拠点事業推進室　　Research Promotion Office

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◇研究目的

当推進室は，生薬・方剤エキスやその含有成分と活性の情報の提供，および生薬をはじめとする天然薬物に関する科学的研究を通して，共同利用・共同研究の推進を支援することを目的とする。

◇活動概要

１）生薬エキスの化学的プロファイリングおよびその情報の提供

当推進室にある約 112 種生薬の水エキスを作製し，ＬＣ－高分解能ＭＳを利用し，化学的プロファイリングを行った。各生薬エキスのＬＣ－ＭＳクロマトグラムを研究者が自由に閲覧できるように和漢薬データベースに掲載している。

２）和漢薬の成分および活性を網羅的に収集し，集積した情報を有機的に繋げることによる和漢薬データベースの充実，発展

和漢薬データベース上の生薬約 180 種類，方剤約 350 種類について，写真や成分データ，処方の方法・用途，薬効・薬理やその主要文献などの情報を収集整理し，随時更新している。

３）生薬の化学成分の研究

和漢薬データベースには生薬の化学成分の情報を重要な項目として記載しているが，化学成分は詳細不明として情報が全くない生薬があった（例：続断）。この情報を提供するため，それらの生薬の水エキスの化学成分を系統的に研究した。その結果，続断の水エキスより新規化合物としてイリドイドグリコシド 2 種，既知化合物としてフェノール類 7 種，サポニン 5 種，アルカロイド 2 種，イリドイドグリコシド 7 種を単利し，構造解析した。これらの結果を纏めて，論文発表ならびに和漢薬データベースにも有用な情報として掲載する。

◇原著論文

1)　Li F., He Y.M., Awale S., Kadota S., Tezuka Y.: Two new cytotoxic phenylallylflavanones from Mexican propolis. Chem. Pharm. Bull., 59: 1194-1196, 2011.

Abstract: Two new phenylallylflavanones, (2R,3R)-6-[1-(4'-hydroxy-3'-methoxyphenyl)prop-2-en-1-yl]pinobanksin (1) and (2R,3R)-6-[1-(4'-hydroxy-3'-methoxyphenyl)prop-2-en-1-yl]pinobanksin 3-acetate (2) were isolated from a methanolic extract of Mexican propolis. Their structures were elucidated with spectroscopic analysis. Both compounds (1, 2) exhibited preferential cytotoxic activity against PANC-1 human pancreatic cancer cells in a nutrient-deprived medium with the concentration at which 50% cells died

preferentially in NDM (PC_{50}) values of 17.9 μM and 9.1 μM, respectively.

2) **Tezuka Y., Morikawa K., Li F., Auw L., Awale S., Nobukawa T., Kadota S.: Cytochrome P450 3A4 inhibitory constituents of the wood of Taxus yunnanensis. J. Nat. Prod., 74: 102-105, 2011.**

Abstract: From the aqueous extract of the wood of Taxus yunnanensis, which showed cytochrome P450 3A4 (CYP3A4) inhibition, a new isoflavan [(3S,4R)-4'-hydroxy-6,3'-dimethoxyisoflavan-4-ol (1)], a new degraded lignin [2,3-bis(hydroxymethyl)-7-hydroxy-6-methoxy-1-tetralone (2)], and a new lignan[(7R)-7-hydroxytaxiresinol (3)] were isolated, together with nine known lignans. Among the isolates obtained, α-conidendrin (12) showed strong CYP3A4 inhibition with an IC50 value of 0.2 μM.

3) **Zhao Q., Matsumoto K., Tsuneyama K., Tanaka K., Li F., Shibahara N., Miyata T., Yokozawa T.: Diabetes-Induced Central Cholinergic Neuronal Loss and Cognitive Deficit Are Attenuated by Tacrine and a Chinese Herbal Prescription, Kangen-Karyu: Elucidation in Type 2 Diabetes db/db Mice. J. Pharmacol. Sci., 117: 230-242, 2011.**

Abstract: We investigated the effect of kangen-karyu (KK), a Chinese herbal prescription, on cognitive deficits and central cholinergic systems of type 2 diabetic db/db mice. Seven-week-old db/db (Y-db/db) mice received daily administration of test drugs during an experimental period of 12 weeks. At 18 weeks of age (O-db/db), the animals underwent the water maze test. Compared with age-matched control strain mice (O-m/m), vehicle-treated O-db/db mice showed impaired learning and memory performance. KK (100 - 200 mg/kg per day) and the reference drug tacrine (THA: 2.5 mg/kg per day) ameliorated the performance of O-db/db mice without affecting their serum glucose level. O-db/db mice had lower levels of brain-derived neurotrophic factor (BDNF) mRNA and its protein in the brain than O-m/m mice. Expression levels of central cholinergic marker proteins in the hippocampus and the number of cholinergic cells in the medial septum and basal forebrain were also significantly lower in O-db/db than in O-m/m mice, whereas no significant differences in the expression levels of these factors and the cell number were found between Y-m/m and Y-db/db mice. KK and THA treatment significantly reversed the down-regulated levels of cholinergic markers, choline acetyltransferase-positive cell number, and BDNF expression in db/db mice. These findings suggest that KK as well as THA prevents diabetes-induced cognitive deficits by attenuating dysfunction of central cholinergic systems.

4) **Tanaka K., Li F., Morikawa K., Nobukawa T., Kadota S.: Analysis of biosynthetic fluctuations of cultured Taxus seedlings using a metabolomic approach. Phytochemistry, 72: 1760-1766, 2011.**

Abstract: Fluctuations in the biosynthesis of taxoids in 1-5 year old cultured seedlings of Taxus chinensis var. mairei were investigated using LC-IT-TOF-MS and a metabolomics approach. In the total ion chromatogram (TIC) of the extracts, 16 prominent peaks were observed. Ten compounds were identified by comparison of retention times and MS/MS spectra with those of reference compounds. An additional 6 taxoids were isolated by preparative HPLC and identified by comparison of their spectroscopic data with those reported in the literature. It was clarified that the relative concentrations of taxoids with 4(20) double bonds are high at early stages of cultivation. On the other hand, relatively higher amounts of 5-acetoxy taxoids oxidized at the 4- and 10- positions and toxoids having 5(20)-oxetane rings were found at later stages of cultivation. This approach provides practical information on the biosynthetic flow of taxoids in cultured yew seedlings.

5) **Awale S., Linn T.Z., Li F., Tezuka Y., Myint A., Tomida A., Yamori T., Esumi H., Kadota S.: Identification of chrysoplenetin from Vitex negundo as a potential cytotoxic agent against PANC-1 and a panel of 39 human cancer cell lines (JFCR-39). Phytother. Res., 25: 1770-1775, 2011.**

Abstract: Human pancreatic cancer is known to be the most deadly disease with the lowest 5-year survival rate and is resistant to well known conventional chemotherapeutic drugs in clinical use.

Screening of medicinal plants from Myanmar utilizing antiausterity strategy led to the identification of Vitex negundo as one of the medicinal plants having potent preferential cytotoxic activity against PANC-1 human pancreatic cancer cells. Bioactivity-guided phytochemical investigation led to the isolation of chrysoplenetin (1) and chrysosplenol D (2) as the active constituents with a PC50 value of 3.4 μg/mL and 4.6 μg/mL, respectively, against PANC-1 cells. Both these compounds induced apoptosis-like morphological changes in PANC-1 cells. Chrysoplenetin was further tested against a panel of 39 human cancer cell lines (JFCR-39) at the Japanese Foundation for Cancer Research,and 25 cell lines belonging to lung, breast, CNS, colon, melanoma, ovarian, prostate cancer and stomach cancer cell lines were found to be highly sensitive to chrysoplenetin at a submicromolar range. In the JFCR-39 panel, lung NCI-H522, ovarian OVCAR-3 and prostate PC-3 cells were found to be most sensitive with GI50 of 0.12, 0.18 and 0.17 μm, respectively. The COMPARE analysis suggested that the molecular mode of action of chrysoplenetin was unique compared with the existing anticancer drugs.

◇学会報告　（*: 特別講演，シンポジウム，ワークショップ等）

1) Shigetoshi Kadota, Feng Li, Yasuhiro Tezuka, Suresh Awale: Recent Trends in Propolis Research. 50th Cerebration symposium for the National Institute of Medicinal Materials (Viet Nam), April 18, 2011, Hanoi, Viet Nam.
2) Li F., Chang J.-C., Dibwe D. F., Awale S., Kadota S., Chiou R.Y.-Y.: Antioxidant and Anti-cancer Activities of Peanut Arahypin-5 and Other Stilbenoids. 43rdAnnual meeting of the American Peanut Research and Education Society. July 11–14, 2011, San Antonio, Texas, USA.
3) 松本欣三，趙琦，常山幸一，田中謙，李峰，宮田健，横澤隆子：加齢・糖尿病に起因する認知行動障害と漢方薬による実験的予防・治療. 第 11 回日本臨床中医薬学会, 2011, 11, 12, 東京.