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◇研究目的　Aims of the research projects

本部門は，化学的手法を応用する和漢薬の基礎研究として，天然薬物を中心とする生理活性分子の医薬化学的及び生物有機化学的研究を行っている。この目的で，天然薬物の成分単離，構造解析，合成等の，和漢薬成分に関する化学的研究を行う。さらに，その過程で構造が明らかとなる天然薬物成分につき，その構造・活性相関，構造・機能相関の化学的解明に取り組んでいる。
本年度の主な研究課題は下記の通りである。

◇研究概要　Research projects

Ⅰ．天然薬物成分の科学的研究

東南アジア（インドネシア，ネパール，ベトナム，タイ，ミャンマー等）の薬用植物

Ⅱ．和漢薬成分の医薬化学

1．羅布麻，プロポリスから単離した生理活性成分の合成
2．肝臓病や骨粗鬆症に有効な天然薬物成分の開発研究
3．マトリックスメタロプロテアーゼ産生阻害を有する天然薬物成分の研究
4．紅豆杉の活性成分の研究

Ⅲ．漢方製剤の品質評価法

1．通関丸，桃核承気湯，当帰飲子など
2．基源植物，修治生薬のLC-MSによる評価

Ⅳ．和漢薬成分の生物有機化学的研究

ベルベリン，ベトナム人参，CAPE類縁体，キナ酸誘導体，カリクシン類，花椒成分など

Ⅴ．天然薬物調査

ベトナム，ミャンマー，ブラジルなど

上記の研究課題によって得られた本年度の成果（原著及び学会報告）は下記の通りである。

◇原　著　original papers

1）**Banskota A. H., Tezuka Y., Adnyana I K., Ishii E., Midorikawa K., Matsushige K., Message D., Huertas A. A. G., and Kadota S.: Hepatoprotective and anti-*Helicobacter pylori* activities of constituents from Brazilian propolis. *Phytomedicine*, 8, 16-23 (2001).**

Abstract: Propolis is a resinous hive product collected by honeybees from various plant sources. It is extensively used in food, beverage and in folk medicine for treating various ailments and reported to have broad spectrum of biological activities. The hepatoprotective activity of propolis and constituents from its MeOH extract belonging to various classes were tested on D-galactosamine (D-GalN)/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes. The result indicated that hepatoprotective activity of alcoholic extract of tropical Brazilian propolis is mainly due to phenolic compounds including flavonoids. All the four isolated flavonoids possessed stronger inhibitory activity (IC_{50}, < 25 1 M) than silibinin (IC50, 39.6 1 M) on TNF-α -induced cell death. The labdane-type diterpenes isolated from the MeOH extract also exhibited significant hepatoprotective activity in the same experimental model. Moreover, the labdane-type diterpenes and some of the prenylated phenolic compounds possessed antibacterial activity against *Helicobacter pylori.*

2）**Tezuka Y., Gewali M. B., Ali M. S., Banskota A. H., and Kadota S.: Eleven Novel Diarylheptanoids and Two Unusual Diarylheptanoid Derivatives from the Seeds of *Alpinia blepharocalyx*. *J. Nat. Prod.*, 64, 208-213 (2001).**

Abstract: An EtOH extract of the seeds of *Alpinia blepharocalyx* afforded eleven novel diarylheptanoids named deoxycalyxin A (**1**), epicalyxin F (**2**), calyxin K (**3**), epicalyxin K (**4**), calyxin I (**5**), epicalyxin I (**6**), calyxin J (**7**), epicalyxin J (**8**), calyxin L (**9**), an epimeric mixture of calyxin M (**10**) and epicalyxin M (**11**), and two unusual diarylheptanoid derivatives, named neocalyxins A (**12**) and B (**13**), together with four known calyxins, calyxins A (**14**), F (**15**), E (**16**) and G (**17**). Structures were elucidated by spectroscopic techniques including 2D NMR spectroscopy. All compounds were examined for cytotoxicity towards murine colon 26-L5 carcinoma and human HT-1080 fibrosarcoma cells. Diarylheptanoids **2**, **3** and **5** were cytotoxic against both cell lines, while **4** and **6-8** were cytotoxic against human fibrosarcoma cells.

3）**Nagaoka T., Tanaka K., Tezuka Y., Namba T., and Kadota S.: Origin of "Koku-oren (黒黄連)", one of the Shosoin Items. *Natural Medicines*, 55, 23-27 (2001).**

Abstract: To identify the origin of a drug, "koku-oren (黒黄連)" in Shosoin (正倉院), 90% acetone extracts of "koku-oren" and of the underground parts of *Picrorhiza kurroa* and *P. scrophulariiflora* were analyzed by GC-MS. A characteristic peak was detected at 10.8 min in the gas chromatograms of "koku-oren" and of the underground parts of *P. scrophulariiflora*, which gave two characteristic ions at *m/z* 154 and 84 in the mass spectrum. Then, this compound, showing the characteristic peaks, was identified as rehmaglutin D by analyzing the NMR spectra. On the basis of this evidence, the "koku-oren" in Shosoin was concluded to be the underground part of *P. scrophulariiflora.*

4）**Ali M. S., Tezuka Y., Awale S., Banskota A. H., and Kadota S.: Six New Diarylheptanoids from the Seeds of *Alpinia blepharocalyx*. *J. Nat. Prod.*, 64, 289-293 (2001).**

Abstract: Chromatographic separation of part of an EtOH extract of the seeds of *Alpinia blepharocalyx* resulted in the isolation of six new (**1-6**) and two known (**7**, **8**) diarylheptanoids together with twelve known compounds. The structures of the new compounds, including their absolute stereochemistry, were elucidated by spectroscopic and chemical methods as (3*S*,5*S*)- (**1**) and (3*S*,5*R*)-3-hydroxy-5-methoxy-1-(4-hydroxyphenyl)-7-phenyl-6*E*-heptene (**2**), (3*S*,5*S*)- (**3**) and (3*S*,5*R*)-3-hydroxy-5-ethoxy-1-(4-hydroxyphenyl)-7-phenyl-6*E*-heptene (**4**), (3*S*)-methoxy-1,7-*bis* (4-hydroxyphenyl)-6*E*-hepten-5-one (**5**) and 1,7-*bis*(4-hydroxyphenyl)hepta-4*E*,6*E*-dien-3-one (**6**). Among the isolated compounds, 5, (3*S*,5*S*)-3,5-dihydroxy-1,7-*bis*(4-hydroxyphenyl)heptane (**8**), 4'-hydroxy-5,6-dehydrokawain

(**14**) and/or phloroglucinol (**20**) showed significant antiproliferative activity against murine colon 26-L5 carcinoma (ED_{50}: **5**, 5.2 μM; **8**, 12.8 μM; **14**, 20.7 μM; **20**, 26.4 μM) and human HT-1080 fibrosarcoma (ED_{50}: **5**, 10.1 μM; **14**, 20.1 μM; **20**, 20.9 μM) cells.

5) **Adnyana I K., Tezuka Y., Banskota A. H., Tran K. Q., and Kadota S.: Three New Triterpenes from the Seeds of *Combretum quadrangulare* and Their Hepatoprotective Activity. *J. Nat. Prod.*, 64, 360-363 (2001).**

Abstract: Three new triterpenes of the lupane type, 2α,6β-dihydroxybetulinic acid (**1**) and 6β-hydroxyhovenic acid (**2**), and an oleanane type, 6β-hydroxyarjunic acid (**3**), together with several known compounds, have been isolated from the MeOH extract of the seeds of *Combretum quadrangulare* Kurz (Combretaceae). The structures of these compounds were elucidated on the basis of spectroscopic analysis, and their hepatoprotective activities were tested for D-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes.

6) **Fan W., Tezuka Y., Ni D. K. M., and Kadota S.: Prolyl Endopeptidase Inhibitors from the Underground Part of *Rhodiola sachalinensis*. *Chem. Pharm. Bull.*, 49, 396-401 (2001).**

Abstract: The methanolic extract of the underground part of *Rhodiola sachalinensis* was found to show inhibitory activity on prolyl endopeptidase (PEP, EC. 3.4.21.26), which is an enzyme playing a role in the metabolism of proline-containing neuropeptidase that have been recognized to be involve in learning and memory processes. From the MeOH extract, five new monoterpenoids named sachalinols A (**24**), B (**25**) and C (**26**) and sachalinosides A (**23**) and B (**27**) were isolated, together with twenty-two known compounds, gallic acid (**1**), *trans-p*-hydroxycinnamic acid (**2**), p-tyrosol (**3**), salidroside (**4**), 6"-*O*-galloylsalidroside (**5**), benzyl β-D-glucopyranoside (**6**), 2-phenylethyl β-D-glucopyranoside (**7**), *trans*-cinnamyl β-D-glucopyranoside (**8**), rosarin (**9**), rhodiocyanoside A (**10**), lotaustralin (**11**), octyl β-D-glucopyranoside (**12**), 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**13**), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**14**), kaempferol (**15**), kaempferol 3-*O*-β-D-xylofuranosyl(1→2)-β-D-glucopyranoside (**16**), kaempferol 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside (**17**), rhodionin (**18**), rhodiosin (**19**), (-)-epigallocatechin (**20**), 3-*O*-galloylepigallocatechin-(4β,8)-epigallocatechin 3-*O*-gallate (**21**) and rosiridin (**22**). Among these, nineteen compounds other than **3**, **4** and **9** have been isolated for the first time from *R. sachalinensis*, and six (**6**, **8**, **13**, **16**, **17**, **20**) are isolated from Rhodiola plants for the first time. Among them, six compounds (**13**, **14**, **18**, **19**, **21**, **22**) showed noncompetitive inhibition against Flavobacterium PEP, with an IC_{50} of 0.025, 0.17, 22, 41, 0.44 and 84 μM, respectively.

7) **Tran Q. L., Adnyana I K., Tezuka Y., Nagaoka T., Tran Q. K., and Kadota S.: Triterpene saponins from Vietnamese ginseng (*Panax vietnamensis*) and their hepatocytoprotective activity. *J. Nat. Prod.*, 64, 456-461 (2001).**

Abstract: The methanol extract of Vietnamese ginseng (*Panax vietnamensis*) was found to possess hepatocytoprotective effects on D-galactosamine (D-GalN)/tumor necrosis factor-alpha (TNF-α)-induced cell death in primary cultured mouse hepatocytes. Further chemical investigation of the extract afforded two new dammarane-type triterpene saponins, ginsenoside Rh_5 (**1**) and vina-ginsenoside R_{25} (**2**), as well as eight known dammarane-type triterpene saponins, majonoside R_2 (**3**), pseudo-ginsenoside RT_4 (**4**), vina-ginsenosides R_1 (**5**), R_2 (**6**) and R_{10} (**7**), ginsenosides Rg_1 (**8**), Rh_1 (**9**) and Rh_4 (**10**), and a known sapogenin protopanaxatriol oxide II (**11**). Their structures were elucidated on basis of spectral analysis. In addition, by using LC-electrospray ionization (ESI)-MS method, five known saponins, ginsenosides Rb_1, Rb_2, Rc, Rd and Re (**12-16**), were also identified in the extract.

Among the compounds isolated, majonoside R2 (**3**), the main saponin in Vietnamese ginseng, showed strong protective activity against D-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes. This demonstrates that the hepatocytoprotective effect of Vietnamese ginseng is due to dammarane-type triterpene saponins that have

an ocotillol-type side chain, a characteristic constituent of Vietnamese ginseng.

8) **Ali M. S., Tezuka Y., Banskota A. H., and Kadota S.: Blepharocalyxins C-E, Three New Dimeric Diarylheptanoids, and Related Compounds from the Seeds of *Alpinia blepharocalyx*. *J. Nat. Prod.*, 64, 491-496 (2001).**

Abstract: Three novel diarylheptanoids blepharocalyxins C-E (**5-7**) together with four new (**1-4**) and one known (**8**) diarylheptanoids bearing a tetrahydropyran ring were isolated from the residual fraction of an EtOH extract of the seeds of *Alpinia blepharocalyx*. The structures and the stereochemistry at the chiral centers of the new diarylheptanoids were elucidated by spectroscopic techniques including 2D NMR spectroscopy. Blepharocalyxins C-E (**5-7**) have a novel carbon framework and are dimeric diarylheptanoids consisting of two diarylheptanoid units. Blepharocalyxin D (**6**) showed potent antiproliferative activity against murine colon 26-L5 carcinoma cells (ED_{50}, 3.61 μM), while against human HT-1080 fibrosarcoma cells, blepharocalyxin E (**7**) showed potent activity (ED_{50}, 9.02 μM).

9) **Fan W., Tezuka Y., and Kadota S.: Effect of Mirabilitum in Formulization: Change of Prolyl Endo-peptidase Inhibitory Activity and of Constituents using the Preparation Method of Tokaku-joki-to (桃核承気湯, Persia and Rhubarb Combination). *Chem. Pharm. Bull.*, 49, 595-600 (2001).**

Abstract: To clarify the effect of Mirabilitum in formularization, change of prolyl endopeptidase inhibitory activity and of constituents using the preparation method of a Kanpo formula Tokaku-joki-to (桃核承気湯, Persia and Rhubarb Combination) was examined by the liquid chromatography-mass spectroscopy (LC-MS) method. Mirabilitum under boiling condition caused qualitative and quantitative change of the constituents through hydrolysis which caused a change of its activity. This was considered to be the main reason the classical Chinese medical book "Shang han lun (傷寒論)" specified that Mirabilitum should be added at a later stage of decoction.

10) **Ali M. S., Banskota A. H., Tezuka Y., Saiki I., and Kadota S.: Antiproliferative Activity of Diarylheptanoids from the Seeds of *Alpinia blepharocalyx*. *Biol. Pharm. Bull.*, 24, 525-528 (2001).**

Abstract: The 95% EtOH extract of the seeds of *Alpinia blepharocalyx* (Zingiberaceae) showed significant antiproliferative activity towards human HT-1080 fibrosarcoma and murine colon 26-L5 carcinoma cells. Chemical investigation of the extract led to the isolation of forty-four new (**1-44**) and one known (**45**) diarylheptanoids, eleven phenolic compounds (**46-56**) together with β-sitosterol glucoside (**57**). Almost all the isolated compounds showed significant antiproliferative activity in a concentration-dependent manner. Among the compounds, epicalyxin F (**17**) exhibited the most potent activity against the proliferation of colon 26-L5 carcinoma cells with an ED_{50} value of 0.89 μM, while calyxin B (**2**) exhibited the most potent activity against human HT-1080 fibrosarcoma cells with an ED_{50} value of 0.69 μM. Moreover, calyxins B (**2**) and K (**11**), epicalyxins F (**17**), I (**20**) and K (**22**), 6-hydroxycalyxin F (**25**), blepharocalyxin B (**27**) and mixtures of **7** and epicalyxin G (**18**) and of calyxin J (**10**) and epicalyxin J (**21**) possessed more potent activity than a clinically used anticancer drug, 5-fluorouracil, towards HT-1080 fibrosarcoma cells. Analysis of the structure activity relationship suggested that the position of the attachment of a chalcone or a flavanone moiety does not affect the activity, although their presence in association causes a substantial enhancement of the antiproliferative activity. Moreover, the conjugated double bond of the chalcone moiety and the phenolic hydroxyl group potentiate the antiproliferative activity of the compounds.

11) **Xiang T., Xiong Q.-B., Adnyana I K., Tezuka Y., Nagaoka T., Wu L.-J., and Kadota S.: Studies on the Hepatocyte Protective Activity and the Structure-Activity Relationships of**

Quinic Acid and Caffeic Acid Derivatives from the Flower Buds of *Lonicera bournei*. *Planta Med.*, 67, 322-325 (2001).

Abstract: 13 quinic acid derivatives along with caffeic acid, methyl caffeate, myo-inositol, bis[5-formylfurfuryl] ether and 6,7-dihydroxycoumarin were isolated from the ethanol extract of the flower buds of *Lonicera bournei* Hemsl., among which 8 compounds were firstly obtained from this genus. The effects of different solvent soluble fractions of the ethanol extract and the pure compounds on hepatocyte death induced by D-galactosamine (D-GalN)/tumor necrosis factor α (TNF-α) were studied, and the structure-activity relationships were also discussed.

12) **Adnyana I K., Tezuka Y., Awale S., Banskota A. H., Tran K. Q., and Kadota S.: 1-*O*-Galloyl-6-*O*-(4-hydroxy-3,5-dimethoxy)benzoyl-β-D-glucose, a New Hepatoprotective Constituent from *Combretum quadrangulare*. *Planta Med.*, 67, 370-371 (2001).**

Abstract: A new gallic acid derivative, 1-*O*-galloyl-6-*O*-(4-hydroxy-3,5-dimethosy)benzoyl-β-D-glucose (*1*) has been isolated from an H_2O-fraction of MeOH extract of *Combretum quadrangulare* seeds. Compound **1** exhibited potent hepatoprotective activity against D-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes with an IC_{50} of 3.3 μM.

13) **Wang J.-H., Li W., Sha Y., Tezuka Y., Kadota S., and Li X.: Triterpenoid Saponins from Leaves and Stems of *Panax quinquefolium* L. J. *Asian Nat. Prod. Res.*, 3, 123-130 (2001).**

Abstract: In the chemical investigation on the saponin composition of leaves and stems of *Panax quinquefolium* L., two new minor dammarane saponins, quinquenoside L_1 (1) and L_2 (2) have been isolated. By means of physico-chemical evidences and spectral analysis their structures were established as 3-*O*-[β-D-glucopyranosyl-dammara-23,25-diene-3β, 12β, 20(*S*)-triol (**1**) and 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-*O*-β-D-glucopyranosyl-(24*Z*)-dammar-24-ene-3β, 12β, 20(*S*), 26-tetraol (**2**).

14) **Awale S., Tezuka Y., Banskota A. H., Kouda K., Tun K. M., and Kadota S.: Five Novel Highly Oxygenated Diterpenes of *Orthosiphon stamineus* from Myanmar. *J. Nat. Prod.*, 64, 592-596 (2001).**

Abstract: Five novel highly oxygenated diterpenes, orthosiphols K (**1**), L (**2**), M (**3**) and N (**4**) and norstaminone A (**5**), were isolated from the aerial part of *Orthosiphon stamineus*, together with three known diterpenes, orthosiphols A (**6**) and B (**7**) and neoorthosiphol A (**8**). Orthosiphol L (**2**) was an isopimarane-type diterpene with a hydroxyl group at C-12, which would support the biogenesis of staminane-type diterpenes, *i.e.*, migration of vinylic group from C-13 of isopimarane to C-12. Norstaminone A (**5**) had a staminane carbon-framework and would support the biosynthetic pathway from staminols to norstaminols via staminolactones. All the isolated compounds showed mild to weak antiproliferative activities towards highly liver metastatic colon 26-L5 carcinoma and human HT-1080 fibrosarcoma cell lines.

15) **Tezuka Y., Irikawa S., Kaneko T., Banskota A. H., Nagaoka T., Xiong Q., Hase K., and Kadota S.: Screening of Chinese herbal drug extracts for inhibitory activity on nitric oxide (NO) production and identification of an active compound of *Zanthoxylum bungeanum*. *J. Ethnopharmacol.*, 77, 209-217 (2001).**

Abstract: Sixty-eight water- and methanol-extracts from thirty-four Chinese herbal drugs, most of which are used for inflammatory diseases, were screened for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated J774.1 macrophages and in LPS/interferon (IFN)-γ-stimulated mouse peritoneal exudate macrophages. Among the extracts, methanol-extracts of *Myristica fragrans*, *Plantago asiatica*, *Rubia cordifolia*, and *Zanthoxylum bungeanum* showed significant inhibition in J774.1 macrophages, while in mouse

peritoneal exudate macrophages, water-extracts of *Ru. cordifolia* and *Scutellaria baicalensis* and methanol-extracts of *Angelica megaphylla*, *My. fragrans*, and *Z. bungeanum* inhibited the NO production. Among them, inhibition of water-extract of *Sc. baicalensis* was found to be mainly due to direct scavenging of NO radicals, through an examination of its scavenging activity on PAPA NONOate-generated NO radicals, while water-extract of *Ru. cordifolia* and methanol-extracts of *An. megaphylla*, *My. fragrans*, *P. asiatica*, and *Z. bungeanum* showed inhibition on iNOS mRNA expression. At last, an inhibitory compound on iNOS mRNA expression was isolated from a methanol-extract of *Z. bungeanum* and identified as 4-*O*-β-D-glucopyranosyldihydroferulic acid by NMR spectral analyses and chemical synthesis.

16) Midorikawa K., Banskota A. H., Tezuka Y., Nagaoka T., Matsushige K., Message D., Huertas A. A. G., and Kadota S.: Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis of Propolis. *Phytochem. Anal.*, 12, 366-373 (2001).

Abstract: The composition of propolis, a resinous hive product collected by honeybees from various plant sources, depends on various factors such as season and vegetation of the area. Based on standards (either isolated from Brazilian propolis or reported from propolis) including chromane, diterpenes and phenolic compounds, different Brazilian propolis were analysed by LC-MS in order to determine their chemical constituents. Dicaffeoylquinic acids were detected in almost all water extracts of Brazilian propolis, whereas diterpenes, flavonoids and prenylated phenolic compounds were found in their methanol extracts. Based on the identified chemical constituents and their biological activities, it was determined that the quality of Brazilian propolis could be directly related to the phenolic constituents. Moreover, *Baccharis dracunculifolia* was concluded to be an important source of Brazilian propolis. Propolis samples from Peru, China and the Netherlands were also studied.

17) Tran Q. L., Tezuka Y., Banskota A. H., Tran Q. K., Saiki I., and Kadota S.: New Spirostanol Steroids and Steroidal Saponins from Roots and Rhizomes of *Dracaena angustifolia*, and Their Antiproliferative Activity. *J. Nat. Prod.*, 64, 1127-1132 (2001).

Abstract: The MeOH extract of Nam ginseng (roots and rhizomes of *Dracaena angustifolia*) afforded nine new compounds, including three spirostanol sapogenins, named namogenins A-C (**1-3**), four spirostanol saponins, named namonins A-D (**4-7**), a furostanol saponin, named namonin E (**8**) and a pregnan glycoside, named namonin F (**9**), along with another eight known steroidal saponins (**10-17**). Their structures were determined on basis of spectral analyses and chemical methods. All compounds were tested for their antiproliferative activity against murine colon 26-L5 carcinoma, human HT-1080 fibrosarcoma and B-16 BL6 melanoma cells. Compounds **4**, **5** and **10** showed potent antiproliferative activity against HT-1080 fibrosarcoma cells, having IC_{50} values of 0.2, 0.3 and 0.6 μM, respectively, comparable to that of doxorubicin.

18) Nagaoka T., Banskota A. H., Xiong Q., Tezuka Y., and Kadota S.: Synthesis and Antihepatotoxic and Antiproliferative Activities of Di- and Tri-*O*-caffeoylquinic Acid Derivatives. *J. Trad. Med.*, 18, 183-190 (2001).

Abstract: Methyl di- and tri-*O*-caffeoylquinates were synthesized by esterification of methyl quinate with di-*O*-acetylcaffeoyl chloride, following deprotection of the acetyl groups. Moreover, 4,5-di-*O*-caffeoylquinic acid was synthesized by esterification of quinide with, followed by a hydrolysis of product quinide. These synthetic compounds were tested for their hepatoprotective activity on D-galactosamine (D-GalN)/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes, which possessed significant hepatoprotective activity concentration-dependently. The activity was enhanced by the presence of caffeoyl group. On the other hand, they showed only weak antiproliferative activities against murine colon 26-L5 carcinoma, human HT-1080 fibrosarcoma, murine B16-BL6 melanoma, and human lung carcinoma A-549 cells.

19) Shrestha K., Banskota A.H., Kadota S., Shrivastava S.P., Strobel G., Gewali M.B.: An antiproliferative norditerpene dilactone, Nagilactone C, from *Podocarpus neriifolius*. *Phytomedicine*, 8, 489-491 (2001).

Abstract: An ethanolic extract of *Podocarpus neriifolius* D. Don (Podocarpaceae) showed antiproliferative activity against two major tumor cell lines, viz. human HT-1080 fibrosarcoma and murine color 26-L5 carcinoma. Bioassay guided fractionation showed the highest antiproliferative activity in chloroformsoluble fraction. Nagilactone C, the major constituent of this fraction was isolated and characterized by using NMR, IR and FAB-MS spectroscopic methods. Nagilactone C possessed potent antiproliferative activity against human fibrosarcoma and murine colon carcinoma tumor cell lines exhibiting ED_{50} values of 2.3 and 1.2 μg/ml, respectively. Hence, nagilactone C could be the active constituent present in this plant.

20) Komatsu K., Zhu S., Fushimi H., Tran K. Q., Cai S., Kadota S.: Phylogenetic Analysis Based on 18S rRNA Gene and Matk Gene Sequences of *Panax vietnamensis* and Five Related Species. *Planta Medica*, 67, 461-465 (2001).

Abstract: *Panax vietnamensis* was discovered recently in Vietnam. Its bamboo-like rhizomes, called Vietnamese Ginseng, have attracted considerable attention because of their specific pharmacological activities. In order to define the taxonomic position of this new species and include it in the molecular authentication of Ginseng drugs, the 18S ribosomal RNA gene and matK gene sequences of *P. vietnamensis* were determined and compared with those of its related taxa, *P. japonicus* var. *major* and *P. pseudo-ginseng* subsp. *himalaicus*, besides previously reported *P. ginseng*, *P. japonicus* and *P. quinquefolius*. The 18S rRNA gene sequences were found to be 1809 bps in length. The sequence of *P. vietnamensis* was identical to that of *P. quinquefolius*, and presented one base substitution from those of both *P. japonicus* var. major and *P. pseudo-ginseng* subsp. *himalaicus*. The matK gene sequences of 6 taxa were found to be 1509 bps in length. The sequence of *P. vietnamensis* differed from those of *P. japonicus* var. *major*, *P. pseudo-ginseng* subsp. *himalaicus*, *P. ginseng*, *P. japonicus* and P. quinquefolius at 4, 5, 9, 9 and 10 nucleotide positions, respectively. The phylogenetic tree reconstructed by the combined 18S rRNA-matK gene analysis using the maximum parsimony method showed that *P. vietnamensis* was sympatric with other *Panax* species and had a close relationship with *P. japonicus* var. *major* and *Panax pseudo-ginseng* subsp. *himalaicus*, molecular taxonomy.

◇総　説　Review

1) Yasuhiro Tezuka：Equality-evaluation of Wakan-yaku (Japanese-Chinese traditional medicines) with liquid chromatography-mass spectrometry (LC-MS): comparison of constituents of original plants for Chinese traditional medicine "Dan-shen (Tan-jin)". *J. Trad. Med.*, 18, 39-47 (2001).

2) Banskota A. H., Tezuka Y., Kadota S.: Recent Progress in Pharmacological Research of Propolis. *Phytother. Res.*, 15, 561-571 (2001).

◇学会報告　Scientific Presentation

1) Suresh Awale, Yasuhiro Tezuka, Arjun H. Banskota, Kyoji Kouda, Kyaw Myint Tun, Shigetoshi Kadota：Highly-Oxygenated Isopimarane-type Diterpenes from *Orthosiphon stamineus*. 日本薬学会第121年会，2001，3/28-30，札幌.

2) Mohammad Shawkat Ali, Arjun H. Banskota, Yasuhiro Tezuka, Ikuo Saiki, Shigetoshi Kadota：Antiproliferative Activity of Diarylheptanoids from the Seeds of *Alpinia blepharocalyx*. 日本薬学会第121年会，2001，3/28-30，札幌.

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◇学位（修士・博士）取得者　Academic degrees and theses

課程博士：

I Ketut Adnyana：「Hepatoprotective Constituents from Vietnamese Medicinal Plant "Tram Bau" (*Combretum quadrangulare* Kurz」

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