細 胞 資 源 工 学

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本部門では，和漢薬資源の恒久的維持，育成を図るため，薬用生物に関する細胞工学的研究を行なうと同時に，動植物細胞の持つ遺伝情報を解析して，その薬用資源開発への応用，あるいは微生物および動物細胞を用いて生理活性物質の探索，和漢薬の薬効発現機構の解明を行なうことを目指している。本年度の主な研究テーマと成果は下記の通りである。

Ⅰ．腸内嫌気性菌によるバイオトランスフォーメーション

腸内細菌による secoisolariciresinol diglucoside の enterolactone（エストロジェン様作用物質）への変換反応を検討し，各種生成物と主要な反応を触媒する菌種の単離，同定を行った。*Peptostreptococcus* は，芳香環のメトキシ基の脱メチル反応を行い，*Eubacterium* はカテコール型のパラ位水酸基の脱離反応を行うことが明らかにされた。また，*C*-配糖体の開裂反応を検討し，*Bacteroides* の一種が *C*-配糖体 mangiferin を norathyriol に変換することを見いだした。

Ⅱ．薬物の代謝活性化に関与する腸内細菌遺伝子

Sennoside および saikosaponin の加水分解に関与する腸内細菌由来の酵素遺伝子の解析を行った。

Ⅲ．抗ウイルス薬の開発

Croton tiglium から種々のホルボール系の化合物を単離し，その抗 HIV 作用および protein kinase C の活性化作用を検討し，12-*O*-acetylphorbol-13-decanoate は protein kinase C を活性化することなく，強い抗 HIV 作用を有することを見いだした。また，*Stephania cepharantha*に含まれる FK-3000の抗 HSV 作用機序を検討した。

Ⅳ．腎疾患における病態の解明と治療薬の開発

増悪因子のフリーラジカルの関与について，細胞，臓器レベルで検討するとともに，Ginsenoside-Rd，茶，地楡，温脾湯などの役割を追求した。また温脾湯を中心とした長期にわたる漢方治療は，慢性腎不全の進行抑制に有効であり，透析導入の遅延効果が期待できる成績を報告した。

◇著　書

1) Kusumoto I. T. and Hattori M.: Searching for Anti-HIV Agents among the Traditional Medicines. ed. by Watanabe H. and Shibuya T., Harwood Academic Publishers, Amsterdum, 1999, pp. 219-235

2) Ienaga K., Mikami H., Takeuchi S., Nakamura K., Yokozawa T., Oura H., Aoyagi K., Nakano K., and Endou H.: NZ-419, an intrinsic antioxidant, as a theapeutic agent against progressive chronic renal failure and guanidino compounds. "Guanidino Compound in Biology and Medicine: 5," eds. by Clark J.F., Mori A. and Ishida M., Blackwell Science Pty Ltd, Australia, 1999, pp.131-138.

◇原　著

1) **Yu Y., Park J., Lee J., Kim G., Jo S., Byun M., Miyashiro H., and Hattori M.: Screening of some plant extracts for inhibitory effects on HIV-1 and its essential enzymes. Kor. J. Pharmacogn., 29 : 338–346, 1998.** (昨年度未記載)

In order to elucidate the relationship between anti-HIV-1 enzyme activity and inhibition of HIV-1 replication by natural sources, extracts from some plants using as foods and oriental medicines were tested for inhibitory effects on the viral replication, reverse transcriptase (RT), protease and α-glucosidase. In the anti-RT test, water extracts of *Ficus carica* (leaf), *Houttuynia cordata* (aerial part) and *Ixeris tamagawaensis* (aerial part) showed more than 79% inhitibion at a concentration of 100 μg/ml. The protease and α-glucosidase inhibiting samples in the screening were water extract of *Syringa dilatata* (leaf) and methanol extract of *Hibiscus syriacus* (leaf and stem), which showed more than 40% inhibition at a concentration of 100 μg/ml. In the primary anti-HIV-1 test, water extracts of *Equisetum arvense* (aerial part), *Hibiscus syriacus* (leaf), *Ixeris tamagawaensis* (aerial part) and *Pueraira thunbergiana* (leaf) showed the potent inhibition against HIV-1 induced cytopathic effects.

2) **Hussein G., Nakamura N., Meselhy M. R., and Hattori M.: Phenolics from *Maytenus senegalensis*. Phytochemistry, 50 : 689–694, 1999.**

Two new methylated flavan-3-ol glucosides and a methylated proanthocyanidin were isolated from the MeOH extract of the stem-bark of *Maytenus senegalensis*, together with five known compounds. The structures of the new compounds were determined as:(−)-4'-methylepigallocatechin 5-*O*-β-D-glucopyranoside, (+)-4'-methylgallocatechin 3'-*O*-β-D-glucopyranoside and (−)-epicatechin (4$\beta\rightarrow$8) (−)-4'-methylepigallocatechin by chemical and spectroscopic means. The MeOH and H_2O extracts showed moderate inhibitory effects against HIV-1 protease.

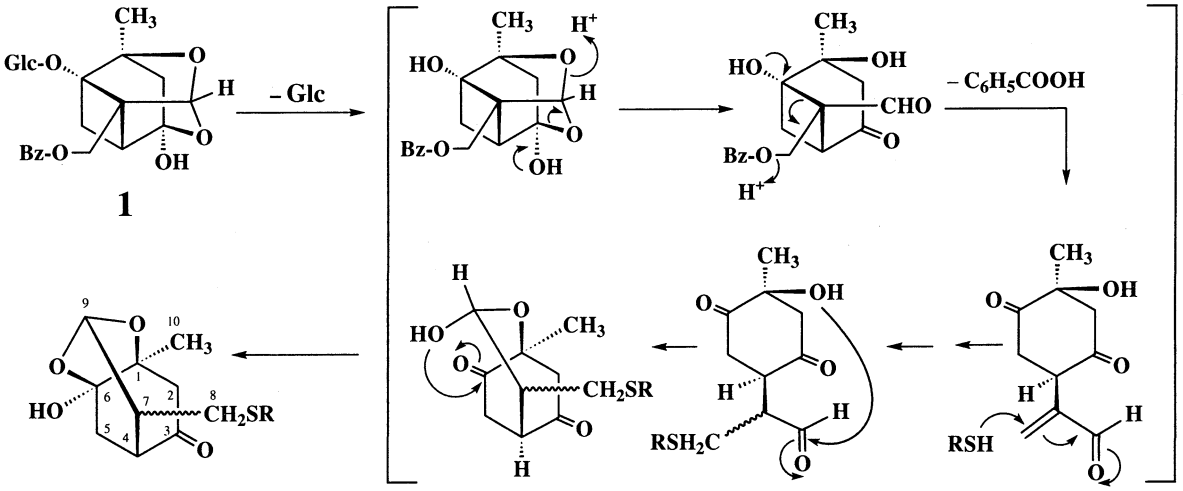
3) **Matsuse I.T., Lim Y.A., Hattori M., Correa M., and Gupta M.P.A.: Search for antiviral properties in Panamanian medicinal plants. The effects on HIV and its essential enzymes. Ethnopharmacology, 64 : 15–22, 1999.**

Aqueous and methanolic extracts of 39 Panamanian medicinal plants were tested for anti-

human immunodeficiency virus (HIV) effects. The extracts were tested for the inhibition of HIV-induced cytopathic effects in cultured cells, HIV-reverse transcriptase (RT) and HIV-protease (PR) enzymes. The water extract of the branches of *Jatropha curcas* (Euphorbiaceae) inhibited strongly the HIV-induced cytopathic effects with low cytotoxicity. On the other hand, the water extracts of the whole plant of *Chamaesyce hyssopifolia* (Euphorbiaceae), the leaves of *Cordia spinescens* (Boraginaceae) and the aerial parts of *Hyptis lantanifolia* (Labiatae), and the methanol extract of the aerial parts of *Tetrapteris macrocarpa* (Malpighiaceae) were potent inhibitors of HIV-RT (IC_{50}: 6-8 μg/ml). Seven out of 39 plants were found to be moderate inhibitors of HIV-PR (IC_{50}: 43-100 μg/ml). Furthermore, we report on the respective inhibitory substances of *J. curcas*, *C. hyssopifolia* and *C. spinescens*, and their possible mechanism of action.

4) **Abdel-Hafez A. A., Meselhy M. R., Nakamura N., Hattori M., Watanabe H., Murakami Y., El-Gendy M. A., Mahfouz N. M. and Mohamed T. A.: Anticonvulsant activity of paeonimetabolin-I adducts obtained by incubation of paeoniflorin and thiol compounds with *Lactobacillus brevis*. Biol. Pharm. Bull., 22 : 491–497, 1999.**

Seventeen thiopaeonimetabolin-I adducts were obtained as mixtures of diastereoisomers after incubation of paeoniflorin with *Lactobacillus brevis* in the presence of various thiols. The anticonvulsant activity of the adducts was investigated in mice using the maximal subcutaneous pentylenetetrazol seizure test and sodium valproate (1.5 mmol/kg) as a positive control. Thirteen adducts showed dose-dependent prolongation of latencies of clonic and tonic convulsions. Maximal protection against convulsions was effectively demonstrated by 8-(*n*-hexylthio)paeonimetabolin I (**8**) and 8-benzoylthiopaeonimetabolin I (**18**) at doses of 0.125 and 0.25 mmol/kg, respectively, while 100% protection was only achieved at 0.5



3, R = *n*-propyl
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10, R = cyclopentyl
11, R = cyclohexyl
12, R = phenyl
13, R = *o*-tolyl
14, R = *m*-tolyl
15, R = *p*-tolyl
16, R = 2-naphthyl
17, R = benzyl
18, R = benzoyl
19, R = acetyl

Chart 1

mmol/kg of 8-cyclopentylthiopaeonimetabolin I and 8-(*p*-tolylthio)paeonimetabolin I. The principal anticonvulsant activity of the diastereoisomers of **8** and **18** was attributed to their 7*S*-isomers [ED_{50} values of 0.09 and 0.12 mmol/kg, and protective indices of 5.0 and 4.0 for **8** (7*S*) and **18** (7*S*), respectively], while the 7*R* counterparts **18** (7*R*) and **18** (7*R*) showed a muscle relaxation eflfect.

5) **Hussein G., Miyashiro H., Nakamura N., Hattori M., Kawahata T., Otake T., Kakiuchi N., and Shimotohno K.: Inhibitory effects of Sudanese plant extracts on HIV-replication and HIV-1-protease. Phytother. Res., 13 : 31–36, 1999.**

Forty-eight methanol and aqueous extracts from Sudanese plants were screened for their inhibitory activity on viral replication. Nineteen extracts showed inhibitory effects on HIV-induced cytopathic effects (CPE) on MT-4 cells. The extracts were further screened against HIV-1 protease (PR) using an HPLC assay method. Of the tested extracts, the methanol extracts of *Acacia nilotica* (bark and pods), *Euphorbia granulata* (leaves), *Maytenus senegalensis* (stem-bark) and aqueous extracts of *A. nilotica* (pods) and *M. senegalensis* (stem-bark) showed considerable inhibitory effects against HIV-1 PR. Inhibitory principles were isolated from *M. senegalensis* and their activities were also discussed.

6) **Nawawi A., Nakamura N., Hattori M., Kurokawa M., Shiraki K.: Inhibitory effects of Indonesian medicinal plants on the infection on herpes simplex virus type 1. Phytother. Res., 13 : 37–41, 1999.**

Water and methanol extracts of 30 traditional medicinal plants, collected in Indonesia, were tested for their anti HSV-1 activity. The extracts of eight plant species showed potent activity on the plaque assay at a concentration of 100 μg/mL. The therapeutic efficacy of seven selected plants was demonstrated by using a mouse HSV-1 infection assay, both the methanol extracts of the fruit of *Melaleuca leucadendron* (Myrtaceae) and the pericarp of *Nephelium lappaceum* (Sapindaceae) significantly prolonged the development of skin lesions and reduced the mortality.

7) **Ma C., Nakamura N., Miyashiro H., Hattori M., and Shimotohno K.: Inhibitory effects of constituents from *Cynomorium songaricum* and related triterpene derivatives on HIV-1 protease. Chem. Pharm. Bull., 47: 141-145, 1999.**

From CH_2Cl_2 and MeOH extracts of the stems of *Cynomorium songaricum* RUPR. (Cynomoriaceae), ursolic acid and its hydrogen malonate were isolated as inhibitors of human immunodeficiency virus type 1 (HIV-1) protease, with 50% inhibitory concentrations (IC_{50}) of 8 and 6 μM, respectively. Amongst various synthesized dicarboxylic acid hemiesters of related triterpenes, inhibitory activity tended to increase in the order of oxalyl, malonyl, succinyl and glutaryl hemiesters, for triterpenes such as ursolic acid, oleanolic acid and betulinic acid. The most potent inhibition was observed for the glutaryl hemiesters, with an IC_{50} of 4 μM.

From the water extract of the stems of *C. songaricum*, flavan-3-ol polymers, consisting of epicatechin as their extender flavan units, were also found to be potent inhibitory principles against HIV-1 protease.

8) **Nawawi A., Ma C., Nakamura N., Hattori M., Kurokawa M., Shiraki K., Kashiwaba N., and Ono M.: Anti-herpes simplex virus activity of alkaloids isolated from *Stephania cepharantha*. Biol. Pharm. Bull., 22 : 268–274, 1999.**

By screening water and MeOH extracts of 30 Chinese medicinal plants for their anti-herpes simplex virus (HSV)-1 activity, a MeOH extract of the root tubers of *Stephania cepharantha* HAYATA showed the most potent activity on the plaque reduction assay with an IC_{50} value of 18.0 μg/ml. Of 49 alkaloids isolated from the MeOH extract, 17 alkaloids were found to be active against HSV-1, including 13 bisbenzylisoquinoline, 1 protoberberine, 2 morphinane and 1 proaporphine alkaloids, while benzylisoquinoline and hasubanane alkaloids were inactive. Although *N*-methylcrotsparine was active against HSV-1, as well as HSV-1 thymidine kinase deficient (acyclovir resistant type, HSV-1 TK^-) and HSV-2 (IC_{50} values of 8.3, 7.7 and 6.7 μg/ml, respectively), it was cytotoxic. FK-3000 was found to be the most active against HSV-1, HSV-1 TK^- and HSV-2 (IC_{50} values of 7.8, 9.9 and 8.7 μg/ml) with *in vitro* therapeutic indices of 90, 71 and 81, respectively. FK-3000 was found to be a promising candidate as an anti-HSV agent against HSV-1, acyclovir (ACV) resistant-type HSV-1 and HSV-2.

9) **Min B., Hattori M., Lee H., and Kim Y.: Inhibitory constituents against HIV-1 protease from *Agastache rugosa*. Arch. Pharm. Res., 22: 75-77, 1999.**

Two diterpenoid compounds, agastanol (**1**) and agastaquinone (**2**), were isolated from the roots of *Agastache rugosa* (Labiatae). Compounds **1** and **2** showed significant inhibitory effects against human immunodeficiency virus type 1 (HIV-1) protease activity with IC_{50} values of 360 and 87 μM, respectively.

10) **Tezuka Y., Terazono M., Kusumoto T. I., Kawashima Y., Hatanaka Y., Kadota S., Hattori M., Namba T., Kikuchi T., Tanaka K., and Supriyatna S.: Helisterculins A and B, two new (7.5', 8.2')-neolignans, and helisorin, the first (6.4', 7.5', 8.2')-neolignan, from the Indonesian medicinal plant *Helicteres isora*. Helv. Chim. Acta, 82 : 408–417, 1999.**

During a chemical study of Indonesian medicinal plants, we examined the constituents of fruits of *Helicteres isora L.* (Sterculiaccae), one of the famoms Jamu medicines. From a water extract of the fruits, we isolated three new neolignans, helisterculins A (**1**) and B (**2**) and helisorin (**3**), and elucidated their structures by spectral analyses. Helisterculins A (**1**) and B (**2**) are (7.5', 8.2')-neolignans with a bicyclo[2.2.2]octene C-framework, while helisorin (**3**) is a (6.4', 7.5', 8.2')-neolignan with a very rare 4,4a,9,9a-tetrahydro-3,9-methano-3*H*-fluorene C-framework. The natural product with the latter C-framework has no literature precedent. The neolignans **1~3** showed weak inhibitory activity against reverse transcriptase

from avian myeloblastosis virus.

11) Kawata Y., Ma C., Meselhy M. R., Nakamura N., Wang H., Hattori M., Namba T., Satoh K., and Kuraishi Y.: Conversion of aconitine to lipoaconitine by human intestinal bacteria and their antinociceptive effects in mice. J. Trad. Med., 16 : 15–23, 1999.

Aconitine was substantially converted to lipoaconitine (LA) after incubation with human intestinal bacteria. The Frit FAB LC/MS and GC/MS indicated that the fatty acid composition of LA was similar to cellular fatty acids of the bacterial strain used; major fatty acids in LA produced by *Bacteroides fragilis* were anteiso C15:0, *n*-C15:0 and *n*-C16:0, while LA produced by *Klebsiella pneumoniae* or by fecal flora contained *n*-C16:0 as the major fatty acid. LA from *Clostridium butyricum* contained C18:1, C18:0, C16:1, C16:0. LA was also obtained after incubation of aconitine with sterile bacterial cells or a precipitate of disrupted bacterial cells in phosphate buffer. Aconitine at a dose of 0.1 mg/kg, significantly increased the nociceptive threshold in mice with nociceptive hypersensitivity, while 8-*O*-oleoylbenzoylaconine (OBA) was active but toxic at a higher dose of 3.0 mg/kg, and 8-*O*-palmitoylbenzoylaconine (PBA) was only active at a dose of 30 mg/kg. These findings suggest that OBA and PBA do not play an important role in the antinociceptive action of aconite tuber and the alkaloid aconitine.

12) Kakiuchi N., Nishikawa S., Hattori M., and Shimotohno K.: A high throughput assay of the hepatitis C virus nonstructural protein 3 serine proteinase. J. Viol. Methods, 80 : 77–84, 1999.

A simple assay was developed based on intramolecular fluorescence resonance energy transfer for detection of the activity of hepatitis C virus (HCV) serine proteinase. Two quenched-fluorogenic substrates, (7-methoxycoumarin-4-yl)acetyl (Mea) Asp-Asp-Ile-Val-Pro-Cys-Ser-Met-Ser-(2,4-dinitrophenyl, Dnp) Lys (Mea-Asp-Asp-Ile-Val-Pro-Cys-Ser-Met-Ser-Lys[Dnp], QF-1) and Mea-Asp-Asp-Ile-Val-Pro-Cys-Ser-Met-Lys(Dnp)-Arg-Arg (QF-2), which derived from the NSSA/5B junction of the HCV polyprotein, were designed. Kinetic studies revealed that QF-1 and QF-2 had high affinity for a recombinant enzyme, which is a fusion protein of maltose binding protein and almost entire nonstructural protein (MBP-NS3), with K_m values comparable to that of longer substrate based on the same cleavage site. QF-1 and QF-2 were cleaved by MBP-NS3 efficiently with k_{cat} values of 7.5 and 4.2 min^{-1}, respectively. QF-2 was also found to be a good substrate of ANS3, which contained serine proteinase part of NS3 with k_{cat} value of 4.3 min^{-1}. The cleavage reaction is detected continuously by the elevation of the fluorescence due to release from quenching. The fluorescence of the substrates increases in proportion to progress of the cleavage reaction under the standard conditions. This method was applied for screening of HCV serine protease inhibitors using a fluorescence multiwell plate reader. A group of naturally occurring products, flavonoids, was subjected to be screened. Two flavonoids out of 25 were found to inhibit the

enzyme moderately at a concentration of 100 μM. The data agreed with those obtained by high-performance liquid chromatography (HPLC). This method is suited to sensitive quantitation of the enzyme reaction as well as the high throughput analysis of the inhibitors.

13) Tanikawa K., Goto H., Nakamura N., Tanaka N., Hattori M., Itoh T., and Terasawa K.: Endotherium-dependent vasodilator effect of tannin extract from Cinnamonomi Cortex on isolated rat aorta. J. Trad. Med., 16 : 45–50, 1999.

Cinnamonomi Cortex (the bark of *Cinnamomum cassia* BLUME) is a crude drug that is widely used in spices and medical products. Although improvement of blood flow by this plant component has long been known, there have been no reports concerning the mechanism involved. We studied the vasodilator actions of this drug especially focusing on the role of endothelium in the isolated vascular bed. Tannin from Cinnamonomi Cortex (TCC) relaxed prostaglandin $F_{2\alpha}$-precontracted ring preparations of rat aorta with intact endothelium. TCC did not cause relaxation of specimens without endothelium, and TCC-induced relaxation was inhibited by pretreatment with 10^{-4} M N^{G}-nitro-*l*-arginine methyl ester. Dimer, trimer, tetramer, and pentamer components of TCC also produced endothelium-dependent vasodilation. Stronger relaxation was caused by higher molecular weight tannins, and endothelium-dependent vasodilation even appeared at low concentrations. In conclusion, we found that TCC exhibits an endothelium-dependent vasodilation in the isolated rat aorta mainly *via* endothelium-derived NO. NO-mediated endothelium-dependent relaxation seems to be more potent for TCC with higher molecular weight than that with lower molecular weight.

14) Tohda C., Nakamura N., Komatsu K., and Hattori M.: Trigonelline-induced neurite outgrowth in human neuroblastoma SK-N-SH cells. Biol. Pharm. Bull., 22 : 679–682, 1999.

Extension of dendrites and axons in neurons may compensate and rescue damaged neuronal networks in the dementia brain. Our aim is to isolate and identify constituents of coffee beans exhibiting neurite outgrowth activity. Among the extracts of raw and roasted coffee beans, a methanol fraction of the ethanol extract (1 μg/ml) of raw beans increased significantly the percentage of cells with neurites in human neuroblastoma SK-N-SH cells. Among subfractions of this methanol fraction was a basic fraction (5 μg/ml) which exhibited significant neurite outgrowth activity. Finally, trigonelline in the basic fraction was identified to be active as far neurite extension was concerned. Treatment with trigonelline (30 μM) increased the percentage of cells with neurites at 3 and 6 d after treatment. In addition, the number of neurites reacting positively to phosphorylated neurofilament-H was increased by treatment with 30 μM trigonelline for 6 d, suggesting enhancement of axonal extension. These results show that trigonelline promotes functional neurite outgrowth.

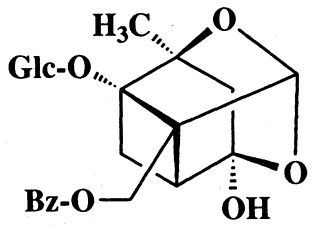
15) **Fan W., Tezuka Y., Xiong Q., Hattori M., Namba T., and Kadota S.: Apocynins A-D: New phenylpropanoid-substituted flavan-3-ol isolated from leaves of *Apocynum venetum* (Luobuma-Ye). Chem. Pharm. Bull., 47 : 1049–1050, 1999.**

Four new phenylpropanoid-substituted flavan-3-ols called apocynins A—D (**1**~**4**) have been isolated from the leaves of *Apocynum venetum* (Apocynaceae), together with two known phenylpropanoid-substituted flavan-3-ols, catechin-[8,7-e]-4 α -(3,4-dihydroxyphenyl)-dihydro-2(3*H*)-pyranone (**5**) and cinchonain Ia (**6**), and four known flavan-3-ols, (−)-epicatechin, (+)-catechin, (−)-epigallocatechin, and (+)-gallocatechin. Their structures were elucidated on the basis of spectral analysis, including 2D NMR and CD spectra. They showed hepatoprotective activity against D-galactosamine (D-GalN)/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes.

16) **Min B., Jung H., Lee J., Kim Y., Bok S., Ma C., Nakamura N., Hattori M., and Bae K.: Inhibitory effect of triterpenes from *Crataegus pinatifida* on HIV-protease. Planta Medica, 65 : 374–375, 1999.**

The methanol extracts of the leaves of *Crataegus pinatifida* showed potent inhibitory activities against HIV-1 protease at a concentration of 100 μg/ml. The subsequent fractionation and isolation of the extract gave two active compounds. Their structures were identified as uvaol (**1**) and ursolic acid (**2**) by spectral data. These active compounds inhibit HIV-1 protease with IC_{50} values of 5.5 and 8.0 μM, respectively.

17) **El-Mekkawy S., Meselhy M. R., Nakamura N., Hattori M., Kawahata T., and Otake T.: 12-*O*-Acetylphorbol-13-decanoate potently inhibits cytopathic effects of human immunodeficiency virus type 1 (HIV-1), without activation of protein kinase C. Chem. Pharm. Bull., 47 : 1346–1347, 1999.**



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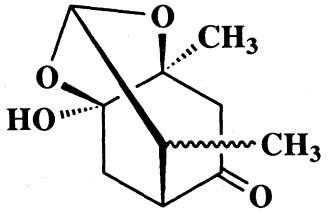

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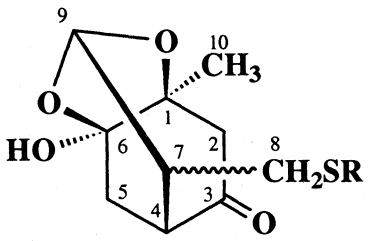

3, **R** = *n*-propyl
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7, **R** = *iso*-butyl
8, **R** = *n*-hexyl
9, **R** = allyl
10, **R** = cyclopentyl
11, **R** = cyclohexyl
12, **R** = phenyl
13, **R** = *o*-tolyl
14, **R** = *m*-tolyl
15, **R** = *p*-tolyl
16, **R** = 2-naphthyl
17, **R** = benzyl
18, **R** = benzoyl
19, **R** = acetyl

Chart 2

Through bioactivity-guided fractionation, eight phorbol diesters, including five new ones (**1~5**), were isolated from the seeds of *Croton tiglium* collected in Egypt. 12-*O*-Acetylphorbol-13-decanoate (**6**) and 12-*O*-decanoylphorbol-13-(2-methylbutyrate) (**4**) potently inhibited the HlV-1-induced cytopathic effect on MT-4 cells (IC_{100} values of 7.6 μg /ml and 7.81 μg/ml, and CC_0 values of 62.5 μg/ml and 31.3 μg/ml, respectively) without activating protein kinase C.

18) Yang X., Zhao J., Cui Y., Liu X., Ma C., Hattori M., and Zhang L.: Anti-HIV-1 protease triterpenoid saponins from the seeds of *Aesculus chinensis*. J. Natural Products, 62: 1510-1513, 1999.

Eight bioactive triterpenoid saponins (**1~8**) were isolated from the seeds of *Aesculus chinensis*, four of which are novel compounds. The major saponins were identified as escin Ia (**1**), Ib (**2**), isoescin Ia (**3**) and Ib (**4**), while the new compounds were identified as 22α-tigloyl-28-acetylprotoaescigenin-3β-*O*-[β-D-glucopyranosyl (1-2)][β-D-glucopyranosyl (1-4)]-β-D-glucopyranosiduronic acid (escin IVc, **5**), 22α-angeloyl-28-acetylprotoaescigenin-3β-*O*-[β-D-glucopyranosyl (1-2)][β-D-glucopyranosyl (1-4)]-β-D-glucopyranosiduronic acid (escin IVd, **6**), 28-tigloylprotoaescigenin-3β-*O*-[β-D-glucopyranosyl (1-2)][β-D-glucopyranosyl (1-4)]-β-D-glucopyranosiduronic acid (escin IVe, **7**), and 28-angeloylprotoaescigenin-3β-*O*-[β-D-glucopyranosyl (1-2)][β-D-glucopyranosyl(1-4)]-β-D-glucopyranosiduronic acid (escin IVf, **8**). The structures were determined by chemical and spectroscopic methods. All the above compounds were evaluated for their inhibitory activity against HIV-1 protease.

19) Min B., Bae K., Kim Y., Miyashiro H., Hattori M. and Shimotohno K.: Screening of Korean plants against human immunodeficiency virus type 1 protease. Phytother. Res., 13 : 680–682, 1999.

With the aim of finding novel anti-human immunodeficiency virus agents from natural products, 93 MeOH extracts of Korean plants were screened for their inhibitory activities against HIV-1 protease. The most potent inhibition was shown by the root of *Rodiola rosea* with 70.4% inhibition at a concentration of 100 μg/mL.

20) Yokozawa T. and Owada S.: Effect of ginsenoside-Rd in cephaloridine-induced renal disorder. Nephron, 81 : 200–207, 1999.

To determine whether ginsenoside-Rd ameliorates the renal injury induced by cephaloridine, the effect of cephaloridine was investigated in rats given ginsenoside-Rd preceding cephaloridine administration and in control rats given no ginsenoside-Rd. In control rats, blood, renal and urinary parameters and the activities of antioxidative enzymes in renal tissue deviated from the normal range, indicating dysfunction of the kidneys. In contrast, when ginsenoside-Rd was given orally for 30 consecutive days prior to cephaloridine injection, the activities of the antioxidation enzymes superoxide dismutase and catalase were

higher, while malondialdehyde levels in serum and renal tissue were lower in the treated rats than in the controls. The urea nitrogen and creatinine levels in serum were decreased in rats given ginsenoside-Rd. Decreased urine volume, increased urinary osmotic pressure, and decreased urinary levels of glucose, protein, sodium and potassium demonstrated a protective action against the renal dysfunction caused by cephaloridine. In addition, it was demonstrated that ginsenoside-Rd affected cultured proximal tubule cells exposed to cephaloridine.

21) Yokozawa T., Dong E., Kawai Y., Gemba M. and Shimizu M.: Protective effects of some flavonoids on the renal cellular membrane. Exp. Toxic. Pathol., 51 : 9–14, 1999.

By assaying lactate dehydrogenase and malondialdehyde leakage from LLC-PK_1 cells in culture, a study was conducted to clarify whether flavonoid compounds ameliorate renal cellular injury The cells were cultured with various concentrations of samples under routine conditions. The results demonstrated that baicalin, cirsimaritin, 6-hydroxyluteolin, luteolin, plantaginin, rhoifolin, sorbarin, afzelin, hyperin, isoquercitrin, isorhamnetin, kaempferitrin, kaempferol 7-glucoside, oxyayanin A, quercetin, quercitrin, rhamnetin and rutin exerted marked protective effects on the cells, whereas acacetin, apigenin, apiin, cirsilineol, genkwanin, pectolinarin and tetramethylquercitin had virtually no effect. In the light of these findings, we propose that the general capability of these compounds is largely decided by the number and position of phenolic hydroxyl groups linked to the structural backbone.

22) Yokozawa T., Liu Z.W., Chen C.P. and Tanaka T.: Evaluation of caffeic acid analogues using a cultured renal epithelial cell line, LLC-PK_1. Pharm. Pharmacol. Commun., 5 : 365–370, 1999.

The effects of several caffeic acid analogues isolated from Salviae Miltiorrhizae Radix (*Salvia miltiorrhiza* Bunge) on the cultured renal epithelial cell line, LLC-PK_1, were studied. Incubation of the cells with different concentrations of these compounds for 48 h markedly suppressed lactate dehydrogenase leakage, which was elicited when the cells were exposed to hypoxia/reoxygenation or cephaloridine. The most active compounds were magnesium lithospermate B and lithospermic acid B, two tetramers of caffeic acid, followed by lithospermic acid (trimer) and rosmarinic acid (dimer). Caffeic acid showed poor activity. These results indicate that caffeic acid analogues, in particular magnesium lithospermate B and lithospermic acid B, exert a direct protective effect on renal cells.

23) Yokozawa T., Lee K.I., Kashiwagi H., Cho E.J. and Chung H.Y.: Antioxidant activity of herbal teas available on the Korean market. J. Food Sci. Nutr., 4 : 92–96, 1999.

The effects of aqueous extracts of Korean commercial teas on excessive free radicals were examined utilizing spin trapping, 1,1-diphenyl-2-picrylhydrazyl(DPPH) radical and lipid peroxidation. A potent scavenging effect of green tea and oriental senna tea was found using spin trapping. The most effective tea against the DPPH radical was green tea,

followed in order by pine leaf tea, Chinese gutta percha tea and oriental senna tea. Similarly to the effects of DPPH radical, green tea, pine leaf tea, Chinese gutta percha tea and oriental senna tea had an inhibitory effect on lipid peroxidation. These findings predict that Korean tea is a promising material for scavenging free radicals, and for curing diseases related to free-radical reactions.

24) Chen C.P., Yokozawa T. and Chung H.Y.: Inhibitory effect of caffeic acid analogues isolated from Salviae Miltiorrhizae Radix against 1,1-diphenyl-2-picrylhydrazyl radical. Exp. Toxic. Pathol., 51 : 59–63, 1999.

Caffeic acid and its four polymers isolated from Salviae Miltiorrhizae Radix were examined for their activity of scavenging free radicals in a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical generating system. The results showed that the polymers of caffeic acid inhibited the DPPH radical more strongly than caffeic acid. The strongest activity was displayed by two tetramers, lithospermic acid B and its Mg^{2+} salt. The trimer (lithospermic acid) and dimer (rosmarinic acid) showed similar efficiency. In comparison, caffeic acid was less efficient in scavenging free radicals. Determination of the activity of caffeic acid derivatives of small molecules revealed that the *o*-dihydroxyl group was the most important active structure of caffeic acid derivatives for scavenging of free radicals. Lack or substitution of this structure resulted in marked reduction or even loss of the activity. Structural modification of the side chain of caffeic acid produced slight changes in activity. The present results demonstrate that a saturated group connected to the aromatic ring has slightly higher inhibitory activity against the DPPH radical than an unsaturated group.

25) Kye I.S., Jeon Y.S., No J.K., Kim Y.J., Lee K.H., Shim K.H., Kim J., Yokozawa T. and Chung H.Y.: Reactive oxygen scavenging activity of green tea polyphenols. Kor. J. Gerontol., 9 : 10–17, 1999.

Reactive oxygen species (ROS), such as O_2^-, H_2O_2, and • OH, mediate reactions which can result in the damage of critical biological molecules including DNA and cellular protein. In the previous studies, green tea polyphenols were found to contain biological and pharmacological activities that many contribute to chemopreventive effects. However, almost no information was known on effects of green tea components against each specific ROS such as • OH, O_2^- and H_2O_2. The purpose of this present study was to investigate the ROS scavenging effects of green tea polyphenols against each species of ROS using DCFDA assay. Green tea polyphenols, (−)-epicatechin 3-*O*-gallate (ECG), (−)-gallocatechin 3-*O*-gallate (GCG), (−)-epigallocatechin 3-*O*-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC) and (+)-catechin (C) significantly showed ROS scavenging activity in liver and kidney homogenate. Especially, ECG, GCG and EGCG showed the potent ROS scavenging activity, which indicates that the galloyl group may be active chemical moiety that is responsible for this scavenging activity. We also examined the effects of ECG, GCG and EGCG on ROS to elucidate the scavenging activity against O_2^-, • OH and/or H_2O_2. As the results, ECG, GCG, and

EGCG remarkably scavenged the ROS. These results suggest that green tea polyphenols, ECG, GCG, and EGCG having multiple galloyl groups and flavan-3-ol, show potent antioxidative action through ROS scavenging activity.

26) Kye I.S., Jeon Y.S., Soung D.Y., Rhee S.H., Kim J., Yokozawa T., Chung H.Y. and Lee K.H.: Cytoprotective mechanisms of green tea polyphenols in cultured liver cells. J. Kor. Assoc. Cancer Prev., 4 : 9–16, 1999.

Oxygen is essential for human life, yet it is dangerous to all aerobic organisms. This is because some oxygen is metabolized to oxygen-derived free radicals and other free radical species which, in excess, can cause the cell injury and be opposed by antioxidant defence systems. Nitric oxide (NO •) is known to the oxidant injury via production of the potent oxidant peroxynitrite ($ONOO^-$). In this study, 3-morpholinosydnonimine (SIN-1), 2,2-azobis[2,4-dimethylvaleronitrile] (AMVN), sodium nitroprusside (SNP), H_2O_2 and *tert*-butyl hydroperoxide (t-BHP) which can produce intracellular free radicals, the cause of tissue damage and lipid peroxidation, were used for elucidating cytoprotective mechanisms of (−)-epicatechin 3-*O*-gallate (ECG), (−)-gallocatechin 3-*O*-gallate (GCG), and (−)-epigallocatechin 3-*O*-gallate (EGCG) extracted from green tea against free radicals. The treatment with ECG, GCG and EGCG markedly reduced cell damage induced by SIN-1, AMVN, H_2O_2 and t-BHP in cultured AcF2 liver cells. On the other hand, in case of SNP, cell viability was not dependent on the concentration and kinds of green tea polyphenols. Therefore, we can postulate that green tea polyphenols inhibitperoxynitrite formation through their O_2^- scavenging as well as directperoxynitrite scavenging.

27) Chen C.P., Yokozawa T. and Tanaka T.: Protective effect of Sanguisorbae Radix against apoptosis and function of renal tissues subjected to ischemia-reperfusion. J. Trad. Med., 16 : 97–101, 1999.

DNA ladders were detected by gel electrophoresis of DNA obtained from rat kidney subjected to 60 min of ischemia followed by 24 h of reperfusion, indicating the involvement of apoptosis in ischemia-reperfusion injury. This ladder formation was significantly inhibited by oral administration of Sanguisorbae Radix extract to rats for 30 days prior to ischemia-reperfusion. In addition, blood levels of urea nitrogen and creatinine, two parameters of renal function, were markedly lower in the Sanguisorbae Radix-treated animals than in the untreated controls. These results suggest that Sanguisorbae Radix has potential for attenuating renal injury and accelerating the recovery of renal function after ischemia-reperfusion injury, which might involve inhibition of apoptosis.

28) Yokozawa T., Kim D.W., Hattori M. and Kaji T: Effect of Luobuma leaves against oxidation of low-density lipoprotein: a cell culture assay. J. Trad. Med., 16 : 141–147, 1999.

In a previous study, we observed an improvement in the atherosclerosis index, together with

a decrease in blood cholesterol, in rats given Luobuma extract orally and fed a high-cholesterol diet. The present study was designed to examine the function of oxidized low-density lipoprotein (LDL) in atherosclerotic lesions, using cultured cells. When endothelial cells were cultured with LDL in the presence of Cu^{2+}, the release of thiobarbituric acid (TBA)-reactive substance and lactic dehydrogenase into the culture medium was increased, with a decrease in cell viability. However, when Luobuma extract was also present in the culture medium, changes in these parameters were more favorable. In another *in vitro* system using macrophages, the levels of TBA-reactive substance, total cholesterol and esterified cholesterol were all significantly lower in the presence of Luobuma extract than in its absence. There was also morphological evidence that foam cell formation through incorporation of oxidized LDL was suppressed. These findings indicate that Luobuma suppresses the progression of atherosclerosis, in which oxidized LDL is involved.

29) No J.K., Soung D.Y., Kim Y.J., Shim K.H., Jun Y.S., Rhee S.H., Yokozawa T. and Chung H.Y.: Inhibition of tyrosinase by green tea components. Life Sciences, 65 : PL241–PL246, 1999.

The pigment melanin in human skin is a major defense mechanism against ultraviolet light of the sun, but darkened skin color, which is the result of increased and redistributed epidermal melanin, could be a serious aesthetic problem. Epidemiologically, it is well known that the consumption of green tea may help prevent cancers in humans and also reduce several free radicals including peroxynitrite. In the present study, to assess the efficacy of the inhibition of mushroom tyrosinase (monophenol monooxygenase EC 1.14.18.1), ten kinds of Korean traditional teas were screened for their tyrosinase inhibitory activity. Green tea was the strongest inhibitor, and the major active constituents in the tea are (−)-epicatechin 3-*O*-gallate (ECG), (−)-gallocatechin 3-*O*-gallate (GCG), and (−)-epigallocatechin 3-*O*-gallate (EGCG). All are catechins with gallic acid group as an active site. The kinetic analysis for inhibition of tyrosinase revealed a competitive nature of GCG with this enzyme for the L-tyrosine binding at the active site of tyrosinase.

30) Yokozawa T., Nakagawa T., Lee K.I., Cho E.J., Terasawa K. and Takeuchi S.: Effects of green tea tannin on cisplatin-induced nephropathy in LLC-PK_1 cells and rats. J. Pharm. Pharmacol., 51 : 1325–1331, 1999.

A study was conducted to clarify whether green tea tannin ameliorates cisplatin-induced renal injury in terms of lactate dehydrogenase and malondialdehyde leakage from LLC-PK_1 cells in culture. Green tea tannin was shown to suppress the cytotoxicity of cisplatin, the suppressive effect increasing with the dose of green tea tannin. The effect of cisplatin was then investigated in rats given green tea tannin for 40 days before cisplatin administration and in control rats given no green tea tannin. In control rats, blood, urinary and renal parameters and the activities of antioxidative enzymes in renal tissue deviated from the normal range, indicating dysfunction of the kidneys. In contrast, rats given green tea tannin

showed decreased blood levels of urea nitrogen and creatinine, and decreased urinary levels of protein and glucose, reflecting less damage to the kidney. In this group, the activity of catalase in the renal tissue was increased, while the level of malondialdehyde was decreased, suggesting the involvement of radicals in the normalizing of kidney function.

31) **Yokozawa T., Lee K.I., Nakagawa T., Cho E.J. and Chung H.Y.: Protective effect of pine leaf against peroxynitrite-mediated renal epithelial cell injury. Pharm. Pharmacol. Commun., 5 : 657–661, 1999.**

The effect of pine leaf extract and its constituent compounds, gallic acid and galloyl gallic acid, on cell injury were determined in the cultured renal epithelial cell line, LLC-PK_1. The cytotoxic effect of 3-morpholinosydnonimine (SIN-1), thought to form peroxynitrite ($ONOO^-$) as a simultaneous superoxide (O_2^-) and nitric oxide (NO) generator, was inhibited by pine leaf extract, and more potently by gallic acid and galloyl gallic acid. The extract inhibited $ONOO^-$ formation by SIN-1 and scavenged $ONOO^-$ directly. Compared with the extract, gallic acid and galloyl gallic acid had potent $ONOO^-$-scavenging activity, indicating that tannin may contribute to the activity of pine leaf extract. Both sodium nitroprusside as a NO generator and pyrogallol as an O_2^- generator, enhanced cytotoxicity in epithelial cells, and this effect was not abolished by the extract, gallic acid or galloyl gallic acid.

The results suggest that pine leaf may exert a protective effect against $ONOO^-$-mediated epithelial cell injury.

32) **Yokozawa T., Wang T.S., Chen C.P. and Hattori M.: *Tinospora tuberculata* suppresses nitric oxide synthesis in mouse macrophages. Biol. Pharm. Bull., 22 : 1306–1309, 1999.**

We have obtained evidence that co-incubation of thioglycollate-elicited peritoneal macrophages with an aqueous extract of *Tinospora tuberculata* inhibits lipopolysaccharide-stimulated excessive production of nitric oxide (NO) *in vitro*. This effect is concentration-dependent and appears to involve suppression of both inducible nitric oxide synthase (iNOS) activity and NADPH-diaphorase activity, thus altering NO production. As NO is one of the critical mediators in various disorders and iNOS inhibitors may have therapeutic potential, these results may explain some aspects of the multifunctional properties of *Tinospora tuberculata*, which has been used in various folk remedies in southeast Asia and China.

33) **Chen C.P., Yokozawa T. and Kitani K.: Beneficial effects of Sanguisorbae Radix in renal dysfunction caused by endotoxin *in vivo*. Biol. Pharm. Bull., 22 : 1327–1330, 1999.**

The effect of Sanguisorbae Radix extract, a traditional crude drug, was investigated in renal dysfunction induced by lipopolysaccharide (LPS) endotoxin. Injection of LPS in rats resulted in a sharp rise in the serum levels of urea nitrogen and creatinine (Cr), indicating impairment of renal function. Nitrite and nitrate levels and the activity of inducible nitric

oxide synthase (iNOS), an enzyme which participates in NO synthesis, were also significantly increased in the serum of LPS-treated rats compared with normal rats. In rats pretreated with Sanguisorbae Radix extract, renal dysfunction was attenuated and the increases in serum urea nitrogen and Cr induced by LPS were significantly reduced. The administration of Sanguisorbae Radix extract also effectively lowered serum nitrite/nitrate level. A similar effect was observed on the iNOS activity. These results indicate that Sanguisorbae Radix extract contributes to the regulation of renal function under conditions where there is excessive generation of NO.

34) Yokozawa T., Chung H.Y., Kim D.W. and Goto H.: Involvement of superoxide and/or nitric oxide in renal tissue injury. Exp. Toxic. Pathol., 51 : 517–521, 1999.

We examined the influence of superoxide (O_2^-) and/or nitric oxide (NO) on renal tissue injury estimated from the levels of lipid peroxidation and sulfhydryl (SH) oxidation. Pyrogallol, an O_2^- generator and precursor of hydrogen peroxide, produced marked tissue injury, but this was prevented by superoxide dismutase (SOD)/catalase (CAT). Hemoglobin (Hb), a NO scavenger, provided protection from tissue injury caused by sodium nitroprusside (SNP). The tissue injury produced by 3-morpholinosydnonimine (SIN-1), which is thought to form peroxynitrite ($ONOO^-$) as a simultaneous O_2^- and NO generator, was blocked by SOD/CAT or Hb. On the other hand, protein-SH and nonprotein-SH were significantly increased by addition of SOD/CAT or Hb. These data suggest that the renal tissue injury induced by O_2^-, NO and $ONOO^-$ can be blocked by SH, suggesting an important protective role against these reactive oxygen species in the mechanism of tissue defense.

35) 三潴忠道，横澤隆子，大浦彦吉，寺澤捷年，成田光陽：慢性腎不全の進行に対する温脾湯を中心とした漢方治療の臨床評価．日腎会誌，41：769–777, 1999.

Previous studies have shown that Kampo (traditional Chinese) prescriptions, mainly Wen-Pi-Tang (Onpi-to, 温脾湯), have a useful effect in patients with chronic renal failure (CRF). We aimed to examine the long-term effect of Kampo prescriptions on serum creatinine (Cr) among patients with CRF. Patients with serum Cr levels of 2 mg/dl more were enrolled if they had at least 4 recordings of serum Cr in the previous 6 months or more, and were followed-up until the start of dialysis. Eight patients aged 24-59 years with serum Cr 4.5 mg/dl were enrolled in the study for 40 to 402 weeks (mean; 228.1 ± 118.8 weeks). The cause of CRF was chronic glomerulonephritis in 7 patients and systemic lupus erythematosus in 1 patient. The end points of the study were the slope of the reciprocal of the serum Cr concentration plot against time using Mitch's method, and the predicted period of pre-dialysis. The predicted pre-dialysis period was defined as an increase in serum Cr by 10 mg/dl. As a result, the individual slopes were improved in 6 of 8 cases, in particular, in 4 of 5 Wen-Pi-Tang-treated cases. The average slope was improved significantly ($p<0.01$) in Wen-Pi-Tang-treated cases, although it showed only a tendency to improve in all 8 cases. The predicted pre-dialysis period was prolonged from 79.2 ± 74.8 weeks to 389.5 ± 355.4 weeks and 55.6

± 37.0 weeks to 262.4 ± 145.8 weeks in all 8 cases and Wen-Pi-Tang-treated cases, respectively. The observed pre-dialysis period was 228.1 ± 118.8 weeks, which showed that Kampo prescriptions prolonged the predicted period for 186 additional weeks. In conclusion, this study demonstrated that the Kampo prescriptions, consisting mainly of Wen-Pi-Tang, retarded the progression of CRF.

36) Yokozawa T., Liu Z.W. and Chen C.P.: Protective effects of Glycyrrhizae Radix extract and its compounds in a renal hypoxia (ischemia)-reoxygenation (reperfusion) model. Phytomedicine, 6 : 439–445, 1999.

Glycyrrhizae Radix water extract (GRWE) and its two compounds, glycyrrhizin and 3-glycyrrhetinic monodesmoside, significantly suppressed LDH leakage and MDA release, whereas glycyrrhetinic acid had no effect. On the other hand, in rats subjected to ischemia-reperfusion, the activities of endogenous antioxidant enzymes including catalase and glutathione peroxidase showed recovery, whereas the levels of urea nitrogen and creatinine in serum were reduced by administration of glycyrrhizin orally for 30 days prior to ischemia-reperfusion. These results indicate that GRWE and its two compounds may be promising for amelioration of hypoxia (ischemia)-reoxygenation (reperfusion) injury and improvement of renal function by acting directly or indirectly as antioxidant and oxygen radical-scavenging agents.

37) Yokozawa T., Chen C.P. and Tanaka T.: Direct scavenging of nitric oxide by traditional crude drugs. Phytomedicine, 6 : 453–463, 1999.

Thirty-one traditional crude drugs and several pure compounds were examined for their possible regulatory effect on nitric oxide (NO) levels using sodium nitroprusside as a NO donor *in vitro*. Most of the crude drugs tested demonstrated direct scavenging of NO. Eight crude drugs including Sanguisorbae Radix, Caryophylli Flos, Gambir, Coptidis Rhizoma, Granati Cortex, Gallae Rhois, Rhei Rhizoma and Cinnamomi Cortex exhibited significant activity (IC_{50} < 1000 μg/ml), and with the exception of Coptidis Rhizoma, all were found to contain tannins as their major constituents. In addition, some crude drugs containing flavonoids or essential oils also appeared to act against NO. Ten major tannins contained in Sanguisorbae Radix and Rhei Rhizoma showed high scavenging activity (IC_{50} < 326.3 μg/ml), and 6 of 8 alkaloids obtained from Coptidis Rhizoma also effectively scavenged the NO radical (IC_{50} < 455.4 μg/ml). It was indicated that these compounds may be the active principles of the crude drugs responsible for NO scavenging. The present results suggest that traditional crude drugs might be potent and novel therapeutic agents for scavenging of NO and the regulation of pathological conditions caused by excessive NO and its oxidation product, peroxynitrite. These findings may also help to explain, at least in part, certain pharmacological activities of crude drugs, especially anti-infection and anti-flammation activities.

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◇総　　説

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◇そ の 他

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4）横澤隆子：シスプラチンによる急性腎不全における薬用人参サポニンRdの役割. The GINSENG REVIEW, No. 27, 7-12, 1999.

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◇海外調査

1）服部征雄，中村憲夫：「中国における漢薬並びに少数民族薬物の比較研究」平成11年度国際学術研究－大学間協力研究，研究代表者　小松かつ子，1999. 7-8.

◇共同研究

1）小松かつ子：薬効解析センター「痴呆脳に対するコーヒーの作用」

2）木谷健一：国立療養所中部病院長寿医療研究センター，「抗老化薬に関する研究」

3）鄭　海泳：釜山大学校薬学大学，「抗酸化物に関する研究」

4）家永和治：日本臓器製薬株式会社，「5-Hydroxy-1-methylhydantoinの創薬研究」

5）田中　隆：長崎大学薬学部，「和漢薬の活性成分に関する研究」

6）三潴忠道：飯塚病院漢方診療科，「腎不全治療薬に関する基礎的，臨床的研究」

◇非常勤講師

1）服部征雄：京都薬科大学非常勤講師，1999，4月～9月.

◇研究費取得状況

1）文部省科学研究費基盤研究（B）（2）（継続，服部代表）「腸内細菌による代謝活性化を利用した新しい薬物の開発」300万円.

2）文部省科学研究費萌芽的研究（継続，服部代表）「HIV由来DNA・RNAハイブリッド分解酵素をターゲットとした天然薬物の探索」50万円.

3）文部省科学研究費基盤研究（A）（1）（新規，服部分担）「新規高次神経変性疾患モデル動物・細胞の開発，神経変性機序の解析と薬効評価法の確立」50万円.

4）財団法人ヒューマンサイエンス振興財団「HIVインテグラーゼおよび複製を制御する蛋白質を標的とする抗ウイルス剤の開発」（新規，服部分担）350万円.

5）全日本コーヒー協会（継続，服部代表）「痴呆脳に対するコーヒーの作用」150万円.

6）文部省科学研究費基盤研究（C）（継続，横澤代表）「抗酸化物としての羅布麻の探索」50万円.

7）厚生省長寿科学総合研究費（新規，横澤分担）「老化・老年病に対する栄養学的・薬理学的・分子遺伝学的手法による干渉に関する総合的研究」250万円.

8）薬用人参研究会（新規，横澤代表）「薬物性腎障害における薬用人参の役割　－分子生物学的手法を用いた検討－」100万円.

9）財団法人日本科学協会（新規，Sahar El-Mekkawy）「発がんプロモーター活性を有しない新しいホルボール系エイズウイルス薬の開発」65万円.

10）財団法人日中医学協会（新規，陳　翠萍）「腎疾患の発症機構とNOの果たす役割と治療薬の検討」60万円.

◇学位（修士・博士）

修士：

大崎茂登喜：「白薬子アルカロイドFK-3000の抗単純ヘルペスウイルス作用機序の解析」

岡　常夫：「ヒト腸内細菌由来サイコサポニン水解 β-glucosidase 遺伝子の探索」

新酒めぐみ：「Sennoside 代謝活性化に関与するヒト腸内細菌 *Bifidobacterium* sp. SEN 遺伝子の解析」

田沢享子：「酵素免疫測定法によるアコニチンの微量定量法の開発と応用」

李　燕：「ヒト腸内細菌による*C*-配糖体の開裂反応の検討」

博士：

Sahar El-Mekkawy：「Anti-HIV-1 Phorhol Esters from *Croton tigrium*」

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