Eur Food Res Technol (2014) 238:527–533
DOI 10.1007/s00217-014-2179-y

REVIEW PAPER

Forsythia suspense leaves, a plant: extraction, purification and antioxidant activity of main active compounds

Jiang-Feng Yuan · Xiao-Qiao Liu · Jian-Xiong Yang · Xiao-Qin Cui

Received: 12 January 2014 / Revised: 28 January 2014 / Accepted: 29 January 2014 / Published online: 20 February 2014

Abstract *Forsythia suspense* leaves (FSL) are applied as tea in Chinese folk, and it is also considered as veterinary medicinal plant. The predominant compound was obtained from FSL by organic reagents extraction, ethanol precipitation and column filter. The crude forsythin, crude forsythiaside, purified forsythin and purified forsythiaside were analysed and quantified by HPLC method. Several biochemical assays were used to screen the antioxidant properties of crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside. Experimental results showed that the four extracts exhibited antioxidant effects in a concentration-dependent manner, and crude forsythiaside and purified forsythiaside had the higher antioxidant activity effect than crude forsythin and purified forsythin, and purified forsythiaside showed the stronger antioxidant activities than positive control in 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, reducing power and lipid peroxidation assay. This study might provide useful information on forsythiaside as a novel potential antioxidant for human health.

Keywords *Forsythia suspense* leaves · Extraction · Forsythin · Forsythiaside · Antioxidant activity

J.-F. Yuan
College of Food and Bioengingeering, Henan University of Science and Technology, Luoyang 471003, China
e-mail: jiangfengyuan@163.com

X.-Q. Liu · J.-X. Yang (✉)
School of Life Sciences, Shaanxi Normal University, Xi'an 710062, China
e-mail: jxyang@snnu.edu.cn

X.-Q. Cui
Department of Dermatology, Heji Hospital Affiliated to Changzhi Medical College, Changzhi 046000, China

Introduction

Oxidative stress is a result of imbalance between the antioxidant defence system and the formation of reactive oxygen species (ROS). It is believed to damage cell membranes and DNA, as well as membrane lipid peroxidation with subsequent decreases in membrane fluidity [1, 2]. Oxidative damage may cause cell injury, death and exacerbate the development of several age-related chronic diseases including cancer, Alzheimer's disease, Parkinson's disease and heart disease [3]. Therefore, antioxidants are considered to be important nutraceuticals with many health benefits. Antioxidants are widely used in the food industry as potential inhibitors of lipid peroxidation [4]. However, many synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and *tert*-butylhydroquinone (TBHQ) have widely been used in retarding lipid oxidation; their safety has recently been questioned due to toxicity and possible carcinogenicity [5]. In recent years, plant-derived antioxidants have raised considerable interest among food scientists, manufactures and consumers. For this reason, development of safer natural and non-toxic antioxidants from extracts of plant materials that can replace synthetic antioxidants has been of interest.

Forsythia suspense (Thunb.) Vahl is a climbing plant, which is widely distributed throughout China, Korea, Japan and many European nations. The fruit of this plant is a famous traditional medicine (lianqiao in Chinese) throughout the world for thousands of years. The fruit of *F. suspense* was reported to have various biological activities, such as antioxidant [6, 7], antibacterial, antiviral, anti-inflammatory [8] and vasorelaxant activities [9]. The main activity compounds from the *F. suspense* leaves (FSL) are higher than the same activity compounds from the *F. suspense* fruits by HPLC method [10]. However, the FSL

have not entered Chinese Pharmacopoeia, the FSL are only applied as tea in Chinese folk, and extract of leaves is usually used as veterinary medicine. Few effective constituents have been isolated from FSL, and its mechanisms of antioxidant activity are still unclear.

Here, some experiments on isolating, identification of forsythin and forsythiaside of FSL and investigating its antioxidant activities were done. The results of this study will provide useful information for human health and contribute to the potential commercial application of forsythin and forsythiaside as natural antioxidants.

Materials and methods

Chemical reagents and plant materials

The dry FSL were picked from the West of Henan Province, China, in September 2012 and authenticated by Professor Xiao-gai Hou, Henan University of Science and Technology, Luoyang 471003. A voucher specimen was deposited in Henan University of Science and Technology, China. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), nitro blue tetrazolium (NBT) salt, BHT, ascorbic acid (Vc), ferrozine, ferric chloride ($FeCl_3{\cdot}6H_2O$), potassium ferricyanide [$K_3Fe(CN)_6$], ferrous chloride ($FeCl_2{\cdot}4H_2O$), $FeSO_4{\cdot}7H_2O$ and linoleic acid were purchased from Sigma Co. (St. Louis, USA). Forsythin and forsythiaside were obtained from National Institutes for Food and Drug Control (China). All chemicals used in this study have a purity of 90 % or greater.

Isolation and purification of forsythiaside and forsythin

The dried and powdered FSL (40 g) were refluxed twice with 55 % ethanol at 74 °C; the ratio of material to liquid was 1:30. The combined extract was concentrated under reduced temperature (50 °C) and pressure, 100 % ethanol (35 ml) was added to this concentrate extract (665 ml), and 5 % ethanol extract was obtained (700 ml). The extract of FSL (FSLE) was loaded onto a macroporous resin column (AB-8, 2.6 cm × 25 cm) eluted with 30 % ethanol and then 50 % ethanol. Two major fractions (FSLE-I and FSLE-II) were made. The FSLE-I was collected by concentration, the deposition was lyophilized, crude forsythin (0.7350 g) was obtained and was further purified by recrystallizing 3 times with 100 % ethanol, and purified forsythin (0.1280 g) was obtained. The FSLE-II was concentrated, the aqueous layer was extracted 3 times with n-butyl alcohol, and crude forsythiaside (4.76 g) was obtained. For the further purifying, the solution of crude forsythiaside was subjected to reverse silica column chromatography (C18, 8 cm × 50 cm) eluted with 30 % ethanol. Based on TLC characteristics, purified forsythiaside (2.5571 g) was made, and the extraction yield of crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside were 1.84, 0.32, 11.9 and 6.39 %, respectively.

HPLC analysis

A waters (Milford, MA, USA) 1525 binary HPLC pump separation module with a Waters 2,996 photodiode array detector was used. The samples were separated on a TC-C18 column (5 μm, 4.6 mm × 250 mm, Agilent Technologies, Santa Clara, CA, USA). All mobile phases for the chromatographic analysis were degassed for at least 15 min and filtered through 0.45-mm filter prior to using. The mobile phase consisted of methanol (A) and a 0.2 % (v/v) acetic acid solution (B). A gradient elution was programmed as follows: 0–3 min, 32 % (v/v) A; 3–25 min, 32–55 % A; 25–35 min, maintaining 55 % A at a flow rate of 1.0 ml/min. The analytes were detected at 235 nm, and the column temperature was set at 30 °C. The injection volume was 20 μl.

The peak area was used to calculate the amount of crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside from the standard curve. All the experiments were conducted in triplicate, and the average values ± standard deviation (SD) were reported.

DPPH radical scavenging activity

The antioxidant activity of crude forsythin, crude forsythiaside and purified forsythiaside was measured on the basis of the scavenging activity of the stable DPPH free radical according to the method [11] with minor modifications. One millilitre of crude forsythin (5, 10, 15, 20, 25, 30, 40, 80, and 100 μg/ml), purified forsythin (1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mg/ml), crude forsythiaside and purified forsythiaside (5, 10, 15, 20, 25 and 30 μg/ml) were added to 3 ml of a 0.004 % MeOH solution of DPPH, respectively. Absorbance at 517 nm was determined after 30 min, and the percent scavenging activity was calculated by the following formula:

$$\text{Scavenging effect } (\%) = \left(1 - A_{\text{sample}}/A_{\text{control}}\right) \times 100$$

where the A_{control} is the absorbance of control (DPPH solution without sample), and A_{sample} is the test sample (DPPH solution plus test sample or positive control).

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the samples was determined according to the deoxyribose assay [12]. One millilitre phosphate buffer (20 mM pH 7.4, containing 0.1 mM ferric chloride, 0.1 mM EDTA, 2.8 mM deoxyribose), 0.1 ml ascorbic acid (1 mM) and 0.5 ml hydrogen

peroxide (20 mM) were added to 1 ml of appropriately diluted sample. Following incubation at 37 °C for 90 min, 1.0 ml of 1 % (w/v) TCA and 0.3 ml of 2.8 % (w/v) TBA were added, and then the reaction mixture was heated in a boiling water bath for 15 min. The absorbance was measured at 532 nm against a blank. A control contained all the reaction reagents except the samples was prepared and measured, and the hydroxyl radical scavenging ratio was calculated as section "DPPH radical scavenging activity".

Superoxide anion scavenging activity

The assay was based on the capacity of the sample to inhibit the photochemical reduction in nitroblue tetrazolium (NBT) in the NADH–NBT–PMS system [13]. The reaction mixture consisted 1.0 ml of NBT (78 μM in 20 mM potassium phosphate buffer pH 7.4), 1.0 ml of NADH (468 μM in 20 mM potassium phosphate buffer pH 7.4) and 1.0 ml of an appropriately sample solution. The reaction was initiated by addition of 0.4 ml of PMS (60 μM in 20 mM potassium phosphate buffer pH 7.4) to the mixture. The tube was incubated at surrounding temperature for 5 min, and the absorbance was measured at 560 nm against a blank. Decreased absorbance of the reaction mixture indicated increasing superoxide anion scavenging activity. Ascorbic acid (Vc) was used as a positive control in the study, and a control contained all the reaction reagents except the samples or positive control was prepared. The percentage inhibition of superoxide anion generation was calculated using the formula similar to that for DPPH radical scavenging activity.

Total antioxidant activity in a linoleic acid system

Total antioxidant activity was determined according to the literature [14] with some modifications. Five milligrams of β-carotene was dissolved in 10 ml chloroform, and the solution was pipetted into a flask containing 250 μl of linoleic acid and 2 g of Tween 20. The chloroform was removed by rotary vacuum evaporator at 50 °C for 10 min, and 500 ml of oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation to form an emulsion. A 5-ml aliquot of the emulsion was added to a tube containing 1.0 ml of the sample solution at 200 μg/ml, and the absorbance was measured at 470 nm, immediately, against a blank emulsion without β-carotene. The tube was placed in a water bath at 50 °C, and the absorbance was monitored at 25 min intervals until 150 min. All determinations were carried out in triplicate.

Reducing power

The reducing power of crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside was determined referring to the method [15] with some modifications. The different concentrations of samples (50, 100, 200, 300, 400 μg/ml) of 1 ml were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1 %). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of TCA (10 %) was added to the mixture that was then centrifuged for 10 min at 3,000*g*. The upper layer of solution (2.5 ml) was mixed with water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %), and the absorbance was measured at 700 nm. Vc and BHT were used as positive controls at the same concentration.

Lipid peroxidation assay

A modified thiobarbituric acid reactive species (TBARS) assay [16] was used to measure the lipid peroxide formed, using mouse liver homogenates as lipid-rich media [17]. Malondialdehyde (MDA), a secondary product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of TBA, yielding a pinkish-red chromogen with an absorbance maximum at 532 nm. Mouse liver suspension (0.5 ml of 10 % v/v) and 0.1 ml of samples (50, 100, 200, 300, 350 and 450 μg/ml) were added to a test tube that was then filled with 1 ml of water. 0.05 ml of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and incubated for 30 min. Then, 1.5 ml of 20 % acetic acid (pH adjusted to 3.5 with NaOH), 1.5 ml of 0.8 % (w/v) TBA in 1.1 % sodium dodecyl sulphate and 0.05 ml 20 % TCA were added, and the resulting mixture was vortexed and heated at 95 °C for 60 min. After cooling the mixture, an equal volume of n-butanol was added to extract the chromogen in the mixture. The absorbance of the n-butanol layer was measured spectrophotometrically at 532 nm. Vc was used as a positive control. The capability to inhibit MDA formation was calculated.

Statistical treatment of data

All the data were expressed as mean ± SD of three replications, and the one-way analysis of variance (ANOVA) was used for the statistical analysis. Values of $P < 0.05$ were considered to be a statistically significant finding.

Results and discussion

HPLC analysis

The crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside from by organic reagents extraction, ethanol precipitation and column filter were analysed and identified by HPLC. Pure commercial forsythin and forsythiaside were used as reference for HPLC

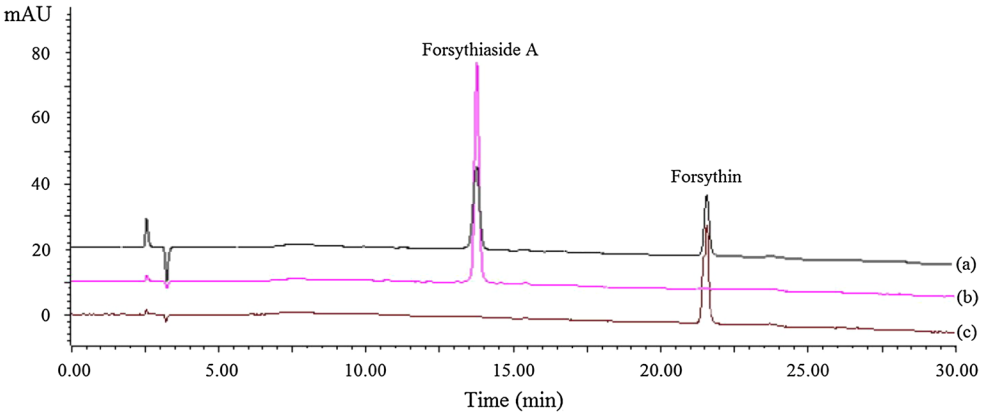

Fig. 1 Chromatograms of forsythiaside and forsythin in the standard solution (*a*), the purified forsythiaside sample (*b*) and the purified forsythin sample (*c*)

identification. The obtained results showed the presence of a single peak corresponding to emerging purified forsythin and purified forsythiaside at the same time as the pure forsythin and pure forsythiaside reference.

The chromatograms of pure forsythin and pure forsythiaside in standard solution and in the sample are shown in Fig. 1. The purified forsythiaside and purified forsythin were eluted at a retention time of 13.7 and 21.6 min, respectively. From the HPLC result, the contents of crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside were 45, 93, 62 and 92 %, respectively.

DPPH radical scavenging activity

DPPH is a useful reagent for investigating the free radical scavenging activities of materials. It is visually noticeable that there is a discolouration from purple to yellow induced by antioxidants. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants according to the decrease in absorbance at 517 nm. Our results found that crude forsythin, crude forsythiaside and purified forsythiaside sample extracted from FSL were effective at reducing the stable radical DPPH to the yellow-coloured diphenylpicrylhydrazine, indicating that these extracts are active in DPPH radical scavenging (Fig. 2a, b). The purified forsythiaside showed obvious higher scavenging effects than those of positive control (BHT and Vc), and the crude forsythiaside had stronger scavenging effects than those of BHT with increasing concentrations in the range of 5–30 μg/ml, suggesting that crude forsythiaside and purified forsythiaside had stronger DPPH radical scavenging activity. However, the scavenging effect of crude forsythin was lower than that of positive control (BHT and Vc), the inhibitory effect of crude forsythin at 100 μg/ml was closely to that of BHT at 30 μg/ml, and the EC_{50} of crude forsythin was 86.77 μg/ml. The scavenging effect of purified forsythin was the lowest activity in the main active combination, and the EC_{50} of purified forsythin was 2.05 mg/ml.

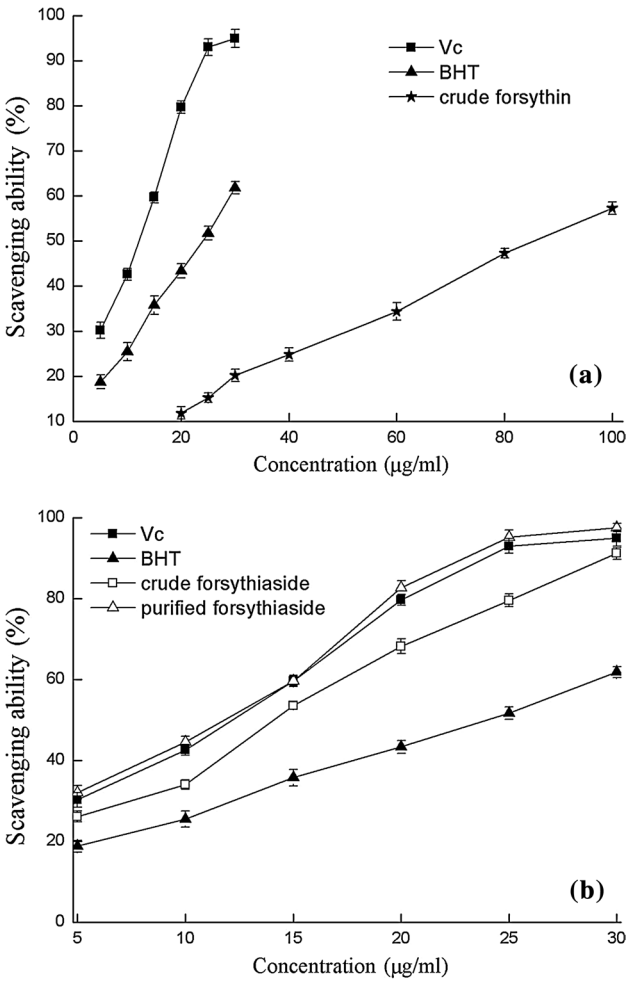

Fig. 2 **a** DPPH radical scavenging activity of crude forsythin from *F. suspense* leaves. **b** DPPH radical scavenging activity of crude forsythiaside and purified forsythiaside from *F. suspense* leaves

Hydroxyl radical scavenging activity

Hydroxyl radicals are extremely reactive free radicals formed in biological systems and have been implicated as a

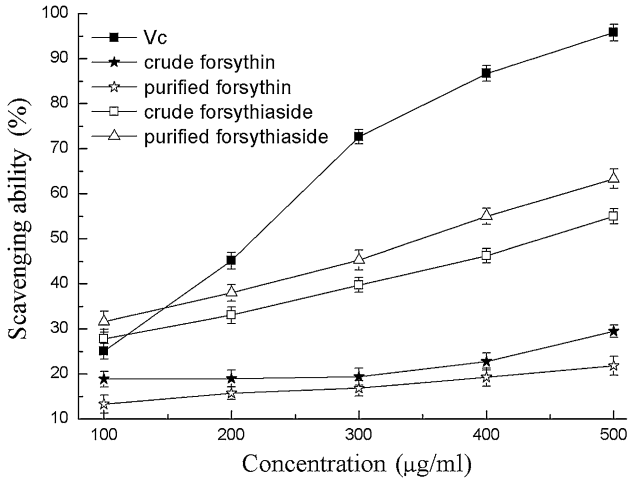

Fig. 3 Hydroxyl radical scavenging activity of crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside measured by deoxyribose assay

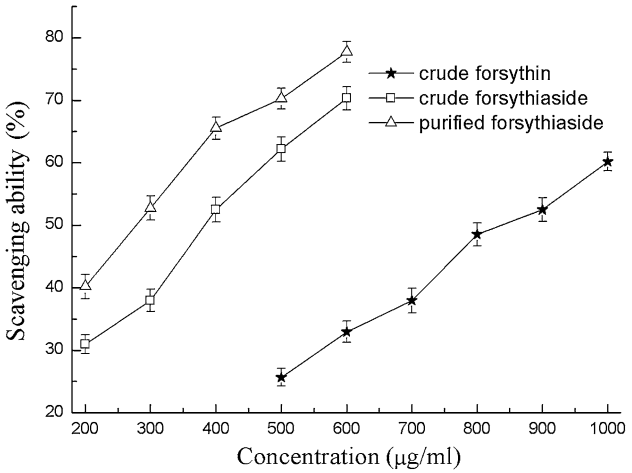

Fig. 4 Superoxide radical scavenging capacity of crude forsythin, crude forsythiaside and purified forsythiaside determined by the PMS/NADH-NBT method

highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [18]. Hydroxyl radicals are very strongly ROS, and there is no specific enzyme to defend against them in humans [19]. Therefore, it is important to discover chemicals with good scavenging capacity for these ROS. Figure 3 shows the percentage ·OH scavenging effect by crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside at the dose of 100, 200, 300, 400 and 500 μg/ml. At the test concentrations, crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside exhibited scavenging activity on hydroxyl radicals in a concentration-dependent manner. The EC_{50} values of crude forsythiaside, purified forsythiaside, and Vc were found to be 484.09, 310.94 and 212.07 μg/ml, respectively. The EC_{50} values of crude forsythin and purified forsythin were found to be 18.52 and 25.62 mg/ml, respectively. The ability of the extracted component to quench hydroxyl radicals seems to be directly related to the prevention of propagation of lipid peroxidation; because crude forsythiaside and purified forsythiaside seems to be a good scavenger of active oxygen species, it will thus reduce the rate of the chain reaction.

Superoxide anion scavenging activity

Superoxide anions play important roles in the formation of ROS such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA [20]. It was therefore proposed to measure the comparative interceptive ability of the antioxidant extracts to scavenge the superoxide radical. In our study, the superoxide radicals were generated in a PMS/NADH system and assayed by the reduction in NBT. A decrease in absorbance at 560 nm indicates the consumption of superoxide anion in the reaction mixture. Figure 4 illustrates the superoxide radical scavenging ability of 200, 300, 400, 500 and 600 μg/ml of crude forsythiaside and purified forsythiaside, and 500, 600, 700, 800, 900 and 1,000 μg/ml of crude forsythin and purified forsythin in comparison with the same doses of Vc. At all the concentrations, the samples exhibited varying degrees of antioxidant activity, although the superoxide anion scavenging activity showed lower than Vc. The percentage inhibition of superoxide generation by 600 μg/ml doses of the crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside was found as 32.93, 2.23, 70.32 and 77.68 %, respectively. The EC_{50} value of crude forsythin, purified forsythin, crude forsythiaside, purified forsythiaside and Vc on superoxide radical scavenging activity was found to be 842.21 μg/ml, 20.96 mg/ml, 333.02 μg/ml, 288.03 μg/ml and 32.23 μg/ml, respectively.

Total antioxidant activity in a linoleic acid system

The mechanism for the bleaching of β-carotene is a free radical-mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. The total antioxidant activity, which reflected the ability of crude forsythin, crude forsythiaside, purified forsythin and purified forsythiaside to inhibit the bleaching of β-carotene, was measured and compared with that of the negative control and BHT, and the result is presented in Fig. 5. It can be seen that all of the crude forsythin, crude forsythiaside, purified forsythin and purified forsythiaside from FSL exhibited definite antioxidant activity compared with the negative control. The crude forsythiaside and purified forsythiaside exhibited significantly higher antioxidant activities than the crude forsythin and

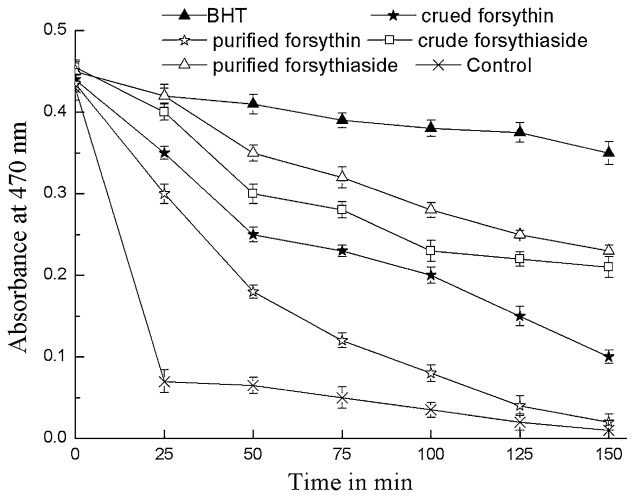

Fig. 5 Total antioxidant activity of crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside

purified forsythin at the experiment system, and crude forsythiaside and purified forsythiaside had antioxidant activities similar to those of BHT at 25 min. The total antioxidant activity of the samples decreased in the order: purified forsythiaside > crude forsythiaside > crude forsythin > purified forsythin; the purified forsythiaside showed a higher ability to prevent the bleaching of β-carotene.

Reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. For the measurements of the reductive ability, we investigated the Fe^{3+}–Fe^{2+} transformation in the presence of samples. In this assay, the reducing power of the tested FSL extracts steadily increased with increasing sample concentration (Fig. 6). The reducing power followed the order: Vc > purified forsythiaside > crude forsythiaside > BHT > crude forsythin > purified forsythin. In the present study, the crude forsythiaside and purified forsythiaside showed higher activity than that of BHT. The data suggest that forsythiaside has a good ability to donate electrons to reactive free radicals, converting them into more stable products and terminating the free radical chain reaction.

Lipid peroxidation assay

Lipid peroxidation is a typical free radical oxidation and proceeds via a cyclic chain reaction. It has been suggested to be an important event in cellular damage, which is strongly associated with ageing, carcinogenesis and other diseases [21]. The antioxidative activities of crude forsythin, purified forsythin, crude forsythiaside, purified forsythiaside and ascorbic acid on MDA formation in the Fe^{2+}/ascorbate-mediated lipid peroxidation in the liposome system are shown in Fig. 7. It was also found that the inhibitory effects on the lipid peroxidation of the tested samples were concentration dependent. The inhibitory effects rose from 47.2 to 72.9 % for Vc, 53.3 to 76.2 % for crude forsythiaside and 58.2–82.7 % for purified forsythiaside with the concentration increasing from 50 to 300 μg/ml. The inhibitory effect of crude forsythin at the dose of 450 μg/ml was slightly stronger than that of Vc at the dose of 300 μg/ml. In the iron-induced mouse microsomal lipid peroxidation system, the antioxidant activities of lipid peroxidation of the samples decreased in the order: purified forsythiaside > crude forsythiaside > Vc > crude forsythin > purified crude forsythin.

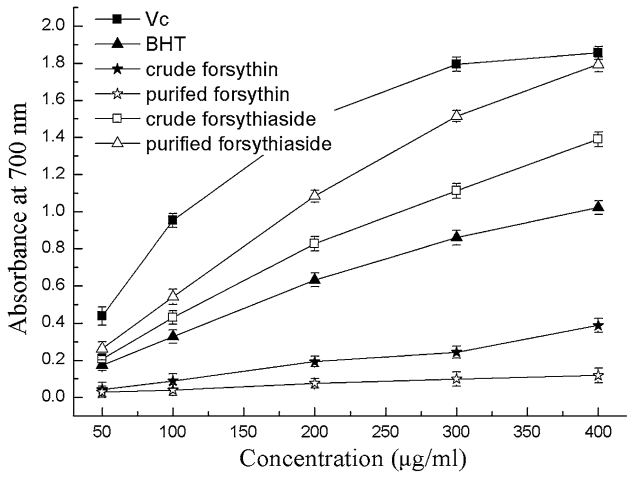

Fig. 6 Reducing power of different concentrations of crude forsythin, purified forsythin, crude forsythiaside, purified forsythiaside, Vc and BHT

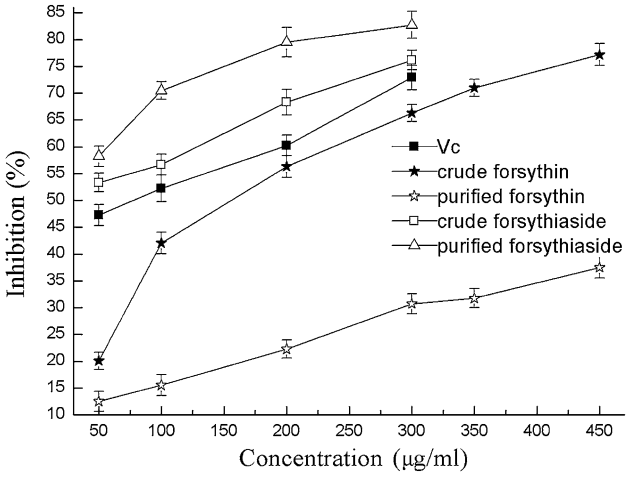

Fig. 7 Inhibition by crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside at different concentrations of $FeSO_4$-induced lipid peroxidation of mouse homogenates

Conclusion

Forsythin and forsythiaside are one of the major active compounds in FSL. From FSL, crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside were obtained by organic reagents extraction, ethanol precipitation and column filter, and the extraction yield of crude forsythin, purified forsythin, crude forsythiaside and purified forsythiaside were 1.84, 0.32, 11.9 and 6.39 %, respectively. The data obtained clearly indicate that forsythiaside possesses potent DPPH radical scavenging activity, reducing power and lipid peroxidation assay, at levels superior to BHT, and forsythiaside and forsythin have the ability of scavenging hydroxyl radicals, superoxide anions and total antioxidant activity, although less effectively than Vc. Compared with crude forsythin and purified forsythin, crude forsythiaside and purified forsythiaside exhibited the higher antioxidant activities, possibly due to forsythin poor water solubility.

All these results have suggested that FSL might be exploited as economic dietary sources of natural antioxidants for improving human health. The forsythiaside possessed good antioxidant properties and can be developed as a novel potential natural antioxidant in the functional food. Overall, we rationally assume that FSL is a new resource partly because of antioxidant of extract component in it. Further investigations are in progress in our laboratory to evaluate in vivo antioxidant potential and therapeutic benefits of forsythiaside and forsythin from FSL in the functional food industry.

Acknowledgments We thank Luoyang Science and Technology Development Plan (1201029A), the Doctors Start Fund of Henan University of Science and Technology (09001544) and the National Nature Science fund of China (21275098) for the financial support to this work.

Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

References

1. Finkel T, Holbrook NJ (2000) Oxidant, oxidative stress and biology of ageing. Nature 408:239–247
2. Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR, Lithgow GJ (2000) Extension of life-span with superoxide dismutase/catalase mimetics. Science 289:1567–1569
3. Raouf O, Patrice AR, Andre B, Jean-Michel W, Yvan T (2000) Evidence of prooxidant and antioxidant action of melatonin on human liver cell line HepG2. Life Sci 68:387–399
4. Scherer R, Godoy HT (2009) Antioxidant activity index (AAI) by the 2, 2-diphenyl-1-picrylhydrazyl method. Food Chem 112:654–658
5. Siriwardhana N, Jeon YJ (2004) Antioxidative effect of cactus pear fruit (*Opuntia ficus-indica*) extract on lipid peroxidation inhibition in oils and emulsion model systems. Eur Food Res Technol 219:369–376
6. Lu T, Piao XL, Zhang Q, Wang D, Piao XS, Kim SW (2010) Protective effects of *Forsythia suspensa* extract against oxidative stress induced by diquat in rats. Food Chem Toxicol 48:764–770
7. Schinella GR, Tournier HA, Prieto JM, De Mordujovich BP, Rios JL (2002) Antioxidant activity of anti-inflammatory plant extracts. Life Sci 70:1023–1033
8. Xia EQ, Aia XX, Zang SY, Guan TT, Xu XR, Li HB (2011) Ultrasound-assisted extraction of phillyrin from *Forsythia suspense*. Ultrason Sonochem 18:549–552
9. Iizuka T, Nagai M (2005) Vasorelaxant effects of forsythiaside from the fruits of *Forsythia suspense*. J Pharmacol Soc Jpn 125:219–224
10. Zhang G, Li FR, Duan F, Ji J, Guo Y, Wang ZZ (2005) Determination of phillyrin, forsythiaside and rutin in folium forsythia gathered in different periods. Nat Prod Res Dev 17:790–793
11. Dutta RK, Maharia RS (2012) Antioxidant responses of some common medicinal plants grown in copper mining areas. Food Chem 131:259–265
12. Halliwell B, Gutteridge JMC, Aruoma OL (1987) The deoxyribose method: a simple 'test tube' assay for determination of rate constants for reactions of hydroxyl radicals. Anal Biochem 165:215–219
13. Kanatt SR, Chander R, Sharma A (2007) Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. Food Chem 100:451–458
14. Yu HH, Liu XG, Xing RE, Liu S, Guo ZY, Wang PB, Li CP, Li PC (2006) In vitro determination of antioxidant activity of proteins from jellyfish *Rhopilema esculentum*. Food Chem 95:123–130
15. Sasipriya G, Siddhuraju P (2012) Effect of different processing methods on antioxidant activity of underutilized legumes, *Entada scandens* seed kernel and *Canavalia gladiata* seeds. Food Chem Toxicol 50:2864–2872
16. Archana B, Nabasree D, Bratati D (2005) In vitro study of antioxidant activity of *Syzygium cumini* fruit. Food Chem 90:727–733
17. Lavania M, Dalal J, Cheema S, Nautiyal CS, Lal B (2011) In vitro study of lipid peroxidation and free radical scavenging activity of cow urine. Eur Food Res Technol 232:703–711
18. Gulcin I (2006) Antioxidant and antiradical activities of L-carnitine. Life Sci 78:803–811
19. Pérez-Tortosa V, López-Orenes A, Martínez-Pérez A, Ferrer MA, Calderón AA (2012) Antioxidant activity and rosmarinic acid changes in salicylic acid-treated *Thymus membranaceus* shoots. Food Chem 130:362–369
20. Pietta PG (2000) Flavonoids as antioxidants. J Nat Prod 63:1035–1042
21. Forman HJ, Davies KJA, Ursini F (2014) How do nutritional antioxidants really work: nucleophilic tone and para-hormesis versus free radical scavenging in vivo. Free Radic Bio Med 66:24–35