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Antioxidant and α-glucosidace inhibitory activities of the *Andrographis paniculata* Nees. ultrasonic leaf extract

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Abstract: Different natural products derived from plants have long been utilized as traditional herbal remedies to treat a variety of ailments, and these substances have inspired the design, discovery, and development of new pharmaceuticals. *Andrographis paniculata* is an annual plant that is frequently grown for therapeutic purposes in Southeast Asian countries. The *A. paniculata* Nees is an Indonesian natural plant that is thought to have antioxidant, anti-diabetic, anti-inflammatory, and anti-hyperlipidemic properties. In this study, ultrasonic-assisted extraction (UAE) was used to optimize extraction conditions by varying time and amplitude. Furthermore, the UAE extract was evaluated for antioxidant activity utilizing 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), iron reducing antioxidant power (FRAP), and copper ion reducing antioxidant capacity (CUPRAC), as well as its anti-diabetic potential via α-glucosidase inhibition. Based on antioxidant activity with an IC_{50} value of 73.71±0.28, extract B (extraction time 35 minutes and amplitude 65%) was considered to have optimum conditions for extraction. In addition, it was found that extract *A. paniculata* (extraction time 30 minutes and amplitude 60%) showed the most active inhibitory activity against α-glucosidase, with an IC_{50} value of 0.87±0.003. The *A. paniculata* ethanolic leaf extract exhibits antioxidant and anti-diabetic properties.

Keywords: α-glucosidase, *Andrographis paniculata* Nees., antidiabetic, antioxidant activity.

INTRODUCTION

Free radicals have been linked to the development of a variety of chronic and degenerative disorders. The human body normally produces free radicals as a result of metabolic activities or immune system responses. Exogenous antioxidants could assist the body resist these radicals (Agrawal and Pandey, 2019; Bhardwaj *et al*., 2019). Antioxidants perform a crucial role in sustaining life as they have the ability to counteract or eliminate harmful free radicals such as reactive oxygen species (ROS). ROS has the ability to cause DNA mutations, disturb cell membranes and harm proteins. Untreated, this condition can result in oxidative damage (Dontha, 2016), which can lead to cancer, inflammation, arthritis, atherosclerosis, Alzheimer's disease, Parkinson's disease, neurological illnesses and diabetes mellitus (Siddeeg *et al*., 2021; Uttara *et al*., 2019; Joon and Takayuki, 2009).

The prevalence of diabetes is projected to increase to 643 million individuals in 2030 and 783 million individuals in 2045, according to the International Diabetes Federation (IDF) (IDF, 2021). Obesity and a lack of physical exercise are the primary causes of Type 2 Diabetes Mellitus (T2DM), which accounts for nearly 90% of all diabetes cases (Goyal and Jialal, 2021). Oral hypoglycemic medicines with α-glucosidase inhibitory action have been shown to be effective in treating high blood sugar levels in people with type T2DM (Dirir *et al*., 2022) There are currently four α-glucosidase inhibitors available: Acarbose, voglibose, miglitol and emiglitate. Among these options, acarbose is the most commonly recommended medication.

The negative effects of these medications encompass flatulence, stomach pains, vomiting and diarrhea. Numerous studies have been carried out to identify natural sources of α-glucosidase inhibitors due to the observed effects (Patil *et al.*, 2015).

Andrographis paniculata Nees. is an erect annual plant of the Acanthaceae family. The *A. paniculata* has been utilized as a folkloric medicine for a range of diseases or as a herbal supplement for health enhancement in Asia for ages (Kiran *et al*., 2023). The complete botanical specimen has been used in numerous formulations intended for the treatment of individuals with diabetes, functioning as an antioxidant and antihyperglycemic agent. (Premanath and Nanjaiah, 2015). Furthermore, the plant is claimed to possess hepatoprotective, immunological, antiinflammatory, hypoglycaemic, antibacterial and hypotensive properties (Pandey, 2011; Nyeem *et al*., 2017). Various ways of extracting *A. paniculata* have been researched in order to determine which of them is most useful in terms of effectivity and efficiency (Tao *et al*., 2014). Typically, UAE is mostly

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utilized for bioactive compound extractions (Liew *et al*., 2016).

Prior studies have not documented the utilization of ultrasound-assisted extraction (UAE) to extract *A. paniculata* leaves for evaluating antioxidant activity through the DPPH, FRAP and CUPRAC methods, as well as α-glucosidase inhibitory activity. This study will utilize the UAE technique to produce the ethanolic extract. The extraction process was carried out under optimal circumstances by changing the length of time and amplitude percentage.

MATERIALS AND METHODS

Sample preparation

The identity of the *Andrographis paniculata* plant had previously been determined at the Bogor Herbarium, National Research and Innovation Agency, Indonesia *A. paniculata* leaves were collected in Sukabumi, West Java. After the sample was ground in a blender, it was sealed, labeled, and kept out of direct sunlight in a dry container to prevent further deterioration.

Sample extraction

The strategy employed in Irawan's study is pertinent and was conducted using the UAE method (Irawan et al., 2021). A total of 7 grams of dry leaf powder was put into a 250mL beaker. The solvent in the form of 70% technical ethanol is added until the dry leaf powder is completely submerged and mixed evenly. The extraction of leaf simplicia was performed using (UAE) with varying time parameters (in minutes) and amplitude levels (in %). The UAE conditions were as follows: 30 minutes at 60% amplitude (A), 35 minutes at 65% amplitude (B), 45 minutes at 60% amplitude (C), and 45 minutes at 65% amplitude (D). After filtration, the liquid extract obtained was separated from the sediment and transferred into a beaker with a known weight. The yield of the leaf extract was obtained by evaporating the ethanolic solvent in an oven set at 40ºC. The dry extract that has been separated from the solvent was then weighed and the recovery value was determined in percentage.

DPPH radical scavenging activity test

The DPPH approach, as employed by Irawan et al (2022), denotes the specific methodology utilized. The study focused on analyzing the ethanolic extracts of A, B, C, and D, which were prepared using the UAE extraction procedure. For pre-analysis, 5mg of extract A was dissolved in methanol to produce a sample solution with a concentration of 1000mg/L. Afterwards, five solutions with various concentrations (40, 80, 160, 320 and 640 mg/L) were prepared in each 5mL measuring flask. Next, 2mL of a DPPH solution with a concentration of 39 mg/L was added to each of 5mL volumetric flasks. After dissolving the DPPH solution in methanol, it was rapidly mixed. A visible light spectrophotometer operating at 515 nm was used to measure the absorbance after the solution had been allowed to remain at room temperature (25ºC) for 30 minutes. Additionally, the same procedure was conducted on extracts B, C, D and BHT. Concentrations of BHT at 4, 32 and 64 mg/L were employed to compare their action against free radicals.

The representation of antioxidant activity is expressed as a percentage inhibition, calculated using the following equation:

$$\%\ \text{Inhibition} = \frac{\left(A_{blank} - A_{sample}\right)}{A_{blank}} \times 100\%$$

Details:
A_{blank} = Absorbance without sample
A_{sample} = Absorbance of sample

A linear equation (Y = bX + a) will be produced when the percent inhibition value is connected with the concentration in ppm (mg/L). When the percentage inhibition was 50%, the IC_{50} value was calculated.

Reducing power

Ferric Reducing Antioxidant Power (FRAP) Test: The FRAP method was employed to conduct antioxidant activity, following the defined procedures by Irawan et al (2021). The ethanol leaf extracts A, B, C and D obtained using the UAE technique were used in this experiment. Ethanol leaf extract solutions with concentrations ranging from 40 to 160mg/L are generated from stock solutions of 1000mg/L. Each solution was poured into different 5mL measuring flask. Then, added 0.4mL of 0.001 M citric acid solution, 0.2mL of 0.002 M Fe^{3+} solution, and 0.4mL of 0.2% o-phenanthroline solution. The mixture was then filtered and homogenized with distilled water. After 35 minutes of incubation at 37ºC elsius, the solution's absorbance was measured with a visible spectrophotometer at 510 nm. The same procedure was followed for standard gallic acid solutions at concentrations of 0.25, 0.5 and 0.75 mg/L. The IC_{50} value can be determined using the same equation as in the CUPRAC approach.

Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Test: Antioxidant activity was tested using the CUPRAC method, which was based on Irawan et al's protocol (2021). The experiment was conducted using ethanol leaf extracts A, B, C and D from the UAE. The ethanol leaf extract solution was created by diluting a 1000 mg/L concentrated solution to a concentration of 20 to 80 mg/L. Each solution was transferred to a 5mL measuring flask, followed by 1mL of CUPRAC solution. Next, each solution was combined with ethanol pa. The solution was incubated for 30 minutes at 37ºC elsius before being measured using a visual spectrophotometer at 459 nm. Utilizing a standardized solution of butylated hydroxytoluene (BHT) in concentrations of 1, 2 and 3

mg/L, the exact same procedures were followed. The subsequent equation can be employed to calculate the decrease in activity:

$$\%\ \text{Reduction Power} = \frac{\left(A_{\text{sample}} - A_{\text{blank}}\right)}{A_{\text{sample}}} \times 100\%$$

The computed values were converted into a linear equation (Y = bX + a) with the concentration (mg/L) represented on the x-axis and the percentage reduction power on the y-axis.. The IC_{50} value was determined at the point where the percentage drop reached 50%.

$$IC50 = \frac{50 - a}{b}$$

α-Glucosidase inhibitory activity tests

The α-glucosidase inhibitory activity test refers to the research that has been done (Barbero *et al*., 2008). The experiments were conducted out using ethanol leaf extracts A, B, C and D. Acarbose was utilized as a reference substance. The various ethanol extract was dissolved in a phosphate buffer at pH 6.8. Next, the acarbose and extract solutions were diluted to various concentrations. Each 30µL solution was mixed with 17µL of 4mM para-nitrophenyl-D-glucopyranoside (PNPG) substrate. After 5 minutes of incubation at 37ºC, 17µL of α-glucosidase solution was added. The solution was incubated for an additional 15 minutes at 37ºC. Next, 100µL of sodium carbonate solution (20mM) was added. The solution's absorbance was then measured using a micro plate reader at 405 nm. The identical procedure was conducted for blank control, with the exception that sodium carbonate was added prior to the addition of α-glucosidase.

STATISTICAL ANALYSIS

The experiment was replicated 3 times and the results are presented as Mean ± Standard Deviation. Mean differences were determined using one-way ANOVA with SPSS software (version 26).

RESULT

Ultrasound-assisted extraction

The application of UAE using 70% ethanol on *A. paniculata* leaves produced a crude extract with the following yields: 1.5419g (7.30%) for treatment A, 1.7477g (8.13%) for treatment B, 1.9120g (8.88%) for treatment C and 1.6797g (7.91%) for treatment D. Extracts B and C yielded almost the same yield (around 8%), whereas extract B just required a short period of time (35 minutes with an amplitude of 65%).

Radical scavenging activity

As shown in fig. 1, the percent inhibition obtained from the antioxidant activity test is then plotted as a curve against a series of samples or standard concentrations. With multiple treatment series, the regression equation for BHT and ethanol extract of *A. paniculata* leaves is as follows: y = 0.1061x - 4.2966; y = 0.0879x + 10.396; y=0.1065x + 6.4073; y = 0.1172x + 11.614 and y = 0.0879x + 10.396. The IC_{50} values for BHT and ethanolic extract from various treatments were determined to be 511.75± 0.31mg/L, 409.32±0.25 mg/L, 327.53±0.21 mg/L and 511.56±0.09 mg/L, respectively, according to the obtained equation. BHT inhibits DPPH activity more effectively than ethanolic extract of *A. paniculata* leaves, on average.

Reducing power using FRAP method

The antioxidant activity measured by the FRAP technique is represented as a percentage of reducing power, which is then connected to a series of sample or standard concentrations to generate a curve, as illustrated in fig. 2. The regression equation for gallic acid and 4 ethanol leaf extract (A, B, C and D) is as follows: y = 55.37x + 16.68; y = 0.2352x - 5.5359; y = 0.1687x + 23.194; y = 0.6578x - 49.327; and y = 0.426x - 8.8166.

From the equation, the IC_{50} values for gallic acid, treatment A, B, C and D were 0.60±0.006, 236.12±1.659, 156.17±2.491, 151.00±0.599 and 136.54±0.980 mg/L, respectively.

Reducing power using CUPRAC method

The CUPRAC method reports antioxidant activity as a percentage of reducing power, which is then paired with a series of sample or standard concentrations to generate a curve, as illustrated in fig. 3. The regression equation for BHT and four sequential ethanol extracts of A. paniculata leaves was y = 19.905x + 9.8499; y = 0.2612x + 38.913; y = 0.5285x + 28.866; y = 0.4445x + 32.384 and y = 0.4723x + 29.693.

The IC_{50} values for BHT, A, B, C and D were 2.02±0.01 mg/L, 113.50±0.55 mg/L, 73.71±0.28 mg/L, 75.03±0.32 mg/L and 111.88±0.49 mg/L, respectively, as determined by the equation. In general, BHT outperforms the ethanolic extract of *A. paniculata* leaves in terms of lowering power against CUPRAC reagent. Because the IC_{50} value was less than 100mg/L, the antioxidant activity of *A. paniculata* leaf extract with treatments B and C was classified as strong antioxidant (Mussard *et al*., 2019).

Potential inhibition of α-Glucosidase activity from ethanolic leaf extract

As shown in fig. 4, α-glucosidase inhibitory activity was represented as percentage inhibition, which was then plotted to a series of sample concentrations to create a curve. The regression equations for acarbose, A, B, C, D ethanolic extract were as follows: y = 0.4946x + 22.38; y =30.471x + 23.486; y =2.0125x + 28.54; y =2.6988x + 24.347 and y = 3.38x + 12.327. The equation yielded IC_{50} values of 55.84±0.2, 0.87±0.003, 10.66±0.024, 9.50± 0.040 and 11.14±0.016 mg/L for acarbose, A, B, C and D, respectively.

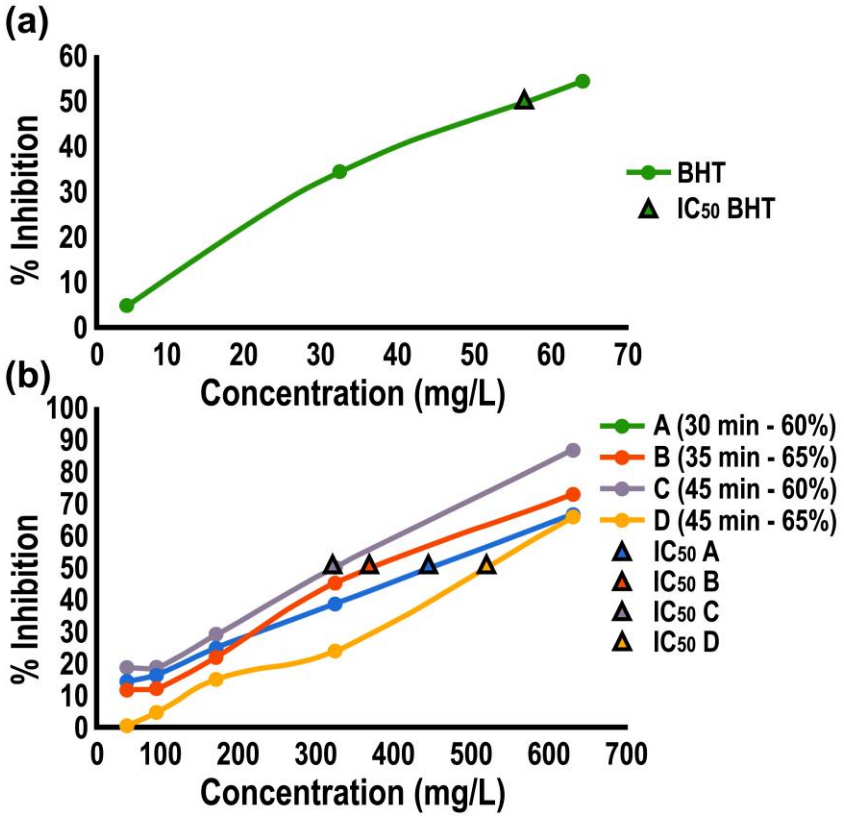

Fig. 1: DPPH Radical Scavenging Activities of (a) BHT and (b) Ethanol leaf extract from *A. paniculata* with time and amplitude variations (A-D).

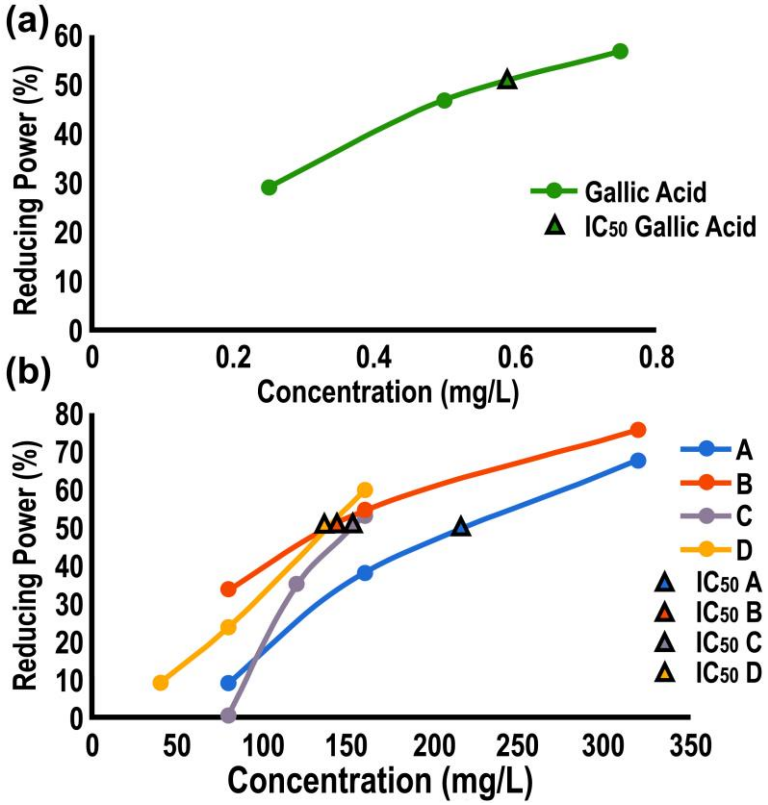

Fig. 2: Antioxidant capacity in the FRAP method of (a) acarbose and (b) ethanol extract of *A. paniculata* leaves (Variations A-D) based on IC_{50} values.

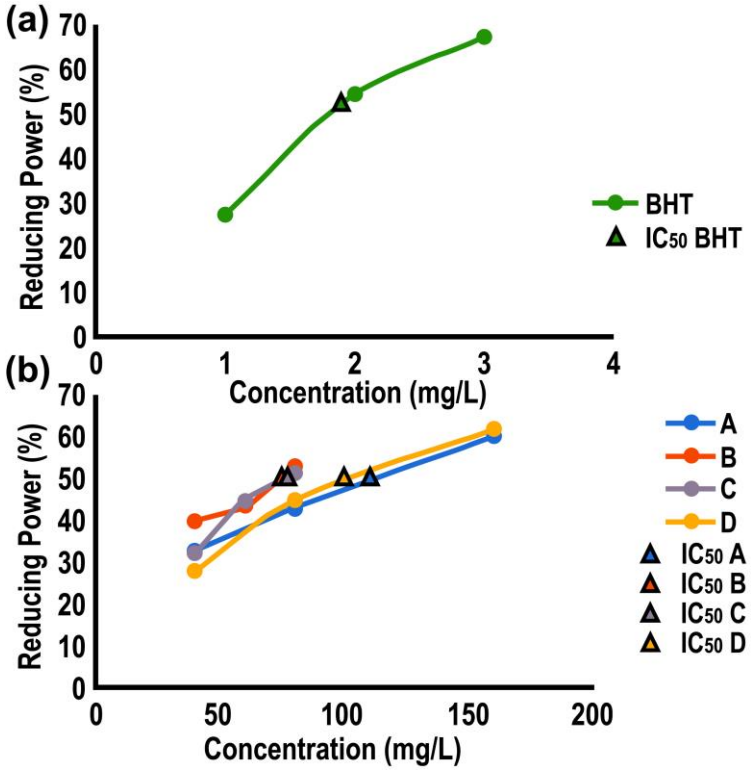

Fig. 3: Antioxidant capacity in the CUPRAC method of (a) acarbose and (b) ethanol extract of *A. paniculata* leaves (Variations A-D) based on IC_{50} values.

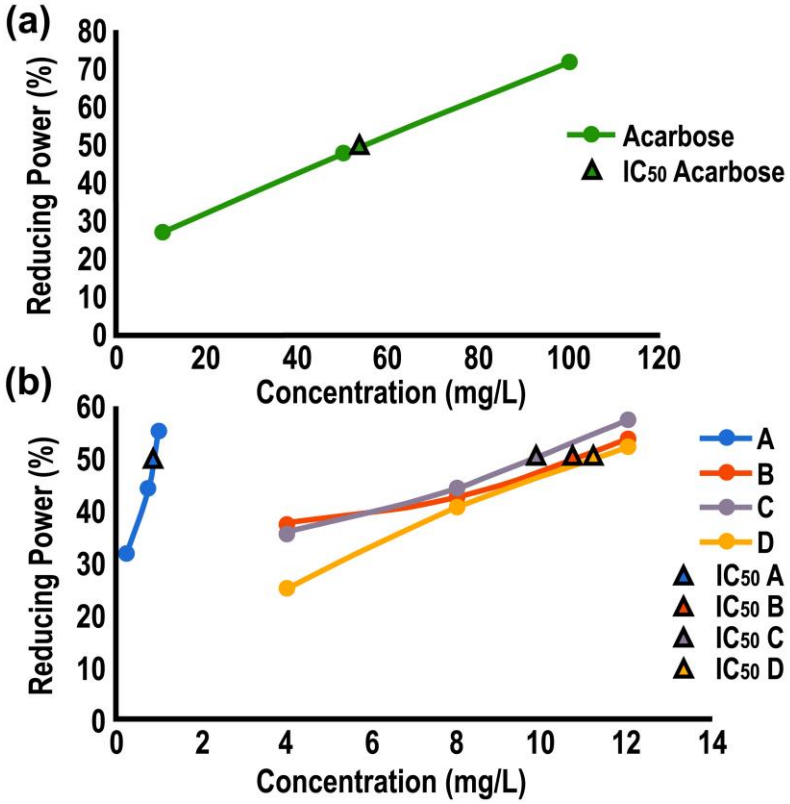

Fig. 4: α-glucosidase inhibitory activity of (a) acarbose and (b) ethanol extract of *A. paniculata* leaves (Variations A-D) based on IC_{50} values.

*The results showed that there was a significant difference between the IC_{50} values for each extract variations ($p<0.05$)

Ethanol leaf extract of *A. paniculata* inhibited α-glucosidase more effectively than acarbose, as indicated by a lower IC_{50} value. According to the research, extract A has the highest activity with an IC_{50} value of 0.87± 0.003, followed by extract C with an IC_{50} value of 9.50± 0.040.

DISCUSSION

UAE is considered to be more effective than other extraction methods including sub critical water, maceration, and microwave assisted extraction (MAE) because it may decrease the amount of damage to phenolic compounds in natural substances (Alara *et al*., 2021; Tzanova *et al*., 2020; Osorio-Tobon, 2020).

DPPH radical quenching assays are commonly used to determine antioxidant activity and an antioxidant candidate that shows promise in DPPH radical scavenging may prevent one of the several pathways through which lipid per oxidation causes oxidative stress (Baliyan *et al*., 2022). This test can be used to determine antioxidant activity by measuring a compound's capacity to serve as a free radical scavenger or hydrogen donor. The reduction of DPPH as a free radical is the basis of the DPPH test method. The maximum DPPH free radical absorption occurs at 517nm and is purple in hue. Antioxidants undergo a reaction with DPPH, resulting in their pairing due to the emergence of a hydrogen donor. This reaction leads to the reduction of DPPH to DPPH-H, thereby causing a decrease in DPPH absorbance. When DPPH-H is formed from a radical, the color changes from purple to yellow (Shekhar and Anju, 2014; Molyneux, 2014).

This colorimetric assay relies on antioxidants' ability to convert the Fe^{3+}-tripyridyltriazine complex (a colorless complex) to its ferrous form at low pH. The end-product, Fe^{2+}-tripyridyltriazine exhibits a vivid blue color. The approach relies on the reduction of Fe^{3+}-tripyridyltriazine complex (a colorless complex) to Fe^{2+}-tripyridyltriazine (a blue complex) through the electron-donating antioxidants at a low pH (Karolina, 2020).

In essence, the influence of chemicals possessing reducing capabilities is manifested through the donation of a hydrogen atom, which causes the free radical chain to be disrupted (Martemucci *et al*., 1999). In FRAP assays, the existence of antioxidants in samples acts as a reducing agent in redox-related colorimetric reactions (Munteanu & Apetrei, 2021).

Plant-derived antioxidant compounds have the ability to engage in oxidation-reduction reactions with FRAP reagents. As a result of these reactions, plant antioxidant compounds are able to extinguish singlets, capture free radicals and neutralise triplet oxygen or peroxide breakdown (Dobrinas *et al*., 2021).

In a previous study conducted on *A. paniculata* leaf extract with variations in time and amplitude, it was found that treatment B had a high total phenolic value compared to others. The same thing was also shown in the CUPRAC assay, treatment B also showed the highest IC_{50} value (Ismail *et al*., 2022). These findings indicate *A. paniculata*'s ethanolic leaf extract could be used as a natural antioxidant supplement. The antioxidative activities of phenolic compounds in natural materials can be attributed to their oxidation and reduction characteristics (Kruk *et al*., 2022; Sushanth and Rajashekhar, 2015).

In this study, the CUPRAC method was the most suitable method to see the antioxidant power of flavonoid compounds, especially the phenolic group, compared to other methods (Apak *et al.*, 2013).

This ethanolic leaf extract's significant α-glucosidase inhibitory action may be connected to its antioxidant activity. Phenolic compounds presence in natural substances is associated with their antioxidant and anti-diabetic properties: these compounds possess the capacity to donate hydrogen atoms to free radicals, thereby reducing their reactivity. Phenolic substances can inhibit α-glucosidase by serving as competitive inhibitors of carbohydrate-digesting enzymes. As a result, carbohydrates take longer to hydrolyze into glucose molecules (Patil *et al*., 2015).

The significant α-glucosidase inhibitory activity observed in both extracts appears to be related to the presence of various chemical components that have a synergistic impact. Numerous studies have documented a correlation between the suppression of α-glucosidase activity in natural substances and the existence of proton-donating compounds, including triterpenoid compounds, flavonoid glycosides, kaempferol, alkaloids and phenolic compounds belonging to the phenylpropanoid category. Previous studies have documented the ability of phenolic compounds to function as α-glucosidase inhibitors, operating through competitive inhibition of enzymes responsible for carbohydrate digestion (Jadalla *et al.*, 2022)

CONCLUSION

According to the study's findings, the CUPRAC technique revealed that the ethanolic leaf extract of *Andrographis paniculata* in all treatments exhibited strong antioxidant activity. In comparison to the other treatments, Treatment B (35 minute extraction time and 65% amplitude) had the highest antioxidant activity, with an IC_{50} of 73.71±0.28 mg/L. Extract A (30 minute extraction time and 60% amplitude) demonstrated the most active inhibitory activity against α-glucosidase, with an IC_{50} value of 0.87±0.003 mg/L. Data reveal that *A. paniculata* ethanol

extract possesses antioxidant and anti-diabetic effects. Because of its natural qualities, this plant can be utilized to treat a variety of ailments and to prevent diabetes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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