EVALUATION OF ANTIOXIDANT CAPACITY IN DENDROCALAMUS ASPER EXTRACTS CULTIVATED IN AGRICULTURAL FIELDS OF AN GIANG PROVINCE, VIETNAM

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Abstract:

This research aims to evaluate the antioxidant capacity of Dendrocalamus asper extracts from different parts of the plant grown in An Giang province, Vietnam, under agricultural conditions. The study utilized leaves, culms, and roots of Dendrocalamus asper, which were extracted using hot water and ethanol through reflux extraction. The antioxidant capacity of the extracts was assessed using the DPPH assay. The results revealed that the extracts from various parts of Dendrocalamus asper exhibited different levels of antioxidant activity, with the ethanol extracts showing the highest activity. The root ethanol extract showed the highest activity (600 mg/ml), followed by the culm extract (534,976 mg/ml) and the leaf extract (476,066 mg/ml). Additionally, the study found that ethanol extracts had higher free radical scavenging activity compared to water extracts.

Keywords: Dendrocalamus asper, Antioxidant capacity, Reflux extraction.

Introduction

A free radical is an atom or molecule containing one or more unpaired electrons in a valence shell or outer orbit and can exist independently. They are formed when a molecule loses or gains electrons; this lack of balance makes them unstable, short-lived, and highly reactive. To become stable again, they can easily donate an electron or accept an electron from other molecules, acting as oxidizing or reducing agents [1]. Free radicals are derived from normal essential metabolic processes in the human body. When an overload of free radicals cannot gradually be destroyed or in case of poor availability of the naturally occurring antioxidant body protection, free radicals can be harmful to live organisms and generate a phenomenon called oxidative damage. Thus, free radicals can cause biological molecule damage, such as DNA, lipids, and protein [2]. The oxidative breakdown of lipids can lead to degenerative diseases like cancer, inflammation, and atherosclerosis [3]. In the body, oxidative stress is an imbalance of free radicals and relates to potential cell damage like aging, cancer, and inflammation [4] [5].

Antioxidants have an important role in counteracting or reducing the negative impact of oxidants in the body caused by free radicals. Significantly, they show versatile health benefits such as anti-inflammatory, antihypertensive, antiatherosclerotic, and antitumor [6] [7]. In recent years, antioxidants have been widely used, either natural or synthetic. However, some studies indicated that the safety issues of synthetic antioxidants have been raised over time and increased the risk of some cancers because of their toxic effects [8]. Therefore, natural products' antioxidants are considered safe alternatives to synthetic antioxidants, as well as growing interest in natural antioxidants [9] [10].

Bamboo is the name given to a group of 1575 perennial evergreen plants in the grass family Poaceae, subfamily Bambusoideae [10]. With about 121 genera and 1662 species, the bamboo population can be divided into three zones geographically: the American zone, the Asian Pacific zone, and the African zone [11]. Bamboo is widely used as multipurpose material with all parts, such as roots, stems, and leaves, used for different purposes, bamboo stalks are used as building materials for houses and for handicrafts [12]. Furthermore, all bamboo parts have been used in traditional treatment for a long time to relieve hypertension, sweating, paralysis, fever, cough, pneumonia, cardiovascular disease, osteoporosis, and arthritis [13] [14]. Some studies indicated that the bamboo extract is safe for medicine because it is low toxicity and has adequate capacity to

scavenge DPPH and other ROS radicals. Moreover, it can prevent cell damage and reduce stress because bamboo contains compounds such as flavonoids, caffeic acids, and phenolic acids. These secondary metabolites promise practical applications to develop products from bamboo extracts to prevent and treat diseases caused by free radicals [15]. A study in 2017 proved that the compounds like flavonoids, saponins, and terpenoids found in D.asper water extracts and ethanol extracts have antioxidant properties. The antioxidant capacity of D.asper shoots is highest at 64.80% [16].

The scientific name of er bamboo is Dendrocalamus asper (Schult.f.) Back. Ex Heyne. Dendrocalamus asper is known as sweet bamboo, which grows in clusters and does not have many thorns [17]. It is found in many countries, such as India, Thailand, Malaysia, Philippines, Indonesia, and Vietnam [18]. The primary nutrients in the Dendrocalamus asper are amino acids (3, 12 g ± 0, 06); vitamin E (0, 91 g ± 0, 31). Those results showed Dendrocalamus asper bamboos have many nutritional benefits for humans [19]. Since ages ago, D.asper shoots have become familiar food in Vietnamese meals or have been widely used in construction. However, some previous Vietnamese studies mainly focused on the nutrition of bamboo shoots. To know more about the importance of D.asper bamboo as a source of natural drugs, this research, D.asper was separated into three parts: roots, stems, and leaves extracted with ethanol and hot water. The purpose of this study was to provide perspectives and directions for future research using D.asper bamboo as a potential source of oxidants.

Materials and Methods

Materials

The parts of D.asper bamboo were collected from mature bamboo growing in the Bay Nui region, An Giang, in the South of Vietnam. Medium-sized stems from 12 m to 15 m high, 5 -11 cm diameter. The fresh D.asper samples were washed properly under tap water and air-dried. Then, cut into small pieces ranging in size from 0.5 cm to 1.0 cm. After that, samples were grounded to a coarse consistency in a grinder mill. Chemicals used (Merck, Germany): ethanol 700, ascorbic acid, methanol, gallic acid, Folin-Ciocalteu reagent, Na2CO3, and DPPH (1,1-diphenyl-2-picrylhydrazyl).

Methods

Extraction of D.asper

Roots stems and leaves extracted with ethanol 70º

25 g of dried powders of each part (roots, stems, and leaves) were soaked in 100 ml of the solvent (1g/4 ml) and refluxed for about three hours in a round flask, using ethanol 700 for 2 hours at 60oC - 70oC [16], [20]. The hot mixture was directly filtered with Whatman filter paper to leave the D.asper residue of roots, stems, and leaves. The D.asper extracts were separated using centrifugation with a speed of 60 rpm, at 45oC, before being stored at 4ºC separately in bottles for further use.

Determination of D.asper extract yield

The loss on drying method was applied to determine the moisture contents of extracts [21].

The moisture content is defined through the following equation:

$$\mathrm{w} = \left(1 - \frac{\mathrm{a}_1}{\mathrm{a}_0}\right) \times 100\ (\%)$$

Where:

W : moisture (%);

a_1 : Weight of the extract before drying (gram)

a_0 : Weight of the extract after drying (gram)

The extractive yields of D.asper extracts of 3 parts were defined through the following equation

[21]:

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

Where:

m_1: weight of the extract after solvent separation (gram)

m_0: the dry weight of D.asper power used for extraction (gram)

H: efficiency (%)

All analyses were run in triplicate.

Preliminary phytochemical screening

The phytochemical screening confirmed the presence of phytoconstituents like fixed oil, carotenoid, alkaloid, flavonoid, steroid, tannin, organic acid, and saponin. Therefore, the presence or absence of the phytochemical constituents of the various parts of D.asper was analyzed using the following methodologies were adapted.

Determination of total polyphenol content

The ethanol extracts' total polyphenol content was estimated using Folin - the Ciocalteu method [22]. Standard gallic acid solution (100 µg/mL) was prepared by dissolving 10 mg of gallic acid in 100 mL of distilled water. Gallic acid (10-60 µg/mL) solution prepared in distilled water was used for making the calibration curve. First, dissolve 10 mg of each D.asper extract (roots, stems, and leaves) in 100 mL of distilled water to obtain 100□g/ml stock solution. Next, 1 mL of stock solution and 5 ml of Folin - Ciocalteu reagent were added to the tube, shaken well, and incubated for 10 minutes at room temperature. Then, 4 mL of 7, 5% sodium carbonate solution was added, shaken well, and stored in the dark for 60 minutes at the temperature. A blank solution was also prepared. Then absorbance was measured at 765 nm. The total phenolic content in the extracts was expressed in terms of gallic acid equivalent (mg/g) using the standard curve equation y=ax+b. Finally, calculating the total polyphenol in each extract was made with respect to the equation of the standard curve of gallic acid.

The concentration of the samples was determined using the calibration curve. Test samples were analyzed in triplicate.

Evaluation of antioxidant capacity

The free radical scavenging activity of different extracts (ethanol. hot water) has been evaluated using the DPPH method [16]. 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 (Prakash, 2000). 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 absorbance of the blank

A_s : The absorbance of sample

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD). Results were analyzed statically by using Microsoft Excel 2007.

Results and Discussion

The moisture content

Table 1 showed the moisture content of samples extracted from ethanol. The moisture content of ethanol extract of various parts of *D.asper* bamboo powders (stems, roots, and leaves) ranged from 14.07% to 14.81%. Analysis of variance (ANOVA) showed that there was no difference value of water content on various bamboo powders (*P>0.05*).

Table 1: The moisture contents of samples extracted from ethanol

Sample	Moisture content (%)
	Ethanol
Stems	14.07 ± 0.48
Roots	14.57 ± 1.05
Leave	14.81 ± 1.19

Table 2 showed the yields of ethanolic extracts of each part of *D.asper* ranged from 8.14% to 12.53%. This indicated that the various extracts had different percentages of extraction yield of leaves. The highest yield in the leaf extract was determined to be 12.53%. Analysis of variance (ANOVA) showed that there was a different value of the percentage of extraction yield on various bamboo powders (*P<0.05*). However, the bamboo stem extract had no difference from the bamboo root extract (*P>0.05*).

Table 2: Percentage of extraction yield

Sample	Moisture content (%)
	Ethanol extract
Stems	8.14 ± 0.2628
Roots	9.8 ± 1.5122
Leave	12.53 ± 1.7325

The details of results for the analysis of phytochemicals in ethanolic extract of various parts of bamboo were presented in Table 3. The major phytochemical detected were found to be fixed oil, carotenoid, flavonoid, saponin in the ethanolic extracts of stem, root and leaf. Flavonoid and saponin was the most common compound in the different parts of bamboo. Previous studies were reported flavonoids has various activities, including antioxidation [23] anti-inflammation [24] and anticarcinogenic activities [25] and so on. By ocular observation, the samples of leaf extract gave highly positive results for carotenoid, saponin and flavonoid (indicated by ++ and +++ in the Table 3). There was no presence of alkaloid in the different extracts. tannin and steroid were found in leaf extract. While organic acid was present in the root extract. The samples of leaf extract also showed highly positive results for carotenoids, flavonoids, and saponins. Similarly, [26] performed the phytochemical analysis on three bamboo species (Bambusa vulgaris, Bambusa ventricosa and Oxytenanthera abyssinica) in which leaves of all the species contained saponins, flavonoids, tannins. However, there were no alkaloids, carotenoids, and steroids in any of those species. [27] showed that phytochemical screening of extracts revealed the presence of flavonoids, terpenoids, alkaloids, tannins, alkaloids and phenolic compounds in the leaf. Detected the presence of saponin, flavonoids in the ethnolic extract of stem (Bambusa balcooa). Organic acid had a wide range of biological effects [28] and also had been found to have allergy-preventive activity [29]. Some studies have revealed the health benefits of saponins on bone health, blood cholesterol levels, blood glucose level, and cancer risk. A diet rich in saponins has been shown to reduce dental caries, inhibit platelet aggregation, treat hypercalciuria, and act as an antidote against heavy metal poisoning [30] [31] Based on the preliminary phytochemical screening of different parts of *D.asper*, the extracts were good sources of different classes of bioactive compounds.

Table 3: Preliminary phytochemical screening of different parts of *D.asper*

Phytochemical	Stem	Root	Leaf
Fixed oil	+	+	+
Carotenoid	+	+	+++
Alkaloid	-	-	-
Flavonoid	+	+	++
Steroid	-	-	+
Tannin	-	-	+
Organic acid	-	+	-
Saponin	++	++	+++

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

Polyphenol content

The results of total polyphenolic contents of various extracts of *D.asper* were given in table 4. The total phenolic content was calculated from regression equation of the calibration curve (y= 0.0081x + 0.1269, R2= 0.9982) and was expressed as mg/ml of gallic acid equivalents (GAE) per 100 mg of differrent extracts.

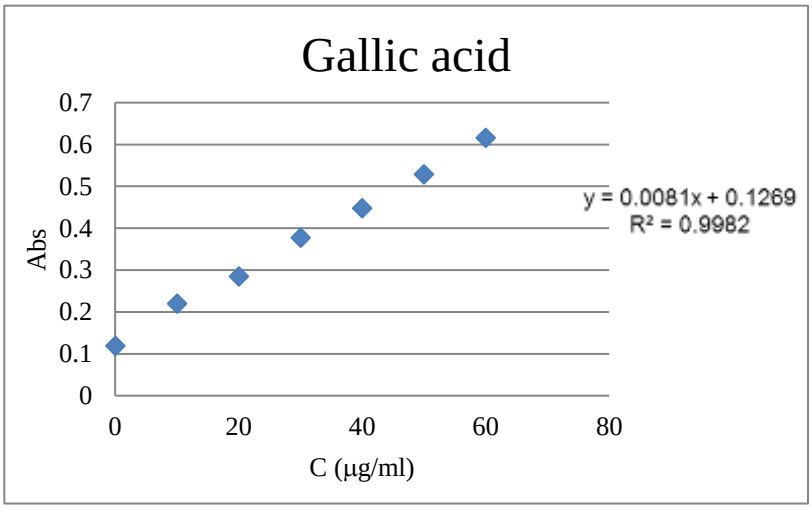

Figure 1: Correlation between absorbance value (Abs) and concentration of gallic acid

Table 4: The total polyphenol of different solvent extracts.

Sample	Polyphenol content (mgGAE/g extract)	
	Ethanol extract	Hot water
Stem	3.89± 0.2785	0.55± 0.323
Root	5.85± 0.604	1.74± 0,8197
Leave	5.85± 0.403	2.13± 0.7626

For water extracts, the polyphenol content of the leaf extract was highest with 2,13± 0,7626, the lowest polyphenol content was in stem extract.with 0,55± 0,323. However, the research indicated the polyphenol content of roots and leaves were the same for ethanol extracts, but the polyphenol content in stem was lowest with 3,89± 0,2785. Compared with the study of [32], the polyphenol content in Manh Tong bamboo leaf extract with ethanol solvent was lower than that in the Bamboo vulgaris leaf extract of the study of [32] .

Antioxidant capacity

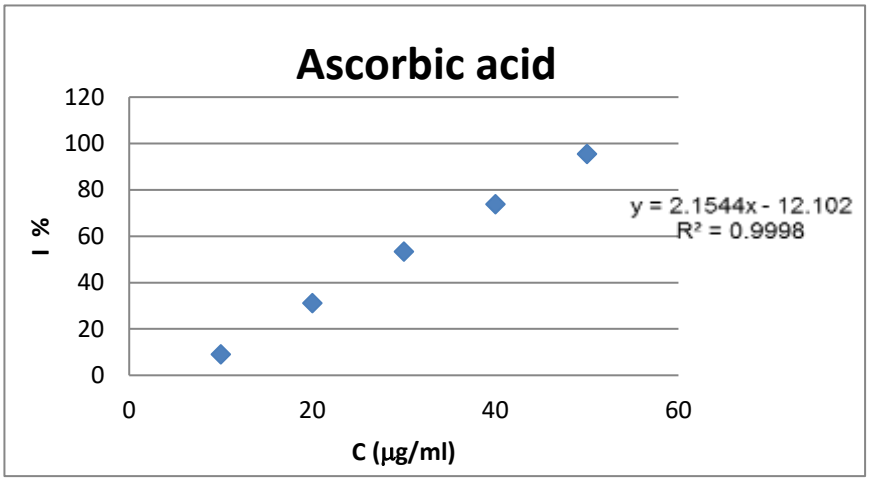

Figure 2: Correlation between percent free radical inhibition (I%) and ascorbic acid concentration

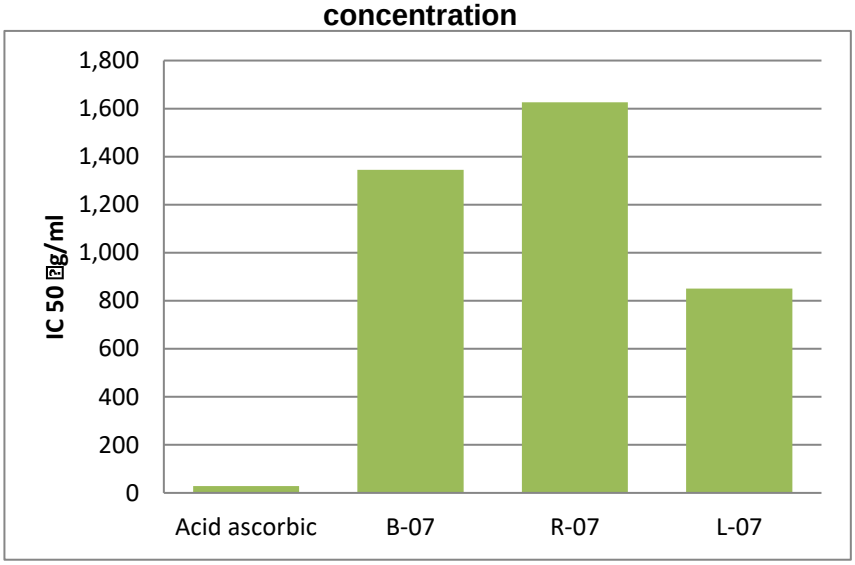

Figure 3: Comparison of antioxidant activity of ascorbic acid and ethanol extracts

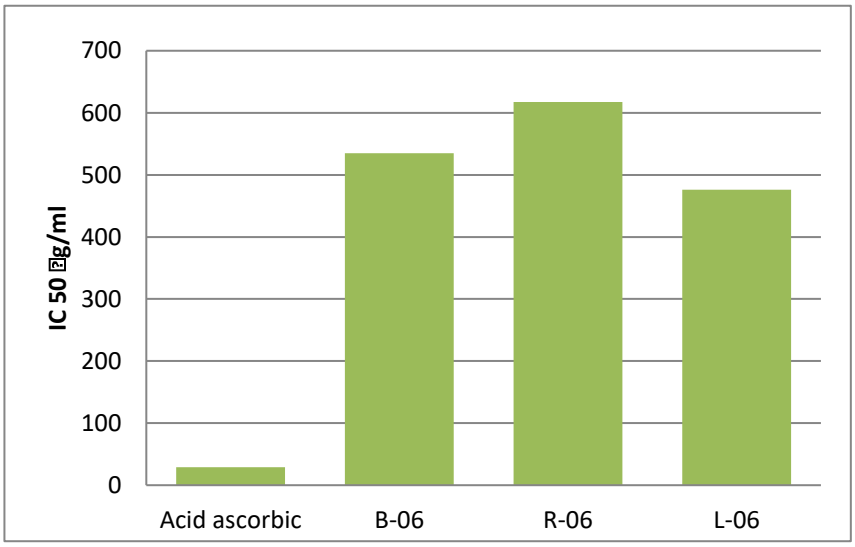

Figure 4: Comparison of antioxidant capacity of ascorbic acid and aqueous extracts

For ethanol extracts, leaves that had the highest antioxidant capacity were used to extract the extract with the lowest IC50 value. Root extract (R-06) has the lowest antioxidant ability because its IC50 value is the highest above 600 g/ml. The antioxidant capacity of ethanol extracts is higher than that of water extracts. Although leaf extract (L-06) has the maximum antioxidant capacity of the high types, it has a lower antioxidant capacity when compared to ascorbic acid. The Manh Tong bamboo leaf extract had a greater antioxidant capacity than the extract from [20] with an IC50 of 1017.20 g/ml when compared to the study of [33].

The root extract (R-03), which was extracted with water solvent, had the lowest extraction efficiency at 6, 11%, while the high extraction efficiency of leaves (L-02) extracted with ethanol solvent had the maximum extraction efficiency at 12,5324%. The leaf extract (L-04) extracted with ethanol yielded the maximum polyphenol content at 5, 8485± 0,403 (mgGAE/g extract).

The leaf extract with alcohol solvent exhibited improved antioxidant activity, but the antioxidant capacity was still lower than ascorbic acid, according to research on the antioxidant activity of the extract using ascorbic acid as a control (IC50 = 28,825 (μg/ml)).

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