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Effect of Extraction Methods on the Antioxidants and Alpha-Glucosidase Inhibitory Activity of *Borassus flabellifer* L. Fruit Fiber

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ABSTRACT

The fruit fiber of Borassus flabellifer (Lontar) is used by the local people in East Nusa Tenggara, Indonesia, to treat various illnesses due to its various bioactive compounds. The extraction methods of bioactive compounds have become one of the factors that significantly affect the activity of a sample. This study aims to identify the extraction method that impacts the antioxidant and alpha-glucosidase inhibitory activity in a 96% ethanol extract from young lontar fruit fiber (YLFF) and old lontar fruit fiber (OLFF). The extraction methods used in this study were maceration (M), soxhlet (S), ultrasonic-assisted extraction (UAE), and microwave-assisted extraction (MAE). Spectrophotometric analysis was performed to determine the total phenolics content, total flavonoid content, the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and alpha-glucosidase inhibitory activity. The result showed that the OLFF sample extracted by Soxhlet (OLFF-S) had the highest total phenolic content of 18.334 mg GAE/g extract and showed more significant activity in inhibiting the alpha-glucosidase enzyme than other samples. At 100 µg/mL, the OLFF-S sample exhibited a 2.441% inhibition of the alpha-glucosidase enzyme, and at 2000 µg/mL, the inhibition increased to 23.087%. Meanwhile, the OLLF sample extracted using the MAE method (OLFF-MAE) had the highest total flavonoid content of 4.99 mg QE/g extract. It also showed the highest DPPH radicals scavenging activity with an IC50 value of 1435.70 µg/mL. In conclusion, the extraction method can impact the phenolic and flavonoid levels of ethanol extracts of both young and old fruit fibers of B. flabellifer, as well as their antioxidant properties and effectiveness as alpha-glucosidase inhibitors.

Keywords: *Antioxidant, Alpha-glucosidase, Borassus flabellifer, Extraction method, Lontar*

Introduction

The palm tree, scientifically known as *Borassus flabellifer* Linn, is a prominent palm species belonging to the Arecaceae family, thriving in arid regions like East Nusa Tenggara (NTT). The palmae group possesses antioxidant and antibacterial properties, making it suitable for medicinal applications.[1,2] Antioxidants are essential for neutralizing highly reactive molecules and free radicals to prevent oxidative effects caused by oxidation reactions. Internal antioxidants can counteract oxidative harm caused by free radicals in the body, while external antioxidants can assist in neutralizing the free radicals produced.[3] A research has explored the antioxidant potency of various constituents of *B. flabellifer* and found *B. flabellifer*'s leaf, flower, root, and seed showed antioxidant properties using DPPH radical scavenging, FRAP Assay, and H_2O_2 methods.[4] Study conducted by Nayak *et al*. (2021)[5] showed that *B. flabellifer* ethanol fruit extract exhibited moderate antioxidant activity with DPPH and nitric oxide assay of 132 and 119 µg/mL, respectively.

Type 2 Diabetes Mellitus (DM) is a chronic and progressive condition characterized by the body's inability to metabolize proteins, lipids, and carbohydrates due to the pancreatic beta cells' failure to produce insulin.[6]

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On the other hand, alpha-glucosidase enzymes are crucial for breaking down carbohydrates into glucose in the human digestive system. Therefore, inhibitors are required to act as antidiabetic drugs by blocking these enzymes. Acarbose, an alpha-glucosidase inhibitor, is used to treat patients with high blood glucose levels. However, most synthetic antidiabetic drugs have been associated with side effects like flatulence, diarrhea, and abdominal discomfort.[7]

Extensive attempts have been made to discover Alpha-glucosidase Inhibitors (AGI) from natural sources for diabetes treatment.[8] Prior studies demonstrated the antidiabetic properties of Lontar fruit fiber extract. The methanol extract of *B. flabellifer* fruit was tested on diabetic rats induced with oral doses of high-fat diets/streptozotocin for 21 days and exhibited a decrease in fasting blood glucose, triglycerides, total cholesterol, free unsaturated fat, fructose 1,6 bisphosphatase, and glucose-6-phosphate compared to diabetic control.[9] The flower and root of *B. flabellifer* also showed potential antidiabetic effects when tested on diabetic rats induced with streptozotocin and alloxan.[10] Combining AGI and antioxidants will be more effective in preventing type 2 diabetes.[11,12]

Secondary metabolites are recognized as the substances responsible for the biological impacts of plants.[8,13] The phytochemical screening of *B. flabellifer* fruit fiber extract revealed the presence of saponins, flavonoids, tannins, alkaloids, steroids, terpenoids, and glycosides.[4,14] Phenols and flavonoids have been found to inhibit the alpha-glucosidase and have antioxidant activity.[15] Various studies have demonstrated that the extraction methods and choice of solvent impact the total phenol and flavonoid content and the activity of these compounds.[16–18]

Recently, some studies have investigated the effect of different collection areas, solvents, and parts of *B. flabellifer* on antioxidant, antibacterial, and antidiabetic activity.[5,19,20,21] However, no studies have

investigated the influence of different extraction methods on the bioactive compound of *B. flabellifer* fruit fibber extract with antioxidant and alpha-glucosidase inhibitory activity.

Extraction is a crucial step in isolating phytochemicals from plant materials. Several extraction techniques, including percolation, maceration, Soxhlet, reflux, supercritical extraction, microwave extraction, and ultrasonic extraction, have been developed to discover plant bioactive compounds. Some compounds may degrade when exposed to heat and light, so choosing appropriate extraction methods is crucial for obtaining pharmacologically active metabolites from herbal plants and standardizing herbal products as they help extract desired soluble compounds.[17,18] The study examines the impact of various extraction methods on the antioxidant and alpha-glucosidase inhibitory properties of 96% ethanol extract obtained from young and old Lontar (*B. flabellifer* L.) fruit fibers and their total phenolic and flavonoid contents.

Materials and Method

Collection and Identification of Plant Material

This study utilized plant samples of young and old Lontar fruit fiber collected in May 2023 from Kupang, East Nusa Tenggara, Indonesia (10º10'20.78"S 123º34'40.45"E). The sample was identified in the Biological Research Center of the Life Science Research Organization of the National Research and Innovation Agency Indonesia, with voucher specimen number B-545/V/DI.05.07/11/2021. Ascorbic acid, ethanol pro analysis, and hydrochloric acid were purchased from Emsure, Indonesia; quercetin and gallic acid from Sigma, Japan; $AlCl_3$, Na_2CO_3, CH_3COONa were purchased from Merck, Germany; Folin-Ciocalteu reagent (Sangon Biotech, China), and DPPH (Aldrich, Singapore).

Preparation of Young and Old Lontar Fruit Fiber Sample

The Lontar fruit, aged 1.5 months and 3 months, were cleaned and peeled, and their fibers separated from the seeds based on their color (pale white for young and orange-yellow for old Lontar fruit fiber). They were chopped, measuring 4x2 cm, and then dehydrated in a Getra FD-30® dehydrator at 400°C. The dried lontar fruit fibers were ground to a coarse powder.

Preparation of Extract

Four different extraction methods were used in this research. The maceration method was conducted for 24 hours at room temperature. The Soxhlet method was performed at a temperature of 70ºC for 4 hours. The MAE method used a modified microwave (Sharp®) on low mode for 10 minutes, and the UAE method used an ultrasonic bath (XUB25 Digital Ultrasonic Bath, Grant, UK) at 30ºC for 60 minutes. An aliquot of 40 g of each sample was used in each procedure, and 96% ethanol was used as the solvent. The filtrate was concentrated using a rotary evaporator (Hei-Vap Core, Heidolph, Germany) at 40°C to obtain a crude extract of young and old Lontar fruit fibers. Each extraction method was repeated twice.[22]

Determination of Total Phenolic Content

The total phenolic content of the extract was determined using the Folin-Ciocalteu method. An aliquot of 25 µL solution (5000 µg/mL) of each sample was mixed with 125 µL 10% Folin-Ciocalteu, shaken constantly, and incubated for 5 minutes in the dark at room temperature. The mixture was then incubated for 60 minutes in the dark at room temperature with 100 µL of 7.5% Na_2CO_3. The absorbance was measured spectrophotometrically at 740 nm using a microplate reader (Infinite 200 M Pro, TECAN, Switzerland). The sample's total phenolic content was expressed as mg Gallic Acid Equivalents (GAE) per gram of dry extract (mg GAE/g DE).[23]

Determination of Total Flavonoids Content

An aliquot of 100 µL (20 mg/mL) of each extract was added to a 96-well microplate. Then, 50 µL of 10% $AlCl_3$ solution, 50 µL of ethanol, and 50 µL of 1 M CH_3COONa were added to the wells. The mixtures were incubated for 30 minutes at room temperature, and the absorbance was measured spectrophotometrically using a microplate reader at a UV wavelength of 425 nm. The total flavonoid content of the sample was expressed as mg Quercetin Equivalents (QE) per gram of dry extract (mg QE/g DE).[24]

Determination of Antioxidant Activity

The antioxidant activity was measured using the DPPH radical scavenging method. An aliquot of 80 µL of each extract solution (500–3500 µg/mL) in ethanol was pipetted into a 96-well microplate, followed by 120 µL of DPPH (100 µg/mL). After 30 minutes of incubation in the dark at room temperature, the absorbance of the sample was measured at a UV wavelength of 515 nm. The positive control used in this study was ascorbic acid. A blank mixture of DPPH and ethanol was used as a control. The percentage of DPPH radical scavenging was measured using the following equation: [23,25]

$$\%DPPH\bullet \text{ Scavenging} = \frac{[\text{Absorbance blank} - \text{Absorbance sample}]}{Absorbance\ blank} x100\%$$

The inhibitor concentration value of the extract on scavenging 50% of DPPH radical (IC_{50}) was measured with the linear regression equation between the sample concentration and %DPPH radical scavenging.

Determination of Alpha-glucosidase Inhibitor

The test was carried out using a previously described method with slight modifications.[26] A test sample volume of 10 µL, 25 µL alpha-glucosidase enzyme solution at a concentration of 0.04 Units/mL, a 0.01 M phosphate buffer with a pH of 6.8, and 25 µL of 0.5 mM 4-nitrophenyl-β-d-glucopyranoside (PNPG) were mixed. This reaction mixture was incubated for 30 minutes at 37°C. 100 µL of a 0.2 M Na_2CO_3 solution was added to stop the reaction. A microplate reader operating at 410 nm wavelength measured the p-nitrophenol produced in the reaction mixture. A solution without a sample was used as a control. Acarbose was used as a positive control.

The percentage of inhibition was determined using the following formula:

$$\%Inhibition = \frac{[Absorbance\ control - Absorbance\ sample]}{Absorbance\ Control} x100\%$$

Statistical Analysis

The data were displayed as means ± standard error of means (SEM) and analyzed using the GraphPad Prism 10.1.1 software.

Results and Discussion

The phenolic and flavonoid contents of the extracts were evaluated using different extraction techniques, and their antioxidant potential and alpha-glucosidase inhibitory activity were assessed in this study.

Phenolic compounds are essential plant components with significant antioxidant activity. Plant extracts facilitate the scavenging of free radicals because of the hydroxyl group substituents contained in the extract. Folin-Ciocalteu reagent was used to determine the phenolic content of each extract. The outcomes were calculated using a calibration curve of gallic acid (20-100 µg/mL) ($y = 0.0059x + 0.0214$, R^2=0.998) and reported as gallic acid equivalents (GAE) per gram dry extract weight. The total phenolic content (TPC) of each sample is shown in Table 1.

The samples were found to have a total phenolic content ranging from 7.79 to 18.33 mgGAE/g. The OLLF-S, OLFF-MAE, and OLFF-M samples showed the highest Total Phenolic Content (TPC) values of 18.33, 18.08, and 17.35 mgGAE/g, respectively. The samples YLFF-UAE and YLFF-M showed the lowest Total Phenolic Content (TPC) with 7.79 and 8.48 mgGAE/g, respectively. The OLFF sample possesses a higher TPC value than YLFF. The age of a sample can impact the extracted compound within it.

Soxhlet and MAE used high temperatures of 80°C and 60°C, respectively, whereas maceration and UAE were conducted at room temperature, ranging from 25°C to 30°C. The data in Table 1 indicates that the Soxhlet and MAE methods were the most efficient techniques for extracting phenolic compounds from Lontar fruit fiber. The total

phenolic content values were 18.33 mgGAE/g and 18.08 mgGAE/g for OLFF, 15.1 mgGAE/g, and 12.25 mgGAE/g for YLFF. Choosing the proper sample and controlling the extraction temperature significantly affect the content of extracted compounds, mainly phenolic compounds from Lontar fruit fiber.

The total flavonoids in the samples were determined using the aluminum chloride colourimetric method. Aluminum chloride complexes with C-4 keto groups and C-3 or C-5 hydroxyl groups of flavones and flavonols causing a shift in wavelength towards the visible spectrum, indicated by yellow colour.[27] The outcomes were calculated using the calibration curve of quercetin (15-50 µg/mL) ($y = 0.016x + 0.0678$, R^2=0.9976) and represented in quercetin equivalents (QE) per gram dry extract weight.[28]

The total flavonoid content of the samples (Table 1) ranged from 0.29 – 4.99 mgQE/g. The result showed that the extract from OLFF had a higher flavonoid content than that from YLFF. In the OLFF sample, the OLFF-MAE sample has the highest TFC value (4.99 mgQE/g), while OLFF-S has the lowest TFC value (3.01 mgQE/g). On the other hand, YLFF-UAE had the highest TFC value (1.04 mgQE/g), and YLFF-MAE had the lowest TFC value (0.29 mgQE/g) in the YLFF sample. The experimental data indicates that the age of a Lontar sample influences the quantity of extracted flavonoid compounds in the extract. Mature fruit has a more excellent flavonoid content compared to immature fruit fiber. There was no direct relationship between the temperature applied during extraction and its Total Flavonoid Content (TFC). Varying extraction techniques can impact the Total Flavonoid Content in OLFF and YLFF extracts.

Table 1: TPC, TFC, and DPPH scavenging activity of young and old fruit fibber extract extracted with different extraction methods

Sample	Method	TPC (mgGAE/g DE)	TFC (mgQE/g DE)	IC_{50} of DPPH• Scavenging (µg/mL)
Young Lontar fruit fibber (YLFF)	M	8.48 ± 0.07	0.41 ± 0.01	1995.95
	S	15.1 ± 0.17	0.39 ± 0.01	1706.04
	UAE	7.79 ± 0.10	1.04 ± 0.01	2291.26
	MAE	12.25 ± 0.20	0.29 ± 0.01	1604.49
Old Lontar fruit fibber (OLFF)	M	17.35 ± 0.26	3.49 ± 0.06	1683.17
	S	18.33 ± 0.12	3.01 ± 0.03	1470.29
	UAE	12.71 ± 0.17	3.33 ± 0.03	1945.51
	MAE	18.08 ± 0.33	4.99 ± 0.09	1435.70
Ascorbic acid	nd	nd	nd	25.71

Note: TPC = Total Phenolic Content, TFC = Total Flavonoid Content, ; M = Maceration, S = Soxhlet, UAE = Ultrasonic Assisted Extraction, MAE = Microwave Assisted Extraction, nd = not defined

Similarly, the ability of the sample to scavenge the DPPH radical was used to determine the antioxidant activity of the extract. Based on the data presented in Table 1, the YLFF and the OLFF sample were categorized as weak antioxidants (IC_{50} >500 µg/mL). The antioxidant activity was compared with ascorbic acid as a positive control, with an IC_{50} value of 25.71 µg/mL. The OLFF-MAE sample showed the highest IC_{50} value; meanwhile, the YLFF-UAE sample had the lowest IC_{50} values, 1435.70 µg/mL and 2291.26 µg/mL, respectively.

The study found a correlation between the antioxidant activity and the sample's total phenolic content (TPC).[22] The samples with a higher TPC exhibited more potent antioxidant activity in scavenging the radical DPPH than those with lower TPC values. The sequence of extraction methods that can deliver the highest total phenolic content and antioxidant activity was MAE, followed by Soxhlet, Maceration, and the UAE method.

Shi *et al.* (2022)[15] found that the temperature and duration of heating during the extraction process can influence the quantities of compounds obtained. Typically, higher temperatures enhance the solubility of the extracted active substance. However, it is crucial to consider that raising the extraction temperature can potentially harm the processed material due to excessive heat.[29]

Several studies regarding the effect of extraction methods on antioxidant activity are often related to the total phenol and flavonoid contents.[30] Research conducted by Phuyal *et al*. (2020)[24] shows that different extraction methods can significantly influence the total phenolic content and antioxidant activity of *Zanthoxylum armatum*. In addition, Luliana *et al*. (2019)[31] and Nursamsiar *et al*. (2023)[32] also stated that the extraction method affects antioxidant activity and the levels of compounds that act as antioxidants, such as phenols in *Syzygium polyanthum* leaf extract and flavonoid in Red Betel Extract. Phenol can act as an antioxidant by donating hydrogen atoms from the hydroxyl group and stabilizing free radicals.[33]

In contrast to the result of this study, research conducted by Sudiono *et al*. (2021)[34] reported that the seed coat of *B. flabellifer* has very strong antioxidant activity with an IC_{50} value of 12.29 ppm. However, using the ABTS method, the root methanol extract of *B. flabellifer* gave an IC_{50} value of 2 mg/mL. At the same time, the FRAP assay indicated that the chloroform extract had activity of 129.6 µg BHT/100 mg extract.[35] *B. flabellifer* leaves also showed potential antioxidant activity with an IC_{50} value of 40.19 µg/mL for DPPH radical scavenging.[19]

The alpha-glucosidase inhibitory activity of Lontar fruit fibber was carried out *in vitro* using a microplate reader to determine the inhibition percentage (%) of the tested ethanol extract of YLFF and OLFF. The alpha-glucosidase enzyme hydrolyzes PNPG and then releases p-nitrophenol as a yellow compound. The intensity of the color produced is proportional to the ability of the sample to inhibit the alpha-glucosidase enzyme. The resulting absorption value is proportional to the activity of a sample in inhibiting the alpha-glucosidase enzyme. An increase in absorbance correlates with the activity of the sample.[26,36,37] From this study, YLFF showed negative alpha-glucosidase inhibitory activity at sample concentrations ranging from 100 – 2000 µg/mL. Meanwhile, according to data presented in Table 2, the OLFF sample showed very low alpha-glucosidase inhibitory activity with % inhibition on minimum test concentration of 100 µg/mL ranging from 0.3 – 2.4 % and on maximum test concentration of 2000 µg/mL ranging from 8.6 – 23%. In contrast, acarbose with a concentration of 0.1 µg/mL had a % inhibition of 36.82 0.74%, while a 10 µg/mL concentration had 99.61±0.55%.

The sample with the highest percent of inhibition at a concentration of 2000 µg/mL was the OLFF-S, and the lowest was the OLFF-MAE, with 23% and 8.6% percent, respectively (Figure 1). The graph also demonstrated that the alpha-glucosidase inhibitory activity of OLFF in all extraction methods depended on sample concentration.

The alpha-glucosidase enzyme inhibitory activity in plant extracts was primarily linked to phenolic and flavonoid compounds. As reported by Aleixandre *et al.* (2022)[38], phenolic compounds and flavonoids can inhibit the activity of the alpha-glucosidase enzyme. The % inhibition activity of the OLFF extract showed positive results. Still, the YLFF extract did not show inhibitory activity against alpha-glucosidase at this concentration, possibly due to the very low phenol and flavonoid content of YLFF, which were insufficient to inhibit the activity of the alpha-glucosidase enzyme.

Table 2: Alpha-glucosidase inhibitory activity of Lontar Fruit Fiber

Extraction Method	Concentration (µg/mL)	% Inhibition (%) YLFF	% Inhibition (%) OLFF
	2000	-14.91 ± 0.37	13.40 ± 0.46
	1000	-11.54 ± 0.11	8.84 ± 0.37
M	500	-5.81 ± 0.56	3.63 ± 0.65
	250	-1.72 ± 0.37	2.31 ± 0.09
	100	-0.40 ± 0.19	0.46 ± 0.28
	2000	-11.87 ± 0.00	8.64 ± 0.28
	1000	-24.21 ± 1.59	4.88 ± 0.56
MAE	500	-31.46 ± 2.71	3.36 ± 0.09
	250	-29.02 ± 1.12	2.51 ± 0.37
	100	-16.95 ± 2.89	0.33 ± 0.47
	2000	-10.36 ± 0.28	9.63 ± 0.75
	1000	-9.41 ± 0.62	7.72 ± 0.28
UAE	500	-5.28 ± 0.00	4.02 ± 0.47
	250	-4.75 ± 0.19	1.98 ± 0.19
	100	-1.85 ± 0.19	0.33 ± 0.00
	2000	-15.50 ± 0.09	23.10 ± 0.00
	1000	-14.58 ± 0.09	18.67 ± 0.37
S	500	-12.67 ± 0.37	12.40 ± 0.00
	250	-8.38 ± 0.09	5.28 ± 0.09
	100	-6.07 ± 0.37	2.44 ± 0.00
	10	99.6 ± 0.55	
	5	95.17 ± 0.18	
Acarbose	1	75.07 ± 0.18	
	0.5	61.49 ± 0.92	
	0.1	36.82 ± 0.74	

Note : YLFF : Young Lontar Fruit Fibber; OLFF : Old Lontar Fruit Fibber; M = Maceration, S = Soxhlet, UAE = Ultrasonic Assisted Extraction, MAE = Microwave Assisted Extraction

The findings of this study align with previous research by Das *et al*. (2012)[39], who conducted a study on the fresh fruit aqueous extract of *B. flabellifer*. The extract showed a moderate TPC value of 1.79 µgGAE/mg fresh fruit weight and a low TFC value of 0.08 µgCE/mg fresh fruit weight. It exhibited weak alpha-glucosidase activity with an IC_{50} value of 44.02 µg/mL.

Conclusion

This study revealed that different sample ages and extraction methods can influence the amount of phenolic and flavonoid compounds and the antioxidant and alpha-glucosidase inhibitory properties of *B. flabellifer* fruit fiber extract. Optimizations of the extraction method for OLFF are necessary to determine the most suitable technique for extracting the beneficial secondary metabolites from *B. flabellifer*. This study offers valuable insights for future research on the antidiabetic characteristics of these samples. Further research is needed to identify the optimal extraction method for enhancing the phenol and flavonoid content and the additional mechanisms of action to understand the antidiabetic properties of *B. flabellifer* fruit fiber.

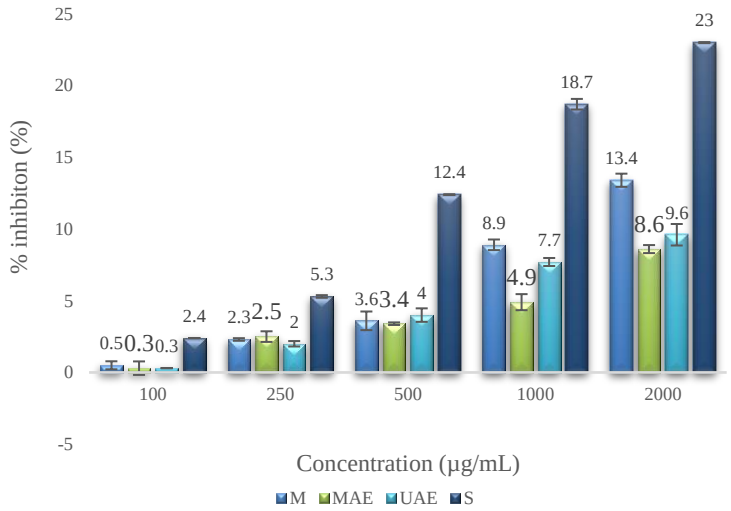

Figure 1: Alpha-glucosidase % inhibition of OLFF extract at various concentrations
Note: M = Maceration, S = Soxhlet, UAE = Ultrasonic Assisted Extraction, MAE = Microwave Assisted Extraction

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating for claims relating to the content of this article will be borne by them.

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