Inhibition of 7,12-Dimethylbenz[a]anthracene-Induced Mouse Skin Carcinogenesis by Artemisia capillaris

Y.S. Kim, K.N. Bahn, C.K. Hah, H.I. Gang, and Y.L. Ha

ABSTRACT: Anticarcinogenic activity of medicinal herbs (Artemisia capillaris, Taxus cuspidata, Anthriscus sylveatris, and Curcuma longa) was examined for 7,12-dimethylbenz[a]anthracene (DMBA)-induced mouse skin carcinogenesis. Four types of solvent fractions (hexane, chloroform, ethyl acetate, and butanol) were prepared from the methanolic extract of medicinal herbs. The cytotoxicity and anticarcinogenic activities of solvent fractions were examined for mouse leukemia L1210 cancer cells and for female ICR mouse epidermal carcinogenesis induced by DMBA, respectively. The chloroform fraction of Artemisia capillaris, Taxus cuspidata, and Anthriscus sylveatris was more toxic to L1210 cells than other solvent fractions. The chloroform fraction of Artemisia capillaris markedly reduced the number of tumors/mouse and tumor incidence relative to that of other medicinal herbs tested. Major active chemical constituents in the chloroform fraction of Artemisia capillaris were found to be camphor, 1-borneol, coumarin, and achillin when analyzed by TLC and GC-MS. These results suggest that Artemisia capillaris was the most effective anticarcinogenic medicinal herb for DMBA-induced mouse epidermal carcinogenesis among 4 medicinal herbs tested, and the effect might be attributed to chemical compounds of camphor, 1-borneol, coumarin, and achillin.

Keywords: anticarcinogenicity, Artemisia capillaris, cytotoxicity, medicinal herbs

Introduction

Over the past decade, intensive research has been focused on the investigation of natural anticarcinogenic compounds. Oriental medicinal herbs have been used as materials for the investigation of cancer agents (Cassady and others 1990; Darzyankiewicz and others 2000; Kummalue 2005; Issa and others 2006; Lee and others 2006). The role of anticancer agents derived from oriental medicinal herbs has been intensively investigated for cancer causing factors such as chemicals, radiation, virus, hormone unbalance, and aging (Wattenberg 1983; Wu and others 2001).

The most popularly used medicinal herbs in Korea were found to be Artemisia capillaris, Anthriscus sylveatris, Taxus cuspidata, and Curcuma longa, among the plenty of oriental herbs. Generally, it is known that the young leaves of Artemisia capillaris have been used as food materials and herbal medicine sources to cure chronic and acute liver cirrhosis and liver cancers (Hu and others 2000; Koo and others 2002; Han and others 2006; Yang and others 2007). The essential oil of Artemisia capillaris exhibited bactericidal, insecticidal, and antimutagenic effects (Kim and others 1991; Kim and others 1992; Cha and others 2005; Shen and others 2007). Taxus cuspidata has been used as a folk medicine to treat inflammation, cancer, and diabetes, and taxol, a chemical compound isolated from stem barks of Taxus cuspidata, is the most effective anticancer agent for breast and ovarian cancers (Vidensek and others 1990; Nicolaou and others 2005; Nishinaka and others 2007). In spite of the biological activity of these medicinal herbs, there is little known scientific data to explain their biological activities. Hence, it is of significance to create scientific data for these medicinal herbs to use as herbal medicines to treat and prevent chronic diseases.

In the present study, the anticarcinogenicity of 4 wild grown medicinal herbs, Artemisia capillaris, Taxus cuspidate, Anthriscus sylveatris, and Curcuma longa, in Korea is investigated against 7,12-dimethylbenz[a]anthracene (DMBA)-induced mouse skin carcinogenesis.

Materials and Methods

Materials

Artemisia capillaris, Taxus cuspidata, Anthriscus sylveatris, and Curcuma longa were purchased in January 2006 from a market in Jinju (Korea). Dimethylsulfoxide (DMSO), DMBA, 12-tetradecanoylphorbal 13-O-acetate (TPA), and trypan blue were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Mouse leukemia 1210 (L1210) and Sarcoma 180 (S-180) cells were obtained from Korean Cell Line Bank (Seoul, Korea). The Fisher’s medium containing 10% horse serum and Dulbecco’s Modified Eagle Medium/Ham’s F-12 nutrient mixture (DMEM/F-12) were obtained from Gibco BRL (Grand Island, N.Y., U.S.A.). Female ICR mice (5 wk of age) and Chow diet were purchased from Life Science...
Anticarcinogenic activity of *Artemisia capillaris*... 

(Deagu, Korea). The TLC plate (Kiesel gel 60 F254 plate) was obtained from Merck (Damstadt, Germany). All other chemicals used were ACS grade.

### Preparation of solvent fractions

Samples of medicinal herbs (< 5% moisture content) were ground to pass a sieve (100 mesh) with an IKA Ultra turrax Mill (Staufen, Germany). The fine sample (50 g) was extracted for 12 h at 80 °C in a refluxing extractor with methanol (500 mL). The methanol extract was concentrated at 50 °C by a vacuum evaporator (EYELA N-1000; Tokyo, Japan) after filtration. The concentrate (10 g) was diluted with distilled water (50 mL), followed by fractionation into hexane, chloroform, ethyl acetate, and butanol fractions according to the method described by Bahn and others (1995).

### Preparation of a terpenoid fraction

Solvent fraction (5 g) was dissolved into 95% ethanol (10 mL), and then reacted with lead acetate, according to the methods mentioned by Guo and others (1992). The reaction mixture was centrifuged at 3000 rpm (4 °C) for 10 min, followed by concentrating under vacuum evaporator (EYELA N-1000; Tokyo, Japan). The concentrate, which is a terpenoid fraction, was further separated on a precoated TLC silica gel plate with a CHCl₃:acetone (4:1 [v/v]) mixture. The separation on TLC plate was visualized under a UV lamp (San Gabriel, Calif., U.S.A.).

### Separation and identification of compounds in a terpenoid fraction

Samples were analyzed by GC (HP 5890 series II; Little Fall, Tex., U.S.A.) equipped with a HP-5 capillary column (50 m × 0.32 μm) and FID detector. Oven temperature was programmed from 50 to 250 °C at 4 °C/min. Nitrogen was used for carrier gas. Flow rate of carrier gas was 2 mL/min. Compounds were tentatively identified by GC-MS (QP2010, Shimadzu; Kyoto, Japan) equipped with a HP-5 capillary column (50 m × 0.32 μm). Identification of compounds was performed by the comparison of mass spectral data with the database of Willeynbs Library.

### Cytotoxicity test

The L1210 were cultured in the Fisher’s medium containing 10% horse serum. The S-180 cells activated in female ICR mouse ascites were cultured in the DMEM/F-12 medium. Cells were cultured at 37 °C in a humidified atmosphere with 5% CO₂. Culture medium was prepared in 1L distilled water by adjusting pH to 7.4, followed by filtering through a sterilized membrane filter (0.20 μm) before use. Both L1210 and S-180 cells were cultured for 48 h to the logarithmic phase (0.8 × 10⁵ cells/mL).

The cells were seeded into a 24-well culture plate at a density of 1.5 × 10⁴ cells/0.5 mL well containing the test sample (0, 15, 30, and 60 μg/60 μL culture medium containing 6 mL DMSO), followed by adjusting total volume to 3 mL with culture medium. At 24 h after incubation, cell culture (50 μL) was treated with 0.2% trypsin blue solution (50 μL), and viable cell numbers were counted by hemocytometer (Bahn and others 1995). The results were expressed as ED₅₀ values (Lee and others 1986).

### Skin carcinogenesis

Mouse skin carcinogenesis induced by DMBA was conducted according to the regimen described by Ha and others (1987). Female ICR mice (25 ± 2 g, 5 wk of age) were randomly assigned to treatment groups of 20 mice each. Mice were housed in polycarbonate cages (5 mice/cage) with sterilized wood chips and maintained in a light- and temperature-controlled room. Mice were fed with pellet diet *ad libitum*. Room temperature was 23 ± 1 °C, and humidity was adjusted to 50%. One week after receipt (3 d before experimentation), the back hair of dorsal skin area of each mouse was shaved. Sample in 0.2 mL acetone or acetone alone was applied on the shaved backs of individual mice 3 times (20 mg/mouse at 7 d, 20 mg/mouse at 3 d, 10 mg/mouse at 5 min) prior to application of DMA (50 nmol/mouse) in 0.2 mL acetone. One week later, twice weekly application of TPA (6 μg/mouse) in 0.2 mL of acetone was commenced, and continued for the remainder of the experiment. The number of papillomas/mouse and tumor incidence was recorded at weekly intervals, beginning at the 1st week of appearance of tumors. Body weight of mouse was measured through whole experimental periods at weekly intervals.

### Statistical analysis

Results were presented as means ± SD. Data were analyzed by Duncan’s multiple range test or *t*-test (Ott 1984).

### Results and Discussion

#### Cytotoxicity of medicinal herbs

Attempts have been made to identify many chemotherapeutic agents such as dactinomycin, mithraycin, bleomycin, and taxol from herbal plants. These agents were directly purified from herbal plants or semisynthesized from precursors of the target agents. The most important step to identify such anticancer agents is to screen active medicinal herbs from a variety of herbal plants. For this purpose, NCI has used L1210 cells (Natl. Cancer Inst. 1972). In the present study, L1210 cell was also used to screen the medicinal herbs for anticarcinogenic activity assay against a DMBA-induced mouse skin carcinogenesis model.

Table 1 shows the cytotoxicity (ED₅₀) of solvent fractions derived from 4 folk medicinal herbs against L1210 cells. Among the fractions tested, the chloroform fractions of *Taxus cuspidata*, *Anthriscus sylveatris*, and *Artemisia capillaris*, and the hexane fraction of *Curcuma longa*, exhibited a strong cytotoxicity with ED₅₀ values of 0.02, 0.02, 1.84, and 0.07 μg/mL, respectively. Taken together the cytotoxicity data, the chloroform fraction of *Taxus cuspidata*, *Anthriscus sylveatris*, and *Artemisia capillaris*, and the hexane fraction of *Curcuma longa*, were selected to investigate anticarcinogenic activities against DMBA-induced mouse skin carcinogenesis.

#### Anticarcinogenic activity of medicinal herbs

Anticarcinogenic activities of chloroform fractions from *Taxus cuspidata*, *Anthriscus sylveatris*, and *Artemisia capillaris*, and hexane fraction from *Curcuma longa*, which exhibited a strong cytotoxicity against L1210, were examined for the mouse skin carcinogenesis induced by DMBA, and the results are shown in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Butanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Artemisia capillaris</em></td>
<td>12.31ab</td>
<td>1.84*</td>
<td>&gt;20.00*</td>
<td>15.20b</td>
</tr>
<tr>
<td><em>Anthriscus sylveatris</em></td>
<td>1.30a</td>
<td>0.02</td>
<td>2.46*</td>
<td>&gt;20.00*</td>
</tr>
<tr>
<td><em>Taxus cuspidata</em></td>
<td>0.23a</td>
<td>0.02</td>
<td>&gt;20.00*</td>
<td>&gt;20.00*</td>
</tr>
<tr>
<td><em>Curcuma longa</em></td>
<td>0.07a</td>
<td>&gt;20.00*</td>
<td>13.55*</td>
<td>&gt;20.00*</td>
</tr>
</tbody>
</table>

*ED₅₀ value (μg/mL) was the average of 3 experimental data and standard deviation (SD) was less than 5% of the average value. Means with same lowercase superscript letters in same row are not statistically different at *P* < 0.05 by Duncan’s multiple test.*
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Figure 1. The cumulative papilloma per mouse was greatly reduced in mice treated with the chloroform fraction of *Artemisia capillaris*. The efficacy of this fraction was much stronger than that of the chloroform fraction of *Taxus cuspidata*, containing a taxol chemical that is a strong anticancer agent for breast cancer. The chloroform fraction from *Anthriscus sylvestris* and hexane fraction from *Curcuma longa* reduced the cumulative papilloma per mouse, but the efficacy of these fractions was lower than that of *Artemisia capillaris* and *Taxus cuspidata*. Similar results were seen in the tumor incidence data as shown in Figure 2. Mice treated with the chloroform fraction of *Artemisia capillaris* showed lower tumor incidence, not statistically different, than mice treated with chloroform fractions of *Taxus cuspidata* and *Anthriscus sylvestris*, and hexane fraction of *Curcuma longa*. Body weight of mice in treatment groups was not significantly different from that of control mice (Table 2).

It is evident that the chloroform fraction of *Artemisia capillaris* exhibited the strongest anticarcinogenic effect, which showed fewer papillomas per mouse and lower tumor incidence over a period of 19 wk than the control and other treatments. The number of tumors per mouse in mice treated with chloroform fraction of *Artemisia capillaris* was reduced to 50% as compared to 20% in mice treated with chloroform fraction of *Taxus cuspidata* (Figure 1). Young leaves of *Artemisia capillaris* have been used as food materials, while old leaves have been used as herbal medicines in Korea, Japan, China, Germany, and many other countries. Previous reports indicate that *Artemisia capillaris* exhibited antiproliferative activity for liver cancers (Hu and others 2000; Koo and others 2002; Yang and others 2007), but no reference on skin carcinogenesis was found in literature. Hence, it is interesting information that the chloroform fraction of *Artemisia capillaris* exhibits a strong anticarcinogenic activity against DMBA-induced mouse skin carcinogenesis.

Active compounds in *Artemisia capillaris*

As a validation of this anticarcinogenic finding, active compounds could be identified from the chloroform fraction of *Artemisia capillaris*. Many bioactive compounds from plant sources are derived from their terpenoid fractions (Guo and others 2012); hence, a terpenoid fraction was isolated from the chloroform fraction of *Artemisia capillaris*. As can be seen in Table 3, the cytotoxicity (ED$_{50}$) of the terpenoid fraction for L1210 and S-180 cells was further elevated, relative to the cytotoxicity of the chloroform fraction. Three fractions (F1, 2, and 3) were separated from the terpenoid fraction by a silica gel TLC, and the cytotoxicity of these fractions against L1210 and S-180 cells is shown in Table 4. ED$_{50}$ values of F1, F2, and F3 fractions for L1210 cells were found to be 0.06, 1.15, and 0.93 µg/mL respectively, indicating that F1 is the most effective cytotoxic fraction. The cytotoxicity of F1 on S-180 cells (ED$_{50}$ = 0.08) was also significantly higher relative to that of F2 and F3 fractions.

The F1 fraction was subjected to GC-MS analysis to identify chemical constituents, and the results are shown in Table 5. Chemical compounds of camphor, borneol, coumarin, and achillin were tentatively identified as major compounds. Based on peak area of GC data, the relative area of achillin and coumarin was found to be 38% and 31%, respectively. Coumarin and its derivatives, including aesculetin, scopoletin, psoralen, angelicin, xanthyletin, seselin, alloxanthoxyletin, demethylsuberisin, ostheno, dicoumarol,

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Table 2—Mouse body weight at week 19 after promotion.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.3 ± 1.5$^{a,b}$</td>
</tr>
<tr>
<td><em>Curcuma longa</em></td>
<td>36.4 ± 1.4$^a$</td>
</tr>
<tr>
<td><em>Taxus cuspidata</em></td>
<td>35.5 ± 1.2$^a$</td>
</tr>
<tr>
<td><em>Anthriscus sylvestris</em></td>
<td>35.8 ± 1.6$^a$</td>
</tr>
<tr>
<td><em>Artemisia capillaris</em></td>
<td>35.8 ± 1.6$^a$</td>
</tr>
</tbody>
</table>

$^{a,b}$Hexane fraction of *Curcuma longa* and chloroform fractions of *Taxus cuspidata*, *Anthriscus sylvestris*, and *Artemisia capillaris*. $^{a}$Mean ± SD of 20 mice. Means with same lowercase superscript letters in the column are not statistically different by Duncan’s multiple test.

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Figure 1—Cumulative papillomas of mice treated with control (closed circles), the chloroform fraction of *Artemisia capillaris* (closed squares), the chloroform fraction of *Taxus cuspidata* (open triangles), the chloroform fraction of *Anthriscus sylvestris* (open circles), and the hexane fraction of *Curcuma longa* (closed triangles). Each data point was the average number of papilloma per mouse in the treatment group, and standard deviation (SD) was less than 5% of the average value. The cumulative papillomas per mouse of the chloroform fraction of *Artemisia capillaris* (closed squares) was significantly different at $P < 0.05$ in the 13th wk of treatment, and significantly different at $P < 0.01$ in the 19th wk from control and other treatments.

Figure 2—Tumor incidence of mice treated with control (closed circles), the chloroform fraction of *Artemisia capillaris* (closed squares), the chloroform fraction of *Taxus cuspidata* (open triangles), the chloroform fraction of *Anthriscus sylvestris* (open circles), and the hexane fraction of *Curcuma longa* (closed triangles). Each data point represented the percentage of tumor-bearing mice to 20 mice in given treatment group.
Anticarcinogenic activity of *Artemisia capillaris* . . .

daphnoretin, surangin B, and isopimpinellin, were also studied for the various biological activities such as anticarcinogenic effect (Leu and Maa 2002; Elinos-Baez and others 2005) and insecticidal and antibacterial effect (Shin and Chi 1979; Jojhi and others 1989). The biological activity of achillin, a sesquiterpene lactone, has been reported as anti-inflammatory radical scavenger (Egelseer and others 1991); however, no reference on the anticarcinogenic activity was available in the literature. At the present time, it is difficult to isolate and obtain a large amount of pure achillin to conduct an animal experiment; hence, further research should be pursued to determine the feasibility of this potentially promising anticarcinogenic compound.

Although the anticarcinogenic activity of *Artemisia capillaris* observed from the present study could be attributed to many chemical constituents, such as scopoletin, capillarins, 6,7-dimethyleucatene, caffeic acid, chlorogenic acid, phenol, cresol, eugenol, and ethylphenol, previously reported in *Artemisia capillaris* (Sheu and others 2001; Elinos-Baez and others 2005), the major contribution would be derived from the efficacy of each of camphor, 1-borneol, coumarin, and achillin, and/or from the combined efficacy of each of 4 compounds identified. Our data prompt the need for future clinical trials for cancer remedy with the purified terpenoid fraction, chloroform fraction, or methanol extract of *Artemisia capillaris*.

### Table 3—ED₅₀ values of chloroform and terpenoid fractions obtained from *Artemisia capillaris* for L1210 and S-180 cells.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>L1210 cell⁷</th>
<th>S-180 cell⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform⁶</td>
<td>1.95 ± 0.12⁷</td>
<td>2.12 ± 0.08⁷</td>
</tr>
<tr>
<td>Terpenoid⁶</td>
<td>1.07 ± 0.05⁷</td>
<td>2.94 ± 0.11⁷</td>
</tr>
</tbody>
</table>

⁶L1210 cell was cultured in Fisher's media.
⁷S-180 cell was cultured in DMEM/F-12 media.
⁶Chloroform fraction was prepared by solvent fractionation from methanol extract of *Artemisia capillaris*.
⁷Mean ± SD. Means with the different lowercase superscript letters in the same column are statistically different at *P* < 0.05 by *t*-test.

### Table 4—ED₅₀ values of the fractions separated from the terpenoid fraction of *Artemisia capillaris* for L1210 and S-180 cells.

<table>
<thead>
<tr>
<th>Fraction⁸ (RI value)</th>
<th>L1210 cell⁷</th>
<th>S-180 cell⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₁(0.83)</td>
<td>0.06§</td>
<td>0.09§</td>
</tr>
<tr>
<td>F₂(0.71)</td>
<td>1.15†</td>
<td>6.54*</td>
</tr>
<tr>
<td>F₃(0.47)</td>
<td>0.93*)</td>
<td>&gt;20.00§</td>
</tr>
</tbody>
</table>

⁸Fractions on a TLC plate were detected under a short wave UV light and the parenthesis represented an RI value.
⁹L1210 cell was cultured in Fisher's media.
⁷S-180 cell was cultured in DMEM/F-12 media.
⁸ED₅₀ value (μg/mL) was the average of 3 experimental data and standard deviation (SD) was less than 5% of the average value. Means with the different lowercase superscript letters in the same column are statistically different at *P* < 0.05 by Duncan’s multiple test.

### Table 5—Compounds identified from F₁ fraction of terpenoid in *Artemisia capillaris*.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Compounds</th>
<th>Area (%)⁹</th>
<th>M⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:33</td>
<td>Camphor</td>
<td>14</td>
<td>152</td>
</tr>
<tr>
<td>11:37</td>
<td>Borneol</td>
<td>17</td>
<td>154</td>
</tr>
<tr>
<td>21:54</td>
<td>Coumarin</td>
<td>31</td>
<td>146</td>
</tr>
<tr>
<td>40:56</td>
<td>Achillin</td>
<td>38</td>
<td>246</td>
</tr>
</tbody>
</table>

⁹Area percent of a given peak area to total peak areas on GC chromatogram.

### Conclusions

*Artemisia capillaris* was found to be a potent anticarcinogenic medicinal herb assayed by DMBA-induced mouse skin carcinogenesis. The cytotoxic terpenoid fraction of *Artemisia capillaris* contained major chemical compounds such as amphot, 1-borneol, coumarin, and achillin. The anticarcinogenic activity of the chloroform fraction of *Artemisia capillaris* observed in the present study might be attributed from the combined effects of these 4 compounds and other minor constituents in *Artemisia capillaris*. Further research should be conducted to clarify the anticarcinogenic activity of *Artemisia capillaris*.

### Acknowledgments

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### References


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