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Antioxidants from a Chinese medicinal herb – *Psoralea corylifolia* L.

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Abstract

The powder and extracts of *Psoralea corylifolia* L. were tested in lard at 100 °C by using the oxidative stability instrument (OSI) and were found to have strong antioxidant effects. Six compounds, bakuchiol, psoralen, isopsoralen, corylifolin, corylin and psoralidin were isolated from the herb and identified by UV, IR, Mass, ^{1}H and ^{13}C NMR spectra and melting point. Their antioxidant activities were investigated individually and compared with butylated hydroxytoluene (BHT) and α-tocopherol by the OSI at 100 °C. The results showed that bakuchiol, corylifolin, corylin and psoralidin had strong antioxidant activities, and especially psoralidin (stronger antioxidant property than BHT), but psoralen and isopsoralen had no antioxidant activities at 0.02% and 0.04% levels. The antioxidant activities of the compounds decrease in the following order: Psoralidin > BHT > α-tocopherol > bakuchiol > corylifolin > corylin > isopsoralen ~ psoralen.

Keywords: Antioxidant activity; *Psoralea corylifolia* L.; Bakuchiol; Psoralen; Isopsoralen; Corylifolin; Corylin; Psoralidin

1. Introduction

Lipid peroxidation not only produces rancid odours and flavours, but also decreases safety and nutritional quality by destruction of essential fatty acids and vitamins in foods during cooking, processing and storing. Lipid peroxidation causes aging, heart disease and carcinogenesis (Edwin, 1996). Oxidation of foods can be retarded in several ways, such as conditions of vacuum, or air replaced by nitrogen or low temperature. In industrial processing, addition of highly effective antioxidants has become a popular and highly effective means to lengthen the shelf life of foods and to reduce nutritional losses and harmful substances formed (Kanner, Harel, & Jeffe, 1991; Tsuda, Ohshima, Kawakishi, & Osawa, 1994).

Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydro-quinone (TBHQ), are widely used in the food industry. However, animal test have demonstrated that BHA and BHT accumulate in the body and result in liver damage and carcinogenesis (Ames, 1983; Baardseth, 1989; Grice, 1986; Ito, Fukushima, Hasegawa, Shibata, & Ogiso, 1983; Ito et al., 1986; Wichi, 1988). Therefore, development and utilization of more effective and non-toxic antioxidants of natural origin are desired (Namiki, 1990).

Psoralea corylifolia L. has been used traditionally as medicine in China and recommended for the treatment of stomachic, deobstruent, anthelmintic, diuretic, vitiligo and also certain skin diseases, e.g., leucoderma, psoriasis and leprosy (Kotiyal & Sharma, 1992; Zhu, 1998). Few reports, however, have addressed the antioxidant activity of *P. corylifolia* L.

In this paper, the antioxidant activity effects of the components isolated from this herb were investigated and their antioxidant effects were compared with those of the most commonly used antioxidants, BHT and α-tocopherol, by OSI, in lard at 100 °C.

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2. Materials and methods

2.1. Materials

P. corylifolia L. was purchased from Shanghai Drug Company, PR China, dried with ventilation at ambient temperature, and stored at 4 °C until use.

2.2. Chemicals

BHT and α-tocopherol were purchased from the Chemical Company, Shanghai, China. Silica gel was bought from Qingdao Ocean Chemical factory, PR China. Lard was rendered in the laboratory from fresh pig fat tissue purchased from Shanghai Slaughter House, PR China. Other chemicals used in this experiment were all AR grade and from Shanghai Chemical Reagent Co.

2.3. Extraction

One kilogramme of air-dried and pulverized *P. corylifolia* L. was exhaustively extracted with petroleum ether and chloroform at room temperature, successively.

2.4. Chromatographing on silica gel column

After removal of the solvents in vacuum, 110 g petroleum ether extract and 65 g chloroform extract, respectively, were obtained . Both of the extracts were tested for their antioxidant activities. Twenty-four grammes of petroleum ether extract were chromatographed on a silica gel column (200–300 mesh, 350 g, 6.0 i.d. × 100 cm), eluting with petroleum ether and EtoAc mixtures of increasing polarity. Five fractions were obtained. From fraction 1 with petroleum, oil was obtained and fractions 2 and 3 were obtained with petroleum ether/EtoAc (9:1). Fractions 2 and 3 were purified by a further silica gel column (200–300, mesh) with petroleum ether/EtoAc (19:1), to give a light-yellow oil-like liquor, compound 1, (200 mg). The latter two fractions were obtained with petroleum ether/EtoAc (8:2) and were recrystallized from MeOH to give isopsoralen (compound 2, 10 mg) and psoralen (compound 3, 62mg).

Thirty-two grammes of chloroform extract were chromatographed on a silica gel column (200–300 mesh, 400 g, 6.0 i.d. × 100 cm) each with 3.0 l of a developing solvent system of petroleum ether/EtoAc (9:1, 8:2, 7:3 and 6:4, v:v) and fractions 1 and fraction 2 were obtained with petroleum ether/EtoAc (8:2). The two fractions were crystallized and recrystallized from MeOH to give isopsoralen (compound 2, 218.3 mg) and psoralen (compound 3, 215.2 mg). Four fractions were obtained with petroleum ether/EtoAc (7:3) and merged into two fractions according to TLC analysis. Corylifolin (compound 4, 10 mg) and corylin (compound 5, 25 mg) was obtained from fraction 2 after recrystallization. Psoralidin (compound 6, 23 mg) was obtained from fractions with petroleum ether/EtoAc (6:4).

2.5. Recording spectra

Mass spectra were recorded with an HP5989 mass spectroscopic instrument. Melting points (MP) were determined on a WRS-1B melting point apparatus, which was not calibrated. Ultraviolet (UV) spectra were recorded with a UV-260 spectroscopic instrument, and methanol was used as solvent and a quartz cuvette was used. IR-spectra were recorded on a Nicolet 5DX IR spectrometer. Samples were prepared for IR spectroscopy by incorporating the crystals into a KBr disc. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AM-400 and tetramethylsilane (TMS) was used as an internal standard.

2.6. Antioxidant activity

The lard without any additives was used as the substrate to evaluate the antioxidant activity of the powder, extracts and components of *P. corylifolia* L. Antioxidant activities of the six compounds and the extracts from the herb were added to lard in OSI sample tubes. The OSI instrument was set at 100 °C and the air flow rate was fixed at 20 l/h; BHT and α-tocopherol were used as comparison samples.

The effect of the structures on activity, interpreted as the protection factor (Pf), was calculated according to the expression: Pf = the induction period (IP) of lard with antioxidant/the IP of lard without antioxidant. All IP results were means of four parallel experiments.

3. Results and discussion

The chemical structure confirmation of the components from the *P. corylifolia* L. was accomplished by comparing the melting point, UV, IR, Mass, ^{1}H and ^{13}C NMR data obtained to those published.

Compound 1 was isolated and rechromatographed on a silica gel column with petroleum ether/EtoAc (19:1) as yellow oil-like liquor. It had maximum absorption at 262 nm in its UV spectrum. Its molecular weight was 256, determined by mass spectrum. Its IR (KBr, cm^{-1}) spectrum had strong absorption at 3397 (OH), 1720 (C=O), 1611, 1591, 1513 (C=C). Its detailed spectral data of IR, MS, ^{1}H and ^{13}C NMR listed in Table 1 agree well with the reported compound, bakuchiol (Labbe, Faini, & Coll, 1996; Mehta, Nayak, & Sukh, 1973).

Compound 2 was isolated and recrystallized from MeOH as white powder, MP: 137–138, MS *m/z* (%): 186(100), 158(85.00), 102(49.00), 130(31.36), 51(20.90),

Table 1
Spectral and melting point data of the compounds isolated from *P. corylifolia* L.

Names of compounds	Data of spectra					MP (°C)
	^{1}H NMR (400 MHz) TMS as int. standard	^{13}C NMR (400 Hz) TMS as int. standard	MS (*m*/*z*)	IR (cm^{-1}) (KBr)	UV (nm) (MeOH)	
1. Bakuchiol	1.19(s,3H),1.46 (t,2H),1.52(s,3H), 1.63(s,3H),1.96(q,2H), 5.10(t,1H),5.89(q,1H), 6.05(d,1H),6.20(d,1H), 6.70(d,2H),7.20(2H), 9.37(s, 1H)	17.61,23.03,25.61, 40.53,41.08,42.20, 111.82,115.4,124.8, 126.6,127.2,128.4, 130.5,134.0,145.9, 156.7	256(13),213(15), 173(100),145(47), 107(50),83(20), 69(27),55(52)	3397,1720, 1611,1591,1513	210.2, 262	
2. Isopsoralen	6.40(d,1H),7.14(m,1H), 7.38(d,1H),7.44(d,1H), 7.70(m,1H),7. 82(d,1H)	160.8,114.1,144.4, 123.8,108.8,157.4, 116.9,148.5,113.5, 145.8,104.1	186(100),158(85), 102(49),130(31), 51 (20), 187(20), 50 (19), 75 (17)		203.1,246.3, 297.7	137–138
3. Psoralen	6.36(d,1H),6.84(d,1H), 7.48(s,1H),7.68(s,1H), 7.70(d,1H),7.80(d, 1H)	160.8,114.5,144.0, 119.8,124.8,156.3, 99.6,151.9,115.3, 106.3,146.8	186(98),158(100), 102(39),130(22), 51(17),76(16), 187 (14), 75 (14)		208.3,244.7, 289.7, 327.9	162–163
4. Corylifolin	1.70(d,6H),2.60(dd,2H), 5.30(t,1H),5.40(dd,2H), 6.41(s,1H),6. 82(d,2H), 7.37(dd,2H), 7.50(s,1H), 9.45(s,1H), 10.47(s,1H)		324(100), 325(44)309(5), 205(48), 149(31),120(20), 91(13)	3282,1654, 1608	219,235,277,322	211–212
5. Corylin	1.40(s,6H),5.78(dd,1H), 6.40(d,1H),6.77(d,1H), 6.90(dd,1H),6. 99(d,1H), 7.32(d,1H), 7.35(dd,1H), 8.00(d,1H), 8.22(s,1H), 10.74(s,1H)	28.29, 76.91,102.9, 115.9,116.3,117.6, 121.4,122.6,124.2, 125.4,127.8,130.4, 131.8,153.3,158.4, 163.6,175.4	320(17),305(100), 306(21),169(5)	3235,2971, 1622,1568,1498	208, 247, 305	228–230
6. Psoralidin	1.75(s,3H),1.77(s,3H), 3.38(d,2H),5.40(t,1H), 6.93(s,1H),7. 00(dd,1H), 7.18(s,1H), 7.67(s,1H), 7.72(d,1H), 9.97(s,1H), 10.7(s,1H)	18.14, 26.13,28.39,99.4,102.9, 103.2,104.7, 114.7,115.6,121.3, 121.6,122.6,127.3, 133.2,153.8,156.8, 158.0,158.4,160.0, 160.3	336(68),281(100)	3448,3349,1720, 1630,1597, 1577	208.4,243.6,305, 347, 362	290–291

187(20.38), 50(18.73), 75(16.68). These data agree well with those of the isopsoralen standard. Compound 2 and standard of isopsoralen had the same R_{fs} in TLC and same MP. Its detailed spectral data, listed in Table 1, agree well with the data reported (Wall, Wani, & Manikumar, 1988; Wang, Yang, & Engelhardt, 1999). So the compound was identified as isopsoralen.

Compound 3 was isolated and recrystallized from MeOH as white needle crystals, MP: 162–163, MS *m*/*z* (%): 186(97.7), 158(100), 102(38.93), 130(22.12), 51(16.96), 76(15.55), 187(14.00), 75(13.93). Compound 3 and standard of psoralen had the same R_{fs} in TLC and the same MP. Its detailed spectral data, listed in Table 1, agree well with the data reported (Zhu, Chen, & Zhou, 1979). This compound was identified as psoralen.

Compound 4 was isolated from petroleum ether/EtoAc (7:3) fraction and recrystallized from acetic acid as white needle crystals. IR (KBr, cm^{-1}): 3282 (OH), 1654 (C=O). Its MP was 211–212 °C. The detailed spectral data of corylifolin are listed in Table 1. This compound was identified as corylifolin by comparing the data of IR, ^{1}H NMR, UV spectra, and MP obtained with data reported (Peng, Yuan, & Wu, 1996).

Compound 5 was isolated from petroleum ether/EtoAc (7:3) as yellow needle crystals. Its MP was 228–230 °C. The IR spectra indicated the presence of hydroxyl (3235 cm^{-1}) and aromatic ring (1622, 1568 cm^{-1}). The molecular weight was 320 in its MS spectrum. Its detailed spectral data of IR, MS, ^{1}H and ^{13}C NMR, listed in Table 1, agree well with the reported compound, corylin (Peng et al., 1996).

Compound 6 was isolated from the petroleum ether/EtoAc (6:4) fraction and recrystallized from acetone as colourless needle crystals. Its MP was 290–291 °C. IR (KBr, cm-1): 3448, 3349 (OH), 1720 (C=O), 1630, 1597, 1577 (C=C). Its molecular weight was 336 in its MS spectrum. Its detailed spectral data by IR, MS, ^{1}H and ^{13}C NMR, listed in Table 1, agree well with the reported

compound, psoralidin (Gupta, Jha, & Gupta, 1990; Khastgir, Dutagupta, & Sengupta, 1961).

The structures and names of all six compounds isolated from *P. corylifolia* L. are shown in Fig. 1.

Several herbs have been studied as sources of natural antioxidants, various compounds have been isolated and many of them are polyphenols. Most common plant phenolic antioxidants include flavonoid compounds, cinnamic acid derivatives, tocopherols (Edwin, Huang, Robert, & Elizabeth, 1996; Gu & Weng, 2001; Shahide & Wanasundara, 1992; Wang, 2000). Figs. 2 and 3 demonstrate that the *P. corylifolia* L. powder and its extracts have strong antioxidant activities and their antioxidant effect increased remarkably with the increase of the percentages added to lard. The antioxidant effect of the petroleum ether extract is stronger than that of the chloroform extract (Fig. 3). So there must be some compounds possessing very strong antioxidant properties occurring in the herb. The two extracts were further separated by column chromatography.

The antioxidant activities of compounds 1–6 and two comparison samples were evaluated using the OSI instrument at 100 °C. Their Pfs in lard are listed in Table 2. The results showed that compounds 1, 4, 5 and 6 at 0.02% and 0.04% levels had different antioxidant activities. Compound 6 was much stronger than that of BHT and α-tocopherol. Compounds 1, 4 and 5 were less effective antioxidants than α-tocopherol at 0.02% and 0.04% levels.

There are no substituted groups on either of the *ortho*-positions of the phenolic hydroxyl group in Compound 1, so it is an unhindered phenol. However, there is a double bond directly connecting the phenol in the *para*-position of the phenolic hydroxyl group. This double bond extends the conjugation system of the phenolic oxygen free radical of compound 1 after donating a hydrogen atom to active free radicals. Hence, this group stabilizes the phenolic oxygen free radical a lot and greatly strengthens the antioxidant activities of the compound. In other words, more resonance structures can form to stabilize the free radical because of the alkenic group (Scheme 1).

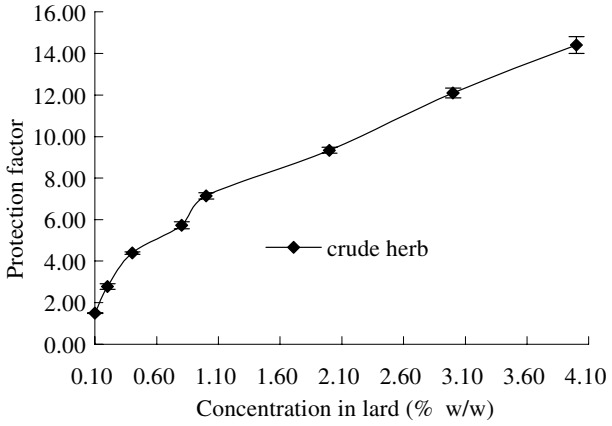


Fig. 2. Antioxidant activity of lard with different crude content of *P. corylifolia* L powder tested in OSI at 100 °C. The data are means of four parallel experiments and error bars indicate SD IP of lard is 5 h.

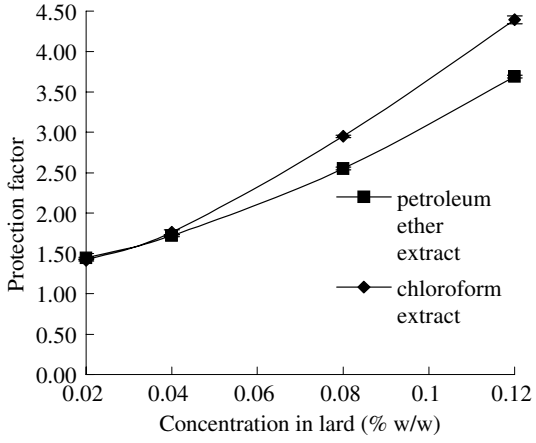


Fig. 3. Antioxidant activity of petroleum ether and chloroform extracts of *P. corylifolia* L. tested in OSI at 100 °C in lard. The data are means of four parallel experiments and error bars indicate SD IP of lard is 5 h.

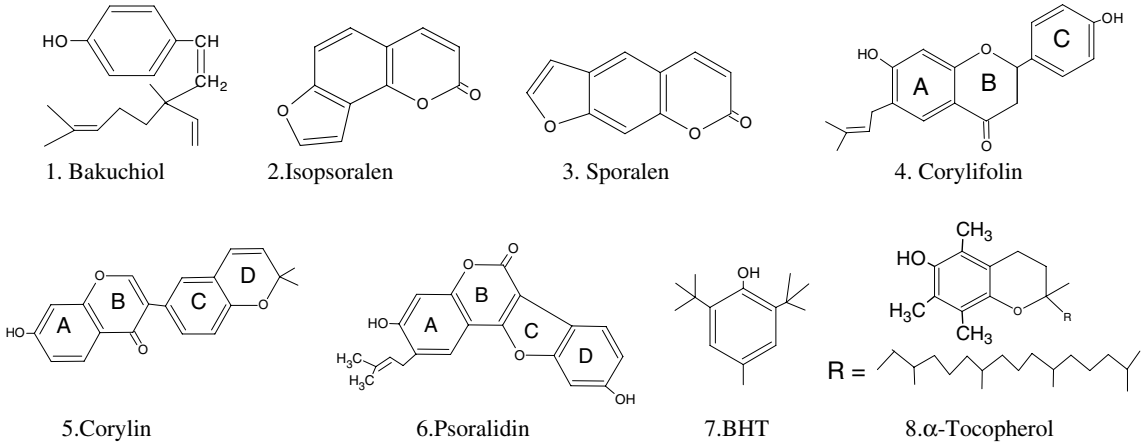
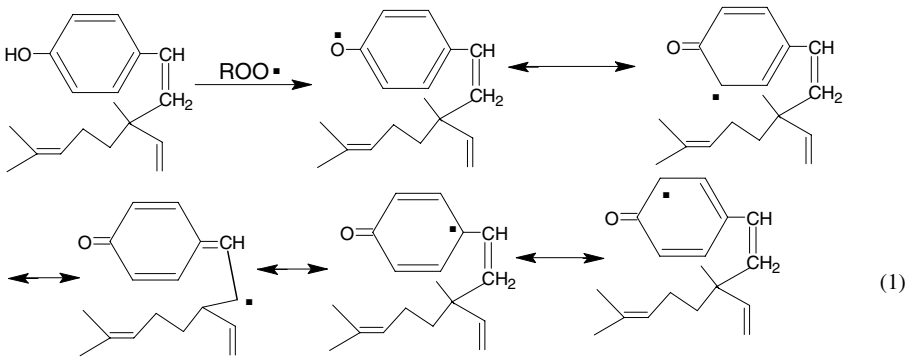


Fig. 1. The structures of the compounds separated from *P. corylifolia* L. and the comparison compounds.

Table 2
Antioxidant effects (Pfs) of compounds isolated from *P. corylifolia* L. in lard at 100 °C[a] ($n = 4$)

Compound	1	2	3	4	5	6	BHT	α-Tocopherol
0.02%	3.18 ± 0.04	0.99 ± 0.01	1.00 ± 0.02	3.08 ± 0.07	2.24 ± 0.03	4.01 ± 0.09	3.92 ± 0. 04	3.77 ± 0.04
0.04%	3.88 ± 0.11	1.00 ± 0.01	1.03 ± 0.01	3. 62 ± 0.11	2.71 ± 0.07	5.23 ± 0.07	5.02 ± 0.02	4.29 ± 0.02

[a] Values are mean ± SD.



(1)

Scheme 1.

Compounds 2 and 3 do not possess any phenolic type of hydroxyl groups, as hydrogen donors, so they do not show any antioxidant activity.

Compound 4 is a dihydroflavone and shows some antioxidant effect. However, its antioxidant effect is slightly weaker than compound 1, although has two antioxidant functional hydroxyl groups, located on rings A and ring C. At the *ortho*-position of the HO-group of ring A, there is an alkyl group, which is an electron-donating group, and has also some stereo-hindering effects. But, at the *para*-position, there is a carbonyl group substituted, which is a strong electron-withdrawing one. On ring C, there is an alkyl group at the *para*-position which is slightly electron-withdrawing because strong electric negative atom, oxygen is attached to the α-position of the group. So the two factors determine compound 4 to be weak antioxidant, even slightly weaker than compound 1, although it has a larger conjugated system and more phenolic OH groups than compound 1.

Compound 5 has the same rings A and B, but shows an absence of an alkyl group at the *ortho*-position hydroxyl group (comparing to compound 4). Yet it does not have any antioxidant functional group on the other rings C or D. These factors result in compound 5 being even less effective than compound 4.

Compound 6 is a lactone phenolic structure with fused aromatic rings. There is a alkyl group at the *ortho*-position of the phenolic hydroxyl group and a furan ring at the *para*-position and a ether bonded oxygen at the *meta*-position of the hydroxyl group on ring A. There is a furan ring fused with ring D. These substituted groups are electron-donating ones. These factors make the free radical stabler after the functional hydroxyl group donates H-atoms to active free radicals. So compound 6 is the most active antioxidant among the compounds listed in Fig. 1.

Although compounds 4 and 6 both carry two phenolic hydroxyl groups, their antioxidant activities are found to be markedly different. However, compound 1 and the comparison samples with only one phenolic hydroxyl group, demonstrate higher antioxidant effects than compound 5. These findings suggest that the antioxidant activities of phenols closely relate to the environment conditions of phenolic hydroxyl groups rather than the numbers of phenolic hydroxyl groups. These findings further confirm three conclusions. First, hydroxyl groups on the aromatic rings are essential for antioxidant activity (Weng, 1993). Second, electron-withdrawing or -donating substituted groups, especially at the *para*-, and *ortho*-positions (Duan et al., 1998; Weng, 1993), greatly affect antioxidant activities of the phenols . Electron-donating groups strengthen antioxidant activities of phenols; electron-withdrawing groups weaken antioxidant effects of phenols. Lastly, *stereo*-hindering groups at the *ortho*-position of antioxidant functional hydroxyl groups will increase antioxidant effects of phenols (Weng, 1993).

The antioxidant activities of the compounds decrease as follows:

Psoralidin > BHT > α-tocopherol > bakuchiol > corylifolin > corylin > isopsoralen ~ psoralen.

P. corylifolia L. is a widespread herb and is easy and cheap to obtain. Also, we found in this research, that it has strong antioxidant activities. So it may be a potential antioxidant resource for food.

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