

Assessment on Antioxidant Properties of *Oplopanax elatus* Nakai *in vitro*

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ABSTRACT : Effects of *Oplopanax elatus* have known to various pharmaceutical therapies. However, chemical prosperities in the plant are rarely investigated. In order to detect biological activity, we evaluated the antioxidant activity of five fractions from methanolic extracts in each part of *O. elatus*. Also, contents of polyphenols and polysaccharides were measured. Five fractions were of sub-fractions using *n*-hexane, ethyl-acetate, *n*-butanol, and water from methanolic extracts. Ethylacetate and *n*-butanol fractions from stem and root exhibited strong antioxidant activity and high total phenolics content. On the HPLC analysis, ten free phenolics, including *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, naringin, hesperidin, quercetin and *trans*-cinnamic acid, were identified from the fractions and were shown to different quantitative proportions. Furthermore, ethylacetate and *n*-butanol fraction had the highest amount of chlorogenic acids, one of the cinnamic acid derivatives possessing pharmacological properties. These results indicated that the fractions of *O. elatus*, as well as methanolic extracts, could be used as natural antioxidative ingredients.

Key words : *Oplopanax elatus*, antioxidant, DPPH, Phenolic compound.

INTRODUCTION

Currently, the potential role of antioxidants and antioxidant enzymes is routinely studied for the treatment and prevention of atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus and several other diseases (Rehman *et al.*, 2004; Coleman *et al.*, 2003). Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu *et al.*, 1998). In order to reduce damage to the human body, synthetic antioxidants are used for industrial processing at the present time. However, negative effects using synthetic antioxidants have been suspected to liver damage and carcinogenesis (Grice, 1988; Qi *et al.*, 2005). Thus, use of natural antioxidants should be a safe method so that they can protect human body from free radicals and retard the progress of many chronic diseases (Kinsella *et al.*, 1993). The protective action of medicinal plants has been attributed to the presence of antioxidants, especially polyphenolic compounds and other phenolic constituents (Ferreira *et al.*, 2006; Çoruh *et al.*, 2007).

Oplopanax elatus which belongs to the Araliceae is a native shrub in Korea, China and Russia. This plant has popularly

used in pharmaceutical area because of the therapeutic properties. It is known that *O. elatus* exhibits a similar pharmacological action to ginseng. This plant has been known to help stamina, heart disease, diabetes mellitus, neurasthenia, blood pressure regulating effect, schizophrenia, antimicrobial effect (Me *et al.*, 1987; Zhang *et al.*, 1993; Wang *et al.*, 2004). Also, *O. elatus* has known to use alleviation of fever, cough, inflammation, alleviation of pain, wound, toothache in the folk. Although *O. elatus* is widely used in everyday life as part of folk medicinal remedies in Korea, the antioxidant activity of this plant has not been assessed in the past time.

In this paper, we evaluate the possible antioxidant activity of extracts and solvent fractions from leaf, stem, root in *O. elatus* and determine principal phenolic phytochemicals. This study may be helpful to understand pharmaceutical behaviour of the plant extract.

MATERIALS AND METHODS

Plant material and extraction

The leaf, stem, and root of *O. elatus* were collected from Hwa-ak mountain, Gyeonggi province, Korea in April, 2004. The plants were identified by Dr. K. Heo (Department of Plant

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Science Program, Faculty of Bio-resource Engineering, Kangwon National University). Voucher specimens of these plants were deposited in The Herbarium of Kangwon Herbarium of Kangwon National University. Dried samples of *O. elatus* were finely ground and extracted by methanol for overnight. The process of methanolic extraction were triplicated. Crude extracts were gained after evaporation of methanol using a rotary evaporator. The crude extracts of leaf, stem and root were partitioned using organic solvents, purified water, *n*-hexane, ethylacetate (EtOAc), and butanol (BuOH).

Apparatus and reagents

Apparatus of this study uses rotary vacuum evaporator (NE-2001, AC-1112A, Eyela Co.), UV/VIS Spectra (V530 spectrophotometer, Jasco Co.), and HPLC (Yonglin Co.).

Chemicals used in this study were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA), with the exception of reagent-grade solvents. Methanol, *n*-hexane, EtOAc, BuOH, and ethanol were purchased from Dongyang (Korea). All chemicals and reagents were of analytical grade.

DPPH assay

Free radical scavenging activity was measured using diphenylpicrylhydrazyl (DPPH) according to the procedure of Blois (Blois, 1958). Reduction of free radical by DPPH was shown to following way. The RC₅₀ values denote the concentration of each sample required to give 50% of the optical density shown by control treatment. The mixture of 4 mL MeOH and 1 mL DPPH solution was used as control treatment. α -Tocopherol, thiobarbituric acid (BHA) and butylated hydroxyl toluene

(BHT) were used as positive controls. All procedure of this analysis were triplicated.

Ferric thiocyanate method (FTC)

The FTC method reported by Inatani (Inatani *et al.*, 1983) was used in this study. A mixture of 0.5 mg of samples (final concentration 0.005% w/v) in 4 mL of 99.5% ethanol were mixed with 2.5% linoleic acid in 99.5% ethanol (2.0 mL), 0.05 M phosphate buffer (pH = 7.0, 4 mL), distilled water (1.9 mL), 10% Tween 20 (0.1 mL) and kept in screw-cap vial (ϕ 30 × 95 mm) under dark condition at 40 °C. In 0.1 mL of the solution, 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate was added. After 3 min, 0.1 mL of 0.02 M ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture. The absorbance of red colour was measured at 500 nm UV. The absorbance was recorded in every 48 h until 4 th day after absorbance of the control reached its maximum value. The control and standard were subjected to the same procedure except for the control composing no addition of sample extract and for the standard adding 0.5 mg of BHA, BHT and α -tocopherol in stead of sample extract, respectively.

Determination of total phenolic compounds and polysaccharides content

Total soluble phenolics in *O. elatus* extracts were determined with Folin-Ciocalteu reagent, according to the method of Slinkard (Slinkard & Singleton, 1977).

The extract of 0.2 g was resuspended in 50 ml 5% (v/v) sulphuric acid and placed in boiling water for 2 h. After acidic hydrolysis, the liquid-solid mixture was diluted to 50 ml with

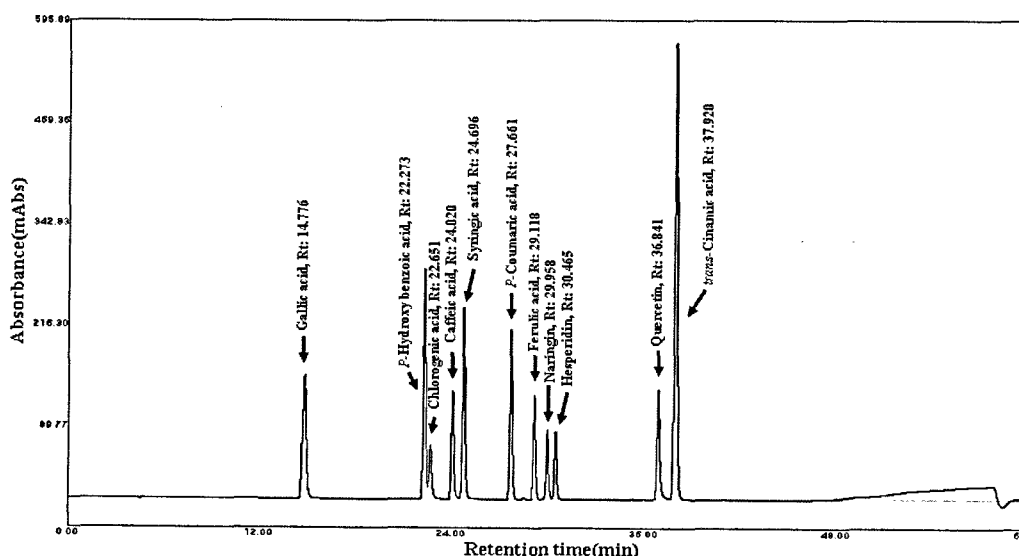


Fig. 1. HPLC profile of phenolic compounds (Detection: UV 270 nm).

Table 1. Calibration curves of 11 phenol compounds

Standard chemicals	Equation	Standard chemicals	Equation
Gallic acid	$Y = 5067.7X - 17.451$	Ferulic acid	$Y = 2844.8X + 11.99$
<i>p</i> -Hydroxy benzoic acid	$Y = 6226.5X + 75.291$	Naringin	$Y = 1801.9X + 14.701$
Chlorogenic acid	$Y = 1675.5X - 17.979$	Hesperidin	$Y = 1853.8X - 13.309$
Caffeic acid	$Y = 2918.2X + 14.292$	Quercetin	$Y = 3568.6X - 30.785$
Syringic acid	$Y = 5306.7X - 27.137$	<i>trans</i> -Cinamic acid	$Y = 12863X + 139.64$
<i>p</i> -Coumaric acid	$Y = 4539X + 29.536$		

distilled water. The supernatant was separated by sedimentation, and the polysaccharide in the supernatant assayed according to the carbazole reaction method of Zhang (Zhang *et al.*, 1996).

Analysis of phenolic compounds

HPLC was conducted using a Yonglin LC Model system (Yonglin Co., Korea). The HPLC system consists of a SP930D pump, a MIDAS auto-sampler, a UV730D ultraviolet-visible detector and a CTS30 column oven. Peak area was calculated using Yonglin autochro-3000 v1.0.0 software. YMC Hydro-sphere C18 column (250 × 4.6 mm I.D. 5 μm) was used. The mobile phase for analysis of 11 phenol compounds was applied to a gradient system (100 : 0-50 : 50 for 0-40 min), with 0.1% formic acid in water: 0.1% formic acid in acetonitrile. The analyses were carried out at a flow-rate of 0.8 mL min⁻¹ with UV detection at 270 nm. Column temperature was kept at 40 °C. A standard solution containing 11 phenol compounds was used to calibrate the retention time and standard curve (Table 1).

RESULTS AND DISCUSSION

Determination of solvent

Certain phenolic compounds were not dissolved in pure water. Since the methanol in crude extracts increases the solubility of these compounds, standards of phenols were diluted in methanol. It has also been reported that the antioxidant activity of certain compounds depends on the solvent used (Vandenberg *et al.*, 1999).

Yield of extracts and fractions

The yields (%) of leaf, stem, and root extracts were 15.9, 8.7, and 12.5% (w/w), respectively. The yields (%) of *n*-hexane, EtOAc, BuOH, and aqueous layer fractions from leaf extract were 22.2, 33.3, 11.1, and 33.3% (w/w), respectively. The yields (%) of *n*-hexane, EtOAc, BuOH, and aqueous layer fractions from stem extract were 53.8, 9.5, 7.6, and 29.1% (w/w), respectively. The yields (%) of *n*-hexane, EtOAc, BuOH, and aqueous layer fractions from *O. elatus* root extract were

Table 2. Yield of extracts and fractions from *Oplopanax elatus*

Plant parts	Extracts and fractions	Weight (g)	Rate of each plant parts (%)
Leaf	Dry weight	225.7	–
	MeOH extract	36.0	15.9
	Hexane layer	8.0	22.2
	EtOAc layer	12.0	33.3
	BuOH layer	4.0	11.1
	Aqueous layer	12.0	33.3
Stem	Dry weight	1800.0	–
	MeOH extract	158.0	8.7
	Hexane layer	85.0	53.8
	EtOAc layer	15.0	9.5
	BuOH layer	12.0	7.6
	Aqueous layer	46.0	29.1
Root	Dry weight	1000.0	–
	MeOH extract	125.4	12.5
	Hexane layer	51.7	41.2
	EtOAc layer	25.5	20.3
	BuOH layer	7.3	5.8
	Aqueous layer	40.9	32.6

41.2, 20.3, 5.8, and 32.6% (w/w), respectively (Table 2).

DPPH free radical scavenging activity

The radical scavenging activities of the samples were determined from the reduction in the optical density (OD) of free radical at 517 nm.

Free radical scavenging activity expressed as RC₅₀ ranged from 8.8 to 400 μg dry extracts and fractions/mL (Table 3). Values are presented in the following order: BuOH fraction and EtOAc fraction > aqueous fraction > MeOH extract. BuOH and EtOAc fractions contained higher phenolic substances, representing intermediate polarity. This is also in accordance with findings by Parejo *et al.* (2002), Maria *et al.* (2006).

Table 3. DPPH¹⁾ free radical scavenging activity of extracts and fractions from *Oplopanax elatus*

Plant parts	Extracts and fractions	RC502) ($\mu\text{g}/\text{ml}$)
Leaf	MeOH extract	40.0 \pm 1.0
	Hexane layer	NE
	EtOAc layer	75.0 \pm 0.0
	BuOH layer	14.0 \pm 0.0
	Aqueous layer	44.0 \pm 1.0
Stem	MeOH extract	150.0 \pm 0.0
	Hexane layer	300.0 \pm 0.0
	EtOAc layer	20.0 \pm 0.0
	BuOH layer	19.5 \pm 0.5
	Aqueous layer	51.7 \pm 1.5
Root	MeOH extract	35.1 \pm 2.8
	Hexane layer	310.0 \pm 0.0
	EtOAc layer	22.0 \pm 0.0
	BuOH layer	8.8 \pm 1.5
	Aqueous layer	26.0 \pm 1.2
	α -Tocopherol	12.0
	BHA ³⁾	14.0
	BHT ⁴⁾	34.0

¹⁾DPPH: 1,1-diphenyl-2-picryl-hydrazyl, ²⁾RC₅₀: Amount required for 50% reduction of DPPH after 30 min. Each value is mean \pm standard deviation of three replicate tests, ³⁾BHA: Butylated Hydroxyanisole, ⁴⁾BHT: Butylated Hydroxytoluene. NE = not examined due to amount of chlorophyll.

Ferric thiocyanate method (FTC)

The amount of peroxide in the initial stages of lipid oxidation was measured every 48 h, over a period of 34 days. The absorbance of positive control was reached to maximum value on the 30th day. As shown in Fig. 2, a low absorbance value represents a high level of antioxidant activity. All parts of *O. elatus* exhibited higher activities than α -tocopherol. Leaf extract was the highest antioxidative activity compared to other plant parts tested. The finding is a similar result with the report of Yen and Hsieh (1998), who found that the antioxidative activity of leaf extract of Du-Zhong (*Eucommia ulmoides*) was higher than the raw and roasted cortex of the plant. On the other hand, Abdul Hamid, Md. Shah, Muse, and Mohamed (2002) reported that ethanol extract of root of *C. asiatica* exhibited the highest activity although it was not significantly different from the leaves. The antioxidative activity of different parts of *O. elatus* may be due to the reduction of hydroperoxides, inactivation of free radicals, chelation of metal ion. From these results, we could therefore suggest that the consumption of this medicine plant could possibly offer some dietary bene-

Table 4. Amounts of total phenolic compounds from *Oplopanax elatus* extracts

Plant parts	Extracts and fractions	Total phenolic compounds [pyrocatechol equivalents ($\mu\text{g}/\text{mg}$)]
Leaf	MeOH extract	62.4 \pm 0.37
	Hexane layer	59.0 \pm 1.09
	EtOAc layer	64.0 \pm 1.93
	BuOH layer	76.4 \pm 1.54
	Aqueous layer	55.2 \pm 0.61
Stem	MeOH extract	65.3 \pm 2.14
	Hexane layer	59.2 \pm 0.22
	EtOAc layer	101.9 \pm 4.46
	BuOH layer	92.9 \pm 3.30
	Aqueous layer	109.8 \pm 4.35
Root	MeOH extract	70.2 \pm 2.51
	Hexane layer	58.9 \pm 1.05
	EtOAc layer	101.1 \pm 6.23
	BuOH layer	97.0 \pm 5.63
	Aqueous layer	55.9 \pm 0.45
	Control	-

Data expressed as means \pm s.e.m. of three samples analysed separately.

fits since they contain constituents, which are able to protect against lipid peroxidation and to scavenge free radicals. The differences in antioxidative activities observed here could be ascribed to several factors, including the different mechanisms involved in the two determination methods, structures of the different phenolic compounds, the antioxidative mechanisms exhibited by the compounds and possibly, also, due to the synergistic effects of interaction with other compounds.

Total phenolic content

The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (Komali *et al.*, 1999; Moller *et al.*, 1999). Table 4 shows the total phenolic compounds found in leaves, stem and roots of *O. elatus*. The results of fractions from leaf were lower than that of the total phenolic compounds from stem and root. Evaluation of phenolic compounds in Korean medicinal plants have popularly established. However the content of total phenolic compounds was very variable in 82 medicinal plants. As an example of low content, *Acanthopanax gracilistylus* showed less than 60 $\mu\text{g}/\text{mg}$ of phenolic compounds (Lim *et al.*, 2004). Total phenolic compounds in all parts of *O. elatus*, compare with another Korean medicinal plants, showed high.

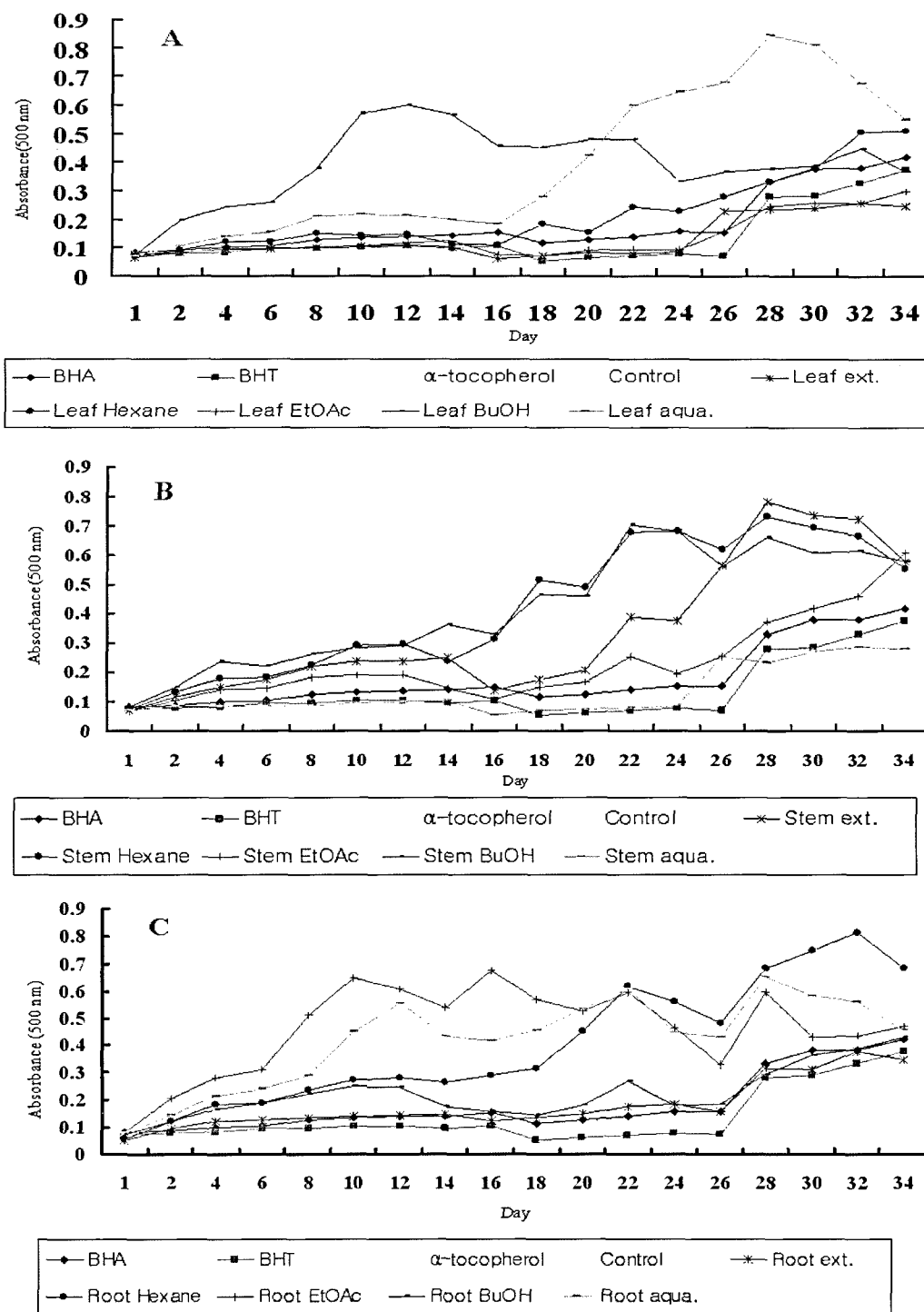


Fig. 2. Lipid oxidation was monitored every other day using FTC method. Low absorbance value indicated strong antioxidant activity. The final concentration of each sample was 0.005% w/v (A: leaf; B: stem; C: root).

Polysaccharides content

The method for colorimetric determination of acidic polysaccharide from *Panax ginseng* in Korean medicinal plant

was established previously (Lee *et al.*, 2006). It is possible to apply the method of carbazole-sulfuric acid to determination of pectin from *O. elatus*, and also to measure the amount of

Table 5. Amounts of polysaccharide from *Oplopanax elatus* extracts

Plant parts	Extracts and fractions	Polysaccharide [D-Galacturonic acid equivalents ($\mu\text{g}/\text{mg}$)]
Leaf	MeOH extract	82.3 \pm 1.02
	Hexane layer	NE
	EtOAc layer	24.8 \pm 0.98
	BuOH layer	113.1 \pm 1.85
	Aqueous layer	109.6 \pm 2.26
Stem	MeOH extract	86.3 \pm 2.65
	Hexane layer	NE
	EtOAc layer	77.3 \pm 0.72
	BuOH layer	113.4 \pm 2.15
	Aqueous layer	112.3 \pm 1.18
Root	MeOH extract	108 \pm 1.81
	Hexane layer	NE
	EtOAc layer	75.4 \pm 1.17
	BuOH layer	122.5 \pm 0.95
	Aqueous layer	114.7 \pm 0.27
	Control	-

Data expressed as means \pm s.e.m. of three samples analysed separately.

NE = not examined due to lack of quantity.

pectin in the mixture of various high molecular compounds such as starch, cellulose and gum. When the method of carbazole-sulfuric acid was applied to determine the amount of acidic polysaccharide, optical density at 530 nm increased linearly with an increase in the concentration of pure acidic polysaccharide.

The amounts of polysaccharide content in the leaves, stem and root of *O. elatus* are presented in Table 5. BuOH fractions of each plant part contained the highest amount of polysaccharide. These results indicated that the amounts of polysaccharide content has a noticeable effect on scavenging free radical. The mechanism of the antioxidant effect by which the amounts of polysaccharide content protect against a hydroxyl radical (OH) may involve radical-scavenging and reducing capability. In addition, D-galacturonic acid, with different -OH group orientation, exhibits stronger activity than glucuronic acid (Rao & Muralikrishna, 2006). The amounts of acidic polysaccharide in stem and root was higher than that of leaf. This is also in accordance with findings by Do *et al.* (1993).

Analysis of phenolic compounds

Some reports described that many phenolic compounds show maximum absorbance between 265 and 335 nm, while excitation and emission spectra are specific (Rodríguez-Delgado *et al.*, 2001). Phenolic determination of this study was

Table 6. Analysis of 11 phenol compounds concentrations in MeOH extract using HPLC

Plant parts	Extracts and fractions	Gallic acid	p-Hydroxy benzoic acid	Chlorogenic acid	Caffeic acid	Syringic acid	p-Coumaric acid	Ferulic acid	Naringin	Hesperidin	Quercetin	trans-Cinnamic acid	Total acid
Leaf	MeOH extract	0.0	0.0	0.0	212.6	93.0	0.0	52.9	0.0	0.0	0.0	0.0	358
	Hexane layer	0.0	0.0	0.0	0.0	0.0	87.5	0.0	0.0	0.0	0.0	0.0	87
	EtOAc layer	0.0	67.1	316.1	179.8	65.8	170.7	125.6	0.0	0.0	0.0	0.0	925
	BuOH layer	0.0	0.0	5527.5	489.0	208.4	0.0	0.0	0.0	0.0	0.0	0.0	6,224
	Aqueous layer	0.0	0.0	0.0	61.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	61
Stem	MeOH extract	0.0	0.0	204.8	193.4	84.3	0.0	313.1	0.0	98.0	93.5	29.1	1,016
	Hexane layer	0.0	0.0	0.0	37.1	0.0	98.1	110.0	0.0	0.0	0.0	9.8	255
	EtOAc layer	0.0	59.5	800.5	3035.2	435.1	1114.7	2613.2	0.0	434.7	588.5	567.2	9,648
	BuOH layer	0.0	0.0	9109.2	0.0	1366.8	0.0	0.0	2827.4	0.0	69.9	0.0	13,373
	Aqueous layer	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	213.0	0.0	0.0	213
Root	MeOH extract	0.0	0.0	6175.4	264.8	0.0	0.0	471.3	0.0	305.0	0.0	33.4	7,249
	Hexane layer	0.0	0.0	0.0	0.0	0.0	36.4	11.8	0.0	0.0	0.0	33.8	82
	EtOAc layer	0.0	46.6	2656.6	896.7	307.6	767.4	1384.2	0.0	558.9	573.8	464.9	7,656
	BuOH layer	0.0	0.0	2515.5	0.0	902.6	819.4	0.0	0.0	0.0	0.0	0.0	4,237
	Aqueous layer	0.0	0.0	12241.2	141.0	647.4	197.1	0.0	0.0	79.3	0.0	0.0	13,306

performed at 270 nm to verify the presence of other phenolic compounds. HPLC determination of phenolic compounds has become one of dominant analytical procedures because of its advantages. Using HPLC analysis, the content of phenolic compounds in parts of *O. elatus* are listed in Table 6. Phenolic compounds were quantified to the highest concentration of *p*-hydroxybenzoic acid in leaf EtOAc fraction (67.1 µg/g), chlorogenic acid in root H₂O fraction (12241.2 µg/g), caffeic acid in stem EtOAc fraction (3035.2 µg/g), syringic acid in stem BuOH fraction (1366.8 µg/g), *p*-coumaric acid in stem EtOAc fraction (1114.7 µg/g), ferulic acid in stem EtOAc fraction (2613.2 µg/g), naringin in stem BuOH fraction (2827.4 µg/g), hesperidin in root EtOAc fraction (558.9 µg/g), quercetin in stem EtOAc fraction (588.5 µg/g), and *trans*-cinnamic acid in stem EtOAc fraction (567.2 µg/g). The components against the antioxidative activity of *O. elatus* are currently unclear. Therefore, it is suggested that further work could be performed on the isolation and identification of the antioxidative components in *O. elatus*.

Association between antioxidative activity and phenolic compounds

EtOAc and BuOH fractions, which exhibited the highest phenolic content, also had high antioxidant activity and reducing power among the four solvent fractions partitioned from the methanolic extracts of *O. elatus*.

Pyo *et al.* (2004) also reported that polyphenols played an important role in the DPPH radical-scavenging activity. Yen *et al.* (1995) found that polyphenols are the most abundant group of compounds in tea leaf and appear to be responsible for antioxidant activity. Thus therapeutic properties of *O. elatus* extracts may possibly be attributed to the phenolic compounds present.

The present study demonstrates that *O. elatus* extracts contained high amount of phenolic compounds and showed high antioxidant activity. Our results may help to the identification of extracts that could contribute to sustain antioxidant status and protect against free radical damage. It provides useful information for the development of safe food products and additives with appropriate antioxidant properties.

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