

Phenolic compounds and antioxidant activity of *Castanopsis phuthoensis* and *Castanopsis grandicaticrata*

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Abstract: In this study, total phenolic and flavonoid contents, antioxidant capacity, and phenolic compositions of *Castanopsis phuthoensis* and *Castanopsis grandicaticrata* (Fagaceae family) were investigated. It was found that bark extracts were rich of phenolic contents, whereas leaf extracts were abundant of flavonoids. The total phenolics varied from 11.20 to 35.47 mg gallic acid equivalent g⁻¹ dry weight (DW), and the total flavonoids were from 2.24 to 12.55 mg rutin equivalent g⁻¹ DW. The results of antioxidant activity evaluation showed that the DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity of the free phenolic extracts were higher than the bound phenolic extracts. Regarding the reducing power and β -carotene bleaching assays, the free phenolic extracts showed remarkably strong antioxidant capacity that were similar to the levels of the standard BHT (dibutyl hydroxytoluene) did. It could be concluded that free phenolic extracts were more effective in antioxidant activities than bound phenolic extracts. Highly significant correlations were observed between phenolic contents and antioxidant activities (0.813 for DPPH and 0.841 for reducing power). By HPLC analysis, seven phenolic acids were detected including gallic, *p*-hydroxybenzoic, vanillic, sinapic, *p*-coumaric, ellagic acids, and vanillin. Of which, gallic, ellagic, and sinapic acids were the most abundant compounds in the two species. The results suggest *C. phuthoensis* and *C. grandicaticrata* contain rich sources of natural antioxidants and phenolic compounds which are probably considered in pharmaceutical use.

1. Introduction

Antioxidants are molecules which can delay or inhibit cellular damages from free radicals existing in biological systems and to be generated in various metabolic processes [1,2]. There are two types of free radicals including reactive oxygen species and nitrogen species. These radicals cause aggregation of protein, degradation of DNA and eventually destroy cell membranes and kill cells. As a result, this process probably increases the possibility of common diseases [3-7]. Antioxidants are believed to play a central role in health care to prevent and scavenge radicals because they can alleviate chronic diseases and degenerate ailments such as cancer, autoimmune disorder, hypertension, atherosclerosis and ageing process [8-14]. In recent years, several plants

have been studied for their antioxidant activity to develop natural antioxidant formulations for food, cosmetic, medicine and other purposes [15,16]. Plants are an excellent source of bioactive compounds with high antioxidant activity such as phenolic acids, flavonoids, coumarins, stilbenes, vitamins, hydrolysable and condensed tannins [17]. Approximately 4,000 to 6,000 antioxidant compounds synthesized by plants as secondary products have been detected [15]. Among these natural compounds, phenolics are the major group which possesses great biological activities including antioxidant, antibacterial, and antifungal activities [18].

Castanopsis belongs to the Fagaceae family which is represented by 52 dominant species in subtropical evergreen forests and mountain moist evergreen broad-leaved forests in Vietnam [19]. *Castanopsis grandicaticata* is a new species recorded in 2014 which distributes in northern and central regions of Vietnam with 20-35 m in height and 50 cm in diameter, cupules range from 6 to 7 cm in diameter and nut scar covering 2/3 of the nut [19]. *Castanopsis phuthoensis* grows in the northern Vietnam with 10-15 m in height and 50-70 cm in diameter [20]. Both two species are timber trees, and seeds are edible. *Castanopsis* species have been known as ornamental trees and are also used as construction materials.

Many pharmacological properties have been reported from different parts *Castanopsis* species like anticancer activity in KB cancer cell and phosphatidylserine targeting antibody system [4]. The extracts from leaves, bark and resin of *Castanopsis* species have been used as herbal medicine for treatment of stomach disorder, headache, diarrhea, chest pain and other diseases related to skin [4]. Several bioactive compounds identified from *Castanopsis* species were classified into phenolic acids, flavonoids, saponins, tannins, galloyl phenolic glucosides, acylated quinic acids, and flavonol glycosides [4]. Thus, *Castanopsis* species are considered as a source of antiseptic agents and can be suitable for the development of food preservatives. However, the studies on antioxidant capacity and phenolic components of *C. phuthoensis* and *C. grandicaticata* have not been reported yet. The purposes of this study were to determine phenolic and flavonoid contents, evaluate antioxidant activities using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging, reducing power and β -carotene bleaching assays, and to identify phenolic constituents of *C. phuthoensis* and *C. grandicaticata*.

2. Materials and methods

2.1. Standards and reagents

Standard phenolics including benzoic acid, caffeic acid, catechol, cinnamic acid, chlorogenic acid, ellagic acid, ferulic acid, gallic acid, protocatechuic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, sinapic acid, syringic acid, rutin, vanillic acid and vanillin were purchased from Kanto Chemical Inc.

Reagents including Folin-Ciocalteu's phenol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), dibutyl hydroxytoluene (BHT), aluminium (III) chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), β -carotene, linoleic acid, polyoxyethylenesorbitan monopalmitate (Tween-40), trichloroacetic acid (CCl_3COOH), iron (III) chloride (FeCl_3), sodium carbonate (Na_2CO_3), disodium hydrogenphosphate (Na_2HPO_4), sodium dihydrogenphosphate (NaH_2PO_4), sodium hydroxide (NaOH), hydrochloric acid (HCl) and all solvents were used of analytical grade and purchased from Kanto Chemical Co., Inc.

2.2. Plant materials

Fresh leaves and barks of *C. phuthoensis* and *C. grandicaticata* were collected from Phu Tho province of Vietnam in April 2015. Voucher specimens have been deposited in the Forest Plant Department, Vietnam Forestry University.

Leaves were collected at lower canopy whereas barks were collected at 1-1.5 m in height by shaving with 3 mm thickness of the outer part from three individual mature trees (with more than 10 m in height and 20-40 cm in diameter) in sunny places. All samples were cut into small pieces and separately dried in a convection oven (MOV- 212F (U), Sanyo, Japan) at 30 °C. Afterward, leaves and barks were pulverized into fine powder using a grinding machine. An amount of ten grams leaf or bark powder of three individual trees of one species were combined and considered one sample.

2.3. Extraction of free phenolics

Dried powdered leaves or barks (3 g) were extracted with 100 mL ethanol (99.5%), stirred for 24 h at room temperature. After extraction, the mixtures were centrifuged at 7,000 rpm for 5 min followed by filtration. The residues were re-extracted using the same procedure once more time. The solvent was removed in a rotary evaporator (SB-350-EYALA, Japan) at 30 °C. The precipitates were weighed, dissolved in methanol and kept in the dark at 4 °C for further analysis.

2.4. Extraction of bound phenolics

Bound phenolics were extracted following the method described previously by Xuan et al. [21] with some modifications. The residues from the free phenolic extraction were hydrolyzed with 50 mL of 4 M NaOH and stirred at 60 °C for 4 h. The suspension was centrifuged at 5,000 rpm for 10 min, filtered by filter papers (Toyo Roshi Kaisha, Ltd., Japan). It was then acidified to pH 2.0 with 37% hydrochloric acid solution. The remaining mixture was extracted five times with ethyl acetate. The ethyl acetate supernatant was evaporated to dryness on a rotary evaporator (SB-350-EYALA, Japan) at 30 °C. The dried extracts were dissolved in methanol and then stored in the dark at 4 °C to further use.

2.5. Determination of total phenolic content

The total phenolic content of the extracts was determined using the Folin-Cicalteau (FC) method described by Ti et al. [22]. In brief, an amount of 0.125 mL of the extracts (free or bound) was mixed with 0.5 mL of distilled water and 0.125 mL of FC reagent was added. After 6 min, 1.25 mL of 7.5% aqueous Na₂CO₃ solution was added into the mixture. Then, 1 mL of distilled water was added to bring the total volume to 3 mL. The mixtures were incubated for 90 min at room temperature. The absorbance of the reaction was measured at 760 nm using a spectrophotometer (HACH DR/4000U, USA). The amount of total phenolic content was expressed as mg of gallic acid equivalent (GAE) per g dry weight (DW).

2.6. Determination of total flavonoid content

The total flavonoid content in extracts was determined using the method described previously by Djeridane et al. [23]. One mL of the extracts (free and bound) was mixed with 1 mL of 2% aluminium (III) chloride hexahydrate. After 15 min, the absorption was measured at 430 nm against methanol as a blank. The amount of total flavonoid content was estimated as mg of rutin equivalent (RE) per g DW.

2.7. Antioxidant properties

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant capacity was determined using DPPH free radical scavenging assay as described in the previous study [24]. The mixture consisted of 0.5 mL sample extracts, 0.25 mL DPPH (0.5 mM) and 0.5 mL of 0.1 M acetate buffer (pH 5.5). After mixing, the reaction was maintained in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. BHT concentrations from 0.01 mg mL⁻¹ to 0.025 mg mL⁻¹ were used as the positive control. The percentage of scavenged DPPH was calculated as follows:

$$\% \text{ radical scavenging} = [(abs_{\text{control}} - abs_{\text{sample}})/abs_{\text{control}}] \times 100$$

The abs_{control} is the absorbance of DPPH solution without extract and abs_{sample} is the absorbance of sample with DPPH solution. IC₅₀ was defined as the concentration of the sample required to scavenge 50% of DPPH. Therefore, a lower IC₅₀ value reflects a higher DPPH radical scavenging activity.

Reducing power

The reducing power of different extracts was determined by using a method described previously [12] with some slight modifications. The reducing power was investigated by observing the transformation of Fe³⁺ to Fe²⁺. The extracts were diluted in methanol to obtain the concentrations from 0.025 to 1 mg mL⁻¹. Two hundred microliters of each extract or BHT was mixed with 0.5 mL phosphate buffer (pH 6.6) and 0.5 mL potassium ferricyanide [K₃Fe(CN)₆] (10 g L⁻¹) in a test tube and incubated in a water bath at 50 °C for 30 min. Afterwards, the test tube was removed from the water bath and 0.5 mL trichloroacetic acid (100 g L⁻¹) was added to the mixture and centrifuged at 4,000 rpm for 10 min. Then 0.5 mL of the supernatant solution was diluted with

0.5 mL of distilled water and 0.1 mL FeCl_3 (1 g L^{-1}) was added. The mixture was measured at 700 nm. The decrease of the reaction mixture absorbance indicated the increase of reducing power and was expressed by IC_{50} values.

Antioxidant assay using the β -carotene bleaching system

Antioxidant activity was evaluated according to a β -carotene bleaching method described by Elzaawely and Tawata [9]. β -Carotene (2 mg) was dissolved in 10 mL of chloroform, and 1 mL of the solution was mixed with 20 μL linoleic acid and 200 mg Tween-40. The chloroform was evaporated under vacuum at 45°C . Afterwards, an aliquot of 50 mL oxygenated water was added, and the emulsion was vigorously shaken until complete homogenization achieved. A volume of 0.120 mL of extracts or BHT ($1000 \mu\text{g mL}^{-1}$) was mixed with 1 mL of the emulsion. An equal amount of methanol was used for negative control. The solutions were incubated at 50°C and measured at 492 nm. All samples were recorded from 0 to 180 min every 15 min intervals. A blank without β -carotene was prepared for background subtraction. Three replicates were prepared for each extract. Lipid peroxidation inhibition (LPI) was calculated using the following equation: LPI inhibition (%) = $A_1/A_0 \times 100$, where A_0 is the absorbance value measured at 0 min for the test sample, while A_1 is the corresponding absorbance value measured after incubation for 180 min.

2.8. Quantification by HPLC

The phenolic compositions of different samples were determined at 254 nm by using HPLC system (LC-Net II/ADC, UV-2075 Plus and PU-2089 Plus, Jasco, Japan), and the column RPC18 (250 mm x 4.6 mm x $5\mu\text{m}$) was employed. The mobile phase was methanol 99.8% (A) and 0.1% acid acetic (v/v) (B) at a flow rate of 1 mL min^{-1} . The gradient elution was performed as follows: 0-5 min (5% A), 5-10 min (20% A), 10-20 min (50% A), 20-30 min (80% A), 30-40 min (100% A), 40-50 (100% A) min, 50-60 min (5% A). Phenolic standards and extracts at 1 mg mL^{-1} were injected to the HPLC column with an amount of 5 μL . Phenolic compositions were identified based on the retention times, and its concentrations were calculated by comparing peak areas of samples with those of the standards.

2.9. Statistical analysis

The results of all assays were expressed as means \pm standard error (SE). Data were analyzed by the Minitab 16 software. Analysis of variance (ANOVA) was used to determine if significant differences existed at a level of confidence of $p < 0.05$.

3. Results

3.1. Total phenolic content

The free, bound and total phenolic contents from leaf and bark extracts of *C. grandicatricata* and *C. phuthoensis* are presented in Table 1. It was found that free extracts had higher content of phenolics. Free phenolic contents of the barks were significantly higher than the leaves. However, bound phenolics of the leaves were markedly greater than the barks. In general, total phenolic contents of both free and bound extracts from barks were significantly higher than leaves.

Table 1. Phenolic contents of *C. grandicatricata* and *C. phuthoensis* leaves and barks.

Tree species	Plant parts	Total phenolics ($\text{mg GAE g}^{-1} \text{ DW}$)		
		Free	Bound	Total phenolics
<i>C. grandicatricata</i>	Barks	$33.24 \pm 0.27\text{b}$	$0.89 \pm 0.08\text{c}$	$34.13 \pm 0.32\text{b}$
	Leaves	$9.72 \pm 0.09\text{d}$	$1.48 \pm 0.07\text{b}$	$11.20 \pm 0.16\text{d}$
<i>C. phuthoensis</i>	Barks	$34.52 \pm 0.31\text{a}$	$0.95 \pm 0.08\text{c}$	$35.47 \pm 0.36\text{a}$
	Leaves	$24.60 \pm 0.00\text{c}$	$3.66 \pm 0.07\text{a}$	$28.27 \pm 0.07\text{c}$

Values represent means \pm standard errors (SE) ($n = 3$)

Different letters in the same column indicate significant differences ($p < 0.05$)

3.2 Total flavonoid content

Free, bound and total flavonoid contents of extracts are presented in Table 2. The results showed that total flavonoid contents in the leaf extracts were extremely higher than the bark extracts in both free and bound forms ($p < 0.05$). In particular, total flavonoid content of *C. phuthoensis* leaf extract was the highest with five folds higher than its bark extract, and four folds higher than bark extract of *C. grandicatricata*. This figure was similar in the case of *C. grandicatricata*, that leaf extract was higher than bark extract.

Table 2. Flavonoid contents of *C. grandicatricata* and *C. phuthoensis* leaves and barks.

Tree species	Plant parts	Total flavonoids (mg RE g ⁻¹ DW)		
		Free	Bound	Total flavonoids
<i>C. grandicatricata</i>	Barks	1.23 ± 0.02c	1.80 ± 0.03a	3.04 ± 0.03c
	Leaves	6.26 ± 0.08b	1.65 ± 0.09a	7.91 ± 0.16b
<i>C. phuthoensis</i>	Barks	1.30 ± 0.04c	0.93 ± 0.03c	2.23 ± 0.02c
	Leaves	11.32 ± 0.19a	1.23 ± 0.03b	12.55 ± 0.16a

Values represent means ± standard errors (SE) (n = 3)

Different letters in the same column indicate significant differences ($p < 0.05$)

3.3. Antioxidant properties

The antioxidant activities of extracts were evaluated using DPPH free radical scavenging, reducing power, and β -carotene bleaching assays. The results of DPPH scavenging activities of extracts showed that the highest activity was found in free extracts of *C. phuthoensis*' bark (Table 3). In bound extracts, leaf extracts of both species were much higher activities than the barks. Interestingly, the levels of antioxidant activities in free extracts were much stronger than bound extracts, and they were close to the antioxidative level of the standard BHT (Table 3).

Table 3. Antioxidant activity of free and bound phenolic extracts of *C. grandicatricata* and *C. phuthoensis* by DPPH radical scavenging (IC₅₀).

Tree species	Plant parts	IC ₅₀ (mg mL ⁻¹)	
		Free	Bound
<i>C. grandicatricata</i>	Barks	0.033 ± 0.000b	0.377 ± 0.003b
	Leaves	0.048 ± 0.000a	0.308 ± 0.001c
<i>C. phuthoensis</i>	Barks	0.030 ± 0.000c	0.447 ± 0.002a
	Leaves	0.031 ± 0.000bc	0.178 ± 0.003d
BHT		0.027 ± 0.001d	0.027 ± 0.001e

Values represent means ± standard errors (SE) (n = 3).

Different letters in the same column indicate significant differences ($p < 0.05$). BHT was used as the positive control

The reducing power capacity of the extracts is represented in Fig. 1 and Table 4. The reducing power was proportional to the examined doses of the extracts and BHT (Fig. 1). The values obtained for all free extracts were excellent because they were similar to the BHT did. At 0.5 mg mL⁻¹ free extracts, the absorbance values of all samples were above 0.6 (Fig. 1A). Bound extracts showed low activities because both leaves and barks exhibited much weaker than the standard BHT (Fig. 1B). The IC₅₀ values were estimated by the sample concentrations at which the absorbance was 0.5 (Table 4). IC₅₀ values of free extracts revealed strong reducing power, especially bark extracts were more pronounced than leaf extracts and similar to that of BHT ($p < 0.05$). By contrast, IC₅₀ values of the bound extracts were significantly lower than that of BHT ($p < 0.05$).

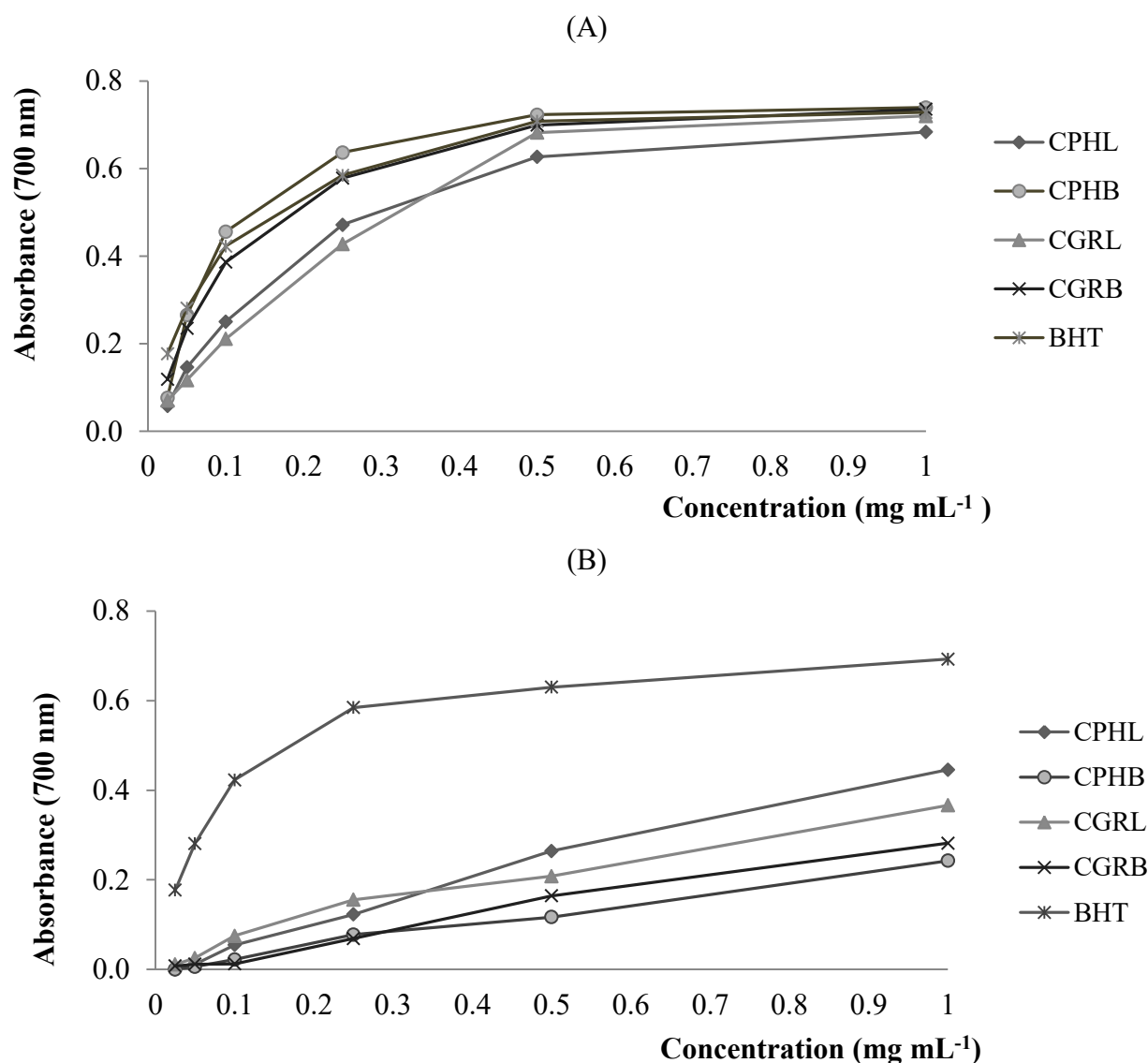


Fig. 1. Reducing powers of (A) free phenolic extracts and (B) bound phenolic extracts of *C. phuthoensis* and *C. grandicatricata*.

CGRL: leaf extract of *C. grandicatricata*, CGRB: bark extract of *C. grandicatricata*
CPHL: leaf extract of *C. phuthoensis*, CPHB: bark extract of *C. phuthoensis*

Table 4. Antioxidant activity of free and bound phenolic extracts of *C. grandicatricata* and *C. phuthoensis* by reducing power assay expressed (IC₅₀)

Tree species	Plant parts	IC ₅₀ (mg mL ⁻¹)	
		Free	Bound
<i>C. grandicatricata</i>	Bark	0.197 ± 0.007c	1.861 ± 0.101ab
	Leaf	0.293 ± 0.005a	1.894 ± 0.266a
<i>C. phuthoensis</i>	Bark	0.170 ± 0.000d	2.294 ± 0.038a
	Leaf	0.257 ± 0.002b	1.270 ± 0.037b
BHT		0.185 ± 0.001cd	0.185 ± 0.001c

Values represent means ± SE (n = 3).

Different letters in the same column indicate significant differences ($p < 0.05$). BHT was used as the positive control

The β -carotene bleaching method is also one among the most common methods to evaluate the antioxidant activities of plants extracts. In general, all the extracts of two species inhibited β -carotene oxidation (Fig. 2). There was a negligible difference of antioxidant activity between the free phenolic extracts of two species and BHT (Fig. 2A). Meanwhile, the bound phenolic extracts

exhibited a weaker inhibition than standard compound BHT. The bound extracts of *C. phuthoensis* exhibited a higher inhibition than that of *C. grandicatricata* (Fig. 2B).

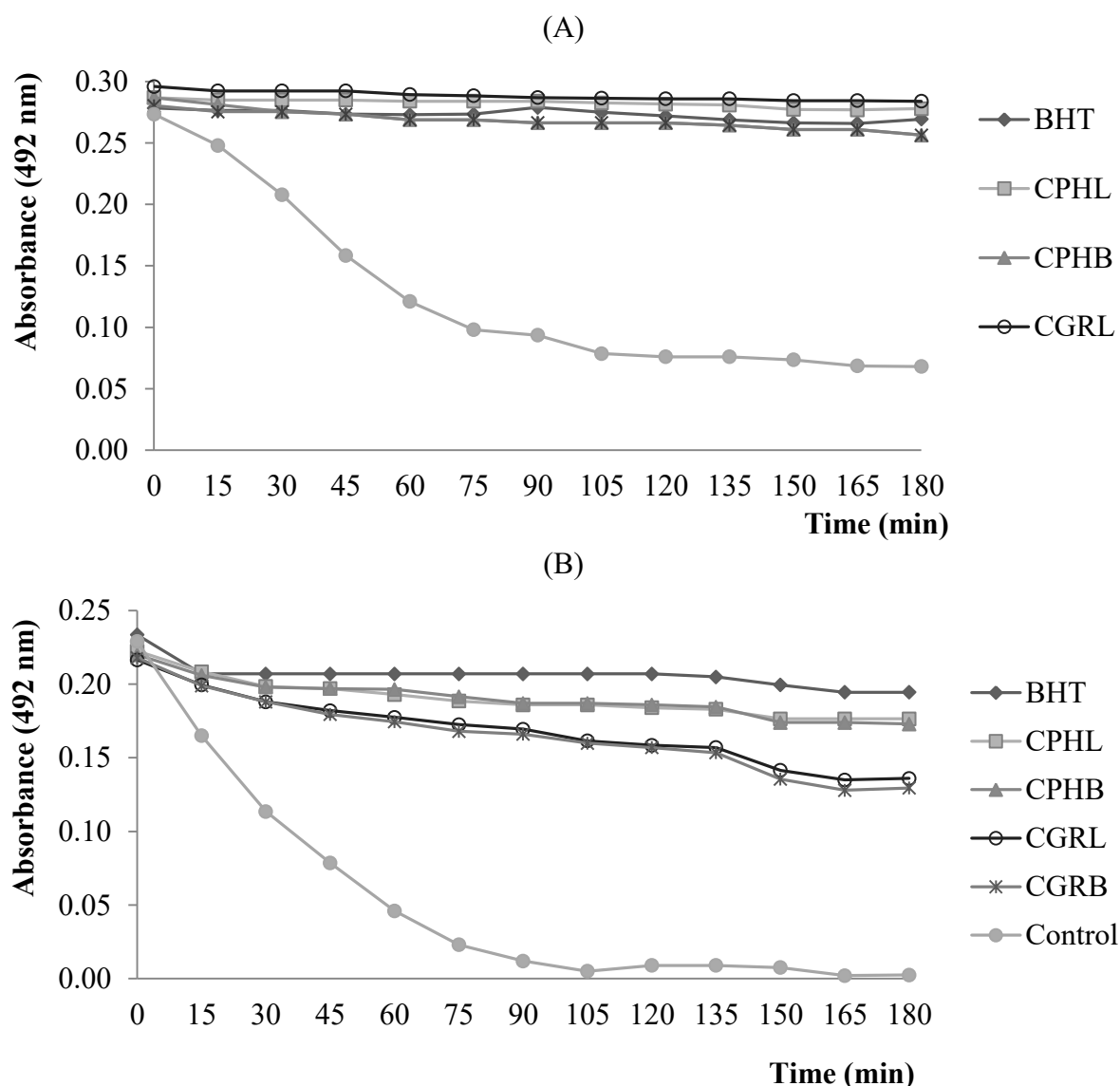


Fig. 2. Antioxidant activity of (A) free phenolic compound and (B) bound phenolic compounds of *C. grandicatricata* and *C. phuthoensis* measured by β -carotene bleaching method.

CGRL: leaf extract of *C. grandicatricata*, CGRB: bark extract of *C. grandicatricata*

CPHL: leaf extract of *C. phuthoensis*, CPHB: bark extract of *C. phuthoensis*

3.4. Correlation between phenolic contents and antioxidant activities

As shown in Table 5, negative correlations were found between the antioxidant activities (IC_{50} values) and phenolic contents. This means a lower IC_{50} value correlating to a higher content of phenolics or flavonoids. Highly significant correlations were observed between phenolic contents and DPPH radical scavenging ability and reducing power, while no significant correlation was found between flavonoid contents and antioxidant activities. Moreover, the scavenging free radical activity positively correlates with the reducing power.

Table 5. Correlation coefficients (r) between antioxidant activities and contents of phenolics and flavonoids of *Castanopsis* extracts

	Phenolic contents	Flavonoid contents	DPPH (IC ₅₀)	Reducing power (IC ₅₀)
Phenolic contents	1			
Flavonoid contents	0.219	1		
DPPH (IC ₅₀)	- 0.813**	- 0.469*	1	
Reducing power (IC ₅₀)	- 0.841**	- 0.470*	0.970**	1

* $p < 0.05$, ** $p < 0.01$.

3.5. Identification and quantification of phenolic compounds using HPLC

The identification of phenolic acids was conducted by comparing peak retention times with the standard compounds. The quantification was determined by using standard curves established from different concentrations (0-100 $\mu\text{g mL}^{-1}$) of the standard phenolics.

Table 6. Phenolic acids (mg g⁻¹DW) of free and bound phenolic extracts of *C. grandicaticata* and *C. phuthoensis* measured by HPLC.

Phenolics	RT	<i>C. grandicaticrica</i>				<i>C. phuthoensis</i>				Total
		Barks		Leaves		Barks		Leaves		
		Free	Bound	Free	Bound	Free	Bound	Free	Bound	
GA	11.9	9.34 ± 0.07a	-	0.20 ± 0.04c	-	0.55 ± 0.03b	-	-	-	10.09
p-HY	16.3	-	-	0.07 ± 0.00a	-	-	-	-	0.04 ± 0.00b	0.11
VA	20.9	-	-	-	-	-	-	-	0.01 ± 0.00	0.01
V	22.4	-	-	-	-	-	0.27 ± 0.00	-	-	0.27
SA	24.3	-	0.71 ± 0.02a	-	0.52 ± 0.01c	-	0.62 ± 0.02b	-	0.32 ± 0.00d	2.17
p-CO	24.6	-	-	-	1.06 ± 0.03	-	-	-	-	1.06
EA	27.5	3.96 ± 0.04a	-	0.70 ± 0.08b	0.32 ± 0.03c	0.90 ± 0.11b	-	0.19 ± 0.01c	0.21 ± 0.02c	6.28

RT: Retention time; GA: gallic acid; p-HY: *p*-Hydroxybenzoic acid; VA: Vanillic acid; V: Vanillin; SA: Sinapic acid; p-CO: *p*-Coumaric acid; EA: Ellagic acid.

Values represent means ± standard errors (SE) (n = 3).

Different letters in the same row indicate significant differences ($p < 0.05$).

(-) Not detected.

There were seven phenolic acids detected including gallic acid, *p*-hydroxybenzoic acid, vanillic acid, vanillin, sinapic acid, *p*-coumaric acid, and ellagic acid with varied quantities and concentrations among the extracts (Table 6). Gallic acid was the major constituent (10.09 mg g⁻¹ DW, followed by ellagic and sinapic acids (6.28 and 2.17 mg g⁻¹ DW, respectively). The amounts of other phenolic substances were lower than 1.06 mg g⁻¹ DW. Vanillic acid and vanillin were found only in *C. phuthoensis*, whereas *p*-coumaric acid was found only in *C. grandicaticata*. Sinapic acid only existed in bound form in all extracts.

4. Discussion

Plants produce an extraordinary diversity of phenolic compounds which are increasingly being used in the food industry. Phenolic compounds show potent antioxidant activity because they can retard or quench oxidative degradation of lipids and play a crucial role in protecting human

body [2,25,26]. In addition, natural antioxidant compounds are considered to be nontoxic and they contain many medical properties [4]. The total phenolic and flavonoid contents are different from species as well as parts of plants. Actually, many plants contain large amount of polyphenols which can act as antioxidants. Previous studies reported that cancer cell growth could be inhibited by some flavonoid components [27]. The results of this study indicated that the barks and leaves of two tree species contain free phenolic compounds which account for around 90% of total phenolics. Total phenolic contents obtained in this study were higher than those in leaf extracts of *Quercus resinosa*, *Q. laeta*, *Q. obtusata* (5.7-7.1 mg GAE g⁻¹ DW) [28], and cork extracts of *Q. suber* (1.7-19.9 mg GAE g⁻¹ DW) [29]. Furthermore, total phenolic contents of the two tree species were similar to that of several other medicinal plants such as *Anthemis arvensis*, *Artemisia campestris*, *Globularia alypum* and *Artemisia herbahalba* [23]. Flavonoids are the most important group of polyphenolic compounds, and they exist widely in leaves and flowers of vascular plants [30]. In this study, the free flavonoid contents were up to 80% in leaf extracts and 40% in bark extracts. The total flavonoids of the two tree species were higher than that of *Q. suber* (0.6-8.2 mg RE g⁻¹ DW) which is a source of high value compounds [5,29].

Natural antioxidants, known as a crucial alternative to the synthetic antioxidants, can be used in the treatment of oxidative damages in food productions [31]. Several previous studies reported that the antioxidant property of plants and their phenolic contents are highly correlation [1,10,32,33]. This study indicated a highly significant correlation between phenolic contents and antioxidant activities in extracts of *Castanopsis* species (Table 5). As shown in Table 3, 4, free phenolic extracts contain higher phenolic contents that properly resulted in stronger free radical scavenging activity, β -carotene bleaching and higher reducing power than bound phenolic extracts. Reducing power of bark extracts were significantly higher than leaf extracts due to the phenolic contents in bark extracts were significantly higher than leaf extracts (Table 1).

Moreover, the amounts of gallic and ellagic acids in bark extracts were much higher than leaf extracts. That was probably the main factor contributing the antioxidant activities of extracts because, gallic acid has three hydroxyl groups in the *ortho* positions bonded to the aromatic ring which is one of the most important factors associated with a strong DPPH scavenging activity [24]. Additionally, ellagic acid consists of four hydroxyl groups which primarily contribute to the strong antioxidant activity of plant extracts [6]. Apart from DPPH radical scavenging and reducing power capacities, ellagic acid also had high antioxidant activities in other assays including hydrogen peroxide scavenging, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and superoxide anion radical scavenging [32]. Furthermore, relating to pharmaceutical studies, ellagic acid was proven to have strong effects on anti-inflammation, anti-proliferation, anti-angiogenesis, anti-carcinogenesis, and event anti-cancer [3].

5. Conclusion

In this study, the antioxidant activity and phenolic compositions of *C. phuthoensis* and *C. grandicatricata* were investigated for the first time. The findings of this study revealed that the phenolic and flavonoid contents were proportional to the levels of antioxidant activities of the two tree species. The antioxidant activities of free phenolic extracts showed similar levels of the standard BHT did. Seven phenolic acids were detected by HPLC, and gallic, ellagic, and sinapic acids were the most abundant compounds in the two species. The results suggest that *C. phuthoensis* and *C. grandicatricata* contain rich sources of natural antioxidants. Therefore, the further research to exploit the potential pharmaceutical properties of the two tree species is indispensable.

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References

- [1] A.A. Soares, C.G.M. de Souza, F.M. Daniel, G.P. Ferrari, S.M.G. da Costa, R.M. Peralta, Antioxidant activity and total phenolic content of *Agaricus brasiliensis* (*Agaricus blazei* Murril) in two stages of maturity, Food Chem. 112(4) (2009) 775-781. doi:10.1016/j.foodchem.2008.05.117.
- [2] G. Sakthidevi, V.R. Mohan, Total phenolic, flavonoid contents and in vitro antioxidant activity of *Dioscorea alata* tuber, J. Pharm. Sci. & Res., 5(5) (2013) 115-119.
- [3] S.A.O. Santos, P.C.R.O. Pinto, A.J.D. Silvestre, C.P. Neto, Chemical composition and antioxidant activity of phenolic extracts of cork from *Quercus suber* L, Ind. Crops Prod. 31 (2010) 521–526. doi:10.1016/j.indcrop.2010.02.001.
- [4] N. Dolai, I. Karmakar, R.B.S. Kumar, B. Kar, A. Bala, P.K. Haldar, Free radical scavenging activity of *Castanopsis indica* in mediating hepatoprotective activity of carbon tetrachloride intoxicated rats, Asian Pac. J. Trop. Biomed. (2012) S242-S251.
- [5] L. Custódio, J. Patarraa, F. Alberício, N.daR. Neng, J.M.F. Nogueira, A. Romanoa, Phenolic composition, antioxidant potential and in vitro inhibitory activity of leaves and acorns of *Quercus suber* on key enzymes relevant for hyperglycemia and Alzheimer's disease, Ind. Crops Prod. 64 (2015) 45–51. <http://dx.doi.org/10.1016/j.indcrop.2014.11.001>.
- [6] L. Sepúlveda, A. Ascacio, R. Rodríguez-Herrera, A. Aguilera-Carbó, C.N. Aguilar, Ellagic acid: Biological properties and biotechnological development for production processes, Afr. J. Biotechnol. 10(22) (2011) 4518–4523. doi:10.5897/AJB10.2201.
- [7] S.B. Nimse, D.K. Pal, Free radicals, natural antioxidants, and their reaction mechanisms, RSC Advances, 5 (2015) 27986-28006. doi:10.1039/c4ra13315c.
- [8] R. Xing, H. Yu, S. Liu, W. Zhang, Q. Zhang, Z. Lia, P. Lia, Antioxidant activity of differently regioselective chitosan sulfates in vitro, Bioorg. Med. Chem. 13(4) (2005) 1387–1392. doi:10.1016/j.bmc.2004.11.002.
- [9] A.A. Elzaawely, S. Tawata, Antioxidant capacity and phenolic content of *Rumex dentatus* L. grown in Egypt, J. Crop Sci. Biotech. 15(1) (2012) 59-64. doi:10.1007/s12892-011-0063-x.
- [10] N. Biswas, P. Balac, S.K. Narlakanti, M.D.E. Haque, M.D.M. Hassan, Identification of phenolic compounds in processed cranberries by HPLC method, J. Nutr. Food Sci. 3(1) (2013) <http://dx.doi.org/10.4172/2155-9600.1000181>.
- [11] M. Mikulic-Petkovsek, J. Samoticha, K. Eler, F. Stampar, R. Veberic, Traditional Elderflower Beverages: A rich source of phenolic compounds with high antioxidant activity, J. Agric. Food Chem. 63(5) (2015) 1477–1487. doi:10.1021/jf506005b.
- [12] R. Singh, N. Kumari, Comparative determination of phytochemicals and antioxidant activity from leaf and fruit of *Sapindus mukorossi* Gaertn.—Avaluable medicinal tree, Ind. Crops Prod, 73 (2015) 1–8. <http://dx.doi.org/10.1016/j.indcrop.2015.04.012>.
- [13] L. Wen, X. Guo, R.H. Liu, L. You, A.M. Abbasi, X. Fu, Phenolic contents and cellular antioxidant activity of Chinese hawthorn *Crataegus pinnatifida*, Food Chem. 186 (2015) 54–62. <http://dx.doi.org/10.1016/j.foodchem.2015.03.017>.
- [14] Y. Xie, Y. Zheng, X. Dai, Q. Wang, J. Cao, J. Xiao, Seasonal dynamics of total flavonoid contents and antioxidant activity of *Dryopteris sorythrosora*, Food Chem. 186 (2015) 113–118. doi:10.1016/j.foodchem.2014.05.024.
- [15] J.C.M. Barreira, I.C.F.R. Ferreira, M.B.P.P. Oliveira, J.A. Pereira, Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit, Food Chem. 107(3) (2008) 1106–1113. doi:10.1016/j.foodchem.2007.09.030.
- [16] Q.D. Do, A.E. Angkawijaya, P.L. Tran-Nguyen, L.H. Huynh, F.E. Soetaredjo, S. Ismadji, Y.H. Ju, Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*, J. Food Drug Anal. 22, (2014) 296 – 302. doi:10.1016/j.jfda.2013.11.001
- [17] C.D. Stalikas, Extraction, separation, and detection methods for phenolic acids and flavonoids, J. Sep. Sci. 30(18) (2007) 3268–3295. doi: 10.1002/jssc.200700261.

-
- [18] E. Oskoueian, N. Abdullah, S. Ahmad, W. Z. Saad, Bioactive Compounds and Biological Activities of *Jatropha curcas* L. Kernel Meal Extract, Int. J. Mol. Sci., 12 (2011) 5955-5970; doi:10.3390/ijms12095955
- [19] D.H. Vuong, N.H. Xia, Two new species in *Castanopsis* (Fagaceae) from Vietnam and their leaf cuticular features, Phytotaxa. 186(1) (2014) 029–041. doi: <http://dx.doi.org/10.11646/phytotaxa.186.1.2>.
- [20] H.H. Pham, An illustrated Flora of Vietnam II, Youth publishing house, Ho Chi Minh, 2003, pp. 612 (in Vietnamese).
- [21] T.D. Xuan, E. Tsuzuki, H. Terao, M. Matsuo, T.D Khanh, Correlation between growth inhibitory exhibition and suspected allelochemicals (Phenolic compounds) in the extract of Alfalfa (*Medicago sativa* L.), Plant Prod. Sci. 6(3) (2003) 165–171. doi:10.1626/pps.6.165.
- [22] H. Ti, R. Zhang, M. Zhang, Q.Li, Z. Wei, Y. Zhang, X. Tang, Y. Deng, L. Liu, Y. Ma, Dynamic changes in the free and bound phenolic compounds and antioxidant activity of brown rice at different germination stages, Food Chem. 161 (2014) 337–344. <http://dx.doi.org/10.1016/j.foodchem.2014.04.024>.
- [23] A. Djeridane, M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker, N. Vidal, Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds, Food Chem. 97(4) (2006) 654–660. doi:10.1016/j.foodchem.2005.04.028.
- [24] A.A. Elzaawely, T.D. Xuan, S. Tawata, Antioxidant and antibacterial activities of *Rumex japonicas* Houtt, Biol. Pharm. Bull. 28(12) (2005) 2225-2230.
- [25] E. Bursal, E. Köksal, Evaluation of reducing power and radical scavenging activities of water and ethanol extracts from sumac (*Rhus coriaria* L.), Food Res. Int. 44(7) (2011) 2217–2221. doi:10.1016/j.foodres.2010.11.001.
- [26] N. Saeed, M.R. Khan, M.R., Shabbir, M, Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L, Bio. Med. Central. 12 (2012) 221. <http://www.biomedcentral.com/1472-6882/12/221>.
- [27] T.M. Elattar, A.S. Virji, The inhibitory effect of curcumin, genistein, quercetin and cisplatin on the growth of oral cancer cells in vitro, Anticancer Res. 20 (2000) 1733–1738.
- [28] J.A. Sánchez-Burgos, M.V. Ramirez-Maresb, M.M. Larrosac, J.A. Gallegos-Infantea, R.F. Gonzalez-Laredoa, L. Medina-Torresd, N.E. Rocha-Guzmana, Antioxidant, antimicrobial, antitopoisomerase and gastroprotective effect ofherbal infusions from four *Quercus* species, Ind. Crops Prod., 42 (2013) 57– 62. doi:10.1016/j.indcrop.2012.05.017.
- [29] S.A.O. Santos, J.J. Villaverde, A.F. Sousa, J.F.J. Coelho, C.P. Neto, A.J.D. Silvestre, Phenolic composition and antioxidant activity of industrial cork by-products, Ind. Crops Prod. 47 (2013) 262-269. <http://dx.doi.org/10.1016/j.indcrop.2013.03.015>.
- [30] J. Zhishen, T. Mengcheng, W. Jianming, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chem. 64(4) (1999) 555–559. doi:10.1016/S0308-8146(98)00102-2.
- [31] R. Touati, S.A.O. Santos, S.M. Rochac, K. Belhamel, A.J.D. Silvestre, The potential of cork from *Quercus suber* L. grown in Algeria as a source of bioactive lipophilic and phenolic compounds, Ind. Crops Prod. 76 (2015) 936–945. <http://dx.doi.org/10.1016/j.indcrop.2015.07.074>.
- [32] D.T. Khang, T.N. Dung, A.A. Elzaawely, T.D. Xuan, Phenolic profiles and antioxidant activity of germinated legumes, Foods 5 (2016) 27. doi:10.3390/foods5020027
- [33] F. Medini, H. Fellah, R. Ksouri, C. Abdelly, Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*, Journal of Taibah University for Science, 8(3) (2014) 216–224. <http://dx.doi.org/10.1016/j.jtusci.2014.01.003>.