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A Study on the Cytotoxic Activity of Korean Red Ginseng Against Cancer Cells

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This study was carried out for the purpose of comparing anticancer activities of *Panax ginseng* of different countries. Petroleum ether-extract including anticancer agents was extracted from Korean Red Ginseng and Chinese Red Ginseng. Then using this extract, *in vitro* tests were performed against various cancer cells, such as mouse leukemic (P_{388}), human rectum (HRT-18), human colon (HT-29, HCT-48), hepatoma (HepG2) and monkey kidney cell (VERO 76), as normal cell.

The results obtained are as follows:

1. The yield of petroleum ehter-extract of Korean Red ginseng (0.64%) was higher than that of Chinese Red Ginseng (0.47%).
2. The growth inhibition rate of each cancer cell was gradually increased in proportion to the concentration of Korean and Chinese Red Ginseng.
3. Growth inhibiton effect against mouse leukemic cell P_{388} was remarkably stronger in the extract of Korean Red Ginseng than in that of Chinese Red Ginseng. In cases of human rectum (HRT-18), human colon cancer cells (HT-29, HCT-48) and hepatoma (HepG2), growth inhibition effects were shown similar tendency to leukemic cells.
4. All of tested ginseng extract inhibited growth of normal cell (VERO 76). But the growth inhibition effect was much lower than in the other human cancer cells (HRT-8, HT-29, HCT-48) and hepatoma (HepG2).
5. The number and size of HepG2 cells cultured in medium containing Korean and Chinese Red Ginseng were significantly reduced and deformed compared with control group and the effects of Korean Red Ginseng was remarkably stronger than that of Chinese Red Ginseng. Judging from these results, it was concluded that growth inhibition effects of Korean Red Ginseng against mouse leukemic cell, human rectum cancer cell and human colon cancer cells, *in vitro* were evidently stronger than those of Chinese Red Ginseng.

Anticarcinogenic Effects of *Panax ginseng* Depending on Types and Ages

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Authors have already shown that 6-year-old red ginseng extract or its powder has remarkable anticarcinogenic effects. In this study, we further investigated whether fresh ginseng or white ginseng has similar anticarcinogenic effect, and also their anticarcinogenic effects are related to the types and ages of ginseng using 9 week medium term bioassay model.

Fresh ginseng at 1.5, 2, 4, 5 and 6 year old was processed in three ways. Fresh ginseng was dried at room temperature and finely powdered for dried fresh ginseng, or processed at the same way after removal of ginseng cortex and fine root. For red ginseng, fresh ginseng was steamed and dried.

Mice were given a single subcutaneous injection of 0.5 mg benzo(a)pyrene within 24 hours after birth. Various ages of dried fresh ginseng, white ginseng and red ginseng respectively at a concentration at 5 mg/ml were orally administered in drinking water for 6 weeks after weaning. All mice were sacrificed at 9th week. Lungs and other main organs were fixed in Tellyesniczky's solution and examined histopathologically. The incidence and multiplicity of lung adenoma were counted with macroscopically.

Following results were obtained :

1. In dried fresh ginseng treated group, the incidence of lung adenoma induced by benzo(a)pyrene was 41.4% and its incidence was reduced to 31.2, 30.0, 31.3, 30.7% and 27.8% after co-treatment of 1.5, 3, 4, 5 and 6 year-dried fresh ginseng, respectively. Significant effect was observed only in 6 year-dried fresh ginseng.
2. In white ginseng treated group, the incidence of lung adenoma induced by benzo(a)pyrene was 45.0% and its incidence was decreased to 41.3, 38.0, 31.6% and 25.3% after co-treatment of 3, 4, 5 and 6 year-white ginseng, respectively. Five and 6 year-ginsengs showed significant inhibition of lung adenoma.
3. In red ginseng treated group, the incidence of lung adenoma induced by benzo(a)pyrene was 48.6% and its incidence was diminished to 37.9, 41.7, 31.7, 28.3% and 25.5% after co-treatment of 1.5, 3, 4, 5 and 6 year-red ginseng, respectively. From 4 year-ginseng, anticarcinogenic effect was prominent. From above results, we concluded that significant anticarcinogenic effect was observed in 6 year-dried fresh ginseng, 5 and 6 year-white ginsengs, and 4, 5 and 6 year-red ginsengs. Further study using three types of ginseng extracts is in progress and more interesting results will be obtained.

Involvement of Steroid Receptor During the Process of Ginsenosides-Inducing Differentiation

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We present evidence that ginseng saponins induce the differentiation of F9 teratocarcinoma stem cells through binding with the steroid receptor. Since ginsenosides and steroid hormone show resemblance in chemical structure, we investigated the possibility of the involvement of a steroid receptor in the differentiation process induced by ginsenosides. According to Southwestern blot analysis, a 94 kDa protein regarded as a steroid receptor was detected in F9 cells cultured in the medium containing ginseng saponin. In addition, F9 stem cells treated with ginseng saponin together with RU 486, a glucocorticoid antagonist with a high affinity for the glucocorticoid receptor, did not differentiate into endoderm cells morphologically and not induce the expression of laminin gene. Gel retardation analysis demonstrate that nuclear extract of F9 cells treated with ginseng saponin contains proteins which exhibit a selective binding affinity for glucocorticoid responsive element (GRE) DNA sequences. Competition assay indicated that when specific binding sites of steroid receptor were filled with ^{3}H-dexametha-

sone, the addition of a 1000-fold excess of non-radioactive ginseng saponin resulted in a reduction of total binding of radiolabeled dexamethasone after 4 hrs. Based on these data, we suggest that differentiation activity of ginsenosides might be exerted *via* binding with a steroid receptor or its analogues nuclear receptor.

Induction of Differentiation of Tumor Cells by Ginsenosides

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The effect of ginsenosides purified from *Panax ginseng* C.A. Meyer on the differentiation of mouse teratocarcinoma stem cells (F9) and human promyelocytic leukemia cell line (HL60) were studied. F9 stem cells cultured in the presence of ginsenoside together with dibutyric cAMP became parietal endoderm-like cells. Moreover, the expression of differentiation marker genes, laminin, type IV collagen, and retinoic acid receptor-β(RAR β) was increased after treatment of ginsenosides. Among various ginsenosides purified from crude ginseng saponin, Rh_1, Rh_2 and Rh_3 caused the differentiation of F9 cells very strongly. In addition, differentiation-inducing activity of ginsenosides on HL60 cells was measured by NBT reduction. After 4 days of incubation in medium containing 50 mM of ginsenoside Rh_1, 25 μM of ginsenoside Rh_2 or 30 μM of ginsenoside Rh_3, NBT reduction of HL60 cells was 9.52%, 11.86% and 37.30%, respectively. We therefore suggest that ginseng saponin, especially ginsenosides Rh_1, Rh_2 and Rh_3 cause the differentiation of both F9 and HL60 cells.

The Effect of Anti-Cancer Drugs and Ginseng Saponin in the γ-GT Activity of Rat Liver Treated with Carcinogen and on the Tumorcidal Activity of Peritoneal Mϕ

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To investigate the effect of anti-cancer drug (CY, γ-IFN) and ginseng saponins (total saponin) on the rat induced cancer by treatment with hepatocarcinogen, the γ-GT (gamma-glutamyl transpeptidase) activity in liver homogenates and the amount of α-FP (alpa-fetoprotein) in peripheral blood system were assayed. The amount of α-FP using anti-α-FP antibody in peripheral blood system was undetectable and the γ-GT activity was decreased upto 60% by ginseng saponin and anti-cancer drug. At early stage of cancer development, those agents suppressed effectively the proliferation of transformed cell into tumor clone. And the effect of these agents on the tumorcidal activity of peritoneal Mϕ showed no cytotoxicity on the target cell by ginseng saponin alone but resulted in the increase of tumorcidal activity through the activation of Mϕ.

Influence of Ginseng Extract (Korea, China and America) on the Cellular Immune Response Against Sarcoma-180 Tumor Cell in Mice

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This experiment was conducted to evaluate the inhibitory effect of ginseng (Korea, China and America) on the growth of cancer through the activation of immunocytes *in vivo*. Lymphocytes were separated from the spleen of tumor bearing mice after oral administration of purified ginseng (Korea, China and America) extract.

Cytotoxicity of NK cells and that of LAK cells were observed by using the method of four-hour ^{51}Cr-release. Inhibition of macrophage migration by MIF were measured by capillary tube method and chemotaxis of leukocytes obtained from the heart puncture were evaluated by using the Bolyden chamber. Tumor mass was measured and different white blood cells were also counted from tumor bearing mice after administrating ginseng.

The results obtained are summarized as following:

1. The chemotaxis of leukocytes from the group treated with Korean red ginseng was significantly higher than control group.
2. .The producing ability of migration inhibition factor from the mice treated with Korean red ginseng was better than those two groups treated with Chinese and American ginseng. Among the subfractions of Korean red ginseng extract, the fraction C appeared to be most effective.
3. The cytotoxic effect of NK cells from the mice treated with Korean red ginseng was significantly increased.
4. The cytotoxicity of IL-2 treated NK cells from the mice treated with ginseng was higher than control group, the Korean red ginseng appeared to be most effective.
5. The cytotoxicity of LAK cells in the mice treated with Korean red ginseng appeared to be significantly increased.
6. Tumor mass was decreased in the Korean red ginseng treated group.
7. The effect of the ginseng extract on differential blood cell counts were not different from the control group. But it tends to be decreased in the group treated with Korean red ginseng, and the values of RBC, hemoglobin and hematocrit were increased.

In summary, ginseng (Korea, China and America) could inhibit the growth of mouse tumor cells through the activation of cytoxicity of NK cells and LAK cells, the inhibition of macrophage migration and the chemotaxis of leukocytes. Among three kinds of ginseng obtained from different sources, the Korean red ginseng appeared to be most effective.

Immunomodulatory Activity of Polysaccharide Fraction *Ginsan* Isolated from *Panax ginseng*

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We isolated an immunomodulatory fraction *ginsan* from *Panax ginseng*. *Ginsan* contained about 83% carbohydrates and 12% protein with molecular weight higher than 50,000. *Ginsan* proliferated murine

splenocytes in higher extend than ConA and also generated activated killer (AK) cells in the presence of adherant macrophages. These AK cells killed both NK sensitive and insensitive tumor target cells without MHC restriction. *Ginsan* showed synergistic activity for the generation of AK cells with IL-2. *Ginsan* did not show any lectin properties such as inhibitory effect of sugar, hemaggutination, and LDCC. Supernatant of cultured splenocytes with *ginsan* contained IL-2 and INFr. Precursor cells of the AK cells was $AGM1^+$ NK cells. Ginsan also activated peritoneal macrophages to kill tumor cells and these activated macrophages produced nitric oxide.

Intraperitonel injection of 25 mg/kg/day for 6 days of *ginsan* inhibited lung metastasis of B16 melanoma cells by 35% and also administration of *ginsan* in drinking water at 0.5 mg/ml for 6 weeks from 3 weeks after birth reduced lung tumor incidence by 64% in mice injected with 0.5 mg of B(a)P within 24 hrs after birth.

The Effect of Red Ginseng Extract on Antioxidant Enzyme Activities and Lipid Peroxidation of the Kidney in γ-Postirradiated Mice

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Effect of red ginseng extract (5.5 mg/mouse; ip) pretreatment on antioxidant enzymes (superoxide dismutase, catalase and peroxidase) activities and lipid peroxidation were studied in the cytosol fraction of kidney. The experimental was carried out on whole-body irradiated (6.0 Gy, ^{60}Co) and non-irradiated ICR mice. In the red ginseng extract treated and irradiated mice, the activities of Cu, Zn-SOD (27.8%), Mn-SOD (31.9%), catalase (17.9%) and peroxidase (15.0%) were significantly enhanced, but the content of malondialdehyde was considerably decreased (81.0%) at 21 day, compared with non-treated mice. The enhanced antioxidant enzymes activities seems to be contributed to scavenge ROS generated by ionizing radiation. These results suggest that red ginseng extract probably plays an important role in radioprotective effect.

Inhibitory Activity of Korean Ginseng and Lipolytic Action of Toxohormone-L from Cancerous Ascites Fluid

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This study was devised to observe the inhibitory activity of the crude acidic polysaccharide, water extract and ginsenosides fraction from Korean white ginseng and red ginseng on a lipolytic action of toxohormone-L.

Toxohormone-L is a lipolytic factor, found in ascites fluid of Sarcoma-180 bearing mice and of patients with hepatoma.

A substance that inhibited the lipolytic action of toxhormone-L was a crude acidic polysaccharide isolated from Korean ginseng. The total inhibitory activity (units) of PG_1 and PG_4 fractions from white

ginseng and that of PG_1, PG_4 and PG_{4-3} fraction in red ginseng were higher than other fraction treatment at the same concentration *in vitro* test.

Each water extract of ginseng was effective *in vitro* test against the inhibition of lipolysis induced by the toxohormone-L at the concentration over 10~100 μg/m*l*. The total inhibitory activity was high at the concentratin of 100 μg/m*l* of the 4-year and 5-year old white ginseng root respectively, while, it was higher in the 6-year old ginseng than other ages regardless of the reaction concentration in the red ginseng.

And the inhibition effect of ginsenoside-Rb_2 on the lipolysis by toxohormone-L was shown the highest percent among other ginsenoside treatment at concentration of 100 μg to 500 μg/m*l* of reaction mixture and total inhibitory activities of ginsenoside-Rb_2 were also the highest among other treatment at the same concentration.

Effect of Ginseng Saponins on the Release of Arachidonic Acid from Plasma Membrane of Human Embryo Fibroblast WI-38 Cell Line and Human Squamous Cell Carcinoma SCC13 Cell Line

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As one of the studies to elucidate biological effect of ginseng in human body, we have studied the effect of ginseng saponins on arachidonic acid (aa) metabolism which is considered to be an important biologic mediator of various homeostatic responses. Since one of rate-limiting steps for aa metabolite formation such as prostagladins and leukotrienes is release of free aa from phospholipid component of plasma membrane, we examined effect of various ginseng saponins on the liberation of aa from plasma membrane of human embro fibroblast WI-38 or human squamous cell carcinoma SCC13 cell line. The cells were labelled with ^{3}H-arachidonic acid for 16 hr and incubated with various concentrations of saponins in the presence of ETYA, which is known to inhibit both cyclooxygenase and lipoxygenase pathway and ultimately accumulate the released aa in cytoplasm. Lipid soluble fractions were extracted and separated by thin layer chromatography followed by autoradiography. Total saponins at concentrations ranging from 10^{-9} g/m*l* to 10^{-4} g/m*l* inhibit aa release from plasma membrane at a dose dependent manner. However, they were not able to block calcium ionophore mediated release of aa from the membrane. Further experiments will elucidate mechanism underlying this response.

Hypoglycemic Action of the Fat Soluble Fraction of *Panax ginseng* C.A. Meyer in Streptozotocin Induced Diabetic Rats

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This study was made to understand a hypoglycemic action of the fat soluble fraction of *Panax ginseng* C.A. Meyer in streptozotocin induced diabetic rats by determining the activities of several enzymes relating to carbohydrate and lipid metabolism as well as several blood component levels such as glucose and ketone bodies, and the results were compared with those of ginsenoside fraction.

Albino rats (Sprague Dawley, 170~200, ♂) were injected once with 70 mg streptozotocin/kg body

weight intraperitoneally and fed with ordinary diet for 7 days, and then the fat soluble fraction (5~15 mg/day/rat) was injected intraperitoneally once a day for three days to rats having high blood glucose level over 300 mg/ml. After a final injection of the fat soluble fraction, rats were starved for 16 hours followed by the analysis of blood sera and liver enzymes.

It was found that increased levels of glucose, ketone bodies and free fatty acids in streptozotocin induced rats were decreased appreciably by administration of the fat soluble fraction. However, the amount of administered fat soluble fraction did not show any significantly different hypoglycemic action. Decreased activities of glucokinase, phosphofructokinase, pyruvate kinase, 6-phosphogluconate dehydrogenase and acetyl CoA carboxylase of the liver of streptozotocin induced diabetic rats were greatly modified suggesting that a hypoglycemic action of the fat soluble fraction was also appreciable as ginseng saponin fraction.

We also compared a hypoglycemic action of the fat soluble fraction prepared from American ginseng and Chinese ginseng with that of Korean *Panax ginseng*. No significant difference of the hypoglycemic activity was observed between the above ginseng fat soluble fractions, suggesting that a study of the fat soluble fraction might be one of the most interesting subjects relating to diabetic hyperglycemia in the near future.

Cellular Distribution and Metabolism of Ginsenosides in Rat Liver

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Natural ginsenoside mixture (0.5 mg) and 0.8 μCi of synthesized ^{14}C-ginsenosides were administered orally to a rat and killed at one hour after the ginsenoside administration and the liver was fractionated into nuclear fraction, mitochondria, microsomes and cytosol fraction.

Radioactivity distribution in subcellular fractions of the liver showed that 32% of total radioactivity absorbed in the liver was in cytosol fraction but a significant portion of the radioactivity was also found in mitochondria (26.6%) and microsomal fraction (18.1%), and 5.8% of the total radioactivity was recovered from the nuclear as well. This suggested that ginsenosides might be distributed into all subcellular fraction.

Activities of mitochondrial aldehyde dehydrogenase, lactate dehydrogenase and malate dehydrogenase of the liver of rat at two hours after the ginsenoside administration were found appreciably stimulated, suggesting that the ginsenoside concentration in the liver might be around 10^{-5}%, since optimum concentrations for most enzyme catalyzed reaction *in vitro* were known to be 10^{-6}~10^{-4}%.

A significant portion of the radioactivity recovered from subcellular fractions of the liver was found in protein fractions, suggesting that proteins might interact with ginsenosides. Examination of protein-ginsenoside interaction by gel filtration, equilibrium dialysis and ammonium sulfate precipitation technique suggested that proteins and ginsenosides do not bind covalently but weakly combine.

When purified ginsenoside Rb_1 and Rg_1 were incubated with cytosolic enzymes for 20 min, ginsenosides were hydrolyzed quickly, suggesting that ginsenosides might be rapidly hydrolyzed and metabolized in the liver.

Inhibitioin Effect of Ginseng Total Saponins on the Development of U-50, 488H-Induced Antinociception is Dependent on Serotonergic Mechanisms

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Purpose : The purpose of this study is to test whether ginseng total saponin (GTS) inhibits the development of tolerance toward U-50, 488H-induced antinociception and to elucidate that GTS is dependent on serotonergic mechanisms.

Method : Antinociceptive effect was measured by the tail flick test, a modified D'Amour and Smith method. U-50, 488H (30 mg/kg) was injected to mice S.C. once a day for 10 days. GTS (100 mg/kg or 200 mg/kg) was injected I.P. 4 hours prior to the administration of U-50, 488H.L-DOPA (100 mg/kg) or 5-HTP (100 mg/kg) was pretreated I.P. 30 min prior to the administration of U-50, 488H. To assess tolerance development, the antinociceptive effect of U-50, 488H measured at day 11th, 24 hours after the final administration of U-50, 488H was expressed as percent of the antinociceptive effect obtained with single administration of U-50, 488H.

Result : The inhibitory effect of GTS on the development of tolerance to U-50, 488H-induced antinociception was reversed by 5-HTP, but not L-DOPA. These finding suggests that the inhibitory effect of GTS on the development of tolerance to U-50, 488H-induced antinociception is dependent on serotonergic mechanisms.

Ginseng Component on DNA Repair and Superoxide Dismutase Activity of UV-Irradiated Cells

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Radioprotective ginseng component purified from Tris-HCl (pH 7, 6) buffer extract of Korean white ginseng was treated to UV irradiated CHO and L5178Y-R cells to determine DNA repair capacity by the method of ^{3}H-thymidine incorporation and superoxide dismutase activity by nitroblue tetrazolium method, respectively.

The results demonstrated significant increase in the DNA repair capacity of damaged cells upon the addition of ginseng component. However, when acid hydrolysates of the component were treated to cells, the enhancing effect was not shown, implying the active component being destroyed by hydrolysis. The activity of superoxide dismutase was augmented in UV irradiated cells, possibly due to the self-protection mechanism of cells in damage. But the addition of ginseng restored the enzyme to normal level suggesting that free radicals produced by UV light were effectively scavenged by the component.

Radioprotection by ginseng may be accomplished by multiple mechanisms, of which DNA repair enhancement and free radical scavenging are confirmed to be at least two of them.

Protective Effect of Ginseng Against Age-Related Increase of SCE Frequencies in Bone Marrow Cells from Senescenes Accelerated Mice (SAM P_1)

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The effect of Korean red ginseng on frequency of sister chromatid exchanges (SCE) was examined in bone marrow cells from SMA P_1 with age. Mice were administered with water extract of ginseng together drinking water (25 mg/kg body weight/day) from the 25th day after birth for their life.

SCE frquencies in bone marrow cells from the untreated mice were progressively increased with age as follows; 3.9± 1.3, 6.5± 1.9 and 7.6± 2.1 at 3, 12 and 15 months of age, respectively. In the mice administered with ginseng extract, SCE frquency was not significantly increased with age and relatively lower as 4.1± 1.3, 5.0± 2.0 and 5.7± 2.1, respectively. However, there was no difference between two groups in chromosomal aberration.

These results suggest that long-term administration of ginseng protects effectively mice from being genetically damaged with age.

Effect of Red Ginseng Saponins on the Psycotropic Action

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In an attempt to identify the funtional role of central dopaminergic processes for the action of red ginseng saponin on apomorphine-induced stereotyped behavior was studied. We have examined the behavioral pharmacological effect of red ginseng saponin as comparared with that on the neutropic drugs in brain damaged rats. The red ginseng saponins, total saponin (50 mg/kg), PT or PD (both; 25 mg/kg), Gb_1 or Gg_1 (both; 12.5 mg/kg) little affected the locomotor activity but high concentrations of PT (100 mg/kg), PD (100 mg/kg) and Gg_1 (50 mg/kg) caused reduction of the locomotor activity in normal rats. However, when 6-hydroxydopamine is treated, total saponin (50 mg/kg) and PT (100 mg/kg) produced a prominent stimulation in locomotor activity. On the other hand, PT (25 mg/kg) and Rg_1 (12.5 mg/kg) showed a significant increase in the apomorphine elicited stereotypy, and total saponin and Rb_1 showed very weak actions but PD showed a significant suppression as compared with neutropic drugs, centropenoxine (CTP) and methylglutamine orotate (MG). The present results suggest that the functional activation of cerebral dopaminergic system plays a possible role in the mode of action of PT and Rg_1. This action could be responsible for the beneficial effects of ginseng saponin on conditions of fatigue and decreased alertness.

Effect of Panaxytriol from Korean Red Ginseng on Breakdown Inhibition of Membrane Phosphoinositides During Activation of Human Platelets with Thrombin

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Panaxatriol (PT) from Korean red ginseng inhibits the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2), phosphatidyllinositol 4-monophosphate (PIP) and phosphatidylinositol (PI) on the platelet aggregation by thrombin.

At the same time, Ca^{2+}-influx and the production of thromboxane A_2(TXA_2) was inhibited by PT. And then, PT also inhibited the release of serotonin by thrombin. These results suggest that PT plays the role of antiplatelets function by inhibiting the breakdown of phosphoinositides (PTP_2, PIP, PI).

Stimulatory Effect of Ginsenosides on $pp60^{c\text{-}src}$ Protein Tyrosine Kinase

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Ginsenosides present in the roots of *Panax ginseng* C.A. Meyer were shown to induce a stimulatory effect on the overexpressed cellular chicken c-src protein tyrosine kinase in NIH 3T3 cells. Among 4 ginsenosides studied (G-Rb_2, G-Rc, G-Re and G-Rg_1), G-Rg_1 showed the most stimulated effect at 16.7 μg/m*l* ginsenoside concentration increasing the activity by 2∼4 times. Inhibitors of protein synthesis or RNA synthesis blocked the activation of c-src protein tyrosine kinase. These results suggest that the c-src kinase activation appears to involve an increase in the amount of protein of the kinase by transcriptional control mechanism rather than an increase in the kinase activity.

Effect of *Panax ginseng* Extract on Histopathological and Biochemical Aspects of Mice Pretreated with Parathion

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In an effort to determine whether administration (0.5 mg/kg BW) of ginseng (*Panax ginseng* C.A. Meyer) extract is effective in normalization of male ICR mice pretreated with repeated oral dose (0.5 mg/kg body weight) of parathion, histopathological, behavioral and biochemical aspects of the four treatment groups (parathion, parathion+ginseng, ginseng and control) were investigated.

Repeated oral dose of parathion affected adverse effect on growth, but administration of ginseng extract to mice pretreated with parathion caused higher weight gain than parathion alone. Differences in food consumption (g/g BW/day) among groups were significant. Mice that were treated with parathion and parathion+ginseng extract consumed more food than that of control. Significant differences in growing activity among four treatment groups were observed. Grawing activity is higher in order of control>parathion>ginseng+parathion>ginseng. These results suggest that sediment action of ginseng may be involved in the phenomenon.

There was no significant difference in weights of liver and kidney among the four treatment groups. Parathion alone caused hepatic necrosis and increase in activities of GPT and GOT, and administration of parathion+ginseng appeared to be effective in normalization of male ICR mice pretreated with parathion. These results suggest that ginseng has influence on the protective action of liver. In male mice administered ginseng, there was a significant increase in hepatic protein. In male mice given parathion and parathion+ginseng showed a slight increase in hepatic protein concentration.

Parathion caused no induction of detoxication enzyme systems such as cytochrome P-450 dependent

monooxygenase system and glutathion S-transferase, but ginseng treatment caused the enzyme induction.

Based upon our data, ginseng may reduce the toxicity in parathion-intoxicated animals.

Maltol, an Antioxidant Component of Korean Red Ginseng, Shows Little Prooxidant Activity

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Plant phenolic compounds have often been demonstrated to show prooxidant properties as well as antioxidant activities. Maltol(3-hydroxyl-2-methyl-4-pyrone) is one of antioxidant components of Korean red ginseng identified in our laboratory. Pro-oxidant activity of a compound is being measured by the tendency of the compound to generate hydroxyl radicals in a given system. Hydroxyl radicals thus generated can be measured by deoxyribose method or DNA-bleomycin assay. Using these methods, prooxidant activity of maltol was compared to other known antioxidants. The results showed that maltol possessed little prooxidant activity. The lack of prooxidant activity of maltol was reinforced by the findings that maltol at 2 mM did not reduce NBT (nitroblue tetrazolium) and showed a diminished Fe^{3+}-reducing ability compared to pyrogallol when reduction of Fe^{3+} ions was measured as the formation of bathophenanthroline sulfonate-Fe^{2+} complex. Therefore, in spite of relatively weak antioxidant activity, maltol having little prooxidant property should be expected to protect the biological system against oxidative stress.

Antigenicity Study of Aqueous Extract of Red Ginseng in Guinea Pigs

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Aqueous extract of red ginseng was examined for the antigenicity in Hartley guinea pigs in comparision with ovalbumin (OVA). The results obtained were as follows:

When guinea pigs were sensitized with aqueous extract of red ginseng emulsified with Freund's complete adjuvant (FCA), these animals showed negative reactions in active systemic anaphylaxis (ASA), active cutaneous anaphylaxis (ACA), passive cutaneous anaphylaxis (PCA), passive hemagglutination (PHA) and Schultz-Dale test.

As positive cotrols, guinea pigs were sensitized with OVA emulsified with FCA. As a result, these animals disclosed positive reactions in ASA, ACA, PCA, PHA and Schultz-Dale test.

As shown above, aqueous extract of red ginseng was considered not to possess antigenic properties in guinea pigs.

In addition, the dose levels of aqueous extract of red ginseng empolyed in the present experiment were confirmed not to suppress immune reactions.

New Season-Specific Saponins from the Leaves of *Panax ginseng* C.A. Meyer

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We investigated the seasonal change of saponins from the leaves of *P. ginseng* and isolated five kinds of season-specific ones. By silica gel column chromatography, prep-HPLC, MS, NMR (^1H, ^{13}C, COSY, DEPT) and chemical reactions, the molecular structures of them were identified as 3β, 6α, 12β, 20(S), 25-pentahydroxydammar-23(24)-ene-(20-O-β-D-glucopyranosyl)-6-O-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside 〈Fe_1〉, 3β, 6α, 12β, 20(S), 24-pentahydroxydammar-2 t(26)-ene-(20-O-β-D-glucopyranosyl)-6-O-α-L-rhamnopyranosyl(1→2)-β-ν-glucopyranoside 〈Fe_2〉, 3β, 6α, 12β, 20(S), 24-pentahydroxydammar-25(26)-ene-(20-O-β-D-glucopyranosyl)-6-O-β-D-glucopyranoside 〈Fg_2〉, 3β, 12β, 20(S), 25-tetrahydroxydammar-23(24)-ene-(20-O-β-D-glucopyranosyl)-3-O-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside 〈Fd_1〉, and 3β, 12β, 20(S), 24-tetrahydroxydammar-25(26)-ene-(20-O-β-D-glucopyranosyl)-3-O-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside 〈Fd_2〉.

It was demonstrated that two of them, Fg_2 and Fe_2, were newly identified saponins. Fe_1 had the same structure as Majoroside-F_6 which had been reported from the leaves of *P. japonicus* and Fd_1 and Fd_2 were identical with F_6a and F_6bc, respectively.

Analysis of Ginseng Saponin by Thermospray LC/MS

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Analysis of ginseng saponins by thermospray (TSP) LC/MS was examined. Capillary temperature, repeller voltage, and concentration of electrolyte were optimised. The TSP LC/MS spectrum of each ginseng saponin was obtained at optimal condition, and the detection limit of each saponin was determined using SIR (selected ion recording) technique. The detection limit of ginsenoside Rg_1 was 10 ng and the correlation coefficient of the calibration curve was 0.999.

Microscale Analysis of Ginseng Saponin By IC/PAD

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Analysis of ginseng saponins by ion chromatographic separation with pulsed amperometric detection (IC/PAD) was examined. Ginseng saponins were separated on Carbopac PA1 or AS4A anion exchange column with 1 M NaOH as mobile phase. The measuring potential (E1), oxidation potential (E2), and reduction potential (E3) were 0.0 + 0.6 V and −0.8 V, respectively, using gold electrode. The dynamic linear range was over three orders and the limit of detection of ginsenoside Re was 1.2 pmol (S/N = 3).

Rapid Hydrolysis of Ginsenosides Using Microwave Oven

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The analysis of ginsenosides by GC contains time-consuming process of the hydrolysis of ginsenoside to panaxadiol or panaxatriol, which usually takes more than 5 hrs. Rapid hydrolysis of ginsenoside was examined to reduce this reaction time. It is based on the microwave oven reaction, which proceeds under high temperature and high pressure. Two kinds of PTFE reaction vessels were tested. The optimum hydrolysis time using 5% H_2SO_4 solution (H_2O : EtOH = 3 : 1) was 10 minutes with PTFE reaction vessel A in microwave oven, which is more then 30 times faster than conventional method. The hydrolysis time was reduced to 80 seconds using reaction vessel B (23 m*l*, Parr). This method was applicable to the analysis of ginsenoside in pharmaceutical preparations of ginseng.

Contents and Gel-Chromatograms of Mucilage and Pectin from *Panax ginseng*

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Contents of mucilage and pectin in the roots of *Panax ginseng* C.A. Meyer were evaluated by the complex formation with Alcian blue dye and the uronic acid analysis, respectively. The mucilage content in red ginseng was about three fold higher than that in fresh ginseng, while the uronic acid contents in both ginsengs were similar.

The determination of mucilage contents in various parts of both ginsengs revealed that main roots contained the component more than fine roots. Gel chromatographic pattern analysis on mucilage and pectin extracted with water from fresh and red ginsengs showed the polysaccharides of both ginsengs quite differed from each other in the aspects of chemical compositions and molecular size distributions.

Sorption Characteristic of Binary Mixture of Red Ginseng Powder and Maltodextrin or Lactose

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A study was designed to investigate the sorption characteristics of binary mixture of red ginseng powder and maltodextrin (DE-9 and DE-17) or lactose stored at various relative humidities ranging from 52% to 92%. At low relative humidities below RH 67%, the sorption equilibrium was easily achieved, whereas at higher relative humidity values over 75%, all of the mixtures tended to absorb moisture continously with an increase in storage time. A linear equation of $\log (dw/dt) = a \log(t) + \log b$ was found to be valid between the sorption rate and storage time with respect to storge humidities. A linearity was also found between $\log (1 - Aw)$ and the amount of water absorbed over the Aw range of 0.52~0.92 and the slope was affected by the kind of sugar added.

Effects of Light on the Ultrastructure of Ginseng Mesophyll Chloroplast and Thylakoid Membrane Protein

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The effect of sun-light exposure on the ultrastructure of chloroplast and proteins of thylakoid membrane has been examined in *Panax ginseng* C.A. Meyer under field conditions using 5% and full sun-light exposures. Leaves from two year old plants were allowed to function for three months after emergence under both conditions.

The control leaves showed good development of the thylakoid membrane with grana stacking. However the full sun-light exposure resulted in remarkable decrease in the grana stacking and the presence of numerous osmophilic globules. Ginseng grown under full sun-light condition has higher chl.a/chl.b ratio.

Protein gel electrophoresis measurement indicated significant decrease, especially in LHCP II* for the leaves from the full sun-light exposure, as compared with the control (5% exposure). Major bands with the molecular weight of 90, 64, 29~30, 22 and 14 kD, as well as minor bands of 59, 58, 54, 52, 49, 46, 44, 35, 23, 21, 18~19 kD were observed by densitometric scanning for proteins.

The exposure to full sun-light resulted significant decrease in all the band intensities, especially for 58~59, 46~47, 23 kD protein.

The Phosphorylation of Cytosolic and Acid-Soluble Proteins in Callus of *Panax ginseng* C.A. Meyer by the Response of Chitosan

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For the investigation of phosphorylation of intracellular proteins in the ginseng cells caused by the interaction between cells and an elicitor chitosan, the two cell lines (PGL 2 and PGL 3) were obtained from the suspension culture by their growth patterns. In both cell lines, *de novo* synthesis of cytosolic proteins were revealed to continue during incubation of inorganic phosphate, in contrast with the case of acid-soluble proteins (incubation time independent amount of proteins).

An identical incubation time (4 hours) for the maximum uptakes of inorganic phosphate was observed in the two cell lines. However, different times for the maximum incorporation of inorganic phosphate to the intracellular proteins were observed in times of cell lines and types of proteins; 1) 50,992 cpm/mg protein in the case of cytosolic proteins in PGL 2 at 8 hours of incubation, 2) 100,000 cpm/mg acid-soluble proteins in PGL 2 at 8 hours of incubation. Further incorporation of phosphate into the acid-soluble proteins than the case of the cytosolic proteins was observed at early time (<1 hour).

When the chitosan was added to the ^{32}Pi-labeled cells (PGL 2), the phosphorylation of cytosolic proteins was fluctuated in the presence of 1 mM $[Ca^{2+}]_o$ only, a predominant phosphorylation of the proteins was revealed at 10 minutes. In contrast, EGTA effect was not appeared in the acid-soluble proteins and faster phosphorylation of the acid-soluble proteins than the one of the cytosolic proteins was also observed in the presence of chitosan and 1 mM $[Ca^{2+}]_o$.

Phosphorylations of individual cytosolic proteins was classified as two groups by the chitosan response in the presence of 1 mM $CaCl_2$; 1) Ca^{2+} dependent phosphorylation (87.1, 47.0 kDa and 24.3 kDa) and

2) Ca^{2+}-dependent dephosphorylation (84.1, 79.4, 50.7, 26.4 and 18.4 kDa).

For identifying whether phosphorylations of the cytosolic protein and acid-soluble protein were dependent or independent on changes of the intracellular calcium ion, the effect of calcium channel blocker (120 μM of verapamil) was studied. Phosphorylations of 47.0 kDa protein and histone fraction I were decreased by the chitosan treatment of verapamil. Although the clear mechanism is not elucitated, it can be supposed that the phosphorylation of 47.0 kDa protein and histone fraction I depend on the intracellular calcium ions at an appropriate time.

Elicitation Activity of the Spore of *Fusarium solani* Eliciting the Phytoalexin from the Root Tissues of *Panax ginseng* C.A. Meyer

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In order to find out the elicitation of the phytoalexin in ginseng root tissues, the spore of *Fusarium solani* was used as an elicitor. The macroscopic symptoms on the sliced root tissues and the measurement of the elicitor activity suggested us that the spore of *Fusarium solani* elicited the phytoalexin, which was not identified yet, from the ginseng root.

The UV spectral data of water and methanol extracts after treatment with the fungal spore were compared with the ones reported in other plants, and it seemed that the phytoalexin formed in ginseng root might be the phenolic compounds. It was observed that phytoalexin started to accumulate from 15 h after infection of elicitors.

Photosynthetic Response of *Panax ginseng* C.A. Meyer to Change of Light Intensity and Leaf Temperature

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This study was conducted with ginseng plants to investigate photosynthetic response to changes of light intensity and temperature.

CO_2 uptake in diural course was highest in the first phase (8 : 00～8 : 30 Am.) on May 30, 1992. In CO_2 uptake related to stomatal conductance these relationship was synchronized in diurnal course, but relationship between CO_2 uptake and intercellular CO_2 concentration in dirunal course was synchronized oppositely. Leaf temperature and light intensity at the highest CO_2 uptake were range of 23～24℃ and 95 $\mu mol \cdot m^{-2} \cdot S^{-1}$ respectively.

In response to an incresing light intensity under constant leaf temperature (18℃), CO_2 uptake was increased throughout the light intensity sequence up to 250 $\mu mol \cdot m^{-2} \cdot S^{-1}$. Proceeding with a series of leaf temperature under constant light intensity (250 $\mu mol \cdot m^{-2} \cdot S^{-1}$), CO_2 uptake was highest at 18℃ as a 4.1 $\mu mol \cdot m^{-2} \cdot S^{-1}$.

These phenomenons were tendency to change similarly in stomatal conductance and intercellular CO_2 concentration.

Evidence from several approaches indicate that synchronization of CO_2 uptake, stomatal conductance

and intercellular CO_2 concentration was strong coupling and changes of leaf temperature influence photoresponse in photosynthetic processes.

Isolation and Characterization of Chloroplast DNA in Korean Ginseng, *Panax ginseng* C.A. Meyer

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In Korean ginseng, *Panax ginseng* C.A. Meyer, it was difficult to isolate chloroplast DNA with classical methods, because of the high polysaccharide content of ginseng chloroplast. The simple and efficient method of chloroplast DNA isolation from ginseng leaves has been developed by modification of recently advanced methods. Also, it can be successfully applied to ct-DNA isolation of chinese cabbage, radish, petunia, tobacco as well as ginseng.

Isolated chloroplast DNA from ginseng was digested with various restriction endonucleases. It was estimated that the molecular weight of Korean ginseng chloroplast DNA was about 142 kb. There was no difference in restriction endonuclease digestion patterns between two variants of Korean ginseng, which are Jakyung-Jong (violet-stem variant) and Hwangsook-Jong (yellow-berry variant).

For construction of chloroplast genomic library of ginseng, ct-DNA was partially digested with *Eco*RI and ligated with pBS(+) vector. The 325 positive colonies were selected. The 4 positive colonies of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (*rbc*L) were cloned by the colony lift hybridization from the library. The clone No. 10 was analysed by restriction mapping, and was estimated as the size of 8.25 kb, and includes the 4 kb homology sequence of *rbc*L probe.

Distributional Patterns of Vicilin and Legumin in Ginseng Endospern Cells

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Vicilin and legumin, the storage proteins of seeds, were purified from ginseng (*Panax ginseng* C.A. Meyer) endosperm cells. Anti-vicilin and anti-legumin antibodies were produced from each rabbit. These two antibodies and 30 nm-colloidal gold conjugated goat anti-rabbit were treated with endosperm cells according to the developmental stages of seed. Using this immunocytochemical method, the site of synthesis and the pathway of storage of vicilin and legumin were observed. Vicilin and legumin were localized in the vicinity of ER and Golgi complexes at the early developmental endosperm cells. Whereas they were distributed over the protein bodies in the endosperm cells of mature seeds. Vicilin and legumin were present in the protein bodies together but they were not observed any other places except protein bodies in mature seeds.

A Novel Biological Control Agent of Root-Rot Disease of Ginseng

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Three species of bacteria (*Arthrobacter* sp., *Bacillus* sp. and *Pseudomonas* sp.) and 3 fungal species (*Aspergillus* sp., *Penicillium* sp., and *Trichoderma* sp.) were isolated from rhizosphere soil of ginseng as antagonistic to the prevailing soil-borne pathogens of root-rot disease of ginseng; *Sclerotinia* sp., *Botrytis* sp., *Rhizoctonia* sp., *Pythium* sp., *Fusarium* sp., and *Cylindrocarpon* sp. Mass culture of these antagonistic microorganisms were combined in two separate groups, bacteria and fungi, both resulting microbial concentration more than 10^9 cells/m*l*. They were formulated mainly through lyophilization.

When multiplied in simple medium developed for farmer's convenience either sterilized or not, the formulated antagonists showed still good growth and high antagonistic activity at an initial inoculation density of 10^9 cells per 10 liter of the media. Irrigation treatments of either bacterial or fungal formulation or both on soil all resulted in successful suppression of growth of the pathogens pre-inoculated.

Characteristics of Early and Late Emergence Group in Inbred Lines of *Panax ginseng* C.A. Meyer

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The characters of aerial parts and root were investigated in early and late emergence groups of ginseng inbred lines to obtain the basic information for ginseng breeding.

Ginseng plants flowered about the middle of May regardless of early and late emergence. The aerial parts were more vigorous in the late emergence lines than in the early emergence lines. And the root characters of late emergence lines were also better than those of early emergence lines. Significant variation for several traits was observed in early and late emergence lines. Especially, significant correlation between root weight and some aerial part characters, such as stem length, stem diameter, and leaf area, was observed. Multi-stem ginseng plants were highly susceptible to the *Alternaria* stem rot as compaired with single stem ginseng plants.

Therefore, the results above-mentioned suggests that it is desirable to select late emergence lines for high yield of ginseng roots.

Development of Agricultural Machinery in Ginseng Cultivation

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The most important problems of rural community in recently were decreased labour population and increased labour cost. There was a corresponding increase in production cost and decrease in supply of raw materials of manufactured goods in Korea.

In order to solve these problems, we really realized the importance of the development of agricultural machinery on the field in 1992.

We developed these agricultural machinery such as Ridger, Ridge-readjuster, Transplanter. Installating machine for shading structure. Ginseng harvester. Clod-pebble remover, Seed sowing machine and seedling harvester.

These machines were generally satisfactory sources of labour saving.

The ridger which used for attachment over 68 Hp tractor was found to be available for ridge form. The labour saving effect was about 91.7 percent as compared with the cultivator's working.

The ridge-readjuster which was used for attachment of 5 Hp cultivator was found to be available for ginseng transplanting.

The installating machine for shading structure was attached to the 5 Hp cultivator equipped with compressor, hammer, nailer and stapler which were used for insertion or propwood, connection of propwood and cohesion of shading materials. It's effect was about 56.7 percent as compared with labour power.

The ginseng harvester which was used for attachment to 68 Hp tractor was found to available for ginseng harvest and clod-pebble remove. It's effect was about 69.4 percent in harvest and was about 98.0 percent in clod-pebble remove as compared with labour power.

The seed sowing machine which put the 2.3 Hp gasoline engine was found to be available for ginseng seedling and the labour saving effect was about 98.0 percent as compared with labour power.

The seedling harvester which was equipped with the 2.3 Hp gasoline engine was found to be available for seedling harvest and the labour saving effect was about 97.6 percent as compared with labour.

The Effects of Korean Ginseng (*Panax ginseng* C.A. Meyer) Extract and Their Fractions on the Growth and Metabolism of *Saccharomyces cerevisiae* and *Saccharomyces uvarus*

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This study was conducted to investigate the effects of Korean ginseng extracts and their fractions on the growth of *Saccharomyces cerevisiae* and *Saccharomyces uvarus* and their glucose consumption and alcohol production.

The growth of both yeasts were stimulated by ginseng extracts and their water soluble fractions, but were suppressed by ether extracts and n-butanol extracts.

Their growth were enhanced considerably by low molecular weight fractions (<1,000) in water solubles.

Similar results were also obtained with glucose consumption by yeasts.

Substances increasing the growth and glucose consumption by yeasts proved to be a low molecular weight fractions (<1,000) in water solubles not saponins.

The production of n-propyl alcohol by yeast was enhanced by adding ginseng extracts into the media, but that of iso-butyl alcohol was suppressed at the same condition.

The Effects of Korean Ginseng (*Panax ginseng* C.A. Meyer) Extracts and Their Fractions on the Growth of *Escherichia coli*

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This study was conducted to investigate the effects of ginseng extracts and their fractions on the growth of *Escherichia coli* and its glucose consumption.

Considerable amount of impurities such as sugar, protein, lipids and minerals other than saponins were contained in n-butanol extracts which are generally referred to be crude saponins.

Sucrose and maltose were contained as major sugars in ginseng extracts and their water soluble fractions.

Arginine and potassium were also contained as major amino acid and mineral in those fractions, respectively.

Though the glucose consumption and growth of *Escherichia coli* were enhanced by ginseng extracts and their water soluble fractions, those were retarded by ether soluble fractions and n-butanol fractions.

Mechanization : A Key to Survival in the Global Ginseng Industry

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Ginseng production is becoming a competitive agricultural industry throughout the world, Ginseng production is extremely labour intensive and labour supply and cost are major determinants in the efficiency and optimization of commodity production. As a consequence of the value of American ginseng (*Panax quinquefolium* L.) root production in North America has now expanded well beyond the native range of the plant in eastern North America. Over the past ten years, a ginseng industry has emerged in the province of British Coumbia, Canada and the interest in the crop and the planting area has grown very rapidly. Employing the new American ginseng industry as a study for detailed analysis, the nature, role and efficiency of mechanization of tillage, planting, crop maintenance, digging, washing and drying operaions were considered in British Columbia. Cost and efficienty analysis indicates that mechanization avoids labour shortages at crucial times during the crop production cycle and assists in the avoidance of escalating labour costs. The results from this study illustrate that not only costs are reduced but there are also increment in the quality of the product. Further, it is illustrated that the merits of mechanization can be applied to any scale of ginseng production in any of the ginseng growing environements found throughout the world. In the global marketplace, survival from increasingly competitive industries will be dependent on the nature, quality and application of technological innovation in facing the demanding challenges of the future.

Physicochemical Behavior of Ginseng Extract Constituents

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The problem in isolation of ginseng peptide has not been solved completely. In this situation it can be proposed to use the one stage method of reversed phase (RP) liquid chromatography. Really it may be apparant for any beforehand-chosen gradient solvent to be the best case of the chromatogram : the interest substituents and the other substances (impurities) will be situated in the different areas.

Ginseng extract contains a many kinds of substances (sugars, free amino acids, ginsenosides, and etc.). The most problem for the peptide isolation is the absent of any concrete information about physi-

cochemical behavior in various solvents system not only for the ginseng peptides, but also impurities. Thus at the first stage we must study physicochemical behavior of the impurities. And then peptide isolation will be established on the basis of data obtained the suitable mode (modes) of the conditions.

Chromatographic behavior of phenylalanine, sucrose, ginsenosides mixture has been studied for RPH-PLC conditions. The dependences of capacity factor and efficiency of this substances on the organic solvent were studied.