Jordan Journal of Pharmaceutical Sciences, Volume 17, No. 1, 2024

Assessment of Extraction Methods Effects on the Biological Activities (Antioxidant and Antiamylase) and Chemistry (Total Phenolics and Flavonoids) of *Guazuma ulmifolia* Leaves

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ABSTRACT

The antioxidant activity was tested using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method. Antiamylase activity was evaluated through a colorimetric assay that employs 3,5-dinitro salicylic acid (DNSA) as a substrate. Total phenolics and flavonoids content were quantified by the colorimetric method. The highest yield from the extraction of G. ulmifolia leaves was obtained from the water extract (9.64%). The infusion showed the most robust antioxidant and antiamylase activities (IC50 = 6.853 ± 0.504 µg/mL and 261.03 ± 6.83 µg/mL, respectively). The highest total phenolics and flavonoids content were found in the ethanolic extract, with 69.848 ± 1.871 mg GAE/g extract and 118.854 ± 1.001 mg QE/g extract respectively. Total phenolics and flavonoids content significantly influenced the antioxidant activity, but not the antiamylase activity. In conclusion, infusions were the best extraction method for obtaining high antiamylase activity, even though they did not yield the highest total phenolics and flavonoids content. Further research is needed to identify the compound in G. ulmifolia leaf infusions that contribute to antioxidant and antiamylase activities.

Keywords: *Guazuma ulmifolia*, extraction methods, antioxidant, antiamylase, phenol, flavonoids.

INTRODUCTION:

In Indonesia, the leaves of Guazuma ulmifolia, locally known as jati belanda, have been used traditionally for anti-obesity and anti-diabetic treatments [1]. The medicine is prepared by boiling several leaves in water until the volume is reduced to one quarter of the original quantity [2]. Existing research supports the use of G. ulmifolia leaves for anti-diabetic treatment, such as the study conducted by Adnyana et al., which showed that a water leaf extract has an anti-diabetic effect [3]. Similar results were shown by combining G. ulmifolia and Tecoma stans to improve the glycaemic profile in patients with type 2 diabetes mellitus [4]. The anti-diabetic mechanism of G. ulmifolia leaves was identified by Alonso-Castro et al. who found that it stimulates glucose uptake [5]. Another anti-diabetic mechanism is related to inhibiting amylase activity, thereby reducing blood glucose levels due to the limitation of saccharide digestion. Therefore, natural products capable of inhibiting amylase have the potential to be developed as anti-diabetic agents, including polyphenols and flavonoids [6]. A relationship exists between the number of hydroxyl groups on the B ring of polyphenol ligands and amylase inhibition potential. This hydroxyl group forms a hydrogen bond with the catalytic residue of the enzyme binding site [7]. Furthermore,

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Received: 28/4/2023 Accepted: 21/12/2023.
DOI: https://doi.org/10.35516/jjps.v17i1.1171

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flavonoids have specific hydroxyl groups at C7 or C4' and conjugated π bonds, forming hydrogen bonds that stabilise the interaction between inhibitors (flavonoids) and the residue of the enzyme. In addition, polyphenols such as chlorogenic acid have also been reported to possess amylase inhibitor activity through the formation of quinone or lactone structures, or compounds with a 4-oxo-pyrane structure [8].

Extraction is the primary step for obtaining bioactive compounds from plant material with the diversity and quantity of the bioactive compounds in the final extract depending substantially on several factors, including the method and solvent used [9]. Traditionally in Indonesia, G. ulmifolia leaves are boiled with water in a decoction process to produce a product known as "Jamu" [2]. Additionally, according to the Indonesian Pharmacopeia 4th Edition, the infusion of leaves is prepared by boiling with water at 90°C for 15 minutes [10]. Both herbal preparations, jamu, and infusion, are intended for use when fresh. However, for the traditional medicine industry, which prefers extracts in a dry form, these methods are seen as inefficient due to the high cost of evaporating water without damaging bioactive compounds. For this purpose, organic solvents are often used to extract bioactive compounds from plant materials to facilitate the drying process, for instance, ethanol [11].

In this research, we examined the impacts of G. ulmifolia leaf preparation methods, water extraction according to "Jamu" preparation used typically in Indonesia [2], infusion as per the Indonesian Pharmacopeia 4th Edition [10], and ethanolic extraction, which is commonly used by the industry [11], on their antioxidant and antiamylase activities, as well as their phenolics and flavonoids content. A previous study on the antioxidant properties of G. ulmifolia leaves utilized organic solvents, specifically n-hexane and ethyl acetate [9], which are not commonly used for traditional Indonesian medicine preparation. Our study focused on extracts commonly used for Jamu preparation in the community, i.e., water, and the industry, i.e., ethanol, to determine not only their antioxidant properties but also antiamylase activity.

MATERIAL AND METHODS:

Sample preparation

The G. ulmifolia leaves were collected from Meru Betiri National Park in Jember, Indonesia, and transported to the University of Jember under the code of GUMB. A voucher sample was sent to the Purwodadi Botanic Garden, National Research and Innovation Agency of Indonesia, in Purwodadi, East Java, for taxonomical identification. The leaves were selected, washed, and dried before being pulverized into a powder. The G. ulmifolia leaves were then extracted using three different methods: water extraction based on the traditional "Jamu" preparation used in Indonesia [2] (termed as water extract), an infusion according to the Indonesian Pharmacopeia 4th Edition[10] (referred to as infus), and an ethanolic extraction typically used by the industry [11] (commonly known as ethanolic extract).

Water extraction

Water extraction was carried out using the traditional method for "Jamu" preparation [2]. Specifically, a quantity of dried leaf powder equivalent to 20 leaves was boiled with three cups of distilled water (approximately 600 mL) until the final volume was reduced to three-quarters of the original. This was then freeze-dried, and the resulting dry extract was used for further tests. The extract obtained was referred to as the water extract.

Infusion

The infusion followed the Indonesian Pharmacopeia 4th Edition [10]. The process began with 10 grams of dried leaf powder, which was extracted with distilled water at 90°C for 15 minutes. Once cool, it was filtered and freeze-dried. This dry extract, known as infus, was used for the subsequent tests.

Extraction with 70% ethanol

To create the ethanolic extract, 200 grams of G. ulmifolia leaf powder was macerated with 1 liter of 70%

ethanol in distilled water for 24 hours, occasionally stirring during this period, before it was filtered. The residue was then re-macerated twice, with the combined filtrate concentrated using a rotary evaporator to produce a crude, dried ethanol extract [11], referred to as the ethanolic extract.

Antioxidant assay

The antioxidant assay was done using DPPH method[12]. A 0.5 mL of the test solution, i.e., the extract or positive control (quercetin) at every concentration, was added to 0.5 mL of DPPH 50 µg/mL. For the negative control, the test solution was replaced with 0.5 mL of methanol. The mixed solution was incubated in a dark place for 15 minutes before its absorbance was measured using a spectrophotometer at a wavelength of 517 nm.

The absorbance data obtained at each concentration was used to calculate the percentage of DPPH scavenging using Equation 1. A regression equation was plotted from the sample concentration (x) to the percentage of DPPH scavenging (y). The regression equation y = bx + a was used to determine the IC50 value using Equation 2.

$$\text{Amylase inhibition (\%)} = \frac{\text{A DPPH} - \text{A Solution test}}{\text{A DPPH}} \times 100\% \quad \text{(Equation 1)}$$

$$IC_{50} = (50 - a)/b \quad \text{(Equation 2)}$$

Antiamylase assay

Evaluation of the antiamylase activity was executed on sample solutions, i.e., the extract and positive control/acarbose, as well as the negative control (without inhibitor) [13]. Each 100 µL of the solution was added to 30 µL of the enzyme solution and incubated at 25°C for 15 minutes. A total of 250 µL of the substrate was added, followed by incubation at the same temperature and time. The enzymatic reaction was stopped by heating the mixture for 1 minute. A portion of 160 µL of the solution was taken, and 80 µL of DNSA reagent was added, heated on a hot plate for 5 minutes [13]. After cooling, 720 µL of distilled water was added. A total of 100 µL of each mixture of test solutions was added to 100 µl of distilled water, and the absorbance was measured using a microplate reader at a wavelength of 540 nm.

The absorbance data obtained at each concentration was adjusted by the corresponding blank absorbance to further calculate the inhibition of amylase using Equation 3. A regression equation was made from the series of sample concentrations in the microplate reader (x-axis) with the inhibition value (y-axis). The regression equation y = bx + a was used to find the IC50 value as in Equation 2.

$$\text{Amylase inhibition (\%)} = \frac{\text{A Control (-)} - \text{A Solution test}}{\text{A Control (-)}} \times 100\% \quad \text{(Equation 3)}$$

The linear regression was constructed by plotting the value of amylase inhibition against the extract concentration. This linear regression was used to calculate the IC50 values of the extracts.

Total Phenolics Content Quantification

The total phenolics content was tested by reacting 50 µL of the extract or gallic acid standard with 1.25 mL of Folin Ciocalteu reagent, which was then incubated for 6 minutes. The mixture was supplemented with 1.25 mL of 7% Na2CO3 and then re-incubated for 1 hour. The mixture was subsequently transferred into a cuvette to measure its absorbance at a wavelength of 725 nm [13, 14].

Total Flavonoids Content Quantification

The quantification of total flavonoids content was performed by reacting 150 µL of the sample (either the extract or quercetin as a standard) with 30 µL of 5% NaNO2, 30 µL of AlCl3 10%, and 400 µL of distilled water. The mixture was incubated for 6 minutes before adding 200 µL of 1N NaOH and 240 µL of distilled water. After homogenization, the absorbance of the solution was measured at a wavelength of 415 nm [14].

Statistical Analysis

The results were reported as mean ± standard deviation (SD). The regression method was used to calculate the IC50 of antiamylase and antioxidant activities. Least Significant Difference (LSD) was used to analyze significant differences among the mean values. Multiple regression analysis was used to evaluate the relationship between total phenolic and flavonoids content on antioxidant and antiamylase activity, and the relationship between total phenolic and flavonoids content and antioxidant activity on antiamylase activity. Pearson correlation was used to determine the contribution of total phenolic and flavonoids content to antioxidant and antiamylase activity. Values of $p < 0.01$ were considered significant.

RESULTS AND DISCUSSION:

The yield of the extract

The yields from the water extract, infus, and ethanolic extract of G. ulmifolia leaves are summarized in Figure 1. Extraction yield (mass of extract/mass of dried leaves powder) was used as an indicator of the efficiency of the extraction methods. The results showed that the highest yield was obtained from water extraction (9.64%), followed by infusion (8.96%) and ethanol extraction (5.92%). These results are consistent with previous reports showing that the yield of extraction with water is higher than that of ethanol [15]. The primary metabolites in G. ulmifolia leaves, such as proteins and carbohydrates (xanthan gum), coexist with secondary metabolites but are produced in larger quantities [16]. These primary metabolites are polar and therefore more soluble in water, which may explain why the yield of water extract and infusion was higher than that of the ethanolic extract [15].

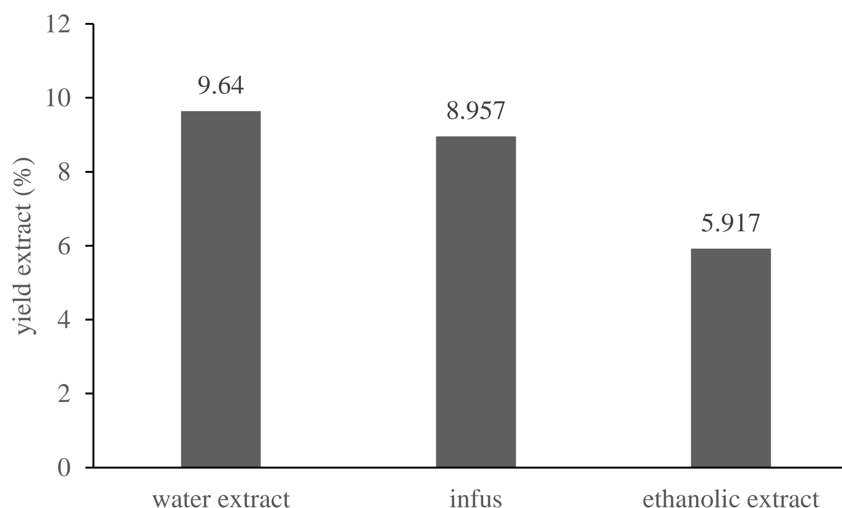


Figure 1. The yield of extract

Antioxidant activity

The results of the antioxidant activity test showed that all three extracts exhibited concentration-dependent antioxidant activity (Figure 2). Although all extracts demonstrated very strong antioxidant activity based on their inhibition of DPPH, infus exhibited the best antioxidant activity (Table 1).

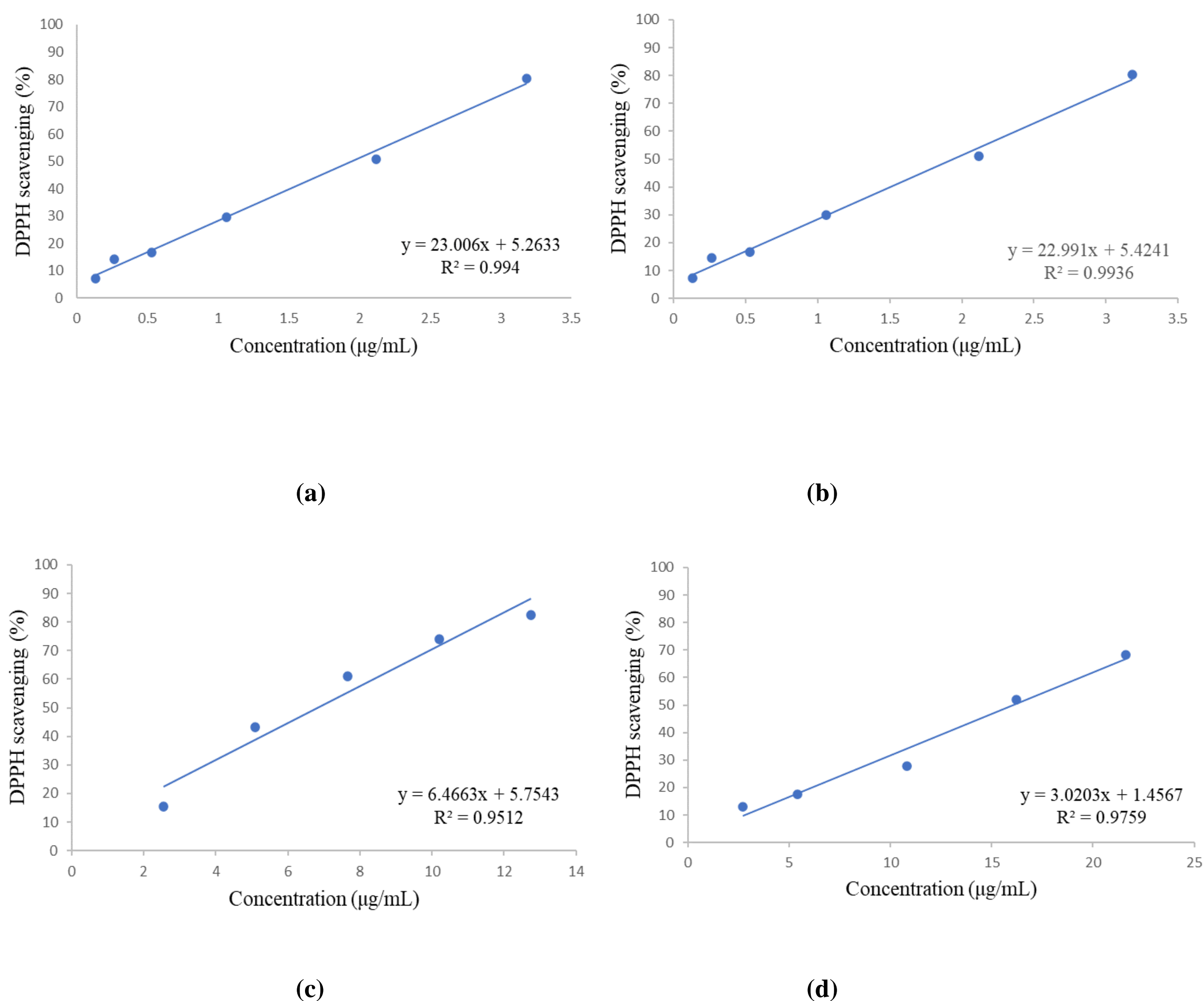


Figure 2.: Correlation concentration of quercetin (a), water extract (b), infus (c), and ethanolic extract (d) of *G. ulmifolia* leaves and DPPH scavenging. All samples are linear, the higher the concentration, the higher the DPPH scavenging activity ($p < 0.01$).

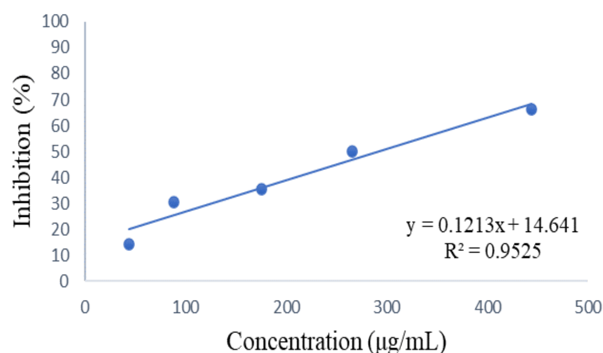
Table 1. IC_{50} of antioxidant activity

Samples	**IC_{50} (µg/mL)***	**Antioxidant activity category[21]**
Quercetin	1,945 ± 0,021[a]	very strong
Water extract of *G. ulmifolia* leaves	9,811 ± 0,805[b]	very strong
Infus of *G. ulmifolia* leaves	6,853 ± 0,504[c]	very strong
Ethanolic extract of *G. ulmifolia* leaves	16.070 ± 0.497[d]	very strong

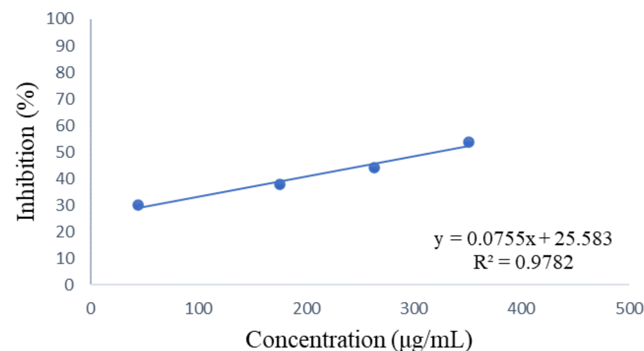
*Data are presented as means ± SD (n = 3), different notation showed significant difference ($p<0.01$)

Antiamyase activity

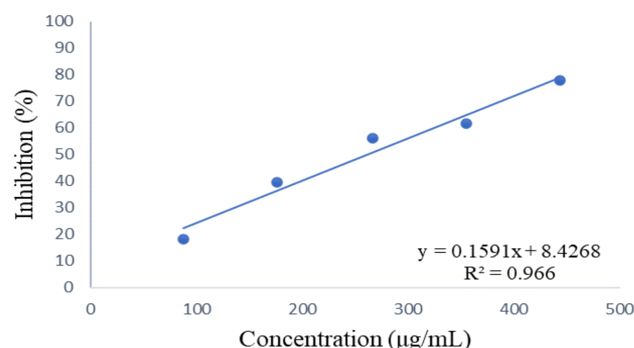
The impact of each extract on amylase inhibitory activity was evaluated. The test results indicated that the percentage of enzyme inhibition increased with the concentration of the extract (Figure 3). Although no significant difference was observed between the water extract and ethanolic extract, infus demonstrated the most potent antiamylase activity (Table 2).



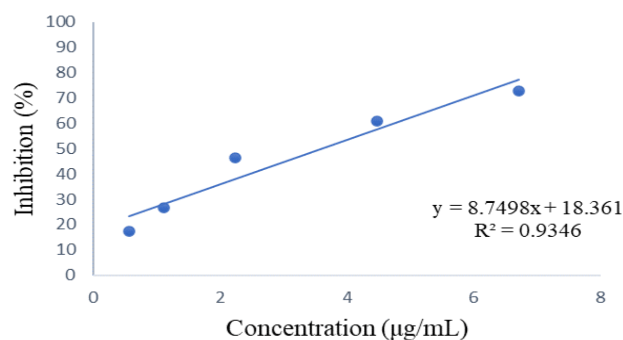
(a)



(b)



(c)



(d)

Figure 3. Correlation concentration of acarbose (a), water extract (b), infusion (c), and ethanolic extract (d) of *G. ulmifolia* leaves and α-amylase inhibition. All samples are linear, the higher the concentration, the higher the amylase inhibition activity ($p < 0.01$).

Table 2. IC_{50} of α-amylase inhibitory activity

Samples	IC_{50} (µg/mL)*
Acarbose	3,609 ± 0,383[a]
Water extract of *G. ulmifolia* leaves	318,273 ± 43,514[b]
Infus of *G. ulmifolia* leaves	261,030 ± 6,829[b]
Ethanolic extract of *G. ulmifolia* leaves	291,674 ± 8,205[b]

*Data were presented as means ± SD (n = 3), different notation showed significant difference ($p<0.01$).

Total Phenolics Content

The total phenolics content of the extracts was calculated from the gallic acid standard curve using the correlation equation $y = 0.1732x + 0.0112$ with a coefficient of determination $R^2 = 0.9916$. Table 3 shows the total phenolics content for the water extract, infus, and ethanolic extract. The ethanolic extract contained the highest amount of total phenolics, followed by infus and the water extract. Ethanol is a selective solvent and extracts more phenolic compounds compared to water [15].

Table 3. Total Phenolics Content

Samples	TPC (mg GAE/g extract)*
Water extract of *G. ulmifolia* leaves	27,503 ± 5,462[a]
Infus of *G. ulmifolia* leaves	40,772 ± 2,504[b]
Ethanolic extract of *G. ulmifolia* leaves	69,848 ± 1,871[c]

*TPC = total phenolics content. Data were presented as means ± SD (n = 3), different notation showed significant difference ($p<0.01$)

Total Flavonoids Content

The total flavonoids content was calculated from the quercetin standard curve using the correlation equation $y = 0.0141x + 0.0966$ with a coefficient of determination $R^2 = 0.9978$. Table 4 shows the total flavonoids content for the water extract, infus, and ethanolic extract. Similar to the total phenolics content, the ethanolic extract had the highest total flavonoids content, followed by infus and the water extract. Ethanol is also more selective in extracting flavonoids compared to water [15].

Table 4. Total Flavonoids Content

Samples	TFC (mg QE/g extract)*
Water extract of *G. ulmifolia* leaves	32,926 ± 0,477[c]
Infus of *G. ulmifolia* leaves	46,203 ± 2,449[b]
Ethanolic extract of *G. ulmifolia* leaves	118,854 ± 1,001[a]

*TFC = total flavonoids content. Data were presented as means ± SD (n = 3), different notation showed significant difference ($p<0.01$)

Correlation between Total Phenolics Content and Total Flavonoids Content on Antioxidant and Antiamylase Activity

Multiple regression analysis (Table 5) showed that total phenolics and flavonoids content did not concurrently affect the antioxidant (F-test was 9.183, less than F-table value of 10.295) nor the antiamylase activity (F-test was 0.137, less than F-table value of 10.295). Similarly, total phenolics, total flavonoids, and antioxidant activity did not affect the antiamylase activity (F-test was 2.123, less than F-table value of 7.519). The t-test of each dependent variable on each independent variable (Table 5) was less than t-table at a 99% confidence level, suggesting that they were not independently affecting or partially affecting the dependent variable.

The Pearson analysis results (Table 6) showed that total phenolics content was strongly correlated with antioxidant activity (Pearson correlation value of 0.868) but did not correlate with antiamylase activity (Pearson correlation value of -0.126). Total flavonoids content strongly correlated with antioxidant activity (Pearson correlation value of 0.864) but did not show correlation with antiamylase activity (Pearson correlation value of -0.145). Furthermore, antioxidant activity was uncorrelated with antiamylase activity (Pearson correlation value of 0.244).

Table 5. Multiple Regression Analysis Results

Dependent variable* / Independent variable	Antioxidant		Antiamylase		Antiamylase	
	F_{test}	F_{table}	F_{test}	F_{table}	F_{test}	F_{table}
Total phenolic content	9.183	10.295	0.137	10.295	2.123	7.591
Total flavonoids content						
Antioxidant						
	t_{test}	t_{table}	t_{test}	t_{table}	t_{test}	t_{table}
Total phenolic content	0.423	2.896	0.377	2.896	0.088	2.718
Total flavonoids content	0.097		-0.418		-0.658	
Antioxidant					2.423	

*Data was analyzed using p 0.01

Table 6. Pearson analysis results

Dependent variable / Independent variable	Antioxidant		Antiamylase	
	Pearson corr.	Sig.	Pearson corr.	Sig.
Total phenolic content	0.868*	0.002	-0.126	0.747
Total flavonoids content	0.864*	0.003	-0.145	0.709
Antioxidant			0.244	0.527

* Correlation is significant at the 0.01 level (2-tailed).

Based on the results, it is clear that infusion is the superior extraction method when looking to achieve the best antioxidant (Table 1) and antiamylase activities (Table 2). The lower the IC50, the higher the activity. Evidence supports that the main components of potential antioxidants are flavonoids and phenols [17]. However, this extraction method did not yield the highest total phenolics (Table 3) and flavonoid content (Table 4), which suggests that the antioxidant and antiamylase activities of the G. ulmifolia leaf infusion may be influenced by other bioactive compounds (Table 5). It has been stated that the presence of alkaloids and saponins in plants is particularly significant in the pharmaceutical field [18]. Alkaloids and saponins present in G. ulmifolia leaves could contribute to its antioxidant property. Some alkaloids, such as carbazomadurin A and B, cordifoline, carquinostatin A, oleracein A, B, and E, have proven scavenging activity against DPPH free radicals. The major factor influencing the antioxidant activity of alkaloids seems to be the number of aromatic hydroxyl groups [19]. The antioxidant activity may also be facilitated by saponins. There is a correlation between saponins and antioxidant activity, including the scavenging activity on DPPH free radicals [20].

The antiamylase activity of G. ulmifolia leaves infus is likely contributed by other metabolites besides phenolics and flavonoids. Alkaloids (such as broussonetine and radicamin) and triterpene saponins (such as arjunolic acid) are examples of non-phenolics and flavonoids secondary metabolites that can inhibit amylase enzymes [21]. According to the results of another study, ethanolic extracts with the highest concentration of total phenolic and flavonoid did not exhibit the best amylase inhibitor activity. Several active phytochemicals, including terpenes and flavonoids, among others, are thought to be responsible for the extract's potential anti-diabetic activity,

although this is not yet certain [22]. It has also been demonstrated that primary metabolites like gum tragacanth can inhibit amylase [23].

Contrarily, the ethanolic extract's total phenolics and flavonoids content was higher than that of the water extract and infus. Since phenolic compounds and flavonoid aglycones are semi-polar and dissolve in ethanol, the ethanolic extract, despite having the lowest yield, has the highest total phenolics and flavonoids content [24]. The yield from water extraction was higher than that from infusion. As previously stated, a water extract was made by heating to 100 ℃ (boiling water), whereas an infusion was produced by heating to 90 ℃ (in a water bath). These findings demonstrate that temperature impacts extraction results. An increase in temperature can improve extraction outcomes by increasing the solubility of secondary metabolites in dried leaf powder [15].

Considering its antioxidant and antiamylase activity, this study justifies the use of G. ulmifolia leaves in traditional medicine for diabetes management. This implies a direct use, as traditionally done in Indonesia, using the infusion method, not with other organic solvent [9]. Therefore, these leaves could serve as an alternative or complementary strategy for managing diabetes and could potentially be a raw material for developing anti-diabetic drugs in the future. Thus, further research is needed to determine which compounds in G. ulmifolia leaves infusion contribute to antioxidant and antiamylase activities.

CONCLUSION:

In conclusion, extraction methods impact the ability to scavenge free radicals, but not the ability to inhibit amylase. These methods also affect the total phenolic and flavonoid content. Infusion is the most effective method to obtain an extract with the best antioxidant and antiamylase activities, although it does not yield an extract high in total phenolic and flavonoid content. Nevertheless, further research is needed to determine which compounds in G. ulmifolia leaves infusion contribute to both antioxidant and antiamylase activities.

ACKNOWLEDGMENTS

This work was supported by Jember University Internal Funds under contract number 6325/UN25.3.1/LT/2022 on October 11, 2022.

CONFLICT OF INTEREST:

The authors hereby declare that regarding the publication of this paper there is no conflict of interests.

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دراسة تأثير طرق الاستخلاص على النشاط البيولوجي ومضاد الأكسدة ومضاد الأميليز (والكيميائي) الفينول الكلي والفلافونويد *Guazuma ulmifolia* (لأوراق نبات)

نوري[1,2*]، إنداه بوسبيتاساري[1,2]، باون ترياتموكو[1,2]، ديوي ديناساري[1,2]، سيتي موسليشاه[1,2]، وآري ساتيا نوجراها[3]

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[2]مجموعة أبحاث تطوير المنتجات الطبيعية النشطة بيولوجيًا، كلية الصيدلة، جامعة جيمبر، إندونيسيا.

[3]المجموعة البحثية لاستخدام الأدوية واكتشافها، كلية الصيدلة، جامعة جيمبر، إندونيسيا.

ملخص

تم تصميم هذه الدراسة لتقييم أنشطة مضادات الأكسدة ومضادات الأميليز، بالإضافة إلى إجمالي محتوى تم تصميم هذه الدراسة لتقييم أنشطة مضادات الأكسدة ومضادات الأميليز، بالإضافة إلى إجمالي محتوى الفينول والفلافونويد في مستخلصات أوراق *G. ulmifolia* المنتجة بطرق استخلاص مختلفة مستخدمة في إندونيسيا. تم اختبار نشاط مضادات الأكسدة باستخدام طريقة الاختزال 2,2-diphenyl-1-picrylhydrazyl (DPPH). تم اختبار نشاط مضاد الأميليز باستخدام اختبار قياس الألوان باستخدام حمض الساليسيليك 3,5-dinitro salicylic acid (DNSA) كركيزة. وفي الوقت نفسه، تم اختبار إجمالي نشاط مضادات الأكسدة تم قياس محتوى الفلافونويد باستخدام الطريقة اللونية. تم الحصول على أعلى عائد لاستخراج أوراق *G. ulmifolia* من مستخلص الماء (9.64%). أظهر التسريب أعلى نشاط مضاد للأكسدة ومضاد الأميليز ($IC_{50} = 6.853 \pm 0.504$ ميكروغرام/مل و 261.03 ± 6.83 ميكروغرام/مل). وفي الوقت نفسه، تم العثور على أعلى محتوى إجمالي من الفينول والفلافونويد في مستخلص الإيثانول، حيث بلغ 69 ± 1.871 ملجم من مستخلص GAE / جرام و $854.118\pm1,001$ ملجم من التيسير الكمي / جرام، على التوالي. تأثر نشاط مضادات الأكسدة بشكل كبير بالمحتوى الكلي للفينول والفلافونويد، لكن نشاط مضاد الأميليز لم يتأثر. في الختام، التسريب هو أفضل طريقة استخلاص للحصول على نشاط مضاد الأميليز العالي، على الرغم من أنه لا يوفر أعلى إنتاج وأعلى محتوى إجمالي من الفينول والفلافونويد. يجب إجراء المزيد من الأبحاث لمعرفة المركبات الموجودة في منقوع أوراق *G. ulmifolia* التي تساهم في نشاط مضادات الأكسدة ومضادات الأميليز.

الكلمات الدالة: *Guazuma ulmifolia*، طريقة الاستخلاص، مضاد الأكسدة، مضاد الأميليز، الفينول، الفلافونويد.

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تاريخ استلام البحث: 2023/4/28 وتاريخ قبوله للنشر 2023/12/21.