Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

Poster Session Abstracts

To cite this article: (2012) Poster Session Abstracts, Pharmaceutical Biology, 50:5, 537-610, DOI: 10.3109/13880209.2012.658723

To link to this article: https://doi.org/10.3109/13880209.2012.658723

Published online: 20 Apr 2012.

Pharmaceutical Biology, 2012; 50(2): 537–610

ISSN 1388-0209 print/ISSN 1744-5116 online
DOI: 10.3109/13880209.2012.658723

informa
healthcare

Poster Session Abstracts

UMU APPLIED FOR SCREENING HERB AND PLANT EXTRACTS OR PURE PHYTOCHEMICALS FOR ANTIMUTAGENIC ACTIVITY

Monique Lacroix, Stéphane Caillet, Stéphane Lessard

INRS-Institut Armand-Frappier, Laval, Quebec H7V1B7, Canada

Antimutagenic activities of twelve herb extracts and twenty two plant extracts or pure phytochemicals assessed using a method based on the umu test system for screening natural antimutagens. All herb extracts tested showed antimutagenic properties except for Italian parsley that had mutagenic activity. Sage, mint, vervaine and oregano were the most antimutagenic. With regard to the metabolites, those from most herb extracts showed antimutagenic properties and those from garlic and thyme showed very strong antimutagenic activities, while those from camomile, rosemary and tarragon showed mutagenic activities, and those from celeriac and sage showed very strong mutagenic activities. Among pure compounds, pycnogenol metabolites showed strong antimutagenic activities.

INSECTICIDAL ACTIVITY OF *DERRIS MALACCENSIS* FROM FRENCH POLYNESIA

Heinui Philippe,[1] Taivini Teai,[1] Maurice Wong,[2] Christian Moretti,[3] Phila Raharivelomanana[1]

[1]Université de la Polynésie Française, Laboratoire BIOTEM, Faa'a, 98702, French Polynesia, [2]Service du Développement Rural, Papeete, 98713, French Polynesia, [3]Institut de Recherche pour le Développement, Papeete, 98713, French Polynesia

Derris malaccensis (G. Bentham) D. Prain, a tropical member of the Fabaceae growing in French Polynesia, was investigated to determine concentrations of metabolites (rotenoids and flavonoids) with pesticidal potential. Comparison of chemical composition of the different plant parts confirmed the prevalence in the roots of rotenone, a rotenoid used as a pesticide and in phytopharmacy. The root extract of *D. malaccensis* exhibited insecticidal activity against major pests in French Polynesia such as aphids (*Toxoptera* spp.), and the ant species *Monomorium destructor* and *Wasmannia auropunctata* (little fire ant). *W. auropunctata*, in particular, is an invasive ant known for its painful stings and impact on the environment; as a result, it is a great nuisance to humans in agriculture areas. The little fire ant is classified among the 100 worst invasive alien species in the world, and could become the greatest ant species threat in the Pacific area.

REPELLENCE OF ESSENTIAL OILS TO *FRANKLINIELLA OCCIDENTALIS* AS AFFECTED BY TYPE OF OIL AND POLYMER RELEASE

Robert Hollingsworth,[1] Monique Lacroix,[2] Isabelle Picard,[2] Stéphane Salmieri[2]

[1]U.S. Pacific Basin Agricultural Research Center, USDA-ARS, Hilo, Hawaii USA 96720, [2]INRS-Institut Armand Frappier, Research Laboratories in Sciences Applied to Food, Laval, Quebec H7V 1B7, Canada

Eight essential oils (at 0.125–1.0% V/V in acetone) were separately deposited on leaf disks to evaluate their potential to repel Western flower thrips [*Franliniella occidentalis* (Pergande)] adult females (WFT). The proportion of thrips counted on control leaf disks in choice bioassays was used as the measure of repellence. The most repellent essential oils were incorporated into polymer matrices, i.e. methylcellulose or alginate (0.5 or 1% W/V) in order to verify the potential of the polymer to extend repellence over time (24–120 h). At a concentration of 0.5%, *Thymus vulgaris* and *Satureja montana* were the most repellent essential oils. For these treatments, no WFT were counted on treated leaf disks 60 minutes after the start of the test. *Thymus serpyllum* and *Origanum compactum* also showed repellence values of 0.9 at this concentration. When *S. montana* and *T. serpyllum* were incorporated within polymer matrices, close to 100% repellence was achieved even after 48 hours regardless of the concentration of the alginate polymer used (0.5 or 1%). This level of

repellence was maintained for 3 days in the presence of *T. serpyllum* and for 4 days in the presence of *S. montana*. Results also showed that the alginate based-coating was repellent by itself.

INHIBITION OF *POTATO VIRUS X* INFECTIVITY BY EXTRACTS OF GREEN TEA

Christopher French, Michel Bouthillier

Agriculture & Agri-Food Canada, Pacific Agri-Food Research Center, Summerland, BC VOH 1ZO, Canada

Infectivity of *potato virus X* in a bioassay using *Chenopodium quinoa* was strongly inhibited by pre-incubation of the purified virus with aqueous solutions of five commercial leaf extracts of green tea, *Camellia sinensis*. Similar results were obtained using equivalent concentrations of the compound epigallocatechin-3-gallate (EGCg), suggesting that this was the active compound in the extracts. This is the first report of antiviral activity of green tea extracts and EGCg on PVX. Natural products, such as green tea extracts, have the potential for development as low toxicity, antiviral, disinfectants in agricultural applications.

ANALYSIS OF GUAVA AS A FORAGE – ORGANIC CONSTITUENTS

Julie Ann Luiz Adrian,[1] James R. Carpenter,[3] Norman Q. Arancon,[2] Bruce Mathews[2]

[1]University of Hawaii at Hilo, College of Pharmacy, Hilo, HI 96720, USA, [2]University of Hawaii at Hilo, College of Agriculture, Forestry and Natural Resource Management, Hilo, HI 96720, USA, [3]University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources, Honolulu, HI 96822, USA

This study was designed to determine the nutrient concentration of guava, *Psidium guajava* L., tree components. The analysis compared various tree tissues, seasons and locations. Nutritional analyses of guava tree parts suggest that guava tree shoots had the highest protein concentration (16.8% CP), while branches were relatively low in protein concentration (2.9% CP). Guava tree branches were high in fiber (65.9% ADF, 76.5% NDF), while the shoots (27.4% ADF, 37.1% NDF) and bark (27.1% ADF, 33.2% NDF) had the least fiber. Leaves were highest in hemicelluloses (11.3%), while the bark (6.1%), immature fruit (6.1%), breaker stage fruit (5.8%) and ripe fruit (5.6%) had the least. Total digestible nutrients were highest in breaker stage (72.6%) and ripe fruit (72.7%) while branches had the lowest value (52.6%). The breaker stage fruit and ripe fruit were highest in energy values (both at NEL 1.7 Mcal/kg, NEM 1.7 Mcal/kg, NEG 1.1 Mcal/kg), while the branches were lowest (NEL 0.7 Mcal/kg, NEM 0.9 Mcal/kg, NEG 0.4 Mcal/kg).

NOVEL PHYTOSIDEROPHORES IN BARLEY

Munkhtsetseg Tsednee,[1,2,3] Yit-Wai Mak,[1] Yet-Ran Chen,[1] Kuo-Chen Yeh[1,2,3]

[1]Agricultural Biotechnology Research Center, Taipei, 115, Taiwan, [2]Molecular and Biological Agricultural Sciences, Taiwan International Graduate Program, National Chung-Hsing University and Academia Sinica, Taipei, 115, Taiwan, [3]Graduate Institute of Biotechnology and Department of Life Sciences, National Chung-Hsing University, Taichung, 402, Taiwan

Iron (Fe) is one of the most important minerals in all organisms as a catalytic cofactor in vital metabolic pathways. Understanding plant Fe acquisition from the environment is critical to improve not only agricultural productivity but also human nutrition as plants are a major nutritional component of the human diet. Under Fe deficiency, graminaceous plants secrete Fe-chelating compounds called phytosiderophores (PSs) into the rhizosphere to take up Fe. Accurate analysis of PSs in grasses could therefore facilitate the use of these natural Fe-chelating compounds to improve Fe availability in plants. For this purpose, we have developed a rapid and highly sensitive LC-ESI-TOF-MS method for direct and simultaneous determinations of free PSs and their ferric complexes. Using this method, we have identified two more PSs, AVA and HAVA, in addition to previously reported PSs, DMA, MA and epi-HMA, in *Hordeum vulgare*, L. cv. Himalaya roots as well as in root exudates under Fe deficiency. MS results of root exudates and PS-ferric complexes suggest that the two PSs identified could be responsible for Fe acquisition.

TRICHODERMA PRODUCES ANTIFUNGAL METABOLITES THAT INHIBIT MYCELIAL GROWTH OF *PHYTOPHTHORA* SPP.

Soo Jung Bae, Vivek Bajpai, Kwang-Hyun Baek, *Hanhong Bae*

Yeungnam University, School of Biotechnology, Gyeongsan, 712–749, South Korea

Trichoderma fungal species, that colonize plant roots, are well-known for their potential to control plant pathogens. *Trichoderma* are ubiquitously distributed soil fungi that produce a variety of antibiotic metabolites. We extracted extracellular metabolites from a liquid culture of 130 *Trichoderma* isolates and screened the metabolites for antifungal

activity against seven *Phytophthora* species. Several metabolites have the potential to control *Phytophthora* pathogens. The structures of the metabolites will be determined later.

EFFECT OF *HELIOPSIS LONGIPES* EXTRACTS ON *MYCOSPHAERELLA FIJIENSIS* MORELET

Eric Tepetate-Arceo,[1] Enrique Ramírez-Chávez,[2] Jorge Molina-Torres,[2] *Patricia Ríos-Chávez*[1]

[1]Universidad Michoacana de San Nicolás de Hidalgo, School of Biology, Morelia, Mich 58000, México, [2]CINVESTAV-IPN, Department of Plant Biotechnology and Biochemistry, Irapuato, Gto 36821, México

Mycosphaerella fijiensis is the most devastating pathogen of plantain and banana worldwide. Since the fungus has developed resistance to several conventional fungicides, it is a necessity to look towards biofungicides. In searching for a biological control agent for this pathogen, we assessed an *in vitro* extract of *Heliopsis longipes,* a plant that contains a high level of affinin. Four extracts, differing in preparation date, were compared. Evaluations were conducted over 15 days by measuring the inhibition radius (mm) and the number of the growing colonies (cm^2). Activity appeared to depend on the date of the ethanolic extract. We found that extracts at 20% and 30% were the most inhibitory.

(+)-PISATIN BIOSYNTHESIS: FROM (–)-ENANTIOMERIC INTERMEDIATES TO A (+)-DERIVATIVE

Rhodesia M. Celoy, Kithsiri Wijeratne, Hans D. VanEtten

University of Arizona, College of Agriculture, Tucson, AZ 85721, USA

(+)-Pisatin, the major phytoalexin of pea (*Pisum sativum* L.), is an isoflavonoid derivative belonging to the pterocarpan family. It was the first chemically identified phytoalexin and subsequent research has demonstrated that most legumes produce pterocarpans with the opposite stereochemistry. Studies have shown that fungal pathogens are often more sensitive to a pterocarpan phytoalexin that has the opposite stereochemistry of its host's phytoalexin. Interestingly, pea produces two pterocarpanoid phytoalexins-minor amounts of (–)-maackiain and large amounts of (+)-pisatin. Studies on the biosynthesis of (+)-pisatin have shown that (–)-enantiomeric compounds are intermediates in its synthesis and are in the same pathway with that of (–)-maackiain biosynthesis. However, the step(s) from the (–)-intermediates to a (+)-derivative is still unknown. Previous studies have shown that (–)-7, 2′-dihydroxy-4′, 5′-methylenedioxy-isoflavanone [(–)-sophorol] is an intermediate in (–)-maackiain and (+)-pisatin biosynthesis. Chemical reduction of (–)-sophorol produces two isomers, *cis* and *trans* (–)-7, 2′-dihydroxy-4′, 5′-methylenedioxyisoflavanol [(–)-DMDI]. However, NMR analysis of the product of (–)-sophorol reduction by sophorol reductase revealed the product to be the *cis* (–)-DMDI isomer and we propose that *cis* (–)-DMDI is the branching point for the production of (–)-maackiain and (+)-pisatin. Time course enzyme assays comparing the proteins from elicited and non-elicited pea tissues using *cis* (–)-DMDI as substrate revealed the early and increasing production of an achiral 7,2′-dihydroxy-4′, 5′-methylenedioxyisoflavene (isoflavene) from the elicited pea tissues as compared to the non-elicited pea tissues. The same protein preparation from elicited tissues also converts the isoflavene into unknown products. We propose that the production of the achiral isoflavene intermediate could serve as the step for the change in the configuration that will ultimately produce a (+)-derivative.

IMPROVING PEPPERMINT ESSENTIAL OIL YIELD AND COMPOSITION THROUGH METABOLIC ENGINEERING

Glenn W. Turner,[1] Bernd Markus Lange,[1] Soheil S. Mahmoud,[4] Mark R. Wildung,[1] Edward M. Davis,[1] Iris Lange,[1] Raymond C. Baker,[2] Richard A. Boydston,[3] Rodney B. Croteau[1]

[1]Washington State University, Institute of Biological Chemistry, Pullman, WA 99164-6340, USA, [2]Washington State University, Irrigated Agriculture research and Extension Center, Prosser, WA 99350, USA, [3]United States Department of Agriculture, Agricultural Research Service, Vegetable and Forage Crops Research Unit, Prosser, WA 99350, USA, [4]University of British Columbia at Okanagan, Department of Biology, Kelowna, BC V1V 1V7, Canada

Peppermint oil production in the United States has been declining in recent years. Disease-resistant, high yielding varieties could help reverse this trend, but peppermint is a sterile hybrid, so conventional breeding is not an option. We conducted a project to evaluate the potential of genetic engineering to produce these badly needed peppermint varieties. We achieved increases in oil yield by overexpressing selected genes from the 2*C*-methyl-D-erythritol 4-phosphate (MEP) pathway. We also analyzed a number of two-gene combinations for their effects on oil yield and oil composition. The most promising results came from coupling expression of an antisense version of (+)-menthofuran synthase with overexpression of the MEP pathway gene 1-deoxy-D-xylulose 5-phosphate reductoisomerase. This line showed an oil yield increase of up to 61% over the yield of wild-type controls with favorable oil composition. In addition, we transformed

peppermint with a gene encoding (+)-limonene synthase which accumulated sufficiently to demonstrate its potential as a marker of transgenic oil. Our study illustrates the utility of metabolic engineering for the sustainable agricultural production of high quality essential oils at a competitive cost.

REGULATION OF GINGER ROOT EXTRACT ON COLONIC INFLAMMATORY SIGNALING IN HUMAN WITH NORMAL AND HIGH RISK OF COLON CANCER

Yan Jiang,[1] Danielle K. Turgeon,[1] Benjamin D. Wright,[2] Mack T. Ruffin,[2] Dean E. Brenner,[1,3,4] *Suzanna M. Zick*[2]

[1]University of Michigan Medical School, Department of Internal Medicine, Ann Arbor, MI 48105, USA, [2]University of Michigan Medical School, Department of Family Medicine, Ann Arbor, MI 48105,USA, [3]University of Michigan MedicalSchool, Department of Pharmacology, Ann Arbor, MI 48105,USA, [4]VA Medical Center, Ann Arbor, MI 48105, USA

Ginger and other zingiberaceous plants have been reported to contain anti-inflammatory activities with significant colon-cancer risk (CRC) preventive potential *in vitro*. Elevated prostaglandin E2 (PGE2) produced by cyclooxygenase (COX) has been shown as an early event of CRC development. In this study, healthy subjects and subjects with high risk of CRC were given 2.0 g/day ginger root extract or placebo for 28 days. The protein levels of COX-1, the constitutive form of COX and 15-hydroxyprostaglandin dehydrogenase (15 PGDH), the rate limiting enzyme in PGE2 catabolism, were measured in colon biopsies obtained from flexible sigmoidoscopy at baseline and the end of the study. Colonic COX-1 was significantly reduced (by 24%+13, $p = 0.03$) from baseline in ginger group in high CRC risk subjects and there was a trend toward significant decreases in COX-1 ($p = 0.055$) in healthy volunteers. On the other hand, 15 PGDH protein was not altered by the intervention in either healthy or high CRC risk subjects. Therefore ginger has the potential to decrease human colonic PGE2 synthesis without affecting PGE2 catabolism. Further investigation in larger studies with longer ginger intervention is necessary to exam the efficacy of ginger in regulation of the tissue level of prostaglandin synthesis.

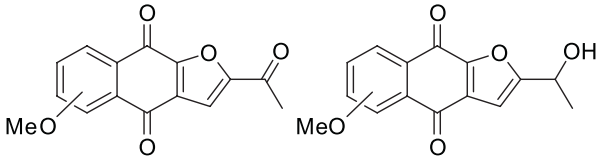
SYNTHESIS OF NATURALLY-OCCURRING FURONAPHTOQUINONES AND CYTOTOXICITY AGAINST HL-60

Ryuta Inagaki, Masayuki Ninomiya, Mamoru Koketsu

Gifu University, Department of Materials Science and Technology, Faculty of Engineering, Gifu, 501–1193, Japan

Naturally-occurring furonaphthoquinones are known as anti-cancer compounds. Especially, 2-acetylnaphtho[2,3-*b*]furan-4,9-dinone and 2-(1-hydroxyethyl)-naphtho[2, 3-*b*]furan-4,9-dinone from *Tabebuia cassinoides* have been used as a folk remedy in South America. Their cytotoxicity against KB, P388 and other cell types are known.

We synthesized several furonaphthoquinones to examine structure-activity relationships. The furonaphthoquinones so obtained were systematically investigated for the cytotoxicity against HL-60 cells.

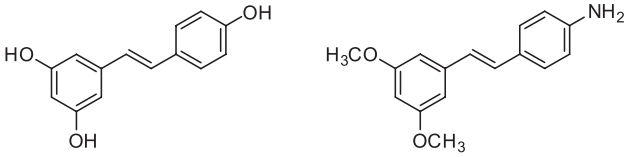


RESVERATROL DERIVATIVE (E)-4-(3,5-DIMETHOXYSTYRYL)ANILINE IS A NOVEL INHIBITOR OF CANCER CELL INVASION

Tamara P. Kondratyuk,[1] Eun-Jung Park,[1] Tyler Hirokawa,[1] Ethyn Leong,[1] Bin Sun,[2] Mark Cushman,[2] John M. Pezzuto[1]

[1]College of Pharmacy, University of Hawaii at Hilo, Hilo, HI 96720, USA, [2]Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, and The Purdue University Center for Cancer Research, Purdue University, West Lafayette, IN 47907, USA

Resveratrol is a structurally simple stilbene that interacts with numerous targets. Following evaluation of scores of structural analogs, the profile of biological responses mediated by (*E*)-4-(3,5-dimethoxystyryl)aniline (**1**) was found to be similar to resveratrol, but *in vivo* absorption and metabolic stability were much greater. Based on *in vitro* matrigel tests conducted with MCF7, MDA-MB-231, PC3 or RPMI 8226 human cancer cells, we now report resveratrol and compound **1** are active inhibitors of cell migration and invasion. Additional mechanistic studies are underway.



EVALUATION OF BLUEBERRY JUICE ON THE PRECARCINOGENIC LESIONS INDUCED BY AZOXYMETHANE IN MOUSE

Isela Alvarez-Gonzalez,[1] Luis Fernando Gacia-Melo,[1] Victor Islas-Islas,[1] Eduardo Osiris Madrigal-Santillan,[2] Eduardo Madrigal-Bujaidar[1]

[1]*Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biologicas, Mexico City, DF 07738, Mexico,* [2]*Universidad Autonoma del Estado de Hidalgo, Instituto de Ciencias de la Salud, Pachuca, Hidalgo, Mexico*

The consumption of fruits and vegetables has been suggested to prevent the development of cancer. Blueberry, in particular, has shown activity against heart disease, inflammation, diabetes mellitus, urinary tract infections and neurodegeneration. Therefore, the objective of this project was to evaluate the chemopreventive effect of blueberry juice (BJ) obtained from *Vaccinium virgatum* in three different doses against the damage caused by azoxymethane (AOM) in mouse colon cells. For the study, we quantified aberrant crypt foci (ACF), which are considered precarcinogenic lesions. In our assay, we found the following results: BJ per se did not alter the weight of the animals, in contrast with AOM, which reduced 20% the weight of the animals at the third week. In regard to precarcinogenic lesions, BJ showed no effect, while AOM induced 104.57 ACF in the colon of the mice. BJ did not show any precarcinogenic damage when administered alone. When we combined the two agents BJ plus AOM, the results showed that the lower and middle doses (0.4 and 1.2 μL/g) significantly decreased the frequency of ACF (70.35 and 88.64%, respectively). However, the high tested dose of BJ (15 μL/g) increased 326.73% the level of ACF. These results establish that the protective effect of BJ corresponds to low doses, while higher ones may act as co-mutagens.

THIAZOLE AND THIADIAZOLE DERIVATIVES OF RESVERATROL AS INDUCERS OF QUINONE REDUCTASE 1

Laura Marler,[1] Abdelrahman S. Mayhoub,[2] Mark Cushman,[2] John M. Pezzuto[1]

[1]*University of Hawaii at Hilo, College of Pharmacy, Hilo, HI 96720, USA,* [2]*Purdue University, Department of Medicinal Chemistry and Molecular Pharmacology, West Lafayette, IN 47907-2091, USA*

Although a number of resveratrol derivatives have been demonstrated to possess potent cancer chemopreventive potential, the stilbene ethylenic bridge is problematic since it is metabolized to the potentially dangerous *trans*-stilbene oxide. To circumvent this problem, a series of derivatives was synthesized with a thiazole or thiadiazole in place of the ethylenic bridge. A number of the resulting derivatives were found to be potent inducers of quinone reductase 1 (QR1), with CD (concentration required to double activity) values as low as 60 nM. Induction of QR1 may indicate activation of the antioxidant responsive element (ARE), which mediates expression of a number of phase II detoxifying enzymes. This activity indicates a reduction in oxidative stress, which could have implications not only for cancer chemoprevention, but for a number of other diseases, including heart disease, diabetes, Alzheimer's, and Parkinson's disease. Structure-activity relationships within the series were analyzed, and a number of assays (e.g., glutathione *S*-transferase induction, glutathione reductase induction, xanthine oxidase inhibition, total antioxidant capacity) were employed to investigate the potential of these compounds to reduce oxidative stress.

SUPPRESSION OF 12-*O*-TETRADECANOYL-PHORBOL-13-ACETATE-INDUCED ORNITHINE DECARBOXYLASE ACTIVITY BY RESVERATROL DERIVATIVES

Suaib Luqman,[1,2] *Tamara P. Kondratyuk*,[1] Juma Hoshino,[3] Mark Cushman,[3] John M. Pezzuto[1]

[1]*College of Pharmacy, University of Hawaii at Hilo, Hilo, HI 96720, USA,* [2]*Molecular Bioprospection Department of Biotechnology Division, Central Institute of Medicinal and Aromatic Plants (Council of Scientific and Industrial Research), Lucknow, 226015, India,* [3]*Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, and the Purdue University Center for Cancer Research, Purdue University, West Lafayette, IN 47907 USA*

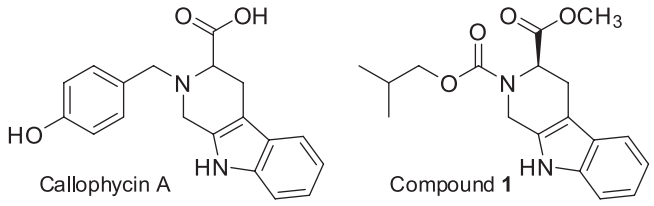
As demonstrated previously, resveratrol (3,4′,5-trihydroxy-*trans*-stilbene) inhibits 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA)-induced ornithine decarboxylase (ODC), the key rate limiting enzyme in mammalian polyamine synthesis. Using human bladder epithelial carcinoma HTB-24 cells in culture, where resveratrol inhibits induction with an IC_{50} of 8.8 μM, we now report potential metabolites [(*E*)-4-(3,5-dihydroxystyryl)phenyl sulfate (IC_{50} 1.2 μM), resveratrol 3,5,4′-trisulfate (IC_{50} 1.8 μM), resveratrol 3,4′-disulfate (IC_{50} 1.8 μM), and resveratrol 3,5-disulfate (IC_{50} 2.3 μM)] demonstrate greater activity. Based on RT-PCR studies, ODC inhibition occurs at the transcriptional level, but this was not due to direct inhibition of protein kinase C (e.g., resveratrol IC_{50}, 79 μM; resveratrol 3,5-disulfate IC_{50}, 49 μM). Additional work is underway to more fully investigate this potentially important observation. [This work was supported by program project P01 CA48112 awarded by the National Cancer Institute. SL acknowledges Indo-US Science and Technology Forum (IUSSTF), New Delhi for a Research Fellowship]

INHIBITORY EFFECT OF A CALLOPHYCIN A DERIVATIVE ON iNOS EXPRESSION IN LIPOPOLYSACCHARIDE-STIMULATED RAW 264.7 CELLS

Eun-Jung Park, Li Shen, Dianqing Sun, Tamara P. Kondratyuk, John M. Pezzuto

College of Pharmacy, University of Hawaii at Hilo, Hilo, HI 96720, USA

Callophycin A, found in the red algae *Callophycus oppositifolius*, has been reported as a potential antitumor agent. In our studies, this compound showed modest inhibition of LPS-induced iNOS activity with RAW 264.7 cells (<50% at 50 μM). However, after chemical modification of callophycin A, several derivatives exerted more potent inhibitory effects. In particular, compound **1** showed the most potent inhibition (IC_{50} = 2.8 μM), and blocked iNOS protein and mRNA expression in a dose-dependent manner. Since compound **1** is of potential value as an anti-inflammatory or cancer chemopreventive agent, further mechanistic studies are underway.



PSAMMAPLIN A INDUCES AUTOPHAGY CELL DEATH IN DOXORUBICIN-RESISTANT HUMAN BREAST CANCER MCF-7/ADR CELLS

Tae Hyung Kim, Young Ju Lee, A Jin Won, Mee Young Ahn, Yu Jin Shin, *Hyung Sik Kim*

Pusan National University, College of Pharmacy, Busan, 609–735, South Korea

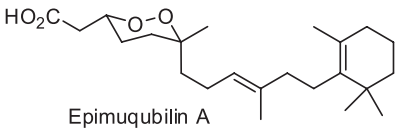
Psammaplin A (PsA) is a natural product isolated from marine sponges, which has been demonstrated to have anticancer activity against several human cancer cell lines through cell cycle arrest and apoptosis. Recently, development of new drugs that are less toxic and more effective against multidrug resistance in cancer patient are needed urgently. Here, we report the anticancer potential of PsA in doxorubicin-resistant human breast cancer MCF-7/Adr cells. PsA significantly inhibited the proliferation of MCF-7/Adr cells in a dose-dependent manner and markedly increased the G2/M phase of cell cycle. PsA significantly decreased SIRT1 enzyme activity, which is more potent than that of nicotinamide, a well known SIRT1 inhibitor. In addition, PsA markedly increased the expression of autophagy-related proteins LC3 and beclin-1 levels. The PsA-induced autophagy cell death was confirmed by acridine orange staining, which is a marker of acidification of autophagic vacuoles.These results suggest that PsA is sufficient to overcome multidrug resistance cancer through SIRT1-mediated autophagy in breast cancer MCF-7/Adr cells, thus indicating therapeutic potential for clinical use.

INHIBITION OF LIPOPOLYSACCHARIDE-INDUCED CYCLOOXYGENASE-2 AND INDUCIBLE NITRIC OXIDE SYNTHASE BY EPIMUQUBILIN A IN RAW 264.7 CELLS

Eun-Jung Park, Sarot Cheenpracha, Leng Chee Chang, Tamara P. Kondratyuk, John M. Pezzuto

College of Pharmacy, University of Hawaii at Hilo, Hilo, HI 96720, USA

Epimuqubilin A, from the marine sponge *Latrunculia* sp., suppressed nitric oxide production with LPS-stimulated RAW 264.7 cells (IC_{50} = 7.6 μM). Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were suppressed at both the mRNA and protein levels in a dose-dependent manner through blockage of the phosphorylation of inhibitor kinase (IKKβ). This resulted in stabilization and inhibition of NF-65 nuclear translocation and DNA binding. This is an unique mechanistic relationship that suggests epimuqubilin A warrants further exploration as a potential therapeutic agent.



AN EXTRACELLULAR ACYLTRANSFERASE CATALYZES CUTIN POLYMERIZATION

Trevor H. Yeats,[1] Laetitia B. B. Martin,[1] Tal Isaacson,[1,2] Yonghua He,[1] Lingxia Zhao,[3] Antonio J. Matas,[1] Gregory J. Buda,[1] David S. Domozych,[4] Jocelyn K. C. Rose[1]

[1]*Cornell University, Dept. of Plant Biology, Ithaca, NY 14853, USA,* [2]*Agricultural Research Organization, Newe Ya'ar Research Center, Ramat Yishay, 30095, Israel,* [3]*Shanghai Jiao Tong University, Plant Biotechnology Research Center,*

Shanghai, 200240, China, [4]Skidmore College, Dept. of Biology and Microscopy Imaging Center, Saratoga Springs, NY, 12866 USA

A waxy cuticle covers the aerial epidermis of plants and provides protection against desiccation and other stresses. It is contiguous with the polysaccharide cell wall and and consists of waxes associated with a polyester matrix of cutin. Previous work has revealed early steps in cutin monomer biosynthesis, but the mechanism of cutin polymerization has remained a mystery. A model system to address this question is provided by tomato (*Solanumlycopersicum*) fruit, which are typically covered with an exceptional amount of cutin composed primarily of esterified 10,16-dihydroxyhexadecanoic acid. We have identified a tomato mutant, *cutin deficient 1* (*cd1*) that has a 90% reduction in polymerized cutin. The *CD1* gene was mapped and its identity confirmed by transgenic complementation of the *cd1* mutant. Immunolocalization showed the protein to localize within the nascent cuticle. GC-MS analysis of soluble surface lipids in the *cd1* mutant showed accumulation of a non-polymerized putative monomeric cutin precursor. Recombinant CD1 showed acyltransferase activity using an analogous substrate as an acyl donor. Taken together, our results indicate that cutin is synthesized via successive transesterification reactions that are catalyzed by CD1. The presence of close orthologs of *CD1* in diverse plant genomes suggests that this is a conserved mechanism of extracellular polyester biosynthesis in plants.

MOLECULAR CLONING AND CHARACTERIZATION OF AN IRIDOID 1-O-GLUCOSYLTRANSFERASE INVOLVED IN SECOLOGANIN BIOSYNTHESIS

Keiske Asada, Mai Nagatoshi, Kazuyoshi Terasaka, Hajime Mizukami

Nagoya City University, Graduate School of Pharmaceutical Science, Nagoya, 467–8603, Japan

Catharanthus roseus is the sole source of commercially important anticancer drugs, vincristine and vinblastine, which are derived from strictosidine, a condensation product of tryptamine and secologanin. Despite an important biogenetic role of secologanin linking terpenes and indole alkaloids, the biosynthetic pathways, especially glucosylation step, leading to secologanin are poorly understood. We attempted to isolate and characterize a cDNA encoding a glucosyltransferase which is involved in secologanin biosynthesis.

Homology-based PCR cloning using the highly conserved motif among plant secondary product glucosyltransferases and the EST database search led us to obtain three full-length glucosyltransferase clones from *C. roseus* cultured cell (CrUGT6) and leaves (CrUGT7 and 8). Enzyme assays using the recombinant proteins revealed that CrUGT8 has high and specific glucosylation activity toward 7-deoxyloganetic acid. To our knowledge, this is first report identifying the glucosylation step in the secologanin pathway in *C. roseus*.

HETEROLOGOUS EXPRESSION AND CHARACTERIZATION OF RECOMBINANT PUTATIVE GLUCOSYLTRANSFERASE CLONE 3 FROM GRAPEFRUIT (*CITRUS PARADISI*)

Deborah Hayford, Daniel K. Owens, Cecilia A. Mcintosh

[1]East Tennessee State University, Biological Sciences, Johnson City, TN 37614, USA, [2]East Tennessee State University, School of Graduate Studies, Johnson City, TN 37614, USA

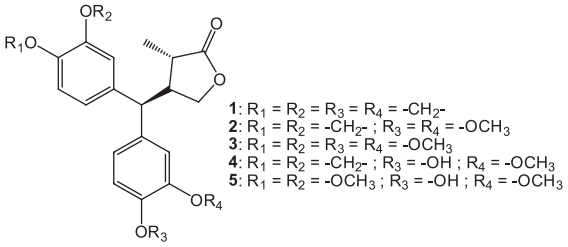
The grapefruit plant, *Citrus paradisi*, tends to accumulate high levels of flavonoid glycosides such as flavanones and flavones. Flavonoids have a vast array of important functions in plants and also in humans. Most naturally occurring flavonoids exist in glycosylated forms and this suggests that glycosylation is a key part of plant biochemical processes. Glucosyltransferases (GT's) involved in secondary metabolism share a loosely conserved UDP sugar binding motif called a PSPG box. Even though there is some degree of homology within the PSPG box, comparison of overall nucleotide or amino acid sequence of these enzymes tends to be low. The use of amino acid sequences alone cannot be used to predict specific functions and biochemical assays remain the only way to conclusively establish function. In our pursuit to study the structure and function of flavonoid GT's, we have used molecular approaches to identify, clone, express, and functionally characterize the enzymes. In this work, clone *PGT3* was obtained through EST mining of a directionally cloned young grapefruit leaf cDNA library. *PGT3* has been modified and cloned into *E. coli* and also into *Pichia pastoris*. Expression of recombinant PGT3 has been confirmed with the *E. coli*, however the majority of the protein was found in insoluble inclusion bodies. Expression with *Pichia pastoris* overcame the challenge of inclusion bodies. Results of expression in *Pichia pastoris* and purification of PGT3 by immobilized metal affinity chromatography are presented. PGT3 has been tested for GT activity with compounds representing the subclasses of flavonoids as well as some simple phenolics.

PEPEROMINS, CHROMENONES, FUNGAL ENDOPHYTES AND MORE FROM *PEPEROMIA GLABELLA* VAR. NERVULOSA

Fabio C. Chaves, Lydia F. Yamaguchi, Camila A. Rodrigues, Massuo J. Kato

University of São Paulo, Chemistry Institute, São Paulo, SP 05508-900, Brazil

In studying the yet unknown biosynthetic origin of secolignans from *Peperomia glabella* var. *nervulosa*, three major (**1**-**3**) and two minor (**4**, **5**) peperomins were characterized by GC/MS and HRESI following isolation of **1**-**3**. Additionally, various chromenones and other polyketides were identified. In order to further understand the biosynthetic pathway leading to peperomin formation, cell cultures were initiated using leaf and stem explants from adult plants. Unexpectedly, a variety of fungi isolated from these plant tissues was identified based on ITS sequences, and their metabolites in culture were determined.



PROBING CHEMICAL EVOLUTION AND DIVERSIFICATION IN THE SUNFLOWER FAMILY (ASTERACEAE)

Trinh-Don Nguyen, Nobuhiro Ikezawa, Dae-Kyun Ro

University of Calgary, Dept. of Biological Sciences, Calgary, AB T2N 1N4, Canada

Asteraceae, the largest and cosmopolitan flowering plant family, is characterized by its contents of sesquiterpene lactone (STL). Thousands of bioactive STLs have been documented, but their biochemistry is poorly understood. Studying STLs in Asteraceae thus provides an insight into the adaptive evolution of enzymes that has led to the family's enormous chemical diversification. Natural variants of closely related enzymes (e.g. terpene synthase and P450) can serve as excellent templates for enzyme evolution studies. We focused on the key P450 enzymes catalyzing three-step oxidations of sesquiterpene backbone at C12. Homologs of *Artemisia annua* amorphadiene C12 oxidase from three major and the basal subfamilies of Asteraceae were functionally characterized to catalyze the conversion of germacrene A (GA) to germacrene A acid (GAA), substantiating that this activity is highly conserved. Interestingly, GA oxidases (i.e., evolutionary predecessors of amorphadiene oxidase) showed promiscuous activities toward various non-natural substrates while amorphadiene oxidase displayed strict substrate specificity. With GA oxidase, we further identified the lactone-forming enzymes. A P450 clone encoding 8-β-GAA hydroxylase from sunflower was functionally identified. Its closest homolog in lettuce, however, was elucidated to catalyze the 6-α-hydroxylation of GAA, which result in the formation of the simplest STL, costunolide. Evolutionary significance of GA oxidases, 8-β- and 6-α-GAA hydroxylases will be discussed in the context of Asteraceae phylogeny.

HETEROLOGOUS EXPRESSION IN YEAST AND BIOCHEMICAL CHARACTERIZATION OF RECOMBINANT PUTATIVE GLUCOSYLTRANSFERASE 9 FROM *CITRUS PARADISI*

Anye Wamucho, Daniel K. Owens, Cecilia A. McIntosh

East Tennessee State University, Biological Sciences, Johnson City, TN 37614, USA, [2]East Tennessee State University, School of Graduate Studies, Johnson City, TN 37614, USA

The wide diversity of plant secondary products is a result of different modifications they undergo among which is glucosylation. Glucosylation of plant secondary metabolites increases their bioavailability, solubility, stability and also affects their organoleptic properties. Due to low homology between the nucleotide and amino acid sequences of plant secondary product glucosyltransferases (GTs), it is not possible to ascribe function based on sequence only. One approach is to identify and isolate putative GT clones, express them heterologously, and biochemically characterize the proteins. Grapefruit has been shown to accumulate high levels of glucosylated flavonoids, predominantly flavanones, flavones and flavonols. Eleven putative GT clones have been isolated from *Citrus paradisi* and some have been biochemically characterized. The hypothesis being tested is that PGT9 is a plant secondary product GT. A PGT9 contig was identified from the harvEST database using the plant secondary product glucosyltransferase (PSPG) box as identifier. It was amplified from young grapefruit leaf cDNA using specific primers. It was cloned into *E. coli* and successfully expressed but was localized to inclusion bodies and could not be tested for GT activity. PGT9 was subsequently cloned into *Pichia pastoris* using the pPICZA vector with zeocin resistance for selection. Expression of recombinant PGT9 in *Pichia pastoris* has been confirmed by Western blot analysis using anti myc antibodies. Enrichment of recombinant PGT9 by IMAC has been achieved and fractions with highly enriched PGT9 were pooled, desalted, concentrated, and used to screen for secondary product GT activity using a variety of flavonoid substrates as well as some simple phenolics.

INHIBITION OF HYDROXYCINNAMOYL-COA THIOESTERASES FROM GINGER PLANTS (*ZINGIBER OFFICINALE*)

I. Josefina Flores-Sanchez, David R. Gang

Washington State University, Institute of Biological Chemistry, Laboratory for Cellular Metabolism and Engineering, Pullman, WA 99164-6340, USA

Ginger (*Zingiber officinale* Rosc.), a plant from the Zingiberaceae, is widely used in traditional Asian cuisine and herbal medicine. Gingerols and diarylheptanoids, important compounds from this plant, are produced by type III polyketide synthases (PKSs), based on *in vitro* characterization of recombinant enzymes identified in our large scale transcriptome sequencing project. Previous efforts to detect PKS activities in ginger tissues were marginally successful because of hydrolysis of the hydroxycinnamoyl-CoA substrates in these assays, presumably due to the presence of thioesterases in these tissues. Three inhibitors of thioesterases were tested in efforts to identify these enzymes in leaf and rhizome crude protein extracts: orlistat, a reduced form of lipstatin and peptide 1 and peptide 2 from hydrolysates of soybean β-conglycinin. Significant differences in enzyme activities for feruloyl-CoA thioesterase and *p*-coumaroyl-CoA thioesterase were detected in rhizome crude extracts ($P<0.05$) while there were no significant differences for the same enzyme activities present in leaf. However, the inhibition of feruloyl-CoA thioesterase and *p*-coumaroyl-CoA thioesterase was significantly different in leaf extracts, with inhibitions of 4-fold or 5-fold, respectively; while no significant differences were found for inhibition of both enzyme activities ($P<0.05$) in the rhizome. The relationship of these thioesterase activities to the biosynthesis of the gingerols and diarylheptanoids in ginger will be discussed.

TRACKING THE BIOSYNTHESIS OF ^{13}C-LABELED GRAPE PHENOLICS *IN SITU*

Alexander W. Chassy, Douglas O. Adams, Andrew L. Waterhouse

University of California, Department of Viticulture and Enology, Davis, CA 95616, USA

Phenylalanine (Phe) is one of the primary building blocks for phenolic compounds in plants, including hydroxycinnamates and flavonoids. While many of the pathways surrounding phenolic biosynthesis have been established, uncertainty remains in the regulation and characteristics of Phe metabolites *in vivo*. The novel method presented here describes a technique able to probe the biosynthetic regulation as well as the nature of Phe metabolites. A ^{13}C-labeled Phe tracer was incorporated into grape berries on the vine (*in situ*), producing similarly ^{13}C-labeled phenolics. Following incubation with the tracer, labeled phenolic compounds were monitored over time by LC-DAD-MS. Labeled Phe was quickly metabolized and the allocation of tracer depends primarily on grape berry maturity. As expected, incorporation into immature grapes resulted in the production of labeled hydroxycinnamate esters whereas incorporation following the onset of ripening resulted in significant concentrations of labeled anthocyanins. This methodology presents a novel technique useful to (1) track the regulation of phenolic biosynthesis following environmental or genetic manipulation, (2) understand the nature of phenolics within their *in vivo* environment, and (3) search for novel Phe metabolites. Future studies using this technique will address the catabolism of flavonoids.

TWO CLASSES OF ENZYMES INVOLVED IN THE BIOSYNTHESIS OF CURCUMINOIDS AND OTHER DIARYLHEPTANOIDS IN GINGER AND TURMERIC

Yuying Sang, *Mark A. Willis*, David R. Gang

Washington State University, Institute of Biological Chemistry, Pullman, WA 99164, USA

Ginger and turmeric have been used for centuries for both culinary and medicinal purposes throughout Asia and now much of the rest of the world. The curcuminoids and other compounds of the diarylheptanoid class found in both of these plants appear to have very important medicinal properties that lead to their application against numerous diseases and ailments. Curcumin in particular has undergone clinical trials for use in treating Alzheimer's disease, diabetes, and various cancers. However, some now believe that tetrahydrocurcumin, which has undergone two double-bond reductions, may be the more bioavailable and relevant diarylheptanoid for human medicinal use. Here we describe the differential production of various diarylheptanoids in ginger and turmeric and two classes of enzymes involved in their production. The first class, type III polyketide synthases, catalyze the formation of the diarylheptanoid backbone. Several different enzymes in two subclasses have now been found to be involved in production of different subgroups of these compounds. Several genes belonging to the second class of enzymes, the double-bond reductase family, have now been cloned from ginger and turmeric. The expression levels of specific genes correlates well with the production of specific diarylheptanoids, which are produced in *in vitro* assays with the corresponding enzymes, suggesting specific roles for specific enzymes in the production of these compounds. We also describe a modeling analysis of these proteins to rationalize their different substrate preferences and catalytic activities.

A STRUCTURE-BASED MECHANISM FOR BENZALACETONE SYNTHASE FROM *RHEUM PALMATUM*

Yoshihiko Shimokawa, Hiroyuki Morita, Ikuro Abe

Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan

Benzalacetone synthase (BAS), a plant-specific type III polyketide synthase (PKS), catalyzes a one-step decarboxylative condensation of malonyl-CoA and 4-coumaroyl-CoA to produce the diketide benzalacetone. We solved the crystal structures of both the wild-type and chalcone-producing I207L/L208F mutant of *Rheum palmatum* BAS at 1.8 Å resolution. In addition, we solved the crystal structure of the wild-type enzyme, in which a monoketide coumarate intermediate is covalently bound to the catalytic cysteine residue, at 1.6 Å resolution. This is the first direct evidence that type III PKS utilizes the cysteine as the nucleophile and as the attachment site for the polyketide intermediate. The crystal structures revealed that BAS utilizes an alternative, novel active-site pocket for locking the aromatic moiety of the coumarate, instead of the chalcone synthase's coumaroyl-binding pocket, which is lost in the active-site of the wild-type enzyme and restored in the I207L/L208F mutant. Furthermore, the crystal structures indicated the presence of a putative nucleophilic water molecule which forms hydrogen bond networks with the Cys-His-Asn catalytic triad. This suggested that BAS employs novel catalytic machinery for the thioester bond cleavage of the enzyme-bound diketide intermediate and the final decarboxylation reaction to produce benzalacetone. These findings provided a structural basis for the functional diversity of the type III PKS enzymes.

MOLECULAR CHARACTERIZATION OF TULIPOSIDE A-CONVERTING ENZYME IN TULIP

Taiji Nomura, Shinjiro Ogita, Yasuo Kato

Toyama Prefectural University, Department of Biotechnology, Faculty of Engineering, Imizu, Toyama 939-0398, Japan

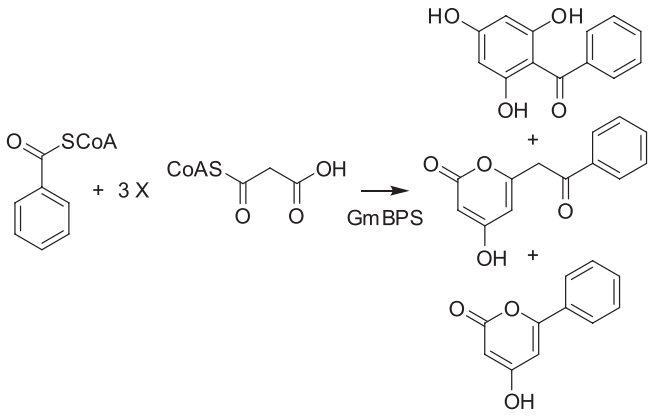
Tuliposides are representative secondary metabolites in tulip (*Tulipa gesneriana*). Their lactonized aglycones, tulipalins, are considered to function as defense chemicals due to their biological activities. We recently found that tuliposides are converted to tulipalins by tuliposide-converting enzyme (TCE), which has been purified from tulip bulbs. Although all parts of tulip possess TCE activity, specific activities in the crude extracts remarkably differ between bulbs and other tulip tissues. In order to investigate the functional diversity of TCE in each tulip tissue, we purified the TCE from petals as a representative of the tissues other than bulbs. The purified enzyme preferentially accepted tuliposides as substrates, with tuliposide A being the best substrate, and exhibited similar characteristics to the bulb enzyme with respect to substrate specificity, temperature and pH optima, and susceptibility to various inhibitors. However, the specific activities and the molecular masses differed greatly between the petal and bulb enzymes. Degenerate RT-PCR and the subsequent RACE PCRs with petal mRNA resulted in the isolation of novel cDNAs (*TgTCEA1* and *TgTCEA2*) encoding the petal TCE. Functional characterization of the *E. coli*-expressed recombinant enzymes confirmed the involvement of TgTCEAs in the conversion of tuliposide A into tulipalin A. *TgTCEA*s were transcribed sufficiently in all tulip tissues, but not in bulbs, showing the presence of another *TgTCEA* homolog expressing specifically in bulbs, as suggested by the distinct enzymatic characters between the petal and bulb enzymes.

CLONING, CHARACTERIZATION AND SITE-DIRECTED MUTAGENESIS OF *GARCINIA MANGOSTANA* BENZOPHENONE SYNTHASE

Natsajee Nualkaew,[1] Hiroyuki Morita,[2] Yoshihiko Shimokawa,[2] Kinjo Keishi,[2] Tetsuo Kushiro,[2,3] Wanchai De-Eknamkul,[4] Yutaka Ebizuka,[2] Ikuro Abe[2]

[1]Faculty of Pharmaceutical Sciences, Khon-Kaen University, Khon-Kaen, 40002, Thailand, [2]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan, [3]School of Agriculture, Meiji University, Kanagawa, 214–8571, Japan, [4]Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, 10330, Thailand

The biosynthesis of xanthones in *Garcinia mangostana* L. (Clusiaceae) involves the first reaction step catalyzed by benzophenone synthase (BPS) which is a type III polyketide synthase. The cDNA of *G. mangostana* BPS (*GmBPS*), cloned from young fruit pericarp, was found to consist of 1176 bp encoding a protein of 391 amino acids (*M*r of 42.7 kDa). The recombinant enzyme produced 2,4,6-trihydroxybenzophenone as the predominant product when using benzoyl CoA as starter. It also accepted other starter substrates and 1-3 units of malonyl CoA to form various phloroglucinol-type and polyketide lactone-type compounds. Site-directed mutagenesis of *GmBPS* led to change in substrate specificity, and increased the ratio of triketide lactone to benzophenone due to the reduce active site cavity by the mutants T135L, and G339S, respectively.



RECOMBINANT EXPRESSION AND CHARACTERIZATION OF AN *ARABIDOPSIS THALIANA* FAD SYNTHETASE

Joseph Lynch, Matthew Holm, Sanja Roje

Washington State University, Institute of Biological Chemistry, Pullman, WA 99164-6340, USA

FMN and FAD are important cofactors for a variety of enzymes involved in a multitude of metabolic processes in all organisms. These cofactors, as well as their inactive precursor riboflavin, are known to be interconverted by a network of enzyme catalyzed reactions. While examples of these enzymes have been characterized in several different organisms, in plants most of those interconverting enzymes have yet to be identified. Our lab has identified an *Arabidopsis* homolog to the known yeast FAD synthetase FAD1, an enzyme which facilitates the ATP-dependent conversion of FMN to FAD, which is non-homologous to other known plant FAD synthetases. Here we describe the recombinant expression, purification, and characterization of this new plant FAD synthetase.

CLONING AND CHARACTERIZATION OF AROMATIC PRENYLTRANSFERASE GENES FROM THAI MEDICINAL PLANTS

Thaniya Wunnakup,[1] Worrawat Promden,[2] Wanchai De-Eknamkul[2]

[1]*Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, 10330, Thailand,* [2]*Department of Pharmocognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Bangkok, 10330, Thailand*

In plants, prenylations of aromatic compounds play a major role in the diversification of natural product groups, such as flavonoids, xanthones, phenylpropanoids, and coumarins. These prenylation reactions are catalyzed by aromatic prenyltransferases which transfer various lengths of prenyl groups to different positions of the aromatic rings of the secondary metabolites. In this study, genes encoding flavonoid prenyltransferases from four Thai medicinal plants, namely *Artocarpuslakoocha* Roxb L., *Clitoria ternatea* L., *Orthosiphon aristatus* Mig. and *Morus alba* L. were amplified from their cDNA preparation using degenerate primers and cloned. The results showed that three different partial genes (*alr1, alr2A* and *alr2B*) were obtained from *A. lakoocha* and one each, *ctl, oam* and *mal*, from *C. ternatea, O. aristatus* and *M. alba*, respectively. These partial genes showed their percentage of identity in the range of 51 - 63, when compared with that of the known *SfN8DT-1* gene. These prenyltransferase genes are being determined for their full-length sequences which will be used for the cloning and functional expression.

METHYL JASMONATE AND YEAST EXTRACT STIMULATE MITRAGYNINE PRODUCTION IN SHOOT CULTURE OF *MITRAGYNA SPECIOSA* (ROXB.) KORTH.

Juraithip Wungsintaweekul, Jutharat Choo-malee, Niwat Keawpradub

Prince of Songkla University, Department of Pharmacognosy and Pharmaceutical Botany, Hat Yai, Songkhla 90112, Thailand

The shoot culture of *Mitragyna speciosa* was established and maintained in McCown woody plant medium (WPM) supplemented with 2 mg/L thidiazuron (TDZ), 1 mg/L benzyladenine (BA) and 2% (w/v) sucrose. The shoot cultures were elicited at exponential phase (14th day of culture) with methyl jasmonate (MJ) and yeast extract (YE). Mitragynine content was determined by HPLC and transcription profiles of tryptophan decarboxylase (*TDC*) and strictosidine synthase (*SSS*) were monitored. The results indicated that MJ at 10 μM, exposed for 24 h and YE at 0.1 mg/L, exposed for 12 h stimulated mitragynine production with a magnitude of 3.4 times and 2 times higher than control. The *TDC* and *SSS* mRNA expressions were related to the ability of mitragynine production in the elicited shoot culture. The results from

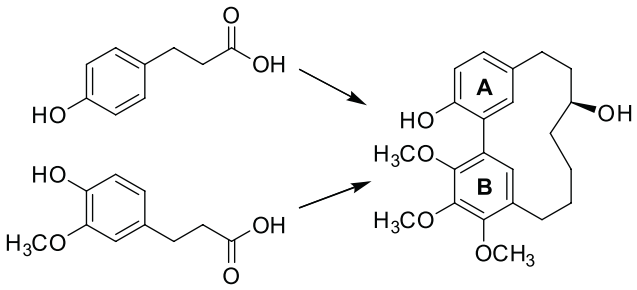
this study demonstrated that mitragynine production in the *M. speciosa* shoot culture was stimulated by MJ and YE. The mechanism of elicitation was up-regulated the *TDC* and *SSS* gene expressions.

DIARYLHEPTANOIDS, MYRICANOL, BIOSYNTHESIS IN *MYRICA RUBRA*: INCORPORATION EXPERIMENTS OF p-HYDROXYCINNAMIC ACID DERIVATIVES

Shingo Kawai, Hiroo Ichizawa, Tomoaki Nishida

Faculty of Agriculture, Shizuoka University, Shizuoka, 422–8529, Japan

To elucidate the hydroxylation and methylation steps in the biosynthesis of myricanol in *Myrica rubra*, we carried out feeding experiments with ^{13}C-labeled *p*-hydroxycinnamic acid dervatives. ^{13}C-NMR studies indicated that 3-(4-hydroxyphenyl)propionic acid and 3-(4-hydroxy-3-methoxyphenyl)propionic acid were preferentially incorporated into the A- and B-rings of myricanol, respectively. A biosynthetic pathway originating from 4-coumaric acid and leading to myricanol is discussed.



EXPRESSION OF 1-DEOXY-D-XYLULOSE 5-PHOSPHATE SYNTHASE, 2C-METHYL-D-ERYTHRITOL 4-PHOSPHATE SYNTHASE AND GERANYLGERANYL DIPHOSPHATE SYNTHASE, KEY ENZYMES OF PLAUNOTOL BIOSYNTHESIS IN *CROTON STELLATOPILOSUS*

Worapan Sitthithaworn,[1] Juraithip Wungsintaweekul,[2] Tanawan Sirisuntipong,[2] Tossaton Charoonratana,[2] Yutaka Ebizuka,[3] Wanchai De-Eknamkul[4]

[1]Faculty of Pharmacy, Srinakharinwirot University, Pharmacognosy, Nakonnayok, 26120, Thailand, [2]Faculty of Pharmaceutical Sciences, Prince of Songkla University, Pharmacognosy and Pharmaceutical Botany, Songkhla, 90112, Thailand, [3]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan, [4]Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pharmacognosy, Bangkok, 10330, Thailand

Plaunotol is an acyclic diterpene alcohol accumulating in the leaves of *Croton stellatopilosus*. Expression levels of genes encoding key enzymes in the plaunotol biosynthetic pathway, namely 1-deoxy-D-xylulose 5-phosphate synthase (*dxs*), 2C-methyl-D-erythritol 4-phosphate synthase (*meps*) and geranylgeranyl diphosphate synthase (*ggpps*), were analysed by measuring transcript levels in leaves of different developmental stages. The results showed that *dxs*, *meps*, and *ggpps* are all active in young leaves prior to full expansion when plaunotol is synthesised from the deoxy-D-xylulose 5-phosphate precursor in chloroplasts. The dense presence of chloroplasts and oil globules in the palisade cells of these leaves support the view that these genes are involved in plaunotol biosynthesis in chloroplast-containing tissues.

IDENTIFICATION AND CHARACTERIZATION OF DITERPENE SYNTHASES IN THE SALVINORIN A BIOSYNTHETIC PATHWAY

Rod Mitchell,[1] Philipp Zerbe,[2] Jörg Bohlmann,[2] Dae-Kyun Ro[1]

[1]University of Calgary, Department of Biological Sciences, Calgary, AB T2N 1N4, Canada, [2]University of British Columbia, Michael Smith Laboratories, Vancouver, BC V6T 1Z4, Canada

Salvinorin A is a hallucinogenic diterpenoid that is found in the glandular trichomes of *Salvia divinorum*. Salvinorin A is the only known hallucinogen to mediate hallucination by binding to and activating the κ-opioid receptor. Derivatives of salvinorin A are candidates for the treatment of hallucination associated disorders such as schizophrenia as a result of this unique receptor binding profile. In the hypothesized pathway of salvinorin A biosynthesis, a class II and a class I diterpene synthase catalyze the conversion of geranylgeranyl pyrophosphate (GGPP) into a novel clerodane diterpene. In order to understand salvinorin A metabolism, *S. divinorum* young leaf cDNA was subjected to pyrosequencing, and the resulting EST database was used to identify five candidate diterpene synthases by sequence homology to known diterpene synthases. Two of these candidates were found to be similar to type II diterpene synthases and were named copalyl pyrophosphate synthase like (CPPSL) 1 and 2. The remaining 3 candidates were found to be similar to type I

diterpene synthases and were named kaurene synthase like (KSL) 1, 2 and 3. CPPSL2, KSL2 and KSL3 are predominantly expressed in the trichomes of *S. divinorum*, indicating that they may play a role in the salvinorin A pathway. Accordingly, recombinant CPPSL2 enzyme was expressed in *Escherichiacoli*, and the purified enzyme was found to catalyze the conversion of substrate GGPP into a novel product. Structural elucidation of this new compound and additional characterizations of the KSLs are in progress.

MITRAGYNINE BIOSYNTHESIS: METABOLITE PROFILING AND mRNA EXPRESSION OF THE EARLY STEPS GENE IN *MITRAGYNA SPECIOSA* (ROXB.)KORTH.

Tossaton Charoonratana, Juraithip Wungsintaweekul

Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

Mitragynine, a terpenoid indole alkaloid, originates from two biosynthetic pathways, the shikimate and the terpenoid pathways. Therefore the genes involved in those pathways are the target in our study. We investigated the mRNA expression level of the 1 month old seedling plant. Tryptophan decarboxylase (*TDC*), 1-deoxy-D-xylulose-5-phosphate synthase (*DXS*), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (*DXR*) and strictosidine syntase (*SSS*) of the *M. speciosa* genes were used to profile genes transcription activities in three organs comprising the whole plant; leaves, roots, and stems. The relative quantitative real-time pcr (qRT-PCR) analysis was used to confirm those upregulated genes. In parallel, the metabolites profiles including secologanin, tryptophan, tryptamine and mitragynine contents were determined using HPLC. For instance, low amounts of tryptophan and tryptamine in *M. speciosa* might be formed by the concerted action of TDC and SSS, which convert the primary metabolite tryptophan to tryptamine and strictosidine in the presence of excess amount of secologanin. In addition, the upstream DXP genes *DXS* and *MEPS* showed lower mRNA expressions, suggested that those genes had a lesser effect in mitragynine biosynthesis. Adding tryptophan and tryptamine to the culture medium also increases the amount of mitragynine in *M. speciosa* culture, so *TDC* and *SSS* play an important role in mitragynine biosynthesis.

SPECIALIZED ROLES FOR THE TWO UDP-GLUCOSYLTRANSFERASES UGT85K2 AND UGT85K3 IN HYDROXYNITRILE GLUCOSIDE METABOLISM IN *LOTUS JAPONICUS*

Camilla Knudsen,[1] Lisbeth Mikkelsen,[1] Fran Robson,[2] Mohammed Saddik Motawie,[1] Carl Erik Olsen,[1] Birger Lindberg Møller,[1] Adam Takos,[1] Fred Rook[1]

[1]*University of Copenhagen, Department of Plant Biology and Biotechnology, Frederiksberg, Denmark 1871,*[2] *John Innes Centre, Department of Metabolic Biology, Norwich, NR4 7UH, United Kingdom*

Cyanogenic glucosides are amino-acid derived plant chemical defense compounds against generalist herbivores. They are α-hydroxynitrile glucosides that are activated by specific β-glucosidases upon tissue disruption. The unstable α-hydroxynitrile will dissociate with the release of hydrogen cyanide. The legume model *Lotus japonicus* contains the cyanogenic glucosides linamarin and lotaustralin, and the non-cyanogenic γ- and β-hydroxynitrile glucosides rhodiocyanoside A and D, which are also thought to function as defense compounds. Glucosylation is a key-step in the biosynthesis of hydroxynitrile glucosides as it stabilizes and detoxifies these compounds, and allows for their storage. Both the UDP-glucosyltransferases UGT85K2 and UGT85K3 are able to catalyze the synthesis of linamarin and lotaustralin, but only UGT85K2 showed significant glucosylation activity for the synthesis of rhodiocyanosides *in vitro*. Mutants in the *UGT85K2* gene, obtained by TILLING, almost lacked rhodiocyanosides and showed severe growth defects. This suggested the toxicity of the rhodiocyanoside aglycones and supports their proposed defense role. The observed specificity of these UGTs further highlights the metabolic flexibility of the hydroxynitrile glucoside based defense pathway in *L. japonicus*.

LAND PLANT ADAPTATION: THE ROLE OF SPECIFIC AROGENATE DEHYDRATASES

Sarah E. Brewer, Oliver R. A. Corea, Laurence B. Davin, Norman G. Lewis

Washington State University, Institute of Biological Chemistry, Pullman, WA 99164-6340, USA

Arogenate dehydratases (ADTs) are enzymes that facilitate the last step in phenylalanine biosynthesis. The six genes encoding these enzymes within *Arabidopsis thaliana*, *ADT1-6*, were recently biochemically characterized, however it is currently unclear whether different ADT isoenzymes act in a functionally redundant manner or whether there are spatial and/or temporal differences in their activity. This is an important area of investigation because ADT enzymes play a crucial role in synthesizing phenylalanine, which has many important downstream products, including lignin. One possibility is that ADTs may be differentially involved in the synthesis of proteins or the production of lignin and other compounds. To investigate their expression patterns over growth and development, *ADT* promoter::*GUS* (*β*-glucuronidase) fusions were constructed then introduced into *Arabidopsis* plants. GUS staining was performed on second-generation transformed (T_2) plants at various growth stages (from three-day-old seedlings to mature plants) and various tissues

(roots, leaves, stems, flowers, siliques and seeds). The results of this staining established the expression patterns of the six *ADTs*, including several major differences, providing insight into the possible functions of each enzyme within plants.

FUNCTIONAL EVOLUTION OF P450S: THE BIOSYNTHESIS OF ALLIARINOSIDE IN *ALLIARIA PETIOLATA*

Tina Frisch, Mohammed Saddik Motawie, Carl Erik Olsen, Nanna Bjarnholt, Birger Lindberg Møller

University of Copenhagen, Department of Plant Biochemistry and Biotechnology, Frederiksberg, DK-1871, Denmark

Nitrile formation in plants involves activity of cytochrome P450s. Hydroxynitrile glucosides are widespread among plants but do generally not occur in glucosinolate (GLS)-producing species. *Alliaria petiolata* (Brassicaceae) is the only species known to produce GLSs as well as a γ-hydroxynitrile glucoside. Furthermore, *A. petiolata* has been described to release cyanide, which indicates an unidentified cyanogenic glucoside. Our research on *A. petiolata* addresses the molecular evolution of P450s. By integrating knowledge of GLS and hydroxynitrile glucoside biosynthesis in other species, we propose a biosynthetic pathway for the γ-hydroxynitrile glucoside, alliarinoside. HomoMet and the corresponding oxime are suggested as shared intermediates in the biosynthesis of alliarinoside and 2-propenyl GLS. The first committed step in the alliarinoside pathway is envisioned to be catalyzed by a P450, which has been recruited to metabolize the oxime. Furthermore, the pathway is suggested to involve enzyme activities common to secondary modification of GLSs. Thus, we argue that biosynthesis of alliarinoside is the first known case of a hydroxynitrile glucoside pathway evolved from the Brassicales-specific GLSs. An intriguing question is if the hydroxynitrile intermediate in the proposed alliarinoside pathway may also be glucosylated into a novel homoMet-derivedcyanogenic glucoside. Elucidating the biosynthesis of alliarinoside and other putative hydroxynitrile glucosides in *A. petiolata* will provide insight into how P450 evolution promotes development of novel natural product pathways.

BIOCHEMICAL ANALYSIS OF A PUTATIVE LIMONOID GLUCOSYLTRANSFERASE FROM *CITRUS PARADISI*

Daniel K. Owens, Cecilia A. McIntosh

East Tennessee State University, Biological Sciences, Johnson City, TN 37614, USA

Limonoids abundantly accumulate in citrus species, and are of particular interest to the marketability of citrus juices as they convey bitterness. As citrus fruit ages, a natural debittering process occurs which has been attributed to the conversion of bitter limonoid aglycones to the corresponding tasteless limonoid glucosides during maturation. Beyond glucosylation's commercial importance, it is also a significant *in planta* modification reaction. Glucosylation serves a number of physiologically important roles such as influencing solubility and thereby transport, regulating bioavailability, and stabilizing structure. The enzymes that catalyze glucosylation, glucosyltransferases (GTs), typically function by transferring a UDP activated glucose to the corresponding aglycone. A loosely conserved 44 amino acid residue motif known as the plant secondary product glucosyltransferase (PSPG) box is thought to encompass the GT UDP-glucose binding moiety. In this work, the PSPG box has been used as a marker to identify putative glucosyltransferase genes using a combination of bioinformatics techniques and "fishing" against grapefruit cDNA libraries. One of the identified putative GTs, PGT8, is highly homologous to a limonoid GT from *Citrus unshiu* (AB033758). PGT8 has been recombinantly expressed in *E. coli* and strongly enriched using metal affinity chromatography. The recombinant enzyme has been screened for activity with synthesized limonoate A-ring lactone as well as a number of flavonoid substrates. Enzymatic activity with quercetin has been demonstrated with this enzyme.

CATALYTIC SITE OF PLANT GLUTATHIONE S-TRANSFERASE, A HERBICIDE DETOXIFICATION ENZYME

Jin-Joo Lee, Ji-Na Kong, Dong-Hyeon Jo, *Kwang-Hoon Kong*

Chung-Ang University, Biomolecular Chemistry Laboratory, Department of Chemistry, College of Natural Sciences, Seoul, 156–756, South Korea

Glutathione *S*-transferase (GST, EC 2.5.1.18) is a family of multifunctional proteins, catalyzing the formation of conjugates between reduced glutathione (GSH) and a wide variety of electrophilic compounds. In plant, this function of GST plays a pivotal role in the detoxification of herbicides, organic pollutants and natural toxins. To gain further insight into herbicide detoxification in plant and GST evolution, we have studied the catalytic mechanism and the relationship between structure and functions of rice GST by the combination of site-directed mutagenesis, X-ray crystallography and in depth kinetic analysis. The substitutions of Tyr8 and Ser13 residue with alanine resulted in approximately 80–90% loss of specific activity. From the pH-log (k_{cat}/K_m^{CDNB}) plot, the pKa values of GSH in enzyme-GSH complex of Y8A and S13A mutants were estimated to be approximately 8.5-8.9, which were about 1.6-2.0 pK units higher than that of the wild-type enzyme. From the 3-dimensional structure of rice GST, we suggested that Ser13 is located in the active site and its side chain is in close proximity to the thiol group of glutathione bound in the enzyme. From these results, we suggest that Ser13 in rice GST is the residue responsible for catalytic activity by lowering the pKa of GSH in enzyme-GSH complex, and by enhancing the nucleophilicity of the thiol of GSH in the active site of plant GST.

PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF SOME HERBS GROWN IN LITHUANIA

Petras Rimantas Venskutonis

Kaunas University of Technology, Department of Food Technology, Kaunas, LT-50254, Lithuania

This presentation reviews phytochemical studies of medicinal plants grown in Lithuania. These studies were focused on the antioxidant properties and phytochemical composition of extracts isolated by using different solvents, as well as their fractions and purified compounds. In some cases genotoxicity and cytotoxicity assays were performed. The plants include such less investigated species as *Hierochloe odorata, Marrubium vulgare, Chrysanthemum balsamita, Rhaponticumcarthamoides, Geranium macrorrhizum, Potentilla fruticosa* and some others. It was found that these plants accumulate strong antioxidants as it was measured by using several radical scavenging assays and some other methods. Flavonoids and phenolic acids were the main phytochemicals in the studied plants, some of them were not previously reported as natural compounds or as constituents of the analyzed plants. However, some other structures were also identified. For instance, two very strong antioxidants were found in sweet grass, namely 5,8-dihydroxybenzopyranone (DHBP) and its 8-*O*-*β*-D-glucopyranoside. Radical scavenging capacity of these compounds were comparable with rosmarinic acid and other well-known strong natural antioxidants. The reactivity of 5,8-DHBP with peroxidase was similar to the reactivity of quercetin; the extract effectively neutralized the effect of singlet oxygen to erythrocytes, however demonstrated the pro-oxidant character of cytotoxicity. 5,8-DHBP inhibited the contractility of arterial smooth muscles and increased spontaneous basal tone of arteries: it was toxic in high concentrations, while low doses only slightly reduced the contraction ability of small arteries.

PHYTOCHEMICAL CHARACTERISATION OF HIGHBUSH BLUEBERRY (*VACCINIUM* × *COVILLEANUM*) AND EUROPEAN CRANBERRY BUSH (*VIBURNUM OPULUS*) ACCESSIONS GROWN IN LITHUANIA

Petras Rimantas Venskutonis,[1] Vilma Kraujalytė,[1] Elicija Stackevièienė,[2] Juozas Labokas,[2] Laima Èesonienė,[3] Remigijus Daubaras[3]

[1]*Kaunas University of Technology, Department of Food Technology, Kaunas, LT-50254, Lithuania,* [2]*Nature Research Centre, Institute of Botany, Vilnius, LT-08406, Lithuania,* [3]*Vytautas Magnus University, Kaunas Botanical Garden, Kaunas, LT-46324, Lithuania*

Growing interest in natural food ingredients exhibiting beneficial effects on human health has been an important factor for expanding the studies of less common horticultural plants in recent years. It is well established that berries accumulate high amounts of bioactive compounds, particularly polyphenolic antioxidants; however, some species remain under investigated. This study presents preliminary phytochemical characterization of different anatomical parts of highbush blueberry (*Vaccinium* × *covilleanum*) and European cranberry bush (*Viburnum opulus*), the species domesticated in Lithuania and being investigated in order to assess the possibilities of their commercial cultivation. In total 13 cultivars of highbush blueberry and 7 cultivars of European cranberry were studied. Antioxidant potential of extracts isolated from different plant parts by various solvents was assessed by using *in vitro* radical scavenging assays and *in situ* antioxidant capacity determination methods. Phytochemical composition was screened by using LC/MS and GC/MS methods. The variations in the concentration of various constituents such as phenolic acids, flavonoids, proanthocyanidins, glycosides, volatile and unidentified compounds between studied cultivars were determined and assessed by using PCA.

SYSTEMATIC STUDIES ON *PANAX GINSENG*

Deqiang Dou, Tingguo Kang, Jiankui Zhang

Liaoning University of Traditional Chinese Medicine, Dalian, 116600, China; doudeqiang2003@yahoo.com.cn

Ginseng, the dried roots of *Panax ginseng*, has been held in high-esteem for thousands years. The biological activities of ginsenosides, and microscopic structures in ginseng with different cultivation modes, were studied. Recently the structure-activity relationships for anti-atherosclerosis, anti-cancer and immunostimulating activities of ginsenosides were studied, indicating protopanaxatriol-type (PPT) ginsenosides are chiefly responsible for the senile dementia, while protopaxadiol-type (PPD) ginsenosides are mainly for the treatment of coronary diseases.

To establish a prediagnostic method for the hemolytic adverse effect of Chinese medicine injection, the interaction rules of ginsenosides on hemolysis were studied. A method called "Fuzzy disemination" was used for the identification of hemolytic constituents of injections and 50% percent hemolytic degree (HD_{50}) was determined for prediagnostic index of hemolytic adverse effect of injections. The method has applied in various Chinese medicine injections.

Ginseng cultivated under mountainous forest is called "Lin-Xia-Shan-Shen" (LXSS). Currently it is a much more popular cultivation mode in that its growing environment and appearance are similar to wild ginseng. The microscopic and chemical characteristics of LXSS were compared with garden grown ginseng, indicating LXSS exhibited unique characteristics in chemicals and microscopy. The adverse growing conditions of LXSS make it possess higher content of ginsenosides and PPT-type ginsenosides.

FUZHUAN TEA: NOVEL PHYTOCHEMICALS AND INITIAL INVESTIGATIONS OF A FERMENTED PREPARATION OF *CAMELLIA SINENSIS*

Amy Keller, Tiffany Weir, Corey Broeckling, Ajay Kumar, Elizabeth Ryan

Colorado State University, Fort Collins, CO 80523, USA

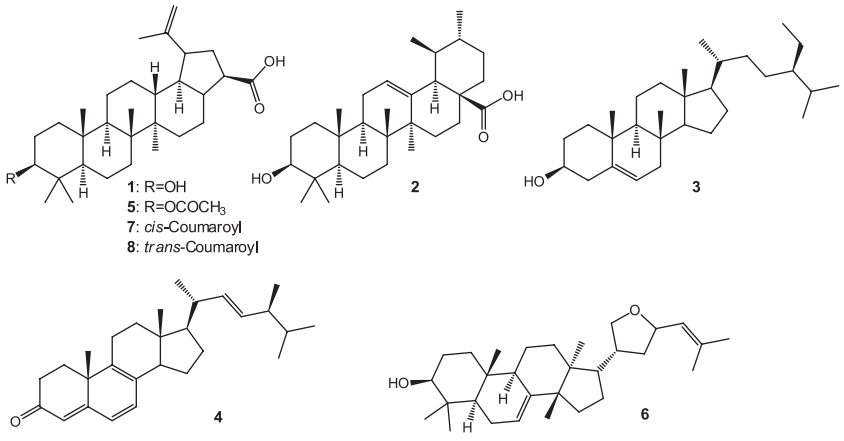
Many fermented foods have been shown to have both unique bioactivity and phytochemicals as a result of the fermentation process. Fuzhuan tea is a traditional preparation of *Camellia sinensis* L. (Theaceae) from Hunan, China that is fermented with the fungus *Eurotium cristatum*. This preparation has had ethnobotanical importance in northern China, Mongolia, and Tibet for centuries to counteract negative effects of high fat diets. Preliminary data shows that Fuzhuan tea significantly inhibited cell viability of the human colon cancer cell lines HT-29 as compared to the control at 750 μg/ml. To explore novel bioactive compounds, metabolomics was performed on Fuzhuan tea extract in comparison with unfermented green tea using UPLC-ToF-MS. Principal Component Analysis shows a unique phytochemical profile for Fuzhuan tea compared to green tea and we have identified candidate fatty acid amide compounds such as linoleamide that, to our knowledge, have not been previously reported in *C. sinensis*. Linoleamide, a fatty acid amide (FAA) that induces sleep, and several related FAAs act as neurological signaling molecules and are thought to have physiological roles in memory, cardiovascular function, cognition, reproduction, and immune function, providing promising new avenues to explore Fuzhuan tea bioactivity. Tissue histology and liver gene expression analysis performed in ICR mice demonstrated the safety of Fuzhuan tea consumption, and further studies of Fuzhuan tea are underway.

ANTIPARASITIC COMPOUNDS FROM *CORNUS FLORIDA* L. WITH ACTIVITIES AGAINST *PLASMODIUM FALCIPARUM* AND *LEISHMANIA TARENTOLAE*

Rocky Graziose,[1] Patricio Rojas-Silva,[1] Thirumurugan Rathinasapathy,[1] Carmen Dekock,[2] Alexander Poulev,[1] Peter Smith,[2] Ilya Raskin[1]

[1]*Rutgers University, Plant Biology and Pathology, New Brunswick, NJ 08901, USA,* [2]*University of Cape Town, Division of Pharmacology, Cape Town, 7925, South Africa*

Cornus florida, a plant traditionally used in North America for the treatment of malaria, was shown to be active *in vitro* against *Plasmodium falciparum* (D10 strain). Antiplasmodial-guided fractionation of the ethanolic extract of bark afforded 8 compounds: betulinic acid (**1**), ursolic acid (**2**), β-sitosterol (**3**), ergosta-4,6,8,22-tetraene-3-one (**4**), 3β-*O*-acetyl betulinic acid (**5**), 3-epideoxyflindissol (**6**), 3β-*O*-*cis*-coumaroyl betulinic acid (**7**), 3β-*O*-*trans*-coumaroyl betulinic acid (**8**), of which **4**, **5**, **6** and **7** are reported for the first time from this genus and **6** is for the first time here isolated from a natural product. *In vitro* IC_{50} values against *Plasmodium falciparum* (D10 strain) (**4**: 61.0 μM; **6**: 128.0 μM, **7**: 10.4 μM, and **8**: 15.3 μM) are shown for the first time. Compounds were also tested for antileishmanial activity against *Leishmania tarentolae*, with IC_{50} values reported here for the first time: **4**: 11.5 μM, **6**: 1.8 μM, **7**: 8.3 μM, and **8**: 2.2 μM). Cytotoxicity toward Chinese Ovarian Hamster cells is discussed for all isolated compounds.



NEUROPROTECTIVE COMPOUNDS ISOLATED FROM THE METHANOLIC EXTRACT OF *LONICERA JAPONICA*

Bohyoung Lee,[1] Jin Bae Weon,[1] Hye Jin Yang,[1] Eun young Mok, [1]*Choong Je Ma*[1,2]

[1]*Kangwon National University, Department of Biomaterials Engineering, School of Bioscience and Biotechnology, Chuncheon, 200–701, South Korea,* [2]*Kangwon National University, Research Institute of Bioscience and Biotechnology, Chuncheon, 200–701, South Korea*

The CH_3Cl fraction of the flower of *Lonicera japonica* (Lauraceae) significantly protected primary cultures of rat cortical cells injured by the excitotoxic amino acid, L-glutamate. loganin, secoxyloganin, caffeic acid rutin, hyperoside, quercetin-3-*O*-glucoside, lonicerin, kaempferol-3-*O*-rutinoside, luteolin-7-*O*-D-glucopyranoside, quercetin, luteolin were isolated by bioactivity-guided fractionation from the CH_3Cl fraction and further separated using chromatographic techniques. Caffeic acid, lonicerin, kaempferol-3-*O*-rutinoside, quercetin and luteolin had significant neuroprotective activities against glutamate-induced neurotoxicity in primary cultures of rat cortical cells at concentrations ranging from 0.1 μM to 10.0 μM.

INHIBITION OF QUORUM SENSING AND BIOFILM FORMATION BY TROPICAL PLANTS

Chieu Anh Ta,[1] Marie Freundorfer,[1] Ammar Saleem,[1] Ana Gargaun,[2] Muhammad Asim,[2] Mario Garcia Quesada,[3] Marco Otarola Rojas,[3] Pablo Sanchez Vindas,[3] Luis Poveda,[3] Victor Cal,[4] Tony Durst,[2] John T. Arnason[1]

[1]*University of Ottawa, Biology, Ottawa, ON K1N 6N5, Canada,* [2]*University of Ottawa, Chemistry, Ottawa, ON K1N 6N5, Canada,* [3]*Universidad Nacional, Herbario Juvenal Valerio Rodriguez, Heredia, 86–3000, Costa Rica,* [4]*Belize Indigenous Training Institute, Punta Gorda, Belize*

This is a new approach to the discovery of new phytochemicals from tropical plants that can interfere with the formation of bacterial biofilms. Bacteria use a cell-to-cell communication system known as quorum sensing (QS) to coordinate gene expression for the formations of these biofilms. Ethanolic extracts of tropical and traditional anti-infective plants were screened for QS interference and biofilm inhibition. Extracts from the Melastomataceae, Meliceae, Sapindaceae, Lepidobotryaceae, Combretaceae, and Euphorbiaceae showed the highest inhibitory activities. QS inhibition ranges from 7.3 + 0.1 mm to 26.1 + 0.3 mm. Inhibition of biofilm growth ranges from 0 to 76.8 + 2.0%. In particular, one Melastomataceae species (*Oxlaju chajom*) was most promising with QS inhibition zone of 25.9 + 0.6 mm and biofilm MIC (minimum inhibitory concentration) of 50 μg/mL. Interestingly, this is the first report of biological activity for this plant and very little is known about its phytochemistry. Bioassay-guided fraction of *Oxlaju chajom* showed that inhibitory activities are in the more polar fractions. Current work is being done on the isolation and identification of the active principles.

ETHNOPHARMACOLOGY OF ANTI-INFLAMMATORY BOTANICALS USED BY THE Q'EQCHI' MAYA OF BELIZE

Brendan Walshe-Roussel,[1] Christine Choueiri,[2] Max London,[1] Ammar Saleem,[1] Federico Caal,[3] Victor Cal,[3] Todd Pesek,[4] Tony Durst,[2] John Thor Arnason[1]

[1]*University of Ottawa, Dept. of Biology, Ottawa, ON K1N6N5, Canada,* [2]*University of Ottawa, Dept. of Chemisty, Ottawa, ON K1N6N5, Canada,* [3]*Belize Indigenous Training Institute, Punta Gorda, Toledo Belize,* [4]*Cleveland State University, Dept of Health Sciences, Center for Healing Across Cultures, Cleveland, OH 44115, USA*

Indigenous pharmacopeias recognize the important role of inflammation in disease, and the Q'eqchi' Maya healers of Belize possess a practical understanding of a large number of immunomodulatory botanicals. Ethnobotanical interviews were held with 5 members of the Q'eqchi' Maya Healers Association using a list of 14 inflammatory symptom categories and one hundred and seven plant species were collected from primary and secondary semi-evergreen rainforest in the Maya Mountains of Belize. Ethanolic extracts of fifty-five species were assayed for anti-inflammatory activity in a LPS-stimulated THP-1 monocyte assay. Of these, 76% demonstrated significant anti-inflammatory activity relative to the vehicle control, and three species displayed activity equal to that of the parthenolide positive control. In addition, several sesquiterpene lactones isolated from *Neurolaena lobata* exhibit potent anti-inflammatory activity. These results demonstrate that plants used by the Q'eqchi' Maya Healers Association for the treatment of inflammatory-related symptoms do indeed possess immunomodulatory properties, and elucidating the active principles of these species can yield compounds with novel bioactivities.

ANTIOXIDANT, ANTIMICROBIAL AND ANTIVEROTOXIC POTENTIALS OF EXTRACTS OF *CURTISIA DENTATA*

James Doughari Hamuel, Patrick Alois Ndakidemi, Izzane Susan Human, Benade Spinney

Cape Peninsula University of Technology, Applied Sciences Faculty, Cape Town, 8000, South Africa

The potentials of *Curtisia dentata* as antimicrobial, antioxidant and antiverotoxin against environmental isolates of *E. coli* and *Acinetobacter* spp., the presence of phytochemicals and some organic compounds was investigated. The highest concentration of phytochemicals and organic compounds such as anthraquinones, alkaloids, essential oils, glycosides, phenols, steroids, saponins and tannins and the organic compounds quinones, anthocyanins, amines and carboxylic acids were found in the stem bark ethanol extracts compared to other parts of the plant or solvent extracts. Antimicrobial activity as relative inhibition zone diameters (%) ranged between 8-28% (MIC values, 100–2500 mg/ml) against *E. coli* serotypes, and 10–28% (MIC, 100–850 mg/ml) and 6-28% (MIC, 150–2500 mg/ml) against *A. lwoffii* and *A. haemolyticus* respectively. The extracts demonstrated inhibitory action against the expression of both Vtx1 and Vtx2 genes in both *E. coli* and *A. haemolyticus* strains with the ethanol extracts demonstrating the highest antiverotoxic activity compared to other solvents. The ethanol root bark extracts consistently showed the highest DPPH radical scavenging activity (62.43%), total phenol content (TPH) (57.62%, 26 mg GAE/g) and reducing power (RP) (41.32%), followed by those of the stem bark and leaf extracts with the respective values of 54.68%, 37.77 mg GAE/g and 21.83%. Ethanol extracts demonstrated the highest values for both DPPH, TPH and RP followed by dichloromethane, hexane, acetone and distilled water in this order. *C. dentata* can be used to source novel antimicrobial agents for the treatment of verotoxic bacterial infections.

ANTI-DIABETIC POTENTIALS OF ETHANOL AND WATER EXTRACTS OF 17 PLANTS USED BY THE EEYOU ISTCHEE CREE FIRST NATIONS OF NORTHERN QUEBEC

Nan Shang,[1,2] Lina Musallam,[1,2] Diane Vallerand,[1,2] Brendan Walshe-Roussel,[2,3] *Arnason John T.*,[2,3] Pierre S. Haddad[1,2]

[1]Natural Health Products and Metabolic Disease laboratory, Department of Pharmacology, University of Montreal, Montréal, Quebec H3T1J4, Canada, [2]Canadian Institutes of Health Research Team in Aboriginal Antidiabetic Medicines and Montreal Diabetes Research Center, Montréal, Quebec H3T1J4, Canada, [3]Medicinal Plant and Ethnopharmacology Laboratory, Department of Biology, University of Ottawa, Ottawa, K1N 6N5, Canada

Our group (CIHR-TAAM) identified 17 plants used by the Cree to treat symptoms of diabetes and screened their 80% hydrated ethanol extracts (EE), using an *in vitro* bioassay platform. However, traditional preparations are often based on hot water extractions (HWE). We thus compared these two extraction methods on the anti-diabetic potential of the 17 Cree plants at equal concentrations. Three main bioassays routinely applied in our laboratory were used: 1) stimulation of glucose transport in muscle cells by measuring 3H-2-deoxyglucose uptake (C2C12 cell line), and 2) inhibition of hepatic glucose production by measuring inhibition of glucose-6-phosphatase activity (G-6Pase; H4IIE cell line), 3) potentiation of adipogenesis by measuring accumulation of triglycerides (3T3-L1 cell line). Our results show that out of the 17 HWE: A) Eight had less or completely lost the effects on glucose transport, B) Five had lower or lost the effects on G6Pase activity, and C) Eleven had lower or lost their effects on adipogenesis in comparison to their EE counterparts. Interestingly, AD01 is the only plant with almost equal anti-diabetic potential between HWE and EE. So the method of extraction is a significant determinant of the biological activity of a medicinal plant. Some HWE plants have comparable anti-diabetic potentials with EE in the bioassays tested here. As EE better extracts phenolics, higher doses of HWE may be necessary to obtain comparable activity. Alternatively, concentrations of components and hence resulting biological activity could be higher in traditional preparations made by aboriginal healers. Changes in the quality and quantity of extract components related to extract preparation as well as underlying mechanisms of action will require further experimentation. Funded by CIHR and the China Scholarship Council.

MYELOPHIL, AN EXTRACT MIX OF ASTRAGALI RADIX AND SALVIAE RADIX, AMELIORATES RESTRAIN-INDUCED STRESS IN MICE MODEL

Hyeong-Geug Kim, *Chang-Gue Son*

Liver and Immunology Research Center, Daejeon Oriental Hospital of Daejeon University, Daejeon, 301–704, South Korea

Myelophil is an extract mix of Astragali Radix and Salviae Radix, which has been prescribed for patients with chronic fatigue symptom. This study evaluated the antioxidant effects of Myelophil in restrain-induced stress model. Six-week Balb/c male mice were orally administered Myelophil (0, 100, 200, or 400 mg/kg for 5 days, and then were given to restrain-stress for six hours. Myelophil pre-treatment significantly ameliorated the alteration of serum alanine aminotransferase, aspartate aminotransferase, total ROS, and total antioxidant capacity compared to control group. In addition, Myelophil pre-treatment showed antioxidant effect such as decreasing lipid peroxidation, restoring glutathione depletion in liver tissue compared to control group. Myelolphil pre-treatment lowered tissue levels of pro-inflammatory cytokine, tumor necrotic factor-Yá.

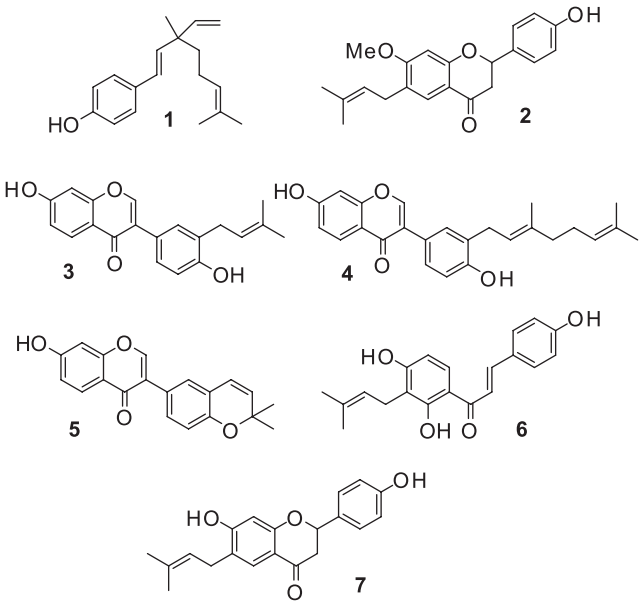
Taken together, Myellophil has potent protective effects against restrain-induced stress via antioxidant actions.

PHENOLIC COMPOUNDS ISOLATED FROM *PSOLALEA CORYIFOLIA* INHIBIT IL-6 INDUCED STAT3 ACTIVATION

Seung Woong Lee, Bo Ra Yun, Hyun-Mee Oh, *Mun-Chual Rho*

Eco-Friendly Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup, 580-185, South Korea

Seven flavonoids (**1**-**7**) were isolated from the methanol extracts of the seeds of *Psoralea corylifolia* by bioactivity-guided fractionation using STAT3-dependent luciferase activity. Compounds **1**-**7** inhibited STAT3 activation by IL-6 in a dose-dependent manner with IC_{50} values of 4.57 ± 0.45, 3.02 ± 0.53, 2.77 ± 0.02, 0.81 ± 0.15, 1.37 ± 0.45, 2.45 ± 0.13 and 4.89 ± 0.05 μM, respectively. They also decreased the level of IL-6-induced STAT3 phosphorylation in Hep3B cells.



ISOLATION OF FLAVONOID DI-C-GLYCOSIDES FROM *NELUMBO NUCIFERO* AND THEIR STRUCTURAL DETERMINATION

Scott Baggett,[1] Hui Yang,[1] Tamara Meragelman,[1] Eugene P. Mazzola,[2] Yan Ling Zhang,[1] Richard E. Staub,[1] Isaac Cohen[1]

[1]*Bionovo, 5858 Horton Street Suite 400, Emeryville, CA 94608,USA,* [2]*University of Maryland, Department of Chemistry, College Park, MD 20742, USA*

Nelumbo nucifero is one of the raw materials in BioNovo's first in class herbal pharmaceutical drug for the treatment of menopausal hot flashes, Menerba®. During our effort to identify marker compounds for each raw material in Menerba® we isolated schaftoside (**1**) and isoshaftoside (**2**) from *N. nucifero*. To our knowledge this is the first reported isolation of these compounds from *N. nucifero*. Dictionary of Natural Product and literature searches showed a number of previously isolated flavonoid di-C-glycosides isomers with MW = 564. Examples include vicenin **1** and **3**, which differs from **1**-**2**, with respect to which sugar is attached at R1 and R2. In addition, a LC-MS trace showed *N. nucifero* contains 10 peaks with MW = 564. To save time and the expense of isolating additional quantities of **1**-**2** for use in our raw material quality control program, we purchased **1**-**2** from various sources. However, given the complexity of these compounds and minor structure differences between known isomers we initiated in-depth 1D and 2D NMR studies on the isolated and purchased compounds. We acquired 1D (^{1}H, ^{13}C) and 2D (COSY, HSQC, H2BC, TOCSY, HMBC) NMR spectrum at 400 and 600 MHz. After detail analysis of the NMR data we able to distinguish between the five schaftoside (**1**) isomers.

ALLEVIATING MEDICINE USAGE AND IMPROVING PULMONARY FUNCTION WITH SUPPLEMENTS OF VEGETABLE AND FRUIT CONCENTRATE, FISH OIL, AND PROBIOTICS IN ASTHMATIC SCHOOL CHILDREN

Lee Shu-Chen,[1,3,4] Yang Yao-Hsu,[2] Chuang Shao-Yuan,[3,4] Pan Wen-Harn[3,4]

[1]*National Taiwan University, Biochemical Science and Technology, Taipei, 10617, Taiwan,* [2]*National Taiwan University, Pediatrics, National Taiwan University Hospital, College of Medicine, Taipei, 10041, Taiwan,* [3]*National Health Research Institutes, Population Health Sciences, Miaoli County, 35053, Taiwan,* [4]*Academia Sinica, Biomedical Sciences, Taipei, 11529, Taiwan*

We designed a 16-week parallel double-blind randomized placebo-controlled school-based intervention trial to investigate the joint effect of the beneficial dietary components on asthma. A total of 192 asthmatic children aged 10–12 yrs were recruited from elementary schools in metropolitan Taipei. The intervention group received vegetable plus fruit capsules, fish oil capsules and probiotic capsules, while the control group received placebos. Asthma symptoms, pulmonary functions, medicine usage and pediatric asthma quality of life questionnaire score (PAQLQ score) were evaluated at baseline, 8 and 16 weeks. Compared to placebo group, the intervention group had significant improvement in pulmonary parameters such as FVC (178 ml *vs.* 91 ml) and FEV1 (107 ml *vs.* 41 ml) and the proportion of children using bronchodilator significantly decreased over the 16 week period. Asthma symptoms and PAQLQ score were not significantly different between the two groups, probably because most children were routinely seen by physicians and medication were adjusted whenever needed. Our study showed that dietary supplements with vegetable and fruit concentrates, fish oil, and probiotics could alleviate bronchodilator usage and increase pulmonary function in asthmatic children.

COMPARING A PHENOMENEX LUNA HPLC-DAD METHOD *VERSUS* A PHENOMENEX KINETEX UPLC-DAD METHOD FOR RAW MATERIAL QUALITY CONTROL FOR MENERBA

Scott Baggett, Richard E. Staub, Isaac Cohen

Bionovo, 5858 Horton Street Suite 400, Emeryville, CA 94608, USA

Menerba®, a mixture of 21 botanical raw materials, is BioNovo's herbal pharmaceutical for the treatment of menopausal hot flashes. Bionovo and the FDA agreed that a HPLC-DAD fingerprint method be included for quality control of raw materials and drug substance (DS). Aqueous herbal extracts are complex mixtures requiring long gradients to provide sufficient resolution of components for HPLC fingerprinting. Therefore, we required a single, rapid, HPLC-DAD method to compare raw material lots, and DS. Three different HPLC methods were employed during development of the quality control program. The first method used a HP 1100 HPLC and a Phenomenex Luna C18 150 × 4.6, 5 μm column. This resulted in a method with a very long analysis time (70 min). To reduce the run time and improve resolution we switched to a Phenomenex Kinetex C18 150 × 4.6, 2.7 μm column. Kinetex columns use a core shell silica partial vs. a traditional silica partial. Using the Kinetex column, on a HP 1100 HPLC, injection cycle time was cut to 50 min and resolution of critical compound pairs was improved. Finally, we adapted this method to a Shimadzu UPLC system using a Kinetex C18 150 × 2.1, 1.7 μm column. This combination reduced the cycle time to 25 min and resolution was slightly improved.

In summary, great improvements were made to the HPLC-DAD fingerprinting method by switching to the Kinetics column technology and utilizing a UPLC system.

HERBAL EXTRACTS OF *CIBOTIUM BAROMETZ*, *GENTIANA SCABRA*, *DIOSCOREA BATATAS*, *CASSIA TORA*, AND *TAXILLUS CHINENSIS* INHIBIT SARS-COV REPLICATION

Chih-Chun Wen,[1,2] Lie-Fen Shyur,[2] Jia-Tsrong Jan,[3] Sheng-Chu Kuo,[1] Ning-Sun Yang[2]

[1]*Graduate Institute of Pharmaceutical Chemistry, China Medical University, Taichung, 404, Taiwan,* [2]*Agricultural Biotechnology Research Center, Academia Sinica, Taipei, 115, Taiwan,* [3]*Genomics Research Center, Academia Sinica, Taipei, 115, Taiwan*

Development of anti-severe acute respiratory syndrome associated coronavirus (SARS-CoV) agents is pivotal to prevent the reemergence of the life-threatening disease, SARS. In this study, more than 200 extracts from Chinese medicinal herbs were evaluated for anti-SARS-CoV activities using a cell-based assay that measured SARS-CoV induced cytopathogenic effect (CPE) *in vitro* on Vero E6 cells. Six herbal extracts, one each from *Gentiana scabra*, *Dioscorea batatas*, *Cassia tora* and *Taxillus chinensis* (designated as GSH, DBM, CTH and TCH, respectively), and two from *Cibotium barometz* (designated as CBE and CBM), were found to be potent inhibitors of SARS-CoV at concentrations between 25 and 200 μg/ml. The concentrations of the six extracts needed to inhibit 50% of Vero E6 cell proliferation (CC_{50}) and 50% of viral replication (EC_{50}) were determined. The resulting selective index values ($SI = CC_{50}/EC_{50}$) of the most effective extracts CBE, GSH, DBM, CTH and TCH were >59.4, >57.5, >62.1, >59.4, and >92.9, respectively. Among these extracts, CBM and DBM also showed significant inhibition of SARS-CoV 3CL protease activity with IC_{50} values of 39 μg/ml and 44 μg/ml, respectively. Our findings suggest that these six herbal extracts may have potential as candidates for future development of anti-SARS therapeutics.

ANTI-WRINKLE POTENTIAL OF STANDARDIZED FLOWER EXTRACT OF *CALENDULA OFFICINALIS* LINN.

Niladri Maity,[1] Neelesh K. Nema,[1] Pulok K. Mukherjee,[1] Birendra K. Sarkar[2]

[1]*Department of Pharmaceutical Technology, School of Natural Product Studies, Jadavpur University, Kolkata, 700032, India,* [2]*Parker Robinson (P) Ltd., 1, Nimak Mahal Road, Kolkata, 700043, India*

Traditionally *Calendula officinalis* L. (Compositae) flower has been claimed for use in inflammation, wound healing, antiseptic, various skin diseases like ulceration, eczema etc. The present study was designed to validate its skin protective activity through inhibition of hyaluronidase, elastase and matrix metalloproteinase-1 (MMP-1). *C. officinalis* flower was extracted with methanol and fractionated with ethyl acetate, *n*-butanol and water. Methanol extract and its fractions were tested for enzyme inhibition assay along with standard oleanolic acid. The extract and fractions were standardized through RP-HPLC using syringic acid as biomarker. *C. officinalis* methanol extract showed significant (cP<0.001) anti-hyaluronidase and anti-elastase activity with IC_{50} of 6.66 ± 1.54 μg mL^{-1} and 2.70 ± 1.73μg mL^{-1} respectively and good MMP-1 inhibition (lower fluorescence reading) compared to standard oleanolic acid (35.55 ± 1.60 & 31.57 ± 0.94 μg mL^{-1}). Among all fractions tested, the ethyl acetate fraction showed significant activity. The RP-HPLC analysis revealed that good amount of syringic acid is present in *C. officinalis* methanol extract (7.3%w/w), which was higher in the ethyl acetate fraction (13.5 %w/w). *C. officinalis* showed potent inhibitory activity on hyaluronidase, elastase and MMP-1. Hence, the traditional claim of *C. officinalis* supports its potential use as an anti-wrinkle agent.

MUSHROOM TYROSINASE INHIBITION AND ANTIOXIDANT PROPERTIES OF *DALBERGIA PARVIFLORA* ROXB.

Worrawat Promden,[1] Orawan Monthakantirat,[2] Kaoru Umehara,[3] Hiroshi Noguchi,[3] Wanchai De-Eknamkul[1]

[1]*Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, 10330, Thailand,* [2]*Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand,* [3]*School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, 422–8526, Japan*

A crude extract of *Dalbergia parviflora* and its constituents of 35 flavonoids isolated as pure compounds were screened for their inhibitory activity against mushroom tyrosinase. Among the flavonoids tested, only four, namely khrinone **(5)**, cajanin **(9)**, (3*RS*)-3-hydroxy-8-methyoxy vestitol **(23)** and (6a*R*,11a*R*)-3,8-dihydroxy-9-methoxy pterocarpan **(33)** were shown to have IC_{50} values lower than 100 μM. These flavonoids were further studied for their inhibition kinetics on the diphenolase activity of the mushroom tyrosinase. The results showed that the inhibition of **(5)**, **(9)**, **(23)** and **(33)** were uncompetitive, non-competitive, mixed and competitive inhibitors, respectively. In addition, the *D. parviflora* extract and the isolates were evaluated for their antioxidant activities: DPPH assay, X/XO assay and ORAC assay. The results revealed that the compounds showed antioxidant activity with, SC_{50} values of 40–400 μM for DPPH assay, 2.5–250 μM for X/XO assay and 2.8–120 μM Trolox equivalent/10 μM flavonoid for ORAC assay. Based on these findings, it was concluded that *D. parviflora* heart wood extract is a potential source of natural antioxidants which might be used as anti-browning agents that can inhibit the enzymatic oxidation of phenols by tyrosinase.

AMERICAN GINSENG ACUTELY REGULATES CONTRACTILE FUNCTION OF RAT HEART

Mao Jiang,[1] Edmund Lui,[2] Earl G. Noble[1]

[1]*University of Western Ontario, School of Kinesiology, Faculty of Health Sciences, London, ON N6A5B9, Canada,* [2]*University of Western Ontario, Department of Physiology and Pharmacology, London, ON N6A5C1, Canada*

Chronic *Panax ginseng* treatments improve the cardiac performance. However reports of acute administration of *Panax ginseng* on cardiovascular function remain controversial and mechanisms are not clear. In this study, we examined effects of acute American ginseng (*Panax quinquefolius*) administration on rat cardiac contractile functions by using electrocardiogram (ECG), non-invasive blood pressure measurement and Langendorff isolated-perfused heart measurements. Eight-week old male Sprague Dawley rats were gavaged with water soluble American ginseng at 300 mg/kg body weight. Heart rate and developed pressure were measured at 1 hr and 24 hr after gavaging. Heart rate was significantly decreased (ECG (6%), non-invasive blood pressure (9–15%) and Langendorff isolated-perfused heart (15–20%)) in water soluble ginseng treated rats comparing with control groups. Markedly decreased developed pressure was observed in ginseng treated Langendorff isolated-perfused hearts but not in non-invasive blood pressure measurements. Direct effect of American ginseng on rat cardiac contractile function was examined by measuring the Langendorff isolated spontaneously beating perfused heart at varying concentrations of water soluble American ginseng. Significantly decreased heart rate and developed force were evidenced after direct ginseng treatments. In our study, we presented the first evidence of depressed cardiac contractile function by acute administration of North American ginseng in rat.

THE ANTI-INFLAMMATORY EFFECT OF SPECIFIC MEDICINAL PLANT EXTRACTS IN DSS-INDUCED COLITIS MODEL

Ning-Sun Yang,[1] Yuh-Ting Huang,[1] Chin-Chun Wen,[1,2] Sheng-Chu Kuo,[2] Shu-Hui Lin[3]

[1]*Academia Sinica, Agricultural Biotechnology Research Center, Taipei, 115, Taiwan,* [2]*China Medical University, Graduate Institute of Pharmaceutical Chemistry, Taichung, 404, Taiwan,* [3]*One Power Biotechnology Co. LTD, Taipei, 100, Taiwan*

Approaches for developing evidence-based application of traditional Chinese medicinal (TCM) herbs have received high attention internationally. Recent studies also revealed that induced or chronic inflammation is strongly associated with carcinogenesis. MP, a specific medicinal plant commonly used as TCM in Taiwan, has been shown by our own laboratory and others to confer high anti-inflammatory effects. In this study, we evaluated the effect of the hot water extract of fresh MP (MPHF) in mice with dextran sulphate sodium-induced colitis or colitis-associated colon cancer. We also compared the anti-colitis effects of MP extracts prepared by different extraction methods. MPHF effectively attenuated clinical symptoms of colitis, including loss of body weight, diarrhea and rectal bleeding. It also provided protections against colon-shortening and histopathological changes caused by colon tissue inflammation. MP extracts prepared by different extraction methods showed significantly different levels of anti-colitis activity, and MPHF conferred the best efficacy. In the colitis-associated colon cancer mouse model, mice of the MPHF-treated group showed significantly higher survival rate and better protection on histological manifestations than the cohort mice of non-treated group. In conclusion, we suggest that MP may have good potential for future development into possible adjuvant treatment of colitis and for general protection of gastrointestinal tract.

ANTIOXIDANT EFFECT OF PLAUNOTOL IN HUMAN RENAL CELLS: HK-2

Chatchai Chaotham,[1] Preedakorn Chunhacha,[1] Pithi Chanvorachote,[2] Wanchai De-Eknamkul[3]

[1]Pharma-ceutical Technology (International) Program, Chulalongkorn University, Bangkok, 10330, Thailand, [2]Department of Pharmacology and Physiology, Chulalongkorn University, Bangkok, 10330, Thailand, [3]Department of Pharmacognosy and Pharmaceutical Botany, Chulalongkorn University, Bangkok, 10330, Thailand

Renal injury is often found in various oxidative stress-related pathologies including hypertension, diabetic and toxicity from some medications. Although many ROS (reactive oxygen species) scavengers are proposed for renal protection, most of them are not approved for their safety and efficacy in human. This research work was, therefore, aimed to search for new efficient natural ROS scavengers for the renal protection. Among Thai medicinal plants, *Croton stellaopilosus* is of particular interest. It contains plaunotol which has been manufactured as an anti-peptic ulcer drug for long time. Its extract has also been shown to have antioxidant activity, and is thus a good source for shedding its novel application. In this study, the antioxidant characteristic profile of plaunotol in human renal cells (proximal tubular renal cells: HK-2) was investigated via flow cytometry analysis. It was found that pretreatment with plaunotol for 6 h significantly decreased the endogenous ROS level. The pretreatment could significantly inhibit the rising of ROS from exogenous ROS treatments such as hydrogen peroxide (H_2O_2) and 2,3-dimethoxy-1,4-naphthoquinone (DMNQ). These antioxidant properties might lead to a possibility to develop plaunotol as a safe and effective renopreventive substance for renal damage prevention from oxidative stress.

INSECTICIDAL ACTIVITY OF KOREAN MEDICINAL PLANT EXTRACTS AGAINST *MYZUS PERSICAE*

Ji Su Kim,[1] Ji Young Jung,[1] Jeoung Bin Nam,[1] Seon Jeong Sim,[1] Yoon-Kuen Kim,[2] Myung-Suk Choi,[1] Jae-Kyung Yang[1]

[1]Division of Environmental Forest Science, Gyeongsang National University, Institute of Agriculture & Life Sciences, Jinju, 660–701, South Korea, [2]Sancheong Oriental Medicinal Herb Institute, Sancheong Goon, 666–831, South Korea

Use of natural compounds from plant extracts has been suggested as a viable source of alternative treatments for insect and mite control because many of such compounds have novel modes of action, no or low toxicity to non-target organisms and humans, and are less harmful to the environment. The insecticidal activity of Korean medicinal plant (*Sorbus commixta*, *Akebia quinata* and *Acer tegmentosum*) from ethanol extract, and its fractions using hexane, chloroform, ethyl acetate and water of *Sorbus commixta*, *Akebia quinata* and *Acer tegmentosum* were tested against *Myzus persicae* to examine their effects on mortality.Their composition of volatile substances was determined using GC-MS.

The hexane fraction from *Acer tegmentosum* at a concentration of 1,000 ppm showed 100% *Myzus persicae* mortality after an exposure of 120 min. The results showed that extracts of *Acer tegmentosum* and some of their constituents have potential for development as botanical insecticides.

AD03: AN ALTERNATIVE TREATMENT TO IMPROVING HYPERGLYCEMIA AND HYPERINSULINEMIA IN A DIET-INDUCED OBESE MODEL

Despina Harbilas,[1,2,3] Antoine Brault,[1,2,3] Diane Vallerand,[1,2,3] Ammar Saleem,[4] John T. Arnason,[4] Lina Musallam,[1,2,3] Pierre S. Haddad[1,2,3]

[1]Natural Health Products and Metabolic Diseases Laboratory, Université de Montréal, Montréal, QC H3T1J4, Canada, [2]Montreal Diabetes Research Center, Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montréal, QC H1W 4A4 Canada, [3]Institute of Nutraceuticals and Functional Foods (INAF), Université Laval, Quebec, QC G1K 7P4, Canada, [4]Department of Biology and Center for Research in Biopharmaceuticals and Biotechnology, University of Ottawa, Ottawa, ON K1H 8M5, Canada

Our team (CIHR-TAAM) conducted ethanobotanical studies in the Cree of Eeyou Istchee communities of Northern Quebec and identified 17 plants with anti-diabetic potential. Previous *in vitro* screening studies revealed that one of these plants, AD03, produced strong anti-diabetic effects. Therefore, we hypothesized that AD03 would exert an anti-diabetic effect, in a mouse model of diet-induced obesity and T2D, by lowering glycemia and insulin resistance. We conducted a prevention study where C57BL/6 mice were subjected to high fat (HF) diet for eight weeks to which AD03 was incorporated at 125 and 250 mg/kg. In comparison, in the treatment study, the mice were subjected to HF diet for sixteen weeks. AD03 was introduced in the HF diet for the last eight weeks and tested at 125 and 250 mg/Kg. Results: In the prevention study, AD03 at 250 mg/Kg gradually but significantly decreased whole body and retroperitoneal fat pad weights and improved circulating adipokine levels as compared to HF cognates. No significant effects were observed on glycemia and insulinemia. In the treatment study: AD03 significantly and dose-dependently improved glycemia and insulinemia levels, circulating adipokine levels as well as the G/I index (indicator of insulin resistance) when compared to HF controls. However, AD03 improvement effect on body weight or retroperitoneal fat pad weight was not as pronounced as in the prevention study. In both the prevention and treatment study, no statistical difference was observed in water or food intake. AD03 thus exhibits promising anti-diabetic and slight anti-obesity effects. Mechanisms remain to be elucidated in the liver, muscle and adipose tissue, all targeted by T2D and obesity. Funded by the CIHR.

INHIBITION EFFECT OF FLAVONOLIGNANS AND LIGNAN GLYCOSIDES FROM THE AERIAL PARTS OF *ORYZA SATIVA* L. ON NO PRODUCTION AND TYROSINASE ACTIVITY

Rak-Hun Jeong,[1] Dae-Young Lee,[1] Jin-Gyeong Cho,[1] Hee-Cheol Kang,[2] Woo-Duck Seo,[3] Nam-In Baek[1]

[1]*Graduate School of Oriental Medicinal Materials & Processing, Kyung Hee University, Yongin, 446–701, South Korea,* [2]*R&D center, GFC Co., Ltd., Suwon, 443–813, South Korea,* [3]*Department of Functional Crop, National Institute of Crop Science, RDA, Milyang, 627–830, South Korea*

Rice (*Oryza sativa* L.) is the principal cereal crop in Asia ingested by the majority of the population. Because there are few reports on constituents or pharmacological activities of the plant, isolation and identification of the bioactive constituents of *O. sativa* are still required. The aerial parts of *Oryza sativa* L. were extracted with 80% aqueous MeOH and the concentrated extract was successively partitioned with *n*-hexane, EtOAc, *n*-BuOH, and H_2O. The phytochemical study on *O. sativa* (leave and stem) led to isolation of four new flavonolignans and two new lignan glycosides along with a known flavonolignan, salcolin B. The structures of the isolated compounds were determined on the basis of EI-MS, FAB-MS, ^1H and ^{13}C-NMR, DEPT, and 2D-NMR (COSY, HSQC, HMBC) experiments. New flavonolignans and lignan glycosides were named as salcolin C, salcolinoside A, salcolinoside B, salcolinoside C, oryzanoside A, and oryzanoside B. The inhibition effect of the isolated compounds on NO production and tyrosinase activity was evaluated. It was suggested that some compounds inhibited NO production *in vitro* and tyrosinase activity. The alcohol extracts and the compounds could be useful for functionality of cosmetics.

IN VITRO ANTIPLASMODIAL AND CYTOTOXIC ACTIVITY OF PHYSALINB, EPOXYPHYSALINB FROM *PHYSALIS ANGULATA* L.

Mariano Lusakibanza,[1,3] Gauthier Mesia,[3] Gaston Tona,[3] Piet Kimpende,[2] Luc Vanmeervelt,[2] Monique Tits,[1] Luc Angenot,[1] Michel Frederich[1]

[1]*University of Liege, Laboratory of Pharmacognosy, Liege, 4000, Belgium,* [2]*Katholieke Universiteit Leuven, Laboratory of Biomolecular Architecture, Leuven, 3000, Belgium,* [3]*University of Kinshasa, Faculty of Pharmaceutical Sciences, Laboratory of Pharmacology, Kinshasa, 00243, Democratic Republic of Congo*

P. angulata is widely used in popular medicine in many tropical countries to treat various diseases. Crude extracts (aqueous, CH_3OH, EtOH and CH_2Cl_2) were prepared by maceration. Physalin B and epoxy-physalin B were obtained by bio-guided fractionation using Si_{60} liquid chromatography (*n*-hexane - ethyl acetate) followed by RP_{18} preparative-HPLC in MeOH-H_2O. Crude extracts and pure compounds were then tested *in vitro* against the 3D7 (chloroquine sensitive) strain of *P. falciparum* and against the human normal fetal lung fibroblasts WI-38, which allowed determination of selectivity index. Physalin B and epoxy-physalin B were identified by X-ray diffraction. The CH_2Cl_2 and MeOH extracts had a high antiplasmodial activity (IC_{50}: 1.25μg/ml and 1.85μg/ml). Epoxy-physalin B and physalin B gave IC_{50} values of 0.31 μg/ml and 1.52 μg/ml. The aqueous extract had moderate activity (IC_{50}: 10.05μg/ml). Epoxy-physalin B and physalin B had a selectivity index of 4.5 and 2.5, respectively. In conclusion, physalin B and epoxy-physalin B could explain the antiplasmodial activity of *P. angulata*.

CYTOTOXIC CONSTITUENTS OF *STEMPHYLIUM SOLANI*, A FUNGAL ENDOPHYTE OF *MORINDA CITRIFOLIA* L. (NONI)

Crispin D. Sesaazi,[1,4] Anthony D. Wright,[1] *Sisay Girmay*,[1] Chad Higa,[2] Fred M. Sebisubi,[3] Jasper Ogwal-Okeng,[4] Brian Perry,[2] Ghee T. Tan[1]

[1]*University of Hawaii at Hilo, Department of Pharmaceutical Sciences, College of Pharmacy, Hilo, HI 96720, USA,* [2]*University of Hawaii at Hilo, Department of Biology, Hilo, HI 96720, USA,* [3]*Ministry of Health, Division of Pharmaceutical Sciences, Kampala, 6523, Uganda,* [4]*Makerere University, Department of Pharmacology and Therapeutics, Kampala, 6523, Uganda*

Morinda citrifolia L. (noni) (Rubiaceae) is a popular medicinal plant indigenous to many pan-tropical regions of the world. The juice from the fermentation of its fruits is claimed to have anticancer properties, but this has remained largely unverified under rigorous pharmacological criteria. The present study investigated the anticancer potential of the metabolites of *Stemphylium solani* (Ascomycota), an endophytic fungus isolated from the leaves of the noni plant. *S. solani* was identified during the screening of 30 pure endophyte isolates from the fruits and leaves of noni for cytotoxic activity. The total ethyl acetate extract of 5-week old malt extract fermentation broth cultures demonstrated IC_{50} values of 5 and 7 μg/mL against human lung carcinoma (LU-1) and human prostate carcinoma (LNCaP) cell lines, respectively. Bioassay-guided flash chromatography of the total extract yielded several major cytotoxic fractions. Preliminary purification of one of these fractions using MPLC and reverse phase semi-preparative HPLC led to the isolation of two cytotoxic compounds whose structures are being determined by state-of-the-art techniques. Since *S. solani* is not known to be pathogenic to noni, these cytotoxic constituents may be the product of symbiotic interactions between the fungal endophyte and its host plant. Such cryptic metabolic contributions could conceivably play a role in the purported usefulness of noni in the management of a variety of diseases including cancer.

ANTI-INFLAMMATORY EFFECT OF NORTH AMERICAN (NA) GINSENG

Chike Godwin Azike, Edmund Man King Lui, Jirui Hou, Hua Pei

University of Western Ontario, Physiology and Pharmacology, London, Ontario N6A 5C1, Canada

North American (NA) ginseng extracts exert immuno-modulatory effects *in vitro*. Aqueous (AQ) extract induced immuno-stimulatory effect, while alcoholic (ALC) extract suppressed lipopolysaccharide (LPS)-stimulated macrophage response. The immuno-stimulatory effect of the AQ extract has been attributed to the presence of polysaccharides (PS). The present study focused on the pro-inflammatory and anti-inflammatory effects of ginseng under *in vivo* and *ex vivo* conditions. Homocysteine treatment in adult rats induced inflammatory responses in aortic tissues as well as in plasma. Concurrent treatment with both types of ginseng extracts suppressed these inflammatory responses, while ginseng treatment alone in control rats produced no apparent effects. Sub-chronic treatment of rats with both types of extracts was found to have no apparent effect on alveolar macrophage function *ex vivo*, but they both suppressed LPS-stimulated NO production in culture. This data suggested that systemically acquired ginseng components derived from both extracts possessed anti-inflammatory effect. To further examine the mechanism underlying the anti-inflammatory effect of AQ extract, macrophages (RAW 264.7) were pre-treated with AQ extract or PS fraction for 24 hr prior to LPS-stimulation in culture. Results showed that pre-treatment desensitized the responsiveness of macrophages to LPS stimulation; and these effects were absent when AQ or PS ginseng treatment was given concurrently with LPS. It is concluded that AQ and ALC ginseng extracts possess anti-inflammatory effect *in vitro* and *in vivo*; and the mode of exposure is an important determinant.

CHEMICAL COMPOSITION OF THE INFUSIONS FROM THE STEMBARK AND LEAVES OF *EXOSTEMA CARIBAEUM*

Araceli Pérez-Vásquez, Erika V. Castillejos, Sol Cristians, Rachel Mata

Universidad Nacional Autónoma de México, Facultad de Química, Farmacia, Mexico City, D.F. 04510, México

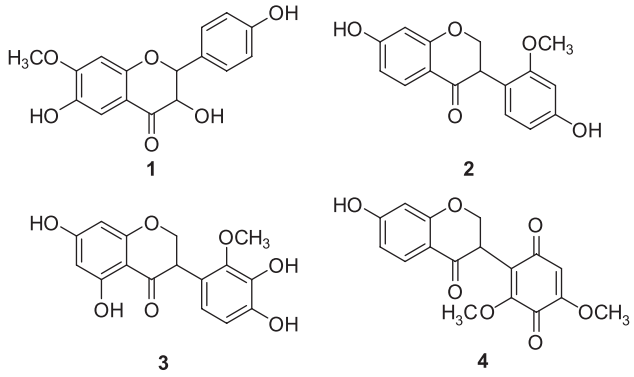
The infusion prepared from the stem-bark of *Exostema caribaeum* (Jacq.) Roemer & Schultes (Rubiaceae) is widely used for the treatment of malaria and diabetes. Previous chemical studies of the stem-bark revealed the presence of several 4-phenylcoumarins. In the present investigation, we describe the isolation and characterization of two additional 4-phenylcoumarins, namely 6′′-*O*-acetyl-5-*O*-*β*-D-glucopyranosyl-7,3′,4′-triihydroxy-4-phenylcoumarin and 5-*O*-*β*-D-glucopyranosyl-7,3′,4′-trihydroxy-4-phenylcoumarin, from an organic extract of the stem-bark. Chemical analyses of the infusions of the leaves and stem-bark established the presence of 4-phenylcoumarins in both preparations. Thus, as in other Mexican copalchis, 4-phenylcoumarins are the antidiabetic principles of *E. caribaeum*. In addition a suitable HPLC-UV procedure for simultaneous quantification of the major 4-phenylcoumarins in the crude drug of *E. caribaeum* was developed. Altogether, these procedures will be valuable for quality control of the crude drug of *E. caribaeum* which is one of the most commercialized medicinal plants in Mexico.

FOUR NOVEL FLAVONOIDS FROM *DALBERGIA PARVIFLORA* ROXB. WITH THE POTENTIAL TO ESTROGENIC AND ANTIESTROGENIC ACTIVITIES

Orawan Monthakantirat,[1] Kaoru Umehara,[2] Wanchai De-Eknamkul,[3] Toshio Miyase,[2] Hiroshi Noguchi[2]

[1]*Faculty of Pharmaceutical Sciences, Khon Kaen University, Pharmaceutical Sciences, Khon Kaen, Khon Kaen 40002, Thailand,* [2]*School of Pharmaceutical Sciences, University of Shizuoka, Pharmacognosy, Shizuoka, 422–8526, Japan,* [3]*Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pharmacognosy, Bangkok, 10330, Thailand*

The heartwood of *Dalbergia parviflora* Roxb. (Leguminosae) has been used to normalize the menstruation in Thai traditional medicine. To support its common use, further investigation on its constituents were carried out. A part of its methanol extract (150 g) of the heartwood was subjected to silica gel column chromatography to yield 26 fractions. In this investigation, fraction R was focused for purification by using HPLC. Estrogenic activity and antiestrogenic activity were evaluated by monitoring cell proliferation of estrogen responsive human breast cancer, MCF-7 and T47D cells with various concentrations of isolates. Novel flavavone (**1**), isoflavanone (**2**-**4**), along with 7 known flavonoids, 3′,4′,6-trihydroxy-7-methoxyflavanone (**5**), 4′,7-dihydroxyflavone (**6**), 4′,7-dihydroxy-8-methoxy-isoflavone (**7**), 4′,5,7-trihydroxy-2′-methoxyisoflavone (**8**) 2′,3′,7-trihydroxy-4′-methoxyisoflavanone (**9**), claussequinone (**10**) and 2′-methoxyisoliquiritigenin (**11**) were isolated and their structures determined. Further purification and their bioactivity will be presented.



DEVELOPMENT OF QUALITY CONTROL PARAMETERS FOR THE MEDICINAL ORCHIDS *CYRTOPODIUM MACROBULBON* AND *SCAPHYGLOTTIS FASCICULATA*

Viridiana Morales-Sánchez,[1] Gerardo Salazar,[2] Rachel Mata[1]

[1]*Universidad Nacional Autónoma de México, Facultad de Química, Farmacia, México City, DF 04510, México,* [2]*Universidad Nacional Autónoma de México, Instituto de Biología, México City, DF 04510, México*

Cyrtopodium macrobulbon and *Scaphyglottis fasciculata* are employed for treating several diseases in Mexican folk medicine. In the present study, we describe quality control parameters for both species including identity and composition tests. The identity tests comprise chromatographic profiles by HPLC and GC, as well as histological studies using electronic microscopy. In addition, headspace analysis (SPME) of the bulb of both plants using different coated fibers revealed that the principal light volatile compounds were hexanal, eucalyptol, isobornyl formate, 1-nonen-3-ol in the case of *C. macrobulbon* and 3,7-dimethyl-1,6-octadien-3-ol, and 1-nonen-3-ol for *S. fasciculata*. Finally, HPLC method for determining the main active principles of the infusions of this species were developed and validated.

CHORIOALLANTOIC MEMBRANE (CAM) OF CHICK EMBRYO ASSAY TO AQUEOUS EXTRACT OF *PTERIDIUM AQUILINUM* EVALUATION

Amanda Leitolis, Fernanda Gimenez de Souza, Kelli Freitas, Ricardo Andrukaitis Moledo, Lucas Maracci, Joceline Franco, *Luiz Fernando Pereira*

Pontifical Catholic University of Parana (PUCPR), Department of Biological Sciences, Curitiba, Paraná 80215-901, Brazil

The goal of this study was to evaluate the response of the chorioallantoic membrane to aqueous extract of *Pteridium aquilinum* (braken fern) and observe its effect on the chick embryo. For the assays (n = 8), the eggs were cleaned and a window was opened in the eggshell for access to CAM. All eggs were kept in a humidified incubator at 37°C and gently agitated manually twice a day. The implants with the *Pteridium aquilinum* extract (0.1, 0.5, 1, 5 and 10 μg/ml) were performed on gel saline (0.5 ml) previously positioned on the CAM of fertile eggs with six days of incubation. After seven

days, the eggs were opened again to the observation on a stereoscopic microscope. The samples were photographed and later, removed for subsequent processing and histological analysis (HE and Masson trichrome). We also evaluated the number of blood vessels and pro- or antiangiogenic response of CAM. The counting was made using a specific program (Image-Pro Plus version 4.5™). Preliminarily, according to our experimental protocol, aqueous extract of *Pteridium aquilinum* had no pro- or antiangiogenic effects on CAM. The histological slides showed collagen fibers as possible indicator of inflammatory disorders and/or reparation signals. The majority of chick embryos treated with Braken Fern suffered severe deformities.

COROSOLIC ACID PRODUCTION FROM *LAGERSTROEMIA SPECIOSA* CALLUS

Prapaporn Wititpanyawong,[1] Soisiri Kongrak,[2] Niwan Intaraksa,[1]*Pimpimon Tansakul*[1,2]

[1]*Prince of Songkla University, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, HatYai, Songkhla 90112, Thailand,* [2]*Prince of Songkla University, Phytomedicine and Pharmaceutical Biotechnology Research Center, Faculty of Pharmaceutical Sciences, HatYai, Songkhla 90112, Thailand*

Corosolic acid, an ursane-type triterpene acid, is found in *Lagerstroemia speciosa* leaves (Lythraceae). Because of its pharmacological activity on hyperglycemia, corosolic acid is used as dietary supplement for reducing blood glucose level. In this study, *L. speciosa* callus was induced from leaves on Murashige and Skoog (MS) medium supplemented with 2 mg/l of 2,4-dichlorophenoxy acetic acid (2,4-D) and 0.5 mg/l of kinetin. Under this culture condition, the callus culture produced biomass of 0.67 ± 0.21 g/callus and corosolic acid with yield of 127.55 ± 13.69 mg. The corosolic acid production curve suggested that callus could produce high yield of corosolic acid at the beginning of stationary phase (24 days of culture), which was 166 times higher than by natural leaves.

NEUROPROTECTIVE EFFECT OF SEED OF *LOTUS PLUMULE* IN THE MOUSE HIPPOCAMPAL HT22 CELL LINE

Eun Sil Kim,[1] Jin Bae Weon,[2] Kyung Jin Lee,[1]*Choong Je Ma*[2]

[1]*National Institute of Biological Resources (NIBR), Wildlife Genetic Resources Center, Incheon, 404–708, South Korea,* [2]*Kangwon National University, Department of Biomaterials Engineering, Division of Bioscience and Biotechnology, Chuncheon, 200–701, South Korea*

Glutamate-induced oxidative injury contributes to neuronal degeneration in many central nervous system diseases, such as Alzheimer's disease. The neuroprotective effects of total extract and its fractions (n-hexane, chloroform, ethyl acetate and *n*-butanol) of seed of *L. plumule* were investigated in glutamate-induced neurotoxicity in the HT22 cell. The ethyl acetate fraction of seed of *L. plumule* showed the potent neuroprotective effects by inhibited ROS production in the HT22 cell. Furthermore, the ethyl acetate fraction of seed of *L. plumule* had DPPH radical (IC_{50} = 90.89 μg/ml) and hydrogen peroxide (IC_{50} = 639.66 μg/ml) scavenging effect, respectively. These results suggested that the ethyl acetate fraction of seed of *L. plumule* could show neuroprotective activity by its anti-oxidative activity.

MARINE NATURAL PRODUCTS AS INGREDIENTS IN TRADITIONAL MEDICINE

Varima Wongpanich, Witaya Lowtangkitcharoen

Faculty of Pharmaceutical Sciences, Khon Kaen University, Division of Pharmacognosy and Toxicology, Khon Kaen, 40002, Thailand

A survey on official Thai Traditional Formularies has revealed the applications of marine natural products as components in various formulated preparations. More than 40 items are those directly obtained or products modified mainly from animal and mineral origins. A small number is obtained from plant sources. Even though medicinal properties of each item are mentioned, none of the items are used as a single therapeutic material. The indications of formulated preparations that contain these marine-derived components comprise treatment on gastro-intestinal disturbance, reproductive disorders, infections, external and chronic wounds, as well as being feverfew and nourishing. It is also noticed that almost all of the items required pre-incorporation processing prior to compounding. Unlike those of the terrestrials, the ethnopharmacological information of marine natural products is not well documented and has not likely been taken into account for drug discovery and development. As research on marine natural products chemistry has received growing interest in recent decades as a potential new source of drug candidates, this presentation may provide a different angle of connection between chemistry and medicine for sustainable benefit to human life.

ANTIMICROBIAL AND POTENTIAL CANCER PREVENTIVE SUBSTANCES FROM MARINE ALGAE AND CYANOBACTERIA COLLECTED IN HAWAII AND THE CARIBBEAN

Monique Lacroix,[1] Dominic Dussault,[1] Tifanie Vansach,[3] Khanh Dang Vu,[1] David Horgen,[3] Clarissa Gerhaeuser[2]

[1]INRS, INRS-Institut Armand-Frappier, Laval, Quebec H7V 1B7, Canada, [2]German Cancer Research Center, Division Epigenomics and Cancer Risk Factors, Heidelberg, 69120, Germany, [3]Hawaii Pacific University, Natural Sciences, Kaneohe, HI 96744, USA

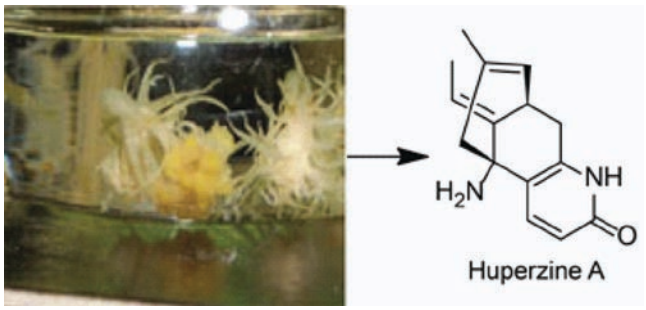
We investigated the effects of a small collection of marine-derived extracts and isolates on the reduction of foodborne illness and prevention of cancer. Ten cyanobacterial isolates and nine organic algal extracts from organisms collected in Caribbean and Hawaiian waters were tested against foodborne pathogens using a broth dilution assay. Results showed that several extracts and compounds showed antimicrobial activity against three Gram positive foodborne pathogens at low concentrations (<500 ppm). Results also showed that extracts of *Gracilaria salicornia*, *Liagora* sp. and *Ulva* sp. demonstrated high potential to suppress carcinogen metabolic activation through induction of NQO1. This research demonstrates the potential of several cyanobacterial isolates as sources of biologically active substances for the control of foodborne pathogens and for potent inducers of an important enzyme in reducing carcinogen activation.

CANDIDATE HUPERZINE A AND OTHER *LYCOPODIUM* ALKALOIDS IN CULTURED CELLS OF *HUPERZIA* SPECIES

Kanichiro Ishiuchi, Jeong-Jin Park, David R Gang

Washington State University, Institute of Biological Chemistry, Pullman, WA 99164, USA

Tissue culture of various *Huperzia* species has been achieved and production of huperzine A (HupA), an anti-Alzheimer's disease drug candidate isolated from the traditional Chinese medicine Qian Ceng Ta (*Huperzia serrata*), has been confirmed in the callus of several species, especially in *H. pinifolia*. The accumulation of various Lycopodium alkaloids, particularly those related to HupA biosynthesis, was also monitored in these tissues using high resolution Q-IMS-TOFMS and ion trap-based MS^n analysis.



HYPOLIPIDEMIC ACTIVITY OF TRITERPENES FROM BURSERACEAE OLEORESINS

Deborah S. Braz,[1,2] André Luis Rüdiger,[1] Emerson Silva Lima,[2] *Valdir F. Veiga Junior*[1]

[1]Universidade Federal do Amazonas, Chemistry Department, Manaus, AM 69079000, Brazil, [2]Universidade Feral do Amazonas, School of Pharmaceutical Science, Manaus, AM 69079000, Brazil

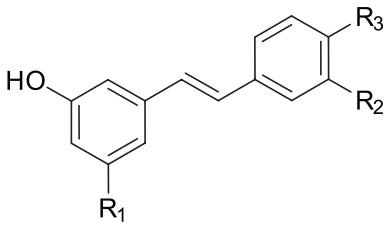
The phytochemical study of Amazonian Burseraceae oleoresins provided four enriched fractions, named HT, OT, DHT and AT. These fractions had their components identified and these were found to be constituted by isomeric triterpenes that were evaluated for lipase, α-amylase and α-glycosidase activities. The mixture OT showed higher percentages of lipase inhibition, presenting IC_{50} (mg/mL) of 3.97 (±0.41), whereas the TA fraction was the only one with significant α-amylase inhibition, showing IC_{50} (mg/mL) of 23.03 (±0.5). Given the results for α-glucosidase enzymatic inhibition, all compounds tested showed an inhibition greater than 80% and a promise for development of alternative drugs for metabolic syndrome prevention.

CELL ADHESION INHIBITORY ACTIVITIES OF STILBENE DERIVATIVES ISOLATED FROM *RHEUM UNDULATUM*

Hyun-Mee Oh, Seung Woong Lee, Mi-Hwa Kim, Woo Song Lee, Mun-Chual Rho

Eco-Friendly Biomaterial Research Center, Korea Research Inst. Bioscience and Biotechnology, Jeongeup, 580-185, South Korea

Six stilbenes were isolated from the methanol extracts of *Rheum undulatum* rhizomes by bioactivity-guided fractionation. Compounds **1**-**4** inhibited direct binding between sICAM-1 and LFA-1 of THP-1 cells in a dose-dependent manner with IC_{50} values of 50.1, 25.4, 33.4 and 45.9 μM, respectively. In addition, the methoxyl group, glycoside, double bond and *trans* configuration of stilbene compounds might be modulatory factors on the binding of LFA-1 and ICAM-1.



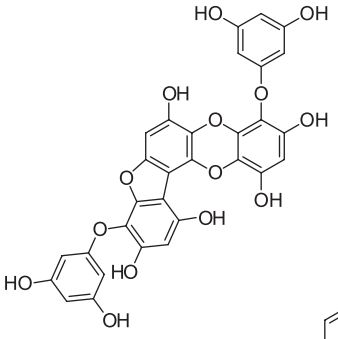
1. R_1=OH, R_2=H, R_3=OCH_3, Desoxyrhapontigenin
2. R_1, R_2=OH, R_3=OCH_3, Rhapontigenin
3. R_1=OH, R_2=H, R_3=OH, *trans*-Resveratrol
4. R_1, R_2, R_4=OH, Piceatannol
5. R_1=OH, R_2=OGlc, R_3=OH, Piceatannol-3-*O*-β-glucopyranoside
6. R_1=OGlc, R_2=OH, R_3=OCH_3, Isorhapontin

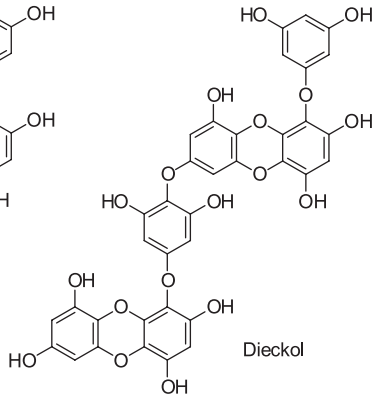
IN VITRO INHIBITORY ACTIVITY OF *ECKLONIA CAVA* AGAINST PORCINE EPIDEMIC DIARRHEA CORONAVIRUS INFECTION

JinA Ko,[1] Hyung-Jun Kwon,[1] Young Bae Ryu,[1] Young Min Kim,[1] Kyoung-Oh Cho,[2] Woo Song Lee,[1] Su-Jin Park[1]

[1]*Eco-Friendly Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup, 580-185, South Korea,* [2]*Biotherapy Human Resources Center, College of Veterinary Medicine, Chonnam National University, Gwangju, 500–757, South Korea*

We evaluated the ability of five polyphenols isolated from the *Ecklonia cava* (EC) to porcine epidemic diarrhea virus (PEDV). We assessed the anti-viral activity of pre-treatment, simultaneous treatment, and post treatment. Using the simultaneous treatment assay, we found that EC extracts and fraction and compounds directly blocking viral adsorption to cells. The 50% effective inhibitory concentrations (EC_{50}) of the EC-1 to EC-5 were 12.4-24.5 μg/mL and compounds **1**-**5** were 10.8-22.5 μM, respectively. Moreover, the post treatment assay showed that all extracts and fractions inhibited viral replication with EC_{50} values of 19.5-28.8 μg/mL. Also, two compounds (**4** and **5**) showed EC_{50} values of 12.2 and 14.6 μM, respectively.





REDUCTION OF OVA-INDUCED LUNG INFLAMMATION IN MICE TREATED WITH AYURVEDIC HERBS USING 2-D AND 3-D IMAGING

Kapil K. Soni, Udeshi Patel, Jim Artwolt, Elizabeth Nunamaker, *Gail B. Mahady*

University of Illinois at Chicago, Pharmacy Practice and Biological Resource Center, Chicago, IL 60612, USA

The medicinal plants *Bacopa monnieri* (L.) Penn. (Scrophulariaceae), *Boswellia serrata* Roxb. (Burseraceae) and *Ocimum sanctum* L. (Lamiaceae) were collected in Vidisha (M.P.), India based on traditional use in Ayurvedia to treat asthma. The plant materials were dried, extracted in methanol and tested in three *in vitro* assays, leukotriene-C4-synthase, leukotriene-A4-hydroxylase and cyclooxygenase-2. The extracts inhibited all enzymes indicating anti-inflammatory

and potential anti-asthmatic activities. The extracts were then tested in 24 BALB/c mice (6 arms, 4 animals per arm) sensitized by i.p. injections of ovalbumin (OVA, 50 μg) weekly for 3 weeks. The animals were then treated by gastric lavage for four days with the herbal extracts (100 mg/kg bw) after intranasal OVA challenge. Animals were subjected to 2-D *in vivo* imaging and 3-D tomography in a Xenogen IVIS 2000 Imaging System to assess lung inflammation induced by OVA. Animals sensitized and challenged with OVA had significant lung and peritoneal inflammation. Treatment of the mice with dexamethasone reduced OVA-induced inflammation by 50–60%, as compared with the PBS control group. Treatment of the mice with the Ayurvedic herbal extracts also reduced inflammation, with the activities of the extracts being in the order of *Ocimum sanctum>Boswellia serrata>Bacopa monnieri*.

EFFECT OF *ABIES KAWAKAMII* LEAF EXTRACTS ON LIFE SPAN EXTENSION IN *DROSOPHILA MELANOGASTER*

Hui-Ting Chang[1], Jui-Hua Chu[2], Chau-Ti Ting[2], Shu Fang[3], Li-Sheng Hsu[1], Shang-Tzen Chang[1]

[1]*National Taiwan University, School of Forestry and Resource Conservation, Taipei, 106, Taiwan,* [2]*National Taiwan University, Department of Life Science, Taipei, 106, Taiwan,* [3]*Academia Sinica, Biodiversity Research Center, Taipei, 115, Taiwan*

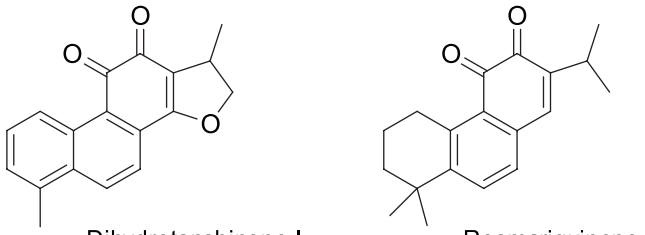
Abies species (Pinaceae) have been used as folk medicines. *Abies kawakamii* (Hayata) Ito is an evergreen conifer tree in Taiwan with the potential of being a medicinal plant. Antioxidant activity and prolongevity effect in the fruit fly (*Drosophila melanogaster*) lifespan of ethanolic extract and hot water extract from *A. kawakamii* leaf were investigated in this study. Both ethanolic extract and hot water extract exhibited anti-oxidative activity including scavenging activity of DPPH radicals, reducing power and trolox equivalent antioxidant capacity. Hot water extract showed better antioxidant efficacy than ethanolic extract. Different dosages of hot water extract and ethanolic extracts have various life span extension effects on different sex of *Drosophila melanogaster*. Lifespan extension is longer on fruit flies fed with hot water extract than with ethanol extract, the result was consistent with the antioxidant performance. Prolongevity effect (56.1% and 65.7% for male and female fruit fly respectively) was found in fruit flies fed with hot water extract at a dosage of 0.1 mg/mL dosages. In conclusion, *A. kawakamii* extracts showed a beneficial effect in extension the mean lifespan of fruit fly.

TANSHINONES FROM *SALVIA MILTIORRHIZA* DISPLAYING POTENT 3CL^{pro} INHIBITION

Ji-Young Park,[1] Young Min Kim,[1] Kyoung-Oh Cho,[2] Su-Jin Park,[1] Woo Song Lee,[1] *Young Bae Ryu*[1]

[1]*Eco-Friendly Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup, 580-185, South Korea,* [2]*Biotherapy Human Resources Center, College of Veterinary Medicine, Chonnam National University, Gwangju, 550–757, South Korea*

Bioactivity-guided fractionation of the ethanol extract yielded seven tanshinones, identified as tanshinone I (**1**), tanshinone II A (**2**), tanshinone II B (**3**), crytotanshinone (**4**), dihydrotanshinone I (**5**), methyl tanshinonate (**6**), and rosmariquinone (**7**). The inhibitory activities of these compounds (**1**-**7**) against 3CL^{pro}s from SARS, bovine (KWD3) CoV and porcine epidemic diarrhea virus (PEDV) were evaluated to determine potencies. Analyses using various *in vitro* coronavirus 3CL^{pro} assays showed that all seven tanshinones were selective 3CL^{pro} inhibitors. Of the tanshienones, rosmariquinone (**7**) exhibited the most potent inhibitory activity toward SARS-CoV 3CL^{pro} (IC_{50} = 10.2 μM), whereas dihydrotanshinone I (**5**) potently inhibited KWD (IC_{50} = 0.7 μM) and PEDV (IC_{50} = 6.1 μM).



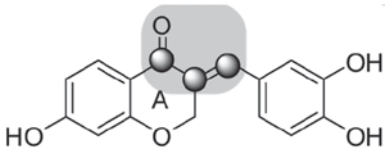
Dihydrotanshinone I Rosmariquinone

CHARACTERISTIC OF VIRAL NEURAMINIDASES INHIBITORY HOMOISOFLAVONOIDS FROM *CAESALPINIA SAPPAN*

Young Min Kim, Ji-Young Park, Su-Jin Park, Young Bae Ryu, Woo Song Lee

Eco-Friendly Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup, 580-185, South Korea

In this study, twelve neuraminidase inhibitory compounds **1**-**12** were isolated from the leaves of *Caesalpinia sappan* on the basis of their biological activities against three types of viral NAs. Of isolated homoisoflavonoids, sappanone A (**2**) showed the most potent NAs inhibitory activities with IC_{50} values of 0.7 μM [H1N1], 1.1 μM [H3N2], and 1.0 μM [H9N2], respectively, whereas saturated homoisoflavonoids such as **3** did not show significant inhibition. The data revealed that the α,β-unsaturated carbonyl group in the A-ring was key requirement for viral NAs inhibitory activity. In our enzyme kinetic study, all NA inhibitors screened were found to be reversible noncompetitive types.



SYSTEMATIC STUDIES ON ARCTII FRUCTUS

Tingguo Kang, *Deqiang Dou*

Liaoning University of Traditional Chinese Medicine, Dalian, Liaoning 116600, China

Arctii fructus is the dry seeds of *Arctium lappa* and generally used as an herbal medicine in traditional Chinese medicine. The pharmacognosy and anticancer constituents of Arctii fructus as well as ecological suitability of *Arctium lappa* L. and its suitable cultivation regions in China were studied.

RECOVERY OF ENDOGENOUS PHENOLIC COMPOUNDS FROM POTATO TUBER USING CONVENTIONAL AND HIGH-PRESSURE EXTRACTION METHODS

Marya Aziz, Sarya Aziz, Selim Kermasha

McGill University, Department of Food Science and Agricultural Chemistry, Ste-Anne de Bellevue, Quebec, H9X 3V9, Canada

The recovery of endogenous phenolic compounds (PC) from potato tuber (*Solanum tuberosum*), using conventional and high-pressure (HP) extraction methods, was investigated. Mixtures of hexane/ethyl acetate and ethyl acetate/chloroform were used as solvents for the conventional extraction method, whereas ethyl acetate and water/ethanol were investigated with the HP one. The experimental results from the conventional method showed that the hexane/ethyl acetate extraction method was 1.6 times more efficient than that of ethyl acetate/chloroform, resulting in 63.5 mg PC/g extract as compared to 39.4 mg PC/g extract, respectively. To optimize the HP extraction method, selected parameters, including the effect of temperature, number of flushing (cycle) and the potato homogenate/Ottawa sand ratio, were studied. The optimum temperature for the HP extraction by ethyl acetate and water/ethanol HP, was determined to be 50 and 40, respectively. Using the HP method, the results showed that the majority of phenolic compounds (65%) were recovered during the first flush. In addition, the optimum ratio of potato homogenate/Ottawa sand (w/w) was determined to be 5:4 and 5:1 for ethyl acetate and water/ethanol extraction methods, respectively. The overall results the experimental results indicated that the HP extraction with ethyl acetate was 3.5 times more efficient than that with ethanol/water, resulting in 43.0 mg PC/g extract as compared to 12.3 mg PC/g extract.

HOW TO SEPARATE THE WHEAT FROM THE CHAFF? EXPLORING BRAZILIAN BIODIVERSITY USING NMR DEREPLICATION TECHNIQUES

Ian Castro-Gamboa, Fausto Carnevale Neto, Rafael Freire, Alan Cesar Pilon, Patricia Cardoso, Vanderlan Bolzani

Institute of Chemistry, São Paulo State University – UNESP, Organic Chemistry, Araraquara, SP 14800900, Brazil

The establishment of new and innovative analytical methods that may shed information towards the composition of complex natural mixtures is critical on bioprospection programs. Our research group NuBBE has incorporated the use of molecular virtual design using NMR aiming to increase the understanding of molecular relationships on dynamic natural matrixes and synergism effects of highly active crude extracts, previously screened using *in vitro* human cell lineages such as HL-60 (leukemia), MDA-MB435 (melanoma), HCT-8 (colon) and SF-295 (glioblastoma). From several Fabaceae and Asteraceae species, NMR data was acquired, processed and compared with a molecular virtual designed NMR environment containing all known reported metabolites. From the multivariate analysis we detected a recurring array of flavonoids, such as 8-methylnaringenin, 4,5,7-trihydroxy-3′,6-dimethoxy-8-methylflavanone, 3′,4′,5,6,7-pentahydroxy-8-methyl-dihydroflavonol, 4′,5,7-trihydroxy-3-methoxy-6,8-dimethylflavanone, 4′,7-

dihydroxy-5-methoxy-6-methyldihydroflavonol, 5,6,7-trihydroxy-3′,4′-dimethoxy-8-methylflavanone and, 3′,4′-dimethoxy-5,7-dihydroxy-6,8-dimethyldihydroflavonol. The occurrence of flavonoids may be associated to the original activity revealed in the *in vitro* assays. However, further studies must be performed in order to establish molecular synergism effects since there is no significant antineoplasic activity reported for those metabolites once isolated.

SIMULTANEOUS EXTRACTION AND QUANTIFICATION OF CAROTENOIDS AND TOCOPHEROLS IN *BRASSICA* SPECIES

Ivette Guzman, Gad Yousef, Allan Brown

North Carolina State University, Plants for Human Health Institute, Kannapolis, NC 28081, USA

Brassica vegetables, like broccoli and cauliflower, are consumed worldwide and are known to contain an array of bioactive compounds. Among these are two classes of photosynthetic lipid soluble compounds: carotenoids and tocopherols. They are isoprenoids with a common precursor. Carotenoids are yellow, orange and red pigments; some of which are vitamin A precursors. Tocopherols have vitamin E activity. As essential vitamins to the mammalian diet, their activities involve protecting membrane lipids from oxidative damage by quenching reactive oxygen species and protecting against degenerative diseases. *Brassica* species accumulate both carotenoids and tocopherols in the edible floret tissue. Due to genetic and environmental variables, carotenoid and tocopherol amounts are not constant. In order to aid breeders in the development of *Brassica* cultivars with high pro-vitamin A and vitamin E activity, a more efficient method was created to quantify the major accumulating carotenoids and tocopherols in broccoli and cauliflower. The novel UPLC method separates 5 carotenoids and 2 tocopherols in a 30 minute run, cutting the run time by half compared to previously published chromatographic runs. This data collected allowed us to compare amounts of β-carotene, lutein, α-tocopherol, and γ-tocopherol in *Brassica* to previous studies. We also report amounts for neoxanthin, violaxanthin, and epoxylutein. The goal is to develop a fast effective extraction and quantification method in order to screen large collections of *Brassica* germplasm, thus aiding breeders in producing a high pro-vitamin A and vitamin E broccoli or cauliflower.

DETECTION OF ADULTERATED NATURAL PRODUCT EXTRACTS CONTAINING SILDENAFIL (VIAGRA) DERIVATIVES

Kimberly L. Colson,[1] Joshua M. Hicks,[1] Sarah M. Luchsinger,[1] Hui Li,[2] Basil Desousa[1]

[1]Bruker BioSpin, Billerica, 01821, USA, [2]Bruker Optics, Billerica, 01821, USA

Adulteration of natural products with sildenafil (Viagra), sulfoaildenafil, tadalafil, vardenafil or other derivatives is common. To elude detection, adulterators regularly change the derivative supplied in the natural product sample in hopes that the presence of such adulterants go undetected due to the use of analytical methods using targeted analysis. Regenerect, which targets an audience for erectile dysfunction, was voluntarily recalled in April 2011 as a result of FDA lab analysis detecting Sulfoaildenafil in two lots (100521 and 112850). In this work, we evaluated Regenerect samples, both from a recalled lot and unrecalled lot, using ^{1}H NMR spectroscopy. Lot-to-lot comparisons, detection and quantification of sulfoaildenafil and component reconstruction was undertaken. Methods for automated detection of sildenafil derivatives through substructure analysis were appraised. Evaluation by NIR and IR was also performed.

PROBING MEDICINAL PLANT PHYTOCHEMICAL FACTORIES THROUGH *IN SITU* MALDI TISSUE IMAGING USING QUADRUPOLE ION MOBILITY TIME-OF-FLIGHT MASS SPECTROMETRY

David R. Gang,[1] Laurence B. Davin,[1] Mwafaq Ibdah,[1] B. Marcus Lange,[1] Norman G. Lewis,[1] Glenn W. Turner,[1] Henry Shion,[2] Greg Witkop,[2] Don Harris,[2] and Alan Millar[2]

[1]Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, [2]Waters Corporation, Milford, MA, USA

Plant cells are well known as "chemical factories", capable of producing a truly amazing diversity of compounds with properties important both to plants and humans. Regulation of metabolic processes at the individual cellular level in plants is poorly understood, in large part due to a previous lack of methodology able to comprehensively probe metabolism at the single cell level in an intact differentiated organism. This study utilized a hybrid quadrupole, ion mobility time-of-flight mass spectrometer equipped with MALDI source (MALDI SYNAPT™ G2 HDMS). Tissues from plants of medicinal interest were flash frozen or used fresh and cut into sections of varying thickness, e.g. 10 μm, and analyzed on the MALDI SYNAPT™ G2 HDMS in order to establish methods to detect the spatial distribution of important medicinal compounds in specific plant tissues. Plant species (and compounds) analyzed included, among others, *Zingiber officinale* (gingerols) and *Larrea tridentata* (nordihydroguaiaretic acid, NDGA). Various sectioning methods were required

for different tissues and plants. Ion mobility spectrometry (IMS) was used in tandem with TOFMS both to filter sample data to remove noise/increase sensitivity and to also help characterize specific compounds for better identification/selectivity. Extracts from these tissues were analyzed by UPLC-IMS/MS/MS in a separate Synapt G2 to verify MS and MS/MS data and establish parameters for ion mobility analysis. Analysis of these plant tissue samples demonstrated localization of different metabolite classes and even specific compounds to specific cell types in complex plant anatomies, such as rhizomes and leaf cross-sections. For example, specific gingerols were shown to accumulate in different cells in ginger rhizomes than was found for terpenoids and disaccharides, and NDGA was found to accumulate differentially across creosote bush leaf anatomies. These results provide a foundation for truly novel insights into medicinal plant metabolism in ways that were never possible before, and thus open up exciting new possibilities in defining at the cellular level how biosynthesis of such compounds is regulated.

HEPATOPROTECTIVE ACTIVITY OF *RHODIOLA IMBRICATA* EDGEW ACETONE EXTRACT AGAINST PARACETAMOL INDUCED HEPATOPATHY IN WISTAR RATS

Senthilkumar Ravichandran, Parimelazhagan Thangaraj

Bharathiar University, Coimbatore, Department of Botany, School of Life Sciences, Coimbatore, Tamil Nadu 641046, India

The dried *Rhodiola* rhizome was extracted in Soxhlet extractor successively with organic solvents and concentrated by rotary vacuum evaporator. The evaporated extracts thus obtained were dissolved in the respective solvents and used for antioxidant assay, acute toxicity and hepatoprotective evaluation. Based on the free radical scavenging potential, acetone extract was chosen to investigate the hepatoprotective activity. Rats weighing 150–200 g were divided in to 5 groups of 6 animals in each. Group-I served as normal control received water, Group-II served as negative control, administered with Paracetamol (2 g/kg), Group-III reference control, Silymarin (25 mg/kg), Group-IV & V received acetone extract for 200 and 400 mg/kg once daily for 14 days. On 14th day, blood was obtained from all animals by puncturing retro-orbital plexus for haematogram and sacrificed for biochemical and histopathological evaluation. We can conclude from this study that *Rhodiola* acetone extract inhibit the oxidation and maintained the activity of antioxidant enzymes to the normal level in liver with reference to the controls.

THE ANTIOXIDATIVE AND ANTIMICROBIAL ROLES OF ASSOCIATED FUNGI OF THE LICHEN *USNEA AUSTRALIS* FROM HAWAII

Dovi Kelman,[1] Jonathan D. Awaya,[2] Anthony D. Wright[1]

[1]*University of Hawaii at Hilo, College of Pharmacy, Department of Pharmaceutical Sciences, Hilo, HI 96720, USA,* [2]*University of Hawaii at Hilo, Department of Biology, Hilo, HI 96720, USA*

Lichens are traditionally considered as symbiotic associations between a single fungal species and either an alga or a cyanobacterium. However, it is now known that lichens provide an ecological niche for additional fungi and bacteria. Lichens have the remarkable ability to survive desiccation, extremes of temperature, UV irradiation and oxidative stress. To do this they produce a suite of secondary metabolites. In the current study, *Usnea australis*, commonly found on the Island of Hawaii, was investigated for antioxidant and antimicrobial properties of its microbial associates. Careful microbial isolation attempts afforded a diversity of fungal strains. Organic extracts of these fungi showed them to have considerable antioxidant activity, compared to the lichen host. Fractionation of the extracts led to the isolation of 8-methoxynaphthalen-1-ol (**1**), avellaneol (**2**) and 4-hydroxy-6-methyltetrahydro-2*H*-pyran-2-one (**3**). Compound **1** had an extremely high antioxidant activity, compared to **2** and **3**. SAR results obtained for a variety of mono-methoxyl - monohydroxyl naphthalene derivatives will also be presented. Antimicrobial assays performed with these extracts showed them to selectively inhibit growth of a range of Gram +ve and Gram -ve bacterial pathogens. In contrast, the lichen extract was found to have no antimicrobial activity. In this presentation, aspects of the possible role of the fungal associates in providing defense against oxidative stress and pathogens to the fungal-algal association are discussed.

ANTIOXIDANT ACTIVITY OF HAWAIIAN LICHENS

Dovi Kelman,[1] Kimberley Johns,[1,2] Patrick R. Wright,[1,3] Nicole K. Tabandera,[1] Kehau A. Hagiwara,[1] Anthony D. Wright[1]

[1]*University of Hawaii at Hilo, College of Pharmacy, Department of Pharmaceutical Sciences, Hilo, HI 96720, USA,* [2]*Sheffield Hallam University, Sheffield, S1 1WB, United Kingdom,* [3]*University of Freiburg, Freiburg, 79085, Germany*

Lichens are symbiotic associations between fungi and an algal or a cyanobacterial photosynthetic partner. The symbiotic partners in lichens are subject to increased oxidative stress caused by production of reactive oxygen species (ROS), mainly through photosynthetic related processes. Lichens have the remarkable ability to survive environmental

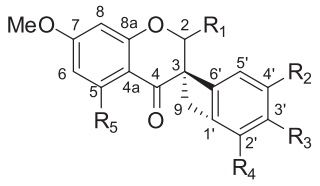
stressors such as desiccation and predation, and to produce secondary metabolites that play important ecological roles in regulation of internal water levels, UV protection, and chemical defense against oxidative stress, pathogens and herbivores. In the current study, we examined the total antioxidative potential of organic extracts of a variety of lichen species found in Hawaii. Of all extracts studied, the ones of *Stereocaulon ramulosum* and *S. vulcani* exhibited the highest antioxidant activity. Bioassay-guided fractionation of the extracts led to the isolation of 2,4-di-*O*-methyldivaric acid (**1**), divaricatic acid (**2**) and perlatolic acid (**3**) as the active principles from the extracts of both lichen species. Compound **1** had extremely high antioxidant activity, compared to **2** and **3**. Together with antioxidant activity of compounds and extracts, structural assignment of **1** will be presented.

COX-2 SPECIFIC INHIBITORS FROM *LEDEBOURIA OVATIFOLIA* AND *LEDEBOURIA SOCIALIS* (HYACINTHACEAE:HYACINTHOIDEAE)

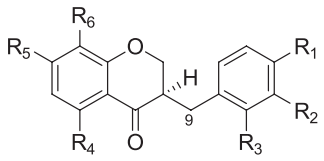
Catherine P. Tunbridge,[1] Neil R. Crouch,[3] Alfred E. Thumser,[2] Dulcie A. Mulholland[1]

[1]*University of Surrey, Chemical Sciences, Guildford, GU2 7XH, United Kingdom,* [2]*University of Surrey, Biological Sciences, Guildford, GU2 7XH, United Kingdom,* [3]*South African National Biodiversity Institute, Ethnobotany Unit, Durban, 4007, South Africa*

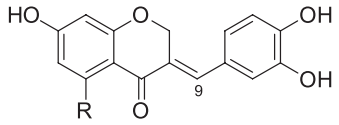
A phytochemical investigation of *Ledebouria ovatifolia* and *Ledebouria socialis* yielded ten novel compounds including a cycloartane derivative, a dihydrochalcone and two xanthones along with the homoisoflavonoids depicted (**1**-**6**). Twenty-three known compounds were also isolated; these included three homoisoflavonoids (**7**-**9**) which had selective COX-2 activity at 10 μM.



1: R_1 = H, R_2, R_3 = OMe, R_4, R_5 = OH
4: R_1 = βOAc, R_2-R_3 = OCH_2, R_4 = H, R_5 = OAc
5: R_1 = αOAc, R_2-R_3 = OCH_2, R_4 = H, R_5 = OAc



6: R_1 = OH; R_2, R_3 = H; R_4, R_6 = OMe;R_5 = OH
9: R_1 = OAc; R_2, R_4 = OMe; R_3, R_6 = H; R_5 = OAc
15: R_1 = OH; R_2, R_3 ,R_6 = H; R_4, R_5 = OH
16: R_1 = OMe; R_2, R_3 ,R_6 = H; R_4, R_5 = OH



10: R = OH
14: R = OMe

MICROTITRE PLATE-BASED ANTIBACTERIAL ASSAY TOWARDS *ASARUM HETEROTROPOIDES* ACTIVE PRINCIPLES AGAINST HUMAN INTESTINAL BACTERIA

Jun-Ran Kim,[1,2] Haribalan Perumalsamy,[2] Young-Joon Ahn[1,2,3]

[1]*Seoul National University, Research Institute for Agricultural and Life sciences, College of Agriculture and Life sciences, Seoul, 151–921, South Korea,* [2]*Seoul National University, Bioactive Natural Products Laboratory, Department of Agricultural Biotechnology, Seoul, 151–921, South Korea,* [3]*Seoul National University, WCU Biomodulation Major, Department of Agricultural Biotechnology, Seoul, 151–921, South Korea*

The growth-inhibiting activity of *Asarum heterotropoides* root derived materials, (–)-asarinin, α-asarone, 1,8-cineole, 3-carene, methyleugenol, pellitorine, pentadecane and safrole, identified in *Asarum heterotropoides* roots toward 10 human intestinal bacteria was evaluated by using microtitre plate-based antibacterial assay compared to those of two commercially available antibiotics, ciprofloxacin and tetracycline. The active principles in *A. heterotropoides* root derived materials were identified by spectroscopic analysis: 3-carene, pellitorine and methyleugenol exhibited very strong growth inhibition and the minimum inhibition concentrations (MIC) ranged from 0.032-0.25 mg/100 μL to almost all bacteria particularly *Escherichia coli* and *Staphylococcus aureus*. The remaining active compounds asarinin, asarone, 1,8-cineole and safrole showed moderate growth inhibition (MIC 0.25-0.5 mg/100 μL) to all bacterial species and weak growth inhibition occurred with pentadecane (4 mg/100 μL). In the control plate well, no adverse effects were observed on growth. Among all compounds isolated, 3-carene, pellitorine and methyleugenol might play an important role in antibacterial activity.

GINSENOSIDES FROM HEAT-PROCESSED KOREAN GINSENG ROOTS, LEAVES AND FLOWER BUDS

Young Ho Kim

College of Pharmacy, Chungnam National University, Daejeon, 305–764, South Korea

Panax ginseng (C.A. Meyer, Araliaceae), an ancient and famous herbal drug in oriental traditional medicine, has been broadly used as a functional food as a boiled extract, powder, tea, tablet, capsule, etc., for thousands of years. These conventional ginseng products are reported to have a wide range of pharmacological and physiological actions, such as antiaging, antidiabetic, anticarcinogenic, analgesic, antipyretic, antistress, antifatigue, and promotion of DNA, RNA, and protein synthesis. Traditionally, ginseng has been processed to make white ginseng (WG, roots air-dried after peeling) and red ginseng (RG, roots steamed at 98–100°C without peeling) to enhance its preservation and efficacy, which is associated with changes in chemical constituents, especially newly formed ginsenosides as a result of the steaming process.

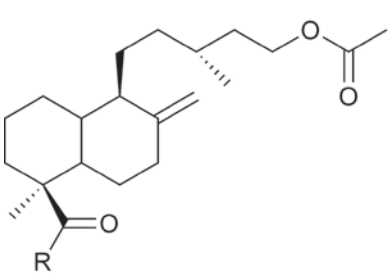
Current studies on the chemical components of the steamed ginseng roots, leaves and flower buds led to the isolation of 22 ginsenosides, including two new ginsenosides from roots, 20 ginsenosides including 6 new compounds from steamed leaves, and 20 ginsenosides, including one new compound from steamed flower buds, respectively. In addition, all of the ginsenosides were biologically evaluated thus establishing their anti-oxidant and anti-inflammatory activity and effects on human leukemia cells. Some of the ginsenosides showed specific biological activities.

NEW DITERPENE AND HETEROCYCLES HYBRID COMPOUNDS: SYNTHESIS AND GASTROPROTECTIVE MECHANISMS OF ACTION USING HUMAN CELL CULTURES

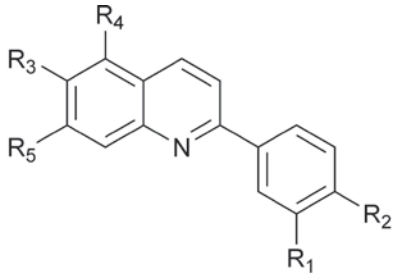
Cristina Theoduloz,[1] Gabriel Vargas,[2] Luis Astudillo,[3] Guillermo Schmeda-Hirschmann[3]

[1]*Universidad de Talca, Facultad de Ciencias de la Salud, Talca, Chile VII Region,* [2]*Universidad Cientifica del Peru, Iquitos, Peru,* [3]*Universidad de Talca, Instituto de Quimica de Recursos Naturales, Talca, Chile VII Region*

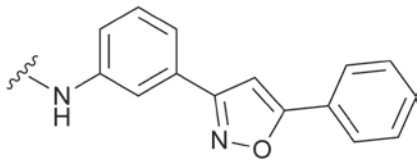
Four new amides were prepared combining the naturally occurring labdane diterpene 15-acetoxyimbricatolic acid and the synthetic heterocycles H1-H4. The activity of the compounds was investigated on human cell culture models including basal cytotoxicity, stimulation of fibroblast and gastric epithelial cell (AGS) proliferation, protection against sodium taurocholate-induced damage on AGS cells and inhibition of the lipoperoxidation induced by tert-butylhydroperoxide in human erythrocyte membranes. Acknowledgements: FONDECYT Project Nr. 1085306.



15-Acetoxyimbricatolic acid



H1: R_1=H, R_2=–NH, R_3=OCH_3, R_4,R_5=H
H2: R_1=H, R_2=–NH, R_3=CH_3, R_4,R_5=H
H3: R_1=–NH, R_2,R_3=H, R_4,R_5=CH_3



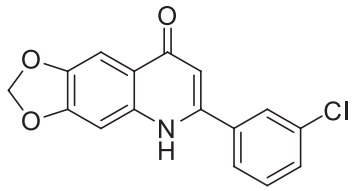
H4

NOVEL QUINOLONE CMQ INDUCES APOPTOSIS AND MITOTIC CATASTROPHE IN PROSTATE CANCER CELLS VIA REACTIVE OXYGEN SPECIES- AND MITOCHONDRIA-DEPENDENT PATHWAYS

Chih-Chun Wen,[1,2] Shin-Hun Juang,[2] Li-Chen Chou,[2] Jing-Gung Chung, Sheng-Chu Kuo,[3] Ning-Sun Yang[2]

[1]*Agricultural Biotechnology Research Center, Academia Sinica, Taipei, 115, Taiwan,* [2]*Graduate Institute of Pharmaceutical Chemistry, China Medical University, Taichung, 404, Taiwan,* [3]*Department of Biological Science and Technology, China Medical University, Taichung, 404, Taiwan*

Quinolone derivatives have been shown to confer various pharmacological activities. In this study, we investigated the effect of 2-(3-chlorophenyl)-6,7-methylenedioxyquinolin-4-one (CMQ) as a drug candidate for antitumor activities in p53-expressing LNCaP cells and p53-null PC-3 cells of prostate cancers. CMQ-1 inhibited tumor cell growth via microtubule-depolymerization and G2/M cell cycle arrest in both cell types. Intriguingly, CMQ triggered a strong apoptotic activity in LNCaP cells but induced mitotic catastrophe in the tested PC-3 cells. The cell cycle blockade in both cell types was found to be associated with an elevated level of reactive oxygen species (ROS), followed by activation of the mitochondrial apoptotic pathway. As a result, cytochrome c, Smac and apoptosis-inducing factor (AIF) were released from mitochondria into the cytosol, with subsequent consecutive activation of caspases-9 and -3. In addition, CMQ significantly activated caspase-8 in LNCaP cells but not in PC-3 cells. Intraperitoneal injection of CMQ significantly suppressed tumor growth in SCID mice bearing LNCaP or PC-3 xenografts. Our findings suggest that CMQ can display differential antitumor activities in different prostate cancers.



2-(3-chlorophenyl)-6,7-methylenedioxyquinolin-4-one (CMQ)

A MECHANISTIC ANALYSIS OF BRYONOLIC ACID TRANSCRIPTIONAL CONTROL: PERTURBATION OF INFLAMMATORY AND ANTIOXIDANT GENES *IN VITRO* AND *IN VIVO*

Tonibelle N. Gatbonton-Schwager, John J. Letterio, Gregory P. Tochtrop

Case Western Reserve University, Departments of Pharmacology, Pediatrics, and Chemistry, Cleveland, OH 44106, USA

The aim of this study is to characterize the mechanisms mediating the anti-inflammatory activity of bryonolic acid (BA) and validate the utility of BA as a tool to explore the relationships between triterpenoid structure and activity. Here we show that BA reduces the inflammatory mediator, nitric oxide (NO) by suppressing the expression of the inflammatory enzyme inducible nitric oxide synthase (iNOS) in LPS-activated RAW 264.7 macrophage cells. In addition, BA robustly induces the antioxidant protein, heme oxygenase-1 (HO-1) *in vitro* and *in vivo* in an Nrf2-dependent manner. Further analysis of Nrf2 target genes (NQO-1, CAT, GCLC and GR) revealed a selectivity for the timing and level of gene induction by BA in treated macrophages with distinct patterns for Nrf2-regulated antioxidant genes. These findings are significant as this is the first study to show mechanistic insights for the anti-inflammatory activity of BA through suppression of iNOS and induction of HO-1. Our study validates the use of BA as a tool to explore the role of the triterpenoid scaffold as a determinant of the anti-inflammatory and chemopreventive properties of these molecules. Further, understanding how selective gene modulation is controlled by the triterpenoid skeletal structure will aid in the design of selective therapeutics that minimize undesirable effects that may often be the consequence of interactions with multiple downstream targets.

ANTIDIABETIC ACTIVE FRACTIONS FROM *MOMORDICA BALSAMINA* FRUIT PULP

Ajudhia Nath Kalia, Shruti Aggarwal, Pradeep Kamboj

ISF College of Pharmacy, Dept. of Pharmacognosy, Moga, 142001, Punjab, India

The present study was designed to isolate and identify the anti-diabetic potential fraction from the methanol extract (ME) of *Momordica balsamina* fruit pulp (MBFP). Charantin and vicine (anti-diabetic compounds) in the *n*-butanol fraction of the ME were identified on the basis of HPTLC and spectral data. Administration of the *n*-butanol fraction in an OGTT-model improved glucose tolerance of normal rats. In STZ induced diabetic rats, a fraction showed significant ($p<0.05$) anti-hyperglycemic and anti-hyperlipidimic activities in a time dependent manner and were on par with the standard anti-diabetic drug metformin (500 mg/kg). In conclusion, the anti-diabetic potential of MBFP may be attributed due to the presence of charantin, vicine and other phenolic compounds.

MELANIN PRODUCTION ENHANCEMENT OF HUMAN TYROSINASE PLASMID (PAH7/TYR) BY TAT AND AN ENTRAPMENT IN ELASTIC CATIONIC NIOSOMES

Narinthorn Khositsuntiwong,[1] Aranya Manosroi,[1,2] Friedrich Goetz,[3] Rolf G. Werner,[4] Worapaka Manosroi,[5] Jiradej Manosroi[1,2]

[1]Faculty of Pharmacy, Chiang Mai University, Chiang Mai, 50200, Thailand, [2]Natural Products Research and Development Center, Science and Technology Research Institute/Faculty of Pharmacy, Chiang Mai University, Chiang Mai, 50200, Thailand, [3]Faculty of Biology, University of Tübingen, Department of Microbial Genetics, Tübingen, 72076, Germany, [4]Boehringer-Ingelheim, Ingelheim am Rhein, 55216, Germany, [5]Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand

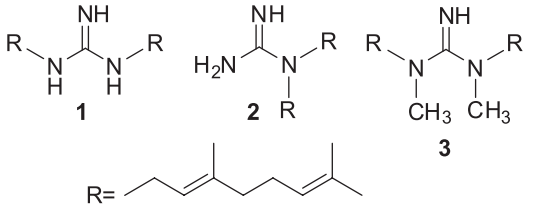
HIV-1 Tat peptide (T), human tyrosinase plasmid (pAH7/Tyr, P) and elastic cationic niosomes (E) complexes (TPE at T/P/E ratio of 0.5:1:160 w/w) exhibited the highest gene expression, as determined by tyrosinase enzyme activity and melanin production in melanoma ($B_{16}F_{10}$) cells of about 12 and 13 fold increases relative to the control, respectively. The remaining plasmid in TPE complexes at 8-week of storage were 45, 32 and 28% for TPE that kept at 4 ± 2, 25 ± 2 and 45 ± 2°C respectively.

SYNTHESIS OF ALKALOIDS FROM *PTEROGYNE NITENS* TUL. AND ITS ANTITUMORAL ACTIVITY IN NUDE MICE BALB/C

Juliana Maria Bozeto,[1]*Mauro Cafundo Morais,*[1] Flavio Campos Monteiro,[1] Luis Octavio Regasini,[2] Dulce Helena Siqueira Silva,[2] Vanderlam Bolzani,[2] Christianne Pienna Soares[1]

[1]School of Pharmaceutical Sciences, [2]Institute of Chemistry, São Paulo State University - UNESP, Araraquara, SP 14801-902, Brazil

The phytochemical study of leaves from *Pterogyne nitens* Tul. (Fabaceae) afforded guanidine alkaloids nitensidine A (**1**), nitensidine B (**2**) and nitensidine C (**3**). Nitensidine A was selected as prototype compound for antitumor activity, and a synthetic geranylated derivative has been prepared. The synthetic guanidine alkaloid was evaluated towards apoptosis in SiHa cells (80% at 0.6 μM) and nude mice xenograft model. Tumor growth was 74.2% in animals treated with saline, whereas animals treated with nitensidine A at 0.244 mg/kg b.w. was 13.8% ($P<0.001$).



WNT/β-CATENIN SIGNALING MEDIATES THE ANTITUMOR ACTIVITY OF MAGNOLOL IN COLORECTAL CANCER CELLS

Hyen Joo Park,[1] You-Jin Kang,[2] Hwa-Jin Chung,[1] Ki-Dae Kim,[1] *Sang Kook Lee*[1]

[1]Seoul National University, College of Pharmacy, Seoul, 151–742, South Korea, [2]Ewha Womans University, College of Pharmacy, Seoul, 120–750, South Korea

Abnormal activation of the canonical Wnt/beta-catenin pathway and up-regulation of the beta-catenin/T-cell factor (TCF) response to transcriptional signaling play a critical role early in colorectal carcinogenesis. Therefore, Wnt/beta-catenin signaling is considered an attractive target for cancer chemotherapeutics or chemopreventive agents. Small molecules derived from the natural products were used in our cell-based reporter gene assay to identify potential inhibitors of Wnt/beta-catenin signaling. Magnolol, a neolignan from the cortex of *Magnolia obovata*, was identified as a promising candidate, as it effectively inhibited beta-catenin/TCF reporter gene (TOPflash) activity. Magnolol also suppressed Wnt3a-induced beta-catenin translocation and subsequent target gene expression in HEK293 cells. To further investigate the precise mechanisms of action in the regulation of Wnt/beta-catenin signaling by magnolol, we performed Western blot analysis, real-time reverse transcriptase-polymerase chain reactions, and an electrophoretic mobility shift assay in human colon cancer cells with aberrantly activated Wnt/beta-catenin signaling. Magnolol inhibited the nuclear translocation of beta-catenin and significantly suppressed the binding of beta-catenin/TCF complexes onto their specific DNA-binding sites in the nucleus. These events led to the down-regulation of beta-catenin/TCF-targeted downstream genes such as c-myc, matrix metalloproteinase (MMP)-7, and urokinase-type plasminogen activator (uPA) in SW480 and HCT116 human colon cancer cells. Magnolol also exhibited antitumor activity in a xenograft-nude mouse model bearing HCT116 cells. These findings suggest that the growth inhibition of magnolol against human colon cancer cells can be partly attributed to the regulation of the Wnt/beta-catenin signaling pathway.

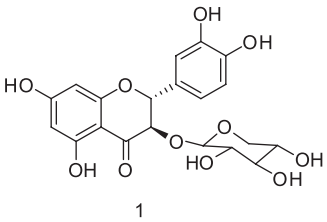
CONSTITUENTS OF *CHAMAECYPARIS OBTUSA* WITH INHIBITORY ACTIVITY ON ALDOSE REDUCTASE AND SORBITOL ACCUMULATION

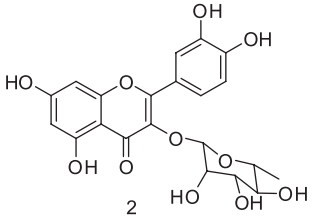
Jin-Kyu Kim,[1] Dong-Joo Kwon,[2,5] Min-Young Lee,[3] Soon-Sung Lim,[1,3,4] Young-Soo Bae,[5] Jun-Hyung Cho[6]

[1]Hallym Universtiy, Institute of Natural Medicine, Chunchon, 200–702, South Korea, [2]Hallym University, Biomedical Science, Chunchon, 200–702, South Korea, [3]Hallym University, Center for Efficacy Assessment and Development of Functional Foods and Drugs, Chunchon, 200–702, South Korea, [4]Hallym University, Food Science and Nutrition, Chunchon, 200–702, South Korea, [5]Kangwon National University, Forest Biomaterials Engineering, Chunchon, 200–701, South Korea, [6]Kangwon National University, Paper Science and Engineering, Chunchon, 200–701, South Korea

Taxifolin-3-O-α-D-xylopyranoside (1) and quercitrin (2) were isolated from an EtOAc-soluble extract of the leaves of *Chamaecyparis obtusa*. Quercitrin was found to possess a potent inhibitory activity of human recombinant aldose reductase *in vitro*, its IC_50 value being 11.5 mM. Kinetic analysis showed that quercitrin exhibited uncompetitive inhibition against DL-glyceraldehyde. Also, quercitrin suppresses sorbitol accumulation in rat lens under high glucose

conditions, demonstrating the potential to prevent sorbitol accumulation *ex vivo*. These results suggest that this compound may be a promising agent in the prevention or treatment of diabetic complications.





RESORCINOL AND FLAVONOIDS COMPOUNDS FROM *ONONIS NATRIX*

Adnan Jathlan Al-Rehaily,[1] Mohammad S. Ahmed,[1] Jamal Mustafa,[1] Muhammad Yousaf,[1] Mohammed A. Al-Yahya,[1] Mansour S. Al-Said,[1] Shabana I. Khan,[2] Ikhlas A. Khan[2]

[1]*King Saud University, Department of Pharmacognosy, Riyadh, 2457, Central Kingdom of Saudi Arabia,* [2]*University of Mississippi, The National Center for Natural Product Research, School of Pharmacy, Oxford, MS 38677, USA*

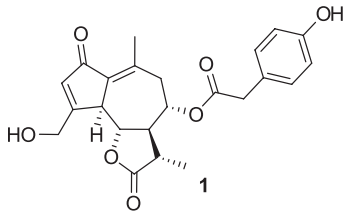
Phytochemical study of the acetonitrile extract of the aerial parts of *Ononis natrix* has resulted in the isolation and identification of 17 resorcinol derivatives and 4 flavonoids. Among these we could succeed in identification of two new compounds. The isolated natural products were evaluated for their antimicrobial, antimalarial, antitrypanosomal, cytotoxic and antioxidant activity.

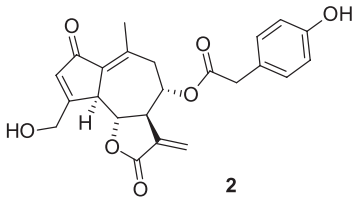
ISOLATION OF SESQUITERPENE LACTONES FROM ROOTS OF *CICHORIUM INTYBUS* L. WITH LEISHMANICIDAL ACTIVITY

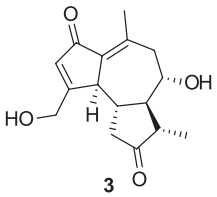
Thirumurugan Rathinasabapathy, *Patricio Rojas-Silva* Alexander Poulev, Slavko Komarnytsky, Ilya Raskin

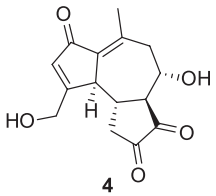
Rutgers, The State University of New Jersey, Plant Biology and Pathology Department, New Brunswick, NJ 08901, USA.

In our effort to seek novel leishmanicidal bioactives from medicinal plants, we isolated four sesquiterpene lactones from the roots of *Cichorium intybus* (Asteraceae), known as chicory, and tested their bioactivity against *Leishmania tarentolae*. Chicory roots infusion has been used as an effective antimalarial treatment in Afghanistan, a country where the prevalence of Leishmaniasis is also very high. Dried and powdered chicory roots were extracted with methanol for 24 hours at room temperature, and then the methanolic extract was filtered, concentrated and dried under vacuum. Next, the methanolic extract was partitioned with *n*-hexane and ethyl acetate. The ethyl acetate extract was then fractionated using fast centrifugal partition chromatography (FCPC) yielding 12 different fractions that were further purified by preparative HPLC. Fraction 5 yielded two compounds identified by LC-MS as 11(S),13-dihydrolactucopicrin (**1**) and lactucopicrin (**2**), respectively. Fractions 8 and 9 were combined and purified together to yield two compounds identified by LC-MS as 11(S),13-dihydrolactucin (**3**) and lactucin (**4**). All the compounds were confirmed by ^{1}H-NMR. The IC_{50} leishmanicidal activity for 2 was the highest (24.8 μM), while the remaining compounds showed low activity >50 μM. This study provides new perspectives on the development of sesquiterpene lactones into novel leishmanicidal drugs and supports lactucopicrin as a candidate for further studies.









METABOLITES FROM THE SILKWORM (*BOMBYX MORI* L.) DROPPINGS PROMOTE THE ACTIVITY OF HO-1 AND SIRT1

Ji-Hae Park, Jin-Gyeong Cho, Dae-Young Lee, Do-Gyeong Lee, Su-Yeon Kim, Nam-In Baek

Graduate School of Biotechnology & Graduate School of Oriental Medicinal Materials & Processing, Kyung Hee University, Yongin, 446–701, South Korea

Silkworm droppings are excrements of the silkworm, *Bombyx mori* L, whose alcohol extracts improve some skin troubles caused by atopy. So, this study was initiated to isolate the principal compounds to manifest the activity of silkworm droppings. Dried and powdered silkworm droppings were extracted with 80% aq. MeOH, and the concentrated extract was partitioned with EtOAc, *n*-BuOH and H_2O, successively. The repeated silica gel and ODS column chromatography of the EtOAc and *n*-BuOH fractions led to isolation of 25 metabolites. From the result of spectroscopic data including NMR, EI/MS, FAB/MS, polarimetry, and IR six lignans, five flavonoids, seven megastigmene norsesquiterpenes, and seven hydroxyl fatty acidswere identified. Three compounds have never been reported in nature, and the other twenty-two compounds were also isolated for the first time from silkworm droppings. Seven compounds among them increased expression of heme oxygenase-1 (HO-1) in HepG2 cells, and two compounds increased the expression of SIRT1 in HepG2 and HEK239 cells, respectively. The enzymes are involved in suppression of inflammatory mediators or factors that may be used to improve atopy-related symptoms.

A BOTANIC LEAD OF ATTENTION DEFICIT HYPERACTIVITY DISORDER

Che-Yi Lin, Jui-Ching Chen, Cheng-Po Huang, Chien-Chang Wu, Yuan-Ling Ku

Medical and Pharmaceutical Industry Technology and Development Center, New Taipei, 24886, Taiwan.

PDC-1421, an extract acquired by a single Traditional Chinese Medicine, demonstrated a great response in tetrabenazine-induced hypothermia. We distinguish the main mechanism of PDC-1421 from the pharmacology in central nervous system. The IC_{50} of norepinephrine transporter (NET), Dopamine transporter (DAT), and Serotonin transporter is 1.27, 76.4, and greater than 300 μg/mL, respectively. The IC_{50} of NE uptake is 0.704 μg/mL in HEK293 cells, but the IC_{50} of both dopamine and serotonin uptake are more than 100 μg/mL. In this study, we identified PDC-1421 as a major NET inhibitor and minor DAT inhibitor for pharmacodynamics. Currently, stimulant drugs that are effective against attention deficit hyperactivity disorder (ADHD) are thought to work by altering the levels in either norepinephrine or the synergistic effect of dopamine and norepinephrine. These results indicated that PDC-1421 may be designated as the botanic lead of ADHD.

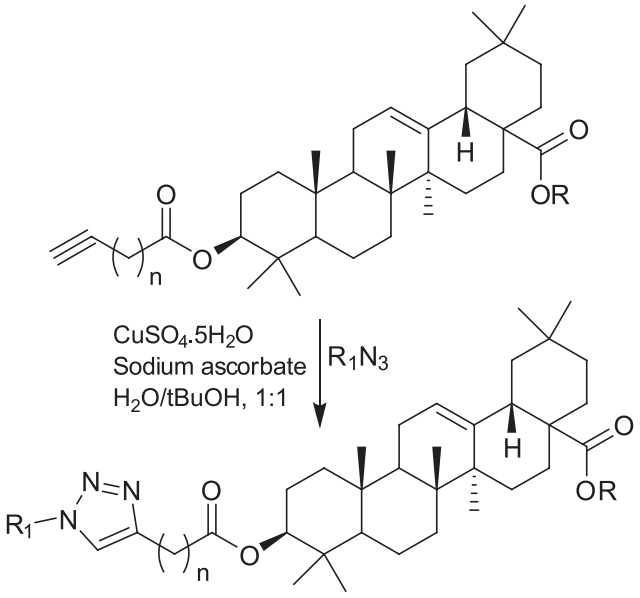
1,2,3-TRIAZOLE-SUBSTITUTED OLEANOLIC ACID DERIVATIVES: SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY

Mariano Walter Pertino,[1] Cecilia López,[1] Cristina Theoduloz,[2] Guillermo Schmeda-Hirschmann[1]

[1]*Universidad de Talca, Instituto de Química de Recursos Naturales, Talca, VII Region, Chile,* [2]*Universidad de Talca, Facultad de Ciencias de la Salud, Talca, VII Region, Chile*

Starting from the naturally occurring triterpene oleanolic acid, alkyl esters were prepared and treated with different aromatic azides to produce "hybrid" compounds using click chemistry. The antiproliferative activity of the new triterpene derivatives was evaluated towards normal lung fibroblasts (MRC-5), gastric epithelial adeno-carcinoma (AGS), promyelocytic leukemia (HL-60), lung cancer (SK-MES-1), and bladder carcinoma (J82) cells.

Acknowledgements: This work was supported by FONDECYT N° 11100046.



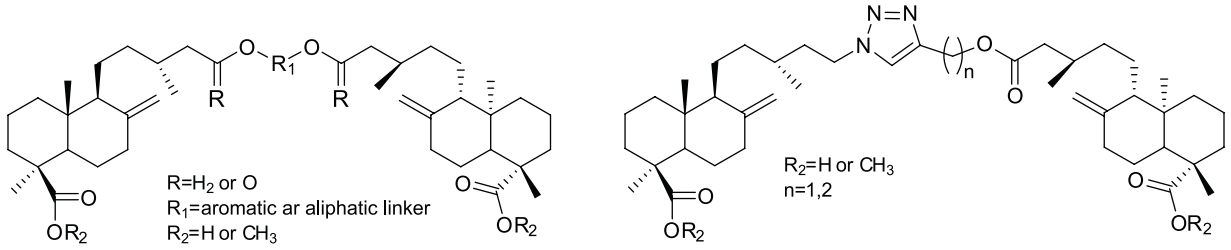
NEW DIMERIC DITERPENES: SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY

Mariano Walter Pertino,[1] Marco Francisco Bastias,[1] Cristina Theoduloz,[2] Guillermo Schmeda-Hirschmann[1]

[1]*Universidad de Talca, Instituto de Química de Recursos Naturales, Talca, VII Region, Chile,* [2]*Universidad de Talca, Facultad de Ciencias de la Salud, Talca, VII Region, Chile*

Several labdane diterpenes have shown biological activity, including antiproliferative effects. Little has been done on the synthesis of dimeric diterpenes using different linkers. The diterpenes labd-8(17)-en-15-hydroxy-19-oic acid (imbricatolic acid) and labd-8(17)-en-15,19-dioic acid (junicedric acid) were used as terpene moieties to prepare dimeric compounds. The new compounds include ethers and esters with different 'linkers' (spacers) as well as 1,2,3-triazole-substitute derivatives prepared by click chemistry. The antiproliferative activity of the new compounds was assessed against normal lung fibroblasts (MRC-5) and four different cancer cell lines including: gastric epithelial adenocarcinoma (AGS), promielocytic leukemia (HL-60), lung cancer (SK-MES-1), and bladder carcinoma (J82) cells.

Acknowledgements: This work was supported by FONDECYT grants 11100046 and 1085306.



HAGININ A, A PTP1B INHIBITOR FROM THE BRANCH OF *LESPEDEZA CYRTOBOTRYA*, STIMULATES GLUCOSE UPTAKE THROUGH AMPK ACTIVATION IN SKELETAL MUSCLE CELLS

Jin Hee Kim,[1] Eun Sook lee,[1] Min Kyun Na[2]

[1]*Daegu Hanny University, Department of Herbal Skin Care, Gyeongsan, 712–715, South Korea,* [2]*Yeungnam University, College of Pharmacy, Gyeongsan, 712–749, South Korea*

Protein tyrosine phosphatase 1B (PTP1B) negatively regulates insulin signaling, and PTP1B inhibitors have been seen as promising therapeutic agents against obesity and type 2 diabetes. We identified haginin A, an isoflavonoid compound, as one of the major components of the branch of *Lespedeza cyrtobotrya*, and an inhibitor of PTP1B by screening an extract library of Korean natural plants. Haginin A inhibits PTP1B activity with IC_{50} values of 3.75 ± 0.6 μM in a competitive inhibitor of PTP1B, with a K_i value of 2.5 μM. Also, haginin A dose-dependently stimulated glucose uptake in differentiated L6 rat myoblast cells. Western blotting analyses revealed that haginin A increased the phophorylation level of AMPK and acetyl-CoA carboxylase (ACC). In addition, it enhanced insulin-mediated Akt activation. In summary, AMPK activation was involved in effects of haginin A on glucose transport activation and insulin sensitivity. Haginin A can be further developed as potential compound for anti-diabetic therapy.

ANTIVIRAL ACTIVITIES OF THE AQUEOUS EXTRACT FROM PEAT MOSS AGAINST INFLUENZA VIRUS *IN VITRO* AND *IN VIVO*

Ha-Na Youn,[1] Yu-Na Lee,[1] Dong-Hun Lee,[1] Jae-Keun Park,[1] Seong-Su Yuk,[1] Yong-Kee Jeong,[2] Joong-Bok Lee,[1] Seung-Yong Park,[1] In-Soo Choi,[1] Chang-Seon Song,[1] Kwon-Ho Jo[3]

[1]*Avian Disease Laboratory, College of Veterinary Medicine, Konkuk University, Seoul, 143–701, South Korea,* [2]*Department of Biotechnology, College of Natural Resources and Life Science, Dong-A University, Busan, 604–714, South Korea,* [3]*Blue Bio Industry Regional Innovation Center (BBI-RIC) Dongeui University, #24 Gaya-dong, Busanjin, Busan, 604–714, South Korea*

Peat moss (PM) is the decomposing, dead, parts of sphagnum moss that usually are found deep in a bog. It has long been used as folk medicine to treat bacterial infection. Here we tested the inhibitory activity of water extract from PM towards influenza A virus *in vitro* and *in vivo*. *In vitro* anti-influenza virus activities of PM extract evaluated using influenza A/NWS/33 (H1N1) virus by the Neutral Red assay on MDCK cells. PM extract exhibited inhibitory activities against A/NWS/33 (H1N1) with 50% effective concentration (EC_{50}) values ranging from 3.2 to 10 μg/ml. The mean 50% cytotoxic concentration (CC_{50}) value of PM extract in the MDCK cells showed 930.30 μg/ml. The antiviral activity of PM extract was further evaluated using murine influenza virus infection model. The mice were infected intranasally with influenza A/NWS/33 (H1N1) virus, and the extracts were orally administered at 10 and 100 mg/kg once daily for 5 days beginning 4 h pre-virus exposure. In this infection model, PM extract was significantly effective at 100 mg/kg in increasing survival rate (40%) of infected mice,

whereas all of the mice in the control group were died. The dose of 10 mg/kg also increased the survival rate (20%) and the survival times of infected mice, although not reaching statistical significance. In the present study, peat moss playing a role as antiviral inhibitor during influenza virus infection was considered to be less toxic and highly protective against influenza infection. Therefore, peat moss may warrant further evaluation as a possible therapy for influenza.

ECKLONIA CAVA EXTRACT PREVENTS AGGREGATION OF β-AMYLOID AND REDUCES β-AMYLOID MEDIATED NEURONAL DEATH.

Young Eun Jeon, Xing Fu Yin, Dan Bi Choi, Min Ju Kim, Il-Jun Kang

Department of Food Science and Nutrition & Hallym International Medical Tourism Education Center, Hallym University, Chuncheon, 200–702, South Korea

β-amyloid (βA) is a major pathogenic peptide for Alzheimers disease (AD) and is generated by the processing of amyloid precursor protein (APP). The βA monomers aggregate into oligomeric and fibrillar forms which have been implicated as the toxic species inducing the neuronal dysfunction. Brown algae *Ecklonia cava* is known for its anti-oxidant and anti-inflammatory functions. Therefore, we tested the effect of *E. cava* extract on the production and aggregation of βA peptides. The extract of *E. cava* reduced βA secretion from HEK293 cells expressing APP with Swedish mutation and increased soluble APPβ and C-terminal fragment-β (CTFβ), of which activity was similar to BACE (β-site of APP cleaving enzyme) inhibitors. Furthermore, the extract inhibited βA oligomerization, particularly mid-size oligomer formation, confirmed by the ultrastructural morphology. Congo red, thioflavin T assays, and electron microscopy showed that the extract inhibited βA fibril formation effectively. Finally, the extract protected primary cortical neurons from various βA-induced cell deaths, especially oligomer-induced death. Although further study is needed to test the effectiveness of the extract *in vivo*, our results demonstrate, for the first time, that the extract of *E. cava* could be used as an anti-βA agent for AD therapeutics.

NELUMBO NUCIFERA RHIZOME EXTRACT AMELIORATES THE SCOPOLAMINE-INDUCED REDUCTIONS OF CELL PROLIFERATION, NEUROBLAST DIFFERENTIATION AND BDNF LEVELS

Xing Fu Yin, Young Eun Jeon, Dan Bi Choi, Il-Jun Kang

Department of Food Science and Nutrition & Hallym International Medical Tourism Education Center, Hallym University, Chuncheon, 200–702, South Korea

This study examined the effects of *Nelumbo nucifera* rhizome extracts (NRE) on cell proliferation and neuroblast differentiation in the hippocampal dentate gyrus (DG) of a rat model of scopolamine-induced amnesia. Immunohistochemical markers included Ki67, an endogenous marker for active cell cycle, and doublecortin (DCX), a marker for immature neurons and migratory neuroblasts. Scopolamine was administered for 28 days via an ALzet minipump (44 mg/mL delivered at 2.5 μL/h). NRE was administered by gavage, 1 g/kg per day for 28 days. The administration of scopolamine significantly reduced the number of Ki67- and DCX-immunoreactive cells in the DG, whereas scopolamine did not induce any significant changes in mature neurons. The administration of NRE significantly ameliorated the scopolamine-induced reduction of Ki67- and DCX-immunoreactive cells in the DG. In addition, the administration of NRE significantly restored the scopolamine-induced reduction of brain-derived neurotrophic factor in DG homogenates. These results suggest that NRE can ameliorate the scopolamine-induced reductions of cell proliferation, neuroblast differentiation and BDNF levels.

ANTIMICROBIAL EVALUATION OF A FOCUSED NARINGENIN AND RESVERATROL CHEMICAL LIBRARY

Dianqing Sun,[1] Julian G. Hurdle,[2] Robin E. Lee,[3] Li Shen,[1] Tamara P. Kondratyuk,[1] Richard E. Lee,[3] Mark Cushman,[4] John M. Pezzuto[1]

[1]University of Hawaii at Hilo, College of Pharmacy, Hilo, HI 96720,USA, [2]University of Texas at Arlington, Department of Biology, Arlington, TX 76019, USA, [3]St Jude Children's Research Hospital, Department of Chemical Biology and Therapeutics, Memphis, TN 38105, USA, [4]Purdue University, College of Pharmacy, West Lafayette, IN 47907, USA

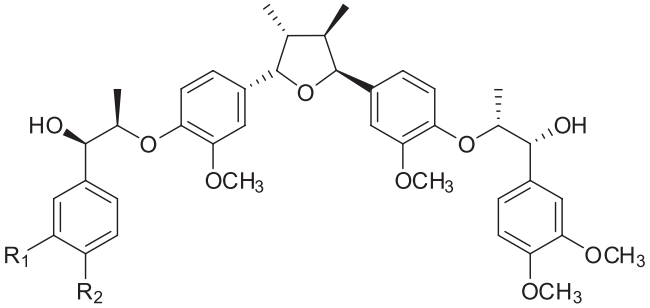
Flavonoids are a class of important naturally occurring secondary metabolites in plants which have been reported to mediate a variety of biological responses. One such compound, (2*S*)-naringenin was recently reported to show good antituberculosis activity (MIC = 2.8 μg/mL) (Chem. Biodivers., 2010, 1814). Inspired by this report, a small focused flavonoid and resveratrol library was screened against a panel of Gram-positive and -negative bacterial pathogens. Among the naringenin, resveratrol, and analogs evaluated, abyssinone II, a naturally occurring flavonoid bearing a lipophilic prenyl group, demonstrated relatively good activity against *M. tuberculosis* (H37Rv), *E. faecalis* (ATCC 29212), *S. aureus* (N315), and *S. pneumoniae* (HM162), with MIC values of 50, 25, 12.5 and 25 μg/mL, respectively. However, racemic naringenin only showed marginal activity in our TB assay (MIC = 200 μg/mL). None of the tested compounds was active against Gram-negative bacteria. Based on these data, abyssinone II was selected as a chemical starting point for further medicinal chemistry optimization in an attempt to identify advanced experimental candidates with antimicrobial therapeutic potential.

NEUROPROTECTIVE EFFECTS OF ETHYL ACETATE EXTRACT OF *CODONOPSIS LANCEOLATA* ON ISCHEMIC DAMAGE IN GERBIL

Dan Bi Choi, Young Eun Jeon, Xing Fu Yin, Il-Jun Kang

Department of Food Science and Nutrition & Hallym International Medical Tourism Education Center, Hallym University, Chuncheon, 200–702, South Korea

We observed the neuroprotective effects of ECLs treatment on ischemic damage in the gerbil hippocampal CA1 region four days after an ischemic insult. Among the 10 ECLs, Ethyl acetate Extracts of Raw and Steamed *Codonopsis lanceolata* (EERCL and EESCL) showed significant neuroprotection: the percentage of neurons remaining after treatment with EERCL and EESCL was 72.7% and 68.4% of that seen in the sham-ischemia group, respectively. The administration of EERCL and EESCL significantly decreased the reactive gliosis of microglia compared with that seen in the vehicle-treated ischemia group. In addition, SOD1 and BDNF immunoreactivity in the EERCL- and EESCL-ischemia groups were markedly increased compared with that in the vehicle-treated ischemia group. These results suggest that the administration of EERCL and EESCL can reduce ischemic neuronal loss potentially by maintaining SOD1 and BDNF immunoreactivity in the ischemic hippocampal CA1 region.



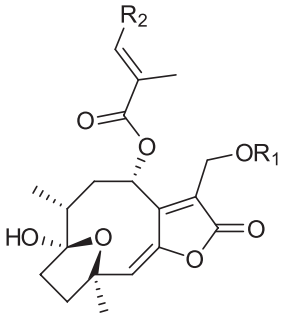
1: $R_1 = R_2 = OCH_3$
2: $R_1 = R_2 = -OCH_2O-$

BIOLOGICALLY ACTIVE CONSTITUTES FROM THE FLOWER OF *VERNONIA CINEREA*

Ui Joung Youn,[1] Eun-Jung Park,[1] Tamara P. Kondratyuk,[1] Onoomar Toyama,[3] Thanapat Songsak,[4] Supakit Wongwiwatthananukit,[2] John M. Pezzuto,[1] Leng Chee Chang[1]

[1]*College of Pharmacy, University of Hawaii, Department of Pharmaceutical Sciences, Hilo, HI 96720, USA,* [2]*College of Pharmacy, University of Hawaii, Department of Pharmacy Practice, Hilo, HI 96720, USA,* [3]*Faculty of Pharmacy, Silpakorn University, Department of Pharmaceutical Chemistry, Nakhon Pathom, 73000, Thailand,* [4]*Faculty of Pharmacy, Rangsit University, Department of Pharmacognosy, Pathumtani, 73000, Thailand*

Bioassay-guided fractionation of the methanol extract of *Vernonia cinerea* (Asteraceae) led to the isolation of three sesquiterpene lactones, 8α-tigloyloxyhirsutinolide-13-*O*-acetate (**1**), 8α-tigloyloxyhirsutinolide (**2**), and 8α-(2-methylacryloyloxy)-hirsutinolide-13-*O*-acetate (**3**), along with two flavonoids (**4** and **5**) and phthalic acid (**6**). The structure and absolute stereochemistry of these compounds (**1**-**3**) were determined on the basis of 1D and 2D NMR experiments. All six compounds inhibited LPS induced NO formation with cultured RAW 264.7; compounds 1-3 were the most active (IC_{50} = 1.9, 6.6, and 5.7 μM, respectively).



1: R_1 = Ac, $R_2 = CH_3$
2: R_1 = Ac, R_2 = H
3: R_1 = H, $R_2 = CH_3$

INHIBITION OF SREBP-1C-DEPENDENT HEPATIC STEATOSIS BY SAUCHINONE, AN AMPK-ACTIVATING LIGNAN IN *SAURURUS CHINENSIS*

Il Je Cho,[1] *Young Woo Kim*,[1] Sang Chan Kim,[1] Sang Geon Kim,[2] Sung Hui Byun[1]

[1]*Daegu Haany University, College of Oriental Medicine, Daegu, 706-060, South Korea,* [2]*Seoul National University, College of Pharmacy, Seoul, 151–742, South Korea*

The building-up of fat in the liver results from delivery of a large amount of fatty acids to hepatocytes and impaired export of triglyceride, which is eventually related with chronic liver diseases in association with systemic metabolic dysfunction. Sauchinone as an AMPK-activating lignan in *Saururus chinensis* has been shown to prevent iron-induced oxidative stress and liver injury. Previously, we identified the role of AMPK in LXRα-mediated SREBP-1c-dependent lipogenesis. Since sauchinone as an antioxidant has the efficacy to activate AMPK, this study investigated its effects on SREBP-1c-dependent lipogenesis in hepatocytes, and high fat diet (HFD)-induced hepatic steatosis and oxidative injury. Sauchinone prevented the ability of T0901317 to activate SREBP-1c, repressing transcription of the *fas*, *acc*, *scd-1*, *abca1*, and *LXRα* genes. Consistently, HFD feeding to mice caused fat accumulation in the liver with SREBP-1c induction, which was attenuated by sauchinone treatment. Also, sauchinone had the ability to inhibit oxidative stress as shown by decreases in TBARS formation, nitrotyrosinylation, and 4-hydroxynonenal production. Moreover, it prevented not only liver injury, but also AMPK inhibition elicited by HFD feeding. These results demonstrate that sauchinone has the capability to inhibit LXRα-mediated SREBP-1c induction and SREBP-1c-dependent hepatic steatosis, thereby protecting hepatocytes from oxidative stress induced by fat accumulation.

EVALUATION OF SMOKING CESSATION AGENTS FROM NATURAL PRODUCTS

Danny Tudor,[1] Devin Harden,[1,2] Aaron Behm,[2] David Montgomerie,[2] Chad Ahia,[1] Supakit Wongwiwatthananukit,[1] Anthony Otsuka,[1] *Leng Chee Chang*[1]

[1]*College of Pharmacy, University of Hawaii Hilo, Hilo, 96720, USA,* [2]*Dept. of Biology, University of Hawaii Hilo, Hilo, HI 96720, USA*

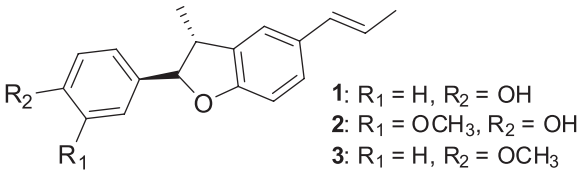
C. elegans is a useful model for the study of nicotine-dependent behaviors. We hypothesized that current smoking cessation drugs (bupropion, varenicline, and mecamylamine) would exhibit similar effects in *C. elegans*. An egg-laying (el) assay was used to study each drug's ability to inhibit the el response in worms after being acutely exposed to nicotine. Egg-laying is dependent on the nicotinic acetylcholine receptor system. All three drugs produced a significant decrease in el behavior when worms were subsequently exposed to nicotine, compared with a control group. The ability of drugs to behaviorally block treated worms from seeking nicotine was studied in a chemotaxis assay. We hypothesized that *C. elegans* cohorts naïve to nicotine will be attracted to the nicotine side of the plate, but worms that were treated with drugs should be equally attracted to both the nicotine and non-nicotine sides of the plate. The nicotine side was significantly favored by naïve worms compared with the non-nicotine side. Compared to naïve worms, those worms treated with all three drugs were less attracted toward the nicotine side of the plate. As a negative control, worms treated with the green tea compound (i.e., EGCG), were significantly attracted to the nicotine side of the plates. We discovered that worms treated with *Phytolaccalatbenia* or *Fagonia critica* crude extracts were significantly less attracted toward the nicotine side of the plate.

CYTOTOXIC DIHYDROBENZOFURANS FROM *MITREPHORA WANGII* HU

Patcharawan Tanamatayarat,[1,2] Uthai Sotanaphun,[2] Siripan Limsirichaikul,[3] Sisay Girmay,[4] *Leng Chee Chang*,[4] Ghee Tan,[4] Onoomar Toyama[1]

[1]*Department of Pharmaceutical Chemistry,* [2]*Department of Pharma-cognosy,* [3]*Department of Biopharmacy, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand 73000,* [4]*Dept. Pharmaceutical Sciences, College of Pharmacy, University of Hawaii Hilo, Hilo, HI 96720, USA*

(+)-(*2R, 3R*)-2,3-Dihydro-2-(4-hydroxyphenyl)-3-methyl-5[1-(*E*)-propenyl] benzofuran or conocarpan (**1**) and two methoxyl derivatives (**2** and **3**) were isolated from the leaf hexane extract of *Mitrephora wangii* Hu. Compounds **1** and **2** exhibited significant inhibitory activity against *Streptomyces* 85E in the hyphae-formation inhibition assay, with clear zones of inhibition of 21 and 11 mm, respectively. Compounds **1** and **2** further inhibited growth of human leukemic monocyte lymphoma (U937) cells with IC_{50} values of 6.3 and 5.9 μg/ml, respectively. Compound **1** also demonstrated a strong inhibitory activity against human lung carcinoma (LU-1) cells with the IC_{50} value <5 μg/ml. Moreover, compound **3** was isolated from plant and from this genus as *2R, 3R* configuration for the first time.



UNCOVERED THERAPEUTIC POTENTIAL OF MARINE RED ALGAE FROM NEW CALEDONIA

Veronique Guillemard,[1,2] Alain Videault,[1,2] Sofia-Elena Motuhi,[2] Claude Payri[2]

[1]*Government of New Caledonia, Department of Health, Noumea, 98800, New Caledonia,* [2]*IRD, Natural Product Research Unit and COREUS Research Unit, Noumea, 98800, New Caledonia*

Marine plants, such as algae, have been investigated for decades and have been shown to be an important source of molecules with therapeutic, agrochemical and botanical potential. To date, little is known regarding bioactivities of red algae from New Caledonia. In order to evaluate their potential, we collected several species of red algae based on availability and easy access. Extractions, partitions and fractionations were successively done and samples were screened for therapeutic potential against a wide range of bacterial pathogens and human cancer cell lines. For the first time, we have uncovered the biological activities of several species with some of them showing cellular selectivity. Our findings may lead to the discovery, identification and isolation of new molecules/leads to serve as candidates for the development of novel therapeutics.

DEVELOPMENT OF POTENTIAL CNS THERAPEUTICS DERIVED FROM THE ALKALOID CYTISINE

Christoph Eibl,[1] Isabelle Tomassoli,[1] Matthias Wulf,[1] Corina Apuakehau,[1,2] Daniela Guendisch[1]

[1]*University of Hawaii at Hilo, College of Pharmacy, Pharmaceutical Sciences, Hilo, HI 96720, USA,* [2]*Hawaii Community College, Hilo, HI 96720, USA*

(–)-Cytisine, a potent nAChR ligand, and its structural frameworks (3,7-diazabicyclo[3.3.1]nonane and 2-pyridone) serve as invaluable templates in the development of novel bioactive compounds. In this study, cytisine isolated from *Laburnum anagyroides* and also its 3,7-diazabicyclo[3.3.1]nonane scaffold were used to develop new compounds applying e.g. the hybrid or twin drug approach with other natural products. The compounds synthesized were tested for their affinities for different nAChR subtypes using radioligand binding assays. A broad spectrum of affinities (e.g. K_i values for $\alpha4\beta2$*: <1 nM to >10,000 nM) provided important insights into structure-affinity relationships. The novel compounds could be useful for further development of therapeutics to treat disorders involving nAChR dysfunction.

Funding: This work was supported by P20RR016467

ANTI-PROLIFERATIVE DINEOLIGNANS FROM *SAURURUS CHINENSIS* AGAINST HUMAN CANCER CELL LINES

You-Jin Lee, Jin-Mu Yi, *Jun Lee*, No Soo Kim, Jinhee Kim, Hae-Jin Kim, Dal-Seok Oh, Se-Mi Oh, Ok Sun Bang

Korea Institute of Oriental Medicine (KIOM), Cancer Research Center, Daejeon, 305–811, South Korea

Activity-guided fractionation of an EtOAc-soluble fraction of *Saururus chinensis* afforded two anti-proliferative di-neolignans, manassantin A (**1**) and B (**2**) along with four flavonoids and four aristolactams. Their chemical structures were identified by spectroscopic methods. Compounds **1** and **2** were evaluated for their anti-proliferation activities against 28 human cancer cell lines and 2 human normal lung cell lines using MTS assay. Compounds **1** and **2** showed a potent anti-proliferation activity against cervical (C33a, IC_{50} = 0.015 μM for **1**; 0.277 μM for **2**) and lung (NCI-H460, IC_{50} = 0.049 μM for **1**; 1.368 μM for **2**) cancer cell lines without any remarkable cytotoxic effect on normal lung cell lines (IC_{50}>10 μM). C33a cells treated with these compounds showed marked ERK inhibition possibly due to Raf inactivation. Together, these data demonstrated the identification of anti-proliferative dineolignans and its possible mechanism of action.

INHIBITION OF LXRα-MEDIATED HEPATIC STEATOSIS BY LIQUIRITIGENIN, A LICORICE FLAVONOID, IN ASSOCIATED WITH NRF2 ACTIVATION

Young Woo Kim,[1] Il Je Cho,[1] Sang Geon Kim,[2] Sang Chan Kim[1]

[1]*Daegu Haany University, College of Oriental Medicine, Daegu, 706-060, South Korea,* [2]*Seoul National University, College of Pharmacy, Seoul, 151–742, South Korea*

Nonalcoholic fatty liver disease is considered as a major hepatic constituent of the metabolic syndrome. LXRα functions a major regulator of lipid homeostasis through activation of SREBP-1c, which promotes hepatic steatosis and

steatohepatitis. Nrf2 is the crucial transcription factor necessary for the induction of anti-oxidant enzymes. This study investigated the potential of liquiritigenin (LQ), a hepatoprotective flavonoid in licorice, to inhibit LXRα-induced hepatic steatosis, and the underlying mechanism of the action. LQ treatment attenuated fat accumulation and lipogenic gene induction in the liver of mice fed a high fat diet. Also, LQ had the ability to inhibit oxidative liver injury, as shown by decreases in thiobarbituric acid reactive substances formation and nitrotyrosinylation. Moreover, LQ treatment antagonized T0901317-mediated SREBP-1c activation, and transactivation of the lipogenic target genes. LQ was found to activate Nrf2, and the ability of LQ to inhibit LXRα-mediated SREBP-1c activation was reversed by a deficiency of Nrf2, which supports the inhibitory role of Nrf2 in LXRα-dependent lipogenesis. Consistently, treatment with other Nrf2 activators or forced expression of Nrf2 also inhibited LXRα-mediated SREBP-1c activation. Our results demonstrate that LQ has an efficacy to activate Nrf2, which contributes to inhibiting the activity of LXRα that leads to SREBP-1c induction and hepatic steatosis.

RESVERATROL PREVENTS MITOCHONDRIAL DYSFUNCTION THROUGH PARP-LKB1 ACTIVATION

Mi Jeong Jo,[1] Sang Mi Park,[1] Il Je Cho,[1] Sang Geon Kim,[2] Seon Young Jee[1]

[1]*Daegu Haany University, Department of Oriental Medicine, Daegu, 706-060, South Korea,* [2]*Seoul National University, Department of Pharmacy, Seoul, 151–742, South Korea*

This study investigated the potential of resveratrol to protect against the mitochondrial impairment induced by arachidonic acid (AA)+iron and the underlying mechanism for this cytoprotection. Resveratrol treatment inhibited apoptosis, ROS production, and GSH depletion elicited by AA+iron in HepG2 cells. Also, resveratrol attenuated superoxide generation and inhibited mitochondrial dysfunction induced by AA+iron. Overall, AMPK activation by resveratrol contributed to cell survival, as supported by the reversal of mitochondrial membrane potential by either overexpression of a dominant negative mutant of AMPK or compound C treatment. Resveratrol increased inhibitory phosphorylation of GSK3beta downstream of AMPK, which contributed to mitochondrial protection and cell survival. Likewise, siRNA knockdown of LKB1 reduced the ability of resveratrol to protect cells from mitochondrial dysfunction. Furthermore, this LKB1-dependent mitochondrial protection resulted from poly(ADP-ribose)polymerase (PARP) activation, but not SIRT1 activation, as supported by the experiment using 3-aminobenzamide, a PARP inhibitor. Other polyphenols, such as apigenin, genistein, and daidzein, did not activate AMPK, nor did they protect mitochondria against AA+iron. Thus, resveratrol protects cells from AA+iron-induced ROS production and mitochondrial dysfunction through AMPK-mediated inhibitory phosphorylation of GSK3beta downstream of PARP-LKB1 pathway.

ANTI-INFLAMMATORY EFFECTS OF ISATIDIS RADIX AND ITS ACTIVE COMPONENT, TRYPTANTHRIN IN LIPOPOLYSACCHARIDE-ACTIVATED RAW264.7 MACROPHAGE CELLS

Mi Jeong Jo, Sang Mi Park, Sook Jahr Park, Jong Rok Lee, Sung Hui Byun, *Sang Chan Kim*

Daegu Haany University, College of Oriental Medicine, Daegu, 706-060, South Korea

Isatidis Radix, the dried root of *Isatis indigotica*, is categorized as a fever-reducing agent in East Asian traditional herbal medicine. The present study was conducted to evaluate the anti-inflammatory effects of Isatidis Radix and its components tryptanthrin and indigo in lipopolysaccharide (LPS)-activated Raw264.7 cells.

LPS-induced nitric oxide (NO) and prostaglandin E2 (PGE2) production were dose dependently decreased by the treatment of Isatidis Radix water extract (IRE) and tryptanthrin, while indigo had no effect. The inhibition of NO production by IRE and tryptanthrin was due to suppression of iNOS expression mediated from the inhibition of nuclear factor-κB (NF-κB) nuclear translocation and inhibitory-κ B α phosphorylation, as determined by Western blot analysis. In case of PGE2 inhibition, IRE and tryptanthrin did not reduce COX-2 expression, but they showed inhibitory effect of COX-2 activity. In addition, IRE inhibited production of inflammatory cytokines IL-1β and TNF-α.

These findings suggest that Isatidis Radix could produce an anti-inflammatory effect through inhibition of iNOS expression and COX-2 activity via NF-κB pathway, and that tryptanthrin might be one component contributing to anti-inflammation of IRE.

RED GINSENG EXTRACT PREVENTS CCl_4-INDUCED LIVER FIBROSIS

Sang Mi Park, Mi Jeong Jo, Sung Hui Byun, Sang Chan Kim, Il Je Cho

Daegu Haany University, Department of Oriental Medicine, Daegu, 706-06, South Korea

Korean Red Ginseng, the processed root of *Panax ginseng* Meyer, has been frequently used for various therapeutic purposes in oriental medicine and is now widely used around the world. The present study investigated the possible preventive effect of Red Ginseng Extract (RGE) for the treatment of liver fibrosis. We injected mice with multiple doses of carbon

tetrachloride (CCl_4) for 4 weeks and then used the animal to determine whether RGE treatment therapeutically improved liver functions and resolved fibers accumulated in the liver. Multiple CCl_4 injections caused elevated levels of ALT, AST and collagen accumulation. In contrast, concomitant treatment with RGE (30, 100, and 300 mg/kg) significantly reduced them in a dose-dependent manner. In histopathological analysis, RGE treatment decreased the percentages of degenerative regions, numbers of degenerative hepatocytes and collagen deposited percentages in hepatic parenchyma. In addition, RGE inhibited the mRNA level of transforming growth factor beta 1, plasminogen activator inhibitor 1 genes in fibrogenic liver. Moverover, RGE dose-dependently reduced the number of alpha smooth muscle actin-positive cells in liver tissue. Taken together, these results demonstrate that RGE can protect the CCl_4-induced liver fibrosis, partly via hepatic stellate cell inactivation.

TRADITIONAL CREE ANTI-DIABETIC MEDICINE: ADVANCED METABOLOMIC ASSESSMENT IN EXPERIMENTAL MODELS OF DIABETES AND ALZHEIMER'S DISEASE

Carolina Cieniak,[1,2,3] Fida Ahmed,[1,2,3] Matthew Taylor,[1,2,3] Camille Juzwik,[2,3] Asim Muhammad,[4] Brian C. Foster,[5] JohnT. Arnason,[1,3] Steffany A.L. Bennett[2,3]

[1]University of Ottawa, Ottawa-Carleton Institute of Biology, Ottawa, ON K1N6N5, Canada, [2]Neural Regeneration Laboratory, Department of Biochemistry, Microbiology, and Immunology, Ottawa, ON K1H8M5, Canada, [3]CIHR Training Program in Neurodegenerative Lipidomics, Ottawa, ON K1H8M5, Canada, [4]University of Ottawa, Dept. Chemistry, Ottawa, ONK1N6N5, Canada, [5]Health Canada, Office of Science, Therapeutic Products Directorate, Ottawa, ON K1A0K9, Canada

Type II diabetes (T2D) may be a risk factor for Alzheimer's Disease (AD), where AD patients have elevated insulin levels and decreased insulin sensitivity. However, the exact mechanism linking the two disease states remains to be elucidated. AD09, a plant used by the Cree of Eeyou Istchee in the treatment of diabetes, was used to treat TgCRND8 transgenic mice ectopically expressing human amyloid precursor protein with Swedish (KM670/671NL) and Indiana (V717F) mutations. TgCRND8 mice and their non-transgenic littermates were fed the AD09 EtOH extract, containing quercetin glycosides, morroniside and goodyeroside as well as other phenolics, at a concentration of 250 mg/kg daily for 2 months. The mice were then exposed to a variety of behavioural tests and glucose and insulin challenge to measure their metabolic response. We found that AD09 may increase insulin resistance in Tg mice while having no impact on glucose mobilization, with no effect observed in NonTg mice with mild behavioural impact on indices of learning, memory, anxiety, and mobility. (Supported by CIHR TGF-96121 and MOP6286 to JTA and SALB).

ANALYSIS OF *GINKGO BILOBA* LEVOPIMARADIENE SYNTHASE (LPS) PROMOTER IN *ARABIDOPSIS THALIANA*

Kim Jin-Hee, Lee Kwang-Il, Kim Soo-Un

Department of Agricultural Biotechnology, Seoul National University, Seoul, 151–921, South Korea

The major constituents of *G. biloba* are diterpeneoid ginkgolides which have potent anti-platelet antagonist factor activity. Levopimaradiene synthase (LPS) catalyzes the first committed step in ginkgolide biosynthesis pathway by converting geranylgeranyl pyrophosphate into levopimaradiene. A 2.2 kb promoter region of *GbLPS* was isolated and fused to a beta-glucuronidase (*GUS*) reporter gene. In *Arabidopsis thaliana*, the *GbLPS* promoter exhibited activities in developing young tissues. When cotyledons were fully open, the growth stage 1, GUS was intensively expressed in cotyledons. When two rosette leaves appeared, the growth stage 1.02, GUS expression was seen in the rosette leaves while GUS expressions in cotyledons disappeared. At 5-rosette leaf stage, growth stage 1.05, GUS expression was observed only in the newly formed leaves. In flower development, GUS was expressed in floral buds, carpel and growing ovaries. However, during the maturation of seed, no GUS expression was observed. In the roots, GUS was constitutively expressed in the vascular tissues before inflorescence emergence. After inflorescence emergence, GUS was expressed in the newly formed roots.

Acknowledgement: Financial Support by Systems and Synthetic Agrobiotech Center is appreciated.

UNRAVELING THE BIOSYNTHETIC CAPACITY OF MONOTERPENES IN SPECIALIZED EPITHELIAL CELLS OF GRAPEFRUIT PEEL

Siau Sie Voo, Glenn Turner, Bernd Markus Lange

Washington State University, Institute of Biological Chemistry, Pullman, WA 99164, USA

The essential oil of citrus peel, which consists primarily of monoterpenes, is synthesized in specialized epithelial cells lining secretory cavities. In order to obtain a quantitative understanding of essential oil formation in the secretory cavities, we have used grapefruit (*Citrus paradisi*) as our model for monoterpenoid essential biosynthesis in the genus

Citrus. We first determined the relationship between distribution and size of secretory cavities and essential oil production at different stages of fruit development. Essential oil biosynthesis starts when secretory cavities are formed at the very early stage of fruit formation. The oil production then enters an exponential phase when secretory cavities are expanding (fruit sizes between 30 and 100 mm), before slowing down to a linear increase. Second, we also investigated the secretory cavity content using micro-capillaries and subsequent chemical analyses. Our results indicate that about 95% of the oil is composed of monoterpenoids (with about 90% of limonene), about 5% is accounted for coumarins and methylated flavonoids, and less than 0.01% is due to the accumulation of fatty acids and sterols. Third, a cell type-specific transcript analysis was performed with isolated epithelial cells that actively synthesize essential oil. Cells were harvested using laser-capture microdissection and global gene expression patterns in these cells were assessed using Affymetrix GeneChip Citrus Genome Arrays. Transcript abundances of selected genes were also evaluated by quantitative real-time PCR. These analyses showed that genes involved in monoterpene biosynthesis are coordinately expressed in epithelial cells, concomitant with developmental changes in the accumulation of essential oil. Our goal is the integration of developmental, biochemical, and anatomical data sets into a comprehensive mathematical model of *Citrus* peel essential oil biosynthesis.

METABOLITE PROFILING OF TRITERPENE SAPONINS IN *MEDICAGO TRUNCATULA* CORE COLLECTION

Dong Sik Yang, Bonnie S. Watson, David Huhman, Shelagh Henson, Maria J. Monteros, Lloyd W. Sumner

The Samuel Roberts Noble Foundation, Ardmore, OK 73401, USA

Medicago truncatula is a model legume species that produces structurally diverse triterpene saponins with a wide range of bioactivities. Triterpene saponins were profiled in an *M. truncatula* core collection containing 63 lines which were determined to represent the major genetic diversity found in the USDA germplasm collection. High resolution metabolic profiling was used to assess the metabolic diversity of triterpene saponins in aerial and root tissues of the diverse lines using UPLC-QTof-MS. Principal component analysis revealed substantial biochemical variation in triterpene saponin content in the core collection. Comparative metabolite analyses revealed the total highest triterpene saponin level in core line 23, which was 7 times higher than that of the lowest line (core line 28). In addition, differential accumulation of 6 specific aglycone classes was observed in the core lines. As a more specific example, the highest accumulation of medicagenic acid saponins was identified in core line 23, which contained 124 times more medicagenic acid than the lowest accumulating line (core line 43). The metabolic profiling results provide high resolution triterpene saponin phenotypes for the core lines. The identified hyper- and hypo-saponin accumulating lines will be used for comparative gene expression analyses to identify putative genes involved in triterpene saponin biosynthesis.

VIRUS-INDUCED GENE SILENCING OF CYTOCHROMES P450 PUTATIVELY INVOLVED IN ALKALOID BIOSYNTHESIS IN OPIUM POPPY

Guillaume Beaudoin, Peter Facchini

University of Calgary, Department of Biological Sciences, Calgary, AB T2N1N4, Canada

Benzylisoquinoline alkaloids are nitrogenous, low-molecular weight compounds found in approximately 20% of plant species. These include the analgesics morphine and codeine, the anti-cancer compound noscapine, the vasodilator papaverine, and the anti-microbial agent sanguinarine. Each of these pathways has been shown or is predicted to involve at least one cytochrome P450 (CYP). However, only one CYP in morphine branch pathway and two CYPs in the sanguinarine branch pathway have been characterized. Several CYP gene candidates showing a correlation between transcript and specific alkaloid accumulation in opium poppy plants and cell cultures have been identified. Virus-induced gene silencing has been used to demonstrate the *in planta* relevance of our candidates. Preliminary results suggest that the silencing of some of these have an effect on the accumulation of sanguinarine, as well as perhaps other alkaloids in the roots of opium poppy. Additional studies used to characterize these enzymes will be discussed.

COMBINED GENOMIC-METABOLOMIC APPROACH FOR THE DIFFERENTIATION OF GEOGRAPHICAL ORIGINS OF NATURAL PRODUCTS

Sunghyouk Park

Inha University, Incheon, 400–712,South Korea and Seoul National University, Seoul, 151–742, South Korea

The correct identification of the geographical origins of natural products is essential to quality control, as their physiological effects correlate with chemical components. In this study, we applied both genomics and metabolomics to the origin identification of 101 deer antler samples from Canada, New Zealand, and Korea. The genomics identified deer

species in each country but failed to categorize all the samples, due to the presence of identical species in different countries. For identical species, NMR-based metabolomics gave clean separations, compounds specific to each country were identified, and the validity was confirmed by prediction analysis. As the genomics provided unambiguous read-outs for different species, and the metabolomics cleanly distinguished among identical species from different countries, their combined use could be a robust method for origin-identification even in difficult cases. We believe the method to be generally applicable to many herbal medicinal products for which various species are grown internationally.

ARABIDOPSIS FIBER–REDUCED (SND1/NST1) MUTANTS: NANOINDENTATION PROBING OF MECHANICAL PROPERTIES IN DISTINCT CELL WALL TYPES

Claudia L. Cardenas[1], Hong Yang[1], Chanyoung Ki[1], Laurence B. Davin[1],David Bahr[2], Norman G. Lewis[1]

[1]*Institute of Biological Chemistry, Washington State University, Pullman, WA 99164, USA,* [2]*School of Mechanical and Materials Engineering, Washington State University, Pullman, WA 99164, USA*

Nanoindentation analyses were used to measure mechanical properties within specific cell walls of wild-type (WT) and several *Arabidopsis* transcription factor mutant lines involved in cell-wall (fiber cell) development. These tests facilitated measurement of elastic modulus (E) and hardness (H) of individual cell wall types [interfascicular fibers (*if*), xylary fibers (*xf*) and xylem vessels (*xv*)] from stem cross- sections. Reductions in E values of *xf* and *if* of *snd1/nst1* were noted at the single cell level as compared to WT E values. Effects on elastic modulus within cell walls were observed from early to mature developmental stages (4, 5 and 8 weeks). Moreover, effects on physiological/mechanical properties were also observed on E values of *xv* of *snd1/nst1* at the mature stage as compared to WT E values. The *snd1/nst1* line displayed a prostrate phenotype with reductions in estimated lignin contents up to 53% of WT level. In addition, E values were determined on complementation of *Arabidopsis* with the NAC homologues from poplar, namely PtNAC1, PtNAC6, PtNAC7, PtNAC13 and PtNAC17. Pyrolysis GC/MS analysis of laser microdissected *vb* and *if* also further confirmed differences in lignin composition in their specific cell wall types.

PROMOTER ANALYSIS OF MULTI COPY *GINKGO BILOBA* 1-HYDROXY-2-METHYL-2-(E)-BUTENYL-4-DIPHOSPHATE REDUCTASE (IDS) GENE

Min-Kyoung Kang, Nargis Sultana, Sang-Min Kim, Soo-Un Kim

Seoul National University, Seoul, 151–921, South Korea

Isoprenoids are synthesized by condensation of five-carbon (isoprene) units, which are derived from two distinct routes in plants: cytosolic mevalonate (MVA) and plastidial 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways. 1-Hydroxy-2-methyl-2-(*E*)-butenyl-4-diphos-phate reductase (IDS) of *Ginkgo biloba* is an enzyme at the final step of the MEP pathway. The gene of the enzyme was cloned as a multi-copy gene in gymnosperms *Ginkgo biloba*. To evaluate the function of each isogene, the role of promoter of the isogene was ex-amined in *Arabidopsis*. Promoters of *GbIDS* series, *GbIDS1, 2,* and *2-1* were cloned and fused to *GUS*. The *GbIDS1*pro::*GUS* fusion showed expression in most tissues except for roots, petals, and stigma. The *GbIDS2*pro::*GUS* fusion showed expression in the young leaves and tissues, and internodes where the flower and shoot branched. There was no GUS expression observed in roots and reproductive tissues of the plants. The results alluded household and specific roles of *GbIDS1*, and *2* respectively.

Acknowledgement: Financial support by Systems and Synthetic Agrobiotech Center is appreciated

FUNCTIONAL CHARACTERIZATION OF *MEDICAGO TRUNCATULA* MYB TRANSCRIPTION FACTORS: MTMYB2 AND MTMYB70

Jihye Do,[1] Hyunjung Jin,[1] Young Im Choi,[2] Eun Woon Noh,[2] Jong-Sug Park,[3] Sun Hee Kim,[3] So Yun Kim,[3] Wook Kim[1] and *Mi Kwon*[1,4]

[1]*Division of Biotechnology, College of Life Science and Biotechnology, Korea University, Seoul 136–713, South Korea;* [2]*Division of Forest Biotechnology, Korea Forest Research Institute, Suwon 441-350, South Korea;* [3]*National Institute of Agricultural Biotechnology, Rural Development Administration, Suwon 441–707, Republic of Korea;* [4]*Institute of Life Science and Natural Resources, College of Life Science and Biotechnology, Korea University, Seoul 136–713, South Korea*

The family of MYB transcription factors is one of the most abundant classes of transcription factors in plants, and the subfamily containing the two-repeat R2R3 DNA-binding domain is the largest. A number of R2R3 MYB proteins have been shown to regulate the biosynthesis of phenolic compounds, including lignin. Researchers are interested in reducing lignin in plants because lignin interferes with cellulosic ethanol production. In order to understand the role of MYB transcription factors on the control of lignin biosynthesis during xylem formation, full-length cDNA of 21 MYB transcription factors of *Medicago truncatula* were isolated using primers specific to the conserved MYB domains via RACE. For

functional characterization of MYB transcription factor 2 and 70, over-expression constructs were individually transformed into the *Arabidopsis thaliana*. Among them, 35S::*MtMYB2* and 35S::*MtMYB70* displayed phenotypic changes relative to wild-type plants, which were alteration stem strength and the lignin contents. So, we generated *MtMYB2* and *MtMYB70* transgenic poplar using hybrid poplar (*Populus alba* x *P. glandulosa*). The existence of a single *MtMYB* gene in the hybrid poplar genome was supported by Southern hybridization. And we selected lines according to Northern blot analysis of *MtMYB* expression. Thus, the expression analysis of the lignin biosynthetic genes could be found genes encoding enzymes specific to lignin biosynthesis. In addition, histochemical analyses would be clearly demonstrated difference of lignin and cellulose deposition. In case of *MtMYB70*, we supposed to *MtMYB70* binding DNA motif using the universal protein binding microarray (PBM). These results suggest that *MtMYB* genes play an important role in the biosynthesis of lignin and the regulation of secondary cell wall formation.

GRANDISIN *IN VITRO* METABOLISM USING BIOMIMETICAL MODELS

Thais Guaratini,[1,2] Leandro Santis Ferreira,[1,2,5] Valeria Priscila Barros,[1] Daniel Roberto Callejon,[2] Marilda das Dores Assis,[3] Massuo Jorge Kato,[4] Hans Humpf,[5] Norberto Peporine Lopes[1]

[1]USP-FCFRP, Departamento de Física e Química, Ribeirão Preto, SP Brazil 14040-903, [2]Lychnoflora, Ribeirão Preto, SP 14040-900, Brazil, [3]USP-FFCLRP, Departamento de Química, Ribeirão Preto, SP 14040-901, Brazil, [4]USP-IQ, São Paulo, SP Brazil 05513-970, [5]Westfälische Wilhelms-Universität Münster, Institut für Lebensmittelchemie, Münster, 48149, Germany

As part of our ongoing project on the biomimetic oxidation reactions of natural products, we present the biomimetic oxidation of grandisin catalyzed by the Jacobsen reagent and the metabolism of this lignan by bacteria from pig ceacum. The samples were analyzed by using a validated UPLC-DAD methodology. The results observed with the Jacobsen reagent indicate the presence of five products, which generally resulted from the addition of hydroxyl groups. The major yielded compound was a di-hydroxylated product. Two minor signals related to a possible water elimination from the hydroxylate and tri-hydroxylate products were also observed. A third minor signal resulted from the oxidative cleavage of grandisin as previously observed with fungus metabolism. A fourth signal was related to a tetra-hydroxylated product. The results noted in pig ceacum model showed that no grandisin was metabolized at any time period studied. These are important findings for the *in vivo* study of metabolism.

Acknowledgements: FAPESP, CAPES and CNPq for the financial support.

PISUM SATIVUM IS A NOVEL BIOINFORMATICS PLATFORM TO STUDY PROANTHOCYANIDIN BIOSYNTHESIS

Kiva Ferraro,[1] Alena Jin,[2] Jocelyn Ozga,[2] Dae-Kyun Ro[1]

[1]University of Calgary, Biological Sciences, Calgary, AB T2N1N4, Canada, [2]University of Alberta, Department of Agricultural, Food and Nutritional Science, Edmonton, AB T6G2P5, Canada

Proanthocyanidins (PAs) are flavonoid polymers with strong antioxidant capabilities. Recent studies suggest human consumption of PAs convey numerous health benefits. The complete biosynthesis of PAs remains to be elucidated. Currently, *Arabidopsis thaliana* and *Medicago truncatula* are the model organisms used to study PAs. In both species, PA polymers are composed of 2,3-*cis*-flavan-3-ol monomers. There are numerous examples in nature of PA polymers composed of mixtures of 2,3-*cis*- and 2,3-*trans*-monomers. We initiated studies of PA metabolism in five *Pisum sativum* (pea) cultivars to understand PA diversity and to unravel unknown aspects of PA biosynthesis. Due to extensive breeding, various pea cultivars with distinctly different PA profiles, or lacking PAs, are readily available. Comparative genomics of different pea cultivars would allow us to gain unique insights into PA biosynthesis. Pyrosequencing and a microarray were performed to study differences in seed coat gene expression of five pea cultivars. Accordingly, quantitative PCR analyses confirmed the reliability of the genomics approach. Our results show comparative genomics can be utilized to explain differences in metabolic profiles naturally occurring within a species. Bioinformatics analysis implicated involvement of several previously unknown genes in PA biosynthesis. Work is currently in progress to characterize the roles of these new genes in PA biosynthesis.

METABOLOMIC CONSEQUENCES IN AROGENATE DEHYDRATASE AND NAC TRANSCRIPTION FACTOR MODIFIED *ARABIDOPSIS THALIANA*

Alan David Budgeon, Syed G.A. Moinuddin, Chanyoung Ki, Laurence B. Davin, Norman G. Lewis

Washington State University, Institute of Biological Chemistry (IBC) and Northwest Advanced Renewable Alliance (NARA), Pullman, WA 99164-6340, USA

Metabolomics represents one of the most powerful tools to probe the overall effects of gene down-regulation and gene knockouts in transgenic plants at all stages of growth and development. This is of particular importance in helping assess the overall effects on metabolism of transgenic lines engineered to be more susceptible for saccharification/fermentation for renewable bioenergy/bioproducts. We thus describe herein the effects of genetically modulating members of the arogenate dehydratase (ADT) gene family, which represent the final step in phenylalanine biosynthesis in higher plants,as well as that of various NAC transcription factors (TF) involved in secondary wall formation in fiber cells of *Arabidopsis*. The purpose was to establish global effects on metabolism when generating transgenic lines altered in lignin compositions and contents. Of these manipulations, ADT is an important upstream biochemical step, whose different members of its gene family differentially modulate carbon flux into the phenylpropanoid pathway, whereas the transcription factors of interest control secondary wall formation in fiber cells. Accordingly, numerous T-DNA knockouts of the six-member ADT family and of various NAC TF genetically modified *Arabidopsis* plant lines were subjected to metabolomic analyses to establish the effects on overall metabolism, particularly in those lines having different levels of lignin reduction, with the results so obtained discussed.

CHARACTERIZATION OF ARABIDOPSIS *THALIANA* SERINE HYDROXYMETHYL TRANSFERASES FROM MITOCHONDRIA AND THE CYTOSOL

Zhaoyang Wei,[1] Kehan Sun,[1] Francisco Sandoval,[2] Sanja Roje[1,2]

[1]*Graduate Program in Molecular Plant Sciences, Washington State University, Pullman, WA 99164, USA,* [2]*Institute of Biological Chemistry, Washington State University, Pullman, WA 99164, USA*

Serine hydroxymethyltransferases (SHMT) catalyze the reversible conversion of L-serine and (6*S*)-H4PteGlun to glycine and (6*S*)-5,10-CH2-H4PteGlun. This reaction is the major one-carbon unit source for a series of essential metabolic processes, and it plays a central role in photorespiration. *Arabidopsis* genome encodes seven SHMT isoforms localized in the cytosol, mitochondria, plastids and nuclei, adapted to their specific physiological functions. Knowledge of the biochemical properties of each isoform is critical to understand and manipulate the one-carbon pathway in plants, which is an important target for nutritional enhancement of crops. We functionally expressed and purified three recombinant *Arabidopsis* SHMTs, two from mitochondria and one from the cytosol. Biochemical properties were studied with respect to enzyme oligomerization state, Michaelis-Menten kinetic parameters, and impact of the folate poly-glutamyl tail length.

MERGER OF PRIMARY AND SECONDARY METABOLISM: ENDOGENOUS TURNOVER PATHWAY OF CYANOGENIC GLUCOSIDES ENABLES SORGHUM TO CHANNEL NITROGEN FROM DEFENSE COMPOUNDS INTO PRIMARY METABOLISM

Nanna Bjarnholt,[1] David P. Dixon,[2] Robert Edwards,[3] Lene Dalsten,[1] Mohammed Saddik Motawia,[1] Kirsten Jørgensen,[1] Birger Lindberg Møller[1]

[1]*Plant Biochemistry Laboratory and the VKR Research Centre Pro-Active Plants, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark,* [2]*School of Biological & Biomedical Sciences, University of Durham, South Road, Durham DH1 3LE, United Kingdom,* [3]*CNAP, Department of Biology (Area 8), University of York, Heslington, YO10 5DD, United Kingdom*

Numerous plants produce cyanogenic glucosides (CGs), which release toxic hydrogen cyanide (HCN) upon cleavage by endogenous plant β-glucosidases as part of the plants' chemical defense. Several crops, including sorghum (*Sorghum bicolor*), contain so high amounts of CGs that they may cause intoxications of humans or animals. As part of our efforts to create plants with reduced amounts of CGs for safe consumption, we aim to understand the role the compounds play in plants. In the germinating sorghum seedling, the total amount of the CG dhurrin initially increases up to 3–5% of plant dry weight, followed by a decrease after 3–4 days. Our hypothesis is that when the seedling has past the earliest vulnerable growth stages where it needs the CGs for defense, it channels the CG nitrogen back into primary metabolism, and we have found that sorghum has the necessary tools for this. At pH > 5 dhurrin and glutathione (GSH) react spontaneously to form a conjugate where one GSH substitutes the glucose moiety of dhurrin. This conjugate is a substrate for glutathione-S-transferases of the lambda class (GSTLs) which perform an unusual reductive cleavage of the conjugate to produce p-hydroxyphenyl acetonitrile (pOHPCN). As we have previously demonstrated, pOHPCN is catabolized by sorghum-specific nitrilase 4 homologs (NIT4s) to release the nitrogen as ammonia. The decrease in dhurrin content during seedling development is accompanied by an increase in total activity of the GSTL+NIT4A/NIT4B2 complex in the developing sorghum seedlings, demonstrating that this is likely to be the pathway acting *in planta*.

BIOAVAILABILITY OF TART CHERRY ANTHOCYANINS

Ara Kirakosyan, E. Mitchell Seymour, Peter Kaufman, Steven Bolling

The University of Michigan, Cardiovascular Center and the Michigan Integrative Medicine Program, Ann Arbor, MI 48109, USA

Tart cherry fruits produce several types of biologically active compounds, including anthocyanins and several other flavonoids. These phytochemicals have been studied for their potential health effects. Therefore, our overall goal was to advance knowledge of the value of tart cherry for their prospective health benefits, including several types of chronic diseases. Here we report the production of anthocyanins in tart cherry fruits and on modes of action of its major phytopharmaceutical compounds at target sites. There are important concerns about bioavailability, preservation and processing of tart cherry fruits for future consumption and uses. Any therapeutic effects that anthocyanins have are dependent on sufficient bioavailability both as exposure to cells and as exposure to a whole organism through the diet. The *in vivo* bioavailability of anthocyanins following ingestion of tart cherry fruits, and their potential biological effects, has not been well characterized. In the present study, the bioavailability of tart cherry anthocyanin compounds was determined in both human subjects and rats. The consumption of tart cherries resulted in the appearance of different proportions of anthocyanin derivatives (unmodified anthocyanins, methylated anthocyanins and glucurono-conjugated derivatives).

METABOLOMIC ANALYSIS OF ANTIOXIDANT PHYTONUTRIENTS IN THE *HIGH PIGMENT* (*hp*-1, *hp*-2) PHOTOMORPHOGENIC MUTANTS OF TOMATO

Ryne Ramaker, James W. Emmet, *Stephen C. Grace*

University of Arkansas at Little Rock, Department of Biology, Little Rock, 72204, USA

We used targeted and untargeted metabolomic analysis to determine antioxidant content across different metabolite classes in three tomato cultivars and two *high pigment* mutants (*hp*-1 and *hp*-2) that exhibit altered photomorphogenic responses. Levels of chlorogenic acid (CGA), rutin, lutein, β-carotene, and lycopene were determined by HPLC-PDA while untargeted metabolic profiling was performed by ESI-LCMS. Green fruits contain substantially higher levels of CGA and rutin, while β-carotene and lycopene were subtantially higher in red fruits, indicating a shift in resource allocation from phenylpropanoids to carotenoids during fruit development. We also observed a strong correlation between CGA and rutin levels across all genotypes, particularly in green fruits, suggesting that synthesis of hydroxycinnamic acids and flavonoids is coordinately regulated. The *hp*-2 mutant contained significantly higher levels of CGA and rutin than the other cultivars, particularly in green fruits, as well as higher levels of β-carotene. The antioxidant content of tomato fruits was strongly correlated with CGA and rutin levels. Correlation and discriminant function analysis were used to identify metabolic relationships among different antioxidant classes in tomato. This study provides a framework for future studies focused on engineering a more nutritious tomato through selective breeding, genetic engineering, and optimal growth conditions.

METABOLOMICS AND PHARMACOGNOSY OF MULLEINS (*VERBASCUM* L.)

Milen Ivanov Georgiev

Institute of Biology, Leiden University, Natural Products Laboratory, Leiden, 2333 CC, The Netherlands

The genus *Verbascum* L. comprises of about 360 species of flowering plants in the Scrophulariaceae family. The leaves, flowers and whole aerial parts have been used in the traditional folk medicine for centuries, for treatment of a wide range of human ailments, inter alia bronchitis, tuberculosis, asthma, and different inflammations. We report the application of ^{1}H NMR metabolic fingerprinting in tandem with principal component analyses in five different *Verbascum* species. *V. xanthophoeniceum* and *V. nigrum* accumulate higher amounts of the pharmaceutically-important harpagoside (~0.5% on dry weight basis) and verbascoside, forsythoside B and leucosceptoside B (in total 5.6–5.8% on dry weight basis), which underlines the possibility for their application in pharmaceutical industry. The anti-inflammatory activities of *V. xanthophoeniceum* were evaluated using several *in vitro* and *in vivo* assays. Based on the obtained results it was concluded that *V. xanthophoeniceum* could serve as a promising source of active compounds with anti-inflammatory action, particularly in complement-mediated disorders.

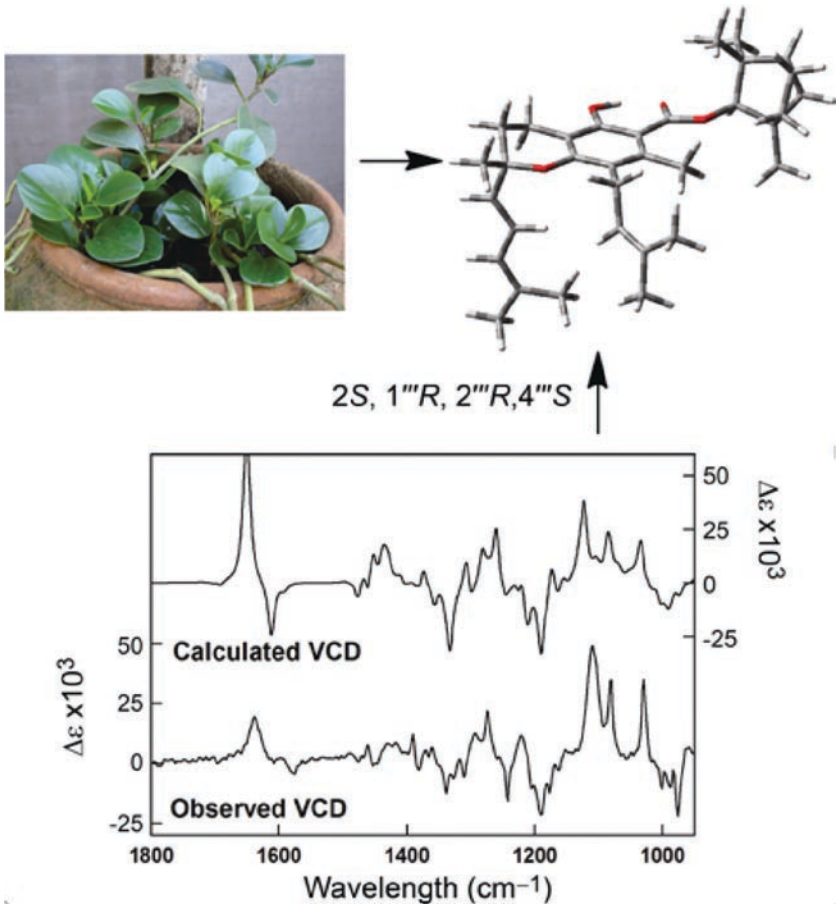
This work has been supported by a Marie Curie Fellowship of the European Community programme "Intra-European Fellowships" project SYSBIOPRO under contract number PIEF-GA-2009–252558.

ABSOLUTE CONFIGURATION DETERMINATION OF COMPLEX CHIRAL NATURAL PRODUCT MOLECULES USING VIBRATIONAL CIRCULAR DICHROISM (VCD): A CASE STUDY

João M. Batista Jr.,[1] Andrea N. L. Batista,[1] Vanderlan S. Bolzani,[1] Massuo J. Kato,[2] Laurence A. Nafie,[3] Silvia N. López,[4] Maysa Furlan[1]

[1]*Institute of Chemistry, São Paulo State University - UNESP, Araraquara, SP 14800-900, Brazil,* [2]*Institute of Chemistry, University of São Paulo, Department of Fundamental Chemistry, São Paulo, SP 05508-000, Brazil,* [3]*Syracuse University, Department of Chemistry, Syracuse, NY 13244-4100, USA,* [4]*College of Biochemical and Pharmaceutical Sciences, Rosario National University, Department of Pharmacognosy, Rosario, S2002LRK, Argentina*

The determination of absolute configuration of chiral molecules is a long-standing problem in natural product chemistry. Herein we report the use of VCD spectroscopy and DFT calculations to assign the absolute configuration of six isomeric monoterpene chromane esters isolated from *Peperomia obtusifolia* (Piperaceae). This work reinforces the capability of VCD to determine unambiguously the absolute configuration of structurally complex molecules in solution, without the need of crystallization, derivatization or UV-Vis chromophores.



BIOORGANIC STUDIES ON JASMONATE GLUCOSIDE, A PUTATIVE TRIGGER FOR ION CHANNEL ACTIVATION IN PLANT

Minoru Ueda

Tohoku University, Sendai, 980–8578, Japan

Jasmonates are ubiquitously occurring plant growth regulators with high structural diversity that mediate numerous developmental processes and stress responses. We identified 12-*O*-α-D-glucopyranosyl-jasmonic acid (GJA) as the bioactive metabolite inducing nyctinastic leaf-closure of *Samanea saman*. We demonstrate that leaf-closure of isolated *Samanea pinnae* is induced upon stereospecific recognition of (–)-GJA. Similarly, rapid and cell-type-specific shrinkage of extensor motor cell protoplasts was selectively initiated by (–)-GJA. (–)-GJA was inactive with respect to activation of typical JA responses, such as induction of JA-responsive genes, accumulation of plant volatiles considered to be mediated

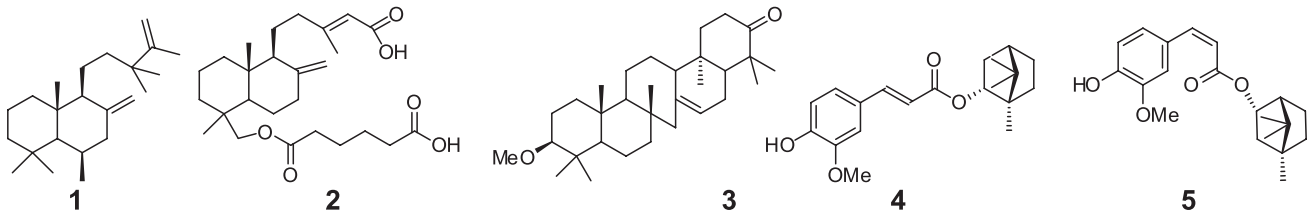
by COI1-dependent fashion. Furthermore, application of selective inhibitors indicated that leaf movement is mediated by potassium fluxes initiated by opening of potassium-permeable channels.Additionally, GJA has been identified as a trap-closing chemical factor of the Venus Flytrap **(4)**: trap-snapping movement of Dionaea can be triggered by GJA without external stimuli. This movement is also known to be classified in an ion-channel regulated behavior. Collectively, our data points to the existence of an ion-channel activation mechanism triggered by GJA in plants.

CHEMICAL CONSTITUENTS OF EAST EUROPEAN FOREST SPECIES

Dorota A. Nawrot, Moses K. Langat, Dulcie A. Mulholland

University of Surrey, Department of Chemistry, Guildford, GU2 7XH, United Kingdom

This work is part of the EU FP7 (FP7-KBB-2008-2B-227239) *ForestSpecs* project whose aim is to utilize diverse types of wood residues from the forestry industry. A comparative analysis was carried out of the performance of the MARS microwave extraction system against traditional extraction methods, Soxhlet extraction and shaker extraction. A number of compounds have been isolated and identified, including the novel labdane diterpenoid 6*β*,13-dihydroxy-14-oxo-8(17)-labdene (**1**) from *L. gmelinii* and the novel pumilanoic acid (**2**) from *P. pumila*, the known and quite unusual serratane triterpenoid, 3*β*-methoxyserrat-14-en-21-one (**3**) as well as *E*- and *Z*-bornyl ferulate (**4**, **5**) also from *P. pumila*.



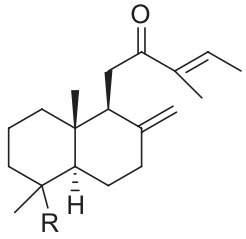
1 **2** **3** **4** **5**

RESIN DITERPENES FROM *AUSTROCEDRUS CHILENSIS*

Guillermo Schmeda-Hirschmann,[1] Veronica Rachel Olate,[1] Olatz Goikoetxeaundia[2]

[1]*Universidad de Talca, Instituto de Quimica de Recursos Naturales, Talca, Chile VII Region,* [2]*Euskal Herriko Unibertsitatea, Facultad de Ciencias Químicas, Donostia-San Sebastián, 20018, Spain*

From the resin of the Chilean gymnosperm *Austrocedrus chilensis* (D. Don) Florin et Boutelje (Cupressaceae), 16 diterpenes belonging to the labdane, abietane and isopimarane skeletons were isolated and identified by spectroscopic and spectrometric methods. Some 14 diterpenes are reported for the first time for the species and the diterpene 12-oxolabda-8(17),13E-dien-19 oic acid is described for the first time as a natural product. Single drop resin samples were collected from female and male adult trees and the diterpene composition analyzed by GC-MS and ^{1}H NMR. Multiple samples from the same individuals were compared according to tree gender and season (late spring, summer, winter) to disclose similarities and differences. Acknowledgements: FONDECYT Nr. 1085306.



14, R = COOH
14a, R = $COOCH_3$
14b, minor (*Z*) isomer

LARVICIDAL ACTIVITY OF COMPOUNDS IDENTIFIED IN *PONGAMIA PINNATA* SEED AGAINST *AEDES AEGYPTI* AND *CULEX PIPIENS PALLENS*

Haribalan Perumalsamy,[1] Jun-Ran Kim,[1,2] Kym Na Ra Hwang,[1] Young-Joon Ahn[1,2,3]

[1]*Seoul National University, Bioactive Natural Products Laboratory, Department of Agricultural Biotechnology, Seoul, 151–921, South Korea,* [2]*Seoul National University, Research Institute for Agricultural and Life sciences, College of Agriculture*

and Life sciences, Seoul, 151–921, South Korea, [3]Seoul National University, WCU Biomodulation Major, Department of Agricultural Biotechnology, Seoul, 151–921, South Korea

The toxicity of materials derived from the seeds of *Pongamia pinnata* Pierre to third instar larvae of *Aedes aegypti* and *Culex pipiens pallens* was examined using a direct contact bioassay. Results were compared with those of the currently used insecticide: fenthion and temephos. The active principles of *P. pinnata* were identified as karanjin (**1**), pongamone (**2**), palmitic acid (**3**) and karanjachromene (**4**), by spectroscopic analysis. The seed steam distillate compounds were identified by GC-MS, as oleic acid, elaidic acid, arachidonic acid, octadecanamide and behenic acid. Based on 24h LC_{50} values, karanjin (14.61 and 16.13 ppm) was the most toxic compound but less effective than fenthion (0.0031 and 0.068 ppm) and temephos (0.016 and 0.056 ppm) against *Ae. aegypti* and *Cx. p. pallens*. Moderate toxicity was shown by pongamone (34.50 and 39.53 ppm), palmitic acid (36.93 and 42.96 ppm), and karanjachromene (43.05 and 48.95 ppm). *P. pinnata* seed derived materials, particularly karanjin, merit further study as potential mosquito larvicides for the control of mosquito populations in light of global efforts to reduce the level of highly toxic synthetic larvicides in the aquatic environment.

QUANTITATIVE ANALYSIS OF COMPOUNDS IN FERMENTED INSAMPAEDOK-SAN AND ITS NEUROPROTECTIVE ACTIVITY IN HT22 CELLS

Bohyoung Lee,[1] Jin Bae Weon,[1] Jin Yeul Ma,[2] Hye Jin Yang,[1] Eun young Mok,[1] Choong Je Ma[1]

[1]Kangwon National University, Department of Biomaterials Engineering, Division of Bioscience and Biotechnology, Chuncheon, 200–701, South Korea, [2]Korea Institute of Oriental Medicine, Daejeon, 305–811, South Korea

Insampaedok-san (IS) is a traditional medicine used as a treatment for colds. We investigated the consituents, neuroprotective activity and anti-oxidative activity in IS and its fermentation proeduct with *Lactobacillus (FIS)*. Contents of four marker compounds (ferulic acid, hesperidin, 6-gingerol and glycyrrhizin) and other compounds in Insampaedok-san (IS) and fermented Insampaedok-san (FIS) were measured and compared by established HPLC-DAD. Neuroprotective activity of IS and FIS was evaluated and compared by cytoprotective effect against glutamate induced neurotoxicity in HT22 cells. Anti-oxidative activity of IS and FIS was compared by DPPH free radical, hydroxyl radical and hydrogen peroxide scavenging activity tests. Contents of three compounds, ferulic acid and glycyrrhizin decreased, but 6-gingerol was increased by fermentation. FIS showed more potent neuroprotective activity than IS. As a results of DPPH, hydroxyl radical and hydrogen peroxide scavenging test, anti-oxidative activity of IS slightly was increased by fermentation. In conclusion, fermentation with *Lactobacillus* could alter contents of compounds in IS and improve neuroprotective activity and anti-oxidative activity of IS.

QUANTITATIVE ANALYSIS OF THE EIGHT MAJOR COMPOUNDS IN THE SAMSOEUM USING A HPLC COUPLED WITH DAD AND ESI-MS

Bohyoung Lee,[1] Jin Bae Weon,[1] Hye Jin Yang,[1] Jin Yeul Ma,[3] Eun young Mok,[1]*Choong Je Ma*[1,2]

[1]Kangwon National University, Department of Biomaterials Engineering, Division of Bioscience and Biotechnology, Chuncheon, 200–701, South Korea, [2]Kangwon National University, Research Institute of Biotechnology, Chuncheon, 200–701, South Korea, [3]Korea Institute of Oriental Medicine, TKM Converging Research Division, Daejeon, 200–701, South Korea

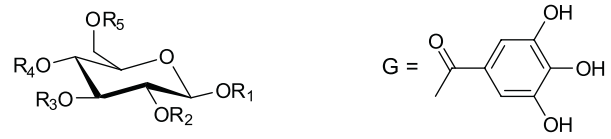
The simultaneous determination of eight major compounds, ginsenoside Rg3, caffeic acid, puerarin, costunolide, hesperidin, naringin, glycyrrhizin and 6-gingerol in the Samsoeum using a high-performance liquid chromatography (HPLC) coupled with diode array detection (DAD) and an electrospray ionization mass spectrometer (ESI-MS) was developed for an accurate and reliable quality assessment. Eight compounds were qualitatively identified based on their mass spectra and by comparing with standard compounds and quantitatively analyzed by HPLC-DAD. Separation of eight compounds was carried out on a LUNA C_{18} column (S-5 μm, 4.6 mm I.D. 250 mm) with gradient elution composed of acetonitrile and 0.1% trifluoroacetic acid (TFA). The data showed good linearity (R^2>0.9996). The limits of detection (LOD) and the limits of quantification (LOQ) were less than 0.53 μg and 1.62 μg, respectively. Inter- and intra- day precisions (expressed as relative standard deviation (RSD) values) were 1.94 and 1.91%, respectively. The recovery of the method was in the range of 94.24–107.90%. The established method is effective and could be applied to quality control of Samsoeum.

POLYPHENOLIC SECONDARY METABOLITES FROM *JUGLANS MANDSHURICA* MAX.

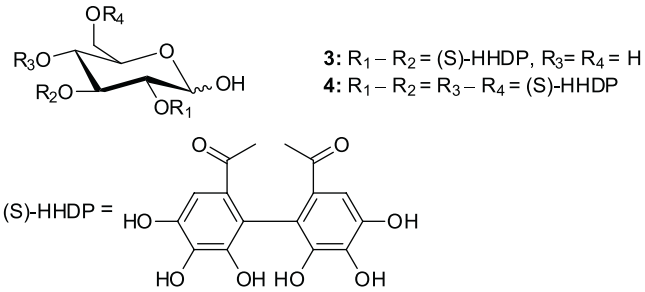
Dong-Joo Kwon[1], Jin-Kyu Kim[3], Se-Yeong Park[2], *Young-Soo Bae*[2]

[1]Hallym University, Biomedical Science, Chunchon, 200–702, South Korea, [2]Kangwon National University, Forest Biomaterials Engineering, Chunchon, 200–701, South Korea, [3]Hallym University, Institute of Natural Medicine, Chunchon, 200–702, South Korea

Juglans mandshurica has a narrow distribution in Korea and northeastern China. Its leaves, roots and seeds have been used as a traditional medicine for esophageal and cardiac diseases, gastroenteritis, diabetes, and lung cancer. The present study reports the isolation of 17 polyphenolic secondary metabolites from the stem bark of *J. mandshurica*. These compounds were identified as aromadendrin, taxifolin, ampelopsin, kaempferol, quercetin, myricetin, afzelin, astragalin, quercitrin, hirsutrin, myricitrin, gallic acid and ellagic acid, including 1,2,4,6-tetra-*O*-galloyl-α-D-glucose (**1**), 1,2,3,4,6-penta-*O*-galloyl-α-D-glucose (**2**), (*S*)-2,3-HHDP-D-glucose (**3**), and pedunculagin (**4**). Their structures were elucidated by means of 1D, 2D-NMR and HR-MS analyses. Compounds **1**, **2**, **3** and **4** were reported from this plant for the first time.



1: $R_1 = R_2 = R_4 = R_5 = G, R_3 = H$
2: $R_1 = R_2 = R_3 = R_4 = R_5 = G$



3: $R_1 - R_2$ = (S)-HHDP, $R_3 = R_4 = H$
4: $R_1 - R_2 = R_3 - R_4$ = (S)-HHDP

CHALCONE GLYCOSIDES FROM *BRASSICA RAPA* L. EHIDABENIF AND THEIR SYNTHETIC ANALOGUES INHIBIT LPS-INDUCED NO PRODUCTION.

Masayuki Ninomiya,[1] Ryosuke Mochizuki,[1] Hirokazu Hara,[2] Yoko Nakamura,[2] Mamoru Koketsu[1]

[1]*Gifu University, Department of Materials Science and Technology, Faculty of Engineering, Gifu, 501–1193, Japan,* [2]*Gifu Pharmaceutical University, Laboratory of Clinical Pharmaceutics, Gifu, 501–1196, Japan*

Phytochemical investigation of the aerial parts of *Brassica rapa* L. ehidabenif resulted in isolation of new chalcone glycosides, 4′-*O*-β-D-glucopyranosyl-4-hydro-xy-3f-methoxychalcone (A1), 4′-*O*-β-D-glucopyranosyl-3′,4-dimethoxychalcone (A2), and 4,4′-di-*O*-β-D-glucopyranosyl-3′-methoxychalcone (A3) along with known glycosides. Among the isolates, chalcone glycoside (A2) inhibited LPS-induced NO production in microglia HAPI cells. Moreover, structure-activity relationship studies on synthesized chalcone glycoside analogues showed that 4′-*O*-β-D-glucopranosyl-3′-methoxychalcone (A11), which has no functional groups in the B-ring, inhibited NO production more potently than A2.

Project supported by the NSF Hawaii EPSCoR Program under National Science Foundation award EPS-0903833.

PHYTOCHEMICAL STUDY OF THE NATIVE HAWAIIAN PLANT, *METROSIDEROS POLYMORPHA*

Swapan Pramanick,[1] Benjamin R. Clark,[1] Norman Q. Arancon,[2] Robert P. Borris[1]

[1]*College of Pharmacy, University of Hawaii at Hilo, Hilo, HI 96720, USA,* [2]*College of Agriculture, Forestry and Natural Resource Management, University of Hawaii at Hilo, Hilo, HI 96720, USA*

Metrosideros polymorpha Gaudich, known in Hawaii as *ʻōhiʻa lehua*, is a flowering, evergreen tree in the myrtle family, Myrtaceae. *M. polymorpha* is endemic to the six largest Hawaiian Islands and is the most common native tree in Hawaii. It is a slow growing species, tolerating a wide range of temperature, rainfall and soil conditions, and is the largest component of lowland and montane wet and mesic forests, dry forests, sub-alpine shrublands and new lava flows. As the name implies, the species is able to assume a variety of forms from scrubby brush to a tree of 20–25 m in height depending on growing conditions. Despite its common occurrence, little is known about the chemistry of this species. In the present investigation, a methanol extract of the dried leaves of *M. polymorpha* was fractionated by solvent partition followed by countercurrent chromatography, preparative thin layer chromatography and HPLC to afford a range of flavonoids and flavonoid glycosides as well as other secondary metabolites.

DIRECT IDENTIFICATION OF PHENOLIC CONSTITUENTS IN *FABIANA IMBRICATA* INFUSIONS BY HPLC-DAD AND HPLC-MSN

Guillermo Schmeda-Hirschmann,[1] Cristina Quispe,[1] Ezequiel Viveros Valdez[2]

[1]*Universidad de Talca, Instituto de Quimica de Recursos Naturales, Talca, Chile VII Region,* [2]*Universidad Autónoma de Nuevo León, Departamento de Química, Facultad de Ciencias Biológicas, Nuevo León, 66450, México*

A simple and direct method was developed for the qualitative analysis of polyphenols in infusions from the Chilean crude drug 'Pichi' or 'Pichi romero' (*Fabiana imbricata* R. et. P., Solanaceae) by HPLC-DAD and HPLC-MS. The phenolic constituents identified in the plant infusions were chlorogenic acid, *p*-hydroxyacetophenone, scopoletin and quercetin derivatives. The glycosides were mainly glucosides of *p*-hydroxyacetophenone and scopoletin while di- and triglycosides of quercetin were the main flavonoids. The results suggest that the medicinal properties reported for this infusion should be attributed to the presence of several phenolics with known anti-oxidant, diuretic and anti-inflammatory activity. The HPLC trace obtained can be useful for a rapid authentication of the crude drug as well as for qualitative analysis and differentiation of plant populations in the plant distribution range.

Acknowledgements: Project PBCT PSD-50 and Programa de Investigacion en Productos Bioactivos, Universidad de Talca.

PIGMENT ANALYSIS OF SPATHES IN *ANTHURIUM* SPECIES

Benjamin R. Clark,[1] Jon Y. Suzuki,[2] Barbara J. Bliss,[2] Robert P. Borris[1]

[1]*College of Pharmacy, University of Hawaii at Hilo, Hilo, HI 96720, USA,* [2]*USDA-ARS-Pacific Basin Agricultural Research Center, Tropical Plant Genetic Resources and Disease Research Unit, Hilo, HI 96720, USA*

Anthurium is a genus of popular ornamental plants in the Araceae that produce highly colored, modified leaf structures known as spathes. While it is known that anthocyanins are the primary pigments responsible for the striking red and orange colors observed in anthuriums, most pigment analyses have relied on older techniques such as thin layer chromatography. We describe here the analysis of pigments from a number of *Anthurium* varieties using LC-MS, which revealed the presence of a range of pelargonidin and cyanidin glycosides. Of particular interest was the detection of numerous pre-pigments in the form of flavonoids, flavonols, proanthocyanidins and their glycosides, which may provide tools for greater control of spathe color through applied molecular genetics.

Project supported in part by the NSF Hawaii EPSCoR Program under National Science Foundation award EPS-0903833

QUANTITATIVE ANALYSIS OF CURCUMIN AND RELATED COMPOUNDS IN *CURCUMA LONGA* BY HPLC AND LC-MS

Kwang-Jin Lee,[1] Kathie Pomeroy,[2] Swapan Pramanick,[1] Robert P. Borris[1]

[1]*College of Pharmacy, University of Hawaii at Hilo, Hilo, HI 96720, USA,* [2]*Kohala Medicinal Herb Farm, Kapa au, HI 96755, USA*

Turmeric, *Curcuma longa* L. (Zingiberaceae), is a well-known medicinal plant in many cultures and is used for a variety of pharmacological activities. Many of these biological activities are attributed to the presence of curcumin and related diphenylheptanoids. Curcumin is found in turmeric along with its desmethoxy and bis-desmethoxy analogs. Like most natural products, the concentration of these interesting compounds is expected to vary depending on growing conditions and time of harvest. In the present work, conditions for the extraction of curcumin and its analogs on an analytical scale have been optimized, and spectrophotometric and chromatographic methods (HPLC, LC-MS) for the determination of total curcumin analogs as well as the individual diphenylheptanoids have been established. These methods were then applied to the analysis of field samples of turmeric grown in a variety of locations on the Island of Hawaii.

Project supported in part by grants from Western Sustainable Agriculture Research & Education (SARE), USDA, award FW10-056 and the NSF Hawaii EPSCoR Program under National Science Foundation award EPS-0903833.

EFFECT OF GYOJYA-NA EXTRACTS ON UVB-INDUCED APOPTOSIS IN HACAT CELL

Atsuyoshi Nishina,[1] Kunihisa Kozawa,[2] Hirokazu Kimura[3]

[1]*Yonezawa Women's Junior College, Health and Nutrition, Yonezawa, Japan 992-0025,* [2]*Gunma Prefectural Institute of Public Health and Environmental Sciences, Maebashi, 371-0052, Japan,* [3]*National Institute of Infectious Diseases, Infectious Disease Surveillance Center, Tokyo, 208-0011, Japan*

The ascetic green (Gyojya-na) is a hybrid plant from ascetic garlic (*Allium victorialis* L. subsp. *Platyphyllum* Hulten) and leek (*Allium tuberosum* Rottler et Spreng), and is cultivated around Nagai City, Yamagata prefecture as a special regional farm product. At present, Gyojya-na is still cultivated at about 3000 kg/year. In order to explore the cell

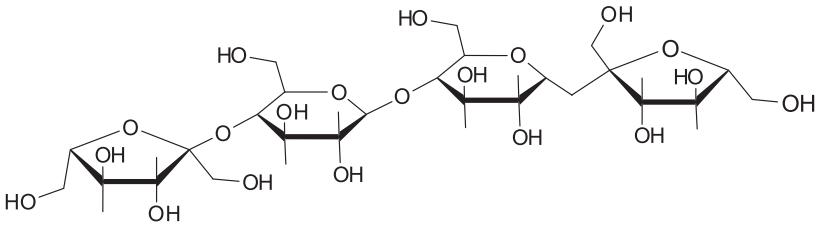
biological effects of Gyojya-na extracts, we examined a rescue effect of UVB-induced apoptosis in human keratinocytes (HaCaT cells). HaCaT cells were UVB irradiated (400 mJ/cm^2) and cell survival was measured by the MTT method. Some Gyojya-na extract fractions extracted by hexane partially inhibited UVB-induced cell death. Thus, a UVB-induced cell death inhibitor may exist in the extracts. Using NMR, MS and IR, we analyzed the component to identify chemical structures at present. Because recent studies suggested that upregulation of MAP kinase or Akt suppressed UVB-induced apoptosis in HaCaT cells, a component extracted from Gyojya-na may be able to activate or inactivate signaling pathways such as ERK1/2, JNK2/3, p-38 MAP kinase or Akt. When the component structure is confirmed, the mechanisms of apoptosis inhibition in HaCaT cells through the intercellular signaling pathways can be studied.

A TETRASACCHARIDE ISOLATED FROM THE FRUITS OF *TAXUS CUSPIDATA*

Se-Yeong Park[1], Chang-Kiu Lee[2], Young-Soo Bae[1]

[1]*Kangwon National University, Forest Biomaterials Engineering, Chunchon, 200–701, South Korea,* [2]*Kang-won National University, Chemistry, Chunchon, 200–701, South Korea*

One tetrasaccharide was isolated from the H_2O soluble extract of the fruits of *Taxus cuspidata* by Sephadex LH-20 column chromatographic purification. Its structure was elucidated by 1D, 2 D-NMR and MALDI-TOF MS, including acid hydrolysis, acetylation and permethylation.



DIFFERENTIAL LIPID PROFILES OF MICROALGAE CO-CULTIVATED WITH MARINE BACTERIA

Dagmar Enss, *Heiko Rischer*

VTT Technical Research Centre of Finland, Espoo, 02044, Finland

Monoalgal cultures isolated from the environment and commonly used for scientific investigations are generally not axenic but contain bacteria. Relatively stable associations (sometimes as symbiosis) of bacteria and algae are known from ecological research, but have not been studied with respect to algal lipid production. However, it is known that the organisms influence each other's physiology or even exchange metabolites. Since mutually beneficial coexistence could even have an economic impact on biotechnological applications we investigated axenic algae, isolated native bacteria and diverse co-cultivations in respect to their respective lipid accumulation.

A first screen based on GC-FID total lipid analysis indeed revealed specific species combinations resulting in higher lipid concentrations compared to the individual cultures alone or the native community. A more thorough LC-MS/MS investigation comprising more than 200 lipids focused on particular differences in the metabolic profiles of these combinations. Qualitative changes in the lipid profiles were recorded suggesting metabolic interaction between the algae and the bacteria.

DIARYLHEPTANOID SULFATES AND RELATED COMPOUNDS FROM THE *MYRICA RUBRA* BARK

Morio Yoshimura, Yoshiaki Amakura, Takashi Yoshida

Department of Pharmacognosy, College of Pharmaceutical Sciences, Matsuyama University, Matsuyama, 790–8578, Japan

The EtOAc and *n*-BuOH soluble portions obtained from the 80% EtOH extract of *Myrica rubra* bark were separately purified by a combination of chromatography over Diaion HP-20, Toyopearl HW40, Sephadex LH-20 and/or Silica gel to yield three new compounds, myricanol 11-*O*-sulfate (**1**), juglanin B 11-*O*-sulfate (**2**), and myricanone 5-*O*-(6′-*O*-galloyl)-glucoside (**3**) together with 11 known compounds. Each structure was elucidated on the basis of spectral analyses (NMR, MS, IR, and $[\alpha]_D$).

CHEMICAL CHARACTERIZATION OF THREE SAMPLES OF BRAZILIAN PROPOLIS BY NMR AND GC/MS

Caroline Cristina Fernandes-Silva,[1] Antonio Salatino,[1] Giuseppina Negri,[2] Maria Luiza Faria Salatino[1]

[1]*University of São Paulo, Botany, São Paulo, SP 05508-090, Brazil,* [2]*Federal University of São Paulo, Psicobiology, São Paulo, SP 04023-062, Brazil*

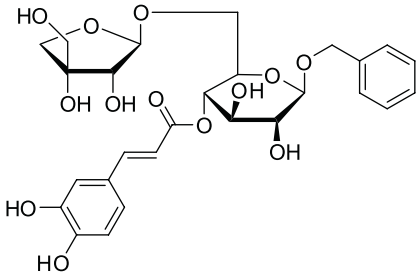
Ethyl acetate fractions of three samples of Brazilian propolis, corresponding to the states of Ceará, Santa Catarina and Paraná were analyzed by NMR and GC/MS. The NMR analysis of propolis from Ceará suggests the presence of carbonylic and aromatic compounds, and methoxylated compounds with long carbon chains. The CG/MS data indicated the presence of triterpenoids such as lupenone and lupeol. The NMR analysis of the propolis from Santa Catarina showed higher diversity of carbonyl compounds and aromatic substances, methylenic and methyl groups linked to oxygen, mainly between 100.4 e 94.6 ppm, indicating the presence of flavonoids or another phenolic compounds, most of them methoxylated, such as ketone esters and/or acids. The CG/MS data are in agreement with NMR evidence, indicating the presence of the cinnamic and ferulic acids (phenylpropanoids), α-amyrine (triterpenoid) and pinocembrin (flavonoid). The NMR analysis of the propolis from Paraná indicated the presence of carbons linked to oxygen, suggesting the presence of other classes of phenolics, perhaps flavonoids and lignans. In the CG/MS analysis no flavonoids were found, however, the presence of methoxylated substances with ketone carbonyl, such as 1-hydroxy-3(4-hydroxy-3-methoxyphenyl) 2-propanone, as suggested by NMR analysis were observed. Thus, the differences in the chemical composition of the three Brazilian propolis studied in the present work are obvious.

PHENOLIC COMPOUNDS FROM SUNFLOWER (*HELIANTHUS ANNUUS*) SEEDS

Yoshiaki Amakura, Morio Yoshimura, *Takashi Yoshida*

Department of Pharmacognosy, College of Pharmaceutical Sciences, Matsuyama University, Matsuyama, 790–8578, Japan

A new compound, benzylalcohol α-D-apiofuranosyl-(1→6)-α-D-(4-*O*-caffeoyl) glucopyranoside, was isolated together with eight known phenolic compounds from the seeds of sunflower (*Helianthus annuus*). The known compounds were characterized by spectroscopic methods as caffeic acid, methyl caffeoate, methyl chlorogenate, chlorogenic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and eriodictyol 5-*O*-α-D-glucoside. The antioxidative effect of these phenolic constituents was also evaluated on the basis of oxygen-radical absorbance capacity (ORAC), and caffeic acid derivatives were shown to be major antioxidants in the seeds.

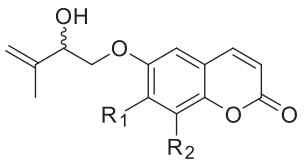


COUMARINS FROM THE MALAGASY *CEDRELOPSIS RAKOTOZAFYI* (PTAEROXYLACEAE)

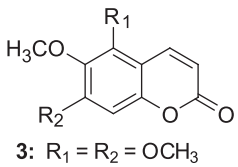
Dorota A Nawrot,[1] Milijaona Randrianarivelojosia,[2] Dulcie A Mulholland[1]

[1]*University of Surrey, Department of Chemistry, Guildford, GU2 7XH, United Kingdom,* [2]*Institute Pasteur de Madagascar, Malaria Research Group, Antananarivo, BP 1274, Madagascar*

The genus *Cedrelopsis* is endemic to Madagascar and comprises eight species, four of which have been examined phytochemically: *C. gracilis*, *C. microfoliata*, *C. grevei* and *C. longibracteata*. The plant *Cedrelopsis rakotozafyi* has been investigated and yielded seven compounds: a new coumarin, 8-hydroxy-7-methoxy-6-(2-hydroxy-3-methylbut-3-enoxy)-2*H*-1-benzopyran-2-one (**1**) along with four known coumarin derivatives (**2**-**5**) and two known triterpenoids, lupeol and α-amyrin.



1: $R_1 = OCH_3$, $R_2 = OH$
2: $R_1 = OH$, $R_2 = H$



3: $R_1 = R_2 = OCH_3$
4: $R_1 = H$, $R_2 = OCH_3$
5: $R_1 = H$, $R_2 = OH$

LUTEOLIN ENHANCES TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND-MEDIATED APOPTOSIS OF SK-BR3 HUMAN BREAST CANCER

An Keun Kim, Eun Young Kim

College of Pharmacy, Sookmyung Women's University, Seoul, 140–742, South Korea

The dietary flavonoid luteolin has been reported to induce apoptosis in various cancer cells, whereas it has no effect on normal cells. Here, we investigated the effect of luteolin on tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis in TRAIL-resistant SK-BR3 human breast cancer cells. Combination of luteolin with TRAIL inhibited SK-BR3 cell growth much more strongly than each agent alone. Long-term toxicity was determined by clonogenic survival assay and enhanced apoptosis was confirmed by PARP-cleavage. Combined treatment with luteolin and TRAIL markedly reduced the clonogenic capacity of cells and activated PARP-cleavage. The apoptotic mechanism induced by combination involved the activation of caspases. The luteolin and TRAIL cooperatively activated caspase-3, -6, -8 and -9. Moreover, combined treatment induced Bid activation, Bcl-2 protein down-regulation. The expression of FLIPs was also down-regulated by TRAIL/luteolin combination. Taken together, the results indicate that luteolin/TRAIL combination could sensitize SK-BR3 human breast cancer cells to TRAIL-induced apoptosis by stimulating caspase-signaling pathway and by regulating the survival proteins.

SYNTHESES OF MACROCYCLIC ENGELHARDIONE ANALOGS AS POTENTIAL ANTITUBERCULOSIS AGENTS

Li Shen, *Dianqing Sun*

University of Hawaii at Hilo, College of Pharmacy, Hilo, HI 96720, USA

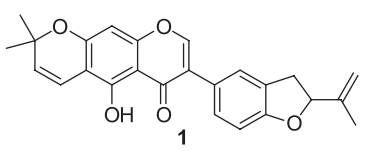
Engelhardione was originally isolated from the roots of *Engelhardia roxburghiana* (Juglandaceae). This naturally occurring macrocyclic compound belongs to a broad family of secondary plant metabolites called diarylheptanoids, which have been shown to mediate diverse biological activities. Engelhardione was reported to have potent *in vitro* activity against *Mycobacterium tuberculosis* strain H37Rv (MIC = 0.2 μg/mL). As part of our continuous effort to develop new antituberculosis agents, we have employed this emerging natural product lead as a chemical starting point for subsequent structure-activity relationship (SAR) studies. Recently, we reported the first total synthesis of engelhardione, and this effort ultimately led to the structural revision of this macrocyclic natural product. The correct structure of the reported engelhardione should be that of pterocarine (L. Shen, D. Sun, Tetrahedron Lett., *in press*). Synthesis of engelhardione was achieved using a series of aldol condensation reactions and selective hydrogenation to generate the key linear building block, 1,7-diphenylheptan-3-one derivative, followed by the macrocyclic Ullmann condensation and appropriate deprotection to afford engelhardione. Using this developed synthetic scheme, diversified macrocyclic engelhardione analogs were subsequently synthesized for antituberculosis screening. Microwave-assisted organic synthesis (MAOS) of this intramolecular macrocyclization will also be presented.

ISOLATION, CHARACTERIZATION, AND BIOACTIVITIES OF PRENYLATED ISOFLAVONOIDS FROM *RHYNCHOSIA* EDULIS

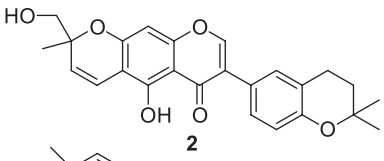
Bernhard W.G. Vogler,[1] Ifedayo V. Ogungbe,[1] Gabrielle M. Hill,[1] Rebecca A. Crouch,[1] Meenakshi Nagarkoti,[1] William A. Haber,[2] William N. Setzer[1]

[1]*University of Alabama in Huntsville, Department of Chemistry, Huntsville, AL 35899, USA,* [2]*Missouri Botanical Garden, St Louis, MO 63166, USA*

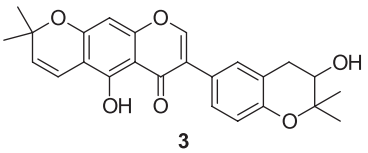
Four new prenylated isoflavones, rhynchosins A-C (1-3) and rhynchosinal (4), were isolated by bioassay-guided fractionation of the dichloromethane bark extract of *Rhynchosia edulis*. Five previously described compounds, scandenal, ulexin B, cajanone, cajanin, and cyclochandalone, were also isolated. These isoflavonoids showed weak inhibitory activity towards rhodesain, the major cathepsin-L like protease in *Trypanosoma brucei*. They also have weak antiproliferative activity towards MCF-7 cells.



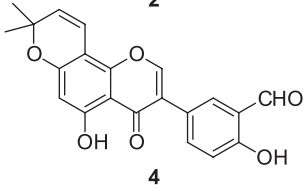
1



2



3



4

PHENOLIC GLYCOSIDES FROM THE STEM OF *STEWARTIA PSEUDOCAMELLIA* MAXIM

Jong Hwan Kwak,[1] Kyoung Hwa Hong,[1] Jong Jin Bae,[1] Ok Pyo Zee,[1] Hana Youn,[2] Yu-na Lee,[2] Chang-seon Song[2]

[1]*Sungkyunkwan University, School of Pharmacy, Suwon, Gyeonggi-do, 440–746, South Korea,* [2]*Konkuk University, College of Veterinary Medicine, Seoul, 143–701, South Korea*

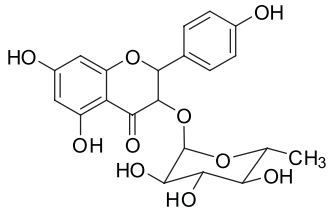
Stewartia pseudocamellia Maxim. is a deciduous tree in the family Theaceae which is distributed in southern Korea. Its fruit or the bark of stems and roots have been used as a folk medicine for treatment of circulatory disorders, paralysis of the limbs, legs and arms, and several pains. In our continuing studies to find bioactive compounds from natural sources, we have found that the MeOH extract of *S. pseudocamellia* has antiviral activity against influenza A(H1N1) virus. The MeOH extract of *S. pseudocamellia* was consecutively partitioned with hexane, CH_2Cl_2, EtOAc and *n*-BuOH to give five fractions. Among these fractions, the *n*-BuOH fraction was subjected to column chromatographic separation. Two new phenolic glycosides were isolated from the *n*-BuOH fraction, and the structures were determined from their spectral data. The tructures of two new compounds, named stewartiaside A and pseudocamelliaside, were established as 1-(2′-methoxy, 4′,6′-dihydroxy-2′-methoxy)phenyl), 3-hydroxy-5-(4′′-hydroxyphenyl) pentan-1-one 3-O-*β*-D-glucopyranoside and 2, 3, 5-trimethoxy 1-*O*-*β*-D-glucopyranoside 6′-*O*-gallate, respectively.

A FLAVONOID GLYCOSIDE FROM *EPERUA GLABRIFLORA* (DUCKE) COWAN

Lidiam Maia Leandro, *Valdir F. Veiga Junior*

Universidade Federal do Amazonas, Department of Chemistry, Manaus, Amazonas 69079000, Brazil

The phytochemical study of the ethyl acetate extract of stem bark of the Amazonian Leguminoseae *Eperua glabriflora* afforded a 3-*O*-rhamnosylflavonol identified as engeletin.

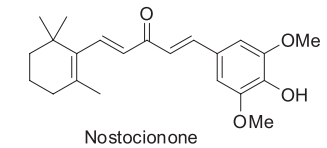


ISOLATION OF SECONDARY METABOLITES FROM MICROALGAE

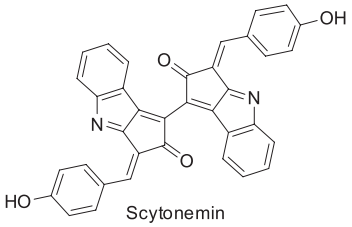
Yuji Muramatsu, Hitoshi Sato, Masayuki Ninomiya, *Mamoru Koketsu*

Gifu University, Department of Materials Science and Technology, Faculty of Engineering, Gifu, 5011193, Japan

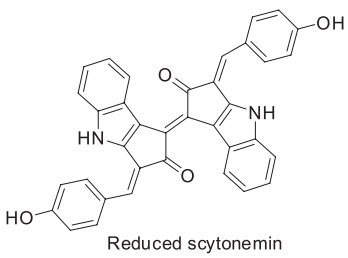
The extracts of two terrestrial alga *Nostoc commune* Vauch. and *Nostoc flagelliforme* showed high anti-oxidant activities. We isolated two β-ionone derivatives, nostocionone and 3-oxo-β-ionone along with four indole alkaloids: scytomemin, reduced scytonemin, *N*-(p-coumaroyl)tryptamine, and *N*-acetyltryptamine from the extract of *N. commune* and *N. flagelliforme*. Nostocionone and reduced scytonemin showed strong anti-oxidant activities.



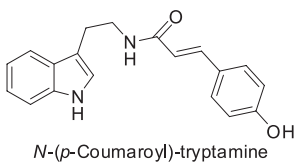
Nostocionone



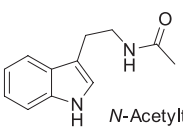
Scytonemin



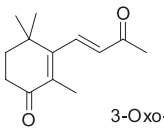
Reduced scytonemin



N-(*p*-Coumaroyl)-tryptamine



N-Acetyltryptamine



3-Oxo-β-ionone

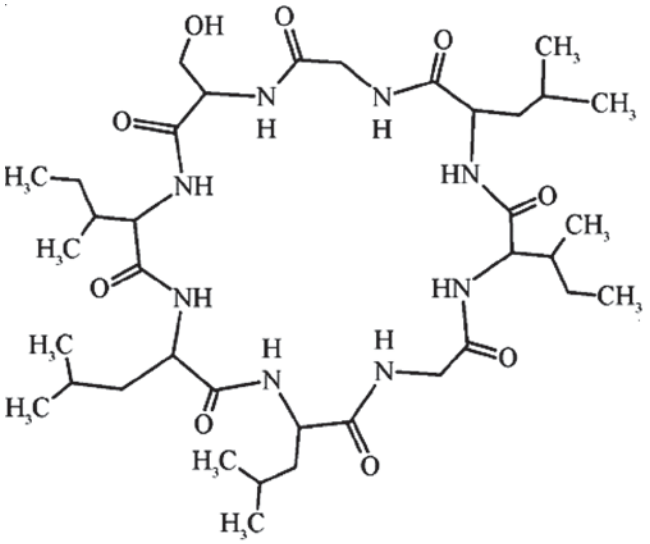
A CYCLOPEPTIDE ISOLATED FROM *JATROPHA RIBIFOLIA* (POHL) BAILL AND ITS SOLID PHASE SYNTHESIS

Meri Emili Pinto Ferreira,[1] Eduardo Maffud Cilli,[1] Joseam Fechine Tavares,[2] Marcelo Sobral da Silva,[2] Vanderlan da Silva Bolzani[1]

[1]*Universidade Estadual Paulista "Júlio de Mesquita Filho", UNESP Cep. 14800-900, Araraquara, São Paulo, Brazil,* [2]*Laboratório de Tecnologia Farmacêutica, UFPB, cx. Postal 5009, João Pessoa, Paraíba, Brazil*

Species of *Jatropha*, Euphorbiaceae family, is well known due to their several biologically active compounds. Their cyclic peptides are also an interesting class of secondary metabolites due to structural features and some pharmacological properties[1]. This work deals with the isolation and structure elucidation of a new cyclic peptide from *Jatropha ribifolia*, collected in the city of João Pessoa, located in the Northeast of Brazil. Two grams of crude extract of *J. ribifolia* were submitted to HPLC, and a new peptide was isolated along with known diterpenes. The synthesis of the new cyclic peptide was accomplished by solid-phase peptide synthesis (SPPS)-Fmoc/tBu, Some biological activities are being evaluated.

[1]Picchi, D.G. Altei, W.F., Saito, M.S., Bolzani, V.S., Cilli, E.M. Cyclic peptide from plant biomass: chemical features and diversity, biosynthesis and biological activities, Química Nova, v. 32, n. 5, p. 1262–1277, 2009.



[*c*(Leu - Gly - Ser - Ile - Leu - Leu - Gly - Ile)]

A THYMOL DERIVATIVE FROM *AGERATINA GLABRATA*

Armando A. Talavera,[1] Guadalupe P. Garcia,[2] Mario H. Gómez,[1] Hugo G. Garcia,[1] Isabel G. Martinez,[2] Thomas Scior,[2] *Rosa del Rio*[1]

[1]*Universidad Michoacana de San Nicolás de Hidalgo, Instituto de Investigaciones Químico Biológicas, Morelia, Michoacán 58030, México,* [2]*Benémerita Universidad Autónoma de Puebla, Facultad de Ciencias Químicas, Puebla, 72570, México*

Ageratina glabrata, H.B.K (syn. *Eupatorium glabratum*, Kunth) is widely distributed throughout Mexico and popularly known as "chamizo blanco" and "hierba del golpe" for its traditional use as external analgesic remedy. NMR and mass analyses identified a thymol derivative. The dichloromethane extract of *A. glabrata* leaves was fractionated by column chromatography affording a pure compound 10-benzoyloxy-6,8,9-trihydroxythymol isobutyrate The ^{13}C NMR spectrum was in agreement with the proposed structure.

T-FBS-02 IS A LIVER X RECEPTOR-α LIGAND AND STIMULATES REVERSE CHOLESTEROL TRANSPORT WITHOUT INDUCING HEPATIC LIPOGENESIS

Minh-Hien Hoang, Hee-jin Jun, Yaoyao Jia, *Sung-Joon Lee*

Korea University, Food Bioscience and Technology, Seoul, 136–713, South Korea

Aim to investigate the ligand activity of T-FBS-02 on liver X receptors (LXRs). Methods and results T-FBS-02 bound-directly to LXR-α but not LXR-β in a reporter gene assay, time-resolved fluorescence resonance energy transfer analysis,

and limited protease digestion experiment. T-FBS-02 stimulation in macrophages stimulated cellular cholesterol efflux dose-dependently, with induction of ATP-binding cassette transporter A1 and G gene and protein expression. In hepatocytes, T-FBS-02 significantly induced Insig-2a levels and delayed nuclear translocation of sterol regulatory element-binding protein 1 (SREBP-1) protein, resulting in a dose-dependent reduction in the expression of genes in fatty acid synthesis and cellular lipid levels.

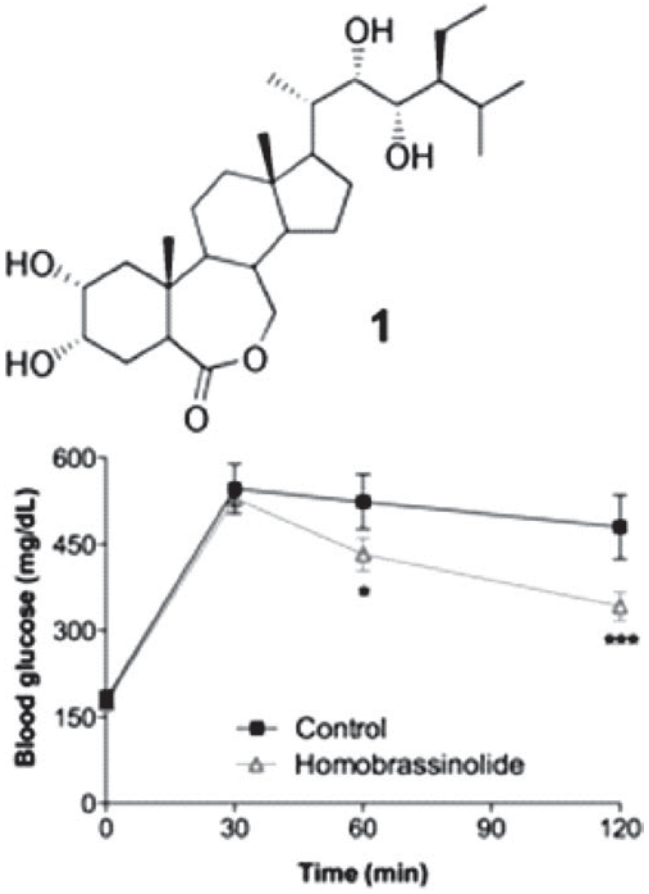
Conclusion: T-FBS-02, a direct ligand for LXR-α, activates reverse cholesterol transport in macrophages without inducing hepatic lipogenesis. The induction of Insig-2a suppressed the nuclear translocation of SREBP-1c.

BRASSINOSTEROID DECREASES HYPERGLYCEMIA IN A DIET-INDUCED OBESITY MOUSE MODEL

Debora Esposito,[1] Slavko Komarnytsky,[1,2] Pablo Kizelsztein,[1] Ilya Raskin[1]

[1]*Rutgers University, Plant Biology/BIOTECH, New Brunswick, NJ 08901, USA,* [2]*NC State University, Plants for Human Health Institute, Kannapolis, NC 28081, USA*

The prevalence of obesity is increasing globally, and obesity is a major risk factor for metabolic syndrome. Previously we observed that daily oral administration of homobrassinolide (**1**) to healthy rats resulted in a slight decrease in fasting blood glucose accompanied by reduction of hepatic expression of PEPCK and G6Pase mRNAs, as well as hepatic AMPK activation. In H4IIE rat hepatoma cells, **1** reduced glucose production and decreased expression of PEPCK and G6Pase in cAMP-stimulated upregulation bioassay. Acute, single-dose administration of 50–300 mg/kg of **1** resulted in a significant reduction in the fasting blood glucose in high fat diet-induced obese C57BL/6J mice. Animals receiving 50 mg/kg of **1** for 8 weeks showed 23% lower fasting blood glucose levels and enhanced insulin sensitivity. Gene expression for hepatic PEPCK and G6Pase was significantly decreased. Thus, the data demonstrate that homobrassinolide improves glucose metabolism and increases insulin sensitivity in an animal model of obesity and insulin resistance.



CAFFEINE ATTENUATES THE CYTOTOXIC ACTIVITIES OF INTERCALATING AROMATIC ALKALOIDS

William N. Setzer,[1] Gabrielle M. Hill,[1] Debra M. Moriarity[2]

[1]*University of Alabama in Huntsville, Department of Chemistry, Huntsville, AL 35899, USA,* [2]*University of Alabama in Huntsville, Department of Biological Sciences, Huntsville, AL 35899, USA*

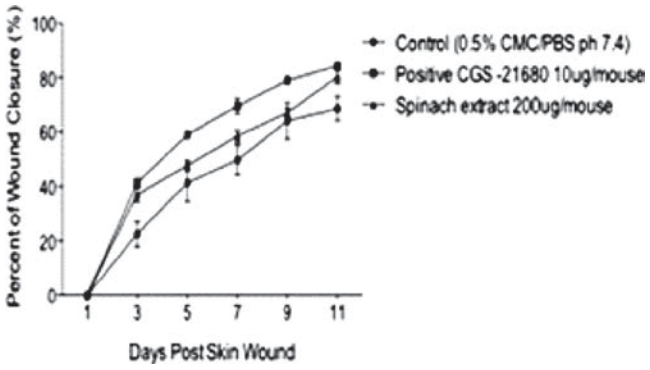
Many anti-tumor drugs function by intercalating into DNA. The xanthine alkaloid caffeine can also intercalate into DNA as well as form π-π molecular complexes with other planar alkaloids and anti-tumor drugs. The presence of caffeine could interfere with the intercalating anti-tumor drug by forming π-π molecular complexes with the drug, thereby blocking the planar aromatic drugs from intercalating into the DNA and ultimately lowering the toxicity of the drug to the cancer cells. The cytotoxic activities of several known DNA intercalators (berberine, camptothecin, chelerythrine, ellipticine, and sanguinarine) on MCF-7 breast cancer cells, both with and without caffeine present (200 μg/mL) were determined. Significant attenuation of the cytotoxicities by caffeine was found. Computational molecular modeling studies involving the intercalating anti-tumor drugs with caffeine were also carried out using density functional theory (DFT) and the recently developed M06 functional. Relatively strong π-π interaction energies between caffeine and the intercalators were found, suggesting an "interceptor" role of caffeine protecting the DNA from intercalation.

WOUND HEALING ACTIVITY OF PHYTOECDYSTEROIDS

Debora Esposito,[1] Brittany Graff,[1] Alexander Poulev,[1] Ilya Raskin[1]

[1]*Rutgers University, BIOTECH Center / Plant Biology, New Brunswick, NJ 08901, USA*

Ecdysteroids are polyhydroxylated derivatives of 5α-cholestane, structurally similar to cholesterol-derived animal steroid hormones. Plants are natural sources of ecdysteroids. Edible plants, such as *Spinacia oleracea* (spinach), contain considerable amounts of ecdysteroids, such as 20-hydroxyecdysone (**1**). *Ajuga turkestanica*, an herb from the basil family native to Uzbekistan, contains high levels of the turkesterone (**2**). Since the increasing interest in the identification of biologically active natural products in traditionally used botanicals, the objective of this investigation was to evaluate extracts and identify biologically active compounds using an *in vitro* skin fibroblast migration and proliferation. Compounds **1** and **2** were the most active among the tested ecdysteroids. We also employed a skin closure model in CD-1 mice to show that spinach extract significantly accelerated cutaneous wound closure. These compounds and the methodologies employed can be used for further studies on the biological role of ecdysteroids, as well as the potential application of crop plants which contain high ecdysteroid levels.



POLYPHENOL SYNERGISM AUGMENTS THERAPEUTIC POTENTIAL OF RESVERATROL: UPDATING OF THE FRENCH PARADOX

Jose Angel Marañon,[1] Arrate Lasa,[3] Sonia Blasco Sancho,[2] Itxiar Txurruka,[3] Ernesto Caballero,[2] Maria P. Portillo Baquedano,[3] Fernando Galan-Estella[2]

[1]*Tradichem Biotech, Madrid, 28040, Spain,* [2]*Universidad Autonoma de Madrid, Quimica Analitica y Analisis Instrumental, Madrid, Spain 28040,* [3]*Universidad del Pais Vasco, Facultad de Farmacia, Nutricion y Bromatologia, Vitoria, 01006, Spain*

Resveratrol, one of the minor components of red wine, has been considered as the molecule responsible of French paradox. Resveradox has also been described as a cardioprotective, anti-inflammatory, antioxidant and chemopreventive agent, however is a molecule with high absorption and very low bioavailability. This molecule seems also to have high therapeutic potential in obesity. Other polyphenols, catechins and flavonols are other major polyphenols found in red wine and grapes. The purpose of this work was to assess if polyphenol blending, mimicking the grape total polyphenol content, could increase the biological activities of resveratrol. The catechin-quercetin-resveratrol blend, upregulates NO synthase expression in a bigger extent than resveratrol stand alone. This blending have more antioxidant effect and a similar lipolytic/anti-obesity effect compared to resveratrol stand alone. Moreover the catechin-flavonoid-resveratrol composition seems to be more bioavailable than resveratrol standalone, because of the inhibition of the sulfation of resveratrol in the liver. The results presented suggest that resveratrol-polyphenol synergism could contribute to the therapeutic potential. Therefore resveratrol synergized by polyphenols may be the true cause of the French Paradox.

CELASTRUS ACULEATUS MERR. SUPPRESSES AUTOIMMUNE ARTHRITIS IN RATS BY INHIBITING PATHOGENIC T-CELL AND ANTIBODY RESPONSES

Shivaprasad H. Venkatesha,[1] Hua Yu,[1] Siddaraju Nanjundaiah,[1] Brian Astry,[1] Rajesh Rajaiah,[1] Li Tong,[1,2] *Kamal D. Moudgil*[1]

[1] *University of Maryland School of Medicine, Department of Microbiology and Immunology, Baltimore, MD 21201, USA,* [2] *Southern Medical University, School of Traditional Chinese Medicine, GuangZhou, 510515, China*

Rheumatoid arthritis (RA) is one of the major autoimmune diseases of global prevalence. The prolonged use of conventionally-used drugs is associated with severe adverse reactions. Therefore, safer and less expensive therapeutic products are continually being sought. *Celastrus aculeatus* Merr. (Celastrus) is a traditional Chinese medicinal herb that has been used in folk medicine for centuries for the treatment of rheumatic conditions. Celastrol represents one of the bioactive components of Celastrus. We examined the anti-arthritic activity of Celastrus and Celastrol, as well as the immunological basis of their action using the rat adjuvant-induced arthritis (AA) model of human RA. Lewis rats were treated with Celastrus (1.5/3g/kg) or Celastrol (1 mg/kg) daily beginning at the onset of arthritis and then continued throughout the observation period. The severity of clinical and histological arthritis was graded, and the levels of specific disease-related parameters were measured. Celastrus/Celastrol inhibited the severity of ongoing AA, along with significant reduction in the level of proinflammatory cytokines: IL-17, IL-6 and IFN-g; transcription factors: STAT3 and ROR-gt; pathogenic antibodies; MMP9 activity; and phospho-ERK. Thus, Celastrus suppresses the mediators of immune pathology in arthritis, and it offers a promising alternative/adjunct treatment for RA.

COMPARISON OF ANTI-HEPATOFIBROTIC EFFECT: *ARTEMISIA CAPILLARIES* VS. *ARTEMISIA IWAYOMOGI*

Jing-Hua Wang, *Chang-Gue Son*

Liver and Immunology Research Center, Daejeon Oriental Hospital of Daejeon University, Daejeon, 301–704, South Korea

Artemisia capillaries and *Artemisia iwayomogi* have been indiscriminately utilized for variously liver disorders as a traditional hepatotherapeutic medicine in Asian countries including Korea. In the present study, anti-hepatofibrotic effect of water extract of *Artemisia capillaries* (AC) and *Artemisia iwayomogi* (AI) were compared in carbon tetrachloride-induced liver fibrosis rat model. AI (50 mg/kg) significantly attenuated the CCl_4-induced excessive release of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and total bilirubin ($p < 0.05$) in serum, and hydroxyproline and malondialdehyde contents ($p < 0.05$) in liver tissue. Further, AI markedly ameliorated total antioxidant capacity (TAC), Glutathione (GSH), Superoxide dismutase (SOD) ($p < 0.05$) in liver tissue. Unexpectedly AC didn't change any of the above parameters. Meanwhile, histopathological and immunohistochemical analysis revealed that AI drastically reduced the inflammation, necrosis, collagen accumulation and activation of hepatic satellite cells in liver tissue rather than AC treatment. Several fibrosis-related genes such as transforming growth factor β, platelet-derived growth factor β and connective tissue growth factor and α-smooth muscle actin were more prominently down-regulated by AI than AC treatment.

Taken together, our results clarified that AI has more potentially hepatoprotective and anti-fibrotic properties rather than AC through enhancing antioxidant capacity and down-regulation of fibrogentic cytokines.

NANOTECHNOLOGY IN PHYTOMEDICINES

Marco Antonio Botelho,[1] Ronaldo Sousa Ruela,[1] Dinalva Brito Queiroz,[4] Eduardo Gomes Azevedo,[2] Leonard Eduward Bannet,[3] Ana Helena Patrus,[3] Aarão Lyra[5]

[1] *Federal Institute of Science and Technology, Research and Innovation, Fortaleza, Ceara 60811-440, Brazil,* [2] *Anna Aslam Medical Center, Biomedical Research, Sao Paulo, SP, 60811-440, Brazil,* [3] *Santé Medical Center, Clinical Medicine, Sao Paulo, SP 01490-000, Brazil,* [4] *Evidence Pharmaceutics, Biotechnology, Fortaleza, Ceara, 60125-100, Brazil,* [5] *University Potiguar, School of Health, Natal, Rio Grande do Norte, 59060600, Brazil*

Objectives: The aim of this study was to compare the antiplaque and antigingivitis effectiveness of an essential oil-containing nanostructured gel.

Material and Methods: Thirty five qualifying subjects, aged 18–65 years, were randomized into two groups: essential oil nanostructured gel (Cepakill® Antiseptic); 0.12% chlorhexidine nanostructured gel (Nano gel®). At baseline, subjects received a complete oral soft tissue examination and scoring of plaque index (PI), gingival index (GI), and gingival bleeding index (GBI). Subjects started locally applying twice daily with their respective nanostructured gel as an adjunct to their usual mechanical oral hygiene procedures. Subjects were reexamined at 7 days. The treatment groups were compared with respect to baseline clinical variables.

Results: From the 35 subjects at baseline, 32 were evaluated after 7 days of treatment. There were no statistically significant differences among the two groups from baseline and after 7 days treatment.

Conclusion: This 1 week controlled clinical study demonstrated that the essential oil nanostructured gel and the chlorhexidine nanostructured gel had comparable antiplaque and antigingivitis activity.

SYNERGISTIC EFFECTS BETWEEN AN AQUEOUS EXTRACT OF *PANAX QUINQUEFOLIUS* AND TOBRAMYCIN AGAINST *PSEUDOMONAS AERUGINOSA* IN A RAT MODEL OF LUNG INFECTION

Misagh Alipour,[1,2,3] Abdel Omri,[2] Zacharias Suntres[1,2,3]

[1]*Northern Ontario School of Medicine, Medical Sciences Division, Thunder Bay, Ontario P7B 5E1, Canada,* [2]*Laurentian University, Programme of Biomolecular Sciences, Sudbury, Ontario P3E 2C6, Canada,* [3]*Ontario Ginseng Innovation & Research Consortium, London, Ontario N6A 5C1, Canada*

This study describes the immunomodulatory and antimicrobial properties of the North American ginseng extract (*Panax quinquefolius*) in synergy with the antibiotic tobramycin to combat *Pseudomonas aeruginosa in vivo*. The infection was induced in male Sprague-Dawley rats under anesthesia by intratracheal instillation of *P. aeruginosa* in agar-beads (107 colony forming units (CFU)). Starting on day 7 post-infection, animals were treated daily, for three consecutive days with saline, tobramycin (300 μg/kg b.wt. intratracheal) and/or aqueous extract of ginseng (100 mg/kg b.wt. subcutaneous); animals were killed 24 h after the third drug instillation. The infection in the control group was successfully maintained after 10 days of infection (6.0×106 CFU). Treatment of infected animals with tobramycin (6.6×104 CFU), ginseng (5.3×104 CFU), or tobramycin and ginseng (2.0×103 CFU) reduced the bacterial infection in the lungs. Cytokine analysis revealed a reduction of pro-inflammatory cytokines (IL-2, IL-4, IL-6, IL-12p70, IFN-α, GM-CSF, TNF-α) in infected animals treated with the ginseng extract. Taken together, the North American ginseng extract promoted the killing of *P. aeruginosa* and attenuated the inflammatory response. (Support provided by the Ministry of Research & Innovation, The Ontario Research Fund-Research Excellence program).

THE ALTERATION OF COMPONENTS IN THE FERMENTED HWANGRYUNHAEDOK-TANG AND ITS NEUROPROTECTIVE ACTIVITY

Bohyoung Lee, Hye Jin Yang, Jin Bae Weon, Eun young Mok, Choong Je Ma

Kangwon National University, Department of Biomaterials Engineering, Division of Bioscience and Biotechnology, Chuncheon, 200–701, South Korea

Hwangryunhaedok-tang is a traditional herbal prescription that has sedative activity, hypotensive and anti-bacterial effects. In this study, we investigated the alteration of contents of components in Hwangryunhaedok-tang, antioxidant activity and neuroprotective activity by fermentation with *Lactobacillus acidophilus* KFRI 128. Contents of three marker compounds (geniposide, berberine and palmatine) and unknown compounds in the Hwangryunhaedok-tang (HR) and the fermented Hwangryunhaedok-tang (FHR) were measured and compared using a photodiode HPLC-DAD. The antioxidant activity of HR and FHR were determined by DPPH free radical and hydrogen peroxide (H_2O_2) scavenging assay. Also, the neuroprotective activities of HR and FHR against glutamate-induced oxidative stress in a mouse hippocampal cell line (HT22) were evaluated by MTT assay. The contents of geniposide and palmatine decreased, but not the content of berberine was increased in the FHR. The contents of unknown compounds (**1**), (**2**), (**3**), (**4**) and (**5**) in the HR were altered by fermentation. Electron donating activity (EDA, %) value of FHR was higher than HR for DPPH radical scavenging activity and H_2O_2 scavenging activity, respectively. In the MTT assay, FHR showed more potent neuroprotective activity than HR by 513.9%. Clearly the fermentation converts compounds in HR and enhances the antioxidant and neuroprotective activity.

IN VITRO AND *IN VIVO* ANTITUMOR EFFECTS OF DEOXYELEPHANTOPIN ON HUMAN BREAST CANCER CELLS

Ning-Sun Yang, Kandan Aravindaram, Yun-Hsiang Chen, Pei-Hsueh Wang, Chun-Wen Lan, Lie-Fen Shyur

Academia Sinica, Agricultural Biotechnology Research Center, Taipei 115, Taiwan

Breast cancers are very common for women and a major cause of death in women worldwide. They can be often highly resistant to current chemotherapy, and thus new therapeutics are desirable. In this study, we evaluated the effect of deoxyelephantopin (DET), a phytocompound extracted from *Elephantopus scaber* (Asteraceae), as a possible antitumor phytomedicine against human breast cancer cells, MDA-MB-231. We observed that DET can effectively suppress the growth of test tumor cells *in vitro* using cell-apoptosis assay. Upon DET treatment, transforming growth factor-beta (TGF-beta) level was significantly decreased in test cells. DET apparently can inhibit cell growth by inducing a G2-M

phase cell cycle arrest and apoptosis in test cells, and the clonogenicity of these cells was also reduced in a concentration-dependent manner. DET can also significantly inhibit the invasion and migration of MDA-MB-231 cells. The effect of DET on suppression of NF-κB, via activation by TNF-α, was examined using electrophoretic mobility shift analysis (EMSA). Decreased level on expression of phospho-NF-kappaB and the downstream molecules of NF-κB signaling pathway, including survivin, Bcl-2, MMP-9 and VEGF, were observed in DET-treated cells. Under *in vivo* conditions, DET significantly inhibited tumor growth and the myeloid derived suppressor cells (MDSCs) population in nude mice experiment. Taken together, our findings suggest that DET may warrant systematic investigation for potential application to chemoprevention or control of breast cancers.

ADJUVANT EFFECT OF SPECIFIC MICROTUBULE-DEPOLYMIRIZING AGENTS ON DENDRITIC CELL-BASED CANCER VACCINES

Chih-Chun Wen,[1,2] Hui-Ming Chen,[2] Swey-Shen Chen,[2] Li-Ting Huang, Li-Chen Chou,[2] Jin-Bin Wu,[1] Sheng-Chu Kuo,[1] Ning-Sun Yang[2]

[1]*Graduate Institute of Pharmaceutical Chemistry, China Medical University, Taichung, 404, Taiwan,* [2]*Agricultural Biotechnology Research Center, Academia Sinica, Taipei, 115, Taiwan*

Damage-associated molecular patterns (DAMPs) are associated with immunogenic cell death (ICD). Specific microtubule-depolymerizing agents (MDAs) such as colchicine have been shown to confer anti-cancer activity and also trigger activation of DCs. In this study, we evaluated the ability of three MDAs (colchicine and two 2-phenyl-4-quinolone analogues) to induce ICD in test tumor cells, and activate DCs. The three test phytochemicals considerably increased the expression of DAMPs including HSP70, HSP90 and HMGB1, but it had no effect on expression of calreticulin (CRT). DC vaccines pulsed with MDA-treated tumor cell lysates (TCLs) had a significant effect on tumor suppression, cytotoxic T-lymphocyte activity, and survival rate of test mice. *In vivo* antibody depletion experiments suggested that CD8+ and NK cells, were the main effector cells responsible for the anti-tumor activity. In addition, culture of DCs with GM-CSF and IL-4 significantly increased the production of IL-12 and decreased production of IL-10. MDAs also induced phenotypic maturation of DCs and augmented $CD4^+$ and $CD8^+$ T-cell proliferation. Specific MDAs including the clinical drug, colchicine, can induce immunogenic cell death in tumor cells, and DCs pulsed with MDA-treated TCLs can generate potent anti-tumor immunity in mice. This approach may warrant future clinical evaluation as a cancer vaccine.

ANTI-UROLITHIATIC SCREENING OF AERIAL PARTS OF *ERYTHRINA STRICTA*

Pradeep Kamboj, Ajudhia Nath Kalia

ISF College of Pharmacy, Dept. of Pharmacognosy, Moga, Punjab 142001, India

The anti-urolithiatic activity of *Erythrina stricta* (aerial parts) was evaluated by a calculi-producing diet model. Calcium oxalate nephrolithiasis was induced by injecting sodium oxalate (7 mg/100 g/day, i.p.) for 7 days. A significant increase in the serum ASAT, ALAT, ALP levels was observed in the control group receiving sodium oxalate. In addition, animals of the control group showed a decrease in the level of serum enzymatic catalase and significant increase in the levels of thiobarbituric acid reactive substances (TBARS) in kidney homogenates. Oral administration of a 70% ethanol extract (500 mg/kg/day, b.w.) and ethyl acetate fraction (200 mg/kg/day, b.w.) along with sodium oxalate in treated groups, showed a significant dose dependent restoration of all altered serum and homogenate enzymatic parameters. Further, histological estimation of kidneys in treated groups strongly inhibited the growth of calculi within the tubule and reduced necrosis of tubular epithelial cell. The results indicate that the aerial parts of *Erythrina stricta* are endowed with anti-urolithiatic activity as evidenced by an inhibitory effect on crystal growth and the improvement of kidney function and architecture.

ANTI-DIABETIC EFFECT OF PICEATANNOL, A STILBENOID, *IN VITRO* AND *IN VIVO*

Miki Minakawa,[1] Yutaka Miura,[2]*Kazumi Yagasaki*[2]

[1]*Tokyo University of Agriculture and Technology, Department of Applied Life Science, Graduate School of Agricultural Science, Tokyo, 183–8509, Japan,* [2]*Tokyo University of Agriculture and Technology, Division of Applied Biological Chemistry, Institute of Agriculture, Tokyo, 183–8509, Japan*

Piceatannnol (PIC) has been found to suppress an increase in fasting blood glucose levels and to improve impaired glucose tolerance in type 2 diabetic model db/db mice. In L6 myotubes, PIC dose-dependently and significantly promoted glucose uptake. It was demonstrated to increase the phosphorylation of AMP-activated protein kinase (AMPK) and the translocation of glucose transporter 4 (GLUT4) to plasma membrane by Western blotting. The stimulatory effect of PIC on the translocation of GLUT4 was also confirmed by transfecting Halo Tag-glut4 vector to L6 cells and visualization. PIC is suggested to show anti-diabetic effect by, at least in part, stimulating AMPK-dependent glucose uptake in muscles.

OCCURRENCE OF LIGNOIDS IN SCHILLERIA AND MACROSTACHYS CLADES OF *PIPER*

Nidia C. Yoshida,[1] Lydia F. Yamaguchi,[1] Antonio Salatino,[2] Juan D. R. Rojas,[3] Gabriel P. Maldonado,[3]Massuo J. Kato[1]

[1]University of São Paulo, Department of Fundamental Chemistry, São Paulo, SP 0550800, Brazil, [2]University of São Paulo, Department of Botany, São Paulo, SP 05422970, Brazil, [3]University of São Paulo, Department of Microbiology, São Paulo, SP 05508900, Brazil

The phylogenetic analysis in *Piper* species using ITS as molecular marker distinguished clades specialized in the production of benzoic acids/chromenes, amides and lignoids. The chemical profiles of these species were determined by analysis of crude extracts by ^{1}H NMR and HPLC combined with principal component analysis (PCA) and hierarchical cluster analysis (HCA). It is remarkable that lignans of dibenzylbutyrolactone and furofuran types occurr in *Macrostachys*, while *Schilleria* contain dimers of propenylphenols such as tetrahydrofurans and conocarpans. The sequences of dirigent protein genes involved in the stereoselectivity of dimerization step indicated consistencies among *Piper* species but without resolution between clades.

THE ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES FROM LEAVES AND STICKS OF *ILLICIUM VERUM*

Li-Yeh Chuang,[1] Cheng-Hong Yang,[2] Ya-Yun Hsieh,[1] Cheng-San Yang[3]

[1]I-Shou University, Institute of Biotechnology and Chemical Engineering, Kaohsiung 840, Taiwan, [2]National Kaohsiung University of Applied Sciences, Department of Electronic Engineering, Kaohsiung 807, Taiwan, [3]Chiayi Christian Hospital, Department of Plastic Surgery, Chiayi 600, Taiwan

In this study, we compared the antibacterial and antioxidant activities of supercritical fluid (SFE) and 95% ethanol extracts from leaves and sticks of *Illicium verum*. The obtained results revealed that the SFE extracts showed better antimicrobial activity than the alcoholic extracts against the clinical antibiotic resistant pathogens with minimum inhibitory concentration (MIC) value at 0.1 mg/mL. The extracts obtained from sticks showed better antimicrobial activity than the extracts of leaves, although the leaf extracts possess a broader antimicrobial spectrum against all the test strains. Moreover, the chemical components of SFE extracts, anethole, anisyl aldehyde and anisyl acetone, provided the antibacterial activity of *Illicium verum* leaves, and anethole was the major antibacterial substance of *Illicium verum* sticks. For the antioxidant activity determination, the alcoholic extracts of leaves and sticks showed better antioxidant activities than the extracts of SFE. Overall, the results revealed that the extracts of *Illicium verum* leaves and sticks have the potential to be developed as natural antibiotics and antioxidants.

HYPOLIPIDEMIC EFFECT OF *DIOSCOREA OPPOSITA* ON DIET-INDUCED OBESITY IN MICE

Jinwoong Kim, Min Hye Yang

Seoul National University, College of Pharmacy, Research Institute of Pharma-ceutical Sciences, Seoul, 151–742, South Korea

Plants belonging to the genus *Dioscorea* have long been used as edible tuber crops in many tropical and subtropical areas, and as a traditional herbal medicine in oriental countries including Korea, China, and Japan. In this study, a number of experiments were carried out to evaluate the hypolipidemic effect of the *n*-BuOH-soluble extract from *Dioscorea opposita* rhizomes against high-fat induced mice *in vivo*. The body weights, parametrial adipose tissue weights, and the levels of TG, TC and LDL-cholesterol in blood serum of female ICR mice were significantly decreased by feeding a high-fat diet with BuOH extract for 8 weeks. In addition, the anti-obesity effects of the phenolic compounds isolated from BuOH extract were assessed using pancreatic lipase *in vitro*. The seventeen compounds isolated from *D. opposita* remarkably reduced pancreatic lipase activities. These results suggested that hypolipidemic effects of *D. opposita* in high-fat diet induced mice may be due to the inhibition of intestinal absorption of dietary fat.

CY-FBS-01, IS A PAN-AGONIST FOR PPARS

Yaoyao Jia, Sung-Joon Lee, Hee-jin Jun, Ji-Hae Lee, *Minh Hien Hoang*

Korea University, Food Bioscience and Technology, Seoul, 136–713, South Korea

Cy-FBS-01, a natural flavonoid, has anti-atherogenic activity both *in vitro* and *in vivo*; however, its molecular target has not been clearly understood. We investigated the ligand binding of CY-FBS-01 to peroxisome proliferator-activated receptors (PPARs) and its effects on lipid metabolism *in vitro*. CY-FBS-01 directly bound to all PPAR subtypes in surface plasmon resonance assay, and induced transactivation activity in reporter gene assay and time-resolved fluorescence resonance energy transfer analyses. CY-FBS-01 significantly reduced cellular lipid concentrations in lipid-loaded

hepatocytes as well. Hepatic transcriptome profiling in lipid-loaded hepatocytes revealed that the net effects of CY-FBS-01 in lipid metabolism pathways were similar to those of fenofibrate and statin, normalizing the expressions in lipid metabolism gene expressions. CY-FBS-01 induced unique target genes of PPARs in the hepatocytes as well. CY-FBS-01, a phytochemical flavonoid abundant in fruit and vegetable and is a pan-agonist for PPARs.

LUTEOLIN SENSITIZES SK-HEP1 HUMAN HEPATOCELLULAR CARCINOMA CELLS TO TRAIL-INDUCED APOPTOSIS THROUGH UPREGULATION OF DEATH RECEPTORS

Eun Young Kim, An Keun Kim

College of Pharmacy, Sookmyung Women's University, Seoul, 140–742, South Korea

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising candidate for cancer treatment since it selectively induces apoptosis in many cancer cells. However, the cytotoxic effect of TRAIL is limited in some TRAIL-resistant cancer cells. Luteolin, a flavonoid found in many plants, exerts various biological and pharmacological activities. In the present study, we examined the effect of luteolin on TRAIL-induced apoptosis in human hepatocellular carcinoma (HCC) SK-Hep1 cells. Combined treatment of luteolin and TRAIL induced marked decrease of cell viability. Cleavage of PARP and activation of effector caspases (caspase-3, 6, and 7) demonstrated that the combined effect of luteolin and TRAIL is mediated through apoptosis. Treatment with luteolin in combination with TRAIL induced the activation of death receptor pathway-related proteins like caspase-8, DR4 and DR5. The synergy effect of TRAIL/luteolin combination was markedly blocked in the presence of DR4/Fc and DR5/Fc chimeric proteins. Our results indicate that luteolin sensitizes SK-Hep1 HCC cells through the death receptor signaling pathway.

ANTIINFLAMMATORY EFFECT OF *PSITTACANTHUS CALYCULATHUS*

Miguel A. Zavala S., Diana E. Martínez, *Salud Pérez G.*, Cuauhtemoc Pérez G.

Department of Sistemas Biológicos. Universidad Autónoma Metropolitana-Xochimilco AP 23–181 México D.F.

Psittacanthus calyculathus (D.C.) G. Don is a large plant with many flowers in crown of an oak. It is used in traditional medicine in the treatment of inflammatory problems.

Chloroform and methanol extracts were prepared by heating and the anti-inflammatory activity of both extracts was tested in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear edema using indomethacin as positive control, at doses of 2 mg/ear. It was found that the methanol extract did not have any effect on this model, however the chloroform extract showed a significant inhibition (53.3 ± 2.4), similar to that obtained with indomethacin (60.2 ± 1.1). Also this extract had activity on the model of mouse ear edema induced by multiple topical applications of TPA (40.7 ± 10.0), and on carrageenan-induced edema. Phytochemical analysis of the chloroform extract gave positive for flavonoids, terpenes and sesquiterpenlactones.

EFFECT OF SMOKING IN THE LEPTIN LEVELS IN OBESITY RATS

Julia Pérez R.[1], Martha Montaño R.[2], Daniel Mercado H.[1], José G. Cisneros L.[2], Carlos Ramos A.[2]

[1]Universidad Autónoma Metropolitana-Xochimilco A.P. 04960 México D.F., [2]Instituto Nacional de Enfermedades Respiratorias Calz.De Tlalpan 14080 México D.F.

The leptin, insulin and triglycerides levels were determined in obesity and smoking Wistar rats for four months. Four groups of 8 rats each were used, one healthy rats as control, the second obesity rats no smoking, third group no obesity and smoking, and last group obesity/smoking. After four months the animals were weighed and a blood sample were obtained, later the lung and the abdominal fat were removed. The triglycerides, leptin, insulin and glucose were analyzed in plasma. Histology showed the presence of lipocytes and fat in the rats obesity and obesity/smoking. The abdominal fat, weighed, level of insulin and leptin were decreased in obesity/smoking, the levels of triglycerides and glucose were not modified in obesity/smoking rats.

EFFECT OF NICOTINIC ACID ON CHOLESTEROL AND TRIGLYCERIDES LEVELS IN OBESITY RATS

Julia Pérez R., Ana L. Esquivel C., M. Cristina Fresan O., Miguel A. Mosqueda C.

Universidad Autónoma Metropolitana-Xochimilco A.P. 04960, México D.F.

The pharmacological effect of niacin on levels of adiponectin, leptin, and soluble leptin receptor in plasma, was evaluated, also the gas exchange was determined, in obesity Zucker-Zucker male rats 53 weeks old. Two groups of eight rats each were used. One group was administered with niacin (2%), for three months, and the other group was used as control. After this time the animals were sacrificed and a blood sample was obtained, the oxygen and CO_2 partial

pressures, oxygen saturation were measured. Also in plasma were evaluated cholesterol, triglycerides, glucose, insulin, adiponectin, liponectin, TNFα, and IL-6 levels. It was found that niacin increases the oxygen saturation and decreased CO_2 partial pressure. This compound also diminishes cholesterol, triglycerides, and IL-6 plasma levels but has no effect on the other parameters. These results suggest that niacin could help the conditions of old people in special of obesity persons.

INSECTICIDE ACTIVITY OF *SALVIA CONNIVENS* AND *SENECIO SALIGNUS*, AGAINST *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE).

Miguel Ramos, Osvaldo Campos, Miguel Zavala, *Salud Pérez,* Diana Martínez.

Universidad Autónoma Metropolitana-Xochimilco, Departamento de Sistemas Biológicos, Calzada del Hueso 1100, Col. Villa Quietud, C.P. 04960, México D.F.

The insecticide activity of chloroform extract of aerial parts of *Salvia connivens* Epling (Lamiaceae) and *Senecio salignus* D.C. (Asteraceae) were tested against first intar larvae from *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae). There were tested six concentrations (5000, 4000, 2000, 1000, 500 and 0 ppm). The larval mortality with *S. connivens* was 91.7 ± 5.8 %, 79.2 ± 8.5%, 70.8 ± 9.8%, 62.5 ± 10.1%, and 37.5 ± 10.1%, at 5000, 4000, 2000, 1000, 500 ppm, and the pupal mortality was 100 ± 0%, 100 ± 0%, 37.5 ± 8.5%, and 30 ± 9.0% at 5000, 4000, 2000, 1000 ppm. The extract of *S. salignus* showed 83.3 ± 7.8%, 66.7 ± 9.8%, 54.2 ± 10.4%, 33.3 ± 9.8%, of larval mortality at 5000, 4000, 2000, 1000 ppm, and the pupal mortality was 40 ± 5.8%, and 25 ± 9.0%, at 5000, and 4000 ppm.

The chloroform extract of *S. connivens* gave positive for flavonoids, sesquiterpenlactones, coumarins, and sterols, and to the extract of *S. salignus* was a positive to flavonoids.

This study demonstrated the insecticide activity of chloroform extract of the aerial parts of *S. connivens* and *S. salignus*.

ANTI-INFLAMMATORY ACTIVITY OF *SALVIA KEERLII*

Salud Pérez[1], Berenice Flores[1], Miguel A. Zavala[1], Norma Cardenas[2]

[1]*Department of Sistemas Biológicos. Universidad Autónoma Metropolitana-Xochimilco AP 23–181 México D.F.;* [2]*Universidad Autónoma de San Luis Potosí. Avenida Dr. Manuel Nava 6, San Luis Potosí, México*

The anti-inflammatory activity of chloroform and metanol extracts of *Salvia keerlii* was tested on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear edema using indomethacin as positive control, at doses of 2 mg/ear. Chloroform and methanol extracts showed similar activity (71.1 and 75.9% respectively), and in both cases the effect was higher than that obtained with indomethacin. The effect of chloroform extract also was tested on Carrageenan an induced rat paw edema, this extract at dose of 400 mg/kg significantly reduced inflammation by 60.1%. This extract also diminished inflammation of the TPA-induced ear edema by multiple administrations (50.6 ± 6.4) at dose of 2 mg/ear, the effect was similar to the indomethacin (59.6 ± 3.4).

A preliminary screening of the chloroform extract gave positive for flavonoids and sesquiterpenlactones.

The present study demonstrated the anti-inflammatory activity of chloroform extract of the leaves of *S. keerlii* in the three models used.

ACTIVITY OF TWO SPECIES OF GENUS *SENNA* ON *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE)

Miguel Ramos, Candy Romero, Miguel Zavala, *Salud Pérez*, Berenice Flores

Universidad Autónoma Metropolitana-Xochimilco, Departamento de Sistemas Biológicos, Calzada del Hueso 1100, Col. Villa Quietud, C.P. 04960, México D.F.

Many species from the genus Fabaceae has been used by their insecticide activity agains many insect pest, so there were tested the chloroform extract of aerial parts of *Senna septemtrionalis* H.S. Irving & Barnebyand *Senna wislizeni* H.S. Irving & Barneby (Fabaceae) against first intar larvae from *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) at six concentrations (5000, 4000, 2000, 1000, 500 and 0 ppm). The larval mortality were 70.8 ± 9.5%, 52.4 ± 10.4%, and 37.5 ± 10.1% at 5000, 4000, and 2000 ppm with *S. septemtrionalis* chloroform extract, and 58.3 ± 10.3%, 45.8 ± 10.4%, and 41.7 ± 10.1% at 5000, 4000, and 2000 ppm with *S. wislizeni*; the pupal mortality were 43 ± 7.8% and 27 ± 9.5% with *S. septemtrionalis*, at 5000, 4000 ppm, and 40 ± 9.0%, and 27.3 ± 10.6% with *S. wislizeni* at same concentrations.

A phytochemical screening of the chloroform extract of *S. septemtrionalis* gave positive for sterols, flavonoids, and sesquiterpenlactones, and the extract of *S. wislizeni* was a positive to flavonoids, lignanes, sterols, limonoids, and sesquiterpenlactones.

ASSESSMENT OF THE WOUND HEALING ACTIVITY OF METHANOLIC AND *n*-HEXANE EXTRACTS OF *GARCINIA KOLA* SEED IN ALBINO RATS

Chinedu Athanasius Eze, Innocent Chima Nwaogu, Emmanuel Nnaemeka Idoko

University of Nigeria, Nsukka, Faculty of Veterinary Medicine, Veterinary Surgery, Nsukka, Enugu +234, Nigeria

High rates of drug resistance in bacteria from wound isolates makes availability and provision of multiple choice/alternative drug sources at reduced costs imperative for effective management of wounds of any type. Medicinal plants are believed to be an important source of the new chemical substances with potential therapeutics. The assessment of wound healing activity of methanolic and *n*-hexane extracts of *Garcinia kola* seeds was studied using full skin thickness excision model on dorsum of albino rats of both sexes. Five and 10% of both extracts were prepared using white soft paraffin (base) as a vehicle. The ointment was topically and aseptically applied on the wound. Group A animals were treated with Cicatrin® powder (positive control), while groups B, C, D, and E received 10%, 5% methanol and 10%, 5% *n*-hexane extracts of *Garcinia kola* seed respectively. Group F rats were treated with White soft paraffin (negative control). The wound healing effect of the extracts was assessed using the wound appearance, mean rate/percentage of wound contraction as well as histopathology. The parameters obtained from the ointment treatment groups were compared with those of Cicatrin® powder (standard) and Base treated (negative controls) group animals respectively. The results showed that the healing rate was faster in group C than in other animal groups. However, the healing rate in group C treatment was comparable to group A animals on post-surgery days 18 and 21. The group D animals showed the least rate of healing throughout the study period. The surfaces of the wound in all groups were found to be wet and inflamed on day 3 post surgery. On day 9 post surgery, the wound surfaces of the animals in groups A, B, C and E were all dry while those of groups D and F were oily and fairly wet. The oily wound surface in group D animals lasted up to day 15 post surgery. However, no suppuration was noticed in all the groups during the period of experiment. Histopathology shows complete epitheliazation which was recorded in groups A, C and E on day 21 post surgery. The sequence of wound healing has shown that both extracts have wound healing activity. The 5% concentration of methanolic extract has better wound healing activity compared with concentrations of 10% methanolic and 5% and 10% *n*-hexane extract treated groups. Therefore, the 5% methanolic extract concentration may favorably be recommended as a topical ointment for incisional wound.

THE ANTI-ULCER ACTIVITIES OF THE OIL EXTRACT OF *BALANITES AEGYTIACA* SEED IN GUINEA PIGS

Chinedu Athanasius Eze, Simon Shuaibu Adamu, Anyogu Davison

University of Nigeria, Nsukka, Nigeria, Veterinary Surgery, Nsukka, Enugu +234, Nigeria, Univeresity of Maiduguri, Nigeria, Veterinary Surgery and Reproduction, Maiduguri, Borno +234, Nigeria

Peptic ulcer is caused by an uncontrollable increase in gastric acid secretion from the gastric glands, which are located in the body of the stomach. This study was carried out to investigate the anti-ulcer activities of the oil extract of *Balanites aegyptiaca* seed in guinea pigs. Thirty guinea pigs weighing between 200 g to 440.8 g were randomly assigned into six groups of five animals each. Gastroduodenal ulceration was induced using pyloric ligation and histamine injection after fasting the animals for 24 h and orally administering both the oil extract and omeprazole 1 h prior to ulcer induction. The anti-ulcer effect of the oil was compared with the standard (control), omeprazole (10 mg/kg body weight) given orally. The severity of the ulcers was scored using standard method following the sacrifice of the animals. The histopathology of the lesion was also studied. *Balanites aegyptiaca* oil significantly ($P < 0.05$) reduced the volume of gastric secretion in histamine induced ulcer model. It also reduced the mean severity of ulcer scores and ulcer index in both pyloric ligation and histamine induced models. The oil extract increased the percentage protection in both models of experiment. However, omeprazole at the dose rate of 10 mg/kg body weight gave better protection. The results showed that *B. aegyptiaca* oil possesses anti-secretory and cytoprotective activity which can be employed in the prevention, treatment and relief of gastric ulcer symptoms.

FUNCTIONAL GENOMICS TO ELUCIDATE NEW ENZYMES IN BENZYLISOQUINOLINE ALKALOID BIOSYNTHESIS

Thu-Thuy T. Dang, Peter James Facchini

University of Calgary, Department of Biological Sciences, Calgary, AB T2N 1N4, Canada

Opium poppy (*Papaver somniferum*) contains more than 80 benzylisoquinoline alkaloids (BIAs) including the pharmacologically active compounds morphine, codeine, noscapine, papaverine and sanguinarine. Of these, the biosynthesis of

morphine and codeine are best understood. However, the biosynthetic pathways leading to most other BIAs are not very well characterized. Stem cDNA libraries of eight opium poppy cultivars displaying different BIA profiles were analyzed using 454 pyrosequencing to generate transcript profile databases. Comparative transcript and metabolite profiling revealed several differentially expressed genes that often correlated with the occurrence of specific alkaloids. Candidate genes were silenced via virus-induced gene silencing approach to test their involvement in specific pathways, and the encoded enzymes produced in *Escherichia coli* were biochemically characterized. The nature of these enzymes and their metabolic functions will be discussed.

TRANSCRIPTION PROFILE OF CYTOCHROME P450-HYDROXYLASES POTENTIALLY INVOLVED IN PLAUNOTOL BIOSYNTHESIS IN *CROTON STELLATOPILOSUS* OHBA

Siriluk Sintupachee,[1] Worrawat Pomden,[2] Nattaya Ngamrojanavanich,[3] Wanchai De-Eknamkul[2]

[1]*Biotechnology Program, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand,* [2]*Department of Phamacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, 10330, Thailand,* [3]*Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand*

Plant cytochromes P450 are involved in a wide range of biosynthetic reactions. In *Croton stellatopilosus* which produces an antipeptic acyclic diterpenoid, plaunotol, a cytochrome P450-dependent geranylgeraniol-18-hydroxylase activity has been shown to catalyze the last step of the plaunotol pathway. In this study, a PCR-based approach was used to isolate cDNA of *C. stellatopilosus* cytochrome P450-hydroxylases. The results showed that two core fragments, namely CYP97_5.4 and CYP79_7.8 belonging to the P450 families of CYP97 and CYP79, respectively, were expressed in high level during the development of leaves. Both gene expressions were correlated with pluanotol content found in same leaves. There results suggested that CYP97_5.4 or CYP79_7.8 might be directly involved in the biosynthesis of plaunotol in *C. stellatopilosus*.

INTEGRATION OF TRANSCRIPT AND METABOLITE PROFILING IN CELL CULTURES OF 18 PLANT SPECIES FROM FOUR FAMILIES THAT PRODUCE BENZYLISOQUINOLINE ALKALOIDS

Scott Cameron Farrow, Jillian M. Hagel, Peter J. Facchini

University of Calgary, Biological Sciences, Calgary, Alberta T2N1N4, Canada

Benzylisoquinoline alkaloids (BIAs) are a large and diverse class of plant secondary metabolites that often possess potent pharmacological properties. In an effort to better understand BIA metabolism, we have generated expressed sequence tag databases for 18 species of BIA-producing cell cultures. We also used HLPC-MS to accurately characterize the alkaloid profiles of each cell culture. The integration of metabolite and transcript profiles provides a valuable repository for the discovery of BIA biosynthetic genes. Using these integrated databases, we have identified numerous gene candidates responsible for most known and several uncharacterized BIA biosynthetic enzymes. The public availability of these resources, and their utility to gene discovery and a better understanding of BIA metabolic networks will be discussed.

ADVANCED PROTEOME ANALYSIS OF AROGENATE DEHYDRATASE KNOCKOUT MUTANTS IN *ARABIDOPSIS THALIANA*

Kim K. Hixson, Laurence B. Davin, Norman G. Lewis

Washington State University, Institute of Biological Chemistry, Pullman, WA 99164-6340, USA

Arogenate dehydratases (ADTs) are a class of enzymes involved in production of phenylalanine (which, in addition to its involvement in protein biosynthesis, is the main starting metabolite in the phenylpropanoid pathway) in plant systems. Our group has recently produced several ADT knockout mutant combinations in *Arabidopsis*. Knockout lines displaying the most significant physiological and lignin compositional changes were further selected for gel-free high throughput proteome analysis. Relative proteome changes were analyzed in isolated chloroplasts as well as at the whole cell level during four different time points (2, 4, 6, and 8 weeks) in leaves, stems, and roots. Initial results have provided evidence that the six ADT isoenzymes in *Arabidopsis* are present at a low abundance in relation to the entire proteome and that the individual expression of each ADT may be regulated in a spatial and temporal manner. These results are discussed in terms of effects overall to the plant proteome that such mutations have.

MICROBIAL PRODUCTION OF PLANT ISOQUINOLINE ALKALOIDS

Fumihiko Sato,[1] Hiromichi Minami[2]

[1]*Graduate School of Biostudies, Kyoto University, Department of Plant Gene and Totipotency, Kyoto, 606–8502, Japan,* [2]*Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, Nonoichi-machi, 921–8836, Japan*

The secondary metabolites of higher plants include diverse chemicals, such as alkaloids, isoprenoids and phenolic compounds (phenylpropanoids and flavonoids). Although these compounds are widely used in human health and nutrition, at present they are mainly obtained by extraction from plants and extraction yields are low because most of these metabolites accumulate at low levels in plant cells. Recent advances in synthetic biology and metabolic engineering have enabled tailored production of plant secondary metabolites in microorganisms, but these methods often require the addition of expensive substrates. Previously we reported the microbial system to produce reticuline, the key intermediate for producing the isoquinoline alkaloids, from dopamine. Isoquinoline alkaloids, such as morphine, codeine, papaverine, berberine and so on, are synthesized from reticuline and used for the medicine. In this technology, reticuline and the related alkaloids, scoulerine and magnoflorine, are produced with the combination of the *Micrococcus luteus* and plant enzymes. Here we report further development of an *Escherichia coli* fermentation system that yields plant alkaloids from simple carbon sources, using selected enzymes to construct a tailor-made biosynthetic pathway. In this system, engineered cells cultured in growth medium without additional substrates produce the plant isoquinoline alkaloids. The fermentation platform offers opportunities for low-cost production of many diverse alkaloids. Recent progresses in molecular characterization of isoquinoline alkaloid biosynthesis are also discussed.

APPLICATION OF HYDROXYPROLINE-O-GLYCOSYLATION FOR ENHANCED PLANT-BASED PRODUCTION

Jianfeng Xu,[1,2] Marcia Kieliszewski[2]

[1]*Arkansas State University, Jonesboro, AR USA 72401,* [2]*Ohio University, Athens, OH 45701, USA*

Hydroxyproline-*O*-glycosylation involves post-translational hydroxylation of proline to hydroxyproline (Hyp) and subsequent glycosylation, a modification that is unique to plants and green algae. Our earlier work with synthetic genes encoding various Hyp-rich glycoproteins (HRGPs) expressed in plant cells elucidated a Hyp-*O*-glycosylation 'code', that is a peptide sequence directs the Hyp-*O*-glycosylation; specifically contiguous Hyp residues, as in X-Hyp-Hyp are sites of oligoarabinosylation; in contrast, clustered non-contiguous Hyp residues, as in X-Hyp-X-Hyp repeats are mainly sites of highly branched arabinogalactan polysaccharide addition, where X is often Ser or Ala. These results demonstrated the feasibility of Hyp-*O*-based glycoprotein design in plants and triggered the following applications: 1) by introducing a Hyp-*O*-glycosylation tag to recombinant proteins expressed in tobacco BY-2 cells, we dramatically enhanced the production of secreted proteins up to 1500 fold; 2) using the same approach, we significantly improved the yields of recombinant proteins transiently expressed in *Nicotiana benthamiana*; 3) by engineering specific Hyp-*O*-based 'designer' biopolymers into plants, we could reconstruct the plant cell walls for improved biomass processability.

TOOLS FOR DEVELOPING GLYCOSYL-TRANSFERASE ASSAYS AND METHODS FOR XYLAN PROFILING IN PLANTS

Vaishali Sharma,[1] Dawn Chiniquy,[1,2] Edward Baidoo,[1] Jay Keasling,[1] Pamela Ronald,[1,2] Henrik V. Scheller[1]

[1]*Joint Bioenergy Institute, Lawrence Berkeley National Labs, Berkeley, CA USA 94720,* [2]*University of California, Davis, 95616, USA*

Hemicelluloses are polysaccharides in plant cell walls that have β-(1→4)-linked backbones and include xyloglucans, xylans, mannans, glucomannans and mixed-linkage glucans. These are synthesized by glycosyltransferases (GTs)that catalyze the transfer of sugar from a glycosyl donor to a suitable acceptor. Most naturally occurring GT acceptors do not contain a chromophore or fluorophore which precludes detection of primary product of GT reaction by spectroscopic techniques. Many GT assays therefore make use of chemically modified donor or acceptor analogues like radiolabeled sugar donors. Availability of such chemically modified analogues is a major problem. We are interested in L-arabinosyltransferases which use UDP-L-arabinofuranose as substrate, a compound that has not been made in radiolabeled form. Here, we present LC-MS as an efficient tool to assay GTs that are heterologously expressed in tobacco leaves. A GT family of particular interest is GT61, which is highly expanded in grasses and proposed to be involved in adding arabinose sidechains onto xylan. We have developed methods to profile xylans using sequential extraction, enzymatic hydrolysis, HPAEC, and LC-MS. With these methods, we have shown specific changes to the profile in some rice GT61 mutants. The key differences in structure of xylan from GT61 mutants and wild type will be discussed.

LIPID, MONOSACCHARIDES AND VITAMIN E CHEMISTRY OF *STICHOCOCCUS BACILLARIS* STRAIN SIVA2011: SOURCE FOR BIODIESEL AND VALUE-ADDED PRODUCTS

Sivakumar Ganapathy,[1] Jennifer Gidden,[2] Jianfeng Xu,[1] Gregory Phillips[1], Jackson Lay[2]

[1]*Arkansas State University, Arkansas Biosciences Institute, Jonesboro, AR United States 72401,* [2]*University of Arkansas, Arkansas Statewide Mass Spectrometry Facility, Fayetteville, AR 72701, USA*

Fossil fuel is currently the accepted primary source of energy in the world. Negative effects from global warming and increased costs have been predicted as we continue to burn this fuel and deplete a diminishing resource. Microalgae are one of the proposed platforms for lipid-based fuel. It consumes large amounts of CO_2 for growth, which makes it an attractive renewable option for next generation energy production. *Stichococcus* sps. are becoming recognized for their potential as biodiesel and bio-based organic product precursors sources. We have isolated a new *S. bacillaris* strain, siva2011, for biofuel production. It is comparable to *S. bacillaris* based on the 18S region of the nuclear rDNA, as well as unique fatty acid methyl esters, monosaccharides and its vitamin E profile. We present growth and vitamin E kinetics, lipid chemistry and bioprocess engineering data for this strain. The mass spectral characterizations of extracts of *S. bacillaris* strain siva2011 have been useful for monitoring the potential of this organism for producing a high quality biofuel and other value-added products.

THE EXTRACTION OF HIGH VALUE PHYTOCHEMICALS IN THE CONTEXT OF A BIOREFINERY

Danielle Julie Carrier, Elizabeth Martin

University of Arkansas, Biological and Agricultural Engineering, Fayetteville, AR 72701, USA

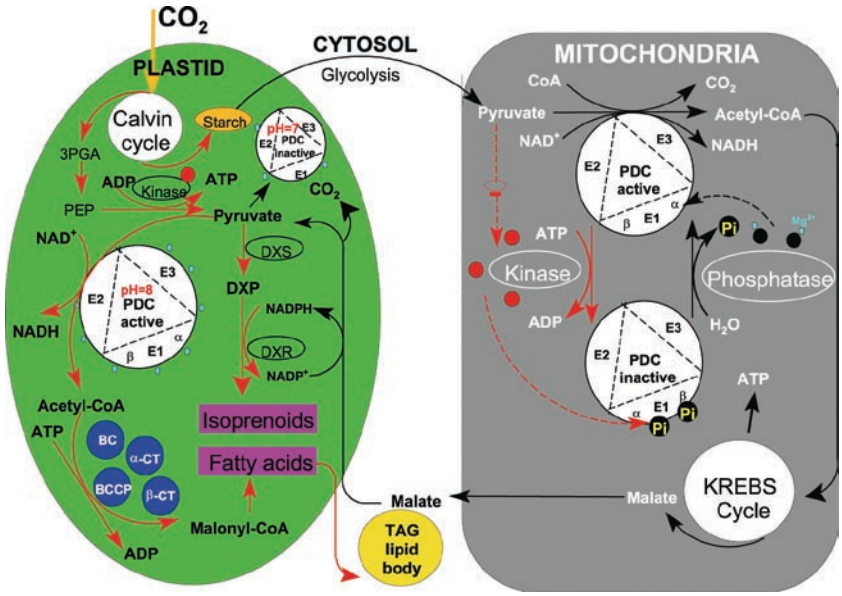
To increase biorefinery revenues, phytochemicals can be extracted prior or even after the biochemical conversion of biomass to biofuels or products. Water, at temperatures between 65°C and 100°C, is an excellent extraction solvent because residual water will not interfere with leading biochemical conversion pretreatments. Herbaceous biomass, switchgrass, and woody biomass, sweetgum, are biorefinery feedstocks that contain valuable phytochemicals. The water from 90°C switchgrasss extracts contained quercitrin and rutin at concentrations of 193 and 186 mg kg^{-1} of dry biomass, respectively. The centrifugal partition chromatography-purified quercitrin and rutin fractions decreased by 78 and 86%, respectively, the oxidation of low density lipoprotein. Sweetgum bark and heartwood extracted in 65°C water yielded 1.7 mg g^{-1} and 0.2 mg g^{-1} of shikimic acid, respectively. The addition of this 65°C water-based extraction step coupled to pretreatment with 0.98% H_2SO_4 at 130°C for 50 min resulted in 21% and 17% increases in xylose percent recovery from bark and heartwood, respectively, as compared to direct pretreatment. These results indicate that, in addition to recovering shikimic acid, the 65°C wash step also increases xylose recovery, demonstrating that this could be integrated to a biorefinery operation.

ENGINEERING OF NON-MEVALONATE PATHWAY FOR THE ENHANCEMENT OF OIL CONTENT IN MICROALGAE *CHLAMYDOMONAS REINHARDTII*

Mahmoud Gargouri, Jeong-Jin Park, David Roger Gang

Institute of Biological Chemistry, Washington State University, Pullman, WA 99164, USA

Microalgae have a strong potential as vectors for biofuels since their oil content may exceed 70% (w/wDW), as compared with 5% for the best agricultural oil crops. Algae accumulate lipids when cultivated under stress conditions, such as nitrogen depletion and high-light. However, while nutrient limitation can effectively increase lipid content, it can



also decrease overall cellular activity leading to reduced cell proliferation. In order to optimize metabolic flux networks that increase the conversion efficiency of solar energy to oil without compromising overall productivity, we aimed to identify and reengineer enzymes that sit at key regulatory pathways. The isoprenoid pathway was chosen in this study since it shares a common intermediate substrate (i.e. pyruvate) with lipid synthesis. Secondly, it uses only the MEP pathway for the biosynthesis of isoprenoids, including sterols in the cytosol (figure). We hypothesized that reducing the utilization of pyruvate in the isoprenoid synthesis pathway can result in the overproduction of fatty acids. In order to test this hypothesis, we produced knockdown mutants using constitutive and inducible promoters in expression RNAi vectors. Experiments are currently underway to test their ability to produce fatty acids under both favorable (low light and nitrogen replete) and stress conditions. Additionally, a complementary chemogenomic approach was conducted to understand the side effects of specific inhibitors of MEP pathway (i.e. ketoclomazone and fosmidomycin) on fatty acids biosynthesis.

OPTIMIZATION OF POTASSIUM HYDROXIDE PRETREATMENT FROM STEAM-EXPLODED SOYBEAN HULL USING RESPONSE SURFACE METHODOLOGY

Ji Young Jung,[1] Ji Su Kim,[1] Myung suk Choi,[1] Young Wun Kim,[2] Byung Tae Yoon,[2] Jae Kyung Yang[1]

[1]*Division of Environmental Forest Science, Gyeongsang National University, Institute of Agriculture & Life Sciences, Jinju, 660–701, South Korea,* [2]*Korea Research Institute of Chemical Technology, Daejeon, 305–600, South Korea*

Soybean hulls represent the major by-product of soybean processing industry and constitutes about 8% of the whole seed. In this study, we have evaluated the potential of soybean hull as a biomass resource as feedstock for the production of bioethanol. Soybean hull is a lignocellulosic material containing about 50.8% cellulose, 14.5% hemicellulose, and 11.5% lignin. The impact of varying pretreatment parameters (temperature, time, and concentration) on acid hydrolysis of soybean hull were investigated. The important independent variables for pretreatment were selected as reaction temperature, reaction time and potassium hydroxide concentration. The pretreatment condition for maximizing the solid recovery, the cellulose recovery and the lignin removal was optimized using RSM (Response Surface Methodology). An optimum cellulose recovery was found with pretreatment conditions of 70°C reaction temperature, 198 min reaction time, 0.6% potassium hydroxide concentration.

COMBINATIONS OF STEAM EXPLOSION AND CHEMICAL PRETREATMENT FOR FERMENTATION SUGAR PRODUCTION FROM *MISCANTHUS SINENSIS*

Jae-Kyung Yang,[1] Myung Suk Choi,[1] Jeoung-Bin Nam,[1] Choon Gil Kim,[2] Young Wun Kim,[3] Byung Tae Yoon,[3] Ji Young Jung,[1] In Ho Cho[2]

[1]*Division of Environmental Forest Science, Gyeongsang National University, Institute of Agriculture & Life Sciences, Jinju, 660–701, South Korea,* [2]*SK Energy Institute of Technology, Daejeon, 305–712, South Korea,* [3]*Korea Research Institute of Chemical Technology, Daejeon, 305–600, South Korea*

Miscanthus sinensis is an interesting raw material for bioethanol production, it is a high yield low maintenance plant with a high cellulose and hemicellulose content. In this study we used steam explosion and chemical pretreatment to evaluate the combination of the pretreatment for bioethanol production with *Miscanthus sinensis*. A combined pretreatment involved sequential treatments by steam explosion (severity log Ro 4.38 and severity log Ro 4.68) and chemical pretreatment (aqueous alkali and organosolv). The steam explosion-organosolv (dioxane:water) pretreatment conditions for *Miscanthus sinensis* were optimized to obtain higher sugar yield. The steam explosion (severity log Ro 4.38)-organosolv (dioxane:water) pretreated material resulted in 79% lignin removal, a cellulose yield of more than 84% and 32% of hemicelluloses recovery. As a result, from 100 g of raw material, 26.8 g (80.7%) of glucose was recovered of 33.2 g available cellulose.

BIOSYNTHESIS OF NOVEL NATURAL NUTRACEUTICALS AND ANTIOXIDANTS: A BIOTECHNOLOGICAL APPROACH

Selim Kermasha

McGill University, Department of Food Science and Agricultural Chemistry, Ste-Anne de Bellevue, Quebec, H9X 3V9, Canada

There has been a growing interest in the use of nutraceuticals as food supplements as well as natural bio-ingredients in food industries. The numerous health benefits of the ω-3 polyunsaturated fatty acids (PUFAs) have been recognized in the modulation of risk of a variety of diseases and disorders. Selected endogenous oils, such as fish oil is rich in ω-3

PUFAs especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are believed to play roles in the prevention of cardiovascular disease and in improving human immune functioning in adults. On the other hand, phenolic compounds represent another important group which possesses antioxidant and functional properties. The incorporation of phenolic acids into triacylglycerols could potentially result in novel structured phenolic lipids, with enhanced anti-oxidative and functional properties. The presented work aimed at the optimization of an environmentally-friendly biotechnological process of selected phenolic lipids in solvent-free medium, by lipase-transesterification of edible oils and endogenous phenolic extracts. The bioconversion yield was determined. In addition, the biosynthesized novel biomolecules of phenolic lipids were characterized in terms of their chemical structures and their antioxidant potentials. This presentation is will cover the overall work carried out in our laboratory that aimed at the development of biotechnological processes, using enzyme technology, for the production of added-value novel nutraceuticals and antioxidant biomolecules.