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Antioxidant and Anticancer Activity of Fractions from *Picrasma quassioides* (D. Don) Benn. Methanolic Extract

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ABSTRACT: The potential antioxidant and anticancer activities of Hexane, EtOAc (Ethyl acetate), BuOH (n-Buthanol) and water fractions from methanolic (MeOH) extract of *Picrasma quassioides* (D. Don) Benn. were evaluated *in vitro*. Tested fractions showed strong antioxidant activity, especially EtOAc fraction had the highest activity (IC_{50} = 114.01 μg/mL), containing high total phenolics and total flavonoids contents, showed 67.59 Tan μg/mg and 64.95 Que μg/mg respectively. Anticancer activity of these fractions was tested by MTT assay on HT-29 (the human colon carcinoma cells) cell line. BuOH fraction not only showed very high anticancer activity, but also had no cytotoxic effect on 293 (the human normal kidney cells) cell line. Considering these results, we used BuOH fraction of MeOH crude extract from *P. quassioides* (D.Don) Benn. to do assessment of apoptosis by flow cytometry and the mRNA expression levels of widely established apoptotic-related genes on HT-29 cell line. All the experiments showed that BuOH fraction can induce apoptosis on HT-29 cell line strongly. Taken together, methanolic extract of *P. quassioides* has potential for antioxidant and anticancer activities products.

Key words : *Picrasma quassioides* (D. Don) Benn., fractions, methanolic extract, antioxidant activity, anticancer activity and apoptosis

INTRODUCTION

Picrasma quassioides (D. Don) Benn. is distributed in China, Korea, India and Japan. Its bark and stem contains a number of medicinal compounds and has been shown to be anthelmintic, antiamoebal, antiviral, bitter, hypotensive and stomachic (Ching Su New Medical College, 1977). It increases the flow of gastric juices. It is used in Korea in the treatment of digestive problems, especially chronic dyspepsia. Many years ago, it was used as herbal medicine in East Asia (Yoshikawa *et al.*, 1995).

The purpose of the present research was to evaluate the effect of MeOH extract of *P. quassioides* on the proliferation of human colon cancer cells. Cell death includes two types, one is necrosis, and the other one is apoptosis. Necrosis is the death of cells or tissues through injury or disease, especially in a localized area of the body. Apoptosis is programmed cell death. Most of compounds can kill cancer cells by necrosis, but they can not be used as anticancer drugs. Apoptosis is a standard to test a compound can be used as an anticancer drug or not (Lavia *et al.*, 2006). So, besides antioxidant of sample assay, we used several methods to demonstrate *P. quassioides* can induce apoptosis on HT-29 cells.

MATERIAL AND METHODS

Preparation of fractions

Picrasma quassioides (D. Don) Benn. was obtained from Chuncheon, Gangwon-do, Korea. Dried *P. quassioides* powder (100 g) was refluxed with methanol for 3 days at room temperature, and the MeOH extract (5 g ×2) was suspended in distilled water (1 L × 2) and partitioned with 1 L of hexane, EtOAc and BuOH in sequence to afford the fractions of Hexane (1.494 g), EtOAc (0.941 g), BuOH (1.584 g) and water (5.972 g) (Yin *et al.*, 2007).

Cell lines and culture medium

HT-29 cells (human colon carcinoma cell line) were purchased from Korean cell line bank. HT-29 cells were cultured in RPMI 1640, supplemented with 10% (v/v) Fetal Bovine Serum (FBS) [Hyclone], 100 U/mL Penicillin-Streptomycin Solution (Hyclone) (Lavia *et al.*, 2006).

DPPH radical scavenging activity

The free radical scavenging of these four fractions was measured as following: 0.02 mM solution of DPPH· (1, 1-diphenyl-2-picrylhydrazol) in methanol was prepared. 2 mL various concentrations of fraction were added to 2 mL DPPH· solu-

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tion. Blanks contained 2 mL distilled water and 2 mL sample solution. While the control contained 2 mL distilled water and 2 mL DPPH·. The mixture was shaken immediately after adding DPPH· and allowed to stand at room temperature in the dark, and the decrease in absorbance at 517 nm was measured after 30 min until the reaction reached a plateau. These experiments were run in triplicate. The inhibitory percentage of DPPH· was calculated according to (Shyu and Hwang, 2002) as follows: Scavenging effect (%) = $[1 - (A_i - A_j) / A_0] \times 100\%$; Where A_0 is the A_{517} of DPPH· without sample (control), A_i is the A_{517} of sample and DPPH·, and A_j is the A_{517} of sample without DPPH· (blank).

Measurement of henolic compounds and flavonoid

Samples of different concentrations (1 mL) were mixed with Folin-Denis reagent (2 mL) and 2 mL of 35% sodium carbonate. The mixtures were shaken thoroughly and made up to 10 mL with distilled water. The absorbance at 765 nm was determined after incubation at room temperature for 30 min. Phenolic content was determined from a standard curve obtained with various concentrations of tannic acid (Tan) (Birt *et al*,. 2001, Lin *et al*,. 2007).

Flavonoid content was determined as follows: one mL of plant extract in methanol (10 mg/mL) was mixed with 1 mL aluminium trichloride in ethanol (20 mg/mL), and the absorption at 415 nm was read after 40 min. Blank samples were prepared from 1 mL of plant extract and 1 mL ethanol. The absorption of a standard Quercetin (Que) solution (0.5 mg/mL) in ethanol was measured under the same conditions. All determinations were carried out in duplicate. The Quercetin equivalents of the flavonoids in plant extracts were calculated from the formula:

$$X = (A \cdot m_0) / (A_0 \cdot m)$$

where X is the flavonoid content in μg/mg plant extract, A is the absorption of the plant extract, A_0 is the absorption of the standard Quercetin solution, m is the weight of plant extract in mg, and m_0 is the weight of Quercetin in the solution in μg (Lin and Tang, 2007).

MTT assay

In this study, cancer cell growth inhibition activity was measured by using MTT assay (Athukorala *et al*., 2006, Wang *et al*,. 2006). Tumor cells were seeded in a 96-well plate at the concentration of 2×10^5 cells/mL. After 4 h (at 37 ℃, in a humidified atmosphere of 5% CO_2), all extracts were treated to the wells at a concentration of 100 μg/mL, 200 μg/mL, 300 μg/mL and 500 μg/mL, respectively. The cells were then further incubated for an additional 72 h at 37 ℃. MTT stock solution (50 μL; 2 mg/mL in PBS) was then added to each well for a total reaction volume of 250 μL. After incubating for 4 h in a humidified atmosphere of 5% CO_2 at 37 ℃, the supernatants of each well were spilt out. The formazan crystals in each well were dissolved in 150 μL of DMSO. The amount of purple formazan was determined by measuring the absorbance at 540 nm after 15-20 min. For treated cells, viability was expressed as a percentage of control cells. All determinations were carried out in triplicate (Popiolkiewicz *et al*,. 2005).

Assessment of apoptosis by Annexin V

Apoptotic cell death of BuOH fraction solution treated with BuOH fraction was measured using FITC-conjugated Annexin V/propidium iodide assay (Biovision, Palo Alto, CA, USA) by flow cytometry (Becton-Dickinson, Franklin Lakes, NJ, USA) (Chiu *et al*., 2006). Briefly, 5×10^5 cells/mL were washed with ice-cold PBS, resuspended in 0.1 mL binding buffer, and stained with 10 μL of FITC-conjugated Annexin V (10 mg/mL) and 10 μL of PI (50 mg/mL). The cells were incubated for 15 min at room temperature in the dark, 400 μL of binding buffer was added, and analyzed by a FACScan flow cytometer (Annexin V excitation 488 nm, emission 515 nm; PI excitation 488 nm, emission 580 nm (Zhang *et al*., 2006).

Determination of the expression level of apoptotic-related genes

The mRNA expression levels of widely established apoptotic-related genes, i.e., c-myc, p53 and caspase 3 were carried out using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) as described (Kousteni *et al*., 1999, Tengku *et al*., 2000). Briefly, the cells were cultured in T-25 flasks and starved in medium with 0.5% (v/v) FCS for 4 h before stimulation. 500 μg/mL of BuOH fraction needed to achieve 50% growth inhibition was used to stimulate the cells over the period of 24 h. Total cellular RNA was isolated from the untreated and treated cells using Trizol Reagent according to manufacturer's protocol. Subsequently, 1 μg RNA was reverse transcribed into cDNA and used as the template for PCR amplication.

PCR was carried out in a final volume of 20 μL containing 1 × PCR buffer and 1 U Taq-polymerase (Corebiosystem), Buffer, dNTP and 0.5 μL of each primer. The template was denaturated for 5 min at 94 ℃, followed by amplification cycles at 94 ℃ for 1 min, 55 ℃ for 1 min and 72 ℃ for 1 min, and terminated with an additional extension step for 7 min at 72 ℃. The oligonucleotide sequences of the PCR primers used herein were designed based on the human mRNA encoding the respective genes (Table 1). The PCR conditions including the quantity of RNA and cDNA samples used to amplify c-

Table 1. The sequence of primers used in RT-PCR

Primer	Sequence (5'-3')
c-myc Forward	GAACAAGAAGATGAGGAAGA
c-myc Reverse	AGTTTGTGTTTCAACTGTTC
p53 Forward	TGTGGAGTATTTGGATGACA
p53 Reverse	GAACATGAGTTTTTTATGGC
Caspase-3 forward	TCACAGCAAAAGGAGCAGTTT
Caspase-3 reverse	CGTCAAAGGAAAAGGACTCAA
β-Actin forward	TCACCCTGAAGTACCCCATC
β-Actin reverse	CCATCTCTTGCTGCAAGTCC

myc, caspase-3, p53 and β-actin genes were in the exponential phase of amplification indicating that the conditions were optimized to be utilised for semi-quantitative studies (Kousteni *et al.*, 1999, Tengku *et al.*, 2000). The mRNA level of β-actin was used as an internal control for template levels. The PCR products were electrophoresed on a 1% (w/v) agarose gel and visualized with ethidium bromide staining.

RESULTS AND DISCUSSION

DPPH· radical scavenging activity

DPPH· is the choice of many scientists to evaluate the free radical scavenging activity of natural compounds (Halliwell, 1995). Table 2 shows IC_{50} of scavenging activity at DPPH· radical scavenging test with different fractions. In this study we used BHA, BHT and vitamin C as commercial standards. According to the results, EtOAc fraction showed the highest DPPH· radical scavenging activity (IC_{50} = 114.01 μg/mL) among all the tested fractions. And water fraction had the lowest scavenging activity (IC_{50} = 1357.28 μg/mL), demonstrate that compounds with high antioxidant activity in *P. quassioides* are not high polar. Under same experimental conditions, IC_{50} of BHA, BHT and Vitamin C are 8.18 μg/mL, 24.3 μg/mL and 7.92 μg/mL, respectively (Gordon, 1990, Halliwell and Gutteridge, 1985).

The content of total phenolics and flovonoids

As phenolic compounds have been shown to possess strong antioxidant activity (Rice-Evans), we decided to measure the phenolic and flavonoid content of fractions as it probably contributes to the antioxidant activity of *P. quassioides* (Le *et al.*, 1998, Zhao *et al.*, 2006). The total phenolic content of extracts was measured by the Folin–Denis method. The Folin– Denis reagent determines total phenols, producing a blue colour by reducing yellow heteropolyphosphomolybdate-tungstate anions (Lu and Foo, 1997). The phenolic contents of the Hexane, EtOAc, BuOH and water fractions were 56.75 μg/mg, 67.59 μg/mg, 42.16 μg/mg and 10.21 μg/mg (Table 3).

Table 2. Antioxidant activity of *P. quassioides* MeOH extract and fractions by DPPH· free radical scavenging test

Fractions	Antioxidant activity (IC_{50} : μg/mL)
Hexane fra.	592.86
EtOAc fra.	114.01
BuOH fra.	341.09
Water fra.	1357.28
Control antioxidant	
Vitamin C	7.92
BHA	8.18
BHT	24.3

Table 3. Total phenolic and flavonoid content in fractions from *P. quassioides* MeOH extract

Fractions	Total phenolics content (Tan μg/mg)	Total flavonoids content (Que μg/mg)
Hexane	56.75 ± 3.17	40.65 ± 1.76
EtOAc	67.59 ± 0.35	64.95 ± 1.72
BuOH	42.16 ± 0.21	22.13 ± 0.10
Water	10.21 ± 0.32	4.86 ± 0.21

Flavonoids are one of the most diverse and widespread group of natural compounds, and compounds such as flavones, isoflavones, flavonones, anthocyanins and catechins, are likely to be the most important natural phenolics (Wang *et al.*, 2005). These compounds possess a broad spectrum of chemical and biological activities including radical scavenging and strong antioxidant capacity. Therefore, the content of flavonoid in the fractions was determined. The flavonoid contents of the Hexane, EtOAc, BuOH and water fractions were 40.65 μg/mg, 64.95 μg/mg, 22.13 μg/mg and 4.86 μg/mg (Table 3). As the results, EtOAc fraction has the highest and water fraction has the lowest phenols and flavonoids contents among four fractions. To consider with antiradical assay (DPPH·), the antioxidant activity of fractions was induced by phenols and flavonoids probably.

Inhibitory effect of *P. quassioides* on the growth of HT-29 cells

In this study, HT-29 cells were chosen to determine the antiproliferative activity of *P. quassioides* fractions (Lee *et al.*, 2006). The MTT assay is a novel method of quantifying metabolically viable cells through their ability to reduce a soluble yellow tetrazolium salt to ble-purple formazan crystals (Mossman, 1983). The crystal are thought to be produced by the mitochondrial enzyme succinate dehydrogenase (Slater *et al.*, 1963) and can be dissolved and quantified by measuring the absorbance of the resultant solution. The absorbance of the solution is related to the number of live cells. By using 96-well micro titer plates and a multiwell spectrophotometer (Enzyme-linked immunosorbent assay plate reader) this assay can be

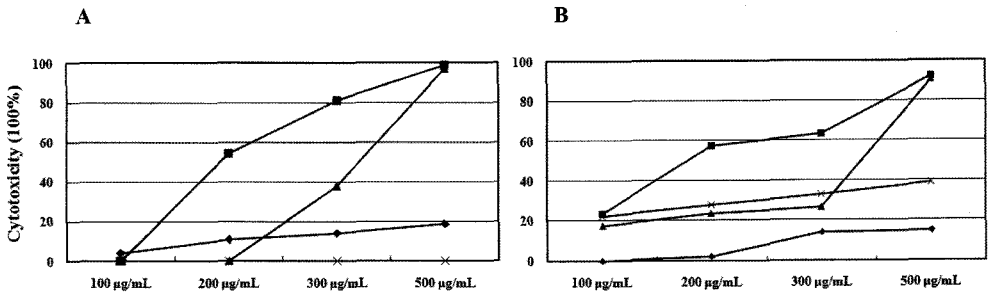

Fig. 1. Cytotoxicity test of fractions from P. quassioides MeOH extract on the human kidney normal cell line (293) (A) and the human colon cancer cell line (HT-29) (B) by MTT assay. The concentrations of each fraction are 100 μg/mL, 200 μg/mL, 300 μg/mL and 500 μg/mL, respectively (◆ : Water fra., ■ : Hexane fra., ▲ : EtOAc fra., × : BuOH fra.).

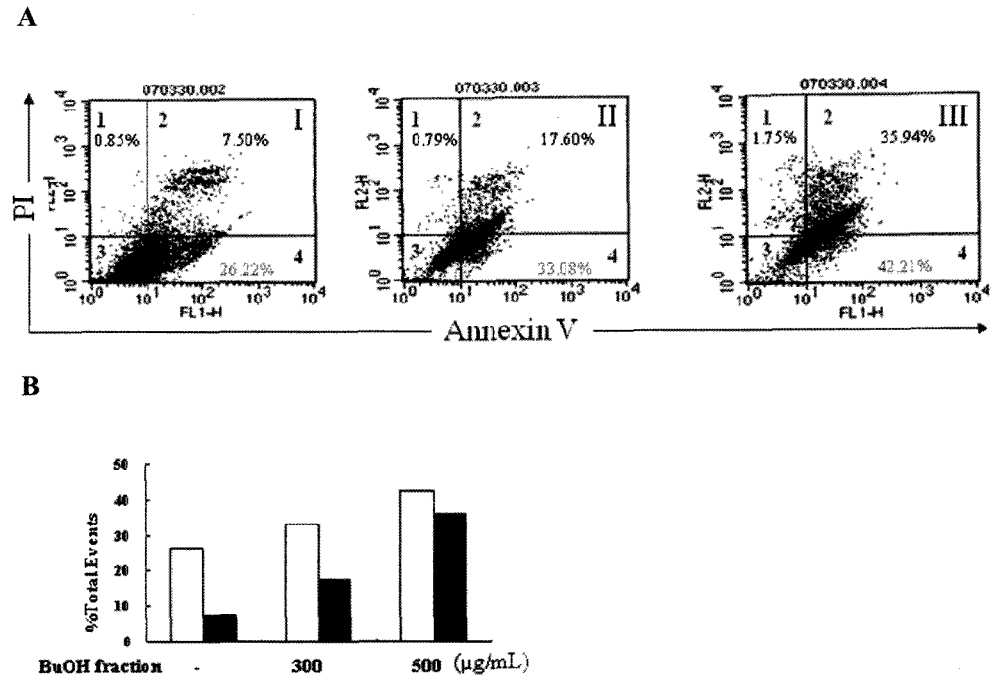

Fig. 2. (A) Assessment of apoptosis by Annexin V on the human colon cancer cell line (HT-29). The cells were pre-treated with (panels II and III) or without (panels I) BuOH fraction 48 h. The necrotic cells lost cell membrane integrity that permits PI entry. Viable cells exhibit Annexin V−/PI− (symbol 3 in the plot); early apoptotic cells exhibit Annexin V+/PI− (symbol 4 in the plot); late apoptotic cells or necrotic cells exhibit Annexin V+/PI+ (symbol 2 in the plot). (B) Percentage of cell death based on the assessment of apoptosis by Annexin V in (A) (□ : Annexin V+/PI−, ■ : Annexin V+/PI−).

semiautomated to process a large number of samples and provide a rapid object measurement of cell number (Kogure *et al.*, 2003). As showed in Fig. 1 B, BuOH fraction has high cytotoxicity on HT-29 cells, but has no cytotoxicity on human normal kidney cells (Fig. 1 A). Meanwhile, other fractions not only have high cytotoxicity on HT-29 cells, but also have high cytotoxicity on human normal kidney cells (Voss *et al.*, 2005).

BuOH fraction promoted cancer cell apoptosis

To study roles of fraction in apoptosis, BuOH fraction was used to set up apoptosis system on HT-29 cells. HT-29 cells were treated with concentration of fraction as 300 μg/mL and 500 μg/mL for 48 h (Wang *et al.*, 2006). Quantitative analysis using Annexin V/PI assay further showed that the proportion of early stage apoptotic cells (Annexin V+/PI-) increased significantly from 26.2% to 42.21%, while the proportion of late stage apoptotic cells (Annexin V+/PI+) decreased significantly from 7.50% to 35.94% (Fig. 2). These results suggested that BuOH fraction can induce apoptosis on HT-29 cells (Pakunlu *et al.*, 2006, Schmitt, 2006).

Expression level of apoptosis-related genes in BuOH fraction-treated cancer cells

In order to determine the expression level of apoptosis

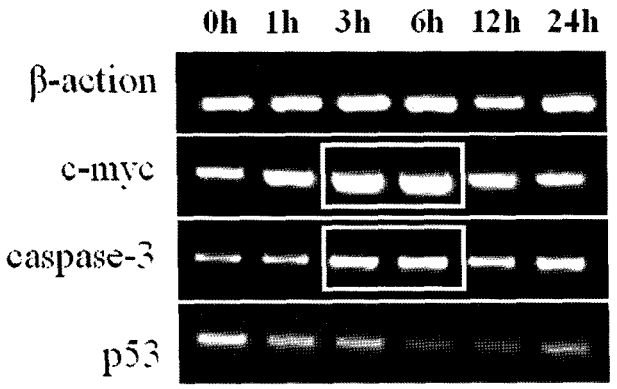

Fig. 3. Time dependency effects of the p53, caspase 3 and c-myc mRNA levels in human colon cancer cell line, HT-29, incubated in the absence or presence of BuOH fraction (500 μg/mL) from P. quassioides MeOH extract. β-actin was used as an internal control for integrity.

related genes induced in the BuOH fraction treated HT-29 cells, the mRNA levels of c-myc, p53 and caspase 3 were evaluated by RT-PCR. Fig. 3 show that the steady state mRNA levels of caspase-3 and c-myc were increased drastically when the cells were treated with the fraction at 3 h, 6 h and 3 h, 6 h on HT-29 cell, respectively (Ju *et al.*, 2004, Qia and Xua, 2006). Thus, the results strongly indicate that the BuOH fraction killed cancer cells through apoptosis mechanism mainly via the activation of caspase-3 and c-myc (Tan *et al.*, 2005, Chen *et al.*, 2006).

The result of DPPH· free radical scavenging assay revealed that the various fractions of *P. quassioides* had significant antioxidant. Total phenolics and flovonoids contents of fractions are very high, especially EtOAc, maybe they have very close relative with the high antioxidant activity of samples. The importance is that BuOH fraction of MeOH extract from *P. quassioides* has very high anticancer activity. All the experiments showed that BuOH fraction can induce apoptosis on HT-29 cells very strongly.

In conclusion, *P. quassioides* could be considered as a functional food ingredient and pharmaceutical. However, the identification of individual compounds and *in vivo* experiments are needed to understand their mechanisms of action.

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LITERATURE CITED

Athukorala Y, Kim KN, Jeon YJ (2006) Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, Ecklonia cava. Food Chem Toxicol. 44:1065-1074.

Birt DF, Hendrich S, Wang WQ (2001) Dietary agents in cancer prevention: flavonoids and isoflavonoids. Pharmacol Ther. 90:157-177.

Chen YL, Lin SZ, Chang JY, Cheng YL, Tsai NM, Chen SP, Chang WL, Harn HJ (2006) In vitro and in vivo studies of a novel potential anticancer agent of isochaihulactone on human lung cancer A549 cells. Biochem Pharmacol. 72:308-319.

Ching Su New Medical College (ed) (1977) Dictionary of Chinese Materia Medica: hanghai Scientific Technological Publishers. Shangai. 1294.

Chiu LCM, Ho TS, Wong EYL, Ooi VEC (2006) Ethyl acetate extract of *Patrinia scabiosaefolia* downregulates anti-apoptotic Bcl-2/Bcl-XL expression, and induces apoptosis in human breast carcinoma MCF-7 cells independent of caspase-9 activation. J Ethnopharmacol. 105:263-268.

Gordon MH (1990) The mechanism of antioxidant action in vitro. In Hudson (Ed.). Food antioxidants. Elsevier. London. 1-18.

Halliwell B (1995) Antioxidant characterization: methodology and mechanism. Biochem Pharmacol. 49:1341-1348.

Halliwell B, Gutteridge JMC (1985) Free radicals in biology and medicine. Oxford University Press. Oxford, 218-313.

Ju EM, Lee SE, Hwang HJ, Kim JH (2004) Antioxidant and anticancer activity of extract from *Betula platyphylla var. japonica*. Life Sci. 74:1013-1026.

Kogure K, Manabe S, Hama S, Tokumura A, Fukuzawa K (2003) Potentiation of anti-cancer effect by intravenous administration of vesiculated a-tocopheryl hemisuccinate on mouse melanoma *in vivo*. Cancer Lett. 192:19-24.

Kousteni S, Tura-Kockar F, Ramji DP (1999) Sequence and expression analysis of a novel Xenpus laevis cDNA that encodes a protein similar to bacterial and chloroplast ribosomal protein L24. Gene. 235:13-18.

Lavia I, Friesemb D, Gereshc S, Hadarb Y, Schwartza B (2006) An aqueous polysaccharide extract from the edible mushroom *Pleurotus ostreatus* induces anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells, Cancer Lett. 244:61-70.

Le Baila JC, Varnata F, Nicolasb JC, Habriouxa G (1998) Estrogenic and antiproliferative activities on MCF-7 human breast cancer cells by flavonoids. Cancer Lett. 130:209-216.

Lee CL, Yang XT, Wan JMF (2006) The culture duration affects the immunomodulatory and anticancer effect of polysaccharopeptide derived from *Coriolus versicolor*. Enzyme Microb Technol. 38:14-21.

Lin JY, Tang CY (2007) Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem. 101:140-147.

Lu YR, Foo LY (1997) Identification and quantification of major polyphenols in apple pomace. Food Chem. 59:187-194.

Mossman T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 65:55-63.

Pakunlu RI, Wang Y, Saad M, Khandare JJ, Starovoytov V, Minko T (2006) In vitro and in vivo intracellular liposomal delivery of antisense oligonucleotides and anticancer drug. J Control Release. 114:153-162.

Popiolkiewicz J, Polkowski K, Skierski JS, Mazurek AP (2005) In vitro toxicity evaluation in the development of new anticancer drugs-genistein glycosides. Cancer Lett. 229:67-75.

Qia LF, Xua ZR (2006) In vivo antitumor activity of chitosan nanoparticles. Bioorg Med Chem Lett. 16:4243-4245.

Schmitt CA (2006) Cellular senescence and cancer treatment. Biochim Biophys Acta. 21:132-136.

Shyu YS, Hwang LS (2002) Antioxidant activity of the crude extract of lignan glycosides fromunroasted Burma black sesame meal. Food Res. Intern. 35:357-365.

Slater TF, Sawyer B, Strauli UD (1963) Studies on succinate-tetrazolium reductase system. III. Points of coupling of four different tetrazolium salts. Biochim Biophys Acta. 77:383-393.

Tan ML, Sulaiman SF, Najimuddin N, Samian MR, Tengku Muhammad TS (2005) Methanolic extract of *Pereskia bleo* (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma, T47-D cell line. J Ethnopharmacol. 96:287-294.

Tengku Muhammad TS, Hughes TR, Ranki H, Cryer A, Ramji DP (2000) Differential regulation of macrophage CCAAT-Enhancer binding protein isoforms by lipopolysaccharide and cytokines. Cytokine. 12:1430-1436.

Voss C, Eyol E, Berger MR (2005) Identification of potent anticancer activity in Ximenia americana aqueous extracts used by African traditional medicine. Toxicol Appl Pharmacol. 12: 312-321.

Wang XJ, Wei YQ, Yuan SL, Liu GJ, Lu YR, Zhang J, Wang WD (2006) Potential anticancer activity of litchi fruit pericarp extract against hepatocellular carcinoma in vitro and *in vivo*. Cancer Lett. 239:144-150.

Wang XJ, Yuan SL, Wang J, Lin P, Liu GJ, Lu YR, Zhang J, Wang WD, Wei YQ (2006) Anticancer activity of litchi fruit pericarp extract against human breast cancer in vitro and *in vivo*. Toxicol Appl Pharmacol. 215:168-178.

Wang Y, Chan FL, Chen Sh, Leung LK (2005) The plant polyphenol butein inhibits testosterone-induced proliferation in breast cancer cells expressing aromatase. Life Sci. 77:39-51.

Yin Y, Heo SI, Jung MJ, Wang MH (2007) Antioxidant Properties of Water Extract from Acorn, J. Appl. Biol. Chem. 50:70-73

Yoshikawa K, Sugawara S, Arihara S (1995) Phenylpropanoids and other secondary metabolites from fresh fruits of *Picrasma Quassioides*. Phytocheraistry. 40:253-256.

Zhang T, Lu H, Shang X, Tian YH, Zheng CY, Wang SW, Cheng HHa, Zhou RJ (2006) Nicotine prevents the apoptosis induced by menadione in human lung cancer cells. Biochem Biophys Res Commun. 342:928-934.

Zhao MM, Yang B, Wang JS, Li BZ, Jiang YM (2006) Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities. Food Chem. 98:539-544.