The Chemopreventive Effects of Saussurea salicifolia through Induction of Apoptosis and Phase II Detoxification Enzyme

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The ethanol extract of the aerial part of the Mongolian medicinal plant Saussurea salicifolia induced a dose-dependent cell growth inhibition in both human gastric adenocarcinoma AGS cells and mouse hepatoma Hepa 1c1c7 cells (IC50=30.22 and 116.96 μg/ml, respectively). The extract induced an apoptosis in AGS cells inference from the externalization of the phosphatidylserine, the increase of the sub G0/G1 content (%) and the apoptotic morphological changes including membrane blebbing, the formation of apoptotic bodies and chromatin condensation. In order to identify active substances causing the apoptosis, we further isolated major compounds present in Saussurea salicifolia and 7 compounds were isolated including a sesquiterpene lactone, cynaropicrin, 3 lignans (trachelogenin, matairesinol and arctigenin) and 3 lignan glycosides (tracheloside, matairesinoside and arctiin). In general the lignan aglycones were more cytotoxic than their lignan glycosides in both AGS cells and Hepa 1c1c7 cells. Cynaropicrin not only showed the most potent cytotoxicity among the 7 major compounds but also it induced an apoptosis and a weak G2/M arrest in AGS cells. Arctigenin had the second-best cytotoxicity among 7 major compounds, and induced an apoptosis. In order to evaluate the induction of the phase II detoxification enzyme, we measured the induction of quinone reductase activity of the extract, fractions and compounds in Hepa 1c1c7 cells. The ethyl acetate fraction and arctigenin showed the strongest cancer chemopreventive activity (chemoprevention index=9.88 and 7.57, respectively). These data suggest that the extract as well as the lignan compounds (especially arctigenin) originated from Saussurea salicifolia may be served as potential cancer chemopreventive agents for prevention or treatment of human cancers.

Key words Saussurea salicifolia; apoptosis; arctigenin; cynaropicrin; quinone reductase; chemoprevention

Apoptosis (or programmed cell death) is one of the most important targets for cancer treatment comprising both chemotherapy and chemoprevention, which is characterized by the peculiar changes in cellular morphology and biochemistry such as membrane blebbing, formation of apoptotic body, chromatin condensation, DNA fragmentation, alternation in membrane symmetry, activation of caspase cascade and loss of mitochondrial membrane potential. Tumorigenesis is a complex and multi-step process, and cancer chemopreventive agents are known to inhibit the transformation of normal cells to premalignant cells or the progression of premalignant cells to malignant cells. Many cancer chemopreventive agents are originated from naturally occurring substances or their synthetic derivatives. These agents are known to modulate cellular processes associated with the xenobiotic biotransformation or the protection from oxidative damage or the promotion of differentiation. A number of chemopreventive agents including retinoids, nonsteroidal anti-inflammatory drugs (aspirin, sulindac, celecoxib, exisulind), polyphenols (resveratrol, epigallocatechin gallate), anti-estrogen/anti-androgen, vanillolinds (capsaicin, curcumin, resiniferatoxin), flavonoids (quercetin, rutin), rotenoids, perillyl alcohol, α-difluoromethylornithine and selenium are known to have the pro-apoptotic activity. 1—4) The induction of phase II detoxification enzyme including quinone reductase (QR, also known as NAD(P)H:quinone oxidoreductase) and glutathione-S-transferases can protect normal cells against toxic and reactive chemicals such as free radicals and electrophiles through detoxifying xenobiotics and blocking the formation of ultimate carcinogen, and thereby it can be an essential biomarker for cancer chemoprevention. 5,6)

Saussurea salicifolia widely spreads in Mongolian rangelands and Mongolian Altai,7,8 which has been used as a traditional Mongolian medicine for a long time to treat various diseases such as gynecological diseases, hepatitis, and gall-bladder disorder (an intramural database according to personal communication with Dr. D. Batsuren). Several major components have been identified from S. salicifolia including sesquiterpene lactones; cynaropicrin and janerin, and lignans; arctigenin, matairesinol, trachelogenin. 8,9) γ-Linoleic acid was also found in S. salicifolia, seed oil.10) In this study, for the first time, we evaluated the cancer chemopreventive effects of a total ethanol extract as well as the compounds obtained from the aerial part of S. salicifolia, through the investigation of antiproliferative, pro-apoptotic and phase II detoxification enzyme activities.

MATERIALS AND METHODS

Plant Material and Extraction Aerial parts of Saussurea salicifolia (L.) DC was collected in the vicinity of Ulaanbaatar, at the 10 km to the west of Nalaikh district, in July, 2003. Aerial parts (5.2 kg) of S. salicifolia have been extracted 3 times by ethanol at room temperature. The ethanol extract solution was evaporated by the vacuum evaporator resulting 275.8 g of the thick extract.

Fractionation and Isolation The ethanol extract (19 g) was suspended in water and partitioned sequentially with n-hexane, dichloromethane (CH2Cl2), ethyl acetate (AcOEt) and n-butanol (n-BuOH). The CH2Cl2 fraction (2 g) was dissolved in methanol (MeOH) and performed semi-preparative HPLC using YMC J-sphere ODS 4 μm 20×250 mm col-
with MeCN–H₂O gradient (30 : 70 in 70 min, 2 ml/min). The

10

many) according to manufacture's instruction. Briefly, AGS

Annexin-V -FLUOS Staining kit (Roche, Mannheim, Ger-

conjugated annexin V and propidium iodide (PI) by using

To evaluate the apoptotic cell death, flow cyto-

and vehicle control cells treated with only DMSO.

solved the extract, fractions and compounds in dimethyl sul-

reader (Bio-Tek Instruments, Winooski, VT, U.S.A.). We dis-

1c1c7 cells were cultured in

well) cells were plated onto 96-well plates, incubated at 37°C

for 24 h, and the cells were treated with the various concen-

units/ml of penicillin and 100

from

mattersinol mixture and 117.7 mg of arcti-

chelogenin and matairesinol mixture and 117.7 mg of arcti-

Torrance, CA, U.S.A.), with MeCN–H₂O gradient (30 : 70 in

Kyoto, Japan). Tracheloside, matairesinoside and arctin were

were separated with acetonitrile

were finally purified by re-chromatography on HPLC using

and detached and ad-

sorbed with MeCN–H₂O gradient (17 : 83 to 27 : 73 in

50 min, 10 ml/min) to yield 51.2 mg of tracheloside and

chelogenin and matairesinol mixture and 117.7 mg of arcti-

Growth was examined using inverted light microscope, Nikon

S. salicifolia

mg/ml of DMSO for 24 h, and the cellular morph-

agarose gel (2.5

M) was used as a positive control. The cells were rinsed

in 500 μl of Hoechst 33258 staining method. After

AGS cells were treated with either 30 or 60 μg/ml of the ex-

for 24 and 48 h, both floating and adherent cells were

Chromatin condensation measurement

The AGS cells were treated with either 30 or 60 μg/ml of the extract for 24 h, and the cellular morphology was examined using inverted light microscope, Nikon TE2000U with differential interference contrast (DIC) module (Nikon, Kanagawa, Japan). The chromatin condensation was assessed through Hoechst 33258 staining method. After AGS cells were treated with either 30 or 60 μg/ml of the extract for 24 and 48 h, both floating and adherent cells were collected by trypsinization. The cells were then washed with DPBS, and incubated in 500 μl of Hoechst 33258 (0.5 μg/ml) solution in DPBS for 20 min in darkness. The stained cells were visualized by using a fluorescent microscope, Nikon TE2000U (Nikon, Kanagawa, Japan). The morphological changes of AGS cells treated with cynaropicrin (1 to 5 μmol/l) and arctigenin (50 to 200 μmol/l) for 24 h were observed by using a phase contrast microscope, OLYMPUS CK40 (Olympus, Tokyo, Japan).

Quinone Reductase Assay The QR activity was measured as described previously1) with minor modifications. Briefly, Hepa1c1c7 cells (1×10⁶ cells per well) were plated onto 96-well plates and incubated for 24 h, then the various concentrations of the extract, fractions and compounds were treated and incubated for an additional 24 h. Sulforaphane (2.5 μM) was used as a positive control. The cells were rinsed with DPBS, lysed with 80 μl of 0.08% digitonin in 2 mM EDTA, incubated for 30 min at room temperature, and QR activity was measured using the cell lysate. A 200 μl of mixed solution [49 ml of 25 mm Tris buffer; 34 mg of BSA; 0.34 ml of 1.5% Tween-20 solution; 0.34 ml of thawed cofactor solution (150 mM glucose-6-phosphate, 4.5 mM NADP, 0.75 mM FAD in Tris buffer); 100 units of glucose-6-phosphate dehydrogenase; 15 mg of MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; and 50 μl of 50 mM phosphate buffered saline (DPBS). The cells were resuspended in 100 μl of binding buffer, which already mixed with 2 μl of annexin V-fluorescein and 2 μl of PI. After 15 min incubation at room temperature, they were analyzed by using FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA, U.S.A.).

Flow Cytometric Analysis of Alteration of Membrane Symmetry To evaluate the apoptotic cell death, flow cyto-

ary 95%, air, 5% CO₂, humidified atmosphere at 37°C.

MTT Cell Proliferation Assay The cell proliferation was evaluated by using the Cell Counting Kit (CCK-8) produced by Dojindo Laboratories (Tokyo, Japan). In brief, AGS cells (5×10³ cells per well) and Hepa 1c1c7 (1×10⁴ per well) cells were plated onto 96-well plates, incubated at 37°C for 24 h, and the cells were treated with the various concentrations of the extract, fractions and compounds acquired from S. salicifolia. After 24 h of treatment, 10 μl of CCK-8 solution was added to the wells, and incubation continued for another 1 h, and then absorbance at 450 nm was measured by using a PowerWave™ XS Microplate Spectrophotometer reader (Bio-Tek Instruments, Winooski, VT, U.S.A.). We dis-

The QR activity was measured using the cell lysate. A 200 μl of mixed solution [49 ml of 25 mm Tris buffer; 34 mg of BSA; 0.34 ml of 1.5% Tween-20 solution; 0.34 ml of thawed cofactor solution (150 mM glucose-6-phosphate, 4.5 mM NADP, 0.75 mM FAD in Tris buffer); 100 units of glucose-6-phosphate dehydrogenase; 15 mg of MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; and 50 μl of 50 mM phosphate buffered saline (DPBS). The cells were resuspended in 100 μl of binding buffer, which already mixed with 2 μl of annexin V-fluorescein and 2 μl of PI. After 15 min incubation at room temperature, they were analyzed by using FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA, U.S.A.).

Flow Cytometric DNA Content Analysis AGS cells (2.5×10⁵) were seeded on 60 mm dishes, and incubated at 37°C for 24 h, then the various concentrations of the extract and compounds acquired from S. salicifolia were treated and incubated for additional 24 h and 48 h. Both detached and ad-

sorbed with MeCN–H₂O gradient (30 : 70 in 70 min, 2 ml/min). The AcOEt fraction (900.0 mg) was dis-

olved in MeOH and performed semi-preparative HPLC using YMC J-sphere ODS 4 μm 10×250 mm column (Phenomenex, Torrance, CA, U.S.A.). We dis-

resolved the extract, fractions and compounds in dimethyl sul-

reader (Bio-Tek Instruments, Winooski, VT, U.S.A.). We dis-

solved the extract, fractions and compounds in dimethyl sul-

ose (150 mM glucose-6-phosphate, 4.5 mM NADP, 0.75 mM FAD in Tris buffer); 15 mg of MTT, 3-(4,5-dimethylthiazo-

and vehicle control cells treated with only DMSO.

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and vehicle control cells treated with only DMSO.
menadione in acetonitrile] was added into a 50 µl aliquot of the cell lysates. Absorbance at 610 nm was measured for five times with 50 s apart, using a PowerWave™ XS Microplate Spectrophotometer microplate reader (Bio-Tek Instruments, Winooski, VT, U.S.A.). Protein content was also measured in a 20 µl aliquot of the cell lysate according to Bradford method by using Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA, U.S.A.). The induction of QR activity was expressed as CD value, concentration required to double QR specific activity. The chemoprevention index (CI) is obtained by dividing IC₅₀ value with CD value.13)

RESULTS

Antiproliferative Activity of the Ethanolic Extract of the Aerial Part of \textit{S. salicifolia} in AGS Cells

The ethanol extract of the aerial part of \textit{S. salicifolia} showed a dose-dependent cell growth inhibition in various human cancer cell lines according to our preliminary study (data not shown). The most potent antiproliferative activity of the extract was observed in human gastric adenocarcinoma AGS cells, with an IC₅₀ value of 30.22 µg/ml (Fig. 1A).

The Ethanol Extract of the Aerial Part of \textit{S. salicifolia} Induces Apoptosis in AGS Cells

In order to examine if this antiproliferative activity of the extract is due to apoptosis, we measured the translocation of phosphatidylserine from the cytoplasm to the extracellular side by means of annexin V-fluorescein and PI double staining assay. The early apoptotic cells (annexin V-fluorescein positive and PI negative, inversed phosphatidylserines without loss of the membrane integrity) were increased to 6.3- and 3.5-fold over the vehicle control at 24 h post-treatment of 30 and 60 µg/ml of the extract, respectively. The necrotic or late apoptotic cells (annexin V-fluorescein positive and PI positive, normal phosphati-
phatidyserines with loss of membrane integrity) were increased to 2.9- and 19.0-fold over the vehicle control at 24 h post-treatment of 30 and 60 μg/ml of the extract, respectively (Fig. 1B). We also examined the sub G0/G1 content (%), which is an indirect clue of apoptosis, by measuring DNA content labeled with PI using flow cytometry. We found that the treatment of 30 and 60 μg/ml of the extract for 24 h increased the sub G0/G1 contents for 2.0 and 2.2-fold, respectively, over the vehicle control. The increase of the sub G0/G1 content was more pronounced at 48 h of the treatments. They were increased to 2.9 and 6.3-fold over the vehicle control at 48 h post-treatment of 30 and 60 μg/ml of the extract, respectively (Fig. 1C). We also investigated the cell cycle of AGS cells treated with 30 and 60 μg/ml of the extract, and the data were evaluated by Modfit LT 3.0 software with PI intensity as a linear scale χ-axis. The treatment of the extract, however, did not induce any apparent cell cycle arrest at a specific phase (data not shown). We also observed that AGS cells underwent dramatic morphological change including membrane blebbing and apoptotic body formation in the cells treated with 30 μg/ml of the extract for 24 h (Fig. 2A). At treatment of 60 μg/ml of the extract for 24 h, the cells were started to detach from the bottom of culture plate forming the various sizes of the round shapes, and the apoptotic bodies were also formed but less frequently than the treatment of 30 μg/ml of the extract. We also examined chromatin condensation which is one of the hallmarks during apoptosis (Fig. 2B). The vehicle control showed normal nuclei with a misty and disperse fluorescence reflect the both of euchromatin and heterochromatin. In contrast, the AGS cells treated with 30 μg/ml of the extract for 24 h and 48 h had the highly condensed nuclei with the bright spherical beads and occasionally the crescent shape.

Antiproliferative Activity of the Compounds Isolated from *S. salicifolia* in AGS Cells To find out the major compounds for causing the antiproliferative and pro-apoptotic activity of the extract, we further performed a chemical purification, and seven major compounds were finally isolated. Seven major compounds were identified including a sesquiterpene lactone, cynaropicrin and three lignans (trachelogenin, matairesinol, arctigenin) and their lignan glycosides (tracheloside, matairesinoside, arctiin). Their chemical structures were identified by NMR spectroscopy as shown in Fig. 3. Cynaropicrin, trachelogenin, matairesinol and arctigenin were isolated from the CH2Cl2 fraction, the others were prepared from the AcOEt fraction. Arctin presented in both CH2Cl2 and AcOEt fraction at the similar proportion. Cynaropicrin and arctigenin among seven major compounds showed the most potent antiproliferative activity in AGS cells (IC50 = 0.68 and 31.90 μg/ml, respectively) in a dose-dependent manner (Fig. 4). While matairesinol and trachelogenin showed moderate cytotoxicity, tracheloside and matairesinoside showed no apparent cytotoxicity. Generally, aglycones are appeared to be more cytotoxic than their lignan glycosides against AGS cells.

Cynaropicrin and Arctigenin Induce Apoptosis in AGS Cells We next investigated the proapoptotic activity of cynaropicrin and arctigenin in AGS cells, which showed the most potent cytotoxicity in AGS cells. Cynaropicrin at 5 μmol/l and arctigenin at 200 μmol/l induced an apparent apoptosis which increased the sub G0/G1 content (%) in a dose- and time-dependent manner in AGS cells. When the
cells were treated with 5 μmol/l of cynaropicrin and 200 μmol/l of arctigenin for 48 h, the sub G0/G1 contents were greatly increased up to 13.5 and 22.7%, respectively (Figs. 5A, B). As a result of cell cycle analysis, treatment of cynaropicrin for any of 24 and 48 h induced a weak increase in G2/M phase accompanied by a decrease of G0/G1 cell population in a dose-dependent manner. The cells in G2/M (%) were increased to 2.0 and 3.1-fold over the vehicle control when the cells were treated with 5 μmol/l of cynaropicrin for 24 and 48 h, respectively (Figs. 5A, C). Treatment of cynaropicrin at 5 μmol/l and arctigenin at 200 μmol/l also induced remarkable morphological changes such as membrane blebbing and formation of apoptotic bodies (Fig. 6).

Quinone Reductase Induction of *S. salicifolia* in Hepa 1c1c7 Cells To evaluate the induction of phase II detoxification enzyme by the extract, fractions and compounds obtained from *S. salicifolia*, we measured induction of QR activity in Hepa 1c1c7 cells which is known to be a biomarker for cancer chemoprevention. We also measured cytotoxicity along with the QR activity so that we can calculate chemoprevention index (CI) values (Table 1). CI value is a well-known screening tool for finding potential chemopreventive agents and is defined as IC50 value/CD value.\(^{13}\) CD value means a concentration for doubling QR activity. The extract induced a dose-dependent cell growth inhibition in Hepa 1c1c7 cells, with an IC50 value of 116.96 μg/ml, and the CD value was 17.79 μg/ml, leading to a CI value of 6.58. The AcOEt fraction among the all five fractions showed the most potent chemopreventive activity with a CI value of 9.88. The CH2Cl2 fraction among the fractions showed the highest QR activity, with a CD value of 2.34 μg/ml, however the CI value turned out to be low due to a strong cytotoxicity with an IC50 value of 8.40 μg/ml. Arctigenin, trachelogenin and matairesinol among the seven major compounds exerted the potent chemopreventive effect showing a high QR activity with a low cytotoxicity. Cynaropicrin had a low chemopreventive effect due to the strong cytotoxicity in spite of the potent QR induction.

DISCUSSION

We found that the ethanolic extract of the aerial part of *S. salicifolia* as well as its compounds had cancer chemopreventive effects including antiproliferation, apoptosis and QR induction. The extract had a potent antiproliferative activity both in AGS cells and Hepa 1c1c7 cells, and it also induced an apoptosis at the concentration of 30 μg/ml. The extract at the concentration of 60 μg/ml increased the necrotic cell population in company with apoptotic cell population in AGS cells. Necrosis, as opposed to apoptosis is a nonspecific mode of cell death, often caused by excessively high concentrations of apoptosis inducing agents.\(^{14,15}\) The cynaropicrin and arctigenin among the seven major compounds showed the most potent antiproliferative activity as well as apoptosis in AGS cells. Thus, cynaropicrin and arctigenin are thought to be responsible for causing anti-proliferation and pro-apoptotic activity of *S. salicifolia* total extract in AGS cells. In this study, we evaluated the chemopreventive activity of the *S. salicifolia* extract and its major compounds according to their CI values which is defined as IC50 value/CD value.
Potential chemopreventive agents show high CI values resulted from strong QR induction with low cytotoxicity in mammalian cells. Although the CH₂Cl₂ fraction caused the highest QR induction in the all five fractions, this fraction had a strong cytotoxicity resulting in the final chemopreventive activity was relatively low. The CH₂Cl₂ fraction contained cynaropicrin, trachelogenin, matairesinol, arctigenin and arctiin as major compounds, and cynaropicrin could be responsible for the cytotoxicity (IC₅₀ = 0.68 μg/ml) of this fraction. Arctigenin, trachelogenin and matairesinol showed a potent chemopreventive activity, and the arctigenin showed the highest CI value among the 7 compounds tested.

Cynaropicrin is known to have cytotoxic effect in various human cancer cell lines and induce apoptosis in leukocyte cancer cells. In our study, cynaropicrin showed a dose dependent inhibition of cell proliferation in both human gastric adenocarcinoma AGS cells and mouse hepatoma Hepa 1c1c7 cells. Cynaropicrin at 5 μmol/l also induced an apparent apoptosis in AGS cells proved by the increase of the sub G0/G1 content (%) and apoptotic morphological changes. Cynaropicrin is also known to induce cell cycle arrest at G1/S phase in hydroxyurea synchronized leukocyte cancer U937 cells. However, in this study, we were not able to find the G1/S phase arrest in AGS cells treated with cynaropicrin in the absence of synchronization. Instead, treatment of cytotoxins...
naropicrin for any of 24 and 48 h induced a little increase in G2/M phase accompanied by a decrease of G0/G1 cell population in a dose-dependent manner. As a similar case, Sausurea lappa extract which contains costunolide as an active compound has been reported to induce G2/M arrest and apoptosis in AGS cells.18) According to the previous reports, arctigenin has a cytotoxicity against to the various cancer cell lines, and induces apoptosis in leukemic cells and colorectal cancer cells in vitro.21—24) Arctigenin also has the ability to eliminate the tolerance of cancer cells to nutrient starvation, and therefore, has anti-tumor promoting activity in vivo.25,26) In our study, arctigenin showed a cytotoxicity against both AGS and Hepa 1c1c7 cells in a dose-dependent manner. Arctigenin at 200 μmol/l also induced an apparent apoptosis by showing the increase of the sub G0/G1 content and apoptotic morphology in AGS cells. We also found that, for the first time, dibenzyl buty lactone plant lignans; arctigenin, trachelogenin and matairesinol present in S. salicifolia induced QR activity. Generally aglycones (trachelogenin, matairesinol and arctigenin) were more cytotoxic than their lignan glycosides (tracheloside, matairesinol and arctiin) isolated from S. salicifolia against both AGS and Hepa 1c1c7 cells. Arctigenin among the lignan aglycones and lignan glycosides showed the most potent antiproliferative activity, and matairesinol and trachelogenin showed moderate cytotoxicity in AGS cells. In addition to cytotoxicity, aglycones also showed a stronger induction of QR compared to glycosides in Hepa 1c1c7 cells. This phenomenon is thought to be due to the difference of membrane permeability originated from the hydrophilicity of glucose connected by O-glycosidic bond. Although lignan glycosides are hydrolyzed to their aglycones by the β-glycosidase is widespread in mammalian intestinal microflora,28,29) membrane permeability of the lignan glycosides still important to explain the pharmacological activity in drug screening methods using in vitro cell culture system. We are currently investigating the membrane permeability and metabolism of lignans and lignan glycosides in various cell lines.

In conclusion, arctigenin alone or combination with other lignan compounds exerted chemopreventive effects including induction of phase II detoxification enzyme and of apoptosis in vitro cellular models. Based on these findings, we suggest that bioactive components of S. salicifolia may serve as potential chemopreventive agents in the prevention or treatment

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Table 1. The Induction of Quinone Reductase Activity of the Extract, Fractions and Compounds Acquired from S. salicifolia in Hepa 1c1c7 Cells

<table>
<thead>
<tr>
<th>Chemical tested</th>
<th>IC50 (μg/ml)</th>
<th>CD50 (μg/ml)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>116.96</td>
<td>17.79</td>
<td>6.58</td>
</tr>
<tr>
<td>Water fraction</td>
<td>&gt;200.00</td>
<td>255.08</td>
<td>&gt;0.78</td>
</tr>
<tr>
<td>n-BuOH fraction</td>
<td>71.19</td>
<td>22.02</td>
<td>3.23</td>
</tr>
<tr>
<td>CH2Cl2 fraction</td>
<td>8.40</td>
<td>2.34</td>
<td>3.60</td>
</tr>
<tr>
<td>AcOEt fraction</td>
<td>116.08</td>
<td>11.75</td>
<td>9.88</td>
</tr>
<tr>
<td>n-Hexane fraction</td>
<td>100.50</td>
<td>15.36</td>
<td>6.54</td>
</tr>
<tr>
<td>Cynaropicrin</td>
<td>1.96</td>
<td>0.51</td>
<td>3.85</td>
</tr>
<tr>
<td>Tracheloside</td>
<td>&gt;200.00</td>
<td>63.68</td>
<td>&gt;3.14</td>
</tr>
<tr>
<td>Matairesinoside</td>
<td>&gt;200.00</td>
<td>111.75</td>
<td>&gt;1.79</td>
</tr>
<tr>
<td>Arctin</td>
<td>&gt;200.00</td>
<td>58.47</td>
<td>&gt;3.42</td>
</tr>
<tr>
<td>Trachelogenin</td>
<td>154.71</td>
<td>21.89</td>
<td>7.07</td>
</tr>
<tr>
<td>Matairesinol</td>
<td>88.29</td>
<td>12.70</td>
<td>6.95</td>
</tr>
<tr>
<td>Arctigenin</td>
<td>115.70</td>
<td>15.27</td>
<td>7.57</td>
</tr>
</tbody>
</table>

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Fig. 6. Morphological Changes of AGS Cells Treated with Cynaropicrin and Arctigenin

Phase contrast microscope images were photographed after treatment of cynaropicrin (1 to 5 μmol/l) and arctigenin (50 to 200 μmol/l) for 24 h. The magnified pictures of apoptotic cells showing membrane blebbing and apoptotic bodies, which were observed in the treatment of cynaropicrin at 5 μmol/l and arctigenin at 200 μmol/l, are also represented on the right side of each original picture (bar=20 μm).
of human cancers. However, further in-depth studies including clinical trials are needed to fully evaluate the value of these active components for cancer chemoprevention.

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