Phytochemical Screening, Total Phenolic Contents and Antioxidant Activity of the Aqueous Extracts of *Dendrocalamus membranaceus* and *Thyrsostachys siamensis*

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Abstract:

This study aims to determine the phytochemical screening, total phenolic contents, and antioxidant activity of leaf and shoot extracts of *Dendrocalamus membranaceus* and *Thyrsostachys siamensis*. Each part of plant was extracted by boiling with distilled water in the solid to liquid ratio of 1 to 5 for 30 min twice. Phytochemical screening involved the methods to detect the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides and triterpenoids. Total phenolic contents were estimated with gallic acid as standard. Antioxidant activity was determined using 2, 2'-azino-bis (3-ethyl benzthiazoline-6-sulfonic acid) (ABTS) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assays. The result showed that flavonoids and saponins were presented in the aqueous extracts of both leaf and shoot of *D. membranaceus* and *T. siamensis*. Total phenolic content was the highest in shoot extract of *D. membranaceus* and the lowest in leaf extract of *D. membranaceus*. The leaf extract of *D. membranaceus* exhibited the highest antioxidant activity in ABTS and DPPH assays, with 24.0 and 503.0 µg/ml, respectively, and was higher activity than its shoot extract.

Keywords: Antioxidant activity, bamboo, phytochemicals, total phenolic contents

Introduction

Free radicals cause human diseases such as cancer, heart disease and cerebrovascular disease through multiple mechanism [1]. Antioxidants are substances which inhibit the oxidation of other molecules in our body and prevent the formation of free radicals by scavenging them leading to prevention of cancer and degenerative diseases, slowing down the aging process and promotion of cardiovascular health [2]. Natural antioxidants present in plant origin protect against these radicals and are therefore important tools in obtaining and preserving good health. Moreover, the different parts of the plant have different bioactivity [3-5]. Therefore, search for new plant sources containing antioxidants is continued for their utilization in cosmetic, functional food and pharmaceutical industries.

Tea from *Camalia sinensis* and herbal tea from the leaves, flowers, seeds, fruits and roots of plants other than *C. sinensis* are some of the most consumed beverages worldwide due to the attractive flavors and antioxidant activity [6]. Other plant infusions have become popular such as mulberry (*Morus alba*), lemon gress (*Cymbopogon citratus*), bamboo gress (*Tiliacora triandra*), sappan (*Caesalpinia sappan*) and bamboo leaf (*Sasa borealis*) [7, 8]. Bamboo is a group of genera of evergreen plants belonging to Poaceae, or grass family and is a multipurpose plant known mostly from its industrial uses but is now being recognized as a source of bioactive compounds and natural antioxidants [4]. There are more than 1250 bamboo species distributed all around the world and all the parts of the bamboo have clinical applications [2]. Wroblewska *et al.* [4] studied phenolic contents and antioxidant activity of five native Brazilian bamboo species such as *Aulonemia aristulata, Chusquea bambusoides, C. capituliflora, C. meyeriana* and *Merostachys pluriflora*. They found that the antioxidant activity which expressed in IC_{50} varied between 137.55-260 µg/ml and phenolic contents ranged from 43.64-87.81 mg of gallic acid equivalents (GAE) per g of plant material. Moreover, bamboo in genus Sasa such as *Sasa borealis* and *Sasa palmata* were developed into herbal tea [7, 8].

Although many bamboo species have already been studied, little is known about the antioxidant activity of bamboo species in the East of Thailand, for example *D. membranaceus* and *T. siamensis*. In addition, water is a universal solvent and environmental friendly. Moreover, water soluble phenolic only important as antioxidant compound [9] and flavonoids and phenolic compounds [10]. For this reason, the aim of this study was to screen the phytochemicals and to determine the total phenolic contents and antioxidant activity of the water extraction of *D. membranaceus* and *T. siamensis*, which was further selected to develop into herbal tea.

Materials and methods

Plant materials

Fresh leaf and shoot of bamboos (*D. membranaceus* and *T. siamensis*) (Figure 1) were collected from Burapha University, Sakaeo Campus. The leaves were washed with tap water, dried at 50˚C for 3 days in tray dryer and grinded to powder. The shoots were also washed with tap water, cut into small pieces, dried at 50˚C for 3 days and grinded to powder. The powder was contained in plastic bag and kept in the desiccator until used.

Figure 1 The figure of *D. membranaceus* (A) and *Thyrsostachys siamemsis* (B)

Plant extraction

The powder sample of 60 g was extracted in boiling water of 300 ml for 30 min twice. The aqueous extract of the bamboo was filtered through filter paper (Whatman No. 1), evaporated at 80˚C using water bath for 12-24 h or until dried, and then kept in refrigerator until used.

Determination of percentage yield (%)

The percentage yield of the extract was determined using the weight of extract after evaporation the solvent (a) and the weight of sample (b) using equation as followed:

$$\text{percentage yield } (\%) = \frac{a}{b} \times 100$$

Phytochemical screening

The crude extracts of leaves and shoots were tested for the present of alkaloids, flavonoids, saponins, tannins, cardiac glycosides and triterpenoids according to the previous report [11-13].

Total phenolic contents

The total phenolic contents were determined using Folin-Ciocalteau reagent as described by Prior *et al.* [14]. Briefly, 100 µl of 2.5 µg/ml extract were added to the test tube and combined with 2 ml of 2% Na_2CO_3. The tubes were vortexed and allowed to react at room temperature 2 min. After that, 100 µl of Folin-Ciocalteu reagent were added to the test tube and incubated in the dark at room temperature for 30

min. The absorbance of the mixture was measured at 750 nm. Standard calibration curve for gallic acid was prepared in the same manner and the results were expressed as miligrams gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract).

Antioxidant activity determination

ABTS radical cation scavenging activity assay

In this method, the radical scavenging capacity was measured by using ABTS radical cation ($ABTS^{\cdot+}$). The assay was carried out according to Re *et al.* [15]. For $ABTS^{\cdot+}$ generation from ABTS, 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) was reacted with 5 ml of 14 mM ABTS in the dark at room temperature for 16 h so that it reached a stable oxidative state. The working solution was prepared by diluting the mixture with ethanol to achieve the absorbance of 0.700±0.020 at 734 nm. The extract of 100 µl at various concentrations (0.2-2.4 mg/ml) was added to 2 ml of $ABTS^{\cdot+}$ solution, mixed well and allowed to react at room temperature for 6 min. The absorbance was measured at 734 nm comparing to the butylated hydroxyl toluene (BHT) as standard.

DPPH radical scavenging activity assay

The DPPH radical scavenging activity was measured using the methods by Bor *et al.* [16]. Briefly, 0.1 ml of different concentrations of the extract (0-50 µg/ml) and 0.9 ml of distilled water were added to 4 ml of 1 mM DPPH in methanol solution, mixed well followed by incubation in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Antioxidant activity was expressed as IC_{50}, which was defined as the concentration of the extract required to inhibit the formation of DPPH radicals by 50%. Ascorbic acid was used as reference standard.

Results and discussion

Percentage of yield

The results showed that the percentage of yield of all extracts were found between 0.83-1.80% in terms of dry weight and the highest yield was associated with leaves of *T. siamensis* (1.80%), followed by shoots of *T. siamensis* (1.32%), shoots of *D. membranaceus* (0.93%) and leaves of *D. membranaceus* (0.83%) as shown in Figure 2.

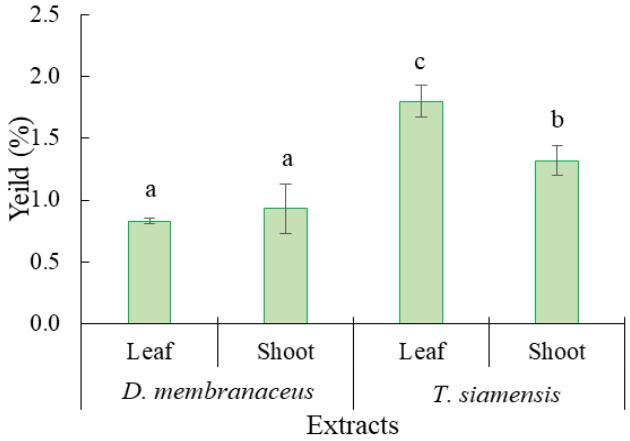

Figure 2 Percentage of yield

Phytochemical screening of extracts of leaves and shoots of bamboo

The leaf and shoot extracts of *D. membranaceus* and *T. siamensis* were subjected to qualitative phytochemical analysis. The result showed that alkaloids, cardiac glycosides and triterpenoids were absent in the leaf and shoot extracts and tannins were absent in the shoot extract of the both bamboos (Table 1). The conclusion is that the different parts of plant could have different phytochemical compound, which may contribute to different pharmacological effect of each part [3, 5]. The presence of flavonoids and

saponins in the bamboo extract were also found in the *Oxytenanthera abyssinica* and *Bambusa ventricosa* in Ghana [17], *Gigantochloa manggong* [18] and *Schizostachyum Lumampao* in Philippine [19].

Total phenolic contents

The total phenolic contents were estimated using gallic acid as standard. The result of total phenolic contents from aqueous extracts of different parts of *D. membranaceus* and *T. siamensis* are shown in Figure 3. Among the four crude extracts, shoot extract of *D. membranaceus* contained the highest (315.2±11.8 mg GAE/g) amount of total phenolic contents followed by shoot (174.0±16.4) and leaf (173.6±6.4) extract of *T. siamensis*, and then leaf (120.7±1.3) extract of *D. membranaceus*. Wroblewska *et al.* [4] reported that the total phenolic contents of five bamboo species were between 43.6-87.8 mg GAE/g, which was lower than total phenolic contents from *D. membranaceus* and *T. siamensis* (120.7-315.2 mg GAE/g).

Table 1 Phytochemical screening of the aqueous extracts of *D. membranaceus* and *T. siamensis*

Phytochemicals	Aqueous extracts			
	D. membranaceus		*T. siamensis*	
	Leaf	Shoot	Leaf	Shoot
Alkaloids	-	-	-	-
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	-	+	-
Cardiac glycosides	-	-	-	-
Triterpenoids	-	-	-	-

Present (+), Absent (-)

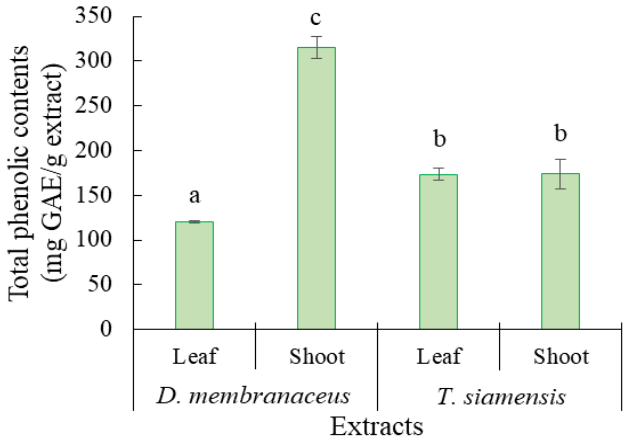

Figure 3 Total phenolic contents of the bamboo extracts

Antioxidant activity

In this study, the antioxidant activity of *D. membranaceus* and *T. siamemsis* was measured using two different assays, namely ABTS and DPPH. The single assay to evaluate the antioxidant activity would not achieve the correct result since the bioactivity of plant extract is influenced by many factors [3]. The antioxidant activity of the extracts which reported in IC_{50} value are shown in Table 2.

ABTS assay depends on the antioxidant ability to scavenge ABTS radical. The antioxidant capacity of lipophilic and hydrophilic compounds could measure in the same sample in this assay [3]. In this study, BHT was as positive control for ABTS radical scavenging activity assay. The leaf extract of *D. membranaceus* was found to be effective in scavenging the ABTS radical than the others. The IC_{50} of the leaf extract of *D. membranaceus* was 24.0 µg/ml while that of BHT was 27.4 µg/ml. This result indicated that the scavenging of the ABTS radical by the leaf extract of *D. membranaceus* was found to be higher

capacity than standard. This result shows that the leaf extract of *D. membranaceus* presents a good ability to scavenge the ABTS radical.

The effect of antioxidants on DPPH radical was thought to be due to their hydrogen donating ability to the free radicals and reducing it to nonreactive substances [20]. In this study, ascorbic acid was as positive control for DPPH radical scavenging activity assay. The DPPH radical scavenging activity of the aqueous leaf and shoot extracts of *D. membranaceus* was 503 and 581 µg/ml, respectively. The result revealed that the extracts of *D. membranaceus* were more activity than the extracts of *T. siamensis* which measured by the lower IC_{50} value, but it has lower antioxidant activity compared to ascorbic acid (1.85 µg/ml). When compared to DPPH scavenging ability of the five native Brazilian bamboo species (137-260 µg/ml) [4], the IC_{50} of the extract of *D. membranaceus* and *T. siamensis* exhibited a lower radical scavenging activity.

Based on the phytochemical screening that has been done, the bamboo extracts contains a various bioactive compounds that have the potential as an antioxidant such as flavonoid and saponins, which might be synergistically work together to prevent oxidative stress and neutralize the negative impact of free radical [18].

Table 2 Antioxidant activity of the bamboo extracts

Bamboo	Parts	Standard	IC_{50}	
			ABTS (µg/ml)	DPPH (µg/ml)
D. membranaceus	leaf		24.0±0.00	503.1±0.01
	shoot		27.6±0.01	581.1±0.00
T. siamensis	leaf		61.2±0.00	1048.0±0.02
	shoot		31.2±0.00	816.0±0.01
Control		ascorbic acid	ND	1.85±0.00
		BHT	27.4±0.00	ND

ND = not determined, IC_{50}: Inhibition concentration means the concentration needed to inhibit 50% of the radical formation.

Conclusions

Phytochemical screening of leaf and shoot extract of *D. membranaceus* and *T. siamensis* revealed that the presence of flavonoids and saponins. Among the extracts, the shoot extract of *D. membranaceus* shows the maximum total phenolic contents. The leaf extract has highest ABTS free radical scavenging activity. The next step of these research will be developing the bamboo leaf into herbal tea.

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