In vitro and *in vivo* antioxidant and antimicrobial activities of extracts from *Platycarya strobilacea* infructescence

Liangliang Zhang*, Yongmei Wang and Man Xu
Jiangsu Province Biomass Energy and Materials Laboratory; Institute of Chemical Industry of Forest Products, CAF, Nanjing 210042, China

Abstract: We determined the *in vitro* antioxidant and antimicrobial activities of *Platycarya strobilacea* infructescence (PSI) from various solvent extracts (methanol, ethyl acetate, water and tannin polymers) and quantified the content of total phenolic of each extract. The ethyl acetate extract was found to have the best radical-scavenging activity and ferric reducing power, as well as antimicrobial activity. In order to investigate the protective effect on oxidative stress *in vivo*, each extract was administered to rats, and the serum, liver and heart were analyzed. The water extract and tannin polymers significantly decreased malonyldialdehyde (MDA) concentrations in serum, liver and heart compared to the control group ($P<0.01$). Furthermore, super oxide dismutase (SOD) and glutathione per oxidase (GSH-Px) activities were increased by administration of all extracts. We concluded that the ethyl acetate extract of PSI has strong antioxidant activity *in vitro* and possesses antimicrobial activity. These results indicated PSI the potential of medicinal uses and food preservation.

Keywords: *Platycarya strobilacea*; Antioxidant activity; Antimicrobial activity; Phenolic compounds.

INTRODUCTION

It is well known that phenolic compounds including tannins found in plants possess antioxidant activity. In recent years, many researchers have focused on the antioxidant activity of phenolic compounds in Chinese traditional medicinal plants, and a positive correlation between high phenolic content and strong antioxidant activity has been observed (Song *et al*., 2010; Molan *et al*., 2012). In preventing cells from injury, these phenolic compounds usually play an important role, such as the cellular and organ damage caused by hydrogen peroxide and lipid peroxides, through the absorption and neutralization of free radicals (Sroka *et al*., 2003). Many of these compounds can possess significant antioxidant capacities and have been associated with decrease of prevalence and mortality rates of some human diseases.

In food industry, foods spoilage which due to the presence of microbial contamination is a significant problem and excessive amounts of food are lost due to microbial-induced spoilage every year. Due to the undesirable effects of synthetic chemicals in foods, non-toxic and natural preservatives have been demand for the people. More and more compounds present in plants have been found to have biologically active and show antimicrobial and allopathic antioxidant activity as well as bio-regulatory properties.

The dried infructescence of *Platycarya strobilacea* Sieb. et Zucc is a traditional medicine in China. Its functions include activating blood circulation, eliminating toxic heat, relieving swelling, reducing pain and evacuating pus (Northwest Institute of Botany, CAS, 1970). Previous studies have shown the infructescence of this plant contained phenolic compounds, flavones, organic acids, tannins and other compounds (Tanaka *et al*., 1993; Tanaka *et al*., 1998; Maeda *et al*., 2011). *P. strobilacea* infructescence has been used as an active ingredient in cosmetics for its medicinal properties (Choi *et al*., 2003; Lee *et al*., 2004; Kim *et al*., 2010). However, few information is available on the antioxidant properties of *P. strobilacea* infructescence (PSI) extracts. In this study, the *in vitro* antioxidant activities of PSI extracts were determined using radical-scavenging and reducing power assays and their antimicrobial activities against different food-borne pathogens were determined to assess their potential as a natural preservative. Furthermore, the effect of extracts on lipid peroxidation and antioxidant enzyme activity in the serum, liver and heart of rats were investigated.

MATERIALS AND METHODS

Materials

P. strobilacea infructescence was collected in Lianyungang, Jiangsu Province, China. Dried *P. strobilacea* infructescence was ground in a knife mill and the powdered sample was stored at -18°C.

Sephadex LH-20 was purchased from Pharmacia LKB Biotec. (Uppsala, Sweden). TPTZ (2,4,6-tripyridyl-*S*-triazine) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich (Shanghai, China). Other chemicals used in these experiments were of analytical grade and obtained from commercial sources.

**Corresponding author:* e-mail: zhll20086@163.com

Preparation of extracts and fractions

The experimental protocol used in the extraction and fractionation of *P. strobilacea* infructescence phenolics is shown in fig. 1. Plant material (150g) was subjected to extraction with 400mL petroleum ether at room temperature for 10h. After drying, the defatted plant material (142g) was subjected to extraction with 400mL aqueous methanol (70%, v/v) in a shaker bath at 40°C for 1h and filtered. The latter extraction step was repeated three times. The filtrates were combined, and the methanol was removed under vacuum at 45°C using a rotary evaporator. The aqueous phase was lyophilized to obtain 14.6g (extract 1). The methanol-derived extract was dissolved in 100mL of water and extracted with 100 mL ethyl acetate three times. The ethyl acetate phases were combined and evaporated to dryness under vacuum at 40°C to yield 2.42 g (extract 2). After ethyl acetate extraction, the aqueous phase remaining was lyophilized to yield 9.89 g (extract 3). Each extract was refrigerated for storage until further experiments.

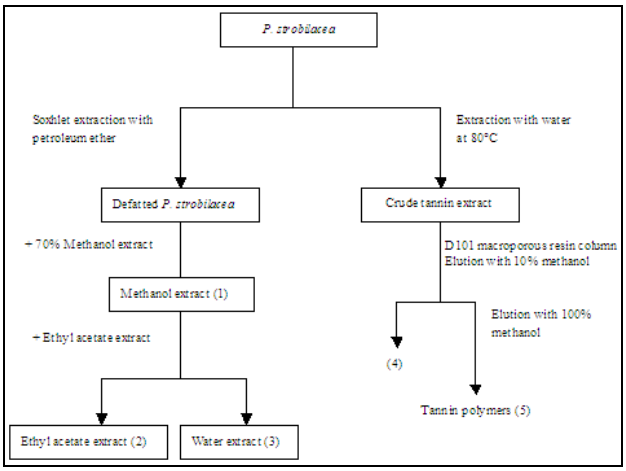

Fig. 1: The extraction and fractionation scheme of *P. strobilacea* infructescence.

Preparation of tannin polymer fraction

Plant material (90g) was subjected to extraction with 600mL water twice at 80°C for 1h. The extract was filtered, pooled and concentrated to 1/4 of its original volume by vacuum evaporation at 55°C. The crude tannin extract was adsorbed onto a D101 macro porous resin column. The column was first eluted with 600mL 10% aqueous methanol solution to remove the impurities and oligomeric tannins and followed by further elution with 600mL methanol solution. The last fraction, which contained the tannin polymers, was concentrated under vacuum to remove the solvent and then freeze-dried to obtain 5.7g of tannin polymers.

Molecular weight distribution of tannin polymers by GPC

The molecular weight distribution of tannin polymers was measured as described by Zhang *et al*. (2014).

Amount of total phenolic compounds

The amounts of total phenolics in the PSI extracts and fractions were measured using the Folin-Ciocalteu reagent method described by Djeridane *et al*. (2006). The final results were expressed as gallic acid equivalents (GAE) in mg per 100 mg extract.

In vitro antioxidant assays

The antioxidant activities of extracts against DPPH free radical were measured as described by Zhang & Lin. (2008). The reducing power of the extracts was measured as described by Benzie & Strain (1996).

Antimicrobial activity

The antimicrobial activities of PSI extracts and sodium benzoate, as the positive control, were tested against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, the Gram-negative bacteria *Escherichia coli*, and the fungus *Aspergillus niger* using the agar double dilution method (Al-Salahi *et al*., 2013) with 1mL of inoculum containing 1×10^4 colony forming units (cfu)/mL of tested bacteria or 1×10^4 spore/mL of fungi. Extract-impregnated discs (50µL per disc) at a concentration of 50mg/mL were placed on the seeded agar and incubated either at 37°C for 24 h for bacteria or at 30°C for three days for fungi. After incubation, antimicrobial activity was measured by determining the zone of inhibition against the test organisms. All of the tests were carried out in triplicate.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits the growth of microorganisms. The MIC was determined by the liquid dilution method (Bonjar-Shahidi, 2004). Approximately 2µL of nutrient broth containing 1×10^4cfu/mL of bacteria or 1×10^4 spore/mL of fungi were mixed separately with the extracts to obtain concentrations of 0.001-50mg/mL. Incubation was carried out at 37°C for 24h for bacteria and at 30°C for three days for fungi. The experiment was repeated in triplicate, and the mean values were determined.

Animal experimental

Animals

The study was carried out on healthy male Sprague Dawley rats weighing 180-210 g. The animals were procured from the Animal Department of China Pharmaceutical University. They were housed in clean cages at room temperature (22±2°C) and 70% humidity with a 12h light/dark cycle throughout the experimental period. The rats were provided with a standard diet and water ad libitum. The animal care procedures and experimental protocol were in accord with institutional ethical guidelines.

Animal experimental design

All animals were weighed and divided randomly into fourteen groups of ten animals each.

- The animals of Group 1 served as a control group and were orally administered with a dose of 1mL/200 g normal saline (NS) for 14 d.
- The animals of Group 2 served as the positive control and were treated with vitamin E dissolved in 0.5% CMC-Na with a dose of 50 mg/kg (p.o.) for 14 d.
- The animals of Group 3-14 were treated with PSI extracts (methanol, ethyl acetate, water and tannin polymer fractions) dissolved in 0.5% CMC-Na at doses of 100, 200, 400 or 50 mg/kg (p.o.) for 14 d.

At the end of the experiment, all animals were fasted for 12 h prior to sacrifice by cervical decapitation. Their blood was collected to obtain serum. The liver and heart were excised immediately, and homogenates were prepared according to the method described by EI-Beshbishy (2008).

Measurement of MDA, SOD and GSH-Px in serum and liver and heart homogenates

The MDA, SOD and GSH-Px levels in the serum and liver and heart homogenates were determined following the instructions provided with the kit. In brief, MDA was determined according to the thiobarbituric acid method. All samples were assayed in triplicate. The level of MDA is expressed as nmol/mg protein (liver and heart homogenate) or nmol/mL (serum). The SOD activity was determined by the xanthine oxidase method, which measures the ability of a compound to inhibit the oxidation of oxyamine by the xanthine-xanthine oxidase system. The red product (nitrite) produced by the oxidation of oxyamine showed absorbance at 550 nm. One unit (U) of SOD activity was defined as the amount that reduced the absorbance at 550 nm by 50%. These samples were assayed in triplicate, and the results were expressed as U/mg protein (liver and heart homogenates) or U/mL (serum). The GSH-Px activity was measured by the DTNB method, and its activity was expressed in U/mg protein (liver and heart homogenates) or U/mL (serum).

HPLC analysis of phenolic acids

Aliquot of the ethyl acetate fraction (10µL) was analyzed using a HPLC system (Shimadzu LC-20AB) equipped with a UV-VIS detector. A Hypersil ODS2 (C18) column (5µm, 250 mm × 4.6 mm) was used. Solvent (A) CH_3CN; and solvent (B) water (0.1% trifluoroacetic acid (TFA), v/v) were used as the mobile phase for the analysis of phenolic acids. The elution profile was: 0-10 min, 90% B (isocratic); 10-15 min, 90%-80% B (linear gradient); 15-45min, 80%B (isocratic); detection at 280, 350 and 357 nm. Extract was dissolved in methanol: water (1:1) and then filtrated through membrane filter with an aperture size of 0.45µm.

STATISTICAL ANALYSIS

Statistical analyses were performed using a one-way ANOVA and Duncan's multiple range test. The results are expressed as the means ± SD. Differences are considered significant when $P<0.05$.

RESULTS

Preparation of extracts and total phenolic content

The PSI was extracted in methanol and subjected to solvent-solvent partitioning to obtain two fractions in ethyl acetate and water fractions. The yield of the ethyl acetate fraction was significantly ($P<0.05$) lower than the water fraction (fig. 2). The phenolic contents of different PSI extracts also varied significantly ($P<0.05$). The ethyl acetate extract had the highest phenolic content, followed by the methanol extract.

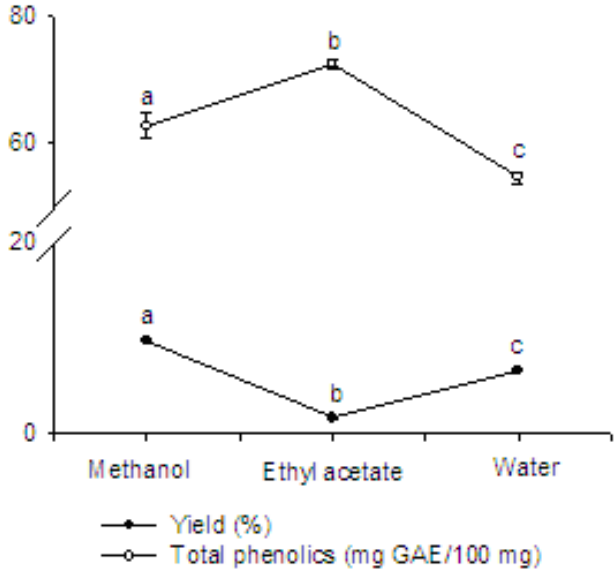

Fig. 2: Yield and total phenolics in PSI various solvent extracts. Mean values ± SD followed by different letter are significantly different ($P<0.05$).

The molecular weight of the tannin fraction is an important characteristic for its properties and applications (Hagerman *et al*., 1998). The molecular weight distribution of the PSI tannin polymers was obtained by GPC. The values of the number-average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersity ($D=M_w/M_n$) of the tannin polymers are 1454, 1587 and 1.091, respectively.

In vitro antioxidant activity

The free radical scavenging abilities of each extract were determined by evaluating the change in absorbance observed upon DPPH reduction. The values of DPPH scavenging activity of each extract are shown in table 1.

Table 1: % Radical-scavenging activity on DPPH and ferric reducing power[a] of PSI extracts

Concentration (μg/mL)	% Radical-scavenging activity [b]				Reducing power [b]			
	Methanol extract	Ethyl acetate extract	Water extract	Tannin polymers	Methanol extract	Ethyl acetate extract	Water extract	Tannin polymers
31.25	13.84±0.40^{b}	19.37±0.55^{c}	11.43±0.50^{a}	13.97±0.40^{b}	0.26±0.00^{e}	0.48±0.00^{g}	0.35±0.01^{f}	0.26±0.00^{e}
62.5	25.46±0.29^{b}	34.98±0.29^{c}	16.44±1.98^{a}	27.05±0.33^{b}	0.46±0.00^{f}	0.70±0.00^{h}	0.51±0.00^{g}	0.43±0.00^{e}
125	47.81±0.50^{b}	67.11±0.40^{d}	39.24±1.25^{a}	52.95±0.19^{c}	0.80±0.01^{f}	1.11±0.02^{e}	0.81±0.00^{f}	0.73±0.01^{e}
250	82.35±0.40^{b}	88.63±0.61^{d}	73.21±0.79^{a}	85.08±0.29^{c}	1.41±0.01^{g}	1.83±0.02^{h}	1.32±0.02^{f}	1.28±0.02^{e}
500	89.52±0.57^{a}	89.65±0.40^{a}	88.25±0.22^{a}	88.63±0.22^{a}	2.39±0.01^{g}	2.93±0.04^{h}	2.24±0.02^{e}	2.30±0.01^{f}
1000	89.71±0.50^{ab}	90.35±0.67^{b}	89.14±0.19^{a}	88.70±0.40^{a}	3.43±0.00^{e}	3.50±0.03^{f}	3.42±0.02^{e}	3.42±0.00^{e}

[a] Ferric reducing power was expressed as absorbance at 593 nm.
[b] Mean values ± SD (n=3) with the same letter in the same line are not significantly different (P<0.05).

Table 2: Antimicrobial activity of PSI extracts against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and fungus *Aspergillus niger* with sodium benzoate as the positive control. (1. *Staphylococcus aureus*, 2. *Bacillus subtilis*, 3. *Escherichia coli*, 4. *Aspergillus niger*. (+) indicates presence of bacterial growth, (-) indicates absence of bacterial growth)

Concentration (mg/mL)	Water extract				Ethyl acetate extract				Methanol extract				Tannin polymers				Sodium benzoate			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
50.00	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
25.00	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
12.50	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
6.25	—	—	—	+	—	—	—	+	—	—	—	+	+	—	—	+	+	+	+	+
3.125	—	+	—	+	—	+	—	+	—	—	—	+	+	+	—	+	+	+	+	+
1.563	+	+	+	+	—	+	—	+	+	+	—	+	+	+	—	+	+	+	+	+
0.780	+	+	+	+	+	+	—	+	+	+	—	+	+	+	+	+	+	+	+	+
0.390	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3: Effects of PSI extracts on MDA, SOD and GSH-Px in rat serum and liver and heart homogenates

Group	Dose (mg/kg b.w.)	Serum			Liver			Heart		
		MDA [a] (nmol/mL)	SOD [a] (U/mL)	GSH-Px [a] (U/mL)	MDA [a] (nmol/mg protein)	SOD [a] (U/mg protein)	GSH-Px [a] (U/mg protein)	MDA [a] (nmol/mg protein)	SOD [a] (U/mg protein)	GSH-Px [a] (U/mg protein)
Control	—	7.76 ±1.36	208.45 ±55.41	382.53 ±31.89	8.05 ±1.22	216.83 ±35.66	52.86 ±9.84	2.31 ±0.76	215.62 ±40.13	142.37 ±21.95
Vitamin E	50	4.73 ±1.04**	375.03 ±65.22**	619.28 ±76.51**	4.49 ±1.15**	398.01 ±50.84**	89.52 ±7.92**	1.22 ±0.34**	326.57 ±46.22**	218.78 ±39.51**
Water extract	200	5.25 ±1.22**	312.24 ±20.04**	625.37 ±81.64**	3.56 ±1.05**	441.26 ±48.86**	90.24 ±23.29**	1.08 ±0.33**	383.18 ±62.01**	298.93 ±53.01**
	100	6.01 ±1.29	276.67 ±32.21*	542.11 ±37.35*	5.11 ±1.24**	419.38 ±55.62**	81.53 ±15.11**	1.19 ±0.41**	315.42 ±39.49**	207.42 ±38.55**
	50	6.35 ±1.56	221.06 ±40.02	403.26 ±30.62	6.37 ±1.82*	318.15 ±50.35*	68.15 ±12. 48*	1.61 ±0.48*	267.25 ±40.19*	193.89 ±32.64*
Ethyl acetate extract	400	5.75 ±1.12*	287.65 ±21.38*	591.58 ±66.12*	6.44 ±1.36*	321.53 ±42.83*	73.66 ±17.87*	1.69 ±0.38*	293.12 ±37.55*	196.04 ±33.58*
	200	6.36± 1.45	229.54 ±26.31	419.42 ±40.37	7.79 ±1.25	244.12 ±50.22	60.52 ±12.54	1.74 ±0.37*	245.82 ±47.21	166.35 ±36.22
	100	7.02 ±1.98	215.32 ±29.06	387.66 ±47.52	8.04 ±1.12	227.65 ±31.89	55.68 ±13.22	2.17 ±0.41	232.27 ±40.11	157.73 ±41.32
Methanol extract	400	5.62 ±1.21*	279.83 ±20.79*	565.62 ±60.28*	4.88 ±1.13**	322.05 ±56.17*	75.13 ±15.29*	1.49 ±0.27*	318.45 ±41.63**	198.34 ±45.19*
	200	6.81 ±1.67	216.28 ±25.44	411.36 ±52.67	5.12 ±1.29**	307.23 ±45.82*	68.77 ±11.91*	1.55 ±0.36*	270.09 ±37.23*	193.41 ±28.77*
	100	7.22 ±2.01	209.85 ±30.12	391.21 ±46.81	6.36 ±1.33*	259.18 ±36.20	57.24 ±12.45	1.69 ±0.34*	236.97 ±40.31	156.42 ±45.08
Tannin polymers	400	5.05 ±1.36**	346.52 ±23.28**	639.41 ±77.28**	4.65± 1.14**	401.33 ±45.57**	82.06 ±19.25**	1.28 ±0.35**	353.22 ±57.86**	216.57 ±43.28**
	200	5.14 ±1.19**	316.81 ±40.11**	602.11 ±44.86*	5.32± 1.36**	312.15 ±50.88*	74.28 ±16.27*	1.59 ±0.46*	285.63 ±44.17*	186.95 ±30.26*
	100	5.81 ±1.88*	297.84 ±45.78*	553.27 ±39.05*	6.51 ±1.93*	258.46 ±41.29	58.24 ±18.21	1.71 ±0.39*	262.15 ±45.23*	161.05 ±38.55

[a] The values are means ± SD (n=10): $^{*}P$<0.05 *vs* control. $^{**}P$<0.01 *vs* control.

Comparison of the scavenging effects of each extract on the DPPH radical showed a significantly higher radical-scavenging activity for the ethyl acetate extract (P<0.05) at concentrations of 31.25, 62.5, 125 and 250μg/mL. The IC_{50} values of the extracts tested in this study are shown in fig. 3A. The free radical scavenging activities of the extracts were in the following order: ethyl acetate > tannin polymers > methanol > water. The ethyl acetate extract (IC_{50}=112.32μg/mL), which had the highest level of total phenolics, had the highest (P<0.05) free radical scavenging activity (or lowest IC_{50} value) followed by the tannin polymers (IC_{50}=146.02μg/mL).

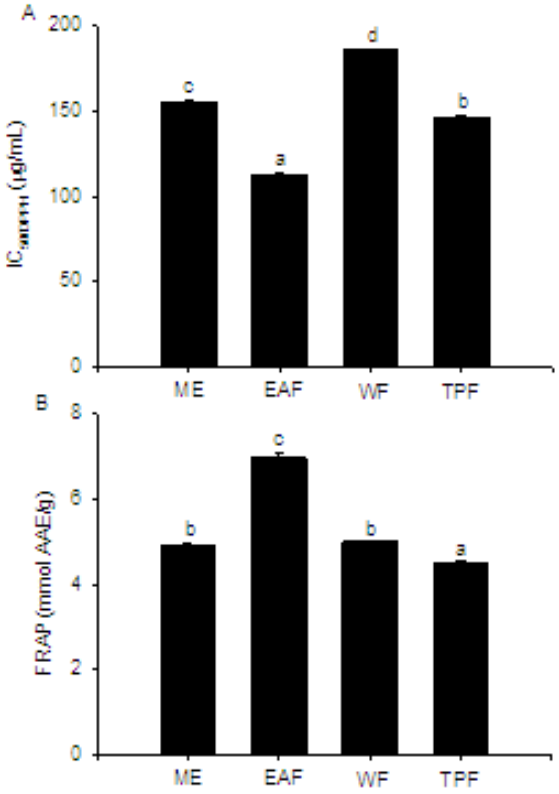

Fig. 3: (A) DPPH free radical-scavenging and (B) ferric reducing power of PSI extracts. ME, methanol extract; EAF, ethyl acetate fraction; WF, water fraction; TPF, tannin polymer fraction. DPPH free radical-scavenging activities were expressed as IC_{50}. The FRAP values were expressed as mmol ascorbic acid equivalents (AAE)/g sample. Mean values ± SD followed by different letter are significantly different (P<0.05).

The ferric reducing antioxidant power (FRAP) of the extracts were in the following order: ethyl acetate > water, methanol > tannin polymers. In accordance with the findings from the DPPH· assay, the highest reducing ability was observed with the ethyl acetate extract. However, the FRAP value of the tannin polymers was significantly lower than those of the water and methanol extracts, which is inconsistent with the DPPH· assay (fig. 3B). All tested extracts showed increased ferric reducing power with increasing concentrations (table 1).

Antimicrobial activity

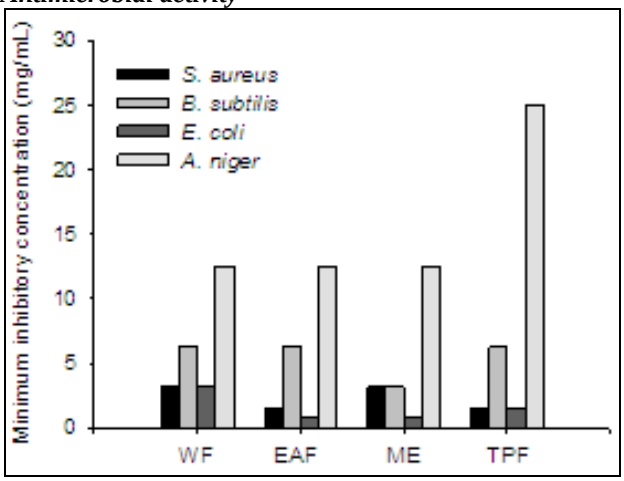

Fig. 4: Effects of PSI various solvent extracts on growth of different bacteria and fungus: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger*. WF, water fraction; EAF, ethyl acetate fraction; ME, methanol extract; TPF, tannin polymer fraction.

The PSI extracts were assayed *in vitro* for their antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* and for their antifungal activity against *Aspergillus niger*. The effect of extracts on the growth of different bacteria and fungus is presented in table 2. The solvent extracts inhibited growth to different extents, depending on the bacterium or fungus in question. Ethyl acetate and methanol extracts exhibited the highest potency against *Escherichia coli* with a low MIC (0.780mg/mL) relative to the reference drug sodium benzoate (MIC=50mg/mL). The ethyl acetate extract was found to be moderately potent against *Bacillus subtilis* with an MIC of 6.25mg/mL (fig. 4). The ethyl acetate extract and tannin polymers showed the highest activity against *Staphylococcus aureus*, with the lowest MIC value of 1.563mg/mL. On the other hand, *Aspergillus niger* was the most resistant to all of the extracts, and higher MIC values were observed for it.

Antioxidant activity in vivo

The effects of dietary PSI extracts on MDA levels and SOD and GSH-Px activities in rat serum and liver and heart homogenates are listed in table 3. All biochemical parameters were significantly different between the extract-treated groups and control group (P<0.05). Serum and liver and heart homogenates in the extract-treated groups showed significantly lower MDA levels than the control group. The SOD and GSH-Px activities were significantly increased in the extract-treated group compared to the control group (P<0.05). The water extract (50mg/kg, 100mg/kg b.w.) and tannin polymers (100, 200, 400mg/kg b.w.) could not only attenuate MDA generation significantly, but increased the SOD and GSH-Px activities in the serum and liver and heart homogenates evidently (table 3). A comparison of the various solvent

extracts of PSI showed that the effects of extracts on MDA, SOD and GSH-Px in rat serum and liver and heart homogenates were in the following order: water > tannin polymers > methanol > ethyl acetate.

HPLC analyses of phenolic compounds

Phenolic acids were present in the PSI ethyl acetate extract as determined by HPLC analysis. The concentrations were determined by calculating the HPLC peak areas, which presented as the mean of three determinations. Gallic acid and ellagic acid were observed at high concentration in the ethyl acetate fraction. Concentrations of gallic acid, ellagic acid and rutin recovered from the PSI ethyl acetate extract were shown in fig. 5. According to our observation, gallic acid and ellagic acid may have important roles in the antioxidant and antimicrobial activities of *P. strobilacea* infructescence extracts.

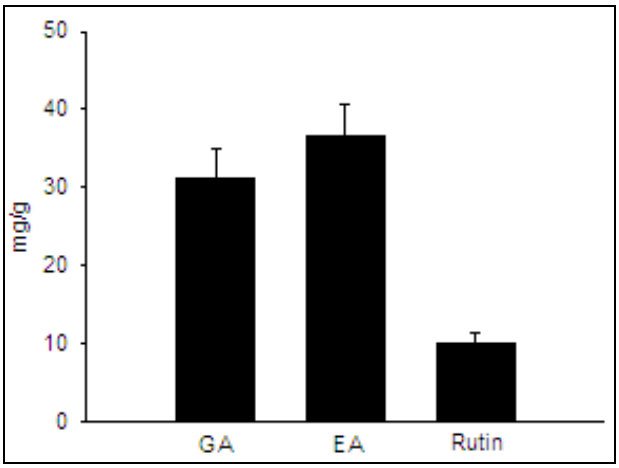

Fig. 5: Concentrations of gallic acid, ellagic acid and rutin recovered from the ethyl acetate fraction of *P. strobilacea* infructescence.

DISCUSSION

Variations in the yields and phenolic contents of the extracts were attributed to different polarities of the compounds present in the PSI. Such differences have been found in the previous literature for the aerial parts of this plant (Choi *et al*., 2003) and other fruit (Negi *et al*., 2005). The result of free radical scavenging activity is consistent with the previous report, which reported that the ethyl acetate fraction of the aerial parts of *P. strobilacea* has high potential as an antioxidant agent (Yang *et al*., 2009). The data showed that the PSI extracts are free radical inhibitors. These results confirmed earlier studies in which the inhibition of DPPH free radicals was reported for *P. strobilacea* stem ethanol extracts (Babu *et al*., 2008). Negi *et al*. (2005) also noted differences in the reducing power of various solvent extracts of seabuckthorn seed. The reducing properties of those extracts are generally associated with the presence of reductones. The antioxidant activity of reductones is believed to break radical chains by donating a hydrogen atom, indicating that the antioxidant properties are concomitant with the reducing power (Gordon, 1990). Therefore, the marked antioxidant activity in the ethyl acetate fraction may be associated with its higher reducing power.

Similar results for inhibition of bacterial growth have been observed in earlier studies with *P. strobilacea* leaf extracts (Lee *et al*., 2004) and *P. strobilacea* ethyl acetate extracts (Yang *et al*., 2009). Yang *et al*. (2009) investigated the antibacterial activity of *P. strobilacea* ethyl acetate extract against various pathogenic skin bacteria, and the results showed that the antibacterial activity of the ethyl acetate fraction was the highest in *S. aureus*.

Evidence supporting an antioxidant function for extracts that are rich in plant polyphenols is usually derived from *in vitro* assays. However, evidence that polyphenols, including PSI extracts, act directly or indirectly as antioxidants *in vivo* is limited. Studies on antioxidant in laboratory animals should be carried out to obtain mechanistic insight and evaluate safety profiles before they are used in humans for intervention trials.

Lipid per oxidation is an autocatalytic process, which may cause per oxidative tissue damage in inflammation, cancer, xenobiotic toxicity and aging (Kurata *et al*., 1993). In the present study, *in vitro* studies showed that the PSI ethyl acetate extract exhibited the highest antioxidant activity. The decreased lipid per oxidation levels in our study may be due to increased antioxidant enzyme activity. In this study, however, the results of *in vivo* antioxidant experiments were inconsistent with the findings of the *in vitro* studies. Similar results were obtained in a previous study, in which black tea did not protect plasma from lipid per oxidation *in vivo* despite its antioxidant efficacy *in vitro* (Cherubini *et al*., 1999). The striking discrepancy between the *in vitro* and *in vivo* data is most likely explained by the insufficient bioavailability of polyphenols in the body, and the presence of other unknown antioxidant compounds in the extracts might also be responsible for their potential antioxidant activity.

CONCLUSIONS

The results in this study revealed that the ethyl acetate extract of *P. strobilacea* infructescence, which contained high amounts of phenolic compounds, showed strong antioxidant and antimicrobial activity. These findings may provide a pharmacological explanation for some of its uses in China folk medicine. It has been shown that free radical scavenging and antioxidant activity of many medicinal plants are responsible for their therapeutic effect against cancer, tissue inflammatory and cardiovascular disease. Thus, the antipyretic and analgesic action of *P. strobilacea* may be associated with its antioxidant and antimicrobial activity.

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