Evaluation of Antioxidant and Anticancer Activity of Steam Extract from The Bamboo Species¹

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ABSTRACT

Natural plant extract has been the subject of intense research aiming in elucidating the underlying mechanisms of their chemopreventive effects upon various forms of human cancers. The objective of our study was to evaluate the natural antioxidants and anticancer agent potential of *Phyllostachys*. The chemical composition of steam extract from *Phyllostachys* was carried out using GC-MS. The steam extract of *Phyllostachys* was dominated by monoterpenes (62.96% - 71.36%) and sesquiterpenes (23.58% - 33.13%) as the main compounds. The antioxidant activities of the steam extract was determined using a DPPH scavenging and hydrogen peroxide scavenging activity test systems. Furthermore, the amounts of total phenolics in steam extract were determined spectrometrically The steam extract of *P. pubescens* and *P. bambusoides* were presented the high activity (69.4% and 64.0%, respectively.). The steam extract from *Pyllostachys* species showed a hydrogen peroxide scavenging activity of approximately 50.4% - 54.6% when compared to that of the standard gallic acid. The anticancer activities of steam extract were determined using a MTT assay. Assessment of the cytotoxic effect of the steam extract on PC-3 cells showed that the *P. bambusoides* (20.85%) and *P. pubescens* (20.41%) were superior in induced cytotoxicity compared with the steam extract of *P. nigra* var. *henonis* (1.15%). Findings from this study indicated that steam extract of *P. bambusoides* and *P. pubescens* possessed potential as medicinal drug especially in prostate cancer treatment.

Keywords: Phyllostachys, antioxidant, anticancer, polyphenol, volatile compounds

1. INTRODUCTION

In recent years, the morbidity and mortality of cancer still reach a high plateau and is a major public health problem worldwide. One among the many reasons for the conversion of normal cells to cancerous cells are the free radicals that are generated (Halliwell *et al.* 1992) in the human body as a consequences of a number of endogeneous metabolic processes involving redox enzymes and bioenergetic

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electron transfer and exposure to a plethora of exogeneous chemicals. *In vivo*, some of these reactive oxygen species (ROS) play positive roles in cell physiology. Under normal circumstances, the ROS generated are detoxified by the innate defense antioxidant enzymes present in the body. However, owing to ROS overproduction and inadequate antioxidant defense, they may cause great damage to cell membranes by lipid peroxidation and induce mutations in DNA leading to cancer and other diseases (Aqil *et al.* 2006). Antioxidants may mediate their effect by directly reacting with ROS (Shukla *et al.* 1997).

Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases like cancer, cardiovascular and inflammatory diseases. Traditionally lipid oxidation controlled by the addition of powerful synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) (Monahan and Troy, 1997). However, new toxicological data on some synthetic antioxidants has led to caution regarding their use, as evidence has shown that they possess toxic, pathogenic and carcinogenic effects (Amarowicz et al. 2000). Hence, many recent investigations have been targeted at the identification of alternative novel antioxidants from natural sources which have similar properties (Hossain et al. 2008). Some components in natural products such as carotenoids, flavonoides, anthocyanins and phenolic compounds are known to function as scavengers in both primary and secondary oxidation process. In particular, it has been reported that potential antioxidants exist in a number of natural plant extract including grapes, green teas, berries, tomatoes and rosemary (Gahler et al. 2003; Nielsen et al. 2003).

Plants are also reported to possess metabolites capable of inducing apoptosis considered to be vital for targeted drug therapies (Amirghofran *et al.* 2007). Natural products are a rich resource of cancer chemotherapy drugs, and primarily target rapidly cycling tumor cells, experimental studies demonstrated that many naturally occurring agents and plant extract have anticancer potential in a variety of bioassay systems and animal models, having relevance to human diseases (Aziz *et al.* 2003), thus the long-standing interest in the identification of medicinal plants and derived natural products for developing cancer therapeutics increased steadily (Dai *et al.* 2011).

Plant extraction compounds have played an important role in the development of several clinically useful anti-cancer agents. The discovery of paclitaxel from the bark of the Pacific yew, *Taxus brevifolia* Nutt. (Taxaceae) is another evidence of the success in natural product drug discovery (Luduena, 1998). Paclitaxel is significantly active against ovarian cancer, advanced breast cancer, small and non-small cell lung cancer (Mehndiratta *et al.* 2011).

Taken together natural plant extract have been the subject of intense research aiming in elucidating the underlying mechanisms of their chemopreventive effects upon various forms of human cancers (Ziech *et al.* 2011).

Bamboo is a perennial plant of the Gramineae family, and is a giant, woody grass that grows in Korea, China, Japan, and Southeast Asia, and represents an important commodity (Choi *et al.* 2008). There are approximately 75 species and 200 varieties of *Phyllostachys*.

Some biologically active components in bamboo extract and their potential health benefits have recently been studied. The ethanol or water extract of bamboo leaves mainly contains flavone glycosides, phenolic acids, coumarone lactones, anthraquinones, and amino acids, and has also been utilized clinically in the treatment of hypertension, arteriosclerosis, cardiovascular disease, and certain forms of

cancer (Shibata et al. 1975). Wang and Ng (2003) reported on the antifungal protein isolated from bamboo shoots. Bamboo shoots are also regarded as a dietary source of natural phenolic antioxidants, particularly exhibiting dose-dependent inhibitory activity on angiotensin-converting enzymes (Park and Jhon 2010). In particular, the bamboo leaves have also been used as a clinical traditional medicine, mainly to lessen or cure stomach ache, diarrhea, and vomiting, chest diaphragm inflammation, restlessness, and excessive thirst (Lu et al. 2005). Many papers have indicated that a bamboo extract has multiple biological effects, such as anti-free radical, anti-oxidation, anti-aging, anti-bacteria, anti-cancer and cosmetic ingredient (Tang and Ding 2000). Although many researchers have attempted to study the biological activities of bamboo extract, only a few studies have reported on the evaluation of an extract from bamboo species.

In the present study, we investigated the utilization of *Phyllostachys* spp. such as *Phyllostachys* bambusoides, *Phyllostachys* pubescens and *Phyllostachys* nigra var. henonis as sources of natural antioxidants and anticancer agents. Therefore, the chemical composition, antioxidant activity (DPPH radical scavenging activity, hydrogen peroxide scavenging activity and total phenolic content) and anticancer activity of steam extracts from isolated *Phyllostachys* species were examined.

2. MATERIALS and METHODS

2.1. Plant Material

Healthy, mature bamboo of *P. bambusoides, P. pubescens* and *P. nigra* var. *henonis* were collected from natural populations in Gyeongsang National University Campus, in Korea. The collection of bamboo was done during July to August in 2012. The

bamboo leaves were cut into $1 \text{ cm} \times 3 \text{ cm}$ pieces with a scissors and preserved in refrigerator to be used within 12 hours.

2.2. Preparation of Extract

The extract was prepared using steam distillation (Yang *et al.* 2009). The fresh bamboo leaves (based on dry weight, 100 g of each) were added with 3 L of distilled water. And extraction was carried out with Gas-Assisted Process (GAP, K22D-10L, Japan) apparatus using liquefied petroleum gas as a heating source for 100 min. The steam extract was obtained by GAP was stored at refrigerator (4, ℃ below) before subsequent experiments. The yield of steam extract of *P. bambusoides*, *P. pubescens* and *P. nigra* var. *henonis* were 9.4%, 11.0% and 3.5%, respectively.

2.3. GC-MS Analysis

The steam extract $(2 \text{ m}\ell)$ was placed in a 22 m ℓ round-bottomed vial; then the vials were sealed with aluminium-rubber septa (Agilent Technologies Inc., USA). GC-MS consisted of an Perkin Elmer Clarus 600 gas chromatograph (Perkin Elmer, USA) equipped with DB-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies Inc., USA), and interfaced with Perkin Elmer Turbomass mass spectrometer (software version 4.1, Perkin Elmer, USA). Head space were as follows: oven temperature, 120°C at 20 min; transfer line temperature 105°C. Temperatures were as follows: injection port, 250°C; oven, programmed at 40°C for 3 min, then raised at 3°C/min to 220°C, and held for 4 min. MS conditions were as follows: capillary direct interface temperature, 250°C; ionization energy, 70 eV; mass range, 50 - 300 amu; scan rate, 2 scans/s. Helium was used as carrier gas at a constant flow rate of 0.96 ml/min. Component identifications were

made by comparing mass spectra from the total ion-chromatogram and retention indices using NIST and Wiley GC-MS libraries.

2.4. Determination of Antioxidant Activity

2.4.1. DPPH Radical Scavenging Activity

The steam extract was tested for their ability to scavenge DPPH radicals by DPPH radical scavenging assay as described by Burits and Bucar (2000). The steam extract from bamboo (0.5 m ℓ) were mixed with 3 m ℓ of 0.1 mM DPPH in ethanol was added to each tube and incubated in dark at room temperature for 30 min. Absorbance was read at 517 nm using a U-3000 UV-Spectrophotometer (Hitachi Inc., Japan). The radical scavenging activity was calculated as a percentage of DPPH discoloration using the following equation:

Radical scavenging activity (%) =
$$\frac{(Ac - A)}{Ac} \times 100$$
 (1)

where $A_{\rm c}$ is the absorbance of the control and A is the absorbance of the steam extract. BHT and quercetin were used as standard compound. All measurements were performed in triplicate.

2.4.2. Hydrogen Peroxide Scavenging Activity

A modified method based on that of Ruch *et al.* (1989) was used to determine the ability of the steam extract to scavenge hydrogen peroxide. Hydrogen peroxide (43 mM) was prepared in phosphate-saline buffer (pH 7.4). Standards (gallic acid) and steam extract solutions were prepared at concentrations of 0.1 to 1.0 mM. Aliquots of standard or steam extract solutions (3.4 m ℓ) were added to 0.6 m ℓ of hydrogen peroxide solution. The reaction mixture was incubated at room temperature for 10 min, and the absorbance was determined at 230 nm.

Blank solution contained the phosphate buffer without H_2O_2 . The percentage of H_2O_2 scavenging was calculated as:

%
$$H_2O_2$$
 scavenging effect (%) = $\frac{(Ac - A)}{Ac} \times 100$ (2)

where $A_{\rm c}$ is the absorbance of the control and A is the absorbance in presence of standard or steam extracts. All measurements were performed in triplicate.

2.4.3. Determination of Total Polyphenol Content

Total polyphenol content was determined by the method described by Lister and Wilson (2001). The steam extract (0.5 m ℓ) were mixed with 2.5 m ℓ of Folin-Ciocalteau reagent and 2 m ℓ of sodium carbonate (7.5% w/v) were added and the tubes incubated at 45°C for 15 min. Absorbance were read at 765 nm using a U-3000 UV-spectrophotometer (Hitachi, Inc., Japan). All measurements were performed in triplicate. Results were expressed in terms of gallic acid equivalence in µg/m ℓ .

2.5. Determination of Anticancer Activity

2.5.1. Cell Cultures

Human prostate adenocarcinoma PC-3 cells (PC-3 cell) were cultured in RPMI-1640 medium supplemented with 10% FBS (v/v) and penicillin (100 U/mℓ)/ streptomycin (100 mg/mℓ). Cultures were maintained in a humidified incubator at 37℃ in 5% CO₂/95% air.

2.5.2. MTT Assay Method

The *in vitro* cytotoxicity of steam extract from bamboo on PC-3 cells was measured with an MTT assay (Prencipe *et al.* 2009), as described. Briefly, cultured PC-3 cells were harvested, counted and plat-

ed (1 \times 10⁴ cells/well) in a 24-well plate and make up to 500 $\mu\ell$ with media.

After 24 hours of incubation to allow for attachment, different concentrations 0 and 40 $\mu\ell$ of steam extract samples were added to each well in duplicate. After the indicated incubation times, the medium was removed and 100 $\mu\ell$ of MTT solution (0.5 μ g/m ℓ in phosphate-saline buffer) was added to each well for 3 hours. Subsequently, MTT solution add 200 $\mu\ell$ of dimethyl sulfoxide (DMSO) transfer to 100 $\mu\ell$ /well of 96 well were measured by absorbance at 570 nm with ELISA reader (Spectra Max 250, USA).

2.6. Statistical Analysis

The means and standard deviations of the data were calculated. Evaluations of each group's significance were performed using SAS ver. 9.1 (SAS Institute, Cary, NC, USA) at the 5% significance level with Duncan's multiple range test.

RESULTS and DISCUSSION

3.1. Chemical Compositions

The steam extract was analyzed by GC-MS and the components were identified on the basis of their RI values and by comparison of their mass spectra with those reported in the literatures. The GC-MS analysis of the steam extract led to the identification of 22 different components, representing 94.46% - 96.57% of total volatile constituents.

Eighteen compounds were identified in steam extract of *P. bambusoides*, which accounted for 94.48% of the total amount; the major constituent was limonene (25.34%), followed by β -pinene (16.30%), (+)-epi-bicyclosesquiphellandrene (12.37%), cadinene (9.57%), γ -terpinene (8.58%) and myrcene (6.07%).

The GC-MS analysis of steam extract from *P. pubescens* showed 12 compounds representing 96.57% of the total amount; limonene was the main constituent (32.25%) followed by (+)-epi-bicyclosesquiphellandrene, γ -terpinene, myrcene and cedrol (21.96%, 13.20%, 11.00% and 7.44%, respectively). In the steam extract of *P. nigra* var. *henonis*, 14 constituents represented 94.94% of the total amount; the major constituent was limonene (27.38%), followed by β -phellandrene (21.87%), (+)-epi-bicyclosesquiphellandrene (14.70%), γ -terpinene (9.74%) and myrcene (7.78%).

The major class of substances in the steam extract of *P. bambusoides, P. pubescens* and *P. nigra* var. henonis were the monoterpene compound (62.96%, 63.44% and 71.36%, respectively), followed by sesquiterpene compounds (31.52%, 33.13% and 23.58%, respectively). Limonene and (+)-epi-bicyclosesquiphellandrene were the main constituents in the all species. Lu *et al.* (2003) reported that limonene showed antiangiogenic and proapoptotic effects on human gastric cancer implanted in nude mice, thus inhibiting tumor growth and metastasis. Misra *et al.* (2013) reported that (+)-epi-bicyclosesquiphellandrene showed antimicrobial and anticancer activities.

Kim et al. (2004) reported that the tea tree (Melaleuca alternifolia) oil has been suggested as a natural antioxidant alternative for BHT with the inherent antioxidant activity attributed mainly to the monoterpenes content such as α -terpinene and γ -terpinene. Tan et al. (2000) reported that significant effect on the treatment of glioma was reported using the sesquiterpenes which is found in small amounts in many essential oils: it prolonged quality survival time of patients with glioma. In addition, in vitro anticancer activity of oxygenated terpinene have been reported (Kim et al. 2004; Wu et al. 2012).

There was a variability in the chemical composi-

Table 1. Chemical composition of steam extract from Phyllostachys species

No	Compound	R.I. ¹⁾	Molecular Formula	Area (%)			
				P. bambusoides	P. pubescens	P. nigra var. henonis	Identification
1	α-pinene	936	C ₁₀ H ₁₆	1.55	3.08	2.32	GC ¹ -MS
2	β -pinene	978	$C_{10}H_{16}$	16.3	-	0.03	GC ¹ -MS
3	myrcene	988	$C_{10}H_{16}$	6.07	11.00	7.78	GC ¹ -MS
4	α -phellandrene	1003	$C_{10}H_{16}$	1.32	-	0.03	GC ¹ -MS
5	α -terpinene	1018	$C_{10}H_{16}$	1.75	-	2.18	GC ¹ -MS
6	<i>p</i> -cymene	1024	$C_{10}H_{14}$	0.66	-	-	GC ¹ -MS
7	limonene	1027	$C_{10}H_{16}$	25.34	32.25	27.38	GC ¹ -MS
8	β -phellandrene	1028	$C_{10}H_{16}$	_2)	0.04	21.87	GC ¹ -MS
9	γ -terpinene	1059	$C_{10}H_{16}$	8.58	13.2	9.74	GC ¹ -MS
10	terpinene-4-ol	1175	$C_{10}H_{18}O$	1.39	3.87	0.03	GC ¹ -MS
11	β -elemene	1391	$C_{15}H_{24}$	0.62	-	-	GC^2 -MS
12	thujopsene	1436	$C_{15}H_{24}$	-	-	4.51	GC ¹ -MS
13	β -humulene	1439	$C_{15}H_{24}$	1.06	-	-	GC ¹ -MS
14	(+)-epi-bicyclosesquiphellandrene	1682	$C_{15}H_{24}$	12.37	21.96	14.70	GC ¹ -MS
15	valencene	1995	$C_{15}H_{24}$	-	0.04	-	GC^2 -MS
16	cadinene	1999	$C_{15}H_{26}$	9.57	-	-	GC ¹ -MS
17	(-)-β-caryophyllene	2003	$C_{15}H_{24}$	0.76	-	-	GC¹-MS
18	oplopenone	1608	$C_{15}H_{24}O$	0.56	0.04	0.03	GC^2 -MS
19	au-cadinol	1635	$C_{15}H_{26}O$	1.65	-	-	GC ¹ -MS
20	au-muurolol	1641	$C_{15}H_{26}O$	-	3.61	0.03	GC^2 -MS
21	solavetivone	1870	$C_{15}H_{22}O$	0.56	0.04	-	GC^2 -MS
22	cedrol	2004	$C_{15}H_{26}O$	4.37	7.44	4.31	GC ² -MS
Monoterpene hydrocarbons			61.57	59.57	71.33		
Oxygenated monoterpenes			1.39	3.87	0.03		
Sesquiterpene hydrocarbons			24.38	22.00	19.21		
Oxygenated sesquiterpenes			7.14	11.13	4.37		
Total identified				94.48	96.57	94.94	

¹⁾ Retention indices relative to C₉-C₂₄ n-alkanes on the DB-5MS column; GC¹: Identification based on retention times of authentic compounds on DB-5MS column; GC²: Retention index according to literature; RI: Kovats retention index according to n-alkanes (C₉-C₂₄) on the DB-5MS column

tion of steam extract obtained from *Phyllostachys* species. These more qualitative, less quantitative differences in the volatile composition are primarily de-

termined by genetic and environmental factors and influenced by the analytical procedures applied (Walden *et al.* 2004).

²⁾ Not detected.

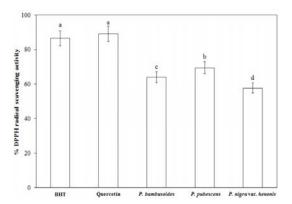


Fig. 1. The DPPH radical-scavenging activity of steam extract from *Phyllostachys* species. Results are expressed as percentage decrease in absorbance at a wavelength of 517 nm with respect to the control. BHT and quercetin were used as a standard. Each value represents mean \pm SD of three experiments, performed in triplicate. Different labels (a - d) indicate a significant difference at p < 0.05 (Control: BHT, quercetin).

3.2. Antioxidant Activity

Antioxidants are believed to have an important role in the maintenance of human health by rendering protection against the constant and unavoidable challenge of reactive oxygen species (Fridovich, 1998). And at least two testing systems have been recommended to deduce the antioxidant activities of crude plant extract owing to the complex nature of phytochemicals present (Schlesier *et al.* 2002). Accordingly, the present study employed two different antioxidant testing systems (DPPH and hydrogen peroxide scavenging) to confirm the antioxidant potentials of the steam extract from *Phyllostachys* species.

The steam extract of *Phyllostachys* species was tested to determine their ability as DPPH scavenging activity was depicted in Fig. 1. All the tested steam extracts more than 57.7% of DPPH scavenging activities. For each *Pyllostachys* species, the DPPH scavenging activity showed the following order: *P*.

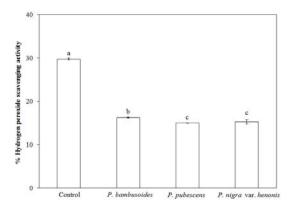


Fig. 2. The hydrogen peroxide scavenging activity of steam extract from *Phyllostachys* species. Results are expressed as percentage decrease in absorbance at a wavelength of 230 nm with respect to the control. Each value represents mean \pm SD of three experiments, performed in triplicate (Control: gallic acid).

pubescens > P. bambusoides > P. nigra var. henonis. The steam extract of P. pubescens and P. bambusoides were presented as the high activity (69.4%, 64.0%) and their DPPH scavenging activities were significantly different from each other (p < 0.05). Our results showed that the steam extraction worked better than the hot water extracting phytochemical from the *Phyllostachys* species leaves (below 4.4%) used in the work of Kim et al. (2001) on DPPH scavengingt activities. Choi et al. (2008) reported on the antioxidant effects of bamboo trees oil from P. nigra var. henonis, P. pubescens and P. bambusoides were investigated, and the results show that the DPPH scavenging rate follows the order of P. bambusoides (100%) > P. nigra var. henonis (94.7%) > P. pubescens (92.8%). The reason where the result is different becomes supposed to influence of different extraction method.

The hydrogen peroxide activities of the steam extract from *Phyllostachys* species compared with gallic acid have been exhibited in Fig. 2. The steam ex-

Table 2. Total polyphenol content of steam extracts from *Phyllostachys* species

Species	Total polyphenol content $(\mu g/m\ell)$
P. bambusoides	$69.2 \pm 3.5a^{1)}$
P. pubescens	$63.3 \pm 2.0 ab$
P. nigra var. henonis	$59.8 \pm 1.2c$

 $^{^{1)}}$ Values with different alphabets in the same column are significantly different at p <0.05.

tract from *Pyllostachys* species showed a hydrogen peroxide scavenging activity of approximately 50.4% - 54.6% when compared to that of the standard gallic acid. The steam extract of *P. bambusoides* has given the higher scavenging activity for hydrogen peroxide. And, the steam extract of *P. pubescens* and *P. nigra* var. *henonis* have not presented any significant differences between them (p < 0.05).

The total polyphenol content present in steam extract was measured using gallic acid as standard samples (Table. 2). According to the results, the total polyphenol contents of steam extract of P. bambusoides and P. pubescens were 69.2 μ g/ml and 63.3 μ g/ml, respectively. While total polyphenol content of P. nigra var. henonis extract was 59.8 μ g/ml showing lower levels than P. bambusoides and P. pubescens steam extract.

Plant phenolic compounds have been found to possess potent antioxidant (Park and Jhon, 2010), antimicrobial (Zaouali *et al.* 2010) and anticancer activities (Mehndiratta *et al.* 2011). Many studies have conclusively shown close relationship between total phenolic contents and antioxidative activity of natural plants (Velioglu *et al.* 1998). DPPH scavenging activities found in the steam extract of *P. bambusoides* and *P. pubescens* (64.0 - 69.4%) imply the role of phenolic compounds in contributing these activities. The determination of total phenolic content is an important parameter to estimate the amount of antioxidants. However, in this study, the total phenolic

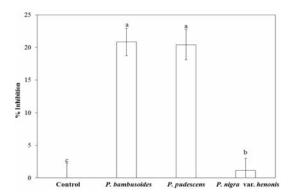


Fig. 3. Cytotoxic activity of steam extracts from *Phyllostachys* species using MTT assay (Control: not treated).

nolic contents was not in agreement with the hydrogen peroxide scavenging activity. This may be explained by the fact that different types of phenolic compounds posses different antioxidant capacities which is related to their chemical structure. For example, the previous researches showed that phenolic compounds with ortho- and para- dihydroxylation or a hydroxy and a methoxy group or both have stronger antioxidant activity than simple phenolics (Hossain et al. 2011). Also, The antioxidant efficiency of volatile compounds was reported in many previous works (Bourgou et al. 2008; Zaouali et al. 2010) and seems to be related to the activity of some kinds of compounds enclosed in such as oxygenated monoterpenes, especially alcohols and phenols than polyphenol content.

3.3. Anticancer Activity (PC-3 cells)

The effect of steam extract from *Phyllostachys* species on the growth of PC-3 cells as determined by the MTT assay was given in Fig. 3. Assessment of the cytotoxic effect of the steam extract on PC-3 cells showed that the *P. bambusoides* (20.85%) and *P. pubescens* (20.41%) were superior in induced cy-

totoxicity compared with the steam extract of *P. ni-gra* var. *henonis* (1.15%).

Interestingly, in the present study, the steam extract of *P. bambusoides* and *P. pubescens* were showed the higher DPPH scavenging activity, hydrogen peroxide scavenging activity and cytotoxic activity. However, the steam extract of *P. nigra* var. *henonis* was showed the higher DPPH scavenging activity and hydrogen peroxide scavenging activity, whereas cytotoxic activity was showed the least value.

This may be explained by the fact that many previous works showed biological (Dorman and Deans, 2000), antioxidant activities (Jirovetz *et al.* 2005) and especially anticancer activities of sesquiterpenes (Banjerdpongchai and Khaw-on, 2013). Sesquiterpenes content of steam extract from *P. nigra* var. *henonis* was 23.58% showed lower levels than steam extract of *P. bambusoides* (31.52%) and *P. pubescens* (33.13%), are shown in Table 1. Moreover, sesquiterpenes has shown anti-inflammatory effect, antibacterial, antifungal and an anticancer activity (Zhang *et al.* 2005) in human melanoma cell lines (M14) (Calcabrini *et al.* 2004) and lung cancer cells have also been showed in recent studies (Wu *et al.* 2012).

Thus, from this study it is likely that steam extract has a high cytotoxic effect in PC-3 cells, suggesting a potential role of *P. bambusoides* and *P. pubescens* as a powerful chemotherapeutic agent. However, further experiments are required to assess the apoptogenic effect of steam extract of *Phyllostachys* species in PC-3 cells and to define the mechanism at the molecular level. This study should help to answer more conclusively, whether *Pyllostachys* species containing sesquiterpenes would be effective in the treatment of advanced prostate cancer.

4. CONCLUSION

Antioxidant activity and anticancer activity were evaluated using steam extract of *Phyllostachys* species. In conclusion, steam extract of *Phyllostachys* species has the highest antioxidant activities as demonstrated by DPPH scavenging and hydrogen peroxide scavenging activities. The steam extract of *P. nigra* var. henonis has low anticancer activity in PC-3 cells; however, the strong anticancer activity was observed with steam extract of *P. bambusoides* and *P. pubescens*. The steam extract containing sesquiterpenes of *Phyllostachys* species has a therapeutic agent potential due to their antioxidant and anticancer activities.

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