生薬資源科学分野　　Division of Pharmacognosy

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◇研究目的

地球環境の変化により、薬用天然資源の減少が危惧される。そこで本分野では、生薬資源の現状の把握と代替生薬の開発、生薬の特徴を把握した効率的利用の促進並びに栽培薬用植物の選択と栽培拡充を目的にして、アジアにおける漢薬資源の調査と薬用生物の遺伝学的、成分化学的、薬理学的多様性の解析を行う。また、天然薬物の標準化を目的にして、遺伝子多型に基づく生薬同定法の開発並びに成分・活性情報の融合による生薬機能の解析を行う。

◇研究概要

Ⅰ）薬用生物及び伝統薬物の調査研究

中国広西壮族自治区、安徽省で桂皮（*Cinnamomum* 属植物）、石菖蒲（*Acorus* 属植物）の資源調査を行った。

Ⅱ）薬用植物・生薬の多様性の解析

1) 大黄の本草書収載の産地で得られた *Rheum* 属植物と市場品の葉緑体 *mat*K 遺伝子型と 28 成分の含量から、四川省北西部～青海省南東部産の *R. palmatum* の I 型が高品質であることを見出した。
2) 精油成分組成・相対含有量及びクルクミノイド組成・含量から、タイ産ウコン類生薬と *Curcuma* 属植物をタイプ分類し、中国産 *C. phaeocaulis* と同じタイプのものを見出した。
3) 百部の基源である *Stemona* 属植物について葉緑体 DNA の *trn*L-*trn*F、*pet*B-*pet*D、*trn*K-*rps*16 及び *trn*H-*psb*A 領域の塩基配列を解析し、4 種の固有配列を明らかにした。これに基づき百部の簡便な同定法として PCR-RFLP 法を開発した。
4) 刺五加（*Eleutherococcus senticosus*）エキスに、*in vitro* の神経障害モデルで神経突起伸展作用が認められ、主な活性成分として eleutheroside B を同定した。

Ⅲ）薬用植物の遺伝子多型に基づく生薬同定法の開発

Panax 属植物の核 18S rRNA 遺伝子の多型性に基づく人参類生薬基原解析用 DNA マイクロアレイを完成させた。

Ⅳ）漢方方剤・生薬・健康食品の品質とレギュレーション

1) ウコン中のクルクミノイドの簡便な定量法として，近赤外分光分析に多変量解析を組み合わせた方法を確立した。
2) 日本薬局方「補中益気湯エキス」収載の生薬配合比が異なる 5 タイプについて LC-MS による網羅的成分解析を行い、各々で違いを示す成分を明らかにした。

Ⅴ）Mass Bank 登録

46 種類の天然化合物の 68 質量スペクトルを登録した。

◇著書

1) 井上堯子，田中　謙：覚せい剤 Q & A，東京法令出版，東京，2008

◇原著論文

1) **Tanaka K. and Komatsu K.: Comparative study on volatile components of Nardostachys Rhizome. *J. Nat. Med.*, 62: 112-116, 2008.**

Abstract: Volatile components in 13 crude drug samples derived from *Nardostachys chinensis* or *Nardostachys grandiflora* were studied by solid phase micro extraction (SPME)-GC and SPME-GC–MS. Twenty-three compounds accounting for 81.3 and 70.0% of volatile components in newly collected samples of two species were identified. β-Maaliene, 9-aristolene, calarene and patchouli alcohol were identified as the major volatile constituents of *N. chinensis*, whereas aromadendrene, cube-11-ene, epi-α-selinene, spirojatamol and valeranone were identified as those of *N. grandiflora.* Using the peaks of β-maaliene and 9-aristolene in GC profiles as the marker, two *Nardostachys* species were clearly distinguished among the samples examined.

2) **Komatsu K., Sasaki Y., Tanaka K., Kuba Y., Fushimi H., Cai S. Q.: Morphological, genetic, and chemical polymorphism of *Curcuma kwangsiensis*. *J. Nat. Med.*, 62: 413-422, 2008.**

Abstract: Previously, Chinese gajutsu available in Japan was identified, from the chloroplast *trn*K gene sequence, to be the rhizomes of *Curcuma phaeocaulis* and two genotypes of *C. kwangsiensis*. Although we defined the two genotypes, the pl and gl types, on the basis of the nucleotide difference, their external features did not correspond to the two phenotypes described in the literature. In this paper, to investigate the relationship between genotype and phenotype of *C. kwangsiensis*, a field investigation was carried out in its main cultivation areas of Guangxi Zhuangzu Autonomous Region and Guangdong Province, China, and sequence analysis of the *trn*K gene and single-nucleotide polymorphism (SNP) analysis of the nuclear 18S rRNA gene were performed on the collected specimens. Four genotypes of *C. kwangsiensis* were recognized from the combined 18S rRNA gene-*trn*K gene sequences: homozygote-K(gl)Wtk type, homozygote-K(pl)Ztk type, heterozygote-K(gl)Wtk type, and heterozygote-Ltk type. Among the four genotypes, *C. kwangsiensis* in a field used for cultivation of gajutsu was of heterozygote-K(gl)Wtk type. Formation of a heterozygote in the 18S rRNA gene might be a result of crossbreeding of *C. kwangsiensis* with several *Curcuma* species which had cytosine at nucleotide position 234. GC analysis of the rhizomes revealed that *C. kwangsiensis* was characterized by camphor and beta-elemene, and by detecting additional components such as curdione and curcumenol *Curcuma* species involved in the formation of the heterozygote might be speculated upon.

3) **Zhu S., Fushimi H., Komatsu K.: Development of a DNA microarray for authentication of ginseng drugs based on 18S rRNA gene sequence. *J. Agric. Food Chem.*, 56: 3953-3959, 2008.**

Abstract: Ginseng drugs, derived from underground parts of *Panax* species (Araliaceae), are the most important group of herbal medicines in the Orient. Previously, the nucleotide sequences of the nuclear 18S rRNA gene of 13 *Panax* taxa were determined, as were the specific polymorphic nucleotides for identification of each species. On the basis of the nucleotide difference, a DNA microarray (PNX array) was developed for the identification of various *Panax* plants and drugs. Thirty-five kinds of specific oligonucleotide were designed and synthesized as probes spotting on a decorated glass slide, which included 33 probes corresponding to the species-specific nucleotide substitutions and 2 probes as positive

and negative controls. The species-specific probes were of 23-26 bp in length, in which the substitution nucleotide was located at the central part. Triplicate probes were spotted to warrant accuracy by correcting variation of fluorescent intensity. Partial 18S rRNA gene sequences amplified from *Panax* plants and drugs as well as their derived health foods were fluorescently labeled as targets to hybridize to the PNX array. After hybridization under optimal condition, specific fluorescent patterns were detected for each *Panax* species, and the analyzed results could be indicated as barcode patterns for quick distinction. The developed PNX array provided an objective and reliable method for the authentication of *Panax* plants and drugs as well as their derived health foods.

4) **Tanaka K., Kuba Y., Sasaki T., Hiwatashi F., Komatsu K.: Quantitation of curcuminoids in Curcuma Rhizome by near-infrared spectroscopic analysis. *J. Agric. Food Chem.*, 56: 8787-8792, 2008.**

Abstract: This study investigated a nondestructive and rapid quantitation method for the curcuminoids, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin, present in turmeric using near-infrared (NIR) spectroscopy and multivariate statistics. In the second derivatives of the NIR spectra of turmeric samples, two characteristic absorptions of curcuminoids were detected around 1700 and 2300-2320 nm. Partial least-squares regression (PLS-R) analysis was applied to the NIR spectra obtained from 34 turmeric samples, and PLS models for the quantitation of curcumin, demethoxycurcumin, bisdemethoxycurcumin, and total curcuminoid contents in the pulverized turmeric samples were constructed. Combination usage of the Standard Normal Variate (SNV) and second derivatives was obviously superior to other preprocessing methods. The lowest root mean squared error of cross-validation (RMSECV) values were detected at 6, 6, 6, and 6 PLS factors, for the quantitative subjects curcumin, demethoxycurcumin, bisdemethoxycurcumin, and total curcuminoid contents. It was clarified that the prediction of the composition by PLS-R analysis showed high correlation with the results of HPLC quantitations.

5) **Tanaka K., Kuba Y., Ina A., Watanabe H., Komatsu K.: Prediction of cyclooxygenase inhibitory activity of Curcuma Rhizome from chromatograms by multivariate analysis. *Chem. Pharm. Bull.*, 56: 936-940, 2008.**

Abstract: The potential use of partial least square regression (PLS-R) models for the prediction of biological activities of a herbal drug based on its liquid chromatography (LC) profile was verified using various extracts of *Curcuma phaeocaulis* and their cyclooxygenase-2 (COX-2) inhibitory activities as the model experiment. The correlation of practically measured inhibitory activities and predicted values by PLS-R analysis was quite good (correlation coefficient=0.9935) and the possibility of transforming chromatographic information into a measure of biological activity was confirmed. In addition, furanodienone and curcumenol were identified as the major active anti-inflammatory constituents of *C. phaeocaulis*, through detailed analysis of the regression vector, followed by isolation of these compounds and their COX-2 inhibitory assays. The selectivity indices (SI), IC(50) of COX-1/IC(50) of COX-2, of both compounds were higher than that of indomethacin and it is considered that furanodienone and curcumenol are the most promising compounds as lead anti-inflammatory agents.

6) **Tanaka K., Tamura T., Fukuda S., Batkhuu J., Sanchir C., Komatsu K.: Quality evaluation of Astragali Radix using a multivariate statistical approach. *Phytochem.*, 69: 2081-2087, 2008.**

Abstract: The quality of 43 Astragali Radix samples collected in China and Mongolia was evaluated using multivariate statistical analysis of data obtained from liquid chromatography-ion trap-time of flight (LC-IT-TOF) mass spectrometry. The samples were classified into four characteristic groups and most of the marker compounds were identified by elemental composition data and the results of MS/MS analysis. The approach provides useful information and gives an overview of the difference between crude drugs originating from different production environments and the genetic nature of the medicinal plants. In

addition, the ease with which particular marker compounds could be identified and the effectiveness of the comparison by means of multivariate statistics, such as principal component analysis (PCA), indicates that this method could be utilized for the establishment of standardization and quality control procedures for crude drugs.

7) **Tohda C., Ichimura M., Bai Y., Tanaka K., Zhu S., Komatsu K.: Inhibitory effects of *Eleutherococcus senticosus* extracts on amyloid beta(25-35)-induced neuritic atrophy and synaptic loss. *J. Pharmacol. Sci.*, 107: 329-339, 2008.**

Abstract: Neurons with atrophic neurites may remain alive and therefore may have the potential to regenerate even when neuronal death has occurred in some parts of the brain. This study aimed to explore effects of drugs that can facilitate the regeneration of neurites and the reconstruction of synapses even in severely damaged neurons. We investigated the effects of *Eleutherococcus senticosus* extracts on the regeneration of neurites and the reconstruction of synapses in rat cultured cortical neurons damaged by amyloid beta (Aβ(25-35). Treatment with Aβ(25-35) (10 microM) induced axonal and dendritic atrophies and synaptic loss in cortical neurons. Subsequent treatment with the methanol extract and the water extract of *E. senticosus* (10 - 1000 ng/ml) resulted in significant axonal and dendritic regenerations and reconstruction of neuronal synapses. Co-application of the extract and Aβ(25-35) attenuated Aβ(25-35)-induced neuronal death. We investigated neurite outgrowth activities of eleutherosides B and E and isoflaxidin, which are known as major compounds in *E. senticosus*. Although eleutheroside B protected against Aβ(25-35)-induced dendritic and axonal atrophies, the activities of eleutheroside E and isofraxidin were less than that of eleutheroside B. Although the contents of these three compounds in the water extract were less than in the methanol extract, restoring activities against neuronal damages were not different between the two extracts. In conclusion, extracts of *E. senticosus* protect against neuritic atrophy and cell death under Aβ treatment, and one of active constituents may be eleutheroside B.

8) **Tohda M., Hayashi H., Sukma M., Tanaka K.: A novel candidate for an intrinsic depression-related factor found in NG108-15 cells treated with Hochu-ekki-to, a traditional oriental medicine, or typical antidepressants. *Neuroscience Res.,* 62: 1-8, 2008.**

Abstract: Wakan-yaku is a type of Japanese and Sino traditional, systematized medical care that has been practiced for hundreds of years. To search for novel intrinsic factors related to the action of antidepressants, we used Hochu-ekki-to (HET), a Wakan-yaku medicine with antidepressive effects. First, we verified the quality of the HET by three-dimensional high-performance liquid chromatography and a cytotoxicity check in NG108-15 cells. We performed a DNA microarray analysis of the gene expression in cells treated with 50 μ/ml HET for more than 20 days. HET enhanced the expression of 125 (2.9%) genes and decreased the expression of 255 (6.0%) genes among the 4277 genes that were tested. The concentration-dependent increase in the expression of BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (BNIP-3) mRNA was particularly remarkable. A concentration-dependent increase in the expression of BNIP-3 mRNA was also observed when cells were treated with imipramine, mianserin, or milnacipran. These results suggest that BNIP-3 is a candidate for an intrinsic factor related to antidepressive effects and that Wakan-yaku theory may be useful for the identification of other intrinsic functional molecules.

9) **Hou X. L., Takahashi K., Tanaka K., Tougou K., Qiu F., Komatsu K., Takahashi K., Azuma J.: *Curcuma* drugs and curcumin regulate the expression and function of P-gp in Caco-2 cells in completely opposite ways. *Int. J. Pharm.*, 358: 224-229, 2008.**

Abstract: Curcumin is a phenolic compound isolated from rhizomes of *C. longa*, *C. aromatica* and other Curcumas except *C. zedoaria*. Recently, both curcumin and Curcumas have become prevalent as supplement. P-gp has been reported as an important determinant for drug absorption in small intestine. In this study, Caco-2 cell monolayers were treated with methanol extracts of Curcumas (0.1 mg/ml) or curcumin (30 microM) for 72h to investigate the relationship between the potential affects of Curcumas and curcumin on P-gp. [(3)H]-digoxin and rhodamine 123 were used to evaluate P-gp activity. All Curcumas significantly increased the activity of P-gp by up-regulating the expressions of P-gp protein and MDR1 mRNA levels. Interestingly, contrary to Curcumas, curcumin treatment inhibited the activity of P-gp with a decrease in P-gp protein and MDR1 mRNA expression levels. Curcumas might alter the pharmacokinetics of co-administrated drugs by up-regulating the function and expression levels of intestinal P-gp. However, curcumin has no relationship with the inductive effect of Curcumas since curcumin showed an opposite effects. Caution should be exercised when Curcumas or curcumin are to be consumed with drugs that are P-gp substrates because Curcumas and curcumin might regulate the function of P-gp in completely opposite ways.

10) **Maruyama T., Kamakura H., Miyai M., Komatsu K., Kawasaki T., Fujita M., Shimada H., Yamamoto Y., Shibata T., Goda Y.: Authentication of the traditional medicinal plant *Eleutherococcus senticosus* by DNA and chemical analyses. *Planta Med.*, 74: 787-789, 2008.**

Abstract: Shigoka (SGK), the rhizome of *Eleutherococcus senticosus*, is a traditional medicine used as a tonic in northeastern Asia and far eastern Russia. We analyzed the nuclear ribosomal DNA internal transcribed spacer (ITS) sequence of the medicine available on the Japanese and Chinese markets and found that at least 3 species were used as the source plant of the commercial SGKs and that only 70% of all samples was made from the correct species. Furthermore, we performed the quantitative determination of 3 marker compounds, eleutheroside B (EB), syringaresinol diglucoside (Syr), and isofraxidin (Iso) by ultraperformance liquid chromatography (UPLC)/mass spectrometry (MS). We found that EB and Iso are specific to the correct source plant of SGK. Of them, EB is thought to be the best marker compound for quality assurance of the SGK from the viewpoint of its pharmacological activity.

11) **Sasaki Y., Komatsu K., Nagumo S.: Rapid detection of *Panax ginseng* by loop-mediated isothermal amplification and its application to authentication of Ginseng. *Biol. Pharm. Bull.* 31: 1806-1808, 2008.**

Abstract: We have developed a novel method called loop-mediated isothermal amplification (LAMP) to detect *Panax ginseng*, the botanical source of Ginseng (Ginseng Radix), and to distinguish *P. ginseng* from *Panax japonicus*. Six allele-specific primers (two outer primers, two inner primers, and two loop primers) were designed based on the 18S ribosomal RNA gene sequence of *P. ginseng*, and LAMP was performed using those primers and total DNA extracted from *P. ginseng* as template. Amplifications were observed from approximately 30 min onwards at DNA concentrations of 0.5 to 10.0 ng. The presence of loop primers shortened the reaction time considerably. In contrast, in the reactions using total DNA from P. japonicus as template, no amplifications were observed. LAMP also enabled us to distinguish Ginseng from Japanese Ginseng (Panacis Japonici Rhizoma). LAMP was proven to be a rapid, highly sensitive, and specific method for the detection of *P. ginseng* and Ginseng.

12) **Cai S. Q., Yu J., Wang X., Wang R. Q., Ran F. X., Shang M. Y., Cui J. R., Komatsu K., Namba T.: Cytotoxic activity of some *Asarum* plants. *Fitoterapia*, 79: 293-297, 2008.**

Abstract: The cytotoxic activity against some tumor cell lines of 16 commonly used species of *Asarum* was evaluated in this study. All of these plants were widely used in Asian countries as traditional medicines or folk medicines. Their inhibitory activities against four tumor cell lines (HL-60, BGC-823, KB and Bel-7402) were compared. It was observed that 10 of the tested extracts (eight ethanol extracts and two water extracts) among 32 extracts of these plants showed cytotoxic activity. Those 95% ethanol

extractions from *A. caudigerellum*, *A. forbesii*, *A. inflatum* and *A. maximum* exhibited the highest cytotoxic activity, and 95% ethanol extracts or water extracts of *A. sieboldii* var. *seoulense*, *A. himalaicum*, *A. splendens* and *A. crispulatum* showed selective activity against one or two cells among the tested tumor cells. This is the first report of *Asarum* plants possessing cytotoxic activity against tumor cell lines.

◇学会報告 （*: 特別講演）

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2) 田中　謙，久場良亮，佐々木哲郎，樋渡史子，小松かつ子：近赤外分光分析による簡便なウコン中クルクミノイドの定量. 日本薬学会第 128 年会，2008，3/26-28，横浜.
3) 三石真生，魏　勝利，田中　謙，Bai Y. J.，Zhu S.，小松かつ子：大黄の基源と品質に関する研究（2）. 日本薬学会第 128 年会，2008，3/26-28，横浜.
4) 伏見裕利，伏谷眞二，小松かつ子，安食菜穂子，御影雅幸，川原信夫，伏見直子 :『日本薬局方』収載生薬類の変遷（第 2 報）. 日本薬学会第 128 年会，2008，3/26-28，横浜.
* 5) 朱　姝，小松かつ子，東田千尋：三七人参及び人参類生薬の系統的研究. 第 1 回三七人参国際シンポジウム，2008，4/1-3，文山，中国雲南省.
* 6) Komatsu K.: Molecular analysis, identification and quality evaluation of *Curcuma* drugs from China and Japan. The First International Symposium of Temulawak，2008，5/27-29，Bogor，Indonesia.
7) 小松かつ子，伏見裕利，民族薬物データベース作成委員会，証類本草データベース作成委員会：民族薬物資料館ポスター. 国立大学博物館等協議会 2008 年大会（第 3 回博物科学会），2008，6/5-6，大阪.
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13) 久場良亮，田中　謙，小松かつ子：タイ産 *Curcuma* 属生薬及び植物の成分化学的多様性の解析. 日本生薬学会第 55 回年会，2008，9/19-20，長崎.
14) Bai Y. J., Tohda C., Tanaka K., Zhu S., Komatsu K.: Effects of *Eleutherococcus senticosus* extracts and the main components on Aβ(25-35)-induced atrophies of axons and dendrites. 日本生薬学会第 55 回年会，2008，9/19-20，長崎.
15) 朱　姝，大家真由子，田中　謙，白　焱晶，小松かつ子，丸山卓郎，合田幸広，川崎武志，藤田正雄：刺五加の基原と品質に関する研究（3）―中国東北地方における遺伝的・成分的多様性. 日本生薬学会第 55 回年会，2008，9/19-20，長崎.
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17) 佐藤直人，馬　超美，小松かつ子，服部征雄：市場品美霊芝の成分研究. 日本生薬学会第 55 回年会，2008，9/19-20，長崎.
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◇その他

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3) 小松かつ子：「医食同源」と薬膳．「グルメやくぜんフェア」，富山新聞社後援，2008，2/16，富山．
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◇研究費取得状況

1) 文部科学省知的クラスター創成事業（第 II 期）「ほくりく健康創造クラスター」広域化プログラム「天然薬物の遺伝子解析等に基づく標準化研究」（代表：小松かつ子，分担：朱姝）「天然薬物の遺伝子解析に基づく標準化」
2) 文部科学省知的クラスター創成事業（第 II 期）「ほくりく健康創造クラスター」広域化プログラム「天然薬物の遺伝子解析等に基づく標準化研究」（分担：田中　謙）「天然薬物

成分の網羅的分析と計量科学的解析」

3) 文部科学省科学研究費，萌芽研究（第 2 年度）（代表：小松かつ子）「神経変性疾患に有効な伝統薬物分子の探索とその治療戦略」，100 万

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7) 学長裁量経費・教育研究支援経費（分担：小松かつ子）「アジア・アフリカ地域の「在来の知」の総合的研究」，20 万

8) 田村科学技術振興財団助成金（代表：田中　謙）「シツリシ（*Tribulus terrestris*）中のステロイドサポニンの吸収及び代謝と血中アンドロジェンに及ぼす影響に関する研究」, 25 万

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研究生：Fan Lanlan（北京大学薬学院，2008, 2/4～8/6）
研究員（広域化プログラム）：佐藤杏子（2008, 12/1～）
協力研究員：高橋京子（2006, 4/1～2008, 3/31）
外国人客員研究員：陳　芳清（三峡大学，2008, 10/7～2009, 3/31）

◇学位（学士，修士，博士）取得者

卒業論文：
　白石史遠：漢薬「芍薬・牡丹皮」の遺伝的多様性の解析
修士論文：
　久場良亮：アジア産ウコン類生薬の成分化学的多様性に関する研究