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Food Chemistry 71 (2000) 45–49

Food Chemistry

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Antioxidant activities of natural phenolic components from *Dalbergia odorifera T. Chen*

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Received 30 November 1999; received in revised form 29 February 2000; accepted 29 February 2000

Abstract

The antioxidant activities on oil of natural phenolic components extracted from *Dalbergia odorifera T. Chen* were investigated. A new benzophenone 2,4-dihydroxy-5-methoxybenzophenone (**1**), together with eight known components, were isolated. The eight components were identified by chemical and spectroscopic methods as 2′,3′,7-trihydroxy-4′-methoxyisoflavanone (**2**), 3′-methoxy-daidzein (**3**), 4′,5,7-trihydroxy-3-methoxyflavone (**4**), vestitol (**5**), medicarpin (**6**), hexanoic acid, 2-propenylester (**7**), hexadecanoic acid, ethyl ester (**8**) and 3,8,-nonadien-2-one (**9**). Their antioxidant activities were investigated and compared with butylated hydroxyluene (BHT) and α-tocopherol. The results showed that components **1**, **3**, **5** and **6** had antioxidant activity and components **2** and **4** had strong antioxidant activity at 0.02 and 0.04% levels. When the individual components (0.02%) were mixed with 0.02% BHT, or 0.02% α-tocopherol, their protection factor was increased, but there was no synergistic effect. When the individual component had 4 ppm added Fe^{3+}, components **1**, **2**, **3** and **4** had antioxidant activity. Their antioxidant activities were tested by an oxidative stability instrument (OSI) at 100°C. Six of the phenolic components showed antioxidant activities. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Suppression of lipid antioxidant reactions in food is a major cause of quality deterioration and off-flavour development (Kanner, Harel & Jeffe, 1991). The antioxidants may be used to preserve food quality from oxidative deterioration of lipid. Therefore, antioxidants play a very important role in the food industry. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and terbutylhydroquinone (TBHQ), are widely used in the food industry, but BHA and BHT have been suspected of being responsible for liver damage and carcinogenesis in laboratory animals (Grice, 1986; Wichi, 1988). Therefore, the development and utilization of more effective antioxidants of natural origin are desired.

A large number of Chinese traditional medicines are plants and 700 species of plants have been found to have obvious antioxidant activities and 24 plant species show strong antioxidant activities (Weng, Ren, Duan & Jiang, 1998). One of the plants is *Dalbergia odorifera T. Chen,* a high tree, named as Jiangxiang in Chinese traditional medicine, which is indigenous in Guangdong, PR China and belongs to the *Leguminosae*. This plant is not only valued for its medicinal properties, but is also antiscabietic. Previous phytochemistry studies reported that this plant contained phenolic components and other components (Shoji et al., 1989; Tetse, Shoji, Reiko, Ryoji & Toshihiro, 1990; Yukihiro, Fumiyuki, Masaak & Ushio, 1992).

The phenolic components have antioxidant activities in vivo (Fuhrman, Lavy & Aviram, 1995; Whitehead, Robinson, Allaway, Syms & Hale, 1995; Rusznyak & Sszent-Gyorgi, 1936; Zloch, 1969), and have been used as natural antioxidants in food (Kroyer, 1986; Pratt & Hudson, 1990). *D*. odorifera contains many phenolic components, but its strong antioxidant activity had not been reported.

We have systematically examined the chemical composition of *D. odorifera*. Nine components, including a new component, have been isolated from the roots of *D. odorifera* (Fig. 1). In this paper, we wanted to describe the antioxidant activity effects of nine components, and to compare their antioxidant effects with those commonly used as food antioxidants, such as BHT and α-tocopherol. The antioxidant activity of nine individual components with added BHT or α-tocopherol was studied.

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PII: S0308-8146(00)00123-0

Fe^{3+} may exacerbate food oxidation and the antioxidant activity of nine components was studied when Fe^{3+} was added. Their antioxidant activities were estimated by the oxidative stability instrument (OSI) at 100°C in order to find a natural antioxidant for use in food, especially in oil. Six phenolic components showed antioxidant activities.

2. Materials and methods

2.1. Plant materials

The root of *D. odorifera* was purchased from the Yantai Drug Company, Shandong Province, PR China in 1998 and stored at 4°C until used. This root was identified by Professor Runeng Zhao (Faculty of Pharmacy, Lanzhou Medical College, PR China).

2.2. Chemicals

Butylated hydroxyluene (BHT) and α-tocopherol, food grade antioxidants, were purchased from the Guangzhou Chemical Company, PR China. Silica gel was obtained from Qingdao Ocean Chemical Factory, PR China. Lard was rendered in the laboratory from fresh pig fat tissue, purchased from Yantai Slaughter House, PR China. $FeCl_3 \cdot 6H_2O$, C, P., was purchased from Jinshan Chemical Factory, PR China.

2.3. Equipment

M.p.s.: X4-micrope (the fourth instrument of Beijing) uncorr. Optical rotation: polarimeter 241 (Perkin Elmer), solvent MeOH. IR spectra were recorded with a Nicolet-5DX. IR spectrometer .^1H,^{13}C and 2D NMR spectra were recorded with a Bruker AM-400, Solvent

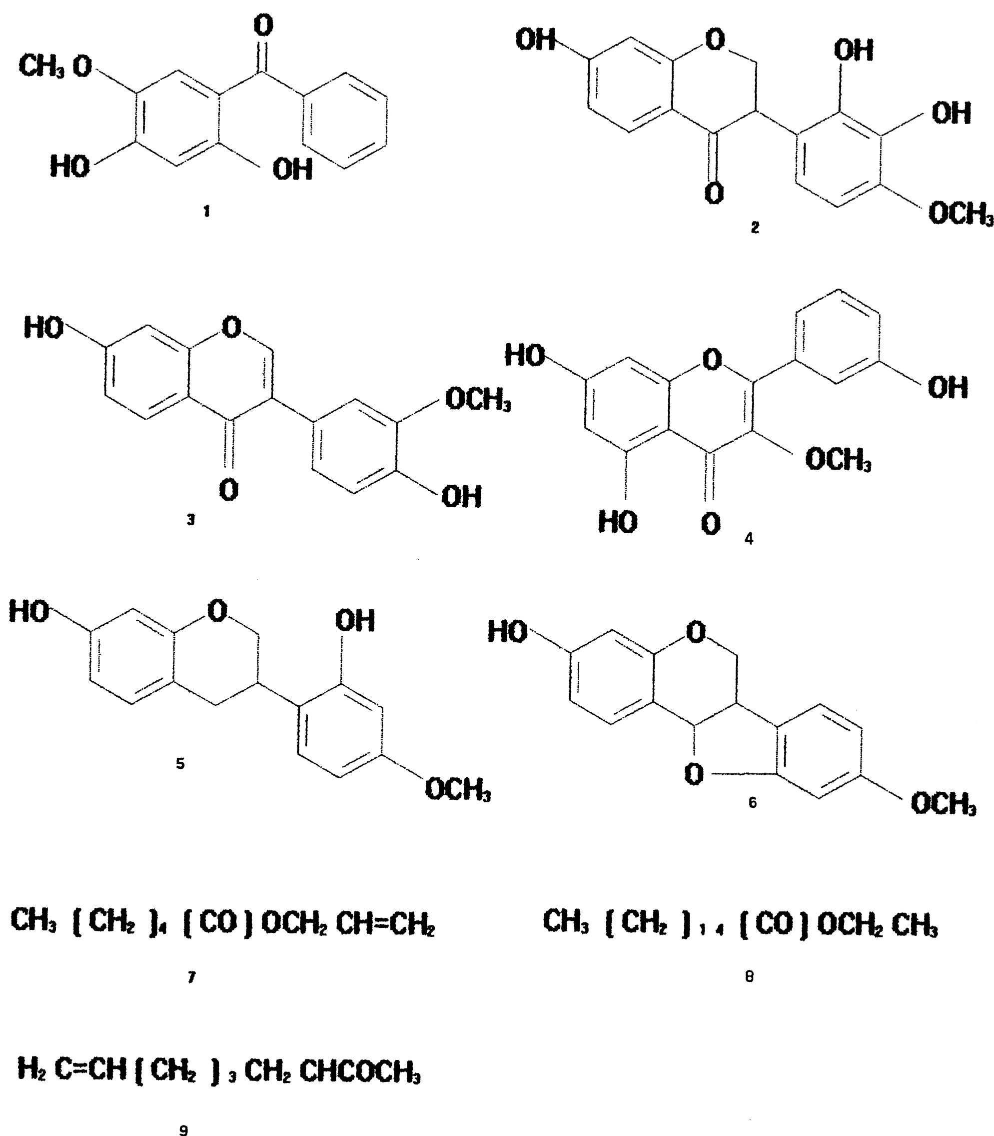

Fig. 1. Nine compound structures.

CDCI$_3$, acetone-d_5, and DMSO, using TMS as internal standard. EI–MS were determined at 70 eV (180°C) on a ZAB-HS mass spectrometer.

2.4. Extraction and isolation

The dried and powdered root of *D. odorifera* (3 kg) was extracted repeatedly with 95% ethanol for 2 weeks at room temperature. The extract was concentrated to dryness under reduced pressure and the residue (318 g) was suspended in water (1 L). The water layer was extracted with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH, respectively. The EtOAc extract was evaporated in vacuo to give a residue of 112 g. The residue was subjected to column chromatography (CC) on a silica gel column, eluted with petroleum ether:EtOAc (10:1–0:10), to give nine fractions. Fractions **1–3** were re-chromatographed on a silica gel column using petroleum ether:EtOAc (20:1–8:1) as eluent to give compound **7** (22 mg), compound **8** (20 mg) and compound **9** (25 mg), respectively. Fraction **4** was subjected to CC on a silica gel column with benzene:acetone (8:1–5:1) to obtain compound **1** (25 mg). Fractions **5–9** were re-chromatographed on a silica gel column eluted with $CHCl_3$:acetone (15:1–8:1) to give compound **2** (25 mg), compound **3** (30 mg), compound **4** (20 mg), compound **5** (21 mg) and compound **6** (20 mg), respectively.

2.5. Antioxidant activity

Antioxidant activities of the nine compounds were studied in lard with an Omnion OSI, MA, USA, at 100°C. The air flow rate was fixed at 20 L/h; Fe^{3+}, BHT and α-tocopherol were used for comparison studies.

The effect of the structure on activity, interpreted as the protection factor (Pf), was calculated according to the expression:

$$\mathrm{Pf} = \frac{\text{Induction period for (lard + antioxidant)}}{\text{Induction period for lard}}$$

The synergy effect (synergy%) was calculated according to the expression:

$$\mathrm{Synergy\%} = \frac{(\mathrm{IP}_{\mathrm{A+B\,combined}}) - \mathrm{IP}_\mathrm{A} - \mathrm{IP}_\mathrm{B}}{\mathrm{IP}_\mathrm{A} + \mathrm{IP}_\mathrm{B}}$$

3. Results and discussion

3.1. Spectrometric identification of isolated compounds 1–9

Compound **1**, yellow powder, mp: 191.2–192.5°C, had the molecular formula $C_{14}H_{12}O_4$ based on EI–MS data (*m*/*z* 244, M^+) and counting carbons and hydrogens from the data of its ^1H and ^{13}C NMR DEPT spectra. The IR of compound **1** showed the presence of hydroxy (3300 cm^{-1}), α,β-unsaturated carbonyl (1637 cm^{-1}) and substituted aromatic ring (1597, 1571, 1512 cm^{-1}). Its UV spectrum showed an absorption maximum at 252, 287 nm due to an aromatic ring. The IR and UV spectra of compound **1** revealed the presence of a partial structure of benzophenone (1637 cm^{-1}, 362 nm). The ^1H NMR spectrum showed the signal of one methoxy group at δ 3.96 (3H, s), and two kinds of aromatic protons. One kind of aromatic group had two signals, H-3′ and H-5′, at δ 7.47 (2H, sdd, 7.6 Hz, 7.3 Hz, 2.2 Hz), H-4′ at δ 7.54 (1H, tt, 7.3 Hz, 2.2 Hz), H-2′ and H-6′ at δ 7.63 (2H, sd, 7.6 Hz, 2.2 Hz) and this means H-2′, H-3′, H-4′, H-5′, H-6′, at one group. The ^{13}C NMR and ^{13}C NMR DEPT spectrum showed a ketonic carbonyl group C-7 at δ 200.0 and C-1′ at δ 138.3, C-3′ and C-5′ at δ 128.7, C-2′ and C-6′ at δ 128.3. The ^1H–^1H COSY and HMQC spectra supported assignment of all proton and carbons signal. The structures of compound **1** was further elucidated from ^{13}C–^1H long-range correlation in the HMBC spectrum. The cross peaks were: H-3 signal with C-1 and C-5 signal, H-6 signal with C-2, C-4 and C-7 signal, H-8 signal with C-5 signal, and H-2′ signal with C-4′, C-6′ and C-7 signal. Thus, the structure of **1** was elucidated as 2,4-dihydroxy-5-methoxybenzophenone. ^1H NMR and ^{13}C NMR data are in Table 1.

Compounds **2** and **3** were identified as the known structures 2,3,7-trihydroxy-4-methoxy-isoflavanone and 3-methoxydaidzin; their MS data, ^1H NMR and ^{13}C NMR data and physical constants agreed completely with those reported (Shoji at al., 1989).

Compounds **5** and **6** were readily identified as the widely known vestitol (Shoji et al., 1989) and medicarpin (Yukihiro et al., 1992) by MS data and ^1H NMR. Compounds **4**, **7**, **8** and **9** were determined to be 4′,5,7-trihydroxy-3-methoxyflavone, hexanoic acid, 2-propenyl

Table 1
^1H NMR and ^{13}C NMR spectral data for component **1**

Component	^1H NMR	^{13}C NMR
1		111.8
2		160.1
3	6.54 (1H,s)	99.9
4		137.8
5		153.6
6	7.10 (1H,s)	116.7
7		200.0
8	4.00 (3H,s)	65.2
−OH	5.24 (1H,s)	
	12.48 (1H,s)	
1′		138.3
2′	7.63 (2H, sd, 7.6 Hz, 2.2 Hz)	128.3
3′	7.47 (2H, sdd, 7.6 Hz, 7.3 Hz, 2.2 Hz)	128.7
4′	7.54 (1H, tt, 7.3 Hz, 2.2 Hz)	131.4
5′	7.47 (2H, sdd, 7.6 Hz, 7.3 Hz, 2.2 Hz)	128.7
6′	7.63 (2H, sd, 7.6 Hz, 2.2 Hz)	128.3

ester, hexadecanoic acid, ethyl ester and 3,8-nonadien-2-one by comparing MS spectral data and IR spectra with reported data (Heller & Milne, 1978a,b,c,d).

3.2. Determination of the antioxidant activities on OSI

The antioxidant activities of the nine components were different, as shown in Table 2; components **1–6** had antioxidant activity at 0.02 and 0.04% while component 9 had antioxidant activity only at 0.04%. Their antioxidant activities were obviously increased with dose. Antioxidant activities of components **1**, **2** and **4** were more than that of BHT or α-tocopherol at 0.02 and 0.04%, and component **3** was more than that of α-tocopherol at 0.02 and 0.04% and more than that of BHT at 0.04%. Components **2** and **4** had strong antioxidant activities due to their structures, (three hydroxy and one methoxy group), so antioxidant activities of components **2** and **4** were more than those of components **3** and **5** which had only two hydroxy and one methoxy group. Antioxidant activity of component **2**, which has three hydroxy groups, moreover two of them adjacent to one methoxy, was more than that of component **4**, in which hydroxy and methoxy were not adjacent, and components **3** and **5** show the same results as shown in Table 3. When 0.02% BHT was added to 0.02% of compounds **1–9** individually, antioxidant activities of components **1**, **3**, **4** and **5** were more than those at 0.02%, and when 0.02% α-tocopherol was added to 0.02% of components **1–9** individually, antioxidant activities of components **3** and **4** were more than those at 0.02%. Their synergy effect was negative, however. When 0.02% BHT or 0.02% α-tocopherol was respectively added to components **7**, **8** and **9**, they showed antioxidant activities. But these were due to the antioxidant activities of BHT or α-tocopherol, and their antioxidant activities were lower than those of 0.02% BHT or of 0.02% α-tocopherol. They, therefore, had an antagonistic effect on BHT or α-tocopherol.

Table 2
The antioxidant protection factor (Pf) of components at 100°C ($n=4$)

Component	0.02%[a,b]		0.04%[a,b]	
1	4.88±0.7*	+ +	6.09±0.08*	+ +
2	12.02±0.09*	+	17.12±0.13*	+
3	4.13±0.08*	+ +	6.52±0.11*	+
4	8.33±0.07*	+	15.07±0.11*	+
5	2.51±0.10*	+	4.11±0.13**	+
6	2.27±0.08*	+	3.46±0.05*	+
7	1.25±0.10*	+	1.0±0.08*	+
8	0.86±0.07*	+	1.75±0.09*	+
9	1.65±0.06*	+	2.07±0.11*	+
BHT	4.21±0.03		5.82±0.04	
α-Tocopherol	3.72±0.06		4.21±0.07	

[a] Compared with α-tocopherol *: $P<0.01$ **: $P<0.05$.
[b] Compared with BHT +: $P<0.01$ + +: $P<0.05$.

Table 3
The synergistic effects between components and BHT or α-tocopherol at 100°C ($n=4$)

Comp.[a]	0.02%comp. + 0.02%BHT	S[b] (%)	0.02%comp. + 0.02% α-Tocopherol	S (%)
1	6.10±0.07	−32.9	4.40±0.09	−48.8
2	11.29±0.11	−30.4	11.32±0.09	−28.1
3	7.27±0.09	−12.8	6.56±0.04	−16.4
4	8.95±0.09	−28.6	8.58±0.07	−28.8
5	4.25±0.08	−36.8	2.69±0.06	−56.8
6	4.14±0.04	−36.1	3.29±0.05	−45.1
7	4.18±0.04	−23.4	3.18±0.02	−36.0
8	3.73±0.02	−26.2	3.01±0.03	−66.8
9	3.79±0.04	−35.5	3.23±0.03	−39.9

[a] Comp.: component.
[b] S.: synergy%.

Table 4
The effects between components and Fe^{3+} at 100°C ($n=4$)

Component	0.02% Component + 4 ppm Fe^{3+}
1	4.67±0.06
2	8.10±0.05
3	2.06±0.02
4	7.92±0.05
5	1.13±0.04
6	1.82±0.02
7	0.85±0.01
8	0.69±0.01
9	0.93±0.01

During production, processing, distribution and storage of food, metal ions, especially Fe^{3+}, cause food deterioration. When there is Fe^{3+} in foods, such as oil, antioxidant activity clearly drops. Table 4 shows effects of 4ppm Fe^{3+} added to 0.02% components **1–9** individually.

Component **1** still appeared to have antioxidant activity and components **2** and **4** showed strong antioxidant activities; however, the antioxidant activities of components **2–9** clearly dropped, while the antioxidant activity of component **1** dropped only a little. This shows that Fe^{3+} had a few effects on the antioxidant activity of component **1**.

Components **1**, **2**, **3** and **4** showed very strong antioxidant activities. They will be useful for the food industry, especially the oil industry.

Acknowledgements

The authors thank the National Natural Science Foundation of China (No. 2972010) for fund support and also thank the National Laboratory of Applied Organic Chemistry and Analysis Centre, Lanzhou University, PR China for measuring ^{1}H and ^{13}C NMR spectra and EI–MS. The work was supported by the

National Laboratory of Applied Organic Chemistry of Lanzhou University of PR China.

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