Research paper

Extractive Contents and DPPH-Scavenging Activities of Bamboo Leaf Extracts from *Gigantochloa atter*, *Dendrocalamus asper*, and *Gigantochloa verticillata*

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【Summary】

In this study, we investigated the extractive contents, phenolic content, and DPPH-scavenging activity of leaf extracts of 3 commonly cultivated bamboo species (*Gigantochloa atter*, *Dendrocalamus asper*, and *G. verticillata*) from Tlogoadi Village, Yogyakarta, Indonesia. Extractions were successively done by reflux, using n-hexane, ethyl acetate, methanol, and hot water. The extracts were subjected to the following procedures: phytochemical screening for alkaloids, saponins, and tannins; a colorimetric assay for total phenolic and flavonoid contents; and a DPPH radical-scavenging capacity assay. Results showed that hot water produced the highest yields of *G. atter* (3.62%) and *D. asper* (3.16%), while n-hexane produced the highest yield of *G. verticillata* (2.46%). Phytochemical screening detected saponins in the hot-water extract of *G. atter*, and tannins were present in the ethanol (70%, v v^{-1}) extract of all 3 bamboo species. Total phenolic and flavonoid contents were highest in the ethyl-acetate extract of *D. asper* (26.09 mg gallic acid equivalents g^{-1}) and *G. verticillata* (92.67 mg quercetin equivalents g^{-1}), respectively. The hot water-soluble extract of *G. verticillata* showed the lowest 50% inhibitory concentration of 662.41 μg ml^{-1}. The present study indicates that the 3 bamboo leaf extracts can potentially be used as natural sources of antioxidants and phytochemicals, especially the polar extract of *G. verticillata* with its high flavonoid content and mild DPPH-scavenging activity.

Key words: phytochemical screening, phenolic, antioxidant, flavonoid, successive extraction.

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研究報告

三竹種(*Gigantochloa atter*, *Dendrocalamus asper*, *Gigantochloa verticillata*)竹葉萃取物含量及清除DPPH自由基活性

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摘 要

本研究旨在調查印度尼西亞日惹特區Tlogoadi村三種常見栽培竹種(*Gigantochloa atter*, *Dendrocalamus asper*, *Gigantochloa verticillata*)竹葉中萃取物與酚類的含量及清除DPPH自由基活性。試驗方法是採用回流法，依序使用正己烷、乙酸乙酯、甲醇和熱水進行萃取。萃取物之生物鹼、皂苷和單寧含量透過植物化學篩選程序測定，總酚、類黃酮含量及和DPPH自由基清除能力則透過比色法測定。研究結果顯示，*G. atter*和*D. asper*以熱水萃取產率最高，*G. verticillata*以正己烷萃取產率最高，產率分別為3.62，3.16和2.46%。植物化學篩選檢測結果，皂苷可在*G. atter*以熱水萃取，以乙醇(70%, v/v)萃取可在三竹種中取得單寧。總酚和類黃酮含量以乙酸乙酯萃取，含量最高分別是*D. asper*的26.09 mg GAE/g及*G. verticillata*的92.67 mg QE/g。*G. verticillata*的熱水溶性萃取產物顯示IC_{50}值最低，為662.41μg/ ml。總結，本研究顯示三種竹葉萃取物具備被用來萃取抗氧化劑和植物化學物質天然來源的潛力，尤其是*G. verticillata*含有高含量的類黃酮和溫和清除DPPH自由基活性的極性萃取產物。

關鍵詞：植物化學篩選、酚類、抗氧化劑、類黃酮、連續提取。

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INTRODUCTION

Bamboos are large lignocellulosic plants in the family Gramineae. They are distributed in many tropical, subtropical, and temperate countries such as Indonesia, Brazil, China, Africa, Australia, Japan, and India. Bamboo is an important non-wood forest product (NWFP), since some parts of the stem are extensively utilized (Agustino et al. 2011). The plant is often used as an ornamental and to provide environmental service; the main stem is used as a construction material for building houses and bridges. Generally, bamboo is used in furniture and handicrafts because it is cheap and lightweight (Benton 2015). In Indonesia, there are about 7 genera and more than 60 known species of bamboo. Legi (*Gigantochloa atter*), Petung (*Dendrocalamus asper*), and Wulung bamboo (*G. verticillata*) are bamboo species cultivated especially on Java island, Indonesia. *Gigantochloa atter* and *D. asper* are mostly utilized for construction, while *G. verticillata* is mostly used for handicrafts and musical instruments (Rifai 1995).

Bamboo has been used as medicine since ancient times. The leaves of some bamboo species are used to treat stomach heat, colds, and arthritis (Hossain and Islamm 2015). Several compounds, such as flavones, phenolic acids, quinones, glycosides, and fatty acids, are found in bamboo leaf extracts (Gong et al. 2014). Previous studies found some bioactivity in leaf extracts of various species. Fatty acids and their derivatives found in *G. apus* leaves exhibited antibacterial activity against pathogenic *Escherichia coli* (Mulyono et al. 2013). Further, fatty acids are never mentioned in term of antioxidant activity. The diverse chemical structures of alkaloids and saponins are thought to contribute to the antioxidant activity. Leaves of *Bambusa arundinacea* are known to exhibit antileprotic and anticoagulation activities, and they are also utilized to alleviate hemoptysis symptoms (Khare 2007). Meanwhile, an extract of leaves of *Phyllostachys nigra* bamboo was shown to exhibit antioxidant activity, and it is used as a standardized food antioxidant in China (Hu et al. 2000, Gong et al. 2014).

Antioxidants are molecules, ions, or stable radicals that are able to prevent the oxidation of other molecules; oxidation can be caused by the activities of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Pinchuk et al. 2012). Some radical reactions are suspected of causing oxidative stress, which can lead to various diseases such as diabetes, arteriosclerosis, aging disorders, and cancers (Halliwell and Aruoma 1991, Apak et al. 2007, Pinchuk et al. 2012). Further, antioxidant agents are often used as additives by the food industry to preserve products for longer periods (Carocho and Ferreira 2013). In recent times, both synthetic and natural antioxidants have been used in many kinds of industries. However, some common synthetic antioxidants, such as butylated hydroxyl anisole (BHA) and butylated hydroxy toluene (BHT), were associated with toxicity and carcinogenicity (Ito et al. 1985, Kahl and Kappus 1993, Caleja et al. 2017). This concern has increased interest in naturally derived antioxidants as safer alternatives than synthetic antioxidants, as well as increasing the popularity of products that use natural antioxidants (Kumar and Pruthi 2014, Carocho and Ferreira 2013).

Due to limited information on leaf extracts of *G. atter*, *D. asper*, and *G. verticillata*, the purpose of this study was to investigate the extractive contents of *G. atter D. asper*, and *G. verticillata* leaves by analyzing total phenolic (TPC) and flavonoid contents (TFC), and qualitatively detecting certain compounds and antioxidant activities. The information obtained by this research is expected to encourage people to explore the possibility of using bamboo leaf extracts as a source of natural antioxidants.

MATERIALS AND METHODS

Preparation of bamboo leaf extracts

Fresh young bamboo leaves of *G. atter*, *D. asper*, and *G. verticillata* were harvested from Tlogoadi Village, Sleman, Yogyakarta, Indonesia (175 m in elevation). Young leaves that had a lighter green color and were located near or at the tip of bamboo stems were selected from 2 m in height of the trunk. These were further dried at room temperature, milled, sieved with a 20~40 mesh, and analyzed for moisture content following the ASTM D 4442-92 (Reapproved 03).

Successive extraction

Powdered leaf samples of each species (50 g in dry weight; DW) were successively extracted by reflux, using the solvents, n-hexane, ethyl acetate, methanol, and hot

water, for 6 h for each solvent. Meanwhile, ethanol (70%, v v^{-1}) extraction (48 h, cold extraction) was conducted separately as a control. The extracts were filtered and evaporated before being stored separately in bottles. The extracted contents were calculated based on the leaf DW, and all analyses were run in triplicate.

Phytochemical screening for alkaloids, saponins, and tannins

The n-hexane, ethyl-acetate, methanol, hot-water, and ethanol 70% extracts were subjected to phytochemical screening following methods described by Pochapski et al. (2011) with slight modifications. Phytochemical screening was then used to identify some classes of secondary metabolites (Table 2). Each test was replicated 3 times. Result are presented as a summary of the replications.

Screening for alkaloids

Alkaloids were screened by adding 50 mg of extract to 5 ml of a chloroform-ammonia mixture; this was then filtered into a test tube. A few drops of 2 M H_2SO_4 were added to the filtrate and shaken to form 2 layers. After that, the top layer (which was clear) was placed in a glass container. Drops of Meyer reagent were added to the top layer. If a white precipitate formed, then the extract was positive for alkaloids.

Screening for saponins

In total, 50 mg of an extract was dissolved in 5 ml of water in a test tube. After heating for about 5 min, the extract was filtered and shaken. After shaking, if a visible froth appeared that did not disappear for 10 min and remained stable after adding 2 N HCl, then the extract was positive for saponins.

Screening for tannins

Methanol (5 ml) was added to dissolve 50 mg of an extract in a test tube. After heating the tube, the extract was filtered through filter paper (8μm pore size). To the filtrate was added 5~10 drops of 1% $FeCl_3$. If the filtrate became dark blue or dark green, then the extract was positive for tannins.

Colorimetric assay for TPC and TFC

The assay for the TPC was carried out using Folin-Ciocalteau reagent according to the method described by Baba and Malik (2015) with slight modifications. An aliquot (0.5 ml) of the extract was diluted with methanol to a concentration 1 mg ml^{-1}. Folin-Ciocalteau reagent at 2.5 ml that had been diluted 10 times was added to diluted extracts, and the solution was further incubated for 2 min at room temperature. After incubation, 2 ml of Na_2CO_3 (7.5%) was added and the solution was incubated for 30 min. The absorbance was analyzed using an ultraviolet-visible (UV-VIS) spectrophotometer at a wavelength of 765 nm. Furthermore, a blank test was also carried out with no extract. A calibration curve was prepared using gallic acid with the same procedure. The TPC was expressed as milligrams gallic acid equivalents (GAE) per gram of sample. All analyses were run in triplicate, and average values are reported.

The TFC was measured using an aluminum chloride assay method described by Diouf et al. (2009) with slight modifications. An aliquot (2 ml) of the extract at a concentration of 1 mg ml^{-1} was added to 2 ml of 2% $AlCl_3.6H_2O$. After being incubated for 1 h at 20℃, the absorbance was analyzed with a UV-VIS spectrophotometer at a wavelength of 415 nm. Furthermore, a blank test was carried out with no extract. A calibration curve was made using quercetin with the same procedure, and the TFC was expressed as mil-

ligrams quercetin equivalents (QE) per gram of sample. All analyses were run in triplicate, and the average is reported.

Scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals

The antioxidant activity was analyzed using a DPPH-scavenging capacity assay described by Gong et al. (2014) with slight modifications. An aliquot (0.1 ml) of an extract at various concentrations (250, 500, 1000, and 2000 μg ml^{-1}) was added to 5 ml of a DPPH solution. The mixture was shaken by hand and incubated in a dark place for 30 min. A blank determination was done without the addition of the extract. The absorbance was analyzed using a UV-VIS spectrophotometer at 516 nm. Inhibition was calculated using the following equation:

$$Inhibition\ (\%) = ((A_0 - A_1) \times 100) / A_0$$

where A_0 is the absorbance of the blank, and A_1 is the absorbance of the sample. The antioxidant activity was expressed as the concentration that can inhibit DPPH radicals by 50% (IC_{50}) using a graph function created for the inhibition. In this experiment, gallic acid and catechin were used as positive controls of natural antioxidants. All analyses were run in triplicate, and their average was calculated.

Statistical analysis

Data were analyzed using SPSS software (vers. 25, IBM, New York, NY, USA). A two-way analysis of variance (ANOVA) at a confidence level of 95% was used to determine the effects of bamboo species (*G. atter*, *G. verticillata*, and *D. asper*) and solvents (n-hexane, ethyl acetate, methanol, and hot water). Thereafter, a post-hoc test was performed using Tukey's honest significant difference (HSD) method to determine the significance level of the data.

RESULTS

Extraction yield, TPC, and TFC

Extractive yields of leaf extracts of the 3 bamboo species (*G. atter*, *D. asper*, and *G. verticillata*) were determined using solvents with successively increasing polarity. Each extract, except for the n-hexane-soluble extract, was analyzed with colorimetric assays. Extraction yields are shown in Table 1. *Gigantochloa verticillata* revealed the highest soluble extract with *n*-hexane (2.46%), ethyl acetate (1.68%), and total extractive yield (8.81%), while *G. atter* had the highest yields of soluble extracts with methanol extraction (2.58%), hot water (3.62%), and 70% ethanol (4.96%). Further, the TPC and TFC are shown in Figures 1 and 2. The ANOVA revealed a significant difference in the interaction of the two factors of TPC ($p < 0.05$) and TFC ($p < 0.01$). The highest TPC

Table 1. Percentage of extraction yield with successive extraction

Bamboo species	Extraction yield (%)					
	n-hexane	ethyl acetate	Methanol	hot water	Total extractive[1)]	ethanol 70%[2)]
G. atter	0.93	0.91	2.58	3.62	8.04	4.96
D. asper	1.76	1.43	2.12	3.16	8.47	4.75
G. verticillata	2.46	1.68	2.40	2.27	8.81	4.58

Data shown are mean from three replication

[1)] Total extractive yield is a sum of the successive extraction yield.

[2)] n-hexane – hot water extractions were done successively, ethanol 70% extraction were done separately.

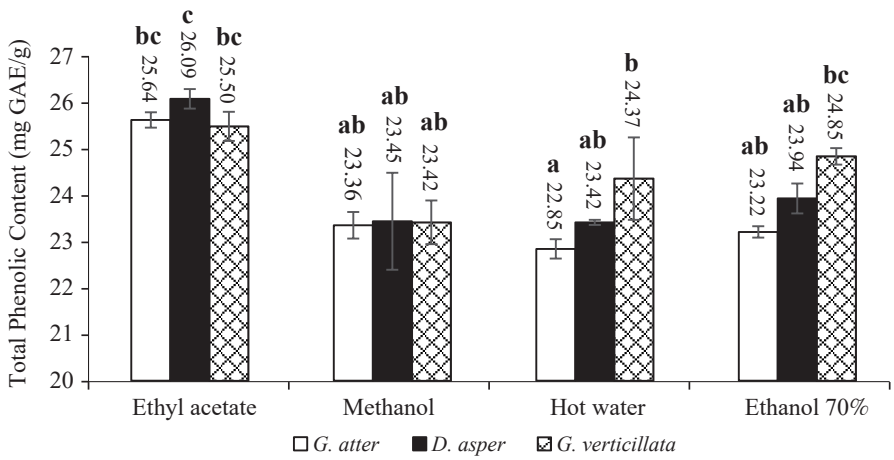

Fig. 1. A: Total phenolic content of ethyl acetate, methanol, hot water, and ethanol 70% soluble extract.

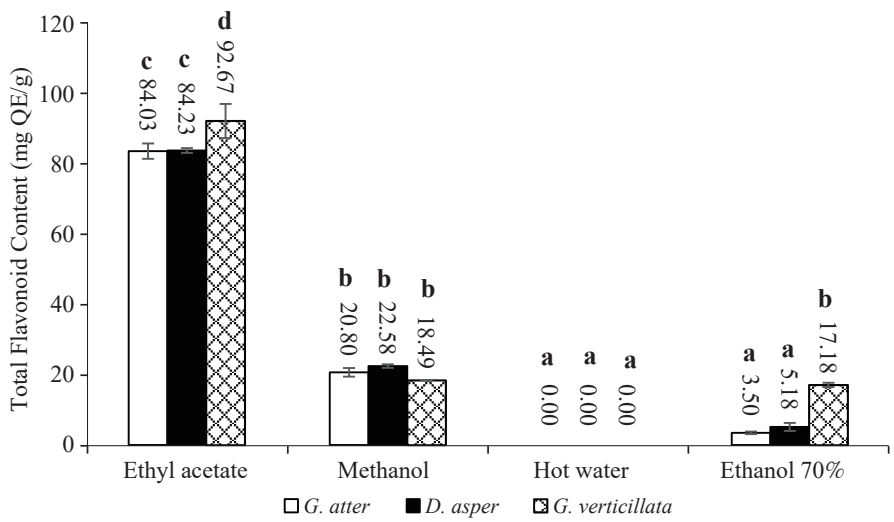

Fig. 2. Total flavonoid content *G. atter*, *D. asper*, and *G. verticillata* on ethyl acetate, methanol, hot water, and ethanol 70% soluble extract.

and TFC were found in the ethyl acetate-soluble extract of *D. asper* (26.09 mg GAE g^{-1}) and *G. verticillata* (92.67 mg QE g^{-1}), respectively. Meanwhile, TPCs were found to be lower in methanol- and hot water-soluble extracts, and no flavonoids were measured in the hot water-soluble extracts.

Phytochemical screening for alkaloids, saponins, and tannins

Amounts of alkaloids, saponins, and tannins detected in leaf extracts of the 3 bamboo species (*G. atter*, *D. asper*, and *G. verticillata*) are shown in Table 2. Saponins, tannins, or alkaloids were not detected in ethyl-acetate or methanol extracts of the 3 bamboo species. The presence of saponins was only detected in the hot-water extract of *G. atter*. Tannins were detected in the ethanol (70%) extracts of all 3 bamboo species.

Table 2. Phytochemical screening for saponin, tannin, and alkaloid

Classes of compounds	Tests	*G. atter*			
		Ethyl acetate	Methanol	Hot water	Ethanol 70%
Saponin	Frothing	(-)	(-)	(+)	(-)
Tannin	Ferric chloride	(-)	(-)	(-)	(+)
Alkaloid	Mayer	(-)	(-)	(-)	(-)
		D. asper			
		Ethyl acetate	Methanol	Hot water	Ethanol 70%
Saponin	Frothing	(-)	(-)	(-)	(-)
Tannin	Ferric chloride	(-)	(-)	(-)	(+)
Alkaloid	Mayer	(-)	(-)	(-)	(-)
		G. verticillata			
		Ethyl acetate	Methanol	Hot water	Ethanol 70%
Saponin	Frothing	(-)	(-)	(-)	(-)
Tannin	Ferric chloride	(-)	(-)	(-)	(+)
Alkaloid	Mayer	(-)	(-)	(-)	(-)

The results are average from three replications. (-) = no presence; (+) = presence of compound.

DPPH radical-scavenging capacity

The DPPH radical-scavenging assay is a method that depends on the ability of a compound to reduce 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 activity of the extract was expressed as the IC_{50} value as shown in Figure 3. The highest antioxidant activity is shown by the lowest IC_{50} value. In general, mild antioxidant activities were exhibited by polar solvents (ethyl acetate, methanol, hot water, and 70% ethanol). Meanwhile, *n*-hexane-soluble extracts showed low antioxidant activity. Gallic acid and quercetin were used as positive controls, and a comparison showed respective values of 92.51 and 169.05 μg ml^{-1}. An ANOVA of the antioxidant activity of various polar solvents showed a significant interaction between the various species and solvent factor ($p = 0.032$).

Tukey's HSD test showed that polar-soluble extracts had no significant antioxidant activity, except for the ethyl acetate-soluble extract of *G. verticillata* which was significantly milder. The ethanol (70%)-soluble extract of *G. verticillata* showed the highest antioxidant activity with an IC_{50} value of 566.79 μg ml^{-1}. Compared to the positive controls, the extract showed mild antioxidant activity (Ethanol soluble extract of G. verticillata IC_{50} was ±6.12 and ±3.35 times the IC_{50} values of gallic acid and quercetin, respectively).

DISCUSSION

Successive extraction is a method used to extract a broad range of compounds using solvents with increasing polarity from non-polar (*n*-hexane) to polar (hot water). *G.atter* showed the highest yield of polar compounds, while *G. verticillata* showed the highest yield of apolar compounds. The apolar n-hexane-soluble extracts showed a dark color, and it was assumed to contain phenolics, albeit to a lesser extent. The highest yield of polar compounds was obtained using 70% ethanol in all species. Further, TPCs of the 3 bamboo spe-

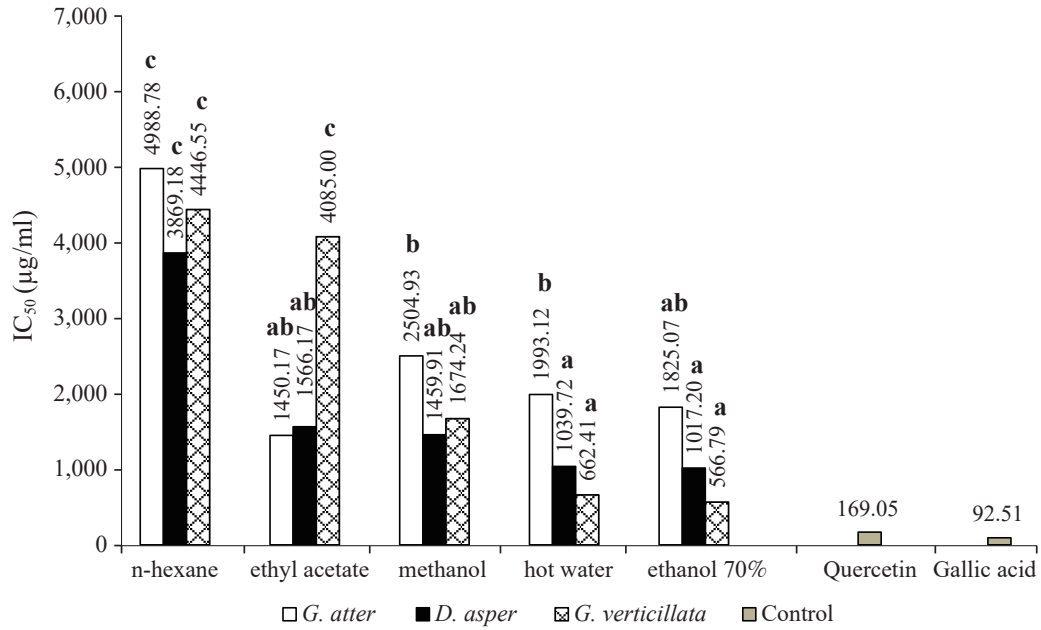

Fig. 3. IC_{50} (inhibitory concentration to scavenge the DPPH radical by 50%) of *G. atter*, *D. asper*, and *G. verticillata* on ethyl acetate, methanol, hot water, and ethanol 70% soluble extract. Quercetin and gallic acid were used as positive control. Lower value indicates stronger antioxidant activity.

cies were higher in semi-polar (ethyl acetate) extracts, while the polar extractive showed lower amounts with no significant difference among the solvents. Meanwhile, TFCs of the 3 species declined as the polarity of the solvent increased, and flavonoids were not present in hot water-soluble extracts. These results indicate that the 3 bamboo leaf extracts with relatively low polarity were dominated by phenolic compounds as seen with quercetin. This was in alignment with a previous study on the ethyl acetate-soluble peel extract of *Citrus hystrix*, where the amount of phenolic compounds varied based on the polarity of the solvent used (Wijaya et al. 2017).

Phytochemical screening detected that saponins were present in the hot-water extract of *G. atter*. Saponins are glycosides that consist of an aglycone, either a choline steroid or triterpenoid, and a sugar side chain that attaches through a bond in C3 (Vicken et al. 2007). Previous studies explained some of the benefits of ingesting saponins by humans because of the hypocholesterolemic, immunostimulatory, antioxidant, and anticarcinogenic properties they possess (Coffie et al. 2014). However, glycosidation of phenolics lowers the antioxidant potency (Merrilon et al. 1997; Hopia and Heinonen 1999). In some cases, saponins are also known to be toxic at high doses, as they have hemolytic properties (Price et al. 1987). Therefore, their detection in the hot-water extract of *G. atter* could indicate additional health benefits to humans. However, their safety and toxicity should be further studied before they are applied to human diets.

Tannins were detected by phytochemical screening in 70% ethanol-soluble extracts. Tannins are hydrophilic polyphenol

compounds with large molecular weights of 500 to 3000. They have the capacity to form insoluble complexes with proteins, cellulose, pectin, and gelatin (Swain and Bate-Smith 1962, Lekha and Lonsane 1997). The tannins present in *G. atter*, *D. asper*, and *G. verticillata* are suggested to be proanthocyanidins (condensed tannins), because hydrolysable tannins are only found in dicotyledonous plants (Sule et al. 2005). Proanthocyanidins are known to have a wide range of beneficial health properties such as antioxidative, anticancer, and health-promoting activities against some diseases such as Alzheimer's disease and cardiovascular disease (Sato and Matsui 2012). Hot-water extracts of the 3 species of bamboo can potentially be used as dietary supplements with those beneficial properties. A portion of the flavonoids found in the successive extraction process was suspected of having a proanthocyanidin monomeric unit (flavan-3-ols), while condensed tannins with larger molecular weights were dissolved in ethanol (70%). This was further detected in the phytochemical screening.

Compared to previous studies, results of the DPPH-scavenging capacity assay showed similarities between the antioxidant activities of the hot-water and ethanol (70%) extracts from *G. verticillata* leaves and the antioxidant activity of ferulic acid (±4.84-fold gallic acid's IC_{50}), a natural antioxidant found in many plant parts including bamboo shoots (Villano et al. 2007, Kumar and Pruthi 2014). This result did not statistically different from the hot water-soluble extracts of *G. atter* and *D. asper*. Therefore, it can be deduced that the polar leaf extracts of the 3 bamboo species, especially that from *G. verticillata*, can potentially be used as a natural source of antioxidants.

Flavonoids are one of the main functional components in leaf extracts of some other bamboo species. Some of the flavonoids were identified as flavone C-glucosides, including isoorientin, vitexin, orientin, and isovitexin (Zhang et al. 2005). However, in this study, although the ethyl acetate-soluble extract of the 3 bamboo species showed high TPC and TFC values, their antioxidant activities were fairly low. Furthermore, the hot water-soluble extracts of the 3 bamboo species generally showed preponderant antioxidant activity even though no flavonoid content was detected. A similar trend was also observed in bark extracts of *Shorea macroptera* and *Neobalanocarpus heimii*, as they had high total phenolics but low antioxidant activity (Kawamura et al. 2011). Pietarinen et al. (2006) who reviewed the antioxidant properties of bark extracts from several species suggested synergism of the polyphenols, or there may be minor compounds that strongly contribute to the activity compared to the predominant compounds. This result therefore indicates that the antioxidant activity of the 3 bamboo species was not attributable to flavonoids that could be measured by the methods applied in this research. In the hot water-soluble extract, we suspect that long-chain proanthocyanidins contributed more to the antioxidative properties of the 3 bamboo extracts because their presence was detected during phytochemical screening. Further identification should be done to determine what compounds are responsible in the antioxidant activity observed in the hot-water extract

CONCLUSIONS

The maximum recovery of polar extracts of *G. atter*, *D. asper*, and *G. verticillata* was achieved using ethanol (70%). The highest yield of polar extracts was shown by *G. atter*, while *G. verticillata* was observed to have the highest yield of apolar extracts. The highest TPC and TFC were shown with the ethyl

acetate-soluble extract. *Dendrocalamus asper* had the highest TPC, while *G. verticillata* had the highest TFC. Phytochemical screening detected saponins in the hot water-soluble extract of *G. atter*, while tannins were detected in the ethanol (70%)-soluble extract of all 3 bamboo species. Higher antioxidant activity was shown by the 70% ethanol-soluble extract of *G. verticillata*, followed by *D. asper*, and the *G. verticillata* leaf extract exhibited effective DPPH radical-scavenging activity. It contained high amounts of TPC and TFC.

Results of this research indicate that leaf extracts from these 3 bamboo species exhibited good concentrations of phenolic compounds, mild DPPH-scavenging activity, and the presence of some beneficial phytochemical compounds. Some bioactivity can be expected especially from the polar (hot water and ethanol 70%) extracts of *G. verticillata* leaves. Hopefully, results of this research can be used as a reference for more in-depth studies of the extracts from these 3 bamboo species as sources of natural antioxidants from non-wood forest products. Future research should be focused on the identification and more-specific quantification of the phenolic compounds, as well as investigation of their antioxidant activities using different types of radicals.

ACKNOWLEDGMENTS

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