17th Annual OXFORD ICSB

April 3rd - 6th 2017

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

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THE UNIVERSITY of MISSISSIPPI
NATIONAL CENTER FOR NATURAL PRODUCTS RESEARCH

April 3, 2017

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 "17th 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 Khan

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PA

HPLC Determination of Phenolics from Different Organs of Calligonum Polygonoides

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An HPLC method was developed for simultaneous determination of ten phenolics in different organs of *Calligonum polygonoides* L. subsp. *Comosum*. The results showed that flavonol glycosides are higher in content compared to the aglycones in all organs. Reverse phase chromatographic analyses was carried out with a ZORBAX eclipse plus C-8 column (21.2 x 250 mm i.d, 7 µm particle size). Flower is the richest organ in flavonols (kaempferol [1], quercetin [2], quercitrin [3], astragalin [4], isoquercitrin [5], kaempferol-3-*O*-glucuronide [6], and quercetin-3-*O*-glucuronide [7]) followed by fruits. Furthermore; leaves, stems and bark are rich in taxifolin and catechin.

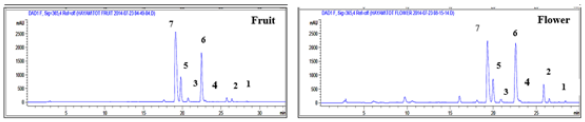

Figure 1: HPLC chromatograms of the fruits and flowers of *Calligonum polygonoides*: kaempferol [1], quercetin [2], quercitrin [3], astragalin [4], isoquercitrin [5], kaempferol-3-*O*-glucuronide [6], and quercetin-3-*O*-glucuronide [7].

The authors want to express their gratitude to the financial support of Beni-Suef University, Beni-Suef, Egypt.

PA

Determination and Characterization of Capsaicinoids and Carotenoids using Ultra-High Performance Liquid Chromatography-PDA-Mass Spectrometry

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Red pepper pod (*Capsicum annum* L.) is commonly used as a vegetable and for imparting of flavor, aroma and color to food. A rapid and sensitive ultra-high performance liquid chromatography-diode array-mass spectrometry method has been developed for the determination of capsaicinoids (capsaicin, dihydrocapsaicin, N-vanillylnonamide) and carotenoids (b-cryptoxanthin, zeaxanthin and b-carotene) present in the oleoresin of *Capsicum annum* fruits. Capsaicin, and dihydrocapsaicin are the main potent capsaicinoids of capsicum oleoresins responsible for the spicy sensation (pungency) while the red color is due to the presence of carotenoids. LC-PDA-MS may provide a method for the rapid screening and structural characterization of bioactive constituents of the capsicum oleoresin. The developed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy and precision. The

limits of detection were found to be 0.01 µg/mL for both capsaicinoids and carotenoids using LC-UV method. LC-mass spectrometry with electrospray ionization (ESI) was used for the identification and confirmation of compounds.

This research is supported by OmniActive Health Technologies, Morristown, NJ07950.

PA

Chemical profiling of Fadogia agrestis using ultra-high performance liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry

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Fadogia agrestis Schweinf. Ex Hiern (known as Black aphrodisiac), family-Rubiaceae is a shrub that is 1-3 feet tall. Ethnomedicinal survey of *F. agrestis* revealed various uses such as aphrodisiac in tropical African countries, antiplasmodial and antimicrobial activities and an aqueous extract of *F. agrestis* stem used to manage complications arising from diabetes. The plant stem has been reported to contain saponins, alkaloids while flavonoids, and anthraquinones were present in small amounts. In this current study, the structural characteristics of chemical constituents in methanolic extracts from dried stem, leaf, root of *F. agrestis* and dietary supplements have been identified and analyzed using UHPLC/QTOF-MS in both negative and positive ion modes. The fragmentation patterns of reference standards were determined and the compounds in the extracts were identified or tentatively characterized from their retention times and mass spectra. The fragments produced by collision induced dissociation (CID) revealed the characteristic cleavage of glycosidic bonds, and the fragmentation pattern provided structural information about the sugars. Fifteen chemical constituents, including nine standard compounds, were detected in the stem, leaf and root of *F. agrestis*. These compounds can be used to determine the presence or absence of *F. agrestis* in commercial products. The analytical method also provided an alternative, fast method for quality control of *Fadogia* in dietary supplements.

This research is supported in part by "Science Based Authentication of Botanical Ingredients" funded by the Food and Drug Administration grant number 2U01FD004246-06, the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No. 58-6060-6-015.

PA

CHEMICAL ANALYSIS OF ANABOLIC STEROIDS FROM BODYBUILDING DIETARY SUPPLEMENTS THAT HAVE BEEN IMPLICATED IN HEPATOTOXICITY

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Herbal dietary supplements (HDS) are widely used, especially for weight loss, bodybuilding and sexual enhancement. There is growing evidence to show that some HDS are capable of causing hepatotoxicity. Forty products categorized as bodybuilding were analyzed for the presence of anabolic steroids using ultra-high performance liquid chromatography-QToF-MS in full scan and targeted MS/MS modes with accurate mass measurement. The compounds were identified in both positive and negative ion modes. Of the 40 products tested, 19 contained steroids including anabolic steroids; eight of these contained labeled and unlabeled steroids; nine of these contained steroids that were different to those indicated on the packaging; two of these contained steroids that were listed on the packaging and 21 products did not contain any steroids. 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. Several of these products contained steroids that may be considered to have considerable pharmacological activity based on their chemical structures and the amounts present which could expose the users to significant risk including liver damage, stroke, kidney failure and death. These dietary supplements that have been implicated in hepatotoxicity through the National Institutes of Health sponsored US Drug Induced Liver Injury Network. In addition, the development of accurate mass time-of-flight mass spectrometer has enabled the calculation of an empirical formula from the molecular ion. Compound structures need to be confirmed with authentic standards, or further verified as far as possible by MS-MS fragmentation patterns together with any other structural information.

This research is supported in part by "Chemical Analysis of Herbal and Dietary Supplements (HDS); Identification of Candidate Hepatotoxins" funded by the National Institute of Diabetes and Digestive and Kidney Diseases, NIH grant number 3U01DK083027-09S2.

PA

Metabolic Profiling of Hoodia, Chamomile, Terminalia Species using Ultra-High Performance-Quadrupole Time of Flight-Mass Spectrometry

Bharathi Avula[1], Yan-Hong Wang[1], Giorgis Isaac[2], Jimmy Yuk[2], Mark Wrona[2], Kate Yu[2] and Ikhlas A. Khan[1,3]

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Ultra-High Performance-Quadrupole Time of Flight Mass Spectrometry (UHPLC-QToF-MS) profiling has become an important tool for identification of marker compounds and generation of metabolic patterns that could be interrogated using chemometric modeling software. Chemometric approaches can be used to analyze small molecule profiles present in plant materials or extracts and their commercial preparations. In this study, UHPLC-QToF-MS was used to generate comprehensive fingerprints of three botanicals (*Hoodia*, *Terminalia* and chamomile) each having different classes of compounds. Detection of a broad range of ions was carried out in full scan mode in both positive and negative mode over *m/z* 100-1500 range using high resolution mass spectrometry. Multivariate statistical analysis was used as a tool to extract relevant chemical information from the data to easily differentiate between *Terminalia* species, chamomile varieties, and quality control of *Hoodia* products. The UHPLC-QToF-MS based chemical fingerprinting with PCA was able to correctly distinguish botanicals and their commercial products. This work can be used as a basis to assure the quality of botanicals and commercial products.

This research is supported in part by "Science Based Authentication of Botanical Ingredients" funded by the Food and Drug Administration grant number 2U01FD004246-06, the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No. 58-6060-6-015.

PA

Pharmaceutical doses of the banned stimulant methylsynephrine (oxilofrine) found in dietary supplements sold in the USA

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Oxilofrine is a pharmaceutical stimulant developed in the 1930s to stimulate the heart and increase blood pressure. It has never been approved for use in the United States (USA), but several athletes have claimed that they inadvertently consumed oxilofrine from sports supplements. Oxilofrine-containing supplements have also been linked to serious adverse events. We designed our study to determine the presence and quantity of oxilofrine in dietary supplements labeled as containing methylsynephrine sold in the USA. A validated ultra-high performance liquid chromatography-quadrupole time of flight-mass spectrometry method was developed for the identification and quantification of oxilofrine. The separation was achieved using a reversed phase column, mass spectrometry detection, and a water/acetonitrile gradient as the mobile phase. The presence of oxilofrine was confirmed using a reference standard. We analyzed 27 brands of supplements and found that oxilofrine was present in 14 different brands (51.9%) at dosages ranging from 0.0003 to 74.8 mg per individual serving. Of the supplements containing oxilofrine, 42.9% (6/14) contained pharmaceutical or greater dosages of oxilofrine. Following instructions on the label, consumers could ingest

as much as 245.6 mg of oxilofrine per day. Only one supplement brand (3.7%) listed an accurate quantity of oxilofrine on the label.

PA

Quantification of Anthraquinones from Bulbine natalensis and Dietary Supplements using UHPLC-PDA-MS

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Bulbine natalensis Baker, family Asphodelaceae is widely distributed in South Africa and traditionally used as an aphrodisiac and skin remedies. A validated ultra-high performance liquid chromatography-photodiode-array method was developed for the quantification of seven anthraquinone type of compounds. The separation was achieved using a reversed phase (C-18) column, photodiode array detection, and a gradient of water/acetonitrile as the mobile phase. The seven compounds could be separated within 15 minutes using the developed UHPLC method with detection limits of 25 ng/mL with 2 μL injection volume. The analytical method was validated for linearity, repeatability, accuracy, limits of detection (LOD) and limits of quantification (LOQ). The Relative Standard Deviations (RSD) for intra- and inter-day experiments were less than 5%, and the recovery efficiency was 98-101%. Nine supplements labeled as containing *B. natalensis* were available for sale, five products showed for the profile of *B. natalensis* with total content ranging from 11.3 to 90.4 mg per recommended daily dose. Compounds of *B. natalensis* were not detected in four of nine supplements. The developed method is simple, economic, rapid and especially suitable for quality control analysis of *B. natalensis*. LC-mass spectrometry coupled with electrospray ionization (ESI) method is described for the identification and confirmation of compounds in plant samples and dietary products.

This research is supported in part by "Science Based Authentication of Botanical Ingredients" funded by the Food and Drug Administration grant number 2U01FD004246-06, the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No. 58-6060-6-015.

PA

Determination of Ten Neutral and Acid Cannabinoids in Extracts of Different Strains of Cannabis Sativa Using Gas-Chromatography Coupled with Flame Ionization Detector (GC-FID)

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Cannabis (*Cannabis sativa*) is an annual herbaceous plant that belongs to the family Cannabaceae. *Trans*-(-)-Δ^9-tetrahydrocannabinol (Δ^9-THC) has been proven to be the most psychoactive ingredient in cannabis. Δ^9-THC and cannabidiol (CBD) are the two major phytocannabinoids accounting for over 40% of the plants' extracts depending on the variety. At the University of Mississippi, different strains of *Cannabis sativa* with different concentration ratios of CBD and Δ^9-THC have been cultivated. A GC-FID method has been developed and validated for the qualitative and quantitative analysis of ten neutral and acid cannabinoids in plant extracts. The method involves TMS derivatization of the extracts. These cannabinoids include, tetrahydrocannabivarian (THCV), cannabidiol (CBD), cannabichromene (CBC), Δ^8-*trans*-tetrahydrocannabinol (Δ^8-THC), Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC), cannabigerol (CBG), cannabinol (CBN), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), and Δ^9-tetrahydrocannabinolic acid-A (THCAA). The concentration-response relationship of the method indicated a linear relationship between the concentration and peak area ratio with $r^2 > 0.99$ for all ten cannabinoids. The precision and accuracy of the method were found to be satisfactory. The validated method is simple, sensitive, reproducible, and suitable for detection and quantitation of these cannabinoids in various extracts of the cannabis plant. The method was applied to the analysis of these cannabinoids in different parts of the micropropagated cannabis plants (buds, leaves, roots, and stems).

We are grateful to the Egyptian government for partially funding of this study.

PA

Development and Validation of HPLC-Diode-Array Detection (DAD) Method for Analysis of *In vitro* and *In vivo* Produced Cupressuflavone and Amentoflavone

Elsayed A. Ibrahim[1,3], Samar Y. Desoukey[2], Ghada M. Hadad[3], Randa A. Abdel Salam[3], Amany K. Ibrahim[4], Mohamed M. Radwan[1,5], Amira S. Wanas[1,2] and Mahmoud A. ElSohly[1,6]

An HPLC-DAD method has been optimized and validated to obtain qualitative and quantitative profiles of callus extracts (*in vitro*) produced cupressuflavone (CPF), and amentoflavone (AMF) as well as, extracts of *Cupressus sempervirens* L., species (*in vivo*) collected from two different locations in Egypt; the North Coast (CNC) and Future University (CFU). The HPLC isocratic reversed phase separation was implemented on 5µm C_{18} column 25 cm length, 4.6 mm (internal diameter), (Luna, Phenomenex, USA). Good resolution between CPR and AMT was achieved using a mixture of water (0.1% formic acid), and acetonitrile (0.1% formic acid) (60: 40, v/v) as a mobile phase at flow rate 1 mL/min. Quantitation was achieved with

photodiode array detector at the wavelength of maximum absorbance (330) nm based on peak area. The proposed method for determination of CPF and AMF was effective in determination of the analytes of interest without any interference of the other compounds and matrix. The method was linear over the concentration range 0.5 – 40 µg/mL (R^2 = 0.9999) for both of the two biflavonoids. Limit of detection is 0.15 and 0.14 µg/ mL and Limit of quantitation is 0.47 and 0.43 µg/mL for CPF and AMF, respectively. The precision and accuracy of the method were found to be satisfactory. The procedure was relatively simple, precise, rapid, and reproducible and for that reason it is appropriate for the determination and quantification of these biflavonoids present in both *in vitro* and *in vivo C. sempervirens* extracts from the above mentioned. It is also, clear that *in vitro* production of CPF could reach concentrations higher than *in vivo,* with media supplemented by certain growth regulators, elicitors or precursors.

We are grateful to the Egyptian government for partially funding of this study. We acknowledge Mohamed Reda Abd Almegid Abd Alhady, Reda El-Said Abo El-Fadl, Ghada Abd El-Moneim Hegazi for supplying of tissue culture samples.

Tissue Culture Unit, Genetic Resources Department, Ecology and Dry Land Agriculture Division, Desert Research Center, 11753 El-Matareya, 1 Mathaf El-Matareya St., Cairo, Egypt

PA

Ion Mobility Mass Spectrometry: A New Tool for Complex Natural Product Characterization and Identification

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Natural products chemical profiling and identification is a challenging task because of the sample complexity and the analyses required. NMR, GC/MS and UHPLC/MS are currently used for natural products analysis and lately advancements in ion mobility separation have been used to provide additional analyte selectivity in plant metabolomics and other complex studies. UHPLC ion mobility mass spectrometry (IM-MS) is a combination of accurate mass separations with high resolution mass spectrometry (HRMS) and high efficiency ion mobility based measurements that offer some unique advantages for profiling complex mixtures. IM-MS is a rapid orthogonal gas phase separation technique that allows another dimension of separation to be obtained within an LC timeframe. Ion mobility data were acquired using Vion IMS QTof (Waters Corporation, Milford, MA). Ion mobility allows to differentiate compounds based on size, shape, and charge. The UHPLC-IM-MS spectrometer generates low energy precursor accurate mass and corresponding high energy fragment information, isotope ratios, retention time and averaged collision cross-section (CCS) parameters. The CCS value provides an additional dimension of separation for confident compound identification. Here we present the use of UHPLC separations with IM-MS and novel informatics tools containing natural product CCS database for complex

natural product characterization and confident compound identification. The potential of ion mobility for the separation of isomers and chromatographically co-eluting compounds will also be investigated.

PA

Developing an HPTLC method for general screening, from sample preparation to final fine-tuning, using the example of fruits and fruit extracts

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Development of a chromatographic method in analytical chemistry is not an easy task. In HPTLC, method development is based on 4 steps:

Fig. 1: steps of a method development.

Method developed should be specific for the target substance(s) and based on its (their) unique aspects and specific properties. The sample preparation depends on the nature of the sample matrix as well as on the goal of the method. It has to be fit for the purpose! Applying standard HPTLC conditions such as those for USP <203> as basic parameters the selection of the mobile phase depends on the target substance(s) and optimization can be achieved taking Snyder's classification of solvents into consideration. The fine tuning of the method concerns small modifications such as the evaluation of the application volume, the derivatization step and the evaluation mode.

The aim of the poster is to show step by step a general methodology applicable to method development in general. To illustrate the approach, development of a method for identification and discrimination of fruits and fruit extracts is taken as example.

Fruits and fruits extracts are broadly used in different fields including dietary supplements, flavors, or food. For example, pomegranates and cranberries are often used in dietary supplements because of the antioxidant activity of their polyphenols content. Blueberries containing anthocyanins are used as natural dye in food industries. As flavor additives, volatile compounds of fruits such as ketones, aldehydes, esters are isolated.

The method for identification developed for cranberry, blueberry, kiwi, prune, apple, acai, blackberry, fig, and bilberry is targeting a large range of metabolites and a fit-for purpose sample preparation must be applicable to various kinds of extracts. It must be suitable to cover a broad spectrum of compounds and it has to be as simple as possible. In the given work, the selection and the optimization of the mobile phase is explained step by step.

The proposed methodology was successfully applied to fruits and fruit extracts but it can be generalized for any method development.

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PA

Spectroscopic Determination of Total Phenol, Flavonoid Contents and Antioxidant Activity of Polylophium involucratum (Pall.) Boiss.

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Nature has been a source of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Various medicinal plants have been used for years in daily life to treat various diseases all over the world. They have been used as remedies and for health care preparations [1]. The objective of the present study was to evaluate content of phenolic compounds, flavonoids and the antioxidant activity of the seeds extracts of *Polylophium involucratum* (Pall.) Boiss. using spectrophotometric method. The seeds of P. *involucratum* (Pall.) Boiss. were collected from Ramsar, Iran and methanolic extract prepared by microwave assisted extraction (MAE) method. Then total flavonoids and total phenolic content of methanolic extracts were determined by the Aluminium Chloride Colorimetric and Folin-Ciocalteau method, respectively. Also, *in vitro* antioxidant activity of methanolic extracts were assayed by 1,1-diphenyl-2-picrylhydrazyl (DPPH0) free radical scavenging method. Our data showed total phenolic content of methanolic extracts of P. *involucratum* (Pall.) Boiss. seeds was 9.78±0.03 mg GAE/g dry plant material. Also, total flavonoid content of methanolic extracts was 2.70±0.3 mg QE/g dry plant material. The value IC_{50} of methanolic extract determined 3.63 mg/mL. Therefore, P.*involucratum* (Pall.) Boiss. seeds may be considered a source of important phytochemicals (mainly flavonoids and phenolic acids) with bioactive properties to be explored for pharmaceutical applications.

A*cknowledgements*: We gratefully acknowledge the financial support from the Research Council of Tonekabon Branch Islamic Azad University.

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PA

A Novel Qualitative Analysis of Phytochemicals in the Ethanolic Extracts of Flowers of Asclepias incarnate

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Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, terpenoids, and flavonoids. They are of great importance to the health of individuals and communities. Many of these indigenous medicinal plants are used as spices and food plants [1]. The aim of this study was to evaluate the bioactive compounds of ethanolic extract of flowers of *Asclepias incarnate* belonging to the family *Apocynaceae*. The flowers of *Asclepias incarnate* were collected from Tonekabon, Iran and ethanolic extract prepared by microwave assisted extraction (MAE) method. The present study reveals that the phytochemicals analysis of twelve different chemical compounds alkaloids (MayerÊ¼s Test), alkaloids (WagnerÊ¼s Test), terpenoids (Salkowski Test), flavonoids (Alkaline Reagent Test), phenols (Ferric Chloride Test), coumarins (Sodium hydroxide Test), tannins (Ferric Chloride Test), phlobatannins (HCl Test), cardiac glycosides (Keller-Killani test), quinones (Sulfuric acid Test), di-terpenoids (Copper acetate Test), and saponins (Foam Test) were tested in ethanolic extract. The results of the phytochemical screening of an ethanolic extract of flowers of *Asclepias incarnate* showed that coumarins, terpenoids, flavonoids, saponins (a few), quinones, di-terpenoids, tannins and cardiac glycosides were present. Therefore, the flowers of *Asclepias incarnate* might represent a new phytoconstituents and antibacterial source with stable, biologically active components that can establish a scientific base for modern medicine.

We gratefully acknowledge the financial support from the Research Council of Tonekabon Branch Islamic Azad University.

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PA

Preparative method development of natural products from analytical/semi-preparative scouting gradients

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Determination of a preparative gradient method for C18 from analytical data is difficult because the actual solvent composition that elutes the desired compound is delayed from the value observed from the scouting gradient. An algorithm that calculates a focused gradient for a preparative column from an analytical or semi-preparative scouting gradient is described. The calculated focused gradient saves time and solvent compared to the preparative system default gradient and also eliminates guesswork to produce a fast

gradient manually. The algorithm may be used with other adsorption chromatography techniques such as flash chromatography (normal phase and reverse phase) and supercritical fluid chromatography (SFC).

PA

Integrated Analysis of the True Bay Leaf and Its Substitutes Using GC/MS, GC/QToF, NMR and Chemometric Techniques

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Bay leaf is one of the most well-recognized culinary leaf spices used in flavoring a wide variety of foods due to its pleasant aromatic flavor. Botanically, the true "bay leaf", also known as "bay laurel" or "sweet bay", is the leaf from the tree *Laurus nobilis* distributed mainly in the Mediterranean region. Despite its popularity, the leaves of several other species, such as *Cinnamomum tamala, Litsea glaucescens, Pimenta racemosa, Syzygium polyanthum and Umbellularia californica* are often sold as 'bay leaves'. Efficient analytical methods are essential for authentication, quality control and detection of substitution.

In the present study, an integrated analysis using GC/MS, GC/QToF and NMR techniques was performed for the non-targeted compound analysis of bay leaf and five of its common substitutes. The *n*-hexane extractions were analyzed by GC/MS to obtain the chemical information of volatile compounds. HNMR was performed to provide the comprehensive chemical information. The chromatographic and mass spectroscopic data collected from GC/MS and NMR were subject to chemometric statistical analysis using the Mass Profiler Professional (MPP) and Simca softwares. In total, 50 authentic and commercial samples from the six species of "bay leaves" were analyzed, different clusters were observed by principal component analysis (PCA), and a predictive model was constructed based on partial least square-discriminant analysis (PLS-DA) for classification and differentiation of bay leaf and its substitutes. In addition, GC/QToF with accurate mass data and structure information was used for compound identification and conformation. The proposed method demonstrated the feasibility of using a predictive model to differentiate and classify the different products marketed as "bay leaf". It is concluded that conventional GC/MS combined with NMR and multivariate statistical analysis may provide comprehensive information for characterization, standardization and authentication of traditional medicinal plants.

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PA

Chemical Investigation of Black Pepper (*Piper nigram* L.) Oil from Ten Different Countries

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The essential oil from black pepper (*Piper nigram* L.) is a valuable spice and flavoring material and has been widely used in food and perfumery products. Usually when plant species are used as spices, they are subject to strict chemical evaluation for authentication, quality control and prevention of contamination. The chemical profile of volatile components in pepper from various geographic origins often show chemodiversity. In addition, analysis and determination of enantiomers is necessary for establishing the flavors and fragrance purposes. The relationship between the enantiomeric composition of the chiral constituents of the essential oil and country of origin has been used as a quality measurement.
In the current investigation, 19 authenticated and four commercial black pepper samples from 10 countries were collected. The oils were isolated by steam distillation, and the analyses were carried out by means of GC/MS analysis. The major compounds identified were β-caryophyllene (15.0-54.0%), 3-carene (0-21.7%), limonene (4.0-19.1%), β-pinene (1.8-9.7%), α-pinene (1.7-7.5%) and sabinene (0-21.7%). Chemometrics such as principal component analysis was used for the data analysis to reveal the correlation between the chemical composition and geographic origin. The components responsible for distinguishing the countries of origin were also identified by chemometrics.

Furthermore, the enantiomer-specific phytochemicals of black pepper oil, *viz*. limonene, β-pinene, α-pinene, sabinene and α-phellandrene were determined with a cyclodextrin-based chiral GC column. The enantiomeric ratios of these monoterpenes related to the country of origin were also evaluated. The information from the enantioselective analyses could provide a more rigorous description of characteristics of the essential oils derived from black peppers and provide a novel tool for quality control of pepper products.
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PA

Comparative Study of Volatile Alkylamines in Dietary Supplements Using Conventional, Chiral GC/MS and NMR Techniques

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GC/MS usually is the method of choice for the analysis of low molecular weight, volatile amines. However, the high polarity of these amines showed little or no retention on commonly used stationary phases. Thus,

derivatization is often required. Recently, a so called new "2016 Stimulant", 2-aminoisoheptane (DMHA) has been marketed as the principal, natural, active component of workout supplements. The natural source of this compound has been cited as plants such as *Aconitum kusnezoffii* and *Kigelia africana* based solely on the library search of GC/MS analysis. Therefore, efficient analytical methods to provide confirmatory tests are essential for establishing natural occurrence, quantitative determination of alkylamines in dietary supplements as well as to identify possible adulterants/ substitutes.

In current study, a GC/MS method was developed for the analysis of underivatized volatile amines in plant samples and commercial dietary supplements using a thick films of nonpolar stationary phase with megabore column. A total of 15 *Aconitum* and *Kigelia* plants, 12 herbal supplements and 15 sports supplements were quantitatively analyzed for volatile alkylamines (Fig.1). None of the amines were detected in the plants and plant based herbal supplements. Even though all of the dietary supplements listed/labeled DMHA as a component, it was detected in measurable quantities in only 3 products. The quantification results were further confirmed with NMR analysis and the results are in agreement with GC analysis.

In addition to conventional GC/MS and NMR, chiral GC/MS analysis was also performed. Based on diastereomeric distribution of DMHA isomers in sports supplements, it appears that the DMHA may be originated from a synthetic source. Overall, conventional GC/MS with chiral analysis may serve as a rapid technique for the identification of alkylamines along with possible adulterants/substitutes in sports supplements.

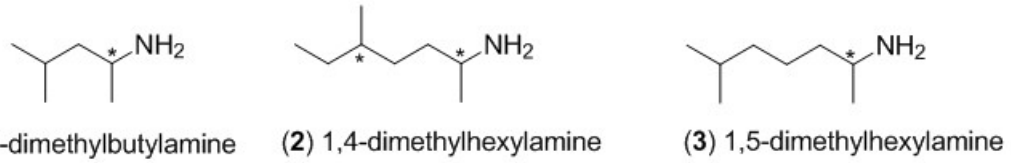

Fig.1. Chemical structures of representative alkylamines and the chiral center is denoted by asterisk.

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PA

Chemical analysis of dream herb (Calea zacatechichi) by UHPLC-UV-MS

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Dream herb is a commonly known Mexican plant *Calea zacatechichi* Schl (syn. *C. ternifolia* Kunth) belonging to the Asteraceae family. This plant is used in folk medicine for a variety of purposes and is currently being marketed in the US for medicinal purposes, including diabetes treatment. The key secondary metabolites of *C. zacatechichi* are germacranolides, one class of sesquiterpene lactones with

antimicrobial and antileishmanial activities [1,2]. However, there is no method reported for the chemical analysis of *C. zacatechichi*.

With the aim of characterization and determination of the marker compounds in botanical ingredients of dietary supplements, an UHPLC-UV-MS method was developed for chemical profile of *C. zacatechichi*. Different types of compounds including organic acids, flavonoids, and sesquiterpenoids were identified 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. Halogenated germacranolides is an unique sub-group of sesquiterpenoids in *C. zacathchichi* and can serve as key markers. Their MS/MS fragmentation pathway has been proposed. The developed method is useful for quality control and quality assurance of products containing *C. zacatechichi*

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PA

The use of SWATHÂ® and High Resolution QTOF Mass Spectrometry for the Investigation of Cannabinoid Extracts

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Current cannabis regulations have a large list of approved pest control products but these lists do not generally contain specific compounds that are commonly used as pesticides. As a result, many regulations have a zero tolerance approach whereby any pesticide found that is not approved causes the material to be out of compliance which may result in removal of the product from distribution. Regulations require that these products are labelled as pesticide free, and this labelling claim must be derived from laboratory analyses. Compliance with regulations is most often performed using LC-MS/MS with a triple quadrupole mass spectrometer. Laboratories typically screen only for a known, or target, list of pesticides and do not screen for unknown pesticides. This leads to growers and producers using other pesticides that are not on the target analyte list in an effort to avoid detection. To detect and identify any potential pesticides a screening technique is required. In addition to providing the ability to find unknown pesticides, a non-targeted screening (NTS) analysis allows the laboratory to investigate the sample for the presence of adulterants such as synthetic cannabinoids or other illicit drugs that may increase or alter the potency or efficacy of the material. It is important to develop analytical strategies that allow laboratories to detect and identify pesticides and other compounds that are not anticipated to be present in the sample. This is most effectively done using a NTS approach with a high resolution LC-MS/MS such as a QTOF instrument. In this work, several matrix types were extracted and analyzed using a SCIEX X500R QTOF mass spectrometer scanning using SWATH®, a data independent technique that provides for identification and quantification of compounds in the sample. This presentation discusses screening methodologies, the SWATH® technique and advantages and disadvantages SWATH®. It is the case for screening methods that the data processing workflow is critical for proper analysis. The software and workflow will be presented along with examples

of different matrices including plant material and edibles that demonstrate how the analytical approach is performed and what was found in the samples.

PB

CALCIUM - MAGNESIUM IMBALANCE IMPLICATED IN BPH AND RESTORATION BY A PHYTOTHERAPEUTIC DRUG – CROTON MEMBRANACEUS MÜLL.ARG

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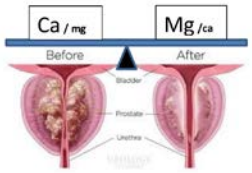
Background: The aetiology of the two main prostate diseases, prostate cancer (PCa) and benign prostatic hyperplasia (BPH) are thought to be multifactorial with different mechanisms involved. Recently, the calcium (Ca)-magnesium (Mg) imbalance has been implicated in the carcinogenesis of PCa, with the understanding that when the Ca/Mg ratio increases, proliferation is promoted. Conversely a decreased ration promoted apoptosis. The Ca/Mg ratio has not been examined in BPH patients.
Aim: The aim of the study therefore was to examine whether Ca/Mg imbalance exist in BPH and the effect of a phytotherapeutic drug treatment choice opted for by BPH patients, on the Ca/Mg ratio.
Methods: This was a retrospective study of 30 BPH patients who used a nutraceutical (ethanolic root extract of Croton membranaceus – 60 mg t.i.d) for 3 months. Blood samples were retrieved and analyzed for Ca, Mg, phosphate, PSA and renal function tests before (BT) and after treatment (AT). Blood samples of a Control group of healthy individuals randomly selected (30) were also run alongside the test group. BT Prostate volume was retrieved from their records.
Results: Renal function test and P for the BT, AT and Controls were normal. Mean PSA was 1.0 ± 0.64, 27.9 ± 19.0 (BT) and 16.2 ± 11.8 ng/mL (AT), respectively; p=0.002. Serum Ca levels did not demonstrate significant differences between the three groups. However, Mg levels were 0.8 ± 0.1 (Control), 0.64 ± 0.18 (BT) and 0.77 ± 0.16 mmol/L (AT) (p=0.0001). Ca/Mg ratio BT, AT and Control were C= 2.87 ± 0.48; 3.81 ± 1.12 and 2.96 ± 0.68, respectively (p=0.0001). The magnesium Ca/Mg ratio showed a strong inverse correlation in the control group (r=-0.755; p<.001). However, the correlation was moderate in the disease group [r= -.493, p=0.001(BT) and r= -.473, p=0.008 (AT)]. There was no correlation between Ca/Mg ratio and prostate volume or PSA. Crude prevalence values of hypomegnesemia/ hypercalcemia for BT, AT and Control were 86.7/36.7%, 13.3/13.3%, 16.7/10%, respectively. The prevalence of Ca/Mg imbalance was 80% (BT), 13.3% (AT) and 3.3% (control group).
Conclusion: A high prevalence of Ca/Mg ratio imbalance is associated with BPH.



Effect of Croton *membranaceus* EtOH root extract

PB

Influence of Gymnena sylvestre on the Pharmacokinetics of Glipizide in Diabetic and normal Rats.

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Background: In Ayurveda *Gymnema sylvester is* used to neutralize the excess of sugar levels present in diabetic patients. But conventionally the sulfonylurea's Glipizide, Glimepiride, and Glibenclamide are commonly prescribed drugs to diabetic patients. There may be chances of using both the drugs at a time which may lead to herb-drug interaction. Hence the present study was undertaken to investigate the possible herb-drug interactions.

Method: G*ymnema sylvestre* leaves were extracted using a solvent (volume ratio of methanol to water was 1:1 ratio) The extract was filtered through 0.45 µm nylon filter (Millipore). The volume was made up to 1000 mL with extraction solvent and the clear supernatant was used for HPLC analysis. A Glipizide standard curve was plotted with different concentrations. The estimation of Glipizide was achieved by HPLC, C_{18} reverse phase column plate used as the stationary phase, The mobile phase consisted of methanol: water: 0. 01M potassium phosphate buffer (60:35:5, v/v/v). The glipizide was estimated as per ICH guidelines the parameters like The concentration in plasma, C_{max}, $AUC_{(o-t)}$ and $AUC_{(0-\infty)}$ (area under the concentration-time curve, AUC) and specificity were determined.

Results: Calibration curve of glipizide was generated, the maximum peak of plasma concentration of Glipizide (µg/mL) was increased significantly in the presence of G*ymnema sylvestre* leaves extract (GSE), GPZ+GSE, from 0.87± 0.08 to 1.42± 0.07 in diabetic rats and 0.44± 0.06 to 1.23± 0.01 in normal rats respectively. Area under the moment curve of Glipizide (µg/ mL) was increased significantly in the presence of extract, GPZ+ GSE, from 5.39± 0.02 to 10.55± 0.18, 2. 53± 0.08 to 8.52± 0.1 in in diabetic and normal rats respectively. The clearance of glipizide decreased significantly i. e. Glipizide , GPZ+ GSE clearances were 6. 19± 0.12 to 2.57± 0.72 in diabetic rats and 9.22± 0.02 to 3.62± 0.69 in normal rats, respectively, but T_{max} values were the same in diabetic and normal rats.**Conclusion**: The present study conclude that hydro alcoholic extract of *Gymnema sylvestre* enhance the bioavailability of Glipizide. So further investigation is necessary to optimize the dose.

Authors are grateful to the management of Netaji Institute of Pharmaceutical Sciences, Toopranpet, BhongiriYadhadhri Dist -508252, Telangana State, India. for providing necessary facilities to carry out the research work

PB

Biochemometrics as a tool for identifying synergists in Hydrastis Canadensis

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Bioassay-guided fractionation, an isolation technique used to identify bioactive molecules in natural product extracts, is often biased by constituents that are most abundant and easiest to isolate. This task can become more challenging when trying to identify synergists, which are often less abundant than the primary bioactive metabolites. Biochemometrics improves upon bioassay and synergy-guided fractionation in that

the entire mass spectral profile is statistically correlated with bioactivity across a set of fractions, resulting in selectivity ratio plots which represent retention time-mass pairs with varying degrees of correlation, indicating the likelihood of contribution to bioactivity. *Hydrastis canadensis* is known to contain berberine, an antimicrobial alkaloid, along with various flavonoids that enhance the potency of berberine against *Staphylococcus aureus*, and serves as a useful case study for evaluating the effectiveness of this approach for identifying synergists. Multiple stages of fractionation were required to reduce the complexity of fractions and produce meaningful statistical results. Third and fourth stage fractions predicted known flavonoids as synergists, and an unknown ion with the mass of 373.1273 $[M+H]^+$ and its ^{13}C isotope had the highest selectivity ratios among stage three fractions. Isolation and structure elucidation are underway, and mass spectral fragmentation patterns indicate that this ion is an analogue of 3,5,3´-trihydroxy-7,4´-dimethoxy-6,8-C-dimethyl-flavone, which was shown to act synergistically with berberine. Isolation, identification, and confirmation of activity are underway for additional correlating unknown ions. Overall, this method has shown to be effective in identifying multiple bioactive candidates at once, which serves as an improvement over existing drug discovery techniques.

PB

Development of a quick DNA authentication protocol for detecting transgene P-35S region in genetically modified papaya

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Papaya (*Carica papaya*) is consumed as both fruit and medicinal material. In many Asian countries, papaya is used to treat aches, pains, and muscle spasms and stiffness. In Hong Kong, over 60% of papaya available in the market are transgenic. They have fusion gene coding coat protein of cauliflower mosaic virus (CaMV) and papaya ringspot virus (PRSV) under the control of the CaMV 35S promoter (P-35S), for the resistance to PRSV. As more consumers prefer organic food and natural products, there is a need in rapid identification of transgenic papaya. We have developed an efficient procedure for the authentication of transgenic papaya based on loop-mediated isothermal amplification (LAMP). LAMP primers are designed based on the transgene P-35S region and the intrinsic papain gene as internal control respectively. The amplification products could be examined by gel electrophoresis and direct visual detection using the DNA binding dye, SYBR green I. Comparing to traditional PCR method, the LAMP detection method provides reliable and rapid screening of transgenic papaya. This LAMP detection method is being streamlined by incorporating experimental steps into a lab-on-a-disc (LOD) to allow efficient on-site detection and result interpretation by laymen.

PB

Biochemometrics as a Strategy to Identify Antimicrobial Constituents in Complex Botanical Mixtures--a Validation Study

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In combination with advanced chromatographic separation systems, mass spectrometry can be utilized to examine hundreds of secondary metabolites concurrently. However, when searching for biologically active constituents in complex botanical mixtures, it is often challenging to assign activity to individual components. Several strategies have been developed for deciphering the biological meaning of biochemometric datasets. S-plots and selectivity ratio plots utilize algorithms to compare the covariance and correlation of individual mixture components with bioactivity, allowing for visual representation of compounds likely to contribute to biological activity. This approach is promising for directing natural products discovery programs towards the most biologically important constituents in a complex mixture. The purpose of this experiment is to assess the prognostic capabilities of this approach for identifying antimicrobial components within a complex botanical mixture. To do this, non-antimicrobial fractions of *Plantago lanceolata* and *Plantago major* were combined with known compounds with varying antimicrobial potency against *Staphylococcus aureus.* In one example, the S-plot and selectivity ratio plot successfully identified cryptotanshinone, a known antimicrobial that was added to selected fractions, as the constituent responsible for antimicrobial activity. Variations of this study are underway, in which active compounds are spiked into an extract before fractionation. Fractionation and bioactivity analysis will be varied, allowing for the assessment of the impact of chromatographic resolution and data format on biochemometric results. This information will provide a validated set of guidelines for subsequent biochemometric analyses on complex natural product extracts.

PB

ABRIN PURIFICATION, CHARACTERIZATION, AND DETECTION THROUGH HIGHLY SENSITIVE IMMUNO-BASED METHODS

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Some natural proteins, including from plant and bacterial sources, are toxic to mammalian cells. Abrin is a type II, heterodimeric 65 kDa glycoprotein from *Abrus precatorius.* Among plant toxins, abrin is so extremely toxic, a single molecule is sufficient to kill a cell – that it is included in the Biological and Toxin Weapons Convention (BTWC). The present study isolated and purified abrin from black *Abrus precatorius* seeds, and characterized it using immunological methods. Abrin was extracted following 30% and 90% ammonium sulphate saturations. After dialysis, crude abrin was purified by lactamyl-sepharose affinity chromatography. The purity of abrin was checked by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Further, abrin was characterized by two dimensional (2D) gel electrophoresis and hemagglutination assay. Antibodies against abrin were raised in both rabbits and rats by immunizing animals with toxoid at various time intervals. Abrin was detected through western blot analysis. The abrin toxin gives a single band in the 60 kDa region under non-reducing conditions and two bands in 30 kDa region under reducing conditions. No other bands were observed. Hemagglutination activity of purified abrin was 1:8 and 1:32 for crude abrin. The 2D pattern of abrin extracted from black *Abrus precatorius* seeds exhibited 10 different spots with pI values of 5.4 to 7.9 at the position of A-chain. Antibody titer checked by

dot ELISA was found to be 1:102,400 in rabbit and 1:6400 in rat. Blot was prominent even at 40 ng/µl of abrin. SDS-PAGE and hemagglutination assays indicate that the abrin purified from our laboratory was of high purity and could be used for screening of antidotes and development of sensitive detection systems.

PB

ABRIN INDUCES OXIDATIVE STRESS, WHICH IS ASSOCIATED WITH HEPATIC AND RENAL TOXICITY IN MICE

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Abrin is one of the most poisonous phytotoxins from the *Abrus precatorious* seeds. It produces various toxic effects and severe damages in different visceral organs. Because of its wide availability and severe toxicity, abrin represents a potential agent for use in bioterrorism, and it is included in the Chemical Weapons Convention. In the present study, we have studied the nephrotoxicity, hepatotoxicity and oxidative stress following intraperitoneal administration of abrin (8µg/kg) in male Swiss albino mice. Results clearly revealed that activities of various enzymes like gamma glutamyl transpeptidase (gamma-GT), lactate dehydrogenase (LDH), glutamic pyruvic transaminase (GPT), and alkaline phosphatase (ALP), were increased in liver, kidney and plasma, indicating damage in the liver and kidney. The level of urea, creatinine, and bilirubin were also found to be increased. Superoxide dismutase activity decreased significantly to 58% in liver and 42% in kidney. Total non-protein sulfhydryl content decreased in plasma (16%), hepatic (36%), and renal (21%) tissues. The activity of glutathione peroxides was also decreased. Lipid peroxidation increased to 68% and 43% in hepatic and renal tissues. A significant increase in catalase activity was observed in the liver (42%), plasma (23%), and kidney (33%). Our findings indicate that abrin causes hepatoxicity, nephrotoxicity, and oxidative damage even at 24 h of post treatment. Effects were more pronounced in the liver than the kidney.

PB

THE EFFECTS OF CNIDISCOLUS ACONITIFOLIUS LEAF EXTRACT ON POLYMERIZATION AND DENSITY OF HBSS RED BLOOD CELLS IN VITRO.

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Sickle cell disorder (SCD) is the commonest hemoglobinopathy amongst the people living in sub-Saharan Africa and Nigeria has the highest prevalence rate in the world with rates varying between 1.5-3%. Medicinal plants have been used in the treatment of painful crises associated with sickle cell disease (SCD) especially among the lower socio-economic class who cannot afford the high cost of western medicine and those who believe in the efficacy of herbs. An ethnomedicinal survey was carried out and *Cnidoscolus aconitifolius* (Mill.) I.M. Johnst. used traditionally in the management of sickle cell anemia was selected for this study to prove this claim, scientifically. The leaves of *C. aconitifolius* (CA) were harvested, dried in the oven at 40°C and extracted by maceration in absolute ethanol for 72 h at room temperature. Inhibitory and reversal antisickling models were employed to determine the effects of CA on HbSS

polymerization while the red cell fractionation assay was carried out to determine its effect on the density of HbSS red blood cells. Ciklavit*, a nutraceutical used in the management of sickle cell disorder was used as positive control. CA gave an antisickling activity significantly higher ($p < 0.05$) than that of Ciklavit*, the positive control and gave 96.82 ± 0.009% rate of decrease in polymerization at 4 mg/mL concentration. The mean percentage change in density of HbSS blood cells treated with CA was 26.46 ± 0.55%.

This result authenticates the traditional use of *C. aconitifolius* leaves as blood booster in treating anemia associated with sickle cell disorder.

Keywords: Sickle cell anemia, Antisickling, Polymerization, Red cell density.
The authors acknowledge Tertiary Education Trust Fund (TETfund) and Obafemi Awolowo University, Ile-Ife, for the grant for the project titled: Sickle Cell: Its Management and Prevention in Nigeria.

PB

Assessment of immunomodulatory activity in ethanolic extract of Albizia procera bark

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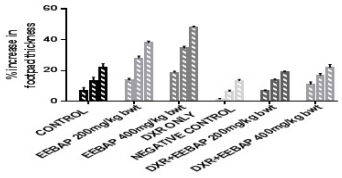
Background: Many species of Indian medicinal plants have been reported to possess active principals with immunomodulating properties, according to the literature and our preliminary studies *Albizia procera* bark is rich in saponins, which are known to be immunomodulatory agents. **Methods**: The present study was carried out for ethanolic extract of *Albizia procera* bark (EEAP) at 200 mg/kg and 400mg/kg by using both *in-vitro* and *in-vivo* models. Nitric oxide synthase activity, nitro-blue tetrazolium reduction assay were performed for *in-vitro* immunomodulatory studies whereas for *in-vivo* model Doxorubicin-induced Immunosuppressive rats were used both cell-mediated immune response and humoral immune response were studied. In cell-medicated immunity, total leukocyte count (TLC) and differential leukocyte count (DLC), neutrophil adhesion and T-cell population test, carbon clearance test, delayed-type hypersensitivity test (DTH) were recorded and for humoral immune response hemagglutination titer assay was performed. **Results and Discussion**: In TLC and DLC counts, EEAP showed a dose-dependent increase in TLC count and the population of neutrophil, monocytes, and lymphocytes. The suppressive effect of doxorubicin on these cells was not reflected in immunosuppressive rats treated with EEAP. It increased the rate of clearance of the carbon particles dose-dependently from the blood circulation in both normal rats and in the immunosuppressive rats. In DTH the results showed a significant increase in footpad thickness of paw in response to the antigen, this potentiating of DTH responses indicates that the EEAP has a stimulatory effect on lymphocytes and accessory cell types required for the expression of reaction and thus increased the cell-mediated immunity. EEAP also showed an enhanced production of circulating antibody titer. This augmentation of a humoral response to SRBC antigen by the increase in Hemagglutination antibody titer indicates the enhanced responsiveness of macrophages, T-lymphocytes and B-lymphocytes subset involved in antibody synthesis. **Conclusion:** The present study concludes that the ethanolic plant bark extract has immunopotentiation activity.



The authors are grateful to the management of Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram, AP, India for providing the facilities to carry out the research work.

PB

Increase in soil organic matter alters plant-associated bacterial community within Echinacea purpurea roots and enhances macrophage activation potential of extracts.

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Previously we reported that changes in the levels of the bacterial components LPS and Braun-type lipoproteins were responsible for ~100-fold variation in the *in vitro* macrophage activation potential of commercially obtained Echinacea material. More recently, we found that mean activity exhibited by *Echinacea purpurea* aerial materials was fully accounted for by the type and prevalence of plant-associated bacteria. In the current study we investigated whether changes in soil conditions (organic matter, nitrogen levels and moisture content) could influence E. *purpurea* associated bacteria and thus influence the macrophage activation potential of this material. Our results indicate that cultivation of E. *purpurea* in soils with increased levels of organic matter significantly enhanced macrophage activation exhibited by root extracts ($p<0.0001$). A 4.2-fold increase in activity was observed with a change in soil organic matter from 5.6% to 67.4%. Samples were further analyzed for community composition to determine whether changes in soil organic matter also influenced the type/amount of plant-associated bacteria. Next generation sequencing of the E. *purpurea* associated bacterial community revealed 8,805 operational taxonomic units spanning 16 bacterial phyla. Analysis using the Jaccard index showed a significant difference in community membership of root material cultivated in soil with different levels of organic matter (AMOVA, $p<0.001$). Thirty taxa representing seven phyla were more abundant in root samples under the various treatment conditions. These results indicate that soil organic matter content alters the bacterial community structure within E. *purpurea* and thereby may contribute to differences in macrophage activation potential exhibited by commercially diverse plant material.

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PB

The antileishmanial artemisinin dimer analogs induce apoptosis in Leishmania donovani promastigotes

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Visceral leishmaniasis (VL) caused by *Leishmania donovani,* a protozoan parasite, is a major global health problem. This disease is fatal if left untreated. About 0.4 million new cases are being added every year and millions of people are at the risk of being exposed to the disease. Current choices of therapies for VL are very limited and suffer from severe drug-toxicities. Most of the available drugs are ineffective due to low efficacy and emerging resistance. Recent studies at our laboratories have identified a few novel artemisinin dimers with outstanding antileishmanial activities against both promastigote and intracellular amastigotes of *L. donovani*. Antileishmanial activities of these dimers are several folds better as compared to the current battery of clinically used antileishmanial drugs. These dimers do not show noticeable toxicity against differentiated THP1 cells (a human monocytic cell line derived from acute monocytic leukemia cells). Selectivity index (SI) has been calculated by comparing toxicity with antileishmanial activity. Dimer morpholine (IC_{50} 0.007 μM, SI >2052), and dimer GABA (IC_{50} 0.013 μM, SI >1086) have been selected as promising leads for extended evaluation. Recent reports have demonstrated the apoptotic response in *L. donovani* cells. The *L. donovani* promastigtes were treated with dimer morpholine, dimer GABA and the parent drug (artemisinin). The treated/untreated cells were stained with annexin V and propidium iodide and analyzed flow cytometry for the apoptotic response. Both dimer morpholine and dimer GABA showed a time-dependent induction of apoptosis in *L. donovani* promastigotes. Artemisinin, the parent drug from this class, neither showed significant antileishmanial activity nor any apoptotic effect up to 35 μM concentration. The artemisinin dimers have shown significant bioavailability when administered through oral route in mice. Further characterization of the molecular mechanism of action will promise these artemisinin dimers as potential new oral treatments for visceral leishmaniasis.

US Army MRMC CDMRP IIRA grant # W81XWH-09-2-0093 and USDA-ARS cooperative scientific agreement.

References: [1] Jain et al. (2016) BMC Complement Altern Med. 2016 May 18; 16 (1):131. [2] Jain et al. (2012) J Vis Exp. 2012 Dec 30; (70). pii: 4054.

PB

Efficacy evaluation of Linium usitatissimum (Linctus of Flax mucilage) in Chronic Obstructive Pulmonary Disease patients

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Chronic obstructive pulmonary disease (COPD) has almost become a global epidemic with an increased morbidity and mortality. So far there is no effective available therapy to better control the inflammatory progression which contributes to this irreversible airflow limitation.
While going through Unani (Greek o Arab Medicine) literature, medicinal uses of Linium usitatissimum in such disorders were observed. Need was felt to explore the potential of this well-known Unani medicine in the management of COPD as safe and efficacious substitute to the existing treatment options based on

spirometric parameters.
A total of 80 patients were enrolled, out of which 60 patients randomly distributed into Test and Control groups were included in the study. All patients in the Test group were given the test drug, a Pharmacopeial Unani formulation "Linctus of Flax mucilage" (Linium usitatissimum L.) in a dose of 10 gm thrice daily for 6 weeks. Similarly, patients in Control group were given standard Theophylline 200 mg thrice daily for 6 weeks.
Based on the spirometry parameters FEV1 and FEV1/FVC, it was observed that the test drug Linctus of Flax mucilage had a significant effect in the management of COPD.Appreciate the efforts of all the contributors of this study. Much thanks to Ms. Tabassum Alam for her dedicated work and untiring efforts ala through the completion of this work.

PB

Active fractions of Caralluma adscendens var. gracilis on Antioxidant and carbohydrate digestive enzymes: *In vitro* studies

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Caralluma are prolific producers of structurally diverse steroidal glycosides, steroids and triterpenes. India is home to many Caralluma species with the Southern parts of India having majority of the species where Tamil Nadu possesses the maximum number of Caralluma. Carallumas have been thoroughly investigated both phytochemically and pharmacologically all around the globe which has resulted in some interesting compounds with significant biological behaviour. The plants fractions of *Caralluma adscendens var. gracilis*and *Caralluma pauciflora* were evaluated for their total phenolic content in relation to their antioxidant activity and inhibitory effect of starch and lipids digestive enzymes. Among all fractions of *C. adscendens var. gracilis* diethyl ether fractions showed highest phenolic content (36.23±1.51 mg of GAE g^{-1} DW, 28.21±3.61 mg of GAE g^{-1} DW), DPPH radical scavenging activity (27.96 ±3.45 μgml^{-1}). The inhibition of starch digestive enzymes α-glucosidase (59.13±1.31 μg ml^{-1}), α-amylase (78.1±3.47μg ml^{-1}) and lipid digestive enzyme Lipase (41.91±3.51μg ml^{-1}).

PB

Antidiabetic activity of hydroalcoholic extract of Prunus persica L. leaves in STZ induced diabetic rats[JP1]

Prunus persica L. Batsch (family: Rosaceae) is commonly known as peach in English and aadoo in Hindi. The aerial parts of the plant are traditionally useful in the treatment of diabetes, but there is no scientific evidence to justify this claim. So, the present study was undertaken to investigate P. *persica* L. leaves for antidiabetic activity to justify its claimed use.

Dried leaves were powdered, sieved and extracted with hydroalcohol (30:70) in Soxhlet apparatus at a temperature not exceeding 60°C. The extract was concentrated under reduced pressure in rotary evaporator to yield a crude semi-solid mass. The extract was analyzed phytochemically to ascertain the presence of different active constituents. Hydroalcoholic extract was screened for its hypoglycemic effect in streptozotocin (60 mg/kg, i.p.) induced diabetes in rats at the doses of 200 and 400 mg/kg respectively.
The extractive value of hydroalcoholic extract was obtained as 28.3% w/w. The preliminary phytochemical screening revealed the presence of cyanogenetic glycosides, flavonoids, steroids, proteins and carbohydrates in the extract. The extract showed significant ($p < 0.01$) antidiabetic activity at both the doses i.e. 200 and 400 mg/kg body weight, which is further evidenced by reduction in blood glucose levels after administering the extract at both the doses for two weeks. The extract also exhibited significant free radical scavenging activity on DPPH free radicals. The antioxidant activity of the P. persica might be due to the presence of flavonoids present in the plant. Previous studies have reported that flavonoids are responsible for the hypoglycemic action of a plant extract. So, the antidiabetic action of this extract might be attributed to its flavonoid content confirmed by the preliminary phytochemical screening.
Conclusion: The results of the study indicates that hydroalcholic extract of *P. persica* L. leaves possess significant antidiabetic effect in STZ induced diabetic rats.
The author is thankful to the Director, Institute of Pharmaceutical Sciences, K.U., Kurukshetra for providing necessary facilities for the study.

PB

An Improved System for Rapid In vitro Regeneration of Stevia rebaudiana Bert.

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A simple and efficient one step regeneration protocol for rapid shoot proliferation and *in vitro* rooting was developed for *Stevia rebaudiana* Bert. using meta-topolin (*m*T), an aromatic natural cytokinin. Cultures were initiated from nodal segments containing axillary buds from selected mother plants. Explants were surface-disinfected using 0.5% NaOCl (15% v/v bleach) and 0.1% Tween 20 for 20 min. and washed in sterile distilled water three times for 5 min each, prior to inoculation on the culture medium. Disinfected explants were inoculated on Murashige and Skoogs medium (MS) supplemented with 500 mg L^{-1}activated charcoal and different concentrations of *m*T ranging from 0.5 to 5.0 μM adjusted to pH 5.7. The best response in terms of production of shoots was achieved in MS medium supplemented with 500 mg L^{-1} activated charcoal and 2μM *m*T. After two subcultures, shoots multiplied on the same medium were able to induce healthy roots within 4 to 6 weeks. As reported previously (Lata et al. 2013), a separate growth regulator for rooting was not required in this system. The rooted plantlets were successfully established in soil in climatic controlled indoor grow room followed by transferring to outdoor and grown to maturity at the 89% survival rate.

Reference

Hemant Lata, Suman Chandra, Yan-Hong Wang, Vijayasankar Raman, Ikhlas A. Khan (2013). TDZ-Induced High Frequency Plant Regeneration through Direct Shoot Organogenesis in *Stevia rebaudiana*

Bertoni: An Important Medicinal Plant and a Natural Sweetener. American Journal of Plant Sciences, 4:117-128

Micropropagation of *Stevia rebaudiana* Bert. *a*: *in vitro* propagated plants, *b*: Well acclimatized *in vitro* propagated plants under climatic controlled grow room conditions

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PB

Will estrogenic Chinese herbal medicines promote breast cancer growth? A preclinical study

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Phytoestrogens were believed to have beneficial effects for women's health. Nevertheless, the potential risks of estrogenic botanicals consumption by breast cancer patients were also aroused over the years. Chinese herbal medicines (CHM) and CHM-containing cuisine are commonly consumed by breast cancer patients in Asia. The safe use of estrogenic CHM in breast cancer patients has long been a concern of Chinese medicine practitioners. The present study aimed to investigate the potential effects of estrogenic CHM on breast cancer using preclinical cell-based and tumor-bearing mouse models.

Human breast cancer cells with different molecular subtypes, MDA-MB-361 (ER+, HER2+), MCF-7 (ER+, HER2-), SKBR3 (ER-, HER2+) and MDA-MB-231 (ER-, HER2-), were used to evaluate the effects of water extracts of selected estrogenic CHM, such as *Astragalus membranaceus* (AM), *Cuscuta chinensis* (CC), *Dioscorea opposita* (DO), *Epimedium brevicornum* (EB), *Glycyrrhiza uralensis* (GU), *Ligusticum chuanxiong* (LC), *Psoralea corylifolia* (PC), *Panax ginseng* (PG) and *Pueraria lobata* (PL). The cells viability and proliferation were assessed by MTT and BrdU assays. The CHM with stimulatory activity was further evaluated for its effect on syngeneic breast tumor-bearing mice.

Our results showed that CC, DO, LC, PC, PG (up to 6.4 mg/mL) could significantly increase cell viability of MDA-MB-361 cells, while DO also slightly increased cell viability and proliferation in MCF-7 and MDA-MB-231 cells. Thus, Balb/c mice bearing 4T1 breast tumors were orally treated with DO extract (0.5 g/kg, human equivalent dose) for 4 weeks. Results showed that there was no difference in tumor size between DO-treated and control groups. Interestingly, DO treatment was shown to modulate immune responses in tumor-bearing mice.

In conclusion, this study demonstrated the differential effects of selected estrogenic CHM on viability and proliferation of breast cancer cells. Among all those tested, only DO (commonly used in Chinese cuisine and herbal medicines) was found to increase breast cancer cell viability and proliferation. However, it did not promote the growth of breast tumor in immunocompetent mice. Further investigation on human breast

cancer xenograft model may consolidate the findings and provide evidences for the safety use of DO in breast cancer patients.

This work was supported by Food and Health Bureau of the Government of the Hong Kong Special Administrative Region, Health and Medical Research Fund no. 12130471.

PB

SUSCEPTIBILITY OF AGS AND NCI-N87 GASTRIC CANCER CELL LINES TO NIGERIAN MEDICINAL PLANTS USED FOR THE TREATMENT OF HELICOBACTER PYLORI INFECTIONS

Lawal TO[1], Patel S[2], Mahady GB[2]

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Three medicinal plants, namely *Anogeissus leiocarpus* (DC.) Guill. & Perr. (African birch, Combretaceae), *Terminalia glaucescens* Planch ex Benth. (Combretaceae) and *Dillenia indica* L. (Elephant apple, Dilleniaceae) were identified from an ethnobotanical survey for the treatment of gastrointestinal diseases in Nigeria. Extracts of each of these plants were active against *Helicobacter pylori* the etiologic agent of gastritis, peptic ulcer disease, gastric MALT lymphoma and gastric cancer. In this study, extracts of the plants were investigated in two gastric cancer lines. The root and stem bark of *A. leiocarpus* and the root bark of *T. glaucescens,* as well as the stem bark and leaves of *D. indica* were collected in Ibadan, Nigeria and extracted using methanol and water. The extracts were tested in AGS and NCI-N87 gastric cancer cell lines at concentrations up to 100 µg/mL. Control cells were treated with vehicle solvent (DMSO 0.02%). Cell viability and cytotoxicity was determined using the CellTiter-Glo® 2.0 assay. Extracts of *A. leiocarpus* root were active in AGS with IC_{50} of 60 µg/mL and 76.00 µg/mL in NCI-N87 while extracts of the stem bark were not active in concentrations up to 100 µg/mL for both cell lines. An ethyl acetate extract of *T. glaucescens* was active in the AGS cell line with an IC_{50} of 57.5 µg/mL, while an aqueous extract of the same plant was weakly active with an IC_{50} of 95.9 µg/mL in NCI-N87. An extract of *D. indica* stembark had IC_{50} of 50 µg/mL in AGS cells, but was not active in NCI-N87 cells. *D. indica* leaf extracts were not active in these cell lines. The results showed that medicinal plants used in Nigeria for GI disorders and that had significant activity against *Helicobacter pylori*, also were cytotoxic to several gastric cancer cell lines. These data support their use in the management of GI disorders.

Acknowledgements: This research was made possible by Schlumberger Foundation Fellowship award to TOL and a First Analysis grant to GBM.

PB

NIGERIAN MEDICINAL PLANT EXTRACTS ARE CYTOTOXIC IN COLON CANCER CELL LINES AND INDUCE APOPTOSIS IN SW480 CELLS THROUGH CASPASE 8

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In Nigeria, plant-based traditional medicines are used to treat a wide range of gastrointestinal (GI) disorders including stomach ulcers and cancers. The stem bark and leaves of *Dillenia indica* L. (Elephant apple, Dilleniaceae) and the root and stem bark of *Anogeissus leiocarpus* (DC.) Guill. & Perr. (African birch, Combretaceae) were collected in Ibadan, Nigeria and extracted using methanol and water. Cell viability and cytotoxicity was determined using the CellTiter-Glo® 2.0 assay that measures the amount of ATP present, an indication of the presence of metabolically active cells in a range of colon cancer cell lines including SW480, HCT116, and Caco2 at concentrations up to 100 µg/mL of the extracts. Control cells were treated with vehicle solvent (0.02% DMSO). Caspase activity for apoptosis was determined with Caspase-Glo® 3/7, Caspase 8, ApoTox-Glo™ Triplex Assay Reagents and flow cytometry. Extracts of *A. leiocarpus* were active against SW480 with IC_{50} of 32.78 µg/mL. The aqueous partition was found to be the most active and explained the activity of the stem bark, with IC50 of 32.94 µg/mL. In SW480, *D. indica* extract had an IC_{50} of 44.12 µg/mL, and in HCT116 of 72.66 µg/ml. In SW480 cells *D. indica* induced apoptosis through the induction of caspase 8. Neither extracts nor the partitions were active against Caco2 cells. From the results it is concluded that aqueous plant extracts used in Nigeria for GI ailments have activity against colon cancer *in vitro*, thereby supporting the traditional medical uses of these plants.

Acknowledgements: This research was made possible by Schlumberger Foundation Fellowship award to TOL and a First Analysis grant to GBM.

PB

AQUEOUS EXTRACTS OF DILLENIA INDICA L. (DILLENIACEAE) STEM BARK INDUCE APOPTOSIS IN MCF-7 CELLS THROUGH THE INDUCTION OF CASPASE 3/7 PATHWAY

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Breast cancer remains one of the deadliest cancer affecting both pre- and postmenopausal women worldwide. The use of traditional medicinal plants in the treatment of breast cancer is common practice in rural populations in the developing countries such as Nigeria. In Nigerian traditional medicine, the mixed juices of bark, leaf and fruits of *Dillenia indica* L. (Elephant apple, Dilleniaceae) are used for the treatment of cancer. In this study, these extracts were investigated in MCF-7 breast cancer cells. The stem bark, and leaves and fresh matured fruits of *D. indica* were collected in Ibadan, Nigeria and extracted using methanol and water. Cell viability and cytotoxicity was determined using the CellTiter-Glo® 2.0 assay in MCF-7 at concentrations up to 100 µg/mL of the extracts. Control cells were treated with vehicle solvent (0.02% DMSO). Caspase activity and apoptosis of the aqueous extract of the stem bark in MCF-7 cells was determined using Caspase-Glo® 3/7, Caspase 8, ApoTox-Glo™ Triplex Assay Reagents and flow cytometry. Aqueous and ethyl acetate extracts of *D. indica* stem bark exhibited cytotoxicity activity on MCF-7 with IC_{50} of 65.3 and 60.6 µg/mL respectively. Extracts of the leaf and fruits were not active up to 100 µg/mL. The aqueous extract of the stem bark induced apoptosis in MCF-7 cells via caspase 3/7 apoptotic pathway, and flow cytometry confirmed these results. The results of this study suggest that extracts of *Dillenia indica* stem bark are cytotoxic in MCF-7 cells and induce apoptosis through the activation of caspase 8. These results support the traditional uses of this plant for the management of breast cancer in Nigeria.

Acknowledgements: This research was made possible by Schlumberger Foundation Fellowship award to TOL and a First Analysis grant to GBM.

PB

BEHAVIORAL ANALYSIS OF JAPANESE MEDAKA LARVAE DEVELOPMENTALLY EXPOSED TO PANAX GINSENG ROOT EXTRACT

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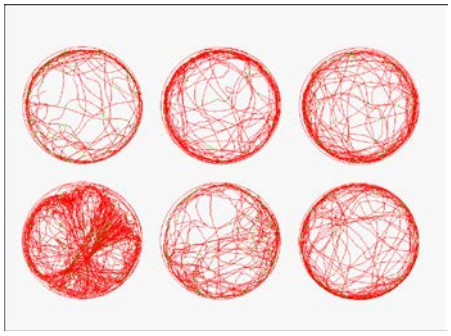
The therapeutic potential of Panax ginseng (PG) in the treatment of neurobehavioral disorders such as Parkinson's disease (PD) prompted us to evaluate the prenatal effect of PG on postnatal prevention in the induction of PD phenotypes. We have used Japanese medaka (Oryzias latipes) as our experimental model. Fertilized eggs were exposed to PG root extract (PGRE; 2-100 µg/ml) 0- 6 days post fertilization (dpf). The heart beats of the embryos were recorded on 3 and 6 dpf and the larvae were used for movement analysis. The larval swimming was tracked for three hours by light-dark cycles (30 min light followed by 30 min dark) in a ZebraBox instrument. The movement threshold was classified as inactive, active, and hyperactive. It was observed that the heart of the embryos on 3 dpf beat slower than the embryos on 6 dpf and PGRE (2-10 µg/ml) has no effect on heart beats either in 3 or 6 dpf. However, embryos exposed to 100 µg/mL significantly reduced the heart beats on 6 dpf not in 3 dpf. All the embryos (both control and 2-10 µg/mL PGRE) were hatched successfully on 8-12 dpf. But there was hatching delay in embryos exposed to 100 µg/mL PGRE. The data on movement analysis showed that the larvae remained more active in presence of light and moved at a constant speed, but in the dark, a gradual adaptation to the environment was noticed. The larvae exposed to PGRE prenatally were less active than controls; however, these larvae travelled greater distances by swimming in comparison to the controls in both light and dark cycles. The pattern of adaptation of the larvae to the dark remained the same as in controls. We conclude that prenatal exposure to PGRE made the fish less active but allowed them to travel greater distances without affecting the adaptive memory (light/dark adaptation).



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Ergocalciferol induces apoptosis in breast and colon cancer cell lines via caspase 3/7 and 8 and has synergistic effects with cholecalciferol and all-trans-retinoic acid

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Vitamin D was first hypothesized to play a role in cancer chemoprevention in 1980 when it was observed that there was a higher rate of colon cancer in the Northern US as compared with populations living in the South. While vitamin cholecalciferol has been tested in many cancer cell lines, published results for ergocalciferol *in vitro* and *in vivo* are lacking for most epithelial cancers and combination studies of both D2 and D3 in the literature is lacking in general. Ergocalciferol (ERGO), also known as vitamin D2, is 9, 10-secoergosta-5,7,10(19),22-tetraen-3-ol,(3 ≤ ,5Z,7E,22E)-; ($C_{28}H_{44}O$) is structurally similar to the active form of vitamin D3, cholecalciferol. In this study we investigated the effects of ERGO and D3, and combinations on the growth of all epithelial cancer cell lines. Cell viability and cytotoxicity was determined using the CellTiter-Glo® 2.0 assay that measures the amount of ATP present, an indication of the presence of metabolically active cells. Caspase activity for apoptosis was determined with Caspase-Glo® 3/7, Caspase 8, ApoTox-Glo™ Triplex Assay Reagents and flow cytometry. Both ERGO and D3 inhibited the growth of all cell lines, except lung and ovarian cancers. The IC_{50} ranged from 19-56 μM. However, when combined together the IC_{50} for the combination of ERGO and D3 was significantly reduced to a range of 5-8.0 μM. All trans-retinoic acid (ATRA) inhibited the growth of all cell lines tested, except lung and ovarian cancers with an IC_{50} range of 1.8 to 16.5 μM. When ATRA was combined with ERGO and D3, the IC_{50}s were significantly reduced to 0.65 to 2.8 μM. Interestingly, ATRA and the combination had the most significant effects on gastric cancer cells. In breast and colon cancer cell lines, ERGO induced apoptosis via caspase 3/7 and 8.
This work was funded in part by a grant from the First Analysis Foundation to GBM, a Schlumberger Foundation Fellowship to TOL, and a Raman Postdoctoral Research Fellowship to NR.

PB

Novel mechanisms of action for black cohosh (Actaea racemosa L.) extracts

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Black cohosh, *Actaea racemosa* L. [syn. *Cimicifuga racemosa* (L.) Nutt., Ranunculaceae] extracts have been used for many years by European and American women for the management of menopausal symptoms, such as hot flashes, depression, and sleep disturbances. One of the primary stumbling blocks to the

progression of the clinical science is the lack of a plausible mechanism of action (MOA) for black cohosh extracts (BCEs). In this investigation, black cohosh extracts a 40% isopropanol extract and a 75% ethanol extract of black cohosh rhizomes were used. Three pure compounds actein (1), 23-epi-26-deoxyactein (2) and cimiracemoside A (3) were purchased from Chromadex (Santa Ana, CA). Granulosa cells (GCs) were cultured in DMEM/F12 media with or without 10% FBS. The results show that BCEs have a biphasic effect on cultured GCs. At very low concentrations (0.5-5 μg/ml) the extracts enhance the growth of GCs, but at 10 μg/ml they inhibit the growth of GCs and induce apoptosis at 10-20 μg/ml. BCEs reverse GC cell cytotoxicity and apoptosis induced by serum starvation, with 0.5 μg/ml restoring cell growth almost as effectively as 10% FBS. Since Actein (AC) and 23-epi-26-deoxyactein (DOAc) are the major triterpenes present in BCEs, and cimiracemoside A is present in commercial BCEs, we tested the effects of these compounds on the viability cultured GCs. Both AC and DOAc have biphasic effects on cultured GCs with very low concentrations (5.0 ng/ml) enhancing the growth of the cells by 35-80%, but at higher concentrations, both compounds (5 μg/ml) reduced cell viability by 40-55%. Our data suggest that concentrations < 5 μg/ml BCEs reduces apoptosis by increasing IGF-1. From this work, we propose that BCEs exerts its pharmacological effects on menopausal symptoms through suppression of ovarian granulosa cell apoptosis via the IGF-1 pathway and indirectly increase both E2 and P4 through this novel mechanism.
This work was supported in part by a Schlumberger Foundation Postdoctoral Fellowship award to TOL and GBM

PB

Ribes nigrum L. (Grossulariaceae) and Sambus nigra L. (Adoxaceae) extracts enhance growth and inhibit apoptosis in rat L6 muscle cells.

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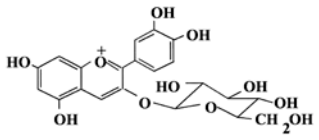
Musculoskeletal disorders affect more than one out of every two persons in the United States over the age of 18, and nearly three out of four over the age of 65. One such disease, sarcopenia is age-related and causes a loss in muscle mass and function in the elderly, causing serious morbidity and mortality. Advanced age is associated with increased enhancement of apoptosis in skeletal myocytes. The extent of apoptosis in aging muscle increases as people age and this increase parallels the loss of both muscle mass and strength. Down-regulation of myocyte apoptosis can be induced by caloric restriction, exercise training, hormone supplements, drugs and various nutrients. In this study we have investigated the effects of berry extracts, specifically Ribes nigrum (blackcurrant) and Sambucus nigra (elderberry) semi-purified extracts on muscle cell apoptosis in L6-rat muscle cells. In this study we investigated the effects of ERGO and D3, and combinations on the growth of all epithelial cancer cell lines. Cell viability and cytotoxicity was determined using the CellTiter-Glo® 2.0 assay that measures the amount of ATP present, an indication of the presence of metabolically active cells. Apoptosis was determined with Caspase-Glo® 3/7, Caspase 8, ApoTox-Glo™ Triplex Assay Reagents. In L6 cells, both R. nigrum and S. nigra ethanol fruit extracts concentration-dependently enhanced the growth of the cells by 300% and 200% respectively. In serum-starved L6 cells,

both extracts prevented L6 cell apoptosis, and in glucose and serum starved cell, the extracts also prevented cell death and apoptosis. Of the anthocyanins present in the extracts, cyanidin-3-glucoside was the most active and enhanced L6 growth by 200%. These data suggest that fruit extracts reduce muscle cell apoptosis and may be useful for development as a preventative treatment for sarcopenia.



Cyanidin 3-*O*-β-D-glucoside

This work was funded in part by a Postdoctoral Fellowship award from the Schlumberger Foundation to TOL and GBM, and a UGC-Raman Postdoctoral Research Fellowship to NR.

PB

Activation of PXR by Bulbine natalensis and its constituents

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Bulbine natalensis (Asphodelaceae) is an indigenous plant of South Africa which is traditionally used for its wound healing and antidiarrheal properties, and as an aphrodisiac. Recently, use of *Bulbine natelensis* in bodybuilding and performance enhancing supplements is on the rise due to its androgenic and aphrodisiac properties. With the increased use of this herb in health products which are often taken along with the conventional drugs, there is always an increased risk for herb drug interactions. This study is aimed to evaluate the effect of *B. natalensis* extract and its constituents on pregnane X receptor (PXR) and its target genes (CYP3A4, CYP1A2, CYP2C9, CYP2B6, and P-gp). The nuclear receptor, PXR, is the master regulator of several cytochrome P450 enzyme isoforms and the efflux transporters, induction of which is known to be one of the mechanisms responsible for clinically relevant herb-drug interactions. Methanolic extract of the stems of *B. natalensis* and its commomnly known constituents (phenylanthraquinones, anthraquinone dimers, and anthraquinone glycosides) were screened for drug interaction potential mediated through PXR mechanism. The extract and three constituents showed significant activation of PXR (2-8 fold increase) that resulted in an increase in the mRNA expression of CYP3A4 (2-10 fold), CYP1A2 (3-6 fold), CYP2C9 (2-4 fold), and CYP2E1 (2-20 fold).

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PB

Pharmacokinetics of aegeline after oral administration in mouse model

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Aegeline is the primary pharmacologically active constituent of *Aegle marmelos* (Rutaceae family). Preclinical studies have shown the therapeutic potential of aegeline against several disorders. In addition, aegeline was added in several weight-loss dietary supplements. However, the FDA has banned aegeline containing supplements such as OxyElite Pro and VERSA-1 due to clinical cases of acute and chronic liver failure. Despite severe toxicity reports, there are no studies documenting the pharmacokinetic properties of aegeline. Accordingly, this study was focused on evaluating the pharmacokinetics of aegeline, after oral administration (30 mg/kg) in mice. The elimination half-life was found to be 1.25 h suggesting that rapid elimination. The C_{max} and AUC was calculated to be 0.92 µg/mL and 2 h*µg/mL, respectively. The volume of distribution (V_d) was found to be 40 L suggesting extensive distribution to tissues. Furthermore, tissue distribution (liver, kidneys, and brain) and metabolic pathways of aegeline will also be presented.

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PB

SAFEY AND EFFICACY OF PELVIGEN SUPPLEMENTATION ON URINARY INCONTINENCE IN PERIMENOPAUSAL WOMEN

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Urinary incontinence (UI) is a significant health problem with considerable social and economic impact. According to the National Association for Continence, UI affects over 25 million people in the U.S., of which 75-80% are women. UI affects women of all ages, and risk factors include pregnancy, childbirth, body mass index, previous hysterectomy and menopause. UI and overactive bladder (OAB) result in significant decrease in quality of life and a number of studies have also indicated that UI results in substantial economic burden. Effective treatment may be economically beneficial as well as improving quality of life. The overall efficacy and tolerability profile of current medications is reported to be less than optimal, as side effects such as constipation and dry mouth are common. The etiology of UI varies between subtype with SUI caused by sphincter weakness and UUI a result of overactivity of the detrusor muscle, the smooth muscle that lines the wall of the bladder. The objectives of this study were to examine the effect of the supplementation of a novel combination of high genistein soy bean extract and pyrogallol plus polyphenols from standardized pumpkin seed extract (Pelvigen) in 82 women with incontinence in perimenopause. In brief: Daily dosage of PELVIGEN was 1g/day from 0 to 4 weeks(T1), following a 500 mg/day daily intake from 4 to 8 weeks (T2). RESULTS: Urgency grade score was reduced to 24.7%.The total urge episodes was reduced to 46%. Nocturia was reduced to 69.35%. Strength Urinary Incontinence (SUI) was also tested showing a remarkable 52.17% reduction. The use of daily pantyliners was reduced to 66.25%.The ICIQ-SFquest(Spanish versión) revealed that 96.2% of subjective satisfaction and a 85.8% objective score in the

improvement of quality of life. CONCLUSION: The combination of high genistin isoflavones and pumpkin seed pyrogallol in (PELVIGEN) tablets seems to be a safe and highly effective supplementation for the relieve of the urinary incontinence symptoms

PB

Can we have an adjuvant for TB- patients from a South African plant?

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South Africa remains one of the countries with the highest burden of Tuberculosis worldwide with an estimated 450 000 active TB cases reported by the WHO in 2013. A large number of these cases are co-infections with HIV/Aids. One of the biggest problems in treating TB patients is compliance to the current drug regime. Patients do not always have easy access to clinics to obtain the required medication. In many instances, patients also prematurely cease the use of the medication, which results in reactivation of the disease and also resistance development of the bacteria, this also promotes the spread of the disease. It is estimated that a patient with active TB will infect/affect on average 1 in 10 individuals. Our study focused on evaluating one indigenous South African plants for antimycobacterial, immune stimulatory and hepatoprotection activities. The plant *Euclea natalensis* (*En*)is from the Ebenaceae family, and the shoots of the plant was used for scientific evaluation.
En is frequently used by various tribes of South Africa for skin lesions in leprosy, for the relief of toothache and headache, respiratory problems such as bronchitis, pleurisy and asthma. The antimycobacterial activity of a semipure fraction from the shoots of the plant was found to be 125 µg/mL. The sample was also found to be a non-mutagen with high antioxidant activity ($IC_{50;}$ 22.55 µg/mL). The fraction showed low cellular toxicity on primary PBMC's ($IC_{50;}$ 131.3µg/ml), secondary U937 monocytes ($IC_{50;}$ 208.9µg/ml) and Chang liver cells ($IC_{50;}$ 95.29µg/mL). *En* exhibited immune stimulatory effects by increasing the production if Interleukin 12, a cytokine important in TB containment, and showed hepatoprotection against acetaminophen toxicity induced damage. The preclinical *in vivo* animal studies have indicated no toxicity at the tested concentrations. The fraction was able to not only decrease the bacterial burden in infected mice, but also to protect the liver against the toxicity induced by ethanol and some of the first line TB drugs. The bioactive compounds have been found to be naphthoquinones.
A PCT patent have been filed on this plant extract and a phase I clinical safety trail is planned for this prototype.
NRF, University of Pretoria, Department of Science and Technology, Medical Research council South Africa

PB

DNA Barcoding for the Identification of Botanicals in Herbal Medicine and Dietary Supplements: Strengths and Limitations

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DNA barcodes, a term introduced by Hebert et al., describe short genomic regions from the nuclear and/or organelle genome used to distinguish animal, plant, fungal, and bacterial species. The use of these short genomic regions for biological species discrimination is called DNA barcoding. The herbal products industry is a multibillion-dollar industry and an important part of the worldÊ¼s economy. However, as the popularity of herbal dietary supplements has increased, so have reports of adulteration; this ad-mixture, or substitution, of herbal products/supplements with materials of substandard quality is a growing concern since it may lead to decreased efficacy and the occurrence of serious adverse events. Price pressure, increased demand, limited availability of medicinal herbs, and greed of unscrupulous suppliers are some of the reasons for the intentional substitution of botanical ingredients. Accurate identification of medicinal herbs is a legal requirement in most countries and prerequisite for delivering a quality product that meets consumer expectations. Traditional identification methods include botanical taxonomy, macroscopic and microscopic examination, and chemical methods. Advances in the identification of biological species using DNA-based techniques have led to the development of a DNA marker based platform for authentication of plant materials. The present work gives an overview on the strengths and limitations of DNA barcoding techniques for identification of botanical ingredient and dietary supplements.

PB

Inhibitory effect of Nymphaea odorata on Gastric Cancer Cell Lines AGS and NCI-N87: Correlation to Helicobacter pylori infection

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Nymphaea odorata Aiton (Nymphaeaceae; American white water-lily) is an aquatic perennial plant root used in First Nation, Ayurvedic and Chinese traditional medicine for the treatment of various ailments. The dried root and rhizome of the white water lily are used orally for the treatment of gastrointestinal, genital, and bronchial diseases. The leaves and roots have also been used externally, as infusions to treat lesions and inflammation associated with mucous membranes.

In this work we have investigated the effects of extracts of *N. odorata* on the growth of *Helicobacter pylori,* the etiologic agent for gastritis, peptic ulcer, gastric MALT lymphoma and gastric cancer, as well as the effects of the active extract on gastric cancer cells *in vitro*. In 17 clinical strains of HP and the ATCC strain 43504, a methanol extract of *N. odorata* inhibited the growth of all HP strains at a concentration of 12.5 mg/ml, with an MIC of 9.25 mg/ml. The second part of the study, the effect of the methanol extract of roots of *N. odorata* was investigated in two gastric cell lines namely, AGS and NCI-N87 at concentrations up to 100 µg/mL. Control cells were treated with vehicle solvent (DMSO 0.02%). Cytotoxicity and cell viability was determined using the CellTiter-Glo® 2.0 assay. The IC_{50} values of methanol extract of *N. odorata* root was found to be 26.79 µg/mL in AGS and 35.44 µg/mL in NCI-N87. The results of this study indicate that extracts of the roots of *N. odorata* inhibit the growth of HP strains providing a plausible of action in the treatment of GI disorders and may be a potential new candidate for the treatment of gastric cancer.

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PB

Methanol extracts of Nymphaea odorata Aiton (Nymphaeaceae) roots are cytotoxic in MCF-7 cell lines and induce apoptosis

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Breast cancer is highly prevalent worldwide and remedies from the herbal origin are always considered safe compared to other treatments for cancer. *Nymphaea odorata* (Nymphaeaceae), the American water-lily is a perennial aquatic plant native to the USA and Canada. Preparations of the roots and leaves of *N. odorata* have been used orally in traditional medicine for the management of cancer, chronic diarrhea, as well as topically for burns and open wounds. In the early 1900s, Eli G. Jones employed *N. odorata* in his treatment of various cancers and he was an early pioneer of America Eclectic Medicine prior to the advent of pharmaceutical treatments. In this work, we assessed the effect of methanol extracts of the roots of *N. odorata* in the estrogen receptor positive human breast adenocarcinoma cell line MCF-7. Coarsely powdered roots of *N. odorata* were extracted with methanol and the extract was air dried. Cytotoxicity and cell viability assay was performed using CellTiter-Glo® 2.0 assay in MCF-7 and IC_{50} value was determined. Control cells were treated with vehicle solvent (0.02% DMSO). Mechanism of cell death was determined by employing Alexa Fluor® 488 annexin V/Dead Cell Apoptosis Kit with Alexa® Fluor 488 Annexin V and PI for Flow Cytometry. The methanol extract of *N. odorata* exhibited cytotoxicity activity on MCF-7 with IC_{50} of 20.6 µg/mL and the results of flow cytometry has revealed that, it induces apoptosis in MCF-7. The results suggest that, *N. odorata* may be an effective remedy for the treatment of breast cancer. Further investigations to isolate the active phytoconstituents and mechanisms of action are currently underway.
This work was funded in part by a Raman Post-Doctoral Fellowship by University Grants Commission, Govt. of India to NAR; a Schlumberger Foundation Postdoctoral Fellowship to TOL and a grant from the First Analysis Foundation to GBM.

PB

PSORALEA CORYLIFOLIA AND EPIMEDIUM BREVICORNUM DO NOT ACTIVATE THE ESTROGEN RECEPTOR Î± OR Î² IN A MANNER SIMILAR TO 17-Î² ESTRADIOL

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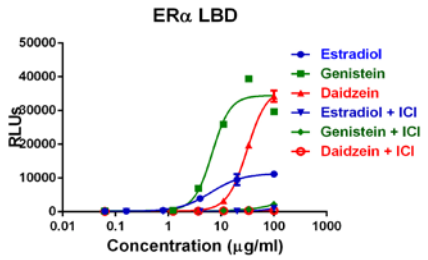
Phytoestrogens are non-steroidal plant-derived compounds that have been shown to have estrogenic activity. Is the estrogenic activity the result of these compounds binding directly to the estrogen receptor (ER) and directly activating it or is it the result of indirect ER activation through other signaling

pathways? This distinction between direct and indirect activation may be important when considering the safety of particular phytoestrogens. Compounds that directly activate the ER, similar to 17-β estradiol, may be less controlled by negative feedback and the lack of negative feedback regulation may be a safety concern. Indirect phytoestrogens may be more negatively regulated and may present less of a concern. To assess direct and indirect ERα or ERβ activation by phytoestrogens, luciferase reporter assays were developed using isolated Ligand Binding Domains (LBD) for ERα and ERβ fused to Gal4. Additionally, full length ERα or ERβ reporter assays were developed. We show that 17-β estradiol activates each of these four assays and we evaluated known phytoestrogens, genistein and diadzein. Next, we selected a number of TCM ingredients that have been described for the treatment of low bone density and assessed their activity. *Psoralea corylifolia* and *Epimedium brevicornum* activated the full length ERα and ERβ. However, only *P. corylifolia* activated the LBD construct. It activated ERβ; it did not activate the LBD of ERα. *E. brevicornum* did not activate either the LBD of ERα or ERβ. Genistein and diadzein were active in each of the 4 assays and ERα or ERβ receptor activation was confirmed using ER inhibitor ICI 182780. Gene activation involved with ERα or ERβ activation are also evaluated and discussed.



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PB

THE USE OF MOLECULAR DOCKING AND COMPUTATIONAL MODELING TO PREDICT HERBAL CONSTITUENT INTERACTIONS WITH CYP450 ENZYMES.

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Herb-drug interactions can result in serious adverse events; associated with an estimated 5% of hospital admissions, and over 100,000 deaths per year. Patients are reticent to disclose herbal product usage to their healthcare providers, and many providers still do not inquire about such usage. Dietary supplements, including herbal products, are not subject to the same regulatory guidelines for pre-market testing as drugs. This lack of a clear definition of risk prevents clinicians and consumers from making informed decisions about the safety of taking herbal products with conventional medications. A framework is needed that describes an integrated and sophisticated tiered approach for assessing HDI potential of dietary supplement ingredients and products. A need exists for an early screening tier to predict potential herb – drug (HDI) and herb – herb interactions (HHI) and prioritize ingredients for second tier testing (e.g., *in vitro* studies). We propose using molecular docking and computational modeling as an initial screening

tier. To do so, our pilot work estimated binding modes and interaction potential of known herbal constituents from milk thistle with human CYP3A4 and CYP2C9. The milk thistle constituents, silybin A, silybin B, isosilybin A, isosilybin B, silychristin, and silibinin were chosen since IC50 and/or Ki data exist for them. Furthermore, clinical data relative to the ability of milk thistle and constituents to cause meaningful inhibition of drug clearance is also available. The milk thistle constituents were examined using the Glide ligand-receptor docking portion of the Schrodinger software package. Because the IC50 values for the milk thistle constituents were highly similar for CYP3A4, their glides scores fell within a tight range of -7.7 to -8.233, as expected. The most potent constituent identified for CYP3A4 *in vitro*, isosilybin B (56μM), resulted in the lowest g-score for this data set indicating the lowest free binding energy. Isosilybin B is not a potent inhibitor of CYP2C9. This was successfully demonstrated *in silico* as this compound failed to dock in the binding site. This approach may provide a rapid and high capacity screen to predict the effects of herbal constituents on CYP450-mediated drug metabolism and transporter activity and potential drug interactions.

PB

Evaluation of 4-O-Methylhonokiol as a modulator of oxidative stress in Japanese medaka embryogenesis

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The present experiment was aimed to study the effect of 4-*O*-methylhonokiol (4-*O*-MH), a neolignan isolated from Magnolia grandiflora seeds, in the modulation of oxidative stress-related enzyme genes during Japanese medaka embryogenesis. Fertilized medaka eggs (Iwamatsu stage 10) were exposed to 1, 2, 5, and 10 μM 4-*O*-MH for 0-6 days post fertilization (dpf) and the heart beats, vessel circulation, thrombus formation, hatching efficiency, and mortality were evaluated. Heartbeats were determined on 3 and 6 dpf embryos. Compared to control embryos, heartbeats were found to be decreased significantly in 2, 5, and 10 μM groups in 6 dpf. Thrombi were seen only in embryos exposed to 5 and 10 μM 4-*O*-MH that can cause significant delay in vessel circulation. The embryos exposed to 5 and 10 μM 4-*O*-MH for 0-6 dpf reduced hatching efficiency in comparison with other groups. The calculated LD_{50} as determined on 10 dpf from three independent experiments is 5.99 μM. We hypothesized that oxidative stress may play a significant role in inducing these toxicological effects. To verify our hypothesis, we analyzed three genes including catalase, glutathione s-transferase (GST) and glutathione reductase (GR) mRNAs at the transcription level. Our data indicate that mRNAs of these antioxidant enzymes were remained unaltered which suggests that the toxic potential of 4-*O*-MH is mediated by mechanisms other than oxidative stress.

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PC

Anticholinesterase Constituents from the leaves of Spondias mombin L. (Anacardiaceae)

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Spondias mombin has been used in traditional medicine for the management of several diseases including memory loss.

This research was carried out to evaluate the cholinesterase inhibitory activity of the methanol extract of the leaves, its derived fractions as well as detailed phytochemical investigations leading to the isolation and characterization of bioactive compounds from the plant. The acetyl cholinesterase (AChE) and butyryl cholinesterase (BUChE) inhibitory activities were evaluated by colorimetric and TLC bioautographic assay techniques.

The results which showed that the ethyl acetate fraction was most active against both enzyme as well as the bioactivity guided analysis of the three compounds isolated from the plant will be presented.

Keywords: Alzheimer's disease (AD), Acetylcholinesterase, butyrylcholinesterase, neurodegenerative, *Spondias mombin.*

PC

Investigation of Bioactive Compounds of Ethanolic Extract of Seeds of Cannabis sativa L. from Sabzevar – Iran

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Using plants as being natural sources have some advantages such as low side effects, low cost and also being easily accessible in comparison to common treatment methods. Therefore, herbs may play an important role in the treatment of the cancer in the future,. In recent years, many attempts have been made to use herbs for the cure of cancer [1,2,3]. The aim of the present study was to investigate the bioactive compounds of ethanolic extract of seeds of *Cannabis sativa* L. belonging to the family *Cannabidaceae*. The seeds of *C. sativa* L. were collected from Sabzevar, Iran and ethanolic extract prepared by microwave assisted extraction (MAE) method. The present study revealed that the phytochemicals analysis of twelve different chemical compounds alkaloids (MayerÊ¼s Test), alkaloids (WagnerÊ¼s Test), terpenoids (Salkowski Test), flavonoids (Alkaline Reagent Test), phenols (Ferric Chloride Test), coumarins (sodium hydroxide Test), tannins (Ferric Chloride Test), phlobatannins (HCl Test), cardiac glycosides (Keller-Killani test), quinones (H_2SO_4 Test), di-terpenoids (copper acetate Test), and saponins (Foam Test) were tested in ethanolic extracts. The results of the phytochemical screening of ethanolic extract of seeds of *Cannabis sativa* L. were alkaloids (both of two test), cardiac glycosides, phenols (a few), Di-terpenoids, coumarins (a few), quinones and tannins presented. Each active compound showed different activities against different types of diseases like cancer, liver disorders, diabetes, atherosclerosis and inflammatory diseases etc. According to their characteristics, they can be involved into medicinal plant category.

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

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PC

Further Constituents of Acacia nilotica Delile with Kinase inhibitory activity

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The genus Acacia (Leguminasae) includes some 1400 species of trees and shrubs widespread throughout the warm arid and semi-arid regions of the world, including Nigeria (1-3). Previously, we have isolated two new Peltogynoids from this plant with activity against DYRK1A (4). In this present study as part of our continuing study on the genus Acacia for protein kinase inhibitory compounds, we report herein the protein kinase inhibitory activity of the fractions and compounds from the stem bark of *A. nilotica*. The pulverised stem bark of this plant was extracted with 70% ethanol and the crude ethanolic extract was suspended in water and partitioned with chloroform, ethyl acetate and n-butanol. A portion of each of these fractions was screened for protein kinase inhibitory activity against a panel of fourteen protein kinases. The ethyl acetate fraction which was the most active was subjected to column chromatography using silica gel and gel filtration over sephadex LH-20 leading to the isolation of ethyl gallate (I) and (+)-catechin (II), the structures of the isolated compounds were elucidated using NMR and MS and compared with the literature. The compounds were screened for protein kinase inhibitory activity. Compound II was more active than compound I by inhibiting nine out of fourteen protein kinases with IC_{50} in the range of 5-32 µg/ml. Compound II also gave the highest activity against CLK1 with IC_{50} of 5 µg/ml, while compound I inhibited the activity of CLK1, GSK3, PIM1, and Haspine kinases with IC_{50} of 27, 30, 35 and 45 µg/ml respectively. The result suggests that the ethyl acetate soluble fraction of *Acacia nilotica* ethanol extract, which contains polyphenols such as catechins and derivatives, has protein kinase inhibitory activity, which can be exploited as leads in the search for anti-cancer agents.

PC

Phytochemical constituents of the stem bark of Pentaclethra macrophylla (Fabaceae)

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Pentaclethra macrophylla (Fabaceae) commonly known as African oil bean tree (locust bean) is a tree which grows up to a height of 21 m and 60 cm girth. It is a plant widely used in African ethnomedicine to treat a

variety of ailments which include wound healing, inflammation and as remedy to treat gonorrhea and convulsion [1]. An infusion of the root bark is use as a laxative and as enema against dysentery, while the seeds are eaten boiled, or roasted. The fermented seed is very popular as condiment in South Eastern Nigeria [2]. Antininoceptive, anti-inflammatory and cytotoxicity of the extracts of this plant have been reported [3], while [4] has reported the antimicrobial activity of the seed extract. The antimicrobial activity of the seed oil and the stem bark extracts of this plant have been reported [5]. In this present study, we report here in the isolation and structure elucidation of three compounds from the ethylacetate and n-butanol soluble fractions of the ethanol extract of the stem bark of this plant namely: Methyl gallate (I), Bergenin (II) and 11-O-galloyl bergenin (III) .The structures were elucidated using NMR and MS and compared with literature. These compounds are being reported for the first time from this plant.\

PC

Progress towards the Synthesis of (-) Mesembrine

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We report our progress towards the synthesis of (-) mesembrine, a naturally occurring alkaloid 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 endorse wellbeing. It displayed a potent serotonin reuptake inhibitory activity in the nanomole range with probable usefulness in treatment of depression and anxiety.[1] It's 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,[2] some synthetic studies have been published to control the construction of the sterically hindered, benzylic quaternary stereogenic center at C-3a[3] since methods for its preparation are limited in number.[4] We utilized Michael addition on chiral enolate derived from a cheap starting material 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.

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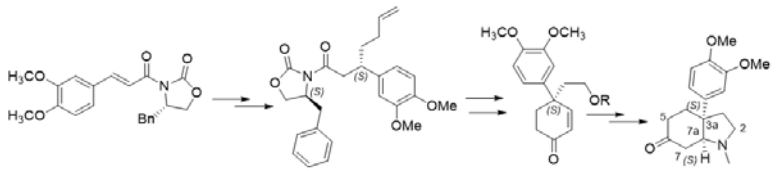

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PC

In chemico evaluation of skin sensitization potential of authentic and non-authentic tea tree oils.

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Tea tree oil (TTO) is obtained after steam distillation of the leaves of *Melaleuca alternifolia*, *M. linariifolia* or *M. dissitiflora*. Tea tree oils has been classified as potential skin sensitizers, and aged oils has been classified as stronger sensitizers compared to fresh ones. Due to the commercial relevance of TTO, substitution or adulteration with other tea tree species (such as cajeput, niaouli, manuka or kanuka oils) is common, although the chemical fingerprint of non-authentic TTOs may be remarkably different from authentic ones. The distinctive nature, qualitative and quantitative compositional variation of these oils is responsible for the various pharmacological activities, and may impact the insurgence of adverse effects
as well.

Regulated *in chemico* methods may be of limited application in the presence of complex and/or unstable mixtures such as TTOs. Chemical sensitizers are usually reactive electrophilic compounds, thus *in chemico* methods can be used to characterize the ability of potential skin allergens to bind to their biological targets. Model nucleophiles have been developed as surrogates to determine the potential of a chemical to covalently bind to skin proteins, which is considered a first key event triggering the sensitization cascade.

In the present study, ten "tea tree" oils and six major TTO constituents have been investigated for chemical stability in correlation to their sensitization potential using a recently developed fluorescence *in chemico* method (HTS-DCYA). Changes in the chemical composition of authentic and non-authentic TTOs were evaluated by GC-MS after 18 months storage. The reactivity of authentic TTOs toward a model thiol (dansyl cysteamine, DCYA) was found to correlate with the age of the oils. Further thio-trapping experiments were performed and potential thio-adducts were identified by UHPLC-DAD-MS. Among pure compounds, major TTO components such as terpinolene, α-terpinene and terpinene-4-ol were found to be unstable upon accelerated aging conditions, which led to formation of several thio-adducts with dansyl cysteamine.

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PC

In chemico evaluation of potential skin sensitizers generated upon radical activation of ascaridole.

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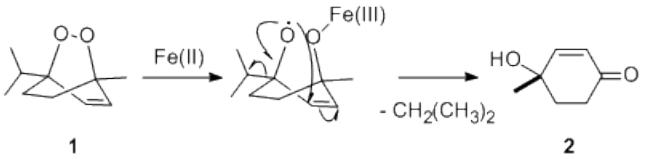
Tea tree oils (TTO) undergo significant alterations in chemical composition after exposure to air, light or heat. Major non-oxygenated monoterpenes are subjected to oxidative and radical reactions leading to the formation of organic peroxides, hydroperoxides, and epoxides among others. Ascaridole (**1**) is one of the major oxidative byproducts accumulating in TTOs after degradation of terpinenes. This small endoperoxide (**1**) has been identified as one of the causative agents responsible for contact allergy observed with TTOs. It

has been hypothesized that a free radical mechanism might be involved in the haptenation process following exposure to ascaridole, although no further information on the radical species formed and/or their correlation to the observed adverse effects is available.

The applicability of *in chemico* methods for the identification and characterization of skin allergens in complex mixtures is the missing element in the battery of regulatory agencies' recommended methods. In the present work, a recently developed high-throughput screening method (HTS-DCYA) was applied to understand the reported enhanced reactivity of ascaridole upon activation with a radical initiator. The reactive, and often elusive, intermediates of the activated ascaridole were identified and characterized in terms of electrophilic reactivity by trapping with dansyl cysteamine (DCYA). As a result of such an approach, a substituted cyclohexenone (**2**) was identified as a potential electrophilic intermediate resulting in complete depletion of DCYA. Along with **2** several nonreactive byproducts of ascaridole were identified. Based on such information, generation of reactive electrophiles via radical mechanisms should be considered as one possible source of the observed increased peptide reactivity of activated ascaridole.



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

PC

Study of cytotoxicity of E. umbellata hexane fraction and euphol against leukemia cell lines

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Euphorbia umbellata (Pax) Bruyns latex is used ethnopharmacologically for the treatment of cancer, including leukemia [1,2]. A preliminary biomonitored in vitro study of hexane fraction (1.25-75 µg/mL) from the latex and the main substance of this material (euphol - 10-50 µg/mL) was executed using MTT assay to determine the cytotoxicity against HL-60 and Jurkat cells. The hexane fraction was also fractionated and the resulting subfractions (ether, diclomethane, ethanol and

methanol) were analyzed by gas chromatography coupled to the mass spectrometer (GC-MS). The cytotoxic assay demonstrated IC50 of 0.061 ± 1.226 μg/mL (HL-60) and 4.624 ± 1.088 μg/mL (Jurkat) for hexane fraction, and IC50 of 21.031 ± 1.778 μg / mL (HL-60) and 35.925 ± 5.011 μg / mL (Jurkat) for euphol [3]. Ten substances were identified from the subfractions: euphol, lanosterol, cycloartenol, tirucallol, taraxasterol, lupeol, phorbol-12,13,20-triacetate, 4-β-phorbol, 3-deoxo-3-16-dihydroxy-12-deoxyphorbol-3-13-16-20-tetracetate, and sitosterol.

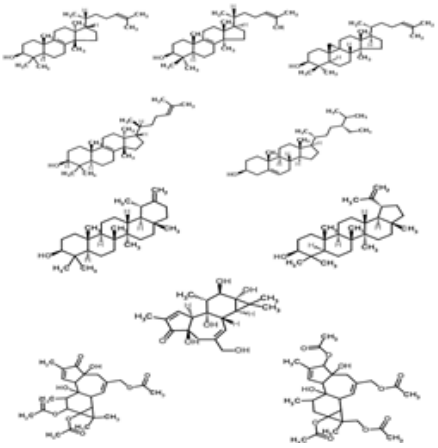

Figure 1: Euphol(A); Lanosterol(B); Cycloartenol(C); Tirucallol(D); Sitosterol(E); Taraxasterol(F); Lupeol(G); Phorbol-12,13,20-triacetate(H); 4-β phorbol(I); 3-deoxo-3-16-dihydroxy-12-deoxyphorbol-3-13-16-20-tetracetate(J).

The preliminary research suggests that E. umbellata hexane fraction presents a cytotoxic effect against leukemic cells and for the isolated substance this effect decrease. This indicates that the compounds present in the hexane fraction can be promising for the treatment of leukemia, possibly by a synergic action.

Acknowledgements: The authors are grateful to Fundação Araucária (Research Grant 234/2014) and technical support of State University of Ponta Grossa (UEPG) and National Centre for Natural Products Research (NCNPR – University of Mississippi). *References: [1] Luz et al. (2016) JEP 183, 29-37, [2] Luz et al. (2015) BJP 25, 344-352, [3] Cruz et al. (2016) poster in VIII SIPM.*

PC

Chemical constituents from the dried fruits of Morus alba and their inhibitory activities against PCSK9 mRNA expression

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Cardiovascular disease (CVD) is leading of the death in the world, caused by behavioral risk habits such as smoking, diet, alcohol and obesity. The risk factor of CVD was affected by elevated low-density lipoprotein cholesterol (LDL-C). 3-Hydroxy-3-methylgataryl-coenzyme A (HMG CoA) reductase inhibitors have been treated to lower LDL-C levels, but they did not fully achieve this goal. Recently it was reported that proprotein convertase subtilisin-kexin type 9 (PCSK9) that binds to the LDLR in the liver leads to high levels of LDL-C. Therefore, inhibition of PCSK9 was emerged as an attractive target and several PCSK9 inhibitors were under clinical trials. Recently, USFDA have been approved two antibody-based PCSK9 inhibitor drugs. Until now, only berberine and curcumin have been shown to inhibit PCSK9 mRNA expression as natural compounds. During the initial screening to monitor PCSK9 mRNA expression in HepG2 cell lines, the chloroform-soluble extract from *Morus alba* fruits was found to be active in inhibiting PCSK9 mRNA expression. From this active fraction, two new and 13 known structures were isolated and

characterized. In the bioassay using HepG2 cells, compound 7 was found to inhibit PCSK9 mRNA expression.

This work was supported by the GRRC program of Gyeonggi province (GRRC DONGGK2016-B01, Development of new health supplements/therapeutics for neurodegenerative diseases and GRRC DONGGUK2016–B03, Development of functional food to alleviating metabolic syndromes and circulatory disorders).

PC

Evaluation of anticancer activities of Lawsonia inermis (Henna) leaves

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This study was aimed to evaluate the inhibitory effect of the two extracts and the active ingredient (lawsone) of *Lawsonia inermis* (Henna) leaves against Ehrlich Ascites Carcinoma (EAC) bearing mice. The anticancer activity of the methanolic (ME) and the diethylether (EE) extracts of *L. inermis* leaves as well as the major metabolite (lawsone) was *in vivo* evaluated. The *in vivo* anticancer activity was determined using Ehrlich ascites carcinoma mice model by monitoring the effect on tumor volume, DNA and RNA concentrations, liver and kidney functions, lipid profile, hemoglobin levels, and status of antioxidant enzymes such as lipid peroxidase, reduced glutathione, superoxide dismutase and catalase activities. The Results showed that the treatment of tumor bearing mice with lawsone, extracts (ME, EE) and 5-FU (positive control) cause significant reduction in tumor volume compared with those of tumor-bearing mice saline treated. This reduction in tumor volume was accompanied with a significant decrease in liver DNA and RNA concentrations, liver function enzymes, urea and total lipids. The extracts prevented lipid peroxidation and restored the antioxidant enzymes as well. The total phenolic contents and flavonoid contents were also determined and the EE was found to have the highest content

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PC

Phytochemical Constituents from *Moringa oleifera*

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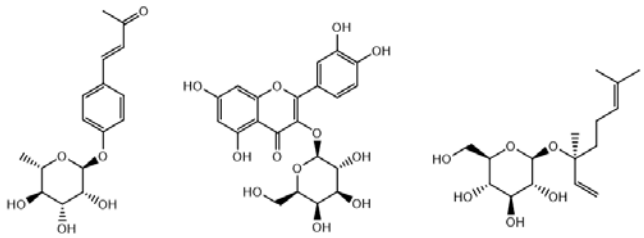
Moringa oleifera Lam. is known as a drumstick tree that widely cultivated in various subtropical and tropical provinces. It has been called the miracle vegetable because all parts of *Moringa* such as pods, leaves, roots and seeds have been exploited to treat a variety of ailments. Beside antioxidant properties, it shows antihypertensive, cardioprotectant, hepatoprotectant, anti-inflammatory, antimicrobial, antidiabetic and

anticancer activities. Phytochemical investigation of methanolic extract for *Moringa oleifera* leaves resulted in the isolation and characterization of eight secondary metabolites including one new compound. Structure elucidation was achieved by 1D, 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.

PC

Absolute Configuration and Therapeutic Potential of Novel and Known Sesquiterpenoids Isolated from Calea urticifolia

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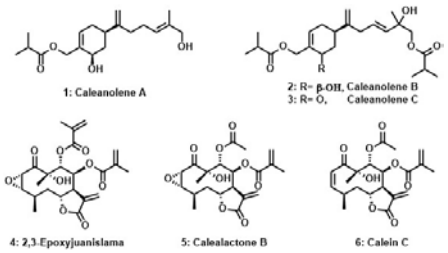
Calea urticifolia is a psychoactive medicinal plant that belongs to the Asteraceae family with known folkloric uses in the treatment of fever, diarrhea, and calming effects. Phytochemical studies of *C. urticifolia* yielded three novel bisabolenes, caleanolenes A-C (**1**-**3**), and three known sesquiterpene lactones: 2,3-epoxyjuanislama **4**, calealactone B **5**, and calein C **6**. The chemical structures of the isolated compounds were determined on the basis of HRMS, IR, UV and from 1D and 2D NMR spectroscopic studies. Electronic circular dichroism (ECD) calculations and probability analysis (DP4) were used to confirm the assignment of absolute configurations of the caleanolenes A-C (**1**-**3**). ECD spectra were calculated using time-dependent density functional theory (TDDFT) at B3LYP/6–31G** level in a polarizable continuum model (PCM) methanol solvent model. The calculated ECD spectra of the *R* stereoisomer of caleanolene A **1** showed a negative cotton peak at ~210 nm, matching that of the experimental result. Caleanolene B **2** and caleanolene C **3** were calculated to have *R* stereochemical configurations. The known sesquiterpene lactones (**4**-**6**) were evaluated for cytotoxicity against the CA46 and Raji lymphona cell lines, and the MCF7 breast cancer cell line, with compound **5** showing the best activity in all cell lines (IC_{50} value range 2.8 to 5.8 μM).



This project was supported in part by grant number P20GM104932 from the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health (NIH). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIGMS or the NIH. Furthermore, this investigation was conducted in a facility constructed with support from research facilities improvement program C06RR14503 from the NIH National Center for Research Resources (NCRR). Ms. Gogineni is thankful to the graduate student council (GSC) for the GSC research award that supported part of this work.

PC

Characterization of eight Cichorium intybus L. landraces via their yield and phytochemical compositions

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The field experiment was conducted on eight *Cichorium intybus* L. landraces at the farm of Horticulture Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt during two successive seasons of 2013/2014 and 2014/2015. The highest chlorophylls were obtained from the landraces which have white green midrib in their leaves. Qena landrace exhibited the highest significant differences in Chl. a (17.92 and 18.69), Chl. b (8.94 and 11.24) and total chlorophyll (23.94 and 29.93 mg/100g fw) in both seasons respectively. On the other side Behiera landrace record the highest carotenoids (2.13 and 2.30 mg/100g fw) and anthocyanins (4.76 and 3.98 mg/g fw) in both seasons respectively. The Yield, ascorbic acid, TSS, total sugars, Inulin and other constituents in the eight landraces were investigated to determine phytochemical variability that lead to differences in yield and phytochemical profiles. The phytochemical screening confirmed that Behiera landrace exhibited the highest nutritional compositions especially flavonoids (7.12 and 6.54 mg/g dw) and antioxidants as DPPH% (76.44 and 87.30 mg/g dw) in both seasons respectively. The highest total phenols were obtained from Alexandria landrace with recorded value 4.12% in the first season and Behiera landrace with register number 4.11% in second season. The SDS-PAGE of the total seed protein showed close relationship among these studied landraces with some genetic diversity. The identified diverse genotypes can be used in future breeding programs for the development of varieties.

Author is grateful to Al-Azhar University, Faculty of Agriculture, Horticulture Department, Government of Egypt for supporting this research project to carry out this work. The author express their gratitude to Prof. Dr. Shamel Ahmed Shanan at vegetable crops, Department of Horticulture, Faculty of Agriculture, Al-Azhar University and Prof. Dr. Lyle Craker at Stockbridge School of Agriculture, Massachusetts University,Amherst, USA for their constructive suggestion, advice, sincere help and valuable guidance throughout the research study

PC

Hydroxylation of alpha-Isomethylionone by Cunninghamella Elegans.

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Ionones, the most investigated C_{13}-apocatenoids, are important constituents of many essential oils containing a trimethylcyclohexane moiety which make them valuable fragrance and flavor material to industry.[1] Their odor ranges from violet to woody notes. a-ionone along with methyl ionones are the main constituents of ionones used in flavor and perfume industry. Among them, the finest orris-violet odor is due to a-isomethyl ionone .[2]

The pale yellow synthetic compound, a-isomethyl ionone , used in cosmetic products is known to cause skin irritation, especially those with sensitive skins which restrict its use as a fragrant ingredient. It is also considered as a possible allergen. However, the Food and Drug Administration has given the approval as a flavor agent.[3]

Since, microbes can mimic mammalian metabolism of xenobiotics it was decided to subject to microbial transformation to obtain metabolites for further study. The organism used was *Cunninghamella elegans.* Two metabolites were isolated and characterized as *cis*-3-hydroxy-a-isomethylionone and *trans*-3-hydroxy-a-isomethylionone by physical methods including NMR spectroscopy .

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Acknowledgements: The authors thank Mr. Frank Wiggers for assistance in obtaining 2D NMR spectra and Dr. Bharathi Avula for conducting HRESIMS analysis. This work was supported, in part, by the United States Department of Agriculture, Agricultural Research Specific Cooperative Agreement No. 58-6060-6-015.

PC

Effects of extracts of Anthemis austriaca in surgically rat endometriosis model and isolation of compounds from the active extracts

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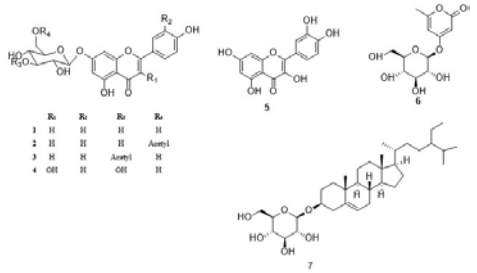
Anthemis austriaca Jacq. is indigenous to Austria, and distributed widely from Europe to Turkey. The plant is used traditionally against abdominal pain, diarrhea, ovarian diseases, hemorrhoids and common colds. The aim of the present study is to determine the effects of this plant in surgically induced endometriosis

model and isolation of compounds responsible for the activity. Hexanes (AAH), ethyl acetate (AAE) and methanol (AAM) extracts were prepared from *A. austriaca* aerial parts, successively. The AAM and AAE extracts displayed significantly decrease in the volumes of endometriotic implants and cytokine levels in treated rats groups compared to the control group. The phytochemical investigation of the AAM extract with the most promising activity led to isolation of apigenin-7-O-*β*-D-glucopyranoside **(1)**, apigenin-7-O-(6-acetyl)-*β*-D-glucopyranoside **(2)**, apigenin-7-O-(3-acetyl)-*β*-D-glucopyranoside **(3)**, quercetin-7-O-*β*-D-glucopyranoside **(4)**, quercetin **(5)**, and, 4-(*β*-D-glucopyranosyloxy)-6-methyl-2*H*-pyran-2-one **(6)**. The phytochemical investigation of AAE extract led to isolation of *β*-sitosterol glucoside **(7)**. All structures were elucidated by NMR spectroscopy and confirmed by HRESIMS.



This study was supported by 2214/A International Research Fellowship Programme, provided by "The Scientific and Technological Research Council of Turkey (TUBITAK)".

PC

Computational approaches towards the design and development of targeted opioid receptor ligands.

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Department of Biomolecular Sciences, Division of Medicinal Chemistry, School of Pharmacy, University of Mississippi, University, MS, USA[1], National Center for Natural Products Research, University of Mississippi, University, MS, USA[2]

Salvinorin A is a neoclerodane diterpenoid and key secondary metabolite isolated from the leaves of *Salvia divinorum*. Furthermore, this natural product is the major metabolite responsible for the perceptiotropic effects observed in *S. divinorum* use. Salvinorin A is also a highly selective κ-opioid receptor (KOR) agonist and the first reported non-nitrogenous opioid receptor agonist.[1] Using the recently published active-state agonist-bound μ-opioid receptor (MOR) crystal structure (PDB ID-5C1M), we developed an *in silico* homology model (**Fig. 1**) of KOR in its active state to aid in the rational design of salvinorin A molecular probes to further explore the three-dimensional structure of the opioid receptors and their interactions.[2,3] We selected the active site by incorporating key residues identified through point-mutation data. Validating our *in silico* model, we established a test database of more than 100 published salvinorin analogues and compared their associated binding affinities to their docking results. To ascertain the predictivity and reliability of our model we designed a series of salvinorin A-based analogues, docked and scored them, synthesizing the prioritized analogues for *in vitro* evaluation. Their binding affinities were determined by competitive radioligand binding assays utilizing stably transfected CHOâ€‘K1 cells expressing human opioid receptor subtypes DOR, KOR, and MOR. This approach generated analogues

possessing greater than 90% radioligand displacement at KOR and MOR (K_i values < 100 nM) in the prioritized ligand sets.

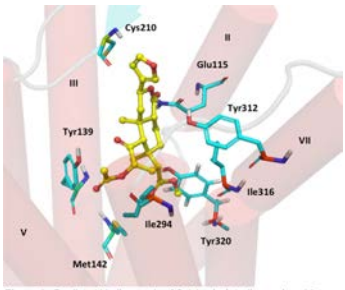

Figure 1. Predicted binding mode of Salvinorin A (yellow carbons) into the active-state KOR model (key residues shown with cyan carbons).

This work was supported, in part, by NIH-COBRE-CORE-NPN grant number P20GM104932 and Grants-in-Aid of Research from the National Academy of Sciences, administered by Sigma Xi, The Scientific Research Society grant number G201503151199208.

PC

Identification of genistein and chrysin in Cytisus villosus as innhibitors on Human Monoamine Oxidases

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Cytisus genus from the Fabaceae family has been used in folk use in different medical problems including to depress the respiration and regulate heart action. We used enzymatic assays to identify natural extracts with inhibition of recombinant human monoamine oxidases (MAO) A and B enzymes An ethanolic extract of *Cytisus villosus* leaves was found to have potent inhibition of MAO-A and B. Bioassay Guided fractionation resulted in the isolation and identification of a flavone, chrysin and an isoflavone, genistein as the bioactive compounds. Genistein was shown to be more potent towards MAO-B than MAO-A with IC_{50} value of 0.65 µM and 2.74 µM, respectively. While chrysin was found to produce more pronounced inhibition against MAO-A than MAO-B with IC_{50} value of 0.25 µM and 1.04 µM, respectively. In addition, molecular recognition studies were carried out to provide insight into the binding mode of chrysin and genistein on the active site of MAO's isoenzymes.

The project described was supported in part by grant number P20GM104932 from the National Institute of General Medical Sciences (NIGMS) a component of the National Institutes of Health (NIH). The content is

solely the responsibility of the authors and does not necessarily represent the official views of the NIGMS or the NIH. Furthermore, this investigation was conducted in a facility constructed with support from research facilities improvement program C06RR14503 from the NIH National Center for Research Resources (NCRR)

PC

Identification of New Antifungal Bisphosphocholines from the Medicinal Plant Gentiana crassicaulis by LC-MS and NMR

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Gentiana crassicaulis (Gentianaceae) is a medicinal plant whose root is used in traditional Chinese medicine (TCM) as "Qin-Jiao" for the treatment of rheumatoid arthritis, allergic inflammations, low-grade fever in chronic diseases, jaundice, and hepatitis. A number of iridoids have been isolated from this plant and some of them demonstrated anti-inflammatory activities through inhibitions on LPS-induced NO and TNF-α production in macrophage RAW264.7 cells. As part of our antifungal discovery efforts for identification of new lead compounds for drug development, we conducted antifungal screening of the methanol extract of the root of *G. crassicaulis* and its column fractions. The active fractions showed in vitro antifungal activities against *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Candida albicans*, and *Candida glabrata*. In particular, the antifungal potency of the most active fraction is equivalent to the positive control amphotericin B with minimum inhibitory concentrations in the range of 0.63–2.5 ug/mL against *A. fumigatus* and *C. neoformans*. Attempt to isolate active compounds from this active fraction using various separation methods failed due to their high polarities and unusual chromatographic behaviors. Subsequently, utilization of the combined LC-MS and NMR analytical tools successfully identified active compounds as bisphosphocholines. So far only one naturally occurring compound, namely irlbacholine, has been identified within this small class of natural products. Irlbacholine was first isolated from the medicinal plants *Irlbachia alata* (Gentianaceae) and *Anthocleista Galonensis* (Loganiaceae) and showed potent in vitro antifungal activities. In the present study, we have identified irlbacholine and four new structurally similar compounds, which accounts for the observed antifungal activities. Identification of this unique class of antifungal compounds in this TCM plant provides scientific information for its medicinal use.

Acknowledgements: This research is supported by the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No. 58-6060-6-015, NIH, NIAID, grant number 27094, and China Scholarship Council.

PC

Sterols as New Acaricides against Rhipicephalus (B.) annulatus Ticks Infecting Cattle in Egypt

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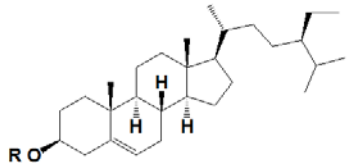
Biologically-guided isolation of natural acaricides to control *Rhipicephalus (Boophilus) annulatus* infesting cattle in Egypt led to the isolation of β-sitosterol and β-sitosterol-3-*O*-glucosid as powerful acaricides. Adult and larval immersion tests of the total alcohol extract of *Mesembryanthemum forsskaolii* Hochst. Ex. Boiss herb in addition to successive solvent extracts of different polarities (*n*-hexane, $CHCl_3$, and MeOH) were performed. The *n*-hexane fraction showed 100% mortality at 5% concentration after 24 hrs exposure. Phytochemical investigation of the *n*-hexane extract led to the isolation β-sitosterol and β-sitosterol-3-*O*-glucosid as major components which were retested for their acaricidal activity. In a concentration of 25 mg/ml; β-sitosterol showed 86.6±5.57% and 91.6±2.88% adulticidal and larvicidal activity respectively while β-sitosterol-3-*O*-glucosid recorded 76.66±5.57% and 98.33±2.88%.



	R
β-sitosterol	H
β-sitosterol-3-*O*-glucosid	β-D-glucose

Fig. 1: Structure of steroids isolated from *M. forsskaolii* herb.

Authors would like to thank Dr. Shawky M Aboelhadid for his advice during the course of the acaricidal study and the veterinarians who helped to get the ticks samples.

PC

Antimicrobial Natural products from Selected Egyptian Plants Extracts

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This study aimed to investigate the antimicrobial potentials of the 17 alcohol extracts of ten plant organs collected from Egypt. The tested organs included the leaves of: (i) *Araucaria columnaris, (ii) Cycas revoluta male plant, (iii) Dioon edule, (iv) Hordeum vulgare;* the seeds and female cones of *Cupressus sempervirens;* the pericarps of *Pisum sativum;* the herbs of *Reaumuria hirtella jaub;* the flowers of *Terminalia arjuna*; the fruits of *Thevetia peruviana;* and the leaves and fruits of *Thuja orientails.* Using the agar well diffusion method, the antimicrobial activities of these extracts were tested against standard strains of *Candida albicans, Sarcina lutea, Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Mycobacterium pheli, Escherichia coli, and Pseudomonas aeruginosa.* Extracts from *Terminalia arjuna* and *Reaumuria hirtella* showed the most potent antibacterial and anti-*Candida* activities, and thus were subjected to solvent-solvent

fractionation using solvents of different polarities (*n*-hexane, dichloromethane (DMC), ethyl acetate (EtOAc) and *n*-butanol saturated with water). Among the tested extracts, the EthOAc extract of *Terminalia arjuna* showed maximum activity against *C. albicans*, Chromatographic isolation of the natural products from the active EtOAc extract of *Terminalia arjuna* afforded the isolation of apigenin, methyl gallate and β-sitosterol-3-*O*-glucoside. Our results indicate that some varieties of the extracts of plants collected from Egypt have the potential to be effective inhibitor of clinically important organisms.

PC

Secondary Metabolites of Colvillea racemose

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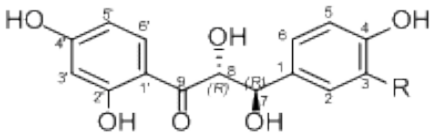
Two new compounds; (7*R*,8*R*)-4,7,8,2′,4′-pentahydroxydihydrochalcone (**3**) and (7*R*,8*R*)-3,4,7,8,2′,4′-hexahydroxydihydrochalcone (**4**) were isolated along with twelve known compounds for the first time from *Colvillea racemosa*, a monotypic genus native to Madagascar. The isolated known compounds include two furofuran lignan glycosides, two flavone-C-glycosides, three flavanones, two flavones, one chalcone, and two triterpenoids. The structures of the isolated compounds were elucidated by spectroscopic analysis, including 1D and 2D NMR, and HRESIMS. The absolute configurations were established by analysis of their electronic circular dichroism ECD and NOESY spectroscopic data.



3 R= H
4 R= OH

The project was supported by Egyptian Government and National Center for Natural Products Research, The University of Mississippi.

PC

A Novel Phytosynthesis, characterisation and antibacterial effect of plant-mediated silver nanoparticles using Atriplex patulum by Microwave Irradiation

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Production of advanced materials from nanomaterials is the main goal of nanotechnology. Silver nanoparticles have attracted tremendous interest due to their applications in bio-sensing, their antimicrobial activity and use in biomedical treatments [1,2,3]. In this study the biosynthesis of silver nanoparticles using *Atriplex atulum* leaves extract as a reducing agent by microwave irradiation method and its antimicrobial properties has been reported. The advantage of using microwave irradiation is it takes less time to reduce the silver ions. The phytosynthesized silver nanoparticles was characterized by FT-IR, UV-Vis, XRD, and TEM analysis. FT-IR spectroscopy revealed that silver nanoparticles were functionalized with biomolecules that have primary amine group ($-NH_2$), carbonyl group, -OH groups and other stabilizing functional groups. The surface plasmon resonance (SPR) band was observed at 465 nm for synthesized silver nanoparticles. 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. The morphological study of silver nanoparticles using TEM suggests that the nanoparticles are spherical in shape with a diameter around 40-nm. 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 zone of inhibition increased with the increase in the concentration of silver nanoparticles. Further, efficient antimicrobial activity of the synthesized silver nanoparticles proves the application potential of green synthesis in the area of nano-medicine.

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

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PC

A Facile One pot of Green synthesis of Silver Nanoparticles using Buxus hyrcana Extract

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Nanomaterials research is an emerging field in the area of medicine, and the biosynthesis of nanoparticles for many applications is an area of current interest. Among these, silver nanoparticles are attractive due to their potential applications in different fields, especially in biosensors, pharmaceuticals, photonics, catalysis, and biomedicine, with potential for utilization as antimicrobial, antiangiogenic, and anticancer agents [1,2,3,4]. In the present study silver nanoparticles were synthesized from aqueous silver nitrate through a simple and eco-friendly route using leaves of *Buxus hyrcana*, which acted as a reductant and stabilizer simultaneously. The formation of silver nanoparticles was observed by the change of colour from colourless to dark brown by the addition the leaves extract. The synthesized silver nanoparticles were characterized using various instrumental techniques including Fourier Transform Infrared Spectroscopy (FT-IR), ultraviolet-visible spectroscopy (UV-Vis), X-ray diffraction (XRD), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). An absorption band centered around 425 nm was observed, this absorption corresponds to the surface plasmon resonance (SPR) of the silver nanoparticles.

The Fourier Transform Infrared Spectroscopy spectral study demonstrates leaves of *Buxus hyrcana* extract acted as the reducing agent. The structure and composition of silver nanoparticles were analysed by XRD and showed that the AgNPs are crystalline in nature and have face-centered cubic (FCC) geometry. The silver nanoparticles showed spherical structure and their sizes were ranging from 30-70 nm under TEM. Furthermore, this green synthesis approach is rapid and better alternative to chemical synthesis and also effective for the large scale synthesis of silver nanoparticles.

Acknowledgements: I gratefully acknowledge the financial support from the Research Council of Tonekabon Branch Islamic Azad University.

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PC

Screening of Novel Secondary Metabolites from Campsis radicans by Phytochemical Analysis

Ojani S[1], Safari L[1], Moghaddam MN[2], Niknejad HR[3], Jahedian S[4]

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Phytochemicals are non-nutritive plant chemicals that have protective or diseases preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases [1]. The aim of this study was to evaluate the secondary metabolites of ethanolic extract of leaves of *Campsis radicans* belonging to the family *Bignoniaceae*. The leaves of *Campsis radicans* were collected from Tonekabon, Iran and ethanolic extract prepared by microwave assisted extraction (MAE) method. The present study reveals that the preliminary phytochemical analysis of eleven different chemical compounds alkaloids (MayerÊ¼s Test), terpenoids (Salkowski Test), flavonoids (Alkaline Reagent Test), phenols (Ferric Chloride Test), coumarins (Sodium hydroxide Test), tannins (Ferric Chloride Test), cardiac glycosides (Keller-Killani test), quinones (H_2SO_4 Test), di-terpenoids (Copper acetate Test), phlobatannins (HCl Test), and saponins (Foam Test) were tested in ethanolic extract. The results of the phytochemical screening of ethanolic extract of leaves of *Campsis radicans* were alkaloids, flavonoids, Di-terpenoids, saponins, tannins, coumarins, cardiac glycosides, quinones and, terpenoids were present. Therefore, presence of above phytochemicals in *Campsis radicans* can be correlated with its medicinal potential. Similar reports on phytochemical composition of various medicinal plants were made earlier by many workers. However, it is very essential to isolate the bioactive fractions from these major groups so that it can be used further in designing specific drugs.

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

References: [1] Nasir R, Chanda S. (2006) plants Ag Biotech News and Information, 2:211-16.

PC

INVESTIGATION INTO SELECTED TERPENES AS ANTICANCER LEADS

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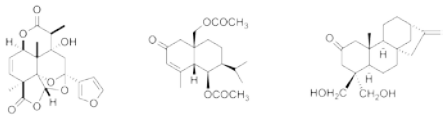
Plants were in the past and still are serving as a good source for providing lots of bioactive compounds including cytotoxic phytochemicals. Doxorubicin, vinca alkaloids and paclitaxel are just few examples.
Three terpenes, saudinolide (**1**), plectranthone (**2**) and psiadin (**3**), were isolated from the dried aerial parts of *Cluytia richardiana*, *Plectranthus cylindraceus*, and *Psiadia arabica*, respectively. The isolated pure compounds were evaluated for their potential antiproliferative activities. Plectranthone (**2**) and psiadin (**3**) exhibited marked growth inhibition on colorectal and hepatocellular cancer cell lines in time- and dose-dependent manner with minimal cytotoxicity against normal human breast cells. The anticancer effects of psiadin on both colorectal and hepatocellular cancer cells were higher than that produced by plectranthone. Saudinolide (**1**) showed very little antimitogenic effects. Comparison with standard antineoplastic drugs indicated that the effects of **2** and **3** were comparable or even better than the tested cytotoxic drugs including 5FU, doxorubicin, camptothecin and ellipticine.



This project was supported by Kuwait University Research Grant PC01/12. Spectral analyses were done at the General Facility, Faculty of Science, Kuwait University, supported by Grant numbers GS01/01 and GS01/03.

PC

Phytochemical and Biological Evaluation of Cichorium intybus L.

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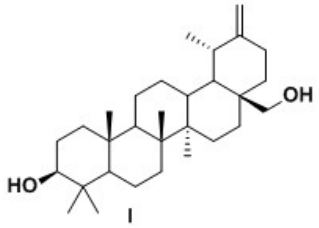
Cichorium intybus L (chichory, Asteraceae family) worldwide grown plant and used as a substitute of coffee. In traditional medicine, this plant is used as diuretic, laxative, anti-inflammatory, digestive, cardiotonic and livertonic. It is also used as an appetizer as well as in the treatment of jaundice, hepatic failure, intermittent fever and mild states of chronic skin diseases. Areal parts of the *C. intybus* was extracted first with super critical fluid (CO_2), followed by maceration with ethanol. Chromatographic purification of the super critical

fluid extract on silica gel column led to isolation of one new triterpene (**I**) and three known compounds: usnic acid, β-sitosterol, β-Sitosterol-3-O-(6′-oleoyl glucoside) (sitoindoside II). Repeated silica gel column chromatography purification of ethanolic extract yielded three known compounds: β-sitosterol-3-O-glucoside, 1,3-diolylglycerate and 11β-13-dihydrolactucin. All the isolated compounds were identified by using ^{1}H and ^{13}C NMR and MS spectral data. All the extracts, fractions and isolated compounds were submitted for biological studies to check for cannabinoid and opioid receptor binding.



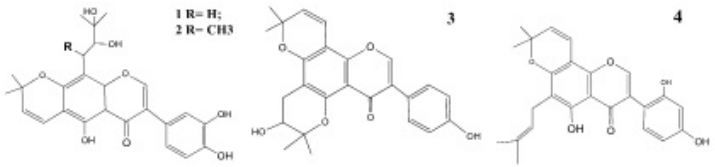
The project was supported by Award Number P20GM104932 from the National Institute of General Medical Sciences and in part by NCNPR.

PC

PRENYLATED ISOFLAVONOIDS FROM MACLURA AURANTIACA GROWING IN KAZAKHSTAN

Yerkebulan Orazbekov[1-3], Serjan Mombekov[2], Ubaidilla Datkhayev[2], Bauyrzhan Makhatov[3], Samir A.Ross[1,4]*
[1]National Center for Natural Products Research and [4]Department of BioMolecular Science The University of Mississippi, University, MS 38677, USA. [2]Kazakh National Medical University, Almaty, Kazakhstan, [3]South Kazakhstan Pharmaceutical Academy, Shymkent, Kazakhstan.

Prenylated isoflavonoids are the most interesting compounds found in *Maclura Aurantiaca* sp. because of their potent pharmacological properties. However, phytochemical or biological properties *Maclura Aurantiaca* growing in Kazakhstan has not been sufficiently examined.
Fourteen prenylated isoflavonoids have been isolated from the ethanolic extract of *Maclura aurantiaca* fruits. Four of them are new (**1-4**). The structures of the isolated compounds were elucidated by spectroscopic analyses, including 1D and 2D NMR.



The project was supported by both Kazakhstan Government and National Center for Natural Products Research, The University of Mississippi, USA.

PC

Cytotoxic activity of ethanol extract of phlomis salicifolia growing in Kazakhstan

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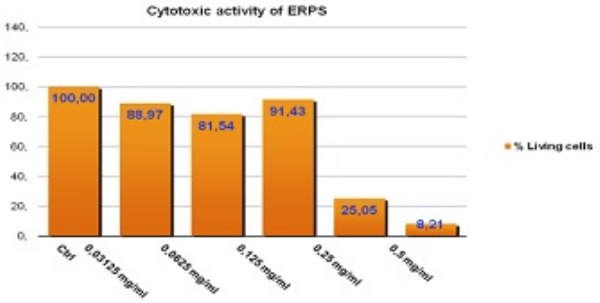
In traditional medicine *Phlomis Salicifolia has been reported* in treatment of arterial hypertension, anemia, cardiovascular disease, respiratory diseases, pneumonia, tuberculosis, and as an antimicrobial. However, phytochemical or biological properties *Phlomis Salicifolia* growing in South Kazakhstan has not been sufficiently examined. Aerial part of the plant and roots of *Phlomis Salicifolia* with different concentration investigated on cytotoxic activity. As a result of research, 96%-ethanol extract of root has shown potent cytotoxic activity against the human HeLa cells with 0,25mg/ml and 0,5mg/ml (**Fig. 1**):



Acknowledgment: The project was supported by Kazakhstan Government and also thanks to Tuscia University, Viterbo, Italy for carrying out the cytotoxic activity test.

PC

MECHANISM OF ACTION AND INHIBITION KINETICS OF THE ENDOCANNABINOID VIRODHAMINE AND OTHER EICOSANOIDS ON HUMAN MONOAMINE OXIDASE (MAO) -A AND -B

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The human endocannabinoid system plays an important role in the pathophysiology of various neurological disorders, such as anxiety, depression, neurodegenerative diseases, and schizophrenia; however, little information is available on the coupling of the endocannabinoid system with the monoaminergic systems in the brain. In the present study, we tested four endocannabinoids and two anandamide analogs for MAO-A and -B activities. Virodhamine inhibited both MAO-A and -B (IC_{50} values of 38.70 μM and 0.71 μM, respectively) with ~54-fold greater inhibition of MAO-B. Two other endocannabinoids (noladin ether and anandamide) also showed greater inhibition of MAO-B with MAO-B IC_{50} values of 18.18 μM and 39.98 μM, respectively. Virodhamine was further evaluated for its kinetic characteristics and mechanism of inhibition

of recombinant human MAO-A and -B. Virodhamine inhibited MAO-B (K_i value of 0.258 ± 0.037 μM) through a mixed mechanism/ irreversible binding. A molecular modeling study with MAO-B predicted virodhamine's terminal $-NH_2$ group would be positioned near the N5 position of flavin adenine dinucleotide (FAD), but that its terminal alkyl group would face towards FAD in MAO-A; this difference could explain virodhamine's higher potency and preference for MAO-B. The docking scores of the computationally-predicted poses also agree with the experimental IC_{50} results. These findings warrant further *in vivo* evaluation of virodhamine for the treatment of neurological disorders.

This publication was made possible by Grant Number P20GM104932 and R15GM119061 from the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health (NIH). Supercomputer support is acknowledged from NSF MRI 1338056 and the Mississippi Center for Supercomputer Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NIGMS or NIH or NSF. This investigation was conducted in part in a facility constructed with support from the Research Facilities Improvements Program (C06RR14503) from the National Institutes of Health (NIH) National Center for Research Resources.

PC

APPLICATION OF MOLECULAR DYNAMICS TO ASSESS THE BINDING ORIENTATION OF THE NEO-CLERODANE DITERPENOID SALVINORIN A WITHIN THE KAPPA-OPIOID RECEPTOR

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Salvinorin A is a non-nitrogenous opioid receptor agonist which selectively and potently activates the kappa-opioid receptor (KOR). Molecular modeling and site-directed mutagenesis studies have been employed to investigate the mechanism of KOR–salvinorin A binding interactions; however, the precise orientation of salvinorin A within KOR is not well established. In the present study, we explored the potential binding-site orientation and interactions of salvinorin A within KOR using computational approaches including homology modeling of the active state of KOR, docking, and molecular dynamics. To gain insight regarding potential binding orientations of salvinorin A within KOR, we performed docking of salvinorin A on an active state model of KOR derived from the active state X-ray crystal structure of the mu-opioid receptor (MOR) (PDB ID: 5C1M). We evaluated two previously-published proposed orientations of salvinorin A within KOR: one in which the furan moiety is directed towards the extracellular region and another in which the furan moiety is directed towards the intracellular region. The docking orientations were compared to previous studies. We performed 200 nanosecond molecular dynamics simulations of the commencing from the best docked pose for each of the two orientations for KOR–salvinorin A, including POPC membrane, water and appropriate ions. The molecular dynamic results combined with accurate estimates of the binding free energy indicated that the intracellular orientation of the furan moiety of salvinorin A within KOR is more stable than the extracellular orientation and matches better to available

experimental mutagenesis data. The results of this work may be helpful for design of new salvinorin A analogs that fit optimally into the KOR binding pocket.

This publication was made possible by Grant Number P20GM104932 and R15GM119061 from the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health (NIH). Supercomputer support is acknowledged from NSF MRI 1338056 and the Mississippi Center for Supercomputer Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NIGMS or NIH or NSF. This investigation was conducted in part in a facility constructed with support from the Research Facilities Improvements Program (C06RR14503) from the National Institutes of Health (NIH) National Center for Research Resources.

PC

New Bioactive Metabolites from the Marine Sponge Monanchora clathrate

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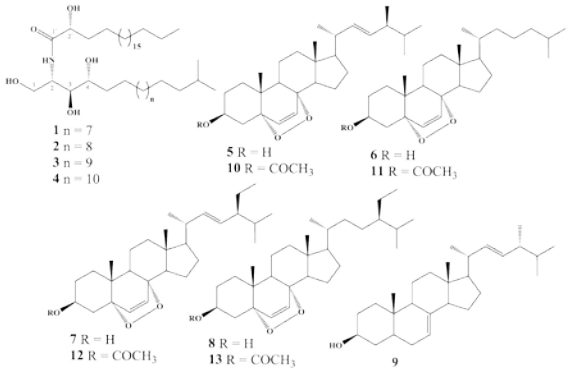
Marine sponges for the past decades have been considered as a very fertile field for the discovery of bioactive natural chemical substances. Chemical investigation of the sponge *Monanchora clathrata* afforded four new compounds (**1**-**4**), along with the known compounds (**5**-**9**). Acetylation of compounds **5**, **6**, **7** and **8** yielded two new chemically modified compounds (**12**, **13**) in addition to the known compounds (**10**, **11**). Compounds **5** and **9** showed strong antitrypanocidal activity with IC_{50} values of 4.15 and 7.67 μg/mL, respectively comparing to α-difluoromethylornithine (DMFO).



We are grateful to the Egyptian government for partially funding.

PC

Chemical and Biological Studies of the Tunicate Polyclinum constellatum Collected from the Red Sea

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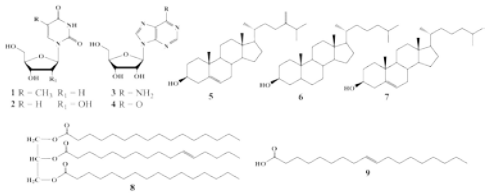
Tunicates are known to be a rich source of chemically diverse secondary metabolites with remarkable biological activities. Chemical investigation of the Red Sea tunicate *Polyclinum constellatum* afforded nine compounds, identified as thymidine (**1**), uridine (**2**), adenosine (**3**), inosine (**4**), 24-methylene cholesterol (**5**), dihydrocholesterol (**6**), cholesterol (**7**), 1,3-palmityl-2-palmitoleoylglycerol (**8**) and oleic acid (**9**). Compound **5** showed potent antitrypanocidal activity with IC_{50} and IC_{90} values of 3.45 and 6.75 µg/mL, respectively compared to *α*-difluoromethylornithine (DMFO).



We are grateful to the Egyptian government for partially funding.

PC

Isolation and Biological Analysis of Aerial Parts of Salvia Aethiopis

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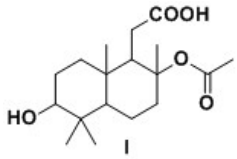
Salvia aethiopis, (Lamiaceae) is a wild herb better known as Mediterranean or African sage. The plant has been historically used in treatment for tumors, and the roots were found to have antimicrobial activity. Its essential oil has antioxidant and antibacterial activity. Although the plant is not native to the United States, it was introduced to many states along the west coast. The ethanolic extract of aerial parts of *S. aethiopis* showed moderate level of inhibition in cannabinoid (CB1-37.0% and CB2- 31.0% displacement) and opioid (Delta -46.3%, Kappa-45.3 % and Mu-32.9% displacement) receptors. Repeated silica gel column chromatography using ethyl acetate/hexane and methanol/dichloromethane solvent system, resulted in isolation of one new (**I**) and three known compounds: spathulenol, *β*-sitosterol, and *β*-sitosterol glucoside, which were identified by using ^{1}H and ^{13}C NMR and MS spectral data. The isolated compounds showed no activity towards cannabinoid and opioid receptor assay.



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PC

Curcumin Nanoparticles : An alternative natural medicine for cancer

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Over recent years major developments and improvements have been made in the field of novel drug delivery systems. Therapeutic benefits of some new drug delivery system include decreasing dosage frequency , controlling the site of release and maintaining constant drug levels. Curcumin, a natural polyphenolic phytoconstituent, is isolated from *Curcuma longa* Linn. (Zingiberaceae) and has numerous pharmacological activities. In this research article we have emphasized the novel part of delivery system of hydrophobic natural drug i.e. ,Curcumin and have discussed its antioxidant property .Curcumin polymeric nanoparticles by solvent evaporation method were formed by using Eudragit S100 as polymer. As curcumin is hydrophobic in nature, its solubility has been one of the main issues for its formulation and development. Chloroform has been used as a solvent for solubility of curcumin and it was found that in chloroform 48.2mg/ml of curcumin was soluble in it. Curcumin possess various therapeutic effects and antioxidant property is one. We have performed antioxidant property of all formulations using DPPH method. The conclusion made from this work was that nanoparticles of curcumin of best batch show size range of 98.32 nm ,the CDR was 78.64 % and surface morphological smoothness of SEM images of the formulated nanoparticles clearly depicted uniformity in the sizes of the processing variables used. Also,the free radical scavenging activity of the optimized batch was found to be 93.3% which ensures about their potential antioxidant property and make these curcumin nanosphere as sustained release alternative treatment for cancer effectively.

I would like to acknowledge our Director, Prof.AC Rana for his valuable suggestions time to time for stimulating our research work through ensuring the availability of chemicals, animal cadavers and equipment. Moreover, we also convey our sincere thanks to our worthy Vice-Chancellor for providing us well equipped labs and library facilities with advanced open access journals.

PC

Phytochemical and biological investigations of Orobanche crenata Forssk., Fam.: Orobanchaceae growing in Egypt

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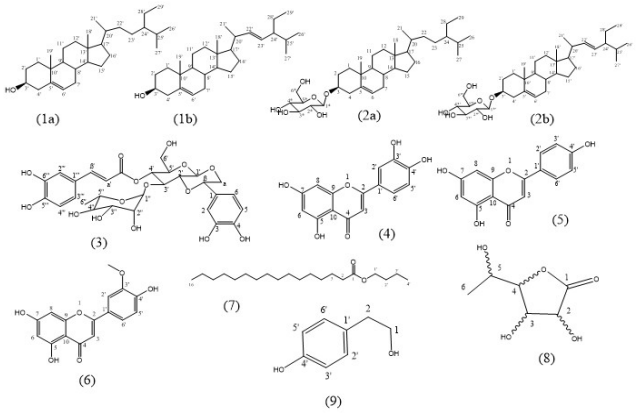
Orobanche crenata Forssk. is a root parasitic plant that depend on host completely, attacking roots of some plants belonging to solanaceae, leguminose and other families. Phytochemical investigation of the whole plant afforded the isolation of a mixture of β-sitosterol and stigmasterol(1a and 1b) and a mixture of their glycosides(2a and 2b), phenyl propanoid glycoside crenatoside (3),three flavonoids, luteoline(4), apigenin(5),chrysoeriol(6) first reported in genus and n-butylpalmitate(7) of first occurrence in family, rhamnonic acid 1,4 lactone(8) and tyrosol(9).The isolated compounds were identified by physical and chemical analyses as well as intensive spectroscopic investigation (1D, 2D NMR and UV). The calculated LD_{50} for the total extract was 3 g/kg indicating that it is safe and doses used were 1.5, 3, 5 g/kg.The total methanolic extract, ethyl acetate, aqueous, and butanol fractions of *O. Crenata* were found to have hepatoprotective activity against CCl_4 induced liver toxicity and showed a decrease in liver enzymes and bilirubin levels in blood and that the ethyl acetate fraction (300 mg/kg) was the most active, compared to silymarin (100 mg/kg) as reference. These results were confirmed by histopathological examination of liver tissue treated with above mentioned fractions. Concomitantly, the ethyl acetate and hexane fractions showed anti-inflammatory activity in carrageenan-induced paw edema in rats with 40.75 and 36.85percentages of inhibition, respectively, persisting for 5 hours in correspondence to 8 mg/kg indomethacin (48.73 % inhibition).On the other hand, the *n*- hexane fraction (300 mg/kg)demonstrated higher antipyretic activity using yeast-induced pyrexia method in comparison with acetyl salicylic acid (150 mg/kg).These findings promote the future perspective of *Orobanche crenata* as hepatoprotictive, anti-inflammatory and anti-pyretic agent.



PC

Antimicrobial activity of isolated compounds from Abutilon hirtum (Lam.) sweet leaves cultivated in Egypt.

Alshymaa A. Gomaa[1], Mamdouh N. Samy[1], Amira S. Wanas[1,2], Samar Y. Desoukey[1], Mohamed S. Kamel[1.]

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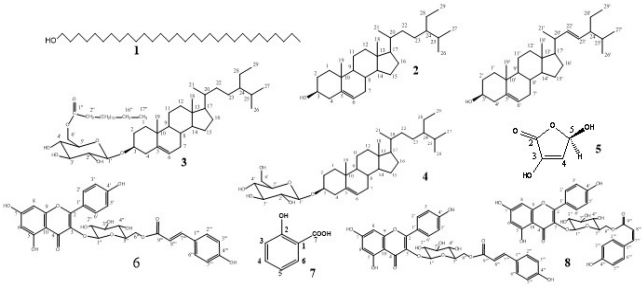
Eight compounds were isolated from the leaves of *Abutilon hirtum* (Lam.) Sweet, and identified as: triacontanol (**1**), β-sitosterol and stigmasterol (**2**), β-sitosterol 3-*O*-(6'-O-heptadecanoyl)-β-glucopyranoside (**3**), β-sitosterol 3-*O*-β- glucopyranoside (**4**), 3,5-dihydroxyfuran-2(5H)-one (**5**), Kaempferol 3-*O*-β- (6"-*E*-*p*-coumaroyl) glucopyranoside(**6**), Salicylic acid (**7**) and kaempferol 3-*O*-β-(6"- *E* -*p*-coumaroyl)-glucopyranoside and kaempferol 3-*O*-β-(6"- *Z* -*p*-coumaroyl)-glucopyranoside (**8**) using MS and NMR spectral analyses. All the isolated compounds were reported for the first time from this plant, and compounds (**3**) and (**5**) were isolated for the first time from family Malvaceae. Compounds **6,7&8** showed antifungal and moderate antibacterial activities which could be promising antimicrobial agents from a natural source known for wide safety margins and lower side effects.



PC

Effect of Salicylic acid and drought stress on the secondary metabolites using gene expression of key enzymes involved in flavonoid and coumarins biosynthesis pathway and UPLC/MS for alkaloids in the leaves of Rutagraveolens L.

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The objective of this study is to evaluate the effect of foliar application of 0.0 and 1.5 mM salicylic acid (SA) under normal [100 % field capacity (FC)] and reduced irrigation (75, 50 and 25% FC) on the expression level of genes of key enzymes involved in biosynthesis pathway of flavonoids; flavonol synthase (FLS) and coumarins; prenyl transferase (PT) in addition to the secondary metabolites of the leaves of *R. graveolens* L. in field condition. The HPLC/ UV analysis showed that the highest yield of rutin (84.42 mg /g dried extract) was obtained from the leaves of plants grown in 25% FC treated with 1.5 mM SA. Similarly, coumarins from the leaves of plants maintained under the same conditions provided the highest percentage of umbelliferone, xanthotoxin and bergapten (34.181, 37.176 and 1.223 mg/g dried extract) respectively. Semi-quantitative RT-PCR analysis showed that drought stress increased the expression level of FLS and PT genes. As maximum relative expression level was observed following1.5 mM SA treatment under 25 % FC, where *FLS* and PT relative expression levels were3.8 and 1.85 times as compared to control, respectively. Moreover, UPLC/MS analysis indicated that biologically active alkaloids were affected by %FC and not by

SA, where the majority of the alkaloids were increased with 75 % FC and decreased with 50 and 25 % FC,except for chalepin accumulation which was increased at all concentrations, while chalepensin and gravacridonochlorine was decreased at 75%FC as compared to the control.

PC

Metabolomics and dereplication-based identification of natural products from three Malvaceae plants.

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Malvaceae (*Hibiscus* or Mallow family) is a large family of flowering plants containing around 243 genera representing 4,225 species, including herbs, shrubs, and trees. The crude extracts as well as the individual fractions (Pet. ether, DCM, and EtOAc) of three Malvaceae plants (*Malvaviscus arboreus*, *Hibiscus mutabilis*, and *Hibiscus schizopetalus*) cultivated in Egypt, were subjected to metabolomic analysis using analytical techniques of high resolution LC-MS. Principal Component Analysis (PCA) was used to evaluate the HRFTMS data of crude extracts and fractions from the three plants. Several compounds were characterized such asrutin, quercetin, kaempferol 3-xylosylglucoside, cyanidin 3-rutinoside, and peonidin 3-O-glucoside, while some other compounds were not identified by the database. Testing for the anti-trypanosomal activity against *Trypanosoma bruceibrucei* showed that the DCM fraction of *M. Arboreus* and the EtoAc fraction of *H. schizopetalus* exhibited good activities with IC_{50} values of 6.3and9.3µg/ml, respectively. Besides, the EtoAc fraction of *H. schizopetalus* showed higher antioxidant activity (IC_{50}= 25.8 µM) compared to the EtoAc fractions of *H. mutabilis* (IC_{50}= 30.5 µM) and *M. arboreus* (IC_{50}= 40.0 µM) as evaluated by the FRAP assay. These findings demonstrated the feasibility and efficacy of utilizing metabolomic analysis for screening and dereplication of some phytoconstituents from higher plants.

PC

Biomarkers discovery for distinguish acute pancreatitis patients from healthy controls by serum metabolomics approach

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TCM and Ethnomedicine Innovation & Development Laboratory, Sino-Luxemburg TCM Research Center, School of Pharmacy, Hunan University of Chinese Medicine, Changsha, 410208, P. R. China[1]

Acute pancreatitis (AP) is an inflammatory condition associated with a progressive systemic inflammatory response (SIRS), and it is an acute disaster of the abdomen with high morbidity and mortality without specific therapeutic measurements. Nevertheless, the pathogenesis of AP remains unclear and is hard to early and accuracy diagnosis of AP in the clinical, as some reasons would interfere the clinical phenotypes and symptoms. Since biomarker discovery as a key step in metabolomics research could be useful for medical guidance. Therefore, this study investigated the GC-MS-based metabolomic research to identify potential biomarkers to help the diagnosis of AP. All serum samples from 63 Chinese individuals, including 26 acute pancreatis patients and 37 healthy controls were performed to obtain 44 metabolites. The internal standard 2-isopropylmalic acid was used for normalization in relative quantitative analysis. Differences in

serum metabolites between the two groups were detected by Principal component analysis (PCA), Partial least-squares discriminate analysis (PLS-DA) and Random forest (RF). Compared to the HC group, AP has a particular metabolic profile. 3-hydroxybutyric acid, citric acid, D-galactose, D-mannose, D-glucose, hexadecanoic acid, phosphoric acid, glycerol and serotonin could be potential biomarkers of early diagnose AP.

Acknowledgments: This work was supported by Hunan Department of Science and Technology (grant numbers 2014FJ1007 and 2014SK4037) and State Key Laboratory of Chinese Medicine Powder and Medicine Innovation in Hunan (Incubation) are acknowledged (grant number ZYFT201408).

PC

A new regulation strategy with tween 80 for enhancing pachymic acid production and its mechanism

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Poria cocos is a famous medicinal fungi widely used in China due to its effects of eliminating dampness and diuresis, strengthening the spleen and stomach, nourishing heart and tranquilizing mind. However, the unstable quality of cultivated *Poria cocos* has seriously influenced the quality of medicine containing *Poria cocos*, so it is important to improve the active ingredient content in *Poria cocos*. Among the main active ingredients of Poria cocos, pachymic acid is regarded as the most effective ingredient for its significant anti-oxidant, anti-inflammatory and anti-tumor activity. So, the research of enhancing pachymic acid content is very urgent. In previous study, we found high viscosity phenomenon during batch fermentation process of Poria cocos seriously restricted cell growth and triterpenoids biosynthesis. To avoid it, effects of four different chemicals including n-hexane, n-dodecane, tween 80 and triton X-100 used as oxygen carrier were investigated. The data showed that adding 1% (V/V) tween 80 at 48h after inoculation can reach maximum pachymic acid concentration (989.52μg/L), which was 1.25 fold of control system. Real-time quantitative PCR technology was also employed to analyze the transcription level of fps (encode farnesyl pyrophosphate synthase), sqs (encode squalene synthase) and ls (encode lanosterol synthase) affecting pachymic acid biosynthesis. The results suggested that tween 80 can efficiently up-regulation of fps, sqs and ls by 42.1, 48.6 and 20.6 percent, respectively. These experimental data can provide an important basis for rational improving pachymic acid production in both fermentation processes and artificial cultivation of *Poria cocos*.

Acknowledgments: This work was supported by *Chinese Medicine Research Program of Hunan Province, China (201621).*

PC

Anti-oxidant Activities of Extraction parts and its Chemical Constituents from Kadsura coccinea (Lem.) A.C. Smith

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Kadsura coccinea (Lem.) A.C.Smith, belonging to the family Schisandraceae within the genera *kadsura*, is a common folk medicinal plants in China. Lignans and triterpenoids are mainly chemical constituents which some have shown anti-oxidant, anti-tumor, anti-HIV, anti-inflammatory and other pharmacological effects. Using DPPH radical scavenging and hydroxyl radical scavenging, to determine the total extract, petroleum ether, dichloromethane, ethyl acetate, *n*-butanol extracts at different concentrations of antioxidant activities. The results showed a good dose-concentration relationship and each parts also show antioxidant activities. In addition, antioxidant activities of dichloromethane and *n*-butanol extracts were better than other extracts. Dichloromethane extracts have led to the isolation of 14 compounds. Antioxidant capacity of different extracts of the roots of *K. Coccinea* was evaluated *in vitro* for the first time.
Acknowledgments: This work was supported by the Efficient Platform Open Innovation Fund Project of Hunan Province of China (13k077), State Key Laboratory of Chinese Medicine Powder and Medicine Innovation in Hunan (Incubation) (ZYFT201403, ZYFT201408), and Hunan Province Key Subject Open Funded Project (Grant number 07).

PC

Chemical Constituents from Root of Rosa laevigata Michx.

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The genus *Rosaceae* comprises about 200 species all over the world, among them about 95 species occur in China. *Rosa laevigata* Michx. an evergreen climbing shrub, is widely distributed throughout southern China. The roots of *R. laevigata* are used as folk medicine of Hunan, Guangdong and Guangxi provinces to cure pelvic inflammation, ascending infection, irregular vaginal bleeding, cervical erosion, and cervicitis. It has been reported that the extract of *R. laevigata* has many active functions, such as antitumor, anti-inflammatory, antibacterial antipyretic-analgesic increase the hypoxiatolerance, hemostasis and anti-diarrhea activities. Phytochemical studies on *R. laevigata* have reported the existence of triterpenoids, flavonoids and tannins in this plant. Keeping in view of its traditional medicinal importance and a few phytochemical investigations, we carried out the phytochemical studies of this plant. The present chemistry investigation resulted in the isolation and identification of one new triterpenoid saponin from the ethanol extract of the root of *R. Laevigata* together with nine known compounds.
Acknowledgments: This work was supported by the Natural science foundation of Hunan province (Grant number 2016JJ6118), Scientific Research Fund of Hunan Provincial Education Department (15C1036), Administration of Traditional Chinese Medicine of Hunan Province (201673) and Hunan Province TCM Key Subject Open Funded Project (zy201503) .

PC

Phytochemical Constituents from the Flower Buds of Aquilaria sinensis

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Aquilaria sinensis is widely distributed in southern China such as Guangdong, Guangxi, Hainan and Fujian provinces, and the Taiwan district [1]. The resinous wood of A. sinensis which is called 'Cheng-Xiang' in China plays an important role in traditional Chinese medicine for usage as digestive, analgesic, sedative and antiemetic agents [1-3]. Up to date, no phytochemical and biological researches were carried out on the flowers of this species, although this part has been widely used as a healthcare herb tea. In order to comprehensively understand the healthcare function and to fully utilize the plant, a chemical investigation was carried out on the flower buds of A. sinensis, leading to the discovery of nine benzophenone glycosides, including four new ones. Their structures were elucidated on the basis of 1D and 2D NMR and HRESIMS spectra. Antimicrobial, antimalarial, antileishmanial, anticancer, and iNOS inhibitory activities were evaluated for all isolated compounds.
This work was supported in part by "Science Based Authentication of Dietary Supplements" funded by the Food and Drug Administration grant numbers 1U01FD004246-05, the United States Department of Agriculture, Agricultural Research Service, Specific Cooperative Agreement No. 58-6408-1-603-07 and National Natural Science Foundation of China (81374062 and 85673179).

PC

Flavanoids from the Tujia ethnomedicine Ji Xue Qi

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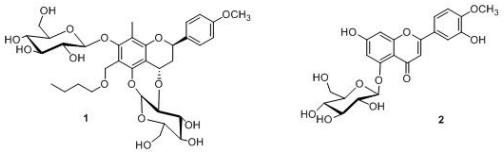
Ji Xue Qi origins from the rhizome of *Pronephrium penangianum*, which belongs to the genus *Pronephrium* (Thlypteridaceae). It has been used as a folk medicine to treat irregular menstruation, rheumatism, dysentery and edema in tujia ethnomedicine for many years. Previous phytochemical investigations showed flavan-4-ol glycosides are the mainly constituents in *P. penangianum.* Further phytochemical research on this plant has been carried out and led to the isolation of one new flavan-4-ol glycoside, named (2*R*,4*S*)-5,7-di-*β*-D-glucopyranosyloxy-6-butoxymethyl-4′-methoxy-8-methyl-4,2″-oxo-flavan (**1**), together with a known flavanone glycoside diosmetin-5-*O*-*β*-D–glucopyranoside (**2**) from Ji Xue Qi (Fig. 1). Their structures have been elucidated by a series of spectroscopic analyses.



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PC

New Sesquiterpenes from Calea zacatechichi Schl.

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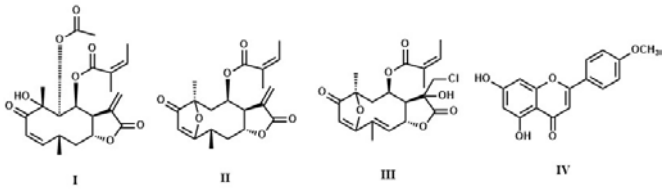
Calea zacatechichi Schl (syn. *C. ternifolia* Kunth) is a flowering plant of the Asteraceae family and well known as dream herb, Mexican calea or bitter grass. This plant has been used in folk medicine to treat cough, asthma, and gastrointestinal tract disorders such as stomachache and diarrhea. Phytochemical studies have identified a group of sesquiterpene lactones and flavonoids that showed anti-inflammatory, antimicrobial, and antileishmanial activities [1-2].

As a part of study on characterization and determination of marker compounds in botanical ingredients of dietary supplements, the methanolic extracts of mixed parts of *C. zacatechichi* was investigated using different chromatographic techniques and resulted in the isolation of two new sesquiterpene lactones along with 11 known germacranolide sesquiterpenoids and 2 flavonoids. The structures of the isolated compounds were elucidated on the basis of extensive analyses of spectroscopic data including 1D and 2D NMR and HR-ESI-MS.

Fig. Typical compounds with different core skeleton in *C. zacatechichi*



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References: [1] Leonti M., Sticher O., *et al.*, (2003) J Ethnopharmacol 88: 119-124. [2] Wu H., Fronczek FR., *et al.*, (2011) Plant Med 77: 749-753.

PC

Bioactive metabolites from *Strumpfia maritima*

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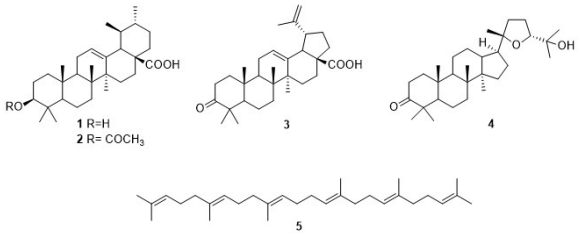
Every year over one million people die from mosquito-borne diseases worldwide. Mosquitoes are vectors of many pathogens that cause serious human diseases including malaria, dengue fever, and yellow fever. In continuation of our efforts to identify new natural repellents that can decrease the incidence of these diseases by reducing mosquito bites. An ethanolic extract of *Strumpfia maritima* was tested for its biting deterrent activities. The extract showed biting deterrent activity in K & D *in vitro* bioassay system. The bio-guided fractionation of this extract led to the isolation of ursolic acid (**1**), acetoxyursolic acid (**2**), betulonic acid (**3**), ocotillone (**4**), and squalene (**5**).



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PC

New 9,10-seco-9,19-cyclolanostane glycosides from *Sutherlandia frutesence*

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Sutherlandia frutescens or cancer-bush belongs to family fabaceae. It is a shrub native to South Africa, Namibia, Botswana and Zimbabwe. In traditional medicine it is known as a medicine for treatment of various diseases [1-3]. The safety of *Sutherlandia* was confirmed through a long term use as traditional medicine with no report of toxicity and the study of acute toxicity of *Sutherlandia* aqueous extract and it passed three clinical trials [4]. Furthermore, double-blind study in 2007 revealed that capsules of *Sutherlandia* leaves powder can be tolerated by healthy adults with a daily dose of 800 mg for three months [5]. The importance of *Sutherlandia frutescens* in folkloric medicine led us for further investigation of phytochemistry of the methanolic extract, which resulted in isolation of five new 9,10-seco-9,19-cyclolanostane glycosides (**1**-**5**). The structures of these compounds were confirmed by using 1D and 2D NMR and HRESIMS.

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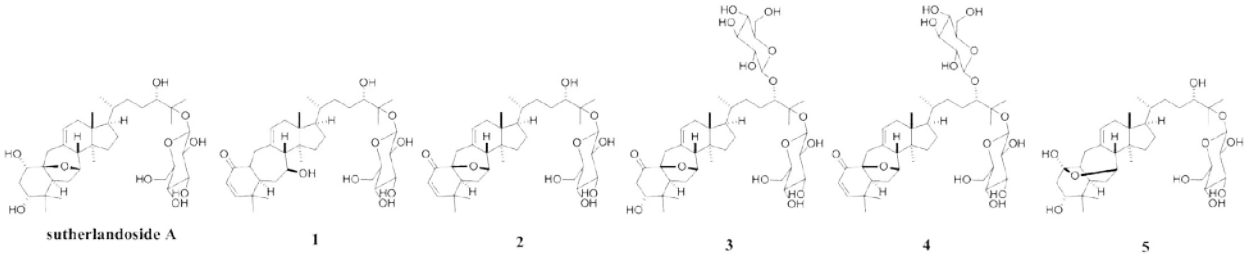

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PC

NMR-based Metabolomics Approach for Investigation of Sceletium tortuosum Variation

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Sceletium tortuosum (L.) N.E. Br. (Mesembryanthemaceae) is indigenous to South Africa and mainly found in the Karroid areas of the Cape region. The plant and its fermented preparation, known as "kanna", "channa" or "kougoed", have been used for centuries by the local communities for treatment of CNS-associated conditions, for quenching thirst, and for relief of hunger. It is also used as a colic remedy for children. *Sceletium* raw materials and various products in different formulations (tablets, extracts, sprays, capsules and tinctures) are currently marketed with claims of psychoactive properties, such as mood uplifting and reduction of anxiety. The aim of this study was to explore the extent of chemotypic variation and the characteristic constituents in *S. tortuosum* specimens collected from different locations in South Africa by using NMR spectroscopy combined with multivariate analysis techniques. Significant metabolite variation was observed in 145 *Sceletium* samples by NMR profiling. The geographic location seemed to be one of factors that affected the metabolite variations in the samples. Pinitol, alkaloids and alkylamines were identified as marker compounds for the differentiation Northern Cape group samples from Western Cape group samples.

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New Benzophenone Glycosides from the Flower Buds of Aquilaria sinensis

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Aquilaria sinensis (Lour.) Gilg (Thymelaeaceae) is an economically important plant and widely planted in southern China. The resinous wood of *A. sinensis,* called 'Cheng-Xiang' in China, plays an important role in TCM for using as digestive, analgesic, sedative and antiemetic agents. The leaves and flowers are consumed as a health tea in China due to their health care function. The extract and constituents from the leaves were found to possess anti-inflammatory activity, laxative effect and inhibitory activity in *vitro* against α-glucosidase. However, no phytochemical and biological studies have been conducted on the flower buds of this plant. In order to comprehensively understand the healthcare function and to fully utilize this plant, a chemical investigation was carried out on the flower buds of *A. sinensis*, leading to the discovery of nine benzophenone glycosides including four new ones.

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PC

Norlignan Glucosides from Hypoxis hemerocallidea (African potato)

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The corm of the *Hypoxis hemeroclladia* has been used as a traditional medicine for centuries in South Africa for the treatment of flu, common cold, adult-onset diabetes mellitus, hypertension, testicular tumors, psoriasis, urinary infections, prostate hypertrophy, internal cancer, HIV/AIDS, and central nervous system disorders. Two new (**1**, **2**), and two known norlignan glucosides (**3**, **4**) were isolated from the hydroalcoholic extract of the corms of *Hypoxis hemeroclladia*. The isolated compounds possess diarylpentanoid carbon skeleton (C6-C5- C6), which were classified as norlignans generated by coupling of C6-C3 and C6-C2 units. Structure elucidation was achieved by means of NMR spectroscopic and mass spectrometric techniques.