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Recent studies on glycosides from plant drugs of Himalaya and south-western China: chemo-geographical correlation of *Panax* species

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Abstract- The geographical relationship of saponin composition of Panax species has been studied extensively. Close similarity in saponin composition was observed for the plants in Eastern Himalaya, South-western China and Southern Japan. The exeptional saponin compositions were found for some plants growing near the western and southern limits of the distribution as well as that in Japan Island. Comparison of the chemical constitents of Ginseng with crude drugs which are used as a Ginseng substitute, was also presented.

Ginseng, the famous plant drug, has been used as an expensive traditional medicine in oriental countries for more than 5,000 years. Our group has elaborated the chemical investigation of secondray metabolites of Ginseng, isolating a number of tetracyclic dammarane saponins named ginsenosides-Ra-h. [ref.1,2] These dammarane saponins are first isolated from Ginseng, being characteristic of Panax species. The pharmacological studies on Ginseng have been centered on these saponins, disclosing a variety of physiological activities for the major dammarane saponins. [ref.2]

Recently, Dr.Kitagawa et al. reported the isolation of unstable acidic malonyl-ginsenosides-Rb1, -Rb2, -Rc and -Rd from Ginseng in relatively high yields. [ref.3,4] Ginsenoside-Ro (=chikusetsusaponin-V) which is an acidic glucuronide sapnoin of oleanolic acid (pentacyclic oleanane triterpene), has been also isolated from Ginseng. [ref.1,2] It has been well-known that saponins of oleanolic acid occur widely in a variety of plants, being not characteristic of Panax species.

In the biosynthesis of triterpenes, dammaranes are formed in the early stage of the cyclization of 2,3-oxido-squalene, while oleananes are the terminal products. A number of Panax species are growing in the Northern hemisphere from Central Himayalas onward through China and Japan to North America. I would like to propose the chemo-geographical correlation of Panax species, mainly based on the differnece in sapogenin, secondarily on the variation in structures of sugar moieties.

Ginseng and American Ginseng The source plant of Ginseng is Panax ginseng C.A.Meyer (Araliaceae) which grows wild extending from North Eastern China, Korea to Far Eastern Siberia. It was revealed that the saponin compositions of Ginseng and P. quinquefolium L. (American Ginseng) are very similar from each other. [ref.1,4] Roots of both the plants contain a variety of dammarane saponins and one oleanolic acid glucuronide saponin, ginsenoside Ro. Recently, Dr.Lee and Dr.Marderosian disclosed that saponin composition of P. trifolius L. (Dwarf-Ginseng) in North America is also similar to those of Ginseng and American Ginseng.

***Panax* species in Eastern Himalaya and south-western China** The close relationship of plants growing between Eastern Himalaya, South-western China and Japan, has been pointed out by many botanists. A variety of plants growing in these area are originated from a common ancestor in the Early Tertiary and now widely separated in the respective regions.

Several Panax species are growing in Eastern Himalaya and designated as P. pseudo-ginseng and its subspecies by late Professor Hara. [ref.5] P.

pseudo-ginseng Wall. subsp. pseudo-ginseng Hara has a carrot-like root like Ginseng, American Ginseng and Dwarf-Ginseng. This species is very rare in nature and Hara described that this would be the original type of Himayalan Panax. We investigated the saponin composition of roots of this species. It was revealed that contents of a Ginseng dammarane saponin, ginsenoside Rg1 and oleanolic acid glucuronide saponins are evidently lower than those of other Himalayan, Chinese and Japanese Panax species-other Panax species.

P. pseudo-ginseng Wall. subsp. himalaicus Hara grows most abundantly in Bhutan and Eastern Nepal. This plant generally has a big rhizome. Another type of P. pseudo-ginsneng subsp. himalaicus grows in Estern Himalaya at relatively high altitude, 3000-4000m. Internodes of the long creeping rhizome of this specimen are elongated and slender. The similar type of plant is also growing in South-western China, designated as P. japonicus C.A.Meyer var. major (Burk.) C.Y.Wu et K.M.Feng.

In South-Western China, Panax japonicus and a variety of the congeners are growing.[ref.7] These plants have a big rhizome, being morphorogically similar to Himalayan and Japanese Panax species. 1) P. japonicus C.A.Meyer (Zhu-jie-shen). 2) P. japonicus C.A.Meyer var. major (Burk.) C.Y.Wu et K.M.Feng. 3) P. japonicus C.A.Meyer var. angustifolius (Burk.) Chen et Chu (very similar to P. pseudo-ginseng Wall. subsp. himalaicus Hara var. angustifolius (Burk.) Li in Eastern Himayala studied by Dr.Shukla, Lucknow, India [ref.8]). 4) P. japonicus C.A.Meyer var. bipinnatifidus (Seem.) C.Y.Wu et K.M.Feng 5) P. zingiberensis C.Y.Wu et K.M.Feng.

By means of the modern techniques of isolation and structure determination of oligo-glycosides,[ref.1] most of the saponin compositions of Panax species growing in Eastern Himalaya [ref.1,9,10] and South-western China[ref. 1,11] have been elucidated under the co-operation with Kunming Institute of Botany, Chinese Academy of Science and other Institutions. It has been demonstrated that saponin compositions of these Chinese species and Himalayan Panax pseudo-ginseng subsp. himalaicus are similar to each other. The rhizomes of these species contains Ginseng saponins, ginsenosides and other characteristic dammarane saponins. The difference of these species from Ginseng and American Ginseng is that these speciese contain a variety of oleanolic acid glucuronide saponins in high contents.

***Panax* species in Japan** P. japonicus growing throughout Japan is named Chikusetsu-ninjin, which is morphologically identical with Chinese Panax japonicus (Zhu-jie-shen). Kyushu island is located in South-western Japan, the nearest district to China. P. japonicus growing in Southern-Kyushu, Japan is called "Satsuma-ninjin" and has been thought to be the better drug than Chikusetsu-ninjin growing in other parts of Japan. It was found that saponin composition of this Satsuma-ninjin [ref.12] is similar to those of Chinese P. japnicus [ref.1]; Rhizomes of this specimen contains two oleanolic acid glucuronide saponins and also dammarane saponins isolated from Ginseng.

The composition of oleanolic acid glucuronide saponins of chikusetsu-ninjin [ref. 1,2] is similar to other Panax species, Satsuma-ninjin, Zhu-jie-shen of South-western China as well as specimens of Eastern Himayala. However, the dammarane saponin composition of Chikusetsu-ninjin is quite different from others. No major dammarane saponins of Ginseng has been identified in rhizomes of this species, while chikusetsusaponin III [ref-sole example of the dammarane saponin of Panax species having a branched sugar chain. This saponin has not been isolated from any other Panax species. It was further demonstrated that the saponin composition of leaves of Chikusetsu-ninjin is also remarkably different from other Panax species. [ref.1]

By means of the application of tissue culture, it was recently proved that this difference in dammarane-saponin composition between Satsuma-ninjin and Chikusetsu-ninjin is not due to the difference in environment but due to the genetic function.[ref.13] Saponins of cullus induced from flower-buds of Chikusetsu-ninjin were compared with those of cullus induced from the same parts of Satsuma-ninjin under the same condition. From callus of Chikusetsu-ninjin, six known oleanolic acid glucuronide saponins such as chikusetsu-saponins IVa and IV were isolated, while no dammarane saponin was detected. On the other hand, from callus of Satsuma-ninjin, two

known-Ginseng dammarane saponins, ginsenoside-Rg1 and -Re were isolated together with oleanolic acid glucuronides saponins, chikusetsusaponin IV and its 28-desglucosyl-compound. This evidence is sufficient enough to prove that the regional strains of P. japonicus, Chikusetsu-ninjin and Satsuma-ninjin must be intrinsic.

Sanchi-Ginseng and *Panax* species in Central Nepal Panax notoginseng (Burk.) F.H.Chen, Chinese name Sanchi-Ginseng or Tien-chi is cultivated in Yunnan, China and the root is a very famous Chinese medicine used as a tonic and a hemostatic. The locality of the wild specimen is still obscure. From roots and rhizomes of Sanchi-ginseng, several Ginseng dammarane saponin, ginsenosides-Rb1, -Rd, -Re and -Rg1 as well as new dammarane saponins named notoginsenosides-R1 and -R2 were isolated in high yields [ref.1,2] The difference of this species from other species is the absence of oleanane saponins.

Recently, by Dr.Namba and his co-workers, Panax species were collected at Chame and Gorapani, around Mt.Annapurna which would be the western limit of the distribution of this genus. The chemical study revealed that the saponin composition of rhizomes of these species is very similar to that of Sanchi-ginseng. The rhizomes contain dammarane saponins exclusively. No oleanane saponins was identified.[ref.14] These results indicated the importance of Panax species growing in Central Nepal not only from chemotaxonomical point of view but also from pharmacological view point.

Panax stipleanatus P. stipleanatus H.T.Tsai grows in Southern Yunnan, China and North Vietnum, near the southern limit of the distribution of Panax species. In contrast to the other Panax species, no dammarane saponins was identified in rhizomes of this species. Further, two oleanolic acid glucuronide saponins named stipleanosides R1 and R2 from the rhizomes of this plant have not been detected in any other Panax species.[ref.15]

***Panax* species in South Vietnam** Very recently, I was informed by Dr. N. T. Nham, Vietnam that from rhizomes and roots of P. vietnamensis Ha et Grushv. collected at the high mountain in Southern Vietnum (the southern limit of the distribution of Panax), Ginseng dammarane-saponins, ginsenosides-Rb1, -Rd and -Rg1 as well as ocotillol-type dammarane-saponins, pseudo-ginsenoside-RT4 and majonoside-R2 were isolated. Contents of oleanolic acid saponins are relatively low like the specimen collected in Nepal by Dr.Kong et al., Hong Kong .[ref. 10]

Ginseng substitutes Finally, I would like to mentioned about the plant drugs which are used as a substitute of Ginseng.

Acanthopanax senticosus(Rupr. et Maxim.) Harms. (=Eleutherococcus senticosus (Rupr. et Maxim.) Maxim.), Araliaceae, the same family as P. Ginseng, grows in Ear East Siberia, North-Eastern China. Roots of this plant, Chinese local name Ciwujia, have been used as a tonic in oriental countries. This drug is recently sold under the name of "Siberian Ginseng". However, chmical constituents of this drug are completely different from Ginseng. From roots of this plant, coumarins, flavonoids and phramacologically active lignanes were isolated by Dr.Elyakov et al. A number of oleanane-saponins were isolated from the leaves [ref.16], while no saponin was identified in the roots.

In South America, roots of Pfaffia species, Amaranthaceae, are named "Brazilian Ginseng" and used as a tonic. From roots of Pfaffia iresinoides Spreng., the known insect hormones, ecdysterone, polypodine B and pterosterone were isolated.[ref.17] From Phaffia paniculata Kuntze, glycosides of a modified triterpene named pfaffic acid were isolated. [ref.18] However, no dammarane saponin was identified in both the plants.

Dr.Takemoto, Dr.Arihara and their co-workers reported the isolation of bioactive Ginseng saponins, ginsenosides-Rb1 and -Rd as well as a number of other dammarane saponins named gypenosides from a Japanese cucurbitaceous herb, Gynostemma pentaphyllum Makino. In Japan, powder and extract of leaves of this plant have been sold as a substitute of Ginseng. However, it was recently demonstrated by Dr.Ohsio and his coworkers that the contents of dammarane saponins vary markedly depend upon the locality where it grows.[ref.19] In the case of 100 specimens collected in Ishikawa-prefecture, 43 samples contained ginsenoside-Rb1, while in the case of 713 samples collected in other areas of Japan, only 15 samples contained ginsenoside-Rb1.

Roots of Codonopsis species, Campanulaceae (Chinese drug name, Dangshen) are very common plant drug in China, being sometimes used as a substitute of Ginseng. Currently, we have investigated the water-soluble constituents of C. tangshen Oliv. cultivated in Sichuan, China. It was disclosed that nearly 90% of the methanolic extract consist of carbohydrates such as glucose, fructose and sucrose. By means of chromatography on the styrene-divinyl-benzene polymer and ion-exchange resin, we have succeeded the saparation of water-soluble secondary metabolites from these mono- and oligo-saccharides. We isolated several hexyl and hexenyl glycosides, a polyacetylene alcohol glucoside and the well-known phenylpropanoid-glucoside, syringin. We also isolated several new phenolic glycosides named tanshenosides I, II, III and IV. [ref.20] Tanshenosides III and IV are the new type neo-lignan glycosides. Anyway, no saponins has been identified in this drug.

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