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Anti-proliferative activities of selected Chinese medicinal herbs against human cancer cell lines

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Abstract

The main objective of this study was to investigate the anti-proliferative properties of selected traditional Chinese medicinal herbs with a view to discover potential candidates for the isolation of anti-cancer compounds and also for designing new anti-cancer herbal formulations. The plants selected for this study have ethno pharmacological importance and currently used in Chinese medicine. The Methylthiazolyldiphenyltetrazolium bromide (MTT) assay was conducted to determine the anti-proliferative properties of the aqueous and ethanol extracts of the selected herbs against one control cell line and 5 human carcinoma cell lines. The key herbs found in this study that are expected to have excellent future potential are: *Ligustrum lucidum, Paeonia suffuticosa, Sarcandra glabra, Scutellaria baicalensis,* and *Sanguisora officinalis*. The study also indicated that the ethanol extracts of the selected herbs were generally more effective than the aqueous extracts. The findings of this study provide strong evidence that some of the medicinal plants examined are potential candidates for the isolation of anti-cancer compounds and also for designning new anti-cancer herbal formulations.

Keywords: Medicinal herbs; Anti-proliferative activity; Lung carcinoma; Breast carcinoma; Colon carcinoma; Hepatocytes carcinoma; Leukemia carcinoma;

Introduction

Cancer is one of the leading causes of death in the modern world (Jemal et al., 2007). Conventionally, surgical procedures, chemotherapy and radiotherapy are the major treatments for cancer. However, these procedures have the drawbacks which include traumatic side effects, expensive diagnosis and treatment (Cho., 2010). Medicinal herbs have therefore received a significant interest in anti-cancer therapy as they do not have these drawbacks.

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In traditional Chinese medicine (TCM), pathological condition of the patient is established by using the four basic diagnostic methods (Cho., 2010; Wang et al., 2004). The origin of cancer is considered to be mainly due to lack of "Zheng Qi" (immune system deficiency) and accumulation of "Xie Qi" (pathogenic factors) (Cho., 2010). After an accurate diagnosis of cancer is established, five TCM principles are then employed to design an appropriate herbal formulation for the treatment. The treatment aims to supplement the "Qi" and the blood to improve the immune system of the patient and to activate circulation. According to TCM theory, "Xie Qi" is recognised as the main factor in the early stage of cancer, where as the deficiency of "Zheng Qi" is considered to be the key factor in the middle and late stages of cancer. Blood stasis is also recognised as one of the factors in the development of tumour (Cho., 2010). Therefore, TCM treatment for early stage cancer is formulated to clear the "Xie Qi" (pathogen), to improve blood circulation and to maintain the immune system ("Zheng Qi") of the patient. However, the TCM treatment during middle and late stages of cancer focuses on further boosting the immune system, eliminating the toxins, softening and dissolving the lumps (Cho., 2010).

Experimental and clinical studies have demonstrated that many herbal formulations are effective for treating cancer in various stages (Cho., 2010; Huang et al., 2008). For example, *Glycyrrhiza uralensis, S. barbate, L. lucidum, H. diffusa*, *A. Macrocephala, S. Miltiorrhiza, Akebia quinata* and *Codonopsis pilosula* have been used in various formulations for the effective treatment of cancer (Cho., 2010; Cragg et al., 2009; Huang et al., 2008; Koehn and Carter, 2005; Nemman and Cragg, 2007).

Scientific screening programs are beneficial to discover potential anti-cancer agents and anti-cancer formulations (Cho., 2010; Takimoto, 2003). Many active compounds isolated from medicinal plants and other natural resources have been used in clinical trials (Cho., 2010; Huang et al., 2008; Zhou et al., 2007). For instance, vinca alkaloids (vinblastine, vincristin), podophyllotoxin and other compounds that have been isolated from plants are used in clinical trials (Cragg et al., 2009; Koehn and Carter., 2005).

The approaches that are generally applied for selecting plants for testing their anti-cancer activity may range from random selection to more guided selection strategies such as an ethno pharmacological approach (Cordell et al., 1991; Cox., 1994) and the experience derived from traditional practice (Cho., 2010; Huang et al., 2008; Zhou et al., 2007). It is anticipated that the latter approach should increase the chance of finding active compounds. Hence, the selection of herbs in this study was based on the ethno pharmacological approach (Cox, 1994) and the traditional practice (Cordell et al., 1991).

There are anti-proliferative assays have been used in oncology research and clinical practice in the assessment of cancer types of individual patients (Edmondson et al., 1988; Fotakis and Timbrell., 2006). Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay has been described as rapid, simple and reproducible method, widely used in the screening anticancer drugs and to measure the tumor cell proliferation (Edmondson et al., 1988; Fotakis and Timbrell., 2006). Hence, in the current study, the anti-proliferative properties of the selected plants were revealed using this assay.

Forty seven (47) medicinal plants (Table 1) were chosen in this study for the measurement of anti-proliferative properties. Many of these plants are part of the formulations cu-

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Table 1. List of the Traditional Chinese Medicinal (TCM) plants that were used in the study.

Plant No	Herbs names	Family	Traditional use
P1	*Acanthopanax senticosus*	Araliaceae	Anti-cancer (Yoon et al., 2004)
P2	*Actinidia arguta* (Sieb.et Zucc.)Flarich.ex Miq.	Actinidiaceae	Anti-tumor (Takano et al., 2003)
P3	*Akebia quinata* (Houtt.)Decne.	Lardizabalaceae	Stage II-III liver cancer (Cho., 2010)
P4	*Andrographis paniculata* (Burm.f.) Wall. ex Nees	Acanthaceae	Anti-cancer (Ajaya et al., 2004)
P5	*Asparagus cochinchinensis* (Lour.) Merr.	Asparagaceae	Lung cancer (Cho., 2010)
P6	*Aster tataricus* L.f.	Asteraceae	Anti-tumor (Ng et al., 2003)
P7	*Atractylodes macrocephala* Koidz.	Compositae	Stage II-III liver cancer (Cho., 2010)
P8	*Codonopsis pilosula* (Franch.) Nannf.	Campanulaceae	Stage II-III liver cancer (Cho., 2010)
P9	*Corydalis yanhusuo* W.	Papaveraceae	Anti-cancer (Cho., 2010)
P10	*Curcuma aromatica* Salisb	Zingiberaceae	Stage II-III liver cancer (Cho., 2010)
P11	*Cynanchum paniculatum* (Bge.) Kitag	Asclepiadaceae	Anti-cancer (Zhou et al., 2007)
P12	*Cyperus rotundus* L.	Cyperaceae	Anti-cancer (Huang et al., 2008)
P13	*Ducheshea indica* (Andr.) Focke.	Rosaceae	Anti-cancer (Zhou et al., 2007)
P14	*Hedyotis diffusa* Willd.	Rubiaceae	Stage II-III liver cancer, Lung, Ovarian, Colon, Bresat, Stomach, Esophageal (Cho., 2010)
P15	*Leonurus japonicus* Houtt.	Labiatae	Anti-tumor (Tao et al., 2009)
P16	*Ligustrum lucidum* Ait. f.	Oleaceae	Anti-tumor (Shoemaker et al., 2005)
P17	*Lobelia chinensis* Lour.	Campanulaceae	Stomach cancer (Cho., 2010)
P18	*Lysinachia christinae* Hance.	Lysimachia	Anti-cancer (Huang et al., 2008)
P19	*Mahonia fortunei* (Lindl.)Fedde	Berberidaceae	Anti-cancer (Huang et al., 2008)
P20	*Paeonia suffuticosa* Andrews.	Ranunculaceae	Anti-tumor (Cho., 2010)
P21	*Paeonia lactiflora* Pall.	Paeoniaceae	Liver cancer (Cho., 2010)
P22	*Pinellia ternate* (Thunb.) Breit.	Araceae	Anti-tumor (Cho., 2010)
P23	*Plantago asiatica* L.	Plantaginaceae	Anti-cancer (Gálvez et al., 2003)
P24	*Pleione bulbocadioides* (Franch.)Rolfe.	Orchidaceae	Anti-cancer (Cho., 2010)
P25	*Pogostemon cablin* Benth.	Asteraceae	Anti-cancer (Park et al., 1998)
P26	*Polygonum aviculare* L.	Polygonaceae	Anti-cancer (Hsu, 2006)
P27	*Polygonum cuspidatum* Sieb. Et Zucc.	Polygonacae	Anti-cancer (Cho., 2010)
P28	*Poria cocos* (Schw.) Wolf	Polyporaceae	Anti-tumor (Cho., 2010)
P29	*Prunella vulgaris* L.	Lamiaceae	Lung adenocarcinoma (Feng et al., 2010)
P30	*Pseudostellaria heterophylla* (Miq.) Pax ex Pax et Hoffm.	Caryophyllaceae	Advanced stage cancers in lung, breast (Cho., 2010)
P31	*Rehmannia glutinosa* (Gaertn.) Steud.	Phrymaceae	Stage II-III liver cancer (Cho., 2010)
P32	*Rheum officinale* Baill.	Polygonaceae	Anti-cancer and diabetes (Wu et al., 2005)
P33	*Alpinae officinarum* (HANCE.)	Zingaberaceae	Anti-tumor (An et al., 2008)
P34	*Salvia miltiorrhiza* Bunge.	caspase	Stage II-III liver cancer (Cho et al., 2010)
P35	*Sanguisorba officinalis* L.	Rosaceae	Anti-cancer (Cho et al., 2010)
P36	*Saposhnikovia divaricata* (Turcz.)Schischk.	Apiaceae	Anti-cancer (Cho et al., 2010)
P37	*Sarcandra glabra* (Thunb.) Nakai	Chloranthaceae	Anti-tumor (Cho et al., 2010)
P38	*Schizandra chinensis* (Turcz.) Baill.	Schisandraceae	Anti-carcinogenic (Hancke et al., 1999)
P39	*Scutellaria baicalensis* Georgi.	Labiatae	Anti-cancer (Cho et al., 2010)
P40	*Scutellaria barbata* D. Don,	Labiatae	Anti-tumor (Cho., 2010)
P41	*Smilax glabra* Roxb.	Smilacaceae	Anti-cancer (Liver cancer) (Cho., 2010)
P42	*Styphnolobium japonica* (L.) Schott.	Fabaceae	Anti-cancer (Huang et al., 2008)
P43	*Solanum lyratum* Thunb.	Solanaceae	Anti-tumor (gastric cancer) (Cho., 2010)
P44	*Solanum nigrum* L.	Solanaceae	Anti-tumor (Ovarian, colon) (Cho., 2010)
P45	*Spatholobus suberectus* Dunn.	Leguminosae	Anti-cancer (Cho., 2010)
P46	*Taxillus chinensis* (DC.) Danser	Loranthaceae	Anti-cancer (Huang et al., 2008)
P47	*Tussilago farfara* L.	Asteraceae	Anti-tumor (Franz, 1969)

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Table 2. Some anti-cancer formulations that are used by Chinese medical practitioners and in clinical studies.

Formulation	Composition of formulation	Medicinal properties required for TCM based anti-cancer prescriptions
#Formulation 1 (Huang et al., 2008)	*Solanum nigrum*	Improve immune system (Cho., 2010; Huang et al., 2008).
	Mahonia fortunei (Lindl.)Fedde	Eliminate heat and toxins, soften and dissolve lumps (Zhou et al., 2007)
#Formulation 2 (Huang et al., 2008)	*Akebia quinata* (Houtt.) Dence.	Improve immune system, improve circulation, relieve pain (Huang et al., 2008)
	Curcuma aromatica Salisb	Improve circulation, relieve pain (Huang et al., 2008; Nayak, 2000)
	Cyperus rotundus L.	Improve circulation, relieve pain, improve immune system (Huang et al., 2008; Seo et al., 2001)
	Corydalis yanhusuo W.	Improve circulation, relieve pain, soften and dissolve lumps (Huang et al., 2008)
#Formulation 3 (Zhou et al., 2007)	*Ducheshea indica* (Andr.) Focke.	Improve immune system, eliminate heat and toxins (Zhou et al., 2007)
	Solanum nigrum	Improve immune system (Huang et al., 2008)
	Solanum lyratum Thunb.	Improve immune system (Kim et al., 1999)
	Lysinachia christinae Hance.	Eliminate heat and toxins, improve immune system, soften and dissolve lumps (Huang et al., 2008; Royo et al., 2003)
	Scutellaria barbata Don.	Eliminate heat and toxin, Improve circulation (Huang et al., 2008)
	Cynanchum paniculatum (Bge.) Kitag	Improve circulation, relieve pain (Zhou et al., 2007)
*Formulation 4 (Cho., 2010)	*Bupleurum chinense*	Relieve pain, Improve immune system (Cho., 2010)
	Angelica sinensis	Relieve pain, Improve immune system improve circulation (Huang et al., 2008; Royo et al., 2003)
	Paeonia lactiflora	Relieve pain, Improve immune system (Huang et al., 2008; Royo et al., 2003)
	Atractylodes macrocephala	Improve immune system, relieve pain (Huang et al., 2008)
	Glycyrrhiza uralensis, etc	Improve immune system, relieve pain, eliminate toxin (Huang et al., 2008)
*Formulation 5 (Cho., 2010)	*Atractylodes macrocephala*	Improve immune system, relieve pain (Huang et al., 2008; Royo et al., 2003)
	Salvia miltiorrhiza	Improve immune system, Improve circulation (Huang et al., 2008; Royo et al., 2003)
	Polyporus umbellantus	Improve immune system (Cho., 2010)
	Poria cocos	Improve immune system, relieve pain (Huang et al., 2008)
	Lycium barbarum	Improve immune system(Cho., 2010; Royo et al., 2003)
	Lingustrum lucidum	Improve immune system (Royo et al., 2003)
	Epimedium grandiflorum	Improve circulation (Cho., 2010)
	Scutellaria barbata, etc	Eliminate heat and toxins, Improve circulation (Cho., 2010)

#Anti-cancer formulations that are used by Chinese medical practitioners
*Anti-cancer formulations that are in clinical studies

rrently used by Chinese medical practitioners and/or in clinical studies (Table 2). Table 2 also gives the medicinal properties of individual herbs that are essential to implement "the five main TCM principles" to design prescriptions for the treatment of cancer (Cho., 2010). Linking these essential medicinal properties with scientific investigations of anti-cancer activities is expected to be of considerable value to create new formulations. This study was, therefore, undertaken to investigate the anti-cancer activities of medicinal herbs to discover potential candidates for the isolation of anti-cancer compounds and also to aid the design of new anti-cancer herbal formulations.

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Materials and Methods

Aqueous and ethanol extracts of the selected plants were used for measuring anticancer properties. In this study, A549 monolayer cells (human lung carcinoma), HepG2 cells (human liver carcinoma), MCF7 cells (human breast carcinoma), HT29 cells (human colon carcinoma), HL60 cell (Human promyelocytic leukemia) and Fa2N4 (immortalized Hepatocyte) were used to conduct anti-cancer tests by employing the Methylthiazolyldiphenyltetrazolium bromide (MTT) assay.

Collection of medicinal plants and preparation of their extracts

The dried plant material was purchased from Beijing Tong Ren Tang Chinese Herbal Medicine shop**,** Sydney, Australia. The scientific names and family names are given in Table 1. The plant materials were ground to a fine powder in a grinder.

In the preparation of the aqueous extracts, approximately 3 g of each powdered plant material was autoclaved in 50 ml water at 121 ℃ for 1 h. The preparations were cooled then centrifuged at 10,447 rpm for 20 min. The supernatant was transferred into a 50 ml volumetric flask and the volume was adjusted to 50 ml with sterile water.

In the preparation of the ethanol extracts, approximately 3 g of powdered plant material was extracted with 95% ethanol on water bath at 70 ℃ for 6 h. The samples were centrifuged and the supernatant was transferred into a 50ml volumetric flask and the volume was adjusted to 50 ml with 95% ethanol. Both aqueous and ethanol extracts were stored at -4 ℃ until analysis. All water and ethanol extracts were filtered and dried to remove the solvent prier to the analysis. Dried extracts have been re-dissolved into appropriate sterile solvent and analysis.

Tumor cell lines

Growth and incubation conditions similar to those reported previously (Tormo et al., 2005; Royo et al., 2003). The five cell lines that were used in the study were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). 1) A549 monolayer cells (human lung carcinoma, CCL-185) were cultured in Ham`s F12 medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. 2) HepG2 cells (human liver carcinoma, CCL-8065) were grown in ATCC-formulated Eagle´s M essential medium (MEM) with 10 % qualified FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, and 100 µM MEM non-essential amino acids. 3) MCF7 cells (human breast HTB-22) were maintained in MEM supplemented with 0.01 mg/ml bovine insulin. 4) HT29 cells (human colon carcinoma HTB-38), were cultured in McCoy`s 5A medium modified with 10 % FBS and 2 mM glutamine. 5) HL60 (human promyeloblast) were maintained in Iscove's Modified Dulbecco's Medium supplemented with 20 % FBS. 6) As a control, normal cells Fa2N4 (immortalized Hepatocyte cell line) were cultured in MFE Essential Support Medium F with MFE Culture Medium Supplement A. All cell cultures were kept at 37ºC under a humidified atmosphere of 5% CO_2.

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Antitumor assay

The Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay test was applied to five cell lines and a control for evaluation of the cytotoxic activity. It is based on the ability of drug-treated cells to reduce the yellow water soluble substrate MTT into a dark blue formazan product that is insoluble in water. Nicotinamide adenine dinucleotide (NADH) is provided directly by the cells which in turn demonstrate proper metabolic function. Therefore, the rate of reduction of MTT is an indicator of the functional integrity of mitochondria and, hence, of cellular viability. For the *in vitro* cytotoxic activity assay, the number of cells per culture well in 96-well plates was 200,000 for MCF7, 200,000 for A549, 100,000 for HL60, 150,000 for HepG2, 150,000 for HT29 and100, 000 for Fa2N4. Samples of plant extracts were incubated with 60-80% of confluent culture of each cell line for 24 hours in an atmosphere of 5% CO_2 at 37ºC. Each extract was tested at 1mg /ml concentration. Absorption at 570 nm (OD_{570}) was measured in a Victor2^{TM} Wallac spectrofluorometer.

The inhibition percentage against the tested cell lines was determined by the equation:

$$Inhibition\ \ percentage = \left(\frac{1-(OD__{Neg\ Contr} - OD__{Pos\ Contr})}{OD__{Neg\ Contr} - OD__{Pos\ Contr}} \right) \times 100$$

Where, $OD__{Neg\ Contr}$ is the optical density of the negative control at 570 nm; $OD__{Pos\ Contr}$ is the optical density of the positive control at 570 nm.

The negative control was the medium with 1% DMSO and the positive control was the medium with 2mM of methyl methane sulfonate (MMS). Internal controls actinomycin D (250 µM, 50 µM, 12.5 µM), doxorubicina (250 µM, 50 µM, 12.5 µM), and rotenone (250 µM, 125 µM) were used.

Data presentation and analysis

Data from the MTT assay were analyzed using the Genedata Screener program (Genedata AG, Switzerland). DMSO at the same concentration at the same concentration as the plant extracts, served as the negative control for all cells assayed and 2mM MMS was used as a positive control. The comparative analysis of various data sets was done using stat-istical correlation. In all results the RZ factor (correlation coefficient) was between 0.7-0.9.

Results

The anticancer activities of the selected 47 Chinese medicinal plant extracts were evaluated against five human cancer cell lines and a normal cell line as the control. These results are discussed below.

Anti-proliferative effects of selected plant extracts against MCF7 cells

As shown in Table 3, the aqueous and ethanol extracts of most of the plants had an inhibitory effect at 1 mg/ml concentration against MCF7 human breast carcinoma cell line. Significant inhibitory activity was exhibited by *L. lucidum, P. suffuticosa, P. asiatica, S. gla-*

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Table 3. In *vitro* cytotoxicity (% of inhibition at 1 mg/ml) of aqueous and ethanol extracts of the plants on five cancer cell lines measured by the MTT assay.

P.No	MCF7		A549		HL60	
	#Ethanol	*Water	#Ethanol	*Water	#Ethanol	*Water
P1	0	69.39±14.78	0	12.97±5.15	0	46.64±8.97
P2	4.34±1.81	15.16±7.95	0	0	75.32±16.18	66.82±16.58
P3	119.2±20.56	30.91±9.78	109.4±22.41	0	96.51±15.54	0
P4	0	16.93±8.78	0	0	0	0
P5	4.99±0.95	0	0	0	0	0
P6	69.22±17.54	44.4±16.47	19.06±5.98	0	39.78±9.89	0
P7	117.8±14.5	0	110.5±16.9	0	86.81±16.54	0
P8	4.88±1.35	0	7.25±3.24	0	0	0
P9	0	0	0	0	0	0
P10	0	0	0	0	0	0
P11	0	0	37.96±10.21	0	0	0
P12	0	52.34±10.58	0	0	0	0
P13	69.54±14.75	36.47±5.89	95.8±14.65	0	57.42±13.52	0
P14	0	68.44±16.85	0	0	0	0
P15	0	70.22±16.58	0	0	0	0
P16	82.31±14.76	67.97±17.43	104.5±28.48	0	59.47±16.24	56.73±16.21
P17	36.09±28.56	3.67±1.50	0	0	9.97±2.05	0
P18	102.8±28.62	0	81.87±18.89	0	60.56±12.17	0
P19	0	21.67±5.89	0	0	0	0
P20	84.88±17.02	54.11±8.82	54.99±12.53	0	64.25±20.51	63.46±13.21
P21	0	23.09±5.84	0	0	0	0
P22	65.25±16.47	0	83.1±16.57	0	85.85±19.18	0
P23	115.8±10.6	57.9±6.5	111.7±20.2	0	84.62±16.50	19.16±5.85
P24	0	40.14±11.52	0	0	0	0
P25	0	43.69±11.62	0	0	0	27.57±5.45
P26	26.33±9.87	75.07±19.89	56.29±19.47	0	0	47.94±9.84
P27	61.93±16.58	43.93±15.52	62.65±16.17	0	0	76.36±19.98
P28	52.49±7.11	21.43±9.85	0	0	14.63±5.21	10.19±1.58
P29	0	110.1±28.59	0	0	0	60.28±16.57
P30	0	0	0	0	0	0
P31	46.81±10.57	0	0	0	0	0
P32	84.56±9.85	5.329±2.10	65.99±19.51	0	58.51±17.19	0
P33	23.75±8.95	27.59±2.99	87.58±14.02	0	86.67±20.04	83.46±16.32
P34	109.3±10.2	22.26±9.95	104.2±24.57	0	0	13.55±2.52
P35	12.28±2.95	63.35±16.52	79.19±16.52	75±21.20	14.9±5.1	63.08±12.02
P36	35.87±6.52	0	0	0	0	0
P37	92.71±16.92	88.22±11.52	41.38±9.85	0	86.26±14.12	73.55±16.62
P38	51.42±12.41	6.16±2.46	88.31±19.47	0	3.69±1.06	0
P39	63.65±16.16	95.09±21.52	44.24±10.20	0	67.26±18.51	49.44±10.01
P40	115±19.88	51.63±11.56	71.93±19.28	0	63.16±19.48	0
P41	47.77±11.52	48.9±15.36	8.8±2.14	0	0	0
P42	7.67±2.65	12.79±3.51	70.31±12.5	0	52.36±12.21	0
P43	119.6±20.48	10.66±4.58	109.2±29.84	0	100.3±18.57	0
P44	123.8±18.4	19.06±8.75	112.5±23.54	0	117.3±21.14	0
P45	0	46.65±11.54	17.43±8.21	53.85±19.44	41.56±9.28	95.79±16.51
P46	37.16±9.62	10.66±1.52	29.82±10.32	11.47±6.51	60.7±16.5	86.45±16.54
P47	120.8±31.20	58.97±19.85	95.8±12.59	0	69.99±14.79	0

Activity of ethanol extracts against the selected cell lines
* Activity of water extracts against selected cell lines
$ Fa2N4 represents normal cell line used as control.

bre, S. baicalensis, S. barbata, T. farfara, A. macrocephala, *L. christinae*, *S. miltiorrhiza, S. lyratum, A. senticosus*, *H. diffusa, L. japonicus, P. aviculare, P. vulgaris* and *S. officinalis* (Table 3 and 5). As can be seen later in this section, a large number of plants studied here exhibited good activity against these cancer cell lines when compared to other cell lines (Table 3 and 4).

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Table 4. In *vitro* cytotoxicity (% of inhibition at 1 mg/ml) of aqueous and ethanol extracts of the plants on five cancer cell lines measured by the MTT assay.

P.No	HepG2		HT29		$Fa2N4	
	#ethanol	*water	#ethanol	*water	#ethanol	*water
P1	0	16.43±4.68	0	0	0	0
P2	0	17.38±5.51	0	0	0	0
P3	97.54±18.25	0	102.4±30.09	0	105.5±23.2	0
P4	0	1.696±0.81	0	0	0	0
P5	0	1.007±0.52	0	0	0	0
P6	36.38±9.58	15.85±5.18	37.29±10.08	0	0	0
P7	20.92±3.25	1.643±0.98	35.97±16.84	0	0	0
P8	7.063±1.02	15.42±3.85	0	0	0	0
P9	0	4.24±1.02		0	0	0
P10	0	2.279±0.51	0	0	0	0
P11	0.4815± 0	0	0	0	0	0
P12	0	0	0	0	0	0
P13	77.96±16.95	4.134±1.53	91.71±16.95	0	21.48 ±4.75	0
P14	0	20.67±9.87	0	0	0	0
P15	0	27.03±9.85	0	0	0	0
P16	46.87±9.57	28.46±10.09	15.3±7.89	0	32.67±6.74	0
P17	0	2.12±1.02	0.84±0.24	0	0	0
P18	70.95±10.02	0	87.43±15.52	0	0	0
P19	0	0	0	0	0	0
P20	47.94±16.51	1.643±0.48	11.81±2.85	0	0	0
P21	0	0.8479±0.11	0	0	0	0
P22	16.16±1.25	1.802±0.96	9.54±4.62	0	28.56±10.11	0
P23	95.77±12.62	2.968±0.87	3.94±0.98	0	44.18±6.71	0
P24	0	2.703±1.09	0	0	0	0
P25	0	17.38±9.85	0	0	13.77±7.18	0
P26	55.91±12.64	11.13±4.32	68.39±15.53	0	0	0
P27	57.84±11.2	11.55±5.86	72.92±17.59	0	0	0
P28		10.33±5.14	2.21±0.52	0	0	0
P29	0	6.571±2.65	0	0	0	0
P30	0	1.749±0.54	0	0	0	0
P31	1.98±0.62	4.928±1.9	0	0	0	0
P32	9.738±1.25	0	3.05±1.05	0	0	0
P33	76.46±16.2	0	84.72±16.57	0	21.02±6.80	0
P34	31.94±9.85	2.173±0.59	0	0	0	0
P35	53.72±14.52	77.53±16.54	70.75±16.29	61.26±16.85	0	0
P36	0	9.751±2.58	0	0	0	0
P37	33.71±8.52	21.73±9.52	104.2±18.97	0	0	0
P38	0	0	0	0	0	0
P39	6.314±1.09	6.412±1.92	0	0	29.67±5.49	0
P40	87.59±15.51	0	82.26±16.59	0	0	0
P41	0	0	2.71±1.08	0	0	0
P42	0	0	0	0	0	0
P43	95.13±13.65	30.05±9.82	99.68±17.29	0	69.18±7.76	0
P44	99.84±14.84	1.219	0	0	127.85±1.77	0
P45	53.13±9.99	71.38±19.82	13.09±6.98	0	0	0
P46	76.35±16.95	23±9.05	0	0	0	0
P47	95.93±16.52	0	88.46±19.52	0	26.32±12.88	0

Activity of ethanol extracts against the selected cell lines
* Activity of water extracts against selected cell lines
$ Fa2N4 represents normal cell line used as control.

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Anti-proliferative effects of selected plant extracts against A549 cells

As shown in Table 3, the ethanol extracts of many of the plants showed inhibitory effect with 1 mg/ml concentration, but only two of the water extracts showed activity against A549 human lung carcinoma cell lines. Amongst all the plants studied, high inhibitory activity was exhibited by the ethanol extracts of *A. macrocephala, D. indica, L. lucidum, L. Christinae, Pinellia ternate, P. asiatica, Alpinae officinarum, S. miltiorrhiza, Schizandra chinensis, T. farfara, S. officinalis* and *S. baicalensis.* On the other hand, the water extracts of only *S. suberectus* and *S. officinalis* showed inhibitory activity against these cell lines (Table 3 and 5).

Anti-proliferative effects of selected plant extracts against HL60 cells

As shown in Table 3, the aqueous and ethanol extracts of most of the plants showed inhibitory effect with 1 mg/ml concentration against HL60 human promyelocytic leukemia cell lines. Amongst all the plants studied, high inhibitory activity was exhibited by *Actinidia arguta, A. macrocephala, L. lucidum, L. christinae, P. suffuticosa, P. ternate, P. asiatica, A. officinarum, S. glabre, S. baicalensis, S. barbata, T. Farfara, P. cuspidatum, P. vulgaris, S. officinalis, S. suberectus* and *T. chinensis* (Table 3 and 5).

Anti-proliferative effects of selected plant extracts against HepG2 cells

As shown in Table 4, the ethanol extracts of many of the plants showed inhibitory effect with 1 mg/ml concentration, but only two of the aqueous extracts showed activity agai-

Table 5. List of anti-cancer medicinal herbs identified in this study.

Herbs names	Activity of aqueous extracts to cell lines	Activity of ethanol extracts to cell lines
Acanthopanax senticosus	MCF7	
Actinidia arguta	HL60	HL60
Aster tataricus		MCF7
Atractylodes macrocephala		MCF7, A549 and HL60
Cyperus rotundus	MCF7	
Ducheshea indica		All cell lines
Hedyotis diffusa	MCF7	
Leonurus japonicus	MCF7	
Ligustrum lucidum	MCF7 and HL60	MCF7, A549 and HL60
Lysinachia christinae		All cell lines
Paeonia suffuticosa	MCF7 and HL60	MCF7, A549 and HL60
Pinellia ternate		MCF7, A549 and HL60
Plantago asiatica	MCF7	MCF7, A549, HL60 and HepG2
Polygonum aviculare	MCF7	A549, HepG2 and HT29
Polygonum cuspidatum	HL-60	MCF7, A549, HepG2 and HT 29
Poria cocos		MCF7
Prunella vulgaris	MCF7 and HL60	
Rheum officinale		MCF7, A549 and HL60
Alpinae officinarum	HL60	A549, HL60, HepG2 and HT29
Salvia miltiorrhiza		MCF7,A549
Sanguisorba officinalis	A549, HL60, HepG2, HT29 and MCF7	A549, HepG2 and HT29
Sarcandra glabra	MCF7 and HL60	MCF7, HL60 and HT29
Schizandra chinensis		MCF7 and A549
Scutellaria baicalensis	MCF7 and HL60	MCF7and HL60

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nst HepG2 human liver carcinoma cell lines. Amongst all the plants studied, high inhibitory activity was exhibited by the ethanol extracts of *D. indica, L. christinae, A. officinarum, S. suberectus, S. officinalis, P. asiatica. S. barbata, T. chinensis, T. farfara, P. aviculare, P. cuspidatum.* In contrast, the water extracts of only *S. suberectus* and *S. officinalis* showed inhibitory activity against these cell lines (Table 4 and 5).

Anti-proliferative effects of selected plant extracts against HT29 cells

As shown in Table 4, the ethanol extracts of most of the plants showed inhibitory effect with 1 mg/ml concentration, but only one of the aqueous extract showed activity against HT29 human breast carcinoma cell lines. Amongst all the plants studied, high inhibitory activity was exhibited by the ethanol extracts of *D. indica, L. christinae, S. officinalis, A. officinarum, S. glabre, S. barbata, T. farfara*, *P. aviculare* and *P. cuspidatum.* However, water extract of only one plant *S. officinalis* showed inhibition against these cell lines (Table 4 and 5).

Anti-proliferative effects of selected plant extracts against Fa2N4 cells

The ethanol and aqueous extracts of the plants were tested against Fa2N4 normal cell lines (immortalized Hepatocyte cell line) as control. As shown in Table 4, the aqueous extracts of the plants did not change the growth of the Fa2N4 cell line (normal cells) indicating that the extracts are non-toxic to the normal cells at the concentration of 1 mg/ml. As shown in Table 3, ethanol extracts of eleven out of forty seven plants showed inhibitory activity against these cell lines indicating their toxicity towards normal cells. The highest toxicity was showed by ethanol extracts of *Akebia quinata, S. lyratum* and *Solanum nigram*. It is important to note that none of the water extracts showed toxicity against these cell lines.

Discussion

Anti-cancer activity of the aqueous and ethanol extracts of selected medicinal plants (Table 1) has been evaluated using the MTT assay against five cancer cell lines (MCF7, A549, HL60, HepG2 and HT29) and one control with normal cell line. Ethanol extracts were generally found to be more active than aqueous extracts.

Of all the plants studied, *A. macrocephala*, *A. officinarum*, *D. indica*, *L. lucidum*, *L. christinae*, *P. suffuticosa*, *P. ternate*, *P. asiatica*, *P. aviculare*, *P. cuspidatum, R. officinale*, *S. miltiorrhiza*, *S. officinalis*, *S. glabre*, *S. baicalensis*, *S. barbata*, *S. lyratum, T. chinensis* and *T. farfara* showed significant anti-cancer activity against two or more cell lines (Table 5). This signifies their broad spectrum of anti-cancer activity. On the other hand, some plant extracts inhibited only one or no cell lines, which indicate their narrow spectrum of activity or lower level of active constituents respectively. Some plants, namely, *A. senticosus*, *Cyperus rotundus*, *H. diffusa* and *L. japonicas* showed their activity with only water extracts against specific cell lines. For instance, the water extracts of the above mentioned plants inhibited exclusively MCF7 cell lines, while the ethanol extracts of these plants did not show any activity against any of the cell lines studied. The findings also revealed that the ethanol extracts of five plants, namely, *T. farfara, S. lyratum, S. barbata, L. christinae* and *D. indica* have shown high anti-cancer activity against several cell lines.

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It is relevant to recapitulate at this point that the medicinal properties required for TCM based anti-cancer prescriptions are immunostimulation, improve blood circulation, eliminate toxins, relieve pain, soften and dissolve lumps (Cho., 2010). As outlined in the section on introduction, the TCM treatment for early stage cancer aims to clear the pathogen, improve blood circulation and maintain the immune system of the patient. In addition, the TCM prescription for the treatment of middle and late stages of cancer is designed to further boost the immune system, eliminate the toxins, soften and dissolve the lumps (Cho., 2010).

A closer observation of the results (Table 3 - 5) presented in this paper indicates that several medicinal herbs possess anti-cancer activity and also have some of the important medicinal properties (see column 3 of Table 2) that are required for TCM prescriptions for the treatment of cancer (Cho., 2010). For example, *A. macrocephala*, *D. indica*, *L. lucidum*, *L. christinae*, *S. miltiorrhiza*, *S. barbata* and *S. lyratum* showed significant anti-cancer activity and also possess most of the properties required for the TCM prescriptions for treating cancer (Cho., 2010; Huang et al., 2008; Wagner, 1999; Zhou et al., 2007). The connection between the measured anti-cancer activities of medicinal herbs and their medicinal properties (Table 2) required for creating TCM prescriptions is very significant and may be of great importance in the discovery of new and effective formulations. Hence, the herbs identified in this study are potential candidates for the isolation of the chemical agents with important medicinal properties such as soften / dissolve lumps, improve immune system, enhance blood circulation and eliminate toxins. Some of the above medicinal herbs could therefore be used to create new and effective TCM formulations for the treatment of cancer.

It should be noted that, anti-cancer agents have been previously isolated (Badami et al., 2003; Kim et al., 2006) from some of the plants studied here. For instance, solamargine isolated from *S. nigram* (Badmai et al., 2003), quercetin 3-*O*-β-L-arabino pyranoside and quercetin 3-*O*-β-D-glucopyranoside isolated from *T. farfara* (Kim et al., 2006) exhibited anti-cancer properties. In addition, the present study revealed a host of new plants that display significant anti-cancer activity that form potential candidates for isolating newer anti-cancer agents. For instance, *A. senticosus, T. chinensis, T. farfara, S. suberectus, R. officinale, P. aviculare* and *L. christinae* displayed substantial anti-cancer activity and hence can be investtigated further to isolate new anti-cancer agents. Research in this direction is underway in our laboratory.

Table 2 gives five important traditional Chinese herbal formulations commonly used for the treatment of cancer and related symptoms. Most of the herbs used in these formulations have been investigated in this study for their anti-cancer property. A close observation of the results presented in Table 4 indicate that the formulation 1 (Table 2) contains a large quantity of toxic plant (*S. nigram*). The only other plant present in this formulation is inactive. It is therefore concluded that the formulation 1 that is used in traditional practice may not be a favourable option for the treatment of cancer. One of the four herbs present in formulation 2 (Table 2) is toxic (*A. quinata*) and the remaining three herbs did not show activity against any of the cancer cell lines studied here. Hence, the results reported in this paper indicate that the formulation 2 may not also be favourable. In the current study, *S. nigram* and *A. quinata* which are the constituents of formulations 1 and 2 respectively displayed high toxicity towards normal cell lines (Table 4). This observation is in agreement with the studies carried out by (Xue et al., 2008) that reported toxic effects of *A. quinata*.

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The results presented in this paper strongly support the anti-cancer activity of formulation 3 (Table 2). Five of the six herbs present in this formulation are very active (Table 5). However, two of the herbs (*S. nigram* and *S. lyratum*) are found to be toxic. It is important to note here that the adverse toxicity effects of many of the Chinese herbal formulations have not been appropriately evaluated (Cho., 2010) and hence their safe use is doubtful. This necessitates further research to evaluate the efficacy and safety of herbal formulations using carefully designed clinical trials supported with scientific data. It may therefore be envisaged that the formulation 3 presents an excellent scope for improvement by systematically reducing the quantity of toxic plants (*S. nigram* and *S. lyratum*) or substituting them with appropriate non-toxic plants. Such a systematically designed clinical trials supported by scientific data are expected to yield improved and safe formulation for the treatment of cancer.

Many of the herbs present in formulations 4 and 5 (Table 2) have been found to be active. Interesting aspect of these two formulations is that, all of the constituent herbs are non-toxic (Table 4). These findings are in support of the fact that both of these herbal formulations are currently in clinical trials (Cho., 2010). It should be mentioned here that a few of the herbal constituents of these two formulations could not be investigated in this study due to their non-availability.

Amongst the forty seven plants investigated in this study, thirty of them showed significant anti-cancer activity against one or several cell lines. The study has also revealed several new plants (*A. senticosus, T. chinensis, T. farfara, S. suberectus, R. officinale P. aviculare* and *L. christinae*) that are favourable candidates for the isolation of novel anti-cancer agents. The results presented in this paper have also provided useful cues to improve existing formulations (Table 2) and to create new formulations. Isolation and characterisation of anti-cancer agents from some of these active plants is in progress in our laboratory.

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Conflict of Interest statement

There is no conflict of interest associated with the authors of this paper, and the funding sponsor did not exert any inappropriate influence on this work.

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