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# Biological Substances from Higher Plants in Asian Region

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**Abstract** - In this review, we summarize the isolation, structural determination, antitumor activity of substances from higher plants which were collected in Asian region.

## 1. Introduction

To date many kinds of compounds have been obtained from plants kingdom as antineoplastic and anticancerous agents. However, there is no special type of compounds for cancer therapy. Various types of substances are effective for various types of cancers and tumors: for instance, alkaloids, lignans, terpenes and steroids etc.<sup>1)</sup> First of all, most important components obtained from higher plants are Vinca alkaloids and Podophyllum lignans. Vinca rosea (= Catharanthus roseus) has been used as inhibiting agent for milk secretion, hypotensor, astringent and emetics as folk medicines in Madagascar. Moreover, native people in West Indian Island have been using Vinca spp. as depression agent of blood sugar. When the extract of this plant was given non-orally, leucopenic and indirect inhibiting action of nuclear division of cells were observed. Above 60 kinds of alkaloids have been isolated from Vinca spp. Vinblastine and vincristine are most active substances among of them. The former is effective to Hodgkin disease and the latter to leukemia. Podophyllotoxin is a representative lignan isolated from the rhizomes of Podophyllum peltatum. Podophyllum rhizome had been used as an emetic and an anthelmintic by American Indians traditionally. Because podophyllotoxin was also found to have inhibiting action for cell-division, antineoplastic activity was noticed.

The others, curcumol obtained from Curcuma aromatica was tested and noticed to be effective against cancer of the uterine cervix clinically. Oridonin isolated from Rabdosia spp. is now investigated for clinical trials in China. Moreover, camptothecin isolated from Camptotheca acuminata is also antineoplastic alkaloid, but is very toxic. Chemical modification has been tried to decrease its toxicity. This compound will be permitted to use as clinical agent later.<sup>2)</sup> Colchicine derivatives are also said to have inhibiting action of cell-division. Demecolcine and colchicine have activity against mammary cancer. Harringtonin was investigated as an anticancerous drug in China. Taxol, a compound with a taxane ring isolated from the bark of Taxus brevifolia,

has been demonstrated to have substantial anticancer activity in patients with solid tumors refractory standard chemotherapy. Supply of this drug has severely limited full exploration of its antineoplastic potential. Some efforts are continued in National Cancer Institute (NCI) Washington for surveying various Taxus species for optimal taxol content, improvement in semi-synthesis from baccatin III, improvement in method of extraction, and development of alternative renewable resources.<sup>3)</sup> Further, there are many compounds which have been reported as antineoplastic agents.

Development of novel clinical useful anticancer agents would be dependent on the screening system and the sample sources for the bioassay. The search for potential anticancer agents from natural sources mainly has been carried out with the guidance of bioassays confirmed by the NCI,<sup>4-10)</sup> because the large number of natural products screened at the NCI program have also been discussed from an overview of the relationship of assessment between experimental animals and clinical patients for drug development, and the screening protocols for each tumor system have been well-established. It is considered that these are "compound-oriented" *in vivo* screenings. These screenings could not lead to develop some new drug for solid cancers.<sup>11-14)</sup>

Recently, NCI has established a "disease-oriented" approach to antitumor activity screening<sup>5,15,16)</sup> and the biological response modifiers (BRM)<sup>17,18)</sup> program from a viewpoint of the diversity and specificity of tumor, and the requirements of novel structure types and novel action-mechanistic types of anticancer agents. These screening system led to isolate many antineoplastic compounds from plants,<sup>19-22)</sup> microorganism<sup>23,24)</sup> and marine metabolites<sup>4,12)</sup> etc. On the other hand, we have screened on higher plants collected in Japan, China, Korea, Southeast Asia and South America<sup>25-27)</sup> for antineoplastic activity, which has been done using Sarcoma 180 ascites in mice, P388 lymphocytic leukemia in mice, Chinese hamster lung V-79 cells, P388 cells and nasopharynx carcinoma (KB) cells in our laboratory, as primary screening. In this review we will describe on antitumor and cytotoxic substances of the higher plants selected from above screening tests.

In 1982, it was given a definition for expression of activity. that is, the word cytotoxicity must be used only for *in vitro* activity, the words antineoplastic and antitumor must be used only for *in vivo* test using animal. We should call anticancer, when it shows activity in clinical trials of human.<sup>12)</sup>

## **2. An Antitumor Morphinane Alkaloid, Sinococuline, from Cocculus trilobus and the Related Compounds<sup>28-30)</sup>**

Cocculus trilobus DC. (Menispermaceae) growing in the mountainous areas of East Asia has been used in folk medicine as a diuretic, analgesic and anti-inflammatory crude drug. When an aqueous solution of the methanolic extract prepared from the stems and rhizomes of C. trilobus was partitioned successively with n-hexane and ethyl acetate, the antitumor activity against Sarcoma 180 ascites in mice was concentrated in the

# CURRENT SITUATION AND PROSPECTS OF HERB CULTIVATION IN JAPAN

YAITIBE TOMITAKA

Herbs have their own peculiar flavors and pungent tastes, hence they are important in enriching the tastes of cooking. Japanese horseradish used with sliced raw fish and 'sushi', and ginger with meat and fish dishes are good examples.

## Drug Discovery Strategy for Anticancer and Cancer Chemopreventive Agents as a Multidisciplinary Approach

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Drug discovery has been a major concern of mankind since prehistoric times drugs of natural product origin are particularly emphasized recently for the treatment of human ailments. Plants are the most abundant resource for the discovery of biologically active natural constituents, which can be important lead compounds for the development of structurally modified derivatives with reduced toxicity and enhanced biological activity. In order to discover new biologically active compounds from natural products, multidisciplinary approach is a useful strategy by collaboration of different scientific area as following : 1) rational plant selection and provision by botanists/collectors 2) extraction, isolation and identification of compounds by pharmacognosist 3) in vitro bioassay based on activity-guided fractionation by biochemists/biologists 4) *in vivo* test to evaluate the efficacy by biochemists/biologists 5) semi-synthesis or total synthesis by chemists for obtaining a large quantity or modify the structure for enhanced activity.

As a part of our ongoing effort to find new anticancer agents from natural sources budmunchiamine alkaloids were discovered from *Albizia amara* using a HPLC-based method combined with DNA-cellulose column chromatography. Antimitotic constituents from *Hyplis verticillata*, *Steganotaenia araliacea*, and *Bursera peruvialis* were isolated by means of bioassay-guided fractionation methods using cultured astrocytoma (ASK) cells, which demonstrated a potent tubulin-mediated cytotoxicity against a panel of cancer cell lines. Bioassay-guided fractionation of *Mundulea sericea* extract led to the isolation of rotenoids and chalcones, which inhibit phorbol ester-induced ornithine decarboxylase (ODC) activity in cell culture system. Rotenoids, which mediate the potent cancer chemopreventive activity through transcriptional regulation of ODC, inhibited the chemically induced preneoplastic lesions in mammary organ culture and papillomas in the two stage mouse skin tumorigenesis model.

In this presentation, an overview of the drug discovery strategy from natural resource will be given, as well as recent results related to anticancer or cancer chemopreventive agents.

## **Research on Biologically Active compounds from Natural Sources**

Prof. Hideji Itokawa, Dept.of Pharm, Tokyo Univ.of Pharm. & Life Sci.

To date, a lot anticancer agents have been isolated from natural sources; from microorganisms, from fungi and from higher plants. At present in our laboratory, we are focussing to higher plants for screenig antitumor agents. From higher plants, many kinds of anticancer agents have been isolated and applied for clinical use;vinca alkloids from *Vinca rosea*, podophyllotoxin from *Podophyllum Peltatum*, camptothecin from *Camptotheca acuminata*, taxol from *Taxus brevifolia* and curcumol from *Curcuma aromatica*. We also collected many materials from all over the world for screening test against Sar coma 180A and some tumor cell lines.

As the results, we observed that various extracts of them revealed antineoplastic activity. For instance, *Curcuma app.* and *Alpinia spp.* of *Zingiberaceae*; *Rubia akane*, *Cocculus trilobus* and so on.

## **The Resources of Higher Fungi In Mt.Paekdu Areas**

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Some 420 species of higher fungi were collected from the Mt.Paekdu, Songwha river and their adjacent areas from 28th July to 12th of August, 1991. These were identified 2 subdivisions, 4 classes 2 subclasses, 12 orders, 51 families, 141 genera and 336 species

The dominant species were 10 species, edible fungi 68, poisonous fungi 18, indistintive fungi of eat and poison 8, wood decay fungi 65, mycorrihzal fungi 29.

Keywords : resorces, Mt.Paekdu, higher fungi.

# **Current Situations and Prospects of Herb Cultivation in Japan**

Yaichibe Tomitaka(Tokyo Univ. of Agr.)

Herbs have their own peculiar flavors and pungent tastes, hence they are important in enriching the tastes of cooking.

Herbs and spices are often mixed up, or sometimes they are altogether referred to as spices. A herb, in a traditional sense, means a medicinal plant in Europe. Naturally, there are great many kinds of herbs, The word 'herbs' can be regarded as equivalent to Chinese medicinal plants. Part of them are used for cooking and these kinds are best classified as condiment herbs or culinary herbs. On the other hand, spices are generally of tropical or subtropical origin.

Among herbs, some kinds have long been grown in Japan and are widely used, Other kinds have been recently introduced from Europe and other parts of the world. We will call the former local varieties and the latter Occidental varieties.

Labiatae is the greatest family, followed by Umbelliferae, Cruciferae and Liliaceae, Garlic, Chinese chive, ginger, mioga and rakkyo show large quantities in local varieties. On the other hand, Basil, herb mixes, chervil, peppermint, corn salad show large quantities in Occidental Varieties.

Herb seeds currently used in Japan are all imported from other countries. The varieties and strains of these seeds are not made clear and therefore their qualities are not consist. To improve the productivity and quality, breeding must be carried out.

Since herbs are shipped as shoot apices or fresh leaves, the freshness can easily be lost. New herb cuisines suited to the taste of the Japanese will lead to larger demands. Herbs were originally medicinal plants and many of them have been widely used in Western and Chinese cooking. Herbs not only enhance the tastes of dishes but also take medicinal effects. Diet using herbs is an effective method of health management. For this, as well as the development of vegetable cultivation. It will be meaningful to promote herb cultivation in Japan

## **The cultural situation and prospect of the medicinal plants in China**

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Chinese medicine in China is the traditional medicine improving the people health and curing the diseases by using the medicinal plants. Thus, with the advent of china history, chinese medicine has been developed for a long time and made a great contribution to promote the chinese's health. China has a wide stretches of land and five thousands of medicinal plants grow naturally throughout the country, and of these plants, five hundreds of medicinal plants were directly used as medicinal materials. Also, dozens of medicinal plants have the anticancer componemts or anticancer effects. Chinese medical science was early developed in china because chinese medicine is cheaper, and has the least harmful side effects and the more curing effects than others.

Recently, two hundred kinds of chinese medicine materials were mainly produced and supplied to consumers by about 200,000 farmers who cultivated medicinal plants. Some cancer-cure reagents from medicinal plants have been already developed and it was known that they have very high cure effects. Cancer is one of the diseases which should be cured. In the future, it would be very desirable that many researches should be done for developing cancer-cure reagents and preventing cancer occurrence.

# Screening for Antitumor Efficacy from the wild plants in Korea(Ⅲ)

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## Cytotoxic activity on P388 cells

The MTT(3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay was performed in a 96-well plate. The blue formazan Produced by the mitochondrial dehydrogenase of viable cells was measured spectro photometrically. 100 $\mu$ l of RPM-1640 medium Supplemented with 5% fetal calf serum and 100 $\mu$ g/ml of kanamycine and containing mouse P388 leukemia cells ( $3 \times 10^4$  cells/ml) was added to each well. After over night incubation (37 $^{\circ}$ C, 5% CO<sub>2</sub>),100, 30, 10, 3, 1, 0, 0.3 and 0.1 $\mu$ g/ml of sample solutions were added to the wells and the plates were incubated for 48h. Then, 20 $\mu$ l of MTT was added to each well and the plates were incubated for 4h. The resulting formazan was dissolved in 100 $\mu$ l of 10% SDS (Sodium dodecyl sulfate) containing 0.01 N HCl. Each well was mixed gently with a pipet for 1 or 2 min and the plate was read on a microplate reader (Tosoh MPR-A4i) at 540nm. The IC<sub>50</sub>( $\mu$ g/ml)value was defined as the concentration of sampic which achieved 50% reduction of viable cells with respect to the control.