Tropical Journal of Pharmaceutical Research June 2014; 13 (6): 849-854
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v13i6.4

Original Research Article

In vitro Antitumor Activities of *Platycarya strobilacea* Sieb et Zucc Infructescence Extracts

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Received: 30 August 2013 *Revised accepted: 15 March 2014*

Abstract

Purpose: *To evaluate the antitumor activities of Platycarya strobilacea infructescence extracts in A549, HepG2, SH-SY-5Y, HCT116, and U2OS-NKFB cell lines.*
Methods: *The total amount of phenolics in P. strobilacea infructescence based on three solvent extracts (methanol, ethyl acetate and water) was measured using Folin-Ciocalteu reagent method. The cytotoxic effect of the various solvent extracts and tannins on five cancerous cell lines (A549, HepG2, SH-SY-5Y, HCT116, and U2OS-NKFB) were assayed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT].*
Results: *Ethyl acetate extract had the highest phenolic content (725.02 mg GAE/g), followed by methanol extract (627.64 mg GAE/g). Among the extracts tested, ethyl acetate extract had effective anticancer activity against A549, HepG2, HCT116 and U2OS-NKFB cells with IC_{50} values of 40.1, 42.6, 40.1 and 73.4 µg/mL, respectively. Methanol extract had the strongest cytotoxic effect on SH-SY-5Y cell with IC_{50} value of 31.2 µg/mL. The number of surviving cancerous cells decreased as the concentration of the ethyl acetate and methanol extracts increased.*
Conclusion: *The study confirms the antitumor activities of ethyl acetate and methanol extracts of P. strobilacea infructescence. Thus, the plant extracts constitute potential therapeutic materials that require further studies and development.*

Keywords: *Platycarya strobilacea, Antitumor, Phenolic compounds, Tannins, MTT assay*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [1]. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives [2]. Some of these phytochemicals can significantly reduce the risk of cancer due to polyphenol antioxidant and anti-inflammatory effects. Some preclinical studies suggest that phytochemicals can prevent colorectal cancer and other cancers [3-5].

Platycarya strobilacea Sieb *et* Zucc has long been recognized in China as a medicinal plant with a variety of medicinal effects. Its dried infructescence has the function of eliminating toxic heat, activating blood circulation, relieving

swelling, evacuating pus and removing pain, etc [6]. The main constituents reported from its infructescence are flavones, ellagic acid, tannins and related compounds [7-9], and the type of tannins from this plant belongs to the ellagitannins [8]. It has been reported that ellagic acid and its derivatives that are present in the plant have numerous biological activities such as antioxidative, antimicrobial, antifungal, and anti-inflammatory activities [10-12]. However, to our knowledge, no information is available on the antitumor properties of *P. strobilacea* infructescence (PSI) extracts.

In the present study, methanol, ethyl acetate and water extracts of PSI as well as tannins from this plant were studied against five cancer cell lines (A549, HepG2, SH-SY-5Y, HCT116 and U2OS-NKFB). It is noteworthy that our study is the first report about cytotoxic activity of *P. strobilacea* infructescence.

EXPERIMENTAL

Plant materials

P. strobilacea infructescence was obtained from Lianyungang, Jiangsu province in August 2012, and authenticated by Professor Jiahong Chen, Institute of Chemical Industry of Forest Products, CAF. A voucher specimen was deposited in the herbarium of our laboratory (ICIFP no. 121). The infructescence was washed with sterile water, dried in shade, finely powdered and stored in air tight plastic bags.

Preparation of plant extract and fractions

Plant material (200 g) was extracted with petroleum ether at room temperature for 10 h. After drying, defatted plant material (190 g) was extracted with 700 mL of 70 % (v/v) aqueous methanol in a shaker bath set at 40 ^{o}C for 1 h and filtered. This extraction step was repeated three times. The filtrates were combined and methanol was evaporated under vacuum at 45 ^{o}C using a rotary evaporator and the aqueous phase was lyophilized to obtain methanol extract. The methanol extract was dissolved in 200 mL of water and extracted with 200 mL ethyl acetate three times. The ethyl acetate phases were combined and evaporated under vacuum at 40 ^{o}C using a rotary evaporator until dryness to give a yield of 7.10 g. The aqueous phase remaining after ethyl acetate extraction was lyophilized to give a yield of 26.5 g. Each extract was refrigerated for further experiments. Yields of extracted matter were calculated as percentage (w/w) of plant material.

Preparation of tannin polymers

The process of water-extraction and macroporous resin adsorption was adopted to separate and purify tannin polymers. Plant material (80 g) was extracted with 500 mL water twice at 80 ^{o}C for 1 h. The extract was filtered and pooled, and the extract was concentrated by vacuum evaporation at 55 °C to 1/4 of its original volume. The crude tannins extract was adsorbed with NKA-9 macroporous resin which was first eluted with 1 L 10 % methanol solution (v/v) to remove oligomeric tannins and then followed by 600 mL methanol solution. The last fraction, containing the tannin polymers, was vacuum-evaporated to remove solvent and freeze-dried to obtain 3.552 g tannin polymer fraction.

Determination of molecular weight distribution of tannin polymers

Gel permeation chromatography (GPC) analysis was performed with Waters 1515 GPC equipment. The column used was an Aglient PI aquagel OH-30 (7.5 mm × 300 mm, 5 μm) column. Tetrahydrofuran (THF) was used as the eluent, and the conditions used were as follows: flow rate, 1 mL/min; column temperature, 30 °C; injection volume, 25 μL; and detection occurred at 270 nm, with a bandwidth of 15 nm. The calibration curve was obtained with polystyrene standards. The tannin polymers were dissolved in THF (4 mg/mL) and analysed by GPC.

Evaluation of total phenolic compounds

The amount of total phenolics in the various PSI various solvent extracts was measured using the Folin-Ciocalteu reagent method described by Djeridane *et al* [13]. The final results were expressed as gallic acid equivalents (GAE) in mg per g extract.

Cell lines

Five tumor cell lines, A549 (non-small cell line carcinoma), HepG2 (hepatocellular carcinoma), SH-SY-5Y (human neuroblastoma), HCT116 (human colon cancer) and U2OS-NKFB (human osteosarcoma) were obtained from the laboratory of pharmacology, China Pharmaceutical University, Nanjing, China. All cell lines were grown in suitable media supplemented with 10 % fetal bovine serum

MTT assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) colorimetric assay is used

to assess cell viability in the presence of different extracts [14]. Cells were seeded into 96-well plates and incubated for 24 h at 37 °C. Then the medium was replaced with fresh medium containing different concentrations of test extracts. After 72 h incubation at 37 °C, the medium was changed with fresh medium containing MTT and incubated for additional 4 h. Thereafter, MTT was removed and the remaining formazan crystals were completely dissolved in DMSO. Afterwards, the absorbance was recorded at 570 nm, using an ELISA reader. Inhibitory rate was calculated using Eq 1.

Inhibitory rate (%) = {1 – (At/Ac)}100 ……..… (1)

where At and Ac are the absorbance of test sample and control, respectively.

IC_{50} value was defined as the concentration of the extract to produce a 50 % reduction in viability of cells relative to the negative control (wells exposed to the solvent without any extract). All experiments were performed in triplicate.

Statistical analysis

The statistical analyses were performed by a one-way ANOVA and the Duncan′s multiple range test. The results were expressed as means ± SD to show variations in the various experiments. The statistical package, SPSS (version 11.0, SPSS Institute, Chicago, IL, USA) was used for the analysis. Difference of $p < 0.05$ was considered to be significant.

RESULTS

The PSI was extracted in methanol and subjected to solvent-solvent partitioning to yield two fractions as ethyl acetate and water. The yield of ethyl acetate fraction (3.55 %) was much lower than that of water fraction (13.25 %). The total phenolic content of each extract was quantified. The phenolic contents in the different extracts also varied significantly ($p < 0.05$). The ethyl acetate fraction had the highest phenolic content (725.02 mg GAE/g), followed by the methanol extract (627.64 mg GAE/g) and the water extract (546.37 mg GAE/g).

The molecular weight distribution of the PSI tannins was obtained by Gel Permeation chromatography (GPC). It was characterized by the presence of high molecular weight species (Figure 1). The values of the number-average molecular weight (Mn), weight-average molecular weight (Mw) and polydispersity (D = Mw/Mn) of the tannin polymer fraction are 1275, 1545 and 1.212, respectively.

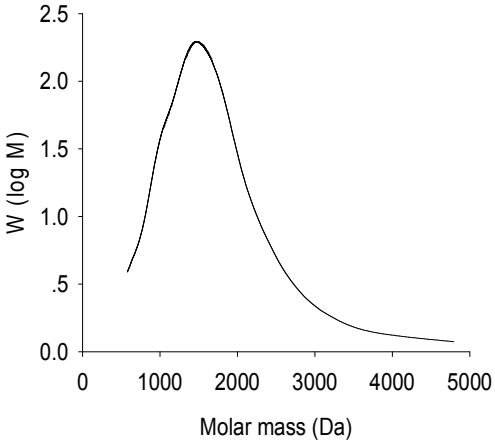

Figure 1: Molecular weight distribution of *P. strobilacea* infructescence tannins

The antitumor activities of the various solvent extracts and tannins of PSI showed significant variations as shown in Tables 1 - 5. A549, HepG2, HCT116 and U2OS-NKFB cells were treated with various solvent extracts and tannins at concentrations ranging from 10 to 160 µg/mL for 72 h at 37 °C and then the percentage of cell viability was calculated. SH-SY-5Y cell was treated with water, ethyl acetate, methanol extracts and tannins at concentrations of 10 - 160, 15 - 240, 10 - 160 and 25 - 400 µg/mL, respectively. The tested malignant cells showed a good response to the effect of ethyl acetate and methanol extracts. Ethyl acetate fraction at 10-160 µg/mL decreased the proliferation of A549, HepG2, HCT116 and U2OS-NKFB cells by 18.4 - 87.6 %, 14.9 - 87.7 %, 15.5 - 95.5 % and 19.4 - 79.4 %, respectively. The IC_{50} values of the four malignant cells were 40.1, 42.6, 40.1 and 73.4 µg/mL, respectively (Figure 2). Also, the methanol extract had the strongest cytotoxic effect on SH-SY-5Y cell. The percentage of dead SH-SY-5Y cell was 86.3 %, after exposure to 160 µg/mL of the methanol extract (Table 5). The IC_{50} for SH-SY-5Y cell viability was 31.2 µg/mL. Compared to the above extracts, water and tannin fractions exerted lower cytotoxic effect on HCT116 and U2OS-NKFB cells as they showed lower degrees of cell death.

Table 1: Anticancer activity of different concentrations of various *P. strobilacea* infructescence solvent extracts and tannins on A549 cell line

Conc	WF		EF		MF		TF	
	$OD_{570\ nm}$	*Inhi*	$OD_{570\ nm}$	*Inhi*	$OD_{570\ nm}$	*Inhi*	$OD_{570\ nm}$	*Inhi*
Control	0.65±0.05	nil	0.65±0.05	nil	0.51±0.04	nil	0.56±0.06	nil
10	0.59±0.03	10.0	0.53±0.06**	18.4	0.49±0.05	2.8	0.47±0.01*	16.3
20	0.58±0.04*	11.8	0.44±0.06**	32.9	0.48±0.02	5.8	0.43±0.01*	23.2
40	0.55±0.05**	15.9	0.49±0.07**	25.4	0.43±0.03*	16.1	0.41±0.02**	25.9
80	0.33±0.07**	49.3	0.11±0.04**	83.8	0.29±0.05**	42.6	0.19±0.05**	66.7
160	0.08±0.03**	88.2	0.08±0.06**	87.6	0.12±0.02**	77.0	0.09±0.04**	84.5

Key: *Conc = concentration (µg/mL); Inhi = inhibition (%); Values are means ± SD (n = 6), *p < 0.05 vs control, **p < 0.01 vs control; WF = water fraction; EF = ethyl acetate fraction; ME = methanol extract; TF = tannin fraction*

Table 2: Anticancer activity of different concentrations of various *P. strobilacea* infructescence solvent extracts and tannins on HepG2 cell line

Conc	WF		EF		MF		TF	
	$OD_{570\ nm}$	*Inhi*	$OD_{570\ nm}$	*Inhi*	$OD_{570\ nm}$	*Inhi*	$OD_{570\ nm}$	*Inhi*
Control	0.66±0.05	nil	0.66±0.05	nil	0.66±0.05	nil	0.64±0.02	nil
10	0.60±0.03	9.6	0.56±0.03**	14.9	0.56±-.07*	14.8	0.59±0.01**	8.1
20	0.59±0.04*	11.4	0.47±0.06**	29.3	0.47±0.02**	28.4	0.52±0.05**	18.5
40	0.56±0.05*	15.4	0.46±0.05**	30.6	0.39±0.03**	40.6	0.39±0.02**	39.0
80	0.34±0.06**	48.9	0.15±0.04**	77.9	0.27±0.01**	58.8	0.31±0.04**	51.0
160	0.09±0.03**	87.1	0.08±0.06**	87.7	0.17±0.01**	74.3	0.28±0.02**	56.8

Key: *Conc = concentration (µg/mL); Inhi = inhibition (%); Values are means ± SD (n = 6), *p < 0.05 vs control, **p < 0.01 vs control; WF = water fraction; EF = ethyl acetate fraction; ME = methanol extract; TF = tannin fraction*

Table 3: Anticancer activity of different concentrations of various *P. strobilacea* infructescence solvent extracts and tannins on HCT116 cell line

Conc	WF		EF		MF		TF	
	$OD_{570\ nm}$	*Inhi*	$OD_{570\ nm}$	*Inhi*	$OD_{570\ nm}$	*Inhi*	$OD_{570\ nm}$	*Inhi*
Control	1.31±0.11	nil	1.31±0.11	nil	1.31±0.11	nil	1.31±0.11	nil
10	1.22±0.14	7.0	1.11±0.15	15.5	1.12±0.13*	14.7	1.29±0.12	15.0
20	1.19±0.11	9.1	1.04±0.17*	20.3	0.88±0.09**	32.6	1.01±0.16*	23.1
40	1.10±0.08	16.3	0.93±0.15**	28.9	0.88±0.22**	33.0	0.87±0.11**	33.3
80	1.01±0.07*	23.1	0.17±0.18**	86.7	0.41±0.13**	69.0	0.84±0.16**	35.9
160	0.59±0.05**	55.0	0.06±0.16**	95.5	0.30±0.12**	77.0	0.80±0.12**	39.2

Key: *Conc = concentration (µg/mL); Inhi = inhibition (%); Values are means ± SD (n = 6), *p < 0.05 vs control, **p < 0.01 vs control; WF = water fraction; EF = ethyl acetate fraction; ME = methanol extract; TF = tannin fraction*

Table 4: Anticancer activity of different concentrations of various *P. strobilacea* infructescence solvent extracts and tannins on U2OS-NKFB cell line

Conc	WF		EF		MF		TF	
	$OD_{570\ nm}$	*Inhi*	$OD_{570\ nm}$	*Inhi*	$OD_{570\ nm}$	*Inhi*	$OD_{570\ nm}$	*Inhi*
Control	1.31±0.10	nil	1.31±0.10	nil	1.31±0.10	nil	1.31±0.10	nil
10	1.23±0.08	6.6	1.06±0.14	19.4	1.10±0.12	15.8	1.28±0.11	2.7
20	1.18±0.15	9.7	1.00±0.14*	23.9	0.99±0.05*	24.2	1.06±0.11*	19.4
40	1.07±0.07*	18.6	0.78±0.16**	40.8	0.89±0.20**	32.1	1.00±0.16*	24.0
80	0.92±0.15*	29.8	0.55±0.14**	57.7	0.52±0.08**	60.5	0.78±0.13**	40.2
160	0.56±0.04**	57.4	0.27±0.11**	79.4	0.38±0.13**	70.7	0.58±0.12**	55.5

Key: *Conc = concentration (µg/mL); Inhi = inhibition (%); Values are means ± SD (n = 6), *p < 0.05 vs control, **p < 0.01 vs control; WF = water fraction; EF = ethyl acetate fraction; ME = methanol extract; TF = tannin fraction*

Table 5: Anticancer activity of different concentrations of various *P. strobilacea* infructescence solvent extracts and tannins on SH-SY-5Y cell line

Conc	WF		Conc	EF		Conc	MF		Conc	TF	
	$OD_{570\ nm}$	*Inhi*		$OD_{570\ nm}$	*Inhi*		$OD_{570\ nm}$	*Inhi*		$OD_{570\ nm}$	*Inhi*
Control	0.110±0.009	nil		0.110±0.009	nil		0.171±0.006	nil		0.212±0.016	nil
10	0.101±0.007	5.3	15	0.094±0.021	11.6	10	0.120±0.034*	30.1	25	0.097±0.016**	54.0
20	0.097±0.015	8.8	30	0.067±0.020*	37.5	20	0.098±0.020**	42.7	50	0.090±0.008**	57.3
40	0.086±0.026*	19.3	60	0.058±0.022**	43.5	40	0.070±0.012**	59.2	100	0.040±0.036**	81.2
80	0.042±0.013**	60.4	120	0.024±0.033**	45.4	80	0.063±0.015**	63.4	200	0.035±0.021**	83.6
160	0.021±0.012**	80.3	240	0.040±0.025**	77.9	160	0.023±0.015**	86.3	400	0.031±0.009**	85.6

Key: *Conc = concentration (µg/mL); Inhi = inhibition (%); Values are means ± SD (n = 6), $^{*}p < 0.05$ vs control, $^{**}p < 0.01$ vs control; WF = water fraction; EF = ethyl acetate fraction; ME = methanol extract; TF = tannin fraction*

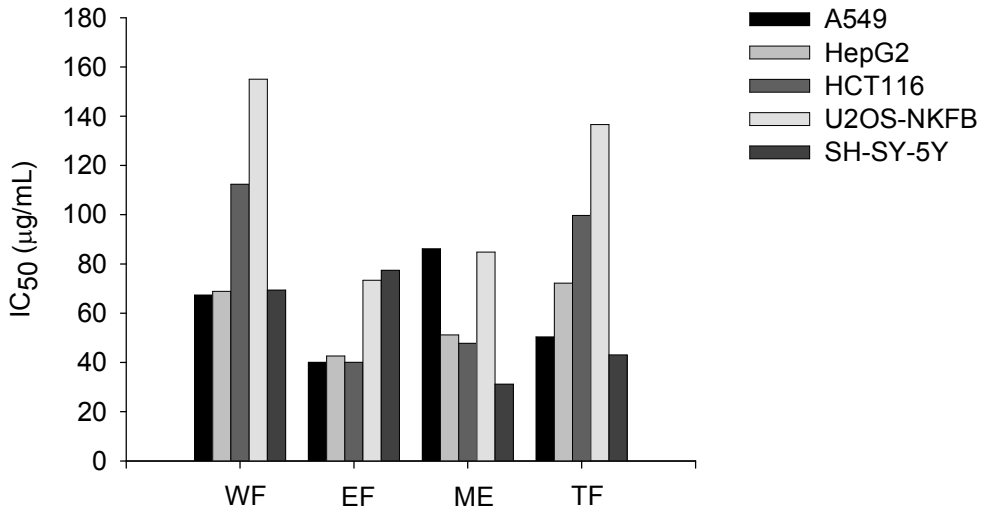

Figure 2: Anticancer activity of various solvent extracts and tannins of *P. strobilacea* infructescence on A549, HepG2, HCT116, U2OS-NKFB and SH-SY-5Y cell lines. ***Key:*** WF = water fraction; EF = ethyl acetate fraction; ME = methanol extract; TF = tannin fraction

DISCUSSION

Variations in the yields and phenolic contents of extracts are attributed to polarities of different compounds present in the PSI and such differences have been reported in the literature for the aerial parts of this plant [12] and other fruits [15].

The relationship between concentration of extracts and their cytotoxic effects on A549, HepG2, SH-SY-5Y, HCT116 and U2OS-NKFB cells was investigated by MTT assay. MTT is a yellow water-soluble tetrazolium salt. Metabolically active cells are able to convert the dye to water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring. The strong cytotoxic activity of ethyl acetate and methanol extracts from PSI may be attributed to the presence of specific components such as polyphenols and flavonoids of these extracts. Besides, some studies have shown that polyphenols and flavonoids are able to influence a variety of cell functions by modulating cell signalling [16], altering proliferation and cytotoxicity in cancer cell lines [17].

CONCLUSION

PSI ethyl acetate and methanol extracts possess cytotoxic effects on the in vitro cancer cell lines tested. The findings also demonstrate that the extracts have low IC_{50} values, and may be a potential source of pharmacologically useful products. Thus, the presence of potent cytotoxic compounds in this plant warrants further investigation.

ACKNOWLEDGEMENT

The authors are grateful to the Natural Science Foundation of Jiangsu, China (BK2012064) for financial support.

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