JBUON 2017; 22(2): 543-551
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

In vitro anticancer activity and cytotoxicity screening of phytochemical extracts from selected traditional Chinese medicinal plants

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Summary

Purpose: *The purpose of this study was to evaluate the anticancer activity of 10 selected traditional Chinese medicinal plants on 4 cancer cell lines, namely MCF-7 (breast carcinoma), BALL-1 (acute lymphoblastic leukemia), Huh-7 (hepatocellular carcinoma), HeLa (cervical carcinoma) and their cytotoxicity on a normal cell line, namely MCF-10A (non-tumorigenic mammary epithelial line), about which there were few reports previously.*

Methods: *Six phytochemical extracts, namely petroleum ether extract (PEE), chloroform extract (CE), ethyl acetate extract (EAE), n-butanol extract (BE), the remainder after extraction (RE) and water extract (WE) from each plant were prepared. These 60 extracts were screened for their cytotoxicity on the aforementioned cell lines using MTT assay.*

Results: *All plant species showed certain anticancer activity against at least one of the 4 cancer cell lines and obvious selective cytotoxicities were observed. Compared with Huh-7 and HeLa cells, MCF-7 and BALL-1 cells were more sensitive to the treatments of the phytochemical extracts. The PEE, CE and EAE of Brassica campestris, the PE and CE of Hibiscus syriacus, as well as the BE of Pittosporum tobira exhibited strong anticancer activity but weak cytotoxicity on the normal cell line.*

Conclusion: *Our results provided new evidence for anticancer activities of these plants which could be useful for developing new anticancer therapies.*

Key words: *anticancer activity, cytotoxicity, MTT assay, phytochemical extracts, traditional Chinese medicinal plants*

Introduction

Cancer had always been the most serious disease in humans around the world due to its high morbidity and mortality [1]. Breast cancer is an important global health problem and one of the principal causes of deaths in females [2]. Hepatocellular carcinoma is the sixth most common cancer and the third leading cause of cancer-related death in the world [3]. Cervical cancer is the third most common cancer of women after breast cancer and colorectal cancer [4]. Acute lymphoblastic leukaemia occurs in both children and adults and has been listed as one of the ten leading cancer types for the estimated new cancer cases and deaths in United States in 2014 [5,6]. Currently, the main therapies for cancer include surgical operation, radiotherapy and chemotherapy. Although these therapies have saved numerous lives of various cancer patients, the severe side effects and high recurrence rate make them only partially effective to cure and control cancers most of the times, and therefore the demand for developing more diverse and effective cancer therapies from different sources is pressing. Compared with synthetic chemotherapeutic drugs, plant-derived nat-

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Received: 07/09//2016; Accepted: 22/09//2016

ural chemicals are relatively low-toxic, possess high target specificity and are generally available in an ingestive form for cancer treatment. Several clinically useful anticancer agents such as vinblastine, vincristine, camptothecin, topotecan, irinotecan, etoposide and paclitaxel were acquired or developed from phytochemicals, which have been proved inevitable and valuable resources to uncover novel anticancer chemical [7].

In the present study, 10 traditional Chinese medicinal plants, namely *Bougainvillea spectabilis, Brassica campestris, Chenopodium ambrosioides, Equisetum hyemale, Euonymus japonicus var.aureomarginata, Fatsia japonica, Hibiscus syriacus, Phytolacca americana , Pittosporum tobira* and *Trifolium repens* were selected for experimentation (Table 1). Only few reports about their anticancer activities have been published so far. Hopefully, our results may provide new information for anticancer drug development from these plants.

Methods

Reagents and chemicals

Absolute ethanol, petroleum ether, chloroform, ethyl acetate and n-butanol were of analytical grade and were purchased from Tianjin JinDong TianZheng Precision Chemical Reagent Factory (Tianjin, P.R. China). Roswell Park Memorial Institute 1640 medium (RPMI-1640), Dulbecco's modified Eagle medium (DMEM), Dulbecco's modified Eagle medium/Nutrient mixture Ham's F-12 (DMEM/Ham's F-12), and phosphate buffered saline (PBS) were purchased from Ther-

Table 1. Detailed information about the selected traditional Chinese medicinal plants

Botanical name	*Family*	*Part(s) used*	*Traditional remedy usage*	*Reported anticancer activities*
Bougainvillea spectabilis Willd.	Nyctaginaceae	Br, L& Fl	Fl: irregular menstruation [8]	None
Brassica campestris L.	Brassicaceae	WP	WP: haemorrhagic dysentery, erysipelas, acute mastitis and hematemesis [9]	Human prostate cancer PC-3 cells [10]
Chenopodium ambrosioides L.	Chenopodiaceae	WP	WP: antipruritic, rheumatalgia, scabies, head louse, parasitic diseases, eczema, menorrhalgia, amenorrhea, sore throat, traumatic injury, snake bite and insect sting [9]	MCF-7 breast cancer cell line [11]; Ehrlich tumor [12]
Equisetum hyemale L.	Equisetaceae	WP	WP: conjunctival congestion, epiphora induced by wind, bleeding haemorrhoids, haemorrhagic dysentery, hypermenorrhea and archoptoma [13]	Murine leukemia L1210 cells [14]
Euonymus japonicus var.aureomarginata Regel.	Celastraceae	WP	None	None
Fatsia japonica (Thunb.) Decne. et Planch.	Araliaceae	Br, L	L,RB: antitussive, expectorant, rheumatalgia, arthrolithiasis and traumatic injury [13]	Rat glioma C6 and human glioma U251 cell lines [15]
Hibiscus syriacus L.	Malvaceae	Br, L& Fl	Fl,R,SB(RB),L&S: antitussive, expectorant, hemoptysis, dysentery, scabies, tuberculosis, empyrosis, bronchitis, eczema, impetigo and abnormal leukorrhea [13]	A549 human lung cancer cells [16,17] H209 and H661 human lung cancer cells [17]
Phytolacca americana L.	Phytolaccaceae	WP	L,S: diuresis, edema, and beriberi [13]	Human glioma U251 cell line [18]; HCT-116 colon cancer cell line [19]
Pittosporum tobira (Thunb.) Ait.	Pittosporaceae	Br, L, Fl &Fr	Br,L: parasiticide, scabies [13]	Colon adenocarcinoma, melanotic melanoma, breast carcinoma, pancreas adenocarcinoma, neuroblastoma and medulloblastoma [20]
Trifolium repens L.	Leguminosae	WP	WP: epilepsy, bleeding haemorrhoids [13]	Crown-gall tumor [21]

Br: branches, Fl: flowers, Fr: fruits, L: leaves, R: roots, RB: root bark, S: seeds, SB: stem bark, WP: whole plant

mo Fisher Scientific (Beijing, P.R. China). Fetal bovine serum (FBS) and horse serum (HS) were purchased from Invitrogen (Auckland, New Zealand). [3-(4,5 dimethylthiazol-2-yl)-2,5,-diphenyl, tetrazolium bromide] (MTT), Trypsin-EDTA (0.25%), penicillin-streptomycin (100 units/ml penicillin and 100 μg/ml streptomycin), epidermal growth factor (EGF), dimethyl sulfoxide (DMSO) and hydrocortisone were obtained from Beijing Solarbio & Technology Co., Ltd. (Beijing, P.R. China), cholera toxin was purchased from Sigma-Aldrich (St. Louis MO, USA), insulin was purchased from Bayer HealthCare (Leverkusen, Germany). Gemcitabine hydrochloride was purchased from Sinopharm Chemical Reagent Co.,Ltd. (Shanghai, P.R. China).

Plant materials

Ten traditional Chinese medicinal plants were collected from two different locations of Kunming, Yunnan Province, P.R. China at different times. *Brassica campestris* was collected from Xishan Mountain Forest Park of Kunming in November 2012; *Bougainvillea spectabilis, Chenopodium ambrosioides, Equisetum hyemale, Euonymus japonicus var.aureomarginata, Fatsia japonica, Hibiscus syriacus, Phytolacca americana, Pittosporum tobira* and *Trifolium repens* were collected from Chenggong campus of Kunming University of Science and Technology in May 2013. Table 1 shows the botanical names, family, plant part(s) used for extraction, the traditional remedy usage and the reported anticancer activities of the collected plants. Plant materials were identified by Dr. Haizhou Li, professor in the field of Natural Medicinal Chemistry. The voucher specimens were deposited in the Laboratory of Medicinal Chemical Biology, Department of Pharmacy, Faculty of Life Science and Technology, Kunming University of Science and Technology.

Preparation of phytochemical extracts and their stock solutions

Plant materials were cut into small pieces and air-dried at room temperature in the dark. For each plant, 100 grams of dry materials were immersed in 1000ml of absolute ethanol and extracted in an ultrasonic bath (60°C, 200W) for 12 hrs. Then, liquid ethanol extract was filtered and concentrated to dryness using a rotary evaporator (EYELA, Japan) under reduced pressure. The resulting dry material was re-dissolved in 500ml of distilled water and then successively extracted with petroleum ether (3×500ml), chloroform (3×500ml), ethyl acetate (3×500ml) and n-butanol (3×500ml). After air-dried, the residual plant materials remained after extraction with absolute ethanol were immersed in 1000ml of distilled water and extracted in an ultrasonic bath (60°C, 200W) for 12 hrs. Finally, all liquid extracts were concentrated to dryness under reduced pressure. Using different solvent extraction, we prepared 6 phytochemical extracts from each plant including petroleum ether extract (PEE), chloroform extract (CE), ethyl acetate extract (EAE), n-butanol extract (BE), the remainder after extraction (RE) and water extract (WE). Totally, 60 phytochemical extracts were acquired from the 10 medicinal plants and their cytotoxic activities against MCF-7 (breast carcinoma cell line), BALL-1 (acute lymphoblastic leukemia cell line), Huh-7 (hepatocellular carcinoma cell line), HeLa (cervical carcinoma cell line) and MCF-10A (non-tumorigenic breast epithelial cell line) were screened by MTT assay. All plant species have shown specific anticancer activity against at least one of the 4 cancer cell lines and obvious selective cytotoxicities were observed. The IC_{50} values of these active extracts were determined.

Stock solutions of each phytochemical extract were prepared in DMSO at 100mg/ml and stored at -20°C until used for further cytotoxic screening.

Cell lines

MCF-7 and HeLa cell lines were kindly provided by the Laboratory of Molecular Genetics of Aging & Tumor, Faculty of Medicine, Kunming University of Science and Technology. Huh-7 cell line was kindly provided by the Laboratory of Molecular Virology, Faculty of Life Science and Technology, Kunming University of Science and Technology. BALL-1 cell line was purchased from Kunming Institute of Zoology, Chinese Academy of Sciences. MCF-10A cell line was kindly provided by the Laboratory of Cancer Biology, Kunming Institute of Zoology, Chinese Academy of Sciences. MCF-7 and BALL-1 cell lines were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin. Huh-7 and HeLa cell lines were cultured in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin. MCF-10A cell line was cultured in DMEM/Ham's F-12, supplemented with 5% HS, 20ng/ml EGF, 100ng/ml cholera toxin, 0.008mg/ml insulin, 500ng/ml hydrocortisone and 1% penicillin-streptomycin. All 5 cell lines were maintained in a humidified incubator at 37 °C in an atmosphere of 5% CO_2.

Cytotoxic screening

The cytotoxicity of phytochemical extracts on the aforementioned cell lines were measured by two rounds of screening procedures in succession using MTT assay. MCF-7, BALL-1, Huh-7 and HeLa cells were seeded in 96-well microplates at different density as follows: 8000 cells /well for MCF-7, 10000 cells /well for BALL-1, 3000 cells /well for Huh-7, 5000 cells /well for HeLa and 5000 cells /well for MCF-10A; the volume of cell culture in each well was 100μl.

In a preliminary screening procedure, the cytotoxicity of all 60 phytochemical extracts were assayed on 4 cancer cell lines (MCF-7, BALL-1, Huh-7 and HeLa),

Table 2. Growth inhibition of MCF-7, BALL-1, Huh-7 and HeLa cells caused by the phytochemical extracts of the ten plants

Botanical name	*Extract*	*Cell growth inhibition rate (%)*			
		MCF-7	*BALL-1*	*Huh-7*	*HeLa*
Bougainvillea spectabilis Willd.	**PEE**	82.70	93.13	94.26	87.90
	CE	82.58	90.44	63.09	61.43
	EAE	3.57	62.31	29.49	9.72
	BE	3.33	6.15	11.84	12.14
	RE	3.51	11.17	7.54	13.85
	WE	4.14	8.30	16.05	6.49
Brassica campestris L.	**PEE**	89.40	87.00	90.12	91.12
	CE	81.32	84.28	64.20	83.31
	EAE	80.21	90.41	72.48	20.86
	BE	0.36	16.75	13.33	1.39
	RE	3.20	16.57	10.37	18.92
	WE	0.23	11.58	8.15	10.63
Chenopodium ambrosioides L.	**PEE**	88.12	91.09	81.35	93.07
	CE	88.36	88.80	82.13	79.99
	EAE	23.70	93.97	22.38	20.12
	BE	14.93	25.08	5.86	11.72
	RE	12.41	27.31	10.85	10.92
	WE	0.95	8.06	4.97	12.92
Equisetum hyemale L.	**PEE**	89.93	84.48	90.56	90.41
	CE	88.42	82.13	67.62	68.51
	EAE	52.21	90.05	40.18	12.10
	BE	6.50	16.25	5.20	6.84
	RE	7.97	22.19	7.28	6.76
	WE	2.37	12.78	3.81	6.52
Euonymus japonicus var.aureomarginata Regel.	**PEE**	87.80	81.31	82.44	88.44
	CE	81.39	85.89	ND	ND
	EAE	30.21	67.76	14.08	12.81
	BE	34.52	42.46	30.01	34.04
	RE	1.64	6.59	15.11	24.72
	WE	6.24	14.92	24.23	27.69
Fatsia japonica (Thunb.) Decne. et Planch.	**PEE**	91.03	91.31	93.28	89.17
	CE	89.52	91.11	92.33	87.02
	EAE	84.12	90.97	91.34	81.59
	BE	2.26	-2.00	-5.62	1.78
	RE	11.56	-6.91	14.08	3.05
	WE	46.08	54.95	18.16	4.89
Hibiscus syriacus L.	**PEE**	89.61	81.59	84.14	90.74
	CE	87.15	87.76	81.61	87.63
	EAE	75.25	94.38	38.93	18.04
	BE	10.85	34.50	15.96	9.54
	RE	12.45	24.88	12.65	14.79
	WE	12.57	21.22	9.04	7.92
Phytolacca americana L.	**PEE**	91.25	92.39	83.05	86.68
	CE	85.09	91.62	77.68	84.79
	EAE	48.79	87.44	5.33	18.83
	BE	1.54	21.89	9.82	8.34
	RE	6.99	15.90	18.39	13.76
	WE	16.00	25.70	3.12	15.04

Continued on the next page

Pittosporum tobira (Thunb.) Ait.	**PEE**	93.68	90.00	91.60	90.63
	CE	86.70	88.84	89.89	40.88
	EAE	79.44	89.96	50.58	37.06
	BE	86.21	83.81	92.94	88.44
	RE	5.42	14.55	5.97	2.90
	WE	86.90	95.16	7.52	88.52
Trifolium repens L.	**PEE**	86.83	81.86	91.25	88.55
	CE	84.08	83.60	68.27	35.18
	EAE	30.57	75.10	28.31	1.04
	BE	6.51	32.29	11.17	16.75
	RE	0.96	24.60	7.02	21.33
	WE	2.23	20.15	12.80	15.46

PEE: petroleum ether extract, CE: chloroform extract, EAE: ethyl acetate extract, BE: n-butanol extract, RE: the remainder after extraction, WE: water extract, ND: not determined. Extracts that caused more than 80% inhibition on at least two cancer cell lines assayed are in bold. Cell growth inhibition rate (%) was presented as the mean of three replicates.

and 0.5% (v/v) DMSO treatment was used as negative control. Briefly, after seeded in culture plate for 24 hrs, cells in each well were cultivated with 1μl stock solution of each plant phytochemical extract or DMSO mixed with another 99μl complete culture medium, and the final concentration of each plant phytochemical extract in each well was 500μg/ml. After 72 hrs incubation, 20μl MTT solution (5mg/ml in PBS) was added into each well and cells were incubated for another 4 hrs. Then the culture medium in each well was replaced with 100μl DMSO and the culture plate was shaken for 10 min with a microoscillator (Haimen Kylin-Bell Lab Instruments Co.,Ltd., P.R.China) to dissolve the precipitated formazan crystals. Afterward, the optical density value of each well was measured on an Automated Microplate Reader (Tecan infinite 200, Switzerland) at the wavelength of 490 nm. Each experiment was performed in triplicate. Cell growth inhibition rate (GI) was calculated based on the following formula: GI(%)=100 - 100×(T/C). C stands for the optical density value of the cell well treated with 0.5% DMSO; T stands for the optical density value of the cell well treated with a phytochemical extract.

An extract showing more than 80% cell GI on at least 2 human cancer cell lines in a preliminary screening was put into the secondary screening to assay its dose-dependent cytotoxic effects on the 4 human cancer cell lines and the normal cell line. The tested concentrations for each phytochemical extract dose effect were 3.9μg/ml, 7.8μg/ml, 15.6μg/ml, 31.25μg/ml, 62.5μg/ml, 125μg/ml, 250μg/ml and 500μg/ml. Gemcitabine hydrochloride of 0.0114μg/ml, 0.114μg/ml, 1.14μg/ml, 11.4μg/ml and 114μg/ml was assayed for its dose effect on the cells and used as positive control. The chemical dose effect results were expressed as IC_{50} values (the chemical concentration that caused 50% cell GI) which were calculated using the GraphPad Prism 5.0 software (GraphPad Software, USA).

Results

To investigate the cytotoxic activities of the 60 phytochemical extracts on MCF-7, BALL-1, Huh-7 and HeLa cells, we firstly assayed the inhibition effects of these extracts on the growth of aforementioned cells using 500μg/ml for each extract. The results indicated that 24 extracts showed more than 80% cell GI on at least 2 human cancer cell lines (Table 2). The PEE and CE of all assayed plants showed more than 80% cell GI on at least 2 human cancer cell lines. The EAE of the partial assayed plants in this study also resulted in more than 80% cell GI against some tested cancer cell lines. For example, for MCF-7 cell line, these EAE were the ones from *Brassica campestris* and *Fatsia japonica*; for BALL-1 cell line, they were the ones from *Brassica campestris, Chenopodium ambrosioides, Equisetum hyemale, Fatsia japonica, Hibiscus syriacus, Phytolacca americana* and *Pittosporum tobira*; for Huh-7 and HeLa cell lines, the only one was from *Fatsia japonica.* In addition, the BE and WE of *Pittosporum tobira* resulted in more than 80% cell GI against all tested cancer cell lines except the WE of *Pittosporum tobira* that was not against Huh-7 cell line. It is worth mentioning that none of the RE from the plants showed any cytotoxicity towards any of the 4 cancer cell lines.

To confirm the activities of the 24 phytochemical extracts showing more than 80% cell GI on at least 2 human cancer cell lines, dose-dependent cytotoxic effects of these extracts on the 4 human cancer cell lines and the normal cell line were assayed and the corresponding IC_{50} values were acquired. The results are shown in Table 3. On the whole, all tested extracts showed stronger cytotoxic activity against MCF-7 than Huh-7

Table 3. Cytotoxicity (IC_{50}) of these active phytochemical extracts on 4 human cancer cell lines (MCF-7, BALL-1, Huh-7 and HeLa) and a normal cell line (MCF-10A)

Botanical name	*Extract*	*IC_{50}(μg/ml)*				
		MCF-7	*BALL-1*	*Huh-7*	*HeLa*	*MCF-10A*
Bougainvillea spectabilis Willd.	PEE	26.04	21.07	149.30	121.70	50.01
	CE	92.24	71.54	>250	>250	152.20
Brassica campestris L.	PEE	40.65	15.44	186.60	>250	111.60
	CE	83.71	13.31	>250	>250	102.60
	EAE	143.70	41.13	>250	>250	>250
Chenopodium ambrosioides L.	PEE	40.83	14.45	>250	>250	51.52
	CE	93.01	ND	>250	>250	43.99
Equisetum hyemale L.	PEE	29.15	22.80	173.9	>250	25.30
	CE	>250	29.22	>250	>250	87.12
Euonymus japonicus var.aureomarginata Regel.	PEE	62.31	8.91	202.90	174.10	55.37
	CE	56.45	28.47	ND	ND	ND
Fatsia japonica (Thunb.) Decne. et Planch.	PEE	32.86	14.49	166.10	112.90	60.93
	CE	29.30	15.46	98.58	87.79	23.14
	EAE	59.18	45.84	78.94	>250	38.23
Hibiscus syriacus L.	PEE	50.69	32.16	>250	>250	107.20
	CE	57.32	34.32	129.10	219.40	105.50
Phytolacca americana L.	PEE	39.12	7.25	143.50	99.55	27.03
	CE	30.68	18.47	>250	186.7	13.05
Pittosporum tobira (Thunb.) Ait.	PEE	22.97	12.16	>250	>250	31.93
	CE	69.60	50.14	>250	>250	72.40
	BE	37.41	ND	36.12	76.67	107.20
	WE	52.75	ND	52.83	85.02	79.29
Trifolium repens L.	PEE	30.00	ND	151.10	163.80	86.63
	CE	53.16	24.80	>250	>250	86.94
Gemcitabine hydrochloride		3.00×10^{-3}	ND	0.05	0.41	1.15

PEE: petroleum ether extract, CE: chloroform extract, EAE: ethyl acetate extract, BE: n-butanol extract, WE: water extract. ND: not determined. Gemcitabine hydrochloride: positive control

and HeLa cell lines. Among all 4 cancer cell lines, BALL-1 was the most sensitive to the phytochemial extracts. All tested extracts showed stronger cytotoxic activity against BALL-1 than MCF-7, Huh-7 and HeLa. For MCF-7 cell line, IC_{50} values of 11 extracts were lower than 50μg/ml and the PEE of *Pittosporum tobira* exhibited the best activity (IC_{50}=22.97μg/ml). For BALL-1 cell line, IC_{50} values of 18 extracts were lower than 50μg/ml except those not determined. The PEE of *Phytolacca americana* (IC_{50}=7.25μg/ml) was the most active; for Huh-7 cell line, IC_{50} value of only one extract was lower than 50μg/ml; it was the BE of *Pittosporum tobira* (IC_{50}=36.12μg/ml). For HeLa cell line, IC_{50} value of all extracts were higher than 50μg/ml, and the BE of *Pittosporum tobira* showed the best activity (IC_{50}=76.67μg/ml). Furthermore, Table 3 shows the cytotoxicity of selected phytochemical extracts against normal cell line MCF-10A. In this context, among all extracts showing strong activity (IC_{50}<50μg/ml) on at least one of the 4 human cancer cell lines, there were 6 extracts showing lower cytotoxicity (IC_{50}>100μg/ml) on MCF10A cell line. They were the PEE, CE and EAE of *Brassica campestris*, and the PEE and CE of *Hibiscus syriacus*, and the BE of *Pittosporum tobira*. The EAE of *Brassica campestris* (IC_{50}>250μg/ml) exhibited the lowest cytotoxicity.

Discussion

Huge number of natural chemicals including alkaloids, cardiac steroids, triterpenoids, diterpenes, flavones, flavonoids and crude extracts from various traditional Chinese medicinal plants were identified to show anticancer activities, which highlights the importance of traditional Chinese medicinal plants as extremely valuable resources for exploring anticancer compounds. Based on this, we initiated a pilot study to firstly evaluate

various phytochemical extracts from 10 traditional Chinese medicinal plants collected in Yunnan Province of P.R. China for their cytotoxic activities against 4 cancer cell lines, MCF-7, BALL-1, Huh-7 and HeLa, and a normal cell line, MCF-10A.

According to the results presented in Table 2, at least one phytochemical extract of each of these 10 plants showed strong cytotoxic activity (cell GI rate >80%) against at least one of the 4 human cancer cell lines. Anticancer activities of all these plants were, for the first time, demonstrated against MCF-7, BALL-1, Huh-7 and HeLa cell lines except *Chenopodium ambrosioides.* It has been reported that the essential oil of *Chenopodium ambrosioides* was an important cytotoxic component to MCF-7 cell line [11], which was confirmed by our results in the present study that the cytotoxicity of *Chenopodium ambrosioides* against MCF-7 was from its PEE (IC_{50} =40.83μg/ml) and from its CE (IC_{50} =93.01μg/ml).

According to the results presented in Table 3, the PEE, CE and EAE from *Brassica campestris*, the PE and CE from *Hibiscus syriacus*, and the BE from *Pittosporum tobira* exhibited strong anticancer activity (IC_{50}<50μg/ml on at least one of the 4 cancer cell line) and weak cytotoxicity (IC_{50}>100μg/ml) on the normal cell line. This suggested promising prospect of these plant extracts for developing anticancer drugs with low side effects.

Our study, for the first time, investigated and discovered the anticancer activities of the phytochemical extracts from *Bougainvillea spectabilis* and *Euonymus japonicus var.aureomarginata. Bougainvillea spectabilis* is a shrub with high ornamental value, it is native to Brazil and now widely grows in tropical and subtropical regions. The flower of this plant is used for treatment of irregular menstruation in China [8]. Antihyperglycemic activity of this plant has also been reported [22]. Pinitol, β-sitosterol, quercetin and quercetin-3-O-α-L-rhamnopyranoside have been isolated from the stem bark of this plant [23]. *Euonymus japonicus var.aureomarginata* is an evergreen shrub native to China, which is usually cultivated as hedge plant. It is one of many varieties of *Euonymus japonicus* which is a traditional medicinal plant for treatment of rheumatism, hematemesis and fractures in China [9], but so far there is no research on the bioactivities related to its chemical components.

Most previous researches on the cytotoxic effects of *Hibiscus syriacus* were focused on the phytochemical extracts from its stem bark. Triterpenoids, pentacyclic triterpene esters and naphthalenes have been isolated from the stem bark of *Hibiscus syriacus* and showed anticancer activities on a panel of human cancer cell lines [16,24,25]. Moreover, Yeung-Leung Cheng et al. reported that acetone extract from the stem bark of *Hibiscus syriacus* could induce apoptosis by activating p53 and AIF in lung cancer cell lines [17]. Different from the previous research, the PEE and CE from branches, leaves and flowers of *Hibiscus syriacus* in our study showed moderate cytotoxicity on MCF-7 cell line (IC_{50}=50.69μg/ml for PEE; IC_{50}=57.32μg/ml for CE) and strong cytotoxicity on BALL-1 cell line (IC_{50}=32.16μg/ml for PEE; IC_{50}=34.32μg/ml for CE). We speculated that possibly there are the same active compounds in stem bark, branches, leaves and flowers of *Hibiscus syriacus.*

The results presented in Table 2 showed that all extracts of *Pittosporum tobira* except its RE exhibited good cytotoxicity on all tested cancer cell lines. This possibly indicated both phytochemicals of weak and strong polarity in *Pittosporum tobira* possessed anticancer activity. The BE and WE of *Pittosporum tobira* showed general anticancer activity on different cancer cells in our study which may be attributed to the presence of triterpenoid estersaponins in *Pittosporum tobira* [20]. Whether anticancer activity of the PEE (IC_{50}=22.97μg/ml on MCF-7; IC_{50}=12.16μg/ml on BALL-1) is related to its volatile oils needs further investigation [26,27]. In addition, pancherins A and B isolated from *Pittosporum pancheri*, another plant species belonging to the genus Pittosporum, displayed a significant activity in an *in vitro* cytotoxic assay against mouth epidermoid carcinoma (KB), suggesting a general potential of the anticancer activity of the genus Pittosporum [28].

Trifolium repens is an ornamental plant as well as a kind of pasture plant of high feeding value. The whole plant is used in the treatment of epilepsy and bleeding haemorrhoids in China [13]. Though the ethanolic extract of *Trifolium repens* showed a growth inhibiting activity in the potato disc assay against crown-gall tumor, a neoplastic disease of plants induced by *Agrobacterium tumefaciens* [21], our study was the first to show whether the phytochemical extracts of *Trifolium repens* was cytotoxic on human cancer cell lines. As a result, the PEE of *Trifolium repens* showed a strong activity (IC_{50}=30.00μg/ml) on MCF-7 cell line, and its CE showed a moderate activity (IC_{50}=53.16μg/ml) on MCF-7 cell line and a strong activity (IC_{50}=24.80μg/ml) on BALL-1 cell line. These effects may be due to the presence of the volatile [29] and phenolic chemicals [30] in *Trifo-*

lium repens.

Brassica campestris, Chenopodium ambrosioides, Equisetum hyemale, Fatsia japonica and *Phytolacca americana* also showed cytotoxic activities in our study. Although previous studies have shown anticancer activities of their phytochemical extracts or pure compounds, the anticancer activities of the majority of these phytochemical extracts still need further investigation and our study provides novel information on this side.

Conclusion

In conclusion, our study is the first to have systematically screened the anticancer activity of the different phytochemical extracts from 10 traditional Chinese medicinal plant species against MCF-7,BALL-1,Huh-7 and HeLa cancer cell lines and cytotoxicity on MCF-10A normal cell line. The results provided valuable information for possible development of novel anticancer drugs based on these medicinal plants in the future.

Acknowledgements

This work was supported by the Key Subject Project Foundation about Natural Product and New Drug Research of Kunming University of Science and Technology (14078183); the Personnel Training Project of Yunnan Province (KKSY201226096), and the Personnel Training Project of Yunnan Province (KKSY201226097).

Conflict of interests

The authors declare no confict of interests.

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