ANTIOXIDANT ACTIVITIES OF ETHANOL AND HOT WATER EXTRACTS FROM *DENDROCALAMUS ASPER* BAMBOO SHOOTS IN AN GIANG, VIETNAM

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Abstract:

This study examined the phytochemical components, extractive contents, and antioxidant capabilities of *D. asper* shoot extracts. Reflux extractions were performed in stages using 70% ethanol and hot water. Total phenolic content, physical-chemical properties, and antioxidant activity (IC_{50}) were examined. To calculate the total phenolic, the Folin-Ciocalteau assay and the aluminum chloride assay were utilized. By using the DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging experiment, the antioxidant activity was measured. The results showed that all examined extracts contained carotenoids, saponins, tannins, and organic acids. In comparison to the hot water extract group, the ethanol extract group had a considerably greater extractive content (11.68%) than the other group (6.9%). After ethanol extraction, the samples' total phenolic content was at its highest (32.3 mg GAE/g). With regard to antioxidant activity, *D. asper* shoot ethanol extracts were at their lowest (IC_{50}: 72.71 mg/ml).

Keywords: *Dendrocalamus asper*; Antioxidant activity; Reflux extraction; Free radical scavenging activity; Bamboo extracts; Bamboo shoots.

Introduction

Due to its well-known advantages and uses, bamboo has long been regarded as one of the most commercially significant plants in the world [1]. Its potential as a component of contemporary functional foods and nutraceuticals has recently attracted the attention of scientists. Every component of bamboo, including the leaves, shoots, culm sheath, and culms, is used in some way, either for food or for therapeutic purposes [2]. According to various studies, they contain less cholesterol and more of the nutrients our bodies need, such as proteins, carbs, vitamins, fibers, phytosterols, amounts of minerals, and carbohydrates [2] [3]. The young shoots are becoming more and more well-known around the world as a healthy food since they taste great and are full of vitamins, antioxidants, and bioactive substances. Shoots may be beneficial for your health in terms of their anti-inflammatory, serum cholesterol-lowering, anti-cancer, and cardiovascular disease prevention properties [4] [5]. Multiple studies have shown that food processing methods reduce the nutrients, bioactive chemicals, and antioxidants in processed meals [6]. In contrast to the remaining 60% that are processed and sold, only around 40% of the bamboo shoots gathered each year are used for fresh consumption [7].

Chain reactions involving free radicals are one of the key components of antioxidant activity. Free radicals, which can cause oxidative damage, are byproducts of routine biological interactions. These pathways aid in the synthesis of energy, the breakdown of lipids, the release of catecholamines in response to stress, and inflammatory activities [8]. The detrimental effects of free radicals, which can cause oxidative damage, would be lessened in the body if a balance between their production and removal was preserved. But if the free radicals are not successfully neutralized, oxidative stress can arise. Numerous diseases, such as cancer, heart disease, depression, and a variety of other ailments, have been shown to be associated with oxidative stress, which is caused by reactive oxygen, or free radicals [9]. The physiologically relevant phytochemicals that are naturally found in plants help them fight off a variety of dangerous microorganisms by exhibiting antimicrobial activity through inhibition or killing processes [10]. Alkaloids, flavonoids, saponins, and tannins are phytochemicals that appear naturally in plants and have been linked to a variety of health benefits,

including antioxidant activity [11] [12]. They have been shown to be anti-diarrheal, anti-aging, anticancer, and tumor-reducing agents. They also control blood pressure, hypertension, and obesity. They also shield our bodies against cardiovascular diseases and potential carcinogens [13] [14]. Antioxidants, which have a number of biological effects including anti-oxidants, anti-inflammatory, anti-hypertensive, anti-aging, anti-atherosclerosis, and anti-tumor, play a vital role in the protective effect of plant foods [15] [16]. Bamboo shoots are an excellent source of phenols, flavonoids, vitamin C, vitamin E, and minerals like selenium, iron, manganese, copper, and zinc. By eliminating anti-nutrients, processing bamboo shoots enhances their flavor, lengthens their shelf life, and detoxifies them [17].

D. asper, sometimes known as sweet bamboo, is a tropical clumping bamboo with a wide range of uses and a high economic value [18] [19]. *D. asper*, commonly known as rough bamboo, black bamboo, or gigantic bamboo, has moderately thick walls and grows to a height of 20 to 30 meters. It has an internode length of 20 to 45 cm and a diameter of 8 to 20 cm [20]. Reference [21] asserts that *D. asper* is widespread throughout Southeast Asia, including Thailand, Vietnam, Malaysia, Indonesia, and the Philippines, despite the fact that its original origins are unknown. Other tropical nations like Ghana, Benin, the Democratic Republic of the Congo, Kenya, and Madagascar have recently received *D. asper* introductions. In tropical Asia, *D. asper* thrives best in humid areas with rich, heavy soils that range in altitude from the lowlands to 1500 m and receive an average annual rainfall of about 2400 mm. With the right care, it may also thrive in semi-arid settings. While the higher internodes are used to produce containers and cooking pots, the mature stems are utilized to make furniture, musical instruments, domestic goods, handicrafts, and paper [22]. Considered to be the finest of all tropical Asiatic bamboos, the sensitive young shoots are eaten as a vegetable. For *D. asper* propagation, the rhizome, stems, and branch cuttings can all be employed. The propagates are grown in a nursery, and after the roots have sprouted, they are planted in the field before or during the first half of the monsoon season. The optimal time to harvest stems is during the dry season. Adult stems should be harvested when they are 5-7 years old, but some adult tillers should still be left in the clump [23]. The bamboo literature contains many outstanding evaluations on the effective use and practical uses of bamboo, with a focus on sustainable bamboo production in the sense of the bioeconomy and the circular economy [24]. Bamboo shoots are abundant in dietary fibers, vitamins, minerals, and dietary fibers while being low in fat [25]. Numerous biological characteristics of phytochemicals have been found to be advantageous to human health [26] [27], including anticancer, antibacterial, anti-inflammatory, and antifungal actions [28]. Despite the great nutritional value of edible bamboo shoots, research on their nutritional makeup is limited [28].

Despite numerous published studies examining the anti-oxidant qualities and phytochemical components of *D. asper* shoots, there are still just a few data reports, particularly in Vietnam. Since the antioxidant capabilities of *D. asper* bamboo shoot extract are important for functional purposes, this study's goal is to investigate them. Free radical scavenging activity and total phenolic content are shown to be related. The knowledge gained from this study is anticipated to inspire people to investigate the possibilities of employing bamboo shoot extracts as a source of natural antioxidants.

Materials and Methods

Materials

The *D. asper* bamboo shoots were gathered from mature bamboo growing in the An Giang province of southern Vietnam's Bay Nui region. The fresh *D. asper* samples were correctly washed with tap water and dried by air. Then, chop into pieces that are between 0.5 and 1.0 cm in size. In a grinder mill, samples were then ground to a coarse consistency. Ethanol 70^0, ascorbic acid, methanol, gallic acid, the Folin-Ciocalteu reagent, sodium carbonate, and 1,1-diphenyl-2-picrylhydrazyl were the chemicals used (Merck, Germany).

Methods

D. asper shoots were extracted using ethanol 70^o

In a round flask, 25 g of dried powders of the *D.asper* shoots were soaked in 100 ml of the solvent (1 g/4 ml), and the mixture was then refluxed for around three hours while using ethanol 70^0 for two hours at 60°C –70°C [2] [29]. Direct filtering using Whatman filter paper was used to remove the

roots of *D. asper* from the heated mixture. The *D. asper* extracts were divided by centrifugation at a speed of 60 rpm and a temperature of 45°C and then stored separately at a temperature of 4°C in bottles for later use.

D. asper extract yield calculation

To calculate the moisture content of extracts, the loss on drying method was used [30].

The following equation defines the water content:

$$W = \left(1 - \frac{W_1}{W0}\right) \times 100\ (\%)$$

Where:

W : water content (%);

W_1: Weight of the extract prior to drying (gram)

W_0: Weight of the dried extract (gram)

The following equation served as a definition for the extraction yields of *D. asper* extracts [30].

$$DEY = \frac{m_1}{m_0} \times 100\ (\%)$$

Where:

m_1: mass of the extract following solvent separation (gram)

m_0: the dry weight of the *D. asper* power extracted (gram)

DEY: Dried extraction yield (%)

Triplicates of each analysis were performed.

Preliminary phytochemical screening

Following a slightly modified version of the standard protocol, the phytochemical analysis of the following components was carried out: Fixed oil, carotenoid, alkaloid, flavonoid, steroid, tannin, organic acid, and saponin [31].

Determination of total polyphenol content

The total polyphenol content of the ethanol extracts was calculated using the Folin-Ciocalteu technique [32]. 10 mg of standard gallic acid was dissolved in 100 mL of distilled water to create a solution with a concentration of 100 µg/mL. The calibration curve was created using pure water and a solution of gallic acid (10–60 µg/mL). First, make a stock solution using 100 mL of distilled water and 10 mg of each *D. asper* extract to achieve a concentration of 100 µg/ml. The tube was then filled with 1 mL of stock solution and 5 mL of Folin-Ciocalteu reagent, thoroughly shaken, and incubated for 10 minutes at room temperature. Then, 4 mL of a 7.5% sodium carbonate solution was added, thoroughly mixed, and kept at the appropriate temperature in the dark for 60 minutes. Additionally, a blank solution was created. At 765 nm, absorbance was then determined. Using the standard curve equation y= ax + b, the total phenolic content in the extracts was calculated in terms of gallic acid equivalent (mg/g). Finally, the total amount of polyphenol in each extract was calculated using the gallic acid standard curve equation. The calibration curve was used to calculate the samples' concentrations. Triplicate test samples were examined.

Antioxidant capacity evaluation

Using the DPPH method, the free radical scavenging ability of several extracts (ethanol and hot water) has been assessed [2]. *D. asper* extracts were diluted to produce solutions at concentrations of 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml, and 600 µg/ml in distilled water. After thoroughly combining three milliliters of each dilution with one milliliter of DPPH solution (0.5 mM/ml in methanol), the mixtures were left at room temperature for 30 minutes in the dark. Using a UV-Vis spectrophotometer and methanol as a blank, the absorbance at 517 nm was determined. The

antioxidant activity was measured using ascorbic acid as a reference standard and positive control. As DPPH radical scavenging activity rises, DPPH absorbance falls. Results were presented as an IC_{50} value, which is the concentration at which the DPPH radical is 50% inhibited. The following formula was used to determine the proportion of DPPH that the extracts were able to scavenge:

The *D.asper* extracts were diluted in distilled water to make 100 μg/ml, 200 μg/ml, 300 μg/ml, 400 μg/ml, 500 μg/ml, and 600 μg/ml dilutions. Three milliliters of each dilution was mixed with 1 ml of DPPH solution (0.5 mM/ml in methanol) and mixed thoroughly, then left in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using UV–Vis spectrophotometer with methanol as blank. Ascorbic acid was used as a positive control and a reference standard in the antioxidant activity. The DPPH absorbance decreases with an increase in DPPH radical scavenging activity. Results were expressed as IC50 concentration where 50% inhibition of the DPPH radical is obtained [33]. The percentage scavenging of DPPH by the extracts was calculated according to the following formula:

$$\%\ \text{DPPH Radical scavenging} = \frac{A_b - A_s}{A_b} x\ 100$$

Where:

A_b : The blank's absorption

A_s : The sample's absorbance

There were three duplicates of each test run. The average and standard deviation (SD) of the data are presented. The statistical analysis of the results was performed using Microsoft Excel 2007.

Results and Discussion

Physicochemical characteristics

Using solvents with progressively increasing polarity, the extractive yields of *D. asper* shoot extracts were determined. According to Table 1, the yield of bamboo shoot powders was about 5.71% [34]. Table 1 presents extraction yields. Ethanol extract showed maximum yield (11.68 %), followed by hot water (6.91%)). The extracts had different percentages of dry yield. The percentage of dry yield is affected by the solvents utilized in this investigation. Ethanol emerged as the more effective extraction solvent among the two employed solvents [35].

Table 1: Physicochemical properties of bamboo shoot powders and extracts

Sample	Bamboo shoots	
	Ethanol	Hot water
Water content of powders (%)	5.7120 ± 0,0270	5.7120 ± 0,0270
Dried extraction yield (%)	11.68±1,91	6.91±0,25
Water content of extracts (%)	15.09±2,94	17.74±1,55

Phytochemical screening

The phytochemical studies of those prepared two extracts were analyzed on the basis of a well establish method reported by [31]. Table 2 displays the results. According to the findings of the phytochemical analyses of the extracts in Table 2, the extracts contain fixed oil, carotenoids, tannin, organic acids and saponins. The extracts of young bamboo shoots are high in fixed oil, carotenoids, and saponins, according to phytochemical screening [34]. These results were in agreement with [34], which reported that the extracts of bamboo shoots did not contain alkaloids, steroids, or some flavonoids.

Table 2: Preliminary phytochemical screening of *D.asper shoots*

Phytochemical	
Fixed oil	++
Carotenoid	++
Alkaloid	-

Flavonoid	-
Steroid	-
Tannin	+
Organic acid	+
Saponin	+++

Lengend: +++= very strong present, ++= strong present, + = Present; − = absent

Total phenolic content

Table 3 displays the total polyphenolic content of various *D. asper* extracts. The calibration curve's regression equation, y = 0.0081x + 0.1269, R^2 = 0.9982, was used to compute the total phenolic content, which was represented as mg/ml of gallic acid equivalents (GAE) per 100 mg of various extracts (Figure 1). Total polyphenol (TP) concentrations from ethanol extract and hot water extract were 32.2972mg/ml and 32.0503 mg/ml, respectively, of GAE per 100 mg of evaporated shoot extracts. This demonstrated that the total phenolic content of bamboo shoot extracts was not different.

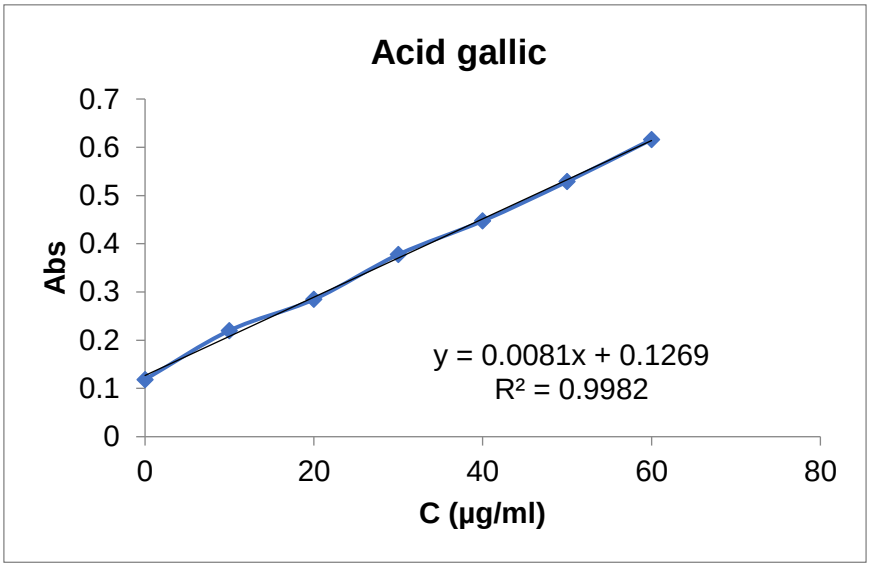

Figure 1: Correlation between absorbance value (Abs) and concentration of gallic acid

Table 3: The total polyphenol of different solvent extracts .

Sample	Polyphenol content (mgGAE/g extract)	
	Ethanol extract	Hot water
Shoot	32.2972±1,9845	32.0503±0,5992

Antioxidant activity

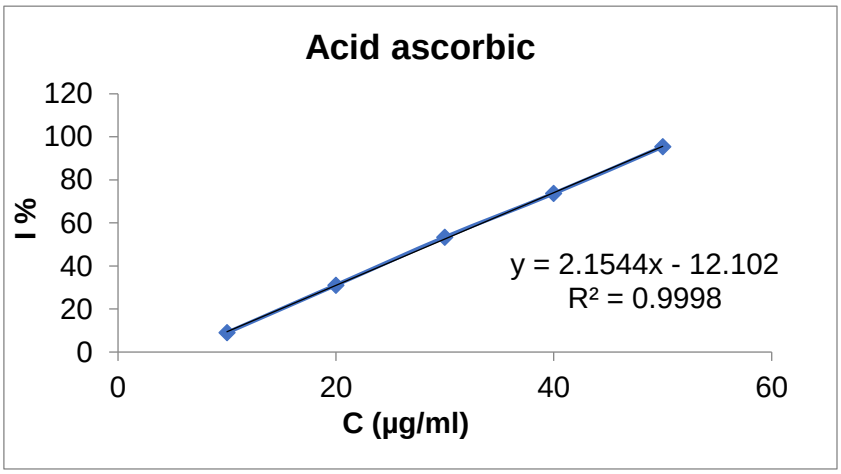

Figure 2: Correlation between percent free radical inhibition (I%) and ascorbic acid

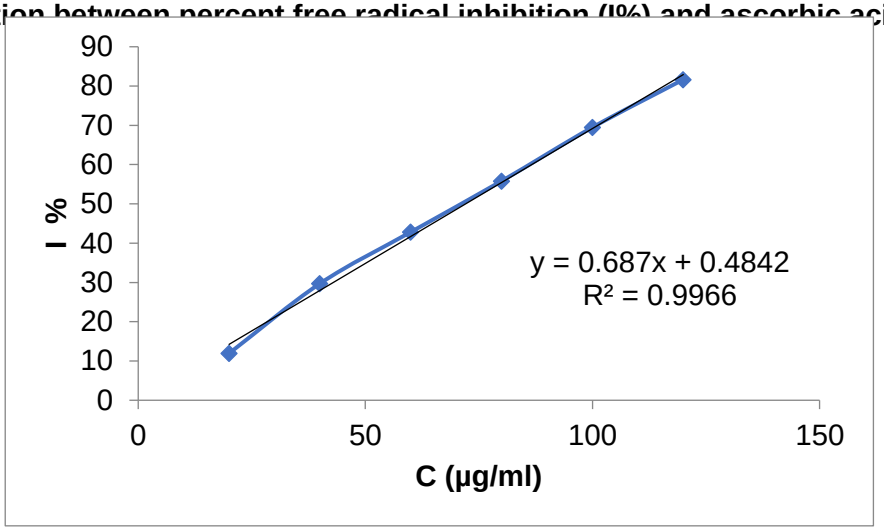

Figure 3: Comparison of antioxidant activity of ascorbic acid and ethanol extracts

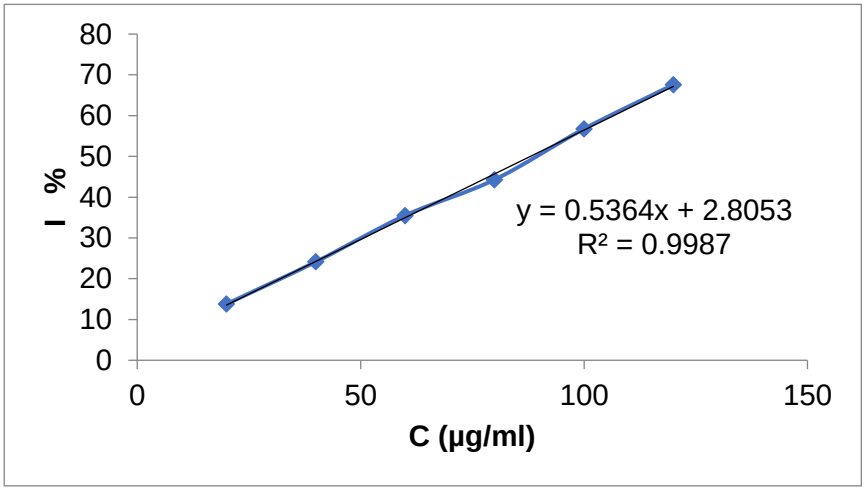

Figure 4: Comparison of antioxidant capacity of ascorbic acid and hot extracts

Table 4: Antioxidant activities of different solvent extracts .

Sample	Antioxidant activity (IC_{50} (µg/mL)	
	Ethanol extract	Hot water
Shoot	72.709	87.984

The DPPH radical-scavenging assay is a procedure that relies on a substance's capacity to lower DPPH radicals. A hydrogen atom that has been donated by a donor compound or antioxidant forms a non-radical form of DPPH-H. The antioxidant activities of the extracts were expressed as the IC_{50} value as shown in Figure 3 and Figure 4. The IC_{50} value with the lowest value represents the strongest antioxidant activity. In general, polar solvents showed modest antioxidant activity (hot water, and 70% ethanol). As positive controls, gallic acid and ascorbic acid were utilized. The results showed that polar soluble extracts had no significant antioxidant activities [35]. The ethanol extract of *D.asper* showed the highest antioxidant activity with an IC_{50} value of 72.709 µg/mL. As a result, it

is inferred that the polar extracts of *D. asper* bamboo shoots may one day serve as a natural source of antioxidants.

Using solvents with increasing polarity, a technique known as successive extraction is used to extract a wide variety of chemicals (ethanol and hot water). Using 70% ethanol, the maximum yield of polar chemicals was produced. This was consistent with a prior investigation of the *Citrus hystrix* peel extract that was soluble in ethyl acetate, which found that the amount of phenolic chemicals changed depending on the polarity of the solvent used [36]. According to the study's results, *D. asper* bamboo shoot extracts had good phenolic compound concentrations, a little amount of DPPH-scavenging action, and some helpful phytochemical substances. Particularly from the polar extracts of *D. asper* shoots (hot water and 70% ethanol), some bioactivity can be anticipated. It is hoped that the results of this study will serve as a guide for more thorough research on the extracts from D. asper bamboo shoots as sources of natural antioxidants from non-wood forest products. Future studies should concentrate on the discovery and more precise quantification of phenolic compounds as well as an analysis of their antioxidant properties when exposed to various radical types.

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