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Comparison of Immuno-stimulatory Activities by Purification Process of *Schizandra chinensis* Baillon Fruits

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ABSTRACT : Two compounds from Gomisin N and Gomisin A were isolated from the fruits of *Schizandra chinensis* Baillon. The highest extraction yield as 21.36% was observed in the ethanol extract, compared to the yield obtained form the water extract. The extraction yields of the single compounds were measured to be 0.13 and 0.014 Gomisin A and Gomisin N, respectively. Approximately, 90% of the growth of human stomach adenocarcinoma cancer cells was inhibited after adding 1.0 g/ℓ of the ethanol extract. The growth of the human normal lung cell was limited to 24% after adding the ethanol extract. The water extract lowered the specific secretion of TNF-á and IL-6 from T cells, 10.3×10^{-4} pg/cell and 12.1×10^{-4} pg/cell, respectively, compared to the ethanol extracts. On the other hand, a treatment with the ethanol extract increased the specific secretion of TNF-á and IL-6 from human T cells, to 11×10^{-4} pg/cell and 14.3×10^{-4} pg/cell, respectively. The crude ethanol extract had the highest effect on the differentiation of human promyelocytic leukemia cells compared to the other extracts and Gomisin A and N. In general, the biological activities of the extracts gradually decreased as the purification process proceeded, which suggests that higher immuno-stimulatory activities can be maintained by adding the crude extracts of the fruits rather than by adding a single compound.

Key words : *Schizandra chinensis* Baillon, Gomisin N, Gomisin A, immuno-stimulatory activities

INTRODUCTION

Schizandra fruit is a part of a vine tree *Schizandra Chinensis* Baillon included into Schizandraceae. This red colored fruit is a major medicinal plant used as a raw medicine and food source, and has been processed into various food products and widely applied to the Oriental medicine or medical science fields (Kim *et al.*, 2002). In the Oriental medicinal field, *Schizandra* fruit has been effectively used for central inhibitory activities such as sedative, antitussive and antipyrectic, liver protection, anti-hypertensive action, alcohol detoxification, and antioxidation, etc. The food products made of *Schizandra* fruit are starch granule using its red colorant, starch paste, *Schizandra* fruit tea bag, *Schizandra* fruit liquor, and so forth (Jang *et al.*, 1996).

The components contained in the *Schizandra* fruit have been reported to be lignans mainly, and also, some other various substances such as crude oil, refined oil, coloring matter, etc. (Tang, 1992; Yang, 1982; Kim, 1994). The studies of biological activities

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are related to antioxidative activation (Toda *et al.*, 1984; Ikeya *et al.*, 1988), liver protection effects (Hiroshi *et al.*, 1984; Yoshinobu *et al.*, 1985; Sadako *et al.*, 1993). This fruit has been also used as raw medicine or food source owing to its various efficacies like hepatic detoxification and so on (Hikino *et al.*, 1984). As for the lignan component in the *Schizandra* fruit, since Kochetkove *et al.* (1961) firstly isolated schizandrin, a kind of lignan in 1961, numerous researchers separated and isolated over 30 kinds of lignans. Among these, schizandrins and schizandrols have been known to be contained relatively highly in the fruit, and especially, schizandrins have been reported as representative lignans showing a liver protection effect. The effect of liver protection by *Schizandra* fruit has been considered to be caused by the high content of schizandrins (Hwang, 1982). However, only few studies are connected to the applicable component biological activities of these pure substances, epigomisin O, gomisin A, gomisin H, gomisin N, -chamigrene, chamigenal, sesquicarene and even, absolutely no study is related to the primary extracts by various solvents and the stimulation on the activity of each isolated component.

Therefore, this study was designed to isolate the effective single components from the extracts of *Schizandra* fruit through consecutive purification processes, with the aim at comparing the synergy effects of pure substance-complicated immune activation functions for making the fruit extracts as functional biomaterials through comparing and examining the immuo-stimulatory activities according to each isolating step.

MATERIALS AND METHODS

Extraction and isolation of Schizandra fruit

The materials collected in the area of Janggi-ri, Anseong-myeon, Muju-gun, (Jeollabuk-do, Korea), cultivated in October 2001. *Schizandra* fruit was dried in shade places and grinded with a mesh grinder. This grinded power was extracted by 95% ethanol (EtOH) at room temperature for 72 hr three times and then concentrated by a rotary vacuum evaporator. The EtOH extracts were continuously extracted and fractionated by orderly using the solvents of petroleum ether, ether (Et2O) and ethyl acetate (EtOAc). Among these, total 300 fractions were obtained by fractionating each 10 ㎖ of the soluble parts of EtOH extracts (195 g) by using silica gel column (7.0×38 ㎝) chromatography with the mobile solution benzene-EtOAc (20:1, v/v), and were divided into 6 fractions named as SCE-1 to SCE-6 by developing each fraction on thin layer chromatography (TLC, silica gel 60 F254, developing solvent: toluene-ethyl formate-formic acid = 5:4:1, v/v/v), and by examining the fraction with UV (254 ㎚) lamp and a coloring agent (50% H_2SO_4).

Separation and identification of each component in the extracts

Compound 1(SCF-1)

The Mass Spectrum (MS) used for the purified compounds was JEOL JMS-600W (Germany). The nuclear magnetic resonance absorption (NMR) spectrum such as 1H-, 13C-NMR, 1H-1H Correlation Spectroscopy (COSY), NOE Spectroscopy (NOESY), 1H Detected Multiple Quantum Coherence (HMQC) and 1H Detected Multiple Bond Connectivity (HMBC) were measured using Varian UI 500(USA).

Total 100 fractions were obtained by fractionating each 10 ㎖ of the SCE-3 fraction (356 ㎎) in the Et2O solution part by using silica gel column (4.5×20 ㎝) with mobile solution hexane-Et2O (4:1, v/v), and were divided into 2 fractions (SCE-3-1~SCE-3-2) through the UV examination on TLC. Compound 1 (270 ㎎) was purified from SCE-3-2 fraction : Pale yellow oil. EI-MS *m/z* : 400 (M+, base ion), 344 (M-56), 312, 235 (M-165), 219 (M-181), 181, 165, 56. 1H-, 13C-NMR : table 1, 2. 1H-1H COSY correlations : H-6↔H-7, H-7↔H-8/CH_3-18, H-8 ↔H-9/CH_3-17. HMBC correlations : CH_3-17↔C-7/C-8, CH_3-18 ↔C-7/C-8, H-4↔C-2/C-3/C-6/C-16, OCH_2O↔C-12/C-13, H-6↔C-4/C-5/C-7/C-8/C-16/C-18, H-9↔C-7/C-8/C-10/C-11/C-15/C-17, H-11 ↔C-9/C-12/C-13/C-15, OCH_3-1↔C-1, OCH_3-2↔C-2, OCH_3-3↔C-3, OCH_3-14↔C-14.

NOESY correlations : H-7/H-9↔CH_3-17/H-11, H-6↔H-8/ CH_3-18/H-4, H-18↔H-4/H-6/H-9)

Compound 2 (SCF-5)

Total 120 fractions were attained by fractionating each 5 mℓ of the SCE-5 fraction (20.8 g) in the Et_2O solution part by using silica gel column (6.0×19 cm) with mobile solution benzene-EtOAc (10:1, v/v), and were divided into 4 fractions (SCE-5-1~SCE-5-4) through the UV examination on TLC. Again, total 300 fractions were acquired by fractionating each 8 mℓ of the SEC-5-4 fraction (15.4 g) by using Sephadex LH-20 (4.5×70 cm) with mobile solution MeOH-H_2O (1:1, v/v), and then, divided into 5 fractions (SCE-5-4-1~SCE-5-4-5) through UV examination. Furthermore, compound 2 (726.6 mg) was isolated from SCE-5-4-2 by using Sephadex LH-20 (3.0×68 cm) with mobile solution MeOH : colorless crystals. EI-MS *m/z* : 416 (M+, base ion), 345 (M-71), 314, 1H-, 13C-NMR : table 1, 2. 1H-1H COSY correlations : H-8↔H-9/CH_3-17. HMBC correlations : CH_3-17↔C-7/C-8/C-9, CH_3-18↔C-6 /C-7/C-8, H-4↔C-2/C-3/C-6/C-16, OCH_2O↔C-12/C-13, H-6↔C-4/C-5/C-7/C-8/C-16/C-18, H-9↔C-7/C-8/C-10/C-11/C-15/C-17, H-11↔C-9/C-12/C-13/C-15, OCH_3-1↔C-1, OCH_3-2↔C-2, OCH_3-3↔C-3, OCH_3-14↔C -14.

NOESY correlations : H-4↔OCH_3-3/H-6/H-8, H-9↔CH_3-17/H-8/H-11, H-6↔H-8/CH_3-18/H-4, H-17↔H-6/H-8/H-9/H-11/CH_3-18

Table 1. Comparison of extract yields from *Schizandra chinensis* Baillon according to extracts solvent.

Solvent	Yields (%, v/v)
Water	15.700 ± 0.1
Ethanol	21.360 ± 0.2
Gomisin N†	0.130 ± 0.4
Gomisin A†	0.014 ± 0.4

† Purified compounds from the ethanol extracts.

Table 2. ^{1}H NMR spectral data for compounds 1-2 (500 MHz, acetone-d_6).

H	1†	2†
4	6.68 *s*	6.64 *s*
6a	2.44 *dd* (2.5, 13.5)	2.70 *d* (13.5)
6b	2.60 *dd* (8.5, 13.5)	2.37 *dd* (3.5, 11.5)
7	1.89 *m*	-
8	1.76 *m*	1.86 *m*
9a	2.05 *m*	2.34 *d* (7.5)
9b	2.22 dd (9.0, 13.5)	2.60 *dd* (1.5, 14.0)
11	6.53 *s*	6.49 *s*
CH_3-17	0.97 *d* (7.5)	0.84 *d* (7.0)
CH_3-18	0.73 *d* (7.5)	1.27 *s*
OMe-1	3.86 *s*	3.53 *s*
OMe-2	3.80 *s*	3.92 *s*
OMe-3	3.48 *s*	3.92 *s*
OMe-12	-	-
OMe-13	-	-
OMe-14	3.76 *s*	3.85 *s*
OCH_2O	5.96 *s*	5.97 *dd* (1.5, 6.0)

† *J* values in parentheses are recorded in Hz.
‡ in chloroform-*d*.

Table 3. ^{13}C-NMR spectral data for compounds 1-2 (125 MHz, acetone-d_6).

C	1†	2†
1	152.21 (*s*)	152.37 (*s*)
2	140.59 (*s*)	141.01 (*s*)
3	152.01 (*s*)	152.56 (*s*)
4	111.13 (*d*)	101.57 (*d*)
5	134.04 (*s*)	132.27 (*s*)
6	38.94 (*t*)	40.78 (*t*)
7	33.83 (*d*)	71.87 (*d*)
8	41.11 (*d*)	42.28 (*d*)
9	35.36 (*t*)	33.98 (*t*)
10	137.91 (*s*)	132.75 (*s*)
11	102.84 (*d*)	106.81 (*d*)
12	149.02 (*s*)	148.14 (*s*)
13	134.89 (*s*)	135.18 (*s*)
14	141.47 (*s*)	141.47 (*s*)
15	121.88 (*s*)	122.12 (*s*)
16	123.59 (*s*)	124.40 (*s*)
CH_3-17	21.10 (*q*)	16.03 (*q*)
CH_3-18	12.37 (*q*)	30.32 (*q*)
OMe-1	55.59 (*q*)	60.83 (*q*)
OMe-2	60.23 (*q*)	61.25 (*q*)
OMe-3	59.96 (*q*)	56.21 (*q*)
OMe-12	-	-
OMe-13	-	-
OMe-14	58.88 (*q*)	59.89 (*q*)
OCH_2O	101.03 (*t*)	101.07 (*t*)

† Assignment are based on DEPT, HSQC and HMBC spectra.
‡ in chloroform-*d*.

Measurement of biological activities

Anticancer activity

Human gastric cancer cell line (AGS, ATCC, USA) and the normal human embryonic lung cell line (HEL299, ATCC, USA) were cultured with 10% FBS and RPMI 1640 medium (GIBCO, USA). 100 ㎕ of each cell culture medium was inoculated onto each 96-well plate with making cell concentration as 4~5 $\times 10^4$ cells/㎖. For the experiments of the inhibition on cancer cell growth and the cytotoxicity on normal cells, the absorbance was measured at 540 ㎚ by using ELISA reader through Sulforhodamine B(SRB) method (Dool & Peto., 1981).

Immuno-stimulatory activities

The amounts of Tumor necrosis factor-á (TNF-á) and Interleukine-6 (IL-6), the cytokines secreted into the medium by human B cells (Raji, ATCC, USA), were estimated through a ELISA kit (Genzyme, USA). The growth and cell density of human B cell were also observed by a Hemacytometer using an inverted microscope. Above all, the supernatant was collected by centrifuging the culture medium and cultured with the variously concentrated standards at 37℃ for 30 min. The absorbance was measured at 450 ㎚ to draw the standard curve. The O.D values obtained from the samples were compared to estimate the amount of cytokines (Han *et al.*, 1998).

Estimation of cell differentiation

Human promyelocytic leukemia cell line (HL-60, ATCC, USA) was maintained in suspension culture under the conditions of 37℃ and 5% CO_2 by using 10% FBS and RPMI 1640 medium. Continuously, 1 ㎖ of 4×10^5 cells/㎖ concentrated cells was inoculated onto 24-well plate for primary culture. After removing the medium at intervals of 24 h, the cell was collected and precipitated by the centrifugation at 320 x g for 10 min., and was ruptured through lysis by adding 200 ㎕ of 0.1% Triton X-100 at 37℃ for 30 min. Predetermined amount of lysate and 0.1% Triton X-100 were moved to be 20 ㎕ into 96 well plate, and 100 ㎕ of 50 mM acetate buffer(pH 0.5) containing 3 ㎎/㎖ of 4-nitrophenyl phosphate was added and reacted at 37℃ for 1 h. After 100㎕ of 0.1 N NaOH was added for stopping the reaction, the absorbance was estimated at 405 ㎚ through the ELISA reader (Yen & Guernsey, 1986; Bang, 1993).

RESULTS AND DISCUSSION

Isolation of each component in the extract

Compound 1 was regarded as one of major components in *Schizandra* fruit, and was purified into a yellow-colored fluid phase. Compound 1 demonstrated *m/z* 400 (M+) of molecular ion (M+) peak on EI-MS spectrum, and its major peaks were *m/z* 344 (M+- 56), 312, 235(M+-165), 219(M+-181), 181, 165, etc. Each of 4 singlet signals shown on ä 3.48 (3H, OMe-3), ä 3.76 (3H, OMe-14), ä 3.80 (3H, OMe-2), and ä 3.86 (3H, OMe-1) of ^{1}H-NMR spectrum was returned to methoxyl replaced on direction nucleus. One multiplet signal present on 3 double doublet signals corresponding to 6H of ä 2.44 (2H, J = 2.5, 13.5 Hz, H-6), ä 2.60 (2H, J = 8.5, 13.5 Hz, H-6) and ä 2.22 (2H, J = 9.0, 13.5 Hz, H-9) is derived from benzylic methylene proton of H-6 and H-9 in the partial structure of cyclootadiene. Two doublet signals corresponding to 6H of ä 0.97 (3H, d, J = 7.5 Hz, CH_3-17) and ä 0.73 (3H, d, J = 7.5 Hz, CH_3-18) were returned to CH_3-17 of equatorial methyl radical and CH_3-18 of axial methyl radical, respectively (Yukinobu *et al.*, 1979; 1980). ^{13}C-NMR spectrum by DEPT method represented total 23 carbon signals. These carbons were 10 quaternary carbons, 4 tertiary carbons, 3 secondary carbons, and 6 primary carbons. ä 102.84 and a 111.13 were returned to methine carbons of C-11 and C-4 based on direction nucleus. HMBC spectrum of compound 1 displayed the cross peaks of dioxymethylene proton with C-12 and C-13, and of H-11 proton with C-13, C-15 and C-9 carbons. Moreover, NOESY spectrum supported these structures from the cross peaks of CH_3-17 proton and dioxymethylene proton. According to the results of comparing of above analyses and reference (Yukinobu *et al.*, 1982), compound 1 was identified as Gomisin N (Fig. 1).

Compound 2 was purified into white crystal. The

molecualr ion (M+) peak was *m/z* 416 on EI-MS spectrum. On ^{1}H-NMR spectrum of compound 2, ä 0.84 (3H, *J* = 7.0 Hz, CH_3-17) and ä 1.27 (3H, CH_3-18) signals were returned to CH_3-17 of axial methyl radical and CH_3-18 of equatorial methyl radical, respectively. One multiplet signal shown on ä 1.86 (1H, *m*, H-8) was caused by H-9 of partial structure of cyclootadiene. Two singlet signals ä 6.64 (1H, s, H-4) and ä 6.49 (1H, s, H-11) present in the low magnetic field were separately returned to methine proton of direction nucleus. In addition, the signal corresponding to 2H of ä 5.97 (2H, *J* = 1.5, 6.0 Hz, OCHO) suggested the existence of dioxymethylene proton, and showed the cross peaks with C-12 and C-13 on HMBC spectrum of compound 2. Four singlet signals shown on ä 3.53 (3H, OMe-1), ä 3.92 (3H, OMe-2), ä 3.92 (3H, OMe-3) and ä 3.85 (3H, OMe-14), separately derived from methoxyl replaced on direction nucleus, showed the cross peaks with C-1, C-2, C-3, and C-14 carbons on HMBC. Furthermore, NOESY spectrum of compound 2 proved the cross peaks with OMe-3 proton of ä 3.92 (3H) and H-4 proton of ä 6.64 (1H). According to the comparison with the above instrumental analyses and reference (Bartlova *et al.*, 2002), compound 2 was identified as Gomisin A (Fig. 1).

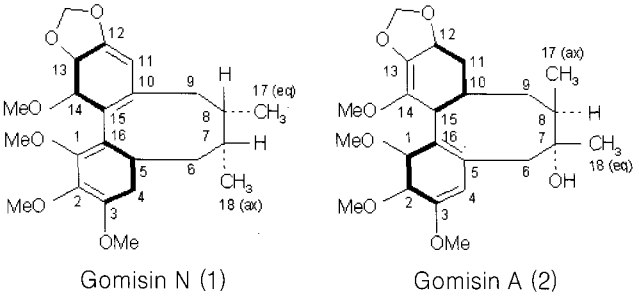

Fig. 1. Chemical structures purified from the fruits of *Schizandra chinensis* Baillon.

Extracting yield of *Schizandra* fruit

Table 1 compares each extracting yield of *Schizandra* fruit. Accoridng to the results, each extracting yield of *Schizandra* fruit was 15.7% in water extraction, 21.36% in ethanol extraction, 0.13% in ethanol extract purified substance, Gomisin N (SCF-1), and 0.014% in Gomisin A (SCF-5). Therefore, ethanol extract showed the highest yield.

Results of estimating biological activities

As illustrated in Fig. 2, 0.2~1.0 g/ℓ of Schizandra fruit juice and ethanol extract was added to the human normal cell line (HEL299) to estimate the growth of these cells. According to the results, the cytotoxicity was relatively not high as showing the inhibition ratio of 13~24% in adding ethanol extract; of 15~21% in adding Gomisin N (SCF-1); of 13~23% in adding Gomisin A (SCF-5); and of 11~21% in adding water extract. The anticancer activities according to the concentrations of Schinzandra fruit and ethanol extracts in the AGS were in Fig. 3. The growth of cancer cells was inhibited with the increase of extract concentration. The growth inhibiting ratios were 91.2% in adding 1 g/ℓ of ethanol extract, 58% and 78% each in adding Gomisin N and Gomisin A that were the purified substances of ethanol extract, and 79.9% in adding 1 g/ℓ of water extract. In the selectivity representing the death ratio of cancer cells to the death ratio of normal cells in the same concentration to show the inhibition ratio of cancer cells to normal cell toxicity of the extracts, ethanol extract had the selectivity 4.7 while water extract had 5.0 in 1 g/ℓ concentration. Therefore, ethanol extract rather than water extract can selectively inhibit the growth of cancer cells, and can have high anticancer activities for the cancer cells.

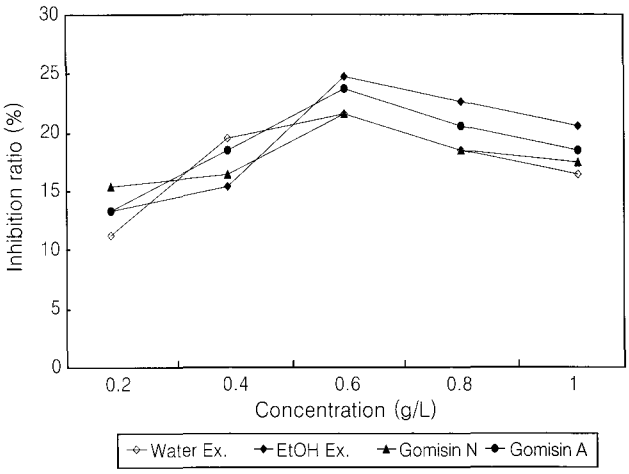

Fig. 2. Cytotoxicity of the extracts (Ex:Extract) from *Schizandra chinensis* Baillon on nomal human cell (HEL299).

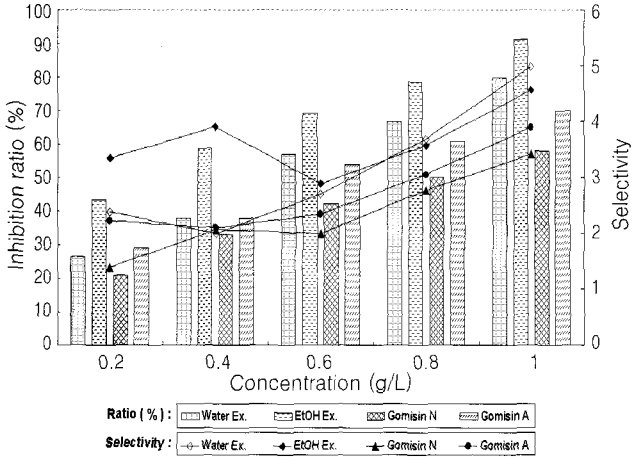

Fig. 3. Inhibition ratio of growth of AGS (bar chart, %) and selectivity (curve line) in adding the extracts (Ex:Extract) from *Schizandra chinensis* Baillon.

In Table 4 demonstrating the secretion amounts of IL-6 and TNF-á from human B cells for 6 days, specific secretion of IL-6 was shown to be 12.7×10^{-4} pg/cell by ethanol-aided extract, 9.0×10^{-4} pg/cell by Gomisin N, 9.7×10^{-4} pg/cell by Gomisin A, and 11.4×10^{-4} pg/cell by water extract, and subsequently, ethanol extract promoted the secretion most effectively, not purified components. The secretion amount of TNF-á in T cells as found in Fig. 4 was 11×10^{-4} pg/cell and 10.3×10^{-4} pg/cell each by ethanol extract and water extract while Gomisin N and Gomisin A secreted TNF-á as much as 8.0×10^{-4} pg/cell and 8.7×10^{-4} pg/cell, respectively. The amounts of TNF-á in adding single components were also lower than those in crude extracts. Moreover, in B cells, TNF-á was secreted as much as 12.2×10^{-4} pg/cell, 10.3×10^{-4} pg/cell, 8.9×10^{-4} pg/cell, and 9.6×10^{-4} pg/cell by each of ethanol extract, water extract, Gomisin N and Gomisin A, respectively. It can be concluded that the crude ethanol extracts promoted the secretion of both IL-6 and TNF-á more effectively rather than single components, Gomisin N and A, and in particular, ethanol extract secreted the most abundant cytokines to be confirmed as the most effective substance.

As confirmed in Fig. 5, the cell differentiating activities were also higher in ethanol extract than water extract. Crude ethanol extract showed the highest differentiability of 159% at the 3rd day of

Table 4. Kinetics of secretion of IL-6 and TNF-α from human B cell cultured in adding the extracts.

S. chinensis	Time (day)	Cell line: B cell($\times10^{-4}$ pg/cell) IL-6	TNF-α
Water Ex.	1	3.3±0.01	3.5±0.06
	2	3.9±0.01	4.9±0.06
	3	6.7±0.02	6.4±0.05
	4	8.9±0.03	8.5±0.07
	5	10.5±0.08	9.8±0.05
	6	11.4±0.09	10.3±0.01
Ethanol Ex.	1	3.1±0.03	3.3±0.05
	2	4.8±0.04	4.6±0.03
	3	7.5±0.04	6.9±0.03
	4	9.5±0.06	9.7±0.08
	5	11.3±0.07	10.9±0.01
	6	12.7±0.05	12.2±0.05
Gomisin N†	1	2.6±0.09	1.9±0.06
	2	3.8±0.08	2.9±0.07
	3	5.4±0.03	4.6±0.08
	4	7.3±0.07	6.1±0.07
	5	8.2±0.05	7.3±0.09
	6	9.0±0.01	8.1±0.04
Gomisin A†	1	3.0±0.01	2.2±0.01
	2	5.1±0.02	3.9±0.08
	3	6.0±0.08	4.5±0.06
	4	7.3±0.02	6.7±0.07
	5	8.6±0.05	7.5±0.05
	6	9.7±0.03	8.7±0.09

† Purified solvent from ethanol extracts.

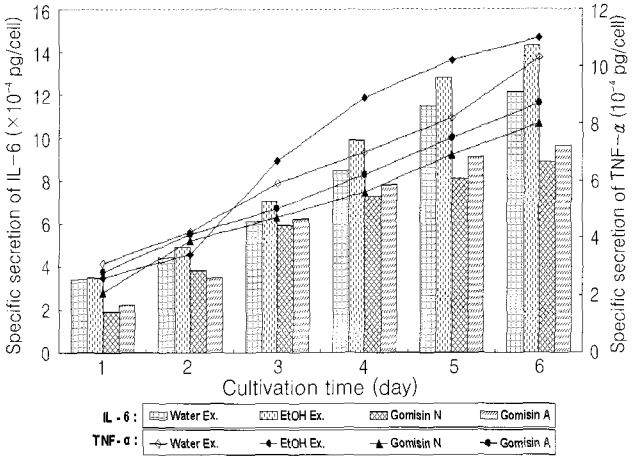

Fig. 4. Kinetics of secretion of cytokine from human T cell cultured in adding 1.0 g/ℓ of the extracts (Ex:Extract) form *Schizandra chinensis* Baillon.

culture while the differentiability was shown to be 134% in water extract, only 119% in Gomisin N, and 123% in Gomisin A, respectively. Thereupon, crude ethanol extract has the higher differentiation of HL-60 cells than water extract having the better differentiability than the isolated substances Gomisin N and Gomisin A. Ethanol extract rather than water extract increased the substances inducing the differentiation to rapidly perform the signal delivery system of the cells, and accordingly, the differentiation was promoted as confirmed in Fig. 6. This result can be associated with the increase of the cell differentiating activity because the differentiation-inducing substances were increased in the ethanol extraction.

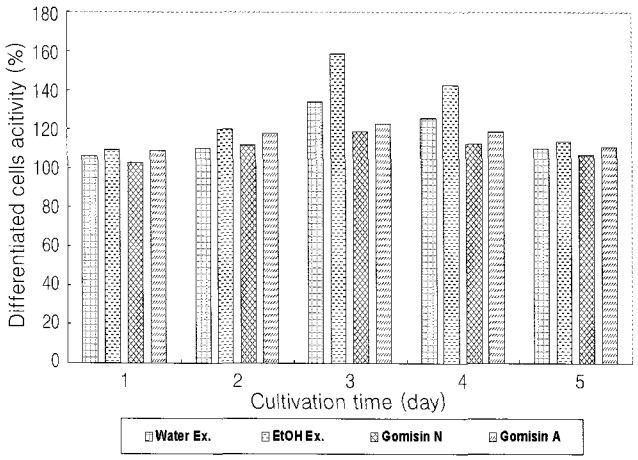

Fig. 5. Comparison of the differentiation of HL-60 cells by adding 1.0 g/ℓ of the extracts (Ex:Extract) from *Schizandra chinensis* Baillon.

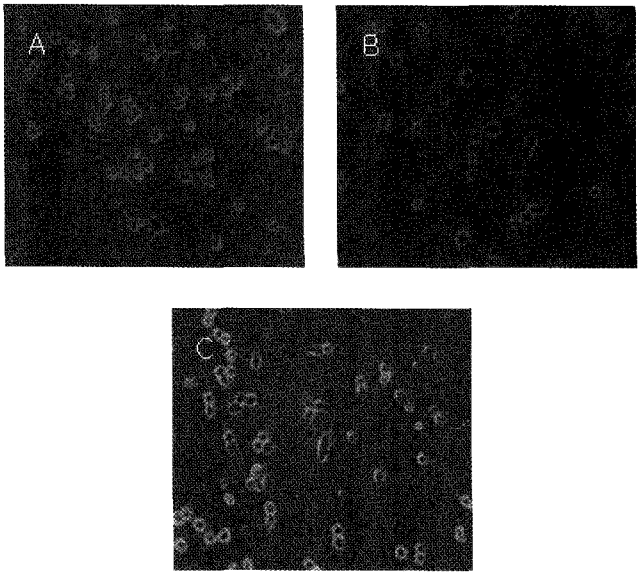

Fig. 6. Comparison of control cells and differentiated cells by adding the ethanol extract from *Schizandra chinensis* Baillon. A : no adding, B : 1st day, C : 3rd day.

The different effects of the immuo-stimulatory activities according to the different purification processes were compared using *Schizandra* fruit. In general the crude ethanol extract had the higher activities than the water extract. That is, the growth inhibiting ratio of growing human gastric cancer cell line was measured as 79.7% in adding 1 g/ℓ of water extract, 91.2% in adding 1 g/ℓ of crude ethanol extract that showed the highest ratio, and only 58% and 78% each inhibiting ratios in adding Gomisin N and Gomisin A. It can tell that the purified compounds obtained through consecutive purification steps showed relatively low anticancer activities, and accordingly, the crude ethanol extracts were proved to have the higher anticancer activity promoting effect than water extract and single components.

As the results of estimating the secretion amounts of cytokines IL-6 and TNF-á for 6 days, 1.0 g/ℓ of crude ethanol extract improved the secretion more, and especially more in T cells as much as 14.3×10^{-4} pg/cell of IL-6 and 11×10^{-4} pg/cell of TNF-á, compared with those in B cells. Similarly, in the HL-60 cell differentiability test, crude ethanol extract showed the higher cell differentiating activity than water extract. It can be concluded that the crude ethanol extract than water extract and single components improved the immune activity together with the high anticancer activity.

Therefore, based on the results according to the separation, the use of crude ethanol extract, instead of the use of isolated components through several purification steps or water extract, can be more effective in developing not only functional foods and health supplementary products using *Schizandra* fruit but also new efficacious active substances. The data shown here may be opposing in generally studying conventional plant chemistry. However, it can imply that the purifying single components from crude extracts through complicated and/or many steps are

not always the best objective in investigating and developing functional foods from natural plant resources. This result can also support the idea of using crude extracts in developing functional foods or relative biomaterials rather than using only purified components in the extracts for ecomical reasons.

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