

## Extraction and Phytochemical Composition of *Ensete glaucum* (Roxb.) Cheesman Fruits in Bu Gia Map National Park, Binh Phuoc Province

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### ARTICLE INFO

Received: 11/5/2022  
Revised: 24/5/2022  
Accepted: 15/6/2022  
Published: 28/6/2022

### ABSTRACT

*Ensete glaucum* (Roxb.) Cheesman fruits have been used in folk medicine to treat kidney stones, diabetes, perforated edema, stomach ulcers, and skin allergies. This study first investigated the phytochemical composition of three parts of *E. glaucum* fruits collected in Bu Gia Map National Park, Binh Phuoc Province. Results showed that *E. glaucum* seeds contain flavonoids, saponins, and tannins. Meanwhile, amino acids, flavonoids, and terpenoids were identified in *E. glaucum* peel; flavonoids, tannins, saponins, and amino acids occur in the pulp. The total content of polyphenols and flavonoids was concentrated in the seeds 59.33 mg GAE/g DW; 70.60 mg CE/g DW), much larger than in the peel (21.09 mg GAE/g DW, 45.43 mg CE/g DW), and pulp (40.75 mg GAE/g DW, 37.82 mg CE/g DW). Until now, the first study researched about general phytochemistry of *E. glaucum* in Bu Gia Map National Park. Thus, further studies on the chemical compositions and biological effects of *E. glaucum* fruits should be conducted to develop this medicinal plant.

### KEYWORDS

*Ensete glaucum*;  
Phytochemicals;  
Polyphenols;  
Flavonoids;  
Bu Gia Map National Park.

Doi: <https://doi.org/10.54644/jte.70B.2022.1209>

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## 1. Introduction

*Ensete glaucum* (Roxb.) Cheesman, also known as Chan Voi banana, Co Don banana, belongs to the Musaceae family, widely distributed in Phuoc Binh National Park (Ninh Thuan province) and Bu Gia Map National Park (Binh Phuoc province). *E. glaucum* seed of wild banana that ethnic minorities have widely used to treat intestinal diseases, diabetes, kidney stones, perforated edema, stomach ulcers, and skin allergies. Studies on the plant's chemical composition, nutritional composition, and biological activities are promoted. Several domestic and foreign studies have shown that all parts of the plant, from the stem to the fruit, contain active ingredients such as saponins, flavonoids, and terpenoids,... and have antioxidant capabilities [1, 2]. However, there have not been many studies on this species's chemical composition and pharmacological effects, especially the studies on peel and pulp fruits.

Some plants in *Ensete* genus, like *E. superbum* and *E. ventricosum* have been studied in compositions and bioactivities [3, 4]. Triterpenoid esters, proanthocyanidin, pro-pelargonidin glucosides, pelargonidin, anigorufone, hydroxyanigorufone,  $\beta$ -carboline alkaloids were major bioactive phytochemicals in *E. superbum*. Pharmacologically, *E. superbum* was non-toxic (LD<sub>50</sub> = 3235.9 mg/kg) and has been reported to possess antiurolithiatic, antidiabetic, antifertility, anti-estrogenic, antiviral, cardiovascular, and anti-inflammatory activities [3]. *E. ventricosum* contains starch and other minors/trace elements, contributing to its nutraceutical applications and improving the country's food and health security [4]. The prospect of more research on the chemical composition and pharmacological effects of *E. glaucum* of this genus suggests the potential.

Therefore, this study was carried out to determine the chemical compositions and quantitative total polyphenol and flavonoid content of the three parts of *E. glaucum* fruits, including peels, pulp, and seeds. With the natural conditions in Bu Gia Map National Park, the chemical composition of the *E. glaucum* fruits was preliminary determined. It is the first study to create a premise for the orientation of chemical composition and biological effects research and the development of medicinal plant and products from parts of this plant for real-life applications. Moreover, this study will also improve the meaning of conservation research on this rare plant.

## 2. Materials and Methods

### 2.1. Plant Materials and Chemicals

*Ensete glaucum* fruits were collected in Bu Gia Map National Park, Binh Phuoc Province, in January 2021. The banana fruits were separated into three parts, including the peels, pulp and seeds, which were dried for the study. Dried raw materials were ground into powder with a diameter of about 1 mm before extracting by maceration method.

Ethanol (Chemsol, Vietnam); Aluminium chloride, sodium hydroxide, ferric chloride, hydrochloric acid, chloroform, sulfuric acid, sodium chloride, potassium iodide, iodine, sodium carbonate, and gelatin was purchased from Xilong Chemical (China). Gallic acid, catechin, ninhydrin, and Folin-Ciocalteu reagent were purchased from Merck (Germany).

### 2.2. Extraction

The powder of peels (0.2 kg), pulp (0.35 kg), and seeds (0.5 kg) of *E. glaucum* fruits was soaked in ethanolic solvent (ethanol: distilled water = 7: 3 v/v) with raw material: solvent ratio of 1: 1.5 (w/v) at room temperature for 24 hours. Liquid extracts from each study sample were collected. The extraction process was repeated until the extract became pale (6 times for the peels and seeds, 7 times for the pulp). The extracts were evaporated under low pressure to get the crude extract. The extracts were stored in a refrigerator at 2-8 °C before using further studies.

The extraction yield of the extracts was estimated using the expression:

$$H(\%) = \frac{m \times (1 - a)}{m' \times (1 - a')} \times 100$$

H: Extraction yield (%)

m: Mass of the extract (g)

m': Mass of the material powder (g)

a: Moisture of the extract (%)

a': Moisture of the material powder (%)

### 2.3. Qualitative phytochemical analysis

The qualitative phytochemical screening of the extracts was performed to identify the main groups of chemical constituents using colour chemical reactions [5]. All experiments were described in detail, including:

#### *Test for flavonoids*

The intense yellow colour on adding a few drops of sodium hydroxide solution to the test solution indicates the presence of flavonoids. The extract is a development of a dark green colour after the addition of a few drops of neutral 1% ferric chloride solution regarded as positive.

#### *Test for saponins*

Each extract was separately shaken with distilled water in a test tube. The formation of frothing shows the presence of saponins.

#### *Test for coumarins*

2 mL of the extract was put into 2 test tubes of equal size. Tube 1 was added with 4 mL of distilled water, and the solution became cloudy. Tube 2 was added with 0.5 mL of 10% NaOH

solution and then boiled for 2-3 minutes in the water bath. Water was added to tube 2 up to the volume of tube 1. Tube 2 was more transparent than tube 1 and continued acidifying the solution in tube 2 with concentrated HCl, the two tubes having approximately equal turbidity indicating the presence of coumarins.

#### ***Test for terpenoids***

The reddish-brown colouration at the interface after adding 2 mL of chloroform followed by 1 mL of concentrated sulfuric acid to 5 mL of the extract indicates the presence of terpenoids.

#### ***Test for tannins***

3 mL of the extract was reacted with a few drops of salted gelatin reagent (1% gelatin and 10% NaCl), shaking gently. The solution appears opalescent, indicating the presence of tannins.

#### ***Test for alkaloids***

Potassium iodide (4 g) and iodine (2 g) were dissolved in distilled water (10 mL), and the solution was diluted to 100 mL with distilled water. Few drops of this solution were added to the filtrate; a brown coloured precipitate indicates the presence of alkaloids.

#### ***Test for amino acids***

A few drops of 3% Ninhydrin reagent were added to a test tube containing the extract and boiled for 10 minutes in a water bath. The reaction is positive when a purple colour appears.

#### ***Test for polysaccharides***

Potassium iodide (2.5 g) and iodine (1 g) were dissolved in distilled water (20 mL), and the solution was diluted to 100 mL with distilled water. Three drops of this solution were added to the filtrate; a dark blue colour appeared, indicating the presence of alkaloids.

### ***2.5. Determination of total polyphenol and flavonoid contents***

#### ***Total polyphenol content (TPC)***

The determination of total polyphenols of the extracts was performed with the Folin-Ciocalteu (FC) reagent according to the previously described method with some modifications [6]. Standard solutions of gallic acid (1-20 µg/mL) were prepared from a stock solution (100 µg/mL). 1300 µL extract was mixed with 1 mL of the FC reagent (10%), after 5 min incubation, 700 µL Na<sub>2</sub>CO<sub>3</sub> (10% (w/v)) was added. The mixture was stirred and incubated room at temperature for 30 min, and the absorbance was measured at 765 nm by a UV spectrophotometer (UH5300, Hitachi, Japan). The results are expressed in mg gallic acid equivalent (GAE)/g dry weight (DW), calculated base on the calibration curve of gallic acid. All experiments were carried out in triplicate.

#### ***Total flavonoid content (TFC)***

Total flavonoid content was determined using the aluminium colourimetric method [6] with some modifications using catechin as the standard. A calibration curve of catechin was prepared in the range of 1–100 µg/mL. Briefly, extract (2.5 mL) or standard (2.5 mL) was reacted with 5% aluminum chloride (0.15 mL), after 5 min incubation, 5% sodium nitrite (0.15 mL) was then added. After 6 min, 1 M NaOH (1 mL) and distilled water (1.2 mL) were added. The mixture was incubated at room temperature for 30 min, and absorbance was taken at 505 nm using a spectrophotometer (UH5300, Hitachi, Japan). The total content of flavonoids was expressed as mg catechin equivalent (CE)/g dry weight (DW), deduced from the calibration curve of catechin. All experiments were measured in triplicate.

### ***2.6. Statistical analysis***

The data obtained from the experiments were calculated, processed with ANOVA statistics and compared according to Fisher's LSD test standards on the statistical analysis software Excel 2010.

### 3. Results and Discussion

#### 3.1. Extraction efficiency

The results of extraction yield are represented in Table 1. The loss in mass relative to the moisture content of the material decreases in the order of peels (90.0%), pulps (86.55%), and seeds (77.7%). The peel extract had the highest extraction efficiency of 10.68%, next to seed extract with 4.74%, and the lowest extraction yield of pulp extract of 4.65%. The results suggested that extraction yield depends on materials containing various compositional characteristics. Moreover, the extracts prepared from *E. glaucum* pulps and seeds were paste forms and the peel extract in dry powder; maybe compositions' pulps and seeds contain more fatty acids than peels.

**Table 1.** Extraction yield of three parts of *E. glaucum* fruits

No	Sample	Refresh weight (kg)	Dry weight (kg)	Raw material moisture (%)	Extraction Yield (%)
1	Peel	3	0.3	90.0	10.68
2	Seed	8.5	1.9	77.7	4.74
3	Pulp	8.5	1.15	86.55	4.65

#### 3.2. Phytochemical qualitative

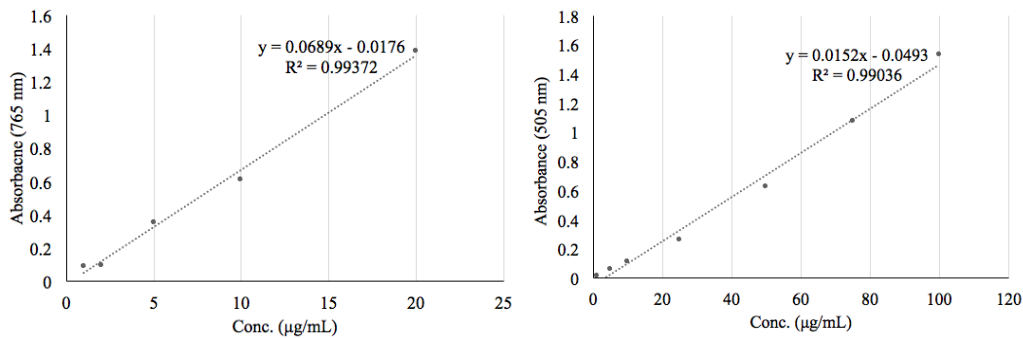
**Table 2.** Summary of phytochemical qualitative results by characteristic reactions

No	Phytochemicals	Seed	Pulp	Peel
1	Flavonoids	+	+	+
2	Saponins	+	+	-
3	Coumarins	-	-	-
4	Amino acids	-	+	+
5	Polysaccharides	-	-	-
6	Terpenoids	-	-	+
7	Tannins	+	+	-
8	Alkaloids	-	-	-

Note: (+) Present, (-) Absent

From Table 2, three parts of *E. glaucum* fruits contained flavonoids but did not contain coumarins, polysaccharides, and alkaloids. *E. glaucum* seed has more saponins and tannins. Meanwhile, *E. glaucum* pulps contain amino acids, saponins, and tannins. Amino acids and terpenoids were detected in *E. glaucum* peels. Interestingly, polysaccharides were not detected in *E. glaucum* pulps. It is suggested that the nutritional value of *E. glaucum* pulps is not as high as that of regular bananas. This is good evidence for using *E. glaucum* fruits for soaking medicine material, not for eating to provide nutrition and energy.

### 3.3. Total polyphenol and flavonoid content



**Figure 1.** Standard calibration curve of gallic acid (Left) and catechin (Right)

The standard calibration curve of gallic acid built from the gallic acid serial solutions from 1 to 20 µg/mL was  $y = 0.0689x - 0.0176$  ( $R^2 = 0.99372$ ) (Figure 1, Left). In Table 3, the TPC in the seed extract was the highest (59.33 mg GAE/g DW), 1.5 times higher than the TPC in the pulp extract (40.75 mg GAE/g DW), and 2.8 times the TPC in the peel extract (21.09 mg GAE/g DW). The amount of polyphenols present in the peels and pulp is still relatively high than the TPC in the fiber part of *E. ventricosum* of the same genus with a TPC of 38.22 mg GAE/g DW [7].

Le et al. research (2022) reported that the 96% ethanol extract of *E. glaucum* seeds at the Phuoc Binh National Park (Ninh Thuan province) contained the TPC of (672.4 mg GAE/g DW) and TFC (8.60 mg QE/g DW) [2]. The results showed that the TPC was high and concentrated in the seeds. However, the difference in TPC between *E. glaucum* seeds collected from two various National Park was due to extraction and growing conditions.

Interestingly, the TPC in three parts of *E. glaucum* fruits was higher than the TPC in some popular fruits like apple peels (15.90 mg GAE/ g DW), blueberries (9.44 mg GAE/ g DW) [8, 9]. Especially, green tea was rich in polyphenol but had a TPC of 26.33 mg GAE/ g DW, smaller than the TPC in *E. glaucum* fruits [10]. It can be seen that these foods have a lower polyphenol content than the seeds and pulp of *E. glaucum* (seed is 2.2 times more polyphenols than green tea, and pulp is 2.6 times larger than the apple peel), even *E. glaucum* peels have a more significant amount of TPC than apple peels and blueberries.

**Table 3.** Total polyphenol and flavonoid contents of *E. glaucum* fruit parts

No	Extract	Total polyphenol content (TPC,	Total flavonoid content (TFC, mg
		mg GAE/g DW)	CE/g DW)
1	Seed	59.33 ± 0.75 <sup>a</sup>	70.60 ± 0.56 <sup>a</sup>
2	Pulp	40.75 ± 0.77 <sup>b</sup>	37.82 ± 1.48 <sup>b</sup>
3	Peel	21.09 ± 0.84 <sup>c</sup>	45.43 ± 1.38 <sup>c</sup>

The data are presented as mean ± standard deviation ( $n = 3$ ). The letters a, b, c in the same column represent a significant difference ( $p < 0.05$ ) in TPC and TFC values of the extracts according to the LSD test.

Similarly, the TFC values were calculated from the standard curve of catechin ( $y = 0.0152x - 0.0493$ ,  $R^2 = 0.99036$ , Figure 1, right). Table 3 showed that the TFC in the seed extract (70.76 mg CE/g DW) was also the highest, nearly twice as high as the TFC in the pulp extract (37.82 mg CE/g DW) and 1.6 times the TFC in the peel extract (45.43 mg CE/g DW). The TFC in *E. glaucum* fruits was greater than the TFC in *E. ventricosum* fiber part, and many foods like green tea, cocoa powder, and citrus fruits are considered to contain high levels of flavonoids [10, 11]. The TFC of *E. glaucum* seeds obtained Phuoc Binh National Park (Ninh Thuan province) was 8.60 mg QE/g DW [2]. Besides, this research showed some biological activities such as DPPH radical scavenging, ABTS radical cation decolorization, reducing power, and xanthine oxidase inhibitory abilities. *E. glaucum* seeds have great potential for antioxidant and xanthine oxidase inhibitory activities based on Pearson's analysis's high total polyphenol and flavonoid contents [2]. Therefore, the high content

of polyphenols and flavonoids in *E. glaucum* fruits may further investigate the chemical composition and biological effects.

#### 4. Conclusions

*Ensete glaucum* fruits contain flavonoids, saponins, tannins, terpenoids, and amino acids. In addition, the existence of high levels of polyphenols and flavonoids in the peels, pulps, and seeds of *E. glaucum* fruits might indicate the potential of the fruits to investigate some biological effects further. This is the first study about seeds, pulps, and peels of *E. glaucum* not only supplies evidence about chemical but also promotes the development of medicinal herbs and products from parts of this plant for real-life applications. Moreover, this study will also improve conservation research on this rare plant.

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