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Enhancement of α-Glucosidase Inhibitory Activity, Antioxidant Activity, and Antioxidant Compounds in Mulberry Leaves Under Salinity Stress

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ABSTRACT

Salinity stress disrupts redox homeostasis in plants, leading to notable changes in the levels of bioactive compounds. These compounds are recognized for their significant role in inhibiting the α-glucosidase enzyme, which is relevant in glycemic control. Detailed insights into how salinity stress modulates α-glucosidase inhibition in mulberry leaves remain scarce. This study aims to investigate the effects of salinity stress on α-glucosidase inhibitory activity in mulberry leaf extracts, and to identify accessions exhibiting the most potent inhibitory properties. *In vitro* assays were employed to conduct qualitative phytochemical analyses and to quantify total phenolic content, total flavonoid content, and α-glucosidase enzyme activity. The results demonstrate that high salinity stress enhances the inhibitory activity of mulberry leaf extracts against the α-glucosidase enzyme. Notably, the MB2-3 sample exhibited the most favorable IC_{50} value (0.59 µg/mL), coupled with a substantial phenolic content (141.9 mg GAE/g extract) and the highest content of flavonoid observed among all tested samples (619.56 mg QE/g extract). Collectively, these findings highlight the capacity of salinity stress to augment both the inhibitory potential of mulberry extracts against α-glucosidase and the accumulation of phenolic and flavonoid compounds, with sample MB2-3 displaying auspicious attributes.

1. Introduction

Diabetes mellitus remains a significant public health challenge globally, with high prevalence and mortality rates observed in both developed and developing nations. The disorder arises from genetic predispositions and lifestyle factors, particularly dietary habits. While the intricate mechanisms underlying diabetes complications remain only partially understood (Sharma *et al.* 2010), oxidative damage has been closely linked to the disease's progression. Consequently, strategies to mitigate oxidative damage, primarily through natural antioxidants, and to manage postprandial hyperglycemia by inhibiting digestive enzymes such as α-glucosidase, are central to current diabetes prevention efforts.

Recent research highlights the potential of plant-derived compounds in inhibiting α-glucosidase activity, thereby supporting their integration into functional foods and therapeutic interventions for diabetes. Notably, flavonoids and phenolic compounds have demonstrated considerable promise in this context (Kumar *et al.* 2011). For example, flavonoids isolated from *Morus alba* L. have exhibited a strong α-glucosidase inhibitory effect (Wang *et al.* 2013). Specifically, compounds such as

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Morin, Luteolin, Baicalein, Kaempferol, and Apigenin have shown notable efficacy (Proenca *et al.* 2017).

Mulberry species serve as valuable sources of bioactive compounds (Ji *et al.* 2009), including alkaloids, phenols, terpenoids, stilbenes, and amino sugars (Iqbal *et al.* 2012; Thakur *et al.* 2019). *Morus nigra*, a wild mulberry species, has a longstanding history of use in traditional Indian medicine for conditions such as rheumatism and arthritis (Pérez-Gregorio *et al.* 2011). In Japan and Korea, mulberry leaves are commonly consumed by individuals with diabetes due to their rich content of antidiabetic compounds, such as 1-Deoxynojirimycin (DNJ), Isobvachalcone, Morachalcon, Fagomine, and Quercetin (Wang *et al.* 2013; Thakur *et al.* 2019). Similarly, in China, mulberry leaves have been utilized for centuries to address a range of ailments, including hypertension, hyperglycemia, inflammation, fever, coughing, and cancer. The leaves are also considered palatable (Thaipitakwong *et al.* 2018) and are often prepared as tea, particularly from young shoots, to regulate blood pressure (Datta 2002; Jian *et al.* 2012).

Salinity stress induces redox imbalances in plants, often resulting in an increased synthesis of bioactive compounds (Rouphael *et al.* 2018). Various forms of abiotic stress, including variations in light (Zapata *et al.* 2020), plant variety (Malarz *et al.* 2021), nutrient availability (Pannico *et al.* 2021), and nitrogen supply (Zhou *et al.* 2021), can enhance the accumulation of phenolic compounds. Notably, applying external compounds such as Omeprazole in combination with salinity has further elevated phenolic content in tomato plants (Rouphael *et al.* 2018).

Despite these insights, there remains limited information on how the stress of salinity affects the accumulation of flavonoid and phenolic compounds and the α-glucosidase inhibitory activity in Indonesian mulberry species. Accordingly, the present study aims to investigate the effects of salinity stress on flavonoid and phenolic content, as well as the inhibitory activity of the α-glucosidase enzyme across various Indonesian mulberry accessions.

2. Materials and Methods

2.1. Experimental Materials and Salinity Treatments

Table 1 displays the accession codes and their respective origins. Salinity treatments were performed according to the protocol outlined by Wulandari *et al.* (2023).

Table 1. Mulberry plant accession codes and origins

Accession code	Accession code
MB1	The SULI-01 variety is maintained at the Forestry Research and Development Agency in Bogor Regency, West Java.
MB2	Collected from Sukamantri Sub-District, Bogor Regency, West Java
MB3	Sourced from Sukamantri Sub-District, Bogor Regency, West Java
MB4	Originated from Muktiharjo Sub-District, Pati Regency, Central Java
MB5	Obtained from Asem Bagus Sub-District, Situbondo Regency, East Java
MB6	Derived from Negara Sub-District, Jembrana Regency, Bali
MB7	Originating from Bili-Bili Sub-District, Gowa Regency, South Sulawesi

Salt treatments were assigned numerical codes from 0 to 3 for clarity in sample labeling. Treatment 0 served as the control group with no added salt, treatment 1 received 0.2% NaCl, treatment 2 was exposed to 0.3% NaCl, and treatment 3 involved 0.4% NaCl. For instance, the code "MB2-3" indicates mulberry accession 2 (MB2) subjected to the 0.4% NaCl treatment. This system simplifies reference and organization throughout the study.

2.2. Preparation of Mulberry Leaf Powder

Fresh mulberry leaves were collected and then dried in an oven at 60°C for six hours. Once thoroughly dried, the leaves were ground using a blender and sifted through an 80-mesh sieve to achieve a consistent powder, following the procedure outlined by Jeszka-Skowron *et al.* (2014).

2.3. Extraction

Mulberry leaf powder was extracted via a standard maceration approach, as previously reported by Jeszka-Skowron *et al.* (2014). Briefly, the powder was immersed in 70% ethanol at a 1:10 (w/v) ratio and maintained under agitation at 125 rpm for the initial 6 hours, followed by a static period to maximize extraction. This process was repeated three times to ensure completeness. The resulting mixture was filtered using Whatman No. 1 filter paper (Cytiva, USA). Filtrates were collected and weighed to determine the preliminary extract mass. A rotary evaporator was then used to remove the solvent, yielding a concentrated paste. The extraction yield was weighed and calculated accordingly.

$$\text{Yield} = \frac{\text{initial weight - final weight}}{\text{initial weight}} \times 100\%$$

2.4. α-Glucosidase Inhibitory Activity Assay

The α-glucosidase inhibitory activity of the mulberry leaf extract was assessed following the method outlined by Batubara *et al.* (2020). Briefly, 10 mg of dried mulberry leaf extract was dissolved in 1 mL of DMSO to obtain a stock solution at 1,000 µg/mL concentration. Subsequently, 50 µL of 0.1 M phosphate buffer (pH 7.0) was added to 10 µL stock solution, followed by 25 µL of 20 mM p-nitrophenyl-α-D-glucopyranoside (pNPG), and 25 µL of α-glucosidase enzyme solution (0.08 U/mL). The resulting mixture was incubated at 37°C for 30 minutes. The reaction was stopped by adding 100 µL of 200 mM Na_2CO_3, after which the absorbance was measured at 410 nm using a microplate reader (Epoch, Biotech, USA). All assays were performed in triplicate to ensure the reliability of the results. Acarbose, used as a positive control, was dissolved in phosphate buffer (pH 7.0) to prepare a series of dilutions (1,000, 100, 10, 5, 1, 0.5, and 0.1 ppm) and subjected to the same assay conditions. The percentage of enzyme inhibition was calculated for each sample, and the IC_{50} value, representing the concentration required to inhibit 50% of α-glucosidase activity, was subsequently determined:

$$\text{Inhibition (\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%$$

2.5. Analysis of Total Phenolic Content

The total phenolic content was evaluated following the method described by Khumaida *et al.* (2019), utilizing gallic acid and the Folin–Ciocalteu reagent as the standard of calibration. In brief, 10 µL of the extract, dissolved in 160 µL of distilled water, was added to 10% Folin–Ciocalteu reagent (10 µL) and 10% sodium carbonate (20 µL). The mixture was allowed to stand at room temperature for 30 minutes. Absorbance measurements were taken at 750 nm using an Epoch Biotech microplate reader. Results are reported as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

2.6. Analysis of Total Flavonoid Content

The total flavonoid content was measured using the aluminum chloride colorimetric assay, following the method outlined by Khumaida *et al.* (2019), with quercetin used as the standard. Specifically, 60 µL of the extract solution was combined with 10 µL of 1 M potassium acetate, 10 µL of 10% aluminum chloride, and 120 µL of distilled water. The mixture was allowed to incubate at room temperature for 30 minutes. Absorbance was then measured at 415 nm using a microplate reader. Results were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW).

2.7. Water Content Analysis

Water content was measured using a conventional gravimetric approach (AOAC 2012). In brief, two grams of mulberry leaf powder were placed in an oven at 105°C for six hours. After drying, the samples were cooled in a desiccator for 15 minutes and weighed to determine the final mass. This process allowed for calculating the water content based on the difference in weight before and after drying.

$$\text{Water content} = \frac{\text{initial weight - final weight}}{\text{initial weight}} \times 100\%$$

2.8. Ash Content Analysis

The ash content was determined according to the Indonesian National Standard (SNI 1992) protocol. In brief, approximately 2 grams of mulberry leaf powder were weighed into a pre-weighed porcelain crucible. The sample was charred over a flame, then transferred to a muffle furnace set at 550°C and incinerated upto only white ash remained. After cooling in a desiccator, the crucible was weighed again. This cycle of incineration, cooling, and reweighing was repeated until the measured weight stabilized, with the variation not exceeding 0.025%. The ash content was then calculated using the standard formula:

$$\text{Ash content} = \frac{w1 - w2}{w} \times 100\%$$

Where:
w : sample weight before ashing (g)
w1 : sample weight + porcelain cup after ashing (g)
w2 : empty porcelain cup (g)

2.9. Qualitative Phytochemical Analysis of Selected Samples

Phytochemical screening was performed following Harborne's (1987) established protocol to identify the presence of various compounds, including alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, and quinones. This method remains widely recognized for its reliability in detecting these specific classes of phytochemicals.

2.9.1. Alkaloid Test

First, 0.25 grams of extract of mulberry leaf was dissolved in approximately 3–5 drops of 28% ammonia solution, ensuring complete dissolution. Afterward, 5 mL of concentrated chloroform ($CHCl_3$) was added to the mixture and filtered. Then, 3 mL were taken from the resulting filtrate and mixed with 5 ml of 2 M sulfuric acid (H_2SO_4). The mixture was vortexed until distinct aqueous and organic layers formed. A small sample from the upper aqueous layer was transferred onto three separate spot plates. Each plate was treated with a different reagent: Mayer's, Wagner's, or Dragendorff's. The presence of alkaloids was confirmed by characteristic precipitates: white with Mayer's, brown with Wagner's, and orange with Dragendorff's.

2.9.2. Phenolic Test (Flavonoid, Tannin, and Saponin)

In the phytochemical screening, 0.25 grams of the extract was combined with 10 mL of distilled water, heated for five minutes, and then filtered. For flavonoid detection, 2 mL of the resulting filtrate was mixed with 100 mg of magnesium powder, five drops of a 1:1 hydrochloric acid (37%) and ethanol (98%), and 1 mL of amyl alcohol. After vortexing and allowing separation of phases, the development of an orange, yellow, or red color in the amyl alcohol layer signified the presence of flavonoids. To test for tannins, 2 mL of the filtrate was treated with 1–2 drops of 10% ferric chloride solution. The appearance of a blackish-green precipitate was considered evidence of tannins. For saponins, 5 mL of the filtrate was shaken vertically for ten seconds and left to stand for ten minutes; the formation of stable foam indicated a positive result for saponins.

2.9.3. Steroid and Triterpenoid Test

The extract, as much as 0.25 gram, is combined with 5 mL of 98% ethanol, then heated and filtered. The resulting filtrate is evaporated until dry, and the residue is dissolved in 1 mL of diethyl ether. After homogenizing the solution, concentrated sulfuric acid and 2 to 3 drops of anhydrous acetic acid are added. A blue or green color indicates the existence of steroids; meanwhile, a red or purple hue points to triterpenoids.

2.9.4. Hidroquinon Test

Mulberry leaf extract (0.25 gram) is dissolved in 5 mL of 98% methanol, heated, and then filtered. Afterwards, 3 mL of the resulting filtrate was combined with three drops of 10% NaOH. The appearance of a red coloration indicated the presence of quinones.

2.10. Data Analysis

All values are presented as mean ± standard error. Statistical analysis was performed using one-way ANOVA in IBM SPSS Statistics version 24. It was followed by Duncan's multiple range test to identify significant differences among treatment means, with significance accepted at $\alpha<0.05$. Determination of the IC_{50} value was conducted through linear regression analysis of percent inhibition against the natural logarithm of extract concentration.

3. Results

3.1. Glucosidase Enzyme Inhibition Activity

The inhibitory activity of mulberry leaf extract on α-glucosidase varied significantly depending on the plant accession and the NaCl concentration, with a notable interaction effect between these two factors ($p<0.05$; see Table 2).

Increasing the NaCl concentration appears to boost α-glucosidase inhibitory activity, as reflected by the declining IC_{50} values. As presented in Table 2, sample MB2-3 recorded the lowest IC_{50} (0.59 µg/mL) among all samples tested, indicating that its inhibitory effect on α-glucosidase is nearly comparable to that of the standard inhibitor, acarbose.

3.2. Total Phenolic Content

Table 3 presents the total phenolic content of each accession under NaCl stress. A significant interaction between accession and NaCl concentration was observed ($p<0.05$), suggesting that both factors influenced phenolic accumulation.

Among the tested samples, MB4-2 exhibited the highest total phenolic content at 154.6 mg GAE/g extract. Notably, this value did not differ significantly ($p>0.05$) from sample MB2-3, which measured 141.9 mg GAE/g extract. Conversely, sample MB1-3 showed the lowest phenolic content, with a value of only 8.57 mg GAE/g extract.

3.3. Total Flavonoid Content

As indicated in Table 4, total flavonoid content was notably affected by the combined influence of plant accession and NaCl concentration ($p<0.05$). This suggests that the response of flavonoid accumulation varies depending on the specific plant accession and the salinity level.

Sample MB2-3 demonstrated the highest total flavonoid content, at 619.56 mg QE/g extract. This value wasn't significantly different from that of

Table 2. IC_{50} values for α-glucosidase inhibition (μg/mL) of mulberry leaf extract cultivated at varying NaCl concentrations in the growth medium

Accession	NaCl concentration (%)			
	0	1	2	3
MB1	5.41^{a-g}	8.46^{a}	1.57^{h}	3.15^{d-h}
MB2	1.60^{h}	5.76^{a-f}	3.54^{d-h}	0.59^{h}
MB3	1.86^{gh}	7.38^{a-c}	1.95^{gh}	2.81^{e-h}
MB4	6.35^{a-e}	7.52^{a-c}	1.28^{h}	1.95^{gh}
MB5	8.76^{a}	6.33^{a-e}	1.89^{gh}	1.43^{h}
MB6	4.19^{c-h}	3.63^{d-h}	1.84^{gh}	1.86^{gh}
MB7	6.52^{a-d}	7.90^{ab}	2.25^{f-h}	4.29^{b-h}

Numbers followed by different letters in the same column are significantly different (Duncan's test, $p<0.05$). 0 = control, 1 = 0.2% NaCl, 2 = 0.3% NaCl, 3 = 0.4% NaCl

Table 3. Total phenolic content (mg GAE/g) of mulberry leaf extract at different NaCl concentrations in planting media

Accession	NaCl concentration (%)			
	0	1	2	3
MB1	65.71^{c-e}	62.54^{c-e}	14.92^{h}	8.57^{h}
MB2	24.44^{gh}	54.60^{d-f}	13.34^{h}	141.90^{a}
MB3	116.51^{b}	57.78^{d-f}	116.51^{b}	86.35^{c}
MB4	45.08^{e-g}	30.79^{f-h}	154.60^{a}	10.16^{h}
MB5	73.65^{cd}	60.95^{c-e}	60.95^{c-e}	41.91^{e-g}
MB6	57.78^{d-f}	45.08^{e-g}	18.10^{gh}	26.03^{gh}
MB7	67.30^{c-e}	30.79^{f-h}	41.91^{e-g}	54.60^{d-f}

Numbers followed by different letters in the same column are significantly different (Duncan's test, $p<0.05$). 0 = control, 1 = 0.2% NaCl, 2 = 0.3% NaCl, 3 = 0.4% NaCl

Table 4. Total flavonoid content (mg QE/g) of mulberry leaf extract in planting media containing different NaCl concentrations

Accession	NaCl concentration (%)			
	0	0.2	0.3	0.4
MB1	295.11^{de}	242.89^{fg}	205.11^{g-j}	184.00^{j}
MB2	184.00^{j}	217.33^{f-j}	187.33^{ij}	619.56^{a}
MB3	376.22^{c}	294.00^{de}	336.22^{cd}	492.89^{b}
MB4	292.89^{de}	208.44^{f-j}	605.11^{a}	176.22^{j}
MB5	256.22^{ef}	346.22^{c}	201.78^{g-j}	212.89^{e}
MB6	215.11^{f-j}	237.33^{f-h}	182.89^{j}	197.33^{g-j}
MB7	298.45^{de}	214.00^{f-j}	234.00^{f-i}	189.55^{h-j}

Numbers followed by different letters in the same column are significantly different (Duncan's test, $p<0.05$). 0 = control, 1 = 0.2% NaCl, 2 = 0.3% NaCl, 3 = 0.4% NaCl

MB4-2 (605.11 mg QE/g extract; $p>0.05$), indicating comparable flavonoid levels between these two samples. In the case of mulberry accession MB2, exposure to 0.4% NaCl led to an increased flavonoid content compared to the untreated control. Due to its strong α-glucosidase inhibitory activity and high level of phenolic and flavonoid content, sample MB2-3 was chosen for more detailed raw material quality assessment and phytochemical analysis.

3.4. Water, Yield, and Ash Content

The findings for extract water, yield, and ash content are displayed in Table 5. Sample MB2-0 contained 8.75% water, 18.7% ash content, and produced a 7.34% extract yield. In comparison, sample MB2-3 exhibited a slightly higher water content (8.84%), alongside a considerably higher extract yield of 11.5%, and ash content of 19.55%.

3.5. Phytochemical Analysis

A summary of the phytochemical test results, including assays for alkaloids, phenolics, steroids, triterpenoids, and quinones, is presented in Table 6 and Figure 1.

In summary, mulberry leaf extract demonstrated the presence of flavonoids, saponins, and steroids, while alkaloids, tannins, quinones, and triterpenoids were not detected (refer to Table 6 for detailed results).

4. Discussion

The water content analysis across all mulberry accessions, including those subjected to salt stress, indicated that levels remained below 10%, aligning with established safety standards (BPOM 2014). Elevated water typically results from inadequate drying (Prasetyo & Inoriah 2013) or reabsorption during storage in humid environments (Saifuddin *et al.* 2011). Maintaining appropriate moisture levels is essential to minimize microbial risks, particularly those associated with fungal contamination (Soetarno & Soediro 1997).

As indicated in Table 2, increasing salinity levels resulted in elevated α-glucosidase inhibitory activity relative to the control. Sample MB2-3 demonstrated the most potent inhibition, with an IC_{50} value of 0.59 μg/mL, remarkably close to that of the standard inhibitor, acarbose (0.44 μg/mL). The moisture content for all selected samples (Table 5) complied with BPOM (2014) regulations. In contrast, the ash content in samples MB2-0 (18.07%) and MB2-3 (19.55%) exceeded the threshold set by the Indonesian Ministry of Health (Depkes 2008) (≤16.6%). This higher ash content likely reflects increased mineral or inorganic

Table 5. The water content, yield, and ash content of selected samples after treatment with various concentrations of NaCl

Sample	Water content (%)	Yield (%)	Ash content (%)
MB2-0	8.75	7.34	18.07
MB2-3	8.84	11.5	19.55

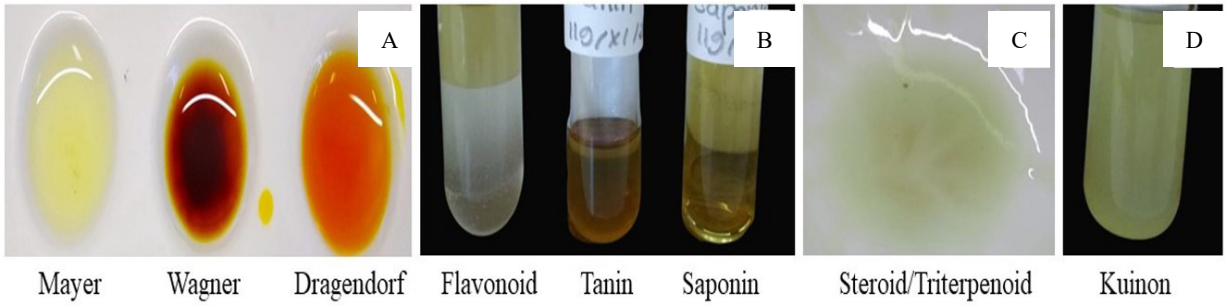

Figure 1. (A) Presents the phytochemical analysis result of mulberry leaf extract, focusing on several compound groups: alkaloids, (B) phenolics, (C)steroids/triterpenoids, and (D) quinones. Alkaloids were not detected, as evidenced by the absence of precipitates in all spot plates. In contrast, flavonoids were identified, indicated by the appearance of a greenish-yellow color in the upper (amyl alcohol) layer. Tannins were not observed-there was no visible sediment formation. The formation of a stable foam layer confirmed the presence of saponins. Steroid compounds were detected, signified by a green coloration in the test solution, while the triterpenoid test was negative due to the lack of red or purple hues. Similarly, the test for quinones returned negative results, with no red coloration noted

Table 6. Phytochemical profile of selected samples after being treated with various NaCl concentrations

Compound	Sample	
	MB2-0	MB2-3
Alkaloid	-	-
Flavonoid	+	+
Tannine	-	-
Saponine	+	+
Steroid	+	+
Triterpenoid	-	-
Quinone	-	-

compound concentrations, potentially affecting the physical properties of the extract (Winarno 1997).

The α-glucosidase inhibitory activity of mulberry leaves is frequently attributed to alkaloids such as 1-deoxynojirimycin (DNJ) (Yatsunami *et al.* 2008; Kwon *et al.* 2011), which are recognized for their efficacy in reducing postprandial blood glucose, even at low doses of 6.5 mg administration (Chen *et al.* 2021). Other alkaloids, including fagomine, isofagomine, 2-O-α-D-Gal-DNJ, and 4-O-β-D-Glc-fagomine, have also been reported to inhibit α-glucosidase (Ji *et al.* 2016). Nevertheless, qualitative phytochemical analysis in the current study did not detect alkaloids (Table 6), possibly due to their low concentrations, as qualitative tests may lack the sensitivity to identify minor constituents, a limitation also noted by Danuri *et al.* (2020).

Conversely, the presence of flavonoids, saponins, and steroids was confirmed in the analyzed samples. Flavonoids, such as quercetin, rutin, and chlorogenic acid, are well-established α-glucosidase inhibitors (Hunyadi *et al.* 2012). Polyphenols may also modulate intestinal α-glucosidase and pancreatic α-amylase activities (Koh *et al.* 2010; Pereira *et al.* 2011). Previous studies (including Hong *et al.* 2013) have shown that flavonoids extracted from Morus atropurpurea display more potent inhibitory activity than acarbose. Reported IC_{50} values for Morus alba extracts range widely, such as 31.04 µg/mL as reported by Yogisha & Raveesha (2009) and 309.82 µg/mL by Danuri *et al.* (2020), while Morus atropurpurea extracts demonstrated even greater potency at 27.05 µg/mL (Hong *et al.* 2013). The low IC_{50} observed in the present study underscores the significant therapeutic potential of mulberry leaf ethanol extract.

Sample MB2-3 and its control tested positively contain phenolic compounds, including flavonoids and saponins. The highest total phenolic content, however, was found in sample MB4-2 (154.6 mg GAE/g extract), closely followed by MB2-3 (141.9 mg GAE/g extract). Most samples showed decreased phenolic content under NaCl stress compared to controls, a trend also observed in lettuce exposed to 40 mM NaCl (Zhang *et al.* 2021). This reduction may indicate insufficient antioxidant production to mitigate oxidative stress. Similar declines in phenolic content were observed in accessions MB1, MB5, and MB6, consistent with findings by Kim *et al.* (2008) and Ahmed *et al.* (2019), which suggest that NaCl disrupts phenolic biosynthesis. The activities of key enzymes involved in phenolic production, such as shikimate dehydrogenase and phenylalanine ammonia-lyase, were also reported to decrease under high salinity in lettuce.

Genetic, environmental, and physiological factors influence the accumulation of phenolic compounds. Although salinity stress can stimulate antioxidant synthesis, enabling plants to counter oxidative

damage, the response appears highly variable. Phenolic compounds function as antioxidants by neutralizing free radicals and enhancing antioxidant capacity. The accumulation of phenolic compounds is significantly influenced by genetic, environmental, and physiological factors (Sanchez *et al.* 2008). Salinity often stimulates the synthesis of antioxidants, helping plants combat oxidative stress (Mahmoudi *et al.* 2010). Phenolic compounds can neutralize free radicals and enhance antioxidant capacity by donating hydrogen atoms (Hanifah & Purwestri 2021).

Sample MB2-3 exhibited the highest flavonoid concentration (619.56 mg QE/g extract), exceeding the control and statistically comparable to MB4-2 (605.11 mg QE/g). This suggests that salt-induced oxidative stress may stimulate the synthesis of antioxidant flavonoids. In contrast, accessions MB1 and MB7 demonstrated reduced flavonoid content, potentially due to varietal sensitivity, stress duration, or other environmental variables.

In conclusion, salinity stress plays a notable role in modulating the biosynthesis of phenolic and flavonoid compounds in mulberry leaves and enhancing α-glucosidase inhibitory activity. The MB2-3 sample from Sukamantri, Bogor, exhibited the highest bioactivity under 0.4% NaCl treatment, indicating a substantial increase in phytochemical accumulation. These findings suggest that targeted salinity treatments could effectively elevate mulberry leaves' functional properties, particularly in developing antidiabetic phytopharmaceuticals.

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