

Development and use of New Materials Derived from Resource Plants

Kosaburo Nishi

Tokyo University of Agriculture

From the time they appeared on this planet until the present time, living things have undergone evolutionary processes. Since the appearance of humanity, people have utilized living things for a wide variety of products, including everything from clothing and food to housing materials.

Today, over 300,000 species of plants have been recognized, but the plants that have a direct connection of human life, such as for food, fragrances, clothing, building materials, fuel, pharmaceuticals, and even plant used for esthetic purposes in the fine arts, only number in the few tens of thousands.

In China, which has practiced Oriental medicine since ancient times, there are several thousands of plants that are used as crude drugs, refined drugs and or home remedies. In fact, ethnic groups around the world use many plants as dietary supplements for health and maintenance and treatment. Whether or not they have proven medical effects.

This report is an introduction to two plants, *Artemisia capillaris* (Kawarayomogi) and *Anoectochilus formosanus* (Kinsennen).

Flow chart from the introduction of new materials to the creation of new products

- 1) Oral interviews with native peoples Purpose of use, distribution of plants, habitat conditions, problems with cultivation, etc.
- 2) Search of the literature Existence or absence of similar plants, distribution, habitat conditions, safety, etc.

3) Introduction of seedlings Introduction of seedling from research institutes, nurseries, etc, from around the world and gather data.

4) Cultivation experiments Preserving materials for component analysis, investigating cultivation conditions, setting breeding targets, selecting superior systems, breeding

5) Component analysis Examination of total composition and effective components, and establishment of analytical methods

6) Animal experiments Experiment on safety and efficacy using rats, mice, etc., searches using animal models that resemble human patients, effective dosages and appropriate recommendations for patients

7) Cultivation experiments Designed to investigate potential for full-scale production

8) Commercialization Investigations regarding commercialization for use as medicines, health food (Specially designated health food, nutritious food stuffs, nutrition supplement food), etc.

***Artemisia capillaris* Thunb (Kawarayomogi)**

Artemisia capillaris is found in riverbeds and coastlines throughout Japan, China (especially the Ussuri region), the Korean Peninsula and Taiwan. Related species include *Artemisia scoparia* (Hamayomogi) and *Artemisia campestris* (Ryukyuyomogi) which grow in Okinawa. It has also been reported that their natural hybrids with *Artemisia*

japonica (Otokoyomogi) grow wild in some zones, but the expression of inherited characters etc., is not yet understood.

In this study, we collected *Artemisia capillaris* exhibiting growth traits found in Japan, China, and Korea, respectively, *Artemisia campestris* of growth traits common to Okinawa and Europe, respectively, as well as some species of the genus *Artemisia* found in Japan, and compared the plants to investigate their morphological and ecological characters, components and DNA.

1) Erect and prostrate types of wild *Artemisia capillaris* growing in Japan. Plants and seeds of *Artemisia capillaris* were collected from eighteen locations covering nine riverbeds and nine coastlines in Japan, and cultivated at the Tsukuba Medical Plant Research Station. We then compared the difference in growth form, flowering date, and content of capillarin (CAP) and 6,7-dimethylscutletin (DME) in flower heads, and carried out analyses using RAPD. From the results, we found that all species collected from riverbeds were of the erect-type, while most (but not all) of the coastline species were of the prostrate-type. No major differences in flowering dates were observed between the two types, but some prostrate-type strains collected from low latitude areas flowered relatively late and were found to be short-day plants.

DME content in the capitulum was significantly higher in the erect-type than in the prostrate-type. CAP content was also higher in the erect-type, but the difference was not significant. Even after cultivation at the Tsukuba Medical Plant Research Station, growth forms of both types were maintained.

From these results, we concluded that the erect-type and the prostrate-type are different ecotypes. Therefore, to compare their differences at the molecular level, DNA was extracted from the leaves of plants that were collected at fifteen locations whose blooming patterns had been confirmed. After amplification using a 10mer

primer, we conducted cluster analyses of observed electrophoretic patterns for PCR products, and found that *Artemisia capillaris* could be classified into two groups: riverbed and coastal.

However, this classification did not completely correspond to groups classified according to their growth form, DME and/or CAP content in the capitulum.

2) *Artemisia campestris* from Okinawa and *Artemisia capillaris* from China and Korea. *Artemisia capillaris* was collected from Japan, China (Yanji-Jilin, Jiangsu) and Korea (Cheon-Buk Do, Kang-Won Do), *Artemisia campestris* from Okinawa and Europe were also used in these experiments. They were cultivated in flowerpots at the Tsukuba and Hokkaido plant research stations to investigate the content of DME and CAP in their capitulum, and capitulum forms.

The Ryukyuyomogi from Okinawa has the same scientific name as the European variety of *Artemisia*. However, of the three strains that we obtained (Kumejima-1, 2, 3), we could tell from the size and shape of the capitulum that Kumejima-2 was very similar to *Artemisia capillaris* from Europe, especially, regarding the secretory sac in the collar lobe. However, the other two strains were completely different from *Artemisia campestris* and *Artemisia capillaris* (Kumejima-1). Kumejima-1 had shape characters somewhere between those of Kumejima-2 and Kumejima-3, indicating that it might be a hybrid. These strains either contained absolutely no DME or CAP, or the amount that did contain was far too little to be of any use as a crude medicine.

The shape and composition of two strains of the Korean Kawarayomogi and one strain of the Chinese Kawarayomogi (from Jilin) were analyzed for two years. As a result, it was found that the shape characters of the capitula were the same as those of the Japanese Kawarayomogi and these strains were classified as *Artemisia capillaris*.

However, it appears that these were compositional varieties where the content of DME and/or CAP was either very low or undetectable.

Anocctachilus formosanus Hayata (Kinsenren)

Kinsenren is a member of the orchid family that has long been held in high esteem in Taiwan. It grows in the understory of broad-leaved forests at elevations of 800-2,000 m asl. The prostrate stalk is arc-shaped, and is anywhere from 5-15 cm in height. The stalk is a brownish-red in color, pulpy and cylindrical in shape. The length of the petiole is ca. 1 cm. The round leaves, which alternate in groups of 4-6, are ca. 5 cm long and 3 cm wide and are sharply pointed at the ends. The surface of the leaves is dark green and contains 5 main veins which may be either gold or silver in color, and branch veins which form a fine net in appearance. The underside of the leaves is a red or dark purple color. Bolting occurs in September, blossoming in October-November. Floral axes develop a thick mat of soft white hairs, that are 10-20 cm in length, and have 2-3 sheath-like bracts on their surface. From 5 to 15 milky white panicle-like flowers blossom at the top of the plant.

Kinsenren gets its name from the fact that there are gold-colored veins running across the surface of the dark green leaves. It is a very beautiful plant.

In its crude form in Taiwan, it is used as a nutritional supplement, as a treatment for stunted growth in young children, and as a treatment for diseases of such organs as the lungs, livers and kidneys. It has been known locally as the "King of Medicines".

Recently, Kinsenren has been attracting attention for its effectiveness in the prevention and treatment of such common afflictions as high blood pressure,

diabetes, clogged arteries (too much fat in the bloodstream), and obesity.

In addition to an abundant amount of Vitamin C, Kinsenren also contains various minerals such as calcium and magnesium, as well as 260 types of amino acids.

At the research lab of the Graduate School of Pharmacy at Kyushu University, Prof. Masahiro Shoyama et al. are investigating a substance called kinsenoside which is a special component of Kinsenren. They performed experiments with mice to determine what sort of effect kinsenoside has on the metabolism of fatty substances. According to their results, 1) in the group of mice that were given kinsenoside, there was suppression of weight gain even when the mice were given a high fat diet, and 2) the livers of mice were removed and the amount of neutral fats they contained was measured. In the mice that were given a high fat diet and kinsenoside, there was no clear increase in fat. Thus it appears that kinsenoside works to improve the metabolism of fat and suppress the build-up of neutral fats in the body.

In the Products Development department of Seiwa Pharmaceutical Company, animal experiments have confirmed that Kinsenren also suppresses the intake of sugars, which can help to control blood insulin values. Thus, we can expect it to be useful in the prevention and treatment of diabetes.

Unfortunately, Kinsenren grows very slowly and is not very resistant to disease, so at the present time it is mostly cultivated in sterilized indoor environments, and is very expensive to produce. Thus, a new cultivation method is being developed to help bring down the cost, and investigations are underway to grow plants that are rich in kinsenoside.

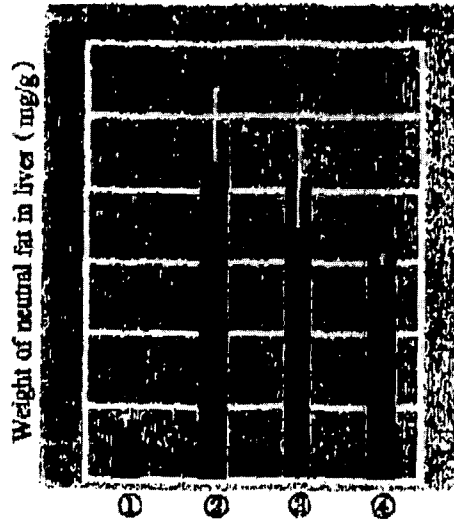


Fig. 1. Effect of Kinsenoside on neutral fat

① Normal feed, ② High fat feed

③ High fat feed + Kinsenoside (50mg/kg)④ High fat feed + Kinsenoside (100mg/kg)

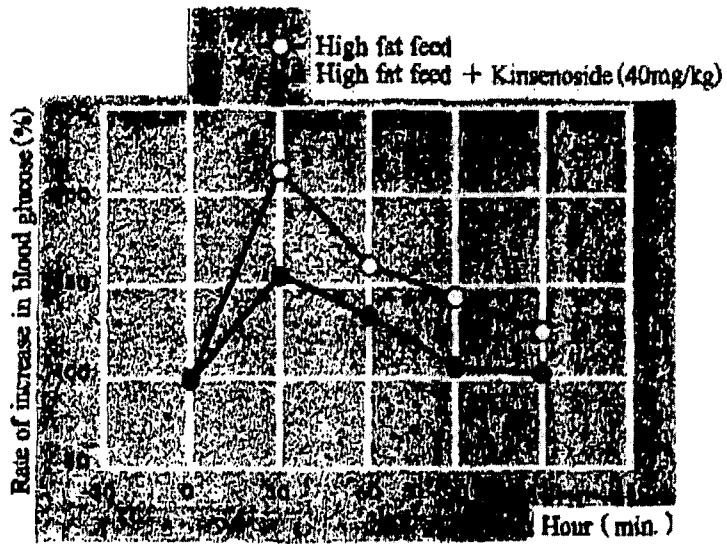


Fig. 2. Effect of Kinsenoside on blood glucose level

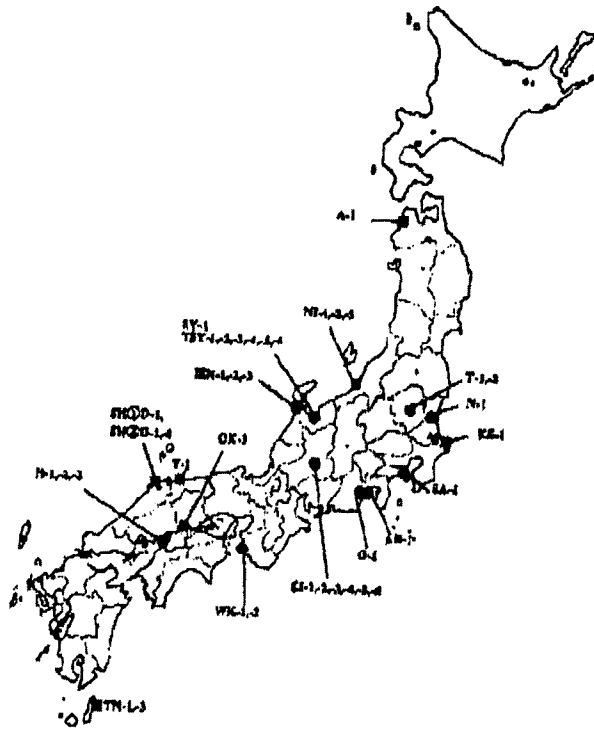


Fig. 3. Habitats of *Artemisia capillaris* collected at eighteen different locations in Japan. Black spots and black squares are marked with the stations of erect- and prostrate-types, respectively.

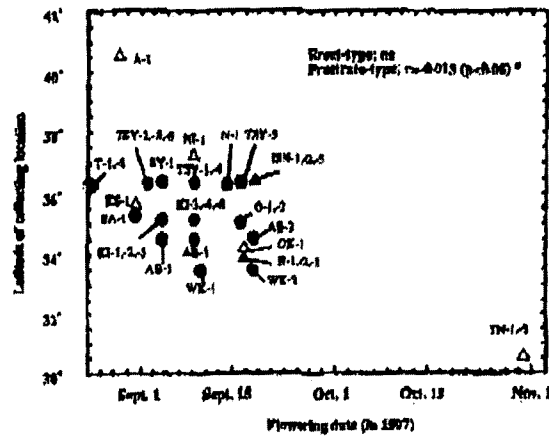


Fig. 4. Correlation between flowering date and latitude of collecting location of thirty six individuals of *Artemisia capillaris* collected at sixteen different locations in Japan.

● : Erect-type collected at riverbed ; ▲ Erect-type collected at coastline; △ : Prostrate-type collected at coastline
 *and ns indicate significant correlation between flowering date and latitude of collecting location and nonsignificant, respectively ($P < 0.05$, correlation coefficient), Abbreviations are shown in Table 1.

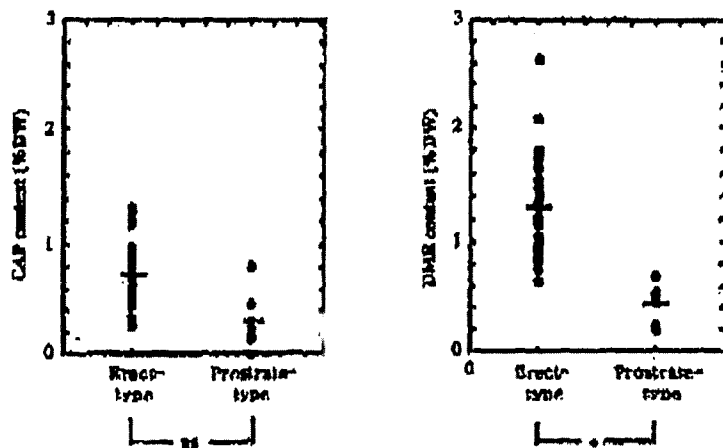


Fig. 5. The contents of capillarin (CAP) and 6,7-dimethylesculetin (DME) in flower head of erect- and prostrate-type of *Artemisia capillaris* at sixteen different locations in Japan. Bar indicates average of each type.

* and ns indicate significantly different from each other and nonsignificant, ($P < 0.05$, t-test).

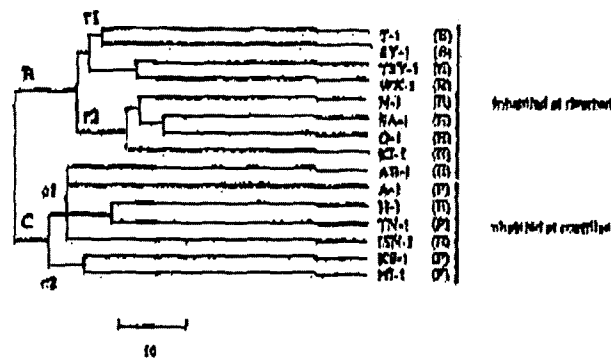


Fig. 6. Dendrogram for clarification of *Artemisia capillaris* collected at fifteen different locations in Japan. The presence or absence of amplified DNA Fragment was treated as a binary character.

A dendrogram was constructed on the basis of euclidian distance by using the unweighted pair group method with arithmetic means (UPGMA),

(E) : Erect-type : (P): Prostrate-type. Abbreviations are shown in Table 1.