和漢薬の科学基盤形成拠点

Joint Usage/Research Center for Science-Based Natural Medicine

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◇研究目的

「和漢薬の科学基盤形成拠点」事業は，本研究所に集積された技術，知識，和漢薬資源および研究環境を活用した共同利用・共同研究の実施を通して，新しい医薬学体系と全人的医療を構築しようとするものである。具体的な取組みとしては，共同利用・共同研究と本研究所での独自研究を通して，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り和漢薬データベースを充実，発展させ，和漢薬に含有される化合物群の構造と活性をケモ・バイオインフォマティクス及びシステムバイオロジー的視点から解析し，複合薬物である和漢薬が複雑系システムである生体内でどのように機能しているかを明らかにする事を目指している。

◇活動概要

１）生薬・方剤エキスの科学的プロファイリング及び生薬をはじめとする天然薬物に関する科学的研究

ウィキを用いた協働のための情報基盤のプロトタイプとして，27 生薬に関する 1600 の画像と各種情報からなるデータベースを作成した。また 118 生薬のＬＣ-ＭＳデータを掲載し，クロマトグラムを自由に閲覧できる仕組みを整えた。

◇原著論文

1) Li F., Awale S., Tezuka Y., and Kadota S.: Cytotoxicity of constituents from Mexican propolis against a panel of six different cancer cell lines. Nat. Prod. Commun., 5: 1601-1606, 2010.

Abstract: The cytotoxicity of 39 compounds, including eighteen flavonoids (flavanones, **1-10**; flavones, **11-17**; flavanol, **18**), sixteen phenolic acid derivatives (aromatic acids, **19-24**; aldehyde, **25**; esters, **26-34**) and five glycerides (**35-39**), isolated from Mexican propolis, were evaluated against a panel of six different cancer cell lines; murine colon 26-L5 carcinoma, murine B16-BL6 melanoma, murine Lewis lung carcinoma, human lung A549 adenocarcinoma, human cervix HeLa adenocarcinoma and human HT-1080 fibrosarcoma. A phenylpropanoid-substituted flavanol, (2*R*,3*S*)-8-[4-phenylprop-2-en-1-one]-4',7-dihydroxy-3',5-dimethoxyflavan-3-ol (**18**), showed the most potent cytotoxicity against A549 cells (IC_{50}, 6.2 μM) and HT-1080 cells (IC_{50}, 3.9 μM), stronger than those of the clinically used anticancer drug, 5-fluorouracil (IC_{50}, 7.5 μM and 5.4 μM, respectively). Based on the observed results, the structure-activity relationships are discussed.

2) Morikawa K., Tanaka K., Li F., Awale S., Tezuka Y., Nobukawa T., and Kadota S.: Analysis of MS/MS fragmentation of taxoids. Nat. Prod. Commun., 5: 1551-1556, 2010.

Abstract: The fragmentation pathways of seven types of taxoids were investigated by using a

LC-MS/MS method, namely: (1) neutral taxoids with a C-4(20) double bond; (2) taxoids with a C-4(20) double bond and oxygenation at C-14; (3) 5-cinnamoyl taxoids with a C-4(20) double bond; (4) a basic taxoid with a C-4(20) double bond; (5) a taxoid with a C-4(20) epoxide; (6) taxoids with an oxetane ring; and (7) taxoids with an oxetane ring and a phenylisoserine C-13 side chain. Depending on the class of core structure and the substitution pattern, each taxoid gave either the molecular adduct ion $[M+NH_4]^+$ or $[M+H]^+$. In the MS/MS, the molecular adduct ion gave characteristic product ions corresponding to the loss of water, acetic acid, benzoic acid, and cinnamic acid or the phenylisoserine group. These could reflect the difference of the substitutions and structural modifications and should be utilized for the structure elucidation of taxoids by LC-MS.

3) **Miyake K., Li F., Tezuka Y., Awale S., and Kadota S.: Cytotoxic activity of quassinoids from *Eurycoma longifolia*. Nat. Prod. Commun., 5: 1009-1012, 2010.**

Abstract: Twenty-four quassinoids isolated from *Eurycoma longifolia* Jack were investigated for their cytotoxicity against a panel of four different cancer cell lines, which includes three murine cell lines [colon 26-L5 carcinoma (colon 26-L5), B16-BL6 melanoma (B16-BL6), Lewis lung carcinoma (LLC)] and a human lung A549 adenocarcinoma (A549) cell line. Among the tested compounds, eurycomalactone (**9**) displayed the most potent activity against all the tested cell lines; colon 26-L5 (IC_{50} = 0.70 μM), B16-BL6 (IC_{50} = 0.59 μM), LLC (IC_{50} = 0.78 μM), and A549 (IC_{50} = 0.73 μM). These activities were comparable to clinically used anticancer agent doxorubicin (colon 26-L5, IC_{50} = 0.76 μM; B16-BL6, IC_{50} = 0.86 μM; LLC, IC_{50} = 0.80 μM; A549, IC_{50} = 0.66 μM).

4) **Li F., Awale S., Tezuka Y., Esumi H., and Kadota S.: Study on the constituents of Mexican propolis and their cytotoxic activity against PANC-1 human pancreatic cancer cells. J. Nat. Prod., 73: 623-627, 2010.**

Abstract: Three new flavonoids, (2*R*,3*R*)-3,5-dihydroxy-7-methoxyflavanone 3-(2-methyl)butyrate (**1**), (7"*R*)-8-[1-(4'-hydroxy-3'-methoxyphenyl)prop-2-en-1-yl]chrysin (**2**), and (7"*R*)-8-[1-(4'-hydroxy-3'-methoxyphenyl)prop-2-en-1-yl]galangin (**3**), together with 41 known compounds (**4-44**) were isolated from a methanolic extract of Mexican propolis. Compounds **2** and **3** are unique natural flavones containing a 1-phenylallyl moiety. The *in vitro* preferential cytotoxicity of all the isolates was evaluated against a PANC-1 human pancreatic cell line. Compound **3** displayed the most potent preferential cytotoxicity (PC_{50} 4.6 μM) in the nutrient-deprived medium (NDM) and triggered apoptosis-like morphological changes in PANC-1 cells.

5) **Miyake K., Tezuka Y., Awale S., Li F., and Kadota S.: Canthin-6-one alkaloids and a tirucallanoid from *Eurycoma longifolia* and their cytotoxic activity against a human HT-1080 fibrosarcoma cell line. Nat. Prod. Commun., 5: 17-22, 2010.**

Abstract: Phytochemical investigation of the stems of *Eurycoma longifolia* Jack led to the isolation of two new canthin-6-one alkaloids, 4,9-dimethoxycanthin-6-one (**1**) and 10-hydroxy-11-methoxycanthin-6-one (**2**), and a new tirucallane-type triterpenoid, 23,24,25-trihydroxytirucall-7-en-3,6-dione (**3**), along with 37 known compounds. Among these, an oxasqualenoid (**4**) was isolated as a natural product for the first time. The structures of the isolates were elucidated by spectroscopic and mass spectrometric means. All the isolates were evaluated for their cytotoxic activity against a HT-1080 human fibrosarcoma cell line. Among them, 9,10-dimethoxycanthin-6-one (**14**, IC_{50} = 5.0 μM), 10-hydroxy-9-methoxycanthin-6-one (**15**, IC_{50} = 7.2 μM), dihydroniloticin (**18**, IC_{50} = 8.2 μM), and 14-deacetyleurylene (**34**, IC_{50} = 3.2 μM) displayed stronger activity than the positive control 5-FU (IC_{50} = 9.2 μM).

◇学会報告　(*: 特別講演，シンポジウム，ワークショップ等)

1) 李峰，Suresh Awale，手塚康弘，門田重利：Constituents of Mexican propolis and their preferential cytotoxicit activity against PANC-1 human pancreatic cancer cells. 日本生薬学会第57回年会，2010, 9, 24-26，徳島.
2) 岡村侑香里，李峰，Suresh Awale，手塚康弘，門田重利：羌活に含まれる抗癌活性物質の

研究．日本生薬学会第 57 回年会，2010, 9, 24-26，徳島．
3) 李峰，Suresh Awale，手塚康弘，門田重利：ミャンマー産プロポリスの細胞毒性成分及び構造活性相関．日本薬学会第 130 年会，2010, 3, 28-30，岡山．
4) 三宅克典，手塚康弘，Suresh Awale，李峰，門田重利：ベトナム生薬 *Euricoma longifolia* Jack の成分研究．日本薬学会第 130 年会，2010, 3, 28-30，岡山．
5) 三宅克典，李峰，手塚康弘，Suresh Awale，門田重利：*Eurycoma longifolia* より単離したカシノイド類の細胞毒性．日本薬学会第 130 年会，2010, 3, 28-30，岡山．