18th Annual OXFORD ICSB

April 9th - 12th 2018

at the Oxford Conference Center | 102 Ed Perry Blvd, Oxford, Mississippi

The Oxford International Conference on the Science of Botanicals is an annual meeting to discuss approaches for post market surveillance, risk and safety assessment, quality control and adverse event reporting (AER) for botanical dietary supplements (BDS) and natural products as well as regulatory aspects with perspectives from government, manufacturers and trade associations

Poster Abstracts

National Center for Natural Products Research
Oxford ICSB
The University of Mississippi

TITLE SPONSORSHIP PROVIDED BY:

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

UNPA
UNITED NATURAL PRODUCTS ALLIANCE

FDA U.S. FOOD & DRUG ADMINISTRATION

http://oxfordicsb.org | https://facebook.com/OxfordICSB | icsb@olemiss.edu | Phone: (662) 915-7821 | Fax: (662) 915-7989

ICSB is supported by a cooperative agreement with the FDA

FDA U.S. FOOD & DRUG ADMINISTRATION

Title Sponshorship Provided by:

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

UNPA
UNITED NATURAL PRODUCTS ALLIANCE®

Additional Sponshorship Provided by:

NUTRILITE™ Exclusively from Amway

THE NATURE'S BOUNTY CO.

DUPONT®

P&G

GA

indena®

W8.CRN

NSF

NUTRA ingredients-usa.com

EXTRASYNTHESE Exclusively Distributed in North America by ALKEMIST LABS

VERDURE SCIENCES®

CAMAG World Leader in Planar Chromatography

Thanks to our local sponsors:

THE CITY OF OXFORD

Hampton Inn

visit OXFORD mississippi

***The DuPont Oval Logo is a trademark of DuPont or its affiliates*

2018 ICSB Exhibitors:

CAMAG
World Leader in Planar Chromatography

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

SCIEX

Agilent Technologies

EXTRASYNTHESE
Exclusively Distributed in North America by
ALKEMIST LABS

TELEDYNE ISCO
Everywhereyoulook™

Special thanks to our co-sponsoring organizations and friends:

This conference is supported by a cooperative agreement between the NCNPR and the Center for Food Safety and Applied Nutrition (CFSAN) at the U.S. Food and Drug Administration (FDA). It is co-sponsored by the Shanghai Institute of Materia Medica/ CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the Ministry of Indigenous Medicine, Sri Lanka; the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant Research (GA); Hunan University, China; the Korean Society of Pharmacognosy (KSP) and the Japanese Society of Pharmacognosy (JSP).

THE UNIVERSITY of MISSISSIPPI
NATIONAL CENTER FOR NATURAL PRODUCTS RESEARCH

April 9, 2018

Dear Friends,

On behalf of the National Center for Natural Products Research, School of Pharmacy, and the University of Mississippi, we would like to welcome you to the "18th International Conference on the Science of Botanicals." With the help of the Oxford Conference Center, we have put together a program of social and entertainment activities to run alongside our rich and informative scientific agenda. The upcoming year's meeting will explore the topic of synergy between natural products and human health. To this end, we will review, discuss, and explore the confluence of current research topics related to natural products research and development as well as topics related to safety, quality and regulatory aspects. Further information regarding this conference can also be found at www.oxfordICSB.org. This conference is supported by a cooperative agreement between the NCNPR and the Center for Food Safety and Applied Nutrition (CFSAN) at the U.S. Food and Drug Administration (FDA). It is co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the Ministry of Indigenous Medicine; Sri Lanka; the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant Research (GA); the Korean Society of Pharmacognosy; The Vietnam Academy of Science and Technology (VAST).

We are excited to present a program featuring a roster of internationally recognized experts and researchers in the field of botanicals. We wish to extend our thanks to our speakers for their willingness to participate in and contribute to the success of this meeting.

We invite you to visit the website of the National Center for Natural Products Research at http://www.pharmacy.olemiss.edu/ncnpr to learn more about our research program. Oxford and the Ole Miss campus are a beautiful setting, and we hope you will get to explore them, especially if this is your first time to visit here. If there is anything, we can do to make your visit more enjoyable, please contact us.

Sincerely,

Ikhlas A. Khan, Ph.D.
Director, National Center for Natural Products Research
Director, FDA Center of Excellence
University of Mississippi

A DIVISION OF THE RESEARCH INSTITUTE OF PHARMACEUTICAL SCIENCES, SCHOOL OF PHARMACY
Thad Cochran Research Center | P.O. Box 1848 | University, MS 38677-1848 | (662) 915-1005 | Fax: (662) 915-1006 | www.olemiss.edu

Organizing Committee

Cara Welch, Ph.D.
Senior Advisor, Division of Dietary Supplement Programs, CFSAN, FDA

Ikhlas Khan, Ph.D.
Director, NCNPR,
The University of Mississippi.

Larry A. Walker, Ph.D.
Emeritus Director, NCNPR,
The University of Mississippi.

Mark Blumenthal
Executive Director
American Botanical Council.

Loren Israelsen, J.D.
Executive Director
United Natural Products Alliance.

Rick Kingston, Ph.D.
President,
Safety Call International

Scientific Program Committee

Cindy Angerhofer, Ph.D.
Executive Director, Botanical Research
Aveda, Minneapolis-St. Paul, MN, USA

Joseph M. Betz, Ph.D.
Office of Dietary Supplements of NIH.

Wolfgang Blaschek, Ph.D.
Professor, Pharmaceutical Biology
University of Kiel

De-an Guo, Ph.D.
Director, Shanghai Research Center for TCM Modernization
SIMM/CAS

Rudolf Bauer, Ph.D.
Institute of Pharmaceutical Sciences
Department of Pharmacognosy
Karl-Franzens-Universitaet Graz.

John Cardellina II, Ph.D.
Distinguished Scientist - Chemistry,
Technical Innovation Center, ReevesGroup Consultations

K. Hüsnü C. Baser, Ph.D.
Professor, Head of the Department of Pharmacognosy, Anadolu University,
Eskisehir, Turkey.

Paula Brown, Ph.D.
Director of Applied Research, Natural Health & Food Products Research Group.
British Columbia Institute of Technology

Sibyl Swift, Ph.D.
Special Assistant, FDA, Office of Dietary Supplement Programs.

Stephen O. Duke, Ph.D.
Research Leader, USDA, ARS, NPURU.

Mahmoud A. ElSohly, Ph.D.
Research Professor NCNPR, Professor of Pharmaceutics, The University of Mississippi.

Edward J. Fletcher
COO/Botanicals Division,
Strategic Sourcing, Inc.

Craig Hopp, Ph.D.
Program Officer, NCCAM, NIH

Jinwoong Kim, Ph.D.
Seoul National University, South Korea.

A. Douglas Kinghorn, Ph.D., D.Sc.
Jack L. Beal Professor and Chair,
Ohio State University,
College of Pharmacy.

Brigitte Kopp, PhD
Professor of Pharmacognosy, Department of Pharmacognosy, University of Vienna, Austria.

G.N. Qazi, Ph.D.
Vice Chancellor
Jamia Hamdard, India.

Steven Musser, Ph.D.
Director, Office of Regulatory Science,
CFSAN, FDA.

Amar Chittiboyina, Ph.D.
Assistant Director, NCNPR,
University of Mississippi

Rachel Mata, Ph.D.
Department of Pharmacy,
National Autonomous University of Mexico.

Robin J. Marles, Ph.D.
Director, Bureau of Clinical Trials and Health Science NHPD, Health Products and Food Branch, Health Canada

Douglas "Duffy" MacKay, N.D.
Vice President, Scientific & Regulatory Affairs, Council for Responsible Nutrition (CRN)

James McChesney, Ph.D.
Ironstone, Inc.

Dan Fabricant, Ph.D.
Natural Products Association

Amy Roe, Ph.D., DABT
The Proctor & Gamble Company

David S. Pasco, Ph.D.
Associate Director, NCNPR
The University of Mississippi.

Guido F. Pauli, Ph.D.
Associate Professor of Pharmacognosy
University of Illinois at Chicago

Eike Reich, Ph.D.
CAMAG Laboratory, Muttenz, Switzerland

Andre Santos, Ph.D.
Americas Market Development Manager
Agilent Technologies, Andover, MA.

Roy Upton
Executive Director, American Herbal Pharmacopoeia.

Ram Vishwakarma, Ph.D.
Director, IIIM, Jammu.

Jimmy Yuk, Ph.D.
Senior Business Development Manager
Waters Corporation, Milford, MA

Daniel S. Marsman, DVM PhD
Head, Product Safety, Global Product Stewardship
P&G Health Care, Worldwide

PA

Chemical-Specific Maximum Allowable Levels (MALs) for Pesticide Residues in Dietary Supplements

Adams R.E.[1], Brickel J.A.[1], and Bhat V.S.[1]*

[1]NSF International, Ann Arbor, MI, USA
**present address: Burdock Group Consultants, Orlando, FL, USA*

More than two-thirds of adults take dietary supplements each year, with the industry contributing more than $120 billion to the US economy. In the US, dietary supplements are regulated as a subset of foods. US FDA regulations involve establishing a tolerance for the maximum amount of a pesticide residue allowed to remain in or on a food commodity. However, most botanicals do not have established pesticide tolerances which proactively results in the enforcement of zero tolerance. Therefore, current pesticide regulations leave many botanical dietary ingredients without allowable limits. For this reason, there is a critical need for science-based pesticide limits for botanical dietary ingredients. The current study developed chemical-specific MALs for 185 pesticides identified on the USDA Prohibited Pesticides List. Three approaches were used to derive MALs: 1) Conversion of existing, authoritative-body derived chronic risk levels, 2) Data-based derivation for pesticides lacking existing chronic risk levels, and 3) Application of the Threshold of Toxicological Concern (TTC) for pesticides lacking existing chronic risk levels and toxicity data. Utilizing the first approach, MALs were derived from existing criteria for 177 (96%) of the listed pesticides. If multiple authoritative values were identified, the lowest, scientifically defensible value was selected, considering both carcinogenic and non-carcinogenic hazards. Five pesticides (o-phenylphenol, pirimicarb, oxadixyl, tetradifon, o,p'-DDT) lacked existing US EPA chronic data, and were evaluated using other international authoritative-body criteria to derive MALs. Criteria were not identified for two pesticides (o,p'-DDD and o,p'-DDE). These chemicals were not candidates for TTC and MALs due to the existing *a prior* exclusion of bioaccumulative chemicals from TTC consideration. Of note, some criteria applied to more than one pesticide for reasons such as technical mixtures, or parent compounds and their metabolites. In such cases, the total concentration of the pesticides were summed and one MAL was derived. The current study utilized health effects criteria derived by international agencies to develop chemical-specific, risk-based MALs that can be applied to all pesticide residues in dietary supplements, regardless of the food commodity on which they are found. This broader applicability eliminates reliance on precautionary zero tolerance by applying a science-based process to protect public health and safety.

The authors appreciate the members who voluntarily participated in the NSF/ANSI 173- Dietary Supplements Joint Committee Pesticides Task Group for providing feedback throughout the development of the MAL derivation process.

PA

How well do label claims for green tea dietary supplements reflect analytically measured phytochemical content and inform photochemical intake assessment?

Andrews KW[1], Gusev PA[1], Savarala S[1], Dang PT[1], Oh L[1], Atkinson R[1], McNeal M[1], Pehrsson PR[1], Dwyer JT[2], Betz JM[2], Kuszak AJ[2], Costello RB[2], Saldanha LG[2]

[1]US Department of Agriculture, Agricultural Research Service, Beltsville Human Nutrition Research Center, Nutrient Data Laboratory, Beltsville, MD, USA, [2]National Institutes of Health, Office of Dietary Supplements, Bethesda, MD, USA

In botanical extracts used in dietary supplements (DS), the amounts of phytochemicals vary widely and may exceed the amounts in foods. Product labels that state only the information required by the FDA (amount of dried material or extract) may not provide accurate estimates of phytochemical constituents. In studies for the Dietary Supplement Ingredient Database, botanical DS containing green tea (GT) were tested for phytochemical content and these levels were compared to label information.

Sixty-nine DS containing GT were purchased in various market channels and in a variety of forms (capsules, liquids, tablets, soft gels and powders) and sent for analysis to multiple laboratories. Contracted laboratories used high-performance or ultra-high performance liquid chromatography with reverse phase columns and either ultraviolet molecular absorbance or mass spectrometric detection for analysis of epigallocatechin gallate (EGCG) and caffeine. Quality control results from all laboratories showed good agreement with certified values provided by several GT Standard Reference Materials.

For products with GT as the only or primary ingredient (GT-1; n=32) and for products in complex matrices containing GT (GT-2; n=37), similar issues and patterns for EGCG and caffeine content were identified. The analytical data revealed a wide range of levels for EGCG and caffeine content, with GT-1 products higher in EGCG content and GT-2 products higher in caffeine content. Products that provided voluntary information about their levels of EGCG (43%) or caffeine (27%), were found to have, on average, more than twice the levels than those that were not labeled. This voluntary labeling of EGCG and caffeine was more accurate for GT-2 products than for GT-1 products. Most importantly, the amount of measured EGCG compared to the total labeled amount of green tea material was highly variable (ranging from 0.2–63% for GT-1; and 2.4–81.8% for GT-2).

In conclusion, required label information does not predict the actual content of GT DS phytochemical constituents. These findings indicate that more complete and accurate label information would be beneficial both for consumers who make decisions on botanical DS use and for researchers who track phytochemical bioactive intakes and their association with health outcomes.

PA

The Hands-on Course in Tablet Technology Department of Pharmaceutics and Drug delivery

Ashour, E

Department of Pharmecutics and Drug Delivery, University of Mississippi, University, MS 38677

The Hands-on Course in Tablet Technology is post graduate educational program that provides an extensive review of conventional and advanced manufacturing technologies, including hot-melt extrusion, that is used to develop a variety of novel delivery and continuous manufacturing systems. Industry and academia experts present lectures and supervise laboratory exercises that provide a scientific overview of the entire tableting operation. Attendees gain extensive hands-on experience with conventional and advanced technologies used to manufacture tablets and other solid oral dosage forms.

Lectures explore the concepts of tablet design using QbD principles, process validation, product evaluation, FDA guidelines for product development and manufacturing. The laboratory portion of the course allows attendees to obtain hands-on practical experience in unit operations.

Attendees include domestic and international cross-functional representatives (e.g. R&D formulation development/process development, QC/QC, regulatory affairs, manufacturing, sales and marketing) from the pharmaceutical, dietary supplement, animal health and food industries; regulatory agencies (e.g. FDA, Health Canada) and raw material/equipment suppliers.

The course was initiated in 1998 at the University of Tennessee College of Pharmacy and moved to the University of Mississippi School of Pharmacy in 2013. The course has been offered more than 50 times over the past 20 years. A total of over 2000 participants have attended the course since its inception.

PA

Quantification of Phenolic Compounds from Roots and Aerial Parts of Fadogia agrestis and Dietary Supplements claiming to contain Fadogia

Avula B[1], Bae J-Y[1], Raman V[1], Wang Y-H[1], Osman AMG[1], Omer F[1], Wang M[1], Ali Z[1], Khan IA[1,2]

[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS 38677, USA. [2]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA.

An UHPLC method was developed for the determination of 11 phenolic compounds from roots and aerial parts of *Fadogia agrestis* Schweinf. ex Hiern (synonym *Vangueria agrestis* (Schweinf. ex Hiern) Lantz). The separation was achieved within 7 minutes by using C-18 column material, a water/acetonitrile mobile phase, both containing 0.1% formic acid gradient system and a temperature of 45°C. The method was validated for linearity, repeatability, limits of detection (LOD) and limits of quantification (LOQ). The limits of detection of phenolic compounds was found to be in the range from 0.025-0.1 µg/mL. The wavelengths used for quantification with the diode array detector were 238, 254, 291 and 325 nm. Twelve of 17 dietary supplements contained phenolic compounds in the range from 0.3-2.5 mg/day. The phenolic compounds were not detected in five dietary supplements. LC-mass spectrometry coupled with electrospray ionization (ESI) interface method is described for the identification and confirmation of compounds from plant samples and dietary supplements claiming to contain *Fadogia*. This method involved the use of

$[M+H]^+$ and $[M+Na]^+$ ions in the positive ion mode and $[M-H]^-$ ions in the negative ion mode with extractive ion monitoring (EIM). The developed method is simple, economic, rapid and especially suitable for quality control analysis of *Fadogia*.

This research is supported in part by "Science Based Authentication of Dietary Supplements" funded by the Food and Drug Administration grant number 1U01FD004246-05, and the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No. 58-6408-1-603-07.

PA

Identification of Unlabeled Hepatotoxins from Dietary Supplements That Have Been Implicated in Liver Injury

Avula B[1], Bae J-Y[1], Wang Y-H[1], Wang M[1], Verma M[2], Navarro VJ[2], Khan IA[1,3]

[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS 38677, USA. . [2]Division of Hepatology, Albert Einstein Medical Center, Philadelphia, PA 19141. [3]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA.

Herbal dietary supplements (HDS) are widely used, especially for weight loss, bodybuilding, bones/joint implications, sexual enhancement, general health, and miscellaneous. There is growing evidence to show that some HDS are capable of causing liver injury. Fifty products categorized as weight loss (10 products), bodybuilding (25 products), bones/joint (5 products), miscellaneous (10 products) were analyzed for the presence of anabolic steroids, pyrrolizidine alkaloids, aflatoxins and pharmaceuticals using ultra-high performance liquid chromatography-QToF-MS in full scan and targeted MSâ€‹/MS modes with accurate mass measurement. The compounds were identified in both positive and negative ion modes. Of the 25 anabolic products tested, 21 products labeled to contain steroids including anabolic steroids; 11 of these contained labeled and unlabeled steroids; 16 contained steroids that were different to those indicated on the packaging; Overall, 21 different steroids were identified; 10 of these were controlled under US Drug Enforcement Administration (DEA) Controlled Substances Schedule III. These controlled substances have been added to the World Anti-Doping Agency (WADA) list of prohibited substances in sport. One of 25 products contained unlabeled pharmaceuticals. One of 5 bones/joint implication products contained unlabeled pharmaceuticals. Three of 10 miscellaneous products contained pyrrolizidine alkaloids and pharmaceuticals. In addition, the development of accurate mass time-of-flight mass spectrometer has enabled the calculation of an empirical formula from the molecular ion. However, the molecular ion present in LC-MS analyses with its mass and elemental formula can be used to search a database of metabolites such as the ForTox database provided by Agilent Technologies, ChemSpider (www.chemspider.com) and customized In-house developed database. In addition, the development of accurate mass time-of-flight mass spectrometers has enabled the calculation of an empirical formula from the molecular ion. Compound structures were confirmed with authentic standards, and further verified by MS-MS fragmentation patterns

This research is supported by "Albert Einstein Healthcare Network/National Institutes of Health" award number # 3U01DKO83027-0952. Supplements were obtained from a repository of products that have been implicated in liver injury.

PA

UHPLC-QToF-MS Chemical Profiling and Characterization of Annonaceous Alkaloids and Acetogenins from Asimina Species

Avula B[1], Bae J-Y[1], Wang Y-H[1], Wang M[1], Ali Z[1], and Khan IA[1,2]

[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS 38677, USA, [2]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

Alkaloids and acetogenins were reported to be the major physiologically active constituents in *Asimina* species. Chemical profiling of alkaloids and acetogenins from methanolic extracts of *Asimina* species (*A. triloba* and *A. parviflora*) and dietary supplement have been analyzed using UHPLC-QToF in positive ion mode. These compounds were tentatively characterized based on the accurate mass and fragment ions. The fragments produced by collision induced dissociation (CID) revealed the characteristic cleavage and the fragmentation pattern provided structural information. The alkaloids of the aporphine, oxoaporphine and benzylisoqionolines type represent the predominant group found in *Asimina* species. The alkaloidal skeleton was determined from the UV absorption spectra and mass spectrometry fragmentation. Acetogenins are an important group of long-chain fatty acid derivatives containing one to three tetrahydrofuran (THF) rings and have a long aliphatic chain on one side (belonging to a series of C_{35}-C_{38} compounds) and aliphatic chain ending in an a, b-unsaturated g-lactone on the other side. These compounds can be used to distinguish *Asimina* species. It also provides an excellent approach for rapid screening of chemical components from plant extracts. Magnoflorine was used as an example to discuss the fragmentation patterns. In (+)-ESI-MS, magnoflorine gave $[M]^+$ ions at *m/z* 342.1705. The fragment ions at *m/z* 297.1127 $[M+H-(CH_3)_2NH]^+$, 282.0886 $[M+H-(CH_3)_3NH]^+$, 265.0865 $[M+H-(CH_3)_2NH-CH_3OH]^+$, 237.0916 $[M+H-(CH_3)_2NH-CH_3OH-CO]^+$, and 222.0681 $[M+H-(CH_3)_2NH-CH_3OH-CO-CH_3]^+$ resulted from the $[M]^+$ molecule. One dietary supplement claiming to contain paw paw was also analyzed and showed similar profile to twigs of *A. triloba*. A total of 120 compounds were identified from the different parts of *A. triloba* and *A. parviflora* samples. However, for definite identification of these unknown components, further investigation is required. This may provide a model for the rapid screening and structural characterization of bioactive constituents from plant extracts.

This research is supported in part by "Science Based Authentication of Dietary Supplements" funded by the Food and Drug Administration grant number 1U01FD004246-05, and the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No. 58-6408-1-603-07.

PA

Identification and Quantification of PrenylFlavones from aerial parts of Epimedium Species using UHPLC-QToF-MS

Bae J-Y[1], *Avula B*[1], *Zulfiqar F*[1], *Wang Y-H*[1], *Wang M*[1], *Ali Z*[1] *& Khan IA*[1,2]

[1]*National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS 38677, USA,* [2]*Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA.*

Epimedium, also known as barrenwort, is a genus of flowering plants in the family Berberidaceae. The majority of the species are endemic to China. According to the Chinese pharmacopoeia [1], the dried aerial parts of *Epimedium brevicornu* Maxim., *Epimedium sagittatum* Maxim., *Epimedium pubescens* Maxim., *Epimedium wushanense* T.S. Ying, and *Epimedium koreanum* Nakai contain the highest concentrations of flavonoids, which are the major compounds present in barrenwort, and have shown multiple beneficial therapeutic effects *in vitro*. UHPLC coupled with PDA was used for the quantitative determination of 16 components from different species of *Epimedium* (*Epimedium brevicornu* Maxim., *Epimedium sagittatum* Maxim., and *Epimedium grandiflorum* C.Morren) and dietary supplements claiming to contain *Epimedium*. These three species of *Epimedium* showed distinct chemical profile and icariin was found to be a major compound in *Epimedium grandiflorum*. LC-mass spectrometry coupled with electrospray ionization (ESI) interface method is described for the identification of compounds and involved the use of $[M+H]^+$, $[M+Na]^+$ and $[M-H]^+$ ions in the positive and negative ion mode with extracted ion chromatogram (XIC).

Ref.: [1] The Pharmacopoeia of People's Republic of China, Editorial Committee of Pharmacopoeia of Ministry of Health PR China, Beijing: China Chemical Industry. Press, 2010, vol. 1, p. 306

This research is supported in part by "Science Based Authentication of Dietary Supplements" funded by the Food and Drug Administration grant number 1U01FD004246-05, and the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No. 58-6408-1-603-07.

PA

"Naturally" sourced food ingredients: Are they always a safe bet?

Brickel JA[1]

[1]*Burdock Group Consultants, Orlando, FL*

Consumer demand for "natural" products with "clean labels" continues to increase, but manufacturers are finding traditional sources of natural ingredients are either exhausted or expensive, resulting in a search for similar ingredients from non-traditional sources. Resveratrol is a great example, as the primary source of resveratrol is no longer winemaking by-products, but the Japanese knot weed. The vexing problem is that only the original extractives, for which the co-extractives and contaminants were determined to be safe, were approved, and little is known about the extractives from these new sources. Currently, there seems to be a wide-spread but unfounded presumption that naturally sourced food ingredients are safe, possibly because of the cultural context of the use of the word "natural". To be sure, the spices and natural seasonings and flavors (21 CFR 182.10) and essential oils, oleoresins, and natural extractives (21 CFR 182.20), which were concluded as Generally Recognized as Safe (GRAS) for their intended use in 1977, based on the information available at the time, contribute to the presumption of safety. However, changes in

sourced raw materials, processing techniques (e.g. supercritical fluid extraction), and intended uses have drastically changed in the last 40 years, warranting a new round of safety assessments for these "naturally" sourced food ingredients. The new and expanded uses of these once sparingly used ingredients now include incorporation into breath and air fresheners, cosmetics, and fragrances, as well as uses as preservatives in foods and substitutes for antibiotics in food production animals. The vast use of these ingredients has surely increased cumulative human exposure levels. These new sources, processes, and uses mandate a reassessment of safety, using modern testing protocols and risks assessed with the new cumulative exposure levels. The safety of "naturally" sourced food ingredients cannot be assumed on the basis of their "natural" origin or their general recognition of safety for past intended uses that may not be applicable for increased use levels or new intended uses.

PA

Detection and Quantification of Cannabinoids in Extracts of Cannabis sativa Roots using LC-MS/MS

ElSohly MA[1,2,3], Gul W[1,2], Ibrahim EA[2,4], Gul SW[1], Chandra S[2] & Lata H[2]

[1]ElSohly Laboratories, Inc., Oxford, MS 38655, USA. [2]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [3]Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, University, MS 38677, USA.[4]Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt.

A liquid chromatography-tandem mass spectrometry single-laboratory validation was performed for the detection and quantification of the 10 major cannabinoids of cannabis, namely, (-)-trans-âˆ†^{9}-tetrahydrocannabinol, cannabidiol, cannabigerol, cannabichromene, tetrahydrocannabivarian, cannabinol, (-)-trans-âˆ†^{8}-tetrahydrocannabinol, cannabidiolic acid, cannabigerolic acid, and âˆ†^{9}-tetrahydrocannabinolic acid-A in the root extract of *Cannabis sativa*. Acetonitrile: Methanol (80: 20, v/v) was used for extraction; d_3-cannabidiol and d_3-tetrahydrocannabinol were used as the internal standards. All 10 cannabinoids showed a good regression relationship with $r^2 > 0.99$. The validated method is simple, sensitive, and reproducible and is therefore suitable for the detection and quantification of these cannabinoids in extracts of cannabis roots. To our knowledge, this is the first report for the quantitation of cannabinoids in cannabis roots.

PA

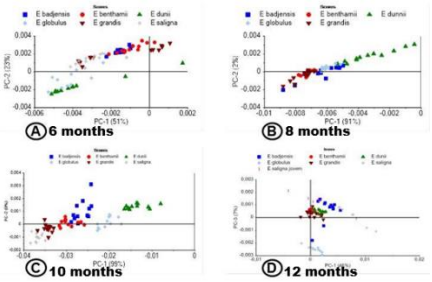
Application of Near Infrared Spectroscopy and Chemometrics for Differentiation of Six Species of Eucalyptus L'Hér. based on their Leaves

Migacz IP[1], Nisgoski S[2], Bolzónde Muniz GI[2], Farago PV[1] & Budel JM[1]

[1]Department of Pharmaceutical Sciences, State University of Ponta Grossa, Paraná, Brazil, [2]Federal University of Paraná, Brazil

Eucalyptus L'Her is one of the more cultivated genus of Magnoliopsida and have been used for producing paper, wood, honey, and volatile oils. However, the morphological similarity of their leaves has resulted in problems for botanical identification. The goal of this work was to investigate the potential use of near infrared spectroscopy (NIR) and chemometrics for differentiation of six *Eucalyptus* species based on their leaves spectra from 6 to 12 months. *E. badjensis*, *E. benthamii*, *E. dunnii*, *E. grandis*, *E. globulus* and *E. saligna* were evaluated. Plants were cultivated at the same environment and their leaves were collected at

20 cm from apex. Analysis was performed using leaves collected at 6, 8, 10 and 12 months. Scans were carried out at adaxial surface in a total of 60 spectra per species at each time interval. Each spectrum was obtained using an infrared spectrometer operating at reflectance mode in a range of 4000-10000 cm^{-1}, resolution of 4 cm^{-1} and 64 scans. In order to eliminate noise and other irregularities in signal, data were preprocessed with second derivative. The score graphics from PCA demonstrated the separation of the groups and revealed changes among the species and collecting times. The best species discrimination was observed at 10 months considering that clusters and no overlapping were obtained. The main component (PC-1) explained 99% of the total variance of the spectra and evidenced a remarkable separation by 6 groupings of scores (Figure 1). When the analysis was performed using leaves of 12 months, an overlapping was observed which can be due to the stabilization of chemical constituents. NIR and chemometrics can be successfully used in discrimination of *Eucalyptus* species based on leaves.



The authors thank to Wood Anatomy Laboratory team at Federal University of Paraná for NIR analyses and CAPES for financial support

PA

Rapid screening of benzoyl diterpene alkaloids from Aconitum herbs by LC-MS/MS using precursor ion scan

Wen Gao, Xin-Guang Liu, Lei Liu, Ping Li, Hua Yang**

State Key Laboratory of Natural Medicines, China Pharmaceutical University, No. 24 Tongjia Lane, Nanjing 210009, China

Herbs from *Aconitum* species have been widely used as painkillers, antirheumatic agents and less commonly as cardiotonics. Diterpene alkaloids are the main bioactive chemical constituentsin which benzoyl diterpene alkaloids are known to possess cardiotoxicity. In order to achieve rapid and accurate identification of the toxic benzoyl alkaloids, an ultra-high performance liquid chromatography method combined with tandem mass spectrometry (UHPLC-MS/MS) was proposed based on precursor ion scan (PreIS) of benzoyl characteristic ion. The diagnostic ion and fragmentation behavior for target alkaloids were summarized by using reference standards. Compared with the conventional total ion chromatography, precursor ion scan spectra by diagnostic ion were more representative and targetable. Then this method was applied to Aconite root, a total of 24 benzoyl alkaloids were discovered and identified, including 10 diester-diterpene alkaloids, 7 monoester-diterpene alkaloids and 7 lipo-diterpene alkaloids. The result revealed that this approach provided an alternative for rapid identification of toxic benzoyl alkaloids.

This study was supported by the National Scientific Foundation of China (No. 81673592),and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

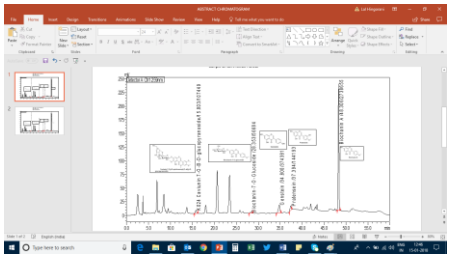
PA

Dalbergia sissoo-a standardized product: a novel approach from traceability to quality control

Aboli Girme [1], Ruchi Singh [1,] Rakesh Maurya [2], Lal Hingorani[1]

[1]Pharmanza Herbal Pvt. Ltd., Dharmaj, Gujarat, India, [2] CSIR-CDRI, Medicinal and Process Chemistry Division, Lucknow, UP, India

Dalbergia Sissoo (DS) (family-Fabaceae) is a large, deciduous tree located in the sub-Himlayan part of southern Asia. It is also known as Indian rosewood or Shisham, particularly in the northern and north-eastern regions of India. *Dalbergia sissoo* is mentioned in classical Ayurvedic literature for diverse clinical conditions as well as specific usage of leaves in traditional practices for conditions associated with osteoporosis. This research presents a specific Dalbergia sissoo extract that has been developed with stringent raw material collection, authentication, and traceability with a sustainable approach with a potential for development as a plant-derived product in bone health. The extract of D. sissoo has been optimized and processed for maximum positive effect with safe, in-vivo activity. The plant has shown presence of compounds Biochanin A, (Caviunin 7-O-β-D-glycopyranoside), 3 Pratensein or 3'-methoxygenistein , Genstein , Quercetin 3-O-rutinoside , Biochanin 7 -O- β-D-glucopyranoside , (Kampferol-3-O- rutinoside), Kaempferol 3-O-β-D-glucopyranoside, Quercetin 3-O-β-D-glucopyranoside, Caviunin 7-O- [β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, Biochanin A 7-O-[β-D-apiofuranosyl-(1→6)-β-D-glycopyranoside ,Biochanin A 7-O[β-D-apiofuranosyl-(1→5) β-D-apiofuranosyl-(1→6) -β- D-glycopyranoside , (Caviunin). However, the extract presented in the optimized extract has been standardized for five marker compounds namely Caviunin 7-O-B-D-glucopyranoside, Biochanin-7-O-Glucoside, Genstein, Pratensein, and Biochanin. The rapid, sensitive RP HPLC method has been developed and validated for these bioactive markers to provide a standardized approach to a scientifically developed extract with health applications.



Initial research and primary development transferred to Pharmanza Herbal Pvt. Ltd by CSIR-CDRI, India.

PA

Morphological and molecular characterization of medicinal plant- Spilanthes acmella L. Murray

Joshi V[1] & Jadhav SK[2]

[1]Center for Basic Sciences, Pt. RSU, Raipur,Chhattisgarh, [2] S.o.S in Biotechnology, Pt. RSU, Raipur,Chhattisgarh

Morphological and molecular characterization is necessary for sustainable management and designing conservation strategies for the plant, also it will enhance understanding in improving the optimal yields of active ingredients. *Spilanthes acmella* (L.) Murray is a miraculous medicinal plant of Asteraceae family possessing antiplasmodial, insecticidal, larvicidal and toothache relieving properties. The active ingredient is an alkamide spilanthol which is responsible for most of its medicinal properties. To assess morphological diversity 25 accessions were collected from different regions of Chhattisgarh state India: Northern Hilly Region, Middle Plain Region and Southern Plateau Region and observed for seven morphological traits (leaf length of third leaf from the top, leaf width of third leaf from the top, leaf margins, leaf colour, flower diameter, flower colour and internodal length) whereas genetic diversity was assessed using RAPD and ISSR markers. On the basis of morphological characters dendrogram was generated which formed two major clusters with 70% polymorphism whereas on the basis of combined RAPD-ISSR analysis 79% polymorphism was obtained. Therefore, DNA polymorphism exceeds the morphological diversity and such high level of genetic variation may have accumulated in the plant during the course of adaptations to varied environmental conditions.

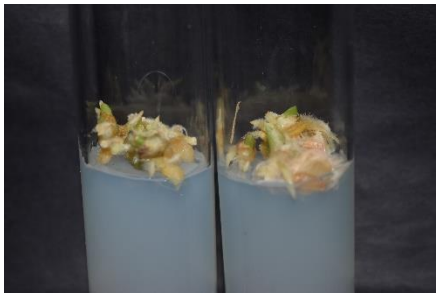
PA

Micropropagation Technology: A Modern Tool for Plant Propagation

Neelu Joshi

School of Biotechnology and Bioinformatics,D.Y. Patil Deemed To Be University,Plot No. 50, Sector-15, CBD Belapur-400614,Navi Mumbai-India

Plants have been conventionally propagated by seed and vegetative methods. Seeds are generally heterozygous and cause undesirable variability in the off-springs. Moreover, methods of vegetative propagation are not available for all the plant species. Further, these methods, wherever available, are slow and able to produce a limited number of propagules. Plant Tissue Culture has become an important alternative where any plant species can be cloned and a large number of genetically uniform disease-free plants can be obtained within a short period of time. The technique enables production of plants in a small laboratory space round the year, independent of season. The controlled physico-chemical culture environment ensures uniform growth and optimum field performance of the plants. The technique has been widely applied for large-scale production of quality plants including fruit plants, ornamentals, plantation crops, tree species, spices and condiments. The demand for tissue culture derived plants has been growing exponentially across the globe. A large number of tissue culture based industries have been engaged in commercial production of selected plant species in India and abroad. Strategies, success and challenges for micropropagation are described in this paper.



The author is grateful to the Director, School of Biotechnology and Bioinformatics,D.Y. Patil Deemed To Be University for providing support and facilities.

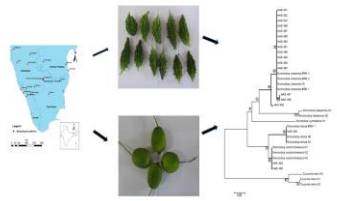
PA

DNA barcoding of Momordica species and assessment of adulteration in Momordica herbal products, an anti-diabetic drug

Santhosh Kumar J U[1, 2], Krishna V[1], Uma Shaanker R[2,3] & Ravikanth G[3]

[1]Department of Post Graduate Studies and Research in Biotechnology, Jnanasahyadri, Kuvempu University, Shankaraghatta, Shimoga-577451, India, [2]School of Ecology and Conservation, Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bangalore-560065, India, [3]Ashoka Trust for Research in Ecology and the Environment, Royal Enclave, Srirampura, Jakkur Post, Bangalore-560064, India

Medicinal plants and their products, since time immemorial, have been used in virtually all cultures as a source of medicine. In India, a number of plant species are used as medicine, and in recent years, the trade in medicinal plants has increased several fold. However, with increasing demand for, and burgeoning trade in, raw herbal products, there has been concern over the safety and the efficacy of the herbal products. In recent years, a number of studies have highlighted the rampant adulteration and species admixtures in raw herbal trade. Here we evaluate the extent of adulteration in the raw herbal trade of *Momordica charantia,* commonly used in the treatment of Type-2 diabetes in south India. Eighteen markets samples representing the raw herbal products of *Momordica,* commonly called as "*karela*" in India, were purchased from local markets. The authenticity of the herbal samples was assessed by evaluating them against the DNA barcode developed for the biological reference standard of *Momordica charantia*. Our results indicate that the market samples sold were most authentic with only three of the eighteen samples containing species other than *M.charantia.* We discuss the implications of the study in the larger context of the concern of adulterations in the raw herbal trade.



This work was supported by Department of Biotechnology, Government of India (Grant number: No.BT/IN/ ISTP-EOI/2011). Authors also acknowledge National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Thrissur, Kerala for providing the plant material for this study. We thank Dr. R. Ganesan, ATREE, Bangalore and Dr. Srikanth Gunaga, Forestry College, Sirsi for the taxonomic identification of the plant samples.

PA

Good Medicinal Plants Practices (GMPP) -Assuring Safety, Efficacy, and Quality of Botanical Products – From Field to Firm

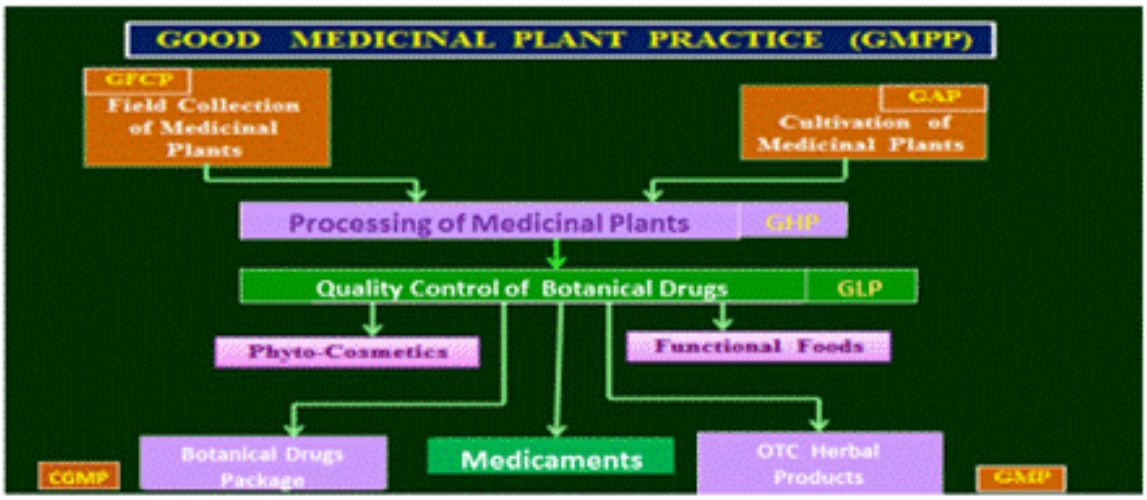
Mohammad Kamil

Zayed Complex for Herbal Research & Traditional Medicine – DHL&ME – Department Of Health- Abu Dhabi, UAE

The challenges are innumerable and enormous, making the global botanical market unsafe. The presentation seeks to enlighten physicians, pharmacists, consumers, researchers and stakeholders in botanical medicine on the need to establish quality parameters in totality i.e. from the birth of the plant until it is dispensed to the patients either in crude form or in form of the finished products, along with highlight on major causes of inconsistency in botanical drugs.

To start with good agricultural practices (GAP), selection of medicinal plants, documentation, seeds and propagation materials, cultivation -which require intensive care and management with details of site selection, ecological environment, soil, irrigation and drainage, plant maintenance & protection, harvest and personnel. Good field collection practices (GFCP) includes technical planning, permission & collection permit, selection of medicinal plants for collection, collecting techniques & procedures and storage. Necessity of identification and severity causes due to taxonomic misidentification, substitution and adulteration and much emphasis on chemical standardization and its advantages. General format for standardization from preliminary examination, microbial contamination, assay, physico- chemical constants, fingerprinting, marker compounds, inter and intra species variation, extract validation in laboratory(GLP), current good manufacturing practices (CGMP), specification of finished botanical products, designing of stability studies, batch to batch reproducibility, adverse interaction, pharmacovigilance, inadvertent substitution and intentional adulteration with specific examples of prescription drugs, the imperceptible use of pharmaceutical analogues and constraints in quality control of botanical products as good marketing practice market (GMP).

This poster is based on complete steps involved in good medicinal plant practices (GMPP) along with modern perspectives of high throughput technology, suggesting random amplified polymorphic DNA finger printing and hyphenated NMR techniques as future of the botanical drugs.



Thanks are due to Department of Health, with special reference to Director-HLME Division- DOH, Under SecretaryDOH and Chairman -Department Of Health-Abu Dhabi-UAE for providing facilities and giving their kind permission to attend this Conference and present this poster.

PA

A Non-Destructive Method for Chemical Analysis of a Garcinia mangostana L. (Mangosteen) Herbarium Specimen

Kao D[1], Henkin JM[2,3], Soejarto DD[2,4], Kinghorn AD[3], Oberlies NH[1]

[1]Department of Chemistry and Biochemistry, The University of North Carolina at Greensboro, Greensboro, NC 27402, USA, [2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, IL, 60612, USA, [3]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, OH 43210, USA, [4]Botany Department, Science and Education, The Field Museum, Chicago, IL 60605, USA

Scientists consider the citation of a voucher herbarium specimen that documents a plant sample being analyzed as mandatory in publications pertaining to the chemical analysis of a plant species. Taxonomic authentication of a specimen is routine when it comes to confirming the validity and reproducibility of research. However, we can sometimes overlook the fact that the process also preserves the scientific findings of a generation of scientists. These stored voucher herbarium specimens can be analyzed by other researchers to document changes over time by phenotype, genotype, and even chemotype. Analyses of the latter two categories have typically required irreversible damage from invasive sampling of specimens. With our approach, which combines droplet-liquid microjunction-surface sampling probe with ultra-performance liquid chromatography (UPLC) coupled to mass spectrometry, we performed a non-destructive chemical analysis on a specimen of *Garcinia mangostana* L. (Clusiaceae) (mangosteen). Metabolites on the surface of the specimen were dissolved via aqueous microextractions, which were chromatographically separated and detected by mass spectrometry. On the voucher herbarium specimen, we were able to identify known cytotoxic prenylated xanthones previously published from this species while maintaining the integrity of the entire specimen with this innovative method.

This research was funded by the National Center for Complementary and Integrated Health, NIH, Bethesda Maryland via grant F31 AT009264-03 and the National Cancer Institute, NIH, Bethesda, Maryland via grant P01 CA125066.

PA

Pesticide Residues in Botanical Dietary Ingredients from India and Data Interpretation using Multivariate Analysis

Koshy Rojison[1-2], Balasubramanian Murali[2], Setty M Manjunath[1] & Agarwal Amit[2]

[1]Manipal Academy of Higher Education, Manipal, Karnataka, India-576104, [2]Natural Remedies Pvt Ltd, Plot 5B, Veerasandra Indl Area, Electronic City Post, Bangalore 560 100, India.

Pesticide residues are a major concern in dietary supplements which are marketed throughout the world. Various regulations are in force which regulate the Maximum Residue Levels (MRL's) of these contaminants. Ideally these MRL's should be based on toxicological studies taking into consideration the recommended dose / average consumption level of the botanical ingredient / supplement. Since dietary

supplements are regulated as foods in US, the EPA limits prescribed for conventional foods become applicable to botanical dietary ingredients. Unfortunately, for most of the Indian botanicals the EPA has not specified any MRLs and as a result "Zero tolerance" becomes applicable to such ingredients. Zero tolerance is often interpreted as 'not detected' which implies 10 ppb in USA.

Pesticide residue method for quantitative determination of more than 100 pesticides comprising majorly of organochlorine, organophosphate and pyrethroids, were developed and optimized for this study. The extraction and clean-up strategies optimized are specific to herbal extracts. Herbal extracts, made using organic solvents are very complex matrices as they are enriched with various secondary metabolites and pigments when compared to parent herbs. Depending on the manufacturing process and plant part used, the composition of herbal extracts varies and consequently, due to high matrix interference, the extraction of analytes and clean-up stages become very important and complicated. The validation requirements were verified and mean recoveries for majority of the pesticides varied from 75-110%. The method was evaluated for five major Indian raw botanicals and their different solvent extracts and was found suitable.

Multivariate analysis was performed to identify similar patterns in pesticide residue levels in the selected five herbal extracts which are dependent on various factors. This study provide detailed analysis for the tested samples based on type of pesticides found, the manufacturing process and geographical origin of the raw materials. This helps to predict occurrence of contaminants in various products based on the factors mentioned before. Further, this approach enables a manufacturer / processer to take adequate preventive measures to avoid the risk of contamination and achieve regulatory compliance to FSMA.

Financial support to the project received from National Medicinal Plants Board, Ministry of Ayush, Govt. of India, is gratefully acknowledged

PA

Proposed protocol for the safety assessment of botanicals as food ingredients

Loeven P[1], Weightman J[1], Rotstein J[1], Marles RJ[2]

[1]Chemical Health Hazard Assessment Division, Bureau of Chemical Safety and [2]Nutrition Premarket Assessment Division, Bureau of Nutritional Sciences, Food Directorate, Health Products Food Branch, Health Canada K1A 0K9

The addition of botanicals, their extracts, and bioactive ingredients to pre-packaged foods and beverages is becoming more prevalent in the food supply. These ingredients either do not have a conventional food purpose, such as nutrition or a technical effect, or they may not be considered to have a conventional food purpose at the levels proposed by manufacturers to be added to food. For example, a substance could be a flavour at low levels but a supplement at higher levels. Health Canada's Food Directorate has developed a proposed protocol for conducting safety assessments for these non-conventional food ingredients to be added to food. The safety assessment of such ingredients will likely differ from those conducted for conventional food ingredients or for dietary supplements. Their use in food may need to be more restrictive because they cannot be consumed safely *ad libitum* by the general population. Cautionary labelling may be required in some cases to alert consumers to the conditions for safe use of these foods. Other challenges to be taken into consideration relate to the ingredients themselves, which may be a mixture of chemicals instead of a single chemical entity. In addition, they may be derived from a variety of sources and may contain adulterants or impurities which can alter the safety of the substance. This presentation outlines Health Canada's proposed approach for conducting safety assessments of such non-conventional botanical food ingredients.

PA

Development of USP Guarana Seed Monograph Family

Maria Monagas[a]; Padmanabharaju P.[a]; Chris Okunji[a]; Satish J.[a]; Eike Reich[b]; Gabriel Giancaspro[a]

[a]United States Pharmacopeial Convention (USP). 12601 Twinbrook Parkway. Rockville, MD 20852, [b]CAMAG. Sonnenmattstrasse 11, 4132 Muttenz. Switzerland

Guarana seed, the dried seeds of *Paullinia cupana* Kunth [syn. *P. cupana* var. *sorbilis* (Mart.) Ducke], is highly valued because of the high caffeine content, which could be up to 6%. Besides its use in soft and energy drinks, approximately 30% of the guarana seed production serves as a raw material for the nutraceutical industry. The present abstract summarizes the work carried out by the United States Pharmacopeial Convention (USP) for the creation of three new Dietary Supplement monographs: Guarana Seed, Guarana Seed Powder and Guarana Seed Dry Extract. The proposal includes tests for identification and composition targeting methylxanthines (caffeine, theobromine, and theophylline) and flavonoids (catechin, epicatechin, epigallocatechin, procyanidin B1 and procyanidin B2) as marker compounds. New HPTLC and HPLC-DAD methods were developed and validated for the analysis of both classes of compounds in a single chromatographic run. In addition, to USP Caffeine RS and USP Catechin RS, two new reference standards (USP Epicatechin RS and USP Procyanidin B2 RS) are proposed. Commercial samples representing the different matrixes were screened to confirm ingredient characteristic fingerprinting and proposed specifications. The HPTLC method provides a fingerprint with reference to the characteristic bands due to caffeine, catechin/epicatechin and procyanidin B2. Content ratios between catechin and epicatechin and between caffeine and total flavonoids were established as acceptance criteria for the HPLC-DAD Identification test. For guarana seed and guarana seed powder, composition limits are based on the content of both caffeine and total flavonoids, whereas for guarana seed dry extract those are based on labeled amount of caffeine. To our knowledge, this is the first attempt to include the full HPLC profile of methylxanthines and flavonoids for the creation of a global public standard for Guarana seed.

PA

Characterization of Fadogia agrestis leaf, stem and root by anatomy and microscopy

Raman V[1], Budel JM[2], Khan IA[1,3]

[1]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, USA; [2]Department of Pharmaceutical Science, State University of Ponta Grossa, Brazil; [3]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, USA.

Fadogia agrestis (Rubiaceae) is a folk medicinal plant in Africa. It has been used as a diuretic and aphrodisiac [1] as well as for the treatment of fevers, kidney pain, diarrhoea, stomach ache, blennorrhoea and toothache [2]. Several products of dietary supplements containing this species as a main ingredient are being sold in the market. The objective of the present work was to study the anatomy and micromorphology of *F. agrestis*. Leaves, stems and roots of this species were investigated by light and scanning electron microscopy. The main anatomical characters observed were: presence of septate non-glandular trichomes in the leaf and stem, paracytic stomata on leaf abaxial epidermis, druse and prismatic crystals in leaf, primary phloem fibers in stem, brachysclereids in stem and root, starch grains in root, and vessels with vestured pits and simple perforated end walls. These features can serve as markers in the identification and quality control of the botanical.

This study was supported by Science Based Authentication of Dietary Supplements and Botanical Dietary Supplement Research funded by the Food and Drug Administration grant # 1U01FD004246-05.

References:
[1] Yakubu MT, Akanji MA, Oladiji AT, 2005. Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. ex Hiern) stem in male albino rats. Asian J. Androl. 7, 399-404.

[2] Odugbemi T, 2008. A textbook of medicinal plants from Nigeria. University of Lagos Press, Lagos, p. 558.

PA

Looks may be deceiving: Authenticating Pelargonium species used in commercial herbal products through DNA barcodes and chemical analyses

Rattray RD[1], *van der Bank M*[1] *& Viljoen AM*[2]

[1]*African Centre for DNA Barcoding, Department of Botany & Plant Biotechnology, Faculty of Science, University of Johannesburg, P. O. Box 524 Auckland Park 2006, South Africa*

[2]*Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria 0001 South Africa*

Globally, the use of Commercial Herbal Products (CHP) has surged in recent years resulting in the phytomedicine industry to come under pressure in the development of rapid, accurate and cost-effective methods for quality control with focus on the positive identification of raw materials. South Africa, like many counterparts, do not enforce any laws that regulate the quality, safety and efficacy of CHP before they enter the consumer market creating a cause for concern. *Pelargonium* species, one of South Africa's key export products, play an immense role in both horticultural and medicinal industries worldwide. Various ethnic groups have made use of root extracts prepared from *P. sidoides* DC. in the treatment of coughs, upper respiratory tract irritations and gastrointestinal conditions. Umkalabo®, an ethanolic extract prepared from the root, is currently successfully marketed in Europe. However, taxonomic delimitation of the allies *P. sidoides* and *P. reniforme* Curtis have been debated in the literature for many years. Majority of consumer products explicitly list *P. sidoides* as the active botanical ingredient leading to quality control concerns as *P. sidoides* and *P. reniforme* have not been proven as pharmacologically equipotent. In this study, DNA sequence and chemical data generated through standardized DNA barcoding techniques and LC-MS chromatography respectively, were used in the authentication of *P. sidoides* herbal products. Results showed that 44% of the products did not contain any traces or chemical marker compounds of *P. sidoides*, indicating potential adulteration of the above-mentioned products; a concerning statistic that may also be the case for many other products produced and sold within the country and globally. This is the first attempt in the compilation of a reference library of DNA barcodes for *Pelargonium* herbal products in South Africa which will provide species-level identification for CHP traded in the country.

PA

Authentication and quality control of concentrated herbal medicine granules by molecular technology

[1]Lo YT, [1]Shaw PC

[1]LDS YYC R&D Centre for Chinese Medicine, Institute of Chinese Medicine and School of Life Sciences, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

Concentrated herbal medicine granules (CCMG) offer patients a convenient option for traditional therapy. However, with morphological and microscopic characteristics lost, it is difficult to authenticate and control the quality of these medicinal products. This study aimed to develop effective and universal methods for species identification and quantification. Species-specific primers which amplified DNA fragments in *Mesobuthus martensii* and *Zaocys dhumnades* CCMG with sizes less than 200 bp were found to be effective for species identification from the adulterants. On the other hand, polymerase chain reaction (PCR) amplification of full-length DNA barcode in processed products is difficult because of severe DNA fragmentation. In order to develop a universal method for species identification, an adaptor ligation-mediated PCR protocol was derived. The specially designed adaptor with asymmetric strands and terminal modification avoided amplification of non-target DNA sequences. Sets of target DNA fragments from *Angelica sinensis* and *Panax notoginseng* CCMG were ligated with the adaptors, amplified by an adaptor primer with a single universal barcode primer to obtain partial internal transcribed spacer 2 (ITS2) sequence, and identified by DNA sequencing. Besides species authentication, determination of the constituent species amount in multi-herb products is important for quality control. Quantitative PCR (qPCR) was used to determine the amount of *Whitmania pigra* and *Zaocys dhumnades* CCMG in mixture solution. Results showed that reproducible quantification results could be obtained (1) using a modified DNA extraction protocol, (2) amongst DNA extracted from the same batch of CCMG and (3) amongst different batches of CCMG from the same company. The above studies extended the application of DNA techniques to concentrated herbal medicine granules and may be further developed for quality assurance and regulatory compliance in the CCMG industry.

PA

How can analytical scouting gradients help us perform prep HPLC?

Jack E. Silver, Chester Bailey

Teledyne Isco, 4700 Superior Street, Lincoln, NE 68504, USA

Analytical scouting gradients provide useful information prior to preparative HPLC while using very little sample. Scouting runs provide an estimate of compound purity. By observation of whether peaks show fronting or tailing, one may determine the best solvent systems and modifiers to purify compounds. Analytical scouting runs also allow calculation of a focused gradient for preparative purification[1]. The potential sample loading on the preparative column can now be determined with little more information than the retention time of the desired compound and nearest impurity, the preparative gradient slope, and dimensions of the preparative column. The calculation determines the resolution between peaks which then determines sample loading. If multiple peaks are of interest for collection, the calculation also suggests whether a gradient can reduce the purification run time.

[1]Preparative method development from analytical columns. Silver, J.E. Presented at the ICSB meeting, Oxford MS, April 2017

PA

Quality control of a flavonoid-rich extract of Glycyrrhiza glabra (GutGard®) using HPLC-PDA–MS/MS

Singh Vineet[1], *Koshy Rojison*[1], *Mundkinajeddu Deepak*[2]

[1]*Manipal College of Pharmaceutical Sciences, Manipal University, Manipal 576104, Karnataka, India,* [2] *Research and Development Centre, Natural Remedies, Bangalore 560 100, Karnataka, India.*

GutGard®, a flavonoid-rich standardized extract derived from *G. glabra* is commercially used for the management of gut health problems. This study aims to develop a quantitative and qualitative evaluation method for the quality control of GutGard® using high-performance liquid chromatography with photo diode array detector coupled with the mass spectrometer (HPLC-PDA–MS/MS). Nine batches of the extract were analysed to establish the chromatographic fingerprint of flavonoids. A total of 53 peaks were assigned using MS/MS as the "common peaks" and nine peaks in the fingerprint of all the nine batches of samples were assigned as "characteristic peaks". Identities of the latter were additionally confirmed by comparing their retention time and mass spectra with those of the reference substances (liquiritin, isoliquiritin, liquiritigenin, isoliquiritigenin, formononetin, isoformononetin, glabridin, glabrol and 4-O-methylglabridin). The similarities of the 9 batches of samples were evaluated by the Pearson correlation coefficient. The results indicated that the samples from different batches had similar HPLC fingerprints. This method was validated for simultaneous determination of the 9 analytes and the the detection wavelength was set at 280 nm. For qualiï¬cation of "common peaks", 53 compounds including 9 quantitative analytes were identiï¬• ed using LC–MS/MS. The results showed that all of these licorice samples were rich in flavonoids, although their contents obviously vary, and the proposed method on combining chromatographic fingerprint with quantitative analysis could serve as a prerequisite for quality control of commercial batches of flavonoid rich *G. glabra* extract.

The research is supported by the Natural Remedies Pvt. Ltd. Bengaluru, India.

PA

Detection of Adulteration in Goldenseal Dietary Supplements via Mass spectrometry-based Metabolomics

E. Diane Wallace[1], *Nicholas H. Oberlies*[1], *Nadja B. Cech*[1], *Joshua J. Kellogg*[1]

[1]*Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, NC 27402*

The consumer usage of herbal dietary supplements has continually increased throughout the past few years. With an increase in popularity comes a higher rate of adulteration as well, commonly for economic benefit. Goldenseal (*Hydrastis canadensis* L.), a well-known herb used to aid digestion, requires years to grow to full maturation for harvest. A large number of goldenseal supplements have been adulterated due to the cost and commitment of growing the plant until maturation. A UPLC-MS metabolomics study was performed on 46 goldenseal commercial products (2 loose-leaf teas, 16 powders, 9 tinctures, 5 capsule extracts, and 14 encapsulated powders), as well as authenticated botanical reference materials. Visual analysis of the chemometric data via principal component analysis (PCA) and, reproduced correlation coefficients (obtained from the PCA model), were used to compare the goldenseal samples and reference materials. The metabolomics analysis revealed that several products were distinct from the main grouping of samples, and subsequent evaluation of contributing metabolites lead to their identification as different species or mixtures of plant material. The obtained results demonstrated the potential of mass spectrometry-based metabolomics

to discriminate between multiple unknown products in a large sample set to determine possible adulteration.

This project was supported by the National Institutes of Health (NIH), specifically the Center of Excellence for Natural Product Drug Interaction Research (NaPDI, U54 AT008909. All mass spectrometry was performed at the Triad Mass Spectrometry Facility at the University of North Carolina at Greensboro.

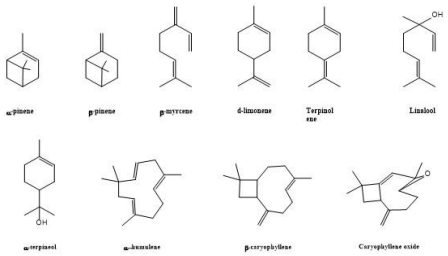
PA

Qualitative and Quantitative Determination of Major Terpenes in Cannabis sativa Using GC/FID

Ibrahim EA[1,2], Wang M[1], Radwan MM[1], Wanas AS[1], Gul W[1,3] Chandra S[1], Lata H [1], Mehmedic Z[1], Majumdar CG[1], Hadad GM[2], Abdel Salam RA[2], Ibrahim AK[4], Ahmed SA[3], Khan IA[1,5] & ElSohly MA[1,3,6]

[1]*National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA.* [2]*Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt.* [3]*ElSohly Laboratories, Inc., 5 Industrial Park Drive, Oxford, MS 38655 USA* [4]*Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt.* [5]*Division of Pharmacognosy, Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA.* [6]*Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, University, MS 38677, USA*

Cannabis sativa L. (Cannabaceae) has been cultivated since ancient times and different cannabis strains possess distinct aromas and flavors, due to the presence of specific terpenes [1, 2]. The analysis of cannabis for terpenes concentration can be applied to strain identification, referred to as fingerprinting, and for concentration accuracy in `` medical marijuana``. In the present study, an analytical method using GC/FID was developed and validated for the determination of ten major terpenes (mono- and sesquiterpenes), namely; ***α*-pinene, *β*-pinene, *β*-myrcene, limonene, terpinolene, linalool, *α*-terpineol, *β*-caryophyllene, *α*-humulene,** and **caryophyllene oxide** in different extracts of *C. sativa*. The GC/FID method was validated according to AOAC guidelines for linearity, limits of quantification and detection, repeatability, intermediate precision, and accuracy. The method was linear over the calibration range of 1-100 µg /mL with R^2 > 0.999 for all terpenes. LOD and LOQ were 0.3 µg/mL and 1.0µg /mL, respectively for all terpenes. Spike recovery studies for all terpenes were carried out on placebo cannabis material and high potency indoor variety with authentic standards. In general, accuracy (recovery) at all levels was in the range of 89-104% and 90-111% for placebo and high potency indoor variety, respectively. The repeatability and intermediate precision of the method were evaluated by quantification of the target terpenes in extracts of three different *C. sativa* varieties, resulting in acceptable RSDs (less than 10%). The developed method is simple, sensitive, reproducible and suitable for the detection and quantitation of mono- and sesquiterpenes in extracts of *C. sativa* and was proposed to the United States Pharmacopoeia for the quality control of *C. sativa* biomass.



This research was partially funded by the National Institute on Drug Abuse (Contract # N01DA-15-7793).

PA

Enantiomeric Distribution as a Tool for Quality Evaluation: Tea Tree and Black Pepper Oils as Examples

Wang M[1], *Chittiboyina AG*[1], *Avula B*[1], *Wang YH*[1], *Parcher JF*[1] *and Khan IA*[1,2]

[1]*National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA.* [2]*Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA.*

The growing demand and commercial value of plant derived essential oils in the market have resulted in considerable interest among producer and consumer industries to develop suitable and cost-effective methods for characterization and quality assessment. Many components of oils are chiral in nature. Because of natural enzymatic processes in plants, biosynthesis of plant volatiles often results in a large excess of one enantiomer, whereas an organic synthesis from achiral starting materials usually produces a racemic mixture. In addition, chiral compounds in specific species have a characteristic enantiomeric distribution that is attributable to stereoselectively-controlled biogenetic formation mechanisms of the respective plant. Therefore, enantiomeric composition of optically active components has been used successfully for authenticity assessment of essential oils, or to differentiate natural compounds from those of synthetic origin, enabling the authenticity of desired products to be guaranteed.

In this study, the enantimeric compositions of selected terpenes present in tea tree (*Melaleuca alternifolia*) and black pepper (*Piper nigrum*) oils were determined by chiral GC/MS. Four chiral components, α-pinene, limonene, terpinen-4-ol and α-terpineol were investigated in 58 known provenance Australian tea tree oils and 47 commercial products. The known provenance tea tree oil samples showed consistent enantiomeric ratios. The average (+) isomer percentages were 92.8±0.4%, 64.58±1.0%, 63.3±0.4% and 79.4±0.8% for α-pinene, limonene, terpinen-4-ol, α-terpineol, respectively. These values were used to evaluate the 47commercial products. The results clearly indicated that 28 commercial products contained excessive (+) isomer or contained the (+) isomer in concentrations below the values measured from the known provenance oils. Similar study was also carried out to determine enantiomeric distribution of β-pinene, sabinene, limonene and terpinen-4-ol in an extensive set of black pepper oils (*n=23*) collected from 9 major producing countries. Interestingly, enantio-selectivity observed for these four monoterpenes was *levo*-isomers to be predominant, unlike tea tree oil, emphasizing the highly conserved enzymatic processes occurring in *P. nigrum*. Like tea tree oil, consistent enantiomeric ratios 92.2±3.0% for (-)-β-pinene, 94.8±2.8% for (-)-sabinene, 60.7±1.1% for (-)-limonene, and 77.2±5.3% for (-)-terpinen-4-ol were observed, independent of geographical location. The results from these two examples demonstrated the potential of using stereospecific compositions as chiral signatures for establishing the authenticity and quality of essential oils.

Acknowledgements: This research is supported in part by "Science Based Authentication of Dietary Supplements" funded by the Food and Drug Administration grant number 1U01FD004246-05, and the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No. 58-6060-6-015.

PA

Analysis of Terpenes in Cannabis sativa L. Using GC/MS: Method Development and Validation to Fulfill US Pharmacopeia Requirements

Wang M[1], *Ibrahim EA*[1,2], *Radwan MM*[1], *Wanas AS*[1], *Avula B*[1], *Wang YH*[1], *Chandra S*[1], *Lata H*[1], *Khan IA*[1,3], *ElSohly MA*[1,4]

[1]*National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA.* [2]*Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt,* [3]*Division of Pharmacognosy, Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA.* [4]*Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, University, MS 38677, USA*

Terpenes are the major components of many fragrant essential oils present in different concentrations in various *Cannabis sativa* L. varieties. These compounds are what impart cannabis varieties with distinctive aromas and flavors. There are some reports to suggest that the synergic action between terpenes and cannabinoids contributed to the overall medical beneficial effects of cannabis, and terpenes may play a key role in differentiating the effects of various cannabis strains. On the other hand, terpenes themselves have also been demonstrated to have therapeutic uses for treatment of inflammation, anxiety and sleep problems. As the legalization of medicinal cannabis continues to grow in the US, in addition to testing for cannabinoids, there is also increasing interest in the detection and quantification of terpenes present in different cannabis varieties.

This investigation was initiated at the request of US Pharmacopeia to develop new analytical methods for accurate and efficient qualitative and quantitative analysis of terpenes in cannabis. The ten most significant terpenes, *viz.* β-caryophyllene, limonene, myrcene, α-pinene, terpinolene, β-pinene, caryophyllene oxide, α-humulene, linalool and α-terpineol were analyzed in samples from three varieties of *C. sativa* (high potency [HP], intermediate, and high CBD) using GC/MS. Good baseline separation was achieved for all the targeted compounds. *n*-Tridecane with a retention time falling between the mono- and sesqui-terpenes was selected as the internal standard. The developed method was validated according to AOAC guidelines with respect to linearity, accuracy (recovery), selectivity, repeatability, intermediate precision, limit of detection (LOD) and limit of quantitation (LOQ). The concentration-response relationship of the developed method was linear within the concentration range of 0.75-50 µg/mL for myrcene, α-pinene, β-pinene, terpinolene, linalool, 0.75-70 µg/mL for limonene, α-terpineol, β-caryophyllene, α-humulene, and 1.0-70 µg/mL for caryophyllene oxide with r^2 values > 0.99 for all terpenes. The average recoveries for all terpenes in the placebo and indoor cultivated HP samples were between 95.0-105.7% with one exception of terpinolene (67.0-70.0%). The measured repeatabilities and intermediate precisions ranged from 0.32-5.89% and 0.50-6.01% for High CBD variety samples, 0.37-8.47% and 1.47-7.07% for HP variety samples, and 0.42-4.00% and 3.43-5.31% for intermediate variety samples, respectively. The LOQs and LODs for all targeted terpenes were determined to be 0.25 and 0.75 µg/mL, respectively.

Acknowledgement: This research was partially funded by the National Institute on Drug Abuse (Contract #: N01DA-15-7793).

PA

Yellow tea (Camellia sinensis L.), a promising Chinese tea: Processing, chemical constituents and health benefits

Xu JY[1,2], Wang M[1], Zhao JP[1], Wang YH[1], Tang Q[2], Khan IA[1,3]

[1]*National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA.* [2]*Tea Department of College of Horticulture Science, Sichuan Agricultural University, Chengdu 611380, Sichuan, China.* [3]*Division of Pharmacognosy, Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA*

Although several different types of teas are derived from the leaves of *Camellia sinensis* L., tea can be classified into six major categories based on their different processing techniques and the characteristics associated with each group, viz. Green tea (non-fermented), yellow and white teas (lightly fermented), Oolong tea (semifermented), black tea (fully fermented) and dark tea (post-fermented). As the rarest and most precious variety of tea, yellow tea has gained increasing popularity in recent years due to its pleasant mellow taste and known health benefits such as anti-oxidation, anti-inflammation and anti-cancer properties. The production process of yellow tea is similar to green tea, but with a unique additional step called "sealed yellowing", in which oxidation is slowed and the grassy smell of green tea is removed. Compared to other types of teas, yellow tea is much less well-known and studied.

In this review, the history and classification of yellow tea, along with the most popular commercial yellow tea products are introduced. The production process, including withering, fixing, rolling, sealed yellowing and drying, is presented (Figure 1). The bioactive chemical compounds common in various types of teas or unique to yellow tea are discussed. Finally, future needs in the research and development of yellow tea are discussed and proposed. As the first review paper for yellow tea, we hope this manuscript can provide comprehensive information about yellow tea and would be a valuable addition to tea science.

Acknowledgement: Dr. Jingyi Xu is the recipient of scholarships offered by the China Scholarship Council (CSC) and Sichuan Agricultural University

PA

Chemical Analysis and Characterization of Calea zacatechichi by UHPLC-UV-MS Using UNIFI Informatics Platform

Yan-Hong Wang[1], Bharathi Avula[1], Mei Wang[1], Ji-Yeong Bae[1], Vijayasankar Raman[1], Ikhlas A. Khan[1,2]

[1] National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA, [2]Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA,

Calea zacatechichi Schl (syn. *C. termifolia* Kunth) is a flowering plant in the Asteraceae family and native to Mexico and Central America. This plant is also known as dream herb and used in folk medicine to treat cough, asthma, and gastrointestinal tract disorders such as stomach ache and diarrhea. In the US, *C. zacatechichi* has been marketed for medicinal purposes including diabetes treatment. Phytochemical studies have found the key secondary metabolites of *C. zacatechichi* are germacranolide type sesquiterpene lactones.

With the aim of developing a strategy on determination of marker compounds in botanical ingredients of dietary supplements, methanol extracts of *C. zacatechichi* were analyzed by UHPLC-UV-MS. The UHPLC-UV and QToF MS data were subjected to UNIFI informatics platform, which can provide fit-for-purpose workflows including encompasses data processing, characterization and identification of potential marker compounds, visualization, and reporting. Different types of compounds such as organic acids, flavonoids, and sesquiterpenoids were identified and characterized. The present work will explore the strategy of marker compounds' determination in *C. zacatechichi* on UNIFI platform.

Acknowledgements: This research is supported in part by "Science Based Authentication of Botanical Ingredients" funded by the Food and Drug Administration grant number 5U01FD004246-07, the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No.58-6408-1-603-07.

PA

Structural Characterization of Lignans with Diphenyl-1-en-4-yne-pentane Skeleton in Hypoxis hemerocallidea by UHPLC-UV-MS/MS

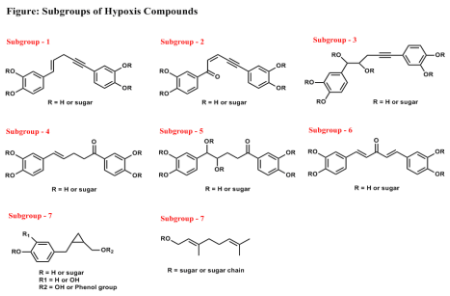
Yan-Hong Wang[1], Yalda Shokoohinia[2], Bharathi Avula[1], Mei Wang[1], Fazila Zulfiqar[1], Zulfiqar Ali[1], Ikhlas A. Khan[1,3]

[1] National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA, [2]Department of Pharmacognosy & Biotechnology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran, [3]Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

Hypoxis hemerocallidea (syn. *H. rooperi*) is a medicinal plant widely distributed in the southern Africa sub-region and commonly known as African potato. Extracts, powders, infusions and decoctions of this plant have been used in folk medicines for the treatment of nervous disorders, immune-related illnesses, heart weaknesses, and urinary tract infections. Phytochemical studies have found that main secondary metabolites

of *H. hemerocallidea* are steroids and lignans with diphenyl-1-en-4-yne-pentane skeleton. There is no analysis has been reported focusing on the chemical analysis of lignans in *Hypoxis*.

With the aim of characterization and determination of marker compounds in botanical ingredients of dietary supplements, an UHPLC-UV-MS/MS fingerprint method has been developed for the analysis methanol extracts of *H. hemerocallidea*. Different types of lignans with diphenyl-1-en-4-yne-pentane skeleton were characterized on the basis of their UV patterns and MS/MS fragmentation pathways. Structures of more than 30 lignans were determined by comparing with reference standards in terms of the retention time, accurate mass, and MS/MS fragment ions or tentatively characterized by analyzing accurate mass and surveying of the literature.



Acknowledgements: This research is supported in part by "Science Based Authentication of Botanical Ingredients" funded by the Food and Drug Administration grant number 5U01FD004246-07 , the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No.58-6408-1-603-07.

PA

Determination of Yohimbine in Standard Reference Material 3383 via Pressurized Liquid Extraction or Solvent Extraction Coupled with Liquid Chromatography/Mass Spectrometry

Wilson WB, Hosbas Coskun S, & Rimmer CA

Chemical Sciences Division, Material Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA

The National Institute of Standards and Technology has developed a variety of dietary supplement Standard Reference Materials (SRMs) for use in validating new and current analytical methodologies and as quality control materials. The candidate SRM analyzed here (SRM 3383) is a fine powder prepared from the grinding, mixing, and sieving of four commercially available yohimbe-containing tablets and/or capsules. Yohimbe bark and/or its extract are well known ingredients in many herbal supplements used for the treatment of sexual disorders in men and improvements in athletic performance. The active compound in yohimbe is the alkaloid yohimbine. Some adverse effects from yohimbe supplements include an increased blood pressure and heart rate making the detection and quantification of yohimbine in commercially available products important to evaluate the potential safety hazards to consumers. In this study, yohimbine was determined in SRM 3383 using two analytical methods. The first method combines pressurized liquid extraction (PLE) and liquid chromatography/mass spectrometry with electrospray ionization (LC-ESI-MS). The second method combines solvent extraction (SE) with LC-MS using atmospheric pressure chemical ionization (APCI). The LC-ESI-MS method measured yohimbine in the PLE extracts using an Ascentis Express RP-amide column. The LC-APCI-MS method used an HALO octadecyl (C_{18}) column to analyze

the SE extracts. The research presented here describes the optimization of the two analytical methods through the investigation of several key extraction and separation parameters.

The authors acknowledge financial support from the Office of Dietary Supplements at the National Institute of Health.

PA

Identification and Characterization of Key Chemical Constituents in Processed Gastrodia elata Using UHPLC-MS/MS and PCA Methods

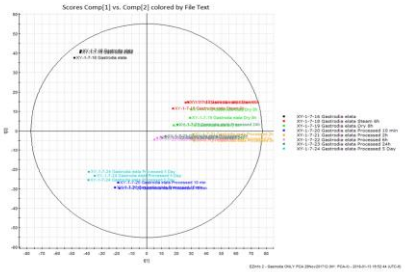
Xide Ye[1], *Yan-Hong Wang*[2], *Jianping Zhao*[2], *Mei Wang*[2], *Bharathi Avula*[2], *Ikhlas A. Khan*[2,3]

[1] *School of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang, Jiangxi Province, P.R. China;* [2] *National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA,* [3]*Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA*

Gastrodia elata is a saprophytic perennial herb in the Orchidaceae family. Dried tuber of *G. elata* (also known as Chi Jian, Ming Tianma) is used in Traditional Chinese Medicine (TCM) for the treatment of headache, dizziness, migraine, limb numbness, infantile convulsion, epilepsy, seizures, and tetanus, but *G. elata* must be processed before using since *G. elata* has shown toxic side effects. The basic processing method is to steam *G. elata* and cut into thin slices. However, the "Jianchang Bang" in Jiangxi has processed *G. elata* with ginger juice in order to enhance the power of *G. elata* for dispelling cold and relieving pain, especially in the treatment of migraine.

With the aim of identification and characterization of key chemical constituents in processed *G. elata*, an UHPLC-MS/MS method has been developed for the analysis of *G. elata* raw material, steamed *G. elata*, and samples that *G. elata* processed with ginger juice for different time periods. The TOF MS and MS/MS data are applied for multivariate statistical analysis using Markerlynx software. From PCA and OPLS-DA results, key chemical components of processed *G. elata* are identified. The structural characterization of marker compounds will be discussed in this work.

Figure: PCA score of *G. elata* raw material and processed samples at different time periods



Acknowledgements: Dr. Xide Ye is the recipient of scholarships offered by the Jiangxi Province Special Funds for Visiting Scholars in the Plan of Young and Mid-Aged Teachers' Development and Jiangxi University of Traditional Chinese Medicine. This work is partially supported by The National Natural Science Fund of China (No. 81760712).

PA

NMR Method for Determination of Phenethylamines in Sports Dietary Supplements

Jianping Zhao[1], *Mei Wang*[1], *Bharathi Avula*[1], *Ikhlas A. Khan*[1,2]

[1]*National Center for Natural Products Research,* [2]*Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA*

Phenethylamines (PEAs) are popular substances found in weight-loss and sports nutrition supplements. They are generally pharmacologically active and primarily affect the sympathetic nervous system. Many PEAs are synthetic chemicals and are on the prohibited list of the World Anti-Doping Agency. In this study, nuclear magnetic resonance (NMR) spectroscopy was applied to detect and identify the presence of PEAs in sports dietary supplements without the need for chromatographic separation or pre-knowledge on formulation. Eight PEAs, *viz*. phenethylamine, synephrine, oxilofrine, hordenine, β-methylphenethylamine, N-methyltyramine, octopamine and deterenol, were identified from 32 dietary supplements sold in the US market. Furthermore, a quantitative NMR method was developed and validated for simultaneous determination of the concentrations of the PEAs. The study demonstrated that NMR could be a potential tool to monitor and detect PEAs or other ingredients in dietary supplements.

This work was supported in part by 'Science Based Authentication of Dietary Supplements' funded by the Food and Drug Administration grant numbers 2U01FD004246-06, and the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No. 58-6060-6-015.

PA

Assessment of Aloe Vera Gel Content in Cosmetics by Using NMR

Jianping Zhao[1], *Cristina Avonto*[1], *Amar Chittiboyina*[1], *Ikhlas A. Khan*[1,2]

[1]*National Center for Natural Products Research,* [2]*Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA*

Aloe vera gel is an important botanical product, and is frequently used in pharmaceutical, food and cosmetics industries. Based on traditional uses and scientific studies, it is believed that aloe gel possesses beneficial properties to skin, including wound healing, anti-acne, anti-aging, anti-inflammation, moisturization, and other effects [1]. A great deal of skin-care cosmetic products on the market claim aloe gel as an ingredient in their formulas. However, the claim is difficult to verify due to the lack of an effective analytical method. In this study, we tried to use nuclear magnetic resonance (NMR) as a tool to detect and quantify the content of acemannan, a characteristic polysaccharide of aloe gel, in cosmetic products. Different methods for sample preparation were explored, and the qNMR method was validated in terms of selectivity, specificity, stability, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and repeatability.

This work was supported in part by "Science Based Authentication of Dietary Supplements" funded by the Food and Drug Administration grant numbers 1U01FD004246-05, and the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No. 58-6408-1-603-07.

PB

Comparative Bioactivity Modeling to Pinpoint Combination Effects in the Botanical Danshen (Salvia miltiorrhiza)

Caesar LK[1], *Nogo S*[1], *Naphen CN*[1], *Cech NB*[1]

[1]*University of North Carolina at Greensboro, Department of Chemistry and Biochemistry, Greensboro NC*

Plants have been utilized as medicines for thousands of years, and today nearly 80% of people worldwide rely on plant-based mixtures as their primary source of medicine. *Salvia miltiorrhiza*, or Danshen, is an important medicinal plant listed in the Chinese, United States, and European pharmacopoeias for its ability to treat a variety of ailments, including microbial infections. Although Danshen possesses several compounds that are active in isolation, the effect of the full complex mixture and its combination effects are poorly understood. To assess the efficacy of complex botanical mixtures such as Danshen, additive and synergistic effects of mixture constituents should be evaluated. Standard biological assays used to assess synergy are time-consuming and require considerable material, and it is infeasible to test every fraction for synergistic activity. As such, methods to prioritize fractions for synergy testing are required. To this end, we have developed a new approach, comparative bioactivity modeling, which allows us to predict which fractions possess combination effects. This simple approach enables us to prioritize fractions for synergy testing and subsequent metabolomic analysis. With this project, Danshen was evaluated for its combination effects against methicillin-resistant *Staphylococcus aureus.* Danshen's most abundant antimicrobial, cryptotanshinone, was quantified in each fraction using mass spectrometry, and a comparative bioactivity model predicting the antimicrobial contribution of cryptotanshinone was produced. In some cases, cryptotanshinone was chromatographically separated from the synergists that potentiated its activity; thus, cryptotanshinone was spiked into samples at sub-inhibitory concentrations for both bioactivity and mass spectral analysis so that a comparative bioactivity model could be produced. In many cases, the observed activity was greater than the predicted activity, indicating the presence of additives and/or synergists in the mixture. Fractions showing such a mismatch were prioritized for follow up testing where synergy was disentanged from additivity. Using this method, we have identified three simplified fractions that contain potent synergistic activity in combination with cryptotanshinone, and identification and isolation of synergists is underway.

The authors would like to acknowledge Richo Cech at Strictly Medicinal Seeds® for his provision of the *S. miltiorrhiza* plant material used for this study. Dr. Olav Kvalheim is also acknowledged for his expertise in biochemometric analysis, without which this project would be impossible. Research reported in this work was supported in part by the National Center for Complementary and Integrative Health of the National Institutes of Health under award numbers 5 T32 AT008929 (fellowship to Lindsay Caesar and Cassandra Naphen), U54 AT008909 (NaPDI, Center of Excellence for Natural Product Drug Interaction Research), and 1R01 AT006860.

PB

DALBERGIA SISSOO FOR BONE HEALTH

Raut A[1], Deshmukh A[2], Hingorani L[2]

[1]Medical & Research Center-Kasturba Health Society (MRC-KHS) Mumbai, India. [2]Pharmanza Herbal Pvt. Ltd. Dharmaj, Gujrat, India.

Osteoporosis is characterized by low bone mass density which increases bone fragility and makes them prone to repeated fracture. Bone mass density decreases with age and women are at greater risk than men. Due to the limitations of conventional therapeutic intervention, attention has turned towards the use of alternative interventions in osteoporosis. *Dalbergia sissoo* is an Ayurvedic herb used traditionally for various ailments. Recent analysis and research on *Dalbergia sissoo* found multiple phytochemicals with bone anabolic activity. *Dalbergia sissoo* extract in animal models was seen to have increased bone formation, reduced bone resorption and accelerated fracture healing [1]. These effects of *Dalbergia sissoo* extract in animal models are attributed to a novel active phyto-compound Caviunin 7-O-[b-Dapiofuranosyl-(1-6)-b-D- glucopyranoside (CAFG) present in its leaves [1]. Activities of *Dalbergia sissoo* encouraged us to take it to clinical evaluation. A 90-day sub-chronic toxicity study in rats revealed that repeated oral exposure to *Dalbergia sissoo* extract up to 1240 mg/kg/d did not produce any toxic effects. An open-labeled one-year clinical study to evaluate the safety, tolerability, and activity of *Dalbergia sissoo* leaves extract in 30 menopausal women with osteoporosis is currently enrolling. Subjects are advised to take one capsule of 300mg *Dalbergia Sissoo* extract twice daily along with one tablet of Calcium and Vit D supplement. 18 subjects have completed 9 months and interim analysis revealed that 12 subjects out of 18 showing positive changes in vertebral bone mass when analyzed for their DEXA scan. Dalbergia sissoo extract is well tolerated and there has been no renal or hepatic toxicity as well. Initial research suggests *Dalbergia sissoo* may be a valued supplement for healthy bone support.

References:

1. Kudhwah P, Caviunin-based isoflavonoid prevents bone loss; Cell Death and Disease (2014) 5, e1422.

PB

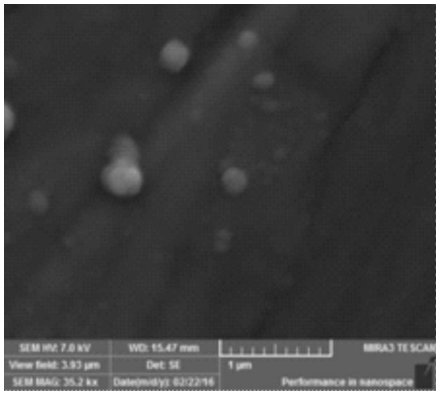
Polymeric Nanoparticles containing Curcumin Improve the Antitumor Effect of Methotrexate against Calu-3 Lung Cancer Cells

Rudnik LAC[1], Kanunfre CC[1], Nadal JM[1], Budel JM[1] & Farago PV[1]

[1]State University of Ponta Grossa, Paraná, Brazil

Curcumin (CUR) is a polyphenolic compound from *Curcuma longa* L. and has a suitable antitumor effect. Methotrexate (MTX) is an antimetabolite drug widely used for the cancer treatment. This drug has a disadvantage of suffering efflux by the P-glycoprotein (P-gp). Tumor cells can develop resistance leading to a failure of chemotherapy during the cancer treatment. In order to improve the use of CUR and MTX on tumor cells, polymer nanoparticles were developed and characterized. In addition, nanoparticles containing CUR and MTX were evaluate in lung adenocarcinoma cells due to the potential synergism of these compounds. Seven formulations were developed from poly(ε-caprolactone) and polyethylene glycol 6000 with different concentrations of CUR and MTX by the interfacial deposition of preformed polymer method.

Calu-3 tumor cell lines were used to assess viability. Nanoparticles showed suitable spherical shapes and sizes (278 to 325 nm) (Figure 1). *In vitro* studies using the CALU-3 strain demonstrated that the formulation containing MTX 0.3 mg/mL and CUR 0.5 mg/mL was the one that presented the most promising results. It was possible to infer that CUR from polymeric nanoparticles was a P-gp inhibitor, allowed the permanence of MTX into Calu-3 cells and led to cell death by its cytotoxic effect. Fluorescence morphological analysis confirmed that early and late apoptosis occurred by nanoparticulate formulation containing CUR and MTX (Figure 2,yellow arrow: early apoptosis; blue arrow: late apoptosis). It was possible to conclude that this formulation showed a synergistic effect between CUR and MTX and can be further used as a novel approach for the lung cancer treatment.



The authors thank to CNPq, CAPES and Fundação Araucária for financial support.

PB

Green tea dietary supplements: disintegration and dissolution testing of commercial products

Gusev PA[1], Andrews KW[1], Savarala S[1], Dang PT[1], Han F[1], Oh L[1], Pehrsson PR[1], Dwyer JT[2], Betz JM[2], Kuszak AJ[2], Costello R[2], Saldanha LG[2]

[1]USDA/ ARS, BHNRC, Nutrient Data Laboratory, Beltsville, MD, [2]National Institutes of Health – Office of Dietary Supplements, Bethesda, MD

The United States Pharmacopeia (USP) develops performance standards designed to detect problems with the release of active ingredients in a variety of dosage forms due to formulation design and manufacturing processes. Meeting these specifications also ensures that different batches of products release active ingredients consistently. In the US, meeting the USP standards is mandatory for drugs but not for dietary supplements (DS). We evaluated whether commercially sold single- and multi-ingredient green tea (GT) DS met the USP general chapter standards for DS disintegration, and whether the single-ingredient DS also passed the USP dissolution tests.

Six units of each DS were individually immersed and agitated in designated solutions. A formulation passed a disintegration test if, after ½ hour, only fragments of coating or capsule shell remained, but not a palpably firm core. Dissolution tests measured the amount of epigallocatechin gallate (EGCG) in the agitation medium. A formulation passed this test if, after 1 hour, > 75% of the EGCG amount present in the capsule or tablet was measured in the solution.

Of 29 single-ingredient products tested in two lots, 16 passed and 12 failed disintegration testing in both lots, and 1 performed inconsistently. Of 36 multi-ingredient products, 21 passed and 10 failed the test in both lots, and 5 exhibited inconsistent performance. Among the single-ingredient products, gelatin capsules

and softgels had higher pass rates than non-gelatin capsules (78% vs. 48%), while among the multi-ingredient products, the pass rates were more similar (92% vs. 77%). Six out of nine immediate release multi-ingredient caplets and tablets failed to disintegrate.

All 22 single-ingredient DS tested for dissolution failed. However, gelatin hard-shell capsules released more EGCG than non-gelatin capsules. We demonstrate significant disparity in performance quality among commercial GT DS. Compliance with the USP or other compendia's standards for DS is currently voluntary, but the wide-spread inadequacies in DS dissolution and disintegration performance raises concerns that DS users may not achieve the health benefits expected from GT DS and jeopardizes confidence in efficacy and safety of DS produced and sold in the US.

Supported by the NIH Office of Dietary Supplements and the USDA Agricultural Research Service

PB

PXR-mediated Induction of CYP Enzymes by Chinese club moss Huperzia serrata

Mona H. Haron[1], Omer Fantoukh[2], Amar Chittiboyina[1], Ikhlas A. Khan[1, 2], and Shabana I. Khan[1, 2]

[1]National Center for Natural Products Research, and [2]Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Huperzia serrata (family Lycopodiaceae, club moss) has been long used in traditional Chinese medicine (TCM) for cognitive and memory enhancement. Huperzine A (a sesquiterpene alkaloid) is considered to be the pharmacologically active constituent of *H. serrata* due to its acetylcholinestrase inhibition activity. In this study we investigated the effects of different concentrations (60, 30, 10 µg/mL) of an ethanolic extract of *H. serrata* and its subfractions on PXR transcriptional activity and subsequent induction of mRNA expression of its target genes (CYP1A2, 3A4 and Pgp) in HepG2 cells. A significant increase in PXR activity (2-4 fold) was observed upon treatment with both ethanolic extract and its ethylacetate fraction and a significant increase in the mRNA expression of CYP1A2 (2-20 fold) and CYP3A4 (2-3.5 fold) was observed with the ethylacetate fraction as determined by qPCR analysis. No significant increase in the mRNA expression of P-gp was detected. Chemical analysis and further fractionation is in progress to identify the constituents responsible for the observed activity of the extract. The activation of PXR by *H. serrata* could pose a concern of affecting the metabolism and transport of co-administered drugs that are the substrates of these CYP enzymes. Further studies are warranted to understand the clinical relevance of these observations and to predict the herb-drug interactions due to increased activity of drug metabolizing enzymes.

This study is supported by the Food and Drug Administration "Science Based Authentication of Dietary Supplements" award number 1U01FD004246-05.

PB

Effect of African potato (Hypoxis hemerocallidea) on PXR and Cytochrome P450 enzymes

Mona H. Haron[1], Olivia R. Dale[1], Fazila Zulfiqar[1], Amar Chittiboyina[1], Ikhlas A. Khan[1, 2], and Shabana I. Khan[1, 2]

[1]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [2]Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

African potato (*Hypoxis hemerocallidea*, AP), indigenous to South Africa, is used traditionally in the treatment of diabetes and high blood pressure. It is also widely used in HIV and cancer patients as an immune booster and anti- inflammatory agent. Several phytochemical constituents of AP are considered to be pharmacologically active such as hypoxoside, acuminoside, sterols, and the stanols. Due to an increased use of alternate therapies in combination with conventional medicine, assessment for the risk of pharmacological drug interaction is necessary. This study was carried out to determine the effects of an alcoholic extract of AP and its butanol and ethylacetate sub-fractions and some isolated compounds (hypoxoside and acuminoside) on the transcriptional activity of PXR and the catalytic activity of CYP enzymes. No induction in the PXR transcriptional activity was observed with the AP extract, sub-fractions or the isolated compounds indicating that AP consumption may not increase the expression of drug metabolizing enzymes and transporters. However the extract and the fractions showed a concentration dependent inhibition of the catalytic activity of CYP 1A2 (up to 85%), 3A4 (up to 95%), 2C19 (up to 91%) and 2D6 (up to 65%). Hypoxoside and acuminoside at 100 µg/ml showed up to 21 and 26% inhibition, respectively, on CYP 3A4 activity but did not show any inhibitory effect on CYP 1A2, 2C19 or 2D6. Taken together the current *in vitro* results are in agreement with the previous reports of CYP inhibition by AP extract. Although hypoxoside and acuminoside alone did not inhibit major CYP isoforms, other constituents present in the AP extract might contribute to the observed CYP inhibition. Chemical analysis of the extract and the sub-fractions is underway to explore further the CYP inhibitory constituents of AP.

This study is supported by the Food and Drug Administration "Science Based Authentication of Dietary Supplements" award number 1U01FD004246-05.

PB

Comparison of Different Cryoprotectants with V-Cryoplate Droplet Vitrification Technique for Cryopreservation of Stevia rebaudiana Bertoni

Lata H[1], Chandra S[1], Uchendu E[1], Khan IA[1,2] and ElSohly MA [1,3]

[1]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA, [2]Division of Pharmacognosy, Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [3]Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, University, MS 38677, USA

This study directly compared the effects of three cryoprotectants with the V-Cryoplate Droplet Vitrification method for effective cryobanking of *Stevia rebaudiana*, a medicinally important plant. Shoot cuttings of 5-6 cm were collected from the field grown plants of *S. rebaudiana* at the University of Mississippi and surface disinfected in 15% commercial bleach with 0.1% Tween 20 for 10 min, rinsed three times with sterile distilled water and also treated with 0.2% mercuric chloride for 3 min. The shoot cuttings were again rinsed three times with sterile distilled water before planting in a multiplication medium. After 4 weeks, shoots

tips (~0.5 mm) with one or two leaf primordia were aseptically dissected and pretreated for 48 hours in MS-agar medium with 0.3 M sucrose and 5% DMSO followed by loading of cells with 2 M glycerol in 0.4 M sucrose MS medium. Cryoprotectants [plant vitrification solutions (PVS) #2, #3 and #4] were initially screened at 25ºC for 10-30 min. Subsequent experiments were based on 15 min exposure duration. Regrowth after a 15 min exposure to PVS2 prior to liquid nitrogen (60%) or PVS4 (61%) were significantly higher than those of other treatments. Regrowth of cryopreserved shoot tips was significantly higher with PVS4 (64%) compared to PVS2 (54%) and PVS3 (3%). These results suggest that the use of PVS4 as a cryoprotectant in the V-Cryoplate Droplet Vitrification technique was most effective for cryopreservation of *Stevia* shoot tips, although PVS2 also produced moderate results. This study provides the first report on these cryoprotectants for cryogenic storage of *Stevia* shoot tips.

This research was partially funded by a grant from the USDA, Agricultural Research Service Specific Cooperative Agreement No. 58-6060-6-015.

PB

Cryopreservation of elite cultivars of Cannabis sativa L. using shoot tips by droplet vitrification

Lata H[1], Uchendu E[1], Chandra S[1], Khan IA[1,2] and ElSohly MA [1,3]

[1]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA[2], Division of Pharmacognosy, Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [3]Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Cannabis sativa L. (marijuana or hemp) is recognized worldwide for its psychoactive properties as well as for fiber production. This study focused on the evaluation of three droplet-vitrification protocols for long term conservation of shoot tips in liquid nitrogen (LN). Shoot tips (~0.5 mm) were excised from 3-4 week old *in vitro*-grown shoots of three cultivars (MX, VI-20 and B-5) and pretreated on 5% DMSO agar plates for 48 hours. The shoot tips were then vitrified in LN using three cryoprotectant (plant vitrification solutions #2, #3, #4) droplets on aluminum cryo-plate. There was no significant difference between the regrowth of cryopreserved shoot tips exposed to PVS2 for 15 min and 20 min but regrowth of all three cultivars significantly declined after 20 min exposure. Exposure duration of 15 min was adapted for subsequent experiments. Regrowth of cryopreserved 'MX' was significantly higher with PVS2 (63%) compared to PVS3 and PVS4 (≤5%). Regrowth of cryopreserved 'V1-20' was highest with PVS2 (57%) and significantly higher than PVS3 and PVS4 (≤ 25%). The regrowth of cryopreserved shoot tips of 'B-5' was significantly different among all three protocols with PVS2>PVS4>PVS3. Both PVS2 and PVS4 produced regrowth above 55% while PVS3 (31%) was significantly lower. These results indicate that 15-20 min exposure to PVS2 is most suitable for cryopreservation of these varieties. This is the first report on protocol development for the cryopreservation of organized tissues of *Cannabis sativa* L. for germplasm conservation.

This work was supported in part by the National Institute on Drug Abuse (NIDA), National Institute of Health (NIH), Department of Health and Human Services, Contract No. NO1DA-15-7793.

PB

Cryopreservation of Cannabis sativa L. using Axillary Buds by V-Cryoplate Droplet Vitrification Technique: The Critical Role of Sucrose Preculture

Lata H[1], Chandra S[1], Uchendu E[1], Khan IA[1,2] and ElSohly MA [1,3]

[1]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA, [2]Division of Pharmacognosy, Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [3]Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, University, MS 38677, USA

The present study aimed to develop an efficient protocol for the cryopreservation of axillary buds of two different screened and selected varieties of *C. sativa* (MX, a high THC and V1-20, a high CBD variety) by the V-cryoplate droplet-vitrification technique. Stem segments (~5 cm in length) with mature axillary buds collected from indoor-grown plants were surface sterilized and then either precultured on MS basal medium with 0.1M sucrose (1st step pre-culture) for 72 hours or non-pre-cultured. All mature axillary buds (~1mm) were aseptically excised from stem segments and pre-cultured for an additional 48 hours on MS basal medium with sucrose (0.3M) and 5% DMSO prior to cryopreservation (2nd step pre-culture). The survival and regrowth rates of cryopreserved axillary buds of cultivar MX following this two-step pre-culture was 45% and 42% respectively while those of cultivar V1-20 was 47 and 44% respectively. A direct pre-culture of axillary buds (2nd step pre-culture) on high sucrose (0.3M sucrose) significantly decreased both the survival and regrowth percentages of axillary buds of cultivar MX (5 and 3% respectively) as well as those of cultivar V1-20 (20 and 17% respectively). These results indicate that two-step sucrose pre-culture or a progressive increase in sucrose pre-culture significantly increased both the survival and regrowth of axillary buds of *C. sativa* cultivars cryopreserved by V-cryoplate droplet-vitrification technique. The resulting plants after cryopreservation appeared normal without any callus formation or morphogenetic variation. This report highlights the role of osmotic stress on recovery of axillary buds of *Cannabis* following cryopreservation and also provides the first protocol for cryopreservation of axillary buds of *Cannabis sativa* cultivars which may be applicable to other important cultivars, plant parts and related medicinal plants.

This work was supported in part by the National Institute on Drug Abuse (NIDA), National Institute of Health (NIH), Department of Health and Human Services, Contract No. NO1DA-15-7793.

PB

Combination of Artemisinin Dimer Oxime and Topotecan for treatment of non-small cell lung cancer in mice

Maqbool MT[1], Abdel-Bakky MS[2], Gul W[3], Elsohly, MA[3] and Ashfaq MK[1]

[1]National Center for Natural Product Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA, [2]Faculty of Pharmacy, Al-Azhar University Cairo, 11884, Egypt, [3]Elsohly Lab Inc, Oxford, MS, 38655, USA

Introduction: Artemisinin dimer oxime - a dimer molecule synthesized from artemisinin possesses high bioavailability and marked *in vitro* anti-cancer activities against tumor-derived cell lines, endothelial cell proliferation, migration and angiogenic processes. Numerous murine models have been developed to study human cancer. The most widely used model is the human tumor xenograft mouse model. This study shows

the chemotherapeutic potential of artemisinin dimer oxime in combination with Topotecan against human tumor cell line NCI-H640 *in vivo*.

Materials and Methods: Human non-small cell cancer cells (NCI H640) grown in tissue culture were injected intrathoracic in nude mice (approximately $5x10^6$ cells). Treatment was initiated 3 days post cancer cell injection and continued for 14 days. Body weights were recorded during the course of the treatment. At the end of the treatment, thoracic cavity (from thoracic vertebrae 1 to 13, T1-T13) was separated and weighed to get a ratio of body weight and thoracic weight as a measure of tumor progression. Animals that died or that lost more than 20% of their body weight were sacrificed and recorded for survivability data.

Results indicate that the combination of Tp (2mg/kg) and Ox (10mg/kg) significantly reduced ($p<0.01$) the thoracic tumor growth compared to untreated and Ox groups. Tp alone also showed significant tumor reduction, but it was less as compared to Tp/Ox ($p<0.05$). Tp and Tp/Ox combination showed improvement in the survivability of animals compared to untreated or Ox alone.

Conclusion: The results show that combination Ox/Tp could provide another option in the treatment of cancer. Similar combinations with other anti-cancer drugs need to be evaluated. These results warrant further investigation to determine the optimal combination doses of Ox and with other known anti-cancer drugs.

The authors thank the vivarium staff of the University of Mississippi for their help in animal care. We also thank Harlan Lab Inc. (now Envigo) for providing mice for these studies.

PB

Chemical composition of essential oil of leaf of Kadsura coccinea (Lem.) A. C. Sm. and its toxicity to the bed bug Cimex lectularius L.

Rehman JU[1], *Wang M*[1], *Wang* W[3], *Chittiboyina AG*[1], *and Khan IA*[1,2]

[1]*National Center for Natural Products Research, The University of Mississippi, University, MS 38677 USA;* [2]*Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677 USA;* [3]*TCM and Ethnomedicine Innovation & Development Laboratory, Sino-Luxemburg TCM Research Center, School of Pharmacy, Hunan University of Chinese Medicine, Changsha, 410208, P. R. China*

Kadsura coccinea (Lem.) A.C.Smith is an evergreen climber with woody stem and widely distributed throughout southwest mainland China. Extracts of this plant is used in traditional Chinese medicine (TCM) for the treatment of various disease, like cancer and dermatosis and as an anodyne to relieve pain while leaves are used to treat eczema. The toxicity of the essential oil from its leaves (EOL) was studied against two strains of bed bug *Cimex lectularius* (Bayonne and Ft. Dix strains). The essential oil was obtained by hydrodistillation and analyzed by GC and GC-MS. β-caryophyllene (27.49%) was the major compound followed by (-)-caryophyllene oxide (5.94%), beta.-himachalene (4.57%) delta.-cadinene(4.34%), α-copaene (3.94%), humulene (3.895) and β-pinene (3.03%). Preliminary screening was performed by topically delivering 1 uL droplet of the treatments dissolved in acetone. The EOL produced 80 and 90% mortality against 5th instar mixed sex bed bugs of Bayonne and Ft. Dix strains, respectively, at both 100 and 50 µg/bug while 64.44% and 68.89% against adults at 100 µg/bug, 24 hr after treatment. Four major compounds viz, β-caryophyllene, (-)-caryophyllene oxide, humulene and β-pinene were selected based on commercial availability. These produced 53.33%, 40.00%, 26.67% and 8.89 % of mortality in Bayonne strain and 55.56%, 53.33%, 42.22% and 24.44% in Ft. Dix strain, respectively, at the dose of 100 µg/adult

bug, 24 hr after the treatment. In dose response test of Deltamethrin (standard) against adult bed bugs, 100% and 67.50% of mortality was recorded at 2.4 ng/bug while no mortality at 0.0375 and 0.075 ng/bug in Ft. Dix and Bayonne strain, respectively, 24 hr after treatment. The four major compounds subjected to fumigation toxicity test did not produce significant mortality in both the strains at 250µg/125mL of air, 24 hr after the treatment.

This research is supported by USDA-Discovery & Development of Natural Products based insect management for medical, veterinary & Urban (58-6066-6-043). The bed bug strains (Bayonne and Ft. Dix) were provided by Dr. Changlu Wang, Department of Entomology, Rutgers University, New Brunswick, NJ.

PB

Anxiolytic Principle from Caesalpinia digyna Rottler Roots

Jitender Singh[1], *Ashwani Kumar*[2] *and Anupam Sharma*[2]

[1]*University Institute of Pharma Sciences, Chandigarh University, Gharuan, Mohali, Punjab-140413, India,*
[2]*University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014, India*

Authors have been reported that antianxiety activity-guided fractionation of ethanol extract of *Caesalpinia digyna* roots yielded an anxiolytic crystalline compound – bergenin. The dose of bergenin was optimized using elevated plus maze (EPM). Bergenin exhibits significant antianxiety activity at 80 mg/kg, po, which is comparable to that of diazepam (2 mg/kg, po). Further, the biomarker was quantified in the plant using TLC-densitometry technique and content of bergenin in *C. digyna* roots was found as 0.589 % w/w. Study on mechanism of action of bergenin revealed that bergenin acts as $GABA_A$ receptor agonist

1. University Grants Commission, New Delhi for funding research (UGC-RFMS) Grant – F.5-94/2007(BSR).
2. Sophisticated Analytical Instrumentation Facility, Central Instrumentation Lab and University Centre for Instrumentation and Microelectronics, Panjab University for spectroscopic analysis.

PB

DNA barcoding of Bay Leaves

Techen N[1], *Parveen I*[1], *Khan IA*[1,2]

[1]*National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, Department of Pharmacognosy, School of Pharmacy, University, Mississippi 38677, United States*
[2]*Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*

Fresh or dried Bay Leaves are used in cooking for their flavor and fragrance. Six different species are considered as Bay Leaves, namely Bay laurel (*Laurus nobilis*, Lauraceae), California Bay Leaf (*Umbellularia californica*, Lauraceae), Indian Bay Leaf (*Cinnamomum tamala*, Lauraceae), Indonesian Bay Leaf (*Syzygium polyanthum*, Myrtaceae), West Indian Bay Leaf (*Pimenta racemosa*, Myrtaceae), and Mexican Bay Leaf (*Litsea glaucescens*, Lauraceae). The FDA only accepts (Laurus nobilis, Lauraceae) and Pimenta racemosa, Myrtaceae as 'Bay Leaf" while *Umbellularia californica* is listed as toxic plant in the FDA Poisonous Plant Database.

As part of an ongoing investigation into the identification of Bay Leaves, we have developed a molecular (DNA) method that can help to identify Bay Leaf species. We have isolated genomic DNA from fresh and dried plant samples and analyzed genomic regions that could be used as mini-barcode helpful to identify/authenticate plant material.

PB

Screening of anti-cell proliferative activity of a collection of Plant Extracts belonging to Annonaceae family and their activity towards NRF-2/ARE pathway

Tran T-T[1], Khan SI [1,2], & Khan IA[1,2]

[1] Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, MS 38677, USA. [2] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, MS 38677, USA.

Annonaceae family or custard apple family has over a hundred genera and thousands of species. This family is distributed worldwide and some species have been used traditionally for medicinal effects. For example, *Annona muricata* is used traditionally to treat arthritis, diarrhea, diabetes, fever, malaria, rheumatism, as well as cancer[1]. The purpose of this investigation is to study the anti-cell proliferative effects of a collection of Annonaceae plant extracts in colon cancer cells (HCT-116 and HT-29) and to determine their effects against inflammation via Nrf-2 activation pathway. Activation of Nrf-2 has been shown to suppress carcinogenesis and inflammatory stress, and Nrf-2 activators such as sulforaphane and curcumin are considered as cytoprotective [2]. A collection of 85 Annonaceae plant extracts (16 genera, 32 species) from the repository of the NCNPR was screened for anti-cell proliferative activity and their effects on the activation of the Nrf-2/ARE pathway. The cell proliferation was determined by a colorimetric method based on tetrazolium salts WST-8 in which viable cells are able to reduce WST-8 salts to water-soluble dye. Reporter gene assay was employed to determine Nrf-2 activation. The results indicated that 26 extracts inhibited cell proliferation of colon cancer cells at the concentration of 50 µg/ml or less. Screening of Nrf-2 activation is in progress. Results will be presented. Further study will be carried out to determine the chemopreventive potential of the selected plant extracts.

1. Moghadamtousi, S. Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. M., & Kadir, H. A. (2015). *Annona muricata* (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities. *International Journal of Molecular Sciences*, *16*(7), 15625–15658.
2. Sporn MB, Liby KT. (2012). NRF2 and cancer: the good, the bad and the importance of context. *Nature reviews Cancer*. 12(8).

Acknowledgment: We would like to thank Ms. Maria Bennett for providing the samples of extracts from the NCNPR repository.

PB

Characterization, *in vitro* and *in vivo* Antimalarial Activities of Triclisia subcordata root and Cycleanine analogues.

Fidelia Ijeoma Uche [ab], Joshua Boating[c] Haddijatou Mbye[a], Imran Ullaha[a], Paul Horrocks[a], Wen-Wu Li[a]

[a] Institute for Science and Technology in Medicine, Keele University, Staffordshire ST5 5BG, United Kingdom; [b] Department of Pharmacognosy, Faculty of Pharmaceutical sciences University of Port Harcourt Rivers state Nigeria; [c]Pharmaceutical Sciences and Formulation Technology University of Greenwich United Kingdom

Triclisia. subcordata Oliv (Fam. Menispermaceae) is used by herbalists in Nigeria to treat malaria. This study aimed to substantiate the use of *Triclisia. subcordata* for malaria treatment in West Africa , to characterize the antimalarial bioactive constituents of this plant and to modify the bioactive component in search of more potency and high selective index bioactive compounds. The evaluation of antimalarial activities of *T. subcordata* was done by *in vitro* and *in vivo* models. While the antimalarial effects of synthetic analogues were investigated *in vitro*. The *in vitro* method was done by Malaria Syber Green 1 Fluorescence assay techniques, to determine both the parasitic antiproliferative effects and 50% inhibitory concentrations (IC_{50}) The *in vivo* antimalarial effects were investigated to evaluate the schizonticidal activity in both early and established infections on mice. Also, the preliminary phytochemical screenings were carried out on crude extracts of this plant, using characteristics colour reactions to identify the presence of bioactive constituents of this plant. The characterization of the bioactive components was investigated by use of spectroscopic data - by analysis of the NMR, GC-MS, LC- MS data. The preliminary results of phytochemical analysis showed the presence of alkaloids, tannins, saponins, flavonoids, cardiac glycosides and terpenes. The results of spectroscopic data analysis revealed the presence of three bisbenzyl isoquinoline alkaloids (BBIQ) - cycleanine, isochondodendrine and 2′-norcocsuline. These alkaloids were demonstrated to exert significant *in vitro* anti-plasmodial activities. The cytotoxicity of the alkaloids was evaluated in normal human ovarian epithelial cells. Cycleanine was shown to exert the highest selective index relative to the other two natural alkaloids. Cycleanine and total alkaloids of this plant were also shown to exhibit schizonticidal activity in both early and established infection $P < 0.05$ (ANOVA) - expressed as in vivo antimalarial activity. GC – MS analysis revealed the presence of other compounds including sterols and some fatty acids. Cycleanine was further modified by semi-synthesis to produce two novel alkaloids which exhibited higher potency and selectivity than cycleanine.This report supports the traditional use of *T. subcordata* root for the treatment of malaria. It is also a novel report of the antimalarial activities of the two novel cycleanine analogues. However, study is ongoing to determine the in vivo antimalarial effects of the cycleanine analogues and to further modify cycleanine structurally by other methods, in search of more improved antimalarial compounds.

PB

Isochondodendrine and 2′-norcocsuline: additional alkaloids from Triclisia subcordata, induced cytotoxicity and apoptosis in ovarian cancer cell lines

Fidelia I. Uche[a,b], Mohammed N. Abed[a], Marwan I. Abdullah[a], Falko P. Drijfhout,[c] James McCullagh,[d] Timothy W.D. Claridge,[d] Alan Richardson[a], Wen-Wu Li[a]*

[a] Institute for Science and Technology in Medicine, Keele University, Stoke-on-Trent, ST4 7QB, United Kingdom; [b] Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria; [c] Chemical Sciences Research Centre, Keele University, Staffordshire, ST5 5BG, United Kingdom; [d]Chemical Research Laboratory, University of Oxford, Oxford, OX1 3TA, United Kingdom

Triclisia subcordata Oliv (Menispermeaceae) is a medicinal plant traditionally used for the treatment of various diseases, including cancer in West Africa. This study aims to isolate minor alkaloids present in this plant and assay their cytotoxic activities. Isochondodendrine and 2′-norcocsuline as two minor alkaloids together with the abundant cycleanine were isolated and identified by mass spectrometry and NMR spectroscopy. Both isochondodendrine and 2′-norcocsuline exhibited *in vitro* cytotoxicity in four ovarian cancer cell lines (A2780, IGROV-1, OVCAR-8, and OVCAR-4) with IC_{50} range of 3.5 - 17 M and 0.8 – 6.2 μM respectively. These alkaloids showed weaker potencies when tested using normal human ovarian epithelial cells, IC_{50} = 10.5 ± 1.2 μM and 8.0 ± 0.2 μM for isochondodendrine and 2′-norcocsuline, respectively. The alkaloids induced apoptosis in ovarian cancer cells because they activated caspases 3/7, induced cleavage of PARP, increased the $subG_1$ population in cell cycle analysis and increased annexin V/propidium iodide staining. These observations suggest that isochondodendrine and 2′-norcocsuline contributing to the cytotoxic activity of *T. subcordata* may be suitable starting points for the future development of novel therapeutics to treat ovarian cancer.

PB

The anti-influenza effects of a four-herb Chinese medicinal prescription

Zhang TB[1], Xiao MJ[2,3], Wong C-K[4], Mok K-P[5], Zhao X[2], Ti HH[2], Shaw P-C[1,6]

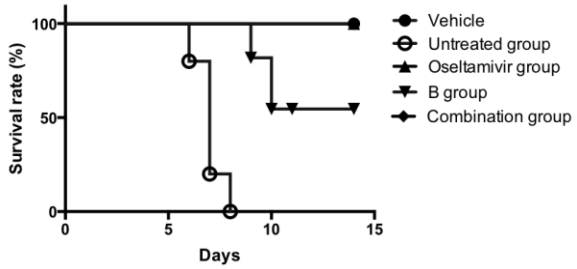
[1] The School of Life Sciences, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong 999077, China, [2] State Key Laboratory of Respiratory Disease, Guangdong Provincial Key Laboratory of Molecular Target & Clinical Pharmacology, School of Pharmaceutical Sciences and The Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou 510632, China, [3] Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Science, Guangzhou 510632, China, [4] Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, N.T., Hong Kong 999077, China, [5] School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pok Fu Lam, Hong Kong 999077, China, [6] Li Dak Sum Yip Yio Chin R & D Centre for Chinese Medicine, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong 999077, China

Many Chinese herbal prescriptions have been used for treating illness over the centuries. A systematic evidence-based and mechanism investigation will promote their popularity in other countries. This study aims to investigate the anti-influenza effect of a classical four-herb prescription and its possible mechanisms.

First, we examined the inhibitory effect of the prescription against different influenza viruses on Madin-Darby canine kidney cells. Second, mice challenged with A/PR/8/34 (H1N1) were orally administrated 1

g/kg/d of extract for seven days and monitored for 14 days. The survival rate, body weight changes, lung index, lung viral load, histopathologic changes and immune-regulation of the mice were measured. Third, the underlying anti-influenza virus mechanism was studied by a series of biological assays.

Results showed that the prescription exerted a broad spectrum of inhibitory effects on influenza A and B strains. The extract also protected 50% of mice from death after infected by influenza virus. The lung index and the lung viral load were significantly reduced. The extract inhibited neuraminidase enzymatic activity to 80% at 2 mg/ml. It up-regulated TNF-α and IFN-α and down-regulated IL-2 in influenza virus induced mice. Thus, the prescription is confirmed to be effective in treating influenza virus infection.



This work was supported by a Theme-based grant (Project No. T11-705/14N) of the Research Grants Council of Hong Kong HKSAR and a grant from the National Natural Science Foundation of China (no. 31401662).

PC

On The Way To The Synthesis Of (-) Mesembrine

Mohamed A. Albadry,[1,2] Sateesh C. Rotte,[2] Pradeep B. Lasonkar,[2] Amar G. Chittiboyina,[2] Ikhlas A. Khan.[1,2]

Mesembrine is a naturally occurring benzylphenethylamine-type alkaloid[1] which has been isolated from the South African plant *Sceletium tortosum,* family Mesembryanthemaceae, known as Kanna. This plant has been used as a stimulant by South African natives to enhance wellbeing. It displayed a potent serotonin reuptake inhibitory activity in the nanomole range with probable usefulness in treatment of depression and anxiety.[2] regardless, many synthetic trials have been published around 40 routes toward mesembrine synthesis,[3] It's still of interest to synthetic chemists due to its thought-provoking chemical features such as a *cis*-3a-aryloctahydroindole moiety with syn configuration at two bridge-head stereogenic centers,[4] some synthetic studies have been published to control the construction of the sterically hindered, benzylic quaternary stereogenic center at C-3a[5]

Our goal here is to design short and efficient synthetic route for (-) mesembrine from an inexpensive starting material 3, 4-Dimethoxycinnamic acid. The simple and efficient route will utilize Michael addition on chiral enolate derived from 3, 4-Dimethoxycinnamic acid to construct the desired stereogenic center at carbon 3a followed by carbene insertion and intramolecular aldol reactions to accomplish the aryloctahydroindole scaffold.

References

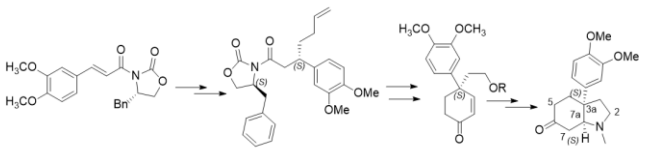
1.Jeffs, P. W.; Archie, W. C.; Hawks, R. L.; Farrier, D. S., Sceletium alkaloids. IV. Biosynthesis of mesembrine and related alkaloids. Amino acid precursors. *Journal of the American Chemical Society* **1971,** 93, (15), 3752-3758.

2.Harvey, A. L.; Young, L. C.; Viljoen, A. M.; Gericke, N. P., Pharmacological actions of the South African medicinal and functional food plant Sceletium tortuosum and its principal alkaloids. *Journal of Ethnopharmacology* **2011,** 137, (3), 1124.

3.Wang, L.-N.; Cui, Q.; Yu, Z.-X., A Concise Total Synthesis of (−)-Mesembrine. *The Journal of Organic Chemistry* **2016,** 81, (21), 10165-10171.

4.Geoghegan, K.; Evans, P., Double Reduction of Cyclic Aromatic Sulfonamides: Synthesis of (+)-Mesembrine and (+)-Mesembranol. *The Journal of Organic Chemistry* **2013,** 78, (7), 3410-3415.

5.Spittler, M.; Lutsenko, K.; Czekelius, C., Total Synthesis of (+)-Mesembrine Applying Asymmetric Gold Catalysis. *The Journal of Organic Chemistry* **2016,** 81, (14), 6100-6105.



PC

Computational Discovery of potential PXR activators from natural sources: The case of anthraquinones

Alhusban M.[1], Ali Z.[2], Khan S.[2], Chittiboyina AG.[2], & Khan IA[1,2]

[1]Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, MS 38677, USA. [2]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences.

The strong growth in herbal supplements consumption is accompanied with the increased potential of herb-drug interaction. In fact, modulation of the Pregnane X Receptor (PXR) is one of the most clinically relevant pharmacokinetic drug interactions, which will put into effect critical downstream events such as modulation of cytochrome P450 enzymes (CYPs) and P-glycoprotein. Among a plethora of secondary metabolites reported to interact with PXR, anthraquinones is one class of compounds involved in such activity. The genus *Bulbine* (Asphodelaceae), which consists of 80 species found in Australia and Africa, is widely used in herbal medicines. Since these herbs are rich in anthraquinones, we are speculating reported adverse effects on liver and kidney function via modulation of xenobiotic mechanisms, specifically activation of PXR. To support our hypothesis, the X-ray crystal structure of PXR was utilized for probing potential ligands from the genus Bulbine with the help of computational tools such as induced fit-docking. Several antharaquinones were identified as potential ligands for PXR, and identified several key ligand-protein interactions. The details of our study, critical interactions in PXR activation and the utility of in-silico predictive models will be presented.

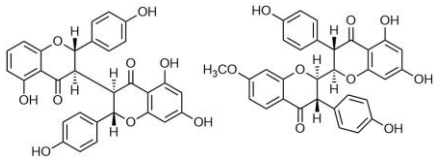
PC

ANTIMALARIAL BIFLAVONONES FROM BRACKENRIDGEA ZANZIBARICA

Issa HH[1], Ali Z[2], Abe N[2], Khan SI[2], Khan IA[2,3]

[1]Department of Natural Sciences, School of Natural and Social Sciences, The State University of Zanzibar, P.O.Box 146, Zanzibar, Tanzania. [2]National Center for Natural Products Research, [3]Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Brackenridgea zanzibarica (Synonym: B. zanguebarica Oliv.) is an endemic species to East Africa and is one among twelve species of the genus Brackenridgea (Ochnaceae), distributed from Africa to Fiji Island. The bark has been traditionally used for the treatment of anaemia, conjunctivitis, ulcers, stomach pains and in snake bite. Two new biflavonones were isolated from the bark of Brackenridgea zanzibarica. The structures were determined using 1D and 2D NMR experiments. The relative configurations have been determined using NOESY experiment and by comparison with NMR data of previously reported biflavonones. The isolated compounds were evaluated for antimalarial activity. Compounds displayed moderate antimalarial activities against chloroquine-sensitive (D6) and/or chloroquine-resistant (W2) Plasmodium falciparum.



PC

Chemical stability and in chemico reactivity of 24 fragrance ingredients of concern for skin sensitization risk assessment

Avonto C[1], Wang M[1], Chittiboyina AG[1], Vukmanovic S[2] & Khan IA[1]

[1]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS, [2]Office of Cosmetics and Colors, Center for Food Safety and Applied Nutrition U.S. Food and Drug Administration, 5001 Campus Drive, College Park, MD 20740, USA

Twenty-four known fragrance ingredients have been identified as potential concern for skin sensitization. Several of these compounds are chemically unstable and convert into reactive species upon exposure to air or light. In the present work, a systematic investigation of the correlation between chemical stability and reactivity has been undertaken. The compounds were subjected to forced photo-degradation for three months and the chemical changes were studied with GC-MS. At the end of the stability study, two-thirds of the samples were found to be unstable. The generation of chemically reactive species was investigated using the *in chemico* HTS-DCYA method. Eleven and fourteen compounds were chemically reactive before and after three months, respectively. A significant increase in reactivity upon degradation was found for isoeugenol, linalool, limonene, lyral, citronellol and geraniol; in the same conditions, the reactivity of hydroxycitronellal decreased. The non-reactive compounds α-isomethyl ionone, benzyl alcohol, amyl cinnamal and farnesol became reactive after photo-oxidative degradation. Overall, forced degradation resulted in four of non-reactive fragrance compounds to display *in chemico* thiol reactivity, while ten out of 24 compounds remained inactive. Chemical degradation thus not necessarily occurs with generation of reactive species. Non-chemical activation may be involved for the 10 stable unreactive compounds.

This research is supported by the Food and Drug Administration "Science Based Authentication of Dietary Supplements" grant number 2U01FD004246.

PC

Non-animal methods for skin sensitization risk assessment. Integrated testing strategies available at the NCNPR.

Avonto C[1],Chittiboyina AG[1], Dale O[1],Khan S[1] & Khan IA[1]

[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677

Skin sensitization is an important toxicological end-point to be assessed for the safety evaluation of chemicals intended for topical use in the formulations of drugs, cosmetics, fragrances and other household products. Skin sensitization is a complex form of immunotoxicity involving numerous molecular pathways which are highly controlled at intra- and inter-cellular levels. For this reason, the development of stand-alone alternatives to animal tests is challenging. The list of standard animal methods includes the Guinea Pig Maximization Test (GPMT), the Buehler's test (BT) and the Local Lymph Node Assay (LLNA).

The need for new, non-animal alternatives has been reinforced by the introduction of new regulations requiring a minimal use of animals for toxicological screenings. In 2012, a new program on the development of Adverse Outcome Pathways (AOP) was launched by the Organisation for Economic Co-operation and Development (OECD). The AOP approach relies on the use of mode-of-action to understand

and predict the potential toxicological effects of chemicals, and on the integration of data from chemical, *in vitro* and computational models for risk assessment. The current vision on skin sensitization AOP identifies four major key events leading to allergic contact dermatitis (ACD) as the clinical outcome caused by exposure to skin sensitizers.

Successfully replacing animal models in skin sensitization risk assessment requires integrated approaches where each key event is assessed independently in targeted non-animal models. Numerous *in chemico*, *in vitro* and *in silico* models have been proposed with the goal to help in replacing, reducing and refining the use of animals in dermatotoxicology. At the present, one *in chemico* method (DPRA) and two *in vitro* methods (KeratinoSens and hCLAT) have been successfully validated and are currently accepted at regulatory levels.

In addition to the three validated methods availble at the UM-NCNPR, two novel *in chemico* methods (HTS-DCYA and NMR-DCYA) have been developed and validated in house.

This research is supported by the Food and Drug Administration "Science Based Authentication of Dietary Supplements" grant number 2U01FD004246. The authors would like to thank the FDA-CFSAN Office of Cosmetics and Colors.

PC

Comparison of Chemical Constituent Profiles of Turmeric (Curcuma longa) Using Different Extraction Processes

Britton ER[1], Sica VP[2], Little J[2], Baker TR[2]

[1]Department of Chemistry and Biochemistry, University of North Carolina Greensboro, NC 27412, United States; [2]Research and Development, Corporate Functions Analytical, The Procter & Gamble Company, Mason Business Center, 8700 Mason-Montgomery Rd, Mason, OH 45040, United States

Curcuma longa, commonly known as turmeric, is a common culinary ingredient that is becoming increasingly popular in the dietary supplement industry due to reports of anti-inflammatory and antioxidant activity. In fact, Nutra Ingredients declared turmeric as the "stand out" botanical in 2015, with annual US sales surpassing $50 million. As companies begin to produce and market turmeric-containing products, it is critical to consider how turmeric raw materials are extracted and processed, such that the desired constituents are present in final products. The chemical constituents from commercial turmeric extracts (ethanolic, aqueous and supercritical CO_2) and in-house extracted raw turmeric root were compared using UHPLC/UV/CAD/HRMS. Specifically, the concentrations of curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) and turmerones (ar-turmerone, α-turmerone and β-turmerone) were used to compare the extracts. Three orthogonal detectors (UV, CAD, and MS) were used to evaluate the samples to best capture the differences of these constituents in the various extracts. While the in-house benchtop prepared extracts contained both compound classes, curcuminoids and turmerones, the commercial extracts were not as comprehensive. Curcuminoids were detected in the aqueous and ethanolic extracts, but were not observed in the CO_2 extract. Alternatively, turmerones were detected in the CO_2 extract, but were not observed in the aqueous and ethanolic extracts. This evaluation suggests that different extraction techniques result in different chemical profiles; therefore, it is critical to determine the desired constituents when selecting an extract for dietary supplement formulation.

PC

Anatomy and Microscopy of Piper caldense, a Folk Medicinal Plant from Brazil

Budel JM[1], Raman V[2], Santos VLP[3], Bobek VB[1], Migacz IP[1], Franco CRC[4], Khan IA[2,5]

[1]Department of Pharmaceutical Science, State University of Ponta Grossa, UEPG, PR 84030-900, Brazil; [2]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA; Department of Cell Biology, Federal University of Paraná, UFPR, PR 81531-900, Brazil; 4 School of Health, Environment, Sustainability and Humanities, Uninter, PR 80021-980, Brazil; [5]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Piper caldense (Piperaceae) is used as a sedative in folk medicine of Brazil. It also has antifungal, antimicrobial and acaricidal properties. The taxonomy of the genus *Piper* is problematic because different species have similar morphologies, making their morphological identification difficult. The present study investigates the anatomical characteristics of the leaves and stems of *P. caldense* by light and scanning electron microscopy in order to provide supporting data for correct identification of the species. The anatomical markers are hypostomatic leaves with a two-layered hypodermis; unicellular pearl glands on the leaf surfaces; flat-convex midrib with about 10 vascular bundles arranged in U-shape; concave-convex petiole with about 12 vascular bundles; circular stem with a continuous ring of sclerenchymatous sheath in the pith; and calcium oxalate sand crystals on the adaxial leaf surface, and raphides in the leaf midrib, petiole and stem.

We acknowledge the Electron Microscopy Center of the LABMU at the State University of Ponta Grossa and the Electron Microscopy Center of the Federal University of Paraná for assistance in SEM analysis.

PC

Foliar Anatomy and Microscopy of Six Species of Baccharis (Asteraceae)

Budel JM[1], Raman V[2], Monteiro LM[1], Almeida VP[1], Bobek VB[1], Heiden G[3], Takeda IJM[4], Khan IA[2,5]

[1]Department of Pharmaceutical Science, State University of Ponta Grossa, UEPG, PR 84030-900, Brazil; [2]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA; [3]Embrapa Clima Temperado, Pelotas, RS 96010-971, Brazil. [4]Department of Environment, State University of Maringá, UEM, PR 87506370, Brazil; [5]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

In Brazil, different species of *Baccharis* are called by the same vernacular name (*vassouras*) and used indiscriminately for the same therapeutic purposes, such as gastroprotective, anti-inflammatory and diuretic. Considering the confusion in the identification of different species of *Baccharis* due to their morphological similarities, the comparative leaf anatomy and micro-morphology of six species namely *B. illinita*, *B. microdonta*, *B. paucifloscul*osa, *B. punctulata*, *B. reticularioides*, and *B. sphenophylla* were investigated by light and scanning electron microscopy. The main distinguishing features as observed during the study are the morphology of the cuticle; type and occurrence of the stomata; presence or absence of glandular trichomes; shape of the flagelliform trichomes; and arrangement of the mesophyll tissues. The findings of the study can be used for species identification as well as quality control of herbal products.

Acknowledgements: JMB is grateful to CAPES (88881.119611/2016-01) and UEPG for financial support and a fellowship as well as the technical support of National Centre for Natural Products Research, The University of Mississippi, MS, USA.

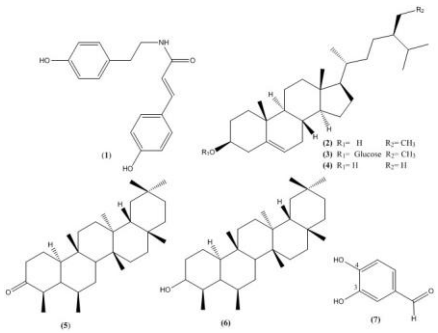
PC

Chemical and Biological Studies of Cannabis sativa Roots

Elhendawy MA[1,2], Wanas AS[1], Radwan MM[1], Ibrahim EA[1,3], Lata H[1], Chandra S[1], Azzaz NA[2], Toson ESA[4]& ElSohly MA[1,5]

[1]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA.[2]Department of Chemistry, Faculty of Agriculture, Damietta University, Damietta, Egypt. Egypt. [3]Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt.[4]Department of Chemistry, Faculty of Science, Damietta University, Damietta, Egypt.[5]Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, MS 38677, USA.

The roots of the cannabis plant have a long history of medical use stretching back millennia. However, the therapeutic potential of cannabis roots has been largely ignored in modern times. Interestingly, cannabis roots are not a significant source of cannabinoids. Therefore, chemical and biological screening of the root extracts were investigated. Chemical study of *Cannabis sativa* roots led to the isolation and identification of seven compounds (**1**-**7**). Their chemical structures were unambiguously established on the basis of 1D and 2D NMR spectroscopy and mass spectrometry as N-(*p*-hydroxy-*b*-phenylethyl)-*p*-hydroxy-*trans*-cinnamamide (**1**), *β*-sitosterol (**2**), *β*-sitosterol-*β*-D-glucoside (**3**), ergost-5-ene-3-ol (**4**), friedelan-3-one (**5**), epifriedelanol (**6**), 3,4 dihydroxybenzaldehyde (**7**), along with other fatty acids and triglycerides. Compound **1** and **4** showed potent antimicrobial activity. Compound **1** was active against *E. Coli* with IC_{50} value of 0.8 µg/ml, while compound **4** was active against *C. neoformans* with IC_{50} value of 13.7 µg/ml. An HPLC method was developed and validated for the detection and quantification of N-(*p*-hydroxy-β-phenylethyl)-*p*-hydroxy-*trans*-cinnamamide (**1**) in extracts of different varieties of *C. sativa* roots.



This research was partially supported by the National Institute on Drug Abuse (N01DA-15-7793) and by the Egyptian and Cultural Bureau, Washington DC, USA.

PC

Secondary Metabolites of Tricholoma caligatum (Viv.) Ricken

Ebru Erol[1,2], Zulfiqar Ali[2], Mehmet Oztürk[1], Mei Wang[2], Ikhlas A. Khan[2-4]

[1] Mugla Sitki Kocman University, Faculty of Science, Department of Chemistry, Mugla 48000, Turkey. [2] National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA. [3] Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677, USA. [4]*Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*

Medicinal mushrooms have an established history of use in traditional oriental therapies. The modern clinical practice in Japan, China, Korea, and other Asian countries has continued to rely on mushroom-derived preparations. Due to their special fragrance and texture, mushrooms have been used for many years in oriental culture as tea and nutritional food as well (Manzi et al., 1999).

In this study, we aimed to investigate secondary metabolities of the *Tricholoma caligatum* which is edible, although fruit bodies are often bitter. It grows in the Mediterranean region fall in Pinus, Abies and Picea forest. (Murata, Ota, Yamada, Yamanaka & Neda, 2013).

The phytochemical investigation of the hexanes, acetone and methanol extracts of *Tricholoma caligatum* (Viv.) Ricken led to isolation of trametenolic acid (**1**), ergosterol (**2**), 5a,6a-epoxy-24-methylcholesta-7,22-dien-3b-ol (**3**), ergosterol peroxide (**4**), lasvicol (**5**), cerebroside (**6**) (Figure 1).

The structures of the isolated compounds were elucidated on the basis of 1D- and 2D-NMR, HR-MS techniques. All the isolated compounds were reported for the first time from this mushroom.

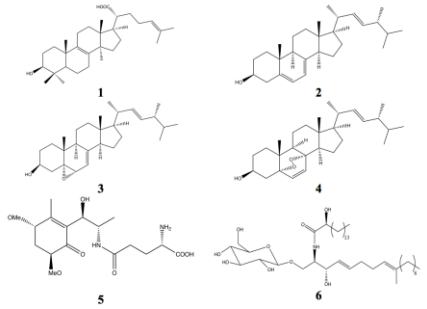


Figure 1: **1**: Trametenolic Acid **2**: Ergosterol **3**: 5α,6α-epoxy-24-methylcholesta-7,22-dien-3β-ol **4**: Ergosterol peroxide **5**: Lasvicol **6**: Cerebroside

Acknowledge: This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK-BIDEB-2214-A).

PC

Phytochemical Study on Tricholoma fracticum (Britzelm.) Kreisel

Ebru Erol[1,2], Zulfiqar Ali[2], Mehmet Oztürk[1], Mei Wang[2], Ikhlas A. Khan[2-4]

[1]Mugla Sitki Kocman University, Faculty of Science, Department of Chemistry, Mugla 48000, Turkey. [2] *National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA.* [3]*Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677, USA.* [4]Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Mushrooms are nutritionally functional foods that have been an integral part of our diet for years. They have not just been consumed for their culinary importance because of their unique taste and flavour (Kalac, 2013) but also because of their potential therapeutic properties which dates back to over 2000 years ago and are recognized as effective to treat and prevent varieties of disorders (Lim et al., 2007; Moro et al., 2012; Silveira et al., 2014).

In this study, two cerebrosides were isolated by recycling preparative HPLC (Figure 1) as a colorless solid. The fatty acid units in cerebrosides were derivatized to their methyl esters and analyzed by GC–MS (Nishimura et al., 2011). Also, three steroids, adenosine, uridine and fumaric acid were isolated by using silica-gel column chromatography from methanol and acetone extract of *Tricholoma fracticum*. The structures were elucidated by using 1D, 2D NMR and HR-MS. All the isolated compounds were reported for the first time from this mushroom.

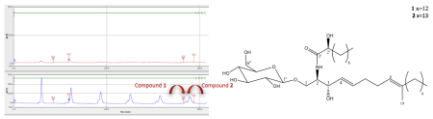


Figure 1. Recycle Preparative HPLC Chromatogram and chemical structure of the Compound 1 and 2

Acknowledge: This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK-BIDEB-2214-A).

PC

Medicinal Importance of Natural Anticancer Pyran Frameworks: An Update

Kumar D[1], Sharma P[1], Singh H[1], Nepali K[1], Gupta GK[2], Jain SK[1], Ntie-Kang F[3,4] & Saini, V[5]

[1]Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, India, [2]Department of Pharmaceutical Chemistry, M.M.College of Pharmacy, Mullana,Ambala, India, [3]Department of Chemistry, Faculty of Science, University of Buea, Buea, Cameroon, [4]Institute for Pharmacy, Martin-Luther-Universität Halle-Wittenberg, Halle, Germany, [5] Vice-Chancellor, Maharishi Markandeshwar University, Solan, India

Pyran is remedially imperative oxygen containing heterocyclic moiety which shows a variety of pharmacological properties. Pyran is also one of the important structural units found in a variety of natural products, such as coumarins, benzopyrans, sugars, flavonoids, and xanthones. The diverse anticancer capabilities of pyrans have been additionally evidenced by a number of recent publications. This review gives an outline of headway made in the field of naturally occurring pyrans as anticancer agents. Along these lines, a framework of the best in class on pyrans and their analogs as anticancer candidates is shown. The discussion will likewise include the structures of the most promising molecules, their biological activities against several human cancer cell lines and mechanistic insights discovered through the pharmacological assessment. The promising activities revealed by these pyran-based scaffolds undoubtedly places them on the front stage for the anti cancer drug discovery.

FNK is currently a Georg Foster fellow of the Alexander von Humboldt Foundation. DK is thankful to UGC, New Delhi for providing research fellowship under UPE (Focus Area-Health Care, Drug Development, and Sports Medicines) Scheme (Sanction No. 25994 /Estt./A-11).

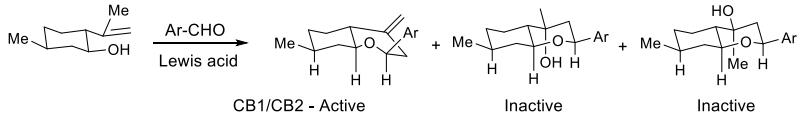
PC

One-step, stereoselective synthesis of octahydrochromenes via the Prins reaction and their cannabinoid activities.

Saqlain Haider[1] , *Amar G. Chittiboyina*[1], *Ikhlas A. Khan*[1,2]

[1]*National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA,* [2]*Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA.*

Novel, functionalized octahydrochromene derivatives were synthesized in a single step *via* the Prins reaction. Enantiomerically pure (+)-isopulegol was reacted with benzaldehyde to stereoselectively yield the corresponding octahydro-2*H*-chromen-4-ol derivative containing five stereocenters. A total of 10 compounds were synthesized by altering the enantiomer of isopulegol and the substituted benzaldehyde, and the resulting enantiopure octahydrochromenes were screened *in vitro* against the cannabinoid receptor, isoforms CB1 and CB2. Compounds containing an olefin at the C4 position [(+)-**3c**, (-)-**3c**, (-)-**7c**, (-)-**9c** and (-)-**11c**] of the octahydrochromene scaffold were found to exhibit reasonable displacement of [^{3}H] CP55,940 from the CB receptors, whereas, the corresponding hydroxy analogs [(+)-**3a**, (+)-**3b**, (-)-**3a**, (-)-**3b** and (+)-**5a**] had very little or no effect.



We would like to thank Prof. Parcher for the editing, proof-reading and valuable suggestions for improving the quality of the manuscript. We are also thankful to Dr. Mei Wang for mass spectroscopy and Ms. Janet A. Lambert for biological screening against cannabinoid receptors. This study was supported in part by United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement 58-6060-6-015. The cannabinoid receptors activity was made possible by Grant Number P20GM104932 from the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health (NIH).

PC

Bio assay-guided isolation and identification of anti-inflammatory compound from Munronia pinnata

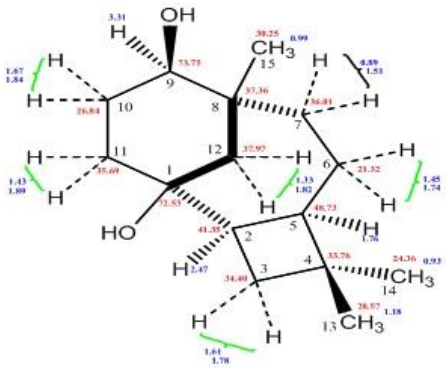
S. D.Hapuarachchi[1], *T.S. Suresh*[2], *W.T.P.S.K. Senarath*[3], *Hadunnetthi SM*[4], *C. Ranasinghe*[5]

[1] *Department of Dravyaguna Vignana, Institute of Indigenous Medicine, University of Colombo, Sri Lanka;*
[2] *Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka;*
[3]*Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka;*
[4]*Institute of Biochemistry, Molecular Biology and Bio Technology, University of Colombo, Sri Lanka;*
[5]*Department of Chemistry, Faculty of Natural Sciences, The Open University of Sri Lanka.*

This study was designed to evaluate the anti-inflammatory effect of methanol extracts of *Munronia pinnata* (MP) in carrageenan-induced, experimental acute inflammatory in Wistar rat model. *M. pinnata* is a highly demanded herb in folklore medicine of Sri Lanka [1]. Experimental procedures and animal care were conducted following World Health Organization guidelines (WHO), 2003 and rules of the Ethics Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka (Ethical clearance

No: 474/09). The crude powder of *M. pinnata* was extracted successively with hexane, chloroform and methanol at room temperature. The methanol extract (MPm) was further partitioned between water and ethyl acetate. Ethyl acetate extract (MPe) was subjected to chromatographic separation using column and thin layer chromatographic techniques. The eluted fractions were screened for bioactivity using carrageenan-induced acute inflammation in Wistar rats. Finally, sub fraction MPe 2-1b (55.0 mg) was identified as senecrassidiol by extensive chromatographic studies. All the tested ethyl acetate fractions of methanol extract were showed a significant inhibitory effect on paw oedema and fraction 2 (MPe 2) exhibited the maximum effect at the 5th hour (67%). Anti-inflammatory effect of the isolated MPe 2-1b (senecrassidiol) also showed the maximum inhibitory effect at the 4th hour (57%). These results of this study provide the scientific rationale for the use of *M. pinnata* as an anti–inflammatory agent in folk medicine.

Key words: *Munronia pinnata*, Anti-inflammatory effect, carrageenan-induced wistar rats, methanol extracts, Ethyl acetate extract, Senecrassidiol.



Acknowledgements and Funding

Financial assistance by University Grant Commission, Sri Lanka – Research Grant -UGC/ICD/045, 2008. This research work was partly supported by The National Center for Advanced Studies in Humanities and Social Sciences (*NCAS*), Sri Lanka.

References:

1. Dassanayake MD, Fosberg FR, Clayton WD. A revised Hand Book to The Flora of Ceylon, 1st ed., Vol ix, Smithonion, Institute and the National Science foundation, Washington DC, Amerind Co.Ltd., New Delhi, 1995, 236-239.

PC

Phytochemical investigation of Mimosa pigra

Mohammed Hawwal[1,2,3], Omer Fantoukh[1,2,3] , Zulfiqar Ali [1], Ikhlas Khan[1,2]

[1]National Center for Natural Products Research, [2]Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677; [3]Department of Pharmacognosy, College of Pharmacy, King Saud University, Alriyadh, Saudi Arabia

Mimosa pigra is native to tropical America from Mexico to Argentina but currently found worldwide. It belongs to the Fabaceae family, which is one of the largest and most important families of flowering plants. *Mimosa pigra* is a leguminus shrub that can reach up to 6 meters in height. It has been used in traditional medicines to treat asthma, diarrhea, typhoid fever, and genitourinary tract infection. Despite its traditional uses in different countries in the world, *M. pigra* was listed in the global invasive species in the database as one of the *One Hundred of the World's Worst Invasive Alien Species*. In 1979, a biological control project was initiated in Australia against *M. pigra,* and this project led to the release of 13 insects and two pathogenic fungi. Unfortunately, both pathogens failed to establish. *M. pigra's* survival ability might come from its unique secondary metabolite constituents. Furthermore, more than 60 species of Southern Brazil were screened for anti-dermatophyte activity and dichloromethane fraction of methanolic extract of *M. pigra* showed lowest MIC values (1.9 µg/mL) without DNA damage at 10 and 50 µg/mL of cell viability of human leukocytes. The present work is aimed at isolating interesting secondary metabolites that have promising antifungal activity with safe human toxicity profile. More than five natural compounds have been isolated and identified so far. Future work will focus on isolating more chemical compounds and evaluating their antifungal activity. Also, the toxicity profile of all isolated compound will be evaluated in *vitro* assays.

PC

Antimicrobial Activity of Chloroform Extract of Albizia odoratissima bark Against Human Pathogens

Sonia Kohli[1], Dinesh Kumar[1], Dr. Sukhbir L. Khokra[1] and Dr. Dhirender Kaushik[1]

[1] Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana - 136119

During the last two decades, various medicinal plants have been studied for their possible antimicrobial activity to discover new antimicrobial agents capable of resolving problems such as the development of drug resistance in pathogenic microorganisms as well as the side effects of some present antibiotics. In this study, the antibacterial activity of chloroform extract of *Albizia odoratissima* bark was investigated by *Agar well diffusion method*. The extract showed antibacterial activity against both Gram positive bacteria with zone of inhibition ranging from 4mm to 19mm and Gram negative bacteria with zone of inhibition ranging from 10mm to 21mm (*Klebsella pneumoniae*) as well as 19mm to 21mm (*Eischeria coli*). Maximum zone of inhibition of 21mm at 30µg /ml concentration was observed against *Klebsella pneumoniae* and *E. coli*. Therefore, chloroform extract of bark showed good activity against Gram-positive as well as Gram-negative bacteria.

PC

Study of antifertility potential of chloroform fraction of L. camara Linn. root and isolated compounds

Kumar D[1], *Kumar A*[1] *& Prakash O*[2],

[1]*Pharmacognosy and Phytochemistry Division, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136119, Haryana (India);* [2]*Deptartment of Chemistry, Kurukshetra University, Kurukshetra-136119, Haryana (India)*

Lantana camara Linn. (Verbenaceae) known as Sage [English] and Raimunia [Hindi] is a terrestrial, evergreen hedge shrub commonly found in tropical, sub tropical and temperate parts of the world including India. The roots of the plants have been traditionally used as oral contraceptives by the women in South Africa, but to date no scientific evidence is reported to justify the claimed use of this plant part in fertility regulation. So, the present study was carried out to study the effect of *L. camara* L. root chloroform fraction (LCC) on implantation and estrogen level in female rats at the doses of 100 & 200 mg/kg body weight respectively. The LCC showed estrogenic effect at both the doses when administered alone, but anti-estrogenic effect at a higher dose (200 mg/kg) when administered alongwith 17α-ethinylestradiol. Three main compounds oleanolic acid, stigmasterol and lantadene A were isolated from the chloroform fraction, purified and characterized by spectral techniques. The antiimplantation effects of chloroform fraction of roots of this plant observed in present study might be due to the estrogenic as well as antiestrogenic potential of the fraction which in turn might be due to presence of one or more of the isolated compounds.

Conclusion: The results of the study indicate antifertility potential of chloroform fraction of *L. camara* Linn. root along with three main compounds oleanolic acid, stigmasterol and lantadene A.

The authors express sincere thanks to UGC, New Delhi for financial support [F.No. 39-955/2010 (SR); dated 12/01/2011] and Professor A. C. Rana, Dean & Director, Institute of Pharmaceutical Sciences, K.U., Kurukshetra for providing necessary facilities for the study.

PC

Isolation, Identification, and Synthesis of Antifungal Chalcone Derivatives from Oxytropis viscida

Suresh G[1], *Jacob MR*[1], *Khan SI*[1], *Wang M*[1], *Khan IA*[1,2] *&* *NCNPR*[1,2]

[1]*National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA.* [2]*Division of Pharmacognosy, Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA.*

Oxytropis is a genus of plants in the legume family, consisting of about 350 species worldwide. *O. viscida* was first discoverd in 1938 and has an alpine habitat in western North America. More than 100 chemical constituents have been isolated from several species of this genus, including chalcones, flavonones, isoflavones, isoflavanones, dihydroflavones, alkaloids, saponins, and lignans. In the course of our discovery of natural product-derived antifungal compounds, a column fraction from the methanol extract of the leaves of *O. viscida* was determined to show strong *in vitro* antifungal actiivty against *Cryptococcus neoformans*. A dereplication analysis of this column fraction by LC-MS indicated the presence of multipe compounds that had close molecular weights (*m/z* 239-254) but distinctly different UV absorptions. Subsequent isolation and purificaiton led to the identification of 2',4'-dihydroxychalcone as the most active compound

against *C. neoformans*, along with other chemotypes of amides and flavonoids that showed little antifungal activity. Using the active chalcone compound as a template, a series of derivatives were synthesized and evaluated for their antifungal, lipometabolic, and cytotoxic activities. Compounds with peroxisome proliferator-activated receptor-alpha activity have been identified.

This research is supported by the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No. 58-6408-1-603.

PC

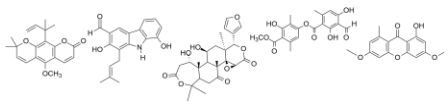
Evaluation of Antioxidant Capacities and Chemical Constituents of Clausena excavata Burm. f.

Pei Cee Lim[1,2], *Zulfiqar Ali*[2], *Nur Kartinee Kassim*[1], *Ikhlas A. Khan*[2,3]

[1]*Department of Chemistry, Faculty of Science, University Putra Malaysia, 43400, Malaysia,* [2]*National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA,* [3]*Division of Pharmacognosy, Department of Biomolecular Science, School of Pharmacy, University of Mississippi, University, MS 38677, USA*

Clausena excavata is widely distributed in Malaysia, Indonesia and Thailand. The leaves are taken raw among the Malays as 'ulam' which means salad. Leaves and stem of *C. excavata* are used in folk medicine for treatment of disorders such as colic, cough, headache, rhinitis, sores, wounds, fever, and detoxification. This plant has been reported to possess various biological properties including anti-inflammatory, antimicrobial, antioxidant, and analgesic properties [1]. In this study, powdered stem bark of *C. excavata* was extracted successively using hexanes, ethyl acetate, ethanol and methanol. Total phenolic contents (TPC) and antioxidant capacities namely 2,2Ê¹-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, oxygen radical absorbance capacity (ORAC) and copper reducing antioxidant capacity assay (CUPRAC) of the extracts were evaluated. Ethanol extract showed highest TPC value as well as antioxidant capacities in DPPH and CUPRAC whereas methanol extract showed highest ORAC value. *Clausena* from the Rutaceae family are known to be an abundant source for secondary metabolites especially carbazole alkaloids and coumarins [2]. Phytochemical investigation of the extracts led to isolation of four coumarins, three carbazole alkaloids, limonoid, xanthone and polyketide. Structure elucidation was achieved by 1D and 2D NMR experiments, LC-MS and DI-MS.

References: [1] Albaayit, S. F. A., Abba, Y., Rasedee, A., & Abdullah, N. (2015). Effect of *Clausena excavata* Burm. f. (Rutaceae) leaf extract on wound healing and antioxidant activity in rats. *Drug Design, Development and Therapy*, *9*, 3507–3518. [2] Auranwiwat, C., Laphookhieo, S., Trisuwan, K., Pyne, S. G. & Ritthiwigrom, T. (2014). Carbazole alkaloids and coumarins from the roots of *Clausena guillauminii*. *Phytochemistry Letters*, 9 113-116.



PC

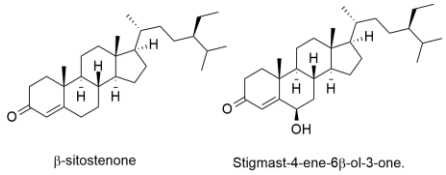
Inhibitory Effect on Streptococcus mutans and Chemical Constituents of Melicope latifolia Leaves Extracts

Pei Cee Lim[1,2], *Zulfiqar Ali*[2], *Nur Kartinee Kassim*[1], *Ikhlas A. Khan*[2,3]

[1]*Department of Chemistry, Faculty of Science, University Putra Malaysia, 43400, Malaysia,* [2]*National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA,* [3]*Division of Pharmacognosy, Department of Biomolecular Science, School of Pharmacy, University of Mississippi, University, MS 38677, USA*

Melicope latifolia, known as Kisampang and Pepau, is distributed in Malaysia, Indonesia and Papua New Guinea. Traditionally, *M. latifolia* leaves are used as remedy for fever and cramps [1]. *Melicope* was reported to possess antiviral [2], anti-inflammatory [3] and antioxidant activities [4]. However, to date, there are limited reports on chemical constituents and biological potential of this species. Therefore, this study is to evaluate the inhibitory activity of *M. latifolia* extracts on *Streptococcus mutans* as well as to investigate the phytochemical constituents from the active extracts. Powdered dried leaves of *M. latifolia* were successively extracted with hexanes, ethyl acetate and methanol. The extracts were subjected to minimum inhibitory concentration (MIC) evaluation on *S. mutans*. Hexanes possess the lowest MIC. Phytochemical studies on the hexanes extracts yielded three compounds which identified as β-sitostenone, phthalic acid isobutyl ester and stigmast-4-ene-6β-ol-3-one. These three compounds were isolated for the first time from this species. The compounds may be potential antimicrobial candidates.

References: [1] Goh, S. H., Chung, V. C., Sha, C. K., & Mak, T. C. W. (1990). *Phytochemistry*, *29*, 1704–1706. [2] Wahyuni, T. S., Tumewu, L., Permanasari, A. A., Apriani, E., Adianti, M., Rahman, A., Hotta, H. (2013). *Virology Journal*, *10*(1), 259. [3] Abas, F., Shaari, K., Israf, D. A., Syafri, S., Zainal, Z., & Lajis, N. H. (2010). *Journal of Food Composition and Analysis*, *23*, 107–112. [4] Kassim, N. K., Rahmani, M., Ismail, A., Sukari, M. A., Ee, G. C. L., Nasir, N. M., & Awang, K. (2013). *Food Chemistry*, *139*(1-4), 87–92.



PC

Chemical and Biological Evaluations of Asimina triloba

Taghreed Majrashi[1,2,3], Zulfiqar Ali[1], Ikhlas A. Khan[1,2]

[1]National Center for Natural Products Research, [2]Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [3]College of Pharmacy, King Khalid University, Abha, Saudi Arabia

Pawpaw, *Asimina triloba*, is native to the eastern United States, and it is mainly distributed in temperate forests. It is a tree species belonging to the Annonaceae family, and it has been reported to contain potent bioactive compounds called acetogenins and a few alkaloids. Its extract is a complex mixture of more than 50 acetogenins that were isolated, identified, and attributed to treatment options for antitumor, antimalarial, anthelmintic, piscicidal, antiviral, and antimicrobial activities. The dietary supplements containing standardized pawpaw twig extracts are consumed as a safe complementary medicine with chemotherapy to treat different types of cancer. However, several epidemiological reports have associated consumption of the fruits and tea leaves of plants from the same family, *Annona muricata* (soursop or graviola), with neurotoxicity including, atypical Parkinsonism. The neurotoxicity of *Annona muricata* is linked to some acetogenins and isoquinoline alkaloids. On the other hand, neurotoxicity has not been reported in people who consume pawpaw fruits or dietary supplements. Although, pawpaw contains chemical constituents similar to those found in graviola. To understand the chemistry and biological differences between the two plants, further studies are needed to identify and isolate the chemical compounds present in each plant and evaluate their biological activities. This research is focused on the isolation and structure determination of alkaloids from *A. triloba* twigs, using different techniques (1D & 2D NMR, HR-ESI-MS, and automatic polarimetry). It also aims to determine whether alkaloids from this plant can cause the neuronal degeneration or can reduce cancer cells proliferation, using cell viability assays (MTS and WST-8). Accordingly, this study has the following goals: isolation of alkaloids from *A. triloba* twigs, structure elucidation, and determination of cytotoxicity and neurotoxicity of the crude extracts and the pure compounds.

PC

A Novel synthesis, Characterization and Antibacterial Effect of Plant-Mediated Silver Nanoparticles using Centaurea cyanus L. by Microwave Irradiation

Shahab Ojani[1,*]

[1,*] *Ph.D Student & Young Researcher and Elite Club, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.*

Nowadays, metal nanoparticles may act both as reducing agents by plant extracts and stabilizing agents in the synthesis of nanoparticles due to their specific chemical and optical properties and are currently used in many high technology areas, such as the medical sector for imaging, faster diagnosis, drug delivery, tissue regeneration, cancer therapeutics, bactericidal and fungicidal agents and antioxidants, as well as the development of new therapeutics [1-3]. In this study silver nanoparticles were synthesized using Centaurea cyanus L. flowers extract as a reducing agent by a microwave irradiation method. The advantage of using microwave irradiation is it takes less time to reduce the silver ions. The synthesized silver nanoparticles were characterized using various instrumental techniques including UV-visible (UV–Vis) spectroscopy, Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and transmission electron microscopy (TEM). Later, the antibacterial activity of the synthesized silver nanoparticles was tested using both gram positive as well as gram negative bacteria i.e. Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922), respectively. The UV–Vis spectra gave surface plasmon resonance (SPR) for synthesized silver nanoparticles at 435 nm. FT-IR spectroscopy revealed that silver nanoparticles were functionalized with biomolecules that have primary amine groups ($-NH^2$), carbonyl group, -OH groups and other stabilizing functional groups. The XRD pattern showed the characteristic Bragg peaks of (111), (200), (220) and (311) facets of the face center cubic (FCC) silver nanoparticles and confirmed that these nanoparticles are crystalline in nature. Also, the silver nanoparticles showed spherical structure and their sizes ranged from 15-50 nm under TEM. The production silver nanoparticles exhibited good antibacterial potential against gram positive and gram negative bacterial strains. Thus, the silver nanoparticles produced by this green synthesis is able to use medical technologies.

We Gratefully Acknowledge the Financial Support from the Research Council of Tonekabon Branch Islamic Azad University.

References: [1] Varahalarao V, Kaladhar DSVGK. (2014) Middle-East J Sci Res, 19(6): 834-842. [2] Pourshamsian K, Ojani S (2016) Planta Medica, 82: 05, PC 62. [3] Kavitha KS, Baker S, Rakshith D, Kavitha HU, Rao HCY, et al. (2013) Int Res J Biological Sci, 2(6): 66-76.

PC

Preliminary Phytochemical: Extraction, Screening and Identification of Bioactive Compounds from Polylophium involucratum (Pall.) Boiss. Extract

Shahab Ojani[1,*]

[1,*] *Ph.D Student & Young Researcher and Elite Club, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.*

Phytochemicals are responsible for medicinal activity of plants. These are non-nutritive chemicals that have protected humans from various diseases. The major constituents consists of phenolic compounds, flavonoids, saponins, alkaloids, cardiac glycosides and terpenoids. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries the constituents play a significant role in the identification of crude drugs [1-2]. The objective of this research was extraction, screening and identification of bioactive compounds from seeds of *Polylophium involucratum* (Pall.) Boiss. in the petroleum ether extract of *Polylophium involucratum* (Pall.) Boiss., a family of *Apiaceae* and seeds of *Polylophium involucratum* (Pall.) Boiss. were collected from the highlands of Javaherdeh (N 36° 52′, E 50° 28′; at ~ 2500 m altitude) Ramsar - Iran, in July 2013 and extract prepared from petroleum ether by maceration method in for 24 hrs. The initial phytochemical experiments may chemical constituents in the plant material, inducing their quantitative estimation and locating the origin of pharmacologically active chemical compound. Qualitative analysis of phytochemical compounds like (di-terpenes, quinones, cardiac glycosides, phenols, coumarins, saponins, flavonoids, terpenoids, tannins, and phlobatannins) were examined in the petroleum ether extract of seeds of *Polylophium involucratum* (Pall.) Boiss. employing standard procedures. In petroleum ether extract of seeds of *Polylophium involucratum* (Pall.) Boiss. Seven tests were positive. In present study, we have found that most of the biologically active phytochemicals were present in the petroleum ether, extract of seeds of *Polylophium involucratum* (Pall.) Boiss. This research has provided insight on the use of secondary metabolites in traditional medicine in maintaining proper human health.

We Gratefully Acknowledge the Financial Support from the Research Council of Tonekabon Branch Islamic Azad University.

References: [1] Pourshamsian K, Ojani S (2016) Planta Medica, 82: 05, PC 62. [2] Peteros NP, Mylene MU (2010) J Med Plant Res, 4: 407-414.

PC

Phytosynthesis of Silver Nanoparticles Using Combination of Allium paradoxum & Origanum vulgare L. & Investigation of its antibacterial properties

Shahab Ojani[1,*]

[1,*] *Ph.D Student & Young Researcher and Elite Club, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.*

Biomolecules present in plant extract can be used to reduce metal ions to nanoparticles in a single step green synthesis process. Synthesis mediated by plant extracts is environmentally benign. Nowadays, nanoparticles of metals have been extensively studied for their potential applications in catalysis, biological labeling, biosensing, drug delivery, antibacterial and antiviral activity and detection of genetic disorders, gene therapy and gene sequencing [1-5]. The aim of this study was phytosynthesis of silver nanoparticles using combination of *Allium paradoxum* and *Origanum vulgare* L. leaves of aqueous extract and investigation of its antibacterial properties. In present study, silver nanoparticles were characterized by UV–Vis absorption spectroscopy with an intense surface plasmon resonance (SPR) band at 445 nm which reveals the formation of nanoparticles. Fourier transmission infrared spectroscopy (FTIR) showed that nanoparticles were capped with plant compounds. Transmission electron microscopy (TEM) showed silver nanoparticles, with a size of 10 nm, were spherical. The X-ray diffraction spectrum (XRD) pattern clearly indicates that silver nanoparticles formed in the present synthesis were crystalline in nature. Later, the silver nanoparticles biosynthesized by combination of *Allium paradoxum* and *Origanum vulgare* L. leaves of aqueous extract showed antibacterial activity against microorganisms *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25619), (Gram Negative) and *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 12711) (Gram Positive) by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods. Also, the synthesized silver nanoparticles exhibited good antibacterial potential against gram positive and gram negative bacterial strains. Furthermore, in the present study the silver nanoparticle of *Allium paradoxum* and *Origanum vulgare* L. were found to be a powerful antibacterial agent and this study can be continued for their structural elucidation and pharmacological activity.

We gratefully acknowledge the financial support from the research council of Tonekabon Branch Islamic Azad University.

References: [1] Iravani S. (2011) Green Chem,13(10):2638-50. [2] Jain KK. (2010) BMC Med, 13(8):83-94. [3] Pourshamsian K, Ojani S (2016) Planta Medica, 82: 05, PC 62. [4] Suri S, Fenniri H, Singh B. (2007) J Occuo Med Toxical, 2(16):1-6. [5] Vankar PS, Shukla D. (2012) Appl Nanosci, 2(2):163-8.

PC

Phytochemical and Biological Evaluation Methanolic Extract of Leaves of Buxus hyrcana

Shahab Ojani[1,*]

[1,*] *Ph.D Student & Young Researcher and Elite Club, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.*

The phytochemicals of the plants are chemical compounds formed during the normal metabolic processes. These chemicals are often referred to as "secondary metabolites". Secondary metabolites are not essential for growth and tend to be strain specific. They have a wide range of chemical structures and biological activities [1-4]. The aims of this study were to investigate the preliminary phytochemical screening, antioxidant activity and antibacterial activity of methanolic extract of leaves of *Buxus hyrcana* belonging to family *Buxaceae*. The dried leaves of *Buxus hyrcana* were collected and subjected to successive extraction by microwave assisted extraction (MAE) method. The present study for phytochemical screening method of phytoconstitute by Trease and Evans, Sofowara and Harbone were followed. Whereas, the total phenolic contents (TPC) and total flavonoid contents (TFC) of the extracts were then measured using Folin Ciocalteu Reagent (FCR) and aluminum chloride colorimetric methods, respectively. And total antioxidant activity was assayed by DPPH free radical scavenging assay method. Later, the antibacterial activity of the leaves of *Buxus hyrcana* was tested using both gram positive as well as gram negative bacteria i.e. *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), respectively. The methanolic extract of leaves of *Buxus hyrcana* contains (alkaloids, flavonoids, terpenoids, phenols, tannins, coumarins, saponins, cardiac glycosides, tri-terpenoids and steroids). Methanolic extract of leaves of *Buxus hyrcana* showed total phenolic contents of (42.51±0.12) mg GAE/g dry plant material respectively. Total flavonoid contents of methanolic extract of leaves of *Buxus hyrcana* was (18.30±0.05) mg QE/g dry plant material, respectively.The antioxidant activity of the investigated methanolic extract of leaves of *Buxus hyrcana* was scavenging ability of DPPH radical scavenging activity (84.65%). Whereas, the IC_{50} of methanolic extract of leaves of *Buxus hyrcana* for DPPH assay was (1.36±0.15) mg/ml respectively. The methanolic extract of leaves of *Buxus hyrcana* showed significant antibacterial activity against both (Gram positive) and (Gram negative) bacteria. Thus, the methanolic extract of leaves of *Buxus hyrcana* would be helpful for the preparation of pharmaceutically useful drugs to destroy pathogenic microbes.

Acknowledgements: We gratefully acknowledge the financial support from the research council of Tonekabon Branch Islamic Azad University.

References: [1] Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Aravindhan P, et al. (2009) Pakisthan Journal of Nutrition, 8: 83-85. [2] Krishnaiah D, Sarbatly R, Bono A (2007) Biotechnol Mol Biol Rev, 1: 97-104. [3] Nostro A, Germanò MP, D'angelo V, Marino A, Cannatelli MA (2000) Lett Appl Microbiol, 30: 379-384. [4] Pourshamsian K, Ojani S (2016) Planta Medica, 82: 05, PC 62.

PC

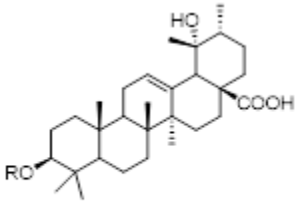
Chemical Constituents from the aerial parts of Vangueria agrestis

Ahmed Galal Osman[1], *Zulfiqar Ali*[1], *Omer Fantoukh*[1], *and Ikhlas A. Khan*[1]

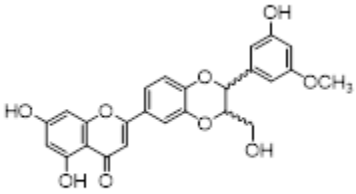
National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS-38677

Vangueria agrestis (Schweinf. Ex Hiem) Lantz, formerly *Fadogia agrestis*, is a traditional herb commonly used in the African traditional medicine for its curative properties. The principal utility of the title herb is treatment of erectile dysfunction and imparting aphrodisiac momentum [1]. In addition, the stem bark of *V. agrestis* showed analgesic and anti-inflammatory properties [2]. It also exhibited a sedative effect comparable to that of acetyl salicylic acid [2].

Herein, we report the isolation and characterization of two ursane-type triterpenoid glycosides, namely 3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranosyl pomolic acid (**1**) and 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl pomolic acid (**2**), one flavolignan (**3**), together with six flavone glycosides, namely narcissin, rutin, hyperoside, quajavarin, 3-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranosyl kaempferol, and 3-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranosyl kaempferol. The identity of each compound was confirmed primarily by 1D and 2D NMR analysis. To the best of our knowledge this is the first report on the isolation of the aforementioned compounds from the genus *Vangueria*.



1 R = -Ara(2→1)Rha
2 R = -Xyl(2→1)Rha



3

References

[1] Yakubu, M.T., Akanji, M.A., Oladiji, A.T., 2005. Asian J Androl 7, 399-404.

[2] Oyekunle, O.A., Okojie, A.K., Udoh, U.S., 2010. Neurophysiology 42, 124-129.

This research is supported in part by "Science Based Authentication of Dietary Supplements" funded by the Food and Drug Administration grant number 1U01FD004246-05.

PC

Secondary Metabolites of Tinospora sinensis and Tinospora crispa

Abidah Parveen[1,3], *Zulfiqar Ali*[2], *Omer Fantoukh*[1], *Yalda Shokoohinia*[2,4], *Yan-Hong Wang*[2], *Vijayasankar Raman*[2] , *Ikhlas A. Khan*[1,2]

[1]*Department of Biomolecular Sciences, Division of Pharmacognosy,* [2]*National Center For Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA,* [3]*Abbottabad University of Science & Technology, Havelian, Abbottabad District, Pakistan,* [4]*Department of Pharmacognosy & Biotechnology, Kermanshah University of Medical Sciences, Kermanshah, Iran.*

Tinospora genus belongs to the family Menispermaceae and consists of 34 species distributed in the tropical and subtropical parts of the world. *Tinospora crispa and Tinospora sinensis* are two medicinally important species that are used in the Chinese, Ayurvedic and Unani systems of traditional medicine since ancient times. The plants are deciduous climbers distributed in South Asia and Southeast Asia. *Tinospora crispa* Miers ex Hook.f. & Thomson is found in Thailand, Vietnam, Indonesia, Malaysia, Philippines, Bangladesh and India. The stems are used in diabetes, rheumatism, malaria, as anti-inflammatory and in various skin conditions. *Tinospora sinensis* (Lour.) Merr., also known as Chinese *Tinospora* is used for the treatment of general debility, diabetes, urinary, skin and liver diseases. The secondary metabolites of *Tinospora sinensis* and *Tinospora crispa* were isolated by repeated column chromatography over normal phase silica, Sephadex LH-20, reverse phase C-18. The phytochemical investigation of the stems of *T. sinensis* yielded cycloeucalenone, and cycloeucalenol, in addition to β- sitosterol and stigmasterol. Sugars and fatty acids constituted bulk of the extract. The stems of *T. crispa* yielded borapetoside B, C and F together with several other compounds. Structures of isolated compounds were elucidated by spectroscopic methods, including 1D and 2D NMR experiments and HRESIMS.

This research is supported by “Science Based Authentication of Dietary Supplements” funded by the Food and Drug Administration grant number 2U01FD004246-06 and Fulbright Graduate Scholarship Program and the United states Educational Foundation of Pakistan (USEFP) are acknowledged for financial support.

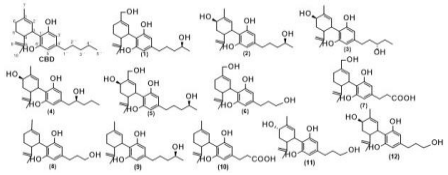
PC

Microbial metabolism of cannabidiol (CBD) from Cannabis sativa

RadwanMM[1], Ahmed SA[2], Ibrahim AK[2], Slade D[1], Khan IA [1,3] & ElSohly MA[1,4]

[1]National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA. [2]Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia, 41522, Egypt. [3]Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [4]Department of Pharmaceutics, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Microbial metabolism of cannabidiol (CBD), with *Mucor ramannianus* (ATCC 9628) yielded 7,4"β-dihydroxycannabidiol (**1**), 6β,4"β-dihydroxycannabidiol (**2**), 6β,3" α -dihydroxycannabidiol (**3**), 6β,2"β -dihydroxycannabidiol (**4**) and 6β,7,4"β-trihydroxycannabidiol (**5**). *Beauveria bassiana (*ATCC 7195) metabolized CBD to afford six metabolites identified as 7,3"-dihydroxycannabidivarin (**6**), 7-hydroxycannabidivarin-3"-carboxylic acid (**7**), 3"-hydroxycannabdiivarin (**8**), 4"β-hydroxycannabidiol (**9**), cannabidivarin-3"-carboxylic acid (**10**), along with compound 1. Incubation of CBD with *Absidia glauca* (22752) yielded three metabolites, 6α,3"-dihyroxycannabidivarin (**11**), 6β,3"-dihyroxycannabidivarin (**12**), and compound 5. All compounds were evaluated for their antimicrobial and antiprotozoal activity.



This research funded in part by the National Institute on Drug Abuse (Contract # N01DA-15-7793).

PC

New Prenylated Isoflavonoid from Limonium Leptophyllum

*Dizamatova A[1], Zhumanova K[1], Zhusupova G[1], Zhussupova A[1], Srivedavyasasri R[2], Ibrahim MA[2] & Ross SA[2,3,]**

[1]Faculty of Chemistry and Chemical Technology, Al-Farabi Kazakh National University, 71 al-Farabi Avenue, Almaty, 050040 Republic of Kazakhstan.[2]National Center for Natural Products Research, [3]Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

Limonium is a genus in Plumbaginaceae consisting of 120 flowering plant species. Species are also known as statice, caspia, sea-lavender, or marsh-rosemary. The genus is widely distributed all over in Asia, Africa, Australia, Europe and North America. *Limonium leptophyllum* (Schrenk) O. Kuntze was collected in August 2016, from Kyzylorda region, Kazakhstan. Crude methanolic extract of aerial parts of *L. leptophyllum* exhibited moderate activity towards CB2 receptor (53.6 % displacement). Further fractionation and purification of the crude extract yielded one new and six known compounds. Using NMR and HR MS data the new compound is identified to be a rare pentacyclic isofavanoid including a five membered dihydrofuran ring between C-5 and C-6. Known compounds are identified as euchrenone b_9,

auriculasin, kaempferol, avicularoside, myricetin-3-arabinoside and β-sitosterol. Auriculasin showed activity towards CB1 receptor (86.7% displacement with IC_{50} 8.923μM).

The project was supported by National Center for Natural Product Research, USA. We acknowledge Award Number P20GM104932 from the National Institute of General Medical Sciences for bioassay results.

PC

ANTIPROTOZOAL AGENTS FROM PILIOSTIGMA THONNINGII, MEDICINAL PLANT GROWN IN NIGERIA

*Afolayan M[1,2,3], Srivedavyasasri R[1], Asekun OT[2], Familoni OB[2], Orishadipe A[3], Ibrahim MA[1] & Ross SA[1,4,]**

[1]National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA. [2]Department of Chemistry, University of Lagos, Lagos, Nigeria. [3]Chemistry Advanced Research Center, Sheda Science and Technology Complex, PMB 186, Garki-Abuja, Nigeria. [4]Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

Leishmaniasis is one of the world's most neglected diseases, caused by a protozoa parasite from over 20 *Leishmania* species and is transmitted to humans by the bite of infected female phlebotomine sandflies. *Piliostigma thonningii*, (Milne-Redhead, Fabaceae) is used for various medicinal purposes in African countries. The decoction of the leaves and bark is used for the treatment of ulcers, wounds, heart pain, arthritis, malaria, pyrexia, leprosy, sore throat, diarrhea, toothache, gingivitis, cough and bronchitis. Phytochemical investigation of *P. thonningii* yielded two new: 2*β*-methoxyclovan-9*α*-ol (**I**), methyl-*ent*-3*β*-hydroxylabd-8(17)-en-15-oate (**II**), and fourteen known compounds which were identified by their NMR, MS and GC-MS spectral analyses as: clovane-2*β*,9*α*-diol (**III**), alepterolic acid (**IV**), anticopalic acid (**V**), (3*S*,5*R*,6*S*,7*E*)-3,5,6-trihydroxy-7-megastigmen-9-one (**VI**), *β*-amyrin (**VII**), vitamin E (**VIII**), piliostigmin (**IX**), (+)-epicatechin (**X**), quercetin (**XI**), quercitrin (**XII**), afzelin (**XIII**), 3-hexenyl-1-O-*β*-D-glucopyranoside (**XIV**), stigmasterol, and *β*-sitosterol glucoside. Compounds **I**, and **IV** selectively showed activity towards leishmanial pathogen *Trypanosoma brucei* with IC_{50} 7.89, 3.42 μM, respectively. The results suggested that hydroxyl group at C-2 is probably responsible for the difference in activity in sesquiterpenes **I** and **III**. Similarly the presence of hydroxyl group at C-3 in labdane diterpenes probably responsible for strong activity towards *T. brucei*. Compound **II**, showed activity towards leishmanial pathogens *T. brucei and Leishmania donovani* Amastigote with IC_{50} 3.84, 7.82 μM, respectively. This is the first report of antileshmanial compounds from this plant.

The project was supported by Sheda Science and Technology Complex, Nigeria and National Center for Natural Product Research, USA. We acknowledge Award Number P20GM104932 from the National Institute of General Medical Sciences for bioassay results.

PC

Cardiocrinum seeds contain novel antitussive phytochemicals

Jia-Wen SHOU[1], Hoi-Yan WU[2], Ren-Wang JIANG[3] Pang-Chui SHAW[4]

[1,2,4]School of Life Sciences, Institute of Chinese Medicine and LDS YYC R & D Centre for Chinese Medicine, The Chinese University of Hong Kong, Hong Kong, China; [2,4]Institute of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong, China; [3]Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou, China; [1]shoujiawen@163.com; [4] pcshaw@cuhk.edu.hk

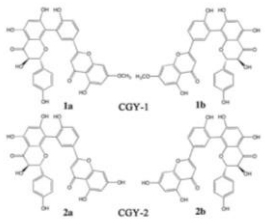
Background: Cough is a world-wide concern and the discovery of new, safe and effective antitussive agents is important. Natural products from Chinese herbal medicine are promising sources for antitussive drugs. Seeds of *Cardiocrinum giganteum* var. *yunnanense*, also known as Doulingzi, have been used as a folk antitussive herb to treat chronic bronchitis and pertussis. The active antitussive phytochemicals in *C. giganteum* seeds are not known.

Aim: Thist work aims at isolating the active phytochemicals in *C. giganteum* seeds and confirming their antitussive effects.

Materials and Methods: Petroleum ether, ethyl acetate, butanol and water were used to separate the methanol extract into different fractions. Active chemicals were isolated their structures identified. Antitussive effects were evaluated with the cough frequency of guinea pigs exposed to citric acid. Electrical stimulation of the superior laryngeal nerve in guinea pigs was performed to differentiate the acting site of the antitussives.

Results: It was shown, among all the fractions, the N-butanol fraction had the strongest effect to inhibit cough induced by inhalation of citric acid in guinea pigs. Two racemic biflavonoids (CGY-1 and CGY-2) were isolated from the N-butanol fraction. CGY-1 was identified as (S)-2″R,3″R- and (R)-2″S,3″S-dihydro-3″-hydroxyamentoflavone-7- methyl ether, which are new compounds and are isolated for the first time from the *C. giganteum* seeds. Racemic CGY-2 was identified as (S)-2″R,3″R- and (R)-2″S,3″S-dihydro-3″-hydroxyamentoflavone. Both CGY-1 and CGY-2 could significantly inhibit cough induced by inhalation of citric acid. Further, they acted on the peripheral reflex pathway to inhibit cough.

Conclusion: Chemicals isolated from *C. giganteum* seeds showed good antitussive effects. The data provide scientific evidence to support the use of *C. giganteum* seeds as an antitussive medicine.



Acknowledgements: This work was supported by the Health and Medical Research Fund of Hong Kong (Ref: 12130851). J.-W. Shou was supported by the Hong Kong Ph.D. Fellowship Scheme.

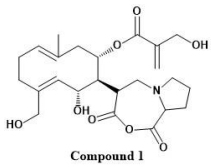
PC

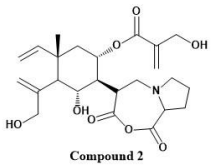
New Sesquiterpene Alkaloids from *Onopordum alexandrinum* Boiss.

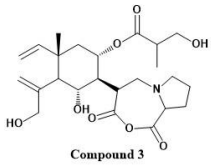
Sachiko Sugimoto,[1] Yoshi Yamano,[1] Amira S. Wanas,[2,3] Samar yehia Desoukey,[3] Hideaki Otsuka,[4] Katsuyoshi Matsunami[1]

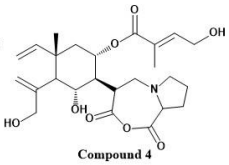
[1]Department of Pharmacognosy, Graduate School of Biomedical and Health Sciences, Hiroshima University, Japan, [2]National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA, [3]Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia 61519, Egypt, [4]Department of Natural Products Chemistry, Faculty of Pharmacy, Yasuda Women's University, Japan

Onopordum is a genus from the family Asteraceae and *O. alexandrinum* that is distributed in Egypt, Palestine and Jordan. Phytochemical studies of this plant have reported the isolation of several steroids, flavonoids and its glycosides. Further investigation on the constituents of the aerial parts of this plant has led to the isolation of four new sesquiterpene alkaloids (**1**-**4**) and one new flavonoid glycoside. Biosynthesis of this new alkaloid skeleton are hypothesized to be germacrane or elemane combined with proline. The structures of the new compounds were elucidated on the basis of spectral and physicochemical evidence.









Acknowledgments: This work was supported in part by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Japan Society for the Promotion of Science (no. 17K15465).

PC

Tujia ethnomedicine Xuetong suppresses the onset and progression of adjuvant-induced arthritis in rats

Yu HH[1,2], Qiu YX[1], Li B[1], Peng CY[1], Jian YQ[1], Cai X[2,3], Zeng R[1*], Wang W[1*]*

[1]TCM and Ethnomedicine Innovation & Development Laboratory, Sino-Luxemburg TCM Research Center, School of Pharmacy, Hunan University of Chinese Medicine, Changsha 410208, People's Republic of China; [2]Hunan Provincial Key Laboratory of Diagnostic and Therapeutic Research in Chinese Medicine, Hunan University of Chinese Medicine, Changsha, Hunan 410208, China; [3]Guangdong Pharmaceutical University, Guangzhou, Guangdong 510006, People's Republic of China;

Kadsura heteroclita (Roxb) Craib (Schizandraceae) is a medicinal plant termed Xuetong in Chinese Tujia ethnomedicine. Xuetong has long been used for the prevention and treatment of rheumatic and arthritic diseases, especially in southern China. The HPLC analysis has shown that the ethanol extract of Xuetong contains lignans and triterpenoids. Our previous studies have shown that KHS exhibits very favorable safety profile and potent anti-inflammatory and analgesic activities.In the present study, we investigated anti-arthritic effects and possible mechanisms of Xuetong on adjuvant-induced arthritis (AIA) in rats. AIA was established in male Sprague-Dawley (SD) rats as described previously, and animals were daily treated by gavage with Xuetong ethanol extract (1.0 g/kg) or vehicle (0.3% CMC-Na) throughout a 30-day experiment[1]. The incidence and severity of arthritis were evaluated using clinical parameters. On day 30, bone destruction of the arthritic joints were assessed by computed tomography (CT) and histopathological analyses. The serum levels of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 were measured by ELISA. Treatment with 1.0 g/kg Xuetong significantly inhibited the onset and progression of AIA. The vehicle-treated rats all developed severe arthritis, while the incidence of AIA in the Xuetong -treated rats was as low as 55% (P=0.035). The Xuetong -treated rats exhibited 1.8- to 2.3-fold reduction of paw swelling, and gained 10 to 20% more body weight than the vehicle-treated AIA rats throughout the experiment. CT and histopathological examinations revealed that Xuetong markedly protected AIA rats from cartilage and bone destruction of joints. Moreover, the serum levels of TNF-α, IL-1β, and IL-6 were significantly decreased in the Xuetong-treated rats than the vehicle-treated AIA rats. These data strongly support the clinical use of Xuetong for rheumatic and arthritic diseases, and suggest that Xuetong is a valuable candidate for further investigation to be a new anti-arthritic drug with favorable safety profile.

This work was supported by the National Natural Science Foundation of China (81673579), Key Project of Scientific Research, Hunan Provincial Department of Education(17A157), Hunan Province TCM Key Subject Open Funded Project (zy201503) and Students Research Innovative Program of Hunan Province(2015-206).

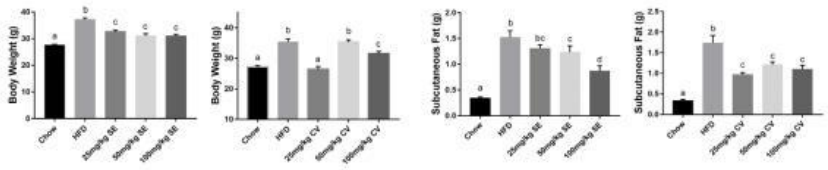
PC

Two Compounds from Tujia Ethnomedicine, Schisanlactone E and Chikusetsusaponin V as Potential Anti-obesity Agents

Qiu YX[1], Yu HH[2], Zeng R[2], Lei XN[2], Liu TB[2], Wang AB[2*],Peng CY[1], Li B[1], Jian YQ[1], Wang W[1*]

[1]TCM and Ethnomedicine Innovation & Development Laboratory, Sino-Luxemburg TCM Research Center, School of Pharmacy, Hunan University of Chinese Medicine, Changsha 410208, PR China; [2]The Key Laboratory of Animal Vaccine & Protein Engineering, College of Veterinary Medicine, Hunan Agricultural University, Changsha 410128, PR China

Tujia ethnomedicine is an important part of the national medicine system in China, and possesses its own unique theories and methods for the treatment and prevention of disease. Two compounds, Schisanlactone E (SE) and Chikusetsusaponin V (CV) are extracted from commonly used Tujia Ethnomedicine *Kadasura heteroclita* Craib. and *Panax japonicus* C.A. Meyer respectively. Intragastrical administration of SE and CV significantly alleviates HFD-induced increase in body, heart and liver weight, epididymal and subcutaneous fat accumulation. The effects of SE and CV on body weight and obesity parameters were not due to reduced food consumption.



This work was supported in part by National Natural Science Foundation of China (81673579 and 81703819), Key Scientific Research Project of Hunan Education Department (17A157), Hunan Provincial Key Laboratory of Dong Medicine (No: 002) and Hunan Key Laboratory of Druggability and Preparation Modification for Traditional Chinese Medicine (No: 004).

PC

Nortriterpenoids and triterpenoids from the stem of Kadsura heteroclita

Cao L[1,2], Li B[1], Zhu XQ[2], Mujeeb-ur-Rahman[3], Choudhary MI[3], Atta-ur-Rahman[3], Wang W[1], Liao DF[1*]*

[1] TCM and Ethnomedicine Innovation & Development Laboratory, Sino-Luxemburg TCM Research Center, School of Pharmacy, Hunan University of Chinese Medicine, Changsha 410208, People's Republic of China; [2]Institute of agriculture environment and agro ecology, Hunan academy of agriculture sciences, Changsha 410125, People's Republic of China; [3]International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.

Kadsura heteroclita (Roxb.) Craib is a climbing species distributed in the southwest part of China. The stem and root of this plant are a kind of "tujia" ethnomedicine called "Xue tong" in local name, traditionally used to treat rheumatism and traumatic injury by the minority people living in the mountain area of southwest China. Phytochemistry research revealed that the compounds from genus Kadsura[1-3] displayed anti-inflammatory, anti-oxidant, anti-tumor activities. Which partly interpret the substance of the traditionally usage. The phytochemistry studies of Kadsura reported mainly on the fractions of chloroform, acetone and ethyl acetate. In this work, we isolated the n-butanol fraction of 95% ethanol extraction produced from stem of Kadsura heteroclita. And for the first time we isolated three new nortriterpenoids (Fig.1 **1-3**) and nine new triterpenoids (Fig.1 **4-12**) from this plant. The structures of the new compounds were established on the ^{1}H, ^{13}C, and 2D NMR spectra and X-ray diffraction analysis. Compounds **1, 2, 7, 8, 9, 10** were tested for cytotoxicity against Hela and HepG-2 human tumor cells by the MTT methods with concentration in 2.5, 5, 10, 15, 20 μM. All compounds showed no inhibitory activity against the tumor cells used and had IC_{50} values of >20 μM.

References: [1] Gao XM, et al. (2008) J. Nat. Prod. 71: 1182-1188. [2] Huang SX, et al.(2007). Chem. Eur. J. 13: 4816–4822. [3] Shi YM, et al. (2014) Tetrahedron 70: 859-868.

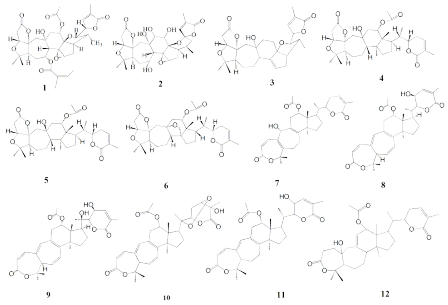


Fig. 1 compounds isolated from Kadsura heteroclite

This work was supported in part by National Natural Science Foundation of China (81673579 and 81703819) and Hunan Natural Science Foundation (2016JJ6062 and 2016JJ6118).

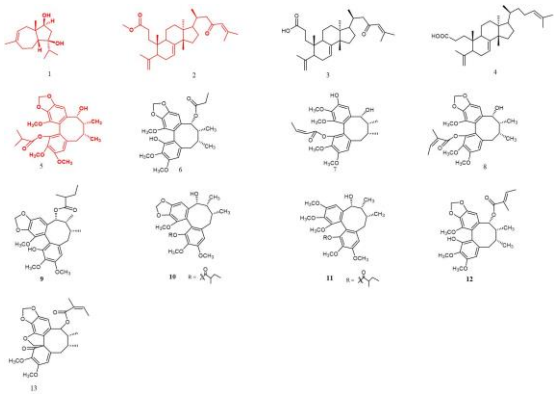
PC

Phytochemical and Biological Evaluation of Kadsura coccinea -- Tujia ethnomedicine“Heilaohu”

Liu YB[1], Yang YP[1], Yuan HW[1], Li MJ[1], Li B[1], Jian YQ[1], Peng CY[1], Wang W[1]*

[1]TCM and Ethnomedicine Innovation & Development Laboratory, Sino-Luxemburg TCM Research Center, School of Pharmacy, Hunan University of Chinese Medicine, Changsha 410208, People’s Republic of China

Phytochemical investigation on ethyl acetate extract from the roots of *Kadsura coccinea* (Heilaohu) led to one new sesquiterpenoid (**1**) and one new triterpenoid (**2**) together with two known lanostane-type triterpenoids (**3-4**), and one new lignan (**5**) with seven dibenzocyclooctadiene lignans (**6-12**) along with one spirobenzofuranoid-dibenzocyclooctadiene (**13**) have been isolated from this extract. Among all compounds, three of them (**6,7,13**) were the first time elucidated from this species. The elucidation of structures were established by extensive spectroscopic analyses and chemical methods. Further bioassay studies would be designed for all pure compounds, including anti-inflammatory, anti-leishmanial, and urease inhibition activity. Anti-malarial and anti- microbial biological activities would be test for all new compounds.



This work was supported by Administration of Traditional Chinese Medicine of Hunan Province (201673 and 201714), College graduate research and innovation projects of Hunan Province (CX2016B362),and the State Key Subject of TCM diagnostics in Hunan University of Chinese Medicine (2015ZYZD06).

PC

Determination of Alkaloids and Phenols in the Husk Products of Areca catechu L. Using HPLC-UV and UHPLC-MS/MS

Yuan HW [1], Cao MR [1], Yi P[1], Zeng T[1], Jian YQ[1], Li B[1], Peng CY[1], Wang W[1]*

[1]TCM and Ethnomedicine Innovation & Development Laboratory, Sino-Pakistan TCM and Ethnomedicine Research Center, School of Pharmacy, Hunan University of Chinese Medicine, Changsha 410208, People's Republic of China.

Areca catechu L. is widely consumed as the fourth most addictive stimulant after caffeine, nicotine, and alcohol [1]. Four characteristic areca alkaloids including guvacoline, arecoline, guvacine, and arecaidine were implicated in both the abuse liability and carcinogenicity of Betel nut (the seeds of Areca catechu) chewing [2]. No investigation on the levels of areca alkaloids in the chewable husk products in Hunan province of China (including *Qingguo* and *Yanguo*) has been conducted. In addition, no information on the levels of major effective phenols in products containing *Areca catechu* was available. In our present study, a robust and accurate method of HPLC-UV, combined with UHPLC-ESI-MS/MS, was developed for quantitative analysis of 5 alkaloids and 8 phenols in the products of *Areca catechu* for the first time. The result revealed substantial variations in the levels of areca alkaloids among *Qingguo*, *Yanguo*, raw husk, and the seeds products. The traditional processing methods for *Qingguo* and *Yanguo* can lower the levels of areca alkaloids in these products and thus reduce adverse effects. The husk products were found to be considerably high in two phenols including (+)-catechin and p-hydroxybenzoic acid among the analytes. Moreover, principle component analysis and random forest were applied to the contents data of 13 analytes in the products.

References: [1] Peng W, Liu YJ, et al. (2015) J Ethnopharmacol, 164: 340-356. [2] Jain V, Garg A, et al. (2017) J Agr Food Chem, 65 (9): 1977-1983.

This work was supported National Natural Science Foundation of China (81673579), Students Research Innovative Program of Hunan Province(2015-206) and Administration of Traditional Chinese Medicine of Hunan Province (201673 and 201714).

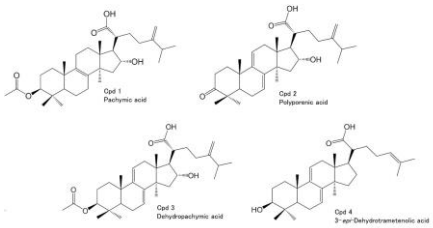
PC

Isolation and mechanism analysis of anti-obesity substances from *Wolfiporia extensa*.

Yoshi Yamano, Moemi Shiga, Sachiko Sugimoto, Toru Hosoi, Koichiro Ozawa, Katsuyoshi Matsunami

Department of Pharmacognosy, Graduate School of Biomedical and Health Sciences, Hiroshima University, Japan

Obesity is an ever-increasing health concern of global importance causing many complications including, high blood pressure, heart diseases and diabetes. In order to explore anti-obesity substances from traditional medicines, we examined anti-obesity activity of several extracts from many herbal medicines by using nematoda *Caenorhabditis elegans*. As a result, we found an extract that has the highest activity and isolated four triterpenes, pachymic acid, polyporenic acid, dehydropachymic acid, 3-epi-dehydrotrametenolic acid from *Wolfiporia extensa*. Further investigation of anti-obesity capability of the extract and isolated compounds, led us to conduct animal tests by using mice in reducing fat amount. In order to determine the mechanism of action of pachymic acid, the effect of pachymic acid on mutant strains of *C. elegans* and the examination of mRNA amounts by RT-PCR were studied. In conclusion, pachymic acid probably activates SKN-1/Nrf2 transcription factor.



Acknowledgments: This work was supported in part by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Japan Society for the Promotion of Science (no. 16K18896).

PC

Diarylpentanoid Glucosides from Hypoxis Hemerocallidea (African Potato)

Zulfiqar F[1], Ross SA[1,2], Ali Z[1], Khan IA[1,2]

[1]National Center for Natural Products Research, [2]Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA

The corms of *Hypoxis hemeroclladia* have been used as a traditional medicine for centuries in South Africa for the treatment of flu, common cold, diabetes, hypertension, testicular tumors, psoriasis, urinary infections, prostate hypertrophy, cancer, HIV/AIDS, and central nervous system disorders. A number of norlignanas were isolated from the hydroalcoholic extract of the corms of *Hypoxis hemeroclladia*. The isolates possess diarylpentanoid carbon skeleton (C_6-C_5-C_6), which were generated by coupling of two phenyl propanoid (C_6-C_3) units. Structure elucidation was achieved by means of NMR spectroscopic and mass spectrometric techniques.