04±0.04e            | 16.43±0.24a                    | 6.89±0.20b              | 11.73±0.26b            | 4.89±0.05c              | 6.08±0.45c             | 10.25±1.58d            |

Values (mean±SD) with different letters in a column indicate significant difference ( $P < 0.05$ ).

against  $\alpha$ -glucosidase, whereas two isolated substances, tyrosol and glucosyl-(6-1)-glycerol, shown moderate and weak  $\alpha$ -glucosidase inhibition, respectively (Dej-adisai et al., 2017). Acetylcholinesterase (AChE) is a key enzyme that catalyzes the hydrolysis of acetylcholine to acetate and choline, serving as an important target of most of the clinically used drugs for Alzheimer's disease treatment (Basnet et al., 2020). The exocarp of *B. flabellifer* L. showed the highest  $\alpha$ -acetylcholinesterase inhibitory activity ( $IC_{50}$  at 157.82mg/ml), followed by leaves ( $IC_{50}$  at 356.14mg/mL) and haustorium ( $IC_{50}$  at 561.88mg/mL) respectively. Some reports regarding the acetylcholinesterase inhibitory activity of *B. flabellifer* L. Abraham et al. (2022) reported haustoria of *B. flabellifer* L. contained the compounds docking affinities against acetylcholinesterase and  $\beta$ -secretase, demonstrating superior binding scores and ADME properties, suggesting their potential as therapeutic agents for Alzheimer's disease. The results showed that the *B. flabellifer* L. possessed  $\alpha$ -glucosidase inhibitory and anticholinesterase activity. In addition, male flowers showed considerable  $\alpha$ -glucosidase inhibitory activities with  $IC_{50}$  at 0.75mg/mL, higher than acarbose ( $IC_{50}$  at 3.0mg/mL).

### Phenolic and Flavonoid Profile

Using the HPLC technique, 7 phenolic compounds were investigated in different parts of *B. flabellifer* L., and the result is shown in Table 4. Protocatechuic acid, vanillic acid, caffeic acid, coumaric acid, ferulic acid, and sinapic acid were identified in differing concentrations across all eight portions of *B. flabellifer* L. However, gallic acid was present only in 6 parts out of 8: endosperm, male flowers, mesocarp, ripe pulp, exocarp, and sap. The most abundant phenolic components were gallic acid, sinapic acid, and caffeic acid, respectively. The male flowers contained the highest amount of gallic acid (224.89µg/g), caffeic acid (38.27µg/g), sinapic acid (36.92µg/g), and ferulic acid (28.07µg/g), while the leaves and sap contained the highest amount of coumaric acid (21.92µg/g) and protocatechuic acid (16.43µg/g), respectively. Vanillic acid was abundant in leaves (8.85µg/g) and ripe pulp (8.31µg/g). The 3 flavonoids, catechin, rutin, and quercetin were determined and shown in Table 5. The most abundant flavonoid compounds were catechin, rutin, and quercetin, respectively. Catechin, rutin, and quercetin were present in all parts of *B. flabellifer* L., in which the male flowers contain the highest amount of catechin (189.26µg/g) and quercetin (134.82µg/g), while the sap contains the highest amount of rutin (239.76µg/g). Our results correspond with previous research, in which

**Table 5:** Flavonoid contents from different parts of *B. flabellifer* L.

|              | Catechin (µg/g) | Rutin (µg/g) | Quercetin (µg/g) |
|--------------|-----------------|--------------|------------------|
| Endosperm    | 113.90±1.30c    | 41.90±0.55e  | 5.75±0.13g       |
| Haustorium   | 165.95±2.23b    | 23.46±0.62f  | 5.85±0.10g       |
| Leaves       | 48.30±0.38f     | 77.38±2.76d  | 94.13±0.64b      |
| Male Flowers | 189.26±8.12a    | 113.33±1.15c | 134.82±1.62a     |
| Mesocarp     | 64.07±1.83e     | 4.29±0.49g   | 13.79±0.25d      |
| Ripe Pulp    | 121.67±2.63c    | 0.63±0.06h   | 9.16±0.22e       |
| Exocarp      | 36.51±0.96g     | 121.70±9.96b | 62.80±2.78c      |
| Sap          | 86.38±2.38d     | 239.76±4.31a | 8.39±0.14f       |

Values (mean±SD) with different letters in a column indicate significant difference ( $P < 0.05$ ).

catechin has been identified as the only phytochemical compound detected in water and ethanol extracts of young and mature male flowers of *B. flabellifer* L. (Fongsuk et al., 2023). Numerous researchers have indicated that medicinal plants and their phenolic and flavonoid content have therapeutic benefits, such as antioxidants and the potential to inhibit both enzymes. ( $\alpha$ -glucosidase and acetylcholinesterase). Tamfu et al. (2021) reported that *T. diversifolia*, *P. Biglobosa* and *C. Febrifuga*, medicinal plants from Chad, exhibited good inhibition against acetylcholinesterase and butyrylcholinesterase, making them potential candidates for the management of oxidative-stress-linked illnesses, such as Alzheimer's disease and diabetes, attributed to the presence of rutin, gallic acid, and protocatechuic acid within their phenolic compounds. Mollaei et al. (2021) reported that the ethyl acetate fractions of *Salsola vermiculata* leaves and the aqueous-acid fraction of its roots had the most significant inhibitory activity against acetylcholinesterase ( $IC_{50}$  at 17.24µg/mL) and  $\alpha$ -glucosidase ( $IC_{50}$  at 62.37µg/mL), respectively, furthermore, vanillic acid, rutin, salsoline, salsoline A, palmitic acid, oleic acid, linoleic acid, cuminaldehyde, and carvone were reported as major components in the roots, while gallic acid, vanillic acid, caffeic acid, rosmarinic acid, rutin, quercetin, limonene, and carvone were identified as major components in the leaves. Eruygur et al. (2022) reported that *Glaucosciadium cordifolium* demonstrated strong antioxidant potential and anti-acetylcholinesterase activity due to the caffeic acid and 1,2-dihydroxy benzene found in the aqueous extract of the roots. Koudoro et al. (2023) reported that *Pteleopsis suberosa* (Combretaceae) leaves possess the potential to mitigate Alzheimer's disease by inhibiting acetylcholinesterase and butyrylcholinesterase, as well as exhibiting antidiabetic activity, attributable the abundant of gallic acid among the fourteen identified phenolic compounds. Saad et al. (2024) reported that the *C. spinosum* L. leaf extracts possessed anti-acetylcholinesterase, antioxidant, and anti-inflammatory



activity, attributed to the significant levels of caffeic acid and coumaric acid in the extracts. According to the referenced research, our findings show that *B. flabellifer* L. has extraordinary potential in the treatment of diabetes and Alzheimer's disease, as it is a substantial source of strong potent antioxidants, anti- $\alpha$ -glucosidase, and anti-acetylcholinesterase compounds.

## Conclusion

This study reported the evaluation of phenolic contents, flavonoids, anthocyanins, carotenoids, antioxidant activities,  $\alpha$ -glucosidase inhibitory activities, and anti-acetylcholinesterase inhibitory activities of the endosperm, haustorium, leaves, male flowers, mesocarp, ripe fruit pulp, exocarp, and sap of *B. flabellifer* L. The male flower exhibited the highest concentrations of phenolic acids, flavonoids, anthocyanins, and tannins among all the different parts, while the ripe pulp showed the highest value of carotenoids among all parts of *B. flabellifer* L. The male flowers exhibited significantly elevated antioxidant activity and  $\alpha$ -glucosidase inhibitory activity, with  $\alpha$ -glucosidase inhibition even greater than that of the positive control, acarbose, a commonly used antidiabetic drug. Moreover, the exocarp of *B. flabellifer* L. showed the highest acetylcholinesterase inhibitory activity, followed by leaves and haustorium, respectively. The results indicate that *B. flabellifer* L. shows antioxidant, anti- $\alpha$ -glucosidase, and anti-acetylcholinesterase activity, making it highly effective for the treatment of diabetes and Alzheimer's disease.

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