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Antioxidative Activity of Phenolic Compounds of Allspice (*Pimenta dioica*)

- Research Note -

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Abstract

The flavonoids kaempherol and epicatechin, and a proanthocyanidin fraction were isolated from the seed of allspice (*Pimenta dioica*). Their structures were determined by chemical and spectral analyses. Antioxidant activities of kaempherol, epicatechin and the proanthocyanidin fraction were tested using the reaction with the stable radical diphenyl-*p*-picrylhydrazyl (DPPH) and exhibited IC_{50} values of 7.83, 4.27 and 2.92 g/mL, respectively. These results demonstrate that allspice proanthocyanidins and flavonoids might act as effective antioxidants.

Key words: allspice, *Pimenta dioica*, kaempherol, epicatechin, proanthocyanidin, antioxidant activity, DPPH

INTRODUCTION

The seed of *Pimenta dioica* is referred to as allspice because it smells like a combination of spices, especially cinnamon, cloves, ginger and nutmeg. Allspice is used in a variety of foods in the bakery and meat industries (1). Synthetic antioxidants such as BHA and BHT have been restricted for use in food because various studies have shown them to be carcinogenic (2). Thus, with the increasing safety concerns of consumers about food additives and the higher manufacturing cost and lower efficiency of natural antioxidants, studies on alternative sources of natural and safe food antioxidants are needed (3). Therefore, studies screening for natural antioxidants, especially of plant origin, have been notably increased in recent years (4).

In the present study, the isolation and the structural characterization of allspice flavonoids and proanthocyanidin fractions having strong antioxidative activity have been reported.

MATERIALS AND METHODS

Experimental

The UV spectra were recorded on a UV-VIS spectrophotometer (HP V-550, Hewlett Packard, CA, USA). The EI-MS was recorded on a mass spectrometer (HP 5985, Hewlett Packard, CA, USA). NMR spectra were recorded by ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) (Gemini 2000, Varian, CA, USA).

Extraction and isolation

Three kilograms of allspice powder were purchased from Pacific Spice Co., Inc., and three times percolated in methanol (40 L × 3) for 3 weeks. The methanol extract (250 g) was fractionated by its solubility in chloroform, ethylacetate and water. The ethylacetate-soluble part (34 g) was subjected to chromatography on a silica gel column chromatography eluted with chloroform containing increasing amounts of methanol. Fractions eluted from the column were rechromatographed on a silica gel column with chloroform-methanol 10:1 and chloroform-methanol 5:1 and yielded compound 1 (5 mg) and compound 2 (30 mg), respectively.

The water-soluble part (180 g) was subjected to chromatography on Sephadex LH-20 eluting with 30% methanol to remove the non-phenolic fraction A1 (115 g), with 70% methanol to remove non-proanthocyanidin fraction A2, and with 100% methanol to afford proanthocyanidin fraction A3. A2 and A3 (1.4 g) were collected by detecting with $FeCl_3$ TLC solution. A3 (10 mg) was heated at 80°C with 7 mL of 5% HCl in *n*-butanol for 2 hrs to observe the UV spectrum of anthocyanidin unit which consists of A3 (4).

Radical scavenging activity on DPPH

Allspice fraction, L-ascorbic acid and samples (1~1000 g) were dissolved in 100 μL MeOH and then added to 100 μL of 2 mM methanolic solution of diphenyl-*p*-

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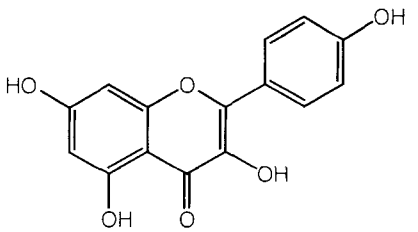
picrylhydrazyl (DPPH). After mixing and 20 min of standing at room temperature, the DPPH radical level was measured spectrophotometrically at 517 nm. The antioxidant activity was expressed as the IC_{50} (concentration in g/mL required to inhibit DPPH radical formation by 50%) determining from the inhibition curve (5).

RESULTS AND DISCUSSION

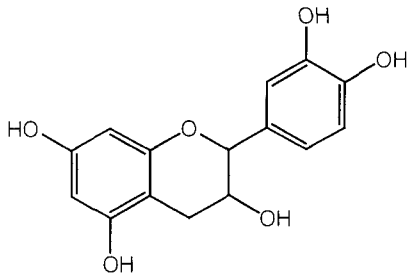
Chemical analysis

Compound 1, mp 276~278°C, was obtained as a yellowish amorphous powder from $CHCl_3$ and MeOH. The EI-MS spectrum of compound 1 showed a $[M]^+$ at *m/z* 286 and the UV spectrum showed absorption maxima at 266 and 367 nm that were characteristic of a flavon. In the ^{1}H-NMR spectrum of compound 1, the 5, 7-disubstitution pattern of A-ring was indicated by the two doublets at δ 6.16 (1H, d, H-6) and δ 6.44 (1H, d, H-8) with a meta coupling constant of 2.4 Hz, a typical A_2B_2 system at δ 8.06 (2H, *J*=9.0 Hz, H-2′, 6′) and 6.89 (2H, d, *J*=9.0 Hz, H-3′, 5′) which indicated the three aromatic protons in the B-ring. Accordingly, the structure of compound 1 was elucidated as kaempherol (Fig. 1) by comparison of the spectral data (Table 1) with an authentic sample as described in the literature (6).

Compound 2 was obtained as a white amorphous powder. The EI-MS gave a qausimolecular ion $[M]^+$ peak at m/z 290. In the ^{1}H-NMR spectrum of compound 2, the 5,7-disubstitution pattern of A-ring was indicated by the two doublets at δ 5.91 (H-6) and 6.01 (H-8) with a meta coupling constant of 2.4 Hz, a typical ABX system at δ 7.04 (1H, d, *J*=1.8 Hz), 6.83 (1H, dd, *J*=8.4, 1.8 Hz) and 6.77 (1H, d, *J*=8.4 Hz) indicated the presence of three aromatic protons in a B-ring. Its identity as epicatechin was apparent from the ^{13}C-NMR spectrum which showed a methylene carbon in the upfield region δ 28.9 ppm and two oxygenated methine carbons in the heterocyclic region δ 66.9 and δ 79.3 ppm which were characteristic of the pyran C-ring of flavanols. The upfield position of the C-2 carbon δ 79.3 ppm was characteristic of the epicatechin chemical shift (7), and this was confirmed by the cis orientation of the C-2 and C-3 substituents on the heterocyclic C-ring as revealed by the small proton-proton couplings between the H-2 and



Compound 1 (kaempherol)



Compound 2 (epicatechin)

Fig. 1. Structure of isolated flavonoids from allspice.

Table 1. Spectrum data of compounds extracted from *Pimenta dioica*

Compound 1	
Yellow powder	
UV	λmax (MeOH) nm: 266, 367
EI-MS	*m/z*: 286 $[M]^+$
^{1}H-NMR	(CD_3OD, 300 MHz) ppm δ 6.16 (1H, d, *J*=2.4 Hz, H-6), 6.44 (1H, d, *J*=2.4 Hz, H-8), 6.89 (2H, d, *J*=9.0 Hz, H-3′, 5′), 8.06 (2H, d, *J*=9.0 Hz, H-2′, 6′)
^{13}C-NMR	(CD_3OD, 75 MHz) ppm 94.45 (C-8), 99.25 (C-6), 104.54 (C-10), 116.29 (C-3′, 5′), 123.72 (C-1′), 130.67 (C-2′, 6′), 137.12 (C-3), 148.02 (C-2), 158.23 (C-9), 160.54 (C-4′), 162.50 (C-5), 165.55 (C-7), 177.34 (C-4)
Compound 2	
White amorphous powder	
EI-MS	*m/z*: 290 $[M]^+$
^{1}H-NMR	(CD_3OD, 300 MHz) ppm δ 2.72 (1H, dd, *J*=3.3, 16.5 Hz, H-4), 2.83 (1H, dd, *J*=4.5, 16.5 Hz, H-4), 4.19 (1H, m, H-3), 4.87 (1H, s, H-2), 5.91 (1H, d, *J*=2.4 Hz, H-6), 6.01 (1H, d, *J*=2.4 Hz, H-8), 6.77 (1H, d, *J*=8.4 Hz, H-5′), 6.83 (1H, dd, *J*=1.8, 8.4 Hz, H-6′), 7.04 (1H, d, *J*=1.8 Hz, H-2′)
^{13}C-NMR	(CD_3OD, 75 MHz) ppm δ 28.5 (C-4), 65.2 (C-3), 94.4 (C-8), 95.3 (C-6), 98.8 (C-10), 115.0 (C-2′), 115.1 (C-5′), 118.2 (C-6′), 130.9 (C-1′), 144.7 (C-4′), 144.8 (C-3′), 156.0 (C-7), 156.5 (C-5), 156.8 (C-9)
Proanthocyanidin fraction	
A tan amorphous powder	
^{1}H-NMR	(DMSO, 300 MHz) spectrum was complicated by rotational isomerism
^{3}C-NMR	(DMSO, 75 MHz) ppm δ 105~115, 145~150, 160

H-3 protons. Compound 2 was identified as epicatechin (Fig. 1) by comparison of spectral data (Table 1) with an authentic sample as described in the literature (8).

A3 tested positive for pheonols using the $FeCl_3$ test (dark blue). ^{1}H-NMR spectrum of A3 did not show any signal and low intensity resonance at 105~115, 145~150, 160 signal derived from the aromatic rings in the ^{13}C-NMR spectrum. After acid hydrolysis of A3 with 5% HCl and *n*-butanol, cyanidin and delphinidin were determined by measurement of their absorptions at 535 and 546 nm, respectively (4). These results suggested that A3 is a proanthocyanidin (Table 1). However, its structure could not be elucidated. Although proanthocyanidins are important, their isolation is difficult because of their high molecular weight as is their identification because of complications introduced by conformational isomerism at ^{1}H-NMR and ^{13}C-NMR spectrum (9). Essential oils, phenolic acids, flavonoids, catechins, tannins and galloylglucosides have also been isolated from allspice (10,11).

The antioxidant activities of two compounds and one fraction from *Pimenta dioica* were tested by using the diphenyl-*p*-picrylhydrazyl (DPPH) method. Their antioxidant activities were defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% [IC_{50} μg/mL] and compared with those of the known antioxidant ascorbic acid. In this study, the antioxidative activities of compound 1 (kaempherol) and 2 (epicathechin), which have been previously reported in the literature (12), were IC_{50}=7.83 and 4.27 μg/mL, respectively. The 2.92 μg/mL value of the antioxidative activity of the proanthocyanidin fraction was nearly identical to the 2.61 μg/mL IC_{50} value of ascorbic acid (Table 2).

Polyphenols such as proantocyanidin are secondary metabolites and are widely distributed throughout the plant kingdom. In general, high-molecular polyphenols are very polar compounds because hydrophobicity decreases with increasing degree of polymerization. These compounds are not only water-soluble antioxidants but also strong inhibitors of numerous physiological enzymatic activities, due to nonspecific (and occasionally specific) protein binding ability. However, separation and determination of these high-molecular polyphenols are very difficult because of the enormous variety of isomers and oligomers with different degrees of polymerization.

Table 2. DPPH radical scavenging effects of L-ascorbic acid and compounds isolated from allspice

Samples	IC_{50} (μg/mL)
L-Ascorbic acid	2.61±0.26[1)]
Kampherol	7.83±0.12
Epicatechin	4.27±0.23
Proanthocyanidin fraction	2.92±0.17

[1)]Values are mean±SD (n=3).

We isolated a small amount of kampherol (5 mg) and epicatechin (30 mg) from allspice (3 kg), but it has a great deal of proanthocyanidin. Allspice has been previously reported to have potent antioxidant activity with eugenol as its main active component (7). These results indicate that the proanthocyanidin fraction might act as effective antioxidants as well as flavonoids and phenylpropanoid in allspice.

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