

Investigation of extraction conditions and evaluation of the ability of antioxidants and lowering blood glucose levels of the *Ensete glaucum* extract

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ABSTRACT

Diabetes is a type of metabolic disorder, which is the cause of many dangerous complications. *Ensete glaucum* (Musaceae family) has active ingredients with antioxidant and hypoglycemic characteristics in some parts of the plant. This study investigates the ability to reduce blood sugar based on the inhibition of α -amylase and α -glucosidase enzymes of *Ensete glaucum* stem extract. The results indicated that 70% ethanol, a material ratio of 1/30, and a temperature of 65°C for 360 minutes are the optimal conditions for the extraction process. Sonication has an extraction efficiency that is 1.8 times greater than that of a thermostatic bath or shaker. With the above conditions, the total polyphenol contents of the upper and the lower stem samples combined with sonication are higher than those without sonication. The ability to capture 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radicals of the extract used with sonication in the upper stem is four times higher than without sonication. In addition, the presence of sonication increases the capacity to inhibit the α -amylase enzyme (IC₅₀ of the upper stem is 29.94 g/mL, the lower stem is 16.42 g/mL), compared to the absence of sonication (IC₅₀ of the upper stem is 36 g/mL, the lower stem is 26.72 g/mL). Similar to the ability to inhibit α -glucosidase enzyme when using sonication (IC₅₀ of the upper stem is 71.04 μ g/mL, the lower stem is 91.91 μ g/mL) is higher than not using sonication (IC₅₀ of the upper stem is 142.99 μ g/mL, the lower stem is 129.88 μ g/mL). In general, the inhibition of α -amylase enzyme was higher than α -glucosidase enzyme in all extracts. In addition, the presence of sonication increased the polyphenol content, DPPH free radical scavenging, and hypoglycemic capacity of *Ensete glaucum* stem extract.

1. Introduction

The study of a plant's chemical composition, nutritional composition, and biological activity has been encouraged as a way to support the growth of the pharmaceutical industry and the manufacture of healthy foods in line with the trend of utilizing products and medicines made from natural sources.

Ensete glaucum, the snow banana, has known as the Chan Voi banana or Co Don banana. *E. glaucum* has also been classified as *Musa nepalensis*, *Ensete giganteum*, or *Ensete wilsonii*, belongs to the Musaceae family (Wang et al., 2020), distributed widely in Phuoc Binh National Park (Ninh Thuan Province) and Bu Gia Mau National Park (Binh Phuoc Province). Currently, the Chan Voi banana has little commercial value and is shrinking in the area quickly, leading to the risk of extinction. The forest banana seeds have been used to treat intestinal diseases, diabetes, kidney stones, perforated edema, stomach ulcers, and skin allergies. *E. glaucum* contains a variety of phytochemical constituents such as amino acids, cardiac glycosides, flavonoids, polyphenols, alkaloids, reducing sugars, starch, saponins, tannins, terpenoids and oils, and fats, ... (Joga, Sangmaa, Karmakara, Lyngdoha, & Aochenb, 2020). Several species of the Ensete genus (*E. superbum*, *E. ventricosum*) have been studied (Tesfaye & Girma, 2017; Venkatesan, Muzemil, Fiche, Grant, & David, 2022). *E. superbum* contains biologically active phytochemicals such as triterpenoid ester, proanthocyanidin, glucoside pro-pelargonidin, pelargonidin, anigorufone, hydroxyl-anigorufone, and β -carboline alkaloids (Vasundharan, Raghunathan, Arunachalam, & Narayana, 2013). According to studies by Sreekutty and Mini (2016), *E. glaucum* seeds extract (ESSE) has been found to be pharmacologically safe with an LD₅₀ value is 3235.9 mg/kg, and to prevent the progression of diabetes treatment, cardiovascular support, anti-inflammatory urinary tract, infertility, anti-estrogen, and antiviral conditions. Besides, *E. ventricosum* exhibits medical potential and research prospects due to the presence of starch and other trace elements with pharmacological and nutritional value, contributing to improving food security and human health (Yemata, 2020).

According to several national and international studies, all parts of *E. glaucum*, from the stem to the fruit and seeds, include active saponins, flavonoids, terpenoids, and other compounds with high antioxidant activity. However, the studies on the chemical composition and pharmacological effects of this plant are not very extensive and are mainly evaluated in seeds (Sethiya, Shekh, & Singh, 2019) there have not been many studies on the stem, a part that accounts for the majority of the plant's mass. Therefore, the purpose of this study was to identify the chemical composition, and ability to lower blood glucose and measure the total polyphenol concentration of the *E. glaucum* stem. The chemical composition of *E. glaucum* has preliminarily been determined under normal conditions at standard temperatures.

Especially, this study provides information about the optimization of extraction to obtain the bioactive extract from the stem. This is the first research about the bioactivity of the *E. glaucum* stem extract to open up the potential of medicinal plants, and create products with high value, provide the basic knowledge for modern ethnopharmacological research which can eventually catalyze the development of new drugs.

2. Materials and methods

2.1. Materials

E. glaucum has been provided by the Center for Biotechnology in Ho Chi Minh City from Lam Dong. The selected *E. glaucum* stem was cut in half, the lower stem is the part from the above

root to the middle, and the upper stem is the part from the middle to the treetop. Wash and chop a banana stem into small pieces. After that, *E. glaucum* stems were thinly sliced, and dried at 60°C for 24 to 48 hours. After that, *E. glaucum* is finely mashed and kept in plastic bags at a temperature of 27 to 28°C.

2.2. Extraction and analysis of polyphenols

The conditions that affect the process of extracting the *E. glaucum* to be investigated consist of the type of solvent, sonication, or non-sonication. Powder from dried *E. glaucum* stems was extracted with water (pure), 70% methanol, and 70% ethanol with a material/solvent ratio of 1:30; sonication or non-sonication; thermostatic bath, shake at room temperature, sonication; and extraction times of 360 min. The *E. glaucum* samples have extracted by Buchi Rotavapor R-300 vacuum concentrator at a temperature of 50°C and pressure (60 - 150rpm). The extracted samples have dried at a temperature of 60°C from 24h to 48h.

2.3. Methods of determining the total polyphenol content TPC

The total polyphenol content was determined by the Folin-Ciocalte method (Vuong et al., 2013). The extracted sample was reconstituted in 70% ethanol to obtain an initial concentration of 1 mg/mL. 0.1mL of extract was added into 0.5ml of 10% Folin-Ciocalte reagent, reacting for 10 minutes. 0.4ml of 10% Sodium Carbonate solution was added and incubated for a further hour at room temperature in the dark, and the absorption was read in a UV-vision spectrophotometer at a wavelength of 765nm. The experiment was repeated three times. The above baseline was plotted using a gallic acid solution diluted at concentrations between 15.625 - 500 µg/mL. Based on the Gallic Acid standard curve to determine the TPC content in the sample. The total polyphenol content is calculated according to the formula:

$$P = \frac{C \times V}{m \times 1000} \quad (1)$$

Where P: Total polyphenol content (mg GAE/g extract).

C: Gallic Acid concentration determined from the standard equation (µg/ml).

V: Volume used during the assay (mL).

m: mass of the extract during the assay (g).

2.4. Method of determining the ability to capture free radicals DPPH

Based on the DPPH's ability to capture free radicals, the substance has an antioxidant effect. DPPH is a stable, violet-colored free radical characterized in solvents and has a maximum absorption at 517nm. In this method, the antioxidants provide the hydrogen atom to reduce the stable free radicals in the DPPH. When antioxidants reduce the activity of free radicals, the characteristic purple color will fade and turn yellow, the more the color changes to yellow, the stronger the antioxidant capacity. Vitamin C is used as a reference substance, the results of which are shown through the equivalent content of vitamin C (ppm). The following equation was used to determine the DPPH scavenging effect (Khaskheli et al., 2017; More & Makola, 2020):

$$DPPH \text{ scavenging effect } (\%) = \frac{A_0 - (A - A_0)}{A_0} \times 100 \quad (2)$$

where A_0 : A_{517} of DPPH without the sample.

A: A_{517} of the sample and DPPH.

Ab: A_{517} of the sample without DPPH.

The sample was repeated three times and the result was represented as an average value \pm SD. Formulate the vitamin C standard line equation: DPPH reagent is contained in a cup with foil on the outside, add methanol to dissolve the reagent and a sealed lid. The reagent solution should be adjusted to have an optical density of 1.00 ± 0.02 at 517nm. Vitamin C standard solutions are prepared with concentrations up to 100ppm. 0.1mL of standard solution was put into foil-sealed test tubes at various concentrations. The DPPH reagent was added to 2.9ml into the solution and measured optical density at 517nm after 30 minutes of incubation in a dark place at room temperature, and thorough shaking. The standard line equation was plotted based on the results of the experiment (Khaskheli et al., 2017; More & Makola, 2020).

2.5. Influence of the extraction method on the ability to reduce blood glucose

2.5.1. Influence of the extraction method on the ability to inhibit the α -amylase of *E. glaucum* extracts

According to the method described by Adisakwattana, Chantarasinlapin, Thammarat, and Yibchok-Anun (2009), the inhibition of starch hydrolysis of the α -amylase by *E. glaucum* extracts was carried out according to the method as follows: 500 μ L mixed solution containing 0.02M sodium phosphate buffer pH 6.9, 6mM sodium chloride, 1 mg/mL starch, 2.5U α -amylase, extract at various dilution concentrations, incubated for 15 minutes at 37°C.

The mixture was measured by spectrophotometer at 540nm after added adding 500mL of DNSA reagent and boiling for 05 minutes. The effectiveness of the α -amylase inhibitors in controlling acarbose positively was assessed concurrently. The percentage of the α -amylase enzyme inhibited (%) is based on the amount of starch initially and the amount of starch remaining after the reaction through the spectral absorption measurement.

$$\text{Reaction efficiency (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \quad (3)$$

Where A_0 : Value of control solution (initial starch amount).

A_1 : The value of the solution after the reaction (the amount of remaining starch).

A standard curve was plotted showing the correlation between the percentage of the inhibitor and sample concentration to determine the IC_{50} value ($y = ax + b$ where y is % inhibition, x is concentration). Samples were evaluated at different concentrations to calculate the IC_{50} value.

2.5.2. Influence of the extraction method on the ability to inhibit α -glucosidase of high extracts from *E. glaucum*

Based on the substrate hydrolysis of the p-nitrophenyl- α -p-glucopyranosid (PP-G) α -glucosidase to produce α -D-glucose and p-nitrophenol PNP, in alkaline media p-nitrophenol converts to p-nitrophenolate measured by spectrophotometer at 400nm (Dean, 2006). The ability to inhibit α -glucosidase is calculated based on the difference in the initial amount of starch and the amount of starch remaining after the hydrolysis reaction to assess the degree of hydrolysis of α -glucosidase. The more starch left after the reaction, the higher the ability to inhibit α -glucosidase.

Inhibition of the starch hydrolysis of the α -glucosidase enzyme was carried out according to the method of Hogan et al. (2010) and Dai, Pham, Tran, and Bui (2012), adjusted as follows: the extracted samples were dissolved with DMSO at a concentration of 1 mg/mL, centrifuged for 10 minutes. The resulting extract was then diluted in pH7 phosphate buffer at different

concentrations, α -glucosidase was diluted with pH7 phosphate buffer at 0.2 U/mL, 100 μ L of enzyme α -glucosidase dissolved with 50 μ L, 50 μ L of 4mM incubation pNPG was added at 37°C, for 20 min. Then 1,000 μ L of 0.2mM Na₂CO₃ was added, and 400nm wavelength spectroscopy was carried out. In addition, an assessment of the α -glucosidase inhibitory efficacy with positive control of acarbose was conducted at concentrations. The percentage of the α -glucosidase enzyme inhibited (%) is calculated based on the amount of p-nitrophenol formed from pNPG in the reaction through spectroscopic absorption measurement.

$$\text{Percentage of enzyme } \alpha\text{-glucosidase inhibited (\%)} = \frac{B-A}{B} \times 100 \quad (4)$$

Where B: Optical value of the control sample.

A: Optical value of the real sample.

2.6. Statistical analysis

Experiments were replicated 03 times to obtain the final results that are presented with mean \pm Standard Deviation (SD). The data is analyzed by Minitab software (17th edition), one-way analysis of variance (ANOVA Statistics with Turkey test) with the acceptable significance at $P < 0.05$.

3. Results

3.1. Investigation of extraction methods

1mg of the *E. glaucum* lower stem extract was diluted into solvents, water, ethanol 70%, and methanol 70% at a ratio of 1:30. The samples were extracted by thermostatic bath, shaken at room temperature, and sonication. The results are recorded in **Figure 1**. From **Figure 1**, it can be seen that the type of solvent and extraction method have an effect on the polyphenol content from the extract from the lower stem of *E. glaucum*. Of which, the amount of polyphenols in the experiment using ethanol solvents and sonicate at 65°C gives the optimal result with a polyphenol content of 18.88mg GAE/g extract, higher than the rest of the experiment.

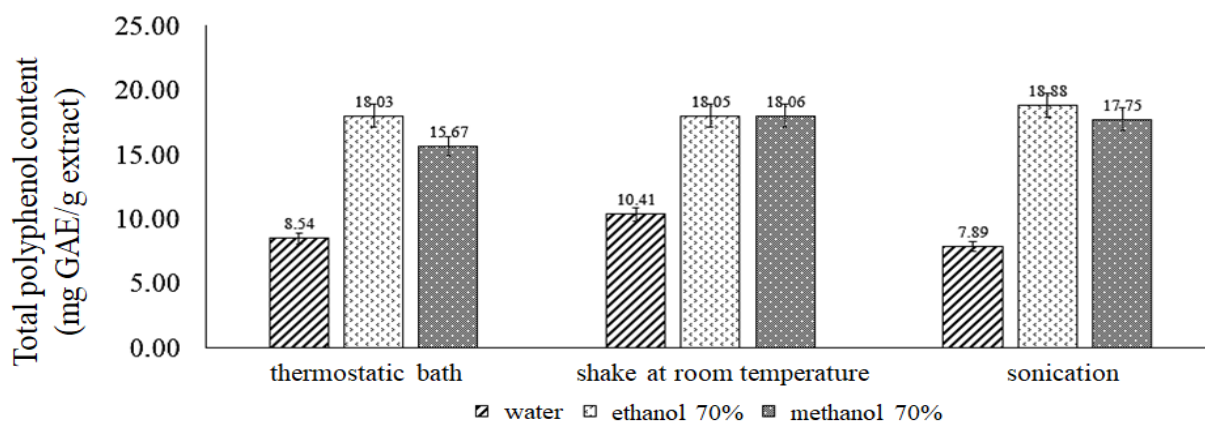


Figure 1. Result of the influence of solvent type and extraction method on total phenolic content

Research continues to investigate the recovery effects of extracts by sonication and non-sonication. The results showed that sonication led to increased extraction recovery efficiency rates. Efficiency was approximately three times higher with sonication than it would have been without it, at 68.2% for the upper stem and 57% for the lower stem (**Figure 2**).

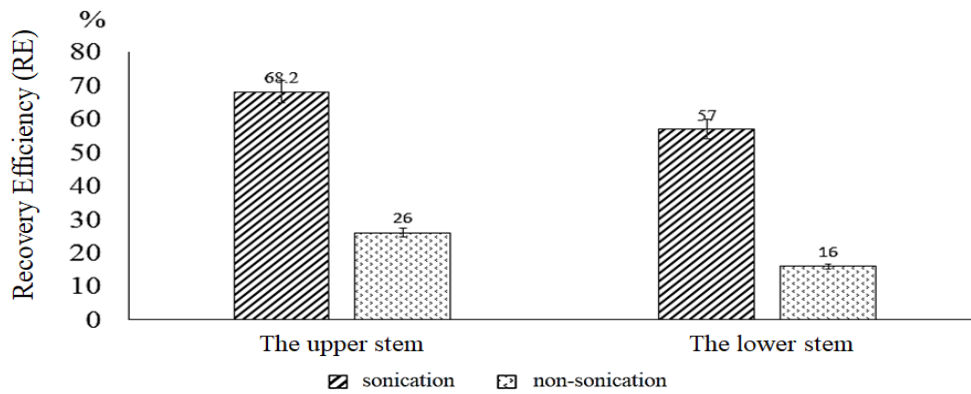


Figure 2. Effect of sonication method on recovery efficiency of the extraction from the *E. glaucum* stem

Subsequent experiments will compare specific investigations conducted with sonication and without sonication. The optimum factor selected is 70% v/v ethanol solvent with a 1/30 raw material/solvent ratio.

3.2. Effect of extraction method on total polyphenol content

When increasing gallic acid concentration from 7.8125 to 500 µg/mL, the Optical Density (OD) increased as well, corresponding to a linear increase in polyphenol content with gallic acid concentration, a statistically significant difference at 5%. The linear equation of gallic acid is $y = 0.0024x + 0.0687$ with a correlation coefficient of $R^2 = 0.9932$.

In order to compare the effect of the extraction method on the stems of *E. glaucum*, in this experiment, 04 samples were diluted from the stem extracts (the upper and lower stems combined with sonication and without sonication) with ethanol 70% and polyphenol content was measured. The total phenolic content is calculated based on the standard equation of gallic acid. **Figure 3** shows the effect of the extraction method on the polyphenol content of *E. glaucum* stem extract.

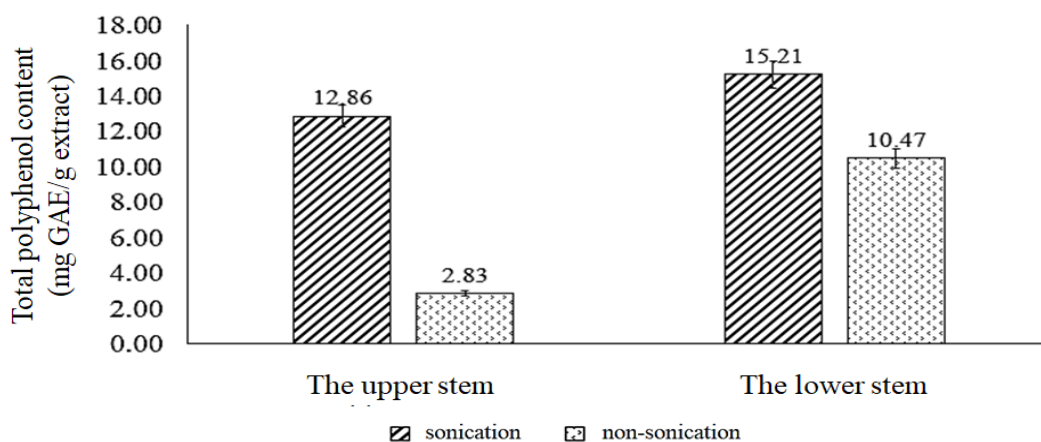


Figure 3. The total polyphenol content is obtained from the extract of *E. glaucum* stem

The result of the total polyphenol content study showed that the banana stems contained a fairly high amount of polyphenols and at most the lower stem had the highest polyphenol content with a maximum value of $p = 15.21^b \pm 2.280$ when extracted by using sonication. In general, in stem extracts from *E. glaucum*, sonication often resulted in higher polyphenol concentrations than

extraction without sonication (**Table 1**). It can be concluded that the extraction method using sonication causes the cell wall structure to break down more, which facilitates the extract's diffusion from the inside of the cell to the solution so that the polyphenol extraction efficiency is higher with sonication than without it.

Table 1

Effect of the extraction method on total polyphenol content of *E. glaucum* stems

No.	Sample type	Extraction method	Polyphenols (mg GAE/g extract)
1	The upper stem	Sonication	12.8556 ^a ± 0.1217
2	The upper stem	Non-sonication	2.833 ^{ab} ± 0.783
3	The lower stem	Sonication	15.21 ^b ± 2.280
4	The lower stem	Non-sonication	10.467 ^s ± 0.211

3.3. Effect of the extraction method on the ability to capture DPPH free radicals

When vitamin C concentrations were increased, increased Optical Density (OD) corresponded with higher efficiency of capturing free radicals, which was statistically significant at 5%. The linear equation showing vitamin C ability to capture DPPH free radicals is $y = 4.4093\ln(x) + 68.026$ with a correlation coefficient of $R^2 = 0.962$ and the IC_{50} of vitamin C is 8.761 $\mu\text{g/mL}$.

The extract samples were investigated to compare the influence of the extraction method on the ability to capture DPPH free radicals. The results indicated that all of the samples showed the ability to capture DPPH free radicals but were much lower than the control of vitamin C (Table 2). The capacity to capture free radicals was 4 times higher with sonication ($IC_{50} = 464.0^b \pm 26.6$) than without sonication ($IC_{50} = 1793.0^a \pm 193.0$). This influence of the extraction method on the ability to capture DPPH free radicals was particularly evident with the sonication approach. For the lower stem samples, sonication extraction has no effect on the ability to capture free radicals, and there was no statistically significant difference between the experiments.

Table 2

Effect of the extraction method on the ability to capture DPPH free radicals of *E. glaucum* stems

No.	Sample type	Extraction method	IC_{50} $\mu\text{g/mL}$ DPPH
1	The upper stem	Sonication	464.0 ^b ± 26.6
2	The upper stem	Non-sonication	1793.0 ^a ± 193.0
3	The lower stem	Sonication	420.6 ^b ± 100.3
4	The lower stem	Non-sonication	447.3 ^b ± 15.5

3.4. Effect of extraction method on the ability to reduce blood glucose

3.4.1. Effect of the extraction method on extracts from *E. glaucum* stems' ability to inhibit the enzyme α -amylase

The effect of extraction on the inhibition of enzyme α -amylase of extracts of *E. glaucum* stem is shown. The ability to inhibit α -amylase is measured based on the difference between the

initial amount of starch and the amount of starch remaining after the hydrolysis reaction to assess the degree of hydrolysis of α -amylase. The more starch left after the reaction, the higher the ability to inhibit α -amylase. Thus, when acarbose concentration was increased, the percentage of inhibited α -amylase also increased linearly with the concentration of acarbose and a statistically significant difference of 5%. The linear equation of α -amylase inhibitory activity is $y = 0.3475x + 4.2062$ with a correlation coefficient of $R^2 = 0.9904$. At 100 $\mu\text{g/mL}$ acarbose, α -amylase inhibitory effect was 38.96%, at 160 $\mu\text{g/mL}$ acarbose inhibitory effect was 64.29%, at 200 $\mu\text{g/mL}$ acarbose inhibition was 70.64%, and an IC_{50} value was of 131.78 $\mu\text{g/mL}$.

Similar to the investigation on positive control, the evaluation was conducted on the extract samples of the stem to compare the influence of the extraction method on the ability to inhibit the α -amylase enzyme. Based on the linear equation standard line of each determined sample, the results of the IC_{50} value of the extracted sample by sonication and non-sonication methods are shown in **Figure 4**.

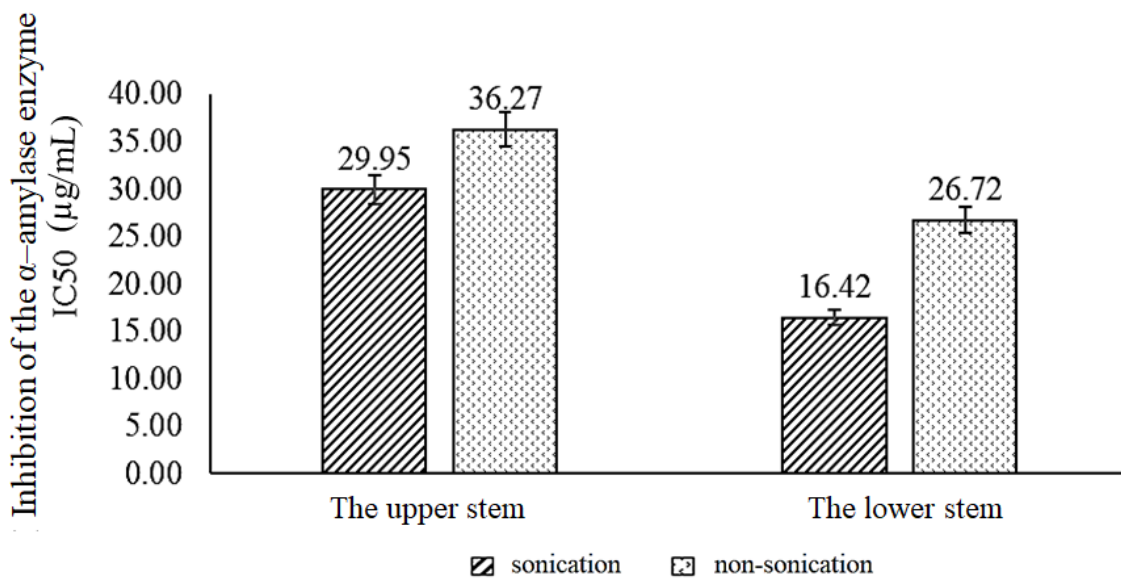


Figure 4. Results of comparing α -amylase inhibition of extract samples

By using sonication, the extracts of both the upper stems and lower stems have lower IC_{50} values than without sonication. The extract of the upper stem extracted by sonication has an IC_{50} value of $29.9538^b \pm 0.1359$, while without sonication, IC_{50} is $36.2736^a \pm 0.0078$. The lower stem sample has a sonicate value of IC_{50} of $16.4246^d \pm 0.0364$, while without sonication, IC_{50} is $26.7219^c \pm 0.0323$, which demonstrates that the extraction method has a great influence on the IC_{50} value of the extract. The lower the inhibitory concentration (IC_{50}) value, the more potent the inhibitor.

In comparison, the lower stem showed a smaller IC_{50} than the upper stem, demonstrating better inhibition of α -amylase in the lower stem than in the upper one.

3.4.2. Effect of the extraction method on extracts from E. glaucum stems' ability to inhibit the α -glucosidase

The effect of the extraction method on the ability of acarbose to inhibit α -glucosidase enzyme. When the acarbose concentration increased from 20-100 $\mu\text{g/mL}$, the inhibition percentage rose linearly with the concentration, a statistically significant difference at 5%. The linear equation

of the α -glucosidase inhibitory activity is $y = 0.9924x + 3.321$ with a correlation coefficient with an R^2 value of 0.9881. At the acarbose concentration of 100 $\mu\text{g/mL}$, the ability to inhibit the α -glucosidase enzyme reaches 102.56%. At the acarbose concentration of 80 $\mu\text{g/mL}$, the ability to inhibit the α -glucosidase enzyme is 82.12%. At the acarbose concentration of 50 $\mu\text{g/mL}$, the ability to inhibit the α -glucosidase enzyme reaches 59.82%, and the IC_{50} value is 47.04 $\mu\text{g/mL}$.

Based on the analysis results of the extract's ability to inhibit the enzyme α -glucosidase, a standard line showing the ability to inhibit the α -glucosidase enzyme at different concentrations was constructed. From the linear equation standard line of each sample, the IC_{50} value of each sample and the difference is statistically significant at 5% (Table 3).

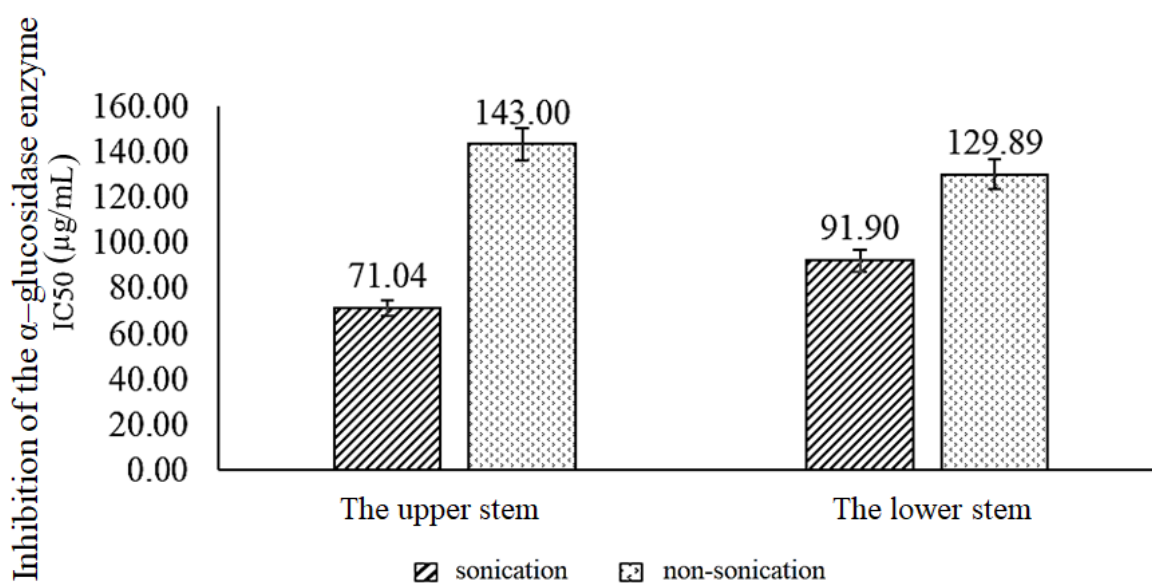


Figure 5. α -glucosidase inhibition of extract samples

Figure 5 shows the IC_{50} values are different among the extract samples using different extraction methods. By using sonication, both the upper and lower stem extracts have lower IC_{50} values than without sonication. The extract of the upper stem with sonication has an IC_{50} value of $71.0415^{\text{d}} \pm 0.0073$, while without sonication, IC_{50} is $143.001^{\text{a}} \pm 0.007$. The extract of the lower stem with sonication has an IC_{50} value of $91.8999^{\text{c}} \pm 0.0145$, while without sonication, IC_{50} is $129.893^{\text{b}} \pm 0.017$. This result shows that the extraction method has a great influence on the IC_{50} value of the extracts, when using sonication, the lower the IC_{50} value the higher the ability to inhibit the enzyme. Comparing the parts of the stem with sonication, it was found that the extract of the upper stem reached a smaller IC_{50} value than one of the lower stem, demonstrating better α -glucosidase inhibitory activity of the upper stem than the lower one.

From the above results, it can be commented that ethanol extracts from the stems of *E. glaucum* are capable of inhibiting α -amylase and α -glucosidase. In general, when using the sonication method, the IC_{50} value is lower than when not using the sonication method. It was shown that sonication increased the inhibition of the α -amylase and α -glucosidase of samples compared with non-sonication. In addition, IC_{50} values of extract samples that inhibit the α -amylase were all lower than α -glucosidase, demonstrating the α -amylase inhibition ability of stems from *E. glaucum* is higher than the α -glucosidase inhibition ability.

Table 3

Comparison of IC₅₀ values of extract samples using different extraction methods

Sample type	Extraction method	IC ₅₀ of α-amylase inhibition (mg/mL)	IC ₅₀ of α-glucosidase inhibition (mg/mL)
The upper stem	Sonication	29.9538 ^b ± 0.1359	71.042 ^d ± 0.0073
The upper stem	Non-sonication	36.2736 ^a ± 0.0078	143.001 ^a ± 0.007
The lower stem	Sonication	16.4246 ^d ± 0.0364	91.899 ^s ± 0.0145
The lower stem	Non-sonication	26.7219 ^s ± 0.0323	129.893 ^b ± 0.017

4. Discussion

E. glaucum has active ingredients with antioxidant and hypoglycemic characteristics in some parts of the plant. The ability to capture DPPH free radicals when combining sonication in the upper stem is four times higher than that of non-sonication. In addition, the presence of sonication increased the total polyphenol content, DPPH free radical scavenging, and hypoglycemic capacity of *Ensete glaucum* stem extract.

In general, the inhibition of α-amylase enzyme was higher than α-glucosidase enzyme in all *E. glaucum* extracts. Diabetes is a type of metabolic disorder, which is the cause of many dangerous complications (Banday, Sameer, & Nissa, 2020). The α-amylase can catalyze the hydrolysis of starch to produce more glucose, leading to increased blood glucose and the risk of diabetes (Joshi, Andhare, Marchawala, Bhattacharya, & Upadhyay, 2021). The α-amylase is an enzyme of humans and other mammals that hydrolyzes alpha bindings of alpha-linked polysaccharides such as starch, glycogen, and other polysaccharides that form glucose and maltose (Li et al., 2021). Therefore, enhancing the inhibition of α-amylase activity is considered a mechanism to reduce blood glucose and the risk of diabetes. Inhibitors like acarbose can heavily influence the effectiveness of the α-amylase enzyme, either through competitive or non-competitive actions, affecting the binding of the active center of the α-amylase to the substrate (Dandekar et al., 2021). Thus, acarbose is used as a control in studies of α-amylase inhibitory activity.

The α-glucosidase enzyme of the small intestine hydrolyzes the α-1,4 bonds of oligosaccharide into glucose and is then absorbed through the small intestine’s lining and into the blood vessels, whereas high blood glucose levels are symptoms of diabetes (Lee et al., 2016). Therefore, the inhibitor of the α-glucosidase enzyme will control blood glucose levels, contributing to the effective treatment of diabetes. Acarbose, as an α-glucosidase inhibitor, is a competitive inhibitor of digestive enzymes and reverses this enzyme in the intestine. Through delayed carbohydrate digestion and extended digestion time, this inhibition decreases the rate of glucose absorption. Acarbose may prevent the development of diabetes symptoms that are commonly used for patients with diabetes mellitus. Acarbose is used as a positive control in studies of α-glucosidase inhibitor activity. From the study results, it can be commented that extracts from the stems of *E. glaucum* are capable of inhibiting α-amylase and α-glucosidase enzymes. The presence of sonication increases the capacity to inhibit the α-amylase enzyme (IC₅₀ of the upper stem is 29.94 g/mL, the lower stem is 16.42 g/mL), compared to the absence of sonication (IC₅₀ of the upper stem is 36 g/mL, the lower stem is 26.72 g/mL). Similar to the ability to inhibit α-glucosidase enzyme when using sonication (IC₅₀ of the upper stem is 71.04 μg/mL, the lower stem

is 91.91 $\mu\text{g/mL}$) is higher than not using sonication (IC_{50} of the upper stem is 142.99 $\mu\text{g/mL}$, the lower stem is 129.88 $\mu\text{g/mL}$).

5. Conclusion

Temperature and solvent removal factors have significant effects on the extraction of antioxidant polyphenols from *E. glaucum*. The most suitable condition for extracting the antioxidant polyphenol analysis from the plant is 70% v/v ethanol, the ratio of raw materials and solvents is 1/30. Analyzing of extracts obtained with the above conditions combined with the sonication method showed that the plant was high in total polyphenol content, resistant to oxidation throughout the stem, and mainly in the lower stem ($\text{IC}_{50} = 420.6^b \pm 100.3$).

The ability to inhibit the α -amylase and α -glucosidase enzymes has also been evaluated and has the lowest IC_{50} values, with values of $71.0415^d \pm 0.0073$ and 47.04 $\mu\text{g/mL}$, respectively. This is the first report on the antioxidant activity and the ability to reduce blood glucose of *E. glaucum* grown in Vietnam. Additionally, the study provides the theoretical basis for future similar studies as well as exploiting and applying *E. glaucum* for use as a source of raw materials containing bioactive polyphenols, and enhancing the value of *E. glaucum*.

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