

# 薬効解析センター

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## ◇研究目的

世界各地の民族薬物に関する資料の収集及び整理，薬効の評価及び解析並びにデータベースの構築を行い，世界の伝統薬物及び薬用植物に関する共同研究を推進する。

## ◇研究概要

### I. 伝統薬物に関するデータベース（ETHMEDmmm）の構築

平成12年度初旬のデータベース公開に向けて，250種類の和漢薬の学術情報，及び約5,000点の民族薬物資料館保有和漢薬標本のデータを整理し，構築した。

### II. 伝統薬物の薬効の評価と解析に関する研究

#### 1) 難治性の神経疾患に対する有効性の検討とそれらの薬理作用の機序に関する研究

ヒト神経モデル細胞の神経突起伸展に有効であった漢薬「地黄」について，活性成分の探索を進めた。コーヒーの生豆に含まれる trigonelline に神経突起伸展活性があることを報告した。

#### 2) 抗糖尿病作用を有する生薬の研究

プロポリス水エキスの抗糖尿病作用を I 型糖尿病モデルの NOD マウスを用いて検討し，糖尿病発症までにかかる期間及び生存率が有意に増加することを明らかにした。

### III. 生薬の品質評価に関する研究

#### 1) 遺伝子解析による生薬の同定法開発に関する研究

中国産ショウガ科 *Curcuma* 属植物10種について *trnK* 遺伝子領域の塩基配列を決定し，この遺伝子領域の配列により各種が系統づけられることを見出した。

#### 2) 生薬の基源と品質に関する研究

ミャンマー黄連 *Coptis teeta* subsp. *lohitisensis* の根茎から6種のアルカロイド成分を単離同定した。

### IV. 世界の伝統医薬学の調査研究

中国北京医科大学薬学院と共同で，雲南省にて三七人参類及び少数民族薬物を調査した。

## ◇原 著

- 1) Chen T., Li J.-X., Cao J.-S., Xu Q., Komatsu K., and Namba T.: A New Flavanone Isolated from *Rhizoma Smilacis Glabrae* and the Structural Requirements of its Derivatives for Preventing Immunological Hepatocyte Damage. *Planta Med.*, **65**: 56-59, 1999.

**Abstract**: From the rhizome of *Smilax glabra* ROXB., a new flavanone was isolated and named as smitilbin (1), together with 6 known compounds, engeletin (2), astilbin (3), dihydroquercetin (4), eurryphin (5), resveratrol (6), and 5-*O*-caffeoylshikimic acid (7). These compounds were applied to the assay of liver nonparenchymal cells (NPC) against hepatocytes (HC) isolated from mice with an immunological liver injury. Against the NPC-caused elevation of ALT (alanine transaminase) in culture supernatant from HC, the pretreatment of NPC with flavanoids (1-3) dose-dependently blocked the ALT release while 4, the aglycone of 3, did not. The chromone 5 showed a much stronger inhibition. Compound 6 also showed the activity. However, 1-7 did not show any suppression of NPC or CCl<sub>4</sub>-induced ALT release when they were used to pretreat HC. These results suggest that compounds 1-3, 5, and 6 could protect the hepatocyte damage from NPC through selectively producing the dysfunction of NPC with an essential requirement of rhamnose, and the chromone part in their structures may be critical for exhibiting the activity rather than through protecting the hepatocyte membranes.

- 2) Fan W.-Z., Tezuka Y., Komatsu K., Namba T., and Kadota S.: Prolyl Endopeptidase Inhibitors from the Underground Part of *Rhodiola sacra* S. H. Fu. *Biol. Pharm. Bull.*, **22**: 157-161, 1999.

**Abstract**: Prolyl endopeptidase (PEP, EC 3.4.21.26) is an enzyme which plays a role in the metabolism of proline-containing neuropeptides, e.g., vasopressin, substance P and thyrotropin-releasing hormone (TRH), which have been suggested to be involved in learning and memory processes. In our systematic screening for PEP inhibitors from traditional Chinese medicines, we found that MeOH extract from the underground part of *Rhodiola sacra* S. H. Fu shows significant inhibitory activity against PEP from *Flavobacterium meningosepticum*. Examination of the constituents of the extract resulted in the isolation of nineteen known compounds, identified as hydroquinone (1), 4-hydroxybenzoic acid (2), caffeic acid (3), 4-hydroxycinnamic acid (4), suberic acid (5), protocatechuic acid (6), gallic acid (7), (-)-epigallocatechin 3-*O*-gallate (8), 2-phenylethyl  $\beta$ -D-glucopyranoside (9), 3-*O*-galloylepigallocatechin-(4  $\beta$   $\rightarrow$  8)-epigallocatechin 3-*O*-gallate (10), 2-phenylethyl  $\alpha$ -L-arabinopyranosyl-(1  $\rightarrow$  6)- $\beta$ -D-glucopyranoside (11), sacranoside A (12),  $\beta$ -D-glucopyranosyl 4-hydroxybenzoate (13), rhodiocyanoside A (14), rhodioctanoside (15), sarmentosin (16), heterodendrin (17), arbutin (18) and 4-*O*-( $\beta$ -D-glucopyranosyl)-gallic acid (19). Among these, 1, 2, 5, 8-10, 13, 16, 18 and 19 have been isolated for the first time from *R. sacra*, among which 5, 9, 10, 13, 16, 18 and 19 have been isolated from *Rhodiola* plants for the first time. On the PEP inhibition, seven compounds (6-8, 10, 12, 18, 19) showed inhibition with an IC<sub>50</sub> of

27.8, 487, 1.47, 0.437, 348, 391 and 215  $\mu\text{M}$ , respectively. The kinetic study of these inhibitors indicated that they are noncompetitive inhibitors, except for 6 which is a competitive inhibitor.

- 3) 山路誠一, 小松かつ子, 谿忠人, 難波恒雄: チベット薬物の生薬学的研究(第13報), *Nardostachys* 属植物に由来する「sPang-spos / パンポェ」, 漢薬「甘松香」, およびアールヴェータ薬物「Jatamansi」について. *Nat. Med.*, 53: 61-71, 1999.

**Abstract:** In this paper, the botanical origins of the commercial crude drugs, "sPang-spos" and related ethnomedicines, such as Chinese "Gansongxiang," and Ayurvedic "Jatamansi," were clarified by anatomical studies of the plant materials, *N. chinensis* and *N. grandiflora*, of the family Valerianaceae growing in Tibet and the neighboring countries. These two species could be distinguished from each other by the difference in the thickness of periclinal epidermal cell wall beneath cuticle in the leaf residue, and by the presence or absence of sclerenchyma cells in the pith of the rhizome. According to the anatomical study, "sPang-spos" and "Jatamansi" were shown to be derived from the rhizome of *N. grandiflora*, and "Gansongxiang" was from that of *N. chinensis*. It is also revealed that one sample of "Gansongxiang" from China contained 25%(w/w) of the whole plant of *Pteroccephalus hookeri* of the family Dipsacaceae, and another "Jatamansi (Jatamansa)," obtained in the market of Sri Lanka, was proved to be derived from the underground part of *Balanophora fungosa* subsp. *indica* var. *indica* of the family Balanophoraceae.

- 4) 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.

**Abstract:** 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 ( $1\ \mu\text{g}/\text{ml}$ ) 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 ( $5\ \mu\text{g}/\text{ml}$ ) 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 ( $30\ \mu\text{M}$ ) 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  $30\ \mu\text{M}$  trigonelline for 6 d, suggesting enhancement of axonal extension. These results show that trigonelline promotes functional neurite outgrowth.

- 5) Ohsugi M., Fan W.-Z., Hase K., Xiong Q.-B., Tezuka Y., Komatsu K., Namba T., Saitoh T., Tazawa K., and Kadota S.: Active-oxygen scavenging activity of

**traditional nourishing-tonic herbal medicines and active constituents of *Rhodiola sacra*. J. Ethnopharmacol., 67 : 111–119, 1999.**

**Abstract :** The active-oxygen scavenging activity of 70 traditional herbal medicines used in China and Japan as nourishing tonics were evaluated by electron spin resonance (ESR) technique, in order to evaluate their effectiveness for anti-aging and to search for new active-oxygen scavengers from natural resources. Most of the 70 herbal medicines showed scavenging activity with various intensities. *Areca catechu* (methanol extract), *Dendrobium plicatile* (methanol extract), *Juglans regia* (water extract), *Paeonia lactiflora* (methanol extract), *Psychotria serpens* (water and methanol extracts), *Rhodiola sacra* (water and methanol extracts) and *Uncaria rhynchophylla* (water extract) especially showed strong scavenging activity against superoxide anion radical ( $\cdot\text{O}_2^-$ ), while *J. regia* (water and methanol extracts), *Morus alba* (water extract) and *Schisandra chinensis* (water extract) revealed strong scavenging activity against hydroxyl radical ( $\text{HO}\cdot$ ). In addition, the active-oxygen scavenging activities of 19 compounds isolated from *R. sacra* were also examined, and hydroquinone (1), caffeic acid (3), protocatechuic acid (6), gallic acid (7), (-)-epigallocatechin 3-*O*-gallate (8), 3-*O*-galloylepigallocatechin-(4 $\beta$ →8)-epigallocatechin 3-*O*-gallate (10), heterodendrin (17) and gallic acid 4-*O*- $\beta$ -D-glucopyranoside (19) were found to show mild or strong inhibitory activity against superoxide anion radical ( $\cdot\text{O}_2^-$ ), while 4-hydroxybenzoic acid (2), 3, 4-hydroxycinnamic acid (4), 6–8 and 19 inhibited hydroxyl radical ( $\text{OH}\cdot$ ). These active-oxygen scavengers may contribute, to different extents, to their anti-aging action.

**6) Tohda C., and Jacobowitz D.M.: The function and expression of sproutin, a novel neurite outgrowth factor. Neuroreport, 10 : 2089–2094, 1999.**

**Abstract :** TA20 cDNA was previously cloned as a neurite outgrowth factor from a hybridoma of mouse and rat cells, NG108-15. To clarify the detailed function and tissue distribution of this gene, homologous sequences of rat and mouse were identified. The cloned sequences had no homology with known genes, and was designated as sproutin. A predicted open reading frame of rat sproutin was transfected into human SK-N-SH cells. The over-expressed protein was distributed in cytoplasm and neurites, and caused an increase in the levels of microtubule associated proteins, but not that of phosphorylated neurofilament-H. The percentage of cells with neurites, the length of neurites and the number of neurites per cell were increased by sproutin transfection. Sproutin mRNA was brain specific. These results suggest that an increase in sproutin promotes dendritic extension.

**7) Kumar A. and Neumann K.-H.: Comparative Investigations on Plastid Development of Meristematic Regions of Seedlings and Tissue Cultures of *Daucus carota* L. J. Appl. Bot.- Angewandte Botanik., 73 : 206–210, 1999.**

**Abstract :** Cells of meristematic regions of the shoot apex of carrot seedlings and of callus cultures exhibit overall architectural similarities with regard to structure of organelles and

other cytoplasmic components. Densely staining bodies presumably proplastids were found in all of these cells. The development of plastids appeared to be synchronous in shoot meristems, but relatively less synchronization in callus cultures was observed. Moreover the callus cultures apparently have developmental pathways leading from chromoplasts (contained in original explants) through amyloplasts or directly from amyloplasts into chloroplasts, besides the usual developmental process of chloroplasts from proplastids. This may account for differences observed in these two meristematic zones as well as for the occurrences of some further developed plastids in meristematic callus culture cells. Besides this, unlike first leaves, the callus cultures did not show the development of prolamellar bodies characteristic of etioplasts if cultured in the dark.

- 8) Kumar A., Bender L., and Neumann K.-H. : Characterization of the Photosynthetic System (Ultrastructure of Plastid, Fluorescence Induction Profiles, Low Temperature Spectra) of *Arachis hypogaea* L. Callus Cultures as Influenced by Sucrose and Various Hormonal Treatments. *J. Appl. Bot.- Angewandte Botanik.*, 73 : 211-216, 1999.

**Abstract :** Freshly cut hypocotyl explants and established *Arachis hypogaea* L. (peanut) callus cultures behaved differentially to the hormonal supplement of the nutritive medium. A hormonal response was more pronounced in case of freshly isolated explants than in long established cultures apparently because the later developed their own hormonal system and thereby responded poorly to exogenous phytohormones. The photosynthetic system of established cultures which proved to be photoautotrophic was characterized in the present paper by ultrastructural investigations, low temperature absorption spectra and fluorescence induction measurements at various hormonal regimes. Whereas influences of exogenous phytohormones were rather small, a supplement of sucrose to the nutrient medium has suppressive effects on the development of the photosynthetic system.

- 9) Prasain J.K., Tezuka Y., Li J.-X., Tanaka K., Basnet P., Dong H., Namba T., and Kadota S.: New Diarylheptanoids from the Seeds of *Alpinia blepharocalyx*. *Planta Med.*, 65 : 196-1997, 1999.

**Abstract :** *Alpinia blepharocalyx* K. Schum (Zingiberaceae) is used in traditional Chinese medicine for the stomach disorder. We have recently reported fifteen novel diarylheptanoids together with six known phenolic compounds. Further investigation on the ether soluble fraction of the seeds of *A. blepharocalyx* has led to the isolation of a new diarylheptanoid, 5,6-dehydro-4"-de-O-methylcentrolabin.

- 10) Kurokawa M., Basnet P., Ohsugi M., Hozumi T., Kadota S., Namba T., Kawana T., and Shiraki K.: Anti-Herpes Simplex Virus Activity of Moronic Acid Purified from *Rhus javanica* *in vitro* and *in vivo*. *J. Pharm. Expt. Ther.*, 289 : 72-78, 1999.

**Abstract :** *Rhus javanica*, a medicinal herb, has been shown to exhibit oral therapeutic anti-

herpes simplex virus (HSV) activity in mice. We purified two major anti-HSV compounds, moronic acid and betulonic acid, from the herbal extract by extraction with ethyl acetate at pH 10 followed by chromatographic separations and examined their anti-HSV activity *in vitro* and *in vivo*. Moronic acid was quantitatively a major anti-HSV compound in the ethyl acetate-soluble fraction. The effective concentrations for 50% plaque reduction of moronic acid and betulonic acid for wild-type HSV type 1 (HSV-1) were 3.9 and 2.6  $\mu\text{g/ml}$ , respectively. The therapeutic index of moronic acid (10.3-16.3) was larger than that of betulonic acid (6.2). Susceptibility of acyclovir-phosphonoacetic acid-resistant HSV-1, thymidine kinase-deficient HSV-1, and wild-type HSV type 2 to moronic acid was similar to that of the wild-type HSV-1. When this compound was administered orally to mice infected cutaneously with HSV-1 three times daily, it significantly retarded the development of skin lesions and/or prolonged the mean survival times of infected mice without toxicity compared with the control. Moronic acid suppressed virus yields in the brain more efficiently than those in the skin. This was consistent with the prolongation of mean survival times. Thus, moronic acid was purified as a major anti-HSV compound from the herbal extract of *Rhus javanica*. Mode of the anti-HSV activity was different from that of ACV. Moronic acid showed oral therapeutic efficacy in HSV-infected mice and possessed novel anti-HSV activity that was consistent with that of the extract.

**11) Hase K., Xiong Q., Basnet P., Namba T., and Kadota S.: Inhibitor Effect of Tetrahydroswertianolin on Tumor Necrosis Factors- $\alpha$ -Dependent Hepatic Apoptosis in Mice. *Biochem. Pharmacol.*, 57 : 1431-1437, 1999.**

**Abstract :** We investigated the effect of tetrahydroswertianolin (THS), a hepatoprotective agent from *Swertia japonica*, on tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )-dependent hepatic apoptosis induced by D-galactosamine (D-GalN) (700 mg/kg, i.p.) and lipopolysaccharide (LPS) (10  $\mu\text{g/kg}$ , i.p.) in mice. Apoptotic symptoms were observed at the initial stage of liver damage. By 5 hr after intoxication, hepatic DNA fragmentation had risen to 2123%, with the value in untreated mice set at 100%, without a significant elevation of serum alanine transaminase (ALT) activity. There was a parallel increase in hepatocytes undergoing chromatin condensation and apoptotic body formation. By 8 hr after intoxication, serum ALT activity had risen to 3707 U/L. Pretreatment with THS (50 mg/kg, p.o.) at 18 and 2 hr before intoxication significantly reduced DNA fragmentation to 821% of that in untreated mice and prevented the emergence of chromatin condensation and apoptotic body formation. A significant and dose-dependent reduction in serum ALT activity at 8 hr also was observed with THS pretreatment. These effects of THS were different from those observed from pretreatment with glycyrrhizin (GCR), which is a clinically used hepatoprotective agent with membrane-stabilizing activity. GCR pretreatment (100 mg/kg, p.o.) did not inhibit hepatic DNA fragmentation (1588% of untreated mice), although this compound significantly protected against serum ALT elevation (1463 U/L). These data suggest that an inhibitory effect on the progression of hepatic apoptosis prior to liver injury may be involved in the

hepatoprotective mechanisms of THS, whereas it appears that GCR affects the processes after apoptosis. In a separate experiment, we found that the concentration of serum TNF- $\alpha$  rose to 2016 pg/mL at 1 hr after intoxication of mice with D-GalN and LPS, but this increase was suppressed by THS pretreatment (10, 50, or 200 mg/kg, p.o.) to 716, 454, or 406 pg/mL, respectively. Further study with a reverse transcriptase-polymerase chain reaction method showed that THS blocked TNF- $\alpha$  production at the transcriptional level. Because TNF- $\alpha$  is a critical mediator to elicit apoptosis in this model, the property of suppressing TNF- $\alpha$  production may be of prime importance for THS inhibition of hepatic apoptosis.

12) Cao H., and But P.P.H.: Molecular Evidences on Separation and Combination of the Genera *Elephantopus* L. and *Pseudelephantopus* Rohr (Compositae) in China. *J. Trop. & Subtrop. Bot.*, 7: 181-190, 1999.

**Abstract:** Two *Elephantopus* species and one *Pseudelephantopus* species have been placed in one single genus *Elephantopus* L. sensu lato by some earlier systematists. The genomic profile obtained by amplifying the DNA with different single primers were distinctive to these three species and two genera. The Similarity Indexes indicated that *Elephantopus mollis* is more closely related to *E. scaber* than to *Pseudelephantopus spicatus*. The present study indicated that the *Elephantopus scaber* L. and its closed species, *E. mollis* as well as *Pseudelephantopus spicatus* were genetically to be distinguished. The estimates of genomic DNA fingerprints based on Similarity Index values calculated from amplified DNA band profiles show a close association with known levels of morphological and histological, cytological relatedness.

◇総説

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#### ◇その他

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- 2) 曹 暉：中国産 *Curcuma* 属植物及び鬱金類の調査研究，1999，7/11-8/23，中国.

#### ◇共同研究

蔡 少青：北京医科大学薬学院

「中国における漢薬並びに少数民族薬物の比較研究」，1999～

磯部正治：富山大学工学部物質生命システム工学科

「遺伝子解析による生薬の同定法開発に関する研究」，1996～

「mRNA の発現動態を指標にした肝障害モデルマウスに対する和漢薬の作用」，1998～

東 純一，高橋京子：大阪大学臨床薬効評価学

「鬱血性心不全に対する活血化瘀生薬の防御作用に関する研究」，1998～

御影雅幸：金沢大学薬学部附属薬用植物園

「茯苓の生薬学的研究」，1999～

高野昭人：昭和薬科大学薬用植物園

「ヒマラヤ産薬物シラジットの基源と抗糖尿病作用」，1999～

服部征雄：富山医科薬科大学和漢薬研究所

「痴呆脳に対するコーヒーの作用」，1998～

「中国における漢薬並びに少数民族薬物の比較研究」，1999～

倉石 泰：富山医科薬科大学薬学部

「生薬からの抗そう痒活性成分の単離，同定」，1998～

#### ◇非常勤講師

- 1) 小松かつ子：金沢大学教養的・総合科目「ヒマラヤ風土記」第10回中国ヒマラヤの自然と文化，第11回チベット医学と仏教，1999，12/9，12/16，金沢.

#### ◇研究費取得状況

- 1) 文部省科学研究費，基盤研究(B)(2)（第1年度）（代表：小松かつ子，分担：東田千尋）「中国における漢薬並びに少数民族薬物の比較研究」，200万
- 2) 文部省科学研究費，研究成果公開促進費（データベース）（代表：渡辺裕司，分担：小松かつ子，東田千尋）「民族薬物データベース」，492万
- 3) 文部省科学研究費，基盤研究（B）（第2年度）（分担：小松かつ子）「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

- 4) 文部省科学研究費, 奨励研究 (A) (第1年度) (代表: 東田千尋) 「新規因子 sproutin の神経ネットワーク賦活化と痴呆の改善に及ぼす作用」, 90万
- 5) 文部省科学研究費, 基盤研究 (B) (第2年度) (分担: 東田千尋) 「アレルギー性の痒みの発生機序」, 400万
- 6) 全日本コーヒー協会 (分担: 小松かつ子, 東田千尋) 「痴呆脳に対するコーヒーの作用」, 150万
- 7) 富山県受託研究 (分担: 小松かつ子) 「和漢薬による慢性疾患の QOL 向上に関する総合的研究」, 40万
- 8) 財内藤記念科学振興財団, 研究成果刊行助成金 (学術図書), (代表: 小松かつ子) 「中国大陸をめぐる仏教医学」, 100万
- 9) 昭和薬科大学共同研究助成金 (分担: 小松かつ子, Basnet P.) 「ヒマラヤ産薬物シラジットの基源と抗糖尿病作用」, 45万

#### ◇研究室在籍者

大学院前期1年(派遣): 朱 妹

研究支援推進員: 曹 暉, 幸 雅子

外国人客員研究員: 劉 玉萍, Unnikrishnan P.M., Konduru Ramachandra Reddy

#### ◇民族薬物資料館記録

##### 1) 一般公開

平成11年10月31日に第2回の民族薬物資料館一般公開を実施した。予約制とし、10時、11時、14時、15時、16時からの5回に分けて各1時間、生薬の解説を加えながら館内を案内した。参加者は73名。

##### 2) 見学者記録 (1999年4月1日~2000年3月23日)

来館者総数: 666名 (日本人 633名, 外国人 33名)

案内総回数: 111回 (日本人 95回, 外国人 16回)

外国人の国名(人数): 韓国 (15), 中国 (11), タイ (2), ドイツ, カナダ, フランス, イタリア, ブラジル (各1)。

##### 3) 資料貸出

富山県立山博物館平成11年度秋季特別企画展 “立山に奇草を求めて—「富山藩薬品会」を通して—”, 1999, 10/2-11/3, 立山町。