Production of Choleric Substances in the Capitulum, Leaf and Stem of *Artemisia capillaris* during the Plant Growth Cycle

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Five *Artemisia capillaris* plants were selected at random from among a bed of twenty plants cultivated in a field for one year. Several branches were collected from each plant once a month, except in August and October, when two collections were made during budding and flowering. Each organ (leaf, stem, and capitulum) was separated from the branch and dried, and later analyzed for capillarisin and 6,7-dimethylesculetin content by high-performance liquid chromatography (HPLC).

The capillarisin and 6,7-dimethylesculetin content reached maximum levels in the leaf just before the appearance of flower buds at end of July. About one month later, at the end of August during budding and flowering, capillarisin content in the capitulum reached a peak and then decreased. On the other hand, 6,7-dimethylesculetin content reached a maximum at the beginning of September, two weeks after the capillarisin maximum.

The results suggest that the most appropriate time to harvest *A. capillaris* for use as a crude drug is between the flower bud stage and early flower stage, from late August to early September.

Keywords *Artemisia capillaris*; choleric substance; Inchinko; crude drug; capillarisin; 6,7-dimethylesculetin

The crude drug Inchinko, consisting of the capitulum of *Artemisia capillaris* THUNB. (Compositae), has been used for anti-inflammatory, antipyretic and diuretic purposes, as well as for liver protection in Oriental medicine.¹⁻³⁾ A number of terpenoid acids, polyenes, phenols, coumarins, chromones and flavonoids have been identified as the constituents of this crude drug.^{2,4,5)} Concerning the pharmacological properties of these constituents, capillarisin and 6,7-dimethylesculetin were reported to exhibit choleric effects.⁶⁻⁸⁾ Recently, the presence of a component promoting root growth in rice was reported.⁹⁾ *A. capillaris* exhibited activity mainly through direct tumoricidal action.^{10,11)}

Although this plant is medicinally useful, it has never been cultivated and bred, and the crude drug has been derived only from wild plants. The present work was initiated to clarify seasonal variations in the content of capillarisin and 6,7-dimethylesculetin, which are the main choleric substances, in the leaves, stems and capitulum at various points throughout the plant's growth cycle to determine the most suitable harvest time for medicinal applications.

MATERIALS AND METHODS

Plant Materials and Agriculture Seeds of *A. capillaris* THUNB. obtained from a field in Nagasaki were sown in a container in March 1988. In May, when the plants had five leaves, they were transplanted to an open field in the Medicinal Botanical Garden of Nagasaki University and cultivated for two years. Five plants among twenty were selected at random and marked. From these, several branches were harvested at different growth stages between April and October 1989. Different parts of the harvested branches—leaf, stem and capitulum at budding and flowering—were separated and dried at 60°C for 48 h in a drying oven for chemical analysis.

Chemical Analysis 0.5 g of pulverized material (leaf,

stem, and capitulum) was dried at 40°C and soaked for about 10 h in 100 ml of methanol at room temperature. After filtration of the extract, the procedure was repeated. The filtrates were combined and evaporated to 10 ml. About 1 ml of this solution was filtered through a 0.45 µm membrane filter (Chromatodisc, #13A, Kurashikibouseki), and 20 µl was subjected to HPLC analysis, as described previously.¹²⁾

HPLC Apparatus: Pump, LC-6A (Shimadzu), injector Model 17125 (Rheodyne); column, Cosmosil C8 (Nakalai) 15 × 0.6 cm i.d.; eluent, CH₃CN–H₂O–H₃PO₄ (30:70:0.1, v/v/v); injection volume, 20 µl; flow rate, 1 ml/min; detection, UV 280 nm (SPD-6A, Shimadzu).

Calibration curves were prepared with a standard methanol solution containing capillarisin (Wako) and 6,7-dimethylesculetin (Wako). Plots were linear over a range of 1–12 µg/injection. Peak heights were plotted against component concentrations. The correlation coefficients for capillarisin and 6,7-dimethylesculetin were

Fig. 1. Capillarisin Content per Dry Weight of Leaf, Stem and Capitulum in *Artemisia capillaris* at Different Stages of Development

●, capitulum; ■, leaf; ▲, stem. Bars indicate standard error.

Fig. 2. 6,7-Dimethylesculetin Content per Dry Weight of Leaf, Stem and Capitulum in *Artemisia capillaris* at Different Stages of Development

●, capitulum; ■, leaf; ▲, stem. Bars indicate standard error.

0.987 and 0.999, respectively.

RESULTS AND DISCUSSION

The capitula of *A. capillaris* appeared in the middle of August, and opened several days later. The time course of capillarisin content in the organs is shown in Fig. 1. Although capillarisin content per dry weight in the leaf, and the capitulum at budding and flowering, varied for each assay, the time course showed almost the same tendency in each organ. Capillarisin content per dry weight in the leaf increased throughout the growth cycle until flower buds were formed, and then it decreased. The content in the stem did not change very much throughout the experimental period. Capillarisin content in the capitulum was significantly less in the immature stage, reaching a maximum concentration about one week after

flower bud formation.

Although 6,7-dimethylesculetin content per dry weight in the leaf began to increase about one month later than capillarisin, it reached a maximum content at same period. In capitulum, it reached a maximum content two weeks later than capillarisin.

The findings suggest that the most suitable time for harvesting the crude drug is in the flower bud stage in the capitulum to the early flower stage, between late August and early September.

It is interesting to note that capillarisin and 6,7-dimethylesculetin both reached maximum concentrations in the leaf at the point when the flower buds were formed in the capitulum. This suggests that flower bud formation might somehow be mediated by these compounds.

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