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Antioxidant Activity of Phenolic Compounds from *Dendropanax dentiger* (*Harms*) Merr.

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Dendropanax dentiger (*Harms*) Merr. is a species of *Dendropanax* from Araliaceae family. It is traditionally used as a folk medicinal plant in China for a variety of diseases. Dried ethanol extract of *D. dentiger* was made and dissolved in distilled water and then fractioned by re-extracting with petroleum ether, ethyl acetate and *n*-butanol. Subsequently, 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity of these fractions and its isolated phenolic compounds was determined. It is observed that the ethyl acetate fraction exhibited the highest 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity with an IC_{50} value of 53.743 ± 0.012 mg/L. Among the isolated phenolic compounds, quercetin, luteolin, caffeic acid showed significent 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity with IC_{50} values of 0.051 ± 0.001, 0.038 ± 0.007 and 0.057 ± 0.020 mmol/L, respectively.

Key Words: *Dendropanax dentiger*, Phenolic compounds, Antioxidant activity.

INTRODUCTION

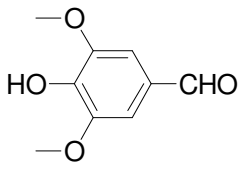
A large body of evidence has been accumulated that an over production of free radicals have been shown to be involved in many biological processes that cause damage to lipids, nucleic acids, membranes and proteins, thus giving rise to a variety of diseases[1], such as atherosclerosis, cancer, ageing, inflammatory and cardiovascular disease[2]. Thus, attention is being focused on the natural antioxidants and recent studies have investigated the potential of plant products to serve as antioxidants against various diseases induced by free radicals[3]. Additionally, it has been determined that the antioxidant effect of plant products is mainly due to phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes[4,5].

The genus *Dendropanax (*Araliaceae*)*, native to tropical American and East Asian regions of the world[6], are traditionally used as medicinal plants for the pharmacological activities such as antirheumatic, liver-prection, anticancer, antimicrobial, antiarrhythmia, anticomplement, antiatherogenic and antifungal[7-11]. Previously, several studies have been demonstrated that *Dendropanax morbifera Lev. (*Araliaceae) from Korea has superior biological activities such as antioxidant[12]. *D. dentiger* is traditionally used as a folk medicinal plant in China for a variety of diseases, such as migraine headache, dysmenorrheal, rheumatoid arthritis and rheumatic arthritis[13] and also applicable to functional health foods, food additives, beverage compositions, fodder additives, *etc*. Therefore, as relatives plant of *D. morbifera*, *D. dentiger* might be a good candidate for antioxidant and further development for antioxidant remedies or as a neutraceutical.

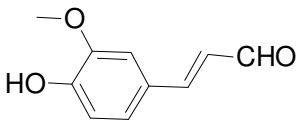
However, there is a little information related to antioxidant activity of *D. dentiger*. In our ongoing activity for identification and development of new anticancer agents for breast cancer from traditional medicinal plant, we explored the antioxidant potential of ethanol extract of stem of *D. dentiger* and its sub-fractions were evaluated by DPPH free radical scavenging assay. It is interesting that we found ethyl acetate and *n*-butanol fractions showed significant DPPH free radical scavenging activity. In our previous works, we have analyzed chemical constituents of ethanol extract of *D. dentiger* and the main phenolic chemical constituents of ethyl acetate and *n*-butanol fractions are syringaldehyde (**1**), coniferylaldehyde (**2**), scopoletin (**3**), sinapaldehyde (**4**), quercetin (**5**), luteolin (**6**), sinapaldehye glucoside (**7**), syrigin (**8**), ferulic acid (**9**), (E)-4-hydroxylcinnamic acid (**10**), caffeic acid (**11**)[14]. In the light of this background, the purpose of the present study was to evaluate the involvement of constituents and to compare their effectiveness in the antioxidant effect of ethanol extract of *D. dentiger* by DPPH free radical scavenging assay.

EXPERIMENTAL

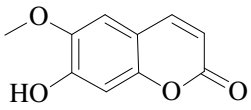
DPPH was purchased from TCI (Japan) and L-Ascorbic was purchased from Sigma (St. Louis, MO). The solvents used for extraction were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other unlabelled chemicals and reagents were of analytical grade.



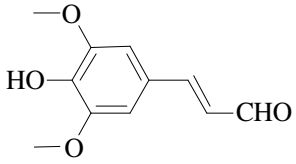
Syringaldehyde (**1**)



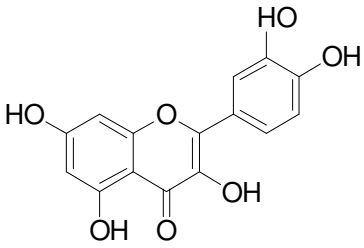
Coniferyl aldehyde (**2**)



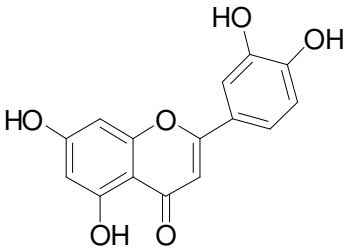
Scopoletin (**3**)



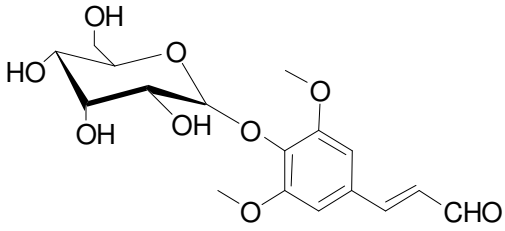
Sinapaldehyde (**4**)



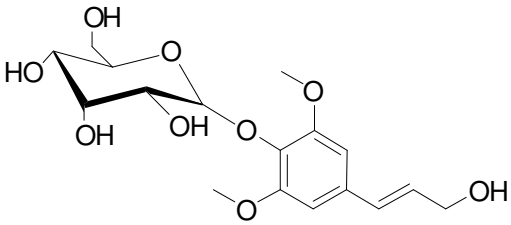
Quercetin (**5**)



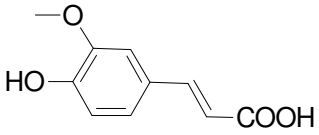
Luteolin (**6**)



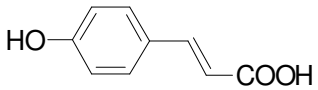
Sinapaldehye glucoside **(7)**



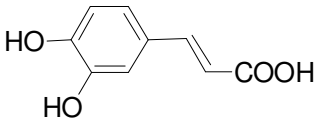
Syrigin (**8**)



Ferulic acid (**9**)



(E)-4-hydroxylcinnamic acid (**10**)



Caffeic acid (**11**)

Scheme-I: Structure of phenolic compounds from *D. dentiger*

D. dentiger stem was collected in May from Xiu Shui of JiangXi Province and identified by Prof. Dr. Cheming Tan from JiuJiang herbarium, Voucher specimens are dried at room temperature and deposited in the herbarium of JiuJiang; herbarium code number: 2004.3-10000.

Extraction and Structure of phenolic compounds

Preparation of extracts: The dried samples (50 kg) were cut into small pieces and soaked in 80 % ethanol at room temperature for 3 days. The extract was decanted, concentrated in a rotary evaporator, dried ethanol extract was dissolved in distilled water and then fractioned by re-extracting with petroleum ether, eethyl acetate and *n*-butanol, in sequence to obtain respective fractions.

Structure of phenolic compounds from *D. dentiger* are given in **Scheme-I**.

DPPH free radical scavenging assay: DPPH free radical scavenging activity of the petroleum ether, ethyl acetate and *n*-butanol fractions of ethanol extract was measured. Then, the activities of pure compounds isolated from these fractions were also determined. Experiments were carried out following the method described by Blois[15] with a slight modification. Briefly, 0.2 mmol/L solution of DPPH radical solution in ethanol was prepared and then, 0.1 mL of this solution was mixed with 0.1 mL of sample solutions in ethanol. Finally, after 0.5 h of incubation at room temperature, the absorbance was measured at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH free radical scavenging activity. This activity is given as % DPPH free radical scavenging that is calculated in the equation:

$$\text{DPPH free radical scavenging}(\%) = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%$$

The DPPH solution without sample solution was used as control and L-ascorbic was used as a positive control.

RESULTS AND DISCUSSION

Statistical analyses of results of activity studies: In this study, the DPPH free radical scavenging capacities of three fractions and phenolic compounds were determined and expressed as IC_{50} values (the concentration required to inhibit radical formation by 50 %). The complete inhibition of DPPH free radical by test samples was observed at a range of 0-200 mg/L (Fig. 1). Among the fractions, the ethyl acetate and *n*-butanol fractions were found to be the most potent DPPH free radical scavengers with IC_{50} values of 53.743 ± 0.012 and 102.824 ± 0.004 mg/L, respectively, while the petroleum ether was the least active scavenger. The IC_{50} value of a well known antioxidant compound used as a reference in this study, IC_{50} values of L-ascorbic acid is 20.169 ± 0.012 mg/L (Table-1).

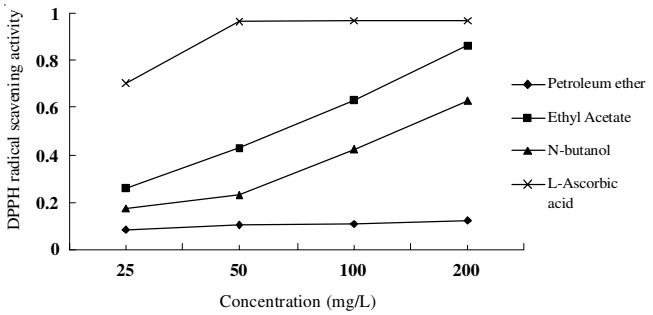


Fig. 1. DPPH free radical scavenging activity of the extracts from *D. dentiger*. Results are mean ± S.D. (n = 4).

TABLE-1
SCAVENGER EFFECT OF *D. dentiger*. EXTRACT: PETROLEUM ETHER FRACTION, ETHYL ACETATE FRACTION AND *n*-BUTANOL FRACTION ON DPPH FREE RADICAL

Material	IC_{50} mg/L
Petroleum ether Fr.	>200
Ethyl acetate Fr.	53.743 ± 0.012
n-Butanol Fr.	102.824 ± 0.004
L-Ascorbic acid	20.169 ± 0.012

The isolated compounds as well as the positive control, L-ascorbic, were also evaluated and compared for their DPPH free radical scavenging capacities. The complete inhibition of DPPH free radical by test samples was observed at a range of 0-1.0 mmol/L (Fig. 2). As shown in Table-2, quercetin (**5**), luteolin (**6**), caffeic acid (**11**) showed significant free radical scavenging capacities with IC_{50} values of 0.051 ± 0.001, 0.038 ± 0.007 and 0.057 ± 0.020 mmol/L, respectively, while the other phenolic compounds had moderate DPPH free radical scavenging activity. The relative order of DPPH free radical scavenging capacity for the isolated phenolic compounds was found to be: **5 > 6 > 11 > 9 > 2 > 3 > 10 > 4 > 1 > 7 > 8**.

It is generally accepted that the DPPH free radical scavenging ability is attributable to their hydrogen donating ability[16].

The number of hydroxyls present in the aromatic ring is likely the crucial factor of the significant activity differences between these phenolic acid derivatives[17]. Though the series of compounds tested was limited. For example, quercetin, luteolin and caffeic acid were disclosed to be the most active

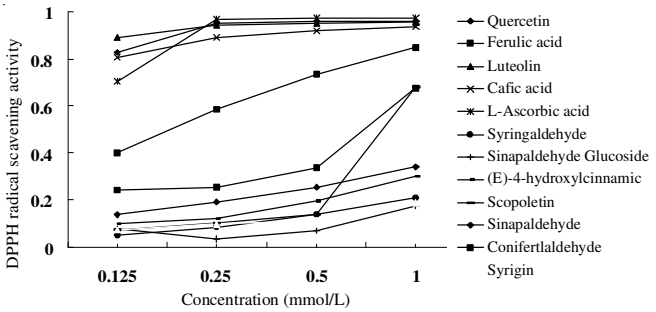


Fig. 2. DPPH free radical scavenging activity of the pure phenolic compounds from D. dentiger. Results are mean ± S.D. (n = 4)

TABLE-2
SCAVENGER EFFECT OF PHENOLIC COMPOUNDS FROM *D. DENTIGER* ON DPPH FREE RADICAL

Phenolic compounds	IC_{50} mmol/L
Syringaldehyde (1)	>1
Coniferyl aldehyde (2)	0.687 ± 0.092
Scopoletin (3)	0.741 ± 0.085
Sinapaldehyde (4)	>1
Quercetin (5)	0.038 ± 0.007
Luteolin (6)	0.051 ± 0.001
Sinapaldehye glucoside (7)	>1
Syrigin (8)	>1
Ferulic acid (9)	0.188 ± 0.039
(*E*)-4-hydroxylcinnamic acid (10)	0.895 ± 0.097
Caffeic acid (11)	0.057 ± 0.020
L-Ascorbic acid	0.059 ± 0.007

free radical scavenger because they have more than two hydroxyl groups, all of which were more active than those with one or none hydroxyl (s) such as ferulic acid, coniferyl aldehyde and sinapaldehye glucoside, other substitutions on the aromatic ring such as methoxyl contributs less DPPH free radical scavenging ability than hydroxyls. However, it is noteworthy that ferulic acid showed a higher antioxidant activity than (E)-4-hydroxyl cinnamic acid, indicating that an adjacent substituted methoxyl group in the aromatic ring enhanced its free radical scavenging capacity. A possible explanation for the result is that the conjugation between the isolated electron pair at O atom in methoxyl group and the π electrons on the benzoic ring framework: p-π conjugation, which enhanced the stability of benzoic-O radical, while two adjacent substituted methoxyl group in the aromatic ring reduced the free radical activity because of the difficulty of quinone formation involved in the reaction mechanism between phenolics and free radicals[18], for example, coniferyl aldehyde showed a higher antioxidant activity compared to sinapaldehyde. The results from this study support the notion that *ortho*-hydroxyl structures are crucial for the enhanced antioxidant activity because the *ortho*-quinone was easily formed[19]. The structure activity relationship evaluation of these phenolic compounds suggested that the number of hydroxyls was the most important factor in determining the antioxidant activity of the phenolic compounds.

Conclusion

In this study, it was demonstrated for the first time that the ethyl acetate and *n*-butanol fractions from *D. dentiger* stem possessed an excellent antioxidant activity based on the DPPH free radical assay. Some of the compounds isolated from *D.*

dentiger exhibited significant radical scavenging activity. In addition, this investigation of antioxidant phenolic compounds suggested that the potency of these compounds could provide a chemical basis for some of the health benefits claimed for *D. dentiger* in foods and folk medicine. The results indicated that the extracts and pure phenolic compounds from *D. dentiger* might be used as potential natural antioxidants and alternatives to synthetic antioxidants. Future studies should focus on the employment of modern medical chemical techniques to modify the structures of certain purified plant ingredients into better agents with high efficiency and activity. In addition, further investigations will focus on *in vivo* pharmacological studies.

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