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ANTIOXIDANT AND XANTHINE OXIDASE INHIBITORY ACTIVITIES OF *ENSETE GLAUCUM* (ROXB.) CHEESMAN SEEDS

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Summary

Antioxidant and Xanthine Oxidase Inhibitory Activities of *Ensete glaucum* (Roxb.) Cheesman Seeds

Ensete glaucum seeds are commonly known as an herbal traditional medicine with many therapeutic potentials. The purpose of this study was to investigate the antioxidant and xanthine oxidase inhibitory activities of *E. glaucum* seed extracts as well as the correlation with total polyphenol content (TPC) and total flavonoid content (TFC) using Pearson's method. The results showed that the 96% ethanol extract of *E. glaucum* seeds by hot extraction (H96) had higher TPC (67.24 mg GAE/100 mg d. w.) and TFC (8.60 mg QE/g d. w.) than other extracts. *E. glaucum* seed extracts presented antioxidant property through their DPPH radical scavenging (IC_{50} of C96 = 5.78 µg/mL), ABTS radical cation decolorization (IC_{50} of H96 = 10.47 µg/mL) and reducing power (EC_{50} of H96 = 27.03 µg/mL) abilities. Furthermore, *E. glaucum* seed extract also showed xanthine oxidase inhibitory activity with the H96 extract having the best effect (IC_{50} = 76.41 µg/mL). There was a remarkably negative correlation between TPC and TFC in *E. glaucum* seed extracts with their IC_{50} of xanthine oxidase, DPPH, ABTS inhibitions, and reducing power activity with correlation coefficient (*r*) of -0.7877, -0.9392, -0.9290, and -0.9329 (TPC), -0.5620, -0.7664, -0.6150, and -0.6169 (TFC), respectively. It was suggested that *E. glaucum* seeds have great potential for antioxidant and xanthine oxidase inhibitory activities based on the high total polyphenol and flavonoid contents. Simultaneously, 96% ethanol extract of *E. glaucum* seeds could be considered as a potential extract for further studies in the direction of antioxidant and anti-hyperuricemic activities.

Keywords: *Ensete glaucum* seeds, *Polyphenols, Flavonoids, Anti-oxidant activity, Xanthine oxidase inhibition.*

1. Introduction

Ensete glaucum (Roxb.) Cheesman, also known as Snow banana, belongs to the Musaceae family, which are widely distributed in South and South -East Asia listed as Myanmar, India, China, Indonesia, Lao, Vietnam, Thailand, and Philippines. In Vietnam, *E. glaucum* is recorded to wildly grows on an open place near dry deciduous forest margins or grassland in the North or Central regions, such as Ninh Thuan, Binh Phuoc, Gia Lai, Kon Tum, Lam Dong, Phu Tho, and Hoa Binh. Due to special reproduction of this species, they grow and develop singly and by seedling. As the plant matured and fruiting, all the nutrition will be supported to their fruits and seeds, so there are many expectations that the pharmaceutical activity of *E. glaucum* seeds might be higher. Vietnamese people traditionally use the dried seed of *E. glaucum* for treating diuretic, anti-inflammation (edema), kidney stone treatment, constipation in children, diabetes treatment supporting...

Oxidation can be a chemical action that will produce free radicals, which in turn leads to chain reactions that will damage the cells of the organism [1]. Free radicals are produced during the normal metabolism of human cells. Antioxidants are also produced by cells to neutralize free radicals. Thus, the body is ready to keep a balance between antioxidants and free radicals [2]. However, the imbalance of free radicals and the ratio of antioxidants in the body leads to an increase in oxidative stress. Long-term suffering to oxidative stress can damage cells, proteins, and even DNA in the body [3]. Excess amounts of free radicals can even accelerate the aging process and have been linked to other serious medical conditions, such as brain stroke, diabetes, gout, autoimmune disease, Parkinson's disease, Alzheimer's disease, cancer… [4].

Based on a previous literature review, studies of chemical composition and biological effects of the *E. glaucum* remain very limit. Therefore, this study was carried out to evaluate the antioxidant activities of *E. glaucum* seed extracts and contribute to providing scientific information for further research on chemical composition, biological effects as well as the use of this medicinal herb in folk medicine.

2. Materials and methods

2.1. Plant materials

Ensete glaucum (Roxb.) Cheesman seeds were collected in April 2020 from Bac Ai District, Ninh Thuan Province, Vietnam. The plant sample were identified and authenticated by MSc. Le Duc Thanh (CGMM) and a voucher specimen (TNDL-CCD-2020) was kept at the Department of Natural Resources and Medicinal Materials Development (CGMM). Seeds were separated from ripe fruits,

then washed and dried (Loss of drying (LOD) ≤ 13%). These dried seeds were ground to a fine powder and kept individually in airtight PVE bag at the CGMM (Sample code: EGS-TTS-2020).

2.2. Chemicals and reagents

Ethanol (96% *v/v*) was purchased from OPC Pharmaceutical Company. Methanol (HPLC ≥ 99.9%), ascorbic acid (vitamin C, HPLC ≥ 99%), Folin-Ciocalteu's phenol reagent (Quality level 200), aluminum chloride (99.999% trace metals basis), quercetin (HPLC ≥ 98%), gallic acid (HPLC ≥ 98%), 1,1-diphenyl-2-picrylhydrazyl reagent (Quality level 200), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Quality level 200, HPLC ≥ 98%), allopurinol (Quality level 200, TLC ≥ 99%), xanthine oxidase (from bovine milk, quality level 300), and xanthine (Quality level 200) were supplied by Sigma-Aldrich® Co. Ltd (USA).

2.3. Extract preparation

The five extracts from *E. glaucum* seeds (500 g) were prepared including 96% and 45% ethanol extracts by maceration method (C96 and C45), 96% amd 45% ethanol extracts by hot extraction (H96 and H45), and water extract by decoction method (WE). *For maceration method:* seed powder was extracted with ethanol (96% and 45% *v/v*) at room temperature for 24 hours, the liquid extract was then filtered, and the extraction was repeated to achieve a ratio of 1/20 (*w/v*). *For hot extraction:* seed powder was extracted with ethanol (96% and 45% *v/v*) at 70°C for 60 minutes, the liquid extract was then filtered, and the extraction was repeated to achieve a ratio of 1/20 (*w/v*). *For decoction method:* seed powder was extracted with distilled water at 100°C for 60 minutes, the liquid extract was then filtered and the extraction was repeated to achieve a ratio of 1/20 (*w/v*). The above liquid extracts were individually concentrated using a rotary evaporator at 70°C under reduced pressure to get corresponding crude extracts. The extraction yield of the extracts was shown in **Table 1**. The crude extracts were stored at 2 - 8°C and dissolved in distilled water to yield a stock solution.

Table 1. Extraction yield of the extracts

Extract	C96	C45	H96	H45	WE
$LOD_{raw\ material}$ (%)	7.96 ± 0.30				
$LOD_{extract}$ (%)	17.24 ± 0.30	18.89 ± 0.08	6.15 ± 0.64	17.48 ± 0.04	15.08 ± 0.66
Extract weight (g)	16.79	22.58	17.21	48.46	31.49
Extraction yield (%)	3.02	3.98	3.51	8.69	5.81

C96 and C45: 96% ethanol and 45% ethanol extracts by maceration, H96 and H45: 96% ethanol and 45% ethanol extracts by hot extraction, WE: water extract by decoction.

2.4. Determination of total polyphenol content (TPC)

The total polyphenol content was determined by Folin-Ciocalteu's method with gallic acid was used as a standard [5]. Briefly, 20 µL test sample was mixed with 100 µL of Folin-Ciocalteu's reagent in 1 mL of double-distilled water. Then 0.3 mL of sodium carbonate solution (20% *w/v*) was poured into this mixture right after 5 min, and with distilled water, the volume reached up to 2 mL. The reaction was kept in less-exposure-to-lights condition for 2 hours at room temperature. The absorbance was measured at 758 nm and all determinations were made in triplicate. The calibration curve was planned to use standard gallic acid. Through the calibration plot, the total polyphenol content was calculated and expressed as milligram of gallic acid equivalent per 100 miligram of dry weight (mg GAE/100 mg d. w.).

2.5. Determination of total flavonoid content (TFC)

The flavonoid content was estimated based on the aluminum chloride colorimetric method with quercetin was used as a reference compound [5]. Briefly, 1 mL of aluminum chloride (2% *w/v*) was added to 1 mL diluted extract or standard quercetin solutions separately, and with methanol, the mixture was made up to 10 mL in quantity. Then, the solution was mixed and incubated for 15 min at room temperature. The absorbance of the reaction mixtures was calibrated at 450 nm with an UV-Vis spectrometer technique. The measurements were carried out in triplicate. The calibration curve was plotted using standard quercetin. The total flavonoid content was estimated from the calibration plot and expressed as milligram of quercetin equivalent per gram of dry weight (mg QE/g d. w.).

2.6. DPPH radical scavenging assay

The DPPH free radical scavenging assay was applied to evaluate the antioxidant activity of the extracts based on a previously described method [5]. Briefly, 1 mL reaction mixture consisting of 125 µL of different concentrations of test extract or positive control and 125 µL of 0.6 mM DPPH reagent in methanol was incubated at room temperature for 30 min in the dark. The absorbance was measured at 515 nm using Spectro UV-2550 spectrophotometer. DPPH inhibitory activity expressed as the percentage inhibition (I%) of DPPH, calculated as $[(A_0 - (A_1 - A_2)/A_0] \times 100$, where A_0, A_1, and A_2 are the absorbance of the blank (without test extract, with DPPH solution), the sample (with test extract, without

DPPH solution), and the sample control (with test extract, without DPPH), respectively. IC_{50} (inhibitory concentration, 50%) values were calculated from the mean values of data from three determinations. Vitamin C (Ascorbic acid) was used as a positive control.

2.7. ABTS radical cation decolorization assay

The ABTS antioxidant test was carried out according to the following description [5]. To start, adding a 7 mM ABTS solution to a 2.45 mM potassium persulfate solution and incubating the solution in the dark for 16 hours at room temperature. Then, the solution was diluted by mixing 1 mL of ABTS solution with 50 mL of methanol to obtain an absorbance of 0.70 ± 0.05 units at 734 nm using a spectrophotometer (Spectro UV-2550). Next, 40 µL of the test sample at various concentrations or positive control was mixed with 1160 µL of ABTS solution, the absorbance was measured at 734 nm after 6 min at room temperature. All samples were done in triplicate and an average of each sample was calculated. The results were expressed as an IC_{50} value for each sample from the proportion of the radical quenching activity, which was calculated by the formula: $\%I = [(A_0 - (A_1 - A_2)/A_0] \times 100$, where A_0, A_1, and A_2 are the absorbance of the blank (without test extract, with ABTS solution), the sample (with test extract, without ABTS solution), and the sample control (with test extract, without ABTS), respectively. Vitamin C was used as a positive control.

2.8. Reducing power (RP) assay

The reducing activity of five extracts was determined by assessing the ability of the extracts to reduce $FeCl_3$ solution as described by Oyaizu [6]. The mixture including 0.2 mL aliquot with 0.5 mL of sodium phosphate buffer 200 mM (pH 6.6) and 0.5 mL potassium ferricyanide 1% (*w/v*) was incubated at 50°C for 30 min, then added 0.5 mL trichloroacetic acid 10% (*w/v*), and centrifuged at 3000 rpm for 10 min. The volume of 0.5 mL of the supernatant was mixed with an equal volume of double-distilled water and 0.1 mL ferric chloride 0.1% (*w/v*). The absorbance was measured at 700 nm. Vitamin C was used as a positive control. The reducing power activity was subsequently analyzed via optical density and EC_{50} values. The lower the optical density value, the weaker the reduction activity of the sample. The EC_{50} value is the concentration of effective antioxidants for the absorbance reaches 0.5, which was calculated through the equation illustrating the correlation between the concentration of the sample and its optical density.

2.9. Xanthine oxidase (XO) inhibitory assay

XO inhibitory activity of *E. glaucum* seed extracts was determined according to the method described by Abu-Gharbieh et al. with some modifications [7]. Briefly, 100 µL of test extract at various concentrations was added to 300 µL of sodium phosphate buffer (50 mM, pH 7.5), and 25 µL of enzyme solution (0.2 units/mL in phosphate buffer, pH 7.5) which was prepared immediately before use and 175 µL of sodium phosphate buffer (50 mM, pH 7.5). After incubation at 25°C for 15 min, the reaction was initiated by the addition of 200 µL of 1.5 mM xanthine solution in the same buffer which acts as a substrate and this reaction mixture were incubated at 25°C for 30 min. The reaction was stopped by addition of 200 µL of 0.5 M of HCl. The absorbance was measured at 295 nm by using a UV spectrophotometer. The assay was done in triplicate. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid per min at 25°C. The XO inhibitory activity was calculated by the formula: $\%I = [(A_0 - A_1) - (A_2 - A_3)/(A_0 - A_1)] \times 100$. Where A_0 was the activity of the enzyme without test extracts, A_1 was the control of A_0 without test extracts and enzyme. A_2 and A_3 were the activities of the test extract solutions with and without enzyme. The values of IC_{50} were calculated from the means of the spectrophotometric data of the test trials repeated three times. Allopurinol was used as a positive control.

2.10. Data analysis

The obtained results were expressed in terms of mean ± SEM (Standard error of the mean). The IC_{50} value was determined based on the equation illustrating the correlation between the test substance concentration and the percentage of antioxidant activity using Graphpad Prism software (version 8.0.1, Inc., La Jolla, CA, USA). Data were analyzed by Graphpad Prism software using t-test and One-way ANOVA. The correlation statistic was based on the Pearson's correlation coefficient *(r)* and coefficient of determination (R^2) for total polyphenols content and total flavonoids content versus IC_{50} values of antioxidant activity ($p < 0.05$ is statistically significant).

3. Results

3.1. Total polyphenol and flavonoid contents

The total polyphenol and flavonoid contents (TPC and TFC) in the *E. glaucum* seed extracts varied considerably as shown in **Table 2**. The highest amount of TPC was found in 96% ethanol extract by hot extraction (H96) with a value of 67.24 ± 2.25 mg GAE/100 mg d. w. whereas the lowest content of polyphenols was 10.96 ± 0.90 mg GAE/100 mg d. w. in water extract (WE). The 96% ethanol extract by maceration (C96) and H96 extract had the similar TFC of 8.3 and 8.6 mg QE/g d.w., respectively and both significant differences compared to C45, H45, and WE extracts.

Table 2. Total polyphenol and flavonoid contents of *E. glaucum* seed extracts ($n = 3$)

Extract	C96	C45	H96	H45	WE
TPC (mg GAE/100 mg d. w.)	59.62 ± 1.59	36.05 ± 2.19****	67.24 ± 0.91$^{\#, \#\#\#\#}$	39.35 ± 0.95****	10.96 ± 0.90
TFC (mg QE/g d. w.)	8.31 ± 0.43$^{***, \#\#\#\#}$	4.66 ± 0.17	8.60 ± 0.09****	5.91 ± 0.16*	5.46 ± 0.16

Total polyphenol content (TPC, mg gallic acid equivalent/100 mg d. w.): $^{\#}p<0.05$ significant difference compared to C96; $^{****}p<0.0001$ significant difference compared to H96, C96, and WE; $^{\#\#\#\#}p<0.0001$ significant difference compared to WE. **Total flavonoid content (TFC, mg quercetin equivalent/g d. w.):** $^{*}p<0.05$ significant difference compared to C45 extract; $^{***}p<0.001$ significant difference compared to H45 extract; $^{****}p<0.0001$ significant difference compared to H45, C45, WE; $^{\#\#\#}p<0.0001$ significant difference compared to C45 and WE. C96 and C45: 96% ethanol and 45% ethanol extracts by maceration, H96 and H45: 96% ethanol and 45% ethanol extracts by hot extraction, WE: water extract by decoction.

3.2. Antioxidant activity of E. glaucum seed extracts

Antioxidant activity of the E. glaucum seed extracts was evaluated through DPPH free radical scavenging, ABTS radical cation decolorization, and reducing power capacities. The IC_{50} values of the antioxidant activity of *E. glaucum* seed extracts in the three assays were presented in **Table 3**. The extracts showed potent DPPH, ABTS quenching and reducing power activities with IC_{50} values in the order of vitamin C < C96 < H96 < H45 < C45 < WE, vitamin C < H96 < C96 < H45 < C45 < WE and vitamin C < H96 < C96 < C45 < H45 < WE, respectively. Among the extraction solvents, 96% ethanol is a better solvent for extracting compounds with antioxidant activity from *E. glaucum* seeds. However, in order to choose extracts from maceration or hot extraction, other activities need to be evaluated.

Table 3. The IC_{50} and EC_{50} values for antioxidant activity of *E. glaucum* seed extracts ($n = 3$)

Extracts	IC_{50} (µg/mL)		EC_{50} (µg/mL)
	DPPH	ABTS	RP
C96	5.78 ± 0.27	12.82 ± 1.73	38.65 ± 2.01
H96	8.64 ± 0.22^{ns}	10.47 ± 2.58	27.03 ± 0.46**
C45	66.77 ± 0.93****	33.65 ± 1.81$^{\#\#\#\#}$	81.84 ± 1.09
H45	43.30 ± 1.41****	20.93 ± 2.32$^{**, ***}$	147.84 ± 2.66
WE	90.33 ± 1.28****	69.62 ± 1.95****	266.62 ± 0.93
Vitamin C	4.39 ± 0.01$^{*/ns}$	9.12 ± 0.87	4.91 ± 0.12

DPPH: $^{ns}p > 0.05$: no significant difference with C96, $^{*}p < 0.05$: significant difference with H96, $^{****}p < 0.0001$: significant difference with H96. **ABTS:** $^{**}p < 0.01$ significant difference with C45 and C95 extracts; $^{***}p < 0.001$ significant difference compared to H96 extract and Vitamin C; $^{****}p < 0.0001$ significant difference compared to other samples; $^{\#\#\#\#}p < 0.0001$ significant difference with C96, H96 and Vitamin C. **RP:** $^{**}p < 0.01$: significant difference with C96, $^{****}p < 0.0001$: one by one significant difference.

3.3. Xanthine oxidase (XO) inhibitory activity of E. glaucum seed extracts

Table 4. The IC_{50} values for xanthine oxidase inhibitory activity of *E. glaucum* seed extracts ($n = 3$)

Extracts	IC_{50} (µg/mL)
C96	192.13 ± 9.12^{ns}
H96	76.41 ± 2.12$^{**, ***, ****}$
C45	192.60 ± 0.90$^{\#, \#\#\#}$
H45	129.03 ± 1.45$^{\#\#}$
WE	270.07 ± 14.35$^{\#\#}$
Allopurinol	0.22 ± 0.01

C96 and C45: 96% ethanol and 45% ethanol extracts by maceration, H96 and H45: 96% ethanol and 45% ethanol extracts by hot extraction, WE: water extract by decoction. $^{ns}p > 0.05$: no significant difference with C45, $^{**}p < 0.01$: significant difference with H45, $^{***}p < 0.001$: significant difference with C96 and C45, $^{****}p < 0.0001$: significant difference with WE, $^{\#}p < 0.05$: significant difference with WE, $^{\#\#}p < 0.01$: significant difference with C96 and H45, $^{\#\#\#}p < 0.001$: significant difference with H45.

The results in **Table 4** showed the XO inhibitory ability of five studied extracts. The xanthine oxidase inhibitory activity of the extracts was arranged in order of H96 > H45 > C96 > C45 > WE. The H96 (96% ethanol extract by hot extraction) was the lowest IC_{50} value; the inhibition of xanthine oxidase from this extract would be higher than other extracts. Combined with the antioxidant capacity in the three mentioned assays, the 96% ethanol extract by hot extraction could be selected for further studies.

3.4. Correlation between total polyphenol and flavonoid contents with antioxidant activity of E. glaucum seed extracts

The lowest IC_{50} or EC_{50} value in xanthine oxidase inhibitory, DPPH, ABTS and reducing power assays will reveal the highest antioxidant activity. The results in **Table 5** showed that the correlation values R^2 in the analyzes were still relatively low but combined with the analytical results of p and r values, it showed that TPC and TFC in *E. glaucum* seed extracts had significant and negative correlation with their IC_{50}/EC_{50} of antioxidant activity. Besides, there was a stronger correlation between total polyphenol content and

investigated activities. It can be predicted that polyphenol and flavonoid compounds may play an important role to antioxidant activity of *E. glaucum* seeds.

Table 5. The results of regression analysis between TPC and TFC, antioxidant activity TPC and TFC of *E. glaucum* seed extracts (IC_{50}/EC_{50})

Phytochemical contents	Antioxidant values	Correlation parameters			
		Linear regression	R^2	*r*	*p*
TPC	IC_{50} of DPPH assay	y=-1.552x+109.1	0.8821	-0.9392	<0.0001****
	IC_{50} of ABTS assay	y=-1.012x+71.88	0.8622	-0.9290	<0.0001****
	EC_{50} of RP assay	y=-4.136x+288.8	0.8703	-0.9329	<0.0001****
	IC_{50} of XO inhibitory assay	y=-0.003235x+0.32	0.6205	-0.7877	0.0005***
	TFC	y=0.06658x+3.749	0.6706	0.8189	0.0002***
TFC	IC_{50} of DPPH assay	y=-15.58x+145.6	0.5874	-0.7664	0.0009***
	IC_{50} of ABTS assay	y=-8.247x+83.08	0.3787	-0.6150	0.0146*
	EC_{50} of RP assay	y=-33.64x+334.0	0.3805	-0.6169	0.0143*
	IC_{50} of XO inhibitory assay	y=-0.02838x+0.3691	0.3158	-0.5620	0.0292*

TPC: total polyphenol content (mg GAE/100 mg d. w.), TFC: total flavonoid content (mg QE/g d. w.), RP: Reducing power, R^2: coefficient of determination, *r*: correlation coefficients, $^{*}p < 0.05$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ were statistically significance.

4. Discussion

In this study, polyphenols and flavonoids were centrally investigated because they are more closely related to antioxidant activity by free radical scavenging, which has been demonstrated in many previous studies [8]. The results showed that the *E. glaucum* seed extracts had a high content of total polyphenols, in which the 96% ethanol extract by hot extraction accounted for the highest proportion. In contrast, the water extract owned the lowest content, and all the other extracts were statistically different than that of water extract. The results also showed that the higher concentration of ethanol, the more polyphenol content obtained. This proved that polyphenol content was dependent on the solvent used and its polarity [9]. Polyphenols are often most soluble in organic solvents which are less polar than water. Besides, using the temperature-affected extraction method seems to have obtained higher polyphenol content. A similar result was found in the total flavonoid content. The TFC in the 96% ethanol extracts was higher than in the other extracts, which mean the solvents that had lower polarity could collect higher flavonoids content than others [10]. However, though the 45% ethanol is less polarity than water, C45 extract still took lower content of flavonoids than WE extract. The assumption for this results could also be explained as the effect of temperature on the extraction However, though the C45 extract is less polarity than WE extract, it still took lower amount of flavonoids than WE extract. The assumption for this results could also be explained as the effect of temperature on the extraction yield of flavonoids.

Moreover, antioxidant activity of *E. glaucum* seed extracts has been determined via radical scavenging capacity using *in vitro* models. In this study, *E. glaucum* seed extracts contained polyphenols and flavonoids with various concentrations which resulted in different antioxidant levels. The 96% ethanol extracts were recorded as potential extracts having stronger than antioxidant properties in all three assays including radical scavenging (DPPH and ABTS) potency and reducing power compared to the lowest of the water extract. The temperature-affected extraction method also showed higher antioxidant activity (2/3 of the assays had a lower IC_{50}). Previous research on *Musa acuminata* (Musaceae family) about extraction methods, maceration method showed the lower antioxidant activity than the hot extraction method [11], strengthening the point that temperature is one of a key for higher yield of antioxidant in extracts. Simultaneously, the results of antioxidant activity were quite consistent with the highest and lowest polyphenol and flavonoid contents of ethanol extract and water extract, accordingly. The high polyphenol and flavonoid contents of *E. glaucum* seed extracts may be a good indicator of their antioxidant activity. The antioxidant activity of polyphenols, which is because of the polyphenol hydroxyl group as electron donor is believed to contribute a part in these activities [12]. Also, flavonoids with strong free radical scavenging activity [13] has been researched as a part of antioxidant activities in polyphenol compounds.

Xanthine oxidase is one of the enzymes required to form the uric acid by breaking down of purine nucleotides [14]. Excessive uric acid production has also determined to be harmful. The urate acts like a pro-oxidant and leads to the formation of free radicals that have an inclination of lipid membranes to be oxidized. Reactive oxygen species naturally tend to break cellular structures, DNA and proteins, and consequently, uric acid is a crucial factors of oxidative stress formation which has links to hypertension,

diabetes, nephrosis, and cardiovascular diseases [15]. Manipulation of xanthine oxidase within the body incorporates a plethora of pharmacological indications, most vital being that of treating the symptoms of gout. Gout could be a painful condition within which there is an excess uric acid metabolism. The surplus then precipitates and forms monosodium urate crystals within the joints and kidneys, causing painful gouty arthritis and uric acid kidney stone [15]. In this study, the *E. glaucum* seed extracts had xanthine oxidase inhibitory activity, in which, an ethanol hot extraction method has stronger xanthine oxidase inhibitory activity in compare with the ethanol maceration method and water decoction. Therefore, the temperature also has a crucial effect on xanthine oxidase inhibitory activity.

Furthermore, in order to contribute to providing more information on the relationship between polyphenols and flavonoids with antioxidant activity, the correlation between them was investigated using Pearson's correlation coefficient. According to correlation coefficient classification (r), the size of a correlation coefficient is indicated as follow: ±0.00 - ±0.19 is "very weak"; ±0.20 - ±0.39 is "weak"; ±0.40 - ±0.59 is "moderate"; ±0.60 - ±0.79 is "strong"; ±0.80 - ±1.00 is "very strong" [16]. The correlation coefficient of TPC and TFC was 0.8189, which was a very strong positive correlation. This result demonstrated that the TFC increased with TPC, which was reasonable because flavonoids was known to be a major component of polyphenols [8]. Furthermore, from results of the correlation between TPC, TFC with IC_{50} values in DPPH, ABTS, xanthine oxidase inhibitory assays and EC_{50} values of RP assay showed that the antioxidant activity of the *E. glaucum* seed extracts in the four used assays was in a moderate and very strong correlation limit with total polyphenol and flavonoid contents. These correlations have proved the relationships between TPC and TFC, as well as TPC, TFC with antioxidant and xanthine oxidase abilities. The highly significant correlations obtained in this study support the hypothesis that polyphenol and flavonoid compounds contribute significantly to the antioxidant capacity of plant extracts, in previous study, a linear correlation between the content of total polyphenol and flavonoid compounds with antioxidant capacity was found of several plant species [17]. The study indicated that polyphenol and flavonoid compounds are the components that play a crucial role in the antioxidant activity of *E. glaucum* seeds. Hence, *E. glaucum* seeds could be a promising candidate for the development of antioxidant products, preventing related-free radical diseases.

On the other hand, different extraction conditions were studied to evaluate the effect on total polyphenol, flavonoid content, antioxidant, and xanthine oxidase inhibitory activities of *E. glaucum* seed extracts. The maceration method was applied with a solvent of 45% ethanol simulating how to soak alcohol in folklore. Besides, ethanol at a higher concentration (96% *v/v*) was also used to evaluate the polarity of the solvent that affects the evaluation criteria. Although this method is time-consuming, it is easy to implement, saves energy, does not require complicated equipment, can recover solvents, and is particularly unaffected temperature-sensitive compounds. Decoction with water was applied to simulate the way of decoction in folk medicine. This method is easy to perform, inexpensive because it does not require organic solvents but requires energy and is only suitable for the extraction of compounds that are stable at elevated temperatures. In addition, this study investigated several other extraction conditions including using hot extraction with solvent of 45% and 96% ethanol, which both applied the extraction solvent of the maceration method and applied extraction conditions have the temperature effect. This method requires the use of energy, organic solvents, more modern equipment, and is only suitable for extracting compounds that are stable at high temperatures but save time. Although the hot extraction with 96% ethanol is more expensive than the others, based on the obtained research results, this extract showed higher bioactive and compound content. In terms of application, hot extraction could also be performed on a larger scale, therefore, this method could be chosen to obtain potential extract for further study.

5. Conclusion

Ensete glaucum (Roxb.) Cheesman seed extracts contain both flavonoids and polyphenols that may contribute to *in vitro* antioxidant and anti-hyperuricemic activities, which was evaluated by DPPH scavenging, ABTS quenching, reducing power, and xanthine oxidase inhibitory mechanism. Substantially, the 96% ethanol extract by hot extraction as it showed the highest TPC, TFC, and the best antioxidant and xanthine oxidase inhibitory effectiveness. Additionally, there was a significant correlation between the studied antioxidant capacity and its estimated total polyphenol and flavonoid contents of *E. glaucum* seed extracts.

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HEPATOPROTECTIVE EFFECT OF STANDARDIZED DRY EXTRACT FROM *PHYLLANTHUS EMBLICA* L. FRUITS ON ETHANOL-INDUCED HEPATOTOXICITY MODEL IN MICE AND HEPATIC STEATOSIS INHIBITION *IN VITRO*

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Summary

Hepatoprotective Effect of Standardized Dry Extract from *Phyllanthus emblica* L. Fruits on Ethanol-Induced Hepatotoxicity Model in Mice and Hepatic Steatosis Inhibition *in vitro*

The study has been undertaken to evaluate the hepatoprotective of extract from *Phyllanthus emblica* L. fruits using the ethanol-induced hepatotoxicity model in mice, as well as the effects on steatosis and lipid accumulation caused by fatty acid *via* regulation of AMPK and ACC signalling in treated HepG2 cells. The results indicated that fruits extract of *P. emblica* L. at doses of 500 and 1000 mg/kg body weight/day exhibited the hepatoprotective effect on ethanol-induced hepatic injury by reducing serum ASAT, ALAT activities, hepatic MDA content, as well as elevating liver S9 fraction SOD activities, GSH content in mouse model. In addition, the extract decreased significantly the fat accumulation and stimulated AMP-activated protein kinase (AMPK) signalling in treated HepG2 cells.

Keywords: *Phyllanthus emblica* L., *Hepatoprotective effect, Ethanol, AMPK, Lipid accumulation.*

1. Introduction

Research studies have shown that *Phyllanthus emblica* L. fruits prevent liver injuries induced by various chemicals or toxins [1],[2],[3]. Our previous study also reported that standardized dry extract from *P. emblica* L. fruits (PEF) at 500, 1000, and 2000 mg/kg/day can reduce the severity of hepatic damage on CCl_4-induced acute hepatotoxicity in mice [4]. Excessive ethanol consumption can increase liver injuries, result in alcoholic liver disease that becomes more and more popular and remains one of serious health problems. Recently, some evidence pointed out that the fruits of *P. emblica* L. can attenuate ethanol-induced oxidative stress in the liver [3],[5]. However, the protective effect of *P. emblica* L. fruits against ethanol-induced liver damage have not been investigated so far in Vietnam. On the other hand, although the hepatoprotective properties of PEF have been reported both from *in vitro* and *in vivo* studies, its mechanism of action still has been unknown. Some studies have revealed that PEF inhibits steatosis and lipid accumulation caused by fatty acid via regulation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) signalling [6]. The activation of AMPK inhibits