Anatomical and Chemical Variations of Medicinal Plants: Related with Geographical Changes

Anatomical and Chemical Variations of Medicinal Plants Related with Geographical Changes

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Keywords: plant histology, plant chemistry, geographical change, cline, Himalayas.

Abstract *To clarify geographical effects on the inner structure and chemical constitution of herbal medicines, Ephedra and Berberis plants from the Himalayas were studied. A histological study of Ephedra plants indicated that they had geoclines in some anatomical characters related to longitude. Besides, the result of chemical study suggested that the differences in chemical constituents was to be attributed not only to genetic factors but also to the differences in the growing habitats, such as altitude, or atmospheric temperature.*

Medicinal plants have wide variations in chemical constituents, and this kind of variation is thought to be genetically controlled. On the other hand, in the Chinese traditional medicine, it has been said that the gathering district of medicinal plant is an important information to evaluate the medicinal power of it.

In this report, to study the geographical effects in anatomical characters and chemical component contents of *Ephedra* plants collected from Himalayas, the correlation between each character and geographical changes such as longitude and altitude of the collecting places of the experimental materials are examined. Moreover, altitudinal variation in berberine content of *Berberis* plants is examined.

《Studies on *Ephedra* Plants》

Ephedrae herba, the herbal stem of *Ephedra* species. of the family Ephedraceae, is well known not only as a Chinese drug "Ma-huang" but also as the plant characteristically containing an alkaloid component, ephedrine, which is used as an asthmatic remedy in Western medicine.

(*Anatomical Study*)

Materials

About 150 plant materials, identified as *Ephedra gerardiana* Wall. ex Stapf and *E. pachyclada* Boiss., were collected mainly by Mikage M. *et al.* and Suzuki M. *et al.* in

Nepal, Sikkim and Bhutan in the summer of 1983, 1993, 1994, and 1995.

Method

The cross sections of three internodes of herbal stems arbitrarily chosen from each plant are observed under the microscope, and the average value of the three is obtained on the variable anatomical characteristics such as diameter of stem, the number of subepidermal fiber bundles, maximum number of fibers in a subepidermal fiber bundle, the number of cortical fiber bundles, and the number of fibers in the peripheral part of pith. And the correlation between these values and the longitude and altitude of the collecting points of experimental materials are examined.

Result and discussions (Fig. 1)

To the result of studying the geographical variations of the inner structures of the herbal stems of *Ephedra gerardiana* related to longitude between Mid. Western Nepal and Bhutan, the geocline that the western samples had bigger stems in diameter and more subepidermal fiber bundles was observed, the correlation coefficient (C.C.) is -0.521 and -0.437, respectively. Moreover, the anatomical local variations were recognized; i.e. the most of the plants growing in Mid. Western Nepal had characteristically more than 40 cortical fibers, and those in Sikkim and Bhutan had more than 50 fibers in a subepidermal fiber bundle. Besides, studying the variation related to altitude on the samples of *E.pachyclada*, the tendencies that the plants growing at higher places had less subepidermal fiber bundles, more fibers in a subepidermal fiber bundle, and more fibers in the pith, were recognized.

The variation related to altitude and latitude is thought to be caused by the change of temperature, beside the factors of the variation with longitude is not sure.

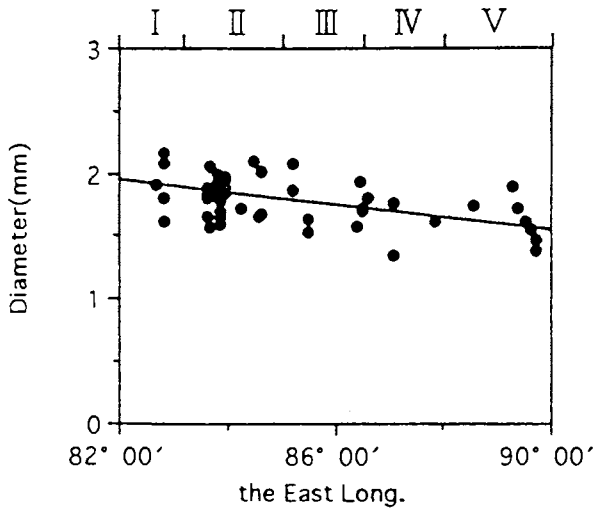
(Chemical Study)

Materials

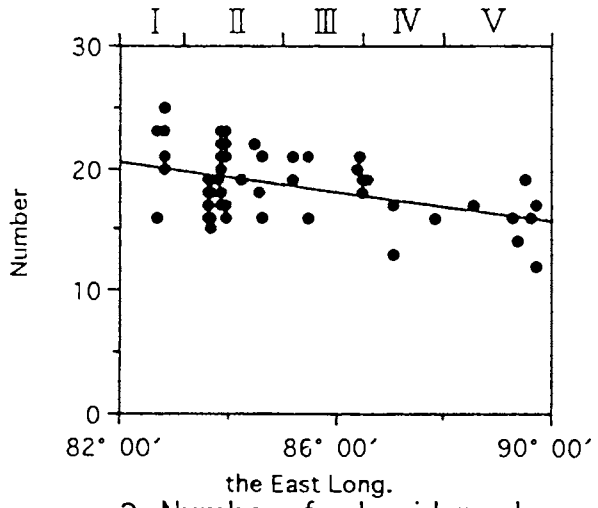
Eighty *Ephedra* plants collected in Nepal in 1994 and 1995 were studied.

Method (Analysis of ephedrine alkaloid)

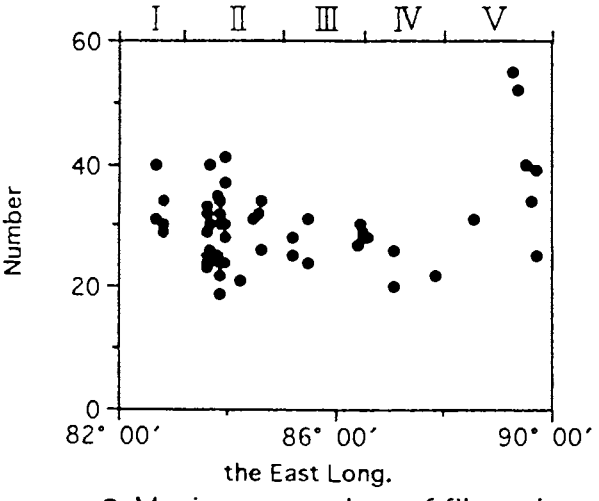
The analytical samples were prepared excluding the internodes at the tip and base of the herbal stem. Analytical conditions are as follows: 30 mL of mobile phase solution of HPLC was added to approximately 150 mg of cut herbal stem of the plant material naturally dried, subjected to a 20-minutes' ultrasonic extraction, and centrifuged at 3,000 revolutions per minutes for 10 minutes. The supernatant fluid was then used for the analysis of four alkaloids, (-)-ephedrine (Eph), (+)-pseudoephedrine (pEph), (-)-norephedrine (nEph) and (±)-methylephedrine (mEph) by HPLC. Column: Chemcosorb 300 5C18 4.6×250mm (Chemco). Mobile phase solution: a mixture of $CH_3CN/H_2O/H_3PO_4$/sodium dodecyl sulfate (SDS) (175: 325:0.2:2). Flow rate: 0.8 mL/min. Detect.: UV 206nm. Column temp.: 30℃.



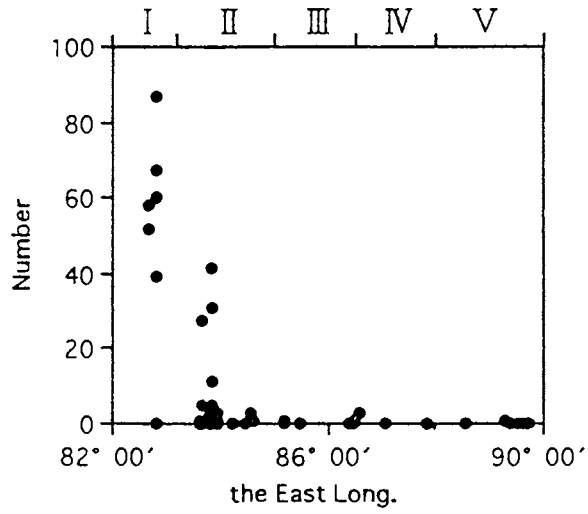
1.Diameter of herbal stem (mm)



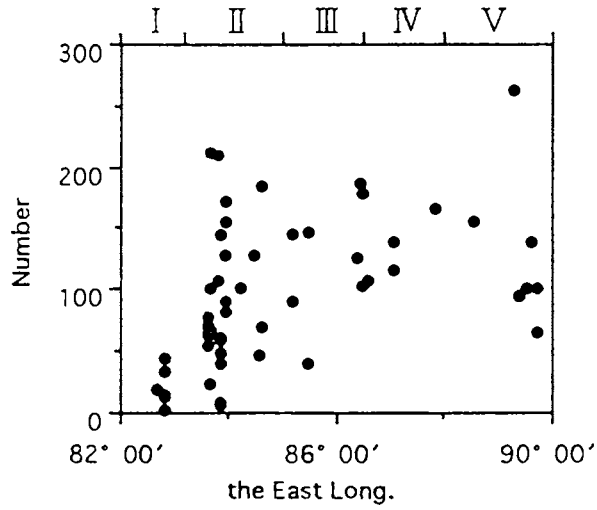
2. Number of subepidermal fiber bundles



3.Maximum number of fibers in a subepidermal fiber bundle



4. Number of cortical fiber bundles



5.Number of pith fibers

Area I : Mid.Western Nepal
Area II : Western Nepal
Area III : Central Nepal
Area IV : Eastern Nepal
Area V : Sikkim and Bhutan

Fig. 1. Geographical variation in anatomical characteristics of herbal stems of *Ephedra gerardiana*. I: Area I. II: Area II. III: Area III. IV: Area IV. V: Area V.

Result and discussions (Fig. 2)

The present study showed that the plants growing in the lowland area had higher ephedrine alkaloid contents than those growing in the highland area, suggesting that the ephedrine alkaloid content was not related to the longitude of the habitat but that it might be affected by the altitude of the area where the stocks grew. In higher places, the climatic temperature is lower. It is reported that the lapse rate of temperature in the monsoon season in a Himalayan high mountain region was around 0.5℃/100m. This ephedrine alkaloid content variation, therefore, might be caused by the changes in temperature: the lower atmospheric temperature may affect the metabolic activity of the plant, which in return may reduce the alkaloid contents.

E. gerardiana and *E. pachyclada* grow at different altitudes, with the former growing in the highlands and the latter in the lowlands. As referred to above results, both species might be observed to differ in alkaloid contents.

These results suggested that the difference in alkaloid content was to be attributed not only to genetic factors but also to difference of environment factors. Therefore, the diversity of quality in the crude drug originating from *Ephedra* plants is considered to be attributed not only the botanical origins generally considered so far, but also the differences in the growing habitats, such as altitude, or atmospheric temperature.

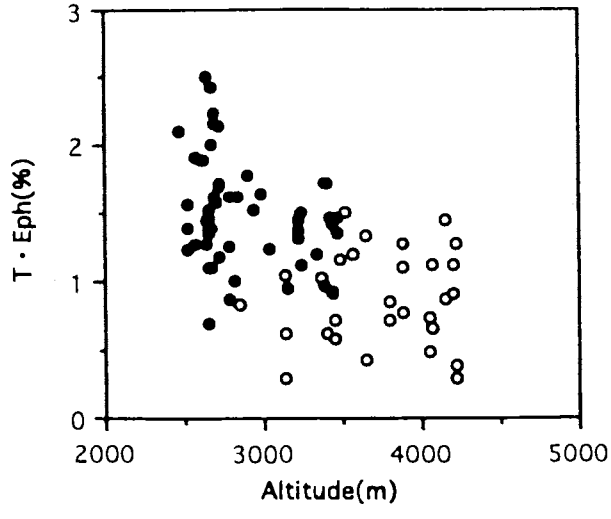


Fig. 2: Geographical variation in alkaloid content of the herbal stem of *Ephedra* Plants with altitudinal changes

《Studies on *Berberis* Plants》

Plants of the genus *Berberis* of the family Berberidaceae contain berberine, an alkaloid component known as an antibacterial reagent. Their bark and wood are generally yellow and taste very bitter due to contained berberine. Decoction of bark or stem of some *Berberis* species has been used as a remedy for curing eye diseases, diarrhea and so on, all over the world including Asian countries. In Nepal Himalayas, 37 taxa of *Berberis* plants including 7 varieties have been recorded from wide altitudinal ranges.

Materials

Fifty six *Berberis* plants were collected in the summer of 1994, 1995, and 1996 in Western Nepal, and were classified into 12 taxa based on the Ahrendt's system for convenience. The botanical names are as follows; **1)** *Berberis hookeri* Lamaire var. *hookeri* , **2)** *B. hookeri* Lamaire var. *microcarpa* Ahrendt, **3)** *B. asiatica* Roxb. ex DC., **4)** *B. umbellata* Wall. ex G. Don, **5)** *B. chitria* Lindl., **6)** *B. angulosa* Wall. ex Hook.f. et Thoms. var. *angulosa,* **7)** *B. tsarica* Ahrendt, **8)** *B. mucrifolia* Ahrendt, **9)** *B. jaeschkeana* Schneid. var. *jaeschkeana,* **10)** *B. jaeschkeana* Schneid. var. *bimbilaica* Ahrendt, **11)** *B. koehneana* Schneid., **12)** *B. zebeliana* Schneid. And, 91 bark samples from these plants are chemically studied.

Method (Quantitative analysis of berberine in the bark)

Barks from the basal part, within 1 meter from the ground, of all the materials were examined. Berberine was determined by the method presented by Yoneda *et al.* with some modifications. Procedures were as follows: sample bark except cork layers was powdered, 0.5g of the powder was extracted three times with MeOH of 30 ml, 30 ml and 20 ml, respectively, for 30 min. at 70℃, and the sum of the extracts was adjusted to 100ml with MeOH and was diluted properly. The solution was filtered through a membrane filter (0.45 μm) and was analyzed with HPLC. Analytical conditions are as follows: Column, Develosil ODS-5 (4.6×150mm); Mobile phase, CH_3CN : H_2O : tartaric acid : SDS = 525 ml : 475 ml : 3.8 g : 1.4 g; Flow rate, 0.8 ml/min; Wave length, 345 nm.

Result and discussions (Fig.3)

As a whole, plants from lower altitudes had more berberine than those from upper altitudes. The correlation coefficient was -0.418, significant level less than 1%.

Among *Berberis* plants examined in this study, those growing at lower altitudes generally contained more berberine. This is the first report of altitudinal trends in chemical contents at the genus level. On the other hand, each Nepalese *Berberis* species had an altitudinally narrow habitat range, mostly within 1000 m. As the result of this habitat segregation, species from lower altitudes seemingly contained more

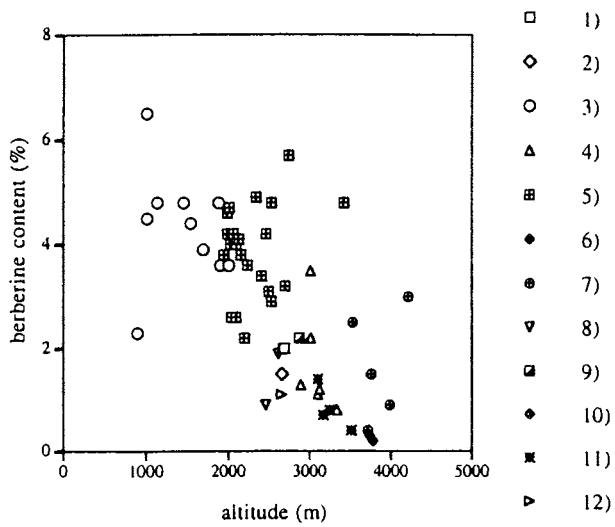


Fig. 3: Elevational variation of berberine contents in the bark of *Berberis* plants (see text for botanical names)

berberine than those from higher altitudes. The difference of habitat temperature controlled by habitat altitude seems to influence the metabolic production of berberine.

These results indicate that the berberine content in the stem bark of *Berberis* plants is probably influenced more by environmental factors, such as temperature, than by genetic one, as like as ephedrine content in *Ephedra* plants.

Acknowledgements: The Ministry of Education, Science, Sports and Culture, Government of Japan, the Grant-in-Aid for International Scientific Research (Field Research Nos. 07041132 and 06041043) supported expeditions, in which most of materials were collected.

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